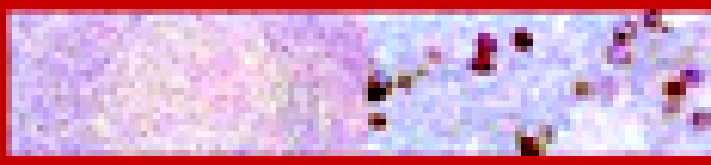
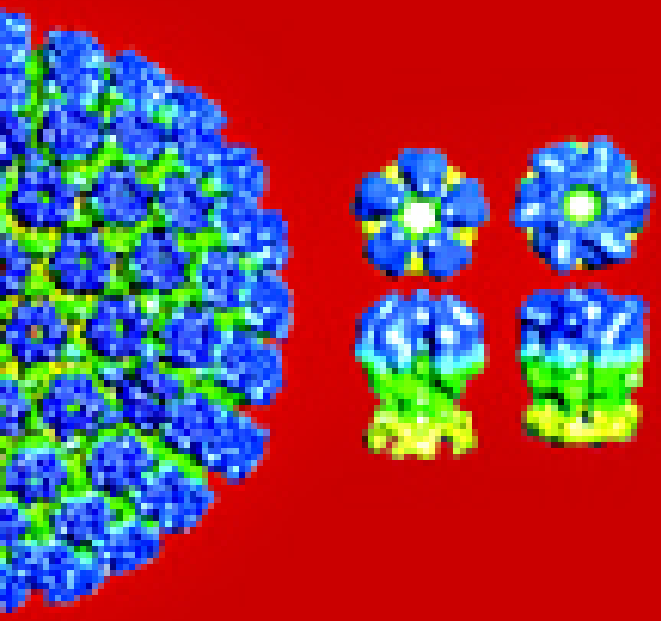


Human Herpesviruses

Biology, Therapy, and Immunoprophylaxis



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Human Herpesviruses

This comprehensive account of the human herpesviruses provides an encyclopedic overview of their basic virology and clinical manifestations. This group of viruses includes human simplex type 1 and 2, Epstein–Barr virus, Kaposi's Sarcoma-associated herpesvirus, cytomegalovirus, HHV6A, 6B, and 7, and varicella-zoster virus. The viral diseases and cancers they cause are significant and often recurrent. Their prevalence in the developed world accounts for a major burden of disease, and as a result there is a great deal of research into the pathophysiology of infection and immunobiology. Another important area covered within this volume concerns antiviral therapy and the development of vaccines. All these aspects are covered in depth, both scientifically and in terms of clinical guidelines for patient care. The text is illustrated generously throughout and is fully referenced to the latest research and developments.

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Preface

Diseases caused by the human herpesviruses were recognized by the earliest practitioners of medicine. Hippocrates, Celsus, Herodotus, Galen, Avicenna and others described cutaneous lesions typical of infections caused by herpes simplex viruses (HSV) 1 and 2, and varicella-zoster virus (VZV). 'Herpes,' the family name of these viruses, is traced to the Greek term for lesions that appeared to creep or crawl over the skin. Among the duties of John Astruc, physician to King Louis XIV, was to understand the diseases of French prostitutes, in Latin, the '*Puellae publicae*', which led to his description of *herpes genitalis*. Distinguishing between genital herpes and syphilis was an obvious concern in this social context as it is now. The modern scientific investigation of HSV can be dated to the work of Gruter, who first isolated the virus and demonstrated its serial transmission in rabbits. During the 19th century, experiments in human subjects showed that HSV and VZV could be transmitted from fluid recovered from HSV and VZV lesions. Demonstrating that Koch's posulates were fulfilled was important but arguably the truly revolutionary discovery about the herpesviruses was made by Andrews and Carmichael in the 1930s who showed that recurrent *herpes labialis* occurred only in adults who already had neutralizing antibodies against HSV. Since our modern understanding of all of the human herpesviruses revolves around latency and reactivation as established facts of their biology, it is important to remember that these concepts are far from obvious and to appreciate the creative insights of Doerr who proposed that recurrent HSV was not an exogenous infection but resulted from stimuli to the cell that caused the endogenous production of a virus-like agent and of Burnet and Williams who perfected the notion that HSV persists for life and "*remains for the most part latent; but under the stimulus of trauma, fever, and so forth it may at any time be called into activity and provoke a visible herpetic lesion.*"

Although their relationships to HSV and VZV were by no means appreciated, the more subtle members of the herpesvirus family began to be discovered after an interval of many hundreds of years. The first of these was human cytomegalovirus (HCMV), which was initially associated with human disease through the detection of enlarged cells containing unusual cytoplasmic inclusions in the urine and organs of infants who were born with signs of intrauterine damage that had been attributed to syphilis. In the early 1950s, HCMV as well as VZV were the first human herpesviruses to be isolated in cultured cells. Within a decade, Epstein-Barr virus (EBV) particles were found in Burkitt's lymphoma cells and EBV was shown to be associated with mononucleosis. By the mid-1990s, three more human herpesviruses, HHV-6A, HHV-6B and HHV-7, which share a tropism for T lymphocytes, were discovered and the etiologic agent of the unusual vascular skin tumor called 'Kaposi's sarcoma, first described in 1872, was identified as "Kaposi's sarcoma-asscoiated herpesvirus (KSHV, HHV8). These four new human herpesviruses were identified during the early years of the human immunodeficiency virus (HIV) epidemic because these viruses caused aggressive disease in HIV-infected patients or were discovered during intensive research on human T cell biology. In each instance, discovery of the human herpesviruses paralleled technologic progress, illustrated by animal models for HSV, cell culture methods for VZV and CMV, the cultivation of B lymphocytes for the detection of EBV and of T lymphocytes for identification of HHV-6 and 7, and differential nucleic acid detection for revealing the existence of HHV8.

Molecular genetics methods demonstrate that the human herpesviruses share a common ancestor. However, each virus has evolved to occupy a particular niche during millions of years of co-evolution with their primate, and eventually human, host. Understanding the nuances of the adaptive strategies that have allowed all of these viruses to be transmitted efficiently and to persist so successfully in the human population, and often in the same individual, constitutes a fascinating enterprise. At the same time, infections caused by these ubiquitous viruses create a substantial global burden of disease affecting healthy and immunocompromised patients and among people living in developed and developing countries. Because of their serious and potentially life-threatening consequences, the human herpesviruses are medically important targets for basic and clinical research.

The goal of this book is to describe the remarkable recent progress towards elucidating the basic and clinical virology of these human pathogens, in conjunction with a summary of the many new insights about their epidemiology, mechanisms of pathogenesis and immune control, approaches to clinical diagnosis and the recognition of the clinical illnesses that result from primary and recurrent herpesvirus infections across the age spectrum. All of the herpesviruses have common genes, structures, replication strategies and mechanisms of defense against the host response but each virus also has unique properties that allow it to find its particular ecological refuge. An unexpected outcome of research over the past decade is the finding that the human herpesviruses have devised many different ways to achieve the same biologic effect, as illustrated by their unique strategies for down-regulation of major histocompatibility complex proteins. Functional similarities exist among these viruses even when they do not share similar genes or infect similar tissues. Each chapter of the book explores these viral themes and variations from the virologic and clinical perspectives. The contributions of the many distinguished authors highlight the basic science aspects of the field, emphasizing the comparative virology of the human herpesviruses and virus-host cell interactions, and the significant clinical developments, including antiviral drugs and vaccines, that are essential for the best practice of medicine in the 21st century. The concluding chapter illustrates how therapies for cancer may emerge from these advances in basic and clinical research, to create a fundamentally new era in the complex history of the relationship between the human herpesviruses and their hosts.

The editors are deeply grateful for the generosity of the authors who have shared their comprehensive knowledge of the human herpesviruses. We hope that this book will serve as a resource for investigators and physicians, and most importantly, that it will motivate a new generation of students and trainees to address the many unresolved questions about these herpesviruses as agents of human disease. Since the genomes of all of these viruses have been sequenced, it is obvious that many genes exist for which functions have not been identified and we now understand that most herpesviral proteins can be expected to have multiple functions. Basic research on the human herpesviruses also reveals fundamental facts about human cellular biology, including surface receptors, metabolic pathways, cell survival mechanisms, malignant transformation as well as innate antiviral defenses. In the clinical realm, every improvement in diagnostic methods expands the spectrum of clinical disorders that are recognized as being caused by these viruses. Clinical interventions exist that could not have been imagined fifty years ago but the need for better therapeutic and preventive measures has become even more apparent as the burden of herpesvirus disease is defined with precision. Given that four human herpesviruses have been discovered in the past 15 years, are there others?

Part I

Introduction: definition and classification of the human herpesviruses

Edited by Bernard Roizman

1

Overview of classification

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Introduction

Taxonomy aims to structure relationships among diverse organisms in order to provide a broader understanding of Nature than is afforded by consideration of organisms in isolation. Since biological systems are shaped by evolution, which is not influenced by the human desire to impose order, any taxonomical scheme is bound to be incomplete and to some extent arbitrary. The criteria applied are necessarily confined to what is technically possible, and thus taxonomy has an important historical component. In addition, taxonomy develops conservatively, since striving for the ideal must be tempered by the need to maintain utility. It is also an unfortunate fact that taxonomy provides fertile soil for debate among a few but is of little interest to most. However, it is beyond dispute that the setting of herpesviruses in a taxonomical framework is vital for understanding the origins and behavior of this fascinating family of organisms.

Historically, herpesvirus taxonomy has been addressed since 1971 by the International Committee on Taxonomy of Viruses (ICTV) (Wildy, 1971). A provisional approach to endowing herpesviruses with formal names (Roizman *et al.*, 1973) was followed by grouping into subfamilies largely on the basis of biological criteria (Roizman *et al.*, 1981). This effort was rather successful, but not free from what turned out in hindsight to be a few misclassifications (Roizman *et al.*, 1992). Further division of the subfamilies into genera utilized molecular data to a greater extent than before, primarily in relation to genome characteristics such as size and structure (Roizman *et al.*, 1992). In the latest report of the ICTV Herpesvirus Study Group (Davison *et al.*, 2005), the family *Herpesviridae* consists of three subfamilies: *Alphaherpesvirinae* (containing the *Simplexvirus*, *Varicellovirus*, *Mardivirus* and *Iltovirus* genera), *Betaherpesvirinae* (containing the *Cytomegalovirus*, *Muromegalovirus* and *Roseolovirus* genera) and *Gammaherpesvirinae* (containing the *Lymphocryptovirus* and *Rhadinovirus* genera). In addition, there is a genus (*Ictalurivirus*) unattached to any subfamily and a large number of species not assigned to genera. The current list is given in Table 1.1. All but one of the viruses assigned to taxa infect mammals or birds, although a substantial number of unassigned herpesviruses have lower vertebrate (reptilian, amphibian and fish) or invertebrate (bivalve) hosts.

Morphological criteria

The primary criterion for inclusion of an agent in the family *Herpesviridae* is that of virion morphology. The virion is spherical, and comprises four major components: the core, the capsid, the tegument and the envelope (see Chapter 3). The diameter of the virion depends on the viral species, but is approximately 200 nm. The core consists of a single copy of a linear, double-stranded DNA molecule packaged at high density into the capsid. The capsid is an icosahedron, and has an external diameter of 125–130 nm. It consists of 162 capsomeres, 12 of which are pentons and 150 hexons, each containing five and six copies, respectively, of the major capsid protein. The capsomeres are joined via the triplexes, each of which contains two copies of one protein and one copy of another. The tegument, which surrounds the capsid, contains perhaps 30 or more viral protein species and is poorly defined structurally. In the tegument, structures positioned with symmetry corresponding to that of the capsid are detectable only in the region close to the capsid. The lipid envelope surrounds the exterior of the tegument, and is studded with at least ten viral membrane

Table 1.1. Herpesvirus taxonomy and nomenclature

Formal name[a]	Abbrev.	Common name[b]	Abbrev.[c]
Subfamily Alphaherpesvirinae			
Genus Simplexvirus			
Ateline herpesvirus 1	AtHV-1	Spider monkey herpesvirus	
Bovine herpesvirus 2	BoHV-2	Bovine mamillitis virus	
Cercopithecine herpesvirus 1	CeHV-1	B-virus	HVB
Cercopithecine herpesvirus 2	CeHV-2	SA8 virus	
Cercopithecine herpesvirus 16	CeHV-16	Herpesvirus papio 2	
Human herpesvirus 1	HHV-1	Herpes simplex virus [type] 1	HSV-1
Human herpesvirus 2	HHV-2	Herpes simplex virus [type] 2	HSV-2
Macropodid herpesvirus 1	MaHV-1	Parma wallaby herpesvirus	
Macropodid herpesvirus 2	MaHV-2	Dorcopsis wallaby herpesvirus	
Saimiriine herpesvirus 1	SaHV-1	Herpesvirus tamarinus	
Genus Varicellovirus			
Bovine herpesvirus 1	BoHV-1	Infectious bovine rhinotracheitis virus	BHV-1
Bovine herpesvirus 5	BoHV-5	Bovine encephalitis virus	BHV-5
Bubaline herpesvirus 1	BuHV-1	Water buffalo herpesvirus	
Canid herpesvirus 1	CaHV-1	Canine herpesvirus	
Caprine herpesvirus 1	CpHV-1	Goat herpesvirus	
Cercopithecine herpesvirus 9	CeHV-9	Simian varicella virus	SVV
Cervid herpesvirus 1	CvHV-1	Red deer herpesvirus	
Cervid herpesvirus 2	CvHV-2	Reindeer herpesvirus	
Equid herpesvirus 1	EHV-1	Equine abortion virus	
Equid herpesvirus 3	EHV-3	Equine coital exanthema virus	
Equid herpesvirus 4	EHV-4	Equine rhinopneumonitis virus	
Equid herpesvirus 8	EHV-8	Asinine herpesvirus 3	
Equid herpesvirus 9	EHV-9	Gazelle herpesvirus	
Felid herpesvirus 1	FeHV-1	Feline rhinotracheitis virus	
Human herpesvirus 3	HHV-3	Varicella-zoster virus	VZV
Phocid herpesvirus 1	PhoHV-1	Harbour seal herpesvirus	
Suid herpesvirus 1	SuHV-1	Pseudorabies virus	PRV
Tentative species in genus Varicellovirus			
Equid herpesvirus 6	EHV-6	Asinine herpesvirus 1	
Genus Mardivirus			
Gallid herpesvirus 2	GaHV-2	Marek's disease virus type 1	MDV-1
Gallid herpesvirus 3	GaHV-3	Marek's disease virus type 2	MDV-2
Meleagrid herpesvirus 1	MeHV-1	Turkey herpesvirus	HVT
Genus Iltovirus			
Gallid herpesvirus 1	GaHV-1	Infectious laryngotracheitis virus	ILTV
Unassigned species in subfamily Alphaherpesvirinae			
Psittacid herpesvirus 1	PsHV-1	Parrot herpesvirus	
Subfamily Betaherpesvirinae			
Genus Cytomegalovirus			
Cercopithecine herpesvirus 5	CeHV-5	African green monkey cytomegalovirus	SCMV
Cercopithecine herpesvirus 8	CeHV-8	Rhesus monkey cytomegalovirus	RhCMV
Human herpesvirus 5	HHV-5	Human cytomegalovirus	HCMV
Pongine herpesvirus 4	PoHV-4	Chimpanzee cytomegalovirus	CCMV
Tentative species in genus Cytomegalovirus			
Aotine herpesvirus 1	AoHV-1	Herpesvirus aotus 1	
Aotine herpesvirus 3	AoHV-3	Herpesvirus aotus 3	

Table 1.1. (*cont.*)

Formal name[a]	Abbrev.	Common name[b]	Abbrev.[c]
Genus Muromegalovirus			
Murid herpesvirus 1	MuHV-1	Mouse cytomegalovirus	MCMV
Murid herpesvirus 2	MuHV-2	Rat cytomegalovirus	RCMV
Genus Roseolovirus			
Human herpesvirus 6	HHV-6		
Human herpesvirus 7	HHV-7		
Unassigned species in subfamily Betaherpesvirinae			
Caviid herpesvirus 2	CavHV-2	Guinea pig cytomegalovirus	GPCMV
Tupaiid herpesvirus 1	TuHV-1	Tree shrew herpesvirus	THV
Subfamily Gammaherpesvirinae			
Genus Lymphocryptovirus			
Callitrichine herpesvirus 3	CalHV-3	Marmoset lymphocryptovirus	Marmoset LCV
Cercopithecine herpesvirus 12	CeHV-12	Herpesvirus papio	
Cercopithecine herpesvirus 14	CeHV-14	African green monkey EBV-like virus	
Cercopithecine herpesvirus 15	CeHV-15	Rhesus lymphocryptovirus	Rhesus LCV
Human herpesvirus 4	HHV-4	Epstein-Barr virus	EBV
Pongine herpesvirus 1	PoHV-1	Herpesvirus pan	
Pongine herpesvirus 2	PoHV-2	Orangutan herpesvirus	
Pongine herpesvirus 3	PoHV-3	Gorilla herpesvirus	
Genus Rhadinovirus			
Alcelaphine herpesvirus 1	AlHV-1	Malignant catarrhal fever virus	AHV-1
Alcelaphine herpesvirus 2	AlHV-2	Hartebeest malignant catarrhal fever virus	
Ateline herpesvirus 2	AtHV-2	Herpesvirus ateles	HVA
Bovine herpesvirus 4	BoHV-4	Movar virus	BHV-4
Cercopithecine herpesvirus 17	CeHV-17	Rhesus rhadinovirus	RRV
Equid herpesvirus 2	EHV-2		
Equid herpesvirus 5	EHV-5		
Equid herpesvirus 7	EHV-7	Asinine herpesvirus 2	
Hippotragine herpesvirus 1	HiHV-1	Roan antelope herpesvirus	
Human herpesvirus 8	HHV-8	Kaposi's sarcoma-associated herpesvirus	KSHV
Murid herpesvirus 4	MuHV-4	Murine gammaherpesvirus 68	MHV-68
Mustelid herpesvirus 1	MusHV-1	Badger herpesvirus	
Ovine herpesvirus 2	OvHV-2	Sheep-associated malignant catarrhal fever virus	
Saimiriine herpesvirus 2	SaHV-2	Herpesvirus saimiri	HVS
Tentative species in genus Rhadinovirus			
Leporid herpesvirus 1	LeHV-1	Cottontail rabbit herpesvirus	
Leporid herpesvirus 2	LeHV-2	Herpesvirus cuniculi	
Leporid herpesvirus 3	LeHV-3	Herpesvirus sylvilagus	
Marmodid herpesvirus 1	MarHV-1	Woodchuck herpesvirus	
Unassigned species in subfamily Gammaherpesvirinae			
Callitrichine herpesvirus 1	CalHV-1	Herpesvirus saguinus	
Unassigned genus Ictalurivirus* in family *Herpesviridae			
Ictalurid herpesvirus 1	IcHV-1	Channel catfish virus	CCV
Unassigned viruses in family Herpesviridae			
Acipenserid herpesvirus 1	AciHV-1	White sturgeon herpesvirus 1	
Acipenserid herpesvirus 2	AciHV-2	White sturgeon herpesvirus 2	
Acciptrid herpesvirus 1	AcHV-1	Bald eagle herpesvirus	
Anatid herpesvirus 1	AnHV-1	Duck plague herpesvirus	
Anguillid herpesvirus 1	AngHV-1	Japanese eel herpesvirus	

(*cont.*)

Table 1.1. (*cont.*)

Formal name[a]	Abbrev.	Common name[b]	Abbrev.[c]
Ateline herpesvirus 3	AtHV-3	Herpesvirus ateles strain 73	
Boid herpesvirus 1	BoiHV-1	Boa herpesvirus	
Callitrichine herpesvirus 2	CalHV-2	Marmoset cytomegalovirus	
Caviid herpesvirus 1	CavHV-1	Guinea pig herpesvirus	
Caviid herpesvirus 3	CavHV-3	Guinea pig herpesvirus 3	
Cebine herpesvirus 1	CbHV-1	Capuchin herpesvirus AL-5	
Cebine herpesvirus 2	CbHV-2	Capuchin herpesvirus AP-18	
Cercopithecine herpesvirus 3	CeHV-3	SA6 virus	
Cercopithecine herpesvirus 4	CeHV-4	SA15 virus	
Cercopithecine herpesvirus 10	CeHV-10	Rhesus leukocyte-associated herpesvirus strain 1	
Cercopithecine herpesvirus 13	CeHV-13	Herpesvirus cyclopis	
Chelonid herpesvirus 1	ChHV-1	Grey patch disease of turtles	
Chelonid herpesvirus 2	ChHV-2	Pacific pond turtle herpesvirus	
Chelonid herpesvirus 3	ChHV-3	Painted turtle herpesvirus	
Chelonid herpesvirus 4	ChHV-4	Argentine turtle herpesvirus	
Ciconiid herpesvirus 1	CiHV-1	Black stork herpesvirus	
Columbid herpesvirus 1	CoHV-1	Pigeon herpesvirus	
Cricetid herpesvirus	CrHV-1	Hamster herpesvirus	
Cyprinid herpesvirus 1	CyHV-1	Carp pox herpesvirus	
Cyprinid herpesvirus 2	CyHV-2	Goldfish herpesvirus	
Elapid herpesvirus 1	EpHV-1	Indian cobra herpesvirus	
Elephantid herpesvirus 1	ElHV-1	Elephant [loxodontal] herpesvirus	
Erinaceid herpesvirus 1	ErHV-1	European hedgehog herpesvirus	
Esocid herpesvirus 1	EsHV-1	Northern pike herpesvirus	
Falconid herpesvirus 1	FaHV-1	Falcon inclusion body diseases	
Gruid herpesvirus 1	GrHV-1	Crane herpesvirus	
Iguanid herpesvirus 1	IgHV-1	Green iguana herpesvirus	
Lacertid herpesvirus	LaHV-1	Green lizard herpesvirus	
Lorisine herpesvirus 1	LoHV-1	Kinkajou herpesvirus	
Murid herpesvirus 3	MuHV-3	Mouse thymic herpesvirus	
Murid herpesvirus 5	MuHV-5	Field mouse herpesvirus	
Murid herpesvirus 6	MuHV-6	Sand rat nuclear inclusion agents	
Ostreid herpesvirus 1	OsHV-1	Pacific oyster herpesvirus	OHV
Ovine herpesvirus 1	OvHV-1	Sheep pulmonary adenomatosis-associated herpesvirus	
Percid herpesvirus 1	PeHV-1	Walleye epidermal hyperplasia	
Perdicid herpesvirus 1	PdHV-1	Bobwhite quail herpesvirus	
Phalacrocoracid herpesvirus 1	PhHV-1	Cormorant herpesvirus	
Pleuronectid herpesvirus 1	PlHV-1	Turbot herpesvirus	
Ranid herpesvirus 1	RaHV-1	Lucké frog herpesvirus	
Ranid herpesvirus 2	RaHV-2	Frog herpesvirus 4	
Salmonid herpesvirus 1	SalHV-1	Herpesvirus salmonis	
Salmonid herpesvirus 2	SalHV-2	*Oncorhynchus masou* herpesvirus	
Sciurid herpesvirus 1	ScHV-2	European ground squirrel cytomegalovirus	
Sciurid herpesvirus 2	ScHV-2	American ground squirrel cytomegalovirus	
Sphenicid herpesvirus 1	SpHV-1	Black footed penguin herpesvirus	
Strigid herpesvirus 1	StHV-1	Owl hepatosplenitis herpesvirus	
Suid herpesvirus 2	SuHV-2	Swine cytomegalovirus	

[a] Type species of genera are in italics.
[b] Some viruses have several common names. Only one is given for each.
[c] The list is restricted to abbreviations used in this publication.
Adapted from Davison *et al.* (2005).

glycoproteins, in addition to some cellular proteins. The protein composition of the tegument and envelope varies widely across the family.

Serological criteria

In contrast to virion morphology, which operates as a criterion at the level of the family, serological relationships are useful only for detecting closely related viruses. Neutralizing antibodies form a subset of serological tools, and are directed against some of the envelope glycoproteins.

Biological criteria

The observation that several distinct herpesviruses have been found in the most extensively studied animals implies that the number of herpesvirus species in Nature must far exceed that catalogued to date. The natural host range of individual viruses is usually restricted to a single species. Occasional transfer to other species can occur, although it could be argued that the settings involved (farms, zoos and keeping pets) are the results of human activities. In experimental animal systems, some members of the *Alphaherpesvirinae* can infect a wide variety of species, whereas *Beta-* and *Gammaherpesvirinae* are very restricted. The same general observation characterizes growth in cell culture.

Herpesviruses are highly adapted to their hosts, and severe symptoms of infection are usually limited to very young or immunosuppressed individuals. Natural transmission routes range from aerosol spread to mucosal contact. Most herpesviruses establish a systemic infection associated with a cell-associated viraemia, although infection with some members of genus *Simplexvirus* is limited to the epithelium at the inoculation site and to innervating sensory neurons. Herpesviruses have elaborate means of modulating the host responses to infection, and are able to establish lifelong latent infections. In simplified, general terms, the cell types involved in latency are the neuron for the *Alphaherpesvirinae*, the monocyte lineage for the *Betaherpesvirinae*, and lymphocytes for the *Gammaherpesvirinae*.

Genomic criteria

Herpesvirus genomes studied to date range in size from about 125 to 240 kbp, and the most extensively characterized contain from about 70 to 165 genes. Prior to the generation of extensive sequence data, genome structures (see Chapter 2) were an aid to classification. However, the usefulness of this criterion is limited, since similar structures have evidently evolved more than once in the family. Nucleic acid hybridization data also provided input and, like serological data, are limited to demonstrating relationships between closely related viruses. As with other groups of organisms, data derived from nucleotide and amino acid sequences have gained increasing prominence and now dominate herpesvirus taxonomy. Figure 1.1 shows one example of such data, a phylogenetic tree based upon amino acid sequence alignments (McGeoch *et al.*, 1994, 1995, 2000). Another approach yielded different schemes of relationships, but was based on analytical criteria not widely accepted in depicting evolutionary relationships (Karlin *et al.*, 1994).

It has long been thought from the apparent adaptation of herpesviruses to their hosts that a substantial degree of co-evolution has occurred. Similarities between the phylogenetic relationships among the viruses and those among their hosts provide strong support for this model, and in some instances indicate that co-speciation has occurred. A number of possible exceptions have been noted and are discussed in further detail in Chapter 2.

Species definition

A virus species is defined as a polythetic class of viruses constituting a replicating lineage and occupying a particular ecological niche (Van Regenmortel, 1989, 1990). Members of a polythetic class share a subset of properties, with each property possessed by several members but no property possessed by all. Herpesviruses are defined as separate species if their nucleotide sequences differ in a readily assayable and distinctive manner across the entire genome and if they occupy different ecological niches by virtue of their distinct epidemiology and pathogenesis or their distinct natural hosts (Roizman *et al.*, 1992; Roizman and Pellett, 2001; Davison *et al.*, 2005). However, genomic data have come to dominate biological properties, with taxa corresponding to genetic lineages defined by sequence comparisons and identification of genes unique to certain lineages. An increasing number of herpesviruses in the tissues of various animals are being inferred from short PCR-derived sequences, usually from a single locus in the genome and often in the absence of any other information. These "virtual viruses" cannot readily be classified under the current species definition. However, their incorporation (perhaps in a special category) could be facilitated by

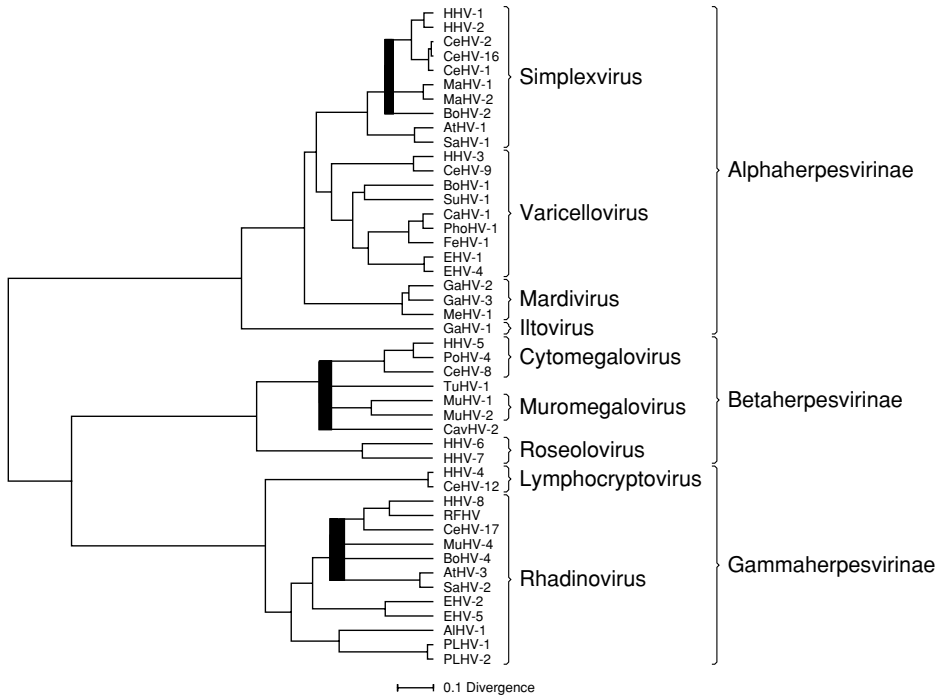

Fig. 1.1. Composite phylogenetic tree for herpesviruses. The tree is based on amino acid sequence alignments of eight sets of homologous genes, constructed from maximum-likelihood trees for subsets of these genes, with molecular clock imposed. Thick lines designate regions of uncertain branching. Formal species abbreviations and designations for genera and subfamilies are given on the right (see Table 1.1). Viruses that are not yet incorporated formally into genera are denoted in italics. Three unclassified viruses are included (RFHV, retroperitoneal fibromatosis herpesvirus of macaques; PLHV-1 and PLHV-2, porcine lymphotropic herpesviruses 1 and 2). Modified from McGeoch *et al.* (2000) with permission from the American Society for Microbiology.

addition to the species definition of a third criterion, that of phylogeny based on the relatedness of conserved genes. Recognition that taxonomy should reflect evolutionary history would also aid rational incorporation of herpesviruses of lower vertebrates and invertebrates into a taxonomy that is currently dominated by herpesviruses of higher vertebrates. Current problems in this area, and a suggested solution, are given in Chapter 2.

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REFERENCES

Davison, A. J., Eberle, R., Hayward, G. S. *et al.* (2005). Family *Herpesviridae*. In *Virus Taxonomy, VIIIth Report of the International Committee on Taxonomy of Viruses*, ed. C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger and L. A. Ball, pp. 193–212. London: Academic Press/Elsevier.

Karlin, S., Mocarski, E. S., and Schachtel, G. A. (1994). Molecular evolution of herpesviruses: genomic and protein sequence comparisons. *J. Virol.*, **68**, 1886–1902.

McGeoch, D. J. and Cook, S. (1994). Molecular phylogeny of the Alphaherpesvirinae subfamily and a proposed evolutionary timescale. *J. Mol. Biol.*, **238**, 9–22.

McGeoch, D. J., Cook, S., Dolan, A., Jamieson, F. E., and Telford, E. A. R. (1995). Molecular phylogeny and evolutionary timescale for the family of mammalian herpesviruses. *J. Mol. Biol.*, **247**, 443–458.

McGeoch, D. J., Dolan, A., and Ralph, A. C. (2000). Toward a comprehensive phylogeny for mammalian and avian herpesviruses. *J. Virol.*, **74**, 10401–10406.

Roizman, B. and Pellett, P. E. (2001). Herpes simplex viruses and their replication. In *Fields Virology*, 4th edn. ed. D. M. Knipe and P. M. Howley, pp. 2399–2459. Philadelphia: Lippincott, Williams and Wilkins.

Roizman, B., Bartha, A., Biggs, P. M. *et al.* (1973). Provisional labels for herpesviruses. *J. Gen. Virol.*, **20**, 417–419.

Roizman, B., Carmichael, L. E., Deinhardt, F. *et al.* (1981). Herpesviridae. Definition, provisional nomenclature, and taxonomy. *Intervirology*, **16**, 201–217.

Roizman, B., Desrosiers, R. C., Fleckenstein, B., Lopez, C., Minson, A. C., and Studdert, M. J. (1992). The *Herpesviridae*: an update. *Arch. Virol.*, **123**, 425–449.

Van Regenmortel, M. H. V. (1989). Applying the species concept to plant viruses. *Arch. Virol.*, **104**, 1–17.

Van Regenmortel, M. H. V. (1990). Virus species, a much overlooked but essential concept in virus classification. *Intervirology*, **31**, 241–254.

Wildy, P. (1971). Classification and nomenclature of viruses. In *Monographs in Virology*, vol. 5, ed. J. L. Melnick, ed., pp. 33–34. Basel: Karger.

2

Comparative analysis of the genomes

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Introduction

Members of the family *Herpesviridae* replicate their genomes in the infected cell nucleus and have a characteristic virion morphology, which consists of the envelope, tegument, capsid and core (Davison and Clements, 1997). An extensive description of virion structure is given in Chapter 3. The present chapter focuses on the viral genome, which occupies the core of the virus particle. Electron microscopy of negatively stained capsids gives the impression that the core consists of the viral DNA molecule wrapped toroidally around a protein spindle (Furlong *et al.*, 1972). Images reconstructed from electron micrographs of virions frozen in ice in the absence of stain, a technique by which morphology is better preserved, show that the core consists of the DNA packed at high density in liquid crystalline form, probably as a spool lacking a spindle (Booy *et al.*, 1991; Zhou *et al.*, 1999).

Herpesvirus genomes consist of linear, double-stranded DNA molecules that range in size from about 125 to 240 kbp and in nucleotide composition from 32 to 75% G+C, depending on the virus species (Honess, 1984). The genome termini are not covalently closed (as in the *Poxviridae;* Moss, 2001) or covalently linked to a protein (as in the *Adenoviridae*; Shenk, 2001). In those herpesvirus genomes that have been examined in sufficient detail, unpaired nucleotides are present at the termini; for example, HSV-1, VZV and HCMV have a single 3′-overhanging nucleotide at each terminus (Mocarski and Roizman, 1982; Davison, 1984; Tamashiro and Spector, 1986). Larger herpesvirus genomes are accommodated in larger capsids, but the relationship is not proportional, as the packing density of the DNA varies somewhat between species (Trus *et al.*, 1999; Bhella *et al.*, 2000). The reasons for the striking range in nucleotide composition of herpesvirus genomes are not clear, but a similar phenomenon is found in other virus families and in cellular organisms. In contrast to the *Alpha-* and *Betaherpesvirinae*, the genomes of most *Gammaherpesvirinae* are generally deficient in the CG dinucleotide (Table 2.1). In vertebrate genomes, this phenomenon is thought to be due to spontaneous deamination of 5-methylcytosine residues in DNA to thymidine residues, followed by fixation through DNA replication. CG depletion in herpesviruses, and concomitant enrichment in TG and CA, has been taken as indicative of latency in dividing cell populations, in which the latent genome is obliged to replicate as host cells divide (Honess *et al.*, 1989). Thus, HSV-1, which is resident in non-dividing neurons, has a CG content consistent with its nucleotide composition, whereas EBV, which latently infects dividing B cell populations, is depleted. Local CG suppression of the major immediate early gene locus of HCMV has also been noted (Honess *et al.*, 1989).

Genome structures

Herpesvirus genomes are not simple lengths of unique DNA, but characteristically contain direct or inverted repeats. The reasons for this are not known, but it is intriguing that similar structures appear to have arisen independently on several occasions during herpesvirus evolution. Herpesvirus genomes are thought to replicate by circularization, followed by production of concatemers and cleavage of unit-length genomes during packaging into capsids (Boehmer and Lehman, 1997). The explanation for the presence of repeats is probably connected in some way with the mode of DNA replication, rather than with any advantage gained by having multiple copies of certain genes. Although greater expression would be a consequence of repeated genes, this appears a simplistic explanation in an evolutionary context, since subtler processes of nucleotide

Table 2.1. Sequenced herpesvirus genomes

Common name	Strain[a]	Abbreviation		Accession	Size (bp)[b]	Composition[c]		Reference
		Common	Formal			G+C	CG	
Mammalian herpesvirus group[d]								
Alphaherpesvirinae								
Simplexvirus								
Herpes simplex virus type 1	17	HSV-1	HHV-1	X14112	152261	68.3	1.01	McGeoch *et al.* (1988)
Herpes simplex virus type 2	HG52	HSV-2	HHV-2	Z86099	154746	70.4	1.06	Dolan *et al.* (1998)
B virus	E2490	HVB	CeHV-1	AF533768	156789	74.5	1.09	Perelygina *et al.* (2003)
SA8	B264	SA8	CeHV-2	AY714813	150715	76.0	1.09	Tyler *et al.* (2005)
Herpes papio 2	X313	HVP2	CeHV-16	DQ149153	156487	76.1	1.08	Tyler and Severini (2006)
Varicellovirus								
Varicella-zoster virus	Dumas	VZV	HHV-3	X04370	124884	46.0	1.14	Davison and Scott (1986)
	Oka vaccine			AB097932	125078			Gomi *et al.* (2002)
	Oka parental			AB097933	125125			Gomi *et al.* (2002)
	MSP			AY548170	124883			Grose *et al.* (2004)
	BC			AY548171	125459			Grose *et al.* (2004)
	Varilrix			DQ008354	124821			Vassilev (2005)
	Varilrix			DQ008355	124815			Vassilev (2005)
	HJO			AJ871403	124928			Fickenscher *et al.* (unpublished)
Simian varicella virus	Delta	SVV	CeHV-9	AF275348	124138	40.4	1.12	Gray *et al.* (2001)
Bovine herpesvirus 1	[Cooper]	BHV-1	BoHV-1	AJ004801	135301	72.4	1.19	Schwyzer & Ackermann (1996)
Bovine herpesvirus 5	SSV507/99	BHV-5	BoHV-5	AY261359	138390	74.9	1.17	Delhon *et al.* (2003)
Pseudorabies virus	[Kaplan]	PRV	SuHV-1	BK001744	143461	73.6	1.12	Klupp *et al.* (2004)
Equine herpesvirus 1	Ab4	EHV-1	EHV-1	AY665713	150224	56.7	0.99	Telford *et al.* (1992)
	V592			AY464052	149430			Nugent *et al.* (2006)
Equine herpesvirus 4	NS80567	EHV-4	EHV-4	AF030027	145597	50.5	0.93	Telford *et al.* (1998)
Mardivirus								
Marek's disease virus type 1	Md5	MDV-1	GaHV-2	AF243438	177874	44.1	1.01	Tulman *et al.* (2000)
	GA			AF147806	174077			Lee *et al.* (2000)
	Md11			[AY510475]	170950			Niikura *et al.* (unpublished)
Marek's disease virus type 2	HPRS24	MDV-2	GaHV-3	AB049735	164270	53.6	1.23	Izumiya *et al.* (2001)
Turkey herpesvirus	FC126	HVT	MeHV-1	AF291866	159160	47.6	1.11	Afonso *et al.* (2001)
Iltovirus								
Infections laryngotracheitis virus	[SA-2]	ILTV	GaHV-1	NC006623	148687	48.2	1.01	Thureen and Keeler (2006)
Psittacid herpesvirus 1[e]	97-0001	PsHV-1	PsHV-1	AY372243	163025	60.9	1.21	Thureen and Keeler (2006)
Betaherpesvirinae								
Cytomegalovirus								
Human cytomegalovirus	Merlin	HCMV	HHV-5	AY446894	235645	57.5	1.19	Dolan *et al.* (2004)
	AD169			X17403	229354			Chee *et al.* (1990)
	AD169			BK000394	230287			Davison *et al.*(2003a)
	AD169			[AC146999]	[233739]			Murphy *et al.* (2003b)
	Towne			[AY315197]	[231236]			Dunn *et al.* (2003)
	Towne			[AC146851]	[229483]			Murphy *et al.* (2003b)
	Toledo			[AC146905]	[226889]			Murphy *et al.* (2003b)
	PH			[AC146904]	[229700]			Murphy *et al.* (2003b)
	TR			[AC146906]	[234881]			Murphy *et al.* (2003b)
	FIX			[AC146907]	[229209]			Murphy *et al.* (2003b)
Chimpanzee cytomegalovirus	–	CCMV	PoHV-4	AF480884	241087	61.7	1.11	Davison *et al.* (2003a)
Rhesus cytomegalovirus	68-1	RhCMV	CeHV-8	AY186194	221454	49.1	0.99	Hansen *et al.* (2003)
Unassigned								
Tupaiid herpesvirus	2	THV	TuHV-1	AF281817	195859	66.6	1.28	Bahr & Darai (2001)
Muromegalovirus								
Murine cytomegalovirus	Smith	MCMV	MuHV-1	U68299	230278	58.7	1.22	Rawlinson *et al.* (1996)
Rat cytomegalovirus	Maastricht	RCMV	MuHV-2	AF232689	230138	61.0	1.25	Vink *et al.* (2000)
Roseolovirus								
Human herpesvirus 6	U1102	HHV-6A	HHV-6	X83413	159321	42.4	1.13	Gompels *et al.* (1995)
	Z29	HHV-6B		AF157706	162114	42.8	1.10	Dominguez *et al.* (1999)
	HST			AB021506	161573			Isegawa *et al.* (1999)

(*cont.*)

Table 2.1. (*cont.*)

Common name	Strain[a]	Abbreviation		Accession	Size (bp)[b]	Composition[c]		Reference
		Common	Formal			G+C	CG	
Human herpesvirus 7	JI	HHV-7	HHV-7	U43400	144861	35.3	0.80	Nicholas (1996)
	RK			AF037218	153080			Megaw *et al.* (1998)
Gammaherpesvirinae								
Lymphocryptovirus								
Epstein–Barr virus	[B95-8]	EBV	HHV-4	AJ507799	171823	59.5	0.61	de Jesus *et al.* (2003)
	GD1			AY961628	171656			Zeng *et al.* (2005)
	AG876			DQ279927	172764			Dolan *et al.* (2006)
	B95-8			V01555	172281			Baer *et al.* (1984)
Marmoset lymphocryptovirus	CJ0149	marmoset LCV	CalHV-3	AF319782	149696	49.3	0.70	Rivailler *et al.* (2002a)
Rhesus lymphocryptovirus	LCL8664	rhesus LCV	CeHV-15	AY037858	171096	61.9	0.68	Rivailler *et al.* (2002b)
Rhadinovirus								
Human herpesvirus 8	BC-1	HHV-8	HHV-8	U75698	[137508]	53.5	0.81	
				U75699	801	84.5	0.92	Russo *et al.* (1996)
	–			U93872	[133661]			Neipel *et al.* (1997)
Rhesus rhadinovirus	17577	RRV	CeHV-17	AF083501	133719	52.2	1.11	Searles *et al.* (1999)
	26-95			AF210726	130733			Alexander *et al.* (2000)
Murine herpesvirus 68[f]	WUMS	MHV-68	MuHV-4	U97553	119450	47.2	0.43	Virgin *et al.* (1997)
	g2.4			AF105037	119550			Nash *et al.* (2001)
Bovine herpesvirus 4	66-p-347	BHV-4	BoHV-4	AF318573	108873	41.4	0.23	Zimmermann *et al.* (2001)
				AF092919	2267	71.2	0.42	
Herpesvirus ateles	73	HVA	AtHV-3	AF083424	108409	36.6	0.40	Albrecht (2000)
				AF126541	1582	77.1	0.79	
Herpesvirus saimiri	A11	HVS	SaHV-2	X64346	112930	34.5	0.33	Albrecht *et al.* (1992)
				K03361	1444	70.9	0.61	
	C488			AJ410493	113027			Ensser *et al.* (2003)
				AJ410494	1458			
Equine herpesvirus 2	86/67	EHV-2	EHV-2	U20824	184427	57.5	0.63	Telford *et al.* (1995)
Alcelaphine herpesvirus 1	C500	AHV-1	AlHV-1	AF005370	130608	46.2	0.42	Ensser *et al.* (1997)
				AF005368	1113	72.0	0.69	
Ovine herpesvirus 2	BJ1035	OvHV-2	OvHV-2	AY839756	135135	52.9	0.58	Stewart *et al.* (unpublished)
Fish herpesvirus group[d]								
Undefined subfamily								
Ictalurivirus								
Channel catfish virus	Auburn 1	CCV	IcHV-1	M75136	134226	56.2	1.1	Davison (1992)
Bivalve herpesvirus group[d]								
Undefined subfamily								
Undefined genus								
Ostreid herpesvirus 1	–	OHV	OsHV-1	AY509253	207439	38.7	0.68	Davison *et al.* (2005)

[a] Square brackets indicate the strain that was used most extensively in assembling a sequence combining data from several strains. A hyphen indicates that the strain was not specified.

[b] Sizes were obtained from the latest version of the accessions, and may differ from those in the references through correction of errors. Square brackets indicate sequences that fall marginally short of full length: for MDV-1 and HCMV strains, sequences are for bacterial artificial chromosomes, the sizes representing a deleted form of the genome plus the vector; for HHV-8 strains, the sequence at the right end of the unique region was not determined. For members of subfamily *Gammaherpesvirinae* other than EHV-2, actual genome sizes are larger than those listed (approximately 150–180 kbp), owing to the presence of variable copy numbers of terminal repeats at both ends of the genome. Where a single value is given, this represents either the unique region flanked by partial terminal repeats or the unique region only. Where two values are given, the first is for the unique region and the second is for the terminal repeat.

[c] G + C content is given as moles %, CG content is given as observed/expected frequency, taking into account overall nucleotide composition. Where multiple strains have been sequenced for a species, values are given for one strain only. For members of the *Gammaherpesvirinae*, values are given for the deposited sequences, which consist either of the unique region flanked by partial terminal repeat sequence, the unique region only, or separate accessions for the unique region and terminal repeat.

[d] This taxon is used for the purposes of discussion, and, unlike the others in the Table, has no formal standing.

[e] PsHV-1 is the closest relative of ILTV and is placed informally in this genus.

[f] MHV-68 strains g2.4 and WUMS are essentially identical, since the latter was derived from the former.

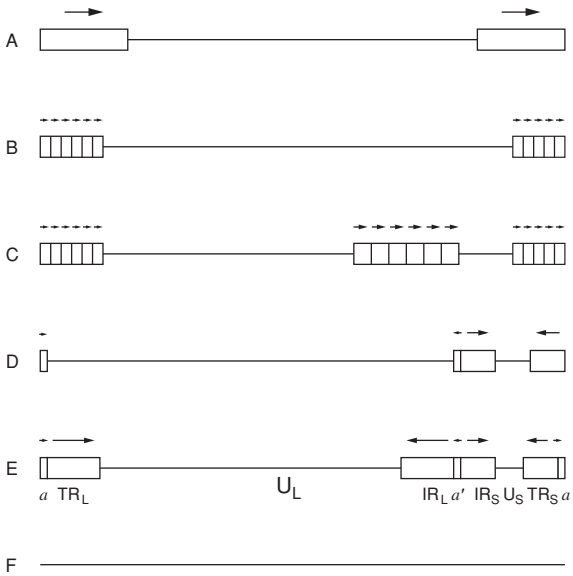

Fig. 2.1. Classes of herpesvirus genome structures (not to scale) as defined by Roizman and Pellett (2001). Unique and repeat regions are shown as horizontal lines and rectangles, respectively. The orientations of repeats are shown by arrows. The nomenclature of unique and repeat regions, including the terminal redundancy (*a*) and its internal, inverted copy (*a*′), is indicated for the class E genome.

substitution can readily alter transcriptional levels over a much greater range. In addition, repeats often do not contain protein-coding regions. As elaborated below, certain genomes exhibit a further structural complexity known as segment inversion, in which unique regions flanked by inverted repeats are found in both orientations in virion DNA. Thus, a genome with two such unique regions would produce either two or four isomers depending on whether one or both regions invert. This phenomenon is probably a consequence of recombination between repeats in concatemeric DNA. Isomers are functionally equivalent (Jenkins and Roizman, 1986), and segment inversion appears to be unrelated to the biology of the virus.

Figure 2.1 shows the major classes of genome structure found among the herpesviruses, as summarized by Roizman and Knipe (2001). The class A genome consists of a unique sequence flanked by a direct repeat. It was first described for CCV (Chousterman *et al.*, 1979), but is also represented among the *Betaherpesvirinae* (HHV-6: Lindquester and Pellett, 1991; Martin *et al.*, 1991; HHV-7: Dominguez *et al.*, 1996; Ruvolo *et al.*, 1996) and one member of the *Gammaherpesvirinae* (EHV-2; Browning and Studdert, 1989). In these examples, the direct repeat is several kbp in size. Other members of the *Betaherpesvirinae* also have this arrangement, but the repeat is smaller, at 504 bp in RCMV (Vink *et al.*, 1996) and 30–31 bp in MCMV (Marks and Spector, 1988; Rawlinson *et al.*, 1996).

Class B genomes also have directly repeated sequences at the termini, but these consist of variable copy numbers of a tandemly repeated sequence of 0.8–2.3 kbp. This arrangement characterizes most *Gammaherpesvirinae* in the *Rhadinovirus* genus, such as HVS and HHV-8 (Bornkamm *et al.*, 1976; Russo *et al.*, 1996). The repeated regions may comprise up to 30% of the DNA molecule (Bornkamm *et al.*, 1976; Lagunoff and Ganem, 1997). The presence of additional terminal repeat sequences in inverse orientation internally in the genome gives rise to a related structure, which is present in another member of the *Gammaherpesvirinae*, cottontail rabbit herpesvirus (Cebrian *et al.*, 1989). The virion DNA of this virus exhibits segment inversion because the two unique regions are flanked by inverted repeats. The class C structure represents another derivative of class B, in which an internal set of direct repeats is present but is unrelated to the terminal set. EBV, a member of the *Gammaherpesvirinae* in the *Lymphocryptovirus* genus, has this arrangement (Given and Kieff, 1979). Segment inversion does not occur because the internal and terminal repeats are not related.

Class D genomes contain two unique regions (U_L and U_S), each flanked by inverted repeats (TR_L/IR_L and TR_S/IR_S). This structure is characteristic of *Alphaherpesvirinae* in the *Varicellovirus* genus, such as PRV and VZV (Rixon and Ben-Porat, 1979; Dumas *et al.*, 1981), and has also evolved separately in salmonid herpesvirus 1 (Davison, 1998). Segment inversion occurs inasmuch as equimolar amounts of genomes containing the two orientations of U_S are found in virion DNA, but U_L is present predominantly or completely in one orientation. The latter feature cannot be explained solely by recombination, and is probably due also to the presence of the cleavage signal solely or largely in the region comprising TR_L/IR_L and one end of U_L (Davison, 1984; Rall *et al.*, 1991).

Class E is the most complex genome structure, and was the first to be described, for HSV-1 (Sheldrick and Berthelot, 1975). It is similar to class D, except that TR_L/IR_L is much larger and segment inversion gives rise to four equimolar genome isomers (Wadsworth *et al.*, 1975; Hayward *et al.*, 1975; Delius and Clements, 1976; Clements *et al.*, 1976; Wilkie and Cortini, 1976). Also, class E genomes are terminally redundant, containing a sequence of a few hundred bp (termed the *a* sequence) that is repeated directly at the genome termini and inversely at the IR_L-IR_S junction (Sheldrick and Berthelot, 1975; Grafstrom *et al.*, 1975a,b; Wadsworth *et al.*, 1976; Hyman *et al.*, 1976).

Minor proportions of genomes contain multiple copies of the *a* sequence at the left terminus or the IR_L-IR_S junction (Wilkie, 1976; Wagner and Summers, 1978; Locker and Frenkel, 1979). The class E arrangement is characteristic of *Alphaherpesvirinae* in the *Simplexvirus* genus, and has evolved independently in the lineage giving rise to HCMV and CCMV, members of the *Betaherpesvirinae* (Weststrate *et al.*, 1980; DeMarchi, 1981; Davison *et al.*, 2003a). A structure similar to both class D and E genomes has also evolved in an invertebrate herpesvirus, OsHV-1 (Davison *et al.*, 2005). This contains two segments, each consisting of a unique region flanked by an substantial inverted repeat, linked via an additional small, non-inverting unique region. As in class E genomes, the two segments undergo inversion, but, like class D, the genome is not terminally redundant.

Class F is represented by a member of the *Betaherpesvirinae*, THV, which apparently lacks the types of inverted and direct repeats that characterize other herpesvirus genomes (Koch *et al.*, 1985; Albrecht *et al.*, 1985). However, since the genome ends of THV have not been analyzed directly, the existence of this unusual structure is considered tentative.

Genome sequences

Table 2.1 lists the 39 herpesvirus species for which genome sequences are currently available in the public databases. Additional strains have been sequenced for some species, yielding a total of 63 sequenced strains. The ease of generating data will continue to expand the number of herpesvirus species and strains sequenced in coming years. Indeed, substantial inroads have been made into large-scale studies of strain variation for certain of the human herpesviruses. It appears that the scale and extent of variation is lineage dependent, with *Betaherpesvirinae* more variable than *Gammaherpesvirinae*, and *Alphaherpesvirinae* the least variable (Murphy *et al.*, 2003b; Dolan *et al.*, 2004; Poole *et al.*, 1999; Midgley *et al.*, 2003; Muir *et al.*, 2002; Gomi *et al.*, 2002). The development of tools to study variation in increasing detail will enhance understanding of viral epidemiology, in terms both of its relation to human evolution and migration and of the changes that are occurring in human populations at the present time.

Gene content

Sequencing herpesvirus genomes is now routine, but the process of describing gene content (annotation) is not trivial. Thus, as with other groups of organisms, the quality of annotation of herpesvirus entries in the public databases varies widely. It is an unfortunate fact that no set of objective criteria is sufficient to interpret the gene content of a sequence completely. Although most genes can be catalogued relatively easily, there are genuine difficulties in identifying all of them, even in the best characterized herpesviruses.

A primary criterion in defining gene content involves identifying open reading frames (ORFs), usually those initiated by methionine (ATG) codons. A tendency to include ORFs that do not encode proteins may be reduced by setting a minimum size. Comparative genomics, which operates on the principle that genes are conserved in evolution, and algorithms that compare sequence patterns within ORFs to the protein-coding regions of known genes, are also useful. However, these tools yield results with least confidence when applied to small, spliced, overlapping or poorly conserved ORFs, and in instances where translation initiates from internal codons, alternative splicing occurs, or esoteric translational mechanisms are employed (e.g., suppression of termination codons and forms of translational editing). In addition to sequence analysis, experimental data on production of an RNA or protein from an ORF provides important imput, although even this falls short of proving functionality. Also, most approaches are aimed at identifying protein-coding genes, and cannot detect genes that encode functional transcripts that are not mRNAs.

The use of different criteria for gene identification may create a degree of uncertainty and debate, and lead to different pictures of gene layout. The case of HCMV provides a contemporary example. In the first analysis of the gene content of HCMV strain AD169, Chee *et al.* (1990) catalogued 189 protein-coding ORFs (counting duplicates once only). Later, the gene number was reduced to 147 by comparing the HCMV and CCMV genomes, allowing, where appropriate, for the presence of genes unique to either genome (Davison *et al.*, 2003a,b). As modified criteria were applied, this number rebounded in a series of increments, first to 157 (Yu *et al.*, 2003), next to 171 (Murphy *et al.*, 2003a), then to 220 (Murphy *et al.*, 2003b) and finally to 232 (Varnum *et al.*, 2004). Although the conservative numbers in this example are more supportable, the existence of unrecognized genes should not be ruled out even in well-characterized genomes, and candidates should be examined rigorously. For example, new genes were identified in previously analysed sequences for VZV (Kemble *et al.*, 2000) and HHV-8 (Glenn *et al.*, 1999).

The genes of HSV-1, presumably like those of all herpesviruses, are transcribed by host RNA polymerase II

(Wagner, 1985; Roizman and Knipe, 2001). Transcription of the first genes to be expressed, the immediate early genes, does not require ongoing protein synthesis, and is enhanced by a tegument protein at low multiplicities of infection (O'Hare, 1993). Some of the immediate early proteins regulate expression of early and late genes (Honess and Roizman, 1974). Early genes, defined as those expressed in the presence of immediate early proteins and before the onset of DNA replication, include enzymes involved in nucleotide metabolism and DNA replication and a number of envelope glycoproteins. Some late genes are expressed at low levels under early conditions, but full expression of "leaky" and "true" late genes is dependent on DNA replication; these genes encode mainly virion proteins. Although the details differ, a similar pattern of regulated gene expression is characteristic of all herpesviruses examined; for example, HCMV (Stinski, 1978), HHV-8 (Sarid *et al.*, 1998) and CCV (Silverstein *et al.*, 1995). In addition, herpesviruses express RNAs whose functions apparently do not involve translation. The best characterized are small RNAs probably transcribed by RNA polymerase III in *Gammaherpesvirinae* such as EBV (Rosa *et al.*, 1981) and MHV-68 (Bowden *et al.*, 1997). Larger noncoding RNAs transcribed by RNA polymerase II include the latency-associated transcripts in HSV-1 (Stevens *et al.*, 1987) and several virion-associated RNAs in HCMV (Bresnahan and Shenk, 2000).

With the exception of a small number of genes that are expressed by splicing from a common 5′-leader, such as the EBNA genes of EBV (Bodescot *et al.*, 1987) and the IE1 and IE2 genes of HCMV (Stenberg *et al.*, 1985), herpesvirus genes have individual promoters. However, it is common for genes to share a polyadenylation site, leading to families of 3′-coterminal transcripts (Wagner, 1985). Apart from families of duplicated genes, there is no pronounced clustering of genes on the basis of function or kinetics of expression. Splicing is uncommon throughout the family, affecting no more than about 20% of the gene number in any genome. Most splicing involves genes that are relatively recent evolutionary developments, and *Beta-* and *Gammaherpesvirinae* have more spliced genes than *Alphaherpesvirinae*.

Genome comparisons and evolution

The availability of extensive sequence data for herpesviruses has facilitated detailed phylogenetic analyses of the family based on amino acid sequence comparisons of conserved genes, as described in Chapter 1. In this section, an overview is given of genetic relatedness at selected levels in the phylogenetic tree, starting with the three major groups that encompass all known herpesviruses, proceeding to the best characterized of these groups, and ending with one subfamily in this group. In chronological terms, this proceeds from earlier to more recent evolutionary events. Detailed information on the gene content of, and the relationships between, the human herpesviruses is available elsewhere in this book.

Three major groups

Three major groups of viruses possess the herpesvirus morphology, including closely similar capsid structures, but share very little genetic similarity (Davison, 1992; Booy *et al.*, 1996; Davison *et al.*, 2005). Viruses in the best characterized group infect mammals, birds and reptiles, viruses in the second group infect amphibians and fish, and the third group contains the single known herpesvirus of an invertebrate, the oyster. Currently, the family *Herpesviridae* comprises the first group classified into three subfamilies and component genera, one member (CCV) of the second group representing an unassigned genus, and the oyster virus is a floating species. The most logical means of accommodating all known herpesviruses taxonomically would be to establish three families under the umbrella of a new order (*Herpesvirales*), containing herpesviruses of mammals, birds and reptiles, of amphibians and fish, and of bivalves, respectively. Since these taxa are presently a proposal and lack any formal standing, the terms mammalian, fish and bivalve herpesvirus groups are used to denote the proposed families in the following discussion.

Only three genes have clear counterparts in all three groups that are detectable by amino acid sequence comparisons. The proteins encoded by two (DNA polymerase and dUTPase) have ubiquitous cellular relatives and could have been captured independently from the host repertoire. The third gene apparently lacks a counterpart in the host cell but has distant relatives in T4 and similar bacteriophages (Davison, 1992; Mitchell *et al.*, 2002). The T4 gene is known to encode the ATPase subunit of a DNA packaging enzyme complex called the terminase (Rao and Black, 1988; Bhattacharyya and Rao, 1993), and the HSV-1 gene has properties that are consistent with a similar function (Yu and Weller, 1998).

The existence of groups of viruses that exhibit close morphological similarities but generally lack detectable genetic relationships is not unique to the herpesviruses, and may be explained as the result either of convergence

from distinct evolutionary sources or as divergence from an ancestor so ancient that sequence similarities have been obliterated. The latter hypothesis is currently favored, but the existence of a common ancestor of all herpesviruses and any contingent dates for divergence of the groups must be viewed cautiously. More speculatively, apparent similarities in aspects of DNA packaging (Booy *et al.*, 1991) and capsid maturation (Newcomb *et al.*, 1996) could be interpreted as supporting an even earlier common evolutionary origin between herpesviruses and certain double-stranded DNA bacteriophages, including T4.

Phylogenetic analyses strongly support the view that herpesviruses have largely co-evolved with their hosts, often co-speciating with them. As would be expected of evolutionary phenomena, a number of problematic observations and exceptions have emerged as data have multiplied, especially in relation to early divergences. From comparisons between the phylogenies of the viruses and their hosts, McGeoch and Cook (1994) proposed an evolutionary timescale for the *Alphaherpesvirinae* in which the *Simplex-* and *Varicellovirus* genera diverged about 73 million years ago, roughly coincident with the period of the mammalian radiation. Even at this stage, potential exceptions to the co-evolution model were apparent. For example, the taxonomical position of avian herpesviruses among the *Alphaherpesvirinae* did not fit well, and prompted the suggestion of ancient interspecies transfers between mammals and birds. In this scheme, a similar argument may be necessary to explain the position of reptilian (turtle) herpesviruses in the same subfamily (Quackenbush *et al.*, 1998; Yu *et al.*, 2001; Coberley *et al.*, 2002), especially given their distance from amphibian herpesviruses (Davison *et al.*, 1999 and unpublished data). Assuming the constancy of the molecular clock derived for the *Alphaherpesvirinae*, McGeoch *et al.* (1995) tentatively dated the divergence of the *Alpha-*, *Beta-* and *Gammaherpesvirinae* at 180–220 million years ago. Given the contrasting lack of relationships between the groups and substantial relationships within them (see below), this date did not fit well qualitatively with a model in which the fish and mammalian herpesvirus groups co-speciated when teleosts separated from other vertebrates. In a recent analysis utilizing improved algorithms and the latest estimates for host divergence dates, McGeoch and Gatherer (2005) pushed back the common ancestor of the *Alpha-*, *Beta-* and *Gammaherpesvirinae* to about 400 million years ago, which permitted a greater degree of support for co-evolution of the *Alphaherpesvirinae*, including avian and reptilian members. In this scheme, a much earlier, non-co-speciative divergence may be indicated for the mammalian and fish herpesvirus groups (along with one of similar or greater antiquity for the bivalve herpesvirus group). However, this would lack the advantage of explaining the segregation of the viral groups to distinct parts of the animal kingdom and necessitate additional arguments involving viral extinction.

The mammalian herpesvirus group

In contrast to the lack of extensive relationships between the three groups, members of the mammalian herpesvirus group are clearly related to each other (Davison, 2002), as are those in the fish herpesvirus group (Bernard and Mercier, 1993; Davison, 1998; Davison *et al.*, 1999; Waltzek *et al.*, 2005, and unpublished data). Figure 2.2 shows the gene layout in representatives of two genera for each of the three subfamilies in the mammalian herpesvirus group. The subfamilies share 43 genes, termed "core genes," which were presumably inherited from a common ancestor (McGeoch and Davison, 1999). This number assumes a small degree of approximation, since amino acid sequence conservation among the set varies from substantial to marginal. The core genes are shaded grey in Fig. 2.2, and are largely confined to the central regions of the genomes, as is especially apparent with HCMV. Accumulation of more recently evolved genes near the termini is a feature of linear, double-stranded DNA genomes from other virus families, such as the *Poxviridae* (Upton *et al.*, 2003; McLysaght *et al.*, 2003; Gubser *et al.*, 2004) and the *Adenoviridae* (Davison *et al.*, 2003c), and also of eukaryotic chromosomes (Kellis *et al.*, 2003). The core genes are ordered similarly in the same subfamily, except for certain members of the *Alphaherpesvirinae* in which different arrangements are apparent: PRV in the *Varicellovirus* genus (Ben-Porat *et al.*, 1983; Davison and Wilkie 1983; Dezelee *et al.*, 1996; Bras *et al.*, 1999) and ILTV in the *Iltovirus* genus (Ziemann *et al.*, 1998). However, as shown in Fig. 2.2, the core genes are arranged differently in the different subfamilies, in the form of blocks, some of which are inverted (Davison and Taylor, 1987; Gompels *et al.*, 1995; Hannenhalli *et al.*, 1995). As indicated in Table 2.2, core genes are involved in vital aspects of herpesvirus growth, and many are involved directly or indirectly in DNA replication, in packaging of replicated DNA into capsids, and in capsid formation and structure. Most of the core genes are essential for growth of virus in cell culture (Ward and Roizman, 1994; Yu *et al.*, 2003; Dunn *et al.*, 2003).

Most core genes are present in all three subfamilies of the mammalian herpesvirus group, but three (encod-

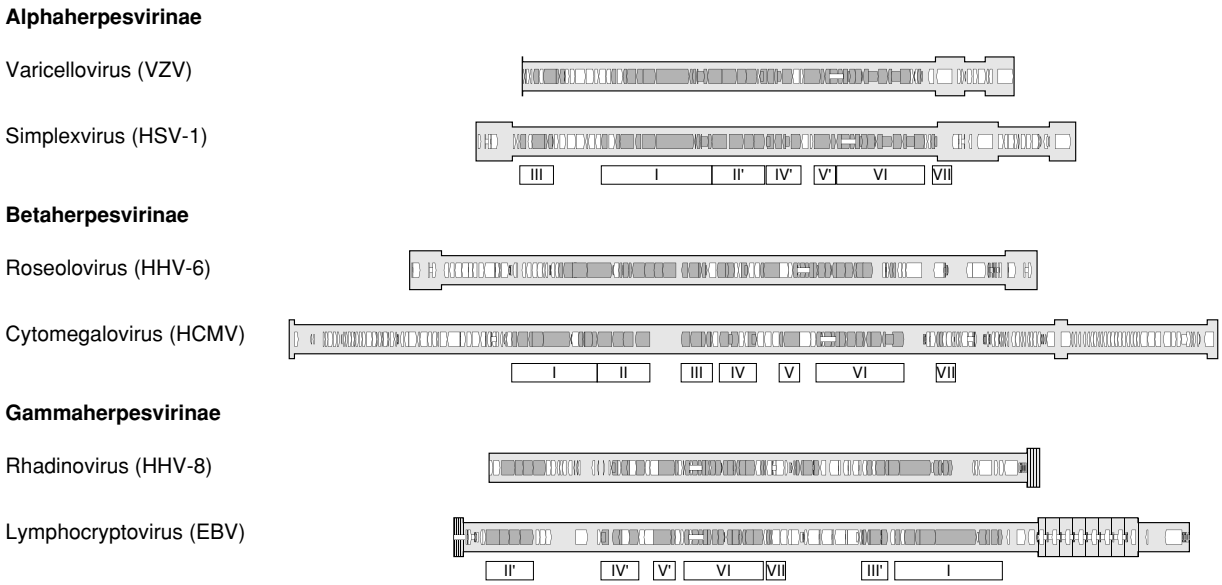

Fig. 2.2. Layout of genes in mammalian herpesvirus genomes. Repeat regions are shown in thicker format than unique regions. Protein-coding regions are shown as arrows shaded grey (core genes) or white (non-core genes), and introns as narrow white bars. Blocks of core genes that are rearranged between the subfamilies are indicated by rectangles I–VII for HSV-1, HCMV and EBV, with inverted blocks marked with a prime (Chee *et al.*, 1990). Block II also contains a local inversion and transposition of one gene (encoding DNA polymerase) that is not indicated. Genome coordinates and gene locations were obtained from accessions X04370 (VZV), X14112 (HSV-1), X83413 (HHV-6) as modified by Megaw *et al.* (1998), AY446894 (HCMV), U93872 (HHV-8) as extended at the right end of the unique region by Glenn *et al.* (1999), and AJ507799 (EBV). Variable numbers of terminal repeats are present in HHV-8 and EBV, but are shown at one end or the other other according to the accessions.

ing thymidine kinase, the small subunit of ribonucleotide reductase and the helicase that binds to the origin of DNA synthesis) have been lost from individual lineages. Thus, the origin-binding helicase gene has been retained in the *Roseolovirus* genus, but lost from other genera in the *Betaherpesvirinae*. This is mirrored in the presence of an origin of lytic DNA replication with similar structure in lineages that have retained this gene (Dewhurst *et al.*, 1993; Inoue *et al.*, 1994).

In addition to protein-coding regions, certain *cis*-acting sequences are conserved. These include the origin of lytic DNA replication, which is located similarly in each subfamily in comparison with adjacent genes, allowing for rearrangement of gene blocks. Certain members of the *Alpha-* and *Gammaherpesvirinae* contain additional lytic origins. Also, short elements near the genome termini that are involved in cleavage and packaging of unit-length genomes are conserved in all subfamilies (Broll *et al.*, 1999).

As well as the part played by the gradual processes of nucleotide substitution, insertion or deletion in generating diversity, acquisition of genes from the cell or from other viruses has played an important role throughout the evolution of the herpesviruses. There are examples of captured genes in all herpesvirus lineages. Among the mammalian herpesvirus group, the *Gammaherpesvirinae* exhibit a particularly impressive number of such genes, ranging from one encoding a product related to an enzyme involved in *de novo* purine biosynthesis (phosphoribosylformylglycineamide amidotransferase; FGARAT; Ensser *et al.*, 1997), which is present in all *Gammaherpesvirinae*, through a cyclin D gene (Nicholas *et al.*, 1992), which features in a subset of the *Rhadinovirus* genus, interferon regulatory factor genes (vIRFs), which are found only in HHV-8 and RRV (Russo *et al.*, 1996; Searles *et al.*, 1999), to a relatively recently captured core 2 β-1,6-*N*-acetylglucosaminyltransferase-mucin gene in BHV-4 (Markine-Goriaynoff *et al.*, 2003).

Duplication of genes, captured or otherwise, followed by divergence, is also apparent in all herpesvirus lineages. For example, up to three copies of the FGARAT gene are present in *Gammaherpesvirinae* (Virgin *et al.*, 1997), and HHV-8 and RRV contain four and eight vIRF genes, respectively (Searles *et al.*, 1999; Jenner *et al.*, 2001; Cunningham *et al.*, 2003). Examples of duplicated genes among other members of the mammalian herpesvirus group include

Table 2.2. Core genes in human herpesviruses, grouped according to functional class. HSV-2 and HHV-7 are not included, since their nomenclatures are the same as those for HSV-1 and HHV-6, respectively. HSV-1 and HCMV genes that are essential for growth in cell culture are marked by asterisks

HSV-1	VZV	HCMV	HHV-6	EBV	HHV-8	Function
DNA replication machinery						
UL30*	28	UL54*	U38	BALF5	9	Catalytic subunit of DNA polymerase complex
UL42*	16	UL44*	U27	BMRF1	59	Processivity subunit of DNA polymerase complex
UL9*	51	–	U73	–	–	Origin-binding protein; helicase
UL5*	55	UL105*	U77	BBLF4	44	Component of DNA helicase-primase complex; helicase
UL8*	52	UL102*	U74	BBLF2/BBLF3	40/41	Component of DNA helicase-primase complex
UL52*	6	UL70*	U43	BSLF1	56	Component of DNA helicase-primase complex; primase
UL29*	29	UL57*	U41	BALF2	6	Single-stranded DNA-binding protein
Peripheral enzymes						
UL23	36	–	–	BXLF1	21	Thymidine kinase
UL39	19	UL45[a]	U28[a]	BORF2	61	Ribonucleotide reductase; large subunit
UL40	18	–	–	BaRF1	60	Ribonucleotide reductase; small subunit
UL50	8	UL72[a]	U45[a]	BLLF3	54	Deoxyuridine triphosphatase
UL2	59	UL114	U81	BKRF3	46	Uracil-DNA glycosylase
Processing and packaging of DNA						
UL12	48	UL98*	U70	BGLF5	37	Deoxyribonuclease; role in DNA maturation and recombination
UL15*	42/45	UL89*	U66	BGRF1/BDRF1	29	Putative ATPase subunit of terminase; capsid-associated
UL28*	30	UL56*	U40	BALF3	7	Putative subunit of terminase; *pac* site-specific binding; capsid-associated
UL6*	54	UL104*	U76	BBRF1	43	Portal protein; forms dodecameric ring at capsid vertex; complexed with terminase
UL25*	34	UL77*	U50	BVRF1	19	Possibly caps the portal after DNA packaging is complete; tegument protein
UL32*	26	UL52*	U36	BFLF1	68	Involved in proper capsid localization in the nucleus
UL33*	25	UL51*	U35	BFRF1A	67A	Interacts with terminase
UL17*	43	UL93*	U64	BGLF1	32	Involved in proper capsid localization in the nucleus; tegument protein
Egress of capsids from nucleus						
UL31*	27	UL53*	U37	BFLF2	69	Nuclear matrix protein; component of capsid docking complex on nuclear lamina
UL34*	24	UL50*	U34	BFRF1	67	Inner nuclear membrane protein; component of capsid docking complex on nuclear lamina
Capsid assembly and structure						
UL19*	40	UL86*	U57	BcLF1	25	Major capsid protein; component of hexons and pentons
UL18*	41	UL85*	U56	BDLF1	26	Component of intercapsomeric triplex between hexons and pentons
UL38*	20	UL46*	U29	BORF1	62	Component of intercapsomeric triplex between hexons and pentons
UL35	23	UL48A*	U32	BFRF3	65	Small capsid protein located on tips of hexons; interacts with dynein and microtubules
UL26*	33	UL80*	U53	BVRF2	17	Maturational protease; generates mature forms of scaffolding proteins
UL26.5	33.5	UL80.5	U53.5	BdRF1	17.5	Scaffolding protein removed from capsid during DNA packaging
Tegument						
UL7	53	UL103	U75	BBRF2	42	Associated with intracellular capsids
UL11	49	UL99*	U71	BBLF1	38	Role in virion egress and secondary envelopment in the cytoplasm; myristylated and palmitylated protein; interacts with UL16 protein
UL14	46	UL95*	U67	BGLF3	34	Interacts with UL11 protein; regulates UL13 protein kinase
UL16	44	UL94*	U65	BGLF2	33	Interacts with UL11 protein; regulates UL13 protein kinase
UL21[b]	38	UL88	U59	BTRF1	23	
UL36*	22	UL48*	U31	BPLF1	64	Huge virion protein; interacts with UL37 protein; influences release of DNA from capsids during entry

Table 2.2. (*cont.*)

HSV-1	VZV	HCMV	HHV-6	EBV	HHV-8	Function
UL37*	21	UL47	U30	BOLF1	63	Interacts with UL36 protein
UL51	7	UL71*	U44	BSRF1	55	
Surface and membrane						
UL27*	31	UL55*	U39	BALF4	8	gB
UL1*	60	UL115*	U82	BKRF2	47	gL; complexed with gH
UL22*	37	UL75*	U48	BXLF2	22	gH; complexed with gL
UL10	50	UL100*	U72	BBRF3	39	gM; complexed with gN
UL49A[c]	9A	UL73*	U46	BLRF1	53	gN; complexed with gM; not glycosylated in some herpesviruses
Control and modulation						
UL13	47	UL97	U69	BGLF4	36	Serine–threonine protein kinase; tegument protein
UL54*	4	UL69	U42	BSLF1/BMLF1	57	Multifunctional regulator of gene expression
Unknown						
UL24	35	UL76*	U49	BXRF1	20	Nuclear protein

[a] Probably not an active enzyme, as catalytic residues are absent.
[b] This assignment is tentative and is excluded from the total of 43 core genes given in the text. It depends on positional, rather than sequence, conservation, and is compromised by that fact that UL21 is not flanked in each subfamily by clear homologues, unlike other core genes assigned on a positional basis.
[c] Also referred to as UL49.5.

a family of glycoprotein genes in the *Alphaherpesvirinae* (McGeoch, 1990) and 12 families, each containing up to 14 genes, in HCMV as a representative of the *Betaherpesvirinae* (Dolan *et al.*, 2004). There are four gene families in the fish herpesvirus, CCV (Davison, 1992), and 12 in the bivalve herpesvirus, OsHV-1 (Davison *et al.*, 2005). Given that gene duplication has been widely employed in host evolution (Prince and Pickett, 2002), and the greater evolutionary rates of herpesviruses, it seems likely that this means for generating diversity has played a greater part in herpesvirus evolution than can be detected by primary sequence comparisons.

The *Alphaherpesvirinae* subfamily

The employment of gene capture and duplication among the *Beta-* or *Gammaherpesvirinae* to generate diversity has received extensive attention in the literature (for details, see Chapters 15 and 22). In contrast, the *Alphaherpesvirinae* have evolved less adventurously in terms of gene content since their divergence from a common ancestor, and it is clear that gene loss has occurred. This mode of survival is considered in the following paragraphs.

The *Alphaherpesvirinae* contain four genera, plus the reptilian herpesviruses. Several complete genomes have been sequenced for the *Simplexvirus*, *Varicellovirus* and *Mardivirus* genera (Table 2.1). Data for the *Iltovirus* genus are more sparse. Limited sequence data are available for reptilian herpesviruses. Figure 2.3 illustrates the genetic content of members of the *Varicello-*, *Simplex-* and *Mardivirus* genera. Three examples (VZV, EHV-1 and BHV-1) were chosen to represent the major lineages in the *Varicellovirus* genus (see Chapter 1), plus SVV as a close relative of VZV. Core genes are shown in grey, and other genes that have counterparts in all three genera are shown in white. All of these genes were presumably present in the common ancestor, which is estimated to have existed 135 million years ago (McGeoch and Gatherer, 2005), and they comprise all or nearly all of the genes in extant *Alphaherpesvirinae*. It seems that only a few genes have developed since that era, and that the most of these are located near the genome termini.

Figure 2.4 shows a scheme of relationships between the genes at the left end of the genome, based on sequence conversation and the observation that two genes (UL56 and ORF1) encode potential membrane proteins. Also included are data for PRV (whose closest sequenced relative is BHV-1), MDV-2 and ILTV. Since U_L inverts in HSV-1, and U_L in the prototype genome orientation turned out to be inverted in comparison with the *Varicellovirus* genus (Hayward *et al.*, 1975; Wadsworth *et al.*, 1975), the genes at the left end of HSV-1 U_L are presented in the reverse order. In Fig. 2.4,

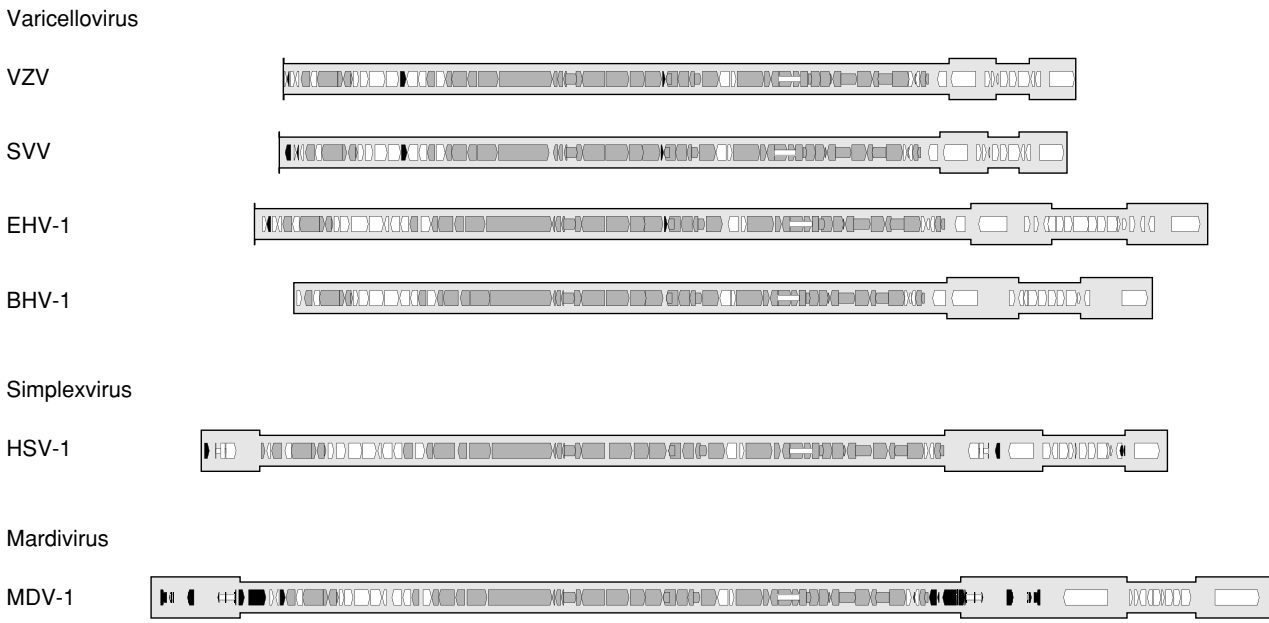

Fig. 2.3. Layout of genes in genomes of the *Alphaherpesvirinae*. Repeat regions are shown in thicker format than unique regions. Protein-coding regions are shown as arrows shaded grey (core genes), white (other genes shared by two or more genera) or black (other genus-specific genes that have presumably evolved more recently), and introns as narrow white bars. Genome coordinates and gene locations were obtained from accessions listed in the legend to Fig. 2.2, and from AF275348 (SVV), M86664 (EHV-1), AJ004801 (BHV-1) and AF243438 (MDV-1).

UL56 and UL55 thus precede UL54, which is a core gene. VZV has two extra genes (ORF1 and ORF2) sandwiched between UL55 and UL56, and the other viruses have between one and four genes in this region. For example, SVV lacks ORF2 and has a partial duplication of UL54 near the end of the genome. A parsimonious approach indicates that the ancestor preceding divergence of the *Iltovirus* genus had at least one of the genes at the left genome terminus (UL56; 180 million years ago; McGeoch and Gatherer, 2005), that the ancestor preceding divergence of the *Mardivirus* genus had at least three (UL56 (since lost), UL55 and ORF2; 135 million years ago), that the ancestor of the *Varicellovirus* and *Simplexvirus* genera had at least three (UL56, UL55 and ORF2; 120 million years ago) and that the ancestor of VZV and EHV-1 had all four genes (82 million years ago). Various of these genes have been lost during subsequent evolution of the mammalian viruses.

Gene loss is also apparent at the right genome terminus (Fig. 2.4), where, again, few genes are specific to one virus or a few closely related viruses. Even the more recently evolved genes may have substantial histories. Of the three HSV-1 genes in this category, two at the right end of U_S (US11 and US12) have counterparts in related viruses of monkeys (HVB and SA8; Ohsawa *et al.*, 2002; Tyler *et al.*, 2005). HSV-1 and HVB are considered to have co-speciated with their hosts about 23 million years ago (McGeoch *et al.*, 2000). The gene at the left genome terminus (RL1; repeated internally) has a counterpart at a similar location in a wallaby herpesvirus genome (Guliani *et al.*, 2002).

Outlook

Investigation of the genome structures, genetic contents and evolution of herpesviruses is a maturing field that undergirds the rest of herpesvirology. As with other complex analytical subjects, future advances will require incisive examination of both new data and the framework into which they are fitted. There is yet room for more surprises.

"It is a mistake to try to look too far ahead. The chain of destiny can only be grasped one link at a time." *Winston Churchill.*

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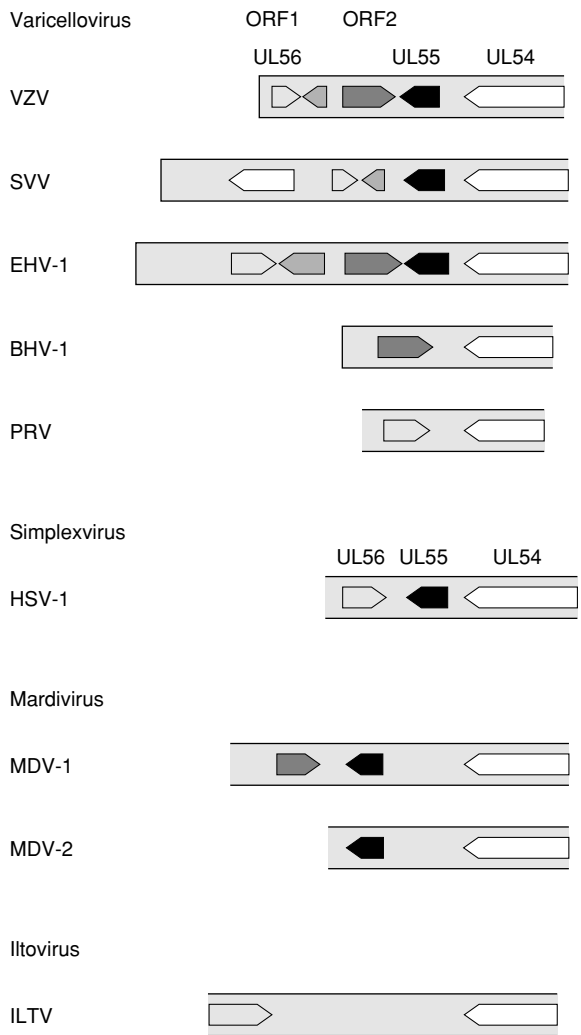

Fig. 2.4. Layout of genes at or near the left terminus in genomes of the *Alphaherpesvirinae*. The left terminus, where included, is shown by a vertical line. Homologous genes are shaded equivalently, and their nomenclature is indicated. An additional non-homologous gene is present in the *Mardi-* and *Iltovirus* genomes down stream from UL55, but is not shown. Gene locations were derived or deduced from accessions listed in the legend to Fig. 2.3, and from BK001744 (PRV), AB049735 (MDV-2) and U80762 (ILTV).

REFERENCES

Afonso, C. L., Tulman, E. R., Lu, Z., Zsak, L., Rock, D. L., and Kutish, G. F. (2001). The genome of turkey herpesvirus. *J. Virol.*, **75**, 971–978.

Albrecht, J. C. (2000). Primary structure of the *Herpesvirus ateles* genome. *J. Virol.*, **74**, 1033–1037.

Albrecht, M., Darai, G., and Flugel, R. M. (1985). Analysis of the genomic termini of tupaia herpesvirus DNA by restriction mapping and nucleotide sequencing. *J. Virol.*, **56**, 466–474.

Albrecht, J. C., Nicholas, J., Biller, D. *et al.* (1992). Primary structure of the herpesvirus saimiri genome. *J. Virol.*, **66**, 5047–5058.

Alexander, L., Denekamp, L., Knapp, A., Auerbach, M. R., Damania, B., and Desrosiers, R. C. (2000). The primary sequence of rhesus monkey rhadinovirus isolate 26–95: sequence similarities to Kaposi's sarcoma-associated herpesvirus and rhesus monkey rhadinovirus isolate 17577. *J. Virol.*, **74**, 3388–3398.

Baer, R., Bankier, A. T., Biggin, M. D. *et al.* (1984). DNA sequence and expression of the B95-8 Epstein–Barr virus genome. *Nature*, **310**, 207–211.

Bahr, U. and Darai, G. (2001). Analysis and characterization of the complete genome of tupaia (tree shrew) herpesvirus. *J. Virol.*, **75**, 4854–4870.

Ben-Porat, T., Veach, R. A., and Ihara, S. (1983). Localization of the regions of homology between the genomes of herpes simplex virus, type 1, and pseudorabies virus. *Virology*, **127**, 194–204.

Bernard, J. and Mercier, A. (1993). Sequence of two Eco RI fragments from salmonis herpesvirus 2 and comparison with ictalurid herpesvirus 1. *Arch. Virol.*, **132**, 437–442.

Bhattacharyya, S. P. and Rao, V. B. (1993). A novel terminase activity associated with the DNA packaging protein gp17 of bacteriophage T4. *Virology*, **196**, 34–44.

Bhella, D., Rixon, F. J., and Dargan, D. J. (2000). Cryomicroscopy of human cytomegalovirus virions reveals more densely packed genomic DNA than in herpes simplex virus type 1. *J. Mol. Biol.*, **295**, 155–161.

Bodescot, M., Perricaudet., M., and Farrell, P. J. (1987). A promoter for the highly spliced EBNA family of RNAs of Epstein–Barr virus. *J. Virol.*, **61**, 3424–3430.

Boehmer, P. E. and Lehman, I. R. (1997). Herpes simplex virus DNA replication. *Annu. Rev. Biochem.*, **66**, 347–384.

Booy, F. P., Newcomb, W. W., Trus, B. L., Brown, J. C., Baker, T. S., and Steven, A. C. (1991). Liquid-crystalline, phage-like packing of encapsidated DNA in herpes simplex virus. *Cell*, **64**, 1007–1015.

Booy, F. P., Trus, B. L., Davison, A. J., and Steven, A. C. (1996). The capsid architecture of channel catfish virus, an evolutionarily distant herpesvirus, is largely conserved in the absence of discernible sequence homology with herpes simplex virus. *Virology*, **215**, 134–141.

Bornkamm, G. W., Delius, H., Fleckenstein, B., Werner, F. J., and Mulder, C. (1976). Structure of *Herpesvirus saimiri* genomes: arrangement of heavy and light sequences in the M genome. *J. Virol.*, **19**, 154–161.

Bowden, R. J., Simas, J. P., Davis, A. J., and Efstathiou, S. (1997). Murine gammaherpesvirus 68 encodes tRNA-like sequences which are expressed during latency. *J. Gen. Virol.*, **78**, 1675–1687.

Bras, F., Dezelee, S., Simonet, B. *et al.* (1999). The left border of the genomic inversion of pseudorabies virus contains genes homologous to the UL46 and UL47 genes of herpes simplex virus type 1, but no UL45 gene. *Virus Res.*, **60**, 29–40.

Bresnahan, W. A. and Shenk, T. (2000). A subset of viral transcripts packaged within human cytomegalovirus particles. *Science*, **288**, 2373–2376.

Broll, H., Buhk, H.-J., Zimmermann, W., and Goltz, M. (1999). Structure and function of the prDNA and genomic termini of the γ_2-herpesvirus bovine herpesvirus type 4. *J. Gen. Virol.*, **80**, 979–986.

Browning, G. F. and Studdert, M. J. (1989). Physical mapping of a genome of equine herpesvirus 2 (equine cytomegalovirus). *Arch. Virol.*, **104**, 77–86.

Cebrian, J., Berthelot, N., and Laithier, M. (1989). Genome structure of cottontail rabbit herpesvirus. *J. Virol.*, **63**, 523–531.

Chee, M. S., Bankier, A. T., Beck, S. *et al.* (1990). Analysis of the protein coding content of the sequence of human cytomegalovirus strain AD169. *Curr. Top. Microbiol. Immunol.*, **154**, 125–169.

Chousterman, S., Lacasa, M., and Sheldrick, P. (1979). Physical map of the channel catfish virus genome: location of sites for restriction endonucleases *Eco*RI, *Hin*dIII, *Hpa*I and *Xba*I. *J. Virol.*, **31**, 73–85.

Clements, J. B., Cortini, R., and Wilkie, N. M. (1976). Analysis of herpesvirus DNA substructure by means of restriction endonucleases. *J. Gen. Virol.*, **30**, 243–256.

Coberley, S. S., Condit, R. C., Herbst, L. H., and Klein, P. A. (2002). Identification and expression of immunogenic proteins of a disease-associated marine turtle herpesvirus. *J. Virol.*, **76**, 10553–10558.

Cunningham, C., Barnard, S., Blackbourn, D. J., and Davison, A. J. (2003). Transcription mapping of human herpesvirus 8 genes encoding viral interferon regulatory factors. *J. Gen. Virol.*, **84**, 1471–1483.

Davison, A. J. (1984). Structure of the genome termini of varicella-zoster virus. *J. Gen. Virol.*, **65**, 1969–1977.

Davison, A. J. (1992). Channel catfish virus: a new type of herpesvirus. *Virology*, **186**, 9–14.

Davison, A. J. (1998). The genome of salmonid herpesvirus 1. *J. Virol.*, **72**, 1974–1982.

Davison, A. J. (2002). Evolution of the herpesviruses. *Vet. Microbiol.*, **86**, 69–88.

Davison, A. J. and Clements, J. B. (1997). Herpesviruses: general properties. In *Topley & Wilson's Microbiology and Microbial Infections, 9th edn*, vol. 1, pp. 309–323. ed. L. Collier, A. Balows and M. Sussman; vol. ed. B. W. J. Mahy and L. Collier. London: Edward Arnold.

Davison, A. J. and Scott J. E. (1986). The complete DNA sequence of varicella-zoster virus. *J. Gen. Virol.*, **67**, 1759–1816.

Davison, A. J. and Taylor, P. (1987). Genetic relations between varicella-zoster virus and Epstein–Barr virus. *J. Gen. Virol.*, **68**, 1067–1079.

Davison, A. J. and Wilkie, N. M. (1983). Location and orientation of homologous sequences in the genomes of five herpesviruses. *J. Gen. Virol.*, **64**, 1927–1942.

Davison, A. J., Sauerbier, W., Dolan, A., Addison, C., and McKinnell, R. G. (1999). Genomic studies of the Lucké tumor herpesvirus (RaHV-1). *J. Cancer Res. Clin. Oncol.*, **125**, 232–238.

Davison, A. J., Dolan, A., Akter, P. *et al.* (2003a). The human cytomegalovirus genome revisited: comparison with the chimpanzee cytomegalovirus genome. *J. Gen. Virol.*, **84**, 17–28.

Davison, A. J., Akter, P., Cunningham, C. *et al.* (2003b). Homology between the human cytomegalovirus RL11 gene family and human adenovirus E3 genes. *J. Gen. Virol.*, **84**, 657–663.

Davison, A. J., Benko, M., and Harrach, B. (2003c). Genetic content and evolution of adenoviruses. *J. Gen. Virol.*, **84**, 2895–2908.

Davison, A. J., Trus, B. L., Cheng, N. *et al.* (2005). A novel class of herpesvirus with bivalve hosts. *J. Gen. Virol.*, **86**, 41–53.

de Jesus, O., Smith, P. R., Spender, L. C. *et al.* (2003). Updated Epstein-Barr virus (EBV) DNA sequence and analysis of a promoter for the BART (CST, BARF0) RNAs of EBV. *J. Gen. Virol.*, **84**, 1443–1450.

Delhon, G., Moraes, M. P., Lu, Z. *et al.* (2003). Genome of bovine herpesvirus 5. *J. Virol.*, **77**, 10339–10347.

Delius, H. and Clements, J. B. (1976). A partial denaturation map of herpes simplex virus type 1 DNA: evidence for inversions of the unique DNA regions. *J. Gen. Virol.*, **33**, 125–133.

DeMarchi, J. M. (1981). Human cytomegalovirus DNA: restriction enzyme cleavage maps and map locations for immediate–early, early, and late RNAs. *Virology*, **114**, 23–38.

Dewhurst, S., Dollard, S. C., Pellett, P. E., and Dambaugh, TR. (1993). Identification of a lytic-phase origin of DNA replication in human herpesvirus 6B strain Z29. *J. Virol.*, **67**, 7680–7683.

Dezelee, S., Bras, F., Vende, P. *et al.* (1996). The *Bam*HI fragment 9 of pseudorabies virus contains genes homologous to the UL24, UL25, UL26, and UL26.5 genes of herpes simplex virus type 1. *Virus Res.*, **42**, 27–39.

Dolan, A., Jamieson, F. E., Cunningham, C., Barnett, B. C., and McGeoch, D. J. (1998). The genome sequence of herpes simplex virus type 2. *J. Virol.*, **72**, 2010–2021.

Dolan, A., Cunningham, C., Hector, R. D. *et al.* (2004). Genetic content of wild type human cytomegalovirus. *J. Gen. Virol.*, **85**, 1301–1312.

Dolan, A., Addison, C., Gatherer, D., Davison, A. J., and McGeoch, D. J. (2006). The genome of Epstein–Barr virus type 2 strain AG876. *Virology*, **350**, 164–170.

Dominguez, G., Black, J. B., Stamey, F. R., Inoue, N., and Pellett, P. E. (1996). Physical and genetic maps of the human herpesvirus 7 strain SB genome. *Arch. Virol.*, **141**, 2387–2408.

Dominguez, G., Dambaugh, T. R., Stamey, F. R., Dewhurst, S., Inoue, N., and Pellett, P. E. (1999). Human herpesvirus 6B genome sequence: coding content and comparison with human herpesvirus 6A. *J. Virol.*, **73**, 8040–8052.

Dumas, A. M., Geelen, J. L., Weststrate, M. W., Wertheim, P., and van der Noordaa, J. (1981). *Xba*I, *Pst*I, and *Bgl*II restriction enzyme maps of the two orientations of the varicella-zoster virus genome. *J. Virol.*, **39**, 390–400.

Dunn, W., Chou, C., Li, H. *et al.* (2003). Functional profiling of a human cytomegalovirus genome. *Proc. Natl Acad. Sci. USA*, **100**, 14223–14228.

Ensser, A., Pflanz, R., and Fleckenstein, B. (1997). Primary structure of the alcelaphine herpesvirus 1 genome. *J. Virol.*, **71**, 6517–6525.

Ensser, A., Thurau, M., Wittmann, S., and Fickenscher, H. (2003). The genome of herpesvirus saimiri C488 which is capable of transforming human T cells. *Virology*, **314**, 471–487.

Furlong, D., Swift, H., and Roizman, B. (1972). Arrangement of herpesvirus deoxyribonucleic acid in the core. *J. Virol.*, **10**, 1071–1074.

Given, D. and Kieff, E. (1979). DNA of Epstein–Barr virus. VI. Mapping of the internal tandem reiteration. *J. Virol.*, **31**, 315–324.

Glenn, M., Rainbow, L., Aurade, F., Davison, A., and Schulz, T. F. (1999). Identification of a spliced gene from Kaposi's sarcoma-associated herpesvirus encoding a protein with similarities to latent membrane proteins 1 and 2A of Epstein–Barr virus. *J. Virol.*, **73**, 6953–6963.

Gomi, Y., Sunamachi, H., Mori, Y., Nagaike, K., Takahashi, M., and Yamanishi, K. (2002). Comparison of the complete DNA sequences of the Oka varicella vaccine and its parental virus. *J. Virol.*, **76**, 11447–11459.

Gompels, U. A., Nicholas, J., Lawrence, G. *et al.* (1995). The DNA sequence of human herpesvirus-6: structure, coding content, and genome evolution. *Virology*, **209**, 29–51.

Grafstrom, R. H., Alwine, J. C., Steinhart, W. L., and Hill, C. W. (1975a). Terminal repetitions in herpes simplex virus type 1 DNA. *Cold Spring Harb. Symp. Quant. Biol.*, **39**, 679–681.

Grafstrom, R. H., Alwine, J. C., Steinhart, W. L., Hill, C. W., and Hyman, R. W. (1975b). The terminal repetition of herpes simplex virus DNA. *Virology*, **67**, 144–157.

Gray, W. L., Starnes, B., White, M. W., and Mahalingam, R. (2001). The DNA sequence of the simian varicella virus genome. *Virology*, **284**, 123–130.

Grose, C., Tyler, S., Peters, G. *et al.* (2004). Complete DNA sequence analyses of the first two varicella-zoster virus glycoprotein E (D150N) mutant viruses found in North America: evolution of genotypes with an accelerated cell spread phenotype. *J. Virol.*, **78**, 6799–6807.

Gubser, C., Hue, S., Kellam P., and Smith, G. L. (2004). Poxvirus genomes: a phylogenetic analysis. *J. Gen. Virol.*, **85**, 105–117

Guliani, S., Polkinghorne, I., Smith, G. A., Young, P., Mattick, J. S., and Mahony, T. J. (2002). Macropodid herpesvirus 1 encodes genes for both thymidylate synthase and ICP34.5. *Virus Genes*, **24**, 207–213.

Hannenhalli, S., Chappey, C., Koonin, E. V., and Pevzner, P. A. (1995). Genome sequence comparison and scenarios for gene rearrangements: a test case. *Genomics*, **30**, 299–311.

Hansen, S. G., Strelow, L. I., Franchi, D. C., Anders, D. G., and Wong, S. W. (2003). Complete sequence and genomic analysis of rhesus cytomegalovirus. *J. Virol.*, **77**, 6620–6636.

Hayward, G. S., Jacob, R. J., Wadsworth, S. C., and Roizman, B. (1975). Anatomy of herpes simplex virus DNA: evidence for four populations of molecules that differ in the relative orientations of their long and short components. *Proc. Natl Acad. Sci. USA*, **72**, 4243–4247.

Honess, R. W. (1984). Herpes simplex and 'the herpes complex': diverse observations and a unifying hypothesis. The Eighth Fleming Lecture. *J. Gen. Virol.*, **65**, 2077–2107.

Honess, R. W. and Roizman, B. (1974). Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. *J. Virol.*, **14**, 8–19.

Honess, R. W., Gompels, U. A., Barrell, B. G. *et al.* (1989). Deviations from expected frequencies of CpG dinucleotides in herpesvirus DNAs may be diagnostic of differences in the states of their latent genomes. *J. Gen. Virol.*, **70**, 837–855.

Hyman, R. W., Burke, S., and Kudler, L. (1976). A nearby inverted repeat of the terminal sequence of herpes simplex virus DNA. *Biochem. Biophys. Res. Commun.*, **68**, 609–615.

Inoue, N., Dambaugh, T. R., Rapp, J. C., and Pellett, P. E. (1994). Alphaherpesvirus origin-binding protein homolog encoded by human herpesvirus 6B, a betaherpesvirus, binds to nucleotide sequences that are similar to *ori* regions of alphaherpesviruses. *J. Virol.*, **68**, 4126–4136.

Isegawa, Y., Mukai, T., Nakano, K. *et al.* (1999). Comparison of the complete DNA sequences of human herpesvirus 6 variants A and B. *J. Virol.*, **73**, 8053–8063.

Izumiya, Y., Jang, H. K., Ono, M., and Mikami, T. (2001). A complete genomic DNA sequence of Marek's disease virus type 2, strain HPRS24. *Curr. Top. Microbiol. Immunol.*, **255**, 191–221.

Jenkins, F. J., and Roizman, B. (1986). Herpes simplex virus 1 recombinants with noninverting genomes frozen in different isomeric arrangements are capable of independent replication. *J. Virol.*, **59**, 494–499.

Jenner, R. G., Albà, M. M., Boshoff, C., and Kellam, P. (2001). Kaposi's sarcoma-associated herpesvirus latent and lytic gene expression as revealed by DNA arrays. *J. Virol.*, **75**, 891–902.

Kellis, M., Patterson, N., Endrizzi, M., Birren, B., and Lander, E. S. (2003). Sequencing and comparison of yeast species to identify genes and regulatory elements. *Nature*, **423**, 241–254.

Kemble, G. W., Annunziato, P., Lungu, O. *et al.* (2000). Open reading frame S/L of varicella-zoster virus encodes a cytoplasmic protein expressed in infected cells. *J. Virol.*, **74**, 11311–11321.

Klupp, B. G., Hengartner, C. J., Mettenleiter, T. C., and Enquist, L. W. (2004). Complete, annotated sequence of the pseudorabies virus genome. *J. Virol.*, **78**, 424–440.

Koch, H. G., Delius, H., Matz, B., Flugel, R. M., Clarke, J., and Darai, G. (1985). Molecular cloning and physical mapping of the tupaia herpesvirus genome. *J. Virol.*, **55**, 86–95.

Lagunoff, M. and Ganem, D. (1997). The structure and coding organization of the genomic termini of Kaposi's sarcoma-associated herpesvirus. *Virology*, **236**, 147–154.

Lee, L. F., Wu, P., Sui, D. *et al.* (2000). The complete unique long sequence and the overall genomic organization of the GA strain of Marek's disease virus. *Proc. Natl Acad. Sci. USA*, **97**, 6091–6096.

Lindquester, G. J. and Pellett, P. E. (1991). Properties of the human herpesvirus 6 strain Z29 genome: G + C content, length, and presence of variable-length directly repeated terminal sequence elements. *Virology*, **182**, 102–110.

Locker, H. and Frenkel, N. (1979). *Bam*I, *Kpn*I, and *Sal*I restriction enzyme maps of the DNAs of herpes simplex virus strains Justin and F: occurrence of heterogeneities in defined regions of the viral DNA. *J. Virol.*, **32**, 429–441.

Markine-Goriaynoff, N., Georgin, J. P., Goltz, M. *et al.* (2003). The core 2 β-1,6-N-acetylglucosaminyltransferase-mucin encoded by bovine herpesvirus 4 was acquired from an ancestor of the African buffalo. *J. Virol.*, **77**, 1784–1792.

Marks, J. R. and Spector, D. H. (1988). Replication of the murine cytomegalovirus genome: structure and role of the termini in generation and cleavage of concatenates. *J. Virol.*, **162**, 98–107.

Martin, M. E., Thomson, B. J., Honess, R. W. *et al.* (1991). The genome of human herpesvirus 6: maps of unit-length and concatemeric genomes for nine restriction endonucleases. *J. Gen. Virol.*, **72**, 157–168.

McGeoch, D. J. (1990). Evolutionary relationships of virion glycoprotein genes in the S regions of alphaherpesvirus genomes. *J. Gen. Virol.*, **71**, 2361–2367.

McGeoch, D. J. and Cook, S. (1994). Molecular phylogeny of the Alphaherpesvirinae subfamily and a proposed evolutionary timescale. *J. Mol. Biol.*, **238**, 9–22.

McGeoch, D. J. and Davison, A. J. (1999). The molecular evolutionary history of the herpesviruses. In *Origin and Evolution of Viruses*, ed. E. Domingo, R. Webster, and J. Holland, pp. 441–465. London: Academic Press.

McGeoch, D. J. and Gatherer, D. (2005). Integrating reptilian herpesviruses into the family *Herpesviridae*. *J. Virol.*, **79**, 725–731.

McGeoch, D. J., Dalrymple, M. A., Davison, A. J. *et al.* (1988). The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. *J. Gen. Virol.*, **69**, 1531–1574.

McGeoch, D. J., Cook, S., Dolan, A., Jamieson, F. E., and Telford, E. A. R. (1995). Molecular phylogeny and evolutionary timescale for the family of mammalian herpesviruses. *J. Mol. Biol.*, **247**, 443–458.

McGeoch, D. J., Dolan, A., and Ralph, A. C. (2000). Toward a comprehensive phylogeny for mammalian and avian herpesviruses. *J. Virol.*, **74**, 10401–10406.

McLysaght, A., Baldi, P. F., and Gaut, B. S. (2003). Extensive gene gain associated with adaptive evolution of poxviruses. *Proc. Natl Acad. Sci. USA*, **100**, 15655–15660.

Megaw, A. G., Rapaport, D., Avidor, B., Frenkel, N., and Davison A. J. (1998). The DNA sequence of the RK strain of human herpesvirus 7. *Virology*, **244**, 119–132.

Midgley, R. S., Bell, A. I., McGeoch, D. J., and Rickinson, A. B. (2003). Latent gene sequencing reveals familial relationships among Chinese Epstein–Barr virus strains and evidence for positive selection of A11 epitope changes. *J. Virol.*, **77**, 11517–11530.

Mitchell, M. S., Matsuzaki, S., Imai, S., and Rao, V. B. (2002). Sequence analysis of bacteriophage T4 DNA packaging/terminase genes 16 and 17 reveals a common ATPase center in the large subunit of viral terminases. *Nucleic Acids Res.*, **30**, 4009–4021.

Mocarski E. S. and Roizman, B. (1982). Structure and role of the herpes simplex virus DNA termini in inversion, circularization and generation of virion DNA. *Cell*, **31**, 89–97.

Moss, B. (2001). Poxviridae: the viruses and their replication. In *Fields Virology*, 4th edn., ed. D. M. Knipe, P. M. Howley, D. E. Griffin *et al.* vol. 2, pp. 2849–2883. Philadelphia: Lippincott, Williams and Wilkins.

Muir, W. B., Nichols, R., and Breuer, J. (2002). Phylogenetic analysis of varicella-zoster virus: evidence of intercontinental spread of genotypes and recombination. *J. Virol.*, **76**, 1971–1979.

Murphy, E., Rigoutsos, I., Shibuya, T., and Shenk, T. E. (2003a). Reevaluation of human cytomegalovirus coding potential. *Proc. Natl Acad. Sci. USA*, **100**, 13585–13590.

Murphy, E., Yu, D., Grimwood, J. *et al.* (2003b). Coding potential of laboratory and clinical strains of human cytomegalovirus. *Proc. Natl Acad. Sci. USA*, **100**, 14976–14981.

Nash, A. A., Dutia, B. M., Stewart, J. P., and Davison, A. J. (2001). Natural history of murine γ-herpesvirus infection. *Phil. Trans. Roy. Soc. Lond. B Biol. Sci.*, **356**, 569–579.

Neipel, F., Albrecht, J. C., and Fleckenstein, B. (1997). Cell-homologous genes in the Kaposi's sarcoma-associated rhadinovirus human herpesvirus 8: determinants of its pathogenicity? *J. Virol.*, **71**, 4187–4192.

Newcomb, W. W., Homa, F. L., Thomsen, D. R. *et al.* (1996). Assembly of the herpes simplex virus capsid: characterization of intermediates observed during cell-free capsid formation. *J. Mol. Biol.*, **263**, 432–446.

Nicholas, J. (1996). Determination and analysis of the complete nucleotide sequence of human herpesvirus 7. *J. Virol.*, **70**, 5975–5989.

Nicholas, J., Cameron, K. R., and Honess, R. W. (1992). Herpesvirus saimiri encodes homologues of G protein-coupled receptors and cyclins. *Nature*, **355**, 362–365.

Nugent, J., Birch-Machin, I., Smith, K. C. *et al.* (2006). Analysis of equid herpesvirus 1 strain variation reveals a point mutation of the DNA polymerase strongly associated with neuropathogenic versus nonneuropathogenic disease outbreaks. *J. Virol.*, **80**, 4047–4060.

O'Hare, P. (1993). The virion transactivator of herpes simplex virus. *Semin. Virol.*, **4**, 145–155.

Ohsawa, K., Black, D. H., Sato, H., and Eberle, R. (2002). Sequence and genetic arrangement of the U_S region of the monkey B virus (*Cercopithecine herpesvirus 1*) genome and comparison with the U_S regions of other primate herpesviruses. *J. Virol.*, **76**, 1516–1520.

Perelygina, L., Zhu, L., Zurkuhlen, H., Mills, R., Borodovsky, M., and Hilliard, J. K. (2003). Complete sequence and comparative analysis of the genome of herpes B virus (*Cercopithecine herpesvirus 1*) from a rhesus monkey. *J. Virol.*, **77**, 6167–6177.

Poole, L. J., Zong, J. C., Ciufo, D. M. *et al.* (1999). Comparison of genetic variability at multiple loci across the genomes of the major subtypes of Kaposi's sarcoma-associated herpesvirus reveals evidence for recombination and for two distinct types of open reading frame K15 alleles at the right-hand end. *J. Virol.*, **73**, 6646–6660.

Prince, V. E. and Pickett, F. B. (2002). Splitting pairs: the diverging fates of duplicated genes. *Nat. Rev. Genet.*, **3**, 827–837.

Quackenbush, S. L., Work, T. M., Balazs, G. H. *et al.* (1998). Three closely related herpesviruses are associated with fibropapillomatosis in marine turtles. *Virology*, **246**, 392–399.

Rall, G. F., Kupershmidt, S., Lu, X. Q., Mettenleiter, T. C., and Ben-Porat, T. (1991). Low-level inversion of the L component of pseudorabies virus is not dependent on sequence homology. *J. Virol.*, **65**, 7016–7019.

Rao, V. B. and Black, L. W. (1988). Cloning, overexpression and purification of the terminase proteins gp16 and gp17 of bacteriophage T4. Construction of a defined in-vitro DNA packaging system using purified terminase proteins. *J. Mol. Biol.*, **200**, 475–488.

Rawlinson, W. D., Farrell, H. E., and Barrell, B. G. (1996). Analysis of the complete DNA sequence of murine cytomegalovirus. *J. Virol.*, **70**, 8833–8849.

Rivailler, P., Cho, Y. G., and Wang, F. (2002a). Complete genomic sequence of an Epstein–Barr virus-related herpesvirus naturally infecting a new world primate: a defining point in the evolution of oncogenic lymphocryptoviruses. *J. Virol.*, **76**, 12055–12068.

Rivailler, P., Jiang, H., Cho, Y. G., Quink, C., and Wang, F. (2002b). Complete nucleotide sequence of the rhesus lymphocryptovirus: genetic validation for an Epstein–Barr virus animal model. *J. Virol.*, **76**, 421–426.

Rixon, F. J. and Ben-Porat, T. (1979). Structural evolution of the DNA of pseudorabies-defective viral particles. *Virology*, **97**, 151–163.

Roizman, B. and Knipe, D. M. (2001). Herpes simplex viruses and their replication. In *Fields Virology*, 4th edn., ed. D. M. Knipe, P. M. Howley, D. E. Griffin *et al.* vol. 2, pp. 2399–2459, Philadelphia: Lippincott, Williams and Wilkins.

Roizman, B. and Pellett, P. E. (2001). The family *Herpesviridae*: a brief introdution. In *Fields Virology*, 4th edn., ed. D. M. Knipe, P. M. Howley, D. E. Griffin *et al.* vol. 2, pp. 2381–2397, Philadelphia: Lippincott, Williams and Wilkins.

Rosa, M. D., Gottlieb, E., Lerner, M. R., and Steitz, J. A. (1981). Striking similarities are exhibited by two small Epstein–Barr virus-encoded ribonucleic acids and the adenovirus-associated ribonucleic acids VAI and VAII. *Mol. Cell. Biol.*, **1**, 785–796.

Russo, J. J., Bohenzky, R. A., Chien, M. C. *et al.* (1996). Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). *Proc. Natl Acad. Sci. USA*, **93**, 14862–14867.

Ruvolo, V. R., Berneman, Z., Secchiero, P., and Nicholas, J. (1996). Cloning, restriction endonuclease mapping and partial sequence analysis of the genome of human herpesvirus 7 strain JI. *J. Gen. Virol.*, **77**, 1901–1912.

Sarid, R., Flore, O., Bohenzky, R. A., Chang, Y., and Moore, P. S. (1998). Transcription mapping of the Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) genome in a body cavity-based lymphoma cell line (BC-1). *J. Virol.*, **72**, 1005–1012.

Schwyzer, M. and Ackermann, M. (1996). Molecular virology of ruminant herpesviruses. *Vet. Microbiol.*, **53**, 17–29.

Searles, R. P., Bergquam, E. P., Axthelm, M. K., and Wong, S. W. (1999). Sequence and genomic analysis of a rhesus macaque rhadinovirus with similarity to Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8. *J. Virol.*, **73**, 3040–3053.

Sheldrick, P. and Berthelot, N. (1975). Inverted repetitions in the chromosome of herpes simplex virus. *Cold Spring Harb. Symp. Quant. Biol.*, **39**, 667–678.

Shenk, T. (2001). Adenoviridae: the viruses and their replication. In *Fields Virology*, 4th edn., ed. D. M. Knipe, P. M. Howley, D. E. Griffin *et al.* vol. 2, pp. 2265–2300, Philadelphia: Lippincott, Williams & Wilkins.

Silverstein, P. S., Bird, R. C., van Santen, V. L., and Nusbaum, K. E. (1995). Immediate–early transcription from the channel catfish virus genome: characterization of two immediate–early transcripts. *J. Virol.*, **69**, 3161–3166.

Stenberg, R. M., Witte, P. R., and Stinski, M. F. (1985). Multiple spliced and unspliced transcripts from human cytomegalovirus immediate–early region 2 and evidence for a common initiation site within immediate–early region 1. *J. Virol.*, **56**, 665–675.

Stevens, J. G., Wagner, E. K., Devi-Rao, G. B., Cook, M. L., and Feldman, L. T. (1987). RNA complementary to a herpesvirus alpha

gene mRNA is prominent in latently infected neurons. *Science*, **235**, 1056–1059.

Stinski, M. F. (1978). Sequence of protein synthesis in cells infected by human cytomegalovirus: early and late virus-induced polypeptides. *J. Virol.*, **26**, 686–701.

Tamashiro, J. C. and Spector, D. H. (1986). Terminal structure and heterogeneity in human cytomegalovirus strain AD169. *J. Virol.*, **59**, 591–594.

Telford, E. A. R., Watson, M. S., McBride, K., and Davison, A. J. (1992). The DNA sequence of equine herpesvirus-1. *Virology*, **189**, 304–316.

Telford, E. A. R., Watson, M. S., Aird, H. C., Perry, J., and Davison, A. J. (1995). The DNA sequence of equine herpesvirus 2. *J. Mol. Biol.*, **249**, 520–528.

Telford, E. A. R., Watson, M. S., Perry, J., Cullinane, A. A., and Davison, A. J. (1998). The DNA sequence of equine herpesvirus-4. *J. Gen. Virol.*, **79**, 1197–1203.

Thureen, D. R. and Keeler, C. L. Jr. (2006). Psittacid herpesvirus 1 and infectious laryngotracheitis virus: comparative genome sequence analysis of two avian alphaherpesviruses. *J. Virol.*, **80**, 7863–7872.

Trus, B. L., Gibson, W., Cheng, N., and Steven A. C. (1999). Capsid structure of simian cytomegalovirus from cryoelectron microscopy: evidence for tegument attachment sites. *J. Virol.*, **73**, 2181–2192.

Tulman, E. R., Afonso, C. L., Lu, Z., Zsak, L., Rock, D. L., and Kutish, G. F. (2000). The genome of a very virulent Marek's disease virus. *J. Virol.*, **74**, 7980–7988.

Tyler, S. D. and Severini, A. (2006). The complete genome sequence of herpesvirus papio 2 (*Cercopithecine herpesvirus 16*) shows evidence of recombination events among various progenitor herpesviruses. *J. Virol.*, **80**, 1214–1221.

Tyler, S. D., Peters, G. A., and Severini, A. (2005). Complete genome sequence of cercopithecine herpesvirus 2 (SA8) and comparison with other simplexviruses. *Virology*, **331**, 429–440.

Upton, C., Slack, S., Hunter, A. L., Ehlers, A., and Roper, R. L. (2003). Poxvirus orthologous clusters: toward defining the minimum essential poxvirus genome. *J. Virol.*, **77**, 7590–7600.

Varnum, S. M., Streblow, D. N., Monroe, M. E. *et al.* (2004). Identification of proteins in human cytomegalovirus (HCMV) particles: the HCMV proteome. *J. Virol.*, **78**, 10960–10966.

Vassilev, V. (2005). Stable and consistent genetic profile of Oka varicella vaccine virus is not linked with appearance of infrequent breakthrough cases postvaccination. *J. Clin. Microbiol.*, **43**, 5415–5416.

Vink, C., Beuken, E., and Bruggeman, C. A. (1996). Structure of the rat cytomegalovirus genome termini. *J. Virol.*, **70**, 5221–5229.

Vink, C., Beuken, E., and Bruggeman, C. A. (2000). Complete DNA sequence of the rat cytomegalovirus genome. *J. Virol.*, **74**, 7656–7665.

Virgin, H. W. 4th, Latreille, P., Wamsley, P. *et al.* (1997). Complete sequence and genomic analysis of murine gammaherpesvirus 68. *J. Virol.*, **71**, 5894–5904.

Wadsworth, S., Jacob, R. J., and Roizman, B. (1975). Anatomy of herpes simplex virus DNA. II. Size, composition, and arrangement of inverted terminal repetitions. *J. Virol.*, **15**, 1487–1497.

Wadsworth, S., Hayward, G. S., and Roizman, B. (1976). Anatomy of herpes simplex virus DNA. V. Terminally repetitive sequences. *J. Virol.*, **17**, 503–512.

Wagner, E. K. (1985). Individual HSV transcripts. In *The Herpesviruses*, ed. B. Roizman, vol. 3, pp. 45–104, New York: Plenum Press.

Wagner, M. J. and Summers, W. C. (1978). Structure of the joint region and the termini of the DNA of herpes simplex virus type 1. *J. Virol.*, **27**, 374–387.

Waltzek, T. B., Kelley, G. O., Stone, D. M. *et al.* (2005). Koi herpesvirus represents a third cyprinid herpesvirus (CyHV-3) in the family *Herpesviridae*. *J. Gen. Virol.*, **86**, 1659–1667.

Ward, P. L. and Roizman, B. (1994). Herpes simplex genes: the blueprint of a successful human pathogen. *Trends Genet.*, **10**, 267–274.

Weststrate, M. W., Geelen, J. L., and van der Noordaa, J. (1980). Human cytomegalovirus DNA: physical maps for restriction endonucleases *Bgl*II, *Hin*dIII and *Xba*I. *J. Gen. Virol.*, **49**, 1–21.

Wilkie, N. M. (1976). Physical maps for herpes simplex virus type 1 DNA for restriction endonucleases *Hind* III, *Hpa*-1, and *X. bad*. *J. Virol.*, **20**, 222–233.

Wilkie, N. M. and Cortini, R. (1976). Sequence arrangement in herpes simplex virus type 1 DNA: identification of terminal fragments in restriction endonuclease digests and evidence for inversions in redundant and unique sequences. *J. Virol.*, **20**, 211–221.

Yu, D. and Weller, S. K. (1998). Genetic analysis of the UL15 gene locus for the putative terminase of herpes simplex virus type 1. *Virology*, **243**, 32–44.

Yu, D., Silva, M. C., and Shenk, T. (2003). Functional map of human cytomegalovirus AD169 defined by global mutational analysis. *Proc. Natl Acad. Sci. USA*, **100**, 12396–12401.

Yu, Q., Hu, N., Lu, Y., Nerurkar, V. R., and Yanagihara, R. (2001). Rapid acquisition of entire DNA polymerase gene of a novel herpesvirus from green turtle fibropapilloma by a genomic walking technique. *J. Virol. Methods*, **91**, 183–195.

Zeng, M. S., Li, D. J., Liu, Q. L. *et al.* (2005). Genomic sequence analysis of Epstein–Barr virus strain GD1 from a nasopharyngeal carcinoma patient. *J. Virol.*, **79**, 15323–15330.

Zhou, Z. H., Chen, D. H., Jakana, J. Rixon F. J., and Chiu W. (1999). Visualization of tegument–capsid interactions and DNA in intact herpes simplex virus type 1 virions. *J. Virol.*, **73**, 3210–3218.

Ziemann, K., Mettenleiter, T. C., and Fuchs, W. (1998). Gene arrangement within the unique long genome region of infectious laryngotracheitis virus is distinct from that of other alphaherpesviruses. *J. Virol.*, **72**, 847–852.

Zimmermann, W., Broll, H., Ehlers, B., Buhk, H. J., Rosenthal, A., and Goltz, M. (2001). Genome sequence of bovine herpesvirus 4, a bovine *Rhadinovirus*, and identification of an origin of DNA replication. *J. Virol.*, **75**, 1186–1194.

3

Comparative virion structures of human herpesviruses

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Introduction

The herpesvirus family consists of a group of viruses distinguished by the large size of their linear double-stranded DNA genomes (~130–250 kbp) and a common architecture of infectious particles (Fig. 3.1) (Chiu and Rixon, 2002; Gibson, 1996; Steven and Spear, 1997). Indeed, before the birth of molecular biology and the availability of genomic sequencing, the common hallmark structural features shared by these viruses were the most important criteria for the classification of a herpesvirus (Roizman and Pellett, 2001). All herpesviruses identified to date, which include eight different types that are known to infect human, and more than 170 other viruses that are found in animals as well as in fish and amphibians (Roizman and Pellett, 2001), exhibit identical structural design as illustrated using human cytomegalovirus shown in Fig. 3.1. These viruses have a highly ordered icosahedral-shape nucleocapsid of about 125–130 nm in diameter, which encases the viral DNA genome. The nucleocapsid is surrounded by a partially ordered proteinaceous layer called the tegument, which in turn is enclosed within the envelope, a polymorphic lipid bilayer containing multiple copies of more than 10 different kinds of viral glycoproteins that are responsible for viral attachment and entry to host cells.

Based on their biological properties such as growth characteristics and tissue tropism, herpesviruses can be further divided into three subfamilies. Among the eight human herpesviruses, the alpha subfamily includes neurotropic viruses and contains the herpes simplex virus (HSV) 1 and 2, and Varicella zoster virus (VZV). The members of the gamma subfamily are lymphotropic viruses and include Epstein–Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV). The viruses of the beta subfamily appear to be able to establish infections in many different types of cells and tissues, and include human cytomegalovirus (HCMV), and human herpesvirus 6 and 7. This subfamily classification system is largely consistent with the extensive genomic information that is now available (McGeoch *et al.*, 2000). While studies have been attempted to investigate the structure and architecture of each of the eight human herpesviruses, virion and virus-related particles of herpes simplex virus 1 (HSV-1), the prototype of all herpesviruses, have been subjected to the most extensive structural studies (Booy *et al.*, 1991; Newcomb *et al.*, 1993, 2000; Schrag *et al.*, 1989; Trus *et al.*, 1996; Zhou *et al.*, 1999, 2000). During the last several years, significant progress has also been made in understanding the structure of cytomegaloviruses (Bhella *et al.*, 2000; Chen *et al.*, 1999; Trus *et al.*, 1999), the prototype of the beta herpesvirus family, and KSHV, a representative of the gamma herpesvirus family (Lo *et al.*, 2003; Nealon *et al.*, 2001; Trus *et al.*, 2001; Wu *et al.*, 2000). Using HSV-1, HCMV, and KSHV as examples for each of the subfamilies, this chapter focuses primarily on the structures of these three viruses, and discusses the recent progress on understanding the structures of human herpesviruses.

Different virus-related particles found in infected cells

Summary of virion assembly pathway

Each of the herpesviruses encodes a specific set of proteins that form the different compartments of the virion (e.g. capsid, Table 3.1). Although many of the primary amino acid sequences of these proteins are not highly conserved among different viruses, the assembly pathway of the virus particles is highly similar (Fig. 3.2) (Gibson, 1996; Roizman and Knipe, 2001; Yu *et al.*, 2003). The nucleocapsid

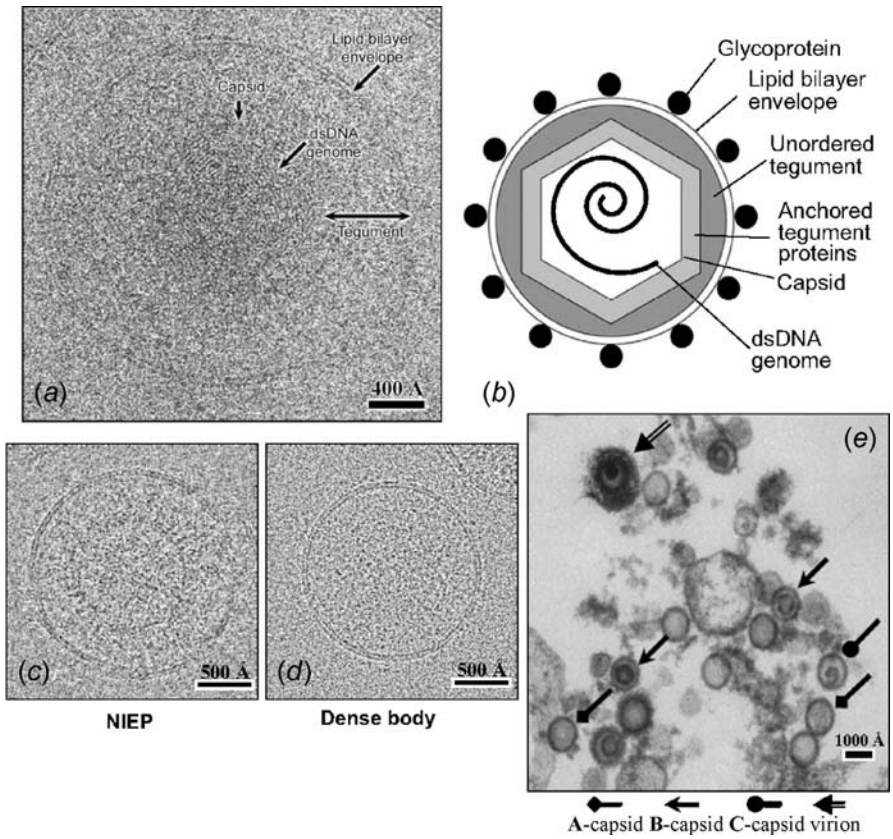

Fig. 3.1. Herpesvirus architecture. (*a*) Electron cryomicrograph of a human cytomegalovirus virion showing the different compartments of a herpes virion. (*b*) Schematic diagram illustrating the multilayer organization of human herpesviruses. Also shown are the electron cryomicrograph of a non-infectious enveloped particle (NIEP) (*c*) and a dense body (*d*) isolated from HCMV virion preparations. (*e*) Virions and different kinds of capsids observed in the thin sections of human foreskin fibroblasts infected with HCMV by negative stain electron microscopy.

is formed in the nucleus and follows a pathway that bears a marked resemblance to those of DNA bacteriophages (Casjens and Hendrix, 1988). First, a procapsid is assembled with the formation of the capsid shell and the internal scaffolding structure. Second, the procapsid is converted into mature nucleocapsid, during which time, the morphogenic internal scaffolding protein is released and replaced by the viral DNA genome, concomitant with a major conformation change of the capsid shell (Newcomb *et al.*, 1999; Yu *et al.*, 2005). Subsequent events, however, differ from the phage assembly pathway (Fig. 3.2). The mature nucleocapsid exits the nucleus and acquires its tegument and envelope, through repeated fusion with and detachment from nuclear membranes and other cellular membranous structures. Eventually, the mature infectious virion particles are released into the extracellular space via cellular secretory pathways. During this assembly process, different virus-related particles and structures, including the mature nucleocapsids and virions as well as the intermediate and aberrant products, can be found in the infected cells and the extracellular media (Figs. 3.1(*c*)–(*e*) and 3.2).

Different virus-like particles secreted from infected cells

Since the discovery of the herpesviruses, it has been long recognized that, in addition to producing infectious virus particles, the infected host cells also generate noninfectious particles such as noninfectious enveloped particles (NIEP, Fig. 3.1(*c*)) and dense bodies (DB) (Figs. 3.1(*d*) and 3.2) (Gibson, 1996; Steven and Spear, 1997). Both NIEP and DB are commonly found in the culture media of cells that are lytically infected with HSV-1 and HCMV. The ratio

Table 3.1. Major virion proteins present in HSV-1, HCMV and KSHV

		HSV-1			HCMV			KSHV			
Location	Common name	Protein name	ORF	Size (aa)	Protein name	ORF	Size (aa)	Protein name	ORF	Size (aa)	
inside the capsid	protease	protease	UL26	635	NP1c	UL80a	708	Pr	ORF17	553	~100
	Scaffolding	VP22a	UL26.5	329	AP	UL80.5	373	AP	ORF17.5	283	~1200 in B-capsid, 0 in A- & C-capsids
	MCP	VP5	UL19	1374	MCP	UL86	1370	MCP	ORF25	1376	960; penton & hexon subunit
on the Capsid shell	TRI-2	VP23	UL18	318	mCP	UL85	306	TRI-2	ORF26	305	640, dimer in triplex
	TRI-1	VP19c	UL38	465	mCBP	UL46	290	TRI-1	ORF62	331	320, monomer in triplex
	SCP	VP26	UL35	112	SCP	UL48.5	75	SCP	ORF65	170	900; hexon tip

Abbreviations: MCP, major capsid protein; TRI-2, triplex dimer protein; TRI-1, triplex monomer protein; SCP, smallest capsid protein; Pr, protease; AP, assembly protein.

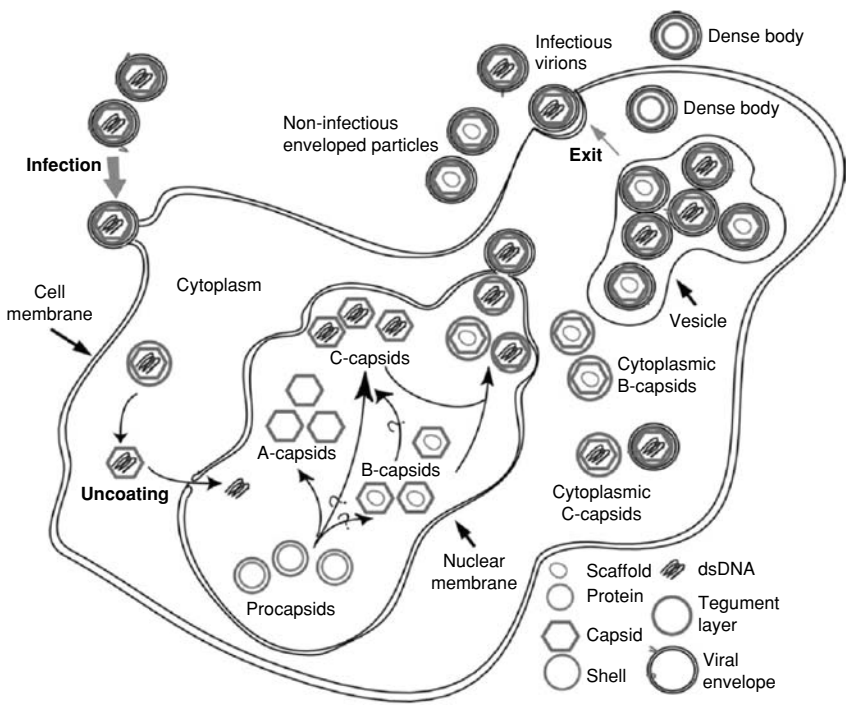

Fig. 3.2. Different virus-like particles and structures during lytic cycle of herpesvirus replication. The infectious virion initializes infection by either endocytosis or fusion with the cell membrane, which releases the nucleocapsid and some tegument proteins into the cytoplasm. The nucleocapsid is uncoated and transported across the cytoplasm (Sodeik *et al.*, 1997), allowing injection of the viral DNA through nuclear pores into the nucleus, where replication and capsid assembly take place. Procapsids mature into the C-capsid by encapsidating the viral dsDNA. Failure of DNA encapsidation results in the abortive A-capsid. Both B-capsid and C-capsid can acquire a layer of tegument proteins at the nuclear membrane of the host cell to become cytoplasmic capsids, and are enveloped and released by exocytosis to become non-infectious (NIEP) and infectious particles, respectively. Dense bodies, which contain a large amount of tegument proteins but no capsids or viral DNA, can also be found in the extracellular media.

of these particles to mature infectious virion particles can sometimes reach 20:1, suggesting that they are produced in great excess (Gibson, 1996; Steven and Spear, 1997). The exact function of these non-infectious particles in viral infection and replication is currently unknown, although they have been proposed to act as decoys that saturate and overwhelm the immune surveillance thereby facilitating the survival of the infectious virions in the hosts (Gibson, 1996; Steven and Spear, 1997).

Structurally, both the NIEP and DB are significantly different from the infectious virion (*cf.* Fig. 3.1(*a*), (*c*), (*d*)). They can be easily distinguished using electron cryomicroscopy (cryoEM) and separated from the mature infectious virions using ultracentrifugation approaches. As described above, all infectious herpesvirus virions share four common structural features (Fig. 3.1(*b*)). First, all herpesviruses contain a large double-stranded DNA (dsDNA) genome. The genomic DNA represents a dense core of ~90 nm in diameter, which can be stained with uranyl acetate and visualized using electron microscopy (Gibson, 1996; Steven and Spear, 1997) and appears as "fingerprint" patterns when examined by electron cryomicroscopy (Fig. 3.1(*a*)) (Booy *et al.*, 1991; Zhou *et al.*, 1999). Second, a capsid of icosahedral shape, which primarily consists of many copies of four different viral proteins, encases the genomic DNA. Third, a protein layer structure, named as the tegument first by Roizman and Furlong (Roizman and Furlong, 1974), surrounds the capsid and occupies the space between the capsid and the envelope. The tegument structure contains many virus-encoded factors that are important for initiating viral gene transcription and expression as well as modulating host metabolism and shutting down host antiviral defense mechanism (for a brief review, see Roizman and Sears, 1996). Finally, a lipid-bilayer envelope constitutes the outermost perimeters of the particles, and contains all the surface virion glycoproteins that are responsible for viral infectivity and entry (Fig. 3.1).

Unlike infectious virion particles, a NIEP does not contain a genomic DNA core and its capsid core appears to be B-capsid-like under electron microscopy (Figs. 3.1(*c*) and 3.2). In contrast, a dense body does not contain a capsid and appears as a cluster of tegument proteins encased by the lipid-bilayer membranous envelope (Fig. 3.1(*d*)). The presence of NIEP and DB indicates that neither packaging of viral genome nor capsid formation is required for viral envelopment.

Different capsid-like structures inside the infected cells

The capsid assembly is a continuous sequential process, leading to the synthesis of the highly ordered capsid structures. In cells that are lytically infected with herpesviruses, several kinds of virus capsid-like structures have been identified as representing stable endpoints or long-lived states (Figs. 3.1 and 3.2). Gibson and Roizman first introduced the terms A-, B-, and C-capsids to describe these intracellular capsid-like structures in HSV-1 infected cells (Gibson and Roizman, 1972). Similar capsid structures have been observed in cells infected with HCMV (Gibson, 1996; Irmiere and Gibson, 1985). Recent work has revealed A-, B- and C-capsids of comparable chemical composition and structural features in the nuclei of gammaherpesvirus infected cells and this suggests that the gammaherpesvirus capsid assembly probably also proceeds in a similar manner (O'Connor *et al.*, 2003; Yu *et al.*, 2003). These capsids all have a distinctive polyhedral shape when examined under electron microscope. Another capsid type, termed procapsid, can be obtained from in vitro assembly experiments using recombinant capsid proteins or from cells infected by a HSV-1 mutant containing a temperature sensitive mutation at the gene encoding the viral protease (Rixon and McNab, 1999; Trus *et al.*, 1996). The procapsid has a distinctive spherical shape and is only transiently stable. They undergo spontaneous structural rearrangement to become the stable angular or polyhedral form similar to the other types of capsids (Heymann *et al.*, 2003; Yu *et al.*, 2005; Zhou *et al.*, 1998b). A-capsids represent empty capsid shells that contain neither viral DNA nor any other discernible internal structure. They are thought to arise from abortive, dead-end products derived from either the inappropriate loss of viral DNA from a C-capsid or the premature release of scaffolding protein from a B-capsid without concurrent DNA packaging (Gibson, 1996). B-capsids are capsid shells containing an inner array of scaffolding protein. C-capsids are mature capsid shells that are packaged with viral DNA and do not contain the scaffolding proteins. B-capsids are believed to be derived from the procapsids upon proteolytic cleavage of the scaffolding protein, and their fate in viral maturation is controversial. Early pulse-chase experiments have suggested that B-capsids can mature to C-capsids, which in turn serve as the infectious virus precursors (Perdue *et al.*, 1976), and recent studies suggest that they might also be a dead-end product in capsid assembly similar to A-capsids (Trus *et al.*, 1996; Yu *et al.*, 2004). It remains unclear whether the spherical procapsids first adapt to the stable angular form before or after the cleavage of its scaffolding protein. The C-capsid buds through the nuclear membrane using an envelopment and de-envelopment process and acquires an additional layer of proteins that forms the tegument in the cytoplasm (for review, see Mettenleiter, 2002). Enveloped virions are then released by exocytosis (Fig. 3.2).

Assembly of viral capsid

A-, B- and C-capsids represent the stable intermediates or the end products of the herpesvirus capsid assembly process (Figs. 3.1(*e*) and 3.2). In HSV-1, capsid assembly begins with the formation of the spherical procapsid through the association of the carboxyl terminus of the scaffolding protein with the amino terminus of the viral major capsid protein (MCP), similar to bacteriophage proheads (Conway *et al.*, 1995; Jiang *et al.*, 2003). Previous experiments have shown that the procapsid can be assembled in vitro from the capsid and scaffolding proteins, in the absence of the viral capsid maturation protease (Newcomb *et al.*, 1999) or from cells infected with viruses containing a temperature-sensitive protease mutant (Heymann *et al.*, 2003). These procapsids can spontaneously rearrange into a large-cored, angular particle resembling the B-capsid, but these large-cored particles do not encapsidate DNA or become mature virions. Past studies have also shown that cells infected with a HSV-1 mutant containing a temperature-sensitive mutation in the protease gene produced capsids that assemble at the non-permissive temperature, similar to the in vitro-assembled procapsids (Rixon and McNab, 1999). The capsids matured when protease activity was restored (Rixon and McNab, 1999), demonstrating that the procapsid is the precursor to the angular capsid (Fig. 3.2). The proteolytic cleavage of the intra-capsid scaffolding proteins at their C-termini by the viral protease (Hong *et al.*, 1996; Liu and Roizman, 1991, 1992; Preston *et al.*, 1992; Welch *et al.*, 1991) interrupts the interactions between the scaffolding proteins and the major capsid proteins (Zhou *et al.*, 1998b). The interactions between the scaffolding protein, the major capsid protein, and viral protease are important targets for antiviral drug design in treating and controlling herpesvirus infections (Flynn *et al.*, 1997; Qiu *et al.*, 1996; Shieh *et al.*, 1996; Tong *et al.*, 1996, 1998). Proteolytic cleavage of the scaffolding protein is followed by the recruitment of the smallest capsid protein, VP26, through an ATP-dependent process (Chi and Wilson, 2000), leading to the formation of the intermediate or B-capsids. The mature procapsids are believed to arise spontaneously by packaging the viral genome DNA, a process that is currently not completely understood (Yu *et al.*, 2005).

Compositions and three-dimensional structural comparisons of alpha, beta and gammaherpesvirus capsids

A-, B-, and C-capsids (Yu *et al.*, 2005) can be isolated from the nucleus of the host cells lytically infected by herpesviruses and they have been subjected to three-dimensional structure studies for HSV-1 (Zhou *et al.*, 1998a, 1994), HCMV (Butcher *et al.*, 1998; Chen *et al.*, 1999; Trus *et al.*, 1999), and KSHV (Nealon *et al.*, 2001; Trus *et al.*, 2001; Wu *et al.*, 2000; Yu *et al.*, 2003). While these three types of capsids have different composition (e.g., viral DNA and internal scaffolding protein), they all have a common shell structure that consists of 150 hexameric (hexon) and 12 pentametric (penton) capsomers, which are connected in groups of three by the triplexes, asymmetric structures that lie on the capsid floor (Fig. 3.3). During the last few years, considerable progress of the three-dimensional structure of the capsids and the assembly of the capsomers and triplexes has been made on the studies.

The capsid, approximately 1250–1300 Å in diameter, is a $T = 16$ icosahedron with 12 pentons forming the vertices, 150 hexons forming the faces and edges, and 320 triplexes interconnecting the pentons and hexons (Rixon, 1993; Steven and Spear, 1997). One of the 20 triangular faces of the icosahedral capsid is indicated by the dotted triangle in Fig. 3.3(*a*) with three fivefold ('5'), a twofold ('2') and threefold (through triplex Tf) symmetry axes labeled. The six fivefold axes pass through the vertices, the ten threefold (3f) axes pass through the centers of the faces, and the 15 twofold (2f) axes pass through the middle of the edges. The structural components in one asymmetric unit are labeled, including 1/5 of a penton ('5'), one P (peri-pentonal) hexon, one C (center) hexons a half E (edge) hexon (Steven *et al.*, 1986), and one each of Ta, Tb, Tc, Td and Te triplex and 1/3 of Tf triplex (Fig. 3.3(*a*)) (Zhou *et al.*, 1994).

HSV-1 is the easiest to grow among all human herpesviruses and has been subjected to the most thorough structural analyses, and its capsid has been reconstructed to 8.5 Å resolution (Fig. 3.3(*a*)) (adapted from Zhou *et al.*, 2000 with permission from the publisher). The capsid shell has a total mass of about 200 MDa. The structural features of the capsid are built from four of the six capsid proteins: 960 copies of the major capsid protein (MCP), VP5; 320 copies of triplex monomer protein (TRI-1), VP19c; 640 copies of triplex dimer protein (TRI-2), VP23; and 900 copies of the smallest capsid protein (SCP), VP26. At this high resolution, details of secondary structure can be resolved that are not visible at lower resolution. Alpha-helices, for example, appear as extended, cylindrical rods of 5–7 Å diameter. The VP5 major capsid protein of HSV-1 was found to contain 24 helices. These assignments of helices to densities were corroborated by docking the cryoEM structure with X-ray crystallographic data which were subsequently obtained for the upper domain of VP5 (Fig. 3.3(*c*)) (Baker *et al.*, 2003; Bowman *et al.*, 2003). A group of seven helices is clustered near the area of the protein that forms the narrowest part of the

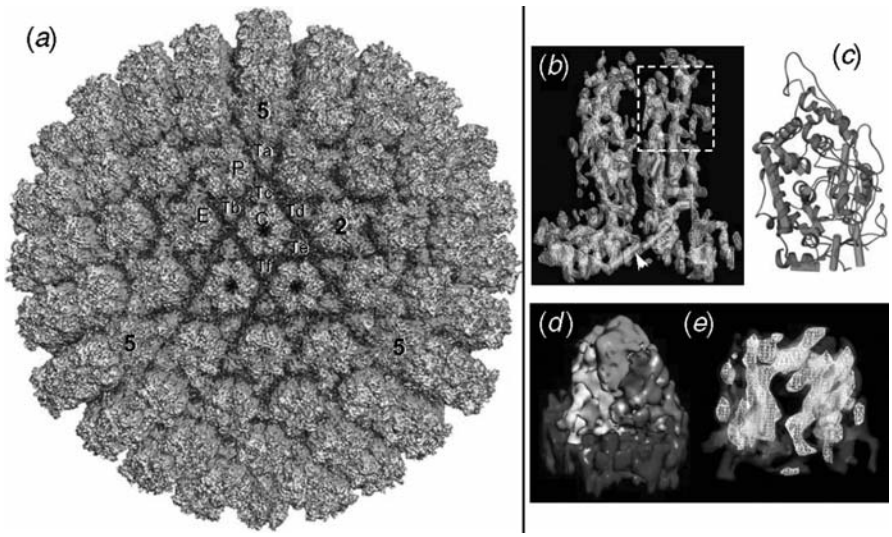

Fig. 3.3. HSV-1 capsid at 8 Å resolution (Zhou *et al.*, 2000) and atomic model of upper domain of the major capsid protein (MCP), VP5 (Bowman *et al.*, 2003). (*a*) Radially color-coded surface representation of the HSV-1 B capsid structure at 8.5 Å. One of the 20 triangular faces is denoted by dashed triangle. The penton and three types of hexons are indicated by '5', P, E and C. Also labeled are the six quasi-equivalent triplexes, Ta, Tb, Tc, Td, Te, Tf. (*b*) Two hexon subunits were shown in wire frame representation with α helices identified in one of the VP5 subunit illustrated by orange cylinders (5 Å in diameter). The red arrowhead points to the 7 helix bundle in the middle domain and the white arrow identifies the long helix in the floor domain that connects adjacent subunits.
(*c*) Ribbon representation of the atomic structure of the HSV-1 MCP upper domain determined by X-ray crystallography (Bowman *et al.*, 2003). The helices identified in the hexon VP5 subunit in the 8.5 Å HSV1 capsid map (Zhou *et al.*, 2000) are shown as cylinders: those in green match with helices present in the X-ray structure and those in yellow are absent in the X-ray model, suggesting possible structural differences of MCP packed in the crystal and inside the virion. (*d*) One single triplex is shown as shaded surface representation with individual subunits in different colors: VP19c in green and the two quasi-equivalent VP23 subunits in light and dark grey, all situated on the capsid shell domains of VP5 (blue). (*e*) α-helices identified in the two quasi-equivalent VP23 molecules (in red and yellow cylinders of 5 Å diameter, respectively). Adapted with permissions from publishers. (See color plate section.)

axial channel of the pentons and hexons (indicated by the red arrowhead in Fig. 3.3(*b*)). Shifts in these helices might be responsible for the constriction that closes off the channel to prevent release of packaged DNA. The floor domain of VP5 also contains several helices, including an unusually long one that interacts with the scaffolding core and may also interact with adjacent subunits to stabilize the capsid (arrow in Fig. 3.3(*b*)). Structural studies of in vitro assembled capsids that are representatives of capsid maturation stages suggest that substantial structural rearrangement at this region is directly related to the reinforcement of penton and hexons during morphogenesis (Heymann *et al.*, 2003).

The higher resolution of this reconstruction also revealed the quaternary structure of the triplexes, which are composed of two molecules of VP23 and one molecule of VP19c (Fig. 3.3(*d*), (*e*)). The lower portion of the triplex, which interacts with the floor of the pentons and hexons, are threefold symmetric with all three subunits roughly equivalent. This arrangement alters through the middle of the triplex such that the upper portion is composed mostly of VP23 in a dimeric configuration. It appears that all three subunits of the triplex are required for the correct tertiary structure to form because VP23 in isolation exists only as a molten globule with no distinct tertiary structure (Kirkitadze *et al.*, 1998).

The capsids of other human herpesviruses have also been studied by electron cryomicroscopy, including HCMV and simian cytomegalovirus (SCMV), and KSHV, members of the beta and gammaherpesviruses, respectively (Fig. 3.4) (Bhella *et al.*, 2000; Chen *et al.*, 1999; Trus *et al.*, 1999, 2001; Wu *et al.*, 2000). The HCMV capsid structure is very similar to HSV-1 in overall organization, with four homologous structural proteins at the same stoichiometries (Fig. 3.4(*a*) and (*b*)). The main difference is that the HCMV capsid had a larger diameter (650 Å) than HSV-1 (620 Å), resulting in a volume ratio of 1.17 (Bhella *et al.*, 2000; Chen *et al.*, 1999; Trus *et al.*, 1999). The increased size of the HCMV

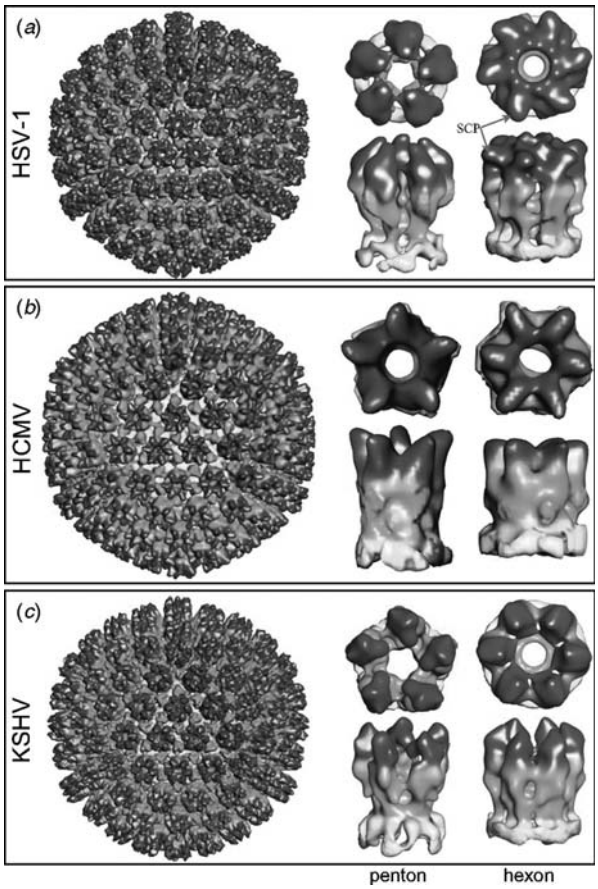

Fig. 3.4. Comparison of the three-dimensional structures of alpha, beta and gammaherpesvirus capsids. The capsid maps of HSV-1 (*a*), HCMV (*b*) and KSHV (*c*) are shown as shaded surfaces colored according to particle radius and viewed along an icosahedral three-fold axis. The resolution of the HSV-1 and KSHV capsid maps is 24 Å and that of the HCMV capsid (Butcher *et al.*, 1998) is 35 Å. The right two columns are detailed comparisons of a penton and an E hexon, which were extracted computationally from each map and shown in their top and side views. (See color plate section.)

capsid despite the similar molecular mass of its component proteins results in a greater center-to-center spacing of the capsomers compared to HSV-1 (Fig. 3.4(*b*)).

The structure of KSHV capsids was also determined by cryoEM to 24 Å resolution and exhibit structural features very similar to those of HSV-1 and HCMV capsids (Fig. 3.4(*c*)) (Trus *et al.*, 2001; Wu *et al.*, 2000). The KSHV and HSV-1 capsids are identical in size and capsomer organization. However, some notable differences are seen upon closer inspection. The KSHV capsid appears slightly more spherical than the HSV-1 capsid, which exhibits a somewhat angular, polyhedral shape. When viewed from the top, the hexons in the KSHV capsid appear flower-shaped, whereas those of HSV-1 have slightly tilted subunits and as a result appear more gear-shaped (see below). Also, the KSHV triplexes are slightly smaller and deviate less from threefold symmetry than the much-elongated triplexes in the HSV-1 capsid. The differences in the upper domains of HSV-1 and KSHV triplexes indicate that the HSV-1 triplexes are slightly taller. The radial density profiles show that the KSHV and HSV-1 capsids have identical inner radii of 460 Å (Wu *et al.*, 2000). Because both viruses also have similar genome sizes, their identical inner radii suggest that their DNA packing densities inside the capsids are similar. In contrast, betaherpesvirus capsids, such as those of HCMV, have a somewhat larger internal volume than HSV-1 or KSHV capsids (Bhella *et al.*, 2000; Chen *et al.*, 1999; Trus *et al.*, 1999). However, the increase in volume is disproportionate to the large increase in the size of the HCMV genome over the HSV-1 and KSHV genomes. This implies that the viral DNA is more densely packed into HCMV virions than into HSV-1 or KSHV virions.

In herpesvirus capsids, both the penton and hexon have a cylindrical shape (about 140-Å diameter, 160-Å height) with a central, axial channel approximately 25 Å in diameter (Fig. 3.4). The penton and hexon subunits both have an elongated shape with multiple domains, including upper, middle, lower, and floor domains. The middle domains of the subunits interact with the triplexes. The lower domains connect the subunits to each other and form the axial channels. While the upper domains of adjacent hexon subunits interact with one another, adjacent penton subunits are disconnected at their upper domains, resulting in the V-shaped side view of the pentons (Fig. 3.4). Another major difference between the penton and hexon concerns their floor domains. These domains play an essential role in maintaining capsid stability, as suggested by the higher-resolution structural studies of the HSV-1 capsid (Zhou *et al.*, 2000), where a long α-helix inserts into the floor domain of the adjacent subunit (Fig. 3.3(*b*)). The relative angle between the floor and lower domains is about 110° in the penton subunit and becomes less than 90° in the hexon subunit, making the penton to appear longer in its side view.

The HSV-1 penton and hexon subunits have the same basic shape as the HCMV and KSHV subunits (Fig. 3.4). Each consists of upper, middle, lower, and floor domains. However, the upper domains of the HSV-1 penton subunits point inward toward the channel, whereas those of the HCMV and KSHV penton subunits point outward. The upper domain of the KSHV subunit has a rectangular

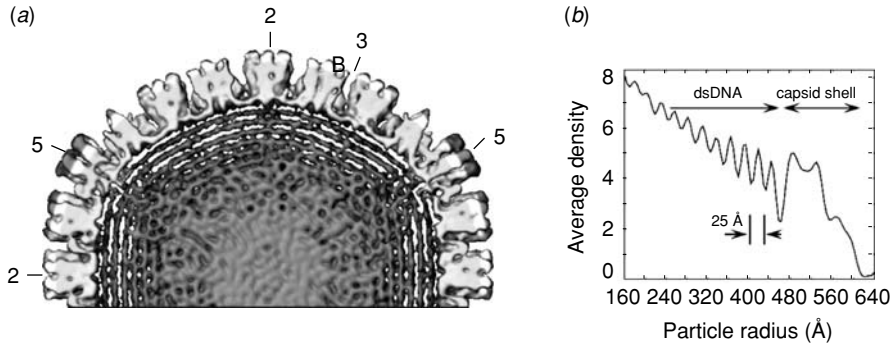

Fig. 3.5. Packing of dsDNA inside herpesvirus capsid (Yu *et al.*, 2003). (*a*) The upper half of a 100-Å thick central slice extracted from the 21 Å resolution reconstruction of the C-capsid of the rhesus rhadinovirus (RRV), a gammaherpesvirus and the closest KSHV homologue. The slice exhibits high-density features organized as multiple spherical shells inside the inner surface of the capsid floor. At least six concentric shells can be distinguished before the pattern becomes indistinct toward the center of the capsid. (*b*) Radial density distribution of the C-capsid obtained by spherically averaging the C-capsid reconstruction and plotted as a function of radius. It is evident that the distance between neighboring peaks is about 25 Å.

shape, while that of the HSV-1 penton subunit appears as a triangle. The most striking difference is that the HSV-1 hexon subunits contain an extra horn-shaped density which is not found in the HSV-1 penton (Fig. 3.4(*a*), arrow in right panel). This extra density binds to the top of each HSV-1 hexon subunit and has been shown to be the SCP, VP26, by difference imaging (Trus *et al.*, 1995; Zhou *et al.*, 1995), which associate with one another to form a hexameric ring around the hexon at a radius of approximately 600 Å. This accounts for the tilted or gear-like appearance of the HSV-1 hexon top view. The KSHV homolog of HSV-1 VP26 is ORF65. Difference map of anti-ORF65 antibody labeled and unlabeled KSHV capsids also showed that ORF65 binds only to the upper domain of the major capsid proteins in hexons but not to those in pentons (Lo *et al.*, 2003). The lack of horn-shaped densities on the hexons indicates that KSHV SCP exhibits substantially different structural features from HSV-1 SCP. The location of SCP at the outermost regions of the capsid suggests a possible role in mediating capsid interactions with the tegument and cytoskeleton proteins during infection.

Structure and packaging of viral genomic DNA

The sizes of the dsDNA genomes of different human herpesviruses vary substantially, e.g., the HCMV genome is 51% longer than HSV-1 (Davison *et al.*, 2003; McGeoch *et al.*, 2000). The major point of interest concerns the packing of their genomes within the capsids. The HCMV capsid is 117% larger than HSV-1. Besides the volume, factors such as DNA density, capsid capacity, and capsid expansion can also influence DNA packaging in viruses. The genome of HCMV might be more densely packed than that of HSV-1, or might induce expansion of the capsid upon packaging. Alternatively, the two viruses might have a similar capacity but differ in the amount of unoccupied space at the center of the capsids.

In HSV-1, the genomic DNA within the nucleocapsid is closely packed into multiple shells of regularly spaced densities, with 26 Å between adjacent DNA duplexes (Zhou *et al.*, 1999). The central slice and radial density plot in Fig. 3.5 indicate that the C-capsid of Rhesus rhadinovirus (RRV), a gammaherpesvirus, has an almost identical pattern of DNA organization to those observed in HSV-1, though slightly more compact, with a 25-Å inter-duplex distance (Yu *et al.*, 2003). Although the RRV capsid, like the KSHV capsid, has nearly the same diameter as the HSV-1 capsid (1250 Å), RRV has a slightly larger genome size than HSV-1, ~165 vs. 153 kb, respectively (Alexander *et al.*, 2000; Lagunoff and Ganem, 1997; Renne *et al.*, 1996; Searles *et al.*, 1999). Therefore, the smaller inter-duplex distance may merely reflect the need to compact this greater amount of DNA into the same volume within the capsid. HCMV has the largest genome (~230 kb) of all human herpesviruses but has a capsid that is only slightly larger (1300 Å diameter), and its DNA was shown to pack with an interduplex distance of only 23 Å (Bhella *et al.*, 2000). Based on the interduplex spacing and the genome sizes, we estimate that the closely packed DNA genomes of HSV-1, RRV, and HCMV would occupy a total volume of 3.52×10^8 Å^3, 3.51×10^8Å^3, and 4.05×10^8Å^3, respectively (Yu *et al.*, 2003). These volumes would measure approximately 92%, 92%, and 90% of the total available spaces inside the HSV-1, RRV, and

HCMV capsids, as estimated on the basis of their inner diameters of 900 Å, 900 Å and 950 Å, respectively. The 23–26 Å packing of strands of herpesvirus dsDNA is very close to the 20-Å diameter of B-type dsDNA, suggesting that herpesvirus genomes are packed as "naked" DNA without any bound histone-like basic proteins. In this regard, SDS-PAGE analyses demonstrated that the A-capsids and C-capsids have the same protein composition (Booy *et al.*, 1991; O'Connor *et al.*, 2003). In the absence of histone-like proteins, close packing of naked DNA would lead to a potentially strong electrostatic repulsion between the juxtaposed negatively charged DNA duplexes. This would make the packaging of DNA into procapsid energetically unfavorable, supporting the need for an energy-dependent DNA packaging machinery such as the bacteriophage-like connector recently reported in HSV-1 capsids (Newcomb *et al.*, 2001). Even so, it is conceivable that the negative charge of DNA may at least be partially neutralized by binding polyamines (Gibson and Roizman, 1971) or some other undiscovered small basic molecules to reduce the strong electrostatic repulsion.

Structure and assembly of tegument

Composition of viral tegument

The tegument occupies the space between the capsid and the envelope. Since the capsid and virion are ~125 nm and ~220 nm in diameter, respectively, the tegument represents a significant part of the virion space and indeed, contains approximately 40% of the herpesvirus virion protein mass (Gibson, 1996;). Since they are components of virions, tegument proteins are delivered to cells at the very initial stage of infection and they have the potential to function even before the viral genome is activated. Extensive studies, including amino acid sequencing and mass spectrometric analyses, have been carried out to determine the protein content of the tegument. These results have revealed the compositions of the teguments of HSV and HCMV, and provided insight into its function.

The tegument of HSV-1 contains more than 20 virus-encoded proteins (Roizman and Knipe 2001). The most notable proteins include the α-trans-inducing factor (αTIF, VP16), the virion host shutoff (vhs) protein (UL41), and a very large protein (VP1–2). VP16 functions as a transcription activator to induce the transcription of viral immediate–early genes, and in addition, plays an essential function as a structural component in the tegument (McKnight *et al.*, 1987; Preston *et al.*, 1988; Weinheimer *et al.*, 1992). The protein vhs is a non-sequence specific RNase that degrades most of the host mRNAs during the initial stage of viral infection, and facilitates the translation of viral mRNAs and viral gene expression (Everly *et al.*, 2002; Read and Frenkel, 1983). VP1–2 is found to be associated with a complex that binds to the terminal *a* sequence of the viral genome, which contains the signal for packaging the genome into the capsid (Chou and Roizman, 1989).

At least 30 virus-encoded proteins have been found in the HCMV tegument (Gibson, 1996; Mocarski and Courcelle, 2001). Significant progress has been made to delineate the function of these HCMV-encoded tegument proteins. For example, the UL69 protein acts to block cell cycle progression, while the UL99-encoded pp28 protein is required for cytoplasmic envelopment of the nucleocapsids (Hayashi *et al.*, 2000; Sanchez *et al.*, 2000; Silva *et al.*, 2003).

There are five predominant protein species found in the HCMV tegument: the high molecular weight protein (HMWP) encoded by UL48, the HMWP-binding protein encoded by UL47, the basic phosphoprotein (BPP or pp150) encoded by UL32, the upper matrix protein (UM or pp71) encoded by UL82, and the lower matrix protein (LM or pp83) encoded by UL83 (Gibson, 1996; Mocarski and Courcelle, 2001). Although their organization within the virion is not completely understood, these abundant proteins are believed to form the structural backbone of the tegument. UL48 and UL32 products, both of which are essential for viral replication (Dunn *et al.*, 2003; Meyer *et al.*, 1997), have been proposed to interact intimately with nucleocapsids (see below). Blocking UL32 expression resulted in accumulation of the nucleocapsid, suggesting that this protein is essential for tegument formation (Meyer *et al.*, 1997).

UL82 is also believed to be involved in direct interaction with the newly synthesized nucleocapsid, and is important for initiation of tegument assembly (Trus *et al.*, 1999). Moreover, the UL82-encoded pp71 protein is a transcriptional activator that helps to induce the transcription of the immediate-early genes within the infected cells (Liu and Stinski, 1992). UL83, the most abundant tegument protein, accounts for more than 15% of the virion protein mass (Gibson, 1996). The encoded pp65 protein has been reported to block major histocompatibility complex class I presentation of a viral immediate–early protein, and more recently, has been implicated to inhibit the induction of host interferon response (Browne and Shenk, 2003; Gilbert *et al.*, 1996). Remarkably, pp65 is not essential for viral replication and infectious virion production (Schmolke *et al.*, 1995). However, UL83 constitutes 90% of the protein mass in the noninfectious dense bodies, which have similar envelope structure, but lack a capsid core (Gibson, 1996). Non-infectious envelope particles, which contain B-capsid like core without the viral DNA genome, have a

reduced amount (30–60% lower) amount of pp65, as do low passage clinical isolates, compared to the laboratory-adapted AD169 and Towne strains (Gibson, 1996; Klages *et al.*, 1989). These observations suggest that UL83 serves as a nonstringent, volume-filling function in facilitating the assembly of virions, non-infectious enveloped particles, and dense bodies. Furthermore, the abundance of this protein in the viral particles and its function in blocking host immune response is believe to allow the virus to escape immune surveillance and significantly contributes to CMV survival (Browne and Shenk, 2003; Gilbert *et al.*, 1996).

Comparative structure of viral tegument

Overview of tegument structure

While significant progress has been made during the last few years to identify tegument proteins and study their functions, little is currently known about the structure of the tegument and the organization of the proteins within the tegument. Equally elusive is the pathway of the assembly and formation of the tegument, which involves the packaging of all the tegument proteins and is certainly a highly regulated, ordered process.

Recent electron cryomicroscopy studies on the virus-related particles of HSV-1, HCMV, and simian CMV (SCMV) provide significant insight into the structure and organization of the herpesvirus tegument (Chen *et al.*, 1999; Trus *et al.*, 1999; Zhou *et al.*, 1999). In these studies, the three-dimensional structures for the infectious virions or cytoplasmic tegumented capsids were reconstructed, and compared to the structures of the intranuclear capsids. The tegument can be seen in the virion as a region of relative low density covering an area in the 60–100 nm radius (Figs. 3.1(*a*), (*c*) and 3.6(*a*), (*c*)) (Chen *et al.*, 1999; Zhou *et al.*, 1999). Although the diameters of the nucleocapsids in different particles appear uniform, the sizes and shapes of the virus particles and the relative locations of nucleocapsids inside the particles vary. These observed variations suggest that most of the tegument proteins do not maintain rigid interactions with the enclosed nucleocapsids, and thus the bulk of the tegument layer does not possess icosahedral symmetry (Chen *et al.*, 1999; Zhou *et al.*, 1999).

The protein densities are also unevenly distributed across the tegument space. Studies on the localization of the tegument proteins have been reported using immunoelectron microscopy with antibodies specifically against tegument proteins and chemical treatment approaches for step-wise removal of layers of virion particles (Gibson, 1996; Steven and Spear, 1997). Several proteins have been found to be located at the tegument space distant to the nucleocapsids. For example, UL23 and UL24 are localized in the HCMV tegument space close to the inner side of the envelope membrane (Adair *et al.*, 2002).

Detailed comparison of the electron cryomicroscopic images of the intact virion particles, the cytoplasmic tegumented capsids, and the nucleocapsids, revealed the unique tegument densities that are present in virion and tegumented capsids but not in capsid preparations. Some of these tegument densities, which are closely associated with nucleocapsid, also exhibit a certain degree of symmetry, and their structures were reconstructed to a resolution of 18–30 Å (Fig. 3.6) (Chen *et al.*, 1999; Trus *et al.*, 1999; Zhou *et al.*, 1999). Since the surface of the nucleocapsid represents the starting site for tegument acquisition and envelopment, these tegument densities are believed to involve specific and direct interactions with capsid proteins and serve as anchors to recruit other tegument proteins for initiation of tegument formation. The tegument densities of HSV1 that are closely associated with the capsids exhibit a dramatic difference from those of HCMV and SCMV (Chen *et al.*, 1999; Trus *et al.*, 1999; Zhou *et al.*, 1999) (Fig. 3.6). This may not be unexpected since there is little evolutionary conservation in the sequence of tegument proteins between HSV-1 and CMV, and many CMV tegument proteins do not have sequence homologues in HSV-1 (Davison *et al.*, 2003; McGeoch *et al.*, 2000).

Tegument structure of HSV-1

Comparison of the maps of HSV-1 intact virion particles and B capsids revealed the marked differences between the two maps in the region of the pentons, which are highlighted in color in the superposition of the difference map on the B-capsid map (Fig. 3.6(*b*)). The most obvious difference is the presence of additional material extending from the surface of the pentons. The extra material has a molecular mass of 170–200 kDa, extends from the interface between the upper domains of two adjacent VP5 subunits in the penton and connects to the nearby triplexes that are made up of VP19C and VP23 proteins (Fig. 3.6(*b*)). The restriction of the tegument contacts to the pentons is consistent with previous observations of tightly attached tegument material at the vertices of capsids in negative stain and freeze-etching images of detergent-treated equine herpevirus virions (Vernon *et al.*, 1982). An identical pattern of tegument protein interaction was observed in a VP26-minus virion mutant (Chen *et al.*, 2001). This result indicates that the lack of tegument association of the HSV-1 hexons is not due to the presence of VP26 on the hexon upper domain, but rather likely due to the inherent structural difference on the upper domains of penton and hexon VP5.

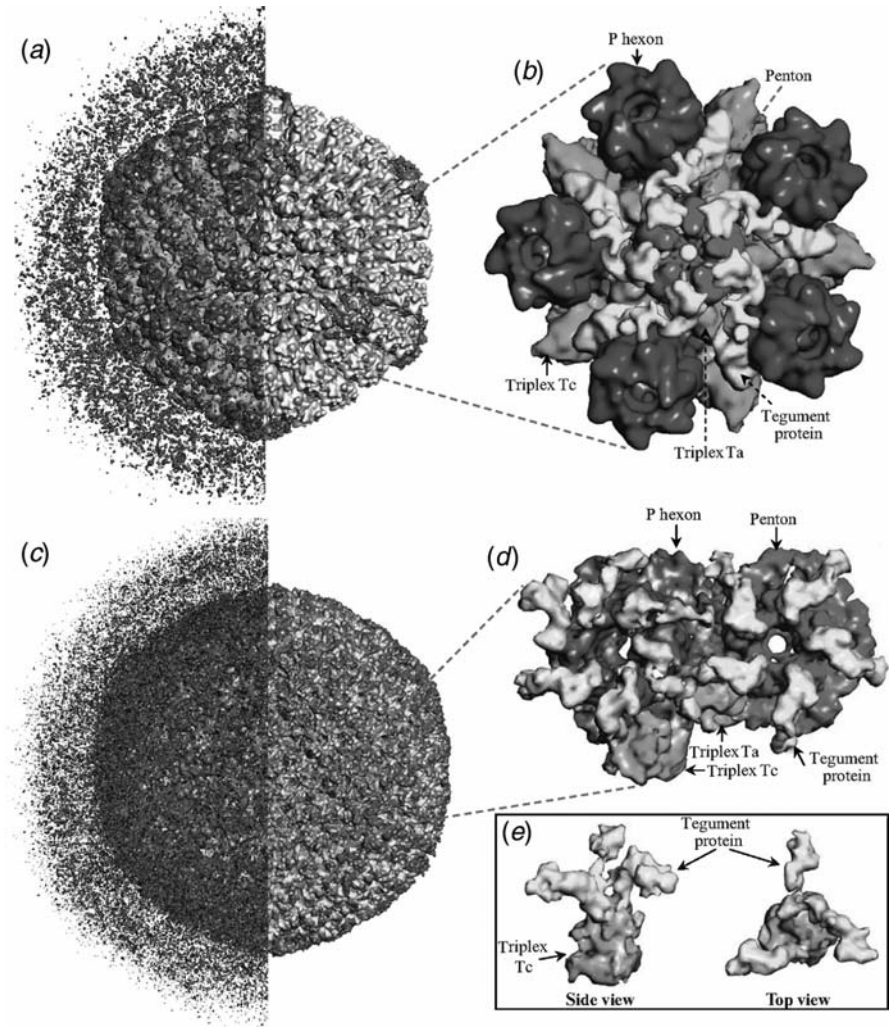

Fig. 3.6. Difference of the anchored tegument proteins between HSV-1 ((*a*) and (*b*)) and HCMV ((*c*)–(*e*)). ((*a*) and (*c*)) Radially color-coded shaded surface views of the three-dimensional reconstruction of HSV-1 (*a*) and HCMV (*c*) virions as viewed along an icosahedral three-fold axis. The bulk of the tegument components and the viral envelope are not icosahedrally ordered or polymorphic, thus appearing as disconnected low densities in the icosahedral reconstruction. These disconnected densities were masked out for the right hemisphere to better reveal the icosahedrally ordered tegument proteins, which are shown in blue to purple colors in (*a*) and in purple in (*c*). ((*b*) and (*d*)) Close-up views of the region indicated in (*a*) and (*c*), respectively, showing the molecular interactions of the tegument proteins (yellow) with the penton (red), P hexon (blue) and triplexes (green). In HSV-1, contrary to the extensive tegument association with all hexons, the tegument densities do not interact with any hexon. (*e*) Extracted triplex HCMV Tc with its attached tegument densities. Three tegument densities interact with the upper domain of each triplex (insert Table 3.1). (See color plate section.)

Based on its close association with the capsid and relative abundance in the tegument, the essential tegument protein VP1–3 has been proposed to constitute a major part of the protein complexes representing the tegument material (Zhou *et al.*, 1999). VP1–3 is an interesting yet poorly characterized protein. It has been shown that VP1–3 is associated with a complex that binds to the terminal *a* sequence of the viral genome, which contains the signal for genome packaging into the capsid (Chou and Roizman, 1989). A temperature-sensitive mutant (*ts* B7) with a mutation in VP1–3 fails to release viral DNA from the infecting capsids into the nucleus during viral decoating process (Batterson and Roizman, 1983). Since the penton has been suggested to be the route by which viral DNA leaves the capsid (Newcomb and Brown, 1994), an interaction between VP1–3 and the penton proteins would place it in an appropriate

position to influence the passage of the viral genome. Further studies are needed to test these hypotheses and completely reveal the identity of the proteins coding for the tegument material.

Tegument structure of CMV

The tegument densities of HCMV that are closely associated with nucleocapsid are dramatically different from those of HSV-1 (*cf.* Fig. 3.6 (*a*) and (*c*)) (Chen *et al.*, 1999). A difference map between the HCMV particles and B-capsids revealed a thin shell of loosely connected filamentous densities, representing the icosahedrally ordered, capsid-proximal portion of the tegument in HCMV (Fig. 3.6(*c*)–(*e*)). Unlike HSV-1, the tegument densities interact with all of the structural components of the nucleocapsid: penton (made up of major capsid protein UL86), hexon (consisted of UL86 and smallest capsid protein UL48.5), and triplex (composed of minor capsid protein UL85 and its binding protein UL46). Figure 3.6(*d*) shows the close-up views of a region from the intact virus reconstruction that includes one penton (red), one P hexon (blue), and two representative adjacent triplexes Ta and Tc (green). Superimposed on the penton and hexon are their associated tegument densities (yellow). Clusters of five and six tegument densities attach to the pentons and hexons, respectively. Moreover, neighboring clusters associate with each by bridging over the intercapsomer space, apparently using triplexes as piers. Each of the filamentous tegument densities, which is about 12 nm in length and 2–3 nm in diameter, acts as the bridge arch. Thus, the capsid appears to act as the scaffold of the ordered tegument protein layer. These results imply that the ordered tegument layer cannot form without the underlying capsid and are consistent with the observations that no such tegument layer was found in dense bodies (Chen *et al.*, 1999).

Detailed examination of the interactions between triplexes and tegument densities further revealed minor differences between the structures of SCMV cytoplasmic tegumented capsids and HCMV particles (Chen *et al.*, 1999; Trus *et al.*, 1999). In SCMV cytoplasmic capsids, two tegument densities were found to be associated with each triplex. In contrast, three densities were shown to be attached to each triplex of the nucleocapsid of the HCMV particles (Fig. 3.6(*e*)). It is conceivable that the extra tegument densities observed in HCMV structure may represent those that were loosely associated with the capsids and probably lost during the purification of the SCMV capsids.

Based on their relative abundance and close association with the nucleocapsids, two CMV tegument proteins, UL32 and UL82, have been proposed to constitute the majority of the observed tegument material that attach to the capsids (Chen *et al.*, 1999; Trus *et al.*, 1999). UL82, which encodes a transcriptional activator (Liu and Stinski, 1992), has a molecular weight of ~70 kDa, similar to the estimated molecule mass of the capsomer-capping tegument protein densities. UL32 has been suggested to be involved in the transport of DNA-containing capsids through nuclear membrane during envelopment or in the stabilization of capsids in the cytoplasm (Meyer *et al.*, 1997). In recent experiments, CMV virion particles were subjected to different chemical conditions, which do not disrupt the integrity of the nucleocapsids, to selectively remove the components not tightly associated with the capsids. These experiments showed that most of the known tegument proteins, including UL99 and UL83, are removed, but UL32 is not affected (Yu, X., Lee, M., Lo, P., Liu, F., and Zhou, Z. H., unpublished results). Thus, these results further suggest that UL99 and UL83 are loosely and distantly associated with capsids and that UL32 is in close proximity and possibly involved in direct interactions with the capsids.

Structure and assembly of viral envelope

The envelope contains most, if not all, of the virion glycoproteins. Each of the herpesviruses encodes a set of 20–80 glycoproteins, very few of which are highly conserved among all the herpesviruses (Kieff and Rickinson, 2001; Mocarski and Courcelle, 2001; Roizman and Knipe, 2001). For example, HSV-1 encodes at least 20 glycoproteins, 11 of which are found in the virions (Roizman and Knipe, 2001). HCMV potentially encodes more than 75 membrane-associated proteins, at least 15 of which are found in the virions (Mocarski and Courcelle, 2001). The exact organization of viral surface glycoproteins in the envelope is not completely understood. Virion glycoproteins are found to aggregate into complexes on the surface of the virion. For example, HCMV glycoproteins gH, gL, and gO are associated to form a heterotrimeric envelope glycoprotein complex (Gibson, 1996; Mocarski and Courcelle, 2001). These proteins may form their complexes in the cellular membrane compartment before trafficking to the viral envelope. However, it remains possible that further higher-order complexes are assembled after these protein components are delivered in the viral envelope membrane.

In addition to the viral encoded glycoproteins, the envelope also contains numerous host proteins or constituents. For example, host proteins associated with HCMV envelope include β2-microglobumin, CD55 and CD59, and annexin II (Grundy *et al.*, 1987a, b; Wright *et al.*, 1995). These molecules may participate in the induction of host cellular responses. It is conceivable that these host proteins, in combination with the viral encoded G protein-coupled

receptors associated with the HCMV envelop, play an important role in modulating host cell response during initial virus attachment, as observed in recent studies (Compton *et al.*, 2003; Zhu *et al.*, 1998).

Herpesvirus envelopment is believed to take place initially at the inner nuclear membrane, and then further proceeds with an envelopment/de-envelopment process that allows the capsid to cross the double nuclear membranes and other cytoplasmic membrane structures (Gibson, 1996; Steven and Spear, 1997). Cytoplasmic envelopment of HSV-1 and CMV capsids can also take place in endosomes as well as Golgi networks (Eggers *et al.*, 1992). Thus, it is not surprising that the envelope contains diverse lipid components that are associated with different parts of the cytoplasmic membrane system in addition to the nuclear membrane. These components include the phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol (Roby and Gibson, 1986). Whether these lipid components may be important in maintaining the integrity of the viral envelope has not been determined and their functional role in stabilizing virion structure is unknown.

The envelopment process appears to be not affected in the absence of a mature capsid since noninfectious enveloped particles and dense bodies can be produced and contain similar envelop contents. In the case of HCMV, pp65 constitutes at least 90% of the dense body protein mass (Gibson, 1996). It is conceivable that this protein may contain signals that promote its own envelopment. There are presumably interactions between proteins of the tegument and envelope that promote the envelopment process. Indeed, recent results indicate that HCMV UL99, a tegument protein, facilitates the cytoplasmic envelopment of the nucleocapsid (Sanchez *et al.*, 2000; Silva *et al.*, 2003). Two HSV-1 membrane proteins, UL34 and UL31, are also implicated to be essential for viral envelopment (Reynolds *et al.*, 2001). Further studies on the organization of the proteins in these particles and their potential interactions with envelope components will provide insight into the process of their assembly.

Other constituents in the virions

Recent studies indicated that viral mRNAs were found in HSV-1 and HCMV virions (Bresnahan and Shenk, 2000; Sciortino *et al.*, 2001). These mRNAs appear to be packaged selectively into the infectious virion particles. They have been proposed to function to facilitate the initiation of viral infection upon viral entry. It is unknown whether these virion mRNAs play a role in maintaining the integrity of the virion structure, as ribosomal RNAs provide the backbone for ribosome assembly.

Depending on the approach of how the virions are prepared and the quality of the preparations being analyzed, numerous host constituents, including lipids, polyamines, and cellular enzyme and structural proteins, are also found to be associated with the viral particles. In particular, two of these host constituents, polyamines and actin-related protein (ARP), may play an important role in stabilizing and maintaining the intact structure of the infectious particles. Two kinds of polyamines, spermidine and spermine, have commonly been found in herpesvirus virions, including HSV-1 and HCMV (Gibson and Roizman, 1971; Gibson *et al.*, 1984). In highly purified HSV-1 virion preparations, there are about 70 000 molecules of spermidine and 40 000 molecules of spermine per virion (Gibson and Roizman, 1971). The functions of these polyamines are believed to provide positive charges to neutralize the highly negatively charged viral DNA genome during the genome replication and packaging. This hypothesis is consistent with the observations that none of the herpesvirus capsid proteins are highly positive charged and addition of arginine facilitates capsid assembly and virion production (Mark and Kaplan, 1971). Spermidine appears to be in the tegument while spermine is localized in the nucleocapsid. It is estimated that the spermine contained in the virion has the capacity to neutralize about 40% of the DNA phosphate, consistent with its role in stabilizing the packed genomic DNA in the nucleocapsid core.

In analyzing highly purified HCMV virions as well as noninfectious enveloped particles and dense bodies, Baldick and Shenk first reported the presence of a substantial amount of a cellular actin-related protein (ARP) in the tegument compartment (Baldick and Shenk, 1996). The exact localization of the ARP is currently unknown, and preliminary studies using stepwise chemical treatment of HCMV virion for removal of different parts of the particles have suggested that ARP is localized in the tegument space distant from the nucleocapsid (Yu, X., Lee, M., Lo, P., Liu, F., and Zhou, Z. H., unpublished results). Based on their roles for providing cytoskeleton and maintaining cellular structure and morphology, it is conceivable that actin-related proteins stabilize the tegument structure. Meanwhile, some ARPs have been implicated in participating dynein-driven microtubule transport system (Lees-Miller *et al.*, 1992; Schroer *et al.*, 1994). Given the fact that viral capsid trafficking from cytoplasm to the nuclear pore complex is driven by the dynein-microtubule system (Dohner *et al.*, 2002; Sodeik *et al.*, 1997), it is possible that these ARPs are specifically incorporated into the teguments and facilitate the transport of the viral particles from the nucleus to

the cytoplasmic membrane during viral envelopment and to the nucleus during post-penetration. Further studies are needed to completely elucidate the function of these proteins in assembly and maintenance of the virus structure.

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REFERENCES

Adair, R., Douglas, E. R., Maclean, J. B. *et al.* (2002). The products of human cytomegalovirus genes UL23, UL24, UL43 and US22 are tegument components. *J. Gen. Virol.*, **83**, 1315–1324.

Alexander, L., Denekamp, L., Knapp, A., Auerbach, M. R., Damania, B., and Desrosiers, R. C. (2000). The primary sequence of rhesus monkey rhadinovirus isolate 26–95: sequence similarities to Kaposi's sarcoma-associated herpesvirus and rhesus monkey rhadinovirus isolate 17577. *J. Virol.*, **74**, 3388–3398.

Baker, M. L., Jiang, W., Bowman, B. R. *et al.* (2003). Architecture of the herpes simplex virus major capsid protein derived from structural bioinformatics. *J. Mol. Biol.*, **331**, 447–456.

Baldick, C. J., Jr. and Shenk, T. (1996). Proteins associated with purified human cytomegalovirus particles. *J. Virol.*, **70**, 6097–6105.

Batterson, W. and Roizman, B. (1983). Characterization of the herpes simplex virion-associated factor responsible for the induction of alpha genes. *J. Virol.*, **46**, 371–377.

Bhella, D., Rixon, F. J., and Dargan, D. J. (2000). Cryomicroscopy of human cytomegalovirus virions reveals more densely packed genomic DNA than in herpes simplex virus type 1. *J. Mol. Biol.*, **295**, 155–161.

Booy, F. P., Newcomb, W. W., Trus, B. L., Brown, J. C., Baker, T. S., and Steven, A. C. (1991). Liquid-crystalline, phage-like packing of encapsidated DNA in herpes simplex virus. *Cell*, **64**, 1007–1015.

Bowman, B. R., Baker, M. L., Rixon, F. J., Chiu, W., and Quiocho, F. A. (2003). Structure of the herpesvirus major capsid protein. *EMBO J.*, **22**, 757–765.

Bresnahan, W. A. and Shenk, T. (2000). A subset of viral transcripts packaged within human cytomegalovirus particles. *Science*, **288**, 2373–2376.

Browne, E. P. and Shenk, T. (2003). Human cytomegalovirus UL83-coded pp65 virion protein inhibits antiviral gene expression in infected cells. *Proc. Natl Acad. Sci., USA*, **100**, 11439–11444.

Butcher, S. J., Aitken, J., Mitchell, J., Gowen, B., and Dargan, D. J. (1998). Structure of the human cytomegalovirus B capsid by electron cryomicroscopy and image reconstruction. *J. Struct. Biol.*, **124**, 70–76.

Casjens, S. and Hendrix, R. (1988). Control mechanisms in dsDNA bacteriophage assembly. In *The Bacteriophages*, ed. R. Celander, New York: Plenum Publishing Corp, pp. 15–91.

Chen, D. H., Jiang, H., Lee, M., Liu, F., and Zhou, Z. H. (1999). Three-dimensional visualization of tegument/capsid interactions in the intact human cytomegalovirus. *Virology*, **260**, 10–16.

Chen, D. H., Jakana, J., and McNab, D. (2001). The pattern of tegument-capsid interaction in the herpes simplex virus type 1 virion is not influenced by the small hexon-associated protein VP26. *J. Virol.*, **75**, 11863–11867.

Chi, J. H. and Wilson, D. W. (2000). ATP-dependent localization of the herpes simplex virus capsid protein VP26 to sites of procapsid maturation. *J. Virol.*, **74**, 1468–1476.

Chiu, W. and Rixon, F. J. (2002). High resolution structural studies of complex icosahedral viruses: a brief overview. *Virus Res.*, **82**, 9–17.

Chou, J. and Roizman, B. (1989). Characterization of DNA sequence-common and sequence-specific proteins binding to *cis*-acting sites for cleavage of the terminal a sequence of the herpes simplex virus 1 genome. *J. Virol.*, **63**, 1059–1068.

Compton, T., Kurt-Jones, E. A., Boehme, K. W. *et al.* (2003). Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-like receptor 2. *J. Virol.*, **77**, 4588–4596.

Conway, J. F., Duda, R. L., Cheng, N., Hendrix, R. W., and Steven, A. C. (1995). Proteolytic and conformational control of virus capsid maturation: the bacteriophage HK97 system. *J. Mol. Biol.*, **253**, 86–99.

Davison, A. J., Dolan, A., Akter, P. *et al.* (2003). The human cytomegalovirus genome revisited: comparison with the chimpanzee cytomegalovirus genome. *J. Gen. Virol.*, **84**, 17–28.

Dohner, K., Wolfstein, A., Prank, U. *et al.* (2002). Function of dynein and dynactin in herpes simplex virus capsid transport. *Mol. Biol. Cell*, **13**, 2795–2809.

Dunn, W., Chou, C., Li, H. *et al.* (2003). Functional profiling of human cytomegalovirus genome. *Proc. Natl Acad. Sci. USA*, **100**, 14223–14228.

Eggers, M., Bogner, E., Agricola, B., Kern, H. F., and Radsak, K. (1992). Inhibition of human cytomegalovirus maturation by brefeldin A. *J. Gen. Virol.*, **73**(Pt 10), 2679–2692.

Everly, D. N., Jr., Feng, P., Mian, I. S., and Read, G. S. (2002). mRNA degradation by the virion host shutoff (Vhs) protein of herpes simplex virus: genetic and biochemical evidence that Vhs is a nuclease. *J. Virol.*, **76**, 8560–8571.

Flynn, D. L., Abood, N. A., and Howerda, B. C. (1997). Recent advances in antiviral research: identification of inhibitors of the herpesvirus proteases. *Curr. Opin. Chem. Biol.*, **1**, 190–196.

Gibson, W. (1996). Structure and assembly of the virion. *Intervirology*, **39**, 389–400.

Gibson, W. and Roizman, B. (1971). Compartmentalization of spermine and spermidine in the herpes simplex virion. *Proc. Natl Acad. Sci. USA*, **68**, 2818–2821.

Gibson, W. and Roizman, B. (1972). Proteins specified by herpes simplex virus. 8. Characterization and composition of multiple capsid forms of subtypes 1 and 2. *J. Virol.*, **10**, 1044–1052.

Gibson, W., van Breemen, R., Fields, A., LaFemina, R., and Irmiere, A. (1984). D,L-alpha-difluoromethylornithine inhibits human cytomegalovirus replication. *J. Virol.*, **50**, 145–154.

Gilbert, M. J., Riddell, S. R., Plachter, B., and Greenberg, P. D. (1996). Cytomegalovirus selectively blocks antigen processing and presentation of its immediate–early gene product. *Nature*, **383**, 720–722.

Grundy, J. E., McKeating, J. A., and Griffiths, P. D. (1987a). Cytomegalovirus strain AD169 binds beta 2 microglobulin in vitro after release from cells. *J. Gen. Virol.*, **68**(Pt 3), 777–784.

Grundy, J. E., McKeating, J. A., Ward, P. J., Sanderson, A. R., and Griffiths, P. D. (1987b). Beta 2 microglobulin enhances the infectivity of cytomegalovirus and when bound to the virus enables class I HLA molecules to be used as a virus receptor. *J. Gen. Virol.*, **68**(Pt 3), 793–803.

Hayashi, M. L., Blankenship, C., and Shenk, T. (2000). Human cytomegalovirus UL69 protein is required for efficient accumulation of infected cells in the G1 phase of the cell cycle. *Proc. Natl Acad. Sci. USA*, **97**, 2692–2696.

Heymann, J. B., Cheng, N., Newcomb, W. W., Trus, B. L., Brown, J. C., and Steven, A. C. (2003). Dynamics of herpes simplex virus capsid maturation visualized by time-lapse cryo-electron microscopy. *Nat. Struct. Biol.*, **10**, 334–341.

Hong, Z., Beaudet-Miller, M., Durkin, J., Zhang, R., and Kwong, A. D. (1996). Identification of a minimal hydrophobic domain in the herpes simplex virus type 1 scaffolding protein which is required for interaction with the major capsid protein. *J. Virol.*, **70**, 533–540.

Irmiere, A. and Gibson, W. (1985). Isolation of human cytomegalovirus intranuclear capsids, characterization of their protein constituents, and demonstration that the B-capsid assembly protein is also abundant in noninfectious enveloped particles. *J. Virol.*, **56**, 277–283.

Jiang, W., Li, Z., Zhang, Z., Baker, M. L., Prevelige, P. E., Jr., and Chiu, W. (2003). Coat protein fold and maturation transition of bacteriophage P22 seen at subnanometer resolutions. *Nat. Struct. Biol.*, **10**, 131–135.

Kieff, E. and Rickinson, A. B. (2001). Epstein–Barr virus and its replication. In *Fields Virology*, ed. D. M. Knipe and P. M. Howley, pp. 2511–2574. Lippincott, Williams & Wilkins, Philadelphia.

Kirkitadze, M. D., Barlow, P. N., Price, N. C. (1998). The herpes simplex virus triplex protein, VP23, exists as a molten globule. *J. Virol.*, **72**, 10066–10072.

Klages, S., Ruger, B., and Jahn, G. (1989). Multiplicity dependent expression of the predominant phosphoprotein pp65 of human cytomegalovirus. *Virus Res.*, **12**, 159–168.

Lagunoff, M. and Ganem, D. (1997). The structure and coding organization of the genomic termini of Kaposi's sarcoma-associated herpesvirus. *Virology*, **236**, 147–154.

Lees-Miller, J. P., Helfman, D. M., and Schroer, T. A. (1992). A vertebrate actin-related protein is a component of a multisubunit complex involved in microtubule-based vesicle motility. *Nature*, **359**, 244–246.

Liu, F. and Roizman, B. (1991). The herpes simplex virus 1 gene encoding a protease also contains within its coding domain the gene encoding the more abundant substrate. *J. Virol.*, **65**, 5149–5156.

Liu, F. and Roizman, B. (1992). Differentiation of multiple domains in the herpes simplex virus 1 protease encoded by the UL26 gene. *Proc. Natl Acad. Sci. USA*, **89**, 2076–2080.

Liu, B. and Stinski, M. F. (1992). Human cytomegalovirus contains a tegument protein that enhances transcription from promoters with upstream ATF and AP-1 cis-acting elements. *J. Virol.*, **66**, 4434–4444.

Lo, P., Yu, X., Atanasov, I., Chandran, B., and Zhou, Z. H. (2003). Three-dimensional localization of pORF65 in Kaposi's sarcoma-associated herpesvirus capsid. *J. Virol.*, **77**, 4291–4297.

Mark, G. E. and Kaplan, A. S. (1971). Synthesis of proteins in cells infected with herpesvirus. VII. Lack of migration of structural viral proteins to the nucleus of arginine-deprived cells. *Virology*, **45**, 53–60.

McGeoch, D. J., Dolan, A., and Ralph, A. C. (2000). Toward a comprehensive phylogeny for mammalian and avian herpesviruses. *J. Virol.*, **74**, 10401–10406.

McKnight, J. L., Kristie, T. M., and Roizman, B. (1987). Binding of the virion protein mediating alpha gene induction in herpes simplex virus 1-infected cells to its *cis* site requires cellular proteins. *Proc. Natl Acad. Sci. USA*, **84**, 7061–7065.

Mettenleiter, T. C. (2002). Herpesvirus assembly and egress. *J. Virol.*, **76**, 1537–1547.

Meyer, H. H., Ripalti, A., Landini, M. P., Radsak, K., Kern, H. F., and Hensel, G. M. (1997). Human cytomegalovirus late-phase maturation is blocked by stably expressed UL32 antisense mRNA in astrocytoma cells. *J. Gen. Virol.*, **78**(Pt 10), 2621–2631.

Mocarski, E. S. and Courcelle, C. T. (2001). Cytomegalovirus and their replication. In *Fields Virology*, ed. D. M. Knipe and P. M. Howley, pp. 2629–2673. Lippincott, Williams & Wilkins, Philadelphia.

Nealon, K., Newcomb, W. W., Pray, T. R., Craik, C. S., Brown, J. C., and Kedes, D. H. (2001). Lytic replication of Kaposi's sarcoma-associated herpesvirus results in the formation of multiple capsid species: isolation and molecular characterization of A, B, and C capsids from a gammaherpesvirus. *J. Virol.*, **75**, 2866–2878.

Newcomb, W. W. and Brown, J. C. (1994). Induced extrusion of DNA from the capsid of herpes simplex virus type 1. *J. Virol.*, **68**, 433–440.

Newcomb, W. W., Trus, B. L., Booy, F. P., Steven, A. C., Wall, J. S., and Brown, J. C. (1993). Structure of the herpes simplex virus capsid. Molecular composition of the pentons and the triplexes. *J. Mol. Biol.*, **232**, 499–511.

Newcomb, W. W., Homa, F. L., Thomsen, D. R. *et al.* (1999). Assembly of the herpes simplex virus procapsid from purified components and identification of small complexes containing the major capsid and scaffolding proteins. *J. Virol.*, **73**, 4239–4250.

Newcomb, W. W., Trus, B. L., Cheng, N. *et al.* (2000). Isolation of herpes simplex virus procapsids from cells infected with a protease-deficient mutant virus. *J. Virol.*, **74**, 1663–1673.

Newcomb, W. W., Juhas, R. M., Thomsen, D. R. *et al.* (2001). The UL6 gene product forms the portal for entry of DNA into the herpes simplex virus capsid. *J. Virol.*, **75**, 10923–10932.

O'Connor, C. M., Damania, B., and Kedes, D. H. (2003). Capsid and virion production in Rhesus monkey rhadinovirus: a model for structure and assembly of Kaposi's sarcoma-associated herpesvirus. *Submitted*.

Perdue, M. L., Cohen, J. C., Randall, C. C., and O'Callaghan, D. J. (1976). Biochemical studies of the maturation of herpesvirus nucleocapsid species. *Virology*, **74**, UNKNOWN.

Preston, C. M., Frame, M. C., and Campbell, M. E. (1988). A complex formed between cell components and an HSV structural polypeptide binds to a viral immediate early gene regulatory DNA sequence. *Cell*, **52**, 425–434.

Preston, V. G., Rixon, F. J., McDougall, I. M., McGregor, M., and Al-Kobaisi, M. F. (1992). Processing of the herpes simplex virus assembly protein ICP35 near its carboxy terminal end requires the product of the whole of the UL26 reading frame. *Virology*, **186**, 87–98.

Qiu, X., Culp, J. S., DeLilla, A. G. *et al.* (1996). Unique fold and active site in cytomegalovirus protease. *Nature (London)*, **383**, 275–279.

Read, G. S. and Frenkel, N. (1983). Herpes simplex virus mutants defective in the virion-associated shutoff of host polypeptide synthesis and exhibiting abnormal synthesis of alpha (immediate early) viral polypeptides. *J. Virol.*, **46**, 498–512.

Renne, R., Lagunoff, M., Zhong, W., and Ganem, D. (1996). The size and conformation of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) DNA in infected cells and virions. *J. Virol.*, **70**, 8151–8154.

Reynolds, A. E., Ryckman, B. J., Baines, J. D., Zhou, Y., Liang, L., and Roller, R. J. (2001). U(L)31 and U(L)34 proteins of herpes simplex virus type 1 form a complex that accumulates at the nuclear rim and is required for envelopment of nucleocapsids. *J. Virol.*, **75**, 8803–8817.

Rixon, F. J. (1993). Structure and assembly of herpesviruses. *Seminars in Virology*, **4**, 135–144.

Rixon, F. J. and McNab, D. (1999). Packaging-competent capsids of a herpes simplex virus temperature-sensitive mutant have properties similar to those of in vitro-assembled procapsids. *J. Virol.*, **73**, 5714–5721.

Roby, C. and Gibson, W. (1986). Characterization of phosphoproteins and protein kinase activity of virions, noninfectious enveloped particles, and dense bodies of human cytomegalovirus. *J. Virol.*, **59**, 714–727.

Roizman, B. and Furlong, D. (1974). The replication of herpes viruses. In *Comprehensive Virology*, ed. H. Fraenkel-Conrat, H., and R. R. Wagner, pp. 229–403. New York, NY: Plenum Press.

Roizman, B. and Knipe, D. M. (2001). Herpes simplex viruses and their replication. In *Fields Virology*, ed. D. M. Knipe and P. M. Howley, vol. 2, pp. 2399–2460. Philadelphia, PA: Lippincott, Williams & Wilkins.

Roizman, B. and Pellett, P. E. (2001). The family herpesviridae: a brief introduction. In *Fields Virology*, ed. D. M. Knipe and P. M. Howley, vol. 2, pp. 2381–2398. Philadelphia, PA. Lippincott, Williams & Wilkins.

Roizman, B. and Sears, A. E. (1996). Herpes simplex viruses and their replication. In Fields, B. N., Knipe, D. M. and Howley, P. M. (eds.), *Fields Virology*, ed. B. N. Fields, D. M. Knipe, and P. M. Howley, vol. 2, pp. 2231–2295. Philadelphia, PA: Lippincott–Raven Publishers.

Sanchez, V., Sztul, E., and Britt, W. J. (2000). Human cytomegalovirus pp28 (UL99) localizes to a cytoplasmic compartment which overlaps the endoplasmic reticulum–golgi–intermediate compartment. *J. Virol.*, **74**, 3842–3851.

Schmolke, S., Kern, H. F., Drescher, P., Jahn, G., and Plachter, B. (1995). The dominant phosphoprotein pp65 (UL83) of human cytomegalovirus is dispensable for growth in cell culture. *J. Virol.*, **69**, 5959–5968.

Schrag, J. D., Prasad, B. V., Rixon, F. J., and Chiu, W. (1989). Three-dimensional structure of the HSV1 nucleocapsid. *Cell*, **56**, 651–660.

Schroer, T. A., Fyrberg, E., Cooper, J. A. *et al.* (1994). Actin-related protein nomenclature and classification. *J. Cell Biol.*, **127**, 1777–1778.

Sciortino, M. T., Suzuki, M., Taddeo, B., and Roizman, B. (2001). RNAs extracted from herpes simplex virus 1 virions: apparent selectivity of viral but not cellular RNAs packaged in virions. *J. Virol.*, **75**, 8105–8116.

Searles, R. P., Bergquam, E. P., Axthelm, M. K., and Wong, S. W. (1999). Sequence and genomic analysis of a Rhesus macaque rhadinovirus with similarity to Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8. *J. Virol.*, **73**, 3040–3053.

Shieh, H.-S., Kurumbail, R. G., Stevens, A. M. *et al.* (1996). Three-dimensional structure of human cytomegalovirus protease. *Nature (London)*, **383**, 279–282.

Silva, M. C., Yu, Q. C., Enquist, L., and Shenk, T. (2003). Human cytomegalovirus UL99encoded pp28 is required for the cytoplasmic envelopment of tegument-associated capsids. *J. Virol.*, **77**, 10594–10605.

Sodeik, B., Ebersold, M. W., and Helenius, A. (1997). Microtubule-mediated transport of incoming herpes simplex virus 1 capsids to the nucleus. *J. Cell Biol.*, **136**, 1007–1021.

Steven, A. C. and Spear, P. G. (1997). Herpesvirus capsid assembly and envelopment. In *Structural Biology of Viruses*, ed. W. Chiu, R. M. Burnett, and R. L. Garcea, pp. 312–351. New York: Oxford University Press.

Steven, A. C., Roberts, C. R., Hay, J., Bisher, M. E., Pun, T., and Trus, B. L. (1986). Hexavalent capsomers of herpes simplex virus type 2: symmetry, shape, dimensions, and oligomeric status. *J. Virol.*, **57**, 578–584.

Tong, L., Qian, C., Massariol, M.-J., Bonneau, P. R., Cordingley, M. G., and Lagace, L. (1996). A new serine-protease fold revealed by the crystal structure of human cytomegalovirus protease. *Nature (London)*, **383**, 272–275.

Tong, L., Qian, C., Massariol, M. J., Deziel, R., Yoakim, C., and Lagace, L. (1998). Conserved mode of peptidomimetic inhibition and substrate recognition of human cytomegalovirus protease. *Nature Struct. Biol.*, **5**, 819–826.

Trus, B. L., Homa, F. L., Booy, F. P. *et al.* (1995). Herpes simplex virus capsids assembled in insect cells infected with recombinant baculoviruses: structural authenticity and localization of VP26. *J. Virol.*, **69**, 7362–7366.

Trus, B. L., Booy, F. P., Newcomb, W. W. *et al.* (1996). The herpes simplex virus procapsid: structure, conformational changes upon maturation, and roles of the triplex proteins VP19c and VP23 in assembly. *J. Mol. Biol.*, **263**, 447–462.

Trus, B. L., Gibson, W., Cheng, N., and Steven, A. C. (1999). Capsid structure of simian cytomegalovirus from cryoelectron microscopy: evidence for tegument attachment sites. *J. Virol.*, **73**, 2181–2192.

Trus, B. L., Heymann, J. B., Nealon, K. *et al.* (2001). Capsid structure of Kaposi's sarcoma-associated herpesvirus, a gammaherpesvirus, compared to those of an alphaherpesvirus, herpes simplex virus type 1, and a betaherpesvirus, cytomegalovirus. *J. Virol.*, **75**, 2879–2890.

Vernon, S. K., Lawrence, W. C., Long, C. A., Rubin, B. A., and Sheffield, J. B. (1982). Morphological components of herpesvirus. IV. Ultrastructural features of the envelope and tegument. *J. Ultrastruct. Res.*, **81**, 163–171.

Weinheimer, S. P., Boyd, B. A., Durham, S. K., Resnick, J. L., and O'Boyle, D. R., 2nd. (1992). Deletion of the VP16 open reading frame of herpes simplex virus type 1. *J. Virol.*, **66**, 258–269.

Welch, A. R., Woods, A. S., McNally, L. M., Cotter, R. J., and Gibson, W. (1991). A herpesvirus maturational proteinase, assemblin: identification of its gene, putative active site domain, and cleavage site. *Proc. Natl Acad. Sci. USA*, **88**, 10792–10796.

Wright, J. F., Kurosky, A., Pryzdial, E. L., and Wasi, S. (1995). Host cellular annexin II is associated with cytomegalovirus particles isolated from cultured human fibroblasts. *J. Virol.*, **69**, 4784–4791.

Wu, L., Lo, P., Yu, X., Stoops, J. K., Forghani, B., and Zhou, Z. H. (2000). Three-dimensional structure of the human herpesvirus 8 capsid. *J. Virol.*, **74**, 9646–9654.

Yu, X.-K., O'Connor, C. M., Atanasov, I., Damania, B., Kedes, D. H., and Zhou, Z. H. (2003). Three-dimensional structures of the A, B and C capsids of Rhesus monkey rhadinovirus: insights into gammaherpesvirus capsid assembly, maturation and DNA packing. *J. Virol.*, **77**, 14182–14193.

Yu, X., Trang, P., Shah, S. *et al.* (2005). Dissecting human cytomegalovirus gene function and capsid maturation by ribozyme targeting and electron cryomicroscopy. *Proc. Natl Acad. Sci. USA*, **102**, 7103–7108.

Zhou, Z. H., Prasad, B. V., Jakana, J., Rixon, F. J., and Chiu, W. (1994). Protein subunit structures in the herpes simplex virus A-capsid determined from 400 kV spot-scan electron cryomicroscopy. *J. Mol. Biol.*, **242**, 456–469.

Zhou, Z. H., He, J., Jakana, J., Tatman, J. D., Rixon, F. J., and Chiu, W. (1995). Assembly of VP26 in herpes simplex virus-1 inferred from structures of wild-type and recombinant capsids. *Nat. Struct. Biol.*, **2**, 1026–1030.

Zhou, Z. H., Chiu, W., Haskell, K. *et al.* (1998a). Refinement of herpesvirus B-capsid structure on parallel supercomputers. *Biophys. J.*, **74**, 576–588.

Zhou, Z. H., Macnab, S. J., Jakana, J., Scott, L. R., Chiu, W., and Rixon, F. J. (1998b). Identification of the sites of interaction between the scaffold and outer shell in herpes simplex virus-1 capsids by difference electron imaging. *Proc. Natl Acad. Sci. USA*, **95**, 2778–2783.

Zhou, Z. H., Chen, D. H., Jakana, J., Rixon, F. J., and Chiu, W. (1999). Visualization of tegument–capsid interactions and DNA in intact herpes simplex virus type 1 virions. *J. Virol.*, **73**, 3210–3218.

Zhou, Z. H., Dougherty, M., Jakana, J., He, J., Rixon, F. J., and Chiu, W. (2000). Seeing the herpesvirus capsid at 8.5 A. *Science*, **288**, 877–880.

Zhu, H., Cong, J. P., Mamtora, G., Gingeras, T., and Shenk, T. (1998). Cellular gene expression altered by human cytomegalovirus: global monitoring with oligonucleotide arrays. *Proc. Natl Acad. Sci. USA*, **95**, 14470–14475.

4

Comparative analysis of herpesvirus-common proteins

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Introduction

Despite the evolutionary and biological divergence represented by the nine human herpesviruses that have been classified into three broad subgroups, a large number of herpesvirus-common (core) gene products are evolutionarily conserved (Table 4.1 see chapter 2). These appear to carry out functions upon which every herpesvirus relies because all exhibit a common virion structure, a core genome replication process, and similar entry and egress pathways. These herpesvirus common functions are most often recognized through deduced protein sequence similarity that extends throughout alpha-, beta-, and gammaherpesviruses subfamilies infecting mammals, reptiles and birds (see Chapter 2, Table 2.2). These herpesviruses exhibit conservation that suggests a shared common ancestor at least 50 million years ago. Other evolutionarily distant herpesviruses infecting fish, amphibians, and invertebrates share less similarity with these better-studied herpesviruses, suggesting a common evolutionary origin dating back over 150 million years. In the more distant relatives, a common virion structure, genome organization and similarity across a small subset herpesvirus-common gene products provide the evidence of a common origin.

A few herpesvirus-common gene products have been recognized via a common enzymatic or binding activity long before any systematic genome sequence analysis became available. The homologous function of envelope glycoprotein B, DNA polymerase, alkaline exonuclease and single strand DNA binding protein, to give a few examples, emerged from biochemical studies in a number of herpesvirus systems. Given the high level of conservation and the importance of DNA synthesis as a target for antiviral inhibitors, these remain among the most broadly studied and best understood of the core gene products. DNA synthesis functions and virion structural components are also among the most highly conserved based on sequence comparisons. Importantly, however, common activity of well-recognized core functions, such as the DNA polymerase processivity factor, the smallest capsid protein or a capsid triplex component, is not based on the level of primary amino acid sequence identity, but rather is supported by a common role in several herpesviruses. Additional core functions initially recognized based on activity have undergone evolutionary divergence to take on new functions. For example, the large subunit of ribonucleotide reductase (HSV-1 *UL39* gene product), which associates with a small subunit to form an active enzyme in the alphaherpesviruses and gammaherpesviruses, is expressed without small subunit in the betaherpesviruses (HCMV, HHV-6, HHV-7) and completely lacks enzymatic activity. This leaves a question as to its true role. Thus, herpesvirus-common proteins may preserve common function as well as sequence homology, function with only limited sequence homology, or sequence homology with distinct function. Homologs may therefore be predicted to carry out similar functions in most situations, but will certainly not behave identically in all settings.

Herpesvirus-common gene products have been recognized as key proteins that form the characteristic herpesvirus virion structure and provide key common functions for the replicative cycle, beginning with entry into cells, continuing through the process of viral DNA synthesis and nucleic acid metabolism and concluding with capsid maturation and egress of virions. The presence of herpesvirus-common genes (see Chapter 2) allows predictions about functional conservation; however, most functional information has been derived from studies in a single or at most two herpesvirus subfamilies. The requirement for herpesvirus-common functions varies considerably with cell type, as well as between viruses of the same

Table 4.1. Identity, function and proposed nomenclature for known and putative functions of herpesvirus-common gene products of human herpesviruses

Common name[a]	Abbrev. name	HSV	VZV	HCMV	HHV 6/7	EBV	KSHV	Key function
Capsid								
major **c**apsid **p**rotein[c]	MCP	UL19	40	UL86	U57	BcLF1	ORF25	hexon, penton, capsid structure
triplex monomer[c]	TRI1	UL38	20	UL46	U29	BORF1	ORF62	TRI1 and TRI2 assoc to form TRI complex, capsid structure
triplex dimer[c]	TRI2	UL18	41	UL85	U56	BDLF1	ORF26	
small **c**apsid **p**rotein[b]	SCP	UL35	23	UL48A	U32	BFRF3	ORF65	capsid transport
portal protein	PORT	UL6	54	UL104	U76	BBRF1	ORF43	penton for DNA encapsidation
portal **c**apping **p**rotein[c]	PCP	UL25	34	UL77	U50	BVRF1	ORF19	covers portal in mature virions
Tegument and cytoplasmic egress								
virion **p**rotein **k**inase	VPK	UL13	47	UL97	U69	BGLF4	ORF36	phosphorylation, regulation
largest **t**egument **p**rotein[c]	LTP	UL36	22	UL48	U31	BPLF1	ORF64	uncoating, secondary envelopment
LTP binding **p**rotein[b]	LTPbp	UL37	21	UL47	U30	BOLF1	ORF63	
encapsidation and **e**gress **p**rotein[c]	EEP	UL7	53	UL103	U75	BBRF2	ORF42	nuclear egress
cytoplamsic **e**gress **t**egument **p**rotein[b]	CETP	UL11	49	UL99	U71	BBLF1	ORF38	secondary envelopment, cytoplasmic egress
CETP binding **p**rotein[b]	CETPbp	UL16	44	UL94	U65	BGLF2	ORF33	
cytoplasmic **e**gress **f**acilitator-**1**[b]	CEF1	UL51	7	UL71	U44	BSRF1	ORF55	cytoplasmic egress
encapsidation **c**haperone **p**rotein[b]	ECP	UL14	46	UL95	U67	BGLF3	ORF34	TERbp chaperone
capsid **t**ransport **t**egument **p**rotein[c]	CTTP	UL17	43	UL93	U64	BGLF1	ORF32	capsid transport in the nucleus
cytoplasmic **e**gress **f**acilitator-**2**[b]	CEF2	UL21	38	UL88	U59	BTRF1	ORF23	egress, interact with CETPbp
		UL24	35	UL76	U49	BXRF1	ORF20	putative membrane or tegument
Envelope								
glycoprotein **B**[c]	gB	UL27	31	UL55	U39	BALF4	ORF8	heparan-binding, fusion
glycoprotein **H**[c]	gH	UL22	60	UL75	U48	BXLF2	ORF22	gH assoc, fusion
glycoprotein **L**[c]	gL	UL1	37	UL115	U82	BKRF2	ORF47	gL assoc, fusion
glycoprotein **M**[b]	gM	UL10	50	UL100	U72	BBRF3	ORF39	gN assoc
glycoprotein **N**[b]	gN	UL49A	9A	UL73	U46	BLRF1	ORF53	gM assoc
Regulation								
multifunctional **r**egulator of **e**xpression[c]	MRE	UL54	4	UL69	U42	BSLF1 BMLF1	ORF57	transcriptional, RNA transport regulation
DNA Replication, recombination and metabolism								
DNA **pol**ymerase[c]	POL	UL30	28	UL54	U38	BALF5	ORF9	DNA synthesis
DNA **p**olymerase **p**rocessivity **s**ubunit[c]	PPS	UL42	16	UL44	U27	BMRF1	ORF59	POL processivity
helicase-**p**rimase **A**TPase subunit[c]	HP1	UL5	55	UL105	U77	BBLF4	ORF44	HP1, HP2 and HP3 assoc to form HP, unwinding and primer synthesis
helicase-**p**rimase RNA pol subunit **B**[c]	HP2	UL52	6	UL70	U43	BSLF1	ORF56	
helicase-primase subunit **C**[c]	HP3	UL8	52	UL102	U74	BBLF2 BBLF3	ORF40 ORF41	
single **s**trand DNA **b**inding protein[c]	SSB	UL29	29	UL57	U41	BALF2	ORF6	DNA fork, recombination
alkaline deoxyribonuclease[b]	NUC	UL12	48	UL98	U70	BGLF5	ORF37	recombination
deoxyuridine triphosphatase[b]	dUTPase	UL50	8	UL72	U45	BLLF3	ORF54	reduce dUTP
uracil-DNA glycosidase[b]	UNG	UL2	59	UL114	U81	BKRF3	ORF46	remove uracil from DNA
ribonucleotide **r**eductase large subunit[b]	RR1	UL39	19	UL45	U28	BORF2	ORF61	active enzyme only in viruses with RR2
Capsid assembly, DNA encapsidation and nuclear egress								
maturational **pr**otease[c]	PR	UL26	33	UL80	U53	BVRF2	ORF17	capsid assembly, scaffolding, DNA encapsidation
assembly **p**rotein[c]	AP (NP)[d]	UL26.5 (UL26)	33.5 (33)	UL80.5 (UL80)	U53.5 (U53)	BdRF1 (BVRF2)	ORF17.5 (ORF17)	

(*cont.*)

Table 4.1. (*cont.*)

Common name[a]	Abbrev. name[a]	HSV	VZV	HCMV	HHV 6/7	EBV	KSHV	Key function
capsid **t**ransport **n**uclear **p**rotein[c]	CTNP	UL32	26	UL52	U36	BFLF1	ORF68	capsid transport to sites of DNA replication
terminase **A**TPase subunit 1[c]	TER1	UL15	42	UL89	U66	BGRF1	ORF29	TER1 and TER2 form TER, packaging machinery
			45			BDRF1		
terminase DNA binding subunit 2[c]	TER2	UL28	30	UL56	U40	BALF3	ORF7	
terminase **b**inding **p**rotein[c]	TERbp	UL33	25	UL51	U35	BFRF1A	ORF67	TER assoc
nuclear **e**gress **m**embrane **p**rotein[c]	NEMP	UL34	24	UL50	U34	BFRF2	ORF66	nuclear egress, primary envelopment
nuclear **e**gress **l**amina **p**rotein[c]	NELP	UL31	27	UL53	U37	BFLF2	ORF69	

[a] proposed.
[b] required for replication in some viruses or some settings.
[c] required for replication in all viruses and settings tested.
[d] AP and NP are related proteins derived from different primary translation products.

or different subfamilies. Currently, 36 of the 40 core functions have an impact on replication in at least one herpesvirus and in at least some experimental setting (Table 4.1), although many are not absolutely essential for replication in any of the herpesviruses where they have been studied. The striking cell type dependence of so many herpesvirus functions suggests that many of these proteins carry out activities that are redundant with other viral or cellular functions. This chapter will seek to deduce the general role of each core function based on data available in different systems. The phenotype of mutants showing even a modest growth defect under any conditions will be an important component for consideration. All core functions are likely to be important for viral replication and pathogenesis in the host even when considered dispensable for replication in cultured cells. While even limited information is useful, experimental confirmation across several distinct viruses builds confidence in the common role of homologs, but this information is limited in many cases.

All herpesviruses form particles that have a characteristic 125–130 nm icosohedral capsid containing a linear DNA genome, surrounded by a protein-containing tegument enclosed in a host-membrane derived lipid bilayer envelope modified by virus-encoded glycoproteins. The overall virion particle size ranges from 200 to 300 nm, depending on the particular virus. This common structure provides the strongest evidence for evolutionary conservation in the replication and maturation processes. The set of core, homologous open reading frames (ORFs) are predominantly involved in the processes of DNA replication through maturation and egress from cells (Fig. 4.1). These also facilitate estimations of the evolutionary relatedness of the three major subfamilies (alpha-, beta-, and gammaherpesviruses) comprising mammalian and avian herpesviruses (see Chapter 2). Recognizable sequence homologs of only three of these proteins (DNA polymerase, DUT and a terminase subunit are conserved in the genomes of every known herpesviruses, including those infecting amphibians, fish, and invertebrates. Of these, the gene for the terminase subunit is remarkable, producing a conserved spliced mRNA that remains a key genomic feature (Davison, 2002) of every herpesvirus genome that has been annotated. The nine distinct human herpesviruses, including three members of alphaherpesviruses (HSV-1, HSV-2 and VZV), four members of the betaherpesviruses (CMV, HHV-6A, HHV-6B and HHV-7) and two members of the gammaherpesviruses (EBV and KSHV/HHV-8) share 40 of the 43 ORFs that have been included in the core set (see Chapter 2 Table 2.2). Although widely distributed amongst alpha- and gammaherpesviruses characterized to date, a thymidine kinase (TK) and a small subunit of ribonuclease (RR2) are absent from all betaherpesviruses. Although all herpesviruses appear to use sequence-specific DNA binding proteins to initiate DNA replication, a homolog of DNA replication origin binding protein (OBP) is not conserved in cytomegaloviruses or gammaherpesviruses.

This chapter stresses common features, while evolution has clearly provided the herpesviruses with a broad canvas on which to evolve the remarkable range of biological properties that characterize individual members. Genome annotation and comparison has suggested a higher level of similarity within subfamilies than between biologically distinct subfamilies. Even though biological properties of the individual subfamily members are distinct, they retain a recognizable evolutionary link.

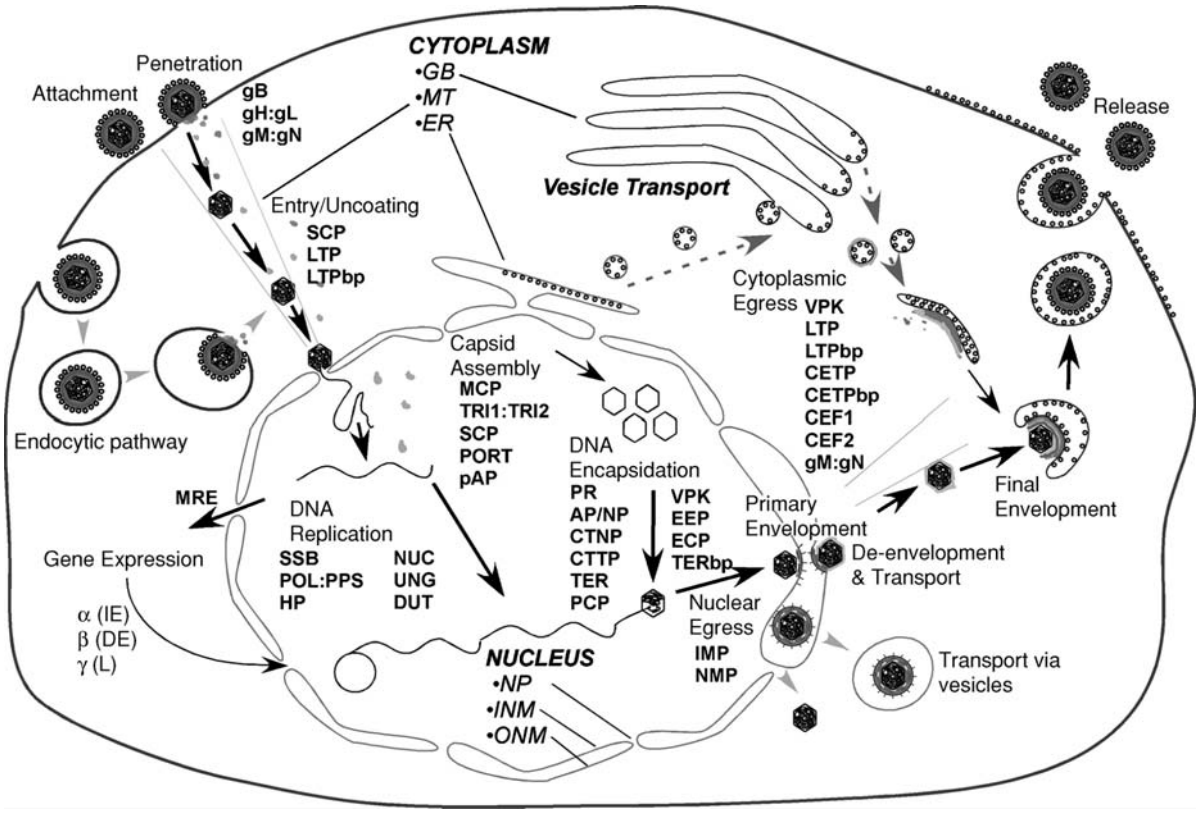

Fig. 4.1. Summary of replication functions carried out by herpesvirus-conserved gene products. Major steps in productive replication are indicated in larger font style and core functions contributing to each step are listed by their proposed abbreviated designations (see Table 4.1). The major entry pathway (black arrows) employs direct fusion at the cell surface (attachment and penetration), which is dependent upon gB and the gH:gL complex, followed by nucleocapsid transport along microtubules to nuclear pores where viral DNA is released into the nucleus. Alternatively, in certain cell types, entry follows the endocytic pathway and virion fusion with an endocytic vesicle (grey arrowheads). Uncoating requires the envelope fusion machinery (gB, gH:gL and in some cases gM:gN) as well as the LTP:LTPbp to direct docking at nuclear pores and release of virion DNA into the nucleus. Following entry and uncoating, one core regulatory protein (MRE) is involved in transcriptional and post-transcriptional regulation. DNA replication depends on several core replication fork proteins (SSB, POL:PPS, HP) as well as accessory functions (NUC, UNG, DUT). Capsid assembly uses MCP, TRI1:TRI2, SCP, and PORT. Pre-formed capsids translocate to sites of DNA replication where PRO, AP/NP (AP and NP are related proteins), CTNP, TER, TERbp and PCP, with possible accessory functions EEP, ECP and CTTP complete the encapsidation of viral DNA. Nuclear egress is controlled by NEMP and NELP. The main pathway of cytoplasmic egress (black arrows) and secondary (final) envelopment is controlled VPK, LTP:LTPbp, CETP, and CETPbp, with possible accessory proteins CEF1, CEF2 and gM:gN. Nucleocapsids are transported on MT and virion envelope glycoproteins follow vesicle transport to sites of final envelopment. Alternative maturation pathways of vesicle formation at the outer nuclear membrane with the mature virion following vesicle transport pathways or release of nucleocapsids directly through nuclear pores into the cytoplasm for transport to sites of final envelopment remain possible (grey arrowheads). Golgi body (GB), microtubules (MT) and endoplasmic reticulum (ER) are identified in the cytoplasm, and nuclear pores (NP), inner nuclear membrane (INM) and outer nuclear membrane (ONM) are identified in the nucleus. The cellular vesicle transport pathway from the ER to GB is also designated (dashed grey arrows).

There is little doubt that herpesviruses encode additional structurally related, functionally similar proteins that are not recognized as sequence homologs and all herpesvirus-common components cannot be recognized through sequence information alone. Other structural and functional properties complement sequence information and may constitute an independent set of criteria on which comparisons can be based. Relative position in a cluster of conserved ORFs, biological activity and the phenotype of mutant viruses all provide important comparative information. The existence of ORFs that fail to show sequence identity but are included in the list of core proteins due to

other information, such as the functional information on DNA polymerase processivity subunit already mentioned, suggests future work will require consideration of a broader set of characteristics. Additional structural and functional homologs will likely emerge as viral proteins are studied in greater detail by X-ray diffraction as by well as computer programs that model primary amino acid sequence on known protein structures.

Available information on the activities of core functions has often been generated in only one of the nine human herpesviruses, sometimes using herpesviruses of veterinary interest (e.g., PRV) and sometimes using rodent herpesviruses (e.g., MCMV). The remarkable diversity in functional organization, replication, latency and disease patterns exhibited by diverse human herpesviruses contrast the common activities of core functions which are key to viral replication. Functions will therefore be presented in relationship to the virus replicative cycle, starting with virion structure and entry, proceeding through regulation of gene expression, DNA synthesis, processing, and packaging and, finally, maturation and egress. Some of the functions involved in entry are structural components that are also involved with maturation and release of progeny virus, but most of the core functions can be implicated in at least one step in the replication pathway, and this is sometimes dependent on cell type. These core replication processes, including entry into cells and viral DNA synthesis, as well as the overall scheme of assembly, maturation and egress occur via evolutionarily conserved proteins and mechanisms. The role of core functions is best understood where they have been subjected to study by a combination of genetics and cell biology, this is often in alphaherpesviruses such as HSV-1 and PRV or in betaherpesviruses such as HCMV and MCMV. There have been many reviews dealing with herpesvirus-common features and evolutionarily common themes that have emerged over the years, both from a biological perspective (Roizman, 1999; Roizman and Pellett, 2001) as well as from a variety of analyses derived from genomic sequence (Davison, 2002; Karlin *et al.*, 1994; McGeoch *et al.*, 2000).

There are several common genome features described in Chapter 2 beyond the ultrastructural appearance of capsids and core proteins described in Chapter 3. All herpesviruses have a linear DNA genome that is cleaved from concatemers formed during replication, which where known, uses a conserved recognition sequence and leaves single base 3 overhanging nucleotide at each genomic terminus (Mocarski and Roizman, 1982). The position of origins of DNA replication that control DNA synthesis during the replicative cycle is conserved in most herpesviruses with a common location adjacent to the conserved single stranded DNA binding protein gene. While exceptions exist, the common evolutionary origin of these viruses is very clear from the range of conserved *cis*-acting elements as well as proteins.

Virion structural proteins

The virion particle of herpesviruses consists of a DNA-containing nucleocapsid with 162 regularly arranged capsomeres arranged in a $T = 16$ icosohedral lattice forming the protein shell. Detailed structural information has been derived from cryo-electron micrograph (cryo-EM) reconstructions involving studies on several different herpesviruses (Chapter 3). In the virion, the nucleocapsid is covered by a protein matrix or tegument that is surrounded by a lipid bilayer envelope derived from host cell membranes into which at least a dozen viral envelope proteins are inserted. Human herpesviruses have been estimated to have as few as 37 (e.g., alphaherpesviruses) and to well over 50 (e.g., cytomegaloviruses) virion proteins (Bortz *et al.*, 2003; Johannsen *et al.*, 2004; Kattenhorn *et al.*, 2004; Varnum *et al.*, 2004; Zhu *et al.*, 2005). About half (22) of the herpesvirus-conserved proteins are components of the virion, providing a genetic basis for the common ultrastructural appearance of all herpesviruses. Additional core proteins collaborate with structural proteins during maturation and egress (see below). Although similar in size, herpesvirus nucleocapsids package double-stranded DNA genomes range from a low of 125 kilobase pairs (VZV) to a high of 240 kilobase pairs (CCMV), which is remarkable given such a uniform capsid shell. The virion provides protection of the viral genome during transmission and mediates a two-stage entry process, first a fusion event between the envelope and cellular membranes that leads to release of the nucleocapsid into the cytoplasm and second a trafficking event that delivers the viral nucleocapsid to the nucleus where the genome is released.

Icosohedral herpesvirus capsids are composed of five herpesvirus-conserved proteins, the major capsid protein (MCP, HSV-1 *UL19* gene product), triplex monomer and dimer proteins (TRI1 and TRI2, HSV-1 *UL38* and *UL18* gene products, respectively), the smallest capsid protein (SCP, HSV-1 *UL35* gene product) and the portal protein (PORT, HSV-1 *UL6* gene product). The 150 hexons that make up the bulk of the capsid consist of six MCP molecules together with six molecules of SCP. Eleven of the 12 capsid pentons consist of five MCP molecules without SCP. One specialized penton consists of 12 molecules of PORT and has an axial channel for entry and exit of viral

DNA. The 125–130 nm diameter capsid has a wall that is 15 nm thick. Although different in shape, each hexon and penton appears cylindrical. Based on cryo-EM studies of HSV-1, HCMV, and KSHV (Chapter 3), the hexons and pentons are held in place by interactions between their bases on the inner side as well as by interconnections via triplexes located midway through the capsid shell. Detailed analyses suggests that beta- and gammaherpesvirus capsids resemble one another more closely than either resembles alphaherpesvirus capsids. Although the least conserved of the structural proteins, SCP is located at hexon tips, and absent from penton tips, in all herpesviruses (Yu *et al.*, 2005). There is a discernible SCP ring around hexons in HSV-1 where this structure contributes to the shape of the outermost capsid surface. Although widely conserved, the SCP is not universally essential for virion maturation and is, for example, dispensable for HSV-1 replication in cell lines (Desai *et al.*, 1998) but essential for HCMV replication (Borst *et al.*, 2001). Thus, the nucleocapsid of every human herpesvirus consists entirely of herpesvirus-conserved proteins, but the functional requirements for capsid maturation vary to some extent.

A large number of tegument proteins are located between the capsid and envelope in herpesviruses. Tegument proteins carry out a remarkably diverse range of activities during infection, although the most well-characterized functions and the most abundant tegument proteins in any herpesvirus are often not in the core set. Eleven proteins are conserved (see Chapter 2). Many are essential for replication in those viruses that have been subjected to systematic study, HSV-1 (Roizman and Knipe, 2001), PRV (Mettenleiter, 2004) and HCMV (Dunn *et al.*, 2003; Yu *et al.*, 2003). In HSV-1, five of the conserved tegument proteins are essential for replication (HSV-1 *UL7*, *UL17*, *UL25*, *UL36*, and *UL37* gene products), including a largest tegument protein (LTP, HSV-1 *UL36* gene product) and the protein that binds to the largest tegument protein (LTPbp) encoded by the adjacent (*UL37*) gene. These play roles in entry as well as in egress. The viral serine–threonine protein kinase (VPK, HSV-1 *UL13* gene product), and five additional tegument proteins (homologues of HSV-1 *UL11*, *UL14*, *UL16*, *UL21*, and *UL51* gene products) exhibit compromised growth, sometimes in specific cell types or under certain growth conditions. In contrast, HCMV requires an overlapping, but distinct set of seven homologs to replicate, without an absolute need for some that are essential in HSV-1. In HCMV, the LTP (UL48), UL77 and UL93 (homologs of HSV-1 *UL36*, *UL25*, and *UL17*, respectively) as well as UL99, UL95, UL94, and UL71 (homologs of HSV-1 *UL11*, *UL14*, *UL16*, and *UL51*, respectively) are required for replication when the entire ORF is eliminated (Dunn *et al.*, 2003). Mutants in the LTPbp (UL47) and UL103 (homologs of HSV-1 *UL37*, and *UL7*, respectively) as well as mutants in VPK and UL88 genes exhibit a reduced level of growth. While it is possible that some of these differences stem from experimental variability or the choice of different host cells in which to study mutants, evolutionary differences are likely to dictate the extent to which each virus relies on overlapping functions as well as the extent to which functional redundancy occurs. HSV-1 UL11 and HCMV UL99 proteins are known as small myristolated tegument proteins, localize to cytoplasm of infected cells, and are involved in the latter stages of virion egress. The HSV-1, *UL11* protein interacts with two other conserved tegument proteins, *UL14* and *UL16* as well as with the envelope glycoprotein, gM, which is part of the gM/gN glycoprotein complex. Although ultrastructural analysis does not provide much information on tegument organization, an association between the LTP:LTPbp complex with the SCP ring or with triplexes in the region of the capsid vertices has been suggested with HSV-1. Investigation of tegument protein activities and capsid : tegument interactions remain important areas for experimental investigation.

One additional protein is conserved amongst herpesviruses, represented by the *UL24* gene of HSV-1 about which little is known. The HCMV homologue (UL76), a minor tegument constitutent that is essential for replication (Dunn *et al.*, 2003), localizes in a pattern that suggests it may be involved in regulating events immediately following infection or during maturation (Wang *et al.*, 2004).

One enzyme contained in this set of conserved proteins, VPK apparently acts in tandem with host cell cycle kinases to regulate a variety of replication events. VPK ensures efficient phosphorylation of other viral proteins, some of which have been reported to increase the efficiency of the host translation machinery as well as other events in gene expression and DNA replication extending from early times during infection to egress. This enzyme is dispensable for replication in alphaherpesviruses as well as in rapidly dividing host cells infected with HCMV where its role is presumably redundant with host protein kinases, possibly including Cdk2. A requirement for VPK is most readily demonstrated in primary, non-dividing host cells that have lower levels of host cell kinases. VPK is also critical for efficient replication in host animals. Interestingly, the homologue in HCMV (UL97) as well as in HHV-6B is a nucleoside kinase required for phosphorylation of the antiviral drug ganciclovir, in addition to being a protein kinase. The UL97-encoded VPK is also the primary target of a candidate antiviral compound, maribavir, which is specifically active against HCMV and EBV, but not other herpesviruses.

Human herpesvirus envelopes are estimated to carry between 12 (HSV-1) and 20 (HCMV) viral integral membrane proteins. Many envelope proteins are specific to each herpesvirus type. Five, gB, gH, gL, gM, and gN, are conserved broadly amongst herpesviruses. Sequence conservation resulted in the adaptation of a common nomenclature for structural glycoproteins, using names originally applied to HSV-1 envelope constituents. Although these names do not imply function, they are now widely used by investigators in the field. One gene product that is an O-glycosylated glycoprotein in some herpesviruses, gN, does not undergo glycosylation in some alphaherpesviruses, such as HSV-1 and VZV. Furthermore, the specific interaction between gH and gL drives stable expression of these proteins, suggesting that they are molecular chaperones as well as functional partners. A similar relationship also appears to occur with gM and gN. Although gB does not form a complex with other viral proteins, complexes of gH:gL and gM:gN form in cells and associate with cellular membranes to be incorporated into the viral envelope of progeny virions during egress. In betaherpesviruses and gammaherpesviruses, gH:gL complexes may be further modified by additional viral glycoproteins that influence cell tropism.

Entry into host cells

Attachment and entry, which typically occurs by fusion with the plasma membrane is followed by translocation of the nucleocapsid through the cytoplasm and delivery of viral genome to the cell nucleus (Fig. 4.1). This process involves a series of distinct steps that have each received varying amounts of attention in different human herpesviruses: (i) binding to specific cell surface receptors, (ii) fusion of envelope with the cellular membrane to release nucleocapsids into the cytoplasm, (iii) nucleocapsid association with cytoskeletal elements and translocation towards the nucleus, (iv) nucleocapsid interaction with nuclear pores and (v) release of the viral genome into the nucleus (see Fig. 4.1). These steps are controlled by unique as well as herpesvirus-common functions. The first step in this process involves multiple cell surface components interacting with viral envelope glycoproteins in a stepwise process that leads to membrane fusion and delivery of the nucleocapsid to the cytoplasm of host cells (Spear, 2004; Spear and Longnecker, 2003) (see specific chapters on individual viruses). Attachment to cells has been studied in most human herpesviruses and usually requires both unique and conserved, sometimes functionally redundant, viral envelope glycoproteins. With the apparent exception of EBV, cell surface proteoglycans such as heparan sulfate play a role for initial contact with cells. Heparan sulfate-dependent entry has been demonstrated in alpha- (HSV-1, VZV), beta- (HCMV, HHV-6A, HHV-6B, HHV-7) and gammaherpesvirus (KSHV/HHV-8) subfamily members (Spear, 2004; Spear and Longnecker, 2003). As a result, many herpesviruses exhibit a broad cell tropism for attachment and entry steps. In these eight human herpesviruses, gB and typically other unique viral envelope proteins exhibit heparan sulfate binding activity and direct the first attachment step in entry. Binding appears to be part of the essential role of gB in the viruses where the process has been dissected. In EBV, gB lacks the domain that controls interaction with the glycosaminoglycan and this step does not seem to be required for entry. In addition to the initial binding step, viruses where entry has been studied in detail engage additional receptors using herpesvirus-conserved as well as unique viral envelope proteins. Attachment steps may rely on unique viral envelope proteins such as EBV gp350/220, which interacts with host CD21 and EBV gH:gL:gp42 complex which interacts with MHC class II protein, KSHV K8.1A which interacts with proteoglycan, or HSV-1 gD, which interacts with nectins as well as a TNF receptor family member. In some herpesviruses conserved envelope glycoproteins are responsible for subsequent steps, such as the role of KSHV gB in binding to integin $\alpha 3\beta 1$ or the role of HCMV gB in binding to the EGF receptor, although these may be more important in the fusion step (Spear and Longnecker, 2003). Thus, except for initial contact with proteoglycan, herpesvirus attachment mechanisms appear unique to each type of virus.

In most cells that have been studied, binding through specific cellular receptors leads to fusion of the viral envelope and plasma membrane, releasing the viral nucleocapsid into the cytoplasm. Fusion typically occurs at the plasma membrane and is under the control of the herpesvirus-conserved envelope glycoproteins, gB and gH:gL which are essential for this step in all studied herpesviruses. In HCMV gH:gL may associate with additional unique proteins that provide receptor specificity, either gO encoded by UL74 or a complex of glycoproteins encoded by UL128, UL130. In HHV-6, either gO encoded by V47 or gQ encoded by U100 provide specificity. There are parallels in human beta- and gammaherpesviruses. In EBV, the presence of gp42-containing complexes reduces epithelial cell tropism, whereas the presence of gp42-free complexes reduces tropism for B lymphocytes (Borza and Hutt-Fletcher, 2002). gM:gN complex is required for entry in some settings, a feature that suggests this complex may also contribute to host cell specificity. Although entry by fusion at the plasma membrane is the most common entry

mechanism, entry via endocytosis has been characterized in some systems (Fig. 4.1). EBV entry into epithelial cells occurs by fusion directly at the cell surface whereas entry into B lymphocytes involves endocytosis (see Chapter 24). The viral functional requirements for different entry processes are still incompletely understood. Thus, both major modes of virus entry may be employed by herpesviruses depending on the setting. Signaling that results from gB or gH:gL binding to cellular receptors has also been implicated as a step in replication but no common themes have emerged from such studies. Essential core glycoproteins seem to play key roles for entry, rather than later in the replication cycle or during egress. All core glycoproteins are incorporated into infected cell membranes as well as into the virion envelope. The evolutionarily conserved manner in which gB and gH:gL control membrane fusion between the viral envelope and plasma membrane has been studied most extensively in the alphaherpesviruses where mutations in each of these gives rise to syncytial viral strains that have provided initial clues to gene products controlling this step.

Herpesviruses exploit normal cytoplasmic transport systems that control cell shape and vesicular traffic, making use of tubulin-containing microtubules and actin-containing microfilaments (Dohner and Sodeik, 2004) to control nucleocapsid transit through the cytoplasm. Like many viruses that traverse the cytoplasm, herpesviruses rely on microtubules to gain access to the nucleus and nuclear pores where uncoating is completed and the viral genome is released into the nucleoplasm. This process was suspected long ago, initially in studies with adenoviruses and herpesviruses (Dales, 1973) and has been the focus of growing attention. Microtubule-destabilizing drugs such as nocadazole and colchacine block transport and entry (Mabit *et al.*, 2002). Net transport proceeds towards microtubule minus ends that terminate at the microtubule organizing center, which is located adjacent to the nucleus. The bidirectional nature of microtubule-directed transport (Welte, 2004) allows these filaments to act as the major highways of virus particle translocation during entry as well as egress (Fig. 4.1). Microfilaments do not play as direct a role during entry; however, evidence suggests depolymerization of the actin-containing cortex may be a requisite event during entry (Jones *et al.*, 1986). Intracellular transport mechanisms that have been intensively studied in neurotropic alphaherpesviruses, predominantly HSV-1, HSV-2 and PRV, because of the requirement to translocate across long expanses of cytoplasm. Herpesvirus-conserved capsid proteins appear to rely on common cellular pathways for movement in neurons as well as all other cell types. Nucleocapsid movement occurs in both directions on microtubules (Smith and Enquist, 2002) and is likely to be regulated in ways similar to vesicle transport (Welte, 2004). Minus-end-directed transport during entry depends on the dynein:dynactin motor complex (Dohner *et al.*, 2002), which is the same motor used for directional vesicle transport. Although still controversial, contact between the SCP of HSV-1 and the cellular constituent of the dynein complex, RP3 (and possibly other proteins) has been implicated in transport (Douglas *et al.*, 2004), suggesting that this herpesvirus-conserved protein may play a similar role in other viruses. Thus, a common bridge may be built between the nucleocapsid and the microtubule to allow an appropriate direction of movement to initiate infection. Thereafter, capsid and tegument proteins act in concert to release viral DNA into the nucleus at nuclear pores although the exact process that occurs once the nucleocapsid reaches the nucleus remains largely unexplored. A conditional, temperature sensitive HSV-1 mutant together with biochemical studies have long implicated the herpesvirus-common LTP in the uncoating process (Chapter 7). The LTP of HSV-1 is needed for uncoating and release of viral DNA at nuclear pores. Studies of other tegument proteins have employed null mutants propagated on cells that complement function in egress, and result in virions that contain the protein. This approach generally masks any role tegument proteins play during entry, leaving this an important area for future exploration.

Regulation of gene expression and replication

Most regulatory proteins encoded by herpesviruses are unique. Only one core protein is purely regulatory, acting as a multifunctional regulator of expression (MRE). MRE has been most extensively studied in HSV-1 (see Chapter 9) where it is the product of *UL54* called ICP27, and EBV (Hiriart *et al.*, 2003) where it is the product of BMLF1 called EB2. MRE binds RNA and localizes to sites of transcription in the nucleus and interacts with components of the RNA polymerase II transcription machinery, the spliceosome complex and pre-mRNA export machinery. MRE stimulates late gene transcription (Jean *et al.*, 2001) and dictates the location of viral transcripts in infected cells (Pearson *et al.*, 2004). MRE is best known for impeding cellular mRNA splicing to allow the mostly intronless viral transcripts to be preferentially exported from the nucleus (Sandri-Goldin, 2001). During the early phase of infection, MRE causes splicing to stall by recruiting host cell kinases to the nucleus to inactivate splicing factors through phosphorylation. In alphaherpesvirus and gammaherpesvirus systems, MRE also recruits an export

adaptor protein (Aly/REF) to sites of viral transcription to facilitate export. This inhibition is relieved during the late phase of infection when splicing and export of host and viral transcripts resumes. Based on studies in HCMV (Lischka et al., 2006; Toth et al., 2006), the function of betaherpesvirus MRE interacts with an RNA helicase, UAP56 to promote cytoplasmic accumulation of unspliced mRNA, and this process is independent of an RNA-binding motif. The MRE may also influence shut-off of the host cell and transcriptional events through a mechanism(s) that remains to be elucidated. Although the level of sequence conservation among MRE homologs is quite limited, others appear to carry out regulatory activities and are sometimes incorporated into the virion tegument, such as in HCMV (Mocarski and Courcelle, 2001).

Viral DNA synthesis and nucleotide metabolism

All herpesviruses encode a core set of six DNA synthesis enzymes that direct the synthesis of viral DNA during productive (lytic) infection. Herpesviruses initiate lytic DNA replication at defined sites on the viral genome that are readily assayed as virus-infection-dependent autonomously replicating sequences. Some herpesviruses have a single origin of DNA replication (ori_{Lyt}), such as in the betaherpesviruses (HCMV, HHV-6A, HHV-6B, HHV-7). Others have either two (VZV, EBV, KSHV) or three (HSV-1, HSV-2) ori_{Lyt} sites that retain sequence homology, although the reason for multiple origins in the biology of viruses that carry them remains a mystery. The relative position of one copy of ori_{Lyt} adjacent to the single stranded DNA binding protein (SSB) gene is conserved in many alpha-, beta- and gammaherpesviruses, even though the primary DNA sequence of ori_{Lyt} is not conserved in all of these settings. All herpesviruses appear to rely on virus-encoded DNA binding proteins to control initiation at ori_{Lyt}. In alphaherpesviruses and the reseolavirus subgroup of betaherpesviruses, a dedicated *ori* binding protein (OBP, HSV-1 *UL9* gene product) is essential for replication. Gammaherpesviruses and cytomegaloviruses rely on DNA binding regulatory proteins that control gene expression and also function during initiation of replication.

In general, DNA replication of herpesviruses, as in other DNA viruses, starts near nuclear structures, called nuclear domain 10, which become disrupted in the course of viral infection. This process overtakes the nucleus and leads to the formation of large, distinct replication compartments where viral replication proteins and DNA accumulate (Wilkinson and Weller, 2003). Viral DNA levels can equal cellular DNA content at late times of infection. Herpesvirus DNA replication proceeds through either of two potential mechanisms that have continued to be the focus of experimental investigation. Initial models of herpesvirus DNA replication have been analogous to bacteriophage lambda (Kornberg and Baker, 1992), starting with genome circularization and theta form replication for which evidence is scant and proceeding to a rolling circle form which has been experimentally well documented (Boehmer and Lehman, 1997; Boehmer and Nimonkar, 2003; Lehman and Boehmer, 1999). This model is based on the existence of ori_{Lyt} sites and site-specific DNA binding proteins and the expectation that the viral genome circularizes upon entry into cells. More recently, a recombination-dependent branching mechanism has been proposed (Wilkinson and Weller, 2003) and supported by the failure to detect circular intermediates during infection as well as by the behavior of HSV-1 mutants that exhibit increased accumulation of circular genomes early after infection (Jackson and DeLuca, 2003). This model has been reinforced by the observation that circular forms of HSV-1 DNA do not correlate with productive replication, but rather with latency. A mechanism analogous to that in the T even bacteriophages (Kornberg and Baker, 1992) has been suggested (Wilkinson and Weller, 2003). Either mechanism of synthesis results in the production of multi-genomic length concatemers that are the substrate for progeny genome packaging using conserved viral gene products. Distinct DNA replication origins separate from ori_{Lyt} sites that are responsible for the synthesis of viral DNA during latent infection have been identified in gammaherpesviruses but not in other subfamilies.

There are two structural categories of ori_{Lyt}. In alphaherpesviruses and non-CMV betaherpesviruses that rely on OBP, the initiation of DNA replication involves a targeted unwinding to enable the assembly of a replication fork complex. This process is best understood in HSV-1. An OBP complex with the viral single stranded DNA binding protein (SSB; HSV-1 *UL29* gene product also called ICP8) unwinds DNA and binds specific sequence motifs (called Box I and Box II in HSV-1) that are symmetrically arranged within ori_{Lyt} (Macao *et al.*, 2004). This allows a more dramatic unwinding at an AT-rich region that is located between Box I and Box II followed by replication fork machinery initiating uni- or bidirectional replication (Boehmer and Lehman, 1997). Beta- and gammaherpesviruses rely on DNA-binding transactivators that are not conserved between subfamilies but act in an analogous fashion to increase transcription across the ori_{Lyt} region which opens the DNA and allows interaction with replication machinery (Xu *et al.*, 2004). Studies in HCMV, EBV and KSHV have all provided evidence for a transcriptional activator-dependent initiation that appears to be distinct

from OBP-dependent initiation in alphaherpesviruses and the betaherpesviruses like HHV-6. The betaherpesvirus HCMV encodes a virion-associated transcript that associates with ori_{Lyt} to form a three-stranded structure whose precise role in DNA synthesis is still under investigation (Prichard *et al.*, 1998). The replication fork machinery includes a highly conserved set of six herpesvirus gene products: SSB, a viral catalytic subunit of DNA polymerase (POL; HSV-1 *UL30* gene product) and associated polymerase processivity subunit (PPS; HSV-1 *UL42* gene product) and a heterotrimeric helicase-primase (HP) consisting of an ATPase subunit (HP1; HSV-1 *UL5* gene product), a primase subunit (HP2; HSV-1 *UL52* gene product) and an accessory subunit (HP3; HSV-1 *UL8* gene product). These proteins direct continuous, leading strand viral DNA replication in a rolling circle mode when used in cell-free assays (Boehmer and Lehman, 1997; Boehmer and Nimonkar, 2003; Lehman and Boehmer, 1999). Following OBP binding to specific sites in ori_{Lyt}, an interaction with single stranded DNA binding protein (SSB) leads to localized unwinding and access of replication fork proteins. Specific interaction of the HP complex with the OBP and synthesis of RNA primers may be an intermediary step leading to DNA replication mediated by the POL–PPS complex. Recombination-directed initiation may be the consequence of SSB and HP activities (Boehmer and Nimonkar, 2003) and may underlie continued viral DNA synthesis (Wilkinson and Weller, 2003; Wilkinson and Weller, 2004). Thus, these six functions provide the core DNA synthesis machinery and mediate homologous recombination during viral replication. Cellular enzymes such as ligase and topoisomerases are highly likely to be required for replication; however, a complete understanding of the steps of herpesvirus DNA replication awaits the development of defined cell-free assay conditions.

Although a high level of recombination has long been associated with herpesvirus replication (Wilkinson and Weller, 2003) and with the isolated biochemical properties of replication proteins (Boehmer and Nimonkar, 2003), only recently has this process received some support as a component of DNA replication (Jackson and DeLuca, 2003). Under conditions where replication is blocked, the HSV-1 genome circularizes more efficiently, suggesting an association of circularization with latency rather than productive replication. Though provocative, this work provides little insight into the steps required for herpesvirus replication. The circumstantial evidence that recombination plays some role either early or late in replication remains strong. In addition to its role in coating single stranded DNA at the replication fork, SSB of HSV-1 appears to direct recombination in a manner similar to *E. coli* RecA (Kornberg and Baker, 1992). Homologous recombination requires SSB as well as another herpesvirus-conserved gene product, alkaline deoxyribonuclease (NUC, HSV-1 *UL12* gene product) (Wilkinson and Weller, 2003; Wilkinson and Weller, 2004). Interestingly, this DNase is absolutely required for HCMV replication (Dunn *et al.*, 2003). In addition to the contribution of sequence specific recombination to DNA replication, circularization of the genome may itself be dependent on recombination and number of herpesviruses that undergo genome isomerization via a recombination event mediated by the *a* sequence which is located at genomic ends and at an internal junction (Mocarski and Roizman, 1982).

Two different nucleotide metabolism enzymes are also broadly conserved, deoxyuridine triphosphatase (DUT; HSV-1 *UL50* gene product) and uracil-DNA glycosidase (UNG; HSV-1 *UL2* gene product). These seem to play accessory roles in replication that are redundant with cellular enzymes. The DUT eliminates pools of dUTP, preventing the incorporation of deoxyuridine into viral DNA and produces dUMP which can be converted to TTP through cellular pathways. The DUT appears to be inactive as an enzyme in betaherpesviruses. UNG cleaves deaminated cytosines (uracil) from the sugar backbone of DNA, leading to base excision and the activation of cellular DNA repair synthesis. In cytomegalovirus, where UNG is required for replication in quiescent cells, the process of uracil incorporation and excision has been proposed to introduce strand breaks that facilitate DNA replication (Courcelle *et al.*, 2001). Functions that are important for replication in nondividing cells where cellular nucleotide metabolism enzymes would be low or absent appear to be critical for replication in the host where differentiated cells lack cellular DNA metabolism enzymes.

Finally, the large subunit of ribonucleotide reductase (RR1, HSV-1 *UL39* gene product) is conserved in all herpesviruses; however, RR1 only forms an active enzyme with a small subunit (RR2, HSV-1 *UL40* gene product) in alphaherpesviruses and gammaherpesviruses. Somewhat surprisingly, betaherpesviruses retain an RR1 that lacks enzymatic activity and do not carry a homolog of RR2 at all. RR1 may have role in cell death suppression in the betaherpesviruses as well as in some alphaherpesviruses.

Capsid assembly and DNA encapsidation

The basic features of herpesvirus capsid maturation common to all herpesviruses have been established through a combination of work with HSV-1 infected cells, recombinant baculovirus-infected cells (Thomsen *et al.*, 1994)

and, importantly, cell-free systems (Newcomb *et al.*, 1996). Assembly employs the herpesvirus-conserved components of the capsid shell (MCP, SCP, TRI1 and TRI2) working in conjunction with a precursor of the assembly protein (pAP, HSV-1 *UL26.5* gene product). Assembly of HSV-1 capsids can proceed without SCP, but at a lower efficiency. A protease (PR, also called assemblin) is required to mature the capsid. This protein is made as a precursor consisting of PR as its amino terminus fused to a longer polypeptide that contains the pAP sequence as its carboxyl end (together called prePR, e.g., HSV-1 UL26 gene product). PR is a serine protease that processes both pAP and prePR. The protease domain self-cleaves in prePR to release PR as well as a variant of pAP, and also processes the carboxyl terminus of PR, pAP, and all variants of pAP (Gibson, 1996). This processing leads to the production of multiple forms of pAP, all colinear at the carboxyl terminus. The presence of pAP is sufficient for capsid assembly, but the presence of prePR, its self-cleavage to PR and variant pAP, and its cleavage of pAP to AP are all necessary for DNA encapsidation to proceed following capsid assembly. In addition, PORT is completely dispensable for the formation of normal appearing capsids, however, this protein is absolutely required for encapsidation of viral DNA. PORT associates with pAP in order to be incorporated into capsids (Newcomb *et al.*, 2003; Singer *et al.*, 2005). During infection, pNP must be cleaved into PR and variant AP for encapsidation to follow capsid formation but both of these proteins, as well as AP, are absent from capsids once encapsidation has occurred. Phosphorylation by VPK has been implicated in the encapsidation step (Wolf *et al.*, 2001).

Once herpesvirus DNA has replicated, encapsidation is controlled by a conserved *cis*-acting element (cleavage/packaging or *pac* site) and a series of seven herpesvirus conserved *trans*-acting functions (Yu and Weller, 1998). Encapsidation has been most extensively studied in alphaherpesviruses but these studies have implications for all herpesviruses. Although initially assigned roles in viral DNA packaging, two of the conserved proteins play roles in transporting preformed capsids to DNA replication compartments, the sites of viral DNA synthesis where packaging also occurs. These two proteins, capsid transport tegument protein (CTTP, HSV-1 *UL17* gene product) and capsid transport nuclear protein (CTNP, HSV-1 *UL32* gene product) are necessary for packaging to proceed. Little is known about the way that these proteins work, except that CTTP is a virion tegument protein and may bind to the capsid (Thurlow *et al.*, 2005). CTNP is a non-structural protein and remains in the nucleus.

Packaging of progeny viral genomes follows a modified head full packaging process reminiscent of bacteriophage λ (Campbell, 1994). Capsid localization, packaging and cleavage of viral DNA are regulated by the conserved VPK as well as by cellular kinases. VPK and cellular kinases may be redundant. The packaging machinery includes a heterodimeric terminase (TER) consisting of an ATPase subunit (TER1; HSV-1 *UL15* gene product) encoded by a conserved spliced gene and a DNA recognition subunit (TER2; HSV-1 *UL28* gene product). This machinery associates with the vertex of the specialized portal penton for the introduction of a free end of a viral DNA concatemer. The PORT protein interacts with TER and brings the packaging machinery and viral DNA to the capsid vertex. This machinery controls the threading of one genome length of DNA into the capsid before scanning for a *pac* site and determining the position of DNA cleavage and therefore the genomic ends. Thus, packaging and cleavage reactions are triggered by *pac* elements located near genomic termini, typically within terminal repeats (*a* sequences). The *pac* signal is composed of at least two elements, referred to as *pac* 1 and *pac* 2, and is broadly conserved among herpesviruses such that the *pac* from HCMV can direct packaging into HSV-1 virions (Spaete and Mocarski, 1985). All studies thus far have suggested that packaging yields single base 3′ extensions at both genomic ends and that packaging proceeds directionally with regard to the viral genome orientation as originally determined for HSV-1 (Mocarski and Roizman, 1982). The conservation of signals, functions and structure strongly suggests that this process is similar across all herpesviruses.

The heterodimeric TER, which is non-structural, and the portal complex, which takes the place of one penton of the capsid, associate with two additional conserved proteins, a TER binding protein (TERbp, HSV-1 *UL33* gene product) that interacts with capsids independent of PORT and a portal capping protein (PCP; HSV-1 *UL25* gene product). PCP remains associated with the nucleocapsid after packaging and thus appears as a minor capsid protein in mature virions. These five proteins are sufficient for recognition of a *pac* site on multi-genome DNA concatamers, docking with the appropriate site on a capsid, threading viral DNA into the caspid, cleavage at a *pac* site and sealing the genome into the progeny nucleocapsid (Fig. 4.1). As such they define a set of viral functions that are likely to be required for genome packaging in all herpesviruses. Once DNA has been packaged and PCP has covered the portal, the packaging stage of replication is completed and the nucleocapsid undergoes initial envelopment at the inner nuclear membrane (Fig. 4.1).

Two additional nuclear proteins, HSV-1 *UL7* and *UL14* gene products contribute to events in capsid maturation or DNA packaging. The *UL14* gene product is a minor tegument protein that appears to act as an encapsidation chaperone protein (ECP) to bring SCP and TERbp into the nucleus (Nishiyama, 2004). The *UL7* gene product appears

to act as an encapsidation and egress protein (EEP), colocalizing with capsids in patterns that suggest a role in DNA encapsidation or egress from the nucleus.

Maturation

Following the formation of the nucleocapsid, the best evidence from several systems suggests that tegument proteins function together with non-structural proteins to control a complex two-stage envelopment and egress process that starts in the nucleus and leads to virion release by exocytosis at the plasma membrane. This two-stage envelopment process has been controversial but strong evidence has accumulated in favor of this pathway in all three herpesvirus subfamilies. The alphaherpesviruses HSV-1 and PRV have been extensively studied, along with the betaherpesviruses HCMV and MCMV (Mettenleiter, 2002; Mettenleiter, 2004). Evidence suggests that nuclear egress starts with primary envelopment at the inner nuclear membrane followed by a de-envelopment event at the outer nuclear membrane, a process that releases the nucleocapsid into the cytoplasm (Fig. 4.1). Secondary envelopment occurs in the cytoplasm at endosomal (or possibly Golgi complex) membranes with resulting vesicles carrying the fully mature virions to the cell surface using the cellular exocytic pathway. Alternatively, the older model of egress has the enveloped viral particle itself following a vesicle transport pathway without deenvelopment (Roizman and Knipe, 2001) (see Chapter 10). These steps, like the initial entry process, rely on membrane fusion events; however, envelopment and egress are relatively independent of viral envelope glycoproteins that play critical roles during entry. Non-replicating mutants in gB or the gH:gL complex mature normally but show defects in entry. Only the gM:gN complex may contribute to secondary, or final, envelopment.

Initial envelopment event occurs at the inner nuclear membrane, following and dependent upon genome packaging that produces nucleocapsids. Capsids lacking DNA do not mature efficiently and nucleocapsids must localize correctly in the nucleus to properly egress. Studies in alphaherpesviruses and betaherpesviruses have shown that two conserved proteins form a nuclear egress complex on the inner nuclear membrane to control egress from the nucleus (Mettenleiter, 2004) and disruption of the nuclear lamina. The nuclear egress membrane protein (NEMP, HSV-1 *UL34* gene product) acts as a type II membrane-spanning protein to anchor the nuclear egress lamina protein (NELP, HSV-1 *UL31* gene product), a phosphoprotein that interacts with the nuclear lamina as well as with membrane-associated NEMP. NEMP and NELP are dependent on one another for proper transport and localization to the inner nuclear membrane. Additional viral proteins or the process of viral maturation itself appear to be necessary for the formation of the nuclear egress complex. Direct binding to nucleocapsids has not been observed, however, the complex recruits viral and/or cellular protein kinases that appear to be important for phosphorylation and disruption of the nuclear lamina to allow egress. Viral mutants in NEMP or NELP are debilitated for egress, likely unable to bud through the inner nuclear membrane (primary envelopment) and so accumulate nucleocapsids inside nuclei. The nuclear egress complex may also participate in membrane fusion events at the outer nuclear membrane that are required to deposit the nucleocapsid in the cytoplasm (de-envelopment). Although NEMP, as a primary envelope protein, and NELP, as a primary tegument protein, are important components of primary virions, neither is retained in fully mature virions.

Primary envelopment delivers viral particles to the perinuclear space between the inner and outer nuclear membranes, a compartment contiguous with the endoplasmic reticulum. Over the past 10 years evidence has accumulated in several different herpesviruses that final herpesvirus envelopment occurs in the cytoplasm at late endosomal or Golgi body membranes. It is likely that primary envelopment is followed by a de-envelopment step that releases the nucleocapsid into the cytoplasm. It remains possible, but topologically unlikely that nucleocapsids move to the cytoplasm without any envelopment step through modified nuclear pores. Either way, movement of nucleocapsids to sites of final envelopment appears to be a microtubule-dependent process. Protein kinases appear to play regulatory roles in primary envelopment as well as de-envelopment occurring at the outer nuclear membrane or endoplasmic reticulum. In alphaherpesvirus, viral US3 kinase facilitates these processes by facilitating phosphorylation of NEMP or other proteins. Primary envelopment and de-envelopment may also rely on cellular kinases. In HCMV, the conserved VPK contributes to nuclear egress (Krosky et al., 2003) whereas in other betaherpesviruses such as MCMV, host protein kinase C has been implicated in nuclear egress (Muranyi *et al.*, 2002). All of this data suggests that there is redundancy and possible interplay between viral and host kinases (presumably in conjunction with host phosphatases) in establishing the appropriate phosphorylation state for egress from the nucleus.

While some virion tegument proteins are associated with nucleocapsids during nuclear egress, many are added in the cytoplasm. Major tegument proteins, though not conserved, localize to the cytoplasm in all herpesviruses that have been studied and it now appears that the bulk of tegument proteins found in mature herpesvirus virions are

added to the nucleocapsid as it traverses the cytoplasm or at sites of final envelopment. There is currently little precise understanding of how nucleocapsids engage microtubules to traverse the cytoplasm, how final envelopment at endosomal (or Golgi complex) membranes occurs or how tegument proteins might be added prior to final envelopment. There is growing suspicion that addition may be nucleated by the conserved, very large LTP:LTPbp protein complex (Mettenleiter, 2004). This and a number of additional interactions between tegument proteins (Vittone et al., 2005) may be important in function. Current evidence on alphaherpesviruses and betaherpesviruses suggests that a conserved cytoplasmic egress tegument protein (CETP, HSV-1 *UL11* gene product) plays a central role in the secondary, or final, envelopment process. In alphaherpesviruses and betaherpesviruses, CETP is a myrisoylated and pamitoylated protein that localizes to the cytoplasmic face of cellular membranes and is known to interact with another herpesvirus-conserved tegument protein, the CETP binding protein (CETPbp, HSV-1 *UL16* gene product). Together, these may form a complex involved in transport. An additional tegument protein called cytoplasmic egress facilitator 1 (CEF1, HSV-1 *UL51* gene product) is a palmitated protein that is conserved amongst herpesviruses and also associates with cytoplasmic membranes (Nishiyama, 2004). In PRV, mutants in CEF1 fail to egress properly. One final tegument protein, called cytoplasmic egress facilitator 2 (CEF2, HSV-1 *UL21* gene product) enhanced maturation and interacts with CETPbp. Limited evidence supports a role for the gM:gN complex in secondary envelopment, particularly when disrupted together with *UL11*(Kopp *et al.*, 2004; Tischer *et al.*, 2002), although this complex is dispensable for replication in a number of other alphaherpesviruses, including HSV-1. Both gM and gN are essential for HCMV replication (Dunn *et al.*, 2003) as well as for assembly in EBV (Lake and Hutt-Fletcher, 2000). gM:gN is a major EBV structural component and has recently been recognized as the major glycoprotein complex on the HCMV envelope (Varnum *et al.*, 2004).

Thus, final envelopment occurs in the cytoplasm and, as a consequence the tegument of virions includes small amounts of cellular proteins, in particular actin (Bortz *et al.*, 2003; Johannsen *et al.*, 2004; Kattenhorn *et al.*, 2004; Varnum *et al.*, 2004; Zhu *et al.*, 2005), as well as RNAs (Sciortino *et al.*, 2001; Terhune *et al.*, 2004) that may represent a quantitative sampling of the cytosol, although a role for any virion component in replication cannot be discounted as yet. Once final envelopment has occurred, exocytosis is believed to carry the mature virion inside of a vesicle to the cell surface for release. Thus, the final step in egress is fusion of an exocytic vesicle with the plasma membrane, a process that is likely to follow cellular vesicle trafficking pathways (Fig. 4.1).

This analysis of the role of herpesvirus-conserved gene products has attempted to evaluate data generated in a wide variety of systems. As a result, some data will certainly have been generalized inappropriately as further investigation reveals unique characteristics or distinguishing activities. While many common names are already in use across the different herpesviruses, the hope is that a common nomenclature for herpesvirus-common functions should emerge to facilitate comprehension of work in otherwise diverse systems. This presentation is intended to provide a starting point for evaluation of data on herpesvirus core functions no matter what virus system is being studied or discussed.

REFERENCES

Boehmer, P. E., and Lehman, I. R. (1997). Herpes simplex virus DNA replication. *Annu. Rev. Biochem.*, **66**, 347–384.

Boehmer, P. E. and Nimonkar, A. V. (2003). Herpes virus replication. *IUBMB Life*, **55**(1), 13–22.

Borst, E. M., Mathys, S., Wagner, M., Muranyi, W., and Messerle, M. (2001). Genetic evidence of an essential role for cytomegalovirus small capsid protein in viral growth. *J. Virol.* **75**(3), 1450–1458.

Bortz, E., Whitelegge, J. P., Jia, Q. *et al.* (2003). Identification of proteins associated with murine gammaherpesvirus 68 virions. *J. Virol.*, **77**(24), 13425–13432.

Borza, C. M. and Hutt-Fletcher, L. M. (2002). Alternate replication in B cells and epithelial cells switches tropism of Epstein–Barr virus. *Nat. Med.*, **8**(6), 594–599.

Campbell, A. (1994). Comparative molecular biology of lambdoid phages. *Annu. Rev. Microbiol.*, **48,** 193–222.

Courcelle, C. T., Courcelle, J., Prichard, M. N., and Mocarski, E. S. (2001). Requirement for uracil-DNA glycosylase during the transition to late-phase cytomegalovirus DNA replication. *J. Virol.*, **75**(16), 7592–7601.

Dales, S. (1973). Early events in cell-animal virus interactions. *Bacteriol. Rev.*, **37**(2), 103–135.

Davison, A. J. (2002). Evolution of the herpesviruses. *Vet. Microbiol.*, **86**(1–2), 69–88.

Desai, P., DeLuca, N. A., and Person, S. (1998). Herpes simplex virus type 1 VP26 is not essential for replication in cell culture but influences production of infectious virus in the nervous system of infected mice. *Virology*, **247**(1), 115–124.

Dohner, K. and Sodeik, B. (2004). The role of the cytoplasm during viral infection. *Curr. Top. Microbiol. Immunol.*, **285,** 67–108.

Dohner, K., Wolfstein, A., Prank, U. *et al.* (2002). Function of dynein and dynactin in herpes simplex virus capsid transport. *Mol. Biol. Cell*, **13**(8), 2795–2809.

Douglas, M. W., Diefenbach, R. J., Homa, F. L. *et al.* (2004). Herpes simplex virus type 1 capsid protein VP26 interacts with dynein light chains RP3 and Tctex1 and plays a role in retrograde cellular transport. *J. Biol. Chem.*, **279**(27), 28522–28530.

Dunn, W., Chou, C., Li, H. *et al.* (2003). Functional profiling of a human cytomegalovirus genome. *Proc. Natl Acad. Sci. USA*, **100**(24), 14223–14228.

Gibson, W. (1996). Structure and assembly of the virion. *Intervirology*, **39**(5–6), 389–400.

Hiriart, E., Bardouillet, L., Manet, E. *et al.* (2003). A region of the Epstein-Barr virus (EBV) mRNA export factor EB2 containing an arginine-rich motif mediates direct binding to RNA. *J. Biol. Chem.*, **278**(39), 37790–37798.

Jackson, S. A. and DeLuca, N. A. (2003). Relationship of herpes simplex virus genome configuration to productive and persistent infections. *Proc. Natl Acad. Sci. USA*, **100**(13), 7871–7876.

Jean, S., LeVan, K. M., Song, B., Levine, M., and Knipe, D. M. (2001). Herpes simplex virus 1 ICP27 is required for transcription of two viral late (gamma 2) genes in infected cells. *Virology*, **283**(2), 273–284.

Johannsen, E., Luftig, M., Chase, M. R. *et al.* (2004). Proteins of purified Epstein–Barr virus. *Proc. Natl Acad. Sci. USA*, **101**(46), 16286–16291.

Jones, N. L., Lewis, J. C., and Kilpatrick, B. A. (1986). Cytoskeletal disruption during human cytomegalovirus infection of human lung fibroblasts. *Eur. J. Cell Biol.*, **41**(2), 304–312.

Karlin, S., Mocarski, E. S., and Schachtel, G. A. (1994). Molecular evolution of herpesviruses: genomic and protein sequence comparisons. *J. Virol.* **68**(3), 1886–1902.

Kattenhorn, L. M., Mills, R., Wagner, M. *et al.* (2004). Identification of proteins associated with murine cytomegalovirus virions. *J. Virol.*, **78**(20), 11187–11197.

Kopp, M., Granzow, H., Fuchs, W., Klupp, B., and Mettenleiter, T. C. (2004). Simultaneous deletion of pseudorabies virus tegument protein UL11 and glycoprotein M severely impairs secondary envelopment. *J. Virol.*, **78**(6), 3024–3034.

Kornberg, A. and Baker, T. A. (1992). *DNA Replication*. 2nd edn. New York: W. H. Freeman and Company.

Krosky, P. M., Baek, M. C., and Coen, D. M. (2003). The human cytomegalovirus UL97 protein kinase, an antiviral drug target, is required at the stage of nuclear egress. *J. Virol.*, **77**(2), 905–914.

Lake, C. M. and Hutt-Fletcher, L. M. (2000). Epstein–Barr virus that lacks glycoprotein gN is impaired in assembly and infection. *J. Virol.*, **74**(23), 11162–11172.

Lehman, I. R. and Boehmer, P. E. (1999). Replication of herpes simplex virus DNA. *J. Biol. Chem.*, **274**(40), 28059–28062.

Lischka, P., Toth, Z., Thomas. M., Mueller, R., and Stamminger, T. (2006). The UL69 transactivator protein of human cytomegalovirus interacts with DEXD&H-Box RNA helicase UAP56 to promote cytoplasmic accumulation of unspliced RNA. *Mol. Cell. Biol.* **26**(5), 1631–1643.

Mabit, H., Nakano, M. Y., Prank, U. *et al.* (2002). Intact microtubules support adenovirus and herpes simplex virus infections. *J. Virol.*, **76**(19), 9962–9971.

Macao, B., Olsson, M., and Elias, P. (2004). Functional properties of the herpes simplex virus type I origin-binding protein are controlled by precise interactions with the activated form of the origin of DNA replication. *J. Biol. Chem.*, **279**(28), 29211–29217.

McGeoch, D. J., Dolan, A., and Ralph, A. C. (2000). Toward a comprehensive phylogeny for mammalian and avian herpesviruses. *J. Virol.*, **74**(22), 10401–10406.

Mettenleiter, T. C. (2002). Herpesvirus assembly and egress. *J. Virol.*, **76**(4), 1537–1547.

Mettenleiter, T. C. (2004). Budding events in herpesvirus morphogenesis. *Virus Res.* **106**(2), 167–180.

Mocarski, E. S., Jr., and Courcelle, C. T. (2001). Cytomegaloviruses and their replication. In *Fields Virology*, 4th edn., ed. D. M. Knipe, P. M. Howley, D. E. Griffin *et al.*, Vol. 2, pp. 2629–2673. Philadelphia: Lippincott, Williams & Wilkins.

Mocarski, E. S. and Roizman, B. (1982). Structure and role of the herpes simplex virus DNA termini in inversion, circularization and generation of virion DNA. *Cell*, **31**(1), 89–97.

Muranyi, W., Haas, J., Wagner, M., Krohne, G., and Koszinowski, U. H. (2002). Cytomegalovirus recruitment of cellular kinases to dissolve the nuclear lamina. *Science*, **297**(5582), 854–857.

Newcomb, W. W., Homa, F. L., Thomsen, D. R. *et al.* (1996). Assembly of the herpes simplex virus capsid: characterization of intermediates observed during cell-free capsid formation. *J. Mol. Biol.*, **263**(3), 432–446.

Newcomb, W. W., Thomsen, D. R., Homa, F. L., and Brown, J. C. (2003). Assembly of the herpes simplex virus capsid: identification of soluble scaffold-portal complexes and their role in formation of portal-containing capsids. *J. Virol.*, **77**(18), 9862–9871.

Nishiyama, Y. (2004). Herpes simplex virus gene products: the accessories reflect her lifestyle well. *Rev. Med. Virol.* **14**(1), 33–46.

Pearson, A., Knipe, D. M., and Coen, D. M. (2004). ICP27 selectively regulates the cytoplasmic localization of a subset of viral transcripts in herpes simplex virus type 1-infected cells. *J. Virol.*, **78**(1), 23–32.

Prichard, M. N., Jairath, S., Penfold, M. E., St Jeor, S., Bohlman, M. C., and Pari, G. S. (1998). Identification of persistent RNA–DNA hybrid structures within the origin of replication of human cytomegalovirus. *J. Virol.*, **72**(9), 6997–7004.

Roizman, B. (1999). HSV gene functions: what have we learned that could be generally applicable to its near and distant cousins? *Acta Virol.* **43**(2–3), 75–80.

Roizman, B., and Knipe, D. M. (2001). Herpes simplex viruses and their replication. In *Fields Virology*, 4th edn., ed. D. M. Knipe, P. M. Howley, D. E. Griffin *et al.*, Vol. 2, pp. 2399–2459. 2 vols. Philadelphia: Lippincott, Williams and Wilkins.

Roizman, B. and Pellett, P. E. (2001). The family Herpesviridae: A Brief Introduction. In *Fields Virology*, 4th edn., ed. D. M. Knipe, P. M. Howley, D. E. Griffin *et al.*, Vol. 2, pp. 2221–2230. 2 vols. Philadelphia: Lippincott, Williams & Wilkins.

Sandri-Goldin, R. M. (2001). Nuclear export of herpes virus RNA. *Curr. Top. Microbiol. Immunol.*, **259**, 2–23.

Sciortino, M. T., Suzuki, M., Taddeo, B., and Roizman, B. (2001). RNAs extracted from herpes simplex virus 1 virions: apparent selectivity of viral but not cellular RNAs packaged in virions. *J. Virol.*, **75**(17), 8105–8116.

Singer, G. P., Newcomb, W. W., Thomsen, D. R., Homa, F. L., and Brown, J. C. (2005). Identification of a region in the herpes simplex virus scaffolding protein required for interaction with the portal. *J. Virol.*, **79**(1), 132–139.

Smith, G. A. and Enquist, L. W. (2002). Break ins and break outs: viral interactions with the cytoskeleton of Mammalian cells. *Annu Rev Cell Dev. Biol.*, **18**, 135–161.

Spaete, R. R. and Mocarski, E. S. (1985). The a sequence of the cytomegalovirus genome functions as a cleavage/packaging signal for herpes simplex virus defective genomes. *J. Virol.*, **54**, 817–824.

Spear, P. G. (2004). Herpes simplex virus: receptors and ligands for cell entry. *Cell Microbiol* **6**(5), 401–410.

Spear, P. G. and Longnecker, R. (2003). Herpesvirus entry: an update. *J. Virol.*, **77**(19), 10179–10185.

Terhune, S. S., Schroer, J., and Shenk, T. (2004). RNAs are packaged into human cytomegalovirus virions in proportion to their intracellular concentration. *J. Virol.*, **78**(19), 10390–10398.

Thomsen, D. R., Roof, L. L., and Homa, F. L. (1994). Assembly of herpes simplex virus (HSV) intermediate capsids in insect cells infected with recombinant baculoviruses expressing HSV capsid proteins. *J. Virol.*, **68**(4), 2442–2457.

Thurlow, J. K., Rixon, F. J., Murphy, M., Targett-Adams, P., Hughes, M., and Preston, V. G. (2005). The herpes simplex virus type 1 DNA packaging protein UL17 is a virion protein that is present in both the capsid and the tegument compartments. *J. Virol.*, **79**(1), 150–158.

Tischer, B. K., Schumacher, D., Messerle, M., Wagner, M., and Osterrieder, N. (2002). The products of the UL10 (gM) and the UL49.5 genes of Marek's disease virus serotype 1 are essential for virus growth in cultured cells. *J. Gen. Virol.*, **83**(Pt 5), 997–1003.

Toth, Z., Lischka, P., and Stamminger, T. (2006). RNA-binding of the human cytomegalovirus transactivator protein UL69, mediated by arginine-rich motifs, is not required for nuclear export of unspliced RNA. *Nucleic Acids Res.* **34**(4), 1237–1249.

Varnum, S. M., Streblow, D. N., Monroe, M. E. *et al.* (2004). Identification of proteins in human cytomegalovirus (HCMV) particles: the HCMV proteome. *J. Virol.*, **78**(20), 10960–10966.

Vittone, V., Diefenbach, E., Triffett, D., Douglas, M. W., Cunningham, A. L., Diefenbach, R. J. (2005). Determination of interactions between tegument proteins of herpes simplex virus type 1. *J. Virol.* **79**(15), 9566–9571.

Wang, S. K., Duh, C. Y., and Wu, C. W. (2004). Human cytomegalovirus UL76 encodes a novel virion-associated protein that is able to inhibit viral replication. *J. Virol.*, **78**(18), 9750–9762.

Welte, M. A. (2004). Bidirectional transport along microtubules. *Curr. Biol.*, **14**(13), R525–R537.

Wilkinson, D. E. and Weller, S. K. (2003). The role of DNA recombination in herpes simplex virus DNA replication. *IUBMB Life*, **55**(8), 451–458.

Wilkinson, D. E., and Weller, S. K. (2004). Recruitment of cellular recombination and repair proteins to sites of herpes simplex virus type 1 DNA replication is dependent on the composition of viral proteins within prereplicative sites and correlates with the induction of the DNA damage response. *J. Virol.*, **78**(9), 4783–4796.

Wolf, D. G., Courcelle, C. T., Prichard, M. N., and Mocarski, E. S. (2001). Distinct and separate roles for herpesvirus-conserved UL97 kinase in cytomegalovirus DNA synthesis and encapsidation. *Proc. Natl Acad. Sci. USA*, **98**(4), 1895–1900.

Xu, Y., Cei, S. A., Rodriguez Huete, A., Colletti, K. S., and Pari, G. S. (2004). Human cytomegalovirus DNA replication requires transcriptional activation via an IE2- and UL84-responsive bidirectional promoter element within oriLyt. *J. Virol.*, **78**(21), 11664–11677.

Yu, D. and Weller, S. K. (1998). Herpes simplex virus type 1 cleavage and packaging proteins UL15 and UL28 are associated with B but not C capsids during packaging. *J. Virol.*, **72**(9), 7428–7439.

Yu, D., Silva, M. C., and Shenk, T. (2003). Functional map of human cytomegalovirus AD169 defined by global mutational analysis. *Proc. Natl Acad. Sci. USA*, **100**(21), 12396–12401.

Yu, X., Shah, S., Atanasov, I. *et al.* (2005). Three-dimensional localization of the smallest capsid protein in the human cytomegalovirus capsid. *J. Virol.*, **79**(2), 1327–1332.

Zhu, F. X., Chong, J. M., Wu, L., and Yuan, Y. (2005). Virion proteins of Kaposi's sarcoma-associated herpesvirus. *J. Virol.*, **79**(2), 800–811.

Part II

Basic virology and viral gene effects on host cell functions: alphaherpesviruses

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5

Genetic comparison of human alphaherpesvirus genomes

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Human herpesviruses 1, *2*, and *3* (herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), and varicella-zoster virus (VZV)) have been classified as alphaherpesviruses based originally upon their biological properties, and subsequently on the sequences of their respective genomes (Minson *et al.*, 2000; Pellett and Roizman, in press). All of these viruses maintain latent infections in sensory ganglia, and can productively infect a variety of human cells, including the living cells of mucous membranes and skin. These epithelial sites also provide exit points for the virus to infect other individuals.

The structure of the genomes of the alphaherpesviruses that infect humans are quite similar at first glance (Fig. 5.1). All have two unique segments that are flanked by repeats of different lengths. The unique segments are designated short (S) and long (L) and the repeats designated as internal (IR) or terminal (TR). Members of the genus simplexvirus (HSV-1 and HSV-2) exist as four roughly equimolar isomers, each isomer differing in the relative orientations of the long and short components. The orientation of one of the HSV isomers has been designated prototypical, and could therefore be designated TRL-U_L-IRL-IRS-U_S-TRS. VZV also produces 4 genomic isomers, but those in which the long component is inverted are significantly reduced in frequency, to about 5% of total genomes. It is tempting to speculate that this is a consequence of the shorter repeats flanking the VZV long component (88.5 bp) as compared to the repeats flanking U_S in human alphaherpesviruses (6000–7400 bp) and U_L in HSV-1 and HSV-2 (around 9,000 bp). The shorter repeat region would be expected to reduce the frequency of homologous recombination events leading to less frequent inversion of the long component in VZV genomes.

The 129 kbp VZV genome is the smallest genome of the human herpesviruses, whereas the HSV-1 and HSV-2 genomes are over 152 kbp in length. The G + C content of the human simplexviruses is around 68% whereas the VZV genome is only 46% G + C. Thus, considerable numbers of mutations have occurred since the two lineages arose from a common progenitor. All three viruses encode well-conserved sequences that guide lytic replication (oriLyt) and genome cleavage and packaging (Frenkel & Roffman 1996). Copies of oriLyt are present in IRS and TRS of all three viruses, and near the center of L for HSV-1 and HSV-2.

Several features are apparent in the global sequence comparisons shown in Fig. 5.2. HSV-1 and HSV-2 are much more closely related to each other than is either to VZV. HSV-1 and HSV-2 genomes are most similar in their U_L components, and least similar in the inverted repeats that bound U_L (TRL and IRL). The VZV sequence is most closely related to HSV in their U_L components. These observations can be extended by measuring the level of sequence identity along the dot plot diagonal, as shown in the nucleotide sequence similarity plot for HSV-1 vs. HSV-2 (Fig. 5.3). As can be seen, the similarity is highest across U_L, with most of the peaks corresponding to protein coding regions, and the valleys to intergenic regions, the exceptions being U_L 42 through U_L 44, and U_L 49. The L component repeats are much less well conserved, with the peak of similarity being in the region encoding ICP0 (RL2). The most highly conserved region in the S component repeats is the ICP4 gene (RS1). Overall, U_S is less well conserved than U_L. This region includes the U_S 4 (gG) gene, which is approximately 1500 bp longer in HSV-2 than in HSV-1 and represents the region of greatest dissimilarity between HSV-1 and HSV-2.

The gene arrangement in the long component of the most common isomer of the VZV genome is inverted relative to the U_L of the prototypical isomer of HSV (Fig. 5.1). With this inversion in mind, the genes in the HSV-1 and HSV-2 U_L segments are mostly collinear with VZV U_L (Fig. 5.2), and all but a few U_L genes are conserved across all human alphaherpesviruses. This consideration also underscores the significance of the overall similarity of the genes,

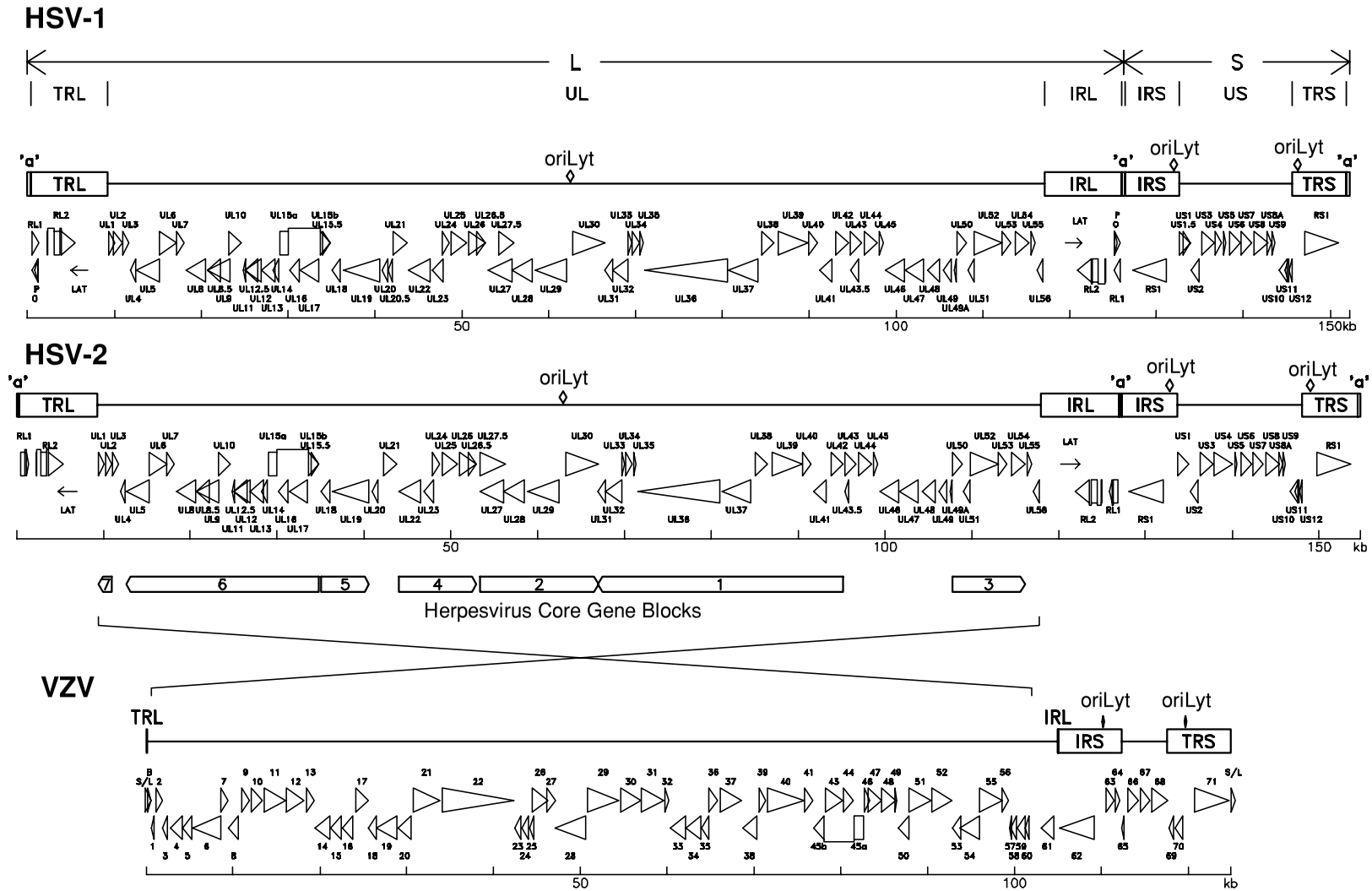

Fig. 5.1. Genomic and genetic architectures of the human alphaherpesviruses. Note that the VZV L component is inverted relative to the HSV prototypic arrangement. The representations are based primarily on annotations in Genbank Accession numbers X14112 (HSV-1 strain 17), Z86099 (HSV-2 strain HG52), and X04370 (VZV strain Dumas), and information in references cited in the text. L, long component; S, short component; TRL, long component terminal repeat; IRL, long component internal repeat; IRS, short component internal repeat; TRS, short component terminal repeat.

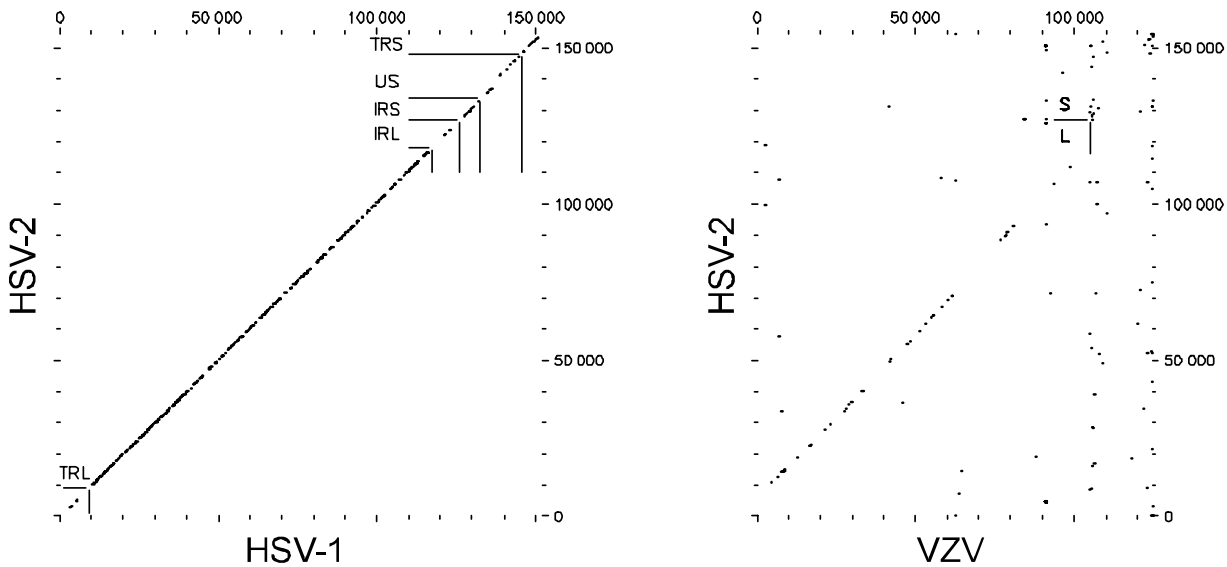

Fig. 5.2. Global genome sequence similarity comparisons among the human alphaherpesviruses. Dot similarity plots were constructed from comparisons of complete genome sequences. For HSV-1 vs. HSV-2, the window was 50 and the stringency was 45 identical residues. For HSV-2 vs. VZV, the window was 50 and the stringency was 25 identical residues. This reduced stringency was needed, because there were no dots when the HSV-2 and VZV were compared at the same stringency used for the HSV-1/HSV-2 comparison. Because the prototypic genomes of HSV-2 and VZV have relatively inverted L components, the HSV-2/VZV comparison was done with a VZV sequence in which the L component was inverted. Sequence sources and abbreviations are the same as for Fig. 5.1.

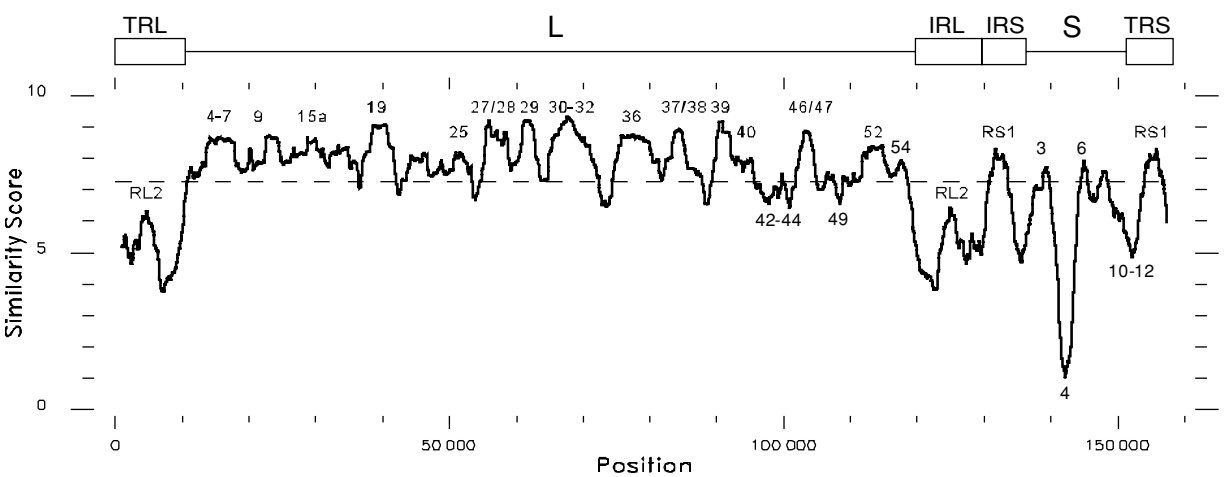

Fig. 5.3. Nucleotide sequence similarity between HSV-1 and HSV-2. HSV-1 and HSV-2 nucleotide sequences were aligned in approximately 20 kb segments, the aligned segments were joined to generate genome-length alignments, and then the similarity along the aligned genomes was plotted as a running percentage of identity in windows of 2000 residues. x-axis positions do not correspond precisely to genomic coordinates because of spaces inserted during the alignment. Boundaries of major architectural features are indicated, as are the locations of various genes along the plot. Sequence sources and abbreviations are the same as for Fig. 5.1.

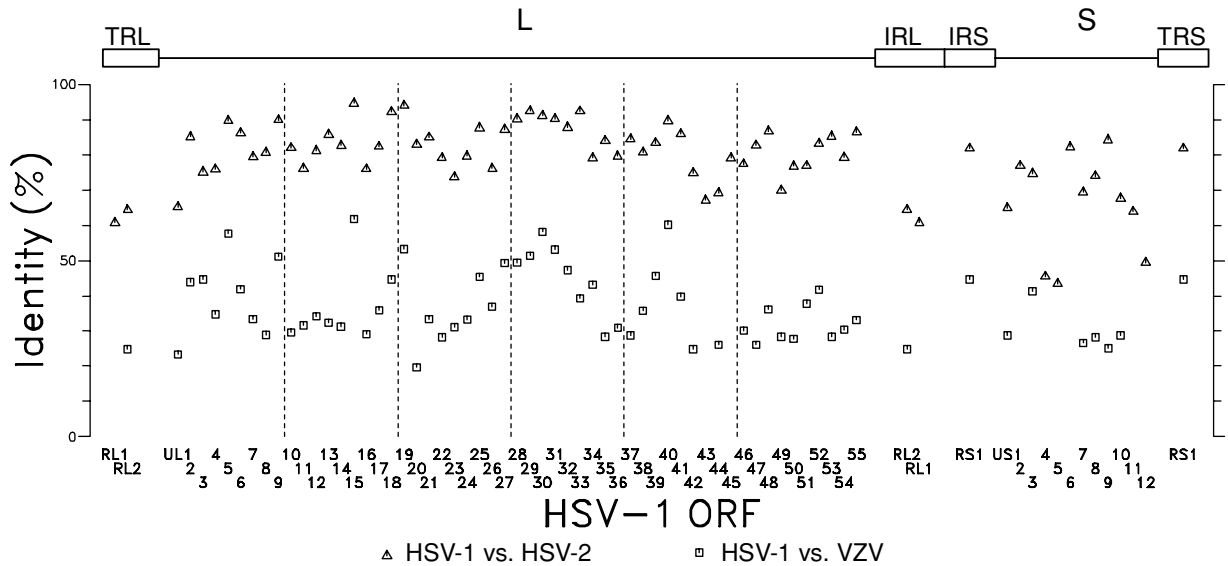

Fig. 5.4. Amino acid sequence identities between proteins encoded by the human alphaherpesviruses. Homologous protein sequences were aligned, and the percentage of identical residues determined in comparisons between HSV-1 and HSV-2 (triangles), and between HSV-1 and VZV (squares). Identity scores were plotted as a function of their location in the HSV-1 genome, relative to the major genomic architectural features. As detailed in the text, some HSV genes do not have identified homologues in VZV, and vice versa, and some genes are encoded within and in frame with others (and are thus not represented individually in this figure). Sequence sources and abbreviations are the same as for Fig. 5.1.

suggesting that they are considerably constrained by their respective functions. The most highly conserved genes between VZV and HSV-1 (HSV U_L 5, U_L 15, U_L 30, and U_L 40) are among the most highly conserved between HSV-1 and HSV-2 (Fig. 5.4). Their encoded proteins are enzymes involved in DNA replication and metabolism. This suggests that there is both little external pressure for these genes to change and little tolerance for accepting the products of random mutation. The least conserved genes between HSV-1 and HSV-2 are encoded in the S component and TRL/IRL; as detailed below, many of these genes do not have homologues between HSV and VZV. These gene products are likely to be important in defining the precise biological niche occupied by each virus.

VZV genes that are absent from HSV genomes

Although homologues of most long component genes exist in HSV-1, HSV-2 and VZV, there are some notable exceptions. Six VZV U_L genes have no homologue in the HSV U_L (ORF1, ORF2, ORF13, ORF32, ORF57 and ORF S/L). All of these genes are dispensable for replication in cell culture in at least some cell types.

ORF1 encodes an integral membrane protein of approximate M_r 17 000 with a C-terminal hydrophobic domain. Its function is not known, but it is completely dispensable for growth of the virus in MeWo cells (Cohen and Seidel, 1995). Equine herpesvirus 1 (EHV-1) and EHV-4 are both members of the genus *Varicellovirus* in the subfamily *Alphaherpesvirinae* (Telford *et al.*, 1992, 1995). Although these viruses encode a gene (termed EHV gene 2) in a position that corresponds to VZV ORF1, it has no obvious similarity with the VZV counterpart.

VZV ORF2 also encodes a phosphorylated membrane-associated protein that is dispensable for growth in cell culture and establishment of latency in dorsal root ganglia of cotton rats (Sato *et al.*, 2002). Genes with very limited homology with VZV ORF2 are present in other Varicelloviruses including gene 3 of EHV-1 and EHV-4. The functions of these genes are not known.

VZV ORF13 encodes thymidylate synthetase. Interestingly, homologues are not present in other alphaherpesviruses, but the genomes of several gammaherpesviruses, including human herpesvirus 8, herpesvirus saimiri, herpesvirus ateles, and equine herpesvirus 2 contain functional homologues. Moreover, human cytomegalovirus upregulates cellular thymidine synthetase (Gribaudo *et al.*, 2002). Thus, incorporation into the viral genome, or other means of upregulation of thymidylate synthetase in the host cell likely confers a selective advantage to these

viruses, possibly as it promotes availability of nucleotides for viral DNA replication in quiescent cells. Within the region corresponding to VZV ORF13, HSV-1 and HSV-2 encode U_L 45, which is encoded on the opposite strand and has no homologue in VZV. The U_L 45 gene encodes a type II membrane protein that is dispensable for replication in cell culture (Cockrell and Muggeridge 1998; Visalli and Brandt 1991).

VZV ORF32 is homologous to gene 34 of EHV-1 and EHV-4, but has no counterparts in other herpesvirus genera. The encoded protein of 16,000–18,000 M_r is posttranslationally modified by the ORF47 protein kinase and is dispensable for change replication in cell culture and the establishment of latency (Reddy *et al.*, 1998; Sato *et al.*, 2003).

VZV ORF57 is located in a region of unusually high diversity among the U_L segments of alphaherpesvirus genomes. VZV ORF57 has no sequence similarity to genes in herpesviridae for which DNA sequence data are available. In HSV, the corresponding region encodes no obvious open reading frame, and in fact has served as an insertion site for the expression of exogenous genes and bacterial artificial chromosome vector sequences without obvious detriment to the virus in cultured cells or animals (Baines and Roizman, 1991; Tanaka *et al.*, 2003). On the other hand, the region between U_L 3 and U_L 4 in the Suid herpesvirus 1 (Pseudorabies virus or PRV) genome encodes gene U_L 3.5 that is necessary for viral egress (Fuchs *et al.*, 1996). Like VZV ORF57, the PRV U_L 3.5 has no obvious homologue in any other herpesvirus.

ORF S/L is also unique to VZV. The encoded protein is located in the cytoplasm of infected cells and varies in size from about 21 000–30 000 apparent M_r among different VZV strains (Kemble *et al.*, 2000). The initiation codon of ORF S/L lies at the right end of TRS with translation crossing into the L component (this requires genome circularization or concatemerization). VZV ORF B is translated from within the same ORF, but initiates within the L component, terminating at the same stop codon as ORF S/L (Mahalingam *et al.*, 2000). The functions of either protein are unknown.

L component genes unique to the simplexviruses

Unless otherwise noted, it should be assumed that the following genes are encoded by HSV-1 and HSV-2, but not VZV.

The 248 residue ORF P lies in the repeats bordering U_L (TRL and IRL). The associated transcript is antisense to the RL1 gene encoding γ_1 34.5, and ends where the full length latency associated transcript (LAT) terminates. The gene is expressed primarily under conditions where other HSV genes, including those of the immediate early or alpha class, are not expressed (Lagunoff and Roizman, 1994). Deletion of an ICP4 binding site in the ORF P promoter augments expression, suggesting that ICP4 normally acts to repress ORF P (Lagunoff *et al.*, 1996). Because HSV alpha genes are not expressed during latent infection, it has been speculated that ORF P might be expressed preferentially in latently infected neurons, although this has not been demonstrated. The predicted HSV-2 counterpart of ORF P is 130 amino acids in length and has substantial similarity over the first 49 codons, after which the similarity drops off significantly at a site corresponding to an intron in HSV-2 RL1, which is encoded on the opposite strand (Dolan *et al.*, 1998). It has not been determined if the HSV-2 counterpart to ORF P is expressed.

ORF O of HSV-1 is shorter than, and entirely contained within, ORF P. The protein is regulated in a similar fashion to ORF P, and appears to be translated from the same inititation codon, but shifts frame by an unknown mechanism after codon 35 to a different reading frame, resulting in translation of a 20 000 apparent M_r protein. The protein can interact with ICP4 and preclude the latter's binding to DNA (Randall *et al.*, 1997). Similar to ORF P, the homology to an HSV-2 counterpart is only convincing at the extreme N-terminus and it is not known if the HSV-2 protein is expressed.

The γ_1 34.5 protein is encoded by RL1 in the repeat region, with the promoter located in the a sequence, and the open reading frame in the b sequence. The protein is a major determinant of neurovirulence and has a number of interesting functions including the recruitment of phosphatase alpha to dephosphorylate elongation initiation factor alpha and thus preserve translation of viral proteins, even in the presence of PKR (He *et al.*, 1997). The HSV-2 counterpart contains an intron, but is otherwise conserved. There is no obvious homologue in VZV or in herpes B virus (Perelygina *et al.*, 2003).

The U_L 8.5 gene is present in both HSV-1 and HSV-2. The gene product, designated OBPC, represents the C terminal 438 amino acids of the U_L 9 gene that encodes the origin binding protein (OBP). OBPC can bind origin sequences and can interfere with DNA replication in vitro (Baradaran *et al.*, 1996; Baradaran *et al.*, 1994).

The U_L 12.5 open reading frame is translated in frame with U_L 12 to yield a 60 000 M_r protein. It retains some of the nuclease activity of U_L 12, but lacks a nuclear localization signal at the N-terminus, perhaps explaining why U_L 12.5 cannot complement a U_L 12 null mutant. The precise function of U_L 12.5 is not known (Martinez *et al.*, 1996; Reuven *et al.*, 2004).

The U_L 15.5 open reading frame is present in VZV, but in contrast to the case with HSV-1, it is not known if the VZV protein is expressed. The open reading frame constitutes most of exon II of U_L 15 and the protein is in frame with U_L 15. The function is not known (Baines *et al.*, 1994, 1997; Yu *et al.*, 1997).

The U_L 20.5 open reading frame is located upstream of U_L 20. The gene product is expressed in infected cells and localizes in discrete sites in the nucleus. The open reading frame is not conserved in HSV-2 or VZV and the function is not known (Ward *et al.*, 2000).

U_L 27.5 is encoded in opposite sense to U_L 27, which encodes glycoprotein B. The 43 000 apparent M_r protein identified in infected cells is much smaller than the 575 codon open reading frame would predict. An HSV-2 protein of similar size that shares epitopes with the HSV-1 gene product is derived from an open reading frame of 985 codons (Chang *et al.*, 1998). The mechanism by which these large open reading frames lead to production of smaller than expected proteins is unknown.

U_L 43.5 is a 311 aa ORF that is encoded completely within U_L 43 coding sequences but is translated in opposite sense to that of U_L 43 (Ward *et al.*, 1996). The genomic region encoding these open reading frames is dispensable for viral growth in cell culture (MacLean *et al.*, 1991). The protein localizes in assemblons, discrete sites within infected cell nuclei that contain a variety of capsid and tegument proteins. The U_L 43.5 open reading frame is not conserved in HSV-2 (Dolan *et al.*, 1998).

The U_L 56 gene is unique to the simplexviruses of humans. The gene lies in a region that is necessary for a virulent phenotype of certain HSV-1 strains, but the gene itself does not contribute substantially to this virulence, at least in mice (Nash and Spivack, 1994). The gene product is associated with virions and is not essential for growth in cultured cells (Rosen-Wolff *et al.*, 1991).

The unspliced latency associated transcript or LAT is around 7–9 kbp in length, and this is extensively spliced. The introns of approximately 1.5 and 2.0 kbp are presumably very stable and accumulate to high levels in the nuclei of latently infected sensory neurons. Both the large and small transcripts are derived from transcriptional units within IRL and TRL and are transcribed in a sense opposite to that of RL1 (encoding ICP0) with which they overlap. Transcription through the repeat regions and antisense to ICP0 is a common theme among many alphaherpesviruses including bovine herpesvirus 1 and PRV, but these transcripts are not greatly similar to HSV LAT, other than in regions that overlap conserved open reading frames (Cheung, 1991; Rock *et al.*, 1987). More extensive discussions of the latency associated transcripts are included elsewhere in this volume.

S component genes unique to the human simplexviruses

The short components of the human alphaherpesviruses are considerably less well conserved than the long components (Figs. 5.1 to 5.4). Perhaps the most striking difference is that homologues of six HSV genes, including U_S 6 encoding the essential glycoprotein D, are not present in the VZV genome (Dolan *et al.*, 1998; McGeoch *et al.*, 1988). In addition, the order of the existing homologues of U_S is considerably rearranged (Davison and McGeoch, 1986).

The U_S1 gene of 420 codon encodes ICP22 whereas the $U_S1.5$ open reading frame of 273 codons is contained within the U_S 1 gene, and is translated in the same reading frame as ICP22 but from a different initiation codon (Carter and Roizman, 1996). A homologous, but highly diverged gene to that encoded by U_S 1.5 is present in HSV-2, but this lacks an obvious start codon. The VZV counterpart is ORF 63/70 (the gene is duplicated at the ends of the short component of VZV); the region of highest similarity with HSV-1 is limited to the C-terminus which includes US1.5.

The U_S2 gene of HSV is present in many other alphaherpesviruses, but is conspicuously absent from the VZV genome. The function of the gene is not known but the gene product is found in HSV-2 virions and can associate with cytokeratin 18 in vitro and in infected cells (Goshima *et al.*, 2001). U_S2 is not essential for growth in cell culture and the HSV-2 gene is dispensable for virulence in mice inoculated by the footpad route (Jiang *et al.*, 1998; Longnecker and Roizman, 1987). The homologous gene product of pseudorabies virus (PRV) is prenylated, a modification that changes its localization from association with membranes to punctate regions in the cytoplasm (Clase *et al.*, 2003). The gene product is associated with virions and this association may be regulated by postranslation modification, inasmuch as the virion-associated gene product is not prenylated.

The U_S 4 gene encodes glycoprotein G (gG). The gene is dispensable for growth in cell culture and differs significantly in sequence such that the HSV-2 gene is approximately 1500 bp larger (Ackermann *et al.*, 1986; Dolan *et al.*, 1998; Longnecker *et al.*, 1987; McGeoch *et al.*, 1988). This difference is exploited in serologic assays to distinguish HSV-2 specific antibodies from those induced by HSV-1

(Lee *et al.*, 1985). The function of gG is not known, but speculation that it has something to do with tropism unique to HSV-2 vs. HSV-1 seems warranted.

U_S5 encodes glycoprotein J (gJ) (Ghiasi *et al.*, 1998). gJ prevents apoptosis in cells infected with HSV-1 gD null mutants, and precludes apoptosis induced by granzyme B and Fas ligation, such as would be expected upon attack of an infected cell by cytotoxic T-lymphocytes (Jerome *et al.*, 2001; Zhou *et al.*, 2000). Interestingly, HSV-2 does not preclude apoptosis induced by ultraviolet radiation or Fas antibody, whereas HSV-1 blocks apoptosis through these stimuli, suggesting a difference in function of anti-apoptotic mechanisms mediated at least partly through gJ (Jerome *et al.*, 1999).

U_S6 encodes gD that is necessary for HSV entry and has been shown to bind a variety of proteinaceous viral receptors (Spear, 2004). The fact that a gene essential for entry of HSV is absent from the VZV genome suggests that the essential steps mediated by gD must be mediated by different VZV proteins. This issue is treated extensively in chapters dealing with herpesvirus entry.

$U_S8.5$ encodes a phosphoprotein that localizes to nucleoli (Georgopoulou *et al.*, 1995). The gene is dispensable for replication in cultured cells and the function is not known. Although $U_S8.5$ is a late gene, the mRNA is packaged into virions and presumably delivered to infected cells upon entry (Sciortino *et al.*, 2002).

The 161 codon U_S11 of HSV-1 is an RNA binding protein that is unique to the simplexviruses of humans. If expressed as an alpha gene, the protein can dephosphorylate EIF2α and thereby rescue the ability of a γ_1 34.5 deletion mutant to replicate in neuronal cell lines (Mohr and Gluzman, 1996; Roller *et al.*, 1996).

U_S12 encodes ICP47, an 88 amino acid immediate early protein. ICP47 can block the transporter associated with antigen transport (TAP) and thus preclude loading of antigenic peptides onto class 1 molecules in the ER (York *et al.*, 1994). Although ICP47 may be unique to human simplexviruses, it can be viewed as functionally conserved inasmuch as interference with antigen presentation is a function common to many viral proteins, including a number from both gamma- and betaherpesviruses (Vossen *et al.*, 2002).

In considering the information outlined herein, the authors would like to include a note of caution. While comparison of the sequences of the human alphaherpesviruses with one another and with other herpesviruses leads to a series of hypotheses regarding functions of individual genes, such an analysis is at once both potentially valuable and misleading. Fortunately, such hypotheses are experimentally testable in the context of the most relevant viral genome. It is anticipated that some surprises will result from these studies. Perhaps this is best illustrated by studies of herpesvirus glycoproteins, as various sequence homologues may have markedly different functions depending on the viral system studied. At the least, the preceding analyses of similarities in the genetic content of the human alphaherpesviruses should be considered with this caveat in mind.

REFERENCES

Ackermann, M., Longnecker, R., Roizman, B., and Pereira, L. (1986). Identification and gene location of a novel glycoprotein specified by herpes simplex virus 1. *Virology*, **150**, 207–220.

Baines, J. D. and Roizman, B. (1991). The open reading frames UL3, UL4, UL10 and UL16 are dispensable for the growth of herpes simplex virus 1 in cell culture. *J. Virol.*, **65**, 938–944.

Baines, J. D., Poon, A. P. W., Rovnak, J., and Roizman, B. (1994). The U_L 15 gene of herpes simplex virus encodes two proteins and is required for cleavage of viral DNA. *J. Virol.*, **68**, 8118–8124.

Baines, J. D., Cunningham, C., Nalwanga, D., and Davison, A. J. (1997). The U_L 15 gene of herpes simplex virus type 1 contains within its second exon a novel open reading frame that is translated in frame with the U_L 15 gene product. *J. Virol.*, **71**, 2666–2673.

Baradaran, K., Dabrowski, C. E., and Schaffer, P. A. (1994). Transcriptional analysis of the region of the herpes simplex virus type 1 genome containing the UL8, UL9, and UL10 genes and identification of a novel delayed-early gene product, OBPC. *J. Virol.*, **68**(7), 4251–4261.

Baradaran, K., Hardwicke, M. A., Dabrowski, C. E., and Schaffer, P. A. (1996). Properties of the novel herpes simplex virus type 1 origin binding protein, OBPC. *J. Virol.*, **70**(8), 5673–5679.

Carter, K. L. and Roizman, B. (1996). The promoter and transcriptional unit of a novel herpes simplex virus 1 alpha gene are contained in, and encode a protein in frame with, the open reading frame of the alpha 22 gene. *J. Virol.*, **70**(1), 172–178.

Chang, Y. E., Menotti, L., Filatov, F., Campadelli-Fiume, G., and Roizman, B. (1998). UL27.5 is a novel gamma2 gene antisense to the herpes simplex virus 1 gene encoding glycoprotein B. *J. Virol.*, **72**(7), 6056–6064.

Cheung, A. K. (1991). Cloning of the latency gene and the early protein 0 gene of pseudorabies virus. *J. Virol.*, **65**(10), 5260–5271.

Clase, A. C., Lyman, M. G., del Rio, T. *et al.* (2003). The pseudorabies virus Us2 protein, a virion tegument component, is prenylated in infected cells. *J. Virol.*, **77**, 12285–12298.

Cockrell, A. S. and Muggeridge, M. I. (1998). Herpes simplex virus 2 UL45 is a type II membrane protein. *J. Virol.*, **72**(5), 4430–4433.

Cohen, J. I. and Seidel, K. E. (1993). Generation of varicella-zoster virus (VZV) and viral mutants from cosmid DNAs: VZV thymidylate synthetase is not essential for replication in vitro. *Proc. Natl Acad. Sci. USA*, **90**(15), 7376–7380.

Cohen, J. I. and Seidel, K. E. (1995). Varicella-zoster virus open reading frame 1 encodes a membrane protein that is dispensable for growth of VZV in vitro. *Virology*, **206**(2), 835–842.

Cox, E., Reddy, S., Iofin, I., and Cohen, J. I. (1998). Varicella-zoster virus ORF57, unlike its pseudorabies virus UL3.5 homolog, is dispensable for viral replication in cell culture. *Virology*, **250**(1), 205–209.

Davison, A. J. and McGeoch, D. J. (1986). Evolutionary comparisons of the S segments in the genomes of herpes simplex virus type 1 and varicella-zoster virus. *J. Gen. Virol.*, **67**, 597–611.

Dolan, A., Jamieson, F. E., Cunningham, C., Barnett, B. C., and McGeoch, D. J. (1998). The genome of herpes simplex virus type 2. *J. Virol.*, **72**, 2010–2021.

Frenkel, N. and Roffman, E. (1996). Human Herpesvirus 7. In *Fields Virology*, 3rd edn, ed. B. N. Fields *et al.*, pp. 2609–2622. Philadelphia: Lippincott-Raven.

Fuchs, W., Klupp, B. G., Granzow, H., Rziha, H.-J., and Mettenleiter, T. C. (1996). Identification and characterization of the pseudorabies virus UL3.5 protein, which is involved in virus egress. *J. Virol.*, **70**, 3517–3527.

Georgopoulou, U., Kakkanas, A., Miriagou, V., Michaelidou, A., and Mavromara, P. (1995). Characterization of the US8.5 protein of herpes simplex virus. *Arch. Virol.*, **140**(12), 2227–2241.

Ghiasi, H., Nesburn, A. B., Cai, S., and Wechsler, S. L. (1998). The US5 open reading frame of herpes simplex virus type 1 does encode a glycoprotein (gJ). *Intervirology*, **41**(2–3), 91–97.

Goshima, F., Watanabe, D., Suzuki, H., Takakuwa, H., Yamada, H., and Nishiyama, Y. (2001). The US2 gene product of herpes simplex virus type 2 interacts with cytokeratin 18. *Arch. Virol.*, **146**(11), 2201–2209.

Gribaudo, G., Riera, L., Rudge, T. L., Caposio, P., Johnson, L. F., and Landolfo, S. (2002). Human cytomegalovirus infection induces cellular thymidylate synthase gene expression in quiescent fibroblasts. *J. Gen. Virol.*, **83**(Pt 12), 2983–2993.

He, B., Gross, M., and Roizman, B. (1997). The gamma(1)34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1 alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. *Proc. Natl Acad. Sci. USA*, **94**(3), 843–848.

Jerome, K. R., Fox, R., Chen, Z., Sears, A. E., Lee, H., and Corey, L. (1999). Herpes simplex virus inhibits apoptosis through the action of two genes, Us5 and Us3. *J. Virol.*, **73**(11), 8950–8957.

Jerome, K. R., Chen, Z., Lang, R. *et al.* (2001). HSV and glycoprotein J inhibit caspase activation and apoptosis induced by granzyme B or Fas. *J. Immunol.*, **167**(7), 3928–3935.

Jiang, Y. M., Yamada, H., Goshima, F. *et al.* (1998). Characterization of the herpes simplex virus type 2 (HSV-2) US2 gene product and a US2-deficient HSV-2 mutant. *J. Gen. Virol.*, **79**, 2777–2784.

Kemble, G. W., Annunziato, P., Lungu, O. *et al.* (2000). Open reading frame S/L of varicella-zoster virus encodes a cytoplasmic protein expressed in infected cells. *J. Virol.*, **74**(23), 11311–11321.

Lagunoff, M. and Roizman, B. (1994). Expression of a herpes simplex virus 1 open reading frame antisense to the gamma(1)34.5 gene and transcribed by an RNA 3 coterminal with the unspliced latency-associated transcript. *J. Virol.*, **68**, 6021–6028.

Lagunoff, M., Randall, G., and Roizman, B. (1996). Phenotypic properties of herpes simplex virus 1 containing a derepressed open reading frame P gene. *J. Virol.*, **70**(3), 1810–1817.

Lee, F. K., Coleman, R. M., Pereira, L., Bailey, P. D., Tatsuno, M., and Nahmias, A. J. (1985). Detection of herpes simplex virus type 2-specific antibody with glycoprotein G. *J. Clin. Microbiol.*, **22**(4), 641–644.

Longnecker, R. and Roizman, B. (1987). Clustering of genes dispensable for growth in culture in the S component of the HSV-1 genome. *Science*, **236**, 573–576.

Longnecker, R., Chatterjee, S., Whitley, R., and Roizman, B. (1987). Identification of a herpes simplex virus 1 glycoprotein gene within a gene cluster dispensable for growth in tissue culture. *Proc. Natl Acad. Sci. USA*, **84**, 4303–4307.

MacLean, C. A., Efstathiou, S., Elliott, M. L., Jamieson, F. E., and McGeoch, D. J. (1991). Investigation of herpes simplex virus type 1 genes encoding multiply inserted membrane proteins. *J. Gen. Virol.*, **72**, 897–906.

Mahalingam, R., White, T., Wellish, M., Gilden, D. H., Soike, K., and Gray, W. L. (2000). Sequence analysis of the leftward end of simian varicella virus (EcoRI-I fragment) reveals the presence of an 8-bp repeat flanking the unique long segment and an 881-bp open-reading frame that is absent in the varicella zoster virus genome. *Virology*, **274**(2), 420–428.

Martinez, R., Shao, L., Bronstein, J. C., Weber, P. C., and Weller, S. K. (1996). The product of a 1.9-kb mRNA which overlaps the HSV-1 alkaline nuclease gene (UL12) cannot relieve the growth defects of a null mutant. *Virology*, **215**(2), 152–164.

McGeoch, D. J., Dalrymple, M. A., Davison, A. J. (1988). The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. *J. Gen. Virol.*, **69**, 1531–1574.

Minson, A., Davidson, A., Eberle, R. *et al.* (2000). Family *herpesviridae*. In *Virus Taxonomy. Seventh report of the International Committee on Taxonomy of Viruses*, ed. M. Van Regenmortel *et al.*, pp. 203–225. New York/San Diego: Academic Press.

Mohr, I. and Gluzman, Y. (1996). A herpesvirus genetic element which affects translation in the absence of the viral GADD34 function. *Europ. Molec. Biol. Org. J.*, **15**(17), 4759–4766.

Nash, T. C. and Spivack, J. G. (1994). The UL55 and UL56 genes of herpes simplex virus type 1 are not required for viral replication, intraperitoneal virulence, or establishment of latency in mice. *Virology*, **204**(2), 794–798.

Pellett, P. E. and Roizman, B. (in press). The Herpesviridae: a brief introduction. In *Fields Virology*, 5th edn, ed. D. M. Knipe *et al.*, vol. 2, chapter 66, Philadelphia: Lippincott, Williams, & Wilkins.

Perelygina, L., Zhu, L., Zurkuhlen, H., Mills, R., Borodovsky, M., and Hilliard, J. K. (2003). Complete sequence and comparative analysis of the genome of herpes B virus (Cercopithecine herpesvirus 1) from a rhesus monkey. *J. Virol.*, **77**(11), 6167–6177.

Randall, G., Lagunoff, M., and Roizman, B. (1997). The product of ORF O located within the domain of herpes simplex virus 1 genome transcribed during latent infection binds to and inhibits in vitro binding of infected cell protein 4 to its cognate DNA site. *Proc. Natl Acad. Sci. USA*, **94**(19), 10379–10384.

Reddy, S. M., Cox, E., Iofin, I., Soong, W., and Cohen, J. I. (1998). Varicella-zoster virus (VZV) ORF32 encodes a phosphoprotein that is posttranslationally modified by the VZV ORF47 protein kinase. *J. Virol.*, **72**(10), 8083–8088.

Reuven, N. B., Antoku, S., and Weller, S. K. (2004). The UL12.5 gene product of herpes simplex virus type 1 exhibits nuclease and strand exchange activities but does not localize to the nucleus. *J. Virol.*, **78**(9), 4599–4608.

Rock, D. L., Beam, S. L., and Mayfield, J. E. (1987). Mapping bovine herpesvirus type 1 latency-related RNA in trigeminal ganglia of latently infected rabbits. *J. Virol.*, **61**(12), 3827–3831.

Roller, R. J., Monk, L. L., Stuart, D., and Roizman, B. (1996). Structure and function in the herpes simplex virus 1 RNA-binding protein U(s)11: mapping of the domain required for ribosomal and nucleolar association and RNA binding in vitro. *J. Virol.*, **70**, 2842–2851.

Rosen-Wolff, A., Lamade, W., Berkowitz, C., Becker, Y., and Darai, G. (1991). Elimination of UL56 gene by insertion of LacZ cassette between nucleotide position 116030 to 121753 of the herpes simplex virus type 1 genome abrogates intraperitoneal pathogenicity in tree shrews and mice. *Virus Res.*, **20**(3), 205–221.

Sato, H., Pesnicak, L., and Cohen, J. I. (2002). Varicella-zoster virus open reading frame 2 encodes a membrane phosphoprotein that is dispensable for viral replication and for establishment of latency. *J. Virol.*, **76**(7), 3575–3578.

Sato, H., Pesnicak, L., and Cohen, J. I. (2003). Varicella-zoster virus ORF47 protein kinase, which is required for replication in human T cells, and ORF66 protein kinase, which is expressed during latency, are dispensable for establishment of latency. *J. Virol.*, **77**(20), 11180–11185.

Sciortino, M. T., Taddeo, B., Poon, A. P., Mastino, A., and Roizman, B. (2002). Of the three tegument proteins that package mRNA in herpes simplex virions, one (VP22) transports the mRNA to uninfected cells for expression prior to viral infection. *Proc. Natl Acad. Sci. USA*, **99**(12), 8318–8323.

Spear, P. G. (2004). Herpes simplex virus: receptors and ligands for cell entry. *Cell Microbiol.*, **6**(5), 401–410.

Tanaka, M., Kagawa, H., Yamanashi, Y., Sata, T., and Kawaguchi, Y. (2003). Construction of an excisable bacterial artificial chromosome containing a full-length infectious clone of herpes simplex virus type 1: viruses reconstituted from the clone exhibit wild-type properties in vitro and in vivo. *J. Virol.*, **77**(2), 1382–1391.

Telford, E. A., Watson, M. S., McBride, K., and Davison, A. J. (1992). The DNA sequence of equine herpesvirus-1. *Virology*, **189**(1), 304–316.

Telford, E. A., Watson, M. S., Aird, H. C., Perry, J., and Davison, A. J. (1995). The DNA sequence of equine herpesvirus 2. *J. Mol. Biol.*, **249**(3), 520–528.

Visalli, R. J. and Brandt, C. R. (1991). The HSV-1 UL45 gene product is not required for growth in Vero cells. *Virology*, **185**(1), 419–423.

Vossen, M. T., Westerhout, E. M., Soderberg-Naucler, C., and Wiertz, E. J. (2002). Viral immune evasion: a masterpiece of evolution. *Immunogenetics*, **54**(8), 527–542.

Ward, P. L., Barker, D. E., and Roizman, B. (1996). A novel herpes simplex virus 1 gene, U_L 43.5, maps antisense to the U_L 43 gene and encodes a protein which colocalizes in nuclear structures with capsid proteins. *J. Virol.*, **70**, 2684–2690.

Ward, P. L., Taddeo, B., Markovitz, N. S., and Roizman, B. (2000). Identification of a novel expressed open reading frame situated between genes $U_{(L)}$20 and $U_{(L)}$21 of the herpes simplex virus 1 genome. *Virology*, **266**(2), 275–285.

York, I. A., Roop, C., Andrews, D. W., Riddell, S. R., Graham, F. L., and Johnson, D. C. (1994). A cytosolic herpes simpelx virus protein inhibits antigen presentation to CD8+ T lymphocytes. *Cell*, **77**, 525–535.

Zhou, G., Galvan, V., Campadelli-Fiume, G., and Roizman, B. (2000). Glycoprotein D or J delivered in *trans* blocks apoptosis in SK-N-SH cells induced by a herpes simplex virus 1 mutant lacking intact genes expressing both glycoproteins. *J. Virol.*, **74**(24), 11782–11791.

6

Alphaherpes viral genes and their functions

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Introduction

In this chapter the emphasis is on viral replication and on the viral gene products that define the outcome of the interaction of the alphaherpesviruses with their host. Viral replicative and host management functions account for some of the RNAs and a large number of proteins encoded by the viruses. There are, however, numerous viral gene products whose functions have not been identified or which do not play a prominent role in viral replication in the systems in which these have been tested. The objective of the table contained in this section is to summarize the functions of all known gene products and provide at least a few references for each product. It should be noted however that: of the three human alphaherpesviruses, we know more about the functions of herpes simplex virus-1 and -2 (HSV-1 and HSV-2) genes than about those of varicella zoster virus (VZV). We have identified in this table the VZV genes that are related to HSV by amino acid sequence homology. We note that partial sequence conservation does not necessarily mean that the homologous HSV and VZV gene products perform identical functions.

The list understates both the number of the products and their functions. The problem is twofold. The HSV genome encodes a large number of open reading frames (ORFs) with 50 or more codons and not all of the ORFs have been probed for to determine whether they are expressed. In addition, standard annotations exclude ORFs that are antisense to known ORFs or that do not have TATA boxes or other motifs that indicate that they encode proteins. HSV encodes several proteins whose ORFs are antisense to each other. An additional problem is that transcripts arising late in infection frequently do not terminate at predicted termination signals. In addition, several viral RNAs either do not encode a protein or the protein is made in undetectable amounts. In several instances, ORFs contain within their domain a transcriptional unit encoding proteins identical to the C terminal domain of the protein encoded by the larger ORF. In essence, we do not know the absolute number of transcripts or proteins encoded by the viral genome.

The third limitation of the table stems from the observation that virtually all viral proteins studied in detail appear to perform multiple functions. Not all of the functions are known and, in some instances, the table lists the most prominent functions of the gene product.

We further note that (i) some alphaherpesvirus genes are found only in HSV-1 (ORF-O and ORF-P), some in HSV-1 and HSV-2 ($\gamma_1$34.5), some in HSV-1, HSV-2 and B virus (the simplexviruses), some in the simplexviruses plus pseudorabies virus, and some are in all of the above, plus VZV. This is annotated in the "Conservation" column. (ii) Some genes have positional homologues (conserved size, orientation, and conserved surrounding genes) for which no functional data are available. We included positional homologues, in cases in which good candidates are present in members of all three herpesviruses subfamilies.

Gene designation	Alternative name	Main properties	Conservation[b]	Gene Block[c]	Homologues[d]				Ref.
					VZV	γ-HV	HCMV	HHV-6	
$\gamma_1$34.5		This ORF encodes a 248-residue γ_1 protein consisting of two unequal domains linked by a variable number of alanine-proline-threonione repeats. The C-terminal domain is homologous to the C-terminal domain of GADD34 and functions as a phosphatase accessory factor which binds phosphatase 1α and redirects it to dephosphorylate the α subunit of the translation initiation factor 2 (eIF-2). In the absence of $\gamma_1$34.5 gene, protein kinase R is activated, eIF-2α is phosphorylated and all protein synthesis ceases. The loss of this function is responsible for the loss of the capacity of mutants to replicate in experimental animal systems (loss of neurovirulence). $\gamma_1$34.5 protein is also involved in the evasion of MHC class 2 responses and encodes other, as yet poorly defined functions. γ_1.[a]	HSV-2, Gadd34 (MyD116)						(100, 101, 230)
ORF-P		A 233-residue ORF encoded on the strand complementary to $\gamma_1$34.5. The transcription of ORF-P is blocked by ICP4 bound to a high affinity transcription initiation site of the ORF. Derepression of ORF-P results in decreased expression of the $\gamma_1$34.5 ORF. ORF-P protein binds p32 and localizes in spliceosomes. Overexpression of ORF-P results in decreased expression of viral products of spliced RNAs. Pre-α.	not in HSV-2 or B virus						(128, 129)
ORF-O		The product of ORF-O is made of 117 residues. The amino terminus is identical to that of ORF-P. The amino acid sequence beyond residue 35 is in an alternate reading frame. Expression of ORF-O is repressed by ICP4. In vitro ORF-O blocks the binding of ICP4 to its cognate high affinity DNA binding sites. Viral gene products encoded by these ORFs have not been detected in murine ganglia harboring latent virus. Pre-α.	not in HSV-2 or B virus						(198)
α0	ICP0	The 3 exons encode a 775-residue protein containing a RING finger domain. ICP0 is dispensable for viral replication in cultured cells. In transfected cells it acts as a promiscuous transactivator of genes introduced by transfection or infection although activation of resident genes has been reported. ICP0 expresses multiple functions, including those of a double ubiquitin ligase targeting promyelocytic leukemia protein to block exogenous interferon, cdc34 to block turnover of cyclin D3, CNP-C, CNP-A, and DNA dependent protein kinase. ICP0 is also associated with the restructuring of chromatin. Additional functions have been described. α	α-HV		ORF61				(99, 171, 222)
U_L1	gL	224 residue soluble glycoprotein. It interacts with and serves as the chaperone of gH. In its absence gH is not transported to plasma membrane and is not fully glycosylated. Essential glycoprotein for virion infectivity and cell-cell fusion. Locus of syncytial mutation. Stimulates neutralizing antibody. γ_1.	α-HV, β-HV, γ-HV	7	ORF60	ORF47	UL115	U82	(78, 106, 203)

(*cont.*)

Gene designation	Alternative name	Main properties	Conservation[b]	Gene Block[c]	Homologues[d]				Ref.
					VZV	γ-HV	HCMV	HHV-6	
U_L2		U_L2 encodes uracil-DNA glycosylase, a highly conserved enzyme associated with the base excision repair pathway. Uracil-DNA glycosylase replaces uracil in G:U base pairs resulting from deamination of cytosine residues in DNA. U_L2 is highly conserved and no other functions have been ascribed it. 334 aa. β or γ_1.	α-HV, β-HV, γ-HV.	7	ORF59	ORF46	UL114	U81	(41, 244)
U_L3		U_L3 is a 235 residue phosphoprotein forming multiple bands in denaturing polyacrylamide gels due in part to phosphorylation mediated by U_L13 protein kinase. It co-localizes with ICP22-$U_S1.5$ proteins in small dense nuclear structures. In the absence of ICP22 or $U_S1.5$ it is diffuse throughout the nucleus. The function is unknown: the gene can be deleted without impairment of viral replication in cultured cells. The protein is transcribed predominantly from the second methionine of its reading frame. γ_2	α-HV Possible positional homologue in γ-HV		ORF58	ORF45			(10, 152, 245)
U_L4		U_L4 is a 199-residue virion protein. In infected cells it localizes in small dense nuclear structures formed prior to the onset of viral DNA synthesis together with ICP22/$U_S1.5$. In the absence of other viral proteins it remains in the cytoplasm. U_L4 and is dispensable for viral replication in cultured cells. γ_2	α-HV		ORF56				(110, 113, 249)
U_L5		U_L5 is a component of the helicase – primase complex. The 882-residue protein contains sequence motifs shared by members of the superfamily of RNA and DNA helicases ranging from bacteria to mammals. Stable U_L5-U_L52, complex has DNA-dependent ATPase and GTPase, DNA primase and DNA helicase activities. A viral mutant in U_L5 exhibiting a neuron specific restriction was shown to produce lower levels of viral DNA in restricted cells. β.	α-HV, β-HV, γ-HV	6	ORF55	ORF44	UL105	U77	(28, 76)
U_L6		The 676-residue protein forms a dodecameric ring located at the vertices of the HSV capsid. The ring structure is similar to the portal for the entry of DNA in bacteriophages. U_L6 has been reported interact with U_L15 and U_L28 (the putative terminase) proteins. Resistance of a class of thiourea drugs that blocks cleavage and packaging of DNA was mapped to U_L6 ORF. Unknown.	α-HV, β-HV, γ-HV	6	ORF54	ORF43	UL104	U76	(176, 232, 242)
U_L7		The 296-residue protein is well conserved among herpesvirus families and may be a component of the tegument. Its function is not known. It is not essential for replication in cell culture. γ_1.	α-HV, β-HV, γ-HV	6	ORF53	ORF42	UL103	U75	(181)
U_L8		The 750-residue U_L8 protein is required for the transport of U_L52 and U_L5 to the nucleus. It interacts with both U_L9 and U_L30. One likely function of U_L8 protein is to facilitate the synthesis of RNA primers on the DNA template. β.	α-HV, β-HV, γ-HV	6	ORF52	ORF41	UL102	U74	(18, 40, 153, 159, 229)

(*cont.*)

Gene designation	Alternative name	Main properties	Conservation[b]	Gene Block[c]	Homologues[d]				Ref.
					VZV	γ-HV	HCMV	HHV-6	
U_L8.5		The 487-residue U_L8.5 protein corresponds to the C-terminal domain of U_L9 protein. U_L8.5 was reported to be synthesized both early and late in infection and to bind to the origin of DNA synthesis. In transient assays the U_L8.5 protein inhibited DNA synthesis. It may play a role in shifting the pattern of synthesis for de novo initiation at origins to a rolling circle model of viral DNA synthesis. γ_1.	α-HV						(15, 16)
U_L9		U_L9, a 851-residue protein, binds to two specific sites flanking the origin of viral DNA synthesis. ICP8 (U_L29) which binds and separates the DNA strands, enables the unwinding of the DNA at the site of the origin by U_L9 protein and allows the entry of the machinery that replicates the DNA. U_L9 is conserved in α-herpesviruses and except for HHV6 and HHV7 it is absent from the genomes of β- or γ-herpesviruses. The function of U_L9 after the initiation of viral DNA synthesis has not been demonstrated. U_L9 protein was reported to interact with two proteins. hTid-1 enhances the binding of a multimer of U_L9 to the origin of DNA synthesis. NFB42, a F box component of the SCF ubiquitin ligase, binds phosphorylated U_L9 protein and targets it for ubiquitin-proteasomal degradation. The phosphorylation and degradation of U_L9 may be a mechanism by which the virus favors a rolling circle type of DNA replication instead of de novo initiation of DNA synthesis at the origins or replication. β.	α-HV, Roseolo-viruses	6	ORF51			U73	(50, 81, 126, 132, 148, 224, 238)
U_L9.5		The transcript of U_L9.5 is coterminal with those of U_L8, U_L8.5, and U_L9. 472 aa. γ_2							(16)
U_L10	gM	Abundant virion glycoprotein of 473 residues, with apparent M_r 53–63,000. Topology predicts six to eight transmembrane segments. A deletion mutant virus has no major phenotype. In other herpesviruses, gM forms a complex with gN, or gN-gO. Based on phenotype of ΔgM-gE-gI PrV and ΔgM EBV viral mutants, gM is thought to play a role in virion maturation and exocytosis, but this function may be redundant. However, a ΔgMgE HSV mutant shows no defect. $\gamma_.$	α-HV, β-HV, γ-HV	6	ORF50	ORF39	UL100	U72	(11, 35)
U_L11		U_L11 is a 96-residue tegument protein that is both myristoylated and palmitoylated. ΔU_L11 mutants exhibit reduced levels of envelopment and egress of virus from infected cells. The protein binds the cytoplasmic face of cellular membranes and is particularly abundant in Golgi. U_L11 protein interacts with and directs U_L16 to the Golgi. The precise function of U_L11 is not known. $\gamma_.$	α-HV, β-HV, γ-HV	6	ORF49	ORF38	UL99	U71	(12, 140, 141, 143, 144)
U_L12	Alkaline nuclease	U_L12 is a 626-residue alkaline nuclease that functions as a resolvase, an enzyme required for processing of replication intermediates-structures defined by their inability to enter pulse field gels and that would interfere with the packaging of viral DNA into capsids. β.	α-HV, β-HV, γ-HV	6	ORF48	ORF37	UL98	U70	(93, 154, 241)

(*cont.*)

Gene designation	Alternative name	Main properties	Conservation[b]	Gene Block[c]	Homologues[d]				Ref.
					VZV	γ-HV	HCMV	HHV-6	
$U_L12.5$	No VZV	The ORF encodes a 500-residue product from a 1.9 kb RNA and corresponds to the C-terminal portion of the U_L12 alkaline nuclease. The protein does not have nuclease activity, does not complement U_L12-null mutants and does not appear to be a structural component of capsids. Its function is not known. Unknown							(154)
U_L13	Protein kinase	U_L13 encodes a 518-residue (M_r 56,000) serine/tyrosine protein kinase for viral and cellular proteins. Its function as a tegument protein is unknown. The gene is essential for viral replication in experimental animal systems but not in cells in culture. U_L13 appears to regulate multifunctional proteins. The substrate specificity of U_L13 protein kinase is similar to that of the mitotic cyclin dependent kinase cdc2. (Y. Kawaguchi, personal communication). γ_1.	α-HV, β-HV, γ-HV	6	ORF47	ORF36	UL97	U69	(25, 67, 116, 117, 196, 217)
U_L14		The ORF encodes a tegument protein of 219 residues. ΔU_L14 mutants replicate well in cell culture but are highly attenuated in the mouse. The only function attributed to U_L14 protein is enhancement of nuclear localization of U_L17 and U_L26. Co-expression of U_L14 enhances nuclear import of the U_L35 (VP26) and U_L33 proteins and increases luciferase expression suggesting that it may facilitate folding of a variety of proteins. γ_2.	α-HV, β-HV, γ-HV	6	ORF46	ORF34	UL96	U67	(65, 250)
U_L15		The ORF consists of two exons. The intron encodes a sequence antisense to U_L16 and U_L17 proteins. U_L15 is a 735-residue (M_r of 83,000) protein that binds U_L28 protein. U_L15 is required for cleavage and packaging of viral DNA and with U_L28 may function as a terminase. U_L15 is cleaved near the amino terminus, a reaction coupled with maturation of viral DNA into unit length molecules. γ_1.	α-HV, β-HV, γ-HV	6	ORF 42/45	ORF29a	UL89ex1	U66 ex1	(9, 64, 206, 208)
$U_L15.5$		$U_L15.5$ Exon 2 or U_L15 encodes a M_r 55,000 protein associated with capsids. 293 aa. Unknown	α-HV, β-HV, γ-HV	6		ORF29b	UL89ex2	U66ex2	(9)
U_L16		The 373-residue U_L16 protein is encoded in the intron located between exon 1 and 2 of U_L15. It is a virion component not essential for viral replication. γ_1.	α-HV, β-HV, γ-HV <positional>	6	ORF44	ORF33	UL94	U65	(174, 208)
UL17		The U_L17 ORF is contained in the intron located between exon 1 and 2 of U_L15, and encodes a 703-residue protein essential for DNA cleavage and packaging. In productive infection U_L17 localizes preformed capsid to the replication compartment. U_L17 has also been reported to be a tegument protein. γ_1.	α-HV, β-HV, γ-HV <positional>	6	ORF43	ORF32	UL93	U64	(206, 207, 227)
U_L18	VP23	The 318-residue (M_r 34,000) protein encoded by U_L18 is a capsid protein designated VP23. Together with the VP19C encoded by U_L38 it forms triplexes consisting of two copies of VP23 and one copy of VP19C. The 320 triplexes connect adjacent hexons (150) and pentons (12). γ_1.	α-HV, β-HV, γ-HV	5	ORF41	ORF26	UL85	U56	(124, 231)

(*cont.*)

Gene designation	Alternative name	Main properties	Conservation[b]	Gene Block[c]	Homologues[d]				Ref.
					VZV	γ-HV	HCMV	HHV-6	
U_L19	ICP5	Major capsid protein made of 1311 residues (M_r 149,000). Capsid hexons appear as tower like complexes containing 6 copies of the protein whereas pentons contain 5 copies. γ_1.	α-HV, β-HV, γ-HV	5	ORF40	ORF25	UL86	U57	(32, 175, 177, 243)
U_L20		U_L20 is a 222 residue polytopic membrane protein, with four predicted transmembrane segments. It localizes to virions, nuclear membranes and Golgi but is absent from plasma membranes. When expressed singly, it localizes mainly to endoplasmic reticulum. Coexpressed with gK, It localizes to the Golgi. Dispensable in cell culture. A deletion mutant in UL20 gene is detective in transport of the virions out of the perinuclear space, particularly in cells with fragmented Golgi. The ΔUL20 mutant is syncytial. Together with gK, inhibits cell-cell fusion. Its postulated role is to prevent fusion of the infected cells with adjacent cells. γ.	α-HV		ORF39				(5, 6, 13, 86)
$U_L20.5$		The $U_L20.5$ ORF is located 5′ to U_L20. The 160-residue protein localizes at small dense nuclear structures containing ICP22, $U_S1.5$, U_L3, and U_L4 proteins and is dispensable for viral replication. Its function is not known. γ_2.	None						(237)
U_L21		The 535-residue U_L21 protein is in tegument and may be weakly associated with capsids. U_L21 binds to microtubules but not to purified tubulin. In overexpressed cells it induces the formation of long cytoplasmic projections possibly due to its interaction with microtubules. It is not required for viral replication in cultured cells. γ_1.	α-HV		ORF38				(8, 226)
U_L22	gH	The 838-residues glycoprotein is essential for virion infectivity and cell-cell fusion. In cells infected with a deletion mutant virus in gH or a *ts* mutant at non permissive temperature, viruses lacking gH egress from the cell but are non infectious. Transport of gH from the endoplasmic reticulum to the Golgi and plasma membranes requires the interaction with the soluble gL. gH is an essential component of the complex enabling cell-cell fusion and induces neutralizing antibodies. VZV gH carries an endocytosis motif in the C-tail, absent from HSV gH. γ. Carries elements typical of viral fusion proteins. Possible fusogen.	α-HV, β-HV, γ-HV	4	ORF37	ORF22	UL75	U48	(36, 73, 79, 85, 94, 184)
U_L23	Thymidine kinase	Although known primarily as a thymidine kinase, it is a wide spectrum nucleoside kinase capable of phosphorylating both purine and pyrimidine nucleosides and their analogues. 376 aa. β.	α-HV, γ-HV		ORF36	ORF21			(63, 89, 158, 170)
U_L24		A 269-residue membrane-associated nuclear protein with an apparent M_r of 30,000. Mutations or deletions result in formation of small polykaryocytes in cell culture, somewhat diminished virus yields and decreased virulence in mice. The bulk of the protein is translated from the first AUG. A shorter transcript containing the second in frame AUG is also translated but the translation of 2 additional 3' co-terminal transcripts encoding C-terminal portions of U_L24 protein is uncertain. The UL24 ORF partly overlaps the TK gene in antisense direction. UL24 is dispensable in cell culture. γ_1.	α-HV, β-HV, γ-HV	4	ORF35	ORF20	UL76	U49	(185)

(*cont.*)

Gene designation	Alternative name	Main properties	Conservation[b]	Gene Block[c]	Homologues[d] VZV	γ-HV	HCMV	HHV-6	Ref.
UL25		The 580 residue product of the U_L25 ORF encodes a capsid protein involved in packaging of viral DNA into capsids. Dispensable for generation of the S terminus but required for correct generation of the L-terminus and possibly for retention of DNA in the capsid. γ_2.	α-HV, β-HV, γ-HV	4	ORF34	ORF19	UL77	U50	(161, 223)
U_L26	capsid scaffolding protein and protease	This ORF encodes a protein of 635 residues that is cleaved in *cis*- or *trans*- by itself at two sites. The N-terminal polypeptide is a protease required for assembly of a scaffolding within capsids for DNA packaging. The larger, middle cleavage product is a component of the scaffolding. γ.	α-HV, β-HV, γ-HV	4	ORF33	ORF17	UL80	U53	(90, 136, 137)
$U_L26.5$		The promoter and coding domain of $U_L26.5$ is contained in its entirety within the U_L26 ORF. The protein product of 328 residues is identical to the corresponding sequence of the U_L26 protein. It is cleaved by the protease at its C-terminus at the same site as the U_L26 protein. γ.	α-HV	4	ORF 33.5			U53.5	(137)
U_L27	gB	A 904-residue glycoprotein with apparent M_r of 110.000. It carries two, or three trasmembrane segments. Essential for virion infectivity and cell-cell fusion. Virions lacking gB egress the cell but are non infectious. The protein binds heparan sulfate and is a component of the cell-cell fusion complex. The cytoplasmic tail carries syncytial mutations, and endocytosis motifs, which mediate gB endocytosis into large vacuoles. Down modulation of gB at cell surfaces may be responsible for negative control of the cell-cell fusion. A *ts* mutation in ectodomain affects virus entry into the cell (*ts* B5). Induces neutralizing antibodies. γ_1. Crystal structure shows a trimer. Possible fusogen.	α-HV, β-HV, γ-HV	2	ORF31	ORF8	UL55	U39	(37–39, 109, 151, 186)
$U_L27.5$		The $U_L27.5$ ORF maps antisense to U_L27 and yields a γ_2 protein with an apparent M_r of 43,000. The predicted size of the coding capacity of the ORF is much larger. The protein accumulates in the cytoplasm. Its function is unknown. 575 aa. γ_2.	HSV-2						(46)
U_L28		A component of cleavage and packaging complex. See Functions of U_L15 and U_L6. 785 aa. Binds *pac1* sequences and is required for correct generation of the L-terminus. See functions of U_L6, U_L14 and U_L33. γ.	α-HV, β-HV, γ-HV	2	ORF30	ORF7	UL56	U40	(23)
U_L29	ICP8, single-stranded DNA binding protein	ICP8 is an essential 1196 residue single stranded DNA binding protein made very early in infection. ICP8 is required for viral DNA synthesis. β.	α-HV, β-HV, γ-HV	2	ORF29	ORF6	UL57	U41	(150, 253)
U_L30	DNA pol.	DNA polymerase. 1235 aa. γ.	α-HV, β-HV, γ-HV	2	ORF28	ORF9	UL54	U38	(29, 114, 234, 239, 240)
U_L31		Nuclear phosphoprotein that requires U_L34 protein for localization at the inner nuclear membrane. Binds lamin A/C in the nuclear lamina and directs envelopment at inner nuclear membrane. Carries a nucleotidylation recognition sequence. 306 aa. γ.	α-HV, β-HV, γ-HV	1	ORF27	ORF69	UL53	U37	(27, 48, 49, 147, 200, 201)
U_L32		U_L32 is 596-residue protein required for cleavage and packaging of viral DNA. The predominant localization of U_L32 in the cytoplasm suggests that either it shuttles between the nucleus and cytoplasm, or that it encodes additional functions. γ_2.	α-HV, β-HV, γ-HV	1	ORF26	ORF68	UL52	U36	(47, 130)

(*cont.*)

Gene designation	Alternative name	Main properties	Conservation[b]	Gene Block[c]	Homologues[d]				Ref.
					VZV	γ-HV	HCMV	HHV-6	
U_L33		A 130-residue capsid associated protein that forms a complex with the U_L15 and U_L28 proteins. Required for packaging of viral DNA into preformed capsids. γ_2.	α-HV, β-HV, γ-HV	1	ORF25	ORF67A	UL51	U35	(22, 199)
U_L34		An essential membrane-associated virion protein of 275 residues was reported to be a type 2 membrane protein phosphorylated by U_S3 kinase. Required for virion envelopment at inner nuclear membrane. Exclusive localization of UL34 protein at the nuclear membrane requires U_L31 protein. Extracellular virions do not contain UL31-UL34 proteins. The U_L34 homologue in murine cytomegalovirus recruits cellular protein kinase C to the nuclear lamina and induces lamin phosphorylation. γ_1.	α-HV, β-HV, γ-HV	1	ORF24	ORF67	UL50	U34	(200, 201, 215)
U_L35	VP26, capsid protein NC7	The 112-residue (M_r 12,000) protein forms a hexameric structure located on the outer surface of each hexon. Previously know as NC7. Dispensable for growth in cell culture but not in vivo. γ_2.	α-HV, β-HV, γ-HV	1	ORF23	ORF65	UL49	U32	(57, 71)
U_L36	VP1/2	The 3165-residue (M_r 270,000) protein essential for viral replication is located in the tegument essential for egress of virions through the cytoplasm. A *ts* mutation in UL36 blocks release of viral DNA into the nucleus at the non permissive temperature. γ_2.	α-HV, β-HV, γ-HV	1	ORF22	ORF64	UL48	U31	(20, 72)
U_L37	tegument protein	A 1123-residue (M_r 120,000) tegument phosphoprotein binds to DNA in the presence of ICP8. It is essential for viral replication. In its absence, nucleocapsids accumulate aberrantly in the nucleus and unenveloped capsids accumulate in the cytoplasm. γ_1.	α-HV, β-HV, γ-HV	1	ORF21	ORF63	UL47	U30	(146, 213, 247)
U_L38	VP19C Capsid protein	This 465-residue protein forms triplexes together with VP23, consisting of two copies of VP23 and one copy of VP19C. The 320 triplexes connect adjacent hexons (150) and pentons. γ_2.	α-HV	1	ORF20	ORF62	UL46	U29	(219, 231)
U_L39	Ribonucleotide reductase, large subunit	This ORF encodes a 1137-residue protein of M_r of 136,000. The protein is anchored in membranes and has protein kinase activity mapping to the N terminus but not required for ribonucleotide kinase activity. U_L39 may play a role in maintaining dTTP pools in infected cells. β.	α-HV, β-HV, γ-HV	1	ORF19	ORF61	UL45	U28	(54, 68, 189)
U_L40	Ribonucleotide-reductase small subunit	This ORF encodes the 340-residue small subunit of ribonucleotide kinase. β.	α-HV, γ-HV		ORF18	ORF60			(108)
U_L41	vhs, virion host shutoff protein	*vhs* (virion host shutoff protein) is a 489-residue protein packaged in the tegument and mediates the degradation of RNA early in infection. The degradation appears to be selective for viral RNAs. At late times after infection it is associated with VP16 (product of U_L48) and no longer exhibits this function. γ_1.	α-HV		ORF17				(82, 83, 211, 216, 225)
U_L42		DNA polymerase accessory protein. Binds double stranded DNA. It also associates with cdc2 and topoisomerase IIa. 488 aa. β.	α-HV, β-HV, γ-HV	1	ORF16	ORF59	UL44	U27	(3, 96)
U_L43		Non-essential protein. Sequence suggests that it is a hydrophobic, myristylated integral membrane protein. 434 residues. γ.	α-HV		ORF15				(44, 144, 145)

(*cont.*)

Gene designation	Alternative name	Main properties	Conservation[b]	Gene Block[c]	Homologues[d]				Ref.
					VZV	γ-HV	HCMV	HHV-6	
U_L43.5		This ORF is located antisense to U_L43. Tagged epitope revealed the synthesis of a M_r 32,000 γ_2 protein, dispensable for growth in cell culture. Its function is not known. γ.	HSV-2						(44, 236)
U_L44	gC	A 511 residue glycoprotein heavily N- and O-glycosylated. Some gC-minus mutants arise in culture, but all primary isolates express gC. A deletion gC mutant attaches to cells with reduced efficiency, but is viable. gC mediates attachment of virions to glycosaminoglycans of heparan sulphate, or chondroitin sulphate. The heparan sulphate binding site on gC maps to the N-terminus of the ectodomain. gC is part of the immune evasion strategy of HSV, as it carries two domains involved in modulating complement activation; one binds C3, and the other is required for blocking C5 and properdin binding to C3. Each region contributes to virulence, as viruses lacking these domains are less virulent than wt-virus. γ_2.	α-HV		ORF14				(88, 103, 122, 142)
U_L45		The 172 residue type 2 membrane protein is dispensable for viral replication in cultured cells. U_L45 is required for herpes simplex virus type 1 glycoprotein B-induced fusion. It is dispensable for growth in cell culture. γ_2.	HSV-2, B virus						(98, 233)
U_L46	VP11/12 tegument protein,	The ORF encodes the 718-residue tegument proteins VP11 and VP12, differentiated solely by their migration in denaturing gels. The ORF is dispensable in cultured cells. Available data suggests that it is a γ_1 protein present along with U_L47 in stechiometric amounts with U_L48 gene product and capable of enhancing its activity. In the absence of U_L48 protein, U_L46 protein inhibits activation of α promoters. γ.	α-HV		ORF12				(157, 252)
U_L47	VP13/14 tegument protein	The two products of the ORF have apparent M_r of 82000 and 81000. They are abundant glycosylated, phosphorylated components of the tegument, dispensable for viral replication in cells in culture. U_L47 binds RNA and is reported to shuttle between the cytoplasm and nucleus. 693 aa. γ_1.	α-HV		ORF11				(77, 123, 164)
U_L48	α-TIF, VP16, ICP25	α-TIF (α-trans-inducing factor), a 491 residue protein, is a multifunctional tegument protein. It induces α-genes by interacting with two cellular proteins, HCF and Oct-1. The complex binds to specific sequences with the consensus GyATGnTAATGArATTCyTTGnGGG-NC. It is also required for virion assembly. Essential for growth in cell culture and has gamma expression regulation. Crystal structure of the conserved core has been solved. γ.	α-HV		ORF10				(59, 138, 172, 187)
U_L49	VP22	Encodes the nonessential tegument protein VP22. VP22 translocates into cells exposed to the protein. Prior to cell division it localizes to microtubules in the cytoplasm. After cell division it is bound to chromatin. VP22 binds RNA and is thought to translocate mRNA from infected to uninfected cells. It has also been reported to bind membranes, and to induce the stabilization and hyperacetylation of microtubules (putative microtubule-associated protein). It is not required for tegument assembly and its role in viral replication is unclear. γ.	α-HV		ORF 9				(33, 80, 194, 214)

(*cont.*)

Gene designation	Alternative name	Main properties	Conservation[b]	Gene Block[c]	Homologues[d]				Ref.
					VZV	γ-HV	HCMV	HHV-6	
U_L49.5	gN	This 91 residues membrane-associated protein with apparent M_r of 6700 is abundant in virions. The PrV homologue is gN, which forms a complex with gM. In other herpesviruses the complex is gM-gN-gO. PrV gN is not accessible in the infected cell plasma membrane. In other herpesviruses, it forms a complex with gM that inhibits fusion. In PrV, gN may be disulphide-linked to the tegument, and gN was absent from gM-negative PrVirions, whereas gM was readily detected in virions in the absence of gN. Thus, gM appears to be required for virion localization of gN. UL49.5 is dispensable for viral replication in cultured cells. γ2.	α-HV, β-HV, γ-HV						(2, 17, 19, 134)
U_L50	deoxyuridine triphosphatase, dUTPase	U_L50 encodes a dUTPase. The ORF is not essential for viral replication in cells in culture. 371 aa. β.	α-HV, β-HV, γ-HV		ORF 8				(14, 17, 205)
U_L51		The 244 residue protein is a component of the virion tegument. Dispensable for growth in cell culture. γ_1.	α-HV, β-HV, γ-HV		ORF 7				(17, 66)
U_L52		U_L52 is a component of the helicase-primase complex. It strongest affinity appears to be for ICP8, but both U_L52 an U_L5 proteins are required for DNA binding. 1058 aa. β.	α-HV, β-HV, γ-HV		ORF6				(26, 62)
U_L53	gK	The 338 amino acid protein encoded by this ORF is a low abundance glycoprotein with M_r 40000. Sequence predicts three or four transmembrane segments. Topology, investigated by epitope mapping, shows an extracellular N-terminus and intracellular C-terminus. In infected cells gK localizes to Golgi apparatus. Plasma membrane localization is debated. gK is the most frequent locus of syncytial (syn) mutations. A deletion mutant in gK is defective in exocytosis of virions, and is syncytial. When expressed singly, gK localizes mainly at ER. Its transport to the Golgi apparatus requires the UL20 protein. gK inhibits fusion in the cell-cell fusion assay. Inhibition is augmented by coexpression with UL20 protein. gK role appears to prevent infected cells from fusing with adjacent cells. γ.	α-HV		ORF5				(4, 5, 70, 87, 107, 167, 193)
U_L54 α27	ICP27	ICP27 is a multifunctional 512-residue protein with two well defined functions. Early in infection it blocks splicing of RNA, thereby enabling the transport of unspliced RNA into the cytoplasm. At late times it acts as an RNA transporter and shuttles between the nucleus and cytoplasm. α.	α-HV, β-HV, γ-HV		ORF4	ORF57	UL69	U42	(21, 51, 52, 173, 210, 220)
U_L55		This ORF encodes a nonstructural 186-residue protein associated with sites of virion assembly. It is dispensable for viral replication in cultured cells. γ_2.	α-HV (not PrV)		ORF 3				

(*cont.*)

Gene designation	Alternative name	Main properties	Conservation[b]	Gene Block[c]	Homologues[d] VZV	γ-HV	HCMV	HHV-6	Ref.
U_L56		This ORF encodes a phosphorylated 234-residue, type II membrane protein associated with virions. The protein is essential for pathogenesis in experimental animal systems but is not required for viral replication in cultured cells. γ_2	HSV-2						(127, 204)
α4	ICP4	ICP4 is an essential regulatory 1298-residue protein. It acts as a transactivator and a repressor of viral gene functions. As a repressor it binds to high affinity sites overlapping the transcription initiation sites of its own ORF and that of ORF P and ORF O. The interaction of ICP4 together with transcriptional factors to low affinity sites -some significantly divergent from the high affinity sites – may account for the trans activating function of ICP4. While the interaction of ICP4 with transcriptional factors has been well documented, the role of low affinity sites remains unclear. α.	α-HV		ORF62/71				(61, 97, 121, 183, 188)
α 22 U_S1	ICP22	This ORF encodes a 420-residue regulatory protein of 420 residues essential in some cells and in experimental animal systems but not in human or primate cells in continuous cultivation. See $U_S1.5$ for details regarding its functions. The protein is extensively phosphorylated by viral and cellular kinases and nucleotidylylated by casein kinase II. α.	α-HV		ORF63/70				(3, 7, 31, 118, 139, 165, 195, 209)
$U_S1.5$		The promoter and coding domain of $U_S1.5$ is contained within the α22 ORF and the α22 met 14 acts as the initiator methionine of $U_S1.5$ protein. Most of the known functions of ICP22 map to the $U_S1.5$ ORF. Thus in the absence of ICP22 or U_L13 a set of late proteins (U_L41, U_S11 and U_L38) accumulates in smaller amounts. The results of two studies may account for this observation. Thus, both ICP22 and U_L13 are required for the activation of cdc2 and degradation of the partners, cyclin A and B. cdc2 partners with U_L42 and together bind and mediate posttranscriptional modification of topoisomerase IIα. In other studies the two proteins have been shown to mediate an "intermediate" phopshorylation of RNA Polymerase II. ICP22 also affects the accumulation of α0 mRNA. 274 aa. VZV ORF63/70 appears to correspond to Us1.5 rather than to ORF Us1. α.	α-HV		ORF63/ 70				(3, 43)
U_S2		The ORF encodes a 291 residue tegument protein not essential for viral replication in culture or in experimental animals. It is conserved among most α-HV. Its function is not known. The PrV homologue is prenylated. γ_2.	HSV-2 and B virus, but not PrV or VZV						(55, 112, 163)
U_S3	Serine/threonine protein kinase	A multifunctional protein kinase that phosphorylates a large number of both cellular and viral proteins. The kinase is not essential for viral replication. U_s3 protein kinase blocks apoptosis induced by viral mutants or exogenous agents. 481 aa. γ_1.	α-HV		ORF66				(24, 102, 119, 120, 133, 197)

(*cont.*)

Gene designation	Alternative name	Main properties	Conservation[b]	Gene Block[c]	Homologues[d]				Ref.
					VZV	γ-HV	HCMV	HHV-6	
U_S4	gG	A 238 residue envelope glycoprotein of unknown function used in serologic assays to differentiate HSV-1 from HSV-2 antibody responses. The HSV-2 gG is larger that HSV-1 gG. γ_1.	α-HV except VZV						(1, 131)
U_S5	gJ	This 92-residue glycoprotein protects the cells from apoptosis induced by gD−/+ and gD−/− virions. Dispensable in cell cultures. γ.	HSV-2, B virus, not PrV or VZV						(111, 254)
U_S6	gD	gD is a virion glycoprotein of 394 residues, with apparent M_r of 56000, essential for virus entry into the cell, and cell-cell fusion. gD is the receptor-binding glycoprotein, and interacts with three alternative receptors, named HVEM, nectin, and modified heparan sulphate. Major determinant of HSV tropism. Crystal structure shows an Ig-folded core with N- and C-terminal extensions. Upon receptor binding, a change in conformation ensues. A deletion gD mutant produces virions that exit the cell, but are non infectious. Soluble gD blocks infectivity. Ectopic expression of full length gD induces in the cell restriction to infection, by sequestering the gD receptor. Mutations at N-terminus allow usage of an alternative receptor. gD protects the cell from apoptosis induced by gD−/+ and gD−/− virions. Antiapoptotic activity is mediated by mannose-phosphate receptor. β-γ.	α-HV except VZV						(42, 53, 56, 92, 135, 180, 254)
U_S7	gI	gI is a 390-residue virion glycoprotein. It forms a heterodimer with gE. The gI-gE complex constitutes a viral Fc receptor for monomeric IgG. The gE-gI complex has basolateral localization in epithelial cells and facilitates basolateral spread of progeny virus in polarized cells. Dispensable in transformed cells, but critical in non transformed cells. γ_1.	α-HV		ORF67				(74, 75, 149, 168, 235)
U_S8	gE	gE is a 550-residue virion glycoprotein. It forms a heterodimer with gI. See, gI. gE is phosphorylated by UL13 protein kinase. γ_1.	α-HV		ORF68				(74, 75, 166, 182, 255)
$U_S8.5$		The 159-residue (M_r 19,000) protein localizes to nucleoli and is dispensable for viral replication. Its mRNA is among the most abundant species packaged in virions. γ_1.	HSV-2, B virus, not PrV or VZV						(91, 214)
U_S9		A dispensable type II membrane-associated protein of 90 residues, reported to play no role in neurovirulence or latency. May be involved in anterograde axonal transport of virions. γ.	α-HV		ORF65				(34, 58, 178, 179)
U_S10		A dispensable tegument phosphoprotein made of 312 residues. Reported to play no role in neurovirulence or latency and to copurify with the nuclear matrix. γ_1.	α-HV		ORF 64/69				(179, 218, 248)
U_S11		This ORF encodes an abundant 161-residues protein expressing multiple functions. Virion associated U_S11 localizes to polyribosomes. Late in infection U_S11 is also found in nucleoli. $U_S11$synthesized under an α promoter blocks phosphorylation of eIF-2α. U_S11 binds RNA in sequence and conformation dependent fashion and is in part responsible for the packaging of RNA in virions. γ_2.	HSV-2						(45, 202, 214)

(*cont.*)

Gene designation	Alternative name	Main properties	Conservation[b]	Gene Block[c]	Homologues[d] VZV	γ-HV	HCMV	HHV-6	Ref.
U_S12, α47	ICP47	The small 88-residues ORF encodes a M_r 9776 protein that binds to TAP1/TAP2 and blocks the transport of antigenic peptides to ER for presentation by MHC class 2 proteins. α.	HSV-2, B virus, not PrV or VZV						(104, 155, 156, 251)
LAT		The primary latency associated transcript is a low abundance transcript 8.5 to 9 kb in size and extends through the length of the ab and b'a' sequences flanking the unique long sequences. Both productively infected and latently infected cells accumulate transcripts 2.0 and 1.5 kb in size thought to be stable introns of the primary transcript. The smaller LAT sequences terminate antisense to and within the coding sequences of α0 gene. Deletion of the sequences encoding LAT reduces the mortality and morbidity in experimental animal systems and the number of neurons harboring latent virus. The decrease in the number of neurons in LAT minus mutants has been linked to pro-apoptotic manifestations of latent virus. Pre-α.							(191, 221, 246)
ORI_S RNA		This RNA originates in the **c** sequences flanking the unique short sequences at or near the transcription initiation sites of α22 or α47 genes, extends across the ORI_S sequence and co-terminates with the transcript encoding ICP4. The RNA is detected after the onset of viral DNA synthesis. Its function is not known.							(105)
αX and βX RNAs		These overlapping RNAs 0.9 and 4.9 Kb in size originate upstream of ORF P and extend across the L-S junction. Their function is not known.							(30)
AL-RNA		RNA reported to be antisense to the 5' sequence of LAT							(192)
VZV ORF1		Membrane protein			ORF1				(60)
VZV ORF 2					ORF2				(212)
VZV ORF 13		Thymidylate synthetase			ORF 13				
VZV ORF 32					ORF 32				
VZV ORF 57					ORF 57				
VZV ORFS/L					ORF S/L				(115)

[a] kinetic class: α, β, γ, Unknown.
[b] Abbreviations: alphaherpesvirinae or α-HV, betaherpesvirinae or β-HV, gammaherpesvirinae or γ-HV
[c] Conserved gene block to which the sequences belongs
[d] Identification of homologues in other herperviruses was based on informations contained in ref.s 69, 95, 125, 162, 169, 190, 228

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REFERENCES

The numbers for each reference refer to the reference numbers in Table 6.1.

1. Ackermann, M., Longnecker, R., Roizman, B., and Pereira, L. (1986). Identification, properties, and gene location of a novel glycoprotein specified by herpes simplex virus 1. *Virology*, **150**, 207–220.
2. Adams, R., Cunningham, C., Davison, M. D., MacLean, C. A., and Davison, A. J. (1998). Characterization of the protein encoded by gene UL49A of herpes simplex virus type 1. *J. Gen. Virol.*, **79**(4), 813–823.
3. Advani, S. J., Weichselbaum, R. R., and Roizman, B. (2003). Herpes simplex virus 1 activates cdc2 to recruit topoisomerase II alpha for post-DNA synthesis expression of late genes. *Proc. Natl Acad. Sci. USA*, **100**, 4825–4830.
4. Avitabile, E., Lombardi, G., and Campadelli-Fiume, G. (2003). Herpes simplex virus glycoprotein K, but not its syncytial allele, inhibits cell–cell fusion mediated by the four fusogenic glycoproteins, gD, gB, gH and gL. *J. Virol.*, **77**, 6836–6844.
5. Avitabile, E., Lombardi, G., Gianni, T., Capri, M., and Campadelli-Fiume, G. (2004). Coexpression of UL20p and gK inhibits cell–cell fusion mediated by herpes simplex virus glycoproteins gD, gH-gL, and wt- gB or an endocytosis-defective gB mutant, and downmodulates their cell surface expression. *J. Virol.*
6. Avitabile, E., Ward, P. L., Di Lazzaro, C., Torrisi, M. R., Roizman, B., and Campadelli-Fiume, G. (1994). The herpes simplex virus UL20 protein compensates for the differential disruption of exocytosis of virions and membrane glycoproteins associated with fragmentation of the Golgi apparatus. *J. Virol.*, **68**, 7397–7405.
7. Baiker, A., Bagowski, C., Ito, H. *et al.* (2004). The immediate-early 63 protein of varicella-zoster virus: analysis of functional domains required for replication in vitro and for T-cell and skin tropism in the SCIDhu model in vivo. *J. Virol.*, **78**, 1181–1194.
8. Baines, J. D., Koyama, A. H., Huang, T., and Roizman, B. (1994). The UL21 gene products of herpes simplex virus 1 are dispensable for growth in cultured cells. *J. Virol.*, **68**, 2929–2936.
9. Baines, J. D., Poon, A. P., Rovnak, J., and Roizman, B. (1994). The herpes simplex virus 1 UL15 gene encodes two proteins and is required for cleavage of genomic viral DNA. *J. Virol.*, **68**, 8118–8124.
10. Baines, J. D. and Roizman, B. (1991). The open reading frames UL3, UL4, UL10, and UL16 are dispensable for the replication of herpes simplex virus 1 in cell culture. *J. Virol.*, **65**, 938–944.
11. Baines, J. D. and Roizman, B. (1993). The UL10 gene of herpes simplex virus 1 encodes a novel viral glycoprotein, gM, which is present in the virion and in the plasma membrane of infected cells. *J. Virol.*, **67**, 1441–1452.
12. Baines, J. D. and Roizman, B. (1992). The UL11 gene of herpes simplex virus 1 encodes a function that facilitates nucleocapsid envelopment and egress from cells. *J. Virol.*, **66**, 5168–5174.
13. Baines, J. D., Ward, P. L., Campadelli-Fiume, G., and Roizman, B. (1991). The UL20 gene of herpes simplex virus 1 encodes a function necessary for viral egress. *J. Virol.*, **65**, 6414–6424.
14. Baldo, A. M. and McClure, M. A. (1999). Evolution and horizontal transfer of dUTPase-encoding genes in viruses and their hosts. *J. Virol.*, **73**, 7710–7721.
15. Baradaran, K., Dabrowski, C. E., and Schaffer, P. A. (1994). Transcriptional analysis of the region of the herpes simplex virus type 1 genome containing the UL8, UL9, and UL10 genes and identification of a novel delayed-early gene product, OBPC. *J. Virol.*, **68**, 4251–4261.
16. Baradaran, K., Hardwicke, M. A., Dabrowski, C. E., and Schaffer, P. A. (1996). Properties of the novel herpes simplex virus type 1 origin binding protein, OBPC. *J. Virol.*, **70**, 5673–5679.
17. Barker, D. E. and Roizman, B. (1990). Identification of three genes nonessential for growth in cell culture near the right terminus of the unique sequences of long component of herpes simplex virus 1. *Virology*, **177**, 684–691.
18. Barnard, E. C., Brown, G., and Stow, N. D. (1997). Deletion mutants of the herpes simplex virus type 1 UL8 protein: effect on DNA synthesis and ability to interact with and influence the intracellular localization of the UL5 and UL52 proteins. *Virology*, **237**, 97–106.
19. Barnett, B. C., Dolan, A., Telford, E. A., Davison, A. J., and McGeoch, D. J. (1992). A novel herpes simplex virus gene (UL49A) encodes a putative membrane protein with counterparts in other herpesviruses. *J. Gen. Virol.*, **73**(8), 2167–2171.
20. Batterson, W. and Roizman, B. (1983). Characterization of the herpes simplex virion-associated factor responsible for the induction of alpha genes. *J. Virol.*, **46**, 371–377.
21. Baudoux, L., Defechereux, P., Rentier, B., and Piette, J. (2000). Gene activation by Varicella-zoster virus IE4 protein requires its dimerization and involves both the arginine-rich sequence, the central part, and the carboxyl-terminal cysteine-rich region. *J. Biol. Chem.*, **275**, 32822–32831.
22. Beard, P. M. and Baines, J. D. (2004). The DNA cleavage and packaging protein encoded by the UL33 gene of herpes simplex virus 1 associates with capsids. *Virology*.
23. Beard, P. M., Taus, N. S., and Baines, J. D. (2002). DNA cleavage and packaging proteins encoded by genes U(L)28, U(L)15, and U(L)33 of herpes simplex virus type 1 form a complex in infected cells. *J. Virol.*, **76**, 4785–4791.
24. Benetti, L., Munger, J., and Roizman, B. (2003). The herpes simplex virus 1 US3 protein kinase blocks caspase-dependent double cleavage and activation of the proapoptotic protein BAD. *J. Virol.*, **77**, 6567–6573.

25. Besser, J., Sommer, M. H., Zerboni, L. (2003). Differentiation of varicella-zoster virus ORF47 protein kinase and IE62 protein binding domains and their contributions to replication in human skin xenografts in the SCID-hu mouse. *J. Virol.*, **77**, 5964–5974.
26. Biswas, N. and Weller, S. K. (2001). The UL5 and UL52 subunits of the herpes simplex virus type 1 helicase-primase subcomplex exhibit a complex interdependence for DNA binding. *J. Biol. Chem.*, **276**, 17610–17619.
27. Blaho, J. A., Mitchell, C., and Roizman, B. (1994). An amino acid sequence shared by the herpes simplex virus 1 alpha regulatory proteins 0, 4, 22, and 27 predicts the nucleotidylylation of the UL21, UL31, UL47, and UL49 gene products. *J. Biol. Chem.*, **269**, 17401–17410.
28. Bloom, D. C. and Stevens, J. G. (1994). Neuron-specific restriction of a herpes simplex virus recombinant maps to the UL5 gene. *J. Virol.*, **68**, 3761–3772.
29. Bludau, H. and Freese, U. K. (1991). Analysis of the HSV-1 strain 17 DNA polymerase gene reveals the expression of four different classes of pol transcripts. *Virology*, **183**, 505–518.
30. Bohenzky, R. A., Lagunoff, M., Roizman, B., Wagner, E. K., and Silverstein, S. (1995). Two overlapping transcription units which extend across the L-S junction of herpes simplex virus type 1. *J. Virol.*, **69**, 2889–2897.
31. Bontems, S., Di Valentin, E., Baudoux, L., Rentier, B., Sadzot-Delvaux, C., and Piette, J. (2002). Phosphorylation of varicella-zoster virus IE63 protein by casein kinases influences its cellular localization and gene regulation activity. *J. Biol. Chem.*, **277**, 21050–21060.
32. Bowman, B. R., Baker, M. L., Rixon, F. J., Chiu, W., and Quiocho, F. A. (2003). Structure of the herpesvirus major capsid protein. *EMBO J.*, **22**, 757–765.
33. Brewis, N., Phelan, A., Webb, J., Drew, J., Elliott, G., and O'Hare, P. (2000). Evaluation of VP22 spread in tissue culture. *J. Virol.*, **74**, 1051–1056.
34. Brideau, A. D., Banfield, B. W., and Enquist, L. W. (1998). The Us9 gene product of pseudorabies virus, an alphaherpesvirus, is a phosphorylated, tail-anchored type II membrane protein. *J. Virol.*, **72**, 4560–4570.
35. Browne, H., Bell, S., and Minson, T. (2004). Analysis of the requirement for glycoprotein m in herpes simplex virus type 1 morphogenesis. *J. Virol.*, **78**, 1039–1041.
36. Buckmaster, E. A., Gompels, U., and Minson, A. (1984). Characterisation and physical mapping of an HSV-1 glycoprotein of approximately 115 X 10(3) molecular weight. *Virology*, **139**, 408–413.
37. Bzik, D. J., Fox, B. A., DeLuca, N. A., and Person, S. (1984). Nucleotide sequence of a region of the herpes simplex virus type 1 gB glycoprotein gene: mutations affecting rate of virus entry and cell fusion. *Virology*, **137**, 185–190.
38. Cai, W. Z., Person, S., DebRoy, C., and Gu, B. H. (1988). Functional regions and structural features of the gB glycoprotein of herpes simplex virus type 1. An analysis of linker insertion mutants. *J. Mol. Biol.*, **201**, 575–588.
39. Cai, W. Z., Person, S., Warner, S. C., Zhou, J. H., and DeLuca, N. A. (1987). Linker-insertion nonsense and restriction-site deletion mutations of the gB glycoprotein gene of herpes simplex virus type 1. *J. Virol.*, **61**, 714–721.
40. Calder, J. M., Stow, E. C., and Stow, N. D. (1992). On the cellular localization of the components of the herpes simplex virus type 1 helicase-primase complex and the viral origin-binding protein. *J. Gen. Virol.*, **73**(3), 531–538.
41. Caradonna, S., Worrad, D., and Lirette, R. (1987). Isolation of a herpes simplex virus cDNA encoding the DNA repair enzyme uracil-DNA glycosylase. *J. Virol.*, **61**, 3040–3047.
42. Carfi, A., Willis, S. H., Whitbeck, J. C. *et al.* (2001). Herpes simplex virus glycoprotein D bound to the human receptor HveA. *Mol. Cell.*, **8**, 169–179.
43. Carter, K. L. and Roizman, B. (1996). The promoter and transcriptional unit of a novel herpes simplex virus 1 alpha gene are contained in, and encode a protein in frame with, the open reading frame of the alpha 22 gene. *J. Virol.*, **70**, 172–178.
44. Carter, K. L., Ward, P. L., and Roizman, B. (1996). Characterization of the products of the U(L)43 gene of herpes simplex virus 1: potential implications for regulation of gene expression by antisense transcription. *J. Virol.*, **70**, 7663–7668.
45. Cassady, K. A., Gross, M., and Roizman, B. (1998). The herpes simplex virus US11 protein effectively compensates for the gamma1(34.5) gene if present before activation of protein kinase R by precluding its phosphorylation and that of the alpha subunit of eukaryotic translation initiation factor 2. *J. Virol.*, **72**, 8620–8626.
46. Chang, Y. E., Menotti, L., Filatov, F., Campadelli-Fiume, G., and Roizman, B. (1998). UL27.5 is a novel gamma2 gene antisense to the herpes simplex virus 1 gene encoding glycoprotein B. *J. Virol.*, **72**, 6056–6064.
47. Chang, Y. E., Poon, A. P., and Roizman, B. (1996). Properties of the protein encoded by the UL32 open reading frame of herpes simplex virus 1. *J. Virol.*, **70**, 3938–3946.
48. Chang, Y. E. and Roizman, B. (1993). The product of the UL31 gene of herpes simplex virus 1 is a nuclear phosphoprotein which partitions with the nuclear matrix. *J. Virol.*, **67**, 6348–6356.
49. Chang, Y. E., Van Sant, C., Krug, P. W., Sears, A. E., and Roizman, B. (1997). The null mutant of the U(L)31 gene of herpes simplex virus 1: construction and phenotype in infected cells. *J. Virol.*, **71**, 8307–8315.
50. Chen, D., Stabell, E. C., and Olivo, P. D. (1995). Varicella-zoster virus gene 51 complements a herpes simplex virus type 1 UL9 null mutant. *J. Virol.*, **69**, 4515–4518.
51. Chen, I. H., Sciabica, K. S., and Sandri-Goldin, R. M. (2002). ICP27 interacts with the RNA export factor Aly/REF to direct herpes simplex virus type 1 intronless mRNAs to the TAP export pathway. *J. Virol.*, **76**, 12877–12889.
52. Cheung, P., Ellison, K. S., Verity, R., and Smiley, J. R. (2000). Herpes simplex virus ICP27 induces cytoplasmic accumulation of unspliced polyadenylated alpha-globin pre-mRNA in infected HeLa cells. *J. Virol.*, **74**, 2913–2919.

53. Chiang, H. Y., Cohen, G. H., and Eisenberg, R. J. (1994). Identification of functional regions of herpes simplex virus glycoprotein gD by using linker-insertion mutagenesis. *J. Virol.*, **68**, 2529–2543.
54. Chung, T. D., Wymer, J. P., Smith, C. C., Kulka, M., and Aurelian, L. (1989). Protein kinase activity associated with the large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10). *J. Virol.*, **63**, 3389–3398.
55. Clase, A. C., Lyman, M. G., del Rio, T. *et al.* (2003). The pseudorabies virus Us2 protein, a virion tegument component, is prenylated in infected cells. *J. Virol.*, **77**, 12285–12298.
56. Cocchi, F., Menotti, L., Mirandola, P., Lopez, M., and Campadelli-Fiume, G. (1998). The ectodomain of a novel member of the immunoglobulin superfamily related to the poliovirus receptor has the attibutes of a *bonafide* receptor for herpes simplex viruses 1 and 2 in human cells. *J. Virol.*, **72**, 9992–10002.
57. Cohen, G. H., Ponce de Leon, M., Diggelmann, H., Lawrence, W. C., Vernon, S. K., and Eisenberg, R. J. (1980). Structural analysis of the capsid polypeptides of herpes simplex virus types 1 and 2. *J. Virol.*, **34**, 521–531.
58. Cohen, J. I., Sato, H., Srinivas, S., and Lekstrom, K. (2001). Varicella-zoster virus (VZV) ORF65 virion protein is dispensable for replication in cell culture and is phosphorylated by casein kinase II, but not by the VZV protein kinases. *Virology*, **280**, 62–71.
59. Cohen, J. I. and Seidel, K. (1994). Varicella-zoster virus (VZV) open reading frame 10 protein, the homolog of the essential herpes simplex virus protein VP16, is dispensable for VZV replication in vitro. *J. Virol.*, **68**, 7850–7858.
60. Cohen, J. I. and Seidel, K. E. (1995). Varicella-zoster virus open reading frame 1 encodes a membrane protein that is dispensable for growth of VZV in vitro. *Virology*, **206**, 835–842.
61. Compel, P. and DeLuca, N. A. (2003). Temperature-dependent conformational changes in herpes simplex virus ICP4 that affect transcription activation. *J. Virol.*, **77**, 3257–3268.
62. Constantin, N. and Dodson, M. S. (1999). Two-hybrid analysis of the interaction between the UL52 and UL8 subunits of the herpes simplex virus type 1 helicase-primase. *J. Gen. Virol.*, **80**(9), 2411–2415.
63. Cook, W. J., Lin, S. M., DeLuca, N. A., and Coen, D. M. (1995). Initiator elements and regulated expression of the herpes simplex virus thymidine kinase gene. *J. Virol.*, **69**, 7291–7294.
64. Costa, R. H., Draper, K. G., Kelly, T. J., and Wagner, E. K. (1985). An unusual spliced herpes simplex virus type 1 transcript with sequence homology to Epstein-Barr virus DNA. *J. Virol.*, **54**, 317–328.
65. Cunningham, C., Davison, A. J., MacLean, A. R., Taus, N. S., and Baines, J. D. (2000). Herpes simplex virus type 1 gene UL14: phenotype of a null mutant and identification of the encoded protein. *J. Virol.*, **74**, 33–41.
66. Daikoku, T., Ikenoya, K., Yamada, H., Goshima, F., and Nishiyama, Y. (1998). Identification and characterization of the herpes simplex virus type 1 UL51 gene product. *J. Gen. Virol.*, **79**(12), 3027–3031.
67. Daikoku, T., Shibata, S., Goshima, F. *et al.* (1997). Purification and characterization of the protein kinase encoded by the UL13 gene of herpes simplex virus type 2. *Virology*, **235**, 82–93.
68. Daikoku, T., Yamamoto, N., Maeno, K., and Nishiyama, Y. (1991). Role of viral ribonucleotide reductase in the increase of dTTP pool size in herpes simplex virus-infected Vero cells. *J. Gen. Virol.*, **72**(6), 1441–1444.
69. Davison, A. (1983). DNA sequence of the US component of the varicella-zoster virus genome. *EMBO J.*, **2**, 2203–2209.
70. Debroy, C., Pederson, N., and Person, S. (1985). Nucleotide sequence of a herpes simplex virus type 1 gene that causes cell fusion. *Virology*, **145**, 36–48.
71. Desai, P., DeLuca, N. A., and Person, S. (1998). Herpes simplex virus type 1 VP26 is not essential for replication in cell culture but influences production of infectious virus in the nervous system of infected mice. *Virology*, **247**, 115–124.
72. Desai, P. J. (2000). A null mutation in the UL36 gene of herpes simplex virus type 1 results in accumulation of unenveloped DNA-filled capsids in the cytoplasm of infected cells. *J. Virol.*, **74**, 11608–11618.
73. Desai, P. J., Schaffer, P. A., and Minson, A. C. (1988). Excretion of non-infectious virus particles lacking glycoprotein H by a temperature-sensitive mutant of herpes simplex virus type 1: evidence that gH is essential for virion infectivity. *J. Gen. Virol.*, **69**(6), 1147–1156.
74. Dingwell, K. S., Brunetti, C. R., Hendricks, R. L. *et al.* (1994). Herpes simplex virus glycoproteins E and I facilitate cell-to-cell spread in vivo and across junctions of cultured cells. *J. Virol.*, **68**, 834–845.
75. Dingwell, K. S. and Johnson, D. C. (1998). The herpes simplex virus gE-gI complex facilitates cell-to-cell spread and binds to components of cell junctions. *J. Virol.*, **72**, 8933–8942.
76. Dodson, M. S. and Lehman, I. R. (1991). Association of DNA helicase and primase activities with a subassembly of the herpes simplex virus 1 helicase-primase composed of the UL5 and UL52 gene products. *Proc. Natl Acad. Sci. USA*, **88**, 1105–1109.
77. Donnelly, M. and Elliott, G. (2001). Nuclear localization and shuttling of herpes simplex virus tegument protein VP13/14. *J. Virol.*, **75**, 2566–2574.
78. Duus, K. M. and Grose, C. (1996). Multiple regulatory effects of varicella-zoster virus (VZV) gL on trafficking patterns and fusogenic properties of VZV gH. *J. Virol.*, **70**, 8961–8971.
79. Duus, K. M., Hatfield, C., and Grose, C. (1995). Cell surface expression and fusion by the varicella-zoster virus gH:gL glycoprotein complex: analysis by laser scanning confocal microscopy. *Virology*, **210**, 429–440.
80. Elliott, G. and O'Hare, P. (1997). Intercellular trafficking and protein delivery by a herpesvirus structural protein. *Cell*, **88**, 223–233.
81. Eom, C. Y. and Lehman, I. R. (2003). Replication-initiator protein (UL9) of the herpes simplex virus 1 binds NFB42 and is degraded via the ubiquitin-proteasome pathway. *Proc. Natl Acad. Sci. USA*, **100**, 9803–9807.

82. Esclatine, A., Taddeo, B., Evans, L., and Roizman, B. (2004a). The herpes simplex virus 1 UL41 gene-dependent destabilization of cellular RNAs is selective and may be sequence-specific. *Proc. Natl Acad. Sci. USA*, **101**, 3603–3608.
83. Esclatine, A., Taddeo, B., and Roizman, B. (2004b). The UL41 protein of herpes simplex virus mediates selective stabilization or degradation of cellular mRNAs. *Proc. Natl Acad. Sci. USA*, **101**, 18165–18170.
84. Farrell, M. J., Dobson, A. T., and Feldman, L. T. (1991). Herpes simplex virus latency-associated transcript is a stable intron. *Proc. Natl Acad. Sci. USA*, **88**, 790–794.
85. Forrester, A., Farrell, H., Wilkinson, G., Kaye, J., Davis Poynter, N., and Minson, T. (1992). Construction and properties of a mutant of herpes simplex virus type 1 with glycoprotein H coding sequences deleted. *J. Virol.*, **66**, 341–348.
86. Foster, T. P., Alvarez, X., and Kousoulas, K. G. (2003). Plasma membrane topology of syncytial domains of herpes simplex virus type 1 glycoprotein K (gK): the UL20 protein enables cell surface localization of gK but not gK-mediated cell-to-cell fusion. *J. Virol.*, **77**, 499–510.
87. Foster, T. P. and Kousoulas, K. G. (1999). Genetic analysis of the role of herpes simplex virus type 1 glycoprotein K in infectious virus production and egress. *J. Virol.*, **73**, 8457–8468.
88. Frink, R. J., Eisenberg, R., Cohen, G., and Wagner, E. K. (1983). Detailed analysis of the portion of the herpes simplex virus type 1 genome encoding glycoprotein C. *J. Virol.*, **45**, 634–647.
89. Gambhir, S. S., Bauer, E., Black, M. E. *et al.* (2000). A mutant herpes simplex virus type 1 thymidine kinase reporter gene shows improved sensitivity for imaging reporter gene expression with positron emission tomography. *Proc. Natl Acad. Sci. USA*, **97**, 2785–2790.
90. Garcia-Valcarcel, M., Fowler, W. J., Harper, D. R., Jeffries, D. J., and Layton, G. T. (1997). Cloning, expression, and immunogenicity of the assembly protein of varicella-zoster virus and detection of the products of open reading frame 33. *J. Med. Virol.*, **53**, 332–339.
91. Georgopoulou, U., Kakkanas, A., Miriagou, V., Michaelidou, A., and Mavromara, P. (1995). Characterization of the US8.5 protein of herpes simplex virus. *Arch. Virol.*, **140**, 2227–2241.
92. Geraghty, R. J., Krummenacher, C., Cohen, G. H., Eisenberg, R. J., and Spear, P. G. (1998). Entry of alphaherpesviruses mediated by poliovirus receptor-related protein 1 and poliovirus receptor. *Science*, **280**, 1618–1620.
93. Goldstein, J. N. and Weller, S. K. (1998). In vitro processing of herpes simplex virus type 1 DNA replication intermediates by the viral alkaline nuclease, UL12. *J. Virol.*, **72**, 8772–8781.
94. Gompels, U. and Minson, A. (1986). The properties and sequence of glycoprotein H of herpes simplex virus type 1. *Virology*, **153**, 230–247.
95. Gompels, U. A., Nicholas, J., Lawrence, G. *et al.* (1995). The DNA sequence of human herpesvirus-6: structure, coding content, and genome evolution. *Virology*, **209**, 29–51.
96. Gottlieb, J., Marcy, A. I., Coen, D. M., and Challberg, M. D. (1990). The herpes simplex virus type 1 UL42 gene product: a subunit of DNA polymerase that functions to increase processivity. *J. Virol.*, **64**, 5976–5987.
97. Grondin, B. and DeLuca, N. (2000). Herpes simplex virus type 1 ICP4 promotes transcription preinitiation complex formation by enhancing the binding of TFIID to DNA. *J. Virol.*, **74**, 11504–11510.
98. Haanes, E. J., Nelson, C. M., Soule, C. L., and Goodman, J. L. (1994). The UL45 gene product is required for herpes simplex virus type 1 glycoprotein B-induced fusion. *J. Virol.*, **68**, 5825–5834.
99. Hagglund, R. and Roizman, B. (2004). Role of ICP0 in the strategy of conquest of the host cell by herpes simplex virus 1. *J. Virol.*, **78**, 2169–2178.
100. He, B., Gross, M., and Roizman, B. (1998). The gamma1 34.5 protein of herpes simplex virus 1 has the structural and functional attributes of a protein phosphatase 1 regulatory subunit and is present in a high molecular weight complex with the enzyme in infected cells. *J. Biol. Chem.*, **273**, 20737–20743.
101. He, B., Gross, M., and Roizman, B. (1997). The gamma(1)34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. *Proc. Natl Acad. Sci. USA*, **94**, 843–848.
102. Heineman, T. C., Seidel, K., and Cohen, J. I. (1996). The varicella-zoster virus ORF66 protein induces kinase activity and is dispensable for viral replication. *J. Virol.*, **70**, 7312–7317.
103. Herold, B. C., WuDunn, D., Soltys, N., and Spear, P. G. (1991). Glycoprotein C of herpes simplex virus type 1 plays a principal role in the adsorption of virus to cells and in infectivity. *J. Virol.*, **65**, 1090–1098.
104. Hill, A., Jugovic, P., York, I. *et al.* (1995). Herpes simplex virus turns off the TAP to evade host immunity. *Nature*, **375**, 411–415.
105. Hubenthal-Voss, J., Starr, L., and Roizman, B. (1987). The herpes simplex virus origins of DNA synthesis in the S component are each contained in a transcribed open reading frame. *J. Virol.*, **61**, 3349–3355.
106. Hutchinson, L., Browne, H., Wargent, V. *et al.* (1992). A novel herpes simplex virus glycoprotein, gL, forms a complex with glycoprotein H (gH) and affects normal folding and surface expression of gH. *J. Virol.*, **66**, 2240–2250.
107. Hutchinson, L., Goldsmith, K., Snoddy, D., Ghosh, H., Graham, F. L., and Johnson, D. C. (1992). Identification and characterization of a novel herpes simplex virus glycoprotein, gK, involved in cell fusion. *J. Virol.*, **66**, 5603–5609.
108. Ingemarson, R., Graslund, A., Darling, A., and Thelander, L. (1989). Herpes simplex virus ribonucleotide reductase: expression in Escherichia coli and purification to homogeneity of a tyrosyl free radical-containing, enzymatically active form of the 38-kilodalton subunit. *J. Virol.*, **63**, 3769–3776.

109. Jacquet, A., Haumont, M., Chellun, D. *et al.* (1998). The varicella zoster virus glycoprotein B (gB) plays a role in virus binding to cell surface heparan sulfate proteoglycans. *Virus Res.*, **53**, 197–207.
110. Jahedi, S., Markovitz, N. S., Filatov, F., and Roizman, B. (1999). Colocalization of the herpes simplex virus 1 UL4 protein with infected cell protein 22 in small, dense nuclear structures formed prior to onset of DNA synthesis. *J. Virol.*, **73**, 5132–5138.
111. Jerome, K. R., Fox, R., Chen, Z., Sears, A. E., Lee, H., and Corey, L. (1999). Herpes simplex virus inhibits apoptosis through the action of two genes, Us5 and Us3. *J. Virol.*, **73**, 8950–8957.
112. Jiang, Y. M., Yamada, H., Goshima, F. *et al.* (1998). Characterization of the herpes simplex virus type 2 (HSV-2) US2 gene product and a US2-deficient HSV-2 mutant. *J. Gen. Virol.*, **79**(11), 2777–2784.
113. Jun, P. Y., Strelow, L. I., Herman, R. C. *et al.* (1998). The UL4 gene of herpes simplex virus type 1 is dispensable for latency, reactivation and pathogenesis in mice. *J. Gen. Virol.*, **79**(7), 1603–1611.
114. Kamiyama, T., Kurokawa, M., and Shiraki, K. (2001). Characterization of the DNA polymerase gene of varicella-zoster viruses resistant to acyclovir. *J. Gen. Virol.*, **82**, 2761–2765.
115. Kemble, G. W., Annunziato, P., Lungu, O. *et al.* (2000). Open reading frame S/L of varicella-zoster virus encodes a cytoplasmic protein expressed in infected cells. *J. Virol.*, **74**, 11311–11321.
116. Kenyon, T. K., Cohen, J. I., and Grose, C. (2002). Phosphorylation by the varicella-zoster virus ORF47 protein serine kinase determines whether endocytosed viral gE traffics to the trans-Golgi network or recycles to the cell membrane. *J. Virol.*, **76**, 10980–10993.
117. Kenyon, T. K., Lynch, J., Hay, J., Ruyechan, W., and Grose, C. (2001). Varicella-zoster virus ORF47 protein serine kinase: characterization of a cloned, biologically active phosphotransferase and two viral substrates, ORF62 and ORF63. *J. Virol.*, **75**, 8854–8858.
118. Kinchington, P. R., Bookey, D., and Turse, S. E. (1995). The transcriptional regulatory proteins encoded by varicella-zoster virus open reading frames (ORFs) 4 and 63, but not ORF 61, are associated with purified virus particles. *J. Virol.*, **69**, 4274–4282.
119. Kinchington, P. R., Fite, K., Seman, A., and Turse, S. E. (2001). Virion association of IE62, the varicella-zoster virus (VZV) major transcriptional regulatory protein, requires expression of the VZV open reading frame 66 protein kinase. *J. Virol.*, **75**, 9106–9113.
120. Kinchington, P. R., Fite, K., and Turse, S. E. (2000). Nuclear accumulation of IE62, the varicella-zoster virus (VZV) major transcriptional regulatory protein, is inhibited by phosphorylation mediated by the VZV open reading frame 66 protein kinase. *J. Virol.*, **74**, 2265–2277.
121. Kinchington, P. R., Hougland, J. K., Arvin, A. M., Ruyechan, W. T., and Hay, J. (1992). The varicella-zoster virus immediate-early protein IE62 is a major component of virus particles. *J. Virol.*, **66**, 359–366.
122. Kinchington, P. R., Ling, P., Pensiero, M., Moss, B., Ruyechan, W. T., and Hay, J. (1990). The glycoprotein products of varicella-zoster virus gene 14 and their defective accumulation in a vaccine strain (Oka). *J. Virol.*, **64**, 4540–4548.
123. Kinoshita, H., Hondo, R., Taguchi, F., and Yogo, Y. (1988). Variation of R1 repeated sequence present in open reading frame 11 of varicella-zoster virus strains. *J. Virol.*, **62**, 1097–1100.
124. Kirkitadze, M. D., Barlow, P. N., Price, N. C. *et al.* (1998). The herpes simplex virus triplex protein, VP23, exists as a molten globule. *J. Virol.*, **72**, 10066–10072.
125. Klupp, B. G., Hengartner, C. J., Mettenleiter, T. C., and Enquist, L. W. (2004). Complete, annotated sequence of the pseudorabies virus genome. *J. Virol.*, **78**, 424–440.
126. Koff, A., Schwedes, J. F., and Tegtmeyer, P. (1991). Herpes simplex virus origin-binding protein (UL9) loops and distorts the viral replication origin. *J. Virol.*, **65**, 3284–3292.
127. Koshizuka, T., Goshima, F., Takakuwa, H. *et al.* (2002). Identification and characterization of the UL56 gene product of herpes simplex virus type 2. *J. Virol.*, **76**, 6718–6728.
128. Lagunoff, M., Randall, G., and Roizman, B. (1996). Phenotypic properties of herpes simplex virus 1 containing a derepressed open reading frame P gene. *J. Virol.*, **70**, 1810–1817.
129. Lagunoff, M. and Roizman, B. (1995). The regulation of synthesis and properties of the protein product of open reading frame P of the herpes simplex virus 1 genome. *J. Virol.*, **69**, 3615–3623.
130. Lamberti, C. and Weller, S. K. (1998). The herpes simplex virus type 1 cleavage/packaging protein, UL32, is involved in efficient localization of capsids to replication compartments. *J. Virol.*, **72**, 2463–2473.
131. Lee, F. K., Coleman, R. M., Pereira, L., Bailey, P. D., Tatsuno, M., and Nahmias, A. J. (1985). Detection of herpes simplex virus type 2-specific antibody with glycoprotein G. *J. Clin. Microbiol.*, **22**, 641–644.
132. Lee, S. S. and Lehman, I. R. (1997). Unwinding of the box I element of a herpes simplex virus type 1 origin by a complex of the viral origin binding protein, single-strand DNA binding protein, and single-stranded DNA. *Proc. Natl Acad. Sci. USA*, **94**, 2838–2842.
133. Leopardi, R., Van Sant, C., and Roizman, B. (1997). The herpes simplex virus 1 protein kinase US3 is required for protection from apoptosis induced by the virus. *Proc. Natl Acad. Sci. USA*, **94**, 7891–7896.
134. Liang, X., Tang, M., Manns, B., Babiuk, L. A., and Zamb, T. J. (1993). Identification and deletion mutagenesis of the bovine herpesvirus 1 dUTPase gene and a gene homologous to herpes simplex virus UL49.5. *Virology*, **195**, 42–50.
135. Ligas, M. W. and Johnson, D. C. (1988). A herpes simplex virus mutant in which glycoprotein D sequences are replaced by beta-galactosidase sequences binds to but is unable to penetrate into cells. *J. Virol.*, **62**, 1486–1494.

136. Liu, F. Y. and Roizman, B. (1991). The herpes simplex virus 1 gene encoding a protease also contains within its coding domain the gene encoding the more abundant substrate. *J. Virol.*, **65**, 5149–5156.
137. Liu, F. Y. and Roizman, B. (1991). The promoter, transcriptional unit, and coding sequence of herpes simplex virus 1 family 35 proteins are contained within and in frame with the UL26 open reading frame. *J. Virol.*, **65**, 206–212.
138. Liu, Y., Gong, W., Huang, C. C., Herr, W., and Cheng, X. (1999). Crystal structure of the conserved core of the herpes simplex virus transcriptional regulatory protein VP16. *Genes Dev.*, **13**, 1692–1703.
139. Long, M. C., Leong, V., Schaffer, P. A., Spencer, C. A., and Rice, S. A. (1999). ICP22 and the UL13 protein kinase are both required for herpes simplex virus-induced modification of the large subunit of RNA polymerase II. *J. Virol.*, **73**, 5593– 5604.
140. Loomis, J. S., Bowzard, J. B., Courtney, R. J., and Wills, J. W. (2001). Intracellular trafficking of the UL11 tegument protein of herpes simplex virus type 1. *J. Virol.*, **75**, 12209–12219.
141. Loomis, J. S., Courtney, R. J., and Wills, J. W. (2003). Binding partners for the UL11 tegument protein of herpes simplex virus type 1. *J. Virol.*, **77**, 11417–11424.
142. Lubinski, J., Wang, L., Mastellos, D., Sahu, A., Lambris, J. D., and Friedman, H. M. (1999). In vivo role of complement-interacting domains of herpes simplex virus type 1 glycoprotein gC. *J. Exp. Med.*, **190**, 1637–1646.
143. MacLean, C. A., Clark, B., and McGeoch, D. J. (1989). Gene UL11 of herpes simplex virus type 1 encodes a virion protein which is myristylated. *J. Gen. Virol.*, **70**(12), 3147–3157.
144. MacLean, C. A., Dolan, A., Jamieson, F. E., and McGeoch, D. J. (1992). The myristylated virion proteins of herpes simplex virus type 1: investigation of their role in the virus life cycle. *J. Gen. Virol.*, **73**, 539–547.
145. MacLean, C. A., Efstathiou, S., Elliott, M. L., Jamieson, F. E., and McGeoch, D. J. (1991). Investigation of herpes simplex virus type 1 genes encoding multiply inserted membrane proteins. *J. Gen. Virol.*, **72**(4), 897–906.
146. Mahalingam, R., Lasher, R., Wellish, M., Cohrs, R. J., and Gilden, D. H. (1998). Localization of varicella-zoster virus gene 21 protein in virus-infected cells in culture. *J. Virol.*, **72**, 6832–6837.
147. Mahalingam, R., Wellish, M., Cabirac, G., Gilden, D., and Vafai, A. (1988). Regulation of varicella zoster virus gene 27 translation in vitro by upstream sequences. *Virus Res.*, **10**, 193–204.
148. Makhov, A. M., Lee, S. S., Lehman, I. R., and Griffith, J. D. (2003). Origin-specific unwinding of herpes simplex virus 1 DNA by the viral UL9 and ICP8 proteins: visualization of a specific preunwinding complex. *Proc. Natl Acad. Sci. USA*, **100**, 898–903.
149. Mallory, S., Sommer, M., and Arvin, A. M. (1997). Mutational analysis of the role of glycoprotein I in varicella-zoster virus replication and its effects on glycoprotein E conformation and trafficking. *J. Virol.*, **71**, 8279–8288.
150. Mapelli, M., Muhleisen, M., Persico, G., van Der Zandt, H., and Tucker, P. A. (2000). The 60-residue C-terminal region of the single-stranded DNA binding protein of herpes simplex virus type 1 is required for cooperative DNA binding. *J. Virol.*, **74**, 8812–8822.
151. Maresova, L., Pasieka, T., Wagenaar, T., Jackson, W., and Grose, C. (2003). Identification of the authentic varicella-zoster virus gB (gene 31) initiating methionine overlapping the 3' end of gene 30. *J. Med. Virol.*, **70** Suppl 1, S64–S70.
152. Markovitz, N. S., Filatov, F., and Roizman, B. (1999). The U(L)3 protein of herpes simplex virus 1 is translated predominantly from the second in-frame methionine codon and is subject to at least two posttranslational modifications. *J. Virol.*, **73**, 8010–8018.
153. Marsden, H. S., McLean, G. W., Barnard, E. C. *et al.* (1997). The catalytic subunit of the DNA polymerase of herpes simplex virus type 1 interacts specifically with the C terminus of the UL8 component of the viral helicase-primase complex. *J. Virol.*, **71**, 6390–6397.
154. Martinez, R., Sarisky, R. T., Weber, P. C., and Weller, S. K. (1996). Herpes simplex virus type 1 alkaline nuclease is required for efficient processing of viral DNA replication intermediates. *J. Virol.*, **70**, 2075–2085.
155. Mavromara-Nazos, P., Ackermann, M., and Roizman, B. (1986). Construction and properties of a viable herpes simplex virus 1 recombinant lacking coding sequences of the alpha 47 gene. *J. Virol.*, **60**, 807–812.
156. Mavromara-Nazos, P., Silver, S., Hubenthal-Voss, J., McKnight, J. L., and Roizman, B. (1986). Regulation of herpes simplex virus 1 genes: alpha gene sequence requirements for transient induction of indicator genes regulated by beta or late (gamma 2) promoters. *Virology*, **149**, 152–164.
157. McKnight, J. L., Pellett, P. E., Jenkins, F. J., and Roizman, B. (1987). Characterization and nucleotide sequence of two herpes simplex virus 1 genes whose products modulate alpha-trans-inducing factor-dependent activation of alpha genes. *J. Virol.*, **61**, 992–1001.
158. McKnight, S. L. (1980). The nucleotide sequence and transcript map of the herpes simplex virus thymidine kinase gene. *Nucl. Acids Res.*, **8**, 5949–5964.
159. McLean, G. W., Abbotts, A. P., Parry, M. E., Marsden, H. S., and Stow, N. D. (1994). The herpes simplex virus type 1 origin-binding protein interacts specifically with the viral UL8 protein. *J. Gen. Virol.*, **75**(10), 2699–2706.
160. McMillan, D. J., Kay, J., and Mills, J. S. (1997). Characterization of the proteinase specified by varicella-zoster virus gene 33. *J. Gen. Virol.*, **78**(9), 2153–2157.
161. McNab, A. R., Desai, P., Person, S. *et al.* (1998). The product of the herpes simplex virus type 1 UL25 gene is required for encapsidation but not for cleavage of replicated viral DNA. *J. Virol.*, **72**, 1060–1070.
162. Megaw, A. G., Rapaport, D., Avidor, B., Frenkel, N., and Davison, A. J. (1998). The DNA sequence of the RK strain of human herpesvirus 7. *Virology*, **244**, 119–132.
163. Meindl, A. and Osterrieder, N. (1999). The equine herpesvirus 1 Us2 homolog encodes a nonessential membrane-associated virion component. *J. Virol.*, **73**, 3430–3437.

164. Meredith, D. M., Lindsay, J. A., Halliburton, I. W., and Whittaker, G. R. (1991). Post-translational modification of the tegument proteins (VP13 and VP14) of herpes simplex virus type 1 by glycosylation and phosphorylation. *J. Gen. Virol.*, **72**(11), 2771–2775.
165. Mitchell, C., Blaho, J. A., McCormick, A. L., and Roizman, B. (1997). The nucleotidylylation of herpes simplex virus 1 regulatory protein alpha22 by human casein kinase II. *J. Biol. Chem.*, **272**, 25394–25400.
166. Mo, C., Lee, J., Sommer, M., Grose, C., and Arvin, A. M. (2002). The requirement of varicella zoster virus glycoprotein E (gE) for viral replication and effects of glycoprotein I on gE in melanoma cells. *Virology*, **304**, 176–186.
167. Mo, C., Suen, J., Sommer, M., and Arvin, A. (1999). Characterization of Varicella-Zoster virus glycoprotein K (open reading frame 5) and its role in virus growth. *J. Virol.*, **73**, 4197–4207.
168. Moffat, J., Ito, H., Sommer, M., Taylor, S., and Arvin, A. M. (2002). Glycoprotein I of varicella-zoster virus is required for viral replication in skin and T cells. *J. Virol.*, **76**, 8468–8471.
169. Montague, M. G. and Hutchison, C. A., 3rd (2000). Gene content phylogeny of herpesviruses. *Proc. Natl Acad. Sci. USA*, **97**, 5334–5339.
170. Mori, H., Shiraki, K., Kato, T., Hayakawa, Y., Yamanishi, K., and Takahashi, M. (1988). Molecular analysis of the thymidine kinase gene of thymidine kinase-deficient mutants of varicella-zoster virus. *Intervirology*, **29**, 301–310.
171. Moriuchi, H., Moriuchi, M., and Cohen, J. I. (1994). The RING finger domain of the varicella-zoster virus open reading frame 61 protein is required for its transregulatory functions. *Virology*, **205**, 238–246.
172. Moriuchi, H., Moriuchi, M., Pichyangkura, R., Triezenberg, S. J., Straus, S. E., and Cohen, J. I. (1995). Hydrophobic cluster analysis predicts an amino-terminal domain of varicella-zoster virus open reading frame 10 required for transcriptional activation. *Proc. Natl Acad. Sci. USA*, **92**, 9333–9337.
173. Moriuchi, M., Moriuchi, H., Debrus, S., Piette, J., and Cohen, J. I. (1995). The acidic amino-terminal region of varicella-zoster virus open reading frame 4 protein is required for transactivation and can functionally replace the corresponding region of herpes simplex virus ICP27. *Virology*, **208**, 376–382.
174. Nalwanga, D., Rempel, S., Roizman, B., and Baines, J. D. (1996). The UL 16 gene product of herpes simplex virus 1 is a virion protein that colocalizes with intranuclear capsid proteins. *Virology*, **226**, 236–242.
175. Newcomb, W. W. and Brown, J. C. (1989). Use of Ar+ plasma etching to localize structural proteins in the capsid of herpes simplex virus type 1. *J. Virol.*, **63**, 4697–4702.
176. Newcomb, W. W., Juhas, R. M., Thomsen, D. R. *et al.* (2001). The UL6 gene product forms the portal for entry of DNA into the herpes simplex virus capsid. *J. Virol.*, **75**, 10923–10932.
177. Newcomb, W. W., Trus, B. L., Booy, F. P., Steven, A. C., Wall, J. S., and Brown, J. C. (1993). Structure of the herpes simplex virus capsid. Molecular composition of the pentons and the triplexes. *J. Mol. Biol.*, **232**, 499–511.
178. Niizuma, T., Zerboni, L., Sommer, M. H., Ito, H., Hinchliffe, S., and Arvin, A. M. (2003). Construction of varicella-zoster virus recombinants from parent Oka cosmids and demonstration that ORF65 protein is dispensable for infection of human skin and T cells in the SCID-hu mouse model. *J. Virol.*, **77**, 6062–6065.
179. Nishiyama, Y., Kurachi, R., Daikoku, T., and Umene, K. (1993). The US 9, 10, 11, and 12 genes of herpes simplex virus type 1 are of no importance for its neurovirulence and latency in mice. *Virology*, **194**, 419–423.
180. Noble, A. G., Lee, G. T., Sprague, R., Parish, M. L., and Spear, P. G. (1983). Anti-gD monoclonal antibodies inhibit cell fusion induced by herpes simplex virus type 1. *Virology*, **129**, 218–224.
181. Nozawa, N., Daikoku, T., Yamauchi, Y. *et al.* (2002). Identification and characterization of the UL7 gene product of herpes simplex virus type 2. *Virus Genes*, **24**, 257–266.
182. Olson, J. K. and Grose, C. (1997). Endocytosis and recycling of varicella-zoster virus Fc receptor glycoprotein gE: internalization mediated by a YXXL motif in the cytoplasmic tail. *J. Virol.*, **71**, 4042–4054.
183. Papavassiliou, A. G., Wilcox, K. W., and Silverstein, S. J. (1991). The interaction of ICP4 with cell/infected-cell factors and its state of phosphorylation modulate differential recognition of leader sequences in herpes simplex virus DNA. *EMBO J.*, **10**, 397–406.
184. Pasieka, T. J., Maresova, L., and Grose, C. (2003). A functional YNKI motif in the short cytoplasmic tail of varicella-zoster virus glycoprotein gH mediates clathrin-dependent and antibody-independent endocytosis. *J. Virol.*, **77**, 4191–4204.
185. Pearson, A. and Coen, D. M. (2002). Identification, localization, and regulation of expression of the UL24 protein of herpes simplex virus type 1. *J. Virol.*, **76**, 10821–10828.
186. Pellett, P. E., Kousoulas, K. G., Pereira, L., and Roizman, B. (1985). Anatomy of the herpes simplex virus 1 strain F glycoprotein B gene: primary sequence and predicted protein structure of the wild type and of monoclonal antibody-resistant mutants. *J. Virol.*, **53**, 243–253.
187. Pellett, P. E., McKnight, J. L., Jenkins, F. J., and Roizman, B. (1985). Nucleotide sequence and predicted amino acid sequence of a protein encoded in a small herpes simplex virus DNA fragment capable of trans-inducing alpha genes. *Proc. Natl Acad. Sci. USA*, **82**, 5870–5874.
188. Peng, H., He, H., Hay, J., and Ruyechan, W. T. (2003). Interaction between the varicella zoster virus IE62 major transactivator and cellular transcription factor Sp1. *J. Biol. Chem.*, **278**, 38068–38075.
189. Peng, T., Hunter, J. R., and Nelson, J. W. (1996). The novel protein kinase of the RR1 subunit of herpes simplex virus has autophosphorylation and transphosphorylation activity that differs in its ATP requirements for HSV-1 and HSV-2. *Virology*, **216**, 184–196.
190. Perelygina, L., Zhu, L., Zurkuhlen, H., Mills, R., Borodovsky, M., and Hilliard, J. K. (2003). Complete sequence and comparative

analysis of the genome of herpes B virus (Cercopithecine herpesvirus 1) from a rhesus monkey. *J. Virol.*, **77**, 6167–6177.
191. Perng, G. C., Jones, C., Ciacci-Zanella, J. *et al.* (2000). Virus-induced neuronal apoptosis blocked by the herpes simplex virus latency-associated transcript. *Science*, **287**, 1500–1503.
192. Perng, G. C., Maguen, B., Jin, L. *et al.* (2002). A gene capable of blocking apoptosis can substitute for the herpes simplex virus type 1 latency-associated transcript gene and restore wild-type reactivation levels. *J. Virol.*, **76**, 1224–1235.
193. Pogue-Geile, K. L., Lee, G. T., Shapira, S. K., and Spear, P. G. (1984). Fine mapping of mutations in the fusion-inducing MP strain of herpes simplex virus type 1. *Virology*, **136**, 100–109.
194. Pomeranz, L. E. and Blaho, J. A. (1999). Modified VP22 localizes to the cell nucleus during synchronized herpes simplex virus type 1 infection. *J. Virol.*, **73**, 6769–6781.
195. Post, L. E., Mackem, S., and Roizman, B. (1981). Regulation of alpha genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with alpha gene promoters. *Cell*, **24**, 555–565.
196. Purves, F. C. and Roizman, B. (1992). The UL13 gene of herpes simplex virus 1 encodes the functions for posttranslational processing associated with phosphorylation of the regulatory protein alpha 22. *Proc. Natl Acad. Sci. USA*, **89**, 7310–7314.
197. Purves, F. C., Spector, D., and Roizman, B. (1991). The herpes simplex virus 1 protein kinase encoded by the US3 gene mediates posttranslational modification of the phosphoprotein encoded by the UL34 gene. *J. Virol.*, **65**, 5757–5764.
198. Randall, G., Lagunoff, M., and Roizman, B. (1997). The product of ORF O located within the domain of herpes simplex virus 1 genome transcribed during latent infection binds to and inhibits in vitro binding of infected cell protein 4 to its cognate DNA site. *Proc. Natl Acad. Sci. USA*, **94**, 10379–10384.
199. Reynolds, A. E., Fan, Y., and Baines, J. D. (2000). Characterization of the U(L)33 gene product of herpes simplex virus 1. *Virology*, **266**, 310–318.
200. Reynolds, A. E., Ryckman, B. J., Baines, J. D., Zhou, Y., Liang, L., and Roller, R. J. (2001). U(L)31 and U(L)34 proteins of herpes simplex virus type 1 form a complex that accumulates at the nuclear rim and is required for envelopment of nucleocapsids. *J. Virol.*, **75**, 8803–8817.
201. Reynolds, A. E., Wills, E. G., Roller, R. J., Ryckman, B. J., and Baines, J. D. (2002). Ultrastructural localization of the herpes simplex virus type 1 UL31, UL34, and US3 proteins suggests specific roles in primary envelopment and egress of nucleocapsids. *J. Virol.*, **76**, 8939–8952.
202. Roller, R. J., Monk, L. L., Stuart, D., and Roizman, B. (1996). Structure and function in the herpes simplex virus 1 RNA-binding protein U(s)11: mapping of the domain required for ribosomal and nucleolar association and RNA binding in vitro. *J. Virol.*, **70**, 2842–2851.
203. Roop, C., Hutchinson, L., and Johnson, D. C. (1993). A mutant herpes simplex virus type 1 unable to express glycoprotein L cannot enter cells, and its particles lack glycoprotein H. *J. Virol.*, **67**, 2285–2297.
204. Rosen-Wolff, A., Lamade, W., Berkowitz, C., Becker, Y., and Darai, G. (1991). Elimination of UL56 gene by insertion of LacZ cassette between nucleotide position 116030 to 121753 of the herpes simplex virus type 1 genome abrogates intraperitoneal pathogenicity in tree shrews and mice. *Virus Res.*, **20**, 205–221.
205. Ross, J., Williams, M., and Cohen, J. I. (1997). Disruption of the varicella-zoster virus dUTPase and the adjacent ORF9A gene results in impaired growth and reduced syncytia formation in vitro. *Virology*, **234**, 186–195.
206. Salmon, B. and Baines, J. D. (1998). Herpes simplex virus DNA cleavage and packaging: association of multiple forms of U(L)15-encoded proteins with B capsids requires at least the U(L)6, U(L)17, and U(L)28 genes. *J. Virol.*, **72**, 3045–3050.
207. Salmon, B., Cunningham, C., Davison, A. J., Harris, W. J., and Baines, J. D. (1998). The herpes simplex virus type 1 U(L)17 gene encodes virion tegument proteins that are required for cleavage and packaging of viral DNA. *J. Virol.*, **72**, 3779–3788.
208. Salmon, B., Nalwanga, D., Fan, Y., and Baines, J. D. (1999). Proteolytic cleavage of the amino terminus of the U(L)15 gene product of herpes simplex virus type 1 is coupled with maturation of viral DNA into unit-length genomes. *J. Virol.*, **73**, 8338–8348.
209. Sato, B., Ito, H., Hinchliffe, S., Sommer, M. H., Zerboni, L., and Arvin, A. M. (2003). Mutational analysis of open reading frames 62 and 71, encoding the varicella-zoster virus immediate-early transactivating protein, IE62, and effects on replication in vitro and in skin xenografts in the SCID-hu mouse in vivo. *J. Virol.*, **77**, 5607–5620.
210. Sato, B., Sommer, M., Ito, H., and Arvin, A. M. (2003). Requirement of varicella-zoster virus immediate-early 4 protein for viral replication. *J. Virol.*, **77**, 12369–12372.
211. Sato, H., Callanan, L. D., Pesnicak, L., Krogmann, T., and Cohen, J. I. (2002). Varicella-zoster virus (VZV) ORF17 protein induces RNA cleavage and is critical for replication of VZV at 37 degrees C but not 33 degrees C. *J. Virol.*, **76**, 11012–11023.
212. Sato, H., Pesnicak, L., and Cohen, J. I. (2002). Varicella-zoster virus open reading frame 2 encodes a membrane phosphoprotein that is dispensable for viral replication and for establishment of latency. *J. Virol.*, **76**, 3575–3578.
213. Schmitz, J. B., Albright, A. G., Kinchington, P. R., and Jenkins, F. J. (1995). The UL37 protein of herpes simplex virus type 1 is associated with the tegument of purified virions. *Virology*, **206**, 1055–1065.
214. Sciortino, M. T., Taddeo, B., Poon, A. P., Mastino, A., and Roizman, B. (2002). Of the three tegument proteins that package mRNA in herpes simplex virions, one (VP22) transports the mRNA to uninfected cells for expression prior to viral infection. *Proc. Natl Acad. Sci. USA*, **99**, 8318–8323.
215. Shiba, C., Daikoku, T., Goshima, F. *et al.* (2000). The UL34 gene product of herpes simplex virus type 2 is a tail-anchored type II membrane protein that is significant for virus envelopment. *J. Gen. Virol.*, **81**, 2397–2405.

216. Smiley, J. R. (2004). Herpes simplex virus virion host shutoff protein: immune evasion mediated by a viral RNaseγ *J. Virol.*, **78**, 1063–1068.
217. Smith, R. F. and Smith, T. F. (1989). Identification of new protein kinase-related genes in three herpesviruses, herpes simplex virus, varicella-zoster virus, and Epstein–Barr virus. *J. Virol.*, **63**, 450–455.
218. Sommer, M. H., Zagha, E., Serrano, O. K. *et al.* (2001). Mutational analysis of the repeated open reading frames, ORFs 63 and 70 and ORFs 64 and 69, of varicella-zoster virus. *J. Virol.*, **75**, 8224–8239.
219. Spencer, J. V., Newcomb, W. W., Thomsen, D. R., Homa, F. L., and Brown, J. C. (1998). Assembly of the herpes simplex virus capsid: preformed triplexes bind to the nascent capsid. *J. Virol.*, **72**, 3944–3951.
220. Spengler, M. L., Ruyechan, W. T., and Hay, J. (2000). Physical interaction between two varicella zoster virus gene regulatory proteins, IE4 and IE62. *Virology*, **272**, 375–381.
221. Stevens, J. G., Wagner, E. K., Devi-Rao, G. B., Cook, M. L., and Feldman, L. T. (1987). RNA complementary to a herpesvirus alpha gene mRNA is prominent in latently infected neurons. *Science*, **235**, 1056–1059.
222. Stevenson, D., Colman, K. L., and Davison, A. J. (1994). Delineation of a sequence required for nuclear localization of the protein encoded by varicella-zoster virus gene 61. *J. Gen. Virol.*, **75**(11), 3229–3233.
223. Stow, N. D. (2001). Packaging of genomic and amplicon DNA by the herpes simplex virus type 1 UL25-null mutant KUL25NS. *J. Virol.*, **75**, 10755–10765.
224. Stow, N. D., Weir, H. M., and Stow, E. C. (1990). Analysis of the binding sites for the varicella-zoster virus gene 51 product within the viral origin of DNA replication. *Virology*, **177**, 570–577.
225. Strom, T. and Frenkel, N. (1987). Effects of herpes simplex virus on mRNA stability. *J. Virol.*, **61**, 2198–2207.
226. Takakuwa, H., Goshima, F., Koshizuka, T., Murata, T., Daikoku, T., and Nishiyama, Y. (2001). Herpes simplex virus encodes a virion-associated protein which promotes long cellular processes in over-expressing cells. *Genes Cells*, **6**, 955–966.
227. Taus, N. S., Salmon, B., and Baines, J. D. (1998). The herpes simplex virus 1 UL 17 gene is required for localization of capsids and major and minor capsid proteins to intranuclear sites where viral DNA is cleaved and packaged. *Virology*, **252**, 115–125.
228. Telford, E. A., Watson, M. S., Aird, H. C., Perry, J., and Davison, A. J. (1995). The DNA sequence of equine herpesvirus 2. *J. Mol. Biol.*, **249**, 520–528.
229. Tenney, D. J., Hurlburt, W. W., Micheletti, P. A., Bifano, M., and Hamatake, R. K. (1994). The UL8 component of the herpes simplex virus helicase-primase complex stimulates primer synthesis by a subassembly of the UL5 and UL52 components. *J. Biol. Chem.*, **269**, 5030–5035.
230. Trgovcich, J., Johnson, D., and Roizman, B. (2002). Cell surface major histocompatibility complex class II proteins are regulated by the products of the gamma(1)34.5 and U(L)41 genes of herpes simplex virus 1. *J. Virol.*, **76**, 6974–6986.
231. Trus, B. L., Booy, F. P., Newcomb, W. W. *et al.* (1996). The herpes simplex virus procapsid: structure, conformational changes upon maturation, and roles of the triplex proteins VP19c and VP23 in assembly. *J. Mol. Biol.*, **263**, 447–462.
232. van Zeijl, M., Fairhurst, J., Jones, T. R. *et al.* (2000). Novel class of thiourea compounds that inhibit herpes simplex virus type 1 DNA cleavage and encapsidation: resistance maps to the UL6 gene. *J. Virol.*, **74**, 9054–9061.
233. Visalli, R. J. and Brandt, C. R. (1991). The HSV-1 UL45 gene product is not required for growth in Vero cells. *Virology*, **185**, 419–423.
234. Visse, B., Huraux, J. M., and Fillet, A. M. (1999). Point mutations in the varicella-zoster virus DNA polymerase gene confers resistance to foscarnet and slow growth phenotype. *J. Med. Virol.*, **59**, 84–90.
235. Wang, Z. H., Gershon, M. D., Lungu, O., Zhu, Z., Mallory, S., Arvin, A. M., and Gershon, A. A. (2001). Essential role played by the C-terminal domain of glycoprotein I in envelopment of varicella-zoster virus in the trans-Golgi network: interactions of glycoproteins with tegument. *J. Virol.*, **75**, 323–340.
236. Ward, P. L., Barker, D. E., and Roizman, B. (1996). A novel herpes simplex virus 1 gene, UL43.5, maps antisense to the UL43 gene and encodes a protein which colocalizes in nuclear structures with capsid proteins. *J. Virol.*, **70**, 2684–2690.
237. Ward, P. L., Taddeo, B., Markovitz, N. S., and Roizman, B. (2000). Identification of a novel expressed open reading frame situated between genes U(L)20 and U(L)21 of the herpes simplex virus 1 genome. *Virology*, **266**, 275–285.
238. Weir, H. M. and Stow, N. D. (1990). Two binding sites for the herpes simplex virus type 1 UL9 protein are required for efficient activity of the oriS replication origin. *J. Gen. Virol.*, **71**(6), 1379–1385.
239. Weisshart, K., Kuo, A. A., Hwang, C. B., Kumura, K., and Coen, D. M. (1994). Structural and functional organization of herpes simplex virus DNA polymerase investigated by limited proteolysis. *J. Biol. Chem.*, **269**, 22788–22796.
240. Weisshart, K., Kuo, A. A., Painter, G. R., Wright, L. L., Furman, P. A., and Coen, D. M. (1993). Conformational changes induced in herpes simplex virus DNA polymerase upon DNA binding. *Proc. Natl Acad. Sci. USA*, **90**, 1028–1032.
241. Weller, S. K., Seghatoleslami, M. R., Shao, L., Rowse, D., and Carmichael, E. P. (1990). The herpes simplex virus type 1 alkaline nuclease is not essential for viral DNA synthesis: isolation and characterization of a lacZ insertion mutant. *J. Gen. Virol.*, **71**(12), 2941–2952.
242. White, C. A., Stow, N. D., Patel, A. H., Hughes, M., and Preston, V. G. (2003). Herpes simplex virus type 1 portal protein UL6 interacts with the putative terminase subunits UL15 and UL28. *J. Virol.*, **77**, 6351–6358.
243. Wingfield, P. T., Stahl, S. J., Thomsen, D. R. *et al.* (1997). Hexon-only binding of VP26 reflects differences between the hexon

and penton conformations of VP5, the major capsid protein of herpes simplex virus. *J. Virol.*, **71**, 8955–8961.
244. Winters, T. A. and Williams, M. V. (1993). Purification and characterization of the herpes simplex virus type 2-encoded uracil-DNA glycosylase. *Virology*, **195**, 315–326.
245. Worrad, D. M. and Caradonna, S. (1993). The herpes simplex virus type 2 UL3 open reading frame encodes a nuclear localizing phosphoprotein. *Virology*, **195**, 364–376.
246. Wu, T. T., Su, Y. H., Block, T. M., and Taylor, J. M. (1996). Evidence that two latency-associated transcripts of herpes simplex virus type 1 are nonlinear. *J. Virol.*, **70**, 5962–5967.
247. Xia, D. and Straus, S. E. (1999). Transcript mapping and transregulatory behavior of varicella-zoster virus gene 21, a latency-associated gene. *Virology*, **258**, 304–313.
248. Yamada, H., Daikoku, T., Yamashita, Y., Jiang, Y. M., Tsurumi, T., and Nishiyama, Y. (1997). The product of the US10 gene of herpes simplex virus type 1 is a capsid/tegument-associated phosphoprotein which copurifies with the nuclear matrix. *J. Gen. Virol.*, **78**(11), 2923–2931.
249. Yamada, H., Jiang, Y. M., Oshima, S. *et al.* (1998). Characterization of the UL4 gene product of herpes simplex virus type 2. *Arch. Virol.*, **143**, 1199–1207.
250. Yamauchi, Y., Wada, K., Goshima, F. *et al.* (2001). The UL14 protein of herpes simplex virus type 2 translocates the minor capsid protein VP26 and the DNA cleavage and packaging UL33 protein into the nucleus of coexpressing cells. *J. Gen. Virol.*, **82**, 321–330.
251. York, I. A., Roop, C., Andrews, D. W., Riddell, S. R., Graham, F. L., and Johnson, D. C. (1994). A cytosolic herpes simplex virus protein inhibits antigen presentation to CD8+ T lymphocytes. *Cell*, **77**, 525–535.
252. Zhang, Y. and McKnight, J. L. (1993). Herpes simplex virus type 1 UL46 and UL47 deletion mutants lack VP11 and VP12 or VP13 and VP14, respectively, and exhibit altered viral thymidine kinase expression. *J. Virol.*, **67**, 1482–1492.
253. Zhou, C. and Knipe, D. M. (2002). Association of herpes simplex virus type 1 ICP8 and ICP27 proteins with cellular RNA polymerase II holoenzyme. *J. Virol.*, **76**, 5893–5904.
254. Zhou, G., Galvan, V., Campadelli-Fiume, G., and Roizman, B. (2000). Glycoprotein D or J delivered in trans blocks apoptosis in SK-N-SH cells induced by a herpes simplex virus 1 mutant lacking intact genes expressing both glycoproteins. *J. Virol.*, **74**, 11782–11791.
255. Zhu, Z., Hao, Y., Gershon, M. D., Ambron, R. T., and Gershon, A. A. (1996). Targeting of glycoprotein I (gE) of varicella-zoster virus to the trans-Golgi network by an AYRV sequence and an acidic amino acid-rich patch in the cytosolic domain of the molecule. *J. Virol.*, **70**, 6563–6575.

7

Entry of alphaherpesviruses into the cell

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Introduction

Herpes simplex virus (HSV) represents the most comprehensive example of virus-receptor interaction in the *Herpesviridae* family, and the prototype virus encoding multipartite entry genes. Whereas small enveloped viruses package the functions required for entry and fusion into one or two fusion glycoproteins, in HSV the same functions are distributed over several distinct glycoproteins, each with a specialized activity. In addition, HSV encodes a highly sophisticated system for promoting and blocking fusion between the viral envelope and cell membrane. Because the most obvious models of virus entry into the cell do not fit with the HSV complexity, and despite our detailed knowledge of the HSV receptors and of the crystal structure of glycoprotein D (gD), the receptor-binding glycoprotein, and of gB, HSV entry is still, in part, a puzzle (WuDunn and Spear, 1989; Cocchi *et al.*, 1998b; Geraghty *et al.*, 1998; Carfi *et al.*, 2001).

The current model of HSV entry envisions that, first, the virus attaches to cell membranes by the interaction of gC, and possibly gB, to glycosaminoglycans (GAGs) (Herold *et al.*, 1991). This binding likely creates multiple points of adhesion, is reversible, and the detached virus maintains its infectivity, indicating that fusion has yet to take place. Penetration requires gD, whose ectodomain contains two physically separate and functionally distinct regions, i.e., the region made of the N-terminus that carries the receptor-binding sites, and the C-terminus that carries the profusion domain (Ligas and Johnson, 1988; Cocchi *et al.*, 2004). The role of gD in entry is to interact with one of the entry receptors, to signal receptor-recognition and thus trigger fusion, by recruiting three additional glycoproteins – gB, gH, gL. The trio of gB, gH, gL, execute fusion with the plasma membrane or endocytic vesicle of the target cell (Fig. 7.1) (Cai, W. H. *et al.*, 1988; Forrester *et al.*, 1992; Roop *et al.*, 1993; Campadelli-Fiume *et al.*, 2000; Spear *et al.*, 2000; Nicola *et al.*, 2003). Of these, gH carries elements characteristic of viral fusion glycoproteins, i.e., a hydrophobic α-helix with attributes of an internal fusion peptide, and two heptad repeats with propensity to form coiled coils (Gianni *et al.*, 2005a,b). gB is a trimer with a coiled coil core; its structure closely resembles that of viral fusion proteins (Heldwein *et al.*, 2006; Roche *et al.*, 2006). Following fusion, the released tegumented nucleocapsid travels along microtubules to the nuclear pore, where the viral DNA is released into the nucleus (Sodeik *et al.*, 1997).

Much less is known about varicella zoster virus (VZV) entry. The process may be very different from that of HSV inasmuch as virion-to-cell infection is inefficient in the VZV system, and the viral genome presents a striking difference from that of HSV, namely the lack of gD (Davison and Scott, 1986).

The membrane proteins

The HSV envelope contains at least eleven glycoproteins (gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM). Additional membrane proteins not detected in the extracellular virion envelope are UL20, UL34, UL45 and possibly US9. The transcripts for UL24, UL43, and UL49.5 ORFs have been recognized, but the proteins have yet to be identified. A summary list with references is presented in Chapter 6.

From a structural point of view, the majority of HSV glycoproteins are type I glycoproteins. Variants include gL, which is soluble (Hutchinson *et al.*, 1992a); gB, which may carry two or three α-helices in the transmembrane (TM) region (Pellett *et al.*, 1985) and gK, gM, and UL20, which carry information for multiple transmembrane segments

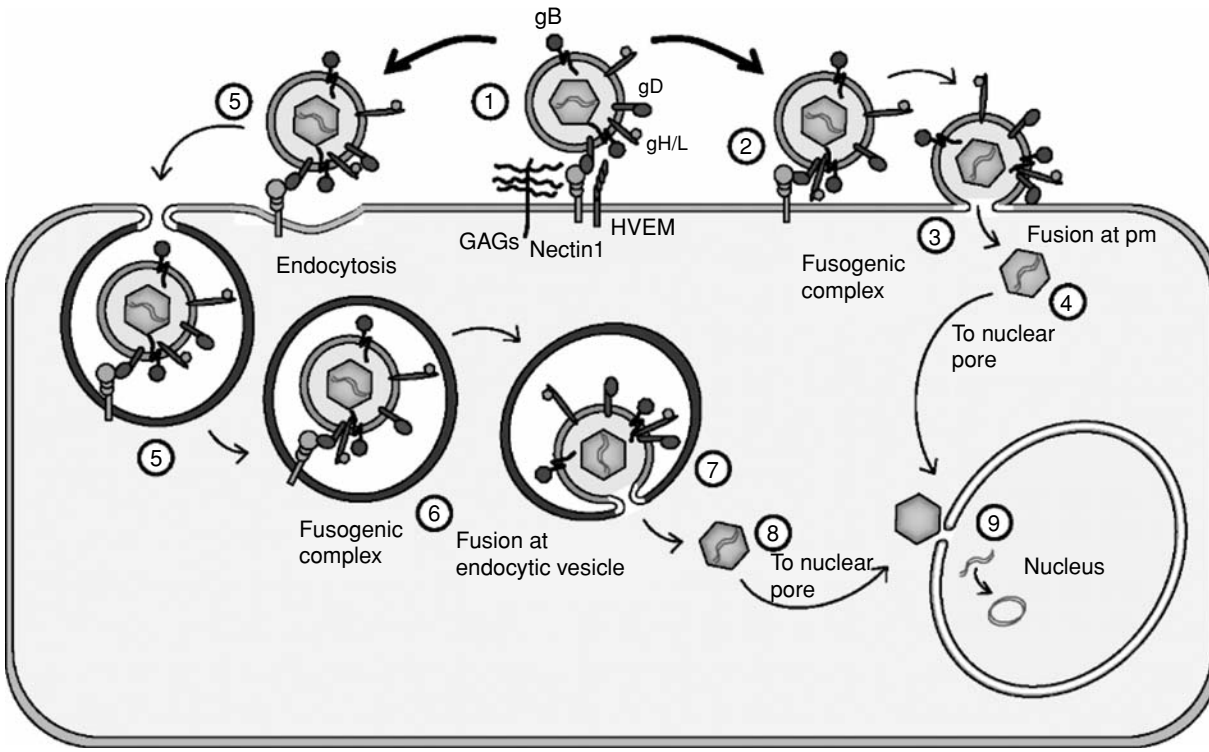

Fig. 7.1. Schematic drawing of HSV entry. Entry can occur either by endocytosis (pathway at the left), or by fusion at the plasma membrane (pathway at the right). Following attachment to cells, gD binds to a cellular receptor (frame 1), and presumably following a conformational change it recruits the glycoproteins B, H and L in an active fusogenic complex (frame 2), triggering the fusion between viral envelope and cellular plasma membrane (frame 3). The naked nucleocapsids are transported to the nucleus (frames 4 and 9). In a cell line-dependent manner or with modified forms of the receptor (see text for details) bound virions can enter cells by endocytosis (frame 5). It is conceivable that at this stage the four fusogenic glycoproteins are in a non-fusion-active form. Following acidification/maturation of the endocytic vesicles, a fusogenic complex may form (frame 6) and fusion ensue between the virion envelope and the vesicle membrane (frame 7). Nucleocapsids delivered to the cytoplasm are transported to the nucleus (frames 8 and 9).

(McGeoch *et al.*, 1988). US9 and HSV-2 UL45 are type II glycoproteins with a C-terminal ectodomain.

At the ultrastructural level, HSV glycoproteins form long thin spikes, each made of a single species. As visualized in cryo-electron tomograms of isolated virions, the envelope contains 600–750 glycoprotein spikes that vary in length, spacings, and in the angle at which they emerge from the membrane. Their distribution in the envelope suggests functional clustering (Grunewald *et al.*, 2003). In contrast, slender spikes have not been seen in varicella zoster virions (VZV) grown in cultured cells. Instead, the virion appears to be covered by an envelope studded with protrusions rather than spikes. An example is shown in Fig. 7.2. Further studies at even higher resolution will be required to determine differences between HSV and VZV envelopes.

HSV

Attachment to cells

Attachment of HSV to cells occurs upon binding of gC to GAGs that decorate heparan sulphate or chondroitin sulphate (Spear *et al.*, 1992) (Fig. 7.1). This step enhances HSV infectivity, but is not an absolute requirement, as cells defective in heparan sulphate and chondroitin sulphate exhibit a 100-fold reduced susceptibility to infection, yet can be infected (Gruenheid *et al.*, 1993). A large variety of viruses use heparan sulphate proteoglycans as receptors; their broad expression argues that they can not be responsible for any specific viral tropism.

The major actor during attachment is gC, a non essential glycoprotein encoded by the UL44 gene. gC is a

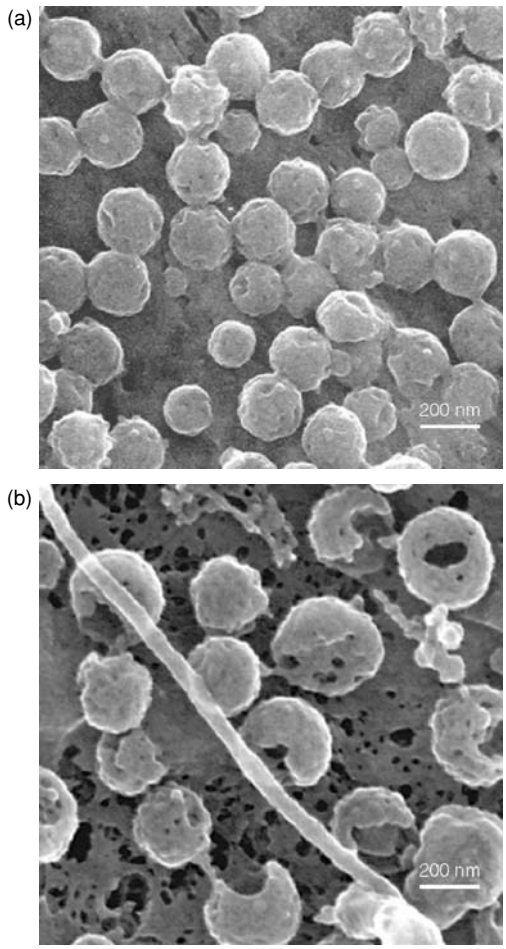

Fig. 7.2. Scanning electron micrographs of HSV-1 and VZV. Images of both viruses were taken by SEM after infection of cultured cells. HSV-1 virions have a more uniform appearance although indentations are seen in an occasional virion envelope (a). In contrast, VZV virions are more aberrant (b). Several of the virions have indentations, while other virions have incomplete envelopes. Since both viruses were examined under the same SEM conditions, it is unlikely that the aberrant nature of the VZV envelope is due to fixation artifacts. Micrographs kindly provided by Dr. Charles Grose.

mucin-type glycoprotein because of its high content in N-linked and O-linked oligosaccharides. Its ectodomain structure is provided in part by 8 cysteines, and harbors two physically separate antigenic regions, antigenic sites I and II, that map at the C- and N-termini of the molecule, respectively (Dolter *et al.*, 1992). Evidence for the role of gC in attachment rests on several lines of evidence. Initially, it was observed that the polycations neomycin and polylysine inhibit attachment of HSV-1, but not HSV-2 to cells, and this differential effect was mapped to gC (Campadelli-Fiume *et al.*, 1990). gC and gB bind heparin-Sepharose columns (Herold *et al.*, 1991). The affinity of binding to heparan sulphate is on the order of 10^{-8} M (Rux *et al.*, 2002). The region important for the interaction with heparan sulphate maps to the N-terminus of gC (Tal-Singer *et al.*, 1995). Virion binding to cells is reduced in HSV-1 mutants lacking gC-type1 gene, but not in HSV-2 mutants lacking gC-type2 (Herold *et al.*, 1991; Gerber *et al.*, 1995). The majority of these studies were performed with mutants constructed in the background of the HSV-1(KOS) strain. In contrast, deletion of gC gene in the genetic background of Sc16 and HFEM strains yielded viruses with unimpaired attachment activity, suggesting that in different virus strains, attachment may be carried out by different proteins (Griffiths *et al.*, 1998). gC has also been implicated in virus attachment to the baso-lateral domain of MDCK polarized epithelial cells, and to the apical domain of polarized human CaCo2 cells.

Interaction of gD with its receptors

gD

The ectodomain of gD is required and sufficient to enable HSV entry into cells. It is made of two separate and distinct regions, i.e., the N-terminus, carrying the receptor-binding sites (approximately contained between residues 1 and 250–260), and the C-terminus carrying the profusion domain (residues 250–260 to 305) (Cocchi *et al.*, 2004).

A breakthrough in our understanding of HSV entry came from resolution of the crystal structure of a soluble form of gD, initially up to amino acid residue 259 (Carfi *et al.*, 2001), and later on up to residue 306 (Krummenacher *et al.*, 2005). The initial structure was determined for gD alone and for gD in complex with one of the gD receptors, HVEM (herpesvirus entry mediator) (Fig. 7.3). The N-terminus consists of three portions, an Ig-folded central core (residues 56–184) made of β-strands forming two antiparallel β-sheets, and two extensions, one N-terminal (residues 1–37) and one C-terminal (Fig. 7.3). The N-terminus, which harbors all the contact residues to HVEM, is disordered in the crystal of gD alone, but forms a hairpin when gD is complexed with HVEM. gD and HVEM thereby form an intermolecular β-sheet, which is believed to stabilize the complex. Formation of the N-terminal hairpin documents a conformational change to gD when it binds HVEM.

The crystal structure of gD in complex with nectin1 has not yet been solved. The nectin1-binding site on gD, determined by means of insertion-deletion or substitution mutants, appears to be more widespread than the HVEM interaction region, (Milne *et al.*, 2003; Yoon *et al.*, 2003; Zhou

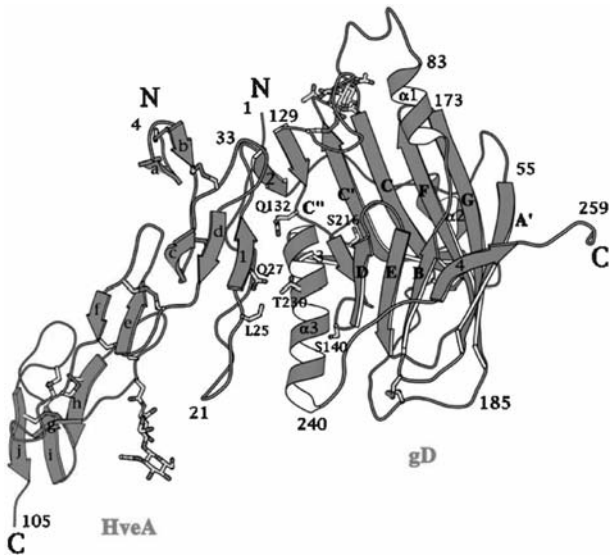

Fig. 7.3. Ribbon diagram of the 3D structure of a soluble truncated form of gD (gD285t, colored in orange) bound to HVEM receptor (HveA, colored in green) as determined by X-ray crystallography. The N-terminus (residues 1–37) of gD is devoid of a specific structure when in the unbound state, but folds into a hairpin when bound to HVEM receptor. The β-strand formed by residues 27–29 (indicated with number 1) forms an intermolecular β-sheet with HVEM residues 35–37 (letter d). The core of gD (residues 56–184) has a V-type immunglobulin domain structure, composed of 9 parallel and antiparallel β-strands (letters A to G) that form two opposing β-sheets, and carries an additional α-helix (α1). The residues 185–259 form two α-helices that fold back to the N-ter (α2 and α3), and two β-strands (numbered 3 and 4). The α3 helix supports gD's N-terminal hairpin. An additional β-strand (number 2) is located in the connector sequence (residues 33–55) that precedes the Ig-like core. Reprinted from (Carfi *et al.*, 2001), with permission. (See color plate section.)

et al., 2003; Jogger *et al.*, 2004; Manoj *et al.*, 2004; Connolly *et al.*, 2005). The only recombinant described so far debilitated for interaction with nectin1 carries the V34S substitution (Zhou and Roizman, 2006).

Receptors

Entry receptors interact with gD. This notion was established long before the actual receptors were identified, and rests on two lines of evidence. First, soluble gD binds in a saturable manner to cells and prevents infection (Johnson, D. C. *et al.*, 1990; Nicola *et al.*, 1997). Second, expression of gD from a transgene renders cells resistant to infection, because of its ability to sequester the receptor, a phenomenon designated restriction to infection or gD-mediated interference (Campadelli-Fiume *et al.*, 1988; Johnson, R. M. and Spear, 1989).

The search for HSV receptors was an active field in the 1990s, and a number of molecules were described as potential receptors. A breakthrough came from Spear and coworkers, who made use of HSV-resistant CHO cells, and identified a HeLa cell cDNA clone that encoded HVEM (Montgomery *et al.*, 1996). However, three observations in that study suggested the existence of additional receptors. First, HVEM appeared to be expressed by a limited number of cell lines. Second, antibodies to HVEM failed to completely block HSV infection. Third, several virus strains were unable to enter CHO cells expressing HVEM but were otherwise viable. This boosted further efforts in the field, which quickly led to the discovery of nectins, and, later on, of modified heparan sulphate. Altogether, the receptors known to date belong to three unrelated molecular families. Their present and past nomenclature, and the viruses for which they serve as receptors are reported in the table.

Nectins are intercellular adhesion molecules

Research in the field of nectins has proceeded in parallel with their characterization as HSV receptors (Takai *et al.*, 2003). Nectins 1–4 form a subfamily of Ca^{2+}-independent immunoglobulin (Ig)-type intercellular adhesion molecules. Together with nectin-like molecules and poliovirus receptor, they share the same overall structure consisting of three Ig-type domains. Splice variant isoforms are designated with Greek letters. Nectins form homo *cis*-dimers on the plasma membranes and *trans*-dimers with nectins present on the adjacent cell. Each nectin has a specialized pattern of *trans*-dimer formation with itself or other nectins (Reymond *et al.*, 2000; Takai *et al.*, 2003). Their main attribute is the formation, together with cadherins, of the adherens junctions of epithelial cells, and in cooperation or not with cadherins, the organization of claudin-based tight junctions. In addition, they are involved in the formation of synapses in neurons and the organization of heterotypic junctions between Sertoli cells and spermatids in the testis (Takai *et al.*, 2003).

Most nectins carry a C-terminal conserved motif that binds afadin, thus anchoring the adhesion molecules to the cytoskeleton (Takai *et al.*, 2003). This domain is absent from nectin1β which is not restricted to adherens junctions. Nectin-mediated signalling activity leads to activation of a variety of extracellular and intracellular molecules, such as scatter factor/hepatocyte growth factor, Ras, Cdc42 and Rac small G proteins (Takai *et al.*, 2003).

Table 7.1. Human HSV receptors and the viral strains for which they serve

		Human α-herpesvirus				
		Unrestricted/*rid*			Animal α-herpesvirus	
Receptor name	Alternative name	HSV-1	HSV-2	mutants	PrV	BHV-1
HVEM	HveA	+	+	−	−	−
Nectin1	PRR1, HIgR, HveC	+	+	+	+	+
Nectin2	PRR2, HveB	−	+/−	+	+	−

Nectin1

Human nectin1 is a broad spectrum receptor for human and animal alphaherpesviruses (Table 7.1). Three isoforms are known, two of which: -α and -β are membrane-bound (Cocchi *et al.*, 1998a,b; Geraghty *et al.*, 1998; Krummenacher *et al.*, 1998; Krummenacher *et al.*, 1999; Campadelli-Fiume *et al.*, 2000). Their main properties as HSV receptors are as follows.

(i) Nectin1 is broadly expressed in human tissues, including tissues and organs targeted by HSV, like CNS, ganglia and muco-epithelia (Cocchi *et al.*, 1998b; Haarr *et al.*, 2001; Matsushima *et al.*, 2003; Richart *et al.*, 2003; Linehan *et al.*, 2004). It is expressed in virtually all human cell lines, including epithelial cells, neurons and fibroblasts (Campadelli-Fiume *et al.*, 2000; Spear *et al.*, 2000). Some of these cells simultaneously express HVEM (Krummenacher *et al.*, 2004).

When HSV initially infects mucosal epithelium, the apical domain of polarized epithelial cells are targeted initially, whereas basolateral domains of epithelial cells are available to the virus only if a lesion disrupts the integrity of the lining. It was therefore of interest to know whether nectin1 can serve as an HSV receptor in polarized epithelial cells. Human CaCo2 cells can be infected with HSV from the apical domain (Griffiths *et al.*, 1998), whereas MDCK cells and primary human keratinocytes are preferentially infected from the basolateral domain (Schelhaas *et al.*, 2003; Marozin *et al.*, 2004). These differences may reflect cell line-dependent differences in the pattern of polarization of a same molecule, or distribution of receptors like nectin1-β and HVEM, which do not appear to be restricted to adherens junctions.

(ii) Nectin1 interacts physically with gD (Krummenacher *et al.*, 1999). The interaction requires the first 250 residues of gD and the V domain of nectin1. The affinity ranges from 10^{-6} to 10^{-8} molar, with the highest affinity observed with forms of gD that were truncated at or after residue 250. Affinity decreases by 100-fold with gD_{306t}, reflecting a folding of the most C-terminal portion of gD towards the core (Whitbeck *et al.*, 1999; Krummenacher *et al.*, 2005). Insertion mutations alter the binding affinity; insertions at the N-terminus (e.g., at residues 34, and 43) modify the binding to HVEM but not to nectin1 (Milne *et al.*, 2003; Jogger *et al.*, 2004). Remarkably, even when the binding affinities are low, or undetectable, the mutant forms of gD maintain the ability to mediate HSV entry and cell–cell fusion, implying that gD functions in virus entry and cell fusion regardless of its receptor-binding affinity and kinetics, and that as long as interaction with a functional receptor occurs, entry takes place (Milne *et al.*, 2003; Zhou *et al.*, 2003).

Human nectin1 also binds isoforms of gD from animal α-herpesviruses. The affinity may be even higher than for HSV-1 gD (in the case of PrV), or very low (in the case of BHV-1) (Connolly *et al.*, 2001). Even when the affinity is very low, human nectin1 is capable of mediating entry (Cocchi *et al.*, 1998b; Geraghty *et al.*, 1998).

(iii) The domain of nectin1 functional in HSV entry and in binding to gD was initially mapped to the N-terminal V domain, and subsequently to the C-C′-C″ ridge (Krummenacher *et al.*, 2000; Cocchi *et al.*, 2001; Menotti *et al.*, 2002b). Critical residues that may be part of the interface with gD are amino acids 77 and 85 (Martinez and Spear, 2002).

(iv) Nectin1-γ is a natural soluble isoform of nectin1 generated by alternative splicing. Although it contains the three Ig domains, it has a narrow distribution in human tissues, unlike nectin1-α and -β. Like soluble recombinant forms of nectin1, it has the capacity to bind to virions and block infectivity. An unexpected property was that the soluble nectin1-γ molecules suffices to mediate virus entry into receptor-negative cells. This may be consequent either to an association to endogenous nectins, or to a direct binding of the soluble receptor to virions (Lopez *et al.*, 2001).

Nectin 2 and the unrestricted or rid *mutations*

The remarkable feature about nectin2 is that a single amino acid substitution in gD confers to HSV the ability to use nectin2 as an alternative receptor, without hampering its ability to use nectin1 (Table 7.1). At the same time, this mutation abolishes the interaction with HVEM (Connolly *et al.*, 2003; Yoon *et al.*, 2003). The end result is that the

host range of the virus is modified. The mutations are L25P, Q27P, or Q27R, and are present in the unrestricted, or *rid* HSV-1 mutants. Nectin2 also serves as a weak receptor for some strains of HSV-2, but is inactive for wt-HSV-1 (Warner *et al.*, 1998; Lopez *et al.*, 2000; Krummenacher *et al.*, 2004). Physical interaction studies were in agreement with these properties (Warner *et al.*, 1998; Lopez *et al.*, 2000; Yoon *et al.*, 2003). The nectin2 residues critical for HSV entry were identified as amino acids 75–81 and 89, which lie adjacent to the predicted C′C″ β-strands, i.e., the region corresponding to the nectin1 region involved in interaction with wild-type gD.

The TNF receptor family

TNFRs (tumor necrosis factor receptors) form a family of signal transduction molecules involved in regulation of cell proliferation, differentiation and apoptotic death. Structurally, their ectodomain is composed of four typical cysteine-rich domains (CRDs). The family includes twenty nine human members, classified into three groups according to their cytoplasmic sequences and signaling properties. Members of the first group (exemplified by Fas) contain a death domain (DD) in the cytoplasmic tail. After binding to their ligands they interact with intracellular adaptors, which, in turn, induce apoptosis by activation of the caspase cascade. Members of the second group (exemplified by TNFR2 and HVEM) lack a death domain, and instead contain one or more TRAF (TNFR-associated factor) interacting motifs (TIMs), which trigger a variety of signal transduction pathways, including those for activation of nuclear factor κB (NF-κB), Jun N-terminal kinase (JNK), p38, extracellular signal-related kinase (ERK) and phosphoinositide 3-kinase (PI3K). Members of the third group (e.g., TNFR3, TNFR4, etc.) lack intracellular signaling motifs, and act as decoy receptors.

The natural ligands of the TNFRs are a family of cytokines whose prototype is TNF, and include lymphotoxin (LT)α, LTβ, and LIGHT. They are biologically active as trimers: their binding to the receptors causes the trimerization of the intracellular domains, which, in turn, interact with high affinity with trimeric cellular adaptors (e.g., TRAFs). Each cytokine interacts with more than one receptor.

HVEM

HVEM was first identified as a HSV receptor and was classified as a novel member of the TNFR family based on structural motifs (Montgomery *et al.*, 1996). The cytoplasmic tail interacts with several members of the TRAF family, leading to the activation of targets like NF-κB, Jun N-terminal kinase, and AP-1, and the consequent induction of T cell activation, proliferation, cytokine release, and expression of cell surface activation markers (Harrop *et al.*, 1998). Its ligands are $LT\alpha_3$ and LIGHT (Mauri *et al.*, 1998). LIGHT–HVEM interactions contribute to the cytotoxic T-lymphocyte-mediated immune response.

The main properties of HVEM as an HSV receptor are as follows.

(i) In early studies HVEM was found to be expressed mainly in cells of the immune system, and in a number of non-hematopoietic tissues and organs. Expression was not observed in brain or skeletal muscle (Montgomery *et al.*, 1996; Kwon *et al.*, 1997).

(ii) HVEM serves as receptor for HSV-1 and HSV-2, as a weak receptor for the U10 HSV mutant (L25P substitution), but not for HSV *rid*-1 and *rid*-2 unrestricted mutants (Connolly *et al.*, 2003; Yoon *et al.*, 2003).

(iii) HVEM binds wild-type gD. The affinity of the binding is of the same order of magnitude as that of nectin1/gD binding, and the interaction requires the same region of gD, i.e., the first 250 residues, or longer (Willis *et al.*, 1998). gD and LIGHT compete with each other for the binding to HVEM; accordingly, LIGHT interferes with HSV entry in HVEM-expressing cells (Mauri *et al.*, 1998).

(iv) The gD contact site on HVEM involves CRD1 and 2, with the majority of contacts lying in CRD1. Residues 35–37 form the intermolecular antiparallel β-sheet with gD (Carfi *et al.*, 2001; Connolly *et al.*, 2003). A systematic structure-based mutagenesis approach revealed that 17 residues in CRD1 and 4 in CRD2 are directly involved in the HVEM-gD interface. Some mutations completely abolish the HVEM binding to gD and its function as an HSV-1 receptor (Connolly *et al.*, 2002).

Modified heparan sulphate

3-O-sulphated heparan sulphate represents the third identified HSV receptor, structurally unrelated to nectins and HVEM (Shukla *et al.*, 1999). It serves as receptor for HSV-1, but not for unrestricted *rid* mutants or HSV-2. Thus, HSV-1 can use heparan sulphate GAGs not only for the initial step of attachment to the target cell, but it also recognizes some specifically modified sites on heparan sulphate (Shukla and Spear, 2001). The physical interaction with gD is in the range of 10^{-6} M, as measured by affinity co-electophoresis, and was not detectable by ELISA.

The sites on heparan sulphate recognized by gD are generated by heparan sulphate D-glucosaminyl 3-O-sulfotransferases (3-OSTs). 3-O-sulphates are rare substitutions in heparan sulphate, generated by at least six 3-OSTs isoforms identified in humans and mice, 3-OST-3_A and 3-OST-3_B, 3-OST-2, 3-OST-4 and 3-OST-5 (Shukla and

Spear, 2001). Of note, 3-OST-3s are not receptors *per se,* consistent with the finding that gD does not bind to the purified enzymes; rather, they catalyze the substitutions, which, in turn, generate the receptors and render CHO cells susceptible to HSV.

The relevance of this potential receptor to HSV infection in human cell lines and in humans remains to be ascertained. In terms of distribution, the 3-OST-3s are broadly expressed isoforms in cells of different origins and tissues; 3-OST-2 and 3-OST-4 expression is mainly in the brain. 3-OST-5 expression is limited to the skeletal muscle.

Receptor preference and usage

The availability of multiple alternative receptors raises a number of questions, such as: What receptor is preferred in cells that coexpress both molecules? Do both serve as *bona fide* receptors in humans? Are they differentially distributed, or employed in different tissues? Limited information is available on these topics.

Two parameters that may guide receptor preference in cell cultures are the affinity of the binding and the cell surface density of the receptors. Neither appears to be relevant in the case of gD, since gD affinity to nectin1 and to HVEM is of the same order of magnitude (Krummenacher *et al.*, 1998). Moreover, cells that appear to be nectin1-negative by fluorescent antibody cell sorting (e.g., EA-1 cells) can be infected and entry is inhibited by antibody to nectin1 (our unpublished result), implying that low density receptors suffice to mediate entry. The same phenomenon is observed with HVEM (Krummenacher *et al.*, 2004).

As far as infection of animal and human tissues is concerned, nectin1 is expressed in nearly every neuron of adult mice, in rat sensory neurons, in some synapses, and serves as the primary receptor for HSV-1 infection in sensory neurons (Haarr *et al.*, 2001; Mata *et al.*, 2001; Richart *et al.*, 2003). Nectin1 is also expressed in the epithelium of the human and murine vagina where it mediates HSV-1 and -2 entry in the genital mucosa of female hosts (Linehan *et al.*, 2004). Nectin1-α is detected at the cell–cell adherens junctions in human skin (Matsushima *et al.*, 2003). Cumulatively, these data are compatible with a major role of nectin1 in the infection of sensory neurons and mucoepithelia in vivo. The tissue distribution of HVEM suggests that it serves as the principal receptor for HSV in activated T lymphocytes, or in lymphoid organs (spleen and thymus), in liver and lung. However, these organs are not target of HSV in the natural history of infection, except in the rare cases of disseminated infection. Recent data show that several clinical/primary isolates can use both receptors, as laboratory strains do (Krummenacher *et al.*, 2004). The fact that viruses of different origin retain the ability to use both receptors suggests that this is a requirement for successful infection and spread in the host (Krummenacher *et al.*, 2004). The interaction with HVEM in particular may have a role in immune evasion (La *et al.*, 2002).

For other viruses, formal proof that a given receptor plays a critical role in humans has rested on correlations between genetic defects in the locus encoding the receptor and a diminished susceptibility to viral infection. A rare truncation of the nectin1 gene has been identified. The phenotypes associated with loss of both alleles include cleft lip/palate, hidrotic ectodermal dysplasia (CLPED1), hair abnormalities, developmental defects of the hands and, in some cases, mental retardation. Studies on the serostatus of these patients for evidence of HSV infection have not been published. A few studies investigated nectin1, nectin2 and HVEM gene polymorphisms and correlations with HSV infection. At present, no relatively common polymorphism has been found to correlate with HSV serostatus or symptoms (Patera *et al.*, 2002; Struyf *et al.*, 2002; Krummenacher *et al.*, 2004). Wild-type HSV can infect CLPED1 fibroblasts through HVEM in vitro, indicating that the ability to exploit redundant receptors may be favorable to the virus in vivo as well (Krummenacher *et al.*, 2004).

The animal orthologues of nectin and HVEM

HSV infects numerous mammalian species, some of which – mice, rabbits and primates – are extensively employed as animal models. The question arises whether they are faithful models in terms of receptor usage.

Porcine and bovine alphaherpesviruses promiscuously use human nectin1, implying that animal orthologs of nectins serve as receptors of these viruses in their hosts (Cocchi *et al.*, 1998b; Geraghty *et al.*, 1998; Warner *et al.*, 1998). Indeed, nectins are conserved among mammals, and nectin1 orthologs have been found in cells derived from mice, hamsters, pigs, cows and monkeys, and nectin2 has a mouse ortholog, suggesting a conserved function in the evolution of these proteins (Milne *et al.*, 2001). A HVEM ortholog is expressed in mice (Yoon *et al.*, 2003). In turn, the murine orthologs of nectin1, nectin2, HVEM, and the porcine and bovine hortologs of human nectin1 can serve as species non-specific HSV receptors when transfected into receptor-negative cells (Menotti *et al.*, 2000; Shukla *et al.*, 2000; Menotti *et al.*, 2001; Milne *et al.*, 2001; Menotti *et al.*, 2002a). The affinity of these potential receptors for gD ranges from as high as that of human nectin1, to very low or undetectable. Because the animal orthologs of nectin, and perhaps also of HVEM serve as receptors of HSV in mice and other animal species, these systems appear to relatively faithfully model HSV receptor usage in humans.

Site of HSV entry into the cell

It has been a long held paradigm that HSV enters cells by fusion at the plasma membrane. Recent evidence indicates that in some cells entry is through an endocytic pathway, and that both the cell type, and the structural features of the receptors are determinants that control the site of entry. Specifically, in cells like HeLa and CHO expressing nectin1 or HVEM, entry is inhibited by drugs that modify the pH of the endosomal compartment (low pH-sensitive entry) (Nicola *et al.*, 2003). However, when nectin1 or HVEM are expressed in J cells, they mediate entry at the plasma membrane. Furthermore, when nectin1 is retargeted to endosomes, by means of a chimeric nectin1-EGFR (epidermal growth factor receptor) chimera, or is sorted to lipid rafts, by means of a nectin1-glycosylphosphatidylinositol anchor chimera, the pathway of entry into J cells becomes endocytic (Gianni *et al.*, 2004). Of note, when HSV infects cells carrying wt-nectin1, neither nectin nor gD localize at lipid rafts, but gB does (Bender *et al.*, 2003). All in all, HSV fusion glycoproteins are well suited to perform two quite different pathways of entry.

Role of gD-receptor interaction in triggering fusion

Crucial to our understanding of how HSV enters cells is the comprehension of how gD binding to its receptor triggers fusion. A hint has come from the unexpected observation that the soluble gD ectodomain is both necessary and sufficient to rescue the infectivity of the non infectious gDnull HSV mutant (Cocchi *et al.*, 2004). Entry mediated by soluble gD requires not only the N-terminus, carrying the receptor-binding sites, but also the C-terminus carrying the pro-fusion domain, required to trigger fusion but not for receptor binding. These findings, together with the observation that a glycosylphosphatidylinositol-anchored form of gD, or substitution of the transmembrane and cytoplasmic tail with heterologous regions leave gD function unaltered (Browne *et al.*, 2003) demonstrate that the transmembrane region and cytoplasmic tail do not play any demonstrable function, except to ensure that gD is delivered to the gD receptor along with the virion, and argue that the role of gD in HSV entry is to signal receptor-recognition to the downstream glycoproteins and to trigger fusion (Cocchi *et al.*, 2004).

Biochemical and structural studies indicate that the receptor-mediated activation of gD takes the form of a conformational change (Cocchi *et al.*, 2004; Fusco *et al.*, 2005; Krummenacher *et al.*, 2005). In the unliganded state the virion gD adopts a conformation in which the flexible C terminus folds back, wraps the N-terminus and masks the receptor binding sites. At receptor binding, the C-terminus is displaced from its binding site on the N-terminus, the receptor binding sites are unmasked and become occupied by the receptor. The binary complex made of receptor plus gD with the displaced C-terminus must create a surface suitable for gB and gH-gL recruitment.

Execution of membrane fusion and its control

gB, gH, gL are essential for entry of all herpesviruses, since they are conserved among all human herpesviruses, with the highest extent of sequence conservation seen in gB. Heterodimer formation between gH and gL is also a conserved feature amongst herpesviruses. Altogether, gB, gH and gL appear to be the executors of fusion and constitute the conserved fusion machinery across the herpesvirus family.

Critical properties of gH and gB have been elucidated recently, and provide an intriguing scenario. On one hand, molecular and biochemical analyses of gH highlighted properties typical of class 1 fusion glycoproteins. Because the gH structure has not yet been solved, these properties wait for confirmation at the structural level. On the other hand, the crystal structure of gB has been solved, it exhibits a remarkable similarity to that of vesicular stomatitis G protein, and to viral fusion glycoproteins in general. Biochemical and mutational confirmation are still to be provided. At present, a most likely scenario is that both gB and gH·gL are fusion executors. How the two glycoproteins cooperate to execute fusion, and why two, and not one, fusion executors are required in the herpesviridae family is unclear. It is worthwhile to note that entry by fusion at plasma membrane, and entry by fusion in endocytic vesicles require all four glycoproteins (gD, gB, gH and gL) (Nicola *et al.*, 2003; Nicola and Straus, 2004). These requirements rule out the possibility that gB serves as fusion executor in one cellular compartment, and gH·gL serves as fusion executor in another cell compartment.

The glycoproteins that execute fusion

gH –gL

gH is a type-1 virion glycoprotein encoded by the UL22 gene (Gompels and Minson, 1986). Soon after its discovery, it was recognized as an essential glycoprotein for virion infectivity, as its deletion produced non infectious progeny and abolished cell–cell fusion (Forrester *et al.*, 1992). Neutralizing antibodies to gH block virus entry but permit attachment, indicating a role at a post-attachment step (Fuller *et al.*, 1989). gH appears to contain elements associated with fusion of membranes, i.e. a hydrophobic α-helix 1 (residues 377-397) with properties typical of a fusion peptide and two heptad repeats with propensity to form a coiled coil. α-Helix 1 is positionally conserved in all the

gH orthologs across the herpesviridae family; in HSV-2 it is located in a loop made of two cysteins. α-Helix 1 is able to interact with biological membranes, can convert a soluble glycoprotein (gD amino acid residues 1-260) into a membrane-bound glycoprotein, and can be functionally replaced by fusion peptides derived from glycoproteins of other, unrelated viruses (Gianni *et al.*, 2005a). A peptide with the sequence of α-helix 1 induces fusion of liposomes and exhibits a strong flexibility documented as ability to adopt an α-helical conformation (Galdiero, S. *et al.*, 2006; Gianni *et al.*, 2006a). These properties strongly argue in favor of α-helix 1 as a candidate fusion peptide loop. Two heptad repeats, capable to form coiled coils and to interact with each other, form a structure of increased α-helical content and are potentially suitable to form a six-helix bundle (Gianni *et al.*, 2005b; Galdiero, S. *et al.*, 2006; Gianni *et al.*, 2006b). Additional elements in gH are a second predicted α-helical domain of lower hydrophobicity than the candidate fusion peptide (aa 513-531), and a pre-transmembrane sequence (aa 626-644) with predicted propensity to partition at membrane interface (Galdiero, S. *et al.*, 2006; Gianni *et al.*, 2006a).

Synthetic peptides corresponding to the heptad repeats inhibit virus infection if present at the time of virus entry into the cell (Gianni *et al.*, 2005b; Galdiero, S. *et al.*, 2006; Gianni *et al.*, 2006b). The presence of coiled coil motifs predicts that gH must undergo profound conformational changes at fusion. Because fusion peptides and coiled coil heptad repeats represent characteristic functional domains in type 1 viral fusion glycoproteins, gH is a candidate fusion executor in HSV.

It remains to be determined whether gH interacts with cellular receptors. The interaction with an integrin is not critical given that mutagenesis of a RGD motif did not reduce virus entry and cell fusion (Galdiero, M. *et al.*, 1997). It is of interest that the transmembrane and C-terminal tail regions of gH can not be exchanged with those of heterologous proteins, in contrast with what happens with gD (Harman *et al.*, 2002; Jones and Geraghty, 2004).

A *ts* mutant, tsQ26, exhibited a phenotype characterized by the production of non-infectious extracellular virions, along with the intracellular retention of gH and of infectious virions (Desai *et al.*, 1988). This phenotype suggested a peculiar mechanism of intracellular retention of gH. A clue to understanding the intracellular trafficking of gH came from the observations that, when expressed from a transgene, gH had a M_r lower than that of mature gH, was not transported to the cell surface, and was retained in the ER unless the cells were superinfected (Gompels and Minson, 1986; Foà-Tomasi *et al.*, 1991; Roberts *et al.*, 1991). The gene product required for gH trafficking and maturation, identified by Johnson and coworkers, is gL (Hutchinson *et al.*, 1992a); gL is required for proper folding and traffiking of gH in all human Herpesviruses (Kaye *et al.*, 1992; Liu *et al.*, 1993).

gL is a soluble glycoprotein encoded by UL1 gene; its presence in the virion envelope is ensured by complex formation with gH (Hutchinson *et al.*, 1992a). In accordance with gH attributes, an HSV mutant unable to express gL could not enter cells, and its particles lacked glycoprotein H (Roop *et al.*, 1993). Both gH and gL are required for fusion in the cell–cell fusion assay (Turner *et al.*, 1998). The first 323 amino acids of gH and the first 161 amino acids of gL can form a stable secreted hetero-oligomer, while the first 648 amino acids of gH are required for reactivity to conformation-dependent antibodies, indicative of correct conformation and oligomerization (Peng *et al.*, 1998). gL is a locus of a *syn* mutation, confirming a role of the gH-gL hetero-oligomer in HSV fusion. The exact role of gL in fusion remains to be elucidated. Because its binding site on gH maps both upstream and downstream of the hydrophobic α-helix, it has been proposed that its role may be to shield the gH hydrophobic sequence, and thus to enable gH water solubility and solvent interface (Gianni *et al.*, 2005a).

gB

gB plays two opposite roles in fusion, i.e. it participates in fusion execution, and it exerts anti-fusion activity. The two functions are physically separated and reside in the ectodomain and the cytoplasmic tail, respectively. gB is a type-1 virion glycoprotein encoded by the UL27 gene (Bzik *et al.*, 1984; Pellett *et al.*, 1985). Its crystal structure reveals a trimer with a coiled coil core. Remarkably, its structure resembles closely that of vesicular stomatitis virus G protein (Heldwein *et al.*, 2006; Roche *et al.*, 2006). Despite the facts that a canonical fusion peptide has not been detected by biochemical, molecular or structural analyses, and that the region homologous to the fusion peptide loop in vesicular stomatitis virus G protein appears to be suboptimal for membrane insertion, the structural similarity between gB and vesicular stomatitis virus G protein strongly relates gB to viral fusion glycoproteins. It has been proposed that the two glycoproteins may represent a novel class of fusion glycoproteins (Heldwein *et al.*, 2006; Roche *et al.*, 2006).

From a structural point of view, gB is a trimeric spike. Each of the three protomers (residues 103–730) appears to be composed of five distinct domains (named I-V), displaying multiple contact sites (Heldwein *et al.*, 2006). Domain I, the "base", is a continous chain with a fold typical of pleckstrin homology domains. Domain II, the "middle", is made of two discontinuous segments, forming a structure

reminiscent of a pleckstrin homology superfold. Domain III, the "core", comprises three discontinuous segments: its prominent feature is a 44-residue α-helix that forms the central coiled coil with its trimeric counterparts. Domain IV, the "crown", adopts a novel structure, and is fully exposed on top of the trimeric spike. Domain V, the "arm", is a long extension spanning the full length of the protomer. Of note, its residues do not contact residues of the same protomer, but instead accommodate into the groove formed by the "cores" of the other two protomers.

The role of gB in virion infectivity and cell–cell fusion is inferred by numerous lines of evidence, including (i) the phenotype of a gB deletion mutant virus which produces non-infectious particles, (ii) the neutralizing activity of antibodies to gB, (iii) gB as a genetic locus of syncytial mutations, and (iv) the requirement for gB in the cell-cell fusion assay (Manservigi *et al.*, 1977; Cai, W. Z. *et al.*, 1987; Turner *et al.*, 1998). Functional domains in the ectodomain were identified by means of two sets of mutations: temperature sensitive mutations for viral growth, and resistance to antibodies with potent neutralizing activity. The first ones, exemplified by the mutants tsB5 and tsJ12, reside in the gB ectodomain, confer a temperature-sensitive phenotype, and affect the rate of virus entry (Bzik *et al.*, 1984). Likely, these mutations affect the gB domain involved in execution of fusion. Following the determination of gB crystal structure, it was recognized that the epitopes of potent neutralizing antibodies, either centered around single amino acid residues or formed by continuous regions, reside on the trimer surface, on the lateral faces of the spike or on the tip of the crown (Pellett *et al.*, 1985; Kousoulas *et al.*, 1988; Highlander *et al.*, 1989; Pereira *et al.*, 1989; Qadri *et al.*, 1991; Heldwein *et al.*, 2006).

The quartet of gD, gB, gH and gL assemble into a complex at virus entry

The nature of the interactions between the complex formed by gD plus its receptor and the executors of fusion is critical to understand the mechanisms by which HSV (and by extension all other herpesviruses) enter cells.

The quartet of glycoproteins essential for HSV entry and fusion (gD, gB, gH and gL) assemble into a complex at virus entry and in infected cells. Complex assembly strictly requires one of the gD receptors, either nectin1 or HVEM. The same complex is assembled also in cells transfected with the quartet, implying that no additional viral protein other than those that participate in the complex itself is required. Because the complex is assembled at virus entry and in transfected cells committed to form polykaryocytes, and fails to be assembled in the absence of either a receptor to gD or of gD, complex assembly appears to be a critical step in the process of virus entry and fusion.

The proteins that negatively control fusion

Cells infected with wt-virus do not form syncytia, despite the fact that they express the fusion glycoproteins at their surface. Syncytia are only formed when the virus carries one of the syncytial (*syn*) mutations, which map to genes encoding gB, gL, gK, UL24, or UL20. By contrast, cells expressing the quartet of gB, gD, gH and gL readily form syncytia (Turner *et al.*, 1998). The paradox may be explained by assuming that the wt-alleles of proteins that are target of *syn* mutations exert a negative control on fusion. This has, in fact, been verified for gB, gK, and UL20 (Fan *et al.*, 2002; Avitabile *et al.*, 2003; Avitabile *et al.*, 2004). As outlined below, HSV has evolved at least two mechanisms by which it blocks fusion. One is exerted through downmodulation of gB cell surface expression, the other is exerted through the concerted action of gK and UL20p. Still other proteins (UL24 and UL45) are likely to exert anti-fusion activity. The evolution of functional redundancy implies that uncontrolled fusion is inimical to HSV-1 replication and spread in nature, and therefore the virus needs to exert a tight control on it.

gB

The anti-fusion activity of gB is located in the cytoplasmic tail, which carries at least two physically distinct functional domains: the *syn* mutation and the endocytosis motifs. Each of them, separately, appears to reduce fusion. Structurally, the cytoplasmic tail carries two predicted α-helices. The *syn* mutations are located immediately downstream of the most N-terminal α-helix. Embedded in the region of the C-terminal α-helix is one, and possibly two functional endocytosis motifs (YTQV889–892 and LL871 (Fan *et al.*, 2002; Avitabile *et al.*, 2004; Beitia Ortiz de Zarate *et al.*, 2004). Deletion of the membrane-proximal α-helix abrogates virus infectivity, implying that this region is critical. Its role, and the molecular mechanism of the *syn3* mutation remain to be elucidated. The membrane-distal α-helix is also implicated in the negative control of fusion, since its deletion increases fusion in the cell fusion assay, and confers a syncytial phenotype upon virus-infected cells (Foster, *et al.*, 2001a; Avitabile *et al.*, 2004). Its antifusion activity is mainly exerted through endocytosis, which acts to decrease the steady state amounts of gB from the cell surface, such that gB becomes a limiting factor in fusion. Of note, the gB-decorated endocytosis vesicles-vacuoles represent the hallmark of gB localization in infected cells.

gK

gK is a polytopic glycoprotein encoded by the UL53 gene, whose topology is still debated (Hutchinson *et al.*, 1992b; Foster, *et al.*, 2001b). It carries an N-terminal extracellular domain, and two or three TM regions connected by loops (Foster, *et al.*, 2003b). Its hydrophobicity, poor immunogenicity and overall problems in its detection have made this glycoprotein a difficult one to study. In infected cells, gK localizes mainly to the Golgi apparatus. One controversial aspect is whether it localizes to the plasma membranes and to virions. When expressed from a transgene, gK is primarily located is at the ER (Hutchinson *et al.*, 1992b; Avitabile *et al.*, 2003; Foster, *et al.*, 2003a). When coexpressed with UL20, both proteins localize to the Golgi apparatus (Avitabile *et al.*, 2004).

gK exerts anti-fusion activity in the cell–cell fusion assay (Avitabile *et al.*, 2003). Mutant viruses carrying a partial or a complete deletion in the gK gene have two major phenotypes (Hutchinson and Johnson, 1995; Foster, and Kousoulas, 1999). First, they form syncytia, arguing that the anti-fusion activity is exerted also in the context of infected cells. Second, they are defective in virus egress, arguing that the anti-fusion activity of gK is exerted not only at the plasma membrane, but also in the membranes of the exocytic compartment. This would provide an explanation as to why these membranes are heavily decorated with fusion glycoproteins, yet do not fuse one with the other. According to this model, the gK role in virion egress may be exerted by maintaining a functional exocytic pathway. It should be stressed that, if indeed gK is also a virion constituent, then, at virus entry into the cell, the trigger to fusion must simultaneously relieve the block to fusion exerted by gK (Avitabile *et al.*, 2004).

UL20

UL20p is a polytopic unglycosylated protein with several analogies to gK. Its hydrophobicity and scarce immunogenicity have hampered its characterization. UL20p is predicted to carry 4 transmembrane segments (McGeoch *et al.*, 1988; Melancon *et al.*, 2004). In the infected cells UL20p localizes at the Golgi apparatus and the nuclear membranes, and is not detectable at the plasma membrane. It has not been detected in virions. When expressed from a transgene, UL20p predominant localization is at the ER (Avitabile *et al.*, 1994; Ward *et al.*, 1994). It relocalizes to the Golgi apparatus, when coexpressed with gK (Foster, *et al.*, 2003b; Avitabile *et al.*, 2004).

Two mutant viruses deleted in UL20 gene have been constructed, both of which are highly defective in secretion of virions to the extracellular space (Baines *et al.*, 1991; Foster, *et al.*, 2004). The first deletion virus was subsequently reported to carry an in-frame fusion between UL20.5 (not known at the time the deletion virus was constructed) and the C-terminus of UL20 gene, and was characterized by syncytia formation and by the entrapping of virions in the perinuclear space (i.e., the space between the inner and outer nuclear membranes) – a phenotype particularly evident in cells whose Golgi apparatus became fragmented following infection (Baines *et al.*, 1991). This phenotype can be interpreted as indication that the UL20p exerts a negative control on fusion. Cells infected with the second deletion virus showed enveloped virions as well as unenveloped nucleocapsids accumulating in the cytoplasm, and occasionally virion envelopes containing multiple capsids within intracytoplasmic vacuoles. These phenotypes were also interpreted to mean that UL20p acts as an inhibitor of membrane fusion, and, interestingly, that UL20p may act to maintain a single nucleocapsid for each envelope and to prevent fusion of enveloped virions among themselves (Foster, *et al.*, 2004). The complexity of these phenotypes reflects both direct and indirect effects of UL20p, including the role of UL20p in the intracellular transport of gK and possibly of the fusion glycoproteins.

The possibility that UL20p exerts an anti-fusion activity was probed in the cell–cell fusion assay, which showed a block to fusion in cells coexpressing UL20p and gK, but not in cells expressing UL20 alone. The block was cell line dependent (Avitabile *et al.*, 2004). The similar behavior of gK and UL20p, their colocalization, their mutual ability to influence each other localization, and their concerted anti-fusion activity make it likely that the two proteins act in a complex, and that they share a common target.

Nucleocapsid transport to the nuclear pore

Virus entry culminates in the release of capsids and approximately twenty tegument proteins into the cytosol. The capsids and some of the tegument proteins, e.g., αTIF, travel to the nuclear pore. Since diffusion of molecules larger than 500 kDa is restricted in the cytoplasm, viruses and nucleocapsids require a transport system. This is particularly true for neurotropic viruses that travel long distances in the axon during retrograde or anterograde transport (Enquist *et al.*, 1998). It has been calculated that in the absence of an active transport mechanism, it would take a herpes virus capsid 231 years to diffuse 10 mm in the axonal cytoplasm (Sodeik, 2000).

Microtubules represent the cytoplasmic highways on which HSV is transported (reviewed in Döhner and Sodeik, 2005). At virus entry, capsids co-localize with microtubules, and their depolymerization reduces capsid transport to

the nucleus (Sodeik *et al.*, 1997; Mabit *et al.*, 2002). Microtubules are polar structures with fast growing plus-ends typically localized in the cell periphery, and less dynamic minus-ends that are usually anchored in close proximity of the nucleus at the microtubule organizing centre (MTOC). Molecular motors use ATP-driven conformational changes to transport cargo along microtubules. Transport to the plus-ends is catalyzed by kinesins and that to minus-end by cytoplasmic dynein and dynactin (Döhner and Sodeik, 2005). Dynein and dynactin mediate capsid transport to the cell centre, since incoming capsids colocalize with these motors (Sodeik *et al.*, 1997; Döhner *et al.*, 2002), and overexpression of dynamitin, a subunit of the dynactin complex, inhibits capsid transport (Döhner *et al.*, 2002). How capsids move further from MTOC to the nuclear pore complex is unclear.

Analysis of HSV-1 entry by digital time-lapse fluorescence microscopy showed that GFP-tagged capsids can move along microtubules both towards and away from the nucleus, with maximal speeds of 1.1 μm/s. The transport is saltatory and bidirectional, but in neuronal processes it shows a retrograde bias towards the cell body (Smith *et al.*, 2001). Efforts are underway to identify the virion proteins that may interact with kinesins as well as dynein or dynactin. Two candidates are UL34p, which, however, is absent from mature virions, and US11, which appears to bind the heavy chain of conventional kinesin (Diefenbach *et al.*, 2002). Once the capsids have reached the proximity of the nucleus, they seem to bind to filaments emanating from the nuclear pores (Batterson *et al.*, 1983; Sodeik *et al.*, 1997). This docking is believed to induce capsid destabilization, release of the viral DNA, and its translocation through the nuclear pore into the nucleoplasm (Ojala *et al.*, 2000). Temperature-sensitive mutants in the UL36 gene accumulate filled viral capsids at the nuclear pore complexes at the non-permissive temperature, suggesting that the large tegument protein VP1–3 is involved in uncoating of the viral genome (Batterson *et al.*, 1983; Ojala *et al.*, 2000).

VZV

There are several remarkable differences between VZV and HSV entry. Because the respective viral glycoproteins undoubtedly influence these differences, the VZV gene products will be briefly summarized.

Is VZV gE a substitute for functions of HSV gD?

All but one of the proteins that have been illustrated above for HSV have a counterpart in VZV. For those glycoproteins for which sufficient information is available, a substantial functional similarity is observed. The single most notable difference between VZV and HSV in terms of the glycoproteins is the absence of gD in the VZV genome. At the same time, VZV is well suited for cell-to-cell spread, which takes place by fusion of the infected cell with an adjacent uninfected cell, whereas HSV is better suited for virion-to-cell infection (at least in cultured cells). So, it is tempting to speculate that the absence of a VZV gD gene may contribute to these differences.

Despite the fact that gD plays such a pivotal role in HSV entry, gD is not conserved throughout the alphaherpesviruses. In the porcine herpesvirus PrV, gD is required for virion-to cell infectivity but not for cell-to-cell spread of the virus. Consistently, gD is not a requirement for the PrV cell-cell fusion assay, although its presence greatly enhances fusion efficiency. Assuming that common basic mechanisms are shared by all of the alphaherpesviruses and given that the triplet gH-gL-gB is conserved, the question then arises: which VZV glycoprotein substitutes for the functions encoded in HSV gD, i.e., receptor recognition and triggering of fusion. The two functions might well be distributed over different entities, but a trigger to fusion consequent to virion interaction with a receptor appears to be essential.

In VZV, four glycoproteins are known to be essential. They are gB, gH, gL and gE (Keller *et al.*, 1984; Montalvo and Grose, 1986; Forghani *et al.*, 1994; Duus *et al.*, 1995; Mallory *et al.*, 1997; Mo *et al.*, 2002). Of note, the HSV gE gene lies in the S component of the genome, proximal to gD; as stated above, VZV lacks the gD gene. Instrumental to our understanding of the role of gE are the results of VZV glycoprotein cell–cell fusion assays. In transfected cells, fusion is induced by coexpression of either gH-gL or of gB-gE (Duus *et al.*, 1995; Duus and Grose, 1996; Maresova *et al.*, 2001). With regard to genome stability, VZV is considered to be one of the more genetically stable herpesviruses. However, viral mutants carrying missense mutations in the gE ectodomain are being isolated from humans; one of them is more fusogenic in cell cultures and in the SCID-hu mice (Santos *et al.*, 1998, 2000). Cumulatively, both circumstantial and genetic evidence supports the possibility that VZV gE subsumes at least some of roles of HSV gD.

Endocytosis of the VZV glycoproteins gE, gB, gH, and the negative regulation of fusion

Three VZV glycoproteins carry functional tyrosine-based endocytosis motifs; they are gE, gB and gH.

The gE cytoplasmic tail has a YAGL sequence beginning with a tyrosine residue 582. As determined by mutagenesis studies, the tyrosine residue is part of a conserved YXXL endocytosis motif. The internalized gE trafficks to the trans-Golgi or is recycled to the cell surface. In addition, the C-tail also contains phosphorylation sites (Kenyon *et al.*, 2002). It has been suggested that serine/threonine and tyrosine phosphorylation of gE may serve as sorting signals for internalized receptors and that formation of a gE–gI complex facilitates gE endocytosis (Olson and Grose, 1997; Olson *et al.*, 1998).

VZV gB contains three predicted endocytosis motifs within its cytoplasmic domain: YMTL (aa 818–821), YSRV (aa 857–860), and LL (aa 841–842). Both tyrosine-based motifs mediate gB internalization, but only the YSRV motif is absolutely required for endocytosis. The YMTL motif functions in trafficking of internalized gB to its subsequent localization in the trans Golgi. The third potential endocytosis motif is a dileucine sequence, whose function is under study (Heineman and Hall, 2001). Of note, VZV gI, the partner of VZV gE, also contains a dileucine endocytosis motif in its C-tail (Olson and Grose, 1998).

Like VZV gE and gB, VZV gH contains a functional but previously unrecognized tyrosine based YNKI motif in its short cytoplasmic tail, which mediates clathrin-dependent and antibody-independent endocytosis. Alignment analysis of the VZV gH cytoplasmic tail with other herpesvirus gH homologues reveals two interesting features: (i) herpes simplex virus types 1 and 2 homologues lack an endocytosis motif while all other alphaherpesvirus gH homologues contain a potential motif, and (ii) the VZV gH C-tail is actually longer than predicted in the original sequence analysis and thus can provide the proper context for a functional endocytosis motif (Pasieka *et al.*, 2003). Surprisingly, the endocytosis-deficient VZV gH mutant plasmid effects greater cell-cell fusion than the wild-type gH plasmid. This result leads to the conclusion that VZV gH endocytosis represents a mechanism through which cell–cell fusion is negatively regulated, i.e., by modulating the amount of fusogenic gH on the cell surface (Pasieka *et al.*, 2004). In this respect, therefore, VZV gH shares a basic mechanism of negative regulation of fusion with HSV gB.

Cumulatively, this comparison of the VZV and HSV-1 systems is very instructive as it highlights that both viruses have evolved an essentially similar mechanism of control of fusion, based on endocytosis and consequent limitation of cell surface expression of the fusion executors themselves. A notable difference between the two viruses is that this type of control appears to be is exerted in HSV-1 mainly by gB and in VZV mainly by gH.

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REFERENCES

Avitabile, E., Ward, P. L., Di Lazzaro, C., Torrisi, M. R., Roizman, B., and Campadelli-Fiume, G. (1994). The herpes simplex virus UL20 protein compensates for the differential disruption of exocytosis of virions and membrane glycoproteins associated with fragmentation of the Golgi apparatus. *J. Virol.*, **68**, 7397–7405.

Avitabile, E., Lombardi, G., and Campadelli-Fiume, G. (2003). Herpes simplex virus glycoprotein K, but not its syncytial allele, inhibits cell–cell fusion mediated by the four fusogenic glycoproteins, gD, gB, gH and gL. *J. Virol.*, **77**, 6836–6844.

Avitabile, E., Lombardi, G., Gianni, T., Capri, M., and Campadelli-Fiume, G. (2004). Coexpression of UL20p and gK inhibits cell–cell fusion mediated by herpes simplex virus glycoproteins gD, gH-gL, and wt-gB or an endocytosis-defective gB mutant, and downmodulates their cell surface expression. *J. Virol.*, **78**, 8015–8025.

Baines, J. D., Ward, P. L., Campadelli-Fiume, G., and Roizman, B. (1991). The UL20 gene of herpes simplex virus 1 encodes a function necessary for viral egress. *J. Virol.*, **65**, 6414–6424.

Batterson, W., Furlong, D., and Roizman, B. (1983). Molecular genetics of herpes simplex virus. VIII. Further characterization of a temperature-sensitive mutant defective in release of viral DNA and in other stages of the viral reproductive cycle. *J. Virol.*, **45**, 397–407.

Beitia Ortiz de Zarate, I., Kaelin, K., and Rozenberg, F. (2004). Effects of mutations in the cytoplasmic domain of herpes simplex virus type 1 glycoprotein B on intracellular transport and infectivity. *J. Virol.*, **78**, 1540–1551.

Bender, F. C., Whitbeck, J. C., Ponce de Leon, M., Lou, H., Eisenberg, R. J., and Cohen, G. H. (2003). Specific association of glycoprotein B with lipid rafts during herpes simplex virus entry. *J. Virol.*, **77**, 9542–9552.

Browne, H., Bruun, B., Whiteley, A., and Minson, T. (2003). Analysis of the role of the membrane-spanning and cytoplasmic tail domains of herpes simplex virus type 1 glycoprotein D in membrane fusion. *J. Gen. Virol.*, **84**, 1085–1089.

Bzik, D. J., Fox, B. A., DeLuca, N. A., and Person, S. (1984). Nucleotide sequence of a region of the herpes simplex virus type 1 gB glycoprotein gene: mutations affecting rate of virus entry and cell fusion. *Virology*, **137**, 185–190.

Cai, W. Z., Person, S., Warner, S. C., Zhou, J. H., and DeLuca, N. A. (1987). Linker-insertion nonsense and restriction-site deletion

mutations of the gB glycoprotein gene of herpes simplex virus type 1. *J. Virol.*, **61**, 714–721.

Cai, W. Z., Gu, B., and Person, S. (1988). Role of glycoprotein B of herpes simplex virus type 1 in viral entry and cell fusion [published erratum appears in *J. Virol.* 1988 Nov;**62**(11):4438]. *J. Virol.*, **62**, 2596–2604.

Campadelli-Fiume, G., Arsenakis, M., Farabegoli, F., and Roizman, B. (1988). Entry of herpes simplex virus 1 in BJ cells that constitutively express viral glycoprotein D is by endocytosis and results in degradation of the virus. *J. Virol.*, **62**, 159–167.

Campadelli-Fiume, G., Stirpe, D., Boscaro, A. *et al.* (1990). Glycoprotein C-dependent attachment of herpes simplex virus to susceptible cells leading to productive infection. *Virology*, **178**, 213–222.

Campadelli-Fiume, G., Cocchi, F., Menotti, L., and Lopez, M. (2000). The novel receptors that mediate the entry of herpes simplex viruses and animal alphaherpesviruses into cells. *Rev. Med. Virol.*, **10**, 305–319.

Carfi, A., Willis, S. H., Whitbeck, J. C. *et al.* (2001). Herpes simplex virus glycoprotein D bound to the human receptor HveA. *Mol. Cell*, **8**, 169–179.

Cocchi, F., Lopez, M., Menotti, L., Aoubala, M., Dubreuil, P., and Campadelli-Fiume, G. (1998a). The V domain of herpesvirus Ig-like receptor (HIgR) contains a major functional region in herpes simplex virus-1 entry into cells and interacts physically with the viral glycoprotein D. *Proc. Natl Acad. Sci. USA*, **95**, 15700–15705.

Cocchi, F., Menotti, L., Mirandola, P., Lopez, M., and Campadelli-Fiume, G. (1998b). The ectodomain of a novel member of the immunoglobulin superfamily related to the poliovirus receptor has the attibutes of a *bonafide* receptor for herpes simplex viruses 1 and 2 in human cells. *J. Virol.*, **72**, 9992–10002.

Cocchi, F., Lopez, M., Dubreuil, P., Campadelli-Fiume, G., and Menotti, L. (2001). Chimeric nectin1-poliovirus receptor molecules identify a nectin1 region functional in herpes simplex virus entry. *J. Virol.*, **75**, 7987–7994.

Cocchi, F., Fusco, D., Menotti, L. *et al.* (2004). The soluble ectodomain of herpes simplex virus gD contains a membrane-proximal pro-fusion domain and suffices to mediate virus entry. *Proc. Natl Acad. Sci. USA*, **101**, 7445–7450.

Connolly, S. A., Whitbeck, J. J., Rux, A. H. *et al.* (2001). Glycoprotein D homologs in herpes simplex virus type 1, pseudorabies virus, and bovine herpes virus type 1 bind directly to human HveC(nectin-1) with different affinities. *Virology*, **280**, 7–18.

Connolly, S. A., Landsburg, D. J., Carfi, A., Wiley, D. C., Eisenberg, R. J., and Cohen, G. H. (2002). Structure-based analysis of the herpes simplex virus glycoprotein D binding site present on herpesvirus entry mediator HveA (HVEM). *J. Virol.*, **76**, 10894–10904.

Connolly, S. A., Landsburg, D. J., Carfi, A., Wiley, D. C., Cohen, G. H., and Eisenberg, R. J. (2003). Structure-based mutagenesis of herpes simplex virus glycoprotein D defines three critical regions at the gD-HveA/ HVEM binding interface. *J. Virol.*, **77**, 8127–8140.

Connolly, S. A., Landsburg, D. J., Carfi, A. *et al.* (2005). Potential nectin-1 binding site on herpes simplex virus glycoprotein D. *J. Virol.*, **79**, 1282–1295.

Davison, A. J. and Scott, J. E. (1986). The complete DNA sequence of varicella-zoster virus. *J. Gen. Virol.*, **67**(9), 1759–1816.

Desai, P. J., Schaffer, P. A., and Minson, A. C. (1988). Excretion of non-infectious virus particles lacking glycoprotein H by a temperature-sensitive mutant of herpes simplex virus type 1: evidence that gH is essential for virion infectivity. *J. Gen. Virol.*, **69**(6), 1147–1156.

Diefenbach, R. J., Miranda-Saksena, M., Diefenbach, E. *et al.* (2002). Herpes simplex virus tegument protein US11 interacts with conventional kinesin heavy chain. *J. Virol.*, **76**, 3282–3291.

Döhner, K. and Sodeik, B. (2005). The role of the cytoskeleton during viral infection. *Curr. Top. Microbiol. Immunol.*, **285**, 67–108.

Döhner, K., Wolfstein, A., Prank, U. *et al.* (2002). Function of dynein and dynactin in herpes simplex virus capsid transport. *Mol. Biol. Cell*, **13**, 2795–2809.

Dolter, K. E., Goins, W. F., Levine, M., and Glorioso, J. C. (1992). Genetic analysis of type-specific antigenic determinants of herpes simplex virus glycoprotein C. *J. Virol.*, **66**, 4864–4873.

Duus, K. M. and Grose, C. (1996). Multiple regulatory effects of varicella-zoster virus (VZV) gL on trafficking patterns and fusogenic properties of VZV gH. *J. Virol.*, **70**, 8961–8971.

Duus, K. M., Hatfield, C., and Grose, C. (1995). Cell surface expression and fusion by the varicella-zoster virus gH:gL glycoprotein complex: analysis by laser scanning confocal microscopy. *Virology*, **210**, 429–440.

Enquist, L. W., Husak, P. J., Banfield, B. W., and Smith, G. A. (1998). Infection and spread of alphaherpesviruses in the nervous system. *Adv. Virus Res.*, **51**, 237–247.

Fan, Z., Grantham, M. L., Smith, M. S., Anderson, E. S., Cardelli, J. A., and Muggeridge, M. I. (2002). Truncation of herpes simplex virus type 2 glycoprotein B increases its cell surface expression and activity in cell–cell fusion, but these properties are unrelated. *J. Virol.*, **76**, 9271–9283.

Foà-Tomasi, L., Avitabile, E., Boscaro, A. *et al.* (1991). Herpes simplex virus (HSV) glycoprotein H is partially processed in a cell line that expresses the glycoprotein and fully processed in cells infected with deletion or ts mutants in the known HSV glycoproteins. *Virology*, **180**, 474–482.

Forghani, B., Ni, L., and Grose, C. (1994). Neutralization epitope of the varicella-zoster virus gH:gL glycoprotein complex. *Virology*, **199**, 458–462.

Forrester, A., Farrell, H., Wilkinson, G., Kaye, J., Davis Poynter, N., and Minson, T. (1992). Construction and properties of a mutant of herpes simplex virus type 1 with glycoprotein H coding sequences deleted. *J. Virol.*, **66**, 341–348.

Foster, T. P. and Kousoulas, K. G. (1999). Genetic analysis of the role of herpes simplex virus type 1 glycoprotein K in infectious virus production and egress. *J. Virol.*, **73**, 8457–8468.

Foster, T. P., Melancon, J., and Kousoulas, K. (2001a). An alpha-helical domain within the carboxyl terminus of herpes simplex virus type 1 (HSV-1) glycoprotein B (gB) is associated with cell fusion and resistance to heparin inhibition of cell fusion. *Virology*, **287**, 18–29.

Foster, T. P., Rybachuk, G. V., and Kousoulas, K. G. (2001b). Glycoprotein K specified by herpes simplex virus type 1 is expressed on virions as a Golgi complex-dependent glycosylated species and functions in virion entry. *J. Virol.*, **75**, 12431–12438.

Foster, T. P., Alvarez, X., and Kousoulas, K. G. (2003a). Plasma membrane topology of syncytial domains of herpes simplex virus type 1 glycoprotein K (gK): the UL20 protein enables cell surface localization of gK but not gK-mediated cell-to-cell fusion. *J. Virol.*, **77**, 499–510.

Foster, T. P., Rybachuk, G. V., Alvarez, X., Borkhsenious, O., and Kousoulas, K. G. (2003b). Overexpression of gK in gK-transformed cells collapses the Golgi apparatus into the endoplasmic reticulum inhibiting virion egress, glycoprotein transport, and virus-induced cell fusion. *Virology*, **317**, 237–252.

Foster, T. P., Melancon, J. M., Baines, J. D., and Kousoulas, K. G. (2004). The herpes simplex virus type 1 UL20 protein modulates membrane fusion events during cytoplasmic virion morphogenesis and virus-induced cell fusion. *J. Virol.*, **78**, 5347–5357.

Fuller, A. O., Santos, R. E., and Spear, P. G. (1989). Neutralizing antibodies specific for glycoprotein H of herpes simplex virus permit viral attachment to cells but prevent penetration. *J. Virol.*, **63**, 3435–3443.

Fusco, D., Forghieri, C., and Campadelli-Fiume, G. (2005). The pro-fusion domain of herpes simplex virus glycoprotein D (gD) interacts with the gD N terminus and is displaced by soluble forms of viral receptors. *Proc. Natl Acad. Sci. USA*, **102**, 9323–9328.

Galdiero, M., Whiteley, A., Bruun, B., Bell, S., Minson, T., and Browne, H. (1997). Site-directed and linker insertion mutagenesis of herpes simplex virus type 1 glycoprotein H. *J. Virol.*, **71**, 2163–2170.

Galdiero, S., Vitiello, M., D'Isanto, M. *et al.* (2006). Analysis of synthetic peptides from heptad-repeat domains of herpes simplex virus type 1 glycoproteins H and B. *J. Gen. Virol.*, **87**, 1085–1097.

Geraghty, R. J., Krummenacher, C., Cohen, G. H., Eisenberg, R. J., and Spear, P. G. (1998). Entry of alphaherpesviruses mediated by poliovirus receptor-related protein 1 and poliovirus receptor. *Science*, **280**, 1618–1620.

Gerber, S. I., Belval, B. J., and Herold, B. C. (1995). Differences in the role of glycoprotein C of HSV-1 and HSV-2 in viral binding may contribute to serotype differences in cell tropism. *Virology*, **214**, 29–39.

Gianni, T., Campadelli-Fiume, G., and Menotti, L. (2004). Entry of herpes simplex virus mediated by chimeric forms of nectin1 retargeted to endosomes or to lipid rafts occurs through acidic endosomes. *J. Virol.*, **78**, 12268–12276.

Gianni, T., Martelli, P. L., Casadio, R., and Campadelli-Fiume, G. (2005a). The ectodomain of herpes simpex virus glycoprotein H contains a membrane alpha-helix with attributes of an internal fusion peptide, positionally conserved in the Herpesviridae family. *J. Virol.*, **79**, 2931–2940.

Gianni, T., Menotti, L., and Campadelli-Fiume, G. (2005b). A heptad repeat in herpes simplex virus gH, located downstream of the alpha-helix with attributes of a fusion peptide, is critical for virus entry and fusion. *J. Virol.*, **79**, 7042–7049.

Gianni, T., Fato, R., Bergamini, C., Lenaz, G., and Campadelli-Fiume, G. (2006a). Hydrophobic alpha-helices 1 and 2 of herpes simplex virus gH interact with lipids, and their mimetic peptides enhance virus infection and fusion. *J. Virol.*, **80**, 8190–8198.

Gianni, T., Piccoli, A., Bertucci, C., and Campadelli-Fiume, G. (2006b). Heptad repeat 2 in herpes simplex virus-1 gH interacts with heptad repeat 1 and is critical for virus entry and fusion. *J. Virol.*, **80**, 2216–2224.

Gompels, U. and Minson, A. (1986). The properties and sequence of glycoprotein H of herpes simplex virus type 1. *Virology*, **153**, 230–247.

Griffiths, A., Renfrey, S., and Minson, T. (1998). Glycoprotein C-deficient mutants of two strains of herpes simplex virus type 1 exhibit unaltered adsorption characteristics on polarized or non-polarized cells. *J. Gen. Virol.*, **79**(4), 807–812.

Gruenheid, S., Gatzke, L., Meadows, H., and Tufaro, F. (1993). Herpes simplex virus infection and propagation in a mouse L cell mutant lacking heparan sulfate proteoglycans. *J. Virol.*, **67**, 93–100.

Grunewald, K., Desai, P., Winkler, D. C. *et al.* (2003). Three-dimensional structure of herpes simplex virus from cryo-electron tomography. *Science*, **302**, 1396–1398.

Haarr, L., Shukla, D., Rodahl, E., Dal Canto, M. C., and Spear, P. G. (2001). Transcription from the gene encoding the herpesvirus entry receptor nectin-1 (HveC) in nervous tissue of adult mouse. *Virology*, **287**, 301–309.

Harman, A., Browne, H., and Minson, T. (2002). The transmembrane domain and cytoplasmic tail of herpes simplex virus type 1 glycoprotein H play a role in membrane fusion. *J. Virol.*, **76**, 10708–10716.

Harrop, J. A., Reddy, M., Dede, K. *et al.* (1998). Antibodies to TR2 (herpesvirus entry mediator), a new member of the TNF receptor superfamily, block T cell proliferation, expression of activation markers, and production of cytokines. *J. Immunol.*, **161**, 1786–1794.

Heineman, T. C. and Hall, S. L. (2001). VZV gB endocytosis and Golgi localization are mediated by YXXphi motifs in its cytoplasmic domain. *Virology*, **285**, 42–49.

Heldwein, E. E., Lou, H., Bender, F. C., Cohen, G. H., Eisenberg, R. J., and Harrison, S. C. (2006). Crystal structure of glycoprotein B from herpes simplex virus 1. Science, **313**, 217–220.

Herold, B. C., WuDunn, D., Soltys, N., and Spear, P. G. (1991). Glycoprotein C of herpes simplex virus type 1 plays a principal role

in the adsorption of virus to cells and in infectivity. *J. Virol.*, **65**, 1090–1098.

Highlander, S. L., Dorney, D. J., Gage, P. J. *et al.* (1989). Identification of mar mutations in herpes simplex virus type 1 glycoprotein B which alter antigenic structure and function in virus penetration. *J. Virol.*, **63**, 730–738.

Hutchinson, L. and Johnson, D. C. (1995). Herpes simplex virus glycoprotein K promotes egress of virus particles. *J. Virol.*, **69**, 5401–5413.

Hutchinson, L., Browne, H., Wargent, V. *et al.* (1992a). A novel herpes simplex virus glycoprotein, gL, forms a complex with glycoprotein H (gH) and affects normal folding and surface expression of gH. *J. Virol.*, **66**, 2240–2250.

Hutchinson, L., Goldsmith, K., Snoddy, D., Ghosh, H., Graham, F. L., and Johnson, D. C. (1992b). Identification and characterization of a novel herpes simplex virus glycoprotein, gK, involved in cell fusion. *J. Virol.*, **66**, 5603–5609.

Jogger, C. R., Montgomery, R. I., and Spear, P. G. (2004). Effects of linker-insertion mutations in herpes simplex virus 1 gD on glycoprotein-induced fusion with cells expressing HVEM or nectin-1. *Virology*, **318**, 318–326.

Johnson, D. C., Burke, R. L., and Gregory, T. (1990). Soluble forms of herpes simplex virus glycoprotein D bind to a limited number of cell surface receptors and inhibit virus entry into cells. *J. Virol.*, **64**, 2569–2576.

Johnson, R. M. and Spear, P. G. (1989). Herpes simplex virus glycoprotein D mediates interference with herpes simplex virus infection. *J. Virol.*, **63**, 819–827.

Jones, N. A. and Geraghty, R. J. (2004). Fusion activity of lipid-anchored envelope glycoproteins of herpes simplex virus type 1. *Virology*, **324**, 213–228.

Kaye, J. F., Gompels, U. A., and Minson, A. C. (1992). Glycoprotein H of human cytomegalovirus (HCMV) forms a stable complex with the HCMV UL115 gene product. *J. Gen. Virol.*, **73**(10), 2693–2698.

Keller, P. M., Neff, B. J., and Ellis, R. W. (1984). Three major glycoprotein genes of varicella-zoster virus whose products have neutralization epitopes. *J. Virol.*, **52**, 293–297.

Kenyon, T. K., Cohen, J. I., and Grose, C. (2002). Phosphorylation by the varicella-zoster virus ORF47 protein serine kinase determines whether endocytosed viral gE traffics to the trans-Golgi network or recycles to the cell membrane. *J. Virol.*, **76**, 10980–10993.

Kousoulas, K. G., Huo, B., and Pereira, L. (1988). Antibody-resistant mutations in cross-reactive and type-specific epitopes of herpes simplex virus 1 glycoprotein B map in separate domains. *Virology*, **166**, 423–431.

Krummenacher, C., Nicola, A. V., Whitbeck, J. C. *et al.* (1998). Herpes simplex virus glycoprotein D can bind to poliovirus receptor-related protein 1 or herpesvirus entry mediator, two structurally unrelated mediators of virus entry. *J. Virol.*, **72**, 7064–7074.

Krummenacher, C., Rux, A. H., Whitbeck, J. C. *et al.* (1999). The first immunoglobulin-like domain of HveC is sufficient to bind herpes simplex virus gD with full affinity, while the third domain is involved in oligomerization of HveC. *J. Virol.*, **73**, 8127–8137.

Krummenacher, C., Baribaud, I., Ponce De Leon, M. *et al.* (2000). Localization of a binding site for herpes simplex virus glycoprotein D on herpesvirus entry mediator C by using antireceptor monoclonal antibodies. *J. Virol.*, **74**, 10863–10872.

Krummenacher, C., Baribaud, I., Ponce De Leon, M. *et al.* (2004). Comparative usage of herpesvirus entry mediator A and nectin-1 by laboratory strains and clinical isolates of herpes simplex virus. *Virology*, **322**, 286–299.

Krummenacher, C., Supekar, V. M., Whitbeck, J. C. *et al.* (2005). Structure of unliganded HSV gD reveals a mechanism for receptor-mediated activation of virus entry. *EMBO J.*, **24**, 4144–4153.

Kwon, B. S., Tan, K. B., Ni, J. *et al.* (1997). A newly identified member of the tumor necrosis factor receptor superfamily with a wide tissue distribution and involvement in lymphocyte activation. *J. Biol. Chem.*, **272**, 14272–14276.

La, S., Kim, J., Kwon, B. S., and Kwon, B. (2002). Herpes simplex virus type 1 glycoprotein D inhibits T-cell proliferation. *Mol. Cells*, **14**, 398–403.

Ligas, M. W. and Johnson, D. C. (1988). A herpes simplex virus mutant in which glycoprotein D sequences are replaced by beta-galactosidase sequences binds to but is unable to penetrate into cells. *J. Virol.*, **62**, 1486–1494.

Linehan, M. M., Richman, S., Krummenacher, C., Eisenberg, R. J., Cohen, G. H., and Iwasaki, A. (2004). In vivo role of nectin-1 in entry of herpes simplex virus type 1 (HSV-1) and HSV-2 through the vaginal mucosa. *J. Virol.*, **78**, 2530–2536.

Liu, D. X., Gompels, U. A., Foà-Tomasi, L., and Campadelli-Fiume, G. (1993). Human herpesvirus-6 glycoprotein H and L homologs are components of the gp100 complex and the gH external domain is the target for neutralizing monoclonal antibodies. *Virology*, **197**, 12–22.

Lopez, M., Cocchi, F., Menotti, L., Avitabile, E., Dubreuil, P., and Campadelli-Fiume, G. (2000). Nectin2a (PRR2a or HveB) and nectin2d are low-efficiency mediators for entry of herpes simplex virus mutants carrying the Leu25Pro substitution in glycoprotein D. *J. Virol.*, **74**, 1267–1274.

Lopez, M., Cocchi, F., Avitabile, E. *et al.* (2001). Novel, soluble isoform of the herpes simplex virus (HSV) receptor nectin1 (or PRR1-HIgR-HveC) modulates positively and negatively susceptibility to HSV infection. *J. Virol.*, **75**, 5684–5691.

Mabit, H., Nakano, M. Y., Prank, U. *et al.* (2002). Intact microtubules support adenovirus and herpes simplex virus infections. *J. Virol.*, **76**, 9962–9971.

Mallory, S., Sommer, M., and Arvin, A. M. (1997). Mutational analysis of the role of glycoprotein I in varicella-zoster virus replication and its effects on glycoprotein E conformation and trafficking. *J. Virol.*, **71**, 8279–8288.

Manoj, S., Jogger, C. R., Myscofski, D., Yoon, M., and Spear, P. G. (2004). Mutations in herpes simplex virus glycoprotein D that prevent cell entry via nectins and alter cell tropism. *Proc. Natl Acad. Sci. USA*, **101**, 12414–12421.

Manservigi, R., Spear, P. G., and Buchan, A. (1977). Cell fusion induced by herpes simplex virus is promoted and suppressed by different viral glycoproteins. *Proc. Natl Acad. Sci. USA*, **74**, 3913–3917.

Maresova, L., Pasieka, T. J., and Grose, C. (2001). Varicella-zoster Virus gB and gE coexpression, but not gB or gE alone, leads to abundant fusion and syncytium formation equivalent to those from gH and gL coexpression. *J. Virol.*, **75**, 9483–9492.

Marozin, S., Prank, U., and Sodeik, B. (2004). Herpes simplex virus type 1 infection of polarized epithelial cells requires microtubules and access to receptors at cell–cell contact sites. *J. Gen. Virol.*, **85**, 775–786.

Martinez, W. M. and Spear, P. G. (2002). Amino acid substitutions in the V domain of nectin-1 (HveC) that impair entry activity for herpes simplex virus types 1 and 2 but not for Pseudorabies virus or bovine herpesvirus 1. *J. Virol.*, **76**, 7255–7262.

Mata, M., Zhang, M., Hu, X., and Fink, D. J. (2001). HveC (nectin-1) is expressed at high levels in sensory neurons, but not in motor neurons, of the rat peripheral nervous system. *J. Neurovirol.*, **7**, 476–480.

Matsushima, H., Utani, A., Endo, H. *et al.* (2003). The expression of nectin-1alpha in normal human skin and various skin tumours. *Br. J. Dermatol.*, **148**, 755–762.

Mauri, D. N., Ebner, R., Montgomery, R. I. *et al.* (1998). LIGHT, a new member of the TNF superfamily, and lymphotoxin alpha are ligands for herpesvirus entry mediator. *Immunity*, **8**, 21–30.

McGeoch, D. J., Dalrymple, M. A., Davison, A. J. *et al.* (1988). The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. *J. Gen. Virol.*, **69**, 1531–1574.

Melancon, J. M., Foster, T. P., and Kousoulas, K. G. (2004). Genetic analysis of the herpes simplex virus type 1 UL20 protein domains involved in cytoplasmic virion envelopment and virus-induced cell fusion. *J. Virol.*, **78**, 7329–7343.

Menotti, L., Lopez, M., Avitabile, E. *et al.* (2000). The murine homolog of human-Nectin1d serves as a species non-specific mediator for entry of human and animal aherpesviruses in a pathway independent of a detectable binding to gD. *Proc. Natl Acad. Sci. USA*, **97**, 4867–4872.

Menotti, L., Avitabile, E., Dubreuil, P., Lopez, M., and Campadelli-Fiume, G. (2001). Comparison of murine and human nectin1 binding to herpes simplex virus glycoprotein D (gD) reveals a weak interaction of murine nectin1 to gD and a gD-dependent pathway of entry. *Virology*, **282**, 256–266.

Menotti, L., Casadio, R., Bertucci, C., Lopez, M., and Campadelli-Fiume, G. (2002a). Substitution in the murine nectin1 receptor of a single conserved amino acid at a position distal from herpes simplex virus gD binding site confers high affinity binding to gD. *J. Virol.*, **76**, 5463–5471.

Menotti, L., Cocchi, F., and Campadelli-Fiume, G. (2002b). Critical residues in the CC′ ridge of the human nectin1 receptor V domain enable herpes simplex virus entry into the cell and act synergistically with the downstream region. *Virology*, **301**, 6–12.

Milne, R. S., Connolly, S. A., Krummenacher, C., Eisenberg, R. J., and Cohen, G. H. (2001). Porcine HveC, a member of the highly conserved HveC/nectin 1 family, is a functional alphaherpesvirus receptor. *Virology*, **281**, 315–328.

Milne, R. S., Hanna, S. L., Rux, A. H., Willis, S. H., Cohen, G. H., and Eisenberg, R. J. (2003). Function of herpes simplex virus type 1 gD mutants with different receptor-binding affinities in virus entry and fusion. *J. Virol.*, **77**, 8962–8972.

Mo, C., Lee, J., Sommer, M., Grose, C., and Arvin, A. M. (2002). The requirement of varicella zoster virus glycoprotein E (gE) for viral replication and effects of glycoprotein I on gE in melanoma cells. *Virology*, **304**, 176–186.

Montalvo, E. A. and Grose, C. (1986). Neutralization epitope of varicella zoster virus on native viral glycoprotein gp118 (VZV glycoprotein gpIII). *Virology*, **149**, 230–241.

Montgomery, R. I., Warner, M. S., Lum, B. J., and Spear, P. G. (1996). Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. *Cell*, **87**, 427–436.

Nicola, A. V. and Straus, S. E. (2004). Cellular and viral requirements for rapid endocytic entry of herpes simplex virus. *J. Virol.*, **78**, 7508–7517.

Nicola, A. V., Peng, C., Lou, H., Cohen, G. H., and Eisenberg, R. J. (1997). Antigenic structure of soluble herpes simplex virus (HSV) glycoprotein D correlates with inhibition of HSV infection. *J. Virol.*, **71**, 2940–2946.

Nicola, A. V., McEvoy, A. M., and Straus, S. E. (2003). Roles for endocytosis and low pH in herpes simplex virus entry into HeLa and Chinese hamster ovary cells. *J. Virol.*, **77**, 5324–5332.

Ojala, P. M., Sodeik, B., Ebersold, M. W., Kutay, U., and Helenius, A. (2000). Herpes simplex virus type 1 entry into host cells: reconstitution of capsid binding and uncoating at the nuclear pore complex in vitro. *Mol. Cell Biol.*, **20**, 4922–4931.

Olson, J. K. and Grose, C. (1997). Endocytosis and recycling of varicella-zoster virus Fc receptor glycoprotein gE: internalization mediated by a YXXL motif in the cytoplasmic tail. *J. Virol.*, **71**, 4042–4054.

Olson, J. K. and Grose, C. (1998). Complex formation facilitates endocytosis of the varicella-zoster virus gE:gI Fc receptor. *J. Virol.*, **72**, 1542–1551.

Olson, J. K., Santos, R. A., and Grose, C. (1998). Varicella-zoster virus glycoprotein gE: endocytosis and trafficking of the Fc receptor. *J. Infect. Dis.*, **178** Suppl 1, S2–S6.

Pasieka, T. J., Maresova, L., and Grose, C. (2003). A functional YNKI motif in the short cytoplasmic tail of varicella-zoster virus glycoprotein gH mediates clathrin-dependent and antibody-independent endocytosis. *J. Virol.*, **77**, 4191–4204.

Pasieka, T. J., Maresova, L., Shiraki, K., and Grose, C. (2004). Regulation of varicella-zoster virus-induced cell-to-cell fusion by the endocytosis-competent glycoproteins gH and gE. *J. Virol.*, **78**, 2884–2896.

Patera, A., Ali, M. A., Tyring, S. *et al.* (2002). Polymorphisms in the genes for herpesvirus entry. *J. Infect. Dis.*, **186**, 444–445.

Pellett, P. E., Kousoulas, K. G., Pereira, L., and Roizman, B. (1985). Anatomy of the herpes simplex virus 1 strain F glycoprotein

B gene: primary sequence and predicted protein structure of the wild type and of monoclonal antibody-resistant mutants. *J. Virol.*, **53**, 243–253.

Peng, T., Ponce de Leon, M., Novotny, M. J. *et al.* (1998). Structural and antigenic analysis of a truncated form of the herpes simplex virus glycoprotein gH-gL complex. *J. Virol.*, **72**, 6092–6103.

Pereira, L., Ali, M., Kousoulas, K., Huo, B., and Banks, T. (1989). Domain structure of herpes simplex virus 1 glycoprotein B: neutralizing epitopes map in regions of continuous and discontinuous residues. *Virology*, **172**, 11–24.

Qadri, I., Gimeno, C., Navarro, D., and Pereira, L. (1991). Mutations in conformation-dependent domains of herpes simplex virus 1 glycoprotein B affect the antigenic properties, dimerization, and transport of the molecule. *Virology*, **180**, 135–152.

Reymond, N., Borg, J., Lecocq, E. *et al.* (2000). Human nectin3/PRR3: a novel member of the PVR/PRR/nectin family that interacts with afadin. *Gene*, **255**, 347–355.

Richart, S., Simpson, S., Krummenacher, C. *et al.* (2003). Entry of herpes simplex virus type 1 into primary sensory neurons in vitro is mediated by Nectin-1/HveC. *J. Virol.*, **77**, 3307–3311.

Roberts, S. R., Ponce de Leon, M., Cohen, G. H., and Eisenberg, R. J. (1991). Analysis of the intracellular maturation of the herpes simplex virus type 1 glycoprotein gH in infected and transfected cells. *Virology*, **184**, 609–624.

Roche, S., Bressanelli, S., Rey, F. A., and Gaudin, Y. (2006). Crystal structure of the low-pH form of the vesicular stomatitis virus glycoprotein G. *Science*, **313**, 187–191.

Roop, C., Hutchinson, L., and Johnson, D. C. (1993). A mutant herpes simplex virus type 1 unable to express glycoprotein L cannot enter cells, and its particles lack glycoprotein H. *J. Virol.*, **67**, 2285–2297.

Rux, A. H., Lou, H., Lambris, J. D., Friedman, H. M., Eisenberg, R. J., and Cohen, G. H. (2002). Kinetic analysis of glycoprotein C of herpes simplex virus types 1 and 2 binding to heparin, heparan sulfate, and complement component C3b. *Virology*, **294**, 324–332.

Santos, R. A., Padilla, J. A., Hatfield, C., and Grose, C. (1998). Antigenic variation of varicella zoster virus Fc receptor gE: loss of a major B cell epitope in the ectodomain. *Virology*, **249**, 21–31.

Santos, R. A., Hatfield, C. C., Cole, N. L. *et al.* (2000). Varicella-zoster virus gE escape mutant VZV-MSP exhibits an accelerated cell-to-cell spread phenotype in both infected cell cultures and SCID-hu mice. *Virology*, **275**, 306–317.

Schelhaas, M., Jansen, M., Haase, I., and Knebel-Morsdorf, D. (2003). Herpes simplex virus type 1 exhibits a tropism for basal entry in polarized epithelial cells. *J. Gen. Virol.*, **84**, 2473–2484.

Shukla, D. and Spear, P. G. (2001). Herpesviruses and heparan sulfate: an intimate relationship in aid of viral entry. *J. Clin. Invest.*, **108**, 503–510.

Shukla, D., Liu, J., Blaiklock, P. *et al.* (1999). A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry. *Cell*, **99**, 13–22.

Shukla, D. Dal Canto, M. C., Rowe, C. L., and Spear, P. G. (2000). Striking Similarity of murine nectin-1alpha to human nectin-1alpha (HveC) in sequence and activity as a glycoprotein D receptor for alphaherpesvirus entry. *J. Virol.*, **74**, 11773–11781.

Smith, G. A., Gross, S. P., and Enquist, L. W. (2001). Herpesviruses use bidirectional fast-axonal transport to spread in sensory neurons. *Proc. Natl Acad. Sci. USA*, **98**, 3466–3470.

Sodeik, B. (2000). Mechanisms of viral transport in the cytoplasm. *Trends Microbiol*, **8**, 465–472.

Sodeik, B., Ebersold, M. W., and Helenius, A. (1997). Microtubule-mediated transport of incoming herpes simplex virus 1 capsids to the nucleus. *J. Cell Biol.*, **136**, 1007–1021.

Spear, P. G., Shieh, M. T., Herold, B. C., WuDunn, D., and Koshy, T. I. (1992). Heparan sulfate glycosaminoglycans as primary cell surface receptors for herpes simplex virus. *Adv. Exp. Med. Biol.*, **313**, 341–353.

Spear, P. G., Eisenberg, R. J., and Cohen, G. H. (2000). Three classes of cell surface receptors for alphaherpesvirus entry. *Virology*, **275**, 1–8.

Struyf, F., Posavad, C. M., Keyaerts, E., Van Ranst, M., Corey, L., and Spear, P. G. (2002). Search for polymorphisms in the genes for herpesvirus entry mediator, nectin-1, and nectin-2 in immune seronegative individuals. *J. Infect. Dis.*, **185**, 36–44.

Takai, Y., Irie, K., Shimizu, K., Sakisaka, T., and Ikeda, W. (2003). Nectins and nectin-like molecules: roles in cell adhesion, migration, and polarization. *Cancer Sci.*, **94**, 655–667.

Tal-Singer, R., Peng, C., Ponce De Leon, M. *et al.* (1995). Interaction of herpes simplex virus glycoprotein gC with mammalian cell surface molecules. *J. Virol.*, **69**, 4471–4483.

Turner, A., Bruun, B., Minson, T., and Browne, H. (1998). Glycoproteins gB, gD, and gHgL of herpes simplex virus type 1 are necessary and sufficient to mediate membrane fusion in a Cos cell transfection system. *J. Virol.*, **72**, 873–875.

Ward, P. L., Campadelli-Fiume, G., Avitabile, E., and Roizman, B. (1994). Localization and putative function of the UL20 membrane protein in cells infected with herpes simplex virus 1. *J. Virol.*, **68**, 7406–7417.

Warner, M. S., Geraghty, R. J., Martinez, W. M. *et al.* (1998). A cell surface protein with herpesvirus entry activity (HveB) confers susceptibility to infection by mutants of herpes simplex virus type 1, herpes simplex virus type 2, and pseudorabies virus. *Virology*, **246**, 179–189.

Whitbeck, J. C., Muggeridge, M. I., Rux, A. H. *et al.* (1999). The major neutralizing antigenic site on herpes simplex virus glycoprotein D overlaps a receptor-binding domain. *J. Virol.*, **73**, 9879–9890.

Willis, S. H., Rux, A. H., Peng, C. *et al.* (1998). Examination of the kinetics of herpes simplex virus glycoprotein D binding to the herpesvirus entry mediator, using surface plasmon resonance. *J. Virol.*, **72**, 5937–5947.

WuDunn, D. and Spear, P. G. (1989). Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. *J. Virol.*, **63**, 52–58.

Yoon, M., Zago, A., Shukla, D., and Spear, P. G. (2003). Mutations in the N termini of herpes simplex virus type 1 and 2 gDs alter functional interactions with the entry/fusion receptors HVEM,

nectin-2, and 3-O-sulfated heparan sulfate but not with nectin-1. *J. Virol.*, **77**, 9221–9231.

Zhou, G. and Roizman, B. (2006). Construction and properties of a herpes simplex virus 1 designed to enter cells solely via the IL-13alpha2 receptor. *Proc. Natl Acad. Sci. USA*, **103**, 5508–5513.

Zhou, G., Avitabile, E., Campadelli-Fiume, G., and Roizman, B. (2003). The domains of glycoprotein D required to block apoptosis induced by herpes simplex virus 1 are largely distinct from those involved in cell-cell fusion and binding to nectin1. *J. Virol.*, **77**, 3759–3767.

8

Early events pre-initiation of alphaherpes viral gene expression

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The regulated transcription of the HSV IE (immediate–early, α) genes has been a model system for elucidating principles and mechanisms of combinatorial-differential regulation, basic RNAPII-directed transcription, and multiprotein assembly specificities. The regulation exemplifies viral mechanisms dedicated to the recruitment of cellular components into complex viral–host interactions that illustrate general parameters of protein–protein, DNA–protein, RNA transcription, and protein complex assembly. Continued studies hold promise of advancing the understanding of the complexities of biochemical interactions in gene expression as well as complex cellular response pathways. The regulation of the IE genes within specific contexts may also lead to the understanding of signals and pathways which modulate viral infection and determine the extent of lytic-latent infection. While HSV has been extensively studied and will represent the focus of this review, the regulatory domain of the VZV IE gene (IE62) contains similar elements and is regulated by similar mechanisms.

The HSV IE regulatory domains: multiple sites for differential regulation

The regulatory domains of the HSV IE genes have been the focus of numerous studies that have defined the sequence elements and their contributions to the basal and induced levels of transcription. These IE domains typically consist of a reiterated inducible enhancer core element (consensus: TAATGARAT) that is flanked by binding sites for members of the ets and kruppel transcription family (Fig. 8.1, left) (Roizman and Sears, 1996; Vogel and Kristie, 2001).

The primary focus has been on the regulated induction of the expression of the IE genes by the HSV IE transactivator (VP16, αTIF, ICP25) via the enhancer core element (Phillips and Luisi, 2000; Roizman and Sears, 1996; Roizman and Sears, 1996; Vogel and Kristie, 2001; Vogel *et al.*, 2001; Wysocka and Herr, 2003). This focus led to the identification of cellular proteins (Oct-1 and HCF-1) that are required for the stable enhancer complex assembly and the induction of the IE gene transcription (Kristie *et al.*, 1989, 1995; Kristie and Sharp, 1993; Roizman and Sears, 1996; Vogel and Kristie, 2001; Vogel *et al.*, 2001; Wilson *et al.*, 1993; Wysocka and Herr, 2003). Extensive characterization of these components illustrates the multiple levels of complex regulatory interactions inherent in this process.

The assembly of the HSV IE enhancer core complex

The assembly of the HSV IE enhancer core complex illustrates the basic elements of the specificities governing the regulatory process. The stages of the assembly are modeled upon in vitro studies of protein–DNA recognition, protein-protein interactions, selective recognition, affinities, and cooperative interactions. As shown in Fig. 8.1 (Right), the cellular Oct-1 POU domain protein recognizes the divergent octamer element in the IE core and provides a nucleation point for the association of the heterodimeric protein complex consisting of VP16 and HCF-1. Specificity for the HSV IE element is determined by selective recognition of the Oct-1 POU-homeobox and the enhancer core DNA sequences by VP16 while the complex is stabilized by HCF-1 interactions. The full enhancer domain complex contains additional cellular transcription factors such as GABP and Sp1. The activation potential is dependent upon the core complex, likely through additional protein–protein interactions. The complexity of the regulatory complex provides for multiple levels of regulation dictated by various protein combinations, interactions, and the specific regulation of the individual components. Therefore, each component

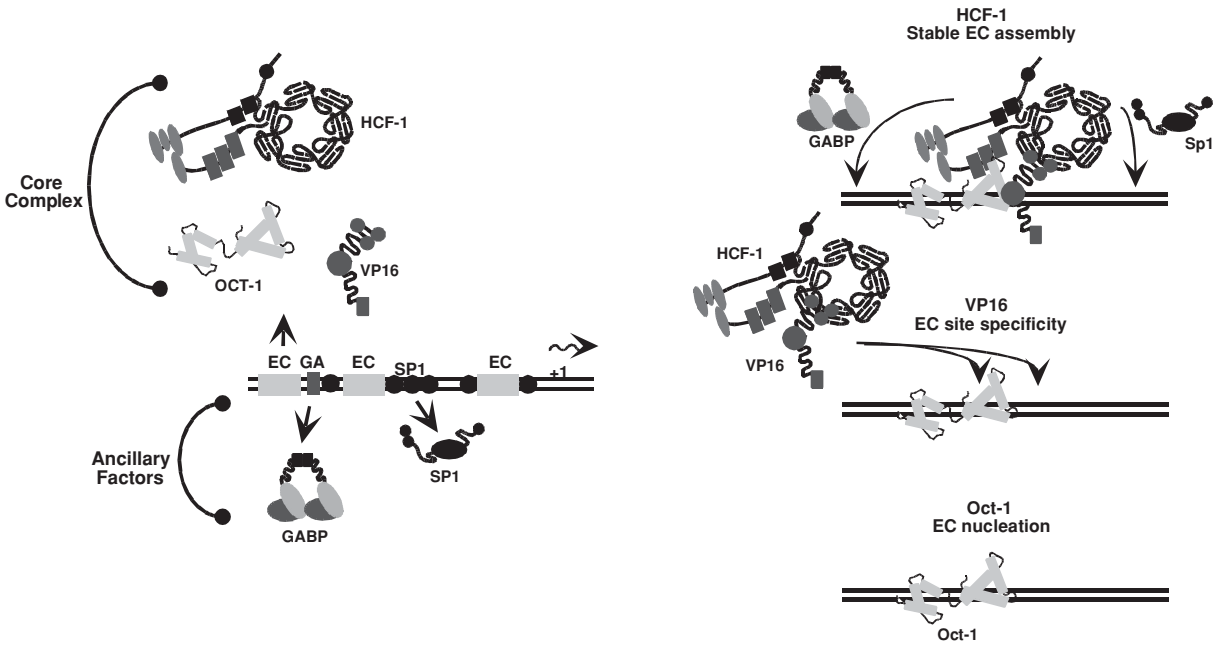

Fig. 8.1. The HSV IE enhancer domain elements, components, and assembly process. (Left) Representation of a typical HSV IE enhancer-promoter domain containing the reiterated enhancer core (EC), GABP (GA), and Sp1 (Sp1) binding sites. The enhancer core components (HCF-1, Oct-1, VP16) and ancillary transcription factors (GABP, Sp1) are represented. (Right) Specificities and interactions in the assembly of the multiprotein enhancer core complex. Oct-1 binds to the octamer element in the 5′ end of the ATGCTAATGARAT enhancer core and nucleates the association of the heteromeric HCF-1/VP16 protein complex. VP16 cooperatively interacts with the Oct-1 POU-homeobox and recognized the 3′ sequences of the core element. The stable enhancer core complex may recruit or interact with the additional factors to form the fully assembled enhancer complex.

will be described with respect to its defined structure, function, interactions, and regulatory mechanisms.

Oct-1

Oct-1 is a representative of the POU family of proteins (Pit, Oct, unc86) containing bipartite DNA binding domains (POU-specific box, POUs and POU-homeobox, POUh) consisting of helix-turn-helix structures that cooperate to recognize the consensus element (ATGCAAAT) (Clerc *et al.*, 1988; Phillips and Luisi, 2000; Sturm *et al.*, 1988). The structure and DNA contacts of the POU-specific box are analogous to the λ repressor in which all four helices make significant network DNA backbone contacts while DNA recognition by the POU-homeobox is characteristic of homeodomains in which helix 3 lies in the major groove and provides sequence recognition. The two DNA binding units recognize bases (POUs, ATGC; POUh, AAAT) in the major groove on opposite sides of the helix and are separated by a flexible and disordered linker region (Fig. 8.2-Oct-1) (Klemm *et al.*, 1994).

In the case of the HSV IE domains, Oct-1 nucleates the enhancer core assembly by binding the divergent octamer sequences of the core consensus (ATGCTAAT). Selective recognition of the Oct-1 POU-homeobox by VP16 was elucidated by comparative analysis of Oct-1 and the highly related Oct-2 protein. While only seven amino acids differ between the exposed homeodomain surfaces of these two proteins, VP16 selectively interacts with Oct-1(Lai *et al.*, 1992; Pomerantz *et al.*, 1992). As shown (Fig. 8.2-Oct-1), four amino acids (K18, S19, E22, E30) in helixes 1 and 2 are required for high affinity recognition by VP16. Strikingly, only the single position 22 (Oct-1, E22) determines the specificity that accounts for the affinity difference in the recognition of Oct-1 over Oct-2 by VP16. This selective recognition by VP16 elucidated a principle of homeobox protein interaction and specificity that has been reflected in multiple Oct-1 interactions in differential positive, negative, and inducible regulatory events. Studies addressing these contextual interactions elucidate the functions of the protein and illustrate the various diverse mechanisms used to regulate these processes:

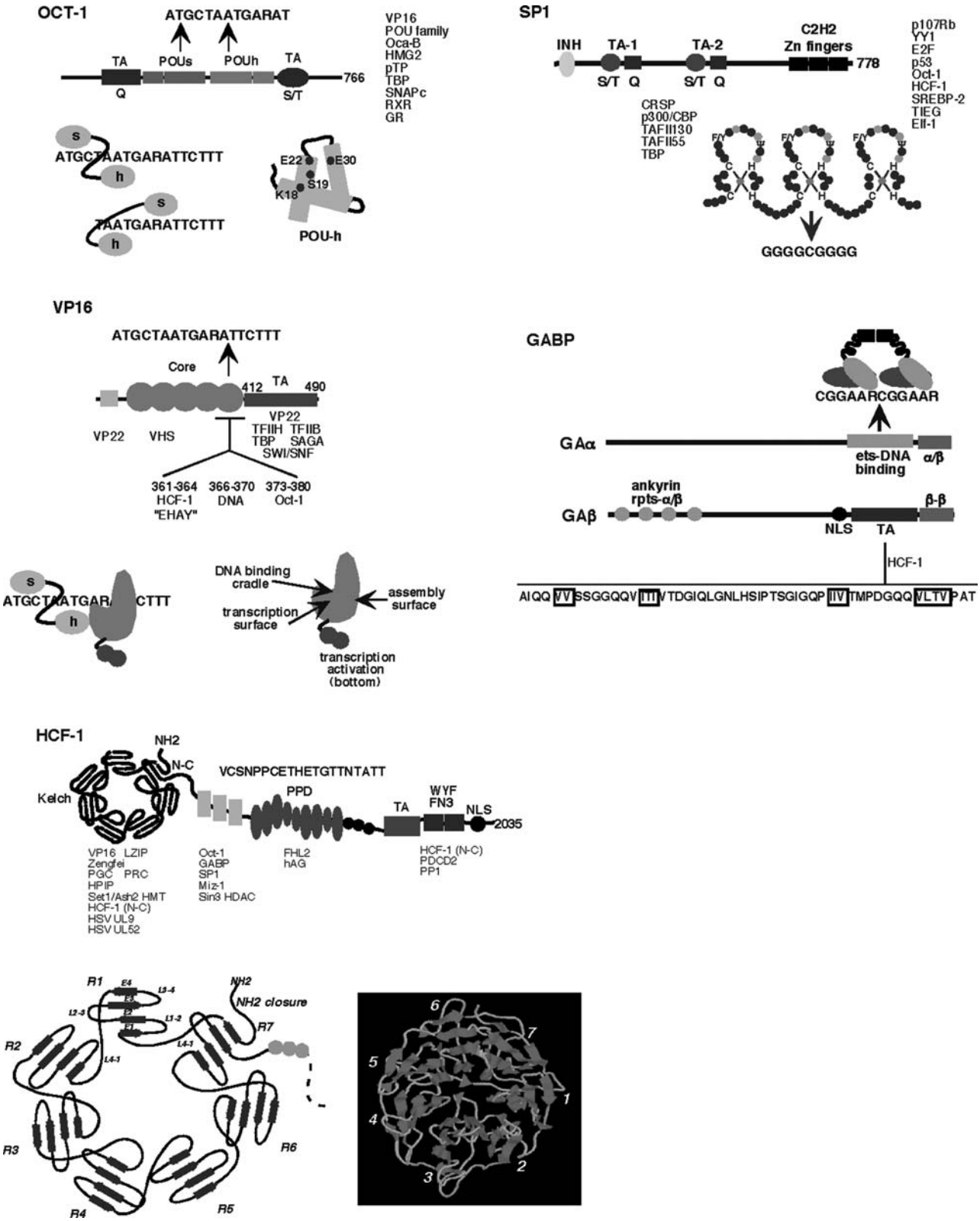

Fig. 8.2. The protein components of the HSV IE enhancer complex.
Oct-1: The transactivation (TA-Q and TA-S/T) and POU (POUs and POUh) domains are shown. The POU-specific box recognizes ATGC while the POU-homeobox recognizes TAAT within the enhancer core element (ATGCTAATGARAT). Proteins that bind to the Oct-1 POU domain are listed. The inherent flexibility of the POU domain and the potential orientations of the POUs box in recognition of the core element are depicted. In the schematic representation of the Oct-1 POU-homeobox, the residues which are important for the recognition by VP16 are indicated. (*cont.*)

(i) The stimulation of transcription preinitiation complex formation via the interaction of the Oct-1 POU domain with TBP in a non-DNA dependent manner has suggested that the interaction enhances TBP-TATA box binding by alleviating TBP autorepression, thus resulting in synergistic activation of octamer containing promoters (Mittal *et al.*, 1999; Zwilling *et al.*, 1994). Similarly, snRNA transcription is regulated via Oct-1/SNAPc interaction in which SNAPc is recruited to the snRNA proximal element via interactions that relieve SNAPc autorepression (Mittal *et al.*, 1999). The interaction of Oct-1 POU with SNAPc utilizes the same POU interaction surface as is bound by specific Oct-1 coactivators illustrating that the DNA site context is a determining factor in the selection of cofactors and coactivators (Hovde *et al.*, 2002).

(ii) Oct-1 stimulates transcription from TATA-less promoters via recruitment of TFIIB, functioning in lieu of TBP to orient the basal factor for RNAPII positioning (Phillips and Luisi, 2000).

(iii) Numerous synergistic interactions with other transcription factors also determine the roles of Oct-1 in positive, negative, and inducible regulation of gene expression. This regulation may be DNA dependent, promoter context dependent, or via DNA-independent protein-protein interactions. At the mouse mammary tumor virus (MMTV) promoter, interaction of the glucocorticoid receptor with the Oct-1 POU domain mediates glucocorticoid induction. Similarly, interaction with the androgen receptor promotes SRC-1 coactivator recruitment and transcription enhancement (Phillips and Luisi, 2000). In contrast, Oct-1 POU-homeobox interaction with the zinc finger domain of RXR disrupts the thyroid hormone receptor TR/RXR heterodimer, leading to repression of TR-dependent transcription (Gonzalez and Robins, 2001).

At the cyclin D1 promoter, interaction of CREB with the Oct-1 POU domain occurs independently of an octamer element and results in CBP coactivator recruitment in the absence of the normally responsive mechanism that depends upon CREB phosphorylation (Boulon *et al.*, 2002). Induction of GADD45 following UV-induced DNA damage is a second example of Oct-1 mediated regulation that is distinct from the defined primary p53 dependent regulatory mechanism (Jin *et al.*, 2001).

(iv) Another level of regulation is exemplified by Oct-1 interactions with cell-type or process-specific coactivators (Phillips and Luisi, 2000; Wysocka and Herr, 2003). The stimulation of immunoglobulin gene transcription by Oct-1 is mediated by binding of the B-cell specific coactivator OCA-B that interacts in a hydrophobic pocket with both subdomains of the Oct-1-POU domain. This interaction requires the Oct-1-DNA assembly, preferentially interacting with Oct-1 bound to the consensus ATGCAAAT vs. the HSV ATGCTAAT. The selection of site-specific Oct-1-DNA complexes by OCA-B is determined by binding a different surface of the Oct-1 POUs/POUh subdomains than VP16 that is positioned to the center of the consensus octamer element and includes OCA-B-DNA contacts (Babb *et al.*, 1997; Chasman *et al.*, 1999). Recent studies have identified a second process-specific coactivator, OCA-S that is involved in the S-phase, cell-cycle dependent stimulation of the histone H2B promoter by Oct-1. Strikingly, this coactivator contains the enzyme GAPDH that may link cellular metabolism to cell growth and division (Zheng *et al.*, 2003). These differential interactions illustrate the structural versatility in Oct-1 protein interactions.

(v) Oct-1 also plays a role in the stimulation of DNA replication as exemplified by the binding of the adenovirus pTP-Pol to the POU-homeobox (Coenjaerts *et al.*, 1994; Phillips and Luisi, 2000). The interaction enhances the association of the pTP-pol with the origin-binding complex. In this case, the interactions are via the DNA binding surface of the

Fig. 8.2. (*continued*) *VP16*: The structure and protein interactions of VP16 are represented. The core structure contains the clustered residues that are critical for the assembly of the IE enhancer complex (HCF-1, Oct-1, DNA) while the transactivation domain (TA, aa 412–490) interacts with a number of basal factors and chromatin modifying components. A schematic representation of the VP16 protein structure is shown (left) indicating the various protein interaction surfaces oriented in recognition of the Oct-1 POU-homeobox/ DNA complex.
HCF-1: The amino-terminal kelch, mid-aminoterminal, proteolytic processing (PPD), autocatalytic (Auto), transactivation (TA), WYF-rich, FN3 repeat, and nuclear localization signal (NLS) regions are represented. The proteins that interact with each region are listed below the appropriate domain. The PPD is represented as a series of consensus (large oval) and divergent (small oval) reiterations of the HCF-1 cleavage sequence shown above. (Bottom left) A stylized representation of the HCF-1 kelch domain is shown illustrating the seven predicted blades (antiparallel sheets, E1 through E4; loops, L1–2 through L4–1). For HCF-1, the predicted ring closure utilizes E4 from the animoterminus and E1-2-3 from the carboxyterminus of the domain (NH2 closure). (Bottom right) The derived molecular model of the HCF-1 kelch domain structure is depicted.
Sp1: The inhibitory domain (INH), transactivation domains (TA-1, TA-2), and DNA binding domains (C2H2, Zn fingers) are represented. Proteins or protein complexes that interact with Sp1 are listed. The structure of the C2H2 Zn finger domain is schematically represented: C, cysteine; H, histadine; F/Y, phenylalanine or tyrosine; y, hydrophobic residue. Light circles represent amino acids that are predicted to make DNA contacts.
GABP: The α subunit contains the ets DNA binding domain recognizing the GA box and the heterodimerization domain (α/β). The β subunit contains ankyrin repeats (α/β heterodimerization region), nuclear localization signals (NLS), transactivation domain (TA), and tetramerization sequences (β−β). The sequence of the transactivation domain is shown and the residues that are critical for both transactivation and interaction with HCF-1 are boxed. (See color plate section.)

POU-homeobox and are distinct from those of the VP16-Oct homeobox complex (de Jong *et al.*, 2002).

Clearly there are a number of distinct mechanisms by which Oct-1 may regulate the basal level expression, induction, or repression of target genes via interactions with specific sites, transcription factors, and coactivators that are mediated through the POU domain. In addition, the protein contains two transactivation domains (Fig. 8.2-Oct-1) that flank the POU domain and function in a promoter context dependent manner, suggesting that additional protein interactions at a given promoter further modulate the regulatory process (Tanaka *et al.*, 1992). The multitude of promoter targets and protein interactions and the diverse regulatory mechanisms involving Oct-1 is reflective of an innate flexibility of the protein conferred by the linker segment separating the POU subdomains (Phillips and Luisi, 2000; Wysocka and Herr, 2003). This flexibility allows for the recognition of sequences and contexts that are divergent from the consensus octamer element. As illustrated by binding to both octamer + and octamer – TAATGARAT elements, the flexibility of the Oct-1 POU domain allows for alternative configurations or positions of the POU-specific box relative to the POU-homeobox (Fig. 8.2-Oct-1). This ability allows for interaction of these domains with proteins in nonconsensus configurations. The configuration is dictated by the sequence of the DNA element and can provide distinct interfaces for various coregulators that are promoter dependent.

The activity of Oct-1 is also regulated by a number of post-transcriptional mechanisms including: (i) enhancement of DNA-binding activity following UV-induced DNA damage (Zhao *et al.*, 2000); (ii) phosphorylation by PKA, PKC, and CKII (Grenfell *et al.*, 1996); (iii) interaction with HMG2 which may function to order the DNA-binding domain for high affinity recognition (Zwilling *et al.*, 1995) and (iv) enhanced phosphorylation of Oct-1 via interaction with MAT1 cyclin dependent kinase activating kinase (Inamoto *et al.*, 1997). Similarly additional levels of regulation exist which impact the function of Oct-1 as illustrated by the modulation of the levels of the OCA-B coactivator by the ring finger protein Siah-1 (Tiedt *et al.*, 2001). The various types of interactions and levels of regulation that impinge on the function of Oct-1 will clearly have implications for the function of the protein in the stimulation of the HSV IE genes in different cellular contexts.

VP16

Vp16 (ICP25, VMW65, αTIF) is the HSV-encoded component of the enhancer core complex that determines the specificity for the HSV elements. Approximately 900 molecules of the protein are packaged within the tegument structure of a virion and are released into the cytoplasm of the cell upon infection (Roizman, 1996). The protein is transported to the nucleus and assembled into the stable enhancer core complex. VP16 is a critical component of the complex as it determines the specificity of the HSV IE core complex by direct recognition of the TAATGARAT DNA element and by selective recognition of the DNA bound Oct-1 (Figs. 8.1 and 8.2) (Kristie and Sharp, 1990; Lai *et al.*, 1992; Pomerantz *et al.*, 1992).

The 490 amino acid protein consists of a conserved structural core (aa 49–403) that contains the specificity surfaces for interaction with Oct-1, DNA, and HCF-1 (Lai and Herr, 1997; Simmen *et al.*, 1997; Wysocka and Herr, 2003) as well as a highly characterized transcription activation domain (aa 412–490) that interacts with both basal transcription factors and chromatin remodeling/nucleosome modification factors (Fig. 8.2-VP16). Surprisingly, the protein lacks a nuclear localization signal and nuclear transport is provided by protein interaction with HCF-1 (La Boissiere *et al.*, 1999). As HCF-1 is a key cell-cycle component, this interaction may play a significant role in the initial "sensing" of the cell state for viral replication.

Advances in the determination of the structure of VP16 have provided an understanding of the protein–protein and protein–DNA interactions that are critical for the protein's selective induction of the HSV IE genes. The highly structured core domain consists of internal symmetry that is dominated by two-stranded antiparallel coiled coils. The resulting structure resembles a "seat" complete with bottom, back, and headrest regions (Fig. 8.2-VP16). Interestingly, the region representing the interaction domain for Oct-1, HCF-1, and DNA (aa 350–394) is disordered in the crystal, suggesting that this domain adopts a structure upon binding these components (Babb *et al.*, 2001; Liu *et al.*, 1999). Correlation of the structure with numerous mutagenesis studies clearly indicates that there are distinct surfaces for interaction with components involved in virion assembly (right surface) vs. transcription (left surface) while the DNA recognition surface is formed by a cleft in the seat structure (Fig. 8.2-VP16, bottom right) (Babb *et al.*, 2001; Liu *et al.*, 1999). As different VP16 orthologs such as those encoded by VZV and BHV recognize different TAATGARAT elements dependent upon the 3′ sequences, the VP16 structure presents a model by which the unstructured region may also contribute to DNA binding by adopting a structure to specifically recognize the GARAT sequences while the "seat" recognizes the 3′ portion of the element. Furthermore, the DNA binding model predicts that the Oct-1 POU-specific box may lie to either side of the TAAT in the enhancer complex

(refer to Fig. 8.2 -VP16) and may modulate the VP16-DNA interaction.

The specific residues that are important for the interactions of VP16 in the IE enhancer core complex have been defined by numerous mutagenesis and peptide inhibition studies (Vogel and Kristie, 2001; Wysocka and Herr, 2003). This work elucidated a clustered core of residues for interaction with HCF-1 (E361, H362, Y364) that is now recognized as the HCF-1 interaction (D/E HXY) motif in a number of cellular proteins that interact with this coactivator protein (Freiman and Herr, 1997; Lu *et al.*, 1998; Wysocka and Herr, 2003). Mutations in residues (Y373, G374, S375) affect the VP16- Oct1 interaction while mutations in residues (R360, R366, R368, K370) affect DNA binding (Lai and Herr, 1997). Interestingly, S375 is a target for CKII-dependent phosphorylation. This residue is normally modified in cell extracts and is required for efficient complex formation, suggesting post-transcriptional modulation of the VP16 interactions required for the IE complex assembly (O'Reilly *et al.*, 1997).

The second unstructured region in the crystal determination is the transcription activation domain (TA, aa 412–490) that is located at the "bottom" of the determined structure (Liu *et al.*, 1999). The VP16 TA domain has been perhaps the most utilized tool in studies of the mechanisms involved in transcription activation. Initially, mutagenesis studies defined the TA domain and determined that this region consisted of two distinct subdomains containing important aromatic and hydrophobic residues (Regier *et al.*, 1993). The effects of substitutions at these positions also suggested that the structure of these subdomains were distinct and were likely to be involved in different stages of transcription stimulation. The unstructured nature of the domain both in solution and in the crystal determination suggests that this region also becomes structurally constrained upon protein-protein interactions (Shen *et al.*, 1996).

Many studies on the mechanisms of transcription activation have utilized the VP16 TA domain, generally in the context of a DNA binding domain fusion protein. These studies have elucidated protein interactions and general principles or mechanisms in the regulation of transcription activation. While early studies demonstrated that activators promote "open complex" formation by enhancing the formation of the RNAPII preinitiation complex and promoter assemblies; later analyses addressed the rate limiting stages, protein interactions, and stepwise activation stages that were affected by the TA domain in given promoter contexts. The data from many such studies have resulted in general models for promoter activation via multiple steps which are not necessarily strictly ordered but can be affected by activators including: activator access and binding, histone modification and chromatin remodeling, binding and assembly of the basal factor-RNAPII complexes, open complex formation, RNAPII promoter escape, RNAPII pausing, transcription elongation, coupled transcription-mRNA splicing, and transcription reinitiation (Cosma, 2002).

Studies that focus upon protein interactions of the VP16 TA domain have suggested that the unstructured domain adopts conformations upon binding specific targets. This flexibility allows for numerous conformations and sequential interactions with factors involved in different stages of promoter activation (basal factors TFIIB, TFIIH, TBP; histone modification SAGA; and chromatin remodeling SWI2/SNF2) (Gold *et al.*, 1996; Hall and Struhl, 2002; Herrmann *et al.*, 1996; Herrera and Triezenberg, 2004; Krumm *et al.*, 1995; Memedula and Belmont, 2003; Nishikawa *et al.*, 1997; Vignali *et al.*, 2000; Walker *et al.*, 1993; Xiao *et al.*, 1994; Yudkovsky *et al.*, 2000). Consistent with early studies, the two subregions of the TA domain interact with distinct factors and function in multiple steps or activation stages including: (i) the ATP-dependent chromatin remodeling and histone modifications that allow factor access and promoter targeting (SWI, SAGA, p300) as demonstrated by the ability of the TA domain to mediate large scale chromatin remodeling; (ii) the assembly of the RNAPII preinitiation complex by alleviating TBP autorepression or by direct recruitment of the protein followed by competition with TBP-basal TAFs interactions to promote enhanced activation; (iii) recruitment of TFIIB leading to RNAPII positioning and open complex formation; (iv) recruitment of TFIIH and CTD kinases that mediate RNAPII promoter escape; (v) regulation of the efficiency of the initiation complex formation that functions to increase the elongation competency of the complexes, thereby affecting the coupled mRNA splicing efficiency; and (vi) stabilization of the reinitiation scaffold (TFIID, TFIIA, TFIIE, mediator and TFIIH) via interactions with TFIIH, leading to efficient reassembly and reinitiation.

These studies have resulted in significant advances in the understanding of general transcription initiation and promoter activation and have suggested mechanisms by which the VP16 TA domain may participate in the activation of the IE gene transcription. It remains, however, to be determined exactly what the inherent rate-limiting steps are for the activation of the IE genes and what the contribution(s) of the VP16 TA domain are within this context. In addition, clearly the contributions of all of the core and ancillary factors will be affected by the interplay of the regulatory proteins and signals within a given cellular milieu.

HCF-1

The last required component for the stable assembly of the core enhancer protein complex is the cellular coactivator HCF-1. Originally identified as a required component derived from extracts of insect or mammalian cells for the formation of a stable core complex in vitro, HCF-1 was subsequently biochemically purified and the gene encoding it was cloned (Kristie *et al.*, 1989, 1995; Kristie and Sharp, 1993; Vogel *et al.*, 2001; Wilson *et al.*, 1993; Wysocka *et al.*, 2003). Interestingly, rather than a single polypeptide, the protein is actually a family of polypeptides ranging from 68–230 kD that are derived from a common precursor via site-specific proteolysis (Kristie *et al.*, 1995; Vogel and Kristie, 2000a; Wilson *et al.*, 1993). The protein is ubiquitously expressed and localized in the nucleus of all cell types with a notable exception (discussed below). Numerous studies in recent years have illuminated both the functions of the protein in HSV IE gene expression as well as its functions in basic cellular processes. The importance of both lies in the strict requirement for HCF-1 in the initiation of HSV lytic cycle as well as the importance of the protein in several basic cellular processes that may impact the viral cycle.

As shown in Fig. 8.2, multiple functional domains and protein interactions have been defined which have identified HCF-1 as a critical component of processes such as cell-cycle control, positive and negative transcription regulation, chromatin modulation, DNA replication, and mRNA splicing. Many studies have focused upon the amino terminal domain of the protein as this region is required for the formation of the HSV IE enhancer core complex as well as for cell cycle progression (Goto *et al.*, 1997; Hughes *et al.*, 1999; LaBoissiere *et al.*, 1997; Wilson *et al.*, 1997). The predicted structure of the amino-terminus of HCF-1 is based upon structural alignments to related "kelch" domain proteins (Adams *et al.*, 2000; Wilson *et al.*, 1997) and a molecular model has been derived based upon the crystal structure of galactose oxidase "kelch" domain (Fig. 8.2 -HCF-1, bottom right) (J.L. Vogel and T.M. Kristie, unpublished data). The domain model consists of seven reiterations of four antiparallel β sheets that form the blades of a propeller-type structure. The ring is closed via the E4 sheet of the aminoterminus with the E1-E2-E3 sheets at the carboxyterminus of the domain to form the 7th blade (Fig. 8.2-HCF-1, bottom left). Kelch domain proteins are involved in a broad range of functions from structural assemblies to signal transduction and the domain presents several distinct protein interaction surfaces formed by the seven L2-3 loops, the L4-1 loops, and the E4 sheets (Adams *et al.*, 2000).

As defined in numerous studies, the kelch domain mediates the high affinity interaction of HCF-1 with VP16 in the assembly of the HSV IE enhancer complex. The analysis of this interaction led to the elucidation of the HCF-1 interaction motif (D/E HXY) recognized in VP16 and subsequently determined to be a common motif found in cellular proteins that interact with the HCF-1 kelch domain (Freiman and Herr, 1997; Lu *et al.*, 1998; Simmen *et al.*, 1997). However, selective mutagenesis has suggested that distinct surfaces within the reiterated kelch structure are involved in the interactions that are mediated by this short common motif (Mahajan and Wilson, 2000). Differences between HCF-1 and the highly related HCF-2 in blades 5 and 6 encode part of the specificity for the preferential binding of VP16 to HCF-1 in a manner analogous to the discrimination between Oct-1 and Oct-2 (Johnson *et al.*, 1999). While the kelch domain is the minimal domain required for the assembly of the VP16 enhancer core complex, it is unlikely to represent the only domain involved in this assembly. Some studies have suggested that additional domains are required, perhaps to constrain or alter the positioning of the VP16 activation domain for stable assembly into the complex (La Boissiere *et al.*, 1997). Notably, multiple other cellular proteins that interact with the kelch domain have also been isolated including transcription factors (LZIP, Zhangfei) E2F1, E2F4, Krox 20 (Freiman and Herr, 1997; Knez *et al.*, 2006; Lu and Misra, 2000b; Lu *et al.*, 1997; Luciano and Wilson, 2002, 2003), transcription coactivators (PGC, PRC) (Lin *et al.*, 2002), a nuclear export protein (HPIP) that may control the nucleo-cytoplasmic pool of HCF-1 (Mahajan *et al.*, 2002), and chromatin modification components (set1/Ash HMT) (Wysocka *et al.*, 2003); suggesting that the protein is involved in numerous or global cellular transcription functions.

In its role during the initial stages of the HSV lytic cycle, the protein is described as the coordinator of the HSV IE enhancer complex assembly as it has interactions with each of the enhancer components (VP16, Oct-1, Sp1, GABP) and may orient the assembled factors for effective activation of the IE gene transcription (Vogel and Kristie, 2000b; Vogel *et al.*, 2001). The interactions may also reflect a central role in the activation of the IE transcription in response to multiple distinct regulatory signals that are mediated by the various factors involved (see below). An additional function of the protein in the regulation of the IE genes is evidenced by its activity as a mediator or coactivator of transcription via interaction with various transcription factors, chromatin modification components and other coactivators (Fig. 8.2 -HCF-1). Several lines of evidence support the proposal that HCF-1 can function as

a general coactivator and mediates transcription activation of the assembled IE complex including: (i) an activation domain in the carboxyterminus of HCF-1 functions synergistically with the VP16 TA domain and may affect a distinct rate-limiting stage (Luciano and Wilson, 2002); (ii) HCF-1 is required to mediate the transcriptional activation potential of LZIP at CRE sites (Lu *et al.*, 1997); (iii) HCF-1 interacts directly with the TA domain of GABP where mutations which affect the transactivation potential correlate directly with affects upon the GABP-HCF-1 interaction (Vogel and Kristie, 2000b); (iv) HCF-1 interacts with chromatin modifying proteins presumably to recruit these enzymes in early stages of transcription activation (Wysocka *et al.*, 2003); and (v) depletion of HCF-1 results in ablation of HSV 1E expression (Narayanan *et al.*, 2005). Significantly, HCF-1 dependent transcription events such as defined for GABP can provide a VP16-independent alternative mechanism for the induction of the HSV IE gene expression outside of the TAATGARAT element core.

In addition to its role in the direct assembly of the IE enhancer core complex, studies delineating the HCF-1 NLS (aa 2015–2035) have demonstrated that HCF-1 is required for the nuclear transport of VP16 during productive infection (La Boissiere *et al.*, 1999). These results suggest that the pool of HCF-1 that is utilized by HSV is likely to be free cytoplasmic protein and that preassociation with VP16 is a critical stage in the enhancer complex assembly. Proteins such as the nuclear export factor HPIP, which interacts with the kelch domain of HCF-1, may play a role in regulating the nucleo-cytoplasmic shuttling of the protein, can therefore have a significant regulatory impact on the availability of cytoplasmic HCF-1 for the HCF-1/VP16 interaction and transport.

HCF-1 is also a critical control component of the cell-cycle as initially demonstrated by the isolation of a ts mutant (P134S) that resulted in G0-G1 cell cycle arrest (Goto *et al.*, 1997). While the exact mechanism(s) of HCF-1 dependent cell-cycle progression remain unclear, studies indicate that the protein has multiple roles in promoting several cell-cycle stages: (i) the dissociation of ts-HCF-1 from cell chromatin at the non-permissive temperature suggests a global effect on cellular transcription (Wysocka *et al.*, 2001); (ii) the interaction of HCF-1 with the TA domain of Miz-1 results in repression of Miz-1 activation of cdk $p15^{INK4b}$ expression, potentiating cell cycle progression (Piluso *et al.*, 2002); (iii) the WYF domain (aa 1793–2005) in the carboxyterminus of HCF-1 interacts with PDCD2 which can suppress complementation of growth arrested ts-HCF-1 cells suggesting that PDCD2 may negatively regulate HCF-1 functions possibly through the association with additional transcription repression components such as NcoR (Scarr and Sharp, 2002); (iv) RNAi-mediated depletion of HCF-1 results in defects in both G_0–G_1 progression and cytokinesis/exit from mitosis which can be rescued by expression of the HCF-1 aminoterminus or carboxyterminus, respectively (Julien and Herr, 2003; and (iv) array studies have implicated HCF-1 in the expression of critical cellular proteins involved in general transcription, cell cycle progression, DNA replication-repair, and signal transduction (Khurana and Kristie, 2004). These studies collectively suggest that HCF-1 regulates cell cycle progression through its multiple roles in the regulation of gene expression. Interestingly, observations that novel HCF-1 aminoterminal polypeptides accumulate in the cytoplasm of cells arrested in G_0 may indicate that specific subdomains of the protein are localized in a regulated manner to control cell-cycle progression (Scarr *et al.*, 2000).

Located in the central region of the 230 kD HCF-1 precursor is one of the more unusual domains consisting of a series of 20 amino acid reiterations that are sites of the specific proteolytic cleavages that results in the family of HCF-1 amino and carboxyterminal polypeptides (PPD domain, Fig. 8.2 -HCF-1). However, the amino and carboxyterminal proteins do not segregate but remain tightly associated via interactions between fibronectin type II repeats in the carboxyterminus (aa 1800–2000) and the 7^{th} blade of the kelch domain (Kristie *et al.*, 1995; Kristie and Sharp, 1993; Wilson *et al.*, 2000). The processing of the protein has been determined to be autocatalytic and requires the PPD and residues carboxyterminal to this domain for efficient processing in vitro (Vogel and Kristie, 2000a). The functional role of the processing remains elusive but may represent a regulatory mechanism for controlling HCF-1-protein interactions and amino-carboxyterminal cooperativity. A novel regulatory function is proposed by the identification of a series of protein-protein interactions within the PPD in which specific reiterations encode an inherent specificity for particular protein binding partners. This model predicts that processing of HCF-1 would regulate the ability of the resulting cleavage products to interact with a specific subset of cofactors, thereby determining the activity of the particular HCF-1 cleavage product (Vogel and Kristie, 2006). Ultimately, the processing may control the protein's nuclear transport, cell-cycle functions, and/or determine its transcription activation or repression potential.

While the emphases of HCF-1 studies have been the roles of the protein in transcriptional regulation and cell cycle progression, the protein has also been implicated in mRNA splicing where it may be a general cofactor (Ajuh

et al., 2002) and in protein modification complexes (HCF-1-Protein Phosphatase 1) (Ajuh *et al.*, 2000) where it may regulate the activity of the phosphatase or determine it's targets. In addition, HCF-1 may play roles in the later stages of HSV lytic replication as suggested by the interaction of the kelch domain with HSV DNA replication proteins (Peng M.L., Nogu eira, and T.M. Kristie 2006, unpublished data).

Clearly HCF represents one of the more complex components of the HSV IE regulatory assembly and has diverse and essential cellular functions. This protein may also be the component that is most critical for viral IE expression suggesting that the evolution of the virus to usurp the functions of this factor has more implications for the regulation of the HSV lytic cycle than is initially readily apparent.

Ancillary factors: Sp1 and GABP

In addition to the enhancer core complex, each IE regulatory domain contains a number of binding sites for cellular transcription factors such as Sp1 and GABP. The expression of the IE genes, even in the absence of VP16, attests to the significance of these components. However, the potential of these elements are dependent upon and enhanced by the presence of the assembled IE enhancer core complex, reflecting an interdependence of the elements.

Sp1

Sp1 is a member of the Zn finger/krupple family and was the first transcription factor to be purified and cloned (Black *et al.*, 2001; Kaczynski *et al.*, 2003; Suske, 1999). The protein recognizes the element GGGCGG (GC box) that is present in multiple copies in the HSV IE regulatory domains (Fig. 8.1) and has been determined to contribute to the basal expression level (Jones and Tjian, 1985). Originally considered to be a ubiquitously expressed, housekeeping factor, the protein is now known to be a member of a large family of related proteins that are subject to and involved in distinct regulatory pathways. Sp1 through Sp6 are highly related proteins which all interact with the GC element although there is some variability in specific binding affinity.

Sp1 is a 778 amino acid protein consisting of a three C2H2 Zn-finger DNA binding domain (C-X_{2-5} C-X_3-(F/Y)-X_5-ψ-X_2-H-X_{3-5}-H) located in the carboxyterminus with an embedded nuclear localization signal (NLS, Fig. 8.2-Sp1). Models that predict the DNA binding contacts are based upon similar structures and suggest that key residues in the various Sp family members determine the specificity for the GC box (Kaczynski *et al.*, 2003). The protein also contains two TA domains [(S/T) and Q-rich] that function in a promoter-dependent context, conceptually similar to those in Oct-1 (Black *et al.*, 2001; Suske, 1999).

Sp1 interacts with a number of known transcription activators (e.g., Oct-1), transcription coactivators (e.g., HCF-1, p300/CBP), and basal transcription factors (e.g., TBP)(Gunther *et al.*, 2000; Kaczynski *et al.*, 2003; Suske, 1999). In a manner analogous to Oct-1, the protein interactions and functional significance can largely be determined by the promoter context. Most significantly, the accumulating data illustrates that this family of proteins is subject to several regulatory signals and pathways in which specific GC boxes mediate responses to particular stimuli via binding proteins involved in those pathways. In addition, the particular regulatory response of a given GC box can also vary depending upon the particular cell context. These proteins interact with factors such as Rb-p107, p53, E2F, and Oct-1 and respond to signals as diverse as growth stimuli, NGF, TGFb, hormones, DNA damage, and apoptosis (Black *et al.*, 2001; Gunther *et al.*, 2000; Kaczynski *et al.*, 2003; Ryu *et al.*, 2003; Suske, 1999; Yan and Ziff, 1997). An additional level of regulation is provided by varying levels of the Sp family within a given cell type and under specific response conditions. As Sp1 and Sp3 are expressed in the same cell types, bind with equal affinity to the GC box, and have distinct functions; the ratio of the proteins can determine the activation vs. repression of given target genes. This regulatory mechanism is exemplified by alterations in the ratios of these proteins during cell differentiation or specific signaling pathways (Black *et al.*, 2001; Gunther *et al.*, 2000; Kaczynski *et al.*, 2003; Suske, 1999). The activation and repression functions of the Sp family are hypothesized to be mediated via interactions with HAT or HDAC complexes, respectively.

Finally, as expected by the involvement of these proteins in response to environmental signals, modifications of the factors also modulate that activity of the family. For example, acetylation of Sp1 in response to neuronal oxidative stress plays a role in neuronal survival pathways (Ryu *et al.*, 2003). In an HSV infection, phosphorylation of Sp1 later in infection decreases the protein's TA potential and may contribute to the down regulation of the IE gene expression (Kim and DeLuca, 2002).

GABP

GA rich sequences adjacent to at least one TAATGARAT element in each of the HSV IE regulatory domains were originally identified in mutagenesis studies and reporter assays where these elements contributed to the VP16-dependent induction of IE expression (Triezenberg *et al.*, 1988). In vitro transcription assays further demonstrated the

significance of the elements for VP16-mediated transcription and suggested that the sites functioned synergistically with the enhancer core (Wu *et al.*, 1994).

The factors binding to these elements (GABP) are related to the ets and notch protein families and consist of an α-subunit containing a carboxyterminal ets DNA binding domain and a β subunit containing the TA domain, NLS, and series of ankyrin repeats that mediate dimerization with the α subunit as well as contributes to DNA binding (Fig. 8.2-GABP) (LaMarco *et al.*, 1991; Thompson *et al.*, 1991). The proteins also form tetrameric structures via carboxyterminal sequences in the β subunits forming helical intertwined coiled coils (de la Brousse *et al.*, 1994). In the β-subunit, the TA domain consists of a series of hydrophobic clusters which are critical for the TA potential (Gugneja *et al.*, 1996; Gugneja *et al.*, 1995). These regions correlate with the ability of the domain to interact with the enhancer core coactivator HCF-1, indicating that HCF-1 mediates the activation potential of the factor (Vogel and Kristie, 2000b).

The focus of many GABP studies has been in elucidating the role of the protein in the activation of nuclear encoded mitochondrial respiration component and assembly genes (Puigserver and Spiegelman, 2003; Scarpulla, 2002). These studies have delineated multiple levels of regulation and have indicated that an important mechanism for regulation of these factors is via regulation of the coactivators which respond to a variety of stimuli resulting in phosphorylation, induction, activation, and stabilization of these coactivators. The coactivators subsequently recruit additional cofactors, coactivators, and histone modification complexes. Two coactivators have been intensely studied in this context: PRC that mediates activation in proliferative responses and PGC that mediates thermogenic effector responses (Scarpulla, 2002). Interestingly, PGC has not been shown to directly interact and modulate GABP activity but may, in fact, do so via its interaction with the kelch domain of HCF-1 (Fig. 8.2-HCF-1). The activity of GABP and its cofactors are modulated by several signaling pathways such as p38 MAPK. For PGC, p38 mediated phosphorylation results in stabilization of the cofactor (Puigserver and Spiegelman, 2003). The phosphorylated PGC is also involved in direct induction of the expression of GABP.

Numerous regulatory response pathways are mediated by GABP including (i) induction of neuregulin expression (Fromm and Burden, 1998); (ii) insulin dependent prolactin gene expression via MAP pathway phosphorylation of GABP (Ouyang *et al.*, 1996); (iii) TPA stimulated IL2 induction which is mediated via JNK activation of GABP (Hoffmeyer *et al.*, 1998); (iv) the HIV LTR induction mediated via Raf-1 kinase activation of GABP (Flory *et al.*, 1996) and (v) the MMTV LTR which is synergistically activated by GABP in the presence of glucocorticoids (Aurrekoetxea-Hernandez and Buetti, 2000). The protein is also involved in numerous regulatory events by synergistic interactions with other transcription factors such as Sp1 and the cAMP responsive proteins CREB and ATF (Bannert *et al.*, 1999; Sawada *et al.*, 1999).

VZV IE gene expression: parallels and divergence

This review has focused on the regulation of HSV IE genes as a representative model of the mechanisms involved in the IE expression of an alpha herpesvirus due to the focus of studies in this area. In contrast, little has been elucidated concerning the mechanisms involved in the regulation of VZV IE gene expression. What has been determined, however, shows striking parallels to the HSV model. In VZV, the expression of the IE gene (IE62) is controlled by an enhancer domain (Fig. 8.3) consisting of multiple defined elements (Bannert *et al.*, 1999; McKee and Preston, 1991; Moriuchi *et al.*, 1995; Sawada *et al.*, 1999).

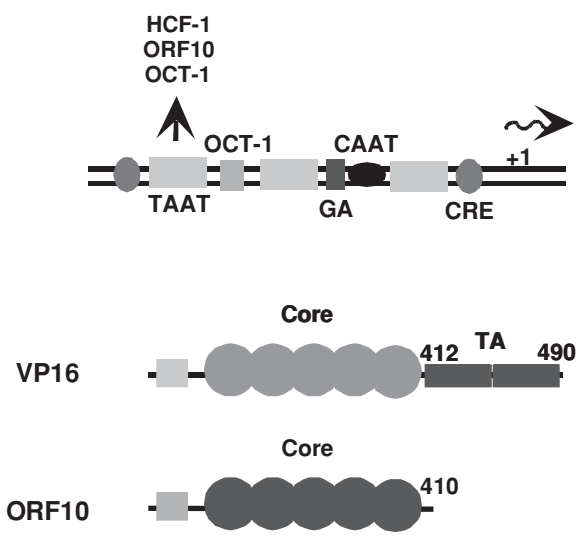

Fig. 8.3. The VZV IE62 regulatory domain. (Top) The arrangement of elements that have been delineated in the VZV IE62 regulatory region are indicated (TAAT, enhancer core element TAATGARAT; Oct, Octamer element; GA, GABP binding site; CAAT, CAT box; CRE, c-AMP responsive element). The assembly of the components HCF-1, Oct-1, and the VZV VP16 ortholog ORF-10 on the TAAT element is indicated. (Bottom) Comparison of the domain structure of HSV VP16 and VZV ORF-10 illustrates conservation of the aminoterminus and core domain and the absence of an ORF-10 transactivation domain.

Similar to HSV, the domain contains reiterations of a TAATGARAT element that is important for induction via the VZV virion component ORF10, the ortholog of the HSV VP16 (Moriuchi *et al.*, 1995). Additional elements include a GABP binding site and c-AMP responsive sites (CRE) that also contribute to the ORF10-mediated stimulation of the IE62 gene expression. As has been demonstrated for HSV, the VZV IE62 TAATGARAT elements can nucleate the assembly of enhancer core complexes that contain Oct-1 and HCF-1 in concert with the ORF10 protein (Moriuchi *et al.*, 1995).

The VZV ORF10 transactivator, while sharing significant homology to VP16, exhibits a striking difference that impacts the regulatory process. In contrast to VP16, ORF10 does not contain a transactivation domain, indicating that the interaction of the protein with other factors and coactivator components (e.g., Oct-1, HCF-1) must provide this function. A second significant difference between the regulatory mechanisms involved in the induction of HSV IE and the VZV IE genes is the autoregulatory response mediated by the major IE gene products (Perera *et al.*, 1992). In HSV, α4/ICP4 functions to down regulate the IE genes in the transition from IE to E gene expression (Roizman, 1996). In VZV, IE62 functions to induce or enhance its own transcription. Interestingly, IE62 is also a component of the virion tegument structure (Kinchington *et al.*, 1992) and may be a significant component of the IE induction response, thus compensating for the lack of the transactivation potential of the ORF10 activator. However, despite minor variations, data on the regulation of the VZV IE62 gene closely parallels the components and mechanisms defined in the regulation of HSV IE genes.

Regulation of the IE genes: multiple levels and response potentials

Studies on the regulation of the IE genes and the various components involved have led to advances in the understanding of enhancer complexes, ordered assembly processes, protein surfaces and interactions, mechanisms of transcriptional activation, and orders of interplay regulation. In addition to the modulation of DNA site recognition, the various transcription factors themselves are subject to modifications, alterations in turnover rates, subcellular localization, and signaling response pathways. Transcription factor synergy (activation or repression) is also dependent upon the cellular milieu and the balance of factors including the availability, competition, activator/repressor ratios, and cell-specific cofactors and coactivators. Additional higher orders of regulation are dependent upon the regulation of the coactivator levels, interactions, and functions. The ability and efficiency of the various assembled complexes to circumvent or alter rate limiting stages in the transcriptional assembly, initiation, elongation, and reinitiation is also likely to depend upon the cell type and state as the consequences of chromatin/nucleosomal structure, available factors, and signal environment will impinge upon a given rate limiting stage and determine the requirements for efficient transcription.

Most strikingly, the complex interactions of the components involved in the regulation of the IE enhancer complexes (e.g., Oct-1, Sp1, GABP, HCF-1) and the ability of these components to respond differentially to multiple environmental signals point to the evolution of the IE regulatory domains to respond to diverse signals.

The regulation of the IE genes: reactivation of HSV from the latent state

The components expressed in specific cell types may impact the normal lytic cycle regulation as suggested by the low levels of Oct-1 expression in sensory neurons. In this situation, other POU proteins such as Oct-2 or members of the Brn family may play a role in suppression or inefficient activation of the IE gene (Brownlees *et al.*, 1999; Latchman, 1996; Latchman, 1999; Lillycrop *et al.*, 1994). For the Brn family, Brn3a functions as an activator while Brn3b functions as a transcriptional repressor and the relative levels of these proteins change upon differentiation or neuronal stimulation (Latchman, 1999). Similarly, the levels of Sp1/Sp3 provide potential for repressor assemblies that may play a role in modulating IE expression in neuronal cells.

The potential of the IE gene domains to respond to diverse environmental stimuli suggests that these domains may respond to latency-reactivation stimuli by targeting distinct elements or components. Significantly, each of the identified IE regulatory factors is commonly linked by its interaction with the HCF-1 coactivator. Therefore, while distinct signals and factors may respond to distinct stimuli, the critical response component may be represented by the common coactivator. Interestingly, HCF-1 itself is uniquely sequestered in the cytoplasm of sensory neurons and is rapidly transported to the nucleus in response to reactivation signals (Fig. 8.4, Top) (Kristie *et al.*, 1999). This regulated transport may well reflect an important element of the activation of the IE genes during the reactivation process. Furthermore, this provides a mechanism for the induction of the IE gene expression in response to signaling events in a VP16-independent manner, utilizing com-