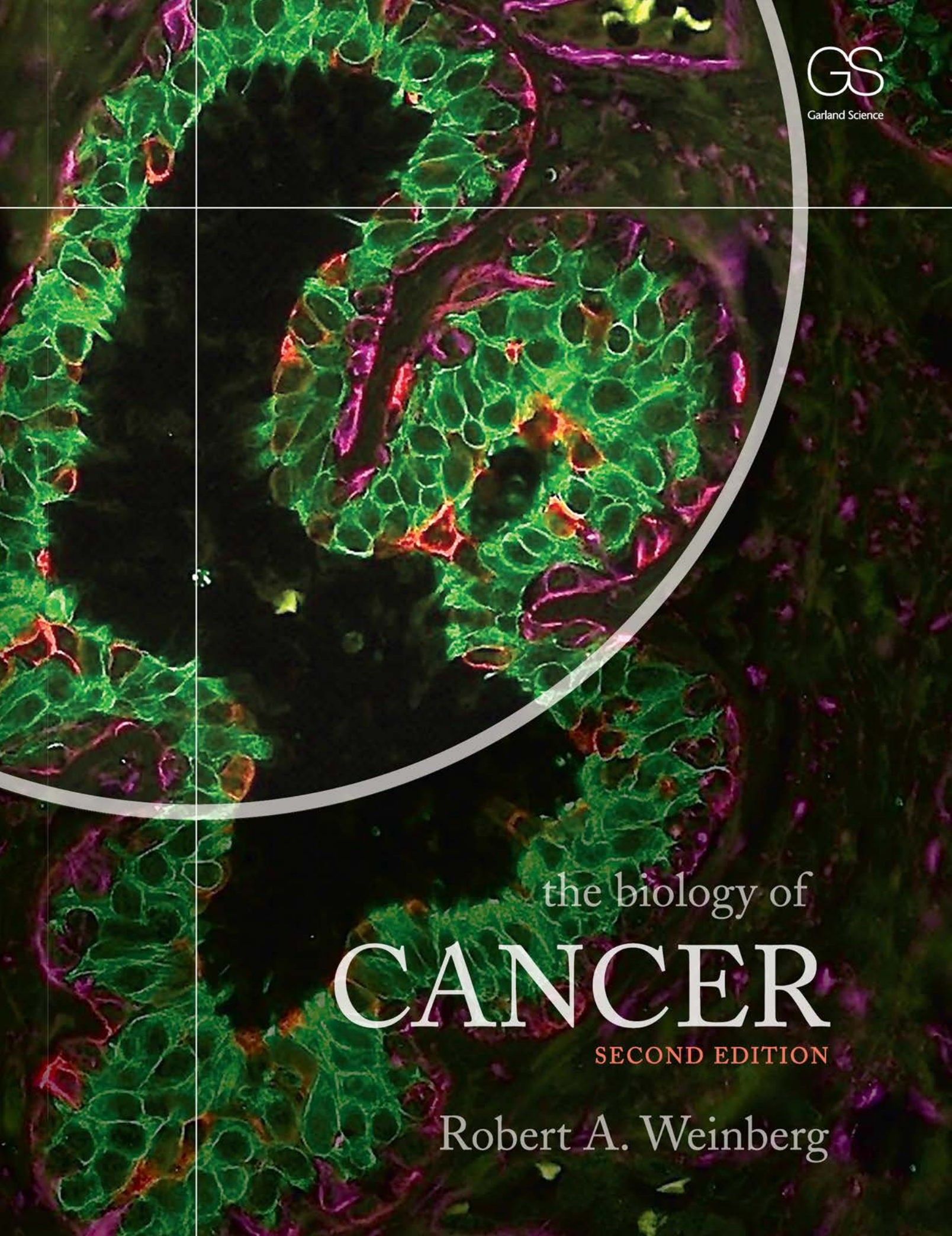

Garland Science

the biology of CANCER

SECOND EDITION

Robert A. Weinberg

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Front Cover

A micrograph section of a human *in situ* ductal carcinoma with α-smooth muscle actin stained in *pink*, cytokeratins 5 and 6 in *red-orange*, and cytokeratins 8 and 18 in *green*. (Courtesy of Werner Böcker and Igor B. Buchwalow of the Institute for Hematopathology, Hamburg, Germany.)

Dedication

I dedicate this second edition, as the first one, to my dear wife, Amy Shulman Weinberg, who endured long hours of inattention, hearing from me repeatedly the claim that the writing of this edition was almost complete, when in fact years of work lay ahead. She deserved much better! With much love.

Preface

Compared with other areas of biological research, the science of molecular oncology is a recent arrival; its beginning can be traced with some precision to a milestone discovery in 1975. In that year, the laboratory of Harold Varmus and J. Michael Bishop in San Francisco, California demonstrated that normal cell genomes carry a gene—they called it a proto-oncogene—that has the potential, following alteration, to incite cancer. Before that time, we knew essentially nothing about the molecular mechanisms underlying cancer formation; since that time an abundance of information has accumulated that now reveals in outline and fine detail how normal cells become transformed into tumor cells, and how these neoplastic cells collaborate to form life-threatening tumors.

The scientific literature on cancer pathogenesis has grown explosively and today encompasses millions of research publications. So much information would seem to be a pure blessing. After all, knowing more is always better than knowing less. In truth, it represents an embarrassment of riches. By now, we seem to know too much, making it difficult to conceptualize cancer research as a single coherent body of science rather than a patchwork quilt of discoveries that bear only a vague relationship with one another.

This book is written in a far more positive frame of mind, which holds that this patchwork quilt is indeed a manifestation of a body of science that has some simple, underlying principles that unify these diverse discoveries. Cancer research is indeed a field with conceptual integrity, much like other areas of biomedical research and even sciences like physics and chemistry, and the bewildering diversity of the cancer research literature can indeed be understood through these underlying principles.

Prior to the pioneering findings of 1975, we knew almost nothing about the molecular and cellular mechanisms that create tumors. There were some intriguing clues lying around: We knew that carcinogenic agents often, but not always, operate as mutagens; this suggested that mutant genes are involved in some fashion in programming the abnormal proliferation of cancer cells. We knew that the development of cancer is often a long, protracted process. And we knew that individual cancer cells extracted from tumors behave very differently than their counterparts in normal tissues.

Now, almost four decades later, we understand how mutant genes govern the diverse traits of cancer cells and how the traits of these individual cells determine the behavior of tumors. Many of these advances can be traced to the stunning improvements in experimental tools. The techniques of genetic analysis, which were quite primitive at the beginning of this period, have advanced to the stage where we can sequence entire tumor cell genomes in several days. (This is in sharp contrast to the state of affairs in 1975, when the sequencing of oligonucleotides represented a formidable task!) Given the critical role of genotype in determining phenotype, we now understand, as least in outline, why cancer cells behave the way that they do. On the one hand, the molecular differences among individual cancers suggest hundreds of distinct types of human cancer. On the other, molecular and biochemical analyses reveal that this bewildering diversity really manifests a small number of underlying common biochemical traits and molecular processes.

Amusingly, much of this unification was preordained by decisions made 600 million years ago. Once the laws and mechanisms of organismic development were established, they governed all that followed, including the behavior of both normal and neoplastic cells. Modern cancer researchers continue to benefit from this rigid adherence to the fundamental, evolutionarily conserved rules of life. As is evident repeatedly throughout this book, much of what we understand about cancer cells, and thus about the disease of cancer, has been learned by studying the cells of worms and fruit flies and frogs. These laws and principles are invoked repeatedly to explain the complex behaviors of human tumors. By providing context and perspective, they can be used to help us understand all types of human cancer.

While these basic principles are now in clear view, critical details continue to elude us. This explains why modern cancer research is still in active ferment, and why new, fascinating discoveries are being reported every month. While they create new perspectives, they do not threaten the solidity of the enduring truths, which this book attempts to lay out. These principles were already apparent seven years ago when the first edition of this book appeared and, reassuringly, their credibility has not been undermined by all that has followed.

In part, this book has been written as a recruiting pamphlet, as new generations of researchers are needed to move cancer research forward. They are so important because the lessons about cancer's origins, laid out extensively in this book, have not yet been successfully applied to make major inroads into the prevention and cure of this disease. This represents the major frustration of contemporary cancer research: the lessons of disease causation have rarely been followed, as day follows night, by the development of definitive cures.

And yes, there are still major questions that remain murky and poorly resolved. We still do not understand how cancer cells create the metastases that are responsible for 90% of cancer-associated mortality. We understand rather little of the role of the immune system in preventing cancer development. And while we know much about the individual signaling molecules operating inside individual human cells, we lack a clear understanding of how the complex signaling circuitry formed by these molecules makes the life-and-death decisions that determine the fate of individual cells within our body. Those decisions ultimately determine whether or not one of our cells begins the journey down the long road leading to cancerous proliferation and, finally, to a life-threatening tumor.

Contemporary cancer research has enriched numerous other areas of modern biomedical research. Consequently, much of what you will learn from this book will be useful in understanding many aspects of immunology, neurobiology, developmental biology, and a dozen other biomedical research fields. Enjoy the ride!

Robert A. Weinberg
Cambridge, Massachusetts
March 2013

A Note to the Reader

The second edition of this book is organized, like the first, into 16 chapters of quite different lengths. The conceptual structure that was established in the first edition still seemed to be highly appropriate for the second, and so it was retained. What has changed are the contents of these chapters: some have changed substantially since their first appearance seven years ago, while others—largely early chapters—have changed little. The unchanging nature of the latter is actually reassuring, since these chapters deal with early conceptual foundations of current molecular oncology; it would be most unsettling if these foundational chapters had undergone radical revision, which would indicate that the earlier edition was a castle built on sand, with little that could be embraced as well-established, unchanging certainties.

The chapters are meant to be read in the order that they appear, in that each builds on the ideas that have been presented in the chapters before it. The first chapter is a condensed refresher course for undergraduate biology majors and pre-doctoral students; it lays out many of the background concepts that are assumed in the subsequent chapters.

The driving force of these two editions has been a belief that modern cancer research represents a conceptually coherent field of science that can be presented as a clear, logical progression. Embedded in these discussions is an anticipation that much of this information will one day prove useful in devising novel diagnostic and therapeutic strategies that can be deployed in oncology clinics. Some experiments are described in detail to indicate the logic supporting many of these concepts. You will find numerous schematic drawings, often coupled with micrographs, that will help you to appreciate how experimental results have been assembled, piece-by-piece, generating the syntheses that underlie molecular oncology.

Scattered about the text are "Sidebars," which consist of commentaries that represent detours from the main thrust of the discussion. Often these Sidebars contain anecdotes or elaborate on ideas presented in the main text. Read them if you are interested, or skip over them if you find them too distracting. They are presented to provide additional interest—a bit of extra seasoning in the rich stew of ideas that constitutes contemporary research in this area. The same can be said about the "Supplementary Sidebars," which have been relegated to the DVD-ROM that accompanies this book. These also elaborate upon topics that are laid out in the main text and are cross-referenced throughout the book. Space constraints dictated that the Supplementary Sidebars could not be included in the hardcopy version of the textbook.

Throughout the main text you will find extensive cross-references whenever topics under discussion have been introduced or described elsewhere. Many of these have been inserted in the event that you read the chapters in an order different from their presentation here. These cross-references should not provoke you to continually leaf through other chapters in order to track down cited sections or figures. If you feel that you will benefit from earlier introductions to a topic, use these cross-references; otherwise, ignore them.

Each chapter ends with a forward-looking summary entitled "Synopsis and Prospects." This section synthesizes the main concepts of the chapter and often addresses

ideas that remain matters of contention. It also considers where research might go in the future. This overview is extended by a list of key concepts and a set of questions. Some of the questions are deliberately challenging and we hope they will provoke you to think more deeply about many of the issues and concepts developed. Finally, most chapters have an extensive list of articles from research journals. These will be useful if you wish to explore a particular topic in detail. Almost all of the cited references are review articles, and many contain detailed discussions of various subfields of research as well as recent findings. In addition, there are occasional references to older publications that will clarify how certain lines of research developed.

Perhaps the most important goal of this book is to enable you to move beyond the textbook and jump directly into the primary research literature. This explains why some of the text is directed toward teaching the elaborate, specialized vocabulary of the cancer research literature, and many of its terms are defined in the glossary. Boldface type has been used throughout to highlight key terms that you should understand. Cancer research, like most areas of contemporary biomedical research, is plagued by numerous abbreviations and acronyms that pepper the text of many published reports. The book provides a key to deciphering this alphabet soup by defining these acronyms. You will find a list of such abbreviations in the back.

Also contained in the book is a newly compiled List of Key Techniques. This list will assist you in locating techniques and experimental strategies used in contemporary cancer research.

The DVD-ROM that accompanies the book also contains a PowerPoint® presentation for each chapter, as well as a companion folder that contains individual JPEG files of the book images including figures, tables, and micrographs. In addition, you will find on this disc a variety of media for students and instructors: movies and audio recordings. There is a selection of movies that will aid in understanding some of the processes discussed; these movies are referenced on the first page of the corresponding chapter in a blue box. The movies are available in QuickTime and WMV formats, and can be used on a computer or transferred to a mobile device. The author has also recorded mini-lectures on the following topics for students and instructors: Mutations and the Origin of Cancer, Growth Factors, p53 and Apoptosis, Metastasis, Immunology and Cancer, and Cancer Therapies. These are available in MP3 format and, like the movies, are easy to transfer to other devices. These media items, as well as future media updates, are available to students and instructors at: http://www.garlandscience.com. On the website, qualified instructors will be able to access a newly created Question Bank. The questions are written to test various levels of understanding within each chapter. The instructor's website also offers access to instructional resources from all of the Garland Science textbooks. For access to instructor's resources please contact your Garland Science sales representative or e-mail science@garland.com.

The poster entitled "The Pathways of Human Cancer" summarizes many of the intracellular signaling pathways implicated in tumor development. This poster has been produced and updated for the Second Edition by Cell Signaling Technology.

Because this book describes an area of research in which new and exciting findings are being announced all the time, some of the details and interpretations presented here may become outdated (or, equally likely, proven to be wrong) once this book is in print. Still, the primary concepts presented here will remain, as they rest on solid foundations of experimental results.

The author and the publisher would greatly appreciate your feedback. Every effort has been made to minimize errors. Nonetheless, you may find them here and there, and it would be of great benefit if you took the trouble to communicate them. Even more importantly, much of the science described herein will require reinterpretation in coming years as new discoveries are made. Please email us at science@garland.com with your suggestions, which will be considered for incorporation into future editions.

Acknowledgments

The science described in this book is the opus of a large, highly interactive research community stretching across the globe. Its members have moved forward our understanding of cancer immeasurably over the past generation. The colleagues listed below have helped the author in countless ways, large and small, by providing sound advice, referring me to critical scientific literature, analyzing complex and occasionally contentious scientific issues, and reviewing individual chapters and providing much-appreciated critiques. Their scientific expertise and their insights into pedagogical clarity have proven to be invaluable. Their help extends and complements the help of an equally large roster of colleagues who helped with the preparation of the first edition. These individuals are representatives of a community, whose members are, virtually without exception, ready and pleased to provide a helping hand to those who request it. I am most grateful to them. Not listed below are the many colleagues who generously provided high quality versions of their published images; they are acknowledged through the literature citations in the figure legends. I would like to thank the following for their suggestions in preparing this edition, as well as those who helped with the first edition. (Those who helped on this second edition are listed immediately, while those who helped with the first edition follow.)

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List of Key Techniques

 Can be found on the DVD-ROM accompanying the book.

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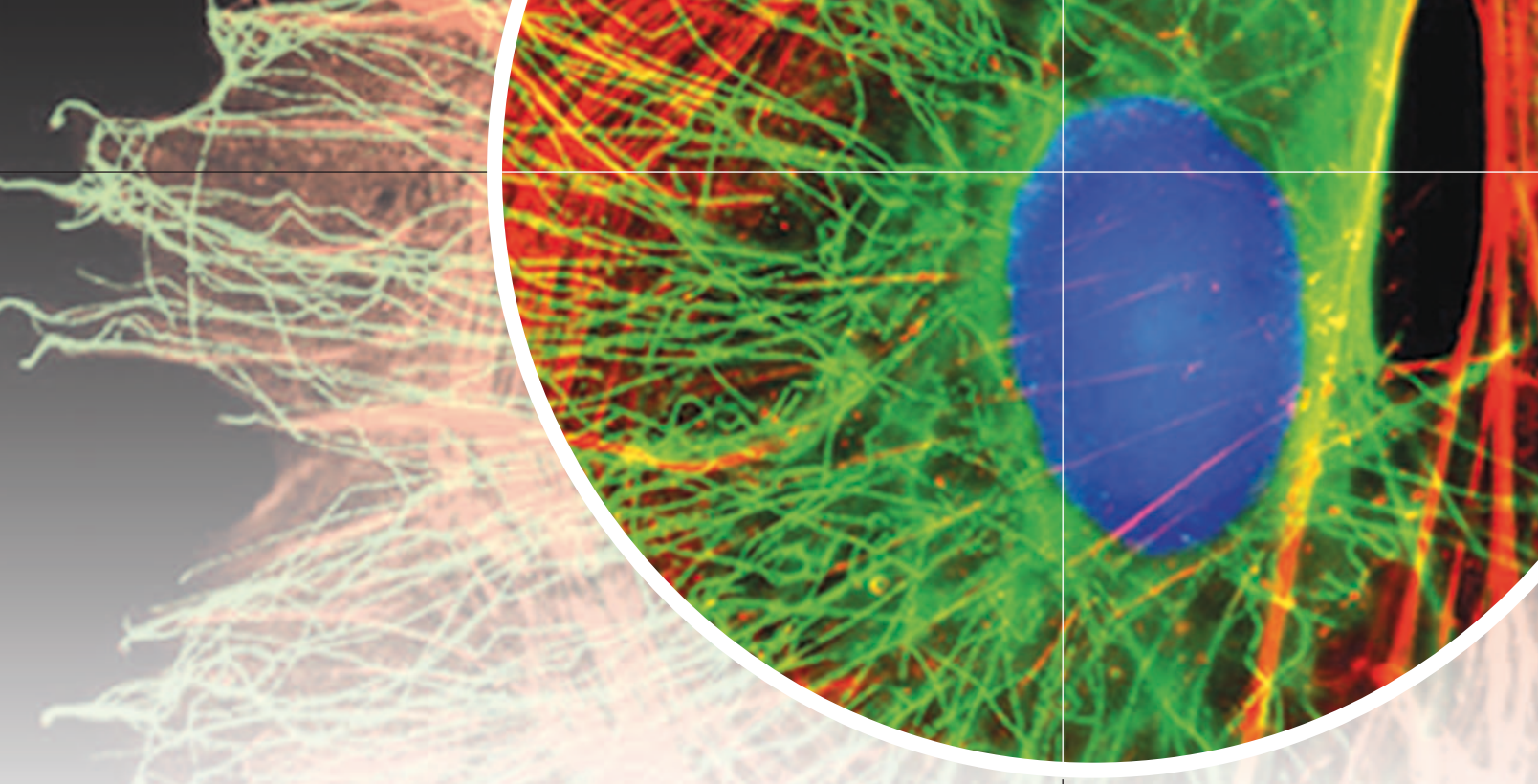


Chapter 1

The Biology and Genetics of Cells and Organisms

> Protoplasm, simple or nucleated, is the formal basis of all life... Thus it becomes clear that all living powers are cognate, and that all living forms are fundamentally of one character. The researches of the chemist have revealed a no less striking uniformity of material composition in living matter.
>
> Thomas Henry Huxley, evolutionary biologist, 1868

> Anything found to be true of *E. coli* must also be true of elephants.
>
> Jacques Monod, pioneer molecular biologist, 1954

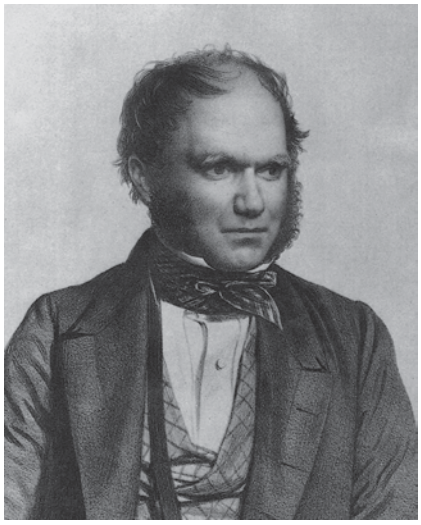
The biological revolution of the twentieth century totally reshaped all fields of biomedical study, cancer research being only one of them. The fruits of this revolution were revelations of both the outlines and the minute details of genetics and heredity, of how cells grow and divide, how they assemble to form tissues, and how the tissues develop under the control of specific genes. Everything that follows in this text draws directly or indirectly on this new knowledge.

This revolution, which began in mid-century and was triggered by Watson and Crick's discovery of the DNA double helix, continues to this day. Indeed, we are still too close to this breakthrough to properly understand its true importance and its long-term ramifications. The discipline of molecular biology, which grew from this discovery, delivered solutions to the most profound problem of twentieth-century biology—how does the genetic constitution of a cell or organism determine its appearance and function?

Without this molecular foundation, modern cancer research, like many other biological disciplines, would have remained a descriptive science that cataloged diverse biological phenomena without being able to explain the mechanics of how they occur.

Movies in this chapter

1.1 Replication I
1.2 Replication II
1.3 Translation I
1.4 Transcription



(A)

(B)

Figure 1.1 Darwin and Mendel
(A) Charles Darwin's 1859 publication of *On the Origin of Species by Means of Natural Selection* exerted a profound effect on thinking about the origin of life, the evolution of organismic complexity, and the relatedness of species. (B) Darwin's theory of evolution lacked a genetic rationale until the work of Gregor Mendel. The synthesis of Darwinian evolution and Mendelian genetics is the foundation for much of modern biological thinking. (A, from the Grace K. Babson Collection, the Henry E. Huntington Library, San Marino, California. Reproduced by permission of The Huntington Library, San Marino, California. B, courtesy of the Mendelianum Museum Moraviae, Brno, Czech Republic.)

Today, our understanding of how cancers arise is being continually enriched by discoveries in diverse fields of biological research, most of which draw on the sciences of molecular biology and genetics. Perhaps unexpectedly, many of our insights into the origins of malignant disease are not coming from the laboratory benches of cancer researchers. Instead, the study of diverse organisms, ranging from yeast to worms to flies, provides us with much of the intellectual capital that fuels the forward thrust of the rapidly moving field of cancer research.

Those who fired up this biological revolution stood on the shoulders of nineteenth-century giants, specifically, Darwin and Mendel (**Figure 1.1**). Without the concepts established by these two, which influence all aspects of modern biological thinking, molecular biology and contemporary cancer research would be inconceivable. So, throughout this chapter, we frequently make reference to evolutionary processes as proposed by Charles Darwin and genetic systems as conceived by Gregor Mendel.

1.1 Mendel establishes the basic rules of genetics

Many of the basic rules of genetics that govern how genes are passed from one complex organism to the next were discovered in the 1860s by Gregor Mendel and have come to us basically unchanged. Mendel's work, which tracked the breeding of pea plants, was soon forgotten, only to be rediscovered independently by three researchers in 1900. During the decade that followed, it became clear that these rules—we now call them Mendelian genetics—apply to virtually all sexual organisms, including **metazoa** (multicellular animals), as well as **metaphyta** (multicellular plants).

Mendel's most fundamental insight came from his realization that genetic information is passed in particulate form from an organism to its offspring. This implied that the entire repertoire of an organism's genetic information—its genome, in today's terminology—is organized as a collection of discrete, separable information packets, now called genes. Only in recent years have we begun to know with any precision how many distinct genes are present in the genomes of mammals; many current analyses of the human genome—the best studied of these—place the number in the range of 21,000, somewhat more than the 14,500 genes identified in the genome of the fruit fly, *Drosophila melanogaster*.

Mendel's work also implied that the constitution of an organism, including its physical and chemical makeup, could be divided into a series of discrete, separable entities. Mendel went further by showing that distinct anatomical parts are controlled by distinct genes. He found that the heritable material controlling the smoothness of peas behaved independently of the material governing plant height or flower color. In

	Seed shape	Seed color	Flower color	Flower position	Pod shape	Pod color	Plant height
One form of trait (dominant)	round	yellow	violet-red	axial	inflated	green	tall
A second form of trait (recessive)	wrinkled	green	white	terminal	pinched	yellow	short

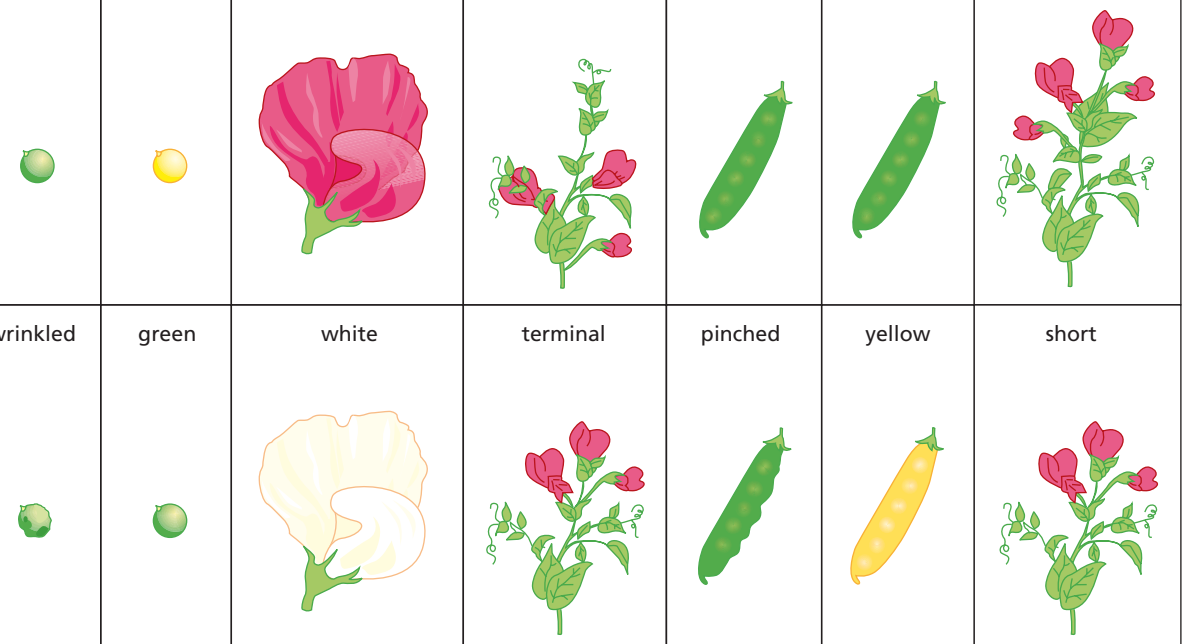


Figure 1.2 A particulate theory of inheritance One of Gregor Mendel's principal insights was that the genetic content of an organism consists of discrete parcels of information, each responsible for a distinct observable trait. Shown are the seven pea-plant traits that Mendel studied through breeding experiments. Each trait had two observable (phenotypic) manifestations, which we now know to be specified by the alternative versions of genes that we call alleles. When the two alternative alleles coexisted within a single plant, the "dominant" trait (*above*) was always observed while the "recessive" trait (*below*) was never observed. (Courtesy of J. Postlethwait and J. Hopson.)

effect, each observable trait of an individual might be traceable to a separate gene that served as its blueprint. Thus, Mendel's research implied that the genetic constitution of an organism (its **genotype**) could be divided into hundreds, perhaps thousands of discrete information packets; in parallel, its observable, outward appearance (its **phenotype**) could be subdivided into a large number of discrete physical or chemical traits (**Figure 1.2**).

Mendel's thinking launched a century-long research project among geneticists, who applied his principles to studying thousands of traits in a variety of experimental animals, including flies (*Drosophila melanogaster*), worms (*Caenorhabditis elegans*), and mice (*Mus musculus*). In the mid-twentieth century, geneticists also began to apply Mendelian principles to study the genetic behavior of single-celled organisms, such as the bacterium *Escherichia coli* and baker's yeast, *Saccharomyces cerevisiae*. The principle of genotype governing phenotype was directly transferable to these simpler organisms and their genetic systems.

While Mendelian genetics represents the foundation of contemporary genetics, it has been adapted and extended in myriad ways since its embodiments of 1865 and 1900. For example, the fact that single-celled organisms often reproduce asexually, that is, without mating, created the need for adaptations of Mendel's original rules. Moreover, the notion that each attribute of an organism could be traced to instructions carried in a single gene was realized to be simplistic. The great majority of observable traits of an organism are traceable to the cooperative interactions of a number of genes. Conversely, almost all the genes carried in the genome of a complex organism play roles in the development and maintenance of multiple organs, tissues, and physiologic processes.

Mendelian genetics revealed for the first time that genetic information is carried redundantly in the genomes of complex plants and animals. Mendel deduced that there were two copies of a gene for flower color and two for pea shape. Today we know that this twofold redundancy applies to the entire genome with the exception of the genes carried in the sex chromosomes. Hence, the genomes of higher organisms are termed **diploid**.

Mendel's observations also indicated that the two copies of a gene could convey different, possibly conflicting information. Thus, one gene copy might specify rough-surfaced and the other smooth-surfaced peas. In the twentieth century, these different versions of a gene came to be called **alleles**. An organism may carry two identical alleles of a gene, in which case, with respect to this gene, it is said to be **homozygous**. Conversely, the presence of two different alleles of a gene in an organism's genome renders this organism **heterozygous** with respect to this gene.

Because the two alleles of a gene may carry conflicting instructions, our views of how genotype determines phenotype become more complicated. Mendel found that in many instances, the voice of one allele may dominate over that of the other in deciding the ultimate appearance of a trait. For example, a pea genome may be heterozygous for the gene that determines the shape of peas, carrying one round and one wrinkled allele. However, the pea plant carrying this pair of alleles will invariably produce round peas. This indicates that the round allele is **dominant**, and that it will invariably overrule its **recessive** counterpart allele (wrinkled) in determining phenotype (see Figure 1.2). (Strictly speaking, using proper genetic parlance, we would say that the phenotype encoded by one allele of a gene is dominant with respect to the phenotype encoded by another allele, the latter phenotype being recessive.)

In fact, classifying alleles as being either dominant or recessive oversimplifies biological realities. The alleles of some genes may be **co-dominant**, in that an expressed phenotype may represent a blend of the actions of the two alleles. Equally common are examples of **incomplete penetrance**, in which case a dominant allele may be present but its phenotype is not manifested because of the actions of other genes within the organism's genome. Therefore, the dominance of an allele is gauged by its interactions with other allelic versions of its gene, rather than its ability to dictate phenotype.

With such distinctions in mind, we note that the development of tumors also provides us with examples of dominance and recessiveness. For instance, one class of alleles that predispose cells to develop cancer encode defective versions of enzymes involved in DNA repair and thus in the maintenance of genomic integrity (discussed again in Chapter 12). These defective alleles are relatively rare in the general population and function recessively. Consequently, their presence in the genomes of many **heterozygotes** (of a wild-type/mutant genotype) is not apparent. However, two heterozygotes carrying recessive defective alleles of the same DNA repair gene may mate. One-fourth of the offspring of such mating pairs, on average, will inherit two defective alleles, exhibit a specific DNA repair defect in their cells, and develop certain types of cancer at greatly increased rates (**Figure 1.3**).

1.2 Mendelian genetics helps to explain Darwinian evolution

In the early twentieth century, it was not apparent how the distinct allelic versions of a gene arise. At first, this variability in information content seemed to have been present in the collective gene pool of a species from its earliest evolutionary beginnings. This perception changed only later, beginning in the 1920s and 1930s, when it became apparent that genetic information is corruptible; the information content in genetic texts, like that in all texts, can be altered. **Mutations** were found to be responsible for changing the information content of a gene, thereby converting one allele into another or creating a new allele from one previously widespread within a species. An allele that is present in the great majority of individuals within a species is usually termed **wild type**, the term implying that such an allele, being naturally present in large numbers of apparently healthy organisms, is compatible with normal structure and function.

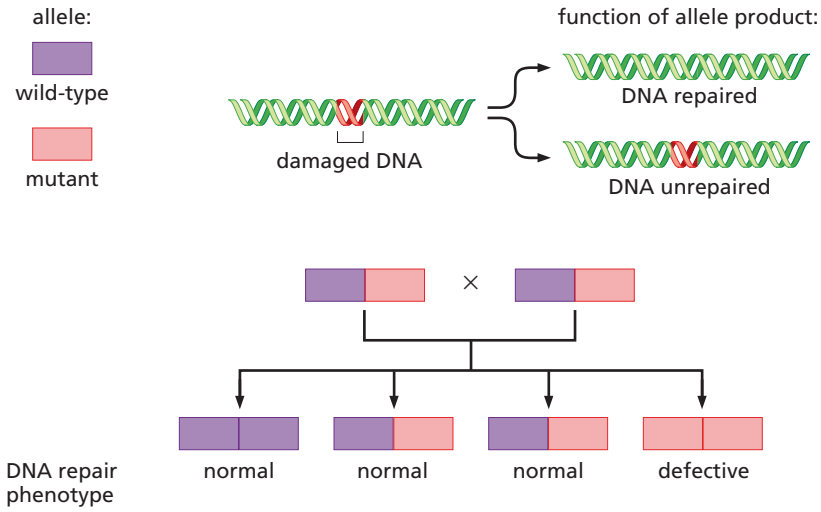


Figure 1.3 Discrepancy between genotype and phenotype The phenotype of an individual often does not indicate genotype. For example, individuals who are phenotypically normal for a trait may nevertheless, at the level of genotype, carry one wild-type (normal) and one mutant (defective) allele of the gene that specifies this trait; this mutant allele will be recessive to the wild-type allele, the latter being dominant. Such individuals are heterozygotes with respect to this gene. In the example shown here, two individuals mate, both of whom are phenotypically normal but heterozygous for a gene specifying a DNA repair function. On average, of their four children, three will be phenotypically normal and their cells will exhibit normal DNA repair function: one of these children will receive two wild-type alleles (be a homozygote) and two will be heterozygotes like their parents. A fourth child, however, will receive two mutant alleles (i.e., be a homozygote) and will be phenotypically mutant, in that this child's cells will lack the DNA repair function specified by this gene. Individuals whose cells lack proper DNA repair function are often cancer-prone, as described in Chapter 12.

Mutations alter genomes continually throughout the evolutionary life span of a species, which usually extends over millions of years. They strike the genome and its constituent genes randomly. Mutations provide a species with a method for continually tinkering with its genome, for trying out new versions of genes that offer the prospect of novel, possibly improved phenotypes. The result of the continuing mutations on the genome is a progressive increase during the evolutionary history of a species in the genetic diversity of its members. Thus, the collection of alleles present in the genomes of all members of a species—the **gene pool** of this species—becomes progressively more heterogeneous as the species grows older.

This means that older species carry more distinct alleles in their genomes than younger ones. Humans, belonging to a relatively young species (<150,000 years old), have one-third as many alleles and genetic diversity as chimpanzees, allowing us to infer that they have been around as a species three times longer than we have.

The continuing diversification of alleles in a species' genome, occurring over millions of years, is countered to some extent by the forces of natural selection that Charles Darwin first described. Some alleles of a gene may confer more advantageous phenotypes than others, so individuals carrying these alleles have a greater probability of leaving numerous descendants than do those members of the same species that lack them. Consequently, natural selection results in a continual discarding of many of the alleles that have been generated by random mutations. In the long run, all things being equal, disadvantageous alleles are lost from the pool of alleles carried by the members of a species, advantageous alleles increase in number, and the overall fitness of the species improves incrementally.

Now, more than a century after Mendel was rediscovered and Mendelian genetics revived, we have come to realize that the great bulk of the genetic information in our own genome—indeed, in the genomes of all mammals—does not seem to specify phenotype and is often not associated with specific genes. Reflecting the discovery in 1944 that genetic information is encoded in DNA molecules, these "noncoding" stretches in the genome are often called **junk DNA** (**Figure 1.4**). Only about 1.5% of a mammal's genomic DNA carries sequence information that encodes the structures of proteins. Recent sequence comparisons of human, mouse, and dog genomes suggest that another ~2% encodes important information regulating gene expression and mediating other, still-poorly understood functions.

Because mutations act randomly on a genome, altering true genes and junk DNA indiscriminately, the great majority of mutations alter genetic information—nucleotide sequences in the DNA—that have no effect on cellular or organismic phenotype. These mutations remain silent phenotypically and are said, from the point of view of natural selection, to be **neutral mutations**, being neither advantageous nor

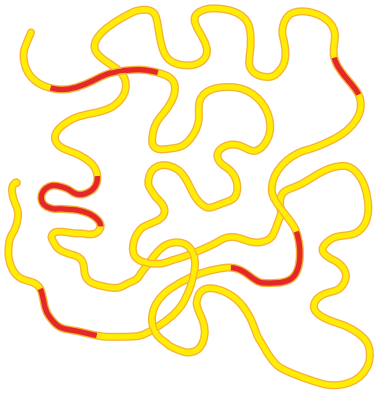


Figure 1.4 Biologically important sequences in the human genome The human genome can be characterized as a collection of relatively small islands of biologically important sequences (~3.5% of the total genome; *red*) floating amid a sea of "junk" DNA (*yellow*). The proportion of sequences carrying biological information has been greatly exaggerated for the sake of illustration. (With the passage of time, genes that appear to play important roles in cell and organismic physiology and specify certain noncoding RNA species have been localized to these intergenic regions; hence the blanket classification of all genomic sequences localized between a human cell's ~21,000 protein-coding genes as useless junk is simplistic.)

disadvantageous (**Figure 1.5**). Since the alleles created by these mutations are silent, their existence could not be discerned by early geneticists whose work depended on gauging phenotypes. However, with the advent of DNA sequencing techniques, it became apparent that hundreds of thousands, even a million functionally silent

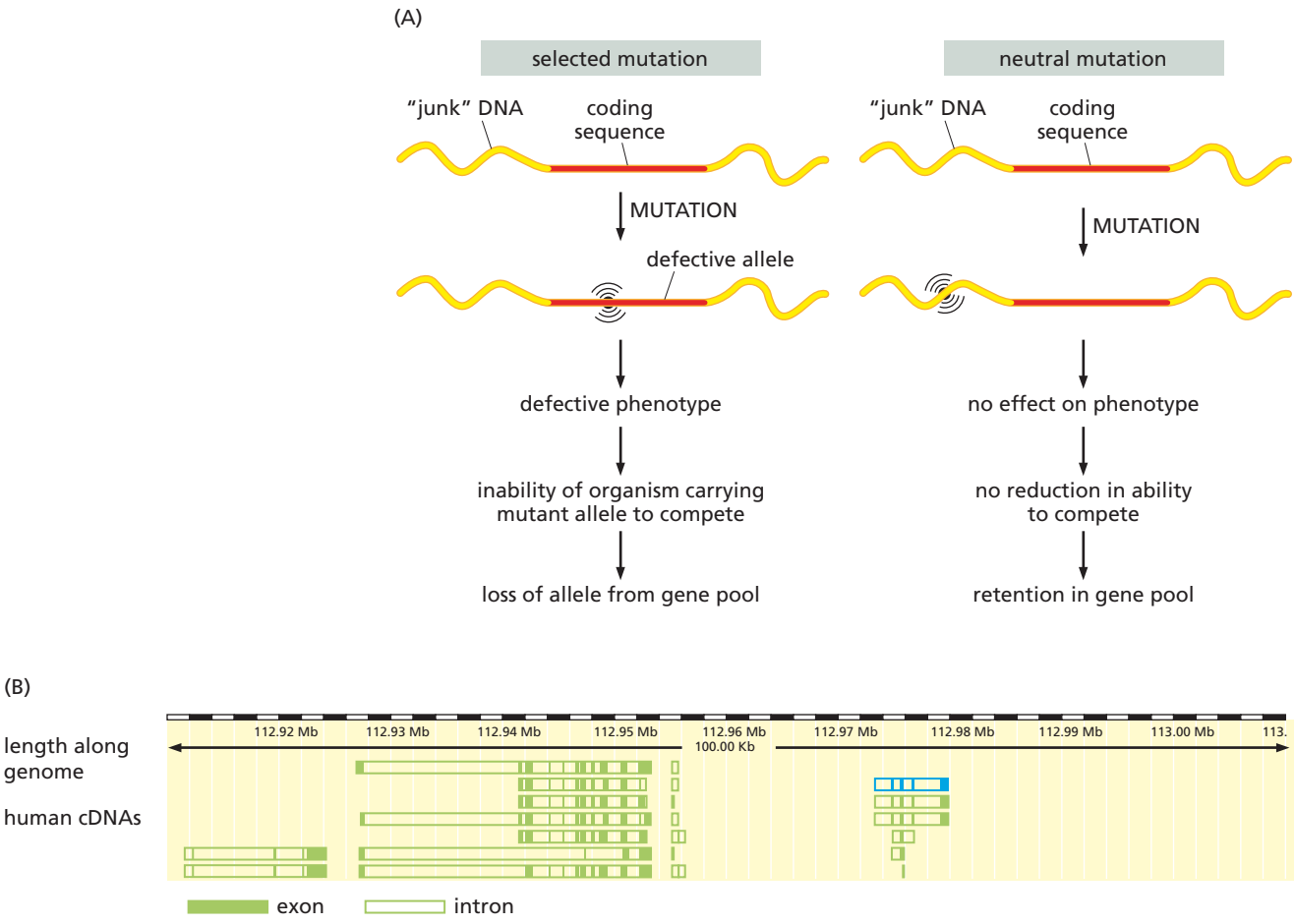


Figure 1.5 Neutral mutations and evolution (A) The coding sequences (*red*) of most genes were optimized in the distant evolutionary past. Hence, many mutations affecting amino acid sequence and thus protein structure (*left*) create alleles that compromise the organism's ability to survive. For this reason, these mutant alleles are likely to be eliminated from the species' gene pool. In contrast, mutations striking "junk" DNA (*yellow*) have no effect on phenotype and are therefore often preserved in the species' gene pool (*right*). This explains why, over extended periods of evolutionary time, coding DNA sequences change slowly, while noncoding DNA sequences change far more rapidly. (B) Depicted is a physical map of a randomly chosen 0.1-megabase segment of human Chromosome 1 (from base pair 112,912,286 to base pair 113,012,285) containing four genes. Each consists of a few islands (*solid rectangles*) that are known or likely to specify segments of mRNA molecules (i.e., exons) and large stretches of intervening sequences (i.e., introns) that do not appear to specify biological information (see Figure 1.16). The large stretches of DNA sequence between genes have not been associated with any biological function. (B, courtesy of The Wellcome Trust Sanger Institute.)

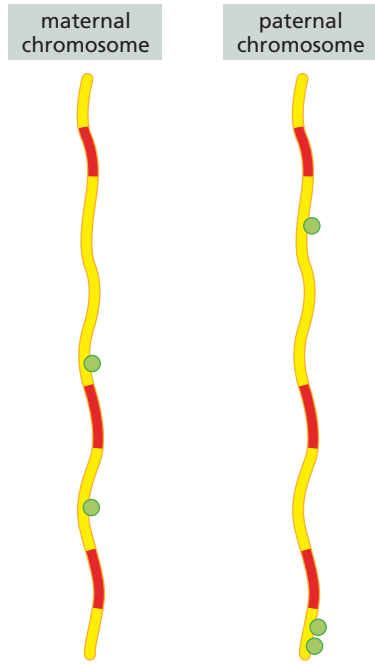


Figure 1.6 Polymorphic diversity in the human gene pool Because the great majority of human genomic DNA does not encode biologically important information (*yellow*), it has evolved relatively rapidly and has accumulated many subtle differences in sequences—polymorphisms—that are phenotypically silent (see Figure 1.5). Such polymorphisms are transmitted like Mendelian alleles, but their presence in a genome can be ascertained only by molecular techniques such as DNA sequencing. The dots (*green*) indicate where the sequence on this chromosome differs from the sequence that is most common in the human gene pool. For example, the prevalent sequence in one stretch may be TAACTGG, while the variant sequence TTACTGG may be carried by a minority of humans and constitute a polymorphism. The presence of a polymorphism in one chromosome but not the other represents a region of heterozygosity, even though a nearby gene (*red*) may be present in the identical allelic version on both chromosomes and therefore be in a homozygous configuration.

mutations can be found scattered throughout the genomes of organisms such as humans. The genome of each human carries its own unique array of these functionally silent genetic alterations. The term *polymorphism* was originally used to describe variations in shape and form that distinguish normal individuals within a species from each other. These days, geneticists use the term **genetic polymorphisms** to describe the inter-individual, functionally silent differences in DNA sequence that make each human genome unique (**Figure 1.6**).

During the course of evolution, the approximately 3.5% of the genome that does encode biological function behaves much differently from the junk DNA. Junk DNA sequences suffer mutations that have no effect on the viability of an organism. Consequently, countless mutations in the noncoding sequences of a species' genome survive in its gene pool and accumulate progressively during its evolutionary history. In contrast, mutations affecting the coding sequences usually lead to loss of function and, as a consequence, loss of organismic viability; hence, these mutations are weeded out of the gene pool by the hand of natural selection, explaining why genetic sequences that do specify biological phenotypes generally change very slowly over long evolutionary time periods (**Sidebar 1.1**).

1.3 Mendelian genetics governs how both genes and chromosomes behave

In the first decade of the twentieth century, Mendel's rules of genetics were found to have a striking parallel in the behavior of the chromosomes that were then being visualized under the light microscope. Both Mendel's genes and the chromosomes were found to be present in pairs. Soon it became clear that an identical set of chromosomes is present in almost all the cells of a complex organism. This chromosomal array, often termed the **karyotype**, was found to be duplicated each time a cell went through a cycle of growth and division.

The parallels between the behaviors of genes and chromosomes led to the speculation, soon validated in hundreds of different ways, that the mysterious information packets called genes were carried by the chromosomes. Each chromosome was realized to carry its own unique set of genes in a linear array. Today, we know that as many as several thousand genes may be arrayed along a mammalian chromosome. (Human Chromosome 1—the largest of the set—holds at least 3148 distinct genes.) Indeed, the length of a chromosome, as viewed under the microscope, is roughly proportional to the number of genes that it carries.

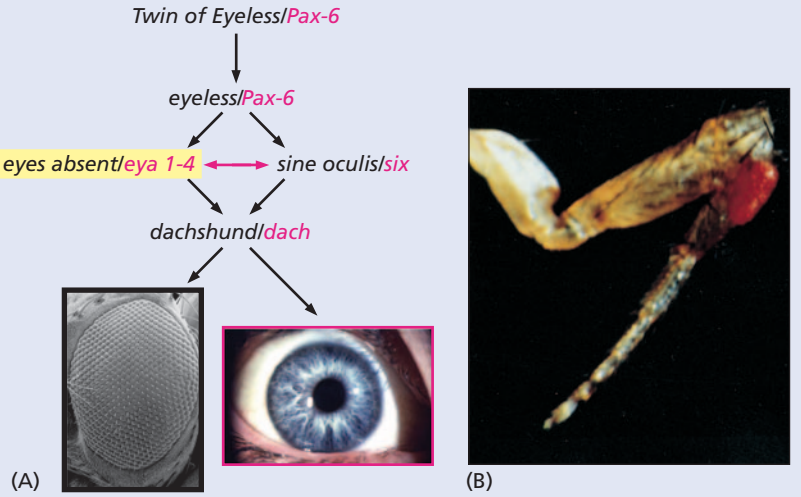
Each gene was found to be localized to a specific site along the length of a specific chromosome. This site is often termed a genetic **locus**. Much effort was expended by geneticists throughout the twentieth century to map the sites of genes—genetic loci—along the chromosomes of a species (**Figure 1.8**).

Sidebar 1.1 Evolutionary forces dictate that certain genes are highly conserved Many genes encode cellular traits that are essential for the continued viability of the cell. These genes, like all others in the genome, are susceptible to the ever-tinkering hand of mutation, which is continually creating new gene sequences by altering existing ones. Natural selection tests these novel sequences and determines whether they specify phenotypes that are more advantageous than the preexisting ones.

Almost invariably, the sequences in genes required for cell and therefore organismic viability were already optimized hundreds of millions of years ago. Consequently, almost all subsequently occurring changes in the sequence information of these genes would have been deleterious and would have compromised the viability of the cell and, in turn, the organism. These mutant alleles were soon lost, because the mutant organisms carrying them failed to leave descendants. This dynamic explains why the sequences of many genes have been highly conserved over vast evolutionary time periods. Stated more accurately, the structures of their encoded proteins have been highly conserved.

In fact, the great majority of the proteins that are present in our own cells and are required for cell viability were first developed during the evolution of single-cell **eukaryotes**. This is indicated by numerous observations showing that many of our proteins have clearly recognizable counterparts in single-cell eukaryotes, such as baker's yeast. Another large repertoire of highly conserved genes and proteins is traceable to the appearance of the first multicellular animals (metazoa); these genes enabled the development of distinct organs and of organismic physiology. Hence, another large group of our own genes and proteins is present in counterpart form in worms and flies (**Figure 1.7**).

By the time the ancestor of all mammals first appeared more than 150 million years ago, virtually all the biochemical and molecular features present in contemporary mammals had already been developed. The fact that they have changed little in the intervening time points to their optimization long before the appearance of the various mammalian orders. This explains why the embryogenesis, physiology, and biochemistry of all mammals is very similar, indeed, so similar that lessons learned through the study of laboratory mice are almost always transferable to an understanding of human biology.

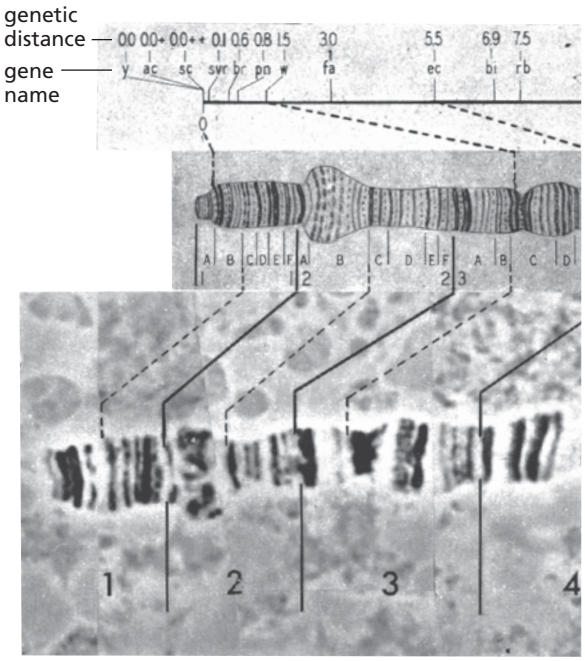


human	FGLARAFGIPIRVYTHEVVTLWYRSPEVLLGS
S. pombe	FGLARSFGVPLRNYTHEIVTLWYRAPEVLLGS
S. cerevisiae	FGLARAFGVPLRAYTHEIVTLWYRAPEVLLGG
human	ARYSTPVDIWSIGTIFAELATKLPLFHGDSEI
S. pombe	RHYSTGVDIWSVGCIFAENIRRSPLFPGDSEI
S. cerevisiae	KQYSTGVDTWSIGCIFAEHCNRLPIFSGDSEI
human	DQLFRIPRALGTPNNEVWPEVESLQDYKNTFP
S. pombe	DEIFKIPQVLGTPNEEVWPGVTLLQDYKSTFP
S. cerevisiae	DQIFKIPRVLGTPNEAIWPDIVYLPDFKPSFP

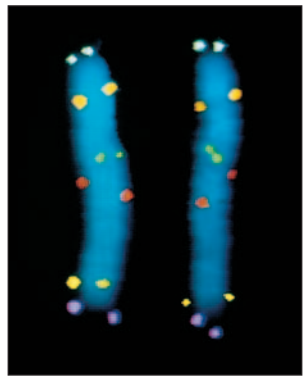
(C)

Figure 1.7 Extraordinary conservation of gene function
The last common ancestor of flies and mammals lived more than 600 million years ago. Moreover, fly (i.e., arthropod) eyes and mammalian eyes show totally different architectures. Nevertheless, the genes that orchestrate their development (*eyeless* in the fly, *Pax-6/small eye* in the mouse) are interchangeable—the gene from one organism can replace the corresponding mutant gene from the other and restore wild-type function. (A) Thus, the genes encoding components of the signal transduction cascades that operate downstream of these master regulators to trigger eye development (*black* for flies, *pink* for mice) are also highly conserved and interchangeable. (B) The expression of the mouse *Pax-6/small eye* gene, like the *Drosophila eyeless* gene, in an inappropriate (*ectopic*) location in a fly embryo results in the fly developing a fly eye on its leg, demonstrating the interchangeability of the two genes. (C) The conservation of genetic function over vast evolutionary distances is often manifested in the amino acid sequences of homologous proteins. Here, the amino acid sequence of a human protein is given together with the sequences of the corresponding proteins from two yeast species, *S. pombe* and *S. cerevisiae*. (A, courtesy of I. Rebay. B, courtesy of Walter Gehring. C, adapted from B. Alberts et al., Essential Cell Biology, 3rd edition New York: Garland Science, 2010.)

The diploid genetic state that reigns in most cells throughout the body was found to be violated in the *germ cells*, sperm and egg. These cells carry only a single copy of each chromosome and gene and thus are said to be **haploid**. During the formation of germ cells in the testes and ovaries, each pair of chromosomes is separated and one of the pair (and thus associated genes) is chosen at random for incorporation into the sperm or egg. When sperm and egg combine subsequently during fertilization,



(A)



(B)

Figure 1.8 Localization of genes along chromosomes (A) The physical structure of *Drosophila* chromosomes was mapped by using the fly's salivary gland chromosomes, which exhibit banding patterns resulting from alternating light (sparse) and dark (condensed) chromosomal regions (*bottom*). Independently, genetic crosses yielded linear maps (*top*) of various genetic loci arrayed along the chromosomes. These loci were then aligned with physical banding maps, like the one shown here for the beginning of the left arm of *Drosophila* chromosome 1. (B) The availability of DNA probes that hybridize specifically to various genes now makes it possible to localize genes along a chromosome by tagging each probe with a specific fluorescent dye or combination of dyes. Shown are six genes that were localized to various sites along human Chromosome 5 by using fluorescence *in situ* hybridization (FISH) during metaphase. (There are two dots for each gene because chromosomes are present in duplicate form during metaphase of mitosis.) (A, from M. Singer and P. Berg, Genes and Genomes. Mill Valley, CA: University Science Books, 1991, as taken from C.B. Bridges, *J. Hered.* 26:60, 1935. B, courtesy of David C. Ward.)

the two haploid genomes fuse to yield the new diploid genome of the fertilized egg. All cells in the organism descend directly from this diploid cell and, if all goes well, inherit precise replicas of its diploid genome. In a large multicellular organism like the human, this means that a complete copy of the genome is present in almost all of the approximately 3×10^{13} cells throughout the body!

With the realization that genes reside in chromosomes, and that a complete set of chromosomes is present in almost all cell types in the body, came yet another conclusion that was rarely noted: genes create the phenotypes of an organism through their ability to act locally by influencing the behavior of its individual cells. The alternative—that a single set of genes residing at some unique anatomical site in the organism controls the entire organism's development and physiology—was now discredited.

The rule of paired, similarly appearing chromosomes was found to be violated by some of the sex chromosomes. In the cells of female placental mammals, there are two similarly appearing X chromosomes, and these behave like the **autosomes** (the nonsex chromosomes). But in males, an X chromosome is paired with a Y chromosome, which is smaller and carries a much smaller repertoire of genes. In humans, the X chromosome is thought to carry about 900 genes, compared with the 78 distinct genes on the Y chromosome, which, because of redundancy, specify only 27 distinct proteins (**Figure 1.9**).

This asymmetry in the configuration of the sex chromosomes puts males at a biological disadvantage. Many of the 900 or so genes on the X chromosome are vital to normal organismic development and function. The twofold redundancy created by the paired X chromosomes guarantees more robust biology. If a gene copy on one of the X chromosomes is defective (that is, a nonfunctional mutant allele), chances are that the second copy of the gene on the other X chromosome can continue to carry out the task of the gene, ensuring normal biological function. Males lack this genetic fail-safe system in their sex chromosomes. One of the more benign consequences of this is color blindness, which strikes males frequently and females infrequently, due to the localization on the X chromosome of the genes encoding the color-sensing proteins of the retina.

This disparity between the genders is mitigated somewhat by the mechanism of X-inactivation. Early in embryogenesis, one of the two X chromosomes is randomly

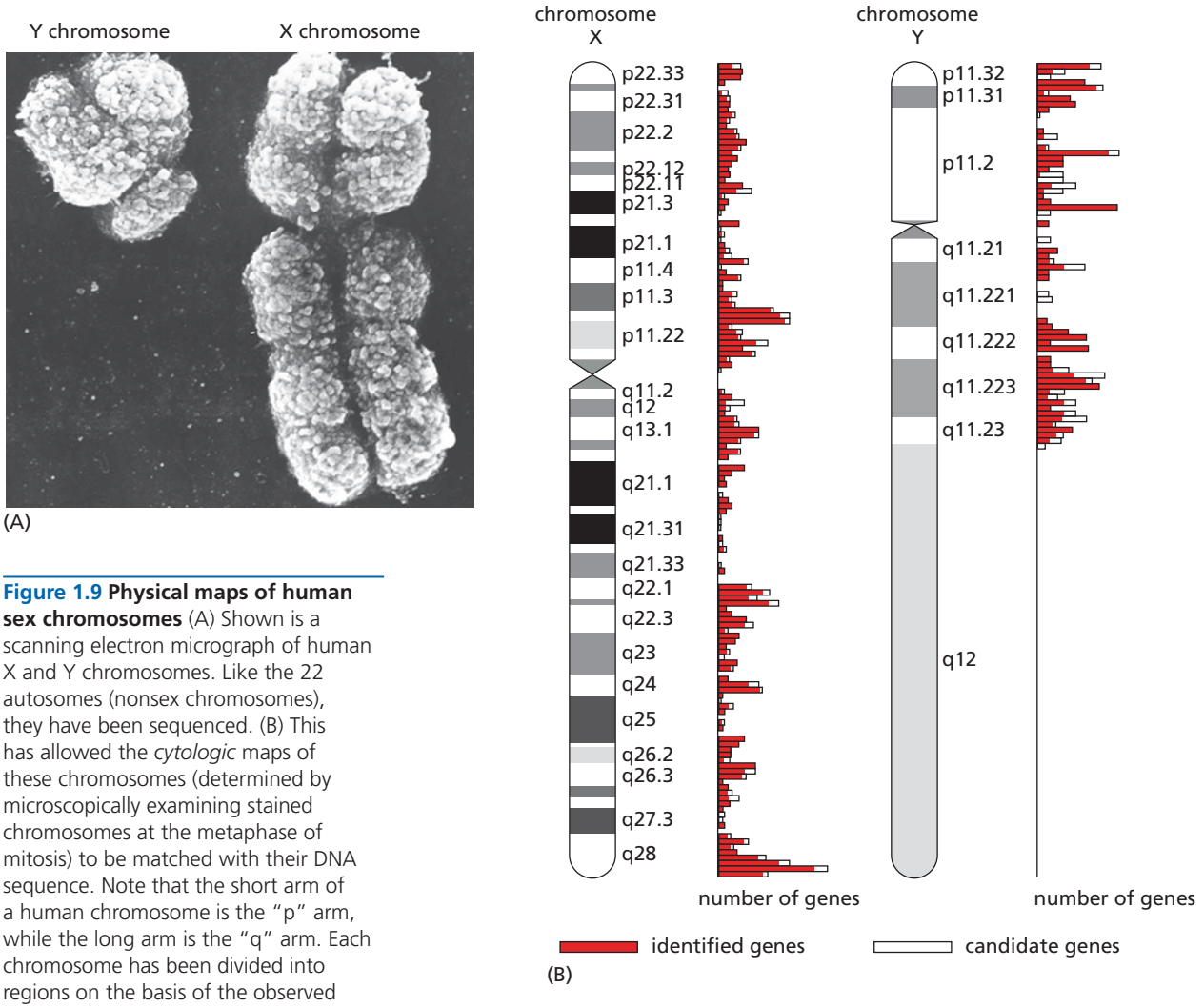


Figure 1.9 Physical maps of human sex chromosomes (A) Shown is a scanning electron micrograph of human X and Y chromosomes. Like the 22 autosomes (nonsex chromosomes), they have been sequenced. (B) This has allowed the *cytologic* maps of these chromosomes (determined by microscopically examining stained chromosomes at the metaphase of mitosis) to be matched with their DNA sequence. Note that the short arm of a human chromosome is the "p" arm, while the long arm is the "q" arm. Each chromosome has been divided into regions on the basis of the observed banding pattern, and distinct genes have been assigned on the basis of the sequence analyses (*histograms to right of each chromosome*). Identified genes are filled bars (*red*), while sequences that appear to encode still-to-be-identified genes are in open bars; in most chromosomal regions the latter represent a small minority. The human Y chromosome is ~57 megabases (Mb) long, compared with the X chromosome's ~155 Mb. (A, courtesy of Indigo® Instruments. B, courtesy of The Wellcome Trust Sanger Institute. Ensembl genome browser http://www.ensembl.org.)

inactivated in each of the cells of a female embryo. This inactivation silences almost all of the genes on this chromosome and causes it to shrink into a small particle termed the **Barr body**. Subsequently, all descendants of that cell will inherit this pattern of chromosomal inactivation and will therefore continue to carry the same inactivated X chromosome. Accordingly, the female advantage of carrying redundant copies of X chromosome–associated genes is only a partial one (Supplementary Sidebar 1.1).

Color blindness reveals the virtues of having two redundant gene copies around to ensure that biological function is maintained. If one copy is lost through mutational inactivation, the surviving gene copy is often capable of specifying a wild-type phenotype. Such functional redundancy operates for the great majority of genes carried by the autosomes. As we will see later, this dynamic plays an important role in cancer development, since virtually all of the genes that operate to prevent runaway proliferation of cells are present in two redundant copies, both of which must be inactivated in a cell before their growth-suppressing functions are lost and malignant cell proliferation can occur.

1.4 Chromosomes are altered in most types of cancer cells

Individual genes are far too small to be seen with a light microscope, and subtle mutations within a gene are smaller still. Consequently, the great majority of the mutations that play a part in cancer cannot be visualized through microscopy. However, the examination of chromosomes through the light microscope can give evidence of

large-scale alterations of the cell genome. Indeed, such alterations were noted as early as 1892, specifically in cancer cells.

Today, we know that cancer cells often exhibit aberrantly structured chromosomes of various sorts, the loss of entire chromosomes, the presence of extra copies of others, and the fusion of the arm of one chromosome with part of another. These changes in overall chromosomal configuration expand our conception of how mutations can affect the genome: since alterations of overall chromosomal structure and number also constitute types of genetic change, these changes must be considered to be the consequences of mutations (**Sidebar 1.2**). And importantly, the abnormal chromosomes seen initially in cancer cells provided the first clue that these cells might be genetically aberrant, that is, that they were mutants (see Figure 1.11).

The normal configuration of chromosomes is often termed the **euploid** karyotypic state. Euploidy implies that each of the autosomes is present in normally structured pairs and that the X and Y chromosomes are present in the numbers appropriate for the sex of the individual carrying them. Deviation from the euploid karyotype—the state termed **aneuploidy**—is seen, as mentioned above, in many cancer cells. Often this aneuploidy is merely a consequence of the general chaos that reigns within a cancer cell. However, this connection between aneuploidy and malignant cell proliferation also hints at a theme that we will return to repeatedly in this book: the acquisition of extra copies of one chromosome or the loss of another can create a genetic configuration that somehow benefits the cancer cell and its agenda of runaway proliferation.

1.5 Mutations causing cancer occur in both the germ line and the soma

Mutations alter the information content of genes, and the resulting mutant alleles of a gene can be passed from parent to offspring. This transmission from one generation to the next, made possible by the germ cells (sperm and egg), is said to occur via the **germ line** (**Figure 1.10**). Importantly, the germ-line transmission of a recently created mutant allele from one organism to its offspring can occur only if a precondition has been met: the responsible mutation must strike a gene carried in the genome of sperm or egg or in the genome of one of the cell types that are immediate precursors of the sperm or egg within the gonads. Mutations affecting the genomes of cells everywhere else in the body—which constitute the **soma**—may well affect the particular cells in which such mutations strike but will have no prospect of being transmitted to the offspring of an organism. Such **somatic mutations** cannot become incorporated into the vehicles of generation-to-generation genetic transmission—the chromosomes of sperm or eggs.

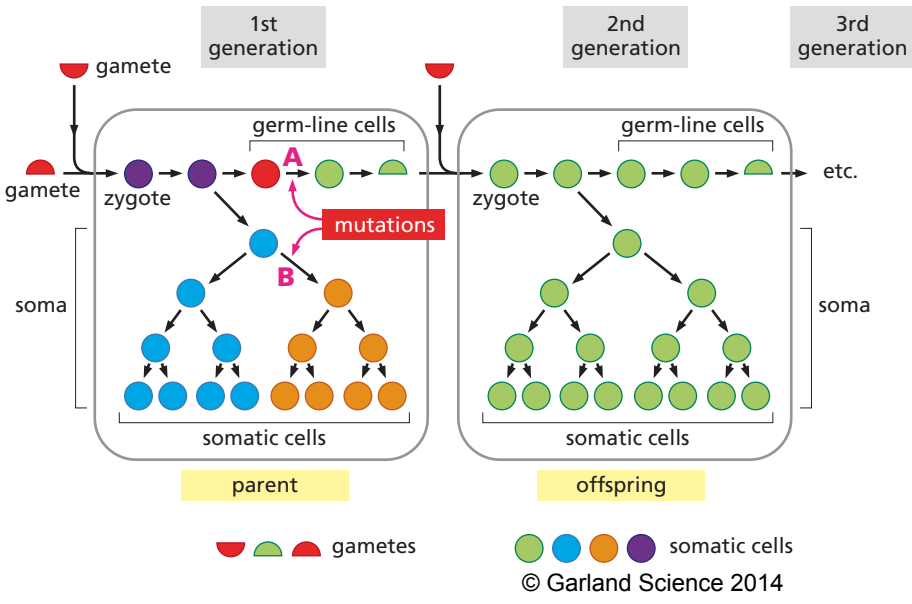


Figure 1.10 Germ-line versus somatic mutations Mutation A, which occurs in the genome of a germ-line cell in the gonads, can be passed from parent (*above left*) to offspring via gametes—sperm or egg (*half circles*). Once incorporated into the fertilized egg (zygote), the mutant alleles can then be transmitted to all of the cells in the body of the offspring (*middle*) outside of the gonads, i.e., its soma, as well as being transmitted via germ-line cells and gametes to a third generation (*not shown*). However, mutation B (*left*), which strikes the genome of a somatic cell in the parent, can be passed only to the lineal descendants of that mutant cell within the body of the parent and cannot be transmitted to offspring. (Adapted from B. Alberts et al., Essential Cell Biology, 3rd ed. New York: Garland Science, 2010.)

Sidebar 1.2 Cancer cells are often aneuploid The presence of abnormally structured chromosomes and changes in chromosome number provided the first clue, early in the twentieth century, that changes in cell genotype often accompany and perhaps cause the uncontrolled proliferation of malignant cells. These deviations from the normal euploid karyotype can be placed into a number of categories. Chromosomes that seem to be structurally normal may accumulate in extra copies, leading to three, four, or even more copies of these chromosomes per cancer cell nucleus (**Figure 1.11**); such deviations from normal chromosome number are manifestations of *aneuploidy*.

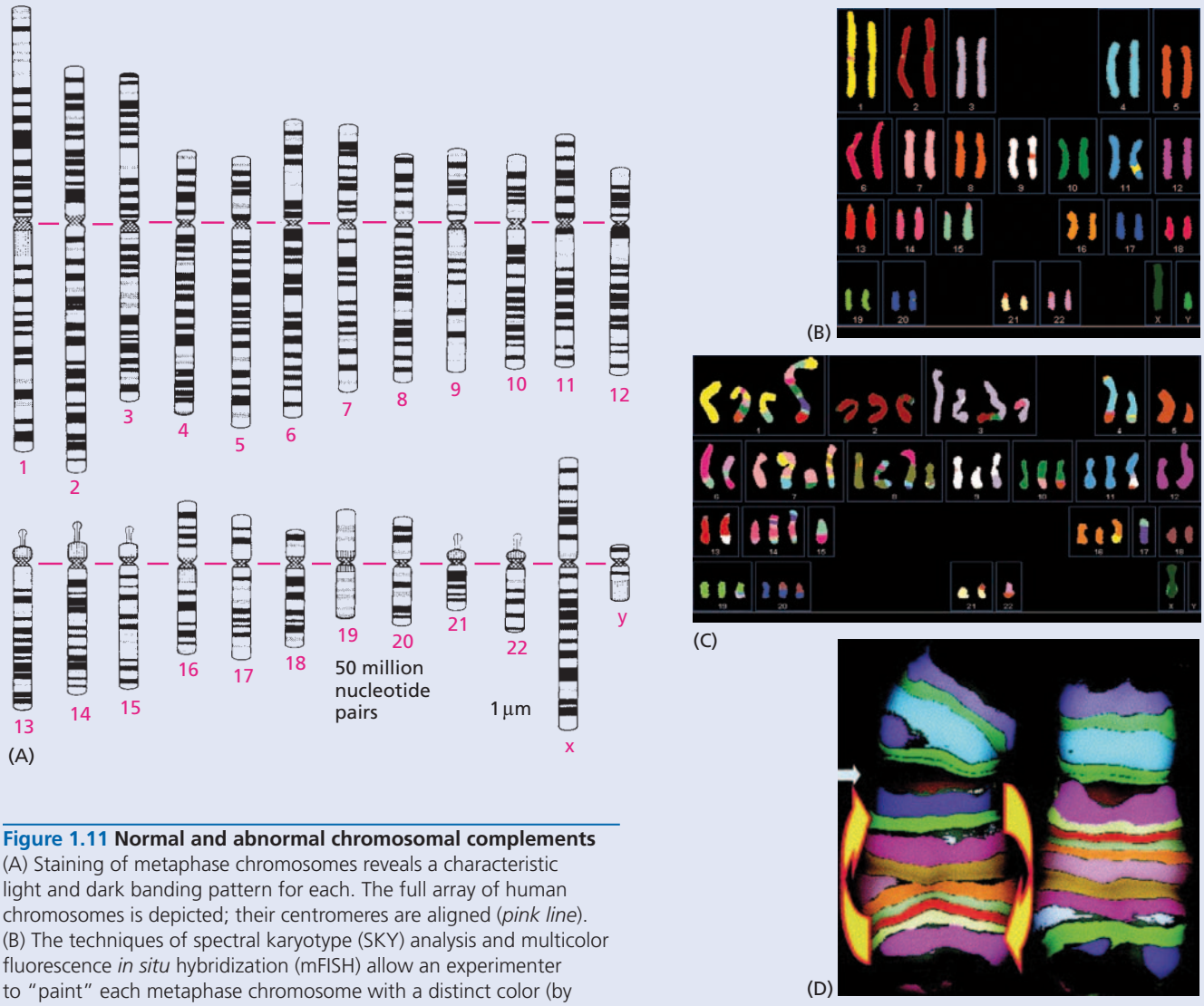
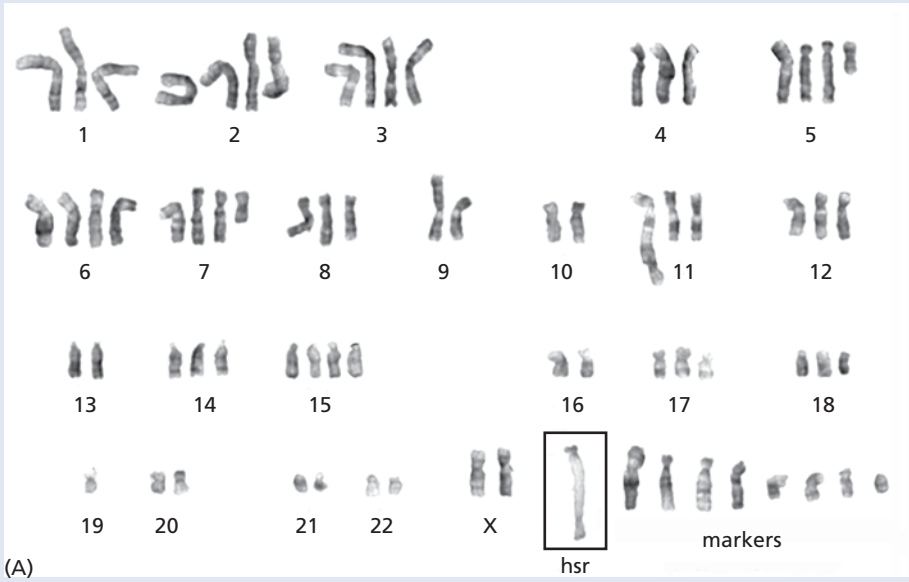
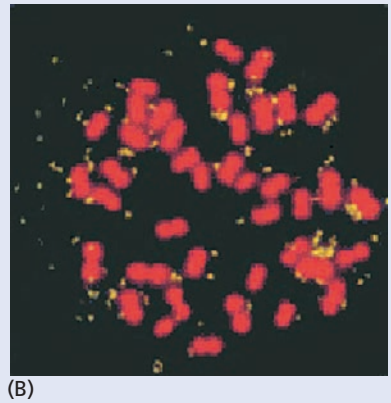


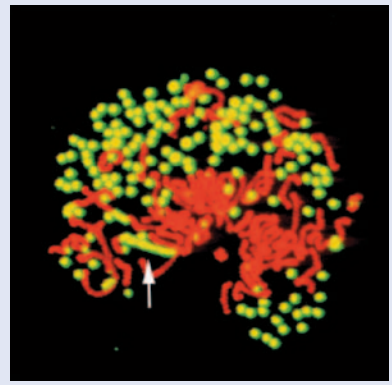
Figure 1.11 Normal and abnormal chromosomal complements (A) Staining of metaphase chromosomes reveals a characteristic light and dark banding pattern for each. The full array of human chromosomes is depicted; their centromeres are aligned (*pink line*). (B) The techniques of spectral karyotype (SKY) analysis and multicolor fluorescence *in situ* hybridization (mFISH) allow an experimenter to "paint" each metaphase chromosome with a distinct color (by hybridizing chromosome-specific DNA probes labeled with various fluorescing dyes to the chromosomes). The actual colors in images such as these are generated by computer. The diploid karyotype of a normal human male cell is presented. (The small regions in certain chromosomes that differ from the bulk of these chromosomes represent hybridization artifacts.) (C) The aneuploid karyotype of a human pancreatic cancer cell, in which some chromosomes are present in inappropriate numbers and in which numerous translocations (exchanges of segments between chromosomes) are apparent. (D) Here, mFISH was used to label intrachromosomal subregions with specific fluorescent dyes, revealing that a large portion of an arm of normal human Chromosome 5 (*right*) has been inverted (*left*) in cells of a worker who had been exposed to plutonium in the nuclear weapons industry of the former Soviet Union. (A, adapted from U. Francke, *Cytogenet. Cell Genet.* 31:24–32, 1981. B and C, courtesy of M. Grigorova, J.M. Staines and P.A.W. Edwards. D, from M.P. Hande et al., *Am. J. Hum. Genet.* 72:1162–1170, 2003.)

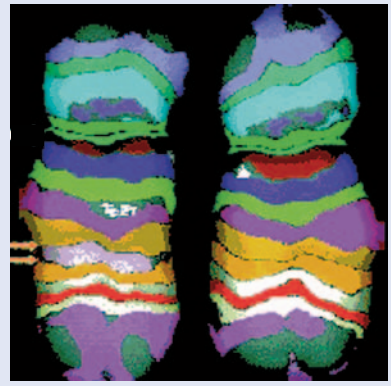
Alternatively, chromosomes may undergo changes in their structure. A segment may be broken off one chromosomal arm and become fused to the arm of another chromosome, resulting in a chromosomal **translocation** (Figure 1.11C). Moreover, chromosomal segments may be exchanged between chromosomes from different chromosome pairs, resulting in **reciprocal translocations**. A chromosomal segment may also become inverted, which may affect the regulation of genes that are located near the breakage-and-fusion points (Figure 1.11D).

A segment of a chromosome may be copied many times over, and the resulting extra copies may be fused head-to-tail in long arrays within a chromosomal segment that is termed an HSR (**homogeneously staining region**; **Figure 1.12A**). A segment may also be cleaved out of a chromosome, replicate









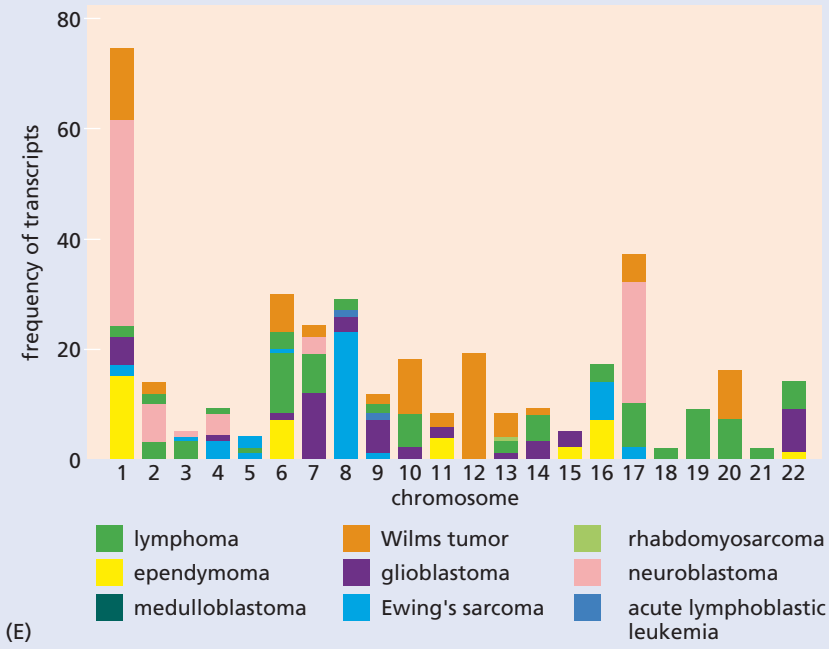


Figure 1.12 Increases and decreases in copy number of chromosomal segments (A) The amplification in the copy number of the *myc* oncogene (see Section 8.9) in a human neuroendocrinal tumor has caused an entire stretch of chromosome to stain *white* (rectangle), creating a homogeneously staining region (HSR). (B) Double-minute chromosomes (DMs) derive from chromosomal segments that have broken loose from their original sites and have been replicated repeatedly as extrachromosomal genetic elements; like normal chromatids, these structures are doubled during metaphase of mitosis. FISH reveals the presence of amplified copies of the *HER2/neu* oncogene borne on DMs (*yellow dots*) in a mouse breast cancer cell. (C) Occasionally, an amplified gene may be found both in an HSR (nested within a chromosome) and in DMs. Here, analysis of COLO320 cells reveals multiple copies of the *myc* oncogene (*yellow*), amid the chromosomes (*red*). One HSR is indicated by the arrow, while many dozens of DMs are apparent. (D) The use of multicolor FISH (mFISH) revealed that a segment within normal human Chromosome 5 (*paired arrows, left*) has been deleted (an interstitial deletion, *right*) following extensive exposure to radiation from plutonium. (E) A survey of nine different types of pediatric cancer indicates that each cancer type has characteristic gene amplification and deletion patterns with corresponding changes in the expression of the altered genes. For example, neuroblastomas (*pink*) often have changes in the copy numbers of genes on chromosomes 1 and 17 and corresponding changes in the levels of the transcripts expressed by these genes. (A, from J.-M. Wen et al., *Cancer Genet. Cytogenet.* 135:91–95, 2002. B, from C. Montagna et al., *Oncogene* 21:890–898, 2002. C, from N. Shimizu et al., *J. Cell Biol.* 140:1307–1320, 1998. D, from M.P. Hande et al., *Am. J. Hum. Genet.* 72:1162–1170, 2003. E, from G. Neale et al., *Clin. Cancer Res.* 14:4572–4583, 2008.)

as an autonomous, extrachromosomal entity, and increase to many copies per nucleus, resulting in the appearance of subchromosomal fragments termed DMs (**double minutes**; Figure 1.12B). These latter two changes cause increases in the copy number of genes carried in such segments, resulting in **gene amplification**. Sometimes, both types of amplification coexist in the same cell (Figure 1.12C). Gene amplification can favor the growth of cancer cells by increasing the copy number of growth-promoting genes.

On some occasions, certain growth-inhibiting genes may be discarded by cancer cells during their development. For example, when a segment in the middle of a chromosomal arm is discarded and the flanking chromosomal regions are joined, this results in an **interstitial deletion** (Figure 1.12D).

These descriptions of copy-number changes in genes, involving both amplifications and deletions, might suggest widespread chaos in the genomes of cancer cells, with gene amplifications and deletions occurring randomly. However, as the karyotypes and genomes of human tumors have been examined more intensively, it has become clear that certain regions of the genome tend to be lost characteristically in certain tumor types but not in others (Figure 1.12E). This suggests a theme that we will pursue in great detail throughout this book—that the gains and losses of particular genes favor the proliferation of specific types of tumors. This indicates that different tumor types undergo different genetic changes as they develop progressively from the precursor cells in normal tissues.

Somatic mutations are of central importance to the process of cancer formation. As described repeatedly throughout this book, a somatic mutation can affect the behavior of the cell in which it occurs and, through repeated rounds of cell growth and division, can be passed on to all descendant cells within a tissue. These direct descendants of a single progenitor cell, which may ultimately number in the millions or even billions, are said to constitute a cell **clone**, in that all members of this group of cells trace their ancestry directly back to the single cell in which the mutation originally occurred.

An elaborate repair apparatus within each cell continuously monitors the cell's genome and, with great efficiency, eradicates mutant sequences, replacing them with appropriate wild-type sequences. We will examine this repair apparatus in depth in Chapter 12. This apparatus maintains genomic integrity by minimizing the number of mutations that strike the genome and are then perpetuated by transmission to descendant cells. One stunning indication of the efficiency of genome repair comes from the successes of organismic cloning: the ability to generate an entire organism from the nucleus of a differentiated cell (prepared from an adult) indicates that this adult cell genome is essentially a faithful replica of the genome of a fertilized egg, which existed many years and many cell generations earlier (Supplementary Sidebar 1.2).

However, no system of damage detection and repair is infallible. Some mistakes in genetic sequence survive its scrutiny, become fixed in the cell genome, are copied into new DNA molecules, and are then passed on as mutations to progeny cells. In this sense, many of the mutations that accumulate in the genome represent the consequences of occasional oversights made by the repair apparatus. Yet others are the results of catastrophic damage to the genome that exceeds the capacities of the repair apparatus.

1.6 Genotype embodied in DNA sequences creates phenotype through proteins

The genes studied in Mendelian genetics are essentially mathematical abstractions. Mendelian genetics explains their transmission, but it sheds no light on how genes create cellular and organismic phenotypes. Phenotypic attributes can range from complex, genetically templated behavioral traits to the **morphology** (shape, form) of cells and subcellular organelles to the biochemistry of cell metabolism. This mystery of how genotype creates phenotype represented the major problem of twentieth-century biology. Indeed, attempts at forging a connection between these two became the obsession of many molecular biologists during the second half of the twentieth century and continue as such into the twenty-first, if only because we still possess an incomplete understanding of how genotype influences phenotype.

Molecular biology has provided the basic conceptual scaffold for understanding the connection between genotype and phenotype. In 1944, DNA was proven to be the

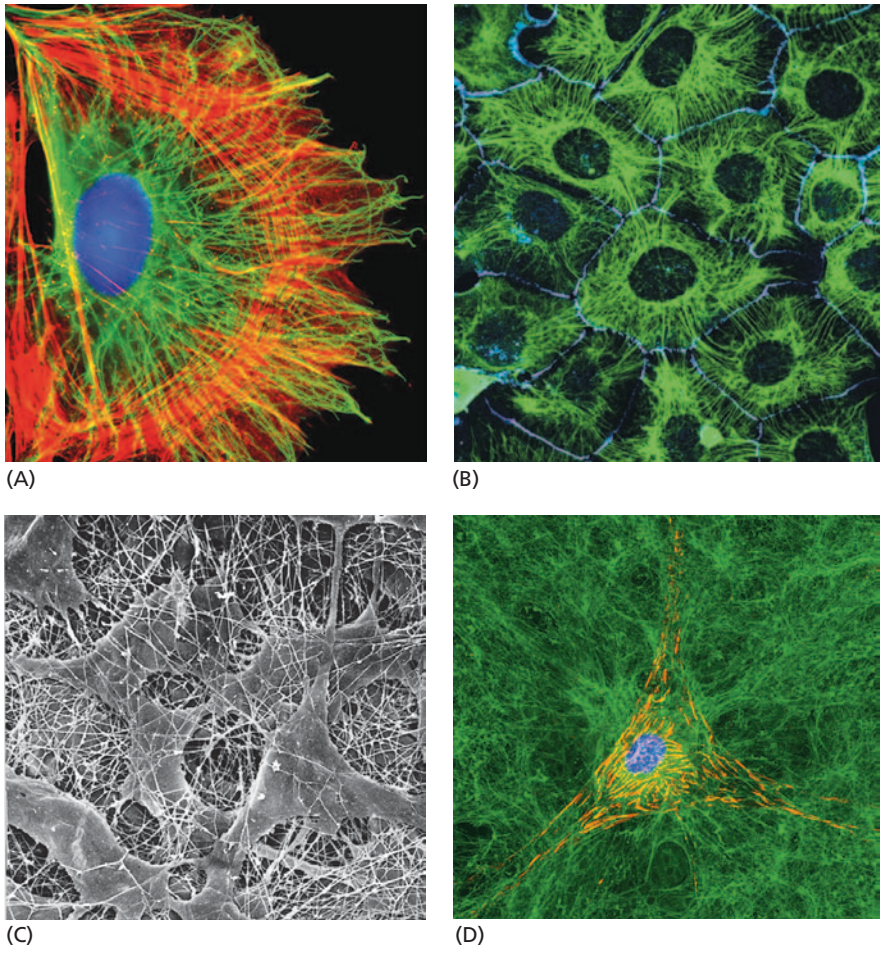


Figure 1.13 Intracellular and extracellular scaffolding The cytoskeleton is assembled from complex networks of intermediate filaments, actin microfilaments, and microtubules. Together, they generate the shape of a cell and enable its motion. (A) In this cultured cell, microfilaments composed of actin (*orange*) form bundles that lie parallel to the cell surface while microtubules composed of tubulin (*green*) radiate outward from the nucleus (*blue*). Both types of fibers are involved in the formation of protrusions from the cell surface. (B) Here, an important intermediate filament of epithelial cells—keratin—is detected using an anti-keratin-specific antibody (*green*). The boundaries of cells are labeled with a second antibody that reacts with a plasma membrane protein (*blue*). (C) Cells secrete a diverse array of proteins that are assembled into the extracellular matrix (ECM). A scanning electron micrograph reveals the complex meshwork of collagen fibers, glycoproteins, hyaluronan, and proteoglycans, in which fibroblasts (connective tissue cells) are embedded. (D) A cell of the NIH 3T3 cell line, which is used extensively in cancer cell biology, is shown amid an ECM network of fibronectin fibers (*green*). The points of cellular attachment to the fibronectin are mediated by integrin receptors on the cell surface (*orange, yellow*). (A, courtesy of Albert Tousson, High-Resolution Imaging Facility, University of Alabama at Birmingham. B, courtesy of Kathleen Green and Evangeline Amargo. C, courtesy of T. Nishida. D, from E. Cukierman et al., *Curr. Opin. Cell Biol.* 14:633–639, 2002.)

chemical entity in which the genetic information of cells is carried. Nine years later, Watson and Crick elucidated the double-helical structure of DNA. A dozen years after that, in 1964, it became clear that the sequences in the bases of the DNA double helix determine precisely the sequence of amino acids in proteins. The unique structure and function of each type of protein in the cell is determined by its sequence of amino acids. Therefore, the specification of amino acid sequence, which is accomplished by base sequences in the DNA, provides almost all the information that is required to construct a protein.

Once synthesized within cells, proteins proceed to create phenotype, doing so in a variety of ways. Proteins can assemble within the cell to create the components of the **cytoarchitecture**, or more specifically, the **cytoskeleton** (**Figure 1.13A and B**). When secreted into the space between cells, such proteins form the **extracellular matrix** (ECM); it ties cells together, enabling them to form complex tissues (Figure 1.13C and D). As we will see later, the structure of the ECM is often disturbed by malignant cancer cells, enabling them to migrate to sites within a tissue and organism that are usually forbidden to them.

Many proteins function as enzymes that catalyze the thousands of biochemical reactions that together are termed **intermediary metabolism**; without the active intervention of enzymes, few of these reactions would occur spontaneously. Proteins can also contract and create cellular movement (**motility**; **Figure 1.14**) as well as muscle contraction. Cellular motility plays a role in cancer development by allowing cancer cells to spread through tissues and migrate to distant organs.

And most important for the process of cancer formation, proteins can convey signals between cells, thereby enabling complex tissues to maintain the appropriate numbers of constituent cell types. Within individual cells, certain proteins receive signals

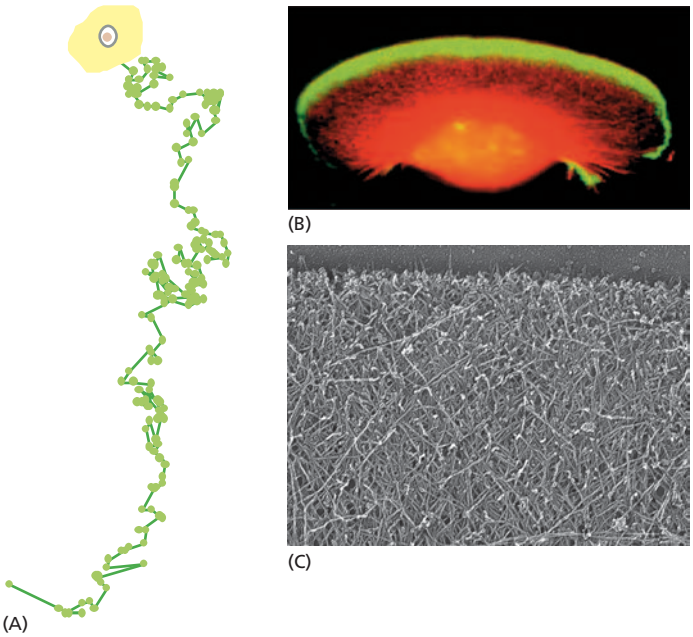


Figure 1.14 Cell motility (A) The movement of individual cells in a culture dish can be plotted at intervals and scored electronically. This image traces the movement of a human vascular endothelial cell (the cell type that forms the lining of blood vessels) toward two attractants located at the bottom—vascular endothelial growth factor (VEGF) and sphingosine-1-phosphate (S1P). Such locomotion is presumed to be critical to the formation of new blood vessels within a tumor. Each point represents a position plotted at 10-minute intervals. This motility is made possible by complex networks of proteins that form the cells' cytoskeletons. (B) The advancing cell is a fish keratocyte; its leading edge (*green*) is pushed forward by an actin filament network, such as the one shown in C. (C) Seen here is the network of actin filaments that is assembled at the leading edge of a motile cell. (A, courtesy of C. Furman and F. Gertler. B and C, from T. Svitkina and G. Borisy, *J. Cell Biol*. 145:1009–1026, 1999. © The Rockefeller University Press.)

from an extracellular source, process these signals, and pass them on to other proteins within the cell; such signal-processing functions, often termed intracellular signal **transduction**, are also central to the creation of cancers, since many of the abnormal-growth phenotypes of cancer cells are the result of aberrantly functioning intracellular signal-transducing molecules.

The functional versatility of proteins makes it apparent that almost all aspects of cell and organismic phenotype can be created by their actions. Once we realize this, we can depict genotype and phenotype in the simplest of molecular terms: genotype resides in the sequences of bases in DNA, while phenotype derives from the actions of proteins. (In fact, this depiction is simplistic, because it ignores the important role of RNA molecules as intermediaries between DNA sequences and protein structure and the recently discovered abilities of some RNA molecules to function as enzymes and others to act as regulators of the expression of certain genes.)

In the complex **eukaryotic** (nucleated) cells of animals, as in the simpler **prokaryotic** cells of bacteria, DNA sequences are copied into RNA molecules in the process termed **transcription**; a gene that is being transcribed is said to be actively **expressed**, while a gene that is not being transcribed is often considered to be **repressed**. In its simplest version, the transcription of a gene yields an RNA molecule of length comparable to the gene itself. Once synthesized, the base sequences in the RNA molecule are **translated** by the protein-synthesizing factories in the cell, its **ribosomes**, into a sequence of amino acids. The resulting macromolecule, which may be hundreds, even thousands of amino acids long, folds up into a unique three-dimensional configuration and becomes a functional protein (**Figure 1.15**).

Post-translational modification of the initially synthesized protein may result in the covalent attachment of certain chemical groups to specific amino acid residues in the protein chain; included among these modifications are, notably, phosphates, complex sugar chains, and methyl, acetyl, and lipid groups (**Sidebar 1.3**). Thus, the extracellular domains of most cell-surface proteins and almost all secreted proteins are glycosylated, having one or more covalently attached sugar side chains; proteins of the Ras family, which are located in the cytoplasm and play important roles in cancer development, contain lipid groups attached to their carboxy termini. An equally important post-translational modification involves the cleavage of one protein by a second protein termed a **protease**, which has the ability to cut amino acid chains at certain sites. Accordingly, the final, mature form of a protein chain may include far fewer amino acid residues than were present in the initially synthesized protein. Following their synthesis, many proteins are dispatched to specific sites within the cell or are exported

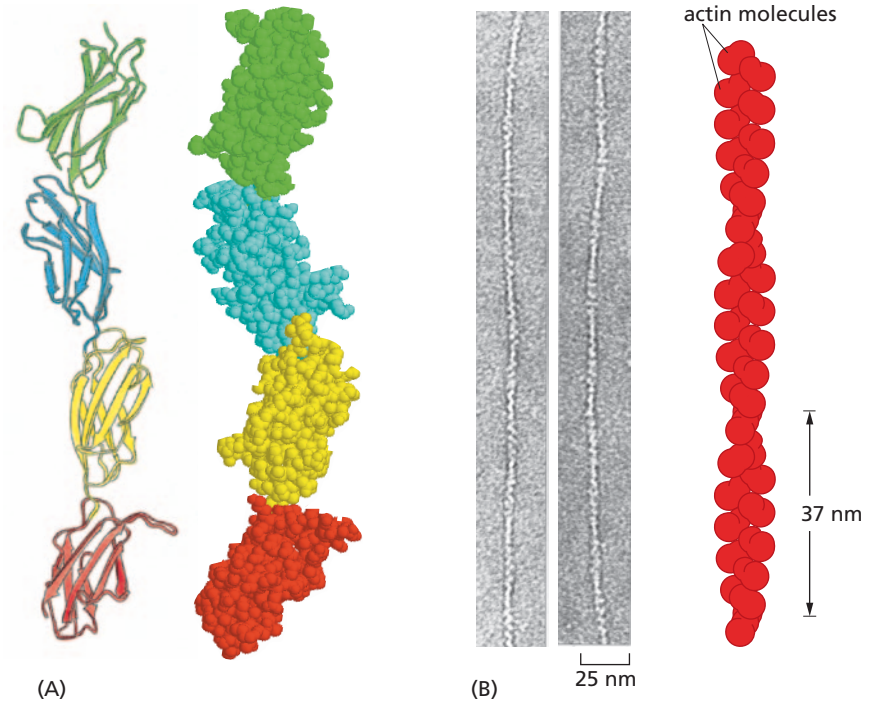


Figure 1.15 Structures of proteins and multiprotein assemblies (A) The three-dimensional structure of part of fibronectin, an important extracellular matrix protein (see Figure 1.13D), is depicted as a ribbon diagram (*left*), which illustrates the path taken by its amino acid chain; alternatively, the space-filling model (*right*) shows the positions of the individual atoms. One portion of fibronectin is composed of four distinct, similarly structured domains, which are shown here with different colors. (B) The actin fibers (*left*), which constitute an important component of the cytoskeleton (see Figures 1.13 and 1.14), are composed of assemblies of individual protein molecules, each of which is illustrated here as a distinct two-lobed body (*right*). (A, adapted from D.J. Leahy, I. Aukhil and H.P. Erickson, *Cell* 84:155–164, 1996. B, left, courtesy of Roger Craig; right, from B. Alberts et al., Molecular Biology of the Cell, 5th ed. New York: Garland Science, 2008.)

from the cell through the process of secretion; these alternative destinations are specified in the newly synthesized proteins by short amino acid (oligopeptide) sequences that function, much like postal addresses, to ensure the diversion of these proteins to specific intracellular sites.

In eukaryotic cells—the main subject of this book—the synthesis of RNA is itself a complex process. An RNA molecule transcribed from its parent gene may initially be almost as long as that gene. However, while it is being elongated, segments of the RNA

Sidebar 1.3 How many distinct proteins can be found in the human body? While some have ventured to provide estimates of the total number of human genes (a bit more than 21,000), it is difficult to extrapolate from this number to the total number of distinct proteins encoded in the human genome. The simplest estimate comes from the assumption that each gene encodes the structure of a single protein. But this assumption is naive, because it ignores the fact that the pre-mRNA transcript deriving from a single gene may be subjected to several *alternative splicing* patterns, yielding multiple, distinctly structured mRNAs, many of which may in turn encode distinct proteins (see Figure 1.16). Thus, in some cells, splicing may include certain exons in the final mRNA molecule made from a gene, while in other cells, these exons may be absent. Such alternative splicing patterns can generate mRNAs having greatly differing structures and protein-encoding sequences. In one, admittedly extreme case, a single *Drosophila* gene has been found to be capable of generating 38,016 distinct mRNAs and thus proteins through various alternative splices of its pre-mRNA; genes having similarly complex alternative splicing patterns are likely to reside in our own genome.

An additional dimension of complexity derives from the post-translational modifications of proteins. The proteins that are exported to the cell surface or released in soluble form into the extracellular space are usually modified by the attachment of complex trees of sugar molecules during the process of **glycosylation**. Intracellular proteins often undergo other types of chemical modifications. Proteins involved in transducing the signals that govern cell proliferation often undergo **phosphorylation** through the covalent attachment of phosphate groups to serine, threonine, or tyrosine amino acid residues. Many of these phosphorylations affect some aspect of the functioning of these proteins. Similarly, the histone proteins that wrap around DNA and control its access by the RNA polymerases that synthesize hnRNA are subject to methylation, acetylation, and phosphorylation, as well as more complex post-translational modifications.

The polypeptide chains that form proteins may also undergo cleavage at specific sites following their initial assembly, often yielding small proteins showing functions that were not apparent in the uncleaved precursor proteins. Later, we will describe how certain signals may be transmitted through the cell via a cascade of the protein-cleaving enzymes termed proteases. In these cases, protein A may cleave protein B, activating its previously latent protease activity; thus activated, protein B may cleave protein C, and so forth. Taken together, alternative splicing and post-translational modifications of proteins generate vastly more distinct protein molecules than are apparent from counting the number of genes in the human genome.

molecule, some very small and others enormous, will be cleaved out of the growing RNA molecule. These segments, termed **introns**, are soon discarded and consequently have no impact on the subsequent coding ability of the RNA molecule (**Figure 1.16**).

Flanking each intron are two retained sequences, the **exons**, which are fused together during this process of **splicing**. The initially synthesized RNA molecule and its derivatives found at various stages of splicing, together with nuclear RNA transcripts being processed from other genes, collectively constitute the **hnRNA** (**heterogeneous**

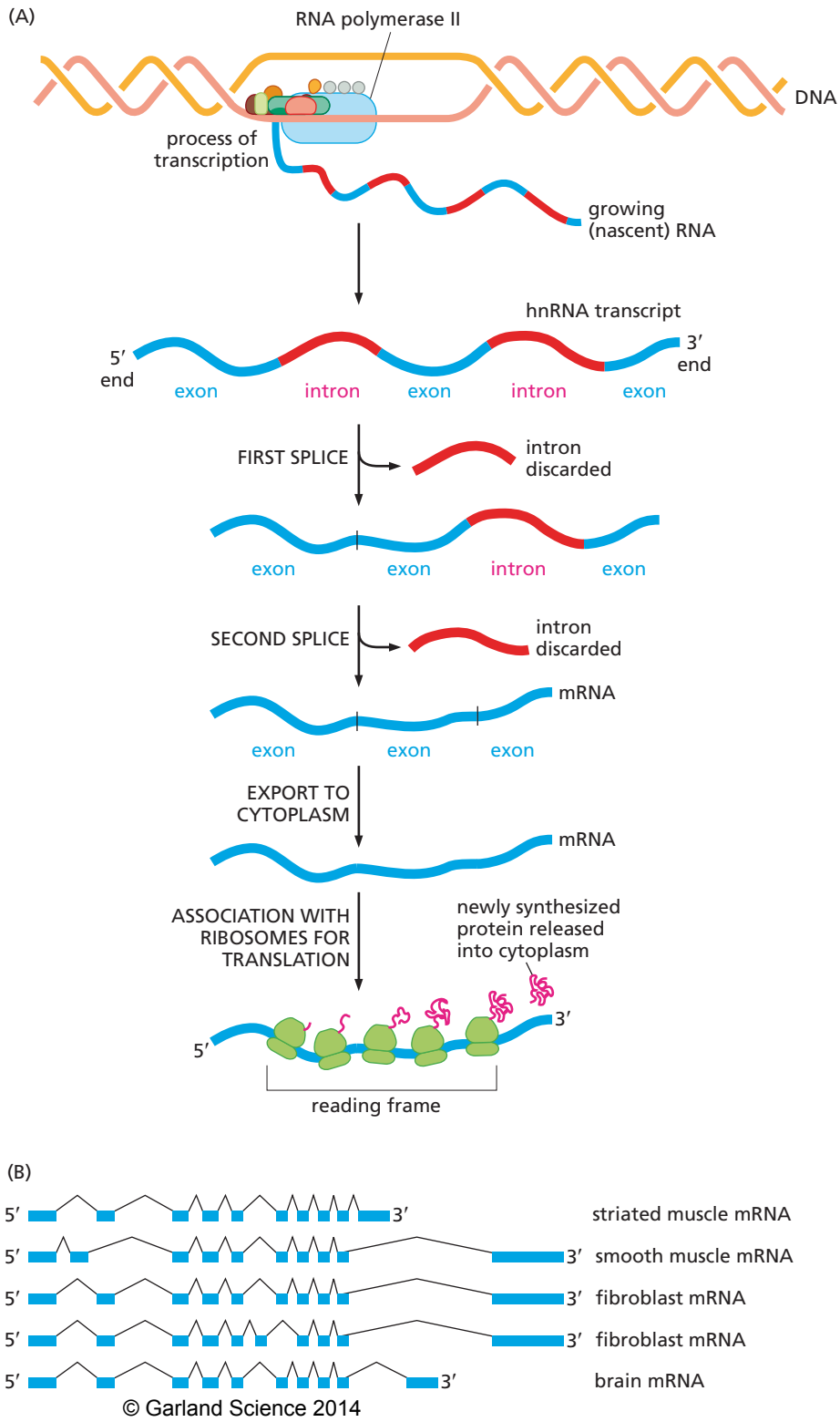


Figure 1.16 Processing of pre-mRNA (A) By synthesizing a complementary RNA copy of one of the two DNA strands of a gene, RNA polymerase II creates a molecule of heterogeneous nuclear RNA (hnRNA) (*red and blue*). Those hnRNA molecules that are processed into mRNAs are termed pre-mRNA. The progressive removal of the introns (*red*) leads to a processed mRNA containing only exons (*blue*). (B) A given pre-mRNA molecule may be spliced in a number of alternative ways, yielding distinct mRNAs that may encode distinct protein molecules. Illustrated here are the tissue-specific alternative splicing patterns of the α-tropomyosin pre-mRNA molecule, whose mRNA products specify important components of cell (and thus muscle) contractility. In this case, the introns are indicated as *black carets* while the exons are indicated as *blue rectangles*. (B, adapted from B. Alberts et al., Molecular Biology of the Cell, 5th ed. New York: Garland Science, 2008.)

nuclear RNA). The end product of these post-transcriptional modifications may be an RNA molecule that is only a small fraction of the length of its initially synthesized, hnRNA precursor. This final, mature RNA molecule is likely to be exported into the cytoplasm, where, as an **mRNA** (**messenger RNA**) molecule, it serves as the template on which ribosomes assemble the amino acids that form the proteins. (The term **pre-mRNAs** is often used to designate those hnRNAs that are known precursors of cytoplasmic mRNAs.) Some mature mRNAs may be less than 1% of the length of their pre-mRNA precursor. The complexity of post-transcriptional modification of RNA and post-translational modification of proteins yields an enormous array of distinct protein species within the cell (see Sidebar 1.3).

Of note, an initially transcribed pre-mRNA may be processed through **alternative splicing** into a series of distinct mRNA molecules that retain different combinations of exons (see Figure 1.16B). Indeed, the pre-mRNAs arising from more than 95% of the genes in our genome are subject to alternative splicing. The resulting alternatively spliced mRNAs may carry altered reading frames, explaining, for example, the distinct isoforms of certain proteins that are found in cancer cells but not in their normal counterparts. Alternatively, these splicing events may affect untranslated regions of mRNAs, such as those targeted by microRNAs (miRNAs; Section 1.10); these interactions with miRNAs can alter the function of an mRNA, by regulating either its translation or its stability. Interestingly, a protein that specifies an alternative splicing pattern of pre-mRNAs has been reported, when expressed in excessively high levels in cells, to favor their **transformation** (conversion) from a normal to a cancerous growth state. Such an effect is surprising, since one might imagine that proteins that regulate splicing would mediate the processing of many or all pre-mRNAs within the cell rather than affecting only a subset of genes involved in a specific cell-biological function, such as cell transformation. Moreover, a 2008 survey of alternatively spliced mRNAs found 41 that showed a distinct pattern of alternative splicing in human breast cancer cells compared with normal mammary cells; indeed, these alternatively spliced mRNAs could be used as diagnostic markers of the cancerous state of these cells. Even more dramatic, in 2010 as many as 1000 pre-mRNAs were found to undergo alternative splicing as cells passed through an epithelial–mesenchymal transition (EMT), an important transdifferentiation step that carcinoma cells utilize to acquire traits of high-grade malignancy, as will be discussed in Chapter 14.

1.7 Gene expression patterns also control phenotype

The 21,000 or so genes in the mammalian genome, acting combinatorially within individual cells, are able to create the extraordinarily complex organismic phenotypes of the mammalian body. A central goal of twenty-first-century biology is to relate the functioning of this large repertoire of genes to organismic physiology, developmental biology, and disease development. The complexity of this problem is illustrated by the fact that there are at least several hundred distinct cell types within the mammalian body, each with its own behavior, its own distinct metabolism, and its own physiology.

This complexity is acquired during the process of organismic development, and its study is the purview of developmental biologists. They wrestle with a problem that is inherent in the organization of all multicellular organisms. All of the cells in the body of an animal are the lineal descendants of a fertilized egg. Moreover, almost all of these cells carry genomes that are reasonably accurate copies of the genome that was initially present in this fertilized egg (see Supplementary Sidebar 1.2). The fact that cells throughout the body are phenotypically quite distinct from one another (e.g., a skin cell versus a brain cell) while being genetically identical creates this central problem of developmental biology: how do these various cell types acquire distinct phenotypes if they all carry identical genetic templates? The answer, documented in thousands of ways over the past three decades, lies in the selective reading of the genome by different cell types (**Figure 1.17**).

As cells in the early embryo pass through repeated cycles of growth and division, the cells located in different parts of the embryo begin to assume distinct phenotypes, this being the process of **differentiation**. Differentiating cells become committed to form

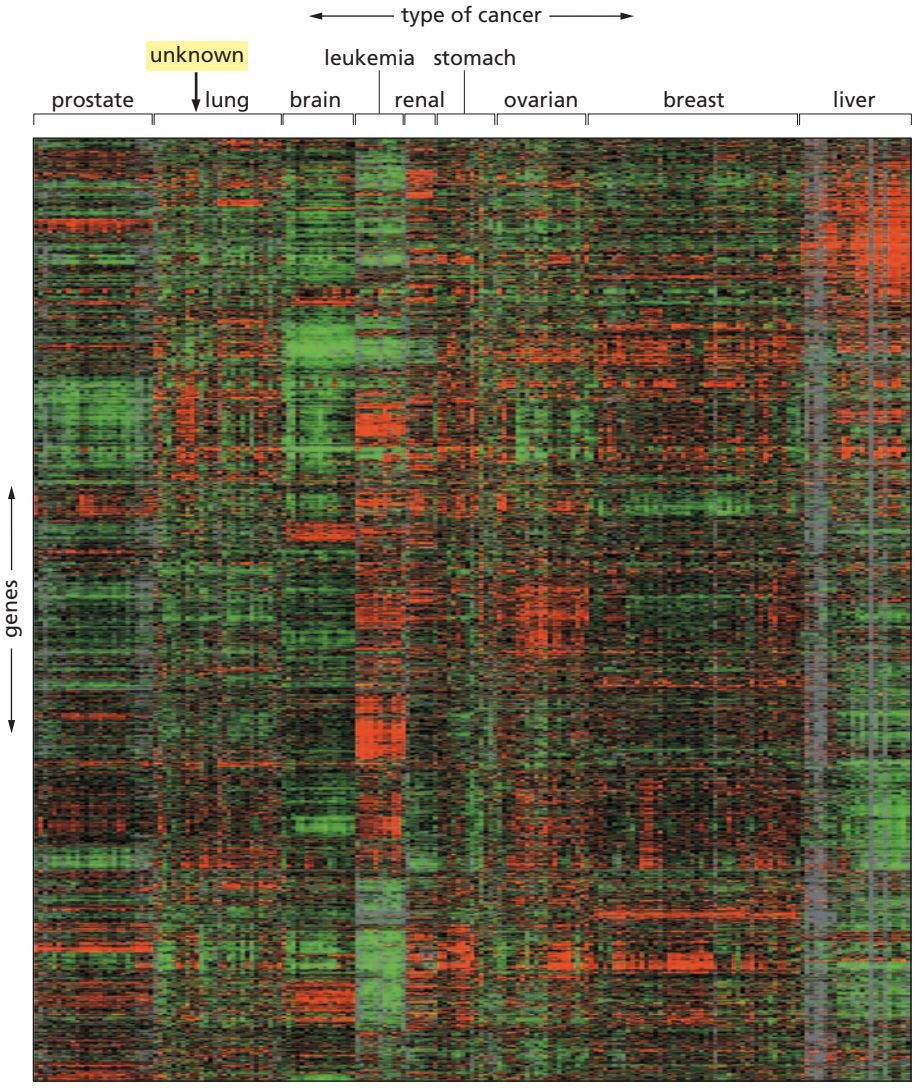


Figure 1.17 Global surveys of gene expression arrays Gene expression microarrays make it possible to survey the expression levels of thousands of genes within a given type of cell. In this image, higher-than-average levels of expression are indicated as *red* pixels, while lower-than-average levels are indicated by *green* pixels. Average-level expression is indicated by *black* pixels. The mRNAs from 142 different human tumors (*arrayed left to right*) were analyzed. In each case, the expression levels of 1800 human genes were measured (*top to bottom*). Each class of tumors has its characteristic spectrum of expressed genes. In this case, a tumor of unknown type (*yellow label*) was judged to be a lung cancer because its pattern of gene expression was similar to those of a series of already-identified lung cancers. (Courtesy of P.O. Brown, D. Botstein and The Stanford Expression Collaboration.)

one type of tissue rather than another, for example, gut as opposed to nervous system. All the while, they retain the same set of genes. This discrepancy leads to a simplifying conclusion: sooner or later, differentiation must be understood in terms of the sets of genes that are expressed (that is, transcribed) in some cells but not in others.

By being expressed in a particular cell type, a suite of genes dictates the synthesis of a cohort of proteins and RNA molecules that collaborate to create a specific cell phenotype. Accordingly, the phenotype of each kind of differentiated cell in the body should, in principle, be understandable in terms of the specific subset of genes that is expressed in that cell type.

The genes within mammalian cells can be grouped into two broad functional classes—the **housekeeping** and the **tissue-specific** genes. Many genes encode proteins that are required universally to maintain viability of all cell types throughout the body or to carry out certain biological functions common to all cell types. These commonly expressed genes are classified as housekeeping genes. Within a given differentiated cell type, housekeeping genes represent the great majority of expressed genes.

A minority of genes within a differentiated cell—the tissue-specific genes—are dedicated to the production of proteins and thus phenotypes that are associated specifically with this cell type. It may be, for example, that 3000–5000 housekeeping genes are expressed by the cell while far fewer than 1000 tissue-specific genes are responsible for the distinguishing, differentiated characteristics of the cell. By implication,

in each type of differentiated cell, a significant proportion of the 21,000 or so genes in the genome are unexpressed, since they are not required either for the cell's specific differentiation program or for general housekeeping purposes.

1.8 Histone modification and transcription factors control gene expression

The foregoing description of differentiation makes it clear that large groups of genes must be coordinately expressed while other genes must be repressed in order for cells to display complex, tissue-specific phenotypes. Such coordination of expression is the job of **transcription factors** (TFs; **Figure 1.18**). Many of these proteins bind to specific DNA sequences in the control region of each gene and determine whether or not the gene will be transcribed. The specific stretch of nucleotide sequence to which the TFs bind, often called a **sequence motif**, is usually quite short, typically 5–10 nucleotides long. In ways that are still incompletely understood at the molecular level, some TFs provide the RNA polymerase enzyme (RNA polymerase II in the case of pre-mRNAs) with access to a gene. Yet other TFs may block such access and thereby ensure that a gene is transcriptionally repressed.

Transcription factors can exercise great power, since a single type of TF can simultaneously affect the expression of a large cohort of downstream responder genes, each of which carries the recognition sequence that allows this TF to bind its promoter (see Figure 1.18). This ability of a single TF (or a single gene that specifies this TF) to elicit multiple changes within a cell or organism is often termed **pleiotropy**. In the case of cancer cells, a single malfunctioning, pleiotropically acting TF may simultaneously orchestrate the expression of a large cohort of responder genes that together proceed to create major components of the cancer cell phenotype. One enumeration of the genes in the human genome that are likely to encode TFs listed 1445 distinct genes (about 7% of the genes carried in the human genome). Not included in this list were variant versions of these proteins arising through alternative splicing of pre-mRNAs.

The transcription of most genes is dependent upon the actions of several distinct TFs that must sit down together, each at its appropriate sequence site (that is, **enhancer**) in or near the gene promoter, and collaborate to activate gene expression. This means that the expression of a gene is most often the result of the combinatorial actions of several TFs. Therefore, the coordinated expression of multiple genes within a cell, often called its gene **expression program**, is dependent on the actions of multiple TFs acting in combination on large numbers of gene promoters.

Figure 1.18 implies that modulation of gene expression is achieved by controlling initiation of transcription by RNA polymerase II (pol II) and that transcription proceeds in one direction. In fact, for many genes, possibly the majority, pol II molecules sit

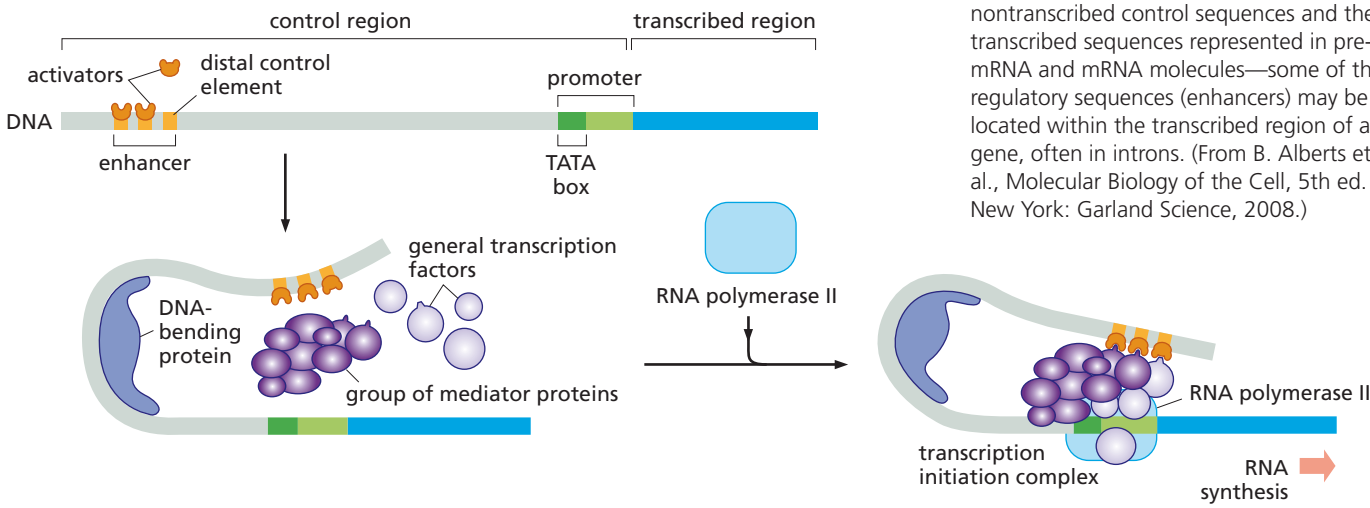


Figure 1.18 Regulation of gene expression The control region of a gene includes specific segments of DNA to which gene regulatory proteins known as transcription factors (TFs) bind, often as multiprotein complexes; in this case TFs, functioning as activators (*light brown*), bind to *enhancer* sequences (*orange*) located some distance upstream of the promoter. In addition, the *promoter* of the gene (*dark, light green*) contains sequences to which RNA polymerase II (pol II) can bind, together with associated general transcription factors. The bound TFs, interacting with the transcription initiation complex via *mediator* proteins, influence the structure of chromatin (notably the histone proteins that package DNA; see Figures 1.19 and 1.20), creating a localized chromatin environment that enables pol II to produce an RNA transcript (*orange-red arrow*). (The general TFs are involved in initiating the transcription of many genes throughout the genome, while the specialized ones regulate the expression of subsets of genes.) Although in general a gene can be separated into two functionally significant regions—the nontranscribed control sequences and the transcribed sequences represented in pre-mRNA and mRNA molecules—some of the regulatory sequences (enhancers) may be located within the transcribed region of a gene, often in introns. (From B. Alberts et al., Molecular Biology of the Cell, 5th ed. New York: Garland Science, 2008.)

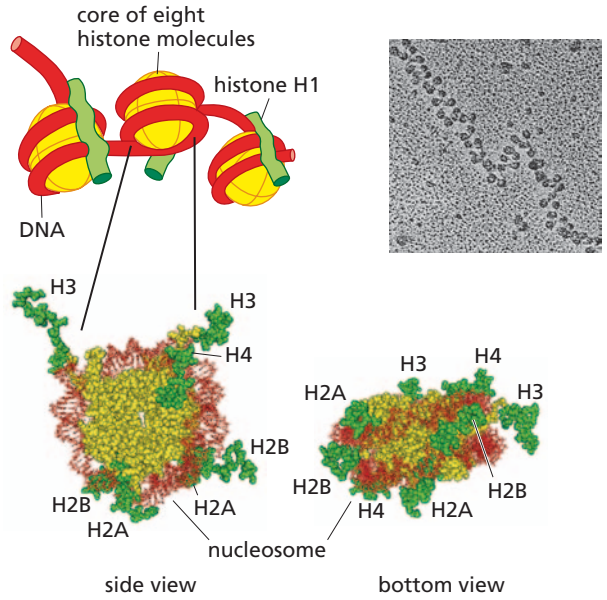


Figure 1.19 Organization of chromatin structure Examination of chromatin under the electron microscope (*above right*) reveals that DNA is associated with small globes of proteins termed nucleosomes, giving the appearance of beads on a string. The DNA double-helix (*above left, red*) is wrapped ~1.7 times around each nucleosome, which consists of a core (*yellow*) formed as an octamer of four different histone molecules (each present in two copies); often an additional histone, H1 (*green*), is located on the outside. X-ray crystallography has revealed (*below*) that the core of the nucleosome (*yellow*) is disc-shaped and that the N-terminal tail (*green*) of each of the four histones extends beyond this core. (Upper schematic, from W.K. Purves et al., Life: The Science of Biology, 5th ed. Sunderland, MA: Sinauer, 1998. Lower schematic from B. Alberts et al., Molecular Biology of the Cell, 5th ed. New York: Garland Science, 2008. Micrograph from F. Thoma, T. Koller and A. Klug, *J. Cell Biol.* 83:403–427, 1979.)

down on the promoter of a gene and proceed to transcribe the DNA in both directions. After extending nascent RNA transcripts for 60–80 nucleotides, pol II halts—the process termed **transcriptional pausing.** A subset of the stalled polymerase complexes that have initiated in the appropriate transcriptional direction are then induced by physiologic signals to resume elongation, resulting in full-length pre-mRNA transcripts, while other pol II complexes remain stalled and never resume transcription. The factors that permit stalled pol II to proceed with elongation of transcripts are incompletely understood but would seem to be as important as the conventionally defined TFs in regulating gene expression. One important cancer-causing protein, termed Myc, has been found to act as an anti-pausing protein whose actions permit thousands of cellular genes to be fully transcribed.

Figure 1.18 also implies that both TFs and RNA polymerase interact only with DNA. In fact, in eukaryotic cells, DNA is packaged in a complex mixture of proteins that, together with the DNA, form the **chromatin** (**Figure 1.19**). These chromatin proteins are responsible for controlling the interactions of TFs and RNA polymerases with DNA and therefore play critical roles in governing gene expression.

The core of chromatin is formed by DNA bound to nucleosomes, the latter being octamers consisting of two copies of each of four distinct histone species (H2A, H2B, H3, and H4) with a fifth histone species—H1—bound to some but not all nucleosome octamers. This basic organization of chromatin structure, which resembles beads on a string, is found throughout the chromosomes.

The globular core of the nucleosome represents the basic scaffold of chromatin that is modified in two ways. First, some of the standard histones, such as histones H2A and H3, may be replaced in a minority of nucleosomes by variant forms, for example, histones H2AZ and H3.3 (specified by genes distinct from those encoding the standard histones). Indeed, a number of such variant histones can be found scattered here and there throughout the chromatin; their precise contributions to the regulation of chromatin structure and transcription remain poorly understood.

Second, chromatin structure and transcription is strongly affected by post-translation modifications of the standard four histones. These modifications do not directly alter the globular core of the nucleosome. Instead, they affect the N-terminal tails of the core histones (**Figure 1.20A**), which extend outward from the globular core and undergo a variety of covalent modifications, prominent among these being methylation, acetylation, phosphorylation, and ubiquitylation. For example, one type of histone phosphorylation is associated with the condensation of chromatin that occurs during mitosis and the related global shutdown of gene expression. At other times in the cell cycle, acetylation of core histones is generally associated with active gene

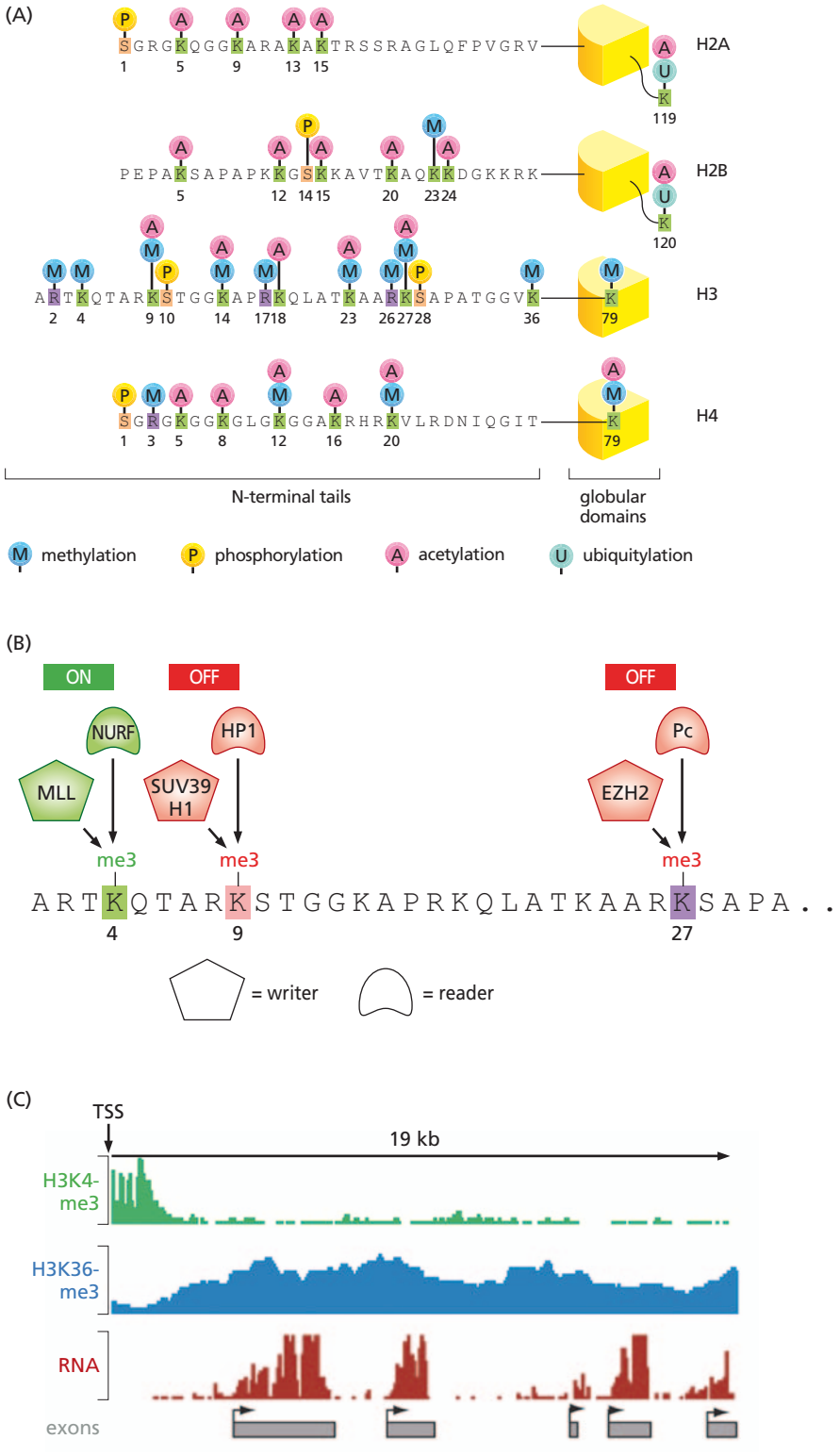


Figure 1.20 Post-translational modification of histone tails (A) Each of these N-terminal histone tails can be modified by the covalent attachment of a variety of chemical groups, most commonly methyl, acetyl, phosphate, and ubiquitin groups. These modifications are attached by histone "writers," which thereby alter the structure and the function of the chromatin, and are removed by histone "erasers." (B) One example of histone modification is provided by three of the lysine (K) residues in the amino-terminal domain of histone H3. (Amino terminus is at *left*; numbers below each K indicate residue number.) Each of these can be trimethylated (indicated by "me3") through the actions of histone methyltransferase writers (HMTs). Trimethylation at the K4 residue is carried out by the MLL1 HMT; the resulting methyl mark is recognized by a NURF (nucleosome remodeling factor) "reader" complex, which contributes to gene activation (*green*). Conversely, trimethylation of the K9 and K27 residues by the SUV39H1 and EZH2 HMT writers, respectively, results in gene repression (*red*). The methylation marks made by the latter two HMTs are recognized by the HP1 and Pc readers, respectively. Once bound, the HP1 reader can trigger the formation of heterochromatin and thereby block transcription. Not shown are other methyltransferase writers that make mono- and dimethyl marks, and histone demethylase erasers that remove the marks made by HMTs on these residues. (C) The locations of various modified histones can be mapped across a gene by using an antibody that specifically immunoprecipitates a modified histone species followed by DNA sequencing of the precipitate. In this fashion, the locations of the nucleosomes containing trimethylated lysine 4 of histone H3 (H3K4me3, *green*) and H3K36me3 (*blue*) have been mapped relative to the transcription start site (TSS) of this gene. Correlations like these indicate that nucleosomes containing H3K4me3 are associated with TSSs, while those containing H3K36me3 are found along the lengths of actively transcribed genes. When the RNA molecules are analyzed (*red*), those that map to known exons of the gene are found in greater abundance, consistent with their long lifetime relative to the short lifetimes of rapidly degraded intron sequences. The function of the gene studied here is not known. (A, from H. Santos-Rosa and C. Caldas, *Eur. J. Cancer* 41:2381–2402, 2005. B, from S.B. Hake, A. Xiao and C.D. Allis, *Brit. J. Cancer* 90:761–769, 2007. C, from M. Guttman et al., *Nature* 458:223–227, 2009.)

expression, while methylation is generally correlated with gene repression. However, as is seen in Figure 1.20B, which presents only one example of a bewildering variety of histone modifications, methylation of histone H3 is correlated with both gene repression and expression, depending on the position of the affected lysine residue.

Rapidly growing evidence indicates that these various histone modifications are functionally important in permitting or preventing transcription by RNA polymerases of specific regions of chromosomal DNA (see Figure 1.20C). Moreover, the modification

state of chromatin can be passed from mother to daughter cells through mechanisms that are still unresolved. This area of research is in great flux: as many as 60 distinct histone-modifying enzymes have been discovered, whose roles in transcriptional regulation and cell biology are largely obscure, and there are likely an even larger number of proteins that form complexes with these enzymes and direct them toward distinct substrates within the chromatin. As more effective sequencing techniques are applied to cancer cell genomes, mutant alleles of the genes encoding these enzymes are being uncovered with ever-increasing frequency.

1.9 Heritable gene expression is controlled through additional mechanisms

The descriptions above of the mechanisms controlling gene expression provide only a partial explanation of how gene expression programs that are established in one human cell are transmitted to its lineal descendants. For example, the specific gene expression program of a fibroblast grown in culture will continue to be expressed by its lineal descendants 10 and 20 cell generations later. Since decisions to express or repress a gene within a fibroblast are not imprinted in the gene's DNA sequence, this implies alternative means of maintaining such decisions in a stable fashion and transmitting them faithfully from one cell generation to the next via biochemical mechanisms that mediate **epigenetic** inheritance.

In addition to the transmission of histone modifications described above, the other key mechanism that enables epigenetic inheritance of gene expression depends on covalent modification of DNA, specifically by DNA methyltransferases—enzymes that attach methyl groups directly to cytosine bases of CpG dinucleotides in the DNA double helix. (The designation CpG indicates that the sequence is a cytidine positioned 5′ immediately before a guanosine.) The affected CpG dinucleotides are often located near transcriptional promoters, and the resulting methylation generally causes repression of nearby genes. The biochemical mechanism of maintenance methylation is well understood: maintenance DNA methyltransferase enzymes recognize **hemi-methylated** segments of recently replicated DNA and proceed to methylate any unmethylated CpG dinucleotides that are complementary to already methylated CpGs in the other DNA strand (**Figure 1.21**).

The mechanism(s) that lead to *de novo* methylation of previously unmethylated CpGs are still elusive. However, recent research reveals how the reverse process occurs: The Tet (ten eleven translocation) enzymes oxidize the methyl group of 5-methyl-cytidine to hydoxymethyl, formyl, and carboxy groups. The altered nucleotides may then be excised by DNA repair enzymes (Chapter 12) and replaced by cytidine; alternatively, when DNA bearing an oxidized cytidine is replicated, the maintenance methylase may fail to methylate the complementary strand. This research has not yet identified how the Tet enzymes are controlled.

The methyl CpG groups do not, on their own, directly block transcription. Instead, they appear to affect the structure of the chromatin proteins that are responsible for packaging chromosomal DNA and presenting it to RNA polymerases for transcription,

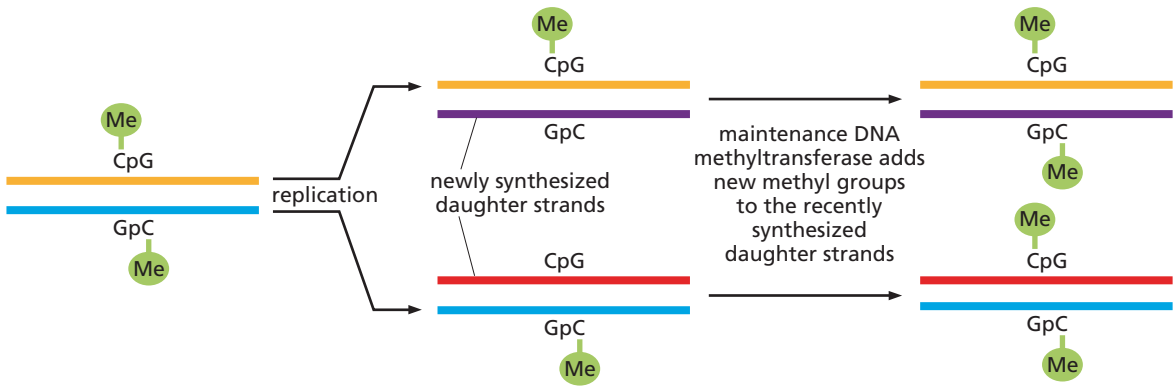


Figure 1.21 Maintenance of DNA methylation following replication When a DNA double helix that is methylated (*green* Me groups, *left*) at complementary CpG sites undergoes replication, the newly synthesized daughter helices will initially lack methyl groups attached to CpGs in the recently synthesized daughter strands (*purple, red*) and will therefore be *hemi-methylated*. Shortly after their synthesis, however, a maintenance DNA methyltransferase will detect the hemi-methylated DNA and attach methyl groups (*green*) to these CpGs, thereby regenerating the same configuration of methyl groups that existed in the parental helix prior to replication. CpG sites that are unmethylated in the parental helix (*not shown*) will be ignored by the maintenance methyltransferase and will therefore remain so in the newly synthesized strands.

as described above. In particular, methyl-CpG–binding proteins associate specifically with methylated dinucleotides and influence the structure of the nearby chromatin in still-poorly understood ways. There is also evidence that the modification of certain histones can operate in the opposite direction to influence the state of DNA methylation.

1.10 Unconventional RNA molecules also affect the expression of genes

The Central Dogma of molecular biology, developed in the decade after the 1953 discovery of the DNA double helix, proposed that information flows in cells from DNA via mRNA to proteins. In addition, non-informational RNA molecules—ribosomal and transfer RNAs—were implicated as components of the translational machinery, and small nuclear RNAs were found to play key roles in the splicing and maturation of pre-mRNAs. In the 1980s, the view of RNA's functions was expanded through the discovery that certain RNA species can act as enzymes, thereby taking their place alongside proteins as catalysts of certain biochemical reactions.

The 1990s revealed an entirely new type of RNA molecule that functions to control either the levels of certain mRNAs in the cytoplasm, the efficiency of translating these mRNAs, or both. These **microRNAs** (miRNAs) are only 21 to 25 nucleotides long and are generated as cleavage products of far larger nuclear RNA precursors. As outlined in **Figure 1.22**, the post-transcriptional processing of a primary miRNA transcript results in the formation in the cytoplasm of a miRNA that is part of a RISC (RNA-induced silencing complex) nucleoprotein. This complex associates with a spectrum of mRNA

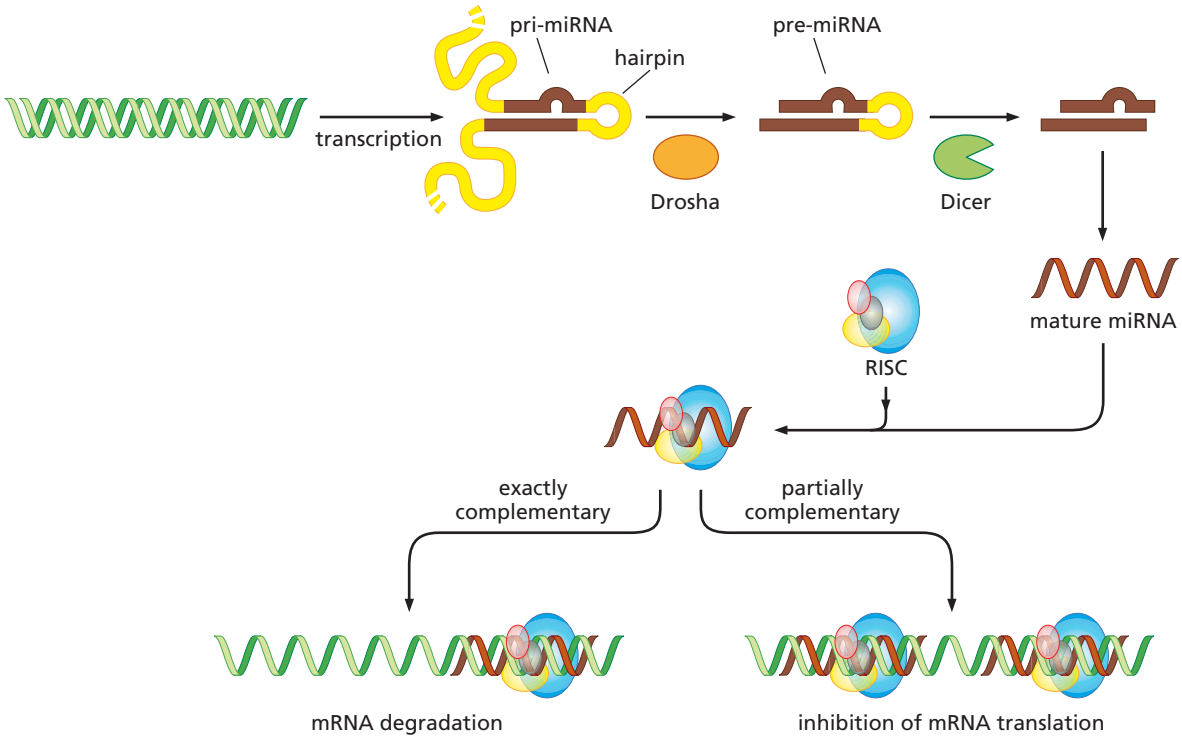


Figure 1.22 MicroRNAs and gene regulation A primary microRNA (pri-miRNA) is transcribed from a gene, and an enzyme complex involving the Drosha protein excises a small segment of the pri-miRNA that has formed a double-stranded RNA hairpin because of the self-complementarity of nucleotide sequences. The resulting pre-miRNA is exported to the cytoplasm, where it is further processed by the Dicer enzyme to generate a mature miRNA of 21 to 25 nucleotides. This miRNA binds to a nucleoprotein complex termed RISC (RNA-induced silencing complex) and associates with mRNAs in the cytoplasm with which it has precise or partial sequence complementarity, resulting in either degradation of the mRNA or inhibition of its translation. Several dozen miRNAs have been found to regulate various steps of tumor formation, either favoring or blocking critical steps of this process. Loss of the Dicer enzyme has been associated with cancer progression, and analyses of miRNA expression patterns, much like expression array analyses of mRNAs (see Figure 1.17), have proved useful in classifying various types of cancer. (Courtesy of P.A. Sharp.)

targets that contain, usually in their untranslated region, a sequence that is partially or completely complementary to the miRNA in the complex. Such association can result in either the inhibition of translation of the mRNA or its degradation, or both.

More than 650 distinct miRNA species have been found in human cells, and this roster continues to grow. Although it is unclear how many of these miRNAs are actually involved in regulating the translation and stability of mRNAs, those that do affect mRNA function are thought to regulate expression of at least one-third of all genes in the human genome. Moreover, a single miRNA species can target and thus regulate the expression of dozens of distinct mRNA species, enabling it to act pleiotropically on a variety of cellular processes.

The potential importance of miRNAs in regulating gene expression is suggested by one survey of mRNAs and corresponding proteins in a group of 76 lung cancers. Only about 20% of the genes studied showed a close correlation between mRNA expression and protein expression levels. Hence, in the remaining 80%, the rate of protein synthesis (which can be strongly influenced by miRNAs) and the post-translational lifetime of proteins (see Supplementary Sidebar 7.4) strongly influenced actual protein levels. Since proteins, rather than mRNA, are responsible for creating cell phenotypes, this also reveals the limitations of studying mRNA levels as indicators of gene activity.

Let-7, an miRNA expressed by the *C. elegans* worm, was one of two initially characterized miRNAs. It was found to suppress expression of the *ras* gene in worms and later in mammals. As we will read later (Chapters 4 through 6), the Ras proteins play critical roles in the development of many types of common human cancers. Since this pioneering work, the overexpression or loss of more than a dozen miRNA species has been associated with the formation of a variety of human cancers and the acquisition by tumors of malignant traits. The list of these miRNAs, which have garnered the term "oncoMiRs," continues to lengthen (see Supplementary Sidebar 1.3). In addition, loss of the Dicer processing enzyme (see Figure 1.22), which is involved in creating mature miRNA, has been found to facilitate the formation of tumors in mice, doing so through still-unknown mechanisms. Interestingly, inheritance of a variant of the *K-ras* gene, which causes a single nucleotide change in the 3′ untranslated region (3′ UTR) of its mRNA, prevents recognition by *Let-7* and is associated with higher levels of the growth-promoting K-Ras protein and as much as a twofold increased risk of certain forms of lung and ovarian cancers.

A decade after the discovery of microRNAs, yet another unusual class of RNAs appeared on the scene: a diverse assay of lncRNA molecules (long non-coding RNAs) were found in the nucleus and cytoplasm to be involved in still-poorly understood ways in regulating gene expression. The discovery of these came from the realization that 4 to 9% of the human genome is transcribed into relatively long (>200 nucleotide) RNA molecules that have no identifiable protein-coding sequences and thus no readily ascertainable functions. Some lncRNAs are polyadenylated while others are not. The few lncRNAs that have been characterized seem to function by associating with proteins that are involved in one fashion or another in regulating transcription, often by serving as scaffolds to hold certain chromatin-modifying proteins together. There may be several thousand distinct lncRNA species encoded by the human genome and they are increasingly viewed as key molecular components of the cell's regulatory machinery.

The role of lncRNAs in cancer development is only beginning to be uncovered. For example, elevated expression of the *HOTAIR* lncRNA has been found to be correlated with metastatic behavior of human breast and colorectal carcinomas. More importantly, forced expression of *HOTAIR* in carcinoma cells causes localization of a transcription-repressing protein complex, termed PRC2, to certain chromosomal sites, altered methylation of histone H3 lysine 27 (see Figure 1.20), and increased cancer invasiveness and metastasis.

The actions of miRNAs and lncRNAs provide a glimpse of the complexity of gene expression and its regulation in mammalian cells. Thus, after the transcription of a gene is permitted, a number of mechanisms may then intervene to control the

accumulation of its ultimate product—a protein that does the actual work of the gene. Among these mechanisms are (1) post-transcriptional processing of pre-mRNA transcripts, including alternative splicing patterns; (2) stabilization or degradation of the mRNA product; (3) regulation of mRNA translation; and (4) post-translational modification, stabilization, or degradation of the protein product. These mechanisms reinforce the notion, cited above, that the rate of transcription of a gene often provides little insight into the levels of its protein product within a cell. Hence, as we will see, distinct patterns of mRNA expression may help us to distinguish various neoplastic cells from one another but, on their own, tell us rather little about how these cells are likely to behave.

1.11 Metazoa are formed from components conserved over vast evolutionary time periods

These descriptions of cell biology, genetics, and evolution are informed in part by our knowledge of the history of life on Earth. Metazoa probably arose only once during the evolution of life on this planet, perhaps 700 million years ago. Once the principal mechanisms governing their genetics, biochemistry, and embryonic development were developed, these mechanisms remained largely unchanged in the descendant organisms up to the present (**Figure 1.23**; see also Figure 1.7). This sharing of conserved traits among various animal phyla has profound consequences for cancer research, since many lessons learned from the study of more primitive but genetically tractable organisms, such as flies and worms, have proven to be directly transferable to our understanding of how mammalian tissues, including those of humans, develop and function.

Upon surveying the diverse organisms grouped within the mammalian class, one finds that the differences in biochemistry and cell biology are minimal. For this reason, throughout this book we will move effortlessly back and forth between mouse biology and human biology, treating them as if they are essentially identical. On occasion, where species-specific differences are important, these will be pointed out.

The complex signaling circuits operating within cells seem to be organized in virtually identical fashion in all types of mammals. Even more stunning is the interchangeability of the component parts. It is rare that a human protein cannot function in place of its counterpart *orthologous* protein (**Sidebar 1.4**) in mouse cells. In the case of many types of proteins, this conservation of both function and structure is so profound that proteins can be swapped between organisms that are separated by far greater evolutionary distances. A striking example of this, noted earlier (see Figure 1.7), is provided by the gene and thus protein that specifies eye formation in mammals and in flies. Extending even further back in our evolutionary history are the histones and the mechanisms of chromatin remodeling discussed earlier. In fact, the counterparts of many molecules and biochemical mechanisms that operate in mammalian cells are already apparent in protozoa.

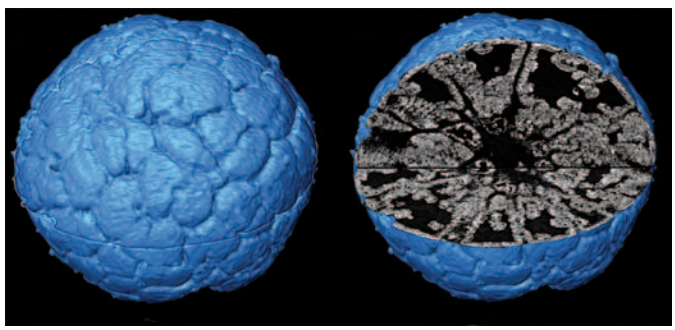


Figure 1.23 Visual evidence of the conservation of metazoan biological traits A stunning visual demonstration that contemporary metazoa develop through pathways that have changed little since the Cambrian era has come from the use of synchrotron-generated X-rays to visualize microscopic fossils at sub-micron resolution, yielding this image of an early Cambrian (~530 million years ago) blastula related either to the modern cnidarian or arthropod phylum. Its resemblance to the blastulas of contemporary metazoa indicates that, in addition to conserved molecular and biochemical mechanisms, certain features of embryonic development have changed relatively little since the emergence of modern metazoan phyla during the Cambrian era. Both the surface (*left*) and the interior cleavage pattern (*right*) are shown. (From P.C.J. Donoghue et al., *Nature* 442:680–683, 2006.)

Sidebar 1.4 Orthologs and homologs All higher vertebrates (birds and mammals) seem to have comparable numbers of genes—in the range of 21,000. Moreover, almost every gene present in the bird genome seems to have a closely related counterpart in the human genome. The correspondence between mouse and human genes is even stronger, given the closer evolutionary relatedness of these two mammalian species.

Within the genome of any single species, there are genes that are clearly related to one another in their information content and in the related structures of the proteins they specify. Such genes form a **gene family**. For example, the group of genes in the human genome encoding globins constitutes such a group. It is clear that these related genes arose at some point in the evolutionary past through repeated cycles of the process in which an existing gene is duplicated followed by the divergence of the two duplicated nucleotide sequences from one another (**Figure 1.24**). More directly related to cancer development are the more than 500 protein kinases encoded by the human genome. Kinases attach phosphate groups to their protein substrates, and almost all of these enzymes are specified by members of a single gene family that underwent hundreds of cycles of gene duplication and divergence during the course of evolution (see Supplementary Figure 16.5).

Genes that are related to one another within a single species' genome or genes that are related to one another in the genomes of two distinct species are said to be **homologous** to one another. Often the precise counterpart of a gene in a human can be found in the genome of another species. These two closely related genes are said to be **orthologs** of one another. Thus, the precise counterpart—the ortholog—of the c-*myc* gene in humans is the c-*myc* gene in chickens. To the extent that there are other *myc*-like genes harbored by the human genome (that is, N-*myc* and L-*myc*), the latter are members of the same gene family as c-*myc* but are not orthologs of one another or of the c-*myc* gene in chickens.

Throughout this book we will often refer to genes without making reference to the species from which they were isolated. This is done consciously, since in the great majority of cases, the functioning of a mouse gene (and encoded protein) is indistinguishable from that of its human or chicken ortholog.

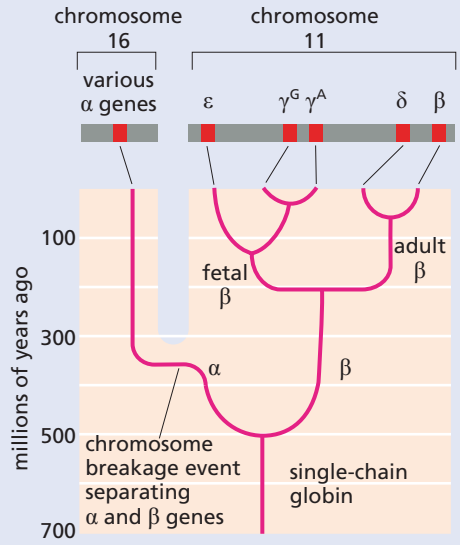


Figure 1.24 Evolutionary development of gene families The evolution of organismic complexity has been enabled, in part, by the development of increasingly specialized proteins. New proteins are "invented" largely through a process of gene duplication followed by diverging evolution of the two resulting genes. Repeated cycles of such gene duplications followed by divergence have led to the development of large numbers of multi-gene families. During vertebrate evolution, an ancestral globin gene, shown here, which encoded the protein component of hemoglobin, was duplicated repeatedly, leading to the large number of distinct globin genes in the modern mammalian genome that are present on two human chromosomes. Because these globins have distinct amino acid sequences, each can serve a specific physiologic function. (From B. Alberts et al., Molecular Biology of the Cell, 5th ed. New York: Garland Science, 2008.)

1.12 Gene cloning techniques revolutionized the study of normal and malignant cells

Until the mid-1970s, the molecular analysis of mammalian genes was confined largely to the genomes of DNA tumor viruses, indeed the viruses described later in Chapter 3. These viruses have relatively simple genomes that accumulate to a high copy number (that is, number of molecules) per cell. This made it possible for biologists to readily purify and study the detailed structure and functioning of viral genes that operate much like the genes of the host cells in which these viruses multiplied. In contrast, molecular analysis of cellular genes was essentially impossible, since there are so many of them (tens of thousands per haploid genome) and they are embedded in a genome of daunting complexity (~3.2 billion base pairs of DNA per haploid cellular genome).

All this changed with the advent of gene cloning. Thereafter, cellular genomes could be fragmented and used to create the collections of DNA fragments known as genomic **libraries**. Various DNA hybridization techniques could then be used to identify the genomic fragments within these libraries that were of special interest to the experimenter, in particular the DNA fragment that carried part or all of a gene under study. The retrieval of such a fragment from the library and the amplification of this

retrieved fragment into millions of identical copies yielded a purified, **cloned** fragment of DNA and thus a cloned gene (see Supplementary Sidebar 1.4). Yet other techniques were used to generate DNA copies of the mRNAs that are synthesized in the nucleus and exported to the cytoplasm, where they serve as the templates for protein synthesis. Discovery of the enzyme **reverse transcriptase** (RT; see Figure 3.18) was of central importance here. Use of this enzyme made it possible to synthesize ***in vitro*** (that is, in the test tube) **complementary DNA** copies of mRNA molecules. These DNA molecules, termed cDNAs, carry the sequence information that is present in an mRNA molecule after the process of splicing has removed all introns. While we will refer frequently throughout this book to DNA clones of the genomic (that is, chromosomal) versions of genes and to cDNAs generated from the mRNA transcripts of such genes, space limitations preclude any detailed descriptions of the cloning procedures *per se*.

For cancer researchers, gene cloning arrived just at the right time. As we will see in the next chapters, research in the 1970s diminished the candidacy of tumor viruses as the cause of most human cancers. As these viruses moved off center stage, cellular genes took their place as the most important agents responsible for the formation of human tumors. Study of these genes would have been impossible without the newly developed gene cloning technology, which became widely available in the late 1970s, just when it was needed by the community of scientists intent on finding the root causes of cancer.

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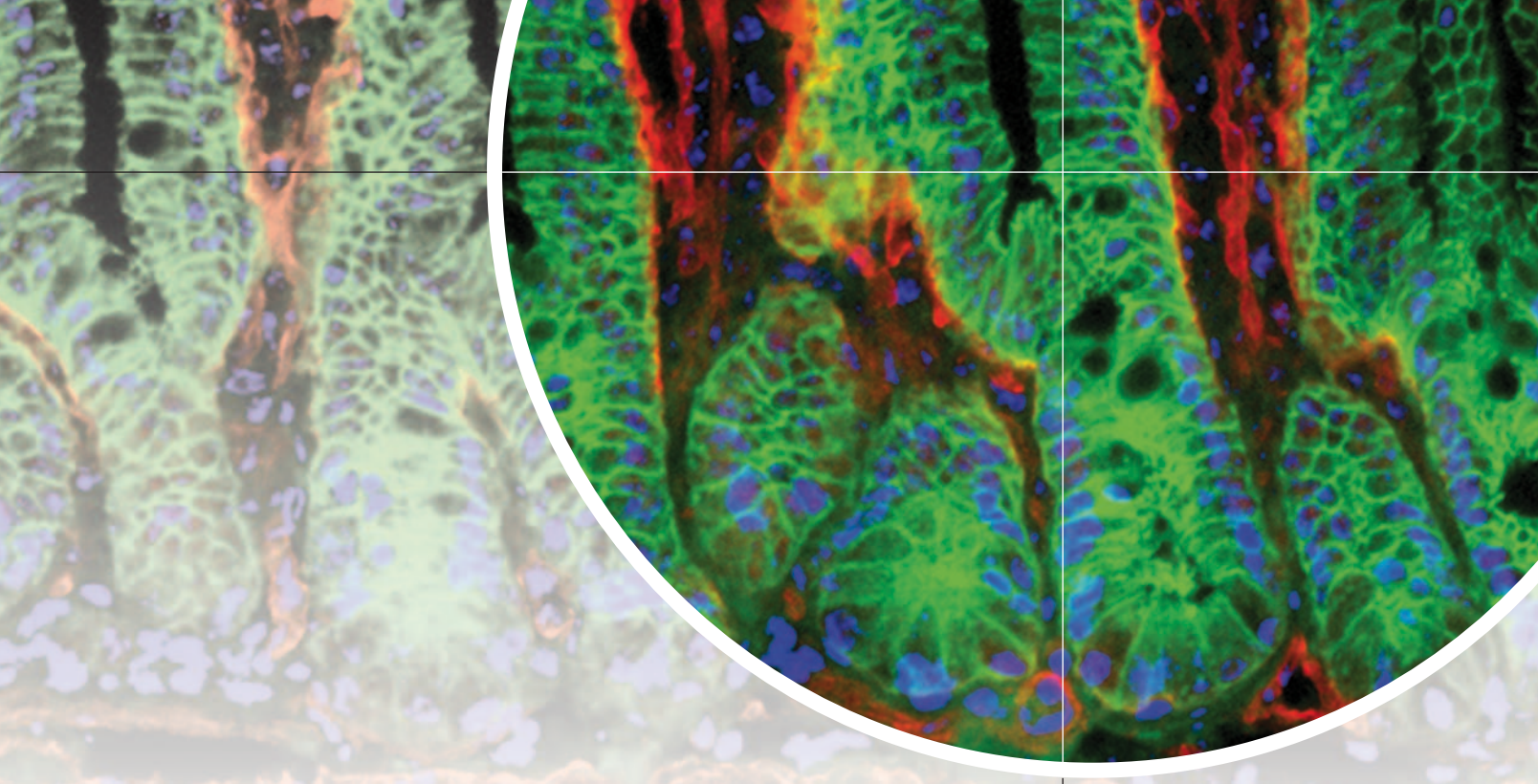


Chapter 2

The Nature of Cancer

> When I published the results of my experiments on the development of double-fertilized sea-urchin eggs in 1902, I added the suggestion that malignant tumors might be the result of a certain abnormal condition of the chromosomes, which may arise from multipolar mitosis. ... So I have carried on for a long time the kind of experiments I suggested, which are so far without success, but my conviction remains unshaken.
>
> Theodor Boveri, pathologist, 1914

> Tumors destroy man in a unique and appalling way, as flesh of his own flesh which has somehow been rendered proliferative, rampant, predatory and ungovernable. They are the most concrete and formidable of human maladies, yet despite more than 70 years of experimental study they remain the least understood.
>
> Francis Peyton Rous, tumor virologist, Nobel lecture, 1966

The cellular organization of metazoan tissues has made possible the evolution of an extraordinary diversity of anatomical designs. Much of this plasticity in design can be traced to the fact that the building blocks of tissue and organ construction—individual cells—are endowed with great autonomy and versatility. Most types of cells in the metazoan body carry a complete organismic genome—far more information than any one of these cells will ever require. And many cells retain the ability to grow and divide long after organismic development has been completed. This retained ability to proliferate and to participate in tissue **morphogenesis** (the creation of shape) makes possible the maintenance of adult tissues throughout the life span of an organism. Such maintenance may involve the repair of wounds and the replacement of cells that have suffered attrition after extended periods of service.

At the same time, this versatility and autonomy pose a grave danger, in that individual cells within the organism may gain access to information in their genomes that is normally denied to them and assume roles that are inappropriate for normal tissue

Movies in this chapter

2.1 Embryonic Origins of Tissues
2.2 Mammary Cancer Cells
2.3 Visualization of Cancer I: Lymphoma

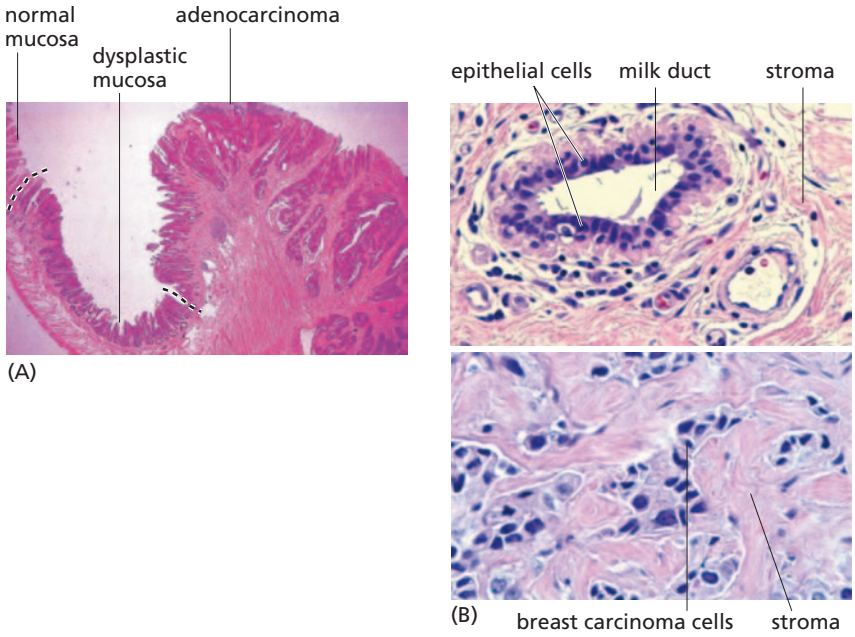


Figure 2.1 Normal versus neoplastic tissue (A) This histological section of the lining of the ileum in the small intestine, viewed at low magnification, reveals the continuity between normal and cancerous tissue. At the far left is the normal epithelial lining, the mucosa. In the middle is mucosal tissue that has become highly abnormal, or "dysplastic." To the right is an obvious tumor—an adenocarcinoma—which has begun to invade underlying tissues. (B) This pair of sections, viewed at high magnification, shows how normal tissue architecture becomes deranged in tumors. In the normal human mammary gland (*upper panel*), a milk duct is lined by epithelial cells (*dark purple nuclei*). These ducts are surrounded by mesenchymal tissue (see Figure 2.7) termed "stroma," which consists of connective tissue cells, such as fibroblasts and adipocytes, and collagen matrix (*pink*). In an invasive ductal breast carcinoma (*lower panel*), the cancer cells, which arise from the epithelial cells lining the normal ducts, exhibit abnormally large nuclei (*purple*), no longer form well-structured ducts, and have invaded the stroma (*pink*). (A, from A.T. Skarin, Atlas of Diagnostic Oncology, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003; B, courtesy of A. Orimo.)

maintenance and function. Moreover, their genomic sequences are subject to corruption by various mechanisms that alter the structure and hence information content of the genome. The resulting mutated genes may divert cells into acquiring novel, often highly abnormal phenotypes. Such changes may be incompatible with the normally assigned roles of these cells in organismic structure and physiology. Among these inappropriate changes may be alterations in cellular proliferation programs, and these in turn can lead to the appearance of large populations of cells that no longer obey the rules governing normal tissue construction and maintenance.

When portrayed in this way, the renegade cells that form a tumor are the result of normal development gone awry. In spite of extraordinary safeguards taken by the organism to prevent their appearance, cancer cells somehow learn to thrive. Normal cells are carefully programmed to collaborate with one another in constructing the diverse tissues that make possible organismic survival. Cancer cells have a quite different and more focused agenda. They appear to be motivated by only one consideration: making more copies of themselves.

2.1 Tumors arise from normal tissues

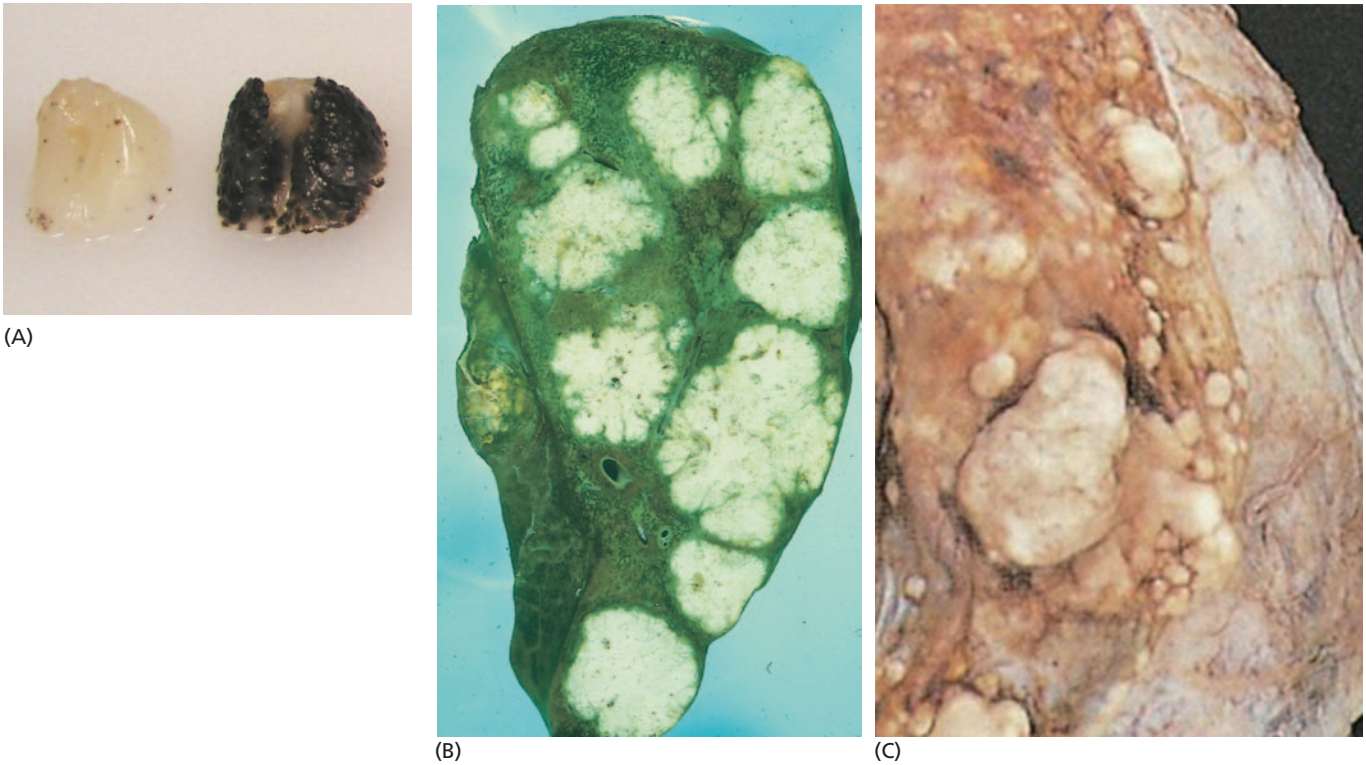
A confluence of discoveries in the mid- and late nineteenth century led to our current understanding of how tissues and complex organisms arise from fertilized eggs. The most fundamental of these was the discovery that all tissues are composed of cells and cell products, and that all cells arise through the division of preexisting cells. Taken together, these two revelations led to the deduction, so obvious to us now, that all the cells in the body of a complex organism are members of cell lineages that can be traced back to the fertilized egg. Conversely, the fertilized egg is able to spawn all the cells in the body, doing so through repeated cycles of cell growth and division.

These realizations had a profound impact on how tumors were perceived. Previously, many had portrayed tumors as foreign bodies that had somehow taken root in an afflicted person. Now, tumors, like normal tissues, could be examined under the microscope by researchers in the then-new science of **histology**. These examinations of tissue **sections** (thin slices) revealed that tumors, like normal tissues, were composed of masses of cells (**Figure 2.1**). Contemporary cancer research makes frequent use of a variety of histological techniques; the most frequently used of these are illustrated in Supplementary Sidebar 2.1.

Evidence accumulated that tumors of various types, rather than invading the body from the outside world, often derive directly from the normal tissues in which they are first discovered. However, tumors did seem to be capable of moving within the confines of the human body: in many patients, multiple tumors were discovered at anatomical sites quite distant from where their disease first began, a consequence of the tendency of cancers to spread throughout the body and to establish new colonies of cancer cells (**Figure 2.2**). These new settlements, termed **metastases**, were often traceable directly back to the site where the disease of cancer had begun—the founding or **primary tumor**.

Invariably, detailed examination of the organization of cells within tumor masses gave evidence of a tissue architecture that was less organized than the architecture of nearby normal tissues (Figure 2.1). These **histopathological** comparisons provided the first seeds of an idea that would take the greater part of the twentieth century to prove: tumors are created by cells that have lost the ability to assemble and create tissues of normal form and function. Stated more simply, cancer came to be viewed as a disease of malfunctioning cells.

While the microarchitecture of tumors differed from that of normal tissue, tumors nevertheless bore certain histological features that resembled those of normal tissue.



(A) (B) (C)

Figure 2.2 Metastasis of cancer cells to distant sites Many types of tumors eventually release cancer cells that migrate to distant sites in the body, where they form the secondary tumors known as metastases. (A) Melanoma metastases can be quickly identified in mice because of their distinctive dark pigmentation. Seen here are the lungs of two mice, in one of which the formation of metastases was almost entirely blocked (*left*) and one in which hundreds of metastases (*black spots*) were allowed to form (*right*), as observed two weeks after B16 mouse melanoma cells were injected into the tail veins of these mice. This injection route causes many of the cells to become mechanically trapped in the lungs, where they seed numerous colonies. (B) Metastases (*white*) in the liver often arise in patients with advanced colon carcinomas. The portal vein, which drains blood from the colon into the liver (see Figure 14.45), provides a route for metastasizing colon cancer cells to migrate directly into the liver. (C) Breast cancer often metastasizes to the brain. Here, large metastases are revealed post mortem in the right side of a brain where the dura (membrane covering; shown intact at *right*) of the brain has been removed. (A, from F. Nimmerjahn et al., *Immunity* 23:41–51, 2005. B, courtesy of Peter Isaacson. C, from H. Okazaki and B.W. Scheithauer, Atlas of Neuropathology. Gower Medical Publishing, 1988.)

This suggested that all tumors should, in principle, be traceable back to the specific tissue or organ site in which they first arose, using the histopathological analyses of tumor sections to provide critical clues. This simple idea led to a new way of classifying these growths, which depended on their presumed tissues of origin. The resulting classifications often united under one roof cancers that arise in tissues and organs that have radically different functions in the body but share common types of tissue organization.

The science of histopathology also made it possible to understand the relationship between the clinical behavior of a tumor (that is, the effects that the tumor had on the patient) and its microscopic features. Most important here were the criteria that segregated tumors into two broad categories depending on their degree of aggressive growth. Those that grew locally without invading adjacent tissues were classified as **benign**. Others that invaded nearby tissues and spawned metastases were termed **malignant**.

In fact, the great majority of primary tumors arising in humans are benign and are harmless to their hosts, except in the rare cases where the expansion of these localized masses causes them to press on vital organs or tissues. Some benign tumors, however, may cause clinical problems because they release dangerously high levels of hormones that create physiologic imbalances in the body. For example, thyroid **adenomas** (premalignant epithelial growths) may cause excessive release of thyroid hormone into the circulation, leading to hyperthyroidism; pituitary adenomas may release growth hormone into the circulation, causing excessive growth of certain tissues—a condition known as **acromegaly**. Nonetheless, deaths caused by benign tumors are relatively uncommon. The vast majority of cancer-related mortality derives from malignant tumors. More specifically, it is the metastases spawned by these tumors that are responsible for some 90% of deaths from cancer.

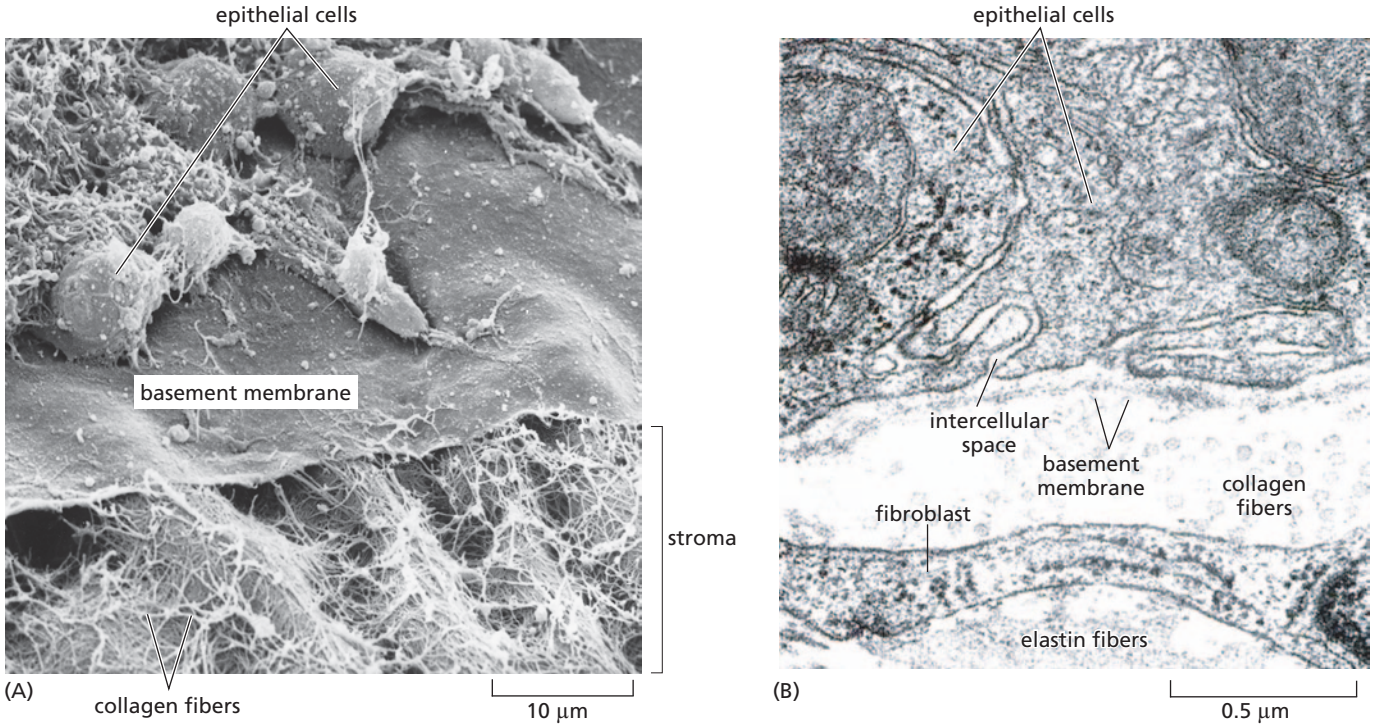
2.2 Tumors arise from many specialized cell types throughout the body

The majority of human tumors arise from epithelial tissues. **Epithelia** are sheets of cells that line the walls of cavities and channels or, in the case of skin, serve as the outside covering of the body. By the first decades of the twentieth century, detailed histological analyses had revealed that normal tissues containing epithelia are all structured similarly. Thus, beneath the epithelial cell layers in each of these tissues lies a **basement membrane** (sometimes called a **basal lamina**); it separates the epithelial cells from the underlying layer of supporting connective tissue cells, termed the **stroma** (**Figure 2.3**).

The basement membrane is a specialized type of extracellular matrix (ECM) and is assembled from proteins secreted largely by the epithelial cells. Another type of basement membrane separates **endothelial** cells, which form the inner linings of capillaries and larger vessels, from an outer layer of specialized smooth muscle cells. In all cases, these basement membranes serve as a structural scaffolding of the tissue. In addition, as we will learn later, cells attach a variety of biologically active signaling molecules to basement membranes.

Epithelia are of special interest here, because they spawn the most common human cancers—the **carcinomas**. These tumors are responsible for more than 80% of the cancer-related deaths in the Western world. Included among the carcinomas are tumors arising from the epithelial cell layers of the gastrointestinal tract—which includes mouth, esophagus, stomach, and small and large intestines—as well as the skin, mammary gland, pancreas, lung, liver, ovary, uterus, prostate, gallbladder, and urinary bladder. Examples of normal epithelial tissues are presented in **Figure 2.4**.

This group of tissues encompasses cell types that arise from all three of the primitive cell layers in the early vertebrate embryo. Thus, the epithelia of the lungs, liver, gallbladder, pancreas, esophagus, stomach, and intestines all derive from the inner cell layer, the **endoderm**. Skin arises from the outer embryonic cell layer, termed the



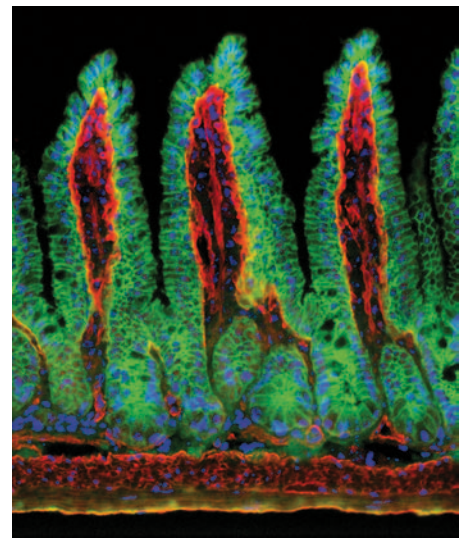

(C)

Figure 2.3 Basement membranes (A) This scanning electron micrograph of a chick corneal epithelium illustrates the basic plan of epithelial tissues, in which epithelial cells are tethered to one side of the basement membrane, sometimes termed "basal lamina." Seen here as a continuous sheet, it is formed as meshwork of extracellular matrix proteins. A network of collagen fibers anchors the underside of the basement membrane to the extracellular matrix (ECM) of the stroma. (B) The epithelium of the mouse trachea is viewed here at far higher magnification through a transmission electron microscope. Several epithelial cells are seen above the basement membrane, while below are collagen fibrils, a fibroblast, and elastin fibers. Note that the basement membrane is not interrupted at the intercellular space between the epithelial cells. (C) While basement membranes cannot be detected using conventional staining techniques, use of immunofluorescence with an antibody against a basement membrane protein—in this case laminin 5 (*red*)—allows its visualization. The epithelial cells coating the villi of the mouse small intestine have been stained with an antibody against E-cadherin (*green*), while all cell nuclei are stained *blue*. Here the convoluted basement membrane separates the outer villus layer of epithelial cells, termed *enterocytes*, from the mesenchymal cells forming the core of each villus (not stained). [A, courtesy of Robert Trelstad. B, from B. Young et al., Wheater's Functional Histology, 4th ed. Edinburgh: Churchill Livingstone, 2003. C, from Z.X. Mahoney et al., *J. Cell Sci.* 121:2493–2502, 2008 (cover image).]

ectoderm, while the ovaries originate embryologically from the middle layer, the **mesoderm** (Figure 2.5). Therefore, in the case of carcinomas, histopathological classification is not informed by the developmental history of the tissue of origin.

The epithelial and stromal cells of these various tissues collaborate in forming and maintaining the epithelial sheets. When viewed from the perspective of evolution, it now seems that the embryologic mechanisms for organizing and structuring epithelial tissues were invented early in metazoan evolution, likely more than 600 million years ago, and that these mechanistic principles have been exploited time and again during metazoan evolution to construct tissues and organs having a wide array of physiologic functions.

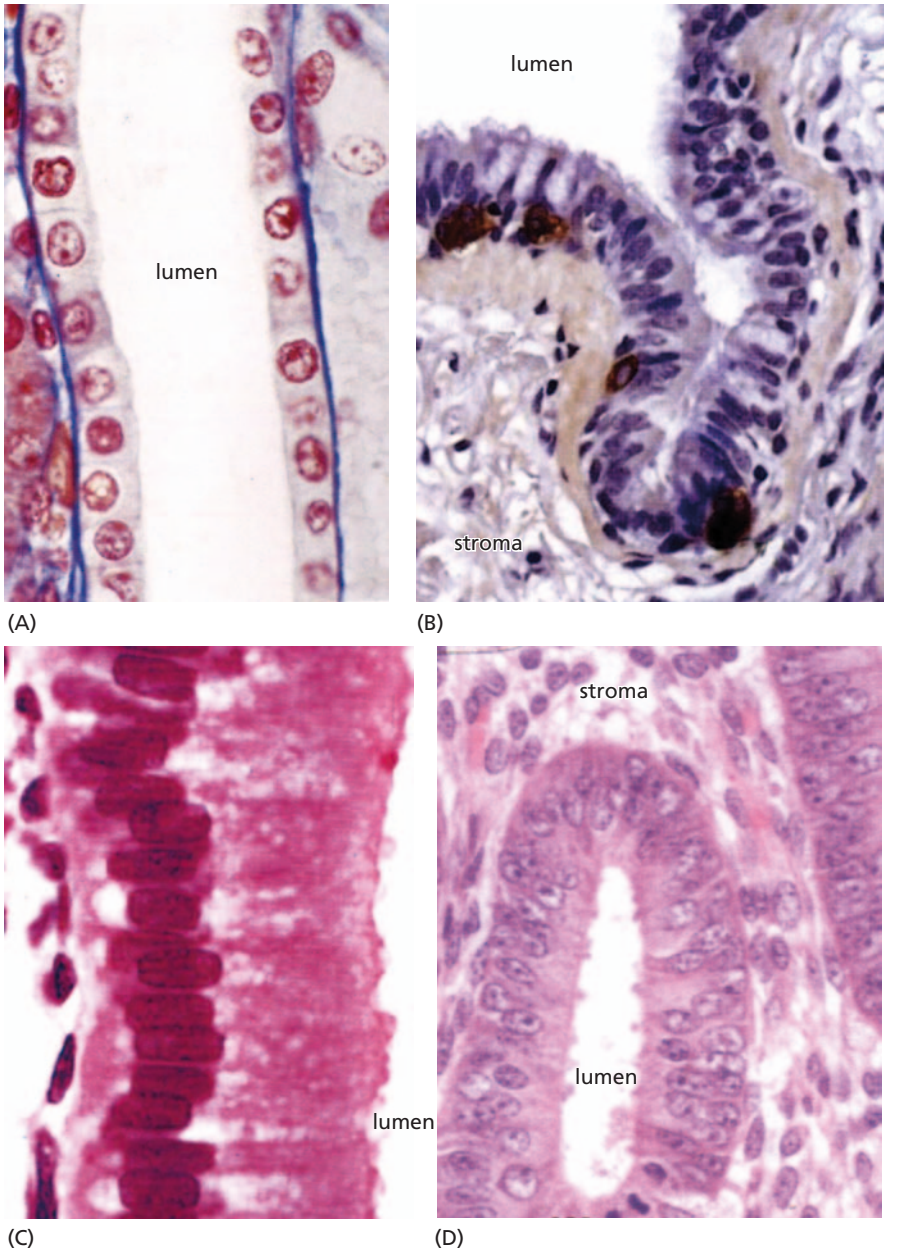


Figure 2.4 Architecture of epithelial tissues A common organizational plan describes most of the epithelial tissues in the body: The mature, differentiated epithelial cells are at the exposed surface of an epithelium. In many tissues, underlying these epithelia are less differentiated epithelial cells, not seen in this figure. Beneath the epithelial cell layer lies a basement membrane (see Figure 2.3), which is usually difficult to visualize in the light microscope. Shown here are epithelia of (A) a collecting tubule of the kidney, (B) the bronchiole of the lung, (C) the columnar epithelium of the gallbladder, and (D) the endometrium of the uterus. In each case, the epithelial cells protect the underlying tissue from the contents of the *lumen* (cavity) that they are lining. Panel C illustrates another property that is characteristic of the epithelial cells forming an epithelium: the state of *apico-basal* polarity, in which individual epithelial cells are organized to present their **apical** surface toward the lumen *(right)* and their **basal** surface toward the underlying basement membrane.This polarization involves the asymmetric localization of the nuclei, which are more basally located, along with hundreds of cell-surface (and associated cytoskeletal) proteins *(not shown)* that are specifically localized either to the apical or basal surfaces of these cells. In addition, the lateral surfaces of the epithelial cells establish several distinct types of junctions with their adjacent epithelial neighbors. (From B. Young et al., Wheater's Functional Histology, 4th ed. Edinburgh: Churchill Livingstone, 2003.)

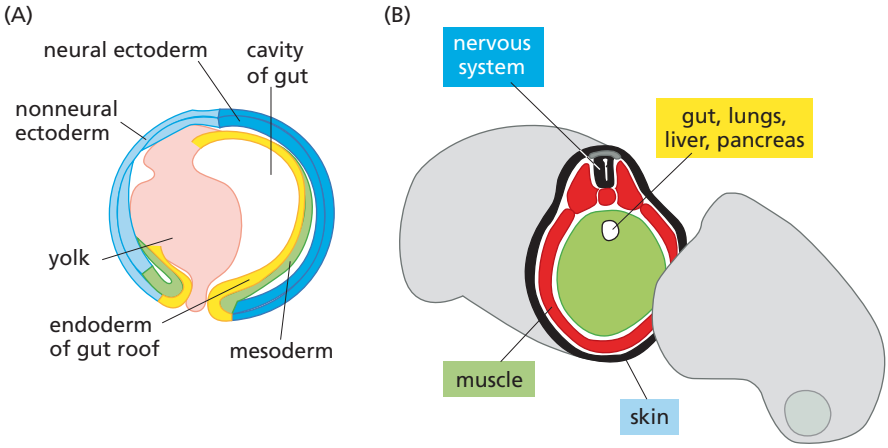


Figure 2.5 Embryonic cell layers (A) The tissues of more complex metazoa develop from three embryonic cell compartments—ectoderm (*blue*), mesoderm (*green*), and endoderm (*yellow*). Each of the three embryonic cell layers is precursor to distinct types of differentiated cells. (B) In an early-stage tadpole, the skin and nervous system develop from the ectoderm (*black*), while the connective tissue, including bone, muscle, and blood-forming cells, develops from the mesoderm (*red*). The gut and derived outpouchings, including lung, pancreas, and liver, develop from the endoderm (*green*). The development of all chordates follows this plan. (Adapted from T. Mohun et al., *Cell* 22:9–15, 1980.)

Most carcinomas fall into two major categories that reflect the two major biological functions associated with epithelia (Table 2.1). Some epithelial sheets serve largely to seal the cavity or channel that they line and to protect the underlying cell populations (Figure 2.6). Tumors that arise from epithelial cells forming these protective cell layers are termed **squamous cell carcinomas**. For example, the epithelial cells lining the skin (**keratinocytes**) and most of the oral cavity spawn tumors of this type.

Many epithelia also contain specialized cells that secrete substances into the ducts or cavities that they line. This class of epithelial cells generates **adenocarcinomas**. Often these secreted products are used to protect the epithelial cell layers from the contents of the cavities (**lumina**) that they surround (see Figure 2.6). Thus, some epithelial cells lining the lung and stomach secrete mucus layers that protect them, respectively, from the air (and airborne particles) and from the corrosive effects of high concentrations of acid. The epithelia in some organs such as the lung, uterus, and cervix have the capacity to give rise to pure adenocarcinomas or pure squamous cell carcinomas; quite frequently, however, tumors in these organs are found in which both types of carcinoma cells coexist.

Table 2.1 Carcinomas

Tissue sites of more common types of adenocarcinoma	Tissue sites of more common types of squamous cell carcinoma	Other types of carcinoma
lung	skin	small-cell lung carcinoma
colon	nasal cavity	large-cell lung carcinoma
breast	oropharynx	hepatocellular carcinoma
pancreas	larynx	renal cell carcinoma
stomach	lung	transitional-cell carcinoma (of urinary bladder)
esophagus	esophagus	
prostate	cervix	
endometrium		
ovary		

The remainder of malignant tumors arise from nonepithelial tissues throughout the body. The first major class of nonepithelial cancers derive from the various connective tissues, all of which share a common origin in the mesoderm of the embryo (Table 2.2). These tumors, the **sarcomas**, constitute only about 1% of the tumors encountered in the oncology clinic. Sarcomas derive from a variety of **mesenchymal** cell types. Included among these are **fibroblasts** and related connective tissue cell

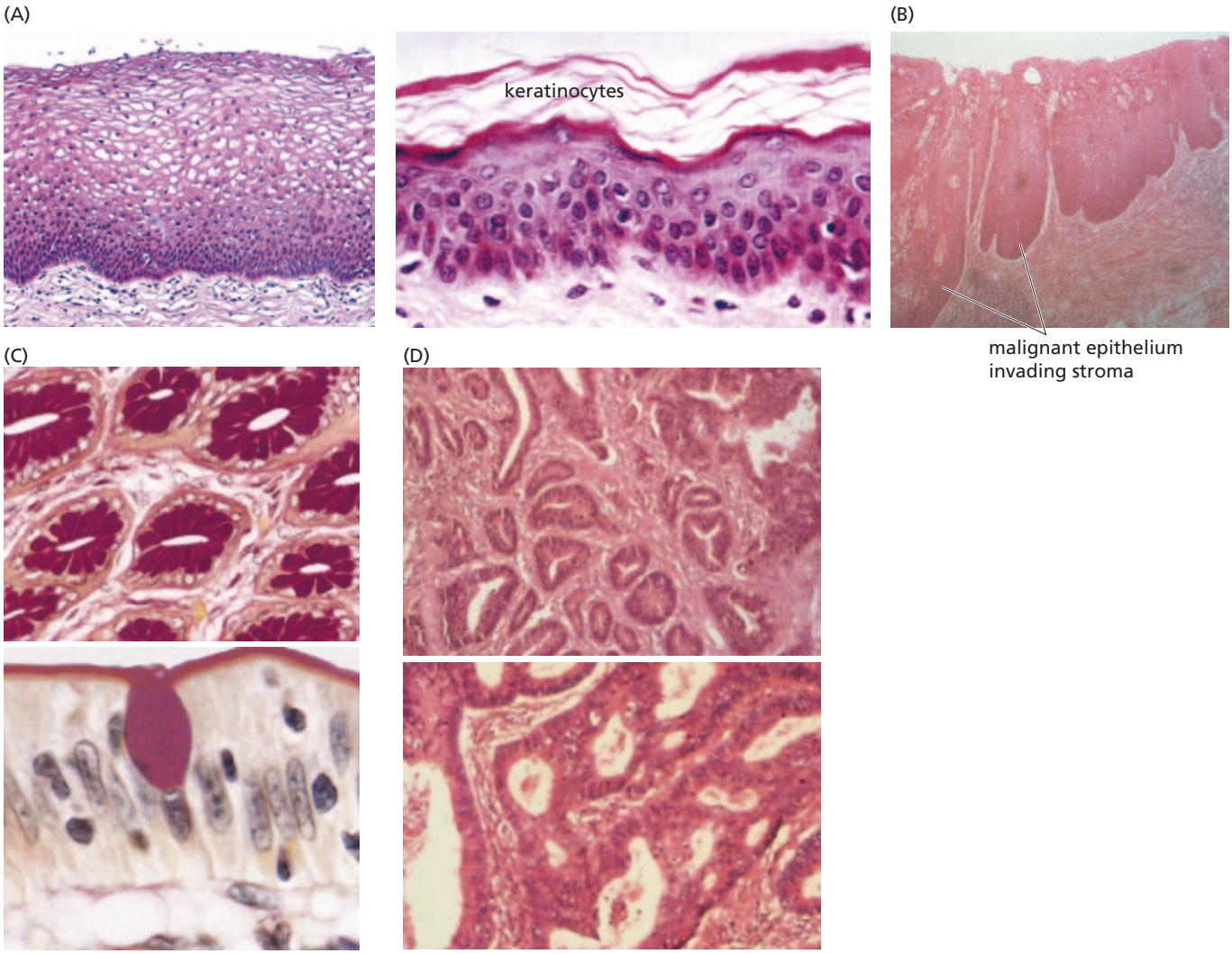


Figure 2.6 Epithelia and derived carcinomas Epithelia can be classified into subtypes depending on the shape and function of the normal epithelial cells and the carcinomas arising from them. The origins of squamous cell carcinomas and adenocarcinomas are seen here. (A) Normal squamous cells are often flattened and function to protect the epithelium and underlying tissue from the contents of the lumen or, in the case of skin, from the outside world. The squamous epithelia of the cervix of the uterus (*left*) and the skin (*right*) are organized quite similarly, with mature flattened cells at the surface being continually shed (for example, the dead keratinocytes of the skin) and replaced by less differentiated cells that move upward and proceed to differentiate. (B) In this carcinoma of the esophagus, large tongues of malignant squamous epithelial cells are invading the underlying stromal/mesenchymal tissue. (C) In some tissues, the glandular cells within epithelia secrete mucopolysaccharides to protect the epithelium; in other tissues, they secrete proteins that function within the *lumina* (cavities) of ducts or are distributed to distant sites in the body. Pits in the stomach wall are lined by mucus-secreting cells (*dark red, upper panel*). In the epithelium of the small intestine (*lower panel*) a single mucus-secreting goblet cell (*purple*) is surrounded by epithelial cells of a third type—columnar cells, which are involved in the absorption of water. (D) These adenocarcinomas of the stomach (*upper panel*) and colon (*lower panel*) show multiple ductal elements, which are clear indications of their derivation from secretory epithelia such as those in panel C. (A and C, from B. Young et al., Wheater's Functional Histology, 4th ed. Edinburgh: Churchill Livingstone, 2003. B and D, from A.T. Skarin, Atlas of Diagnostic Oncology, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

Table 2.2 Various types of more common sarcomas

Type of tumor	Presumed cell lineage of founding cell
Osteosarcoma	osteoblast (bone-forming cell)
Liposarcoma	adipocyte (fat cell)
Leiomyosarcoma	smooth muscle cell (e.g., in gut)
Rhabdomyosarcoma	striated/skeletal muscle cell
Malignant fibrous histiocytoma	adipocyte/muscle cell
Fibrosarcoma	fibroblast (connective tissue cell)
Angiosarcoma	endothelial cells (lining of blood vessels)
Chondrosarcoma	chondrocyte (cartilage-forming cell)

types that secrete collagen, the major structural component of the extracellular matrix of tendons and skin; **adipocytes**, which store fat in their cytoplasm; **osteoblasts**, which assemble calcium phosphate crystals within matrices of collagen to form bone; and **myocytes**, which assemble to form muscle (**Figure 2.7**). Hemangiomas, which are relatively common in children, arise from precursors of the endothelial cells. The stromal layers of epithelial tissues include some of these mesenchymal cell types.

The second group of nonepithelial cancers arise from the various cell types that constitute the blood-forming (**hematopoietic**) tissues, including the cells of the immune system (**Table 2.3** and **Figure 2.8**); these cells also derive from the embryonic mesoderm. Among them are cells destined to form **erythrocytes** (red blood cells), antibody-secreting (**plasma**) cells, as well as T and B **lymphocytes**. The term **leukemia** (literally "white blood") refers to malignant derivatives of several of these hematopoietic cell lineages that move freely through the circulation and, unlike the red blood cells, are nonpigmented. **Lymphomas** include tumors of the **lymphoid** lineages (B and T lymphocytes) that aggregate to form solid tumor masses, most frequently found in lymph nodes, rather than the dispersed, single-cell populations of tumor cells seen in leukemias. This class of tumors is responsible for ~7% of cancer-associated mortality in the United States.

The third and last major grouping of nonepithelial tumors arises from cells that form various components of the central and peripheral nervous systems (**Table 2.4**). These are often termed **neuroectodermal** tumors to reflect their origins in the outer cell

Table 2.3 Various types of more common hematopoietic malignancies

Acute lymphocytic leukemia (ALL)
Acute myelogenous leukemia (AML)
Chronic myelogenous leukemia (CML)
Chronic lymphocytic leukemia (CLL)
Multiple myeloma (MM)
Non-Hodgkin's lymphoma[a] (NHL)
Hodgkin's lymphoma (HL)

[a]The non-Hodgkin's lymphoma types, also known as lymphocytic lymphomas, can be placed in as many as 15–20 distinct subcategories, depending upon classification system.

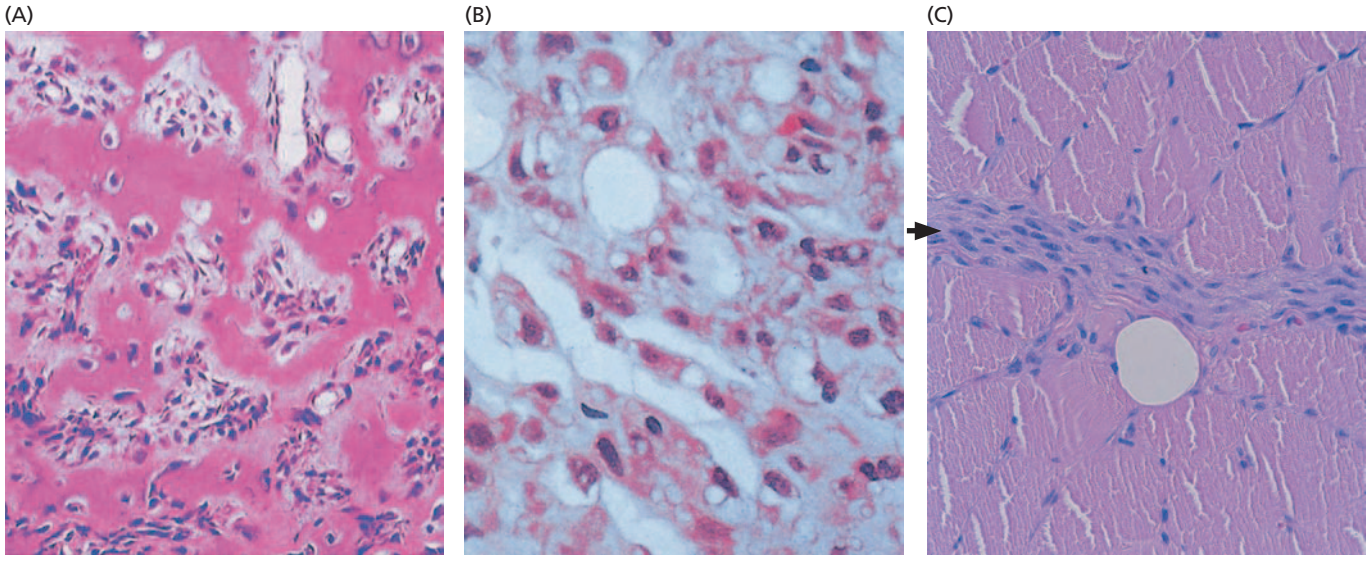


Figure 2.7 Mesenchymal tumors (A) Seen in this osteosarcoma are malignant bone-forming cells—osteoblasts (*dark purple nuclei*)—amid the mineralized bone (*pink*) they have constructed in the surrounding extracellular matrix. (B) A liposarcoma arises from cells closely related to adipocytes, which store lipid globules in various tissues. The presence of these globules throughout this tumor gives it a foamy appearance. (C) This leiomyosarcoma (*arrow, dark purple nuclei*), which arises in cells that form smooth muscle, is dispatching individual tumor cells to grow among adjacent normal muscle fibers (*light purple*). (D) Rhabdomyosarcomas arise from the cells forming striated skeletal muscles; the cancer cells (*dark red nuclei*) are seen here amid several normal muscle cells (*arrows*). (E) Hemangiomas—common tumors in infants—derive from the endothelial cells that form the lining of the lumina of small and large blood vessels. The densely packed capillaries in this particular tumor are formed from endothelial cells with cell nuclei stained *green* and cytoplasms stained *red.* Like epithelial cells, endothelial cells form basement membranes to which they attach, seen here in *blue.* (A–C, from A.T. Skarin, Atlas of Diagnostic Oncology, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003. D, from H. Okazaki and B.W. Scheithauer, Atlas of Neuropathology. Gower Medical Publishing, 1988. E, from M.R Ritter et al., *Proc. Natl. Acad. Sci. USA* 99:7455–7460, 2002.)

layer of the early embryo. Included here are **gliomas**, **glioblastomas**, **neuroblastomas**, **schwannomas**, and **medulloblastomas** (Figure 2.9). While comprising only 1.3% of all diagnosed cancers, these are responsible for about 2.5% of cancer-related deaths.

2.3 Some types of tumors do not fit into the major classifications

Not all tumors fall neatly into one of these four major groups. For example, **melanomas** derive from melanocytes, the pigmented cells of the skin and the retina. The melanocytes, in turn, arise from a primitive embryonic structure termed the **neural crest**. While having an embryonic origin close to that of the neuroectodermal cells,

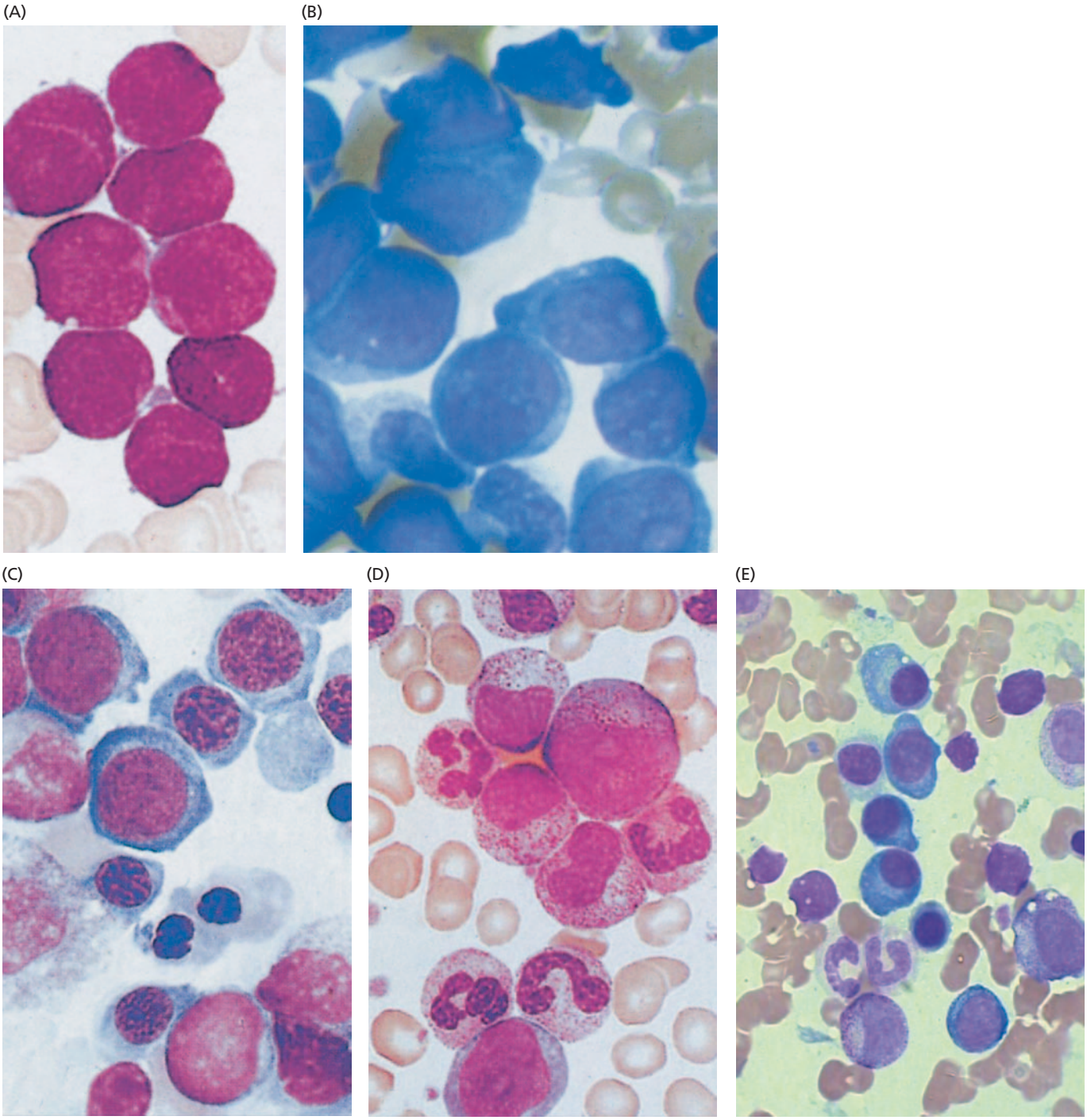


Figure 2.8 Hematopoietic malignancies (A) Acute lymphocytic leukemias (ALLs) arise from both the B-cell (80%) and T-cell (20%) lineages of lymphocytes (see Section 15.1). The cells forming this particular tumor (*red-purple*) exhibited the antigenic markers indicating origin from pre-B cells. (B) As in many hematopoietic malignancies, these acute myelogenous leukemia (AML) cells (*blue*) have only a small rim of cytoplasm around their large nuclei. They derive from precursor cells of the lineage that forms various types of granulocytes as well as monocytes, the latter developing, in turn, into macrophages, dendritic cells, osteoclasts, and other tissue-specific phagocytic cells. (C) The large erythroblasts in this erythroleukemia (*red-purple*) closely resemble the precursors of differentiated red blood cells—erythrocytes. (D) In chronic myelogenous leukemia (CML), a variety of leukemic cells of the myeloid (marrow) lineage are apparent (*red nuclei*), suggesting the differentiation of myeloid stem cells into several distinct cell types. (E) Multiple myeloma (MM) is a malignancy of the plasma cells of the B-cell lineage, which secrete antibody molecules, explaining their relatively large cytoplasms in which proteins destined for secretion are processed and matured. Seen here are plasma cells of MM at various stages of differentiation (*purple nuclei*). In some of these micrographs, numerous lightly staining erythrocytes are seen in the background. (From A.T. Skarin, Atlas of Diagnostic Oncology, 4th ed. Philadelphia: Elsevier Science Ltd., 2010.)

Table 2.4 Various types of neuroectodermal malignancies

Name of tumor	Lineage of founding cell
Glioblastoma multiforme	highly progressed astrocytoma
Astrocytoma	astrocyte (type of glial cell)[a]
Meningioma	arachnoidal cells of meninges[b]
Schwannoma	Schwann cell around axons[c]
Retinoblastoma	cone cell in retina[d]
Neuroblastoma[e]	cells of peripheral nervous system
Ependymoma	cells lining ventricles of brain[f]
Oligodendroglioma	oligodendrocyte covering axons[g]
Medulloblastoma	granular cells of cerebellum[h]

[a]Nonneuronal cell of central nervous system that supports neurons.
[b]Membranous covering of brain.
[c]Constructs insulating myelin sheath around axons in peripheral nervous system.
[d]Photosensor for color vision during daylight.
[e]These tumors arise from cells of the sympathetic nervous system.
[f]Fluid-filled cavities in brain.
[g]Similar to Schwann cells but in brain.
[h]Cells of the lower level of cerebellar cortex (for example, see Figure 2.9B).

the melanocytes end up during development as wanderers that settle in the skin and the eye, provide pigment to these tissues, but acquire no direct connections with the nervous system (**Figure 2.10**).

Small-cell lung carcinomas (SCLCs) contain cells having many attributes of **neurosecretory** cells, such as those of neural crest origin in the **adrenal** glands that sit above the kidneys. Such cells, often in response to neuronal signaling, secrete biologically active peptides. It remains unclear whether the SCLCs, frequently seen in tobacco users, arise from neuroectodermal cells that have insinuated themselves during normal development into the developing lung. According to a more likely alternative, these tumors originate in endodermal cell populations of the lung that have shed some of their epithelial characteristics and taken on those of a neuroectodermal lineage.

This switching of tissue lineage and resulting acquisition of an entirely new set of differentiated characteristics is often termed **transdifferentiation**. The term implies that the commitments cells have made during embryogenesis to enter into one or another tissue and cell lineage are not irreversible, and that under certain conditions, cells can move from one differentiation lineage to another. Such a change in phenotype may affect both normal and cancer cells. For example, at the borders of many carcinomas, epithelial cancer cells often change shape and gene expression programs and take on attributes of the nearby stromal cells of mesenchymal origin. This dramatic shift in cell phenotype, termed the **epithelial–mesenchymal transition**, or simply EMT, implies great plasticity on the part of cells that normally seem to be fully committed to behaving like epithelial cells. As described later (Chapters 13 and 14), this transition may often accompany and enable the invasion by carcinoma cells into adjacent normal tissues.

Of the atypical tumor types, **teratomas** are arguably the most bizarre of all, in part because they defy all attempts at classification. While only ~10,000 cases are diagnosed worldwide annually, teratomas deserve mention because they are unique and shed light on the biology of embryonic stem (ES) cells, which have become so important to biologists; ES cells enable genetic manipulation of the mouse germ line and are central to certain types of stem cell therapies currently under development. Teratomas

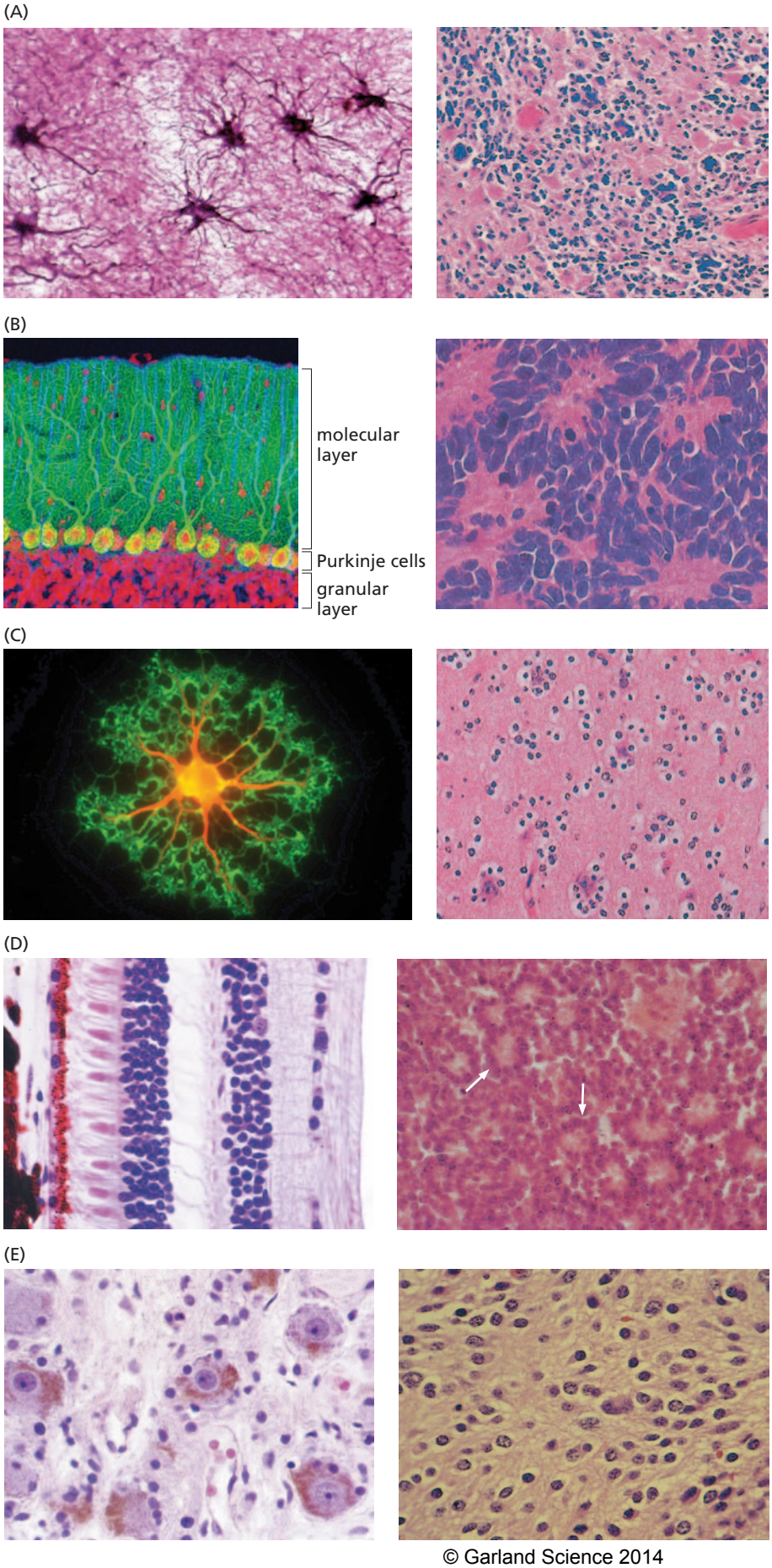


Figure 2.9 Neuroectodermal tumors
(A) Astrocytes—nonneuronal, supporting cells of the brain (*dark purple, left panel*)—are the presumed precursors of astrocytomas and glioblastomas (*right panel*). Glioblastoma multiforme takes its name from the multiple distinct neuroectodermal cell types that constitute the tumor. The tumor cells are seen to have nuclei of various sizes (*purple*). (B) Cells of the granular layer of the cerebellum (*left panel*) reside below Purkinje cells and cells of the molecular layer in the cortex of the cerebellum. The precursors of granular cells yield medulloblastomas (*right panel*), the cells of which are notable for their ability to differentiate into neurons, glial cells, and pigmented neuroepithelial cells (*purple nuclei, pink cytoplasms*). About one-third of these tumors show the rosettes of cells seen here. (C) Shown is an oligodendroglioma (*right*), which derives from oligodendrocytes, nonneuronal cells of ectodermal origin that support and insulate axons in the central nervous system. Each of the neoplastic cell nuclei here has a halo around it, which is characteristic of this tumor. The cultured normal oligodendrocyte shown here (*left*) exhibits a number of branching (dendritic) arms—each of which associates with one or several axons and proceeds to form an insulating myelin sheath around a segment of each of these axons. The cell body has been immunostained (*yellow/orange*) for the O4 oligodendrocyte marker, while the tips of the dendritic arms (*green*) have been stained for CNPase, an enzyme associated with myelination of axons. (D) Rods, cones, and other neuronal cell types (*left panel*) constitute important components of the normal retina. Retinoblastomas (*right panel*) arise from cells with attributes of the cone precursors present in the normal developing retina. Retinoblastomas often show the characteristic rosettes, indicated here with *arrows*. (E) Cells of the sympathetic ganglia of the peripheral nervous system (*larger cells, left panel*) give rise to neuroblastomas (*right panel*), which are usually seen in children. The individual tumor cells here are surrounded by dense fibrillary webs, which are derived from neurites—cytoplasmic processes used by neurons to communicate with one another. (A, D, and E, left panels, from B. Young et al., Wheater's Functional Histology, 4th ed. Edinburgh: Churchill Livingstone, 2003. A–C, right panels, from H. Okazaki, B.W. Scheithauer, Atlas of Neuropathology. Gower Medical Publishing, 1988. B, left panel, Thomas Deerinck, NCMIR/Science Source. C, left panel, courtesy of R. Hardy and R. Reynolds. D, E, right panels, from A.T. Skarin, Atlas of Diagnostic Oncology, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

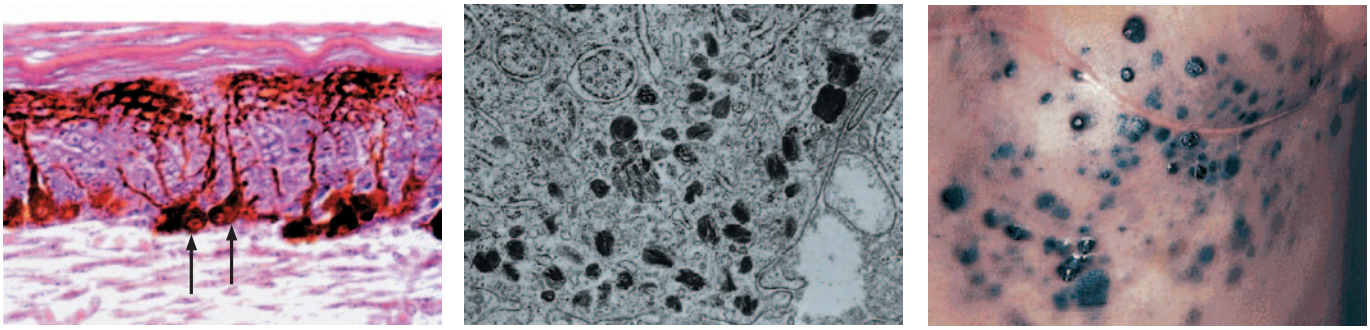


Figure 2.10 Melanocytes and melanomas (A) Melanocytes (*arrows*), which form melanin pigment granules, are normally scattered among the basal keratinocytes of the skin. They extend long, thin cytoplasmic processes through which they deposit these granules in the cytoplasm of keratinocytes, which form the bulk of the epithelium (see Figure 2.6A). Layers of dead keratinocytes at the surface of the skin (*above*) and stroma cells (*below*) are also apparent. (B) The pigment granules, visualized here by transmission electron microscopy, have made melanomas favored objects of research because the metastases that they form are easily visualized. (for example, see Figure 2.2A). Once melanomas have begun to invade vertically from the superficial layers of the skin into the underlying stroma, they have a high tendency to metastasize to distant tissues. (C) This case of cutaneous melanoma dramatizes the metastatic nature of the disease and the readily observed, pigmented metastases. (A, from W.J. Bacha Jr. et al., Color Atlas of Veterinary Histology, 3rd ed. Ames, IA: Wiley–Blackwell, 2012. B and C, from A.T. Skarin, Atlas of Diagnostic Oncology, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

seem to arise from **germ cell** (egg and sperm) precursors (see Section 1.3) that fail to migrate to their proper destinations during embryonic development and persist at **ectopic** (inappropriate) sites in the developing fetus. They retain the **pluripotency** of early embryonic cells—the ability to generate most and possibly all of the tissues present in the fully developed fetus. The cells in different sectors of common "mature" teratomas—which are largely benign, localized growths—differentiate to create tissues that are very similar to those found in a variety of adult tissues (**Figure 2.11**). Typically, representatives of the three cell layers of the embryo—endoderm, mesoderm, and ectoderm (see Figure 2.5)—coexist within a single tumor and often develop into recognizable structures, such as teeth, hair, and bones. Occasionally these tumors progress to become highly malignant and thus life-threatening.

Of special interest is the fact that careful karyotypic and molecular analyses of benign, mature teratomas have indicated that the associated tumor cells are genetically wild type. This suggests that such teratoma cells are unique, being the only type of tumorigenic cell whose genomes are truly wild type, in contrast to the cells of all other tumor types described in this book, which carry multiple genetic aberrations.

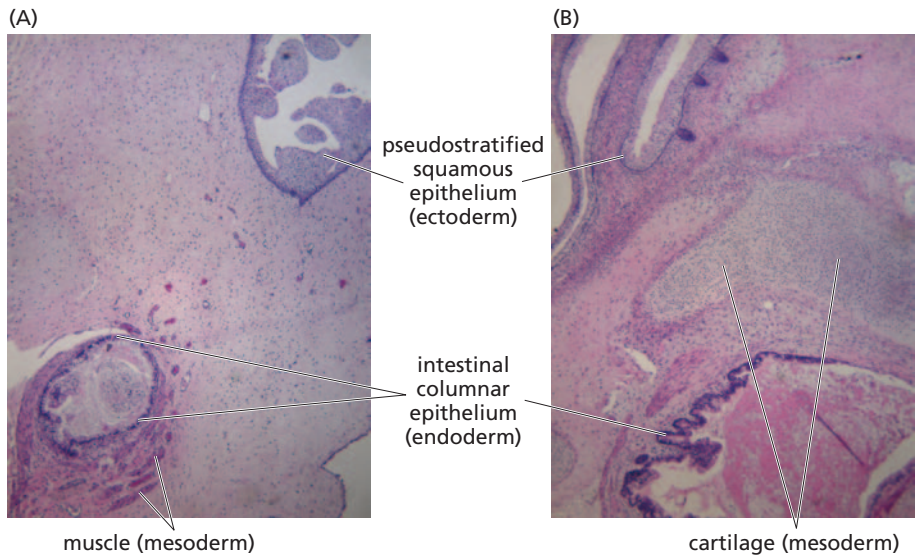


Figure 2.11 Teratomas This teratoma was created by implanting human embryonic stem (ES) cells into a mouse, yielding a tumor that is a **phenocopy** of the spontaneous teratomas found in children; such "mature" teratomas contain fully differentiated cells and are localized, noninvasive tumors. The two sections of this teratoma (A, B) indicate the typical behavior of these tumors, in that different sectors of this tumor have formed differentiated tissues deriving from all three cell layers of the early embryo depicted in Figure 2.5. (Courtesy of Sumita Gokhale.)

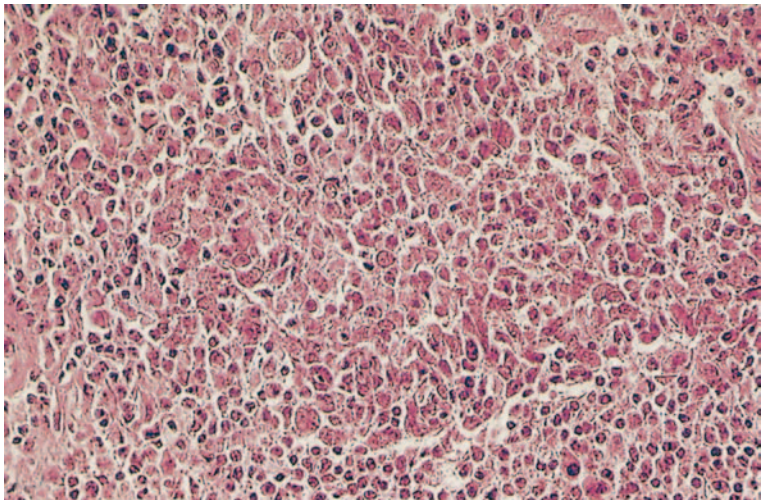


Figure 2.12 Anaplastic tumors of obscure origin The histological appearance of an anaplastic tumor, such as that shown here, gives little indication of its tissue of origin. Attempts to determine the origin of these cells with an antibody stain that specifically recognizes one or another tissue-specific protein marker may also prove uninformative. (From A.T. Skarin, Atlas of Diagnostic Oncology, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

The occasional rule-breaking exceptions, such as those represented by teratomas and the products of the EMT, do not detract from one major biological principle that seems to govern the vast majority of cancers: while cancer cells deviate substantially in behavior from their normal cellular precursors, they almost always retain some of the distinctive attributes of the normal cell types from which they have arisen. These attributes provide critical clues about the origins of most tumors; they enable pathologists to examine tumor biopsies under the microscope and assign a tissue of origin and tumor classification, even without prior knowledge of the anatomical sites from which these biopsies were prepared.

In a small minority of cases (2–4%), the tumors given to pathologists for analysis have shed virtually all of the tissue-specific, differentiated traits of their normal precursor tissues. The cells in such tumors are said to have **dedifferentiated**, and the tumors as a whole are **anaplastic**, in that it is no longer possible to use histopathological criteria to identify the tissues from which they have arisen (**Figure 2.12**). A tumor of this type is often classified as a **cancer of unknown primary** (CUP)**,** reflecting the difficulty of identifying the original site of tumor formation in the patient.

2.4 Cancers seem to develop progressively

Between the two extremes of fully normal and highly malignant tissue architectures lies a broad spectrum of tissues of intermediate appearance. The different gradations of abnormality may well reflect cell populations that are evolving progressively toward greater degrees of aggressive and invasive behavior. Thus, each type of abnormal growth within a tissue may represent a distinct step along this evolutionary pathway. If so, these architectures suggest, but hardly prove, that the development of tumors is a complex, multi-step process, a subject that is discussed in great detail in Chapter 11.

Some growths contain cells that deviate only minimally from those of normal tissues but may nevertheless be abnormal in that they contain excessive *numbers* of cells. Such growths are termed **hyperplastic** (**Figure 2.13**). In spite of their apparently deregulated proliferation, the cells forming hyperplastic growths have retained the ability to assemble into tissues that appear reasonably normal.

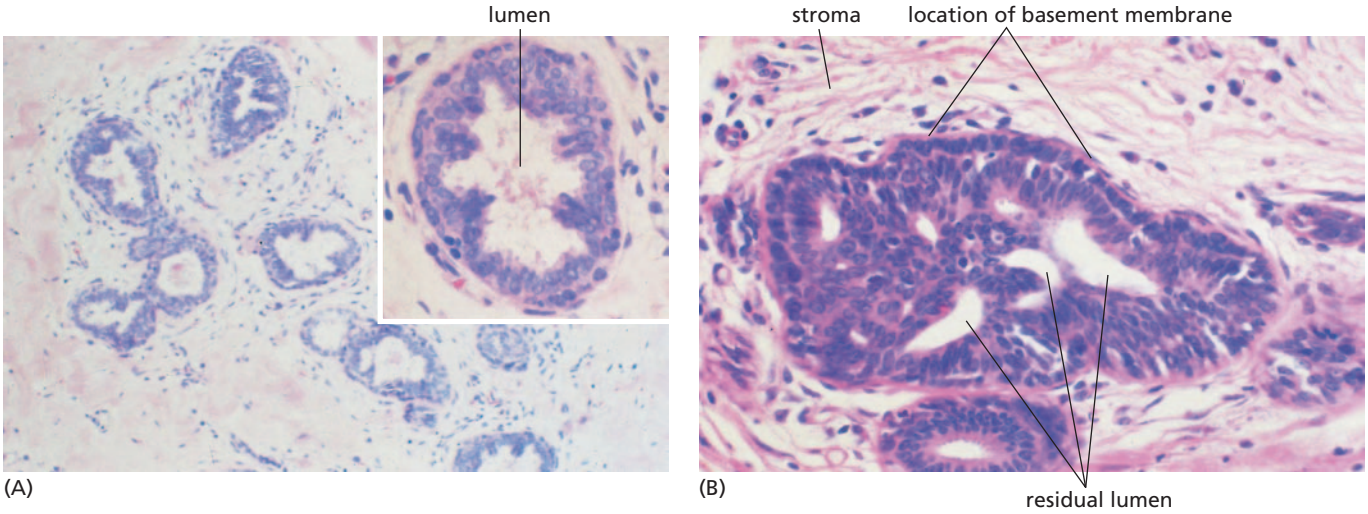


Figure 2.13 Normal versus hyperplastic epithelium The morphology of the normal ductal epithelium of the mammary gland (see Figure 2.1B) can be compared with different degrees of hyperplasia. (A) In these mildly hyperplastic milk ducts, shown at low magnification and high magnification (*inset*), mammary epithelial cells have begun to form piles that protrude into the lumina. (B) A more advanced hyperplastic mammary duct shows epithelial cells that are crowded together and almost completely fill the lumen. However, they have not penetrated the basement membrane (*not visible*) and invaded the surrounding stroma. (From A.T. Skarin, Atlas of Diagnostic Oncology, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

An equally minimal deviation from normal is seen in **metaplasia**, where one type of normal cell layer is displaced by cells of another type that are not normally encountered in this site within a tissue. These invaders, although present in the wrong location, often appear completely normal under the microscope. Metaplasia is most frequent in epithelial transition zones where one type of epithelium meets another. Transition zones like these are found at the junction of the cervix with the uterus and the junction of the esophagus and the stomach. In both locations, a squamous epithelium normally undergoes an abrupt transition into a mucus-secreting epithelium. For example, an early indication of premalignant change in the esophagus is a metaplastic condition termed **Barrett's esophagus**, in which the normally present squamous epithelium is replaced by secretory epithelial cells of a type usually found within the stomach (**Figure 2.14**). Even though these gastric cells have a quite normal appearance, this metaplasia is considered an early step in the development of esophageal adenocarcinomas. Indeed, patients suffering from Barrett's esophagus have a thirty-fold increased risk of developing these highly malignant tumors.

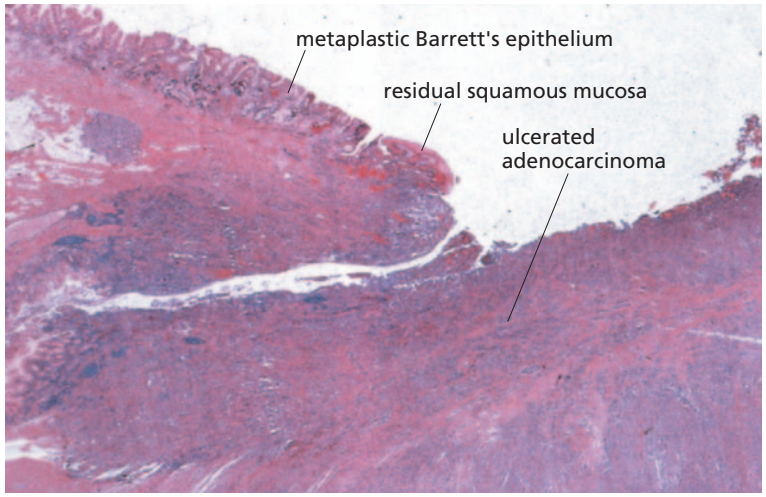


Figure 2.14 Metaplastic conversion of epithelia In certain precancerous conditions, the normally present epithelium is replaced by an epithelium from a nearby tissue—the process of metaplasia. For example, in Barrett's esophagus (sometimes termed Barrett's esophagitis), the squamous cells that normally line the wall of the esophagus (*residual squamous mucosa*) are replaced by secretory cells that migrate from the lining of the stomach (*metaplastic Barrett's epithelium*). This particular metaplasia, which is provoked by chronic acid reflux from the stomach, can become a precursor lesion to an esophageal carcinoma, which has developed here from cells of gastric origin (*ulcerated adenocarcinoma*). (Adapted from A.T. Skarin, Atlas of Diagnostic Oncology, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

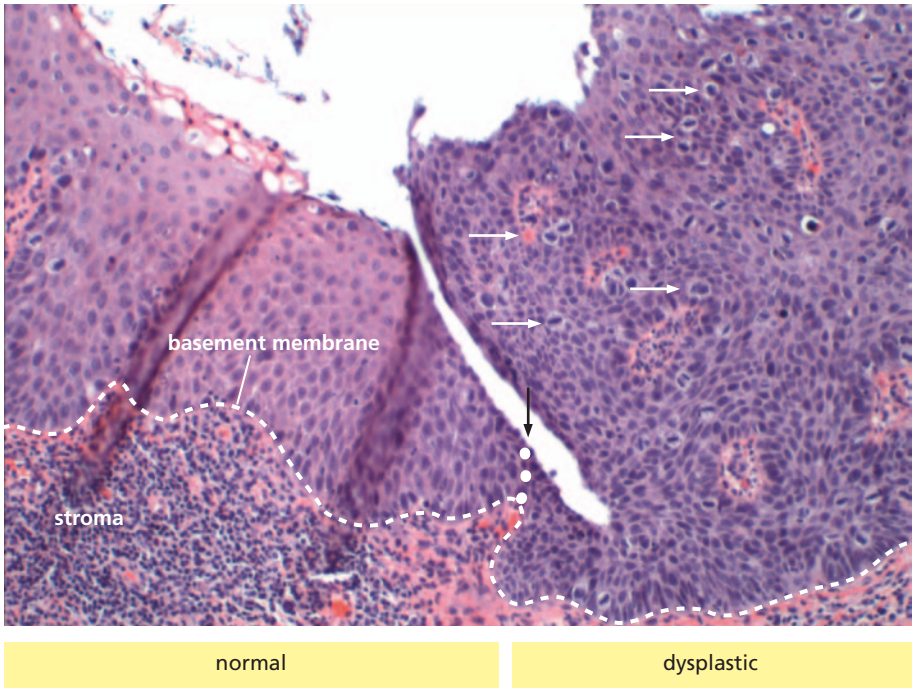


Figure 2.15 Formation of dysplastic epithelium In this intraepithelial squamous neoplasia of the cervix (*to right of white dotted line, black arrow*), the epithelial cells have not broken through the basement membrane (*not visible, indicated by white dashed line*) and invaded the underlying stroma. The cells in this dysplasia continue to be densely packed all the way to the luminal surface (*above*), in contrast to the more diffuse distribution of cells in the normal epithelium (*left*), whose cytoplasms (*light pink*) increase in size as the cells differentiate. Numerous mitotic figures are also apparent in the dysplasia (*white arrows*), indicating extensive cell proliferation. (Courtesy of Tan A. Ince.)

A slightly more abnormal tissue is said to be **dysplastic**. Cells within a dysplasia are usually abnormal **cytologically**; that is, the appearance of individual cells is no longer normal. The cytological changes include variability in nuclear size and shape, increased nuclear staining by dyes, increased ratio of nuclear versus cytoplasmic size, increased mitotic activity, and lack of the cytoplasmic features associated with the normal differentiated cells of the tissue (**Figure 2.15**). In dysplastic growths, the relative numbers of the various cell types seen in the normal tissue are no longer observed. Together, these changes in individual cells and in cell numbers have major effects on the overall tissue architecture. Dysplasia is considered to be a transitional state between completely benign growths and those that are premalignant.

Even more abnormal are the growths that are seen in epithelial tissues and termed variously adenomas, **polyps**, adenomatous polyps, **papillomas**, and, in skin, warts (**Figure 2.16**). These are often large growths that can be readily detected with the naked eye. They contain all the cell types found in the normal epithelial tissue, but this assemblage of cells has launched a program of substantial expansion, creating a macroscopic mass. Under the microscope, the tissue within these adenomatous growths is seen to be dysplastic. These tumors usually grow to a certain size and then stop growing, and they respect the boundary created by the basement membrane, which continues to separate them from the underlying stroma. Since adenomatous growths do not penetrate the basement membrane and invade underlying tissues, they are considered to be benign.

A further degree of abnormality is represented by growths that do invade underlying tissues. In the case of carcinoma cells, this incursion is signaled the moment carcinoma cells break through a basement membrane and invade into the adjacent stroma (**Figure 2.17**). Here, for the first time, we encounter malignant cells that have a substantial potential of threatening the life of the individual who carries them. Clinical oncologists and surgeons often reserve the word **cancer** for these and even more abnormal growths. However, in this book, as in much of contemporary cancer research, the word cancer is used more loosely to include all types of abnormal growths. (In the case of epithelial tissues, the term "carcinoma" is usually applied to growths that have acquired this degree of invasiveness.) This disparate collection of growths—both benign and malignant—are called collectively **neoplasms**, that is, new types of tissue.

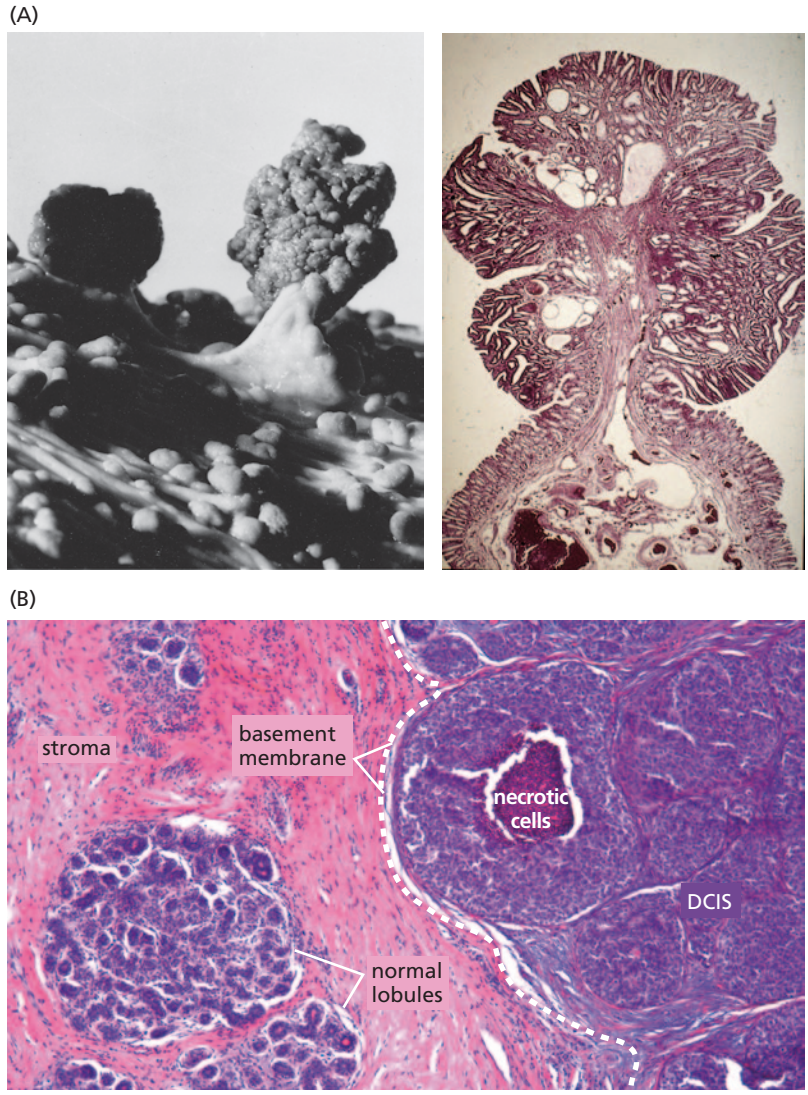


Figure 2.16 Pre-invasive adenomas and carcinomas Adenomatous growths, termed polyps in certain organs, have a morphology that sets them clearly apart from normal and dysplastic epithelium. (A) In the colon, pre-invasive growths appear as either flat thickenings of the colonic wall (sessile polyps, *not shown*) or as the stalk-like growths (pedunculated polyps) shown here in a photograph (*left*) and a micrograph (*right*). These growths, also termed "adenomas," have not penetrated the basement membrane and invaded the underlying stroma. (B) The lobules of the normal human breast (*purple islands, left half of figure*), each containing numerous small alveoli in which milk is produced, are surrounded by extensive fibrous stroma (*pink*). The cells of an intraductal carcinoma, often called a ductal carcinoma *in situ* (DCIS; *purple, to right of dashed line*), fill and distend ducts but have not invaded through the basement membrane surrounding the ducts into the stroma. In the middle of one of these ducts is an island of necrotic carcinoma cells (*dark red*) that have died, ostensibly because of inadequate access to the circulation. (A, left, courtesy of John Northover and Cancer Research, UK; right, courtesy of Anne Campbell. B, courtesy of Tan A. Ince.)

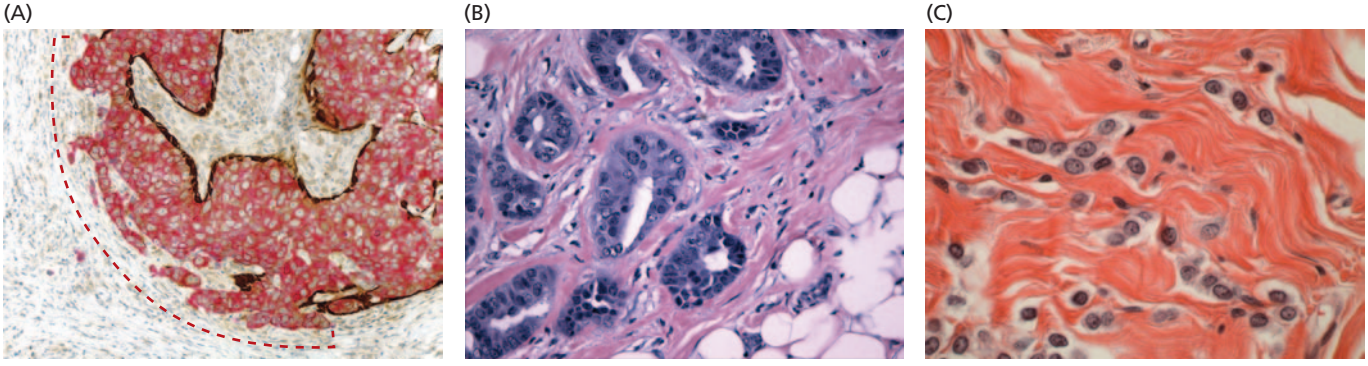


Figure 2.17 Invasive carcinomas Tumors are considered malignant only after they have breached the basement membrane and invaded the surrounding stroma. (A) These breast cancer cells (*dark red*), which previously constituted a ductal carcinoma *in situ* (DCIS; see Figure 2.16B), have now broken through on a broad front (*dashed line*) the layer of myoepithelial cells (*dark brown*) and underlying attached basement membrane (*not visible*) into the stroma; this indicates that they have acquired a new trait: invasiveness. (B) After breaching the basement membrane, invasive cancer cells can appear in various configurations amid the stroma. In this invasive ductal carcinoma of the breast, islands of epithelial cancer cells (*dark purple*) are interspersed amid the stroma (*dark pink*). The ductal nature of this carcinoma is revealed by the numerous rudimentary ducts formed by the breast cancer cells. (C) In this invasive lobular carcinoma of the breast, individual carcinoma cells *(dark purple nuclei)* have ventured into the stroma (*red-orange*), often doing so in single-file formation. (A, from F. Koerner, Diagnostic Problems in Breast Pathology. Philadelphia: Saunders/Elsevier, 2008. B and C, courtesy of Tan A. Ince.)

(Some reserve the term "neoplasm" for malignant tumors.) A summary of the overall pathological classification scheme of tumors is provided in **Figure 2.18**. A short discussion of the organizing principles underlying these classifications can be found in Supplementary Sidebar 2.2.

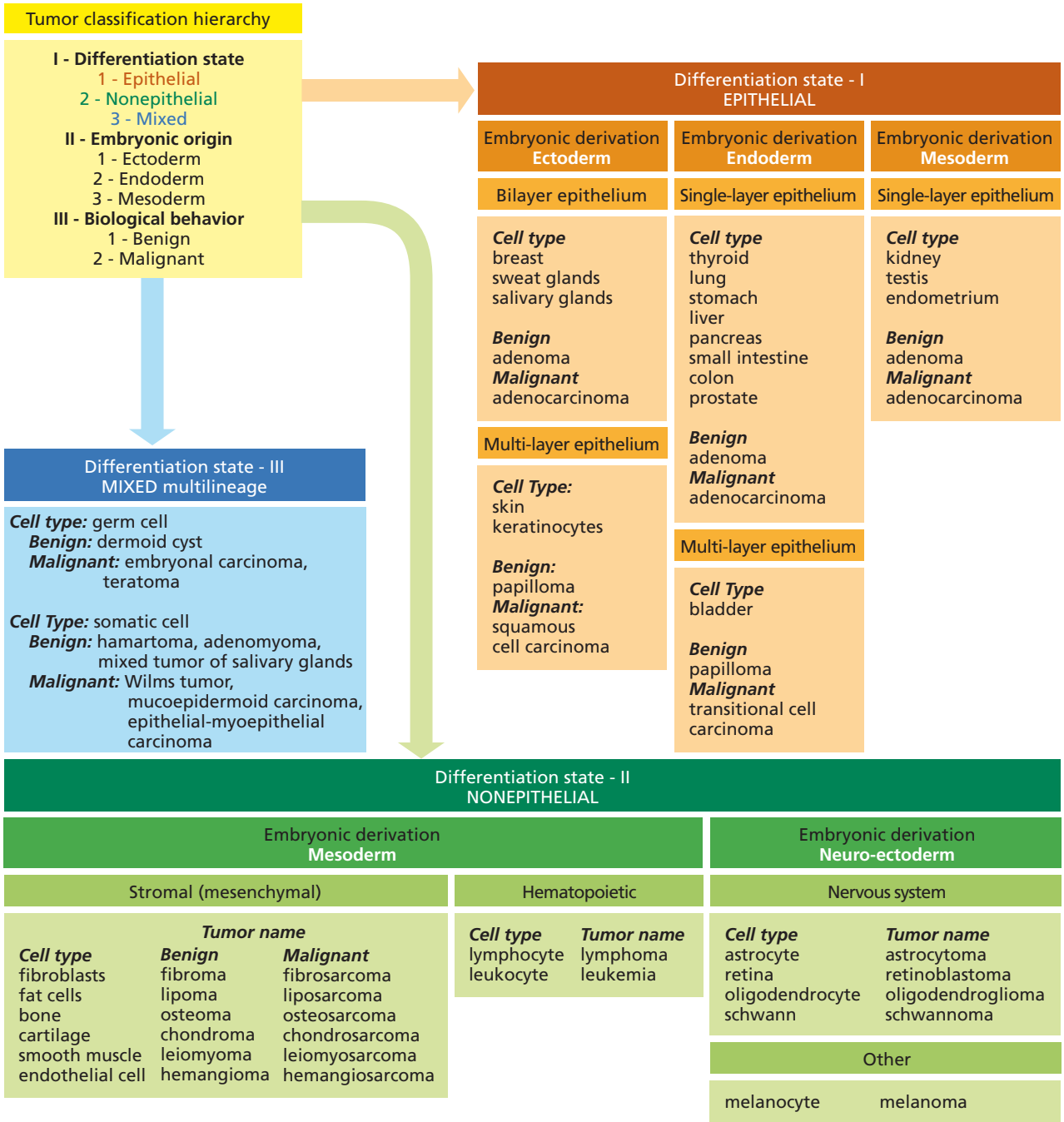


Figure 2.18 Classification scheme of tumors A clear understanding of the histopathological classification of tumors is essential for the study of cancer. However, the entire spectrum of tumors arising in various organs and tissues has been difficult to capture in a single classification scheme that is either purely morphologic or purely molecular. This has necessitated the use of histological features of tumor cells together with information about their respective tissues-of-origin, differentiation states, and biological behaviors; together these make it possible to develop a taxonomy of human tumors that has proven useful for the diagnosis and clinical management of most tumors. The scheme for classifying tumors presented here responds to three critical determinants of tumor biology: the embryonic tissue-of-origin and normal cell-of-origin of the tumor, the phenotype of the cell that has undergone transformation (for example, epithelial vs. mesenchymal), and the extent of progression to a highly malignant state. This scheme allows classification of the great majority of, but not all, human tumors. (Courtesy of Tan A. Ince.)

As mentioned above, cells in an initially formed primary tumor may seed new tumor colonies at distant sites in the body through the process of metastasis. This process is itself extraordinarily complex, and it depends upon the ability of cancer cells to invade adjacent tissues, to enter into blood and lymph vessels, to migrate through these vessels to distant anatomical sites, to leave the vessels and invade underlying tissue, and to found a new tumor cell colony at the distant site. These steps are the subject of detailed discussion in Chapter 14.

Because the various growths cataloged here represent increasing degrees of tissue abnormality, it would seem likely that they are distinct stopping points along the road of **tumor progression**, in which a normal tissue evolves progressively into one that is highly malignant. However, the precursor–product relationships of these various growths (that is, normal → hyperplastic → dysplastic → neoplastic → metastatic) are only suggested by the above descriptions but by no means proven.

2.5 Tumors are monoclonal growths

Even if we accept the notion that tumors arise through the progressive alteration of normal cells, another question remains unanswered: how many normal cells are the ancestors of those that congregate to form a tumor (**Figure 2.19**)? Do the tumor cells descend from a single ancestral cell that crossed over the boundary from normal to abnormal growth? Or did a large cohort of normal cells undergo this change, each becoming the ancestor of a distinct subpopulation of cells within a tumor mass?

The most effective way of addressing this issue is to determine whether all the cells in a tumor share a common, highly unique genetic or biochemical marker. For example, a randomly occurring somatic mutation might mark a cell in a very unusual way. If this particular genetic marker is present in all cells within a tumor, this would suggest that they all descend from an initially mutated cell. Such a population of cells, all of which derive from a common ancestral cell, is said to be **monoclonal**. Alternatively, if the tumor mass is composed of a series of genetically distinct subpopulations of cells that give no indication of a common origin, it can considered to be **polyclonal**.

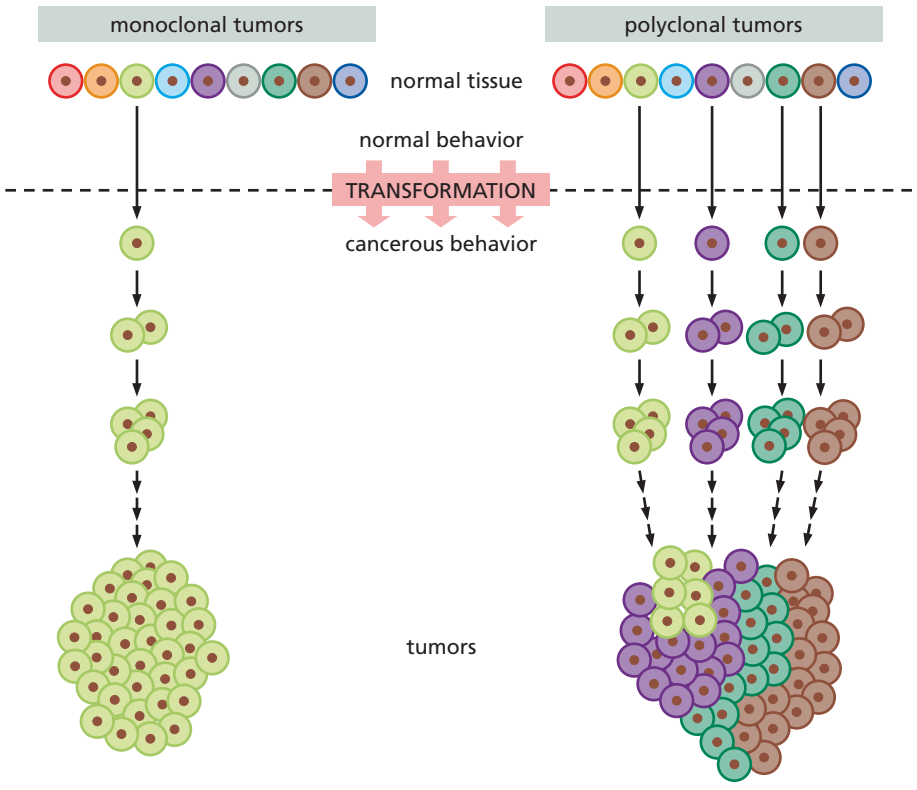


Figure 2.19 Monoclonality versus polyclonality of tumors In theory, tumors may be polyclonal or monoclonal in origin. In a polyclonal tumor (*right*), multiple cells cross over the border from normalcy to malignancy to become the ancestors of several, genetically distinct subpopulations of cells within a tumor mass. In a monoclonal tumor (*left*), only a single cell is transformed from normal to cancerous behavior to become the ancestor of the cells in a tumor mass.

The first experiments designed to measure the clonality of tumor cell populations actually relied on a naturally occurring, nongenetic (**epigenetic**) marking event. As described in Chapter 1, in the somatic cells of early embryos of female placental mammals, one of the two X chromosomes in each cell is selected randomly for silencing. This silencing causes almost all genes on one X chromosome in a cell to be repressed transcriptionally and is manifested karyotypically through the condensation of the silenced X chromosome into a small particle termed the Barr body (see Supplementary Sidebar 1.1). Once an X chromosome (of maternal or paternal origin) has been inactivated in a cell, all descendant cells in adult tissues appear to respect this decision and thus continue to inactivate the same X chromosome.

Thus, the lineage of a cell can be followed *in vivo* from its embryonic ancestor, a term called **lineage tracing**. The gene for glucose-6-phosphate dehydrogenase (G6PD) is located on the X chromosome, and more than 30% of African American women are heterozygous at this locus. Thus, they carry two alleles specifying forms of this enzyme that can be distinguished either by starch gel electrophoresis or by susceptibility to heat inactivation. Because of X-chromosome silencing, each of the cells in these heterozygous women will express only one or the other allele of the *G6PD* gene, which is manifested in turn in the variant of the G6PD protein that these cells synthesize (**Figure 2.20**). In most of their tissues, half of the cells make one variant enzyme, while the other half make the other variant. In 1965, observations were reported on a number of **leiomyomas** (benign tumors of the uterine wall) in African American heterozygotes. Each leiomyoma invariably expressed either one or the other variant form of the G6PD enzyme. This meant that, with great likelihood, its component cancer cells all descended from a single founding progenitor that expressed only that particular allele.

This initial demonstration of the monoclonality of human tumors was followed by many other confirmations of this concept. One proof came from observations of **myelomas**, which derive from the B-cell precursors of antibody-producing plasma

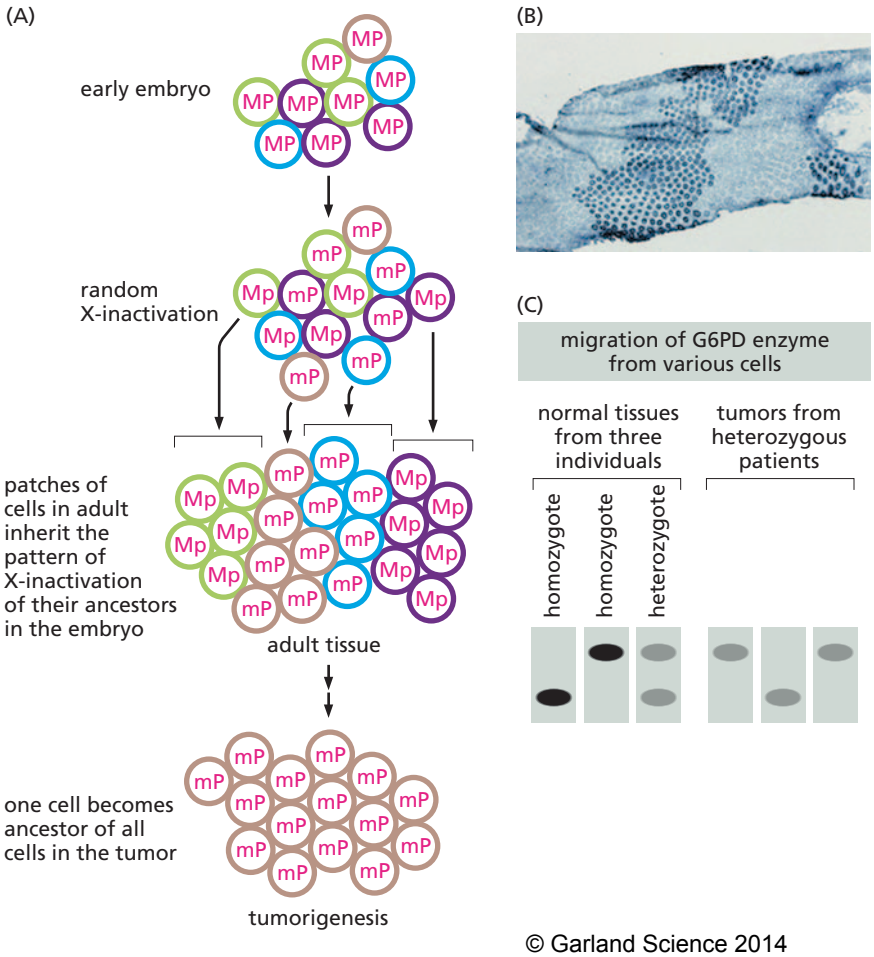


Figure 2.20 X-chromosome inactivation patterns and the monoclonality of tumors (A) While the female embryo begins with both X chromosomes in an equally active state, either the X chromosome inherited from the mother (M) or the one from the father (P) soon undergoes inactivation at random. Such inactivation silences expression of almost all genes on that chromosome. In the adult, all of the lineal descendants of a particular embryonic cell continue to inactivate the same X chromosome. Hence, the adult female body is made of patches (clones) of cells of the type Mp and patches of the type mP, where the *lowercase letter* denotes an inactivated state. (B) The two allelic forms of glucose-6-phosphate dehydrogenase (G6PD), which is encoded by a gene on the X chromosome, have differing sensitivities to heat inactivation. Hence, gentle heating of tissue from a heterozygote—in this case a section of intestine—reveals patches of cells that carry the heat-resistant, still-active enzyme variant (*dark blue spots*) among patches that do not. The cells in each patch are the descendants of an embryonic cell that had inactivated either its maternal or paternal X chromosome. (C) Use of starch gel electrophoresis to resolve the two forms of G6PD showed that all of the cancer cells in a tumor from a *G6PD* heterozygous patient express the same version of this enzyme. This indicated their likely descent from a common ancestral cell that already had this particular pattern of X-inactivation, suggesting that the cancer cells within a tumor mass constitute a monoclonal growth. (B, from M. Novelli et al., *Proc. Natl. Acad. Sci. USA* 100:3311–3314, 2003. C, adapted from P.J. Fialkow, *N. Engl. J. Med.* 291:26–35, 1974.)

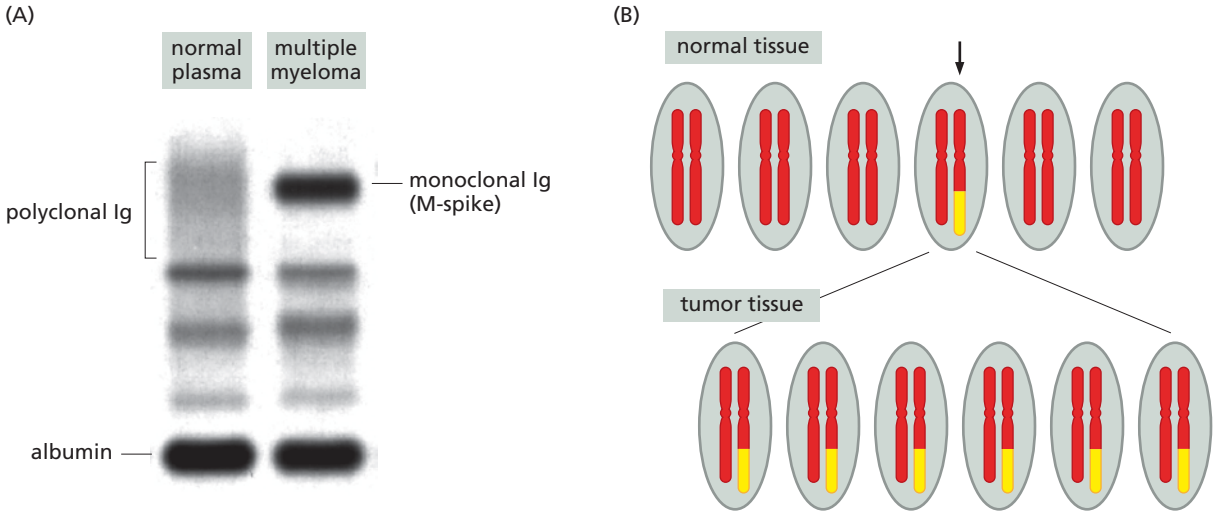


Figure 2.21 Additional proofs of tumor monoclonality (A) In normal plasma, the immunoglobulin (Ig) molecules (for example, antibodies) migrate as a heterogeneous collection of molecules upon gel electrophoresis (polyclonal Ig, *top of left channel*); this heterogeneity is indicative of the participation of a diverse spectrum (a polyclonal population) of plasma cells in antibody production. However, in multiple myeloma, this heterogeneity is replaced by a single antibody species (termed an M-spike) that is produced by a single clonal population of antibody-secreting tumor cells. (B) Illustrated is an unusual translocation (*arrow*) that involves exchange of segments between two different (nonhomologous) chromosomes—a *red* and a *yellow* chromosome. (Only one of the two chromosomal products of the translocation is shown here.) The translocation creates a characteristic "signature" that distinguishes the affected cell from the surrounding population of karyotypically normal cells (*top row*). Since all of the cancer cells within a subsequently arising tumor carry the identical, rare translocation (*bottom row*), this indicates their descent from a common progenitor in which this translocation initially occurred. (A, courtesy of S. Chen-Kiang and S. Ely.)

cells. Normally, the pool of these B-cell precursors consists of hundreds of thousands, likely millions of distinct subpopulations, each expressing its own specific antibody molecules as a consequence of a particular **immunoglobulin** (antibody) gene rearrangement. In contrast, all the myeloma cells in a patient produce the identical antibody molecule, indicating their descent from a single, common ancestor that was present years earlier in this complex, heterogeneous cell population (**Figure 2. 21A**).

Perhaps the most vivid demonstrations of tumor monoclonality have come from cancer cells sporting a variety of chromosomal aberrations that can be visualized microscopically when chromosomes condense during metaphase of mitosis. Often, a very peculiar chromosomal abnormality—the clear result of a rare genetic accident—is seen in all the cancer cells within a tumor mass (see Figure 2.21B). This observation makes it obvious that all the malignant cells within this tumor descend from the single ancestral cell in which this chromosomal restructuring originally occurred.

While such observations seem to provide compelling proof that tumor populations are monoclonal, tumorigenesis may actually be more complex. Let us imagine, as a counterexample, that 10 normal cells in a tissue simultaneously crossed over the border from being normal to being malignant (or at least premalignant) and that each of these cells, and its descendants in turn, proliferated uncontrollably (see Figure 2.19). Each of these founding cells would spawn a large monoclonal population, and the tumor mass, as a whole, consisting of a mixture of these 10 cell populations, would be polyclonal.

It is highly likely that each of these 10 clonal populations varies subtly from the other 9 in a number of characteristics, among them the time required for their cells to double. Simple mathematics indicates that a cell population that exhibits a slightly shorter doubling time will, sooner or later, outgrow all the others, and that the descendants of these cells will dominate in the tumor mass, creating what will appear to be a monoclonal tumor. In fact, many tumors seem to require decades to develop, which is plenty of time for one clonal subpopulation to dominate in the overall tumor cell population. Hence, the monoclonality of the cells in a large tumor mass hardly proves that this tumor was strictly monoclonal during its early stages of development.

A second confounding factor derives from the genotypic and phenotypic instability of tumor cell populations. As we will discuss in great detail in Chapter 11, the population of cells within a tumor may begin as a relatively homogeneous collection of cells (thus constituting a monoclonal growth) but soon may become quite heterogeneous because of the continual acquisition of new mutant alleles by some of its cells, a term called genetic instability. The resulting genetic heterogeneity may mask the true monoclonal origin of this cell population, since many of the genetic markers in these descendant cells will be present only in specific subpopulations of cells within the tumor mass.

These caveats complicate our assessment of the monoclonal origins of tumors. Nonetheless, it is a widespread consensus that the vast majority of advanced human tumors are monoclonal growths descended from single normal progenitor cells that took the first small steps to becoming cancerous. Such progenitors are often termed **cells-of-origin**, and it is increasingly appreciated that the differentiation programs of these cells continue to influence the behavior of derived tumor cell populations decades later. Indeed, in the great majority of human tumor types, one can identify the tissues in which these cells-of-origin resided, but the precise identities of these normal cells, including their state of differentiation, often remain obscure.

2.6 Cancer cells exhibit an altered energy metabolism

The monoclonality of tumor cell populations was first demonstrated in 1965. Another equally interesting peculiarity of tumors was already appreciated more than four decades earlier: the energy metabolism of most cancer cells differs markedly from that of normal cells, a trait first reported in 1924 by Otto Warburg, the Nobelist later honored for discovering the respiratory enzyme now known as cytochrome *c* oxidase. As was documented in the decades that followed, normal cells that experience aerobic conditions break down glucose into pyruvate in the cytosol through the process of glycolysis and then dispatch the pyruvate into mitochondria, where it is broken down further into carbon dioxide in the citric acid cycle (known also as the Krebs cycle; **Figure 2.22A**). Under anaerobic or **hypoxic** (low oxygen tension) conditions, however, normal cells are limited to using only glycolysis, generating pyruvate that is reduced to lactate, which is then secreted from cells. Warburg discovered that even when exposed to ample oxygen, many types of cancer cells rely largely on glycolysis, generating lactate as the breakdown product of glucose (see Figure 2.22B).

The use by cancer cells of "aerobic glycolysis," as Warburg called it, would seem to make little sense energetically, since the breakdown of one molecule of glucose yields only two molecules of ATP through glycolysis. In contrast, when under aerobic conditions glycolysis is followed by oxidation of pyruvate in the citric acid cycle, as many as 36 ATPs per glucose molecule are generated. In fact, most types of normal cells in the body have continuous access to O_2 conveyed by the blood and therefore metabolize glucose through this energetically far more efficient route. The tendency of cancer cells to limit themselves to glycolysis, even when provided with adequate oxygen, stands out as exceedingly unusual behavior.

The fact that cancer cells metabolize glucose so inefficiently requires them to compensate by importing enormous amounts of glucose. This behavior is seen in many types of cancer cells, including both carcinomas and hematopoietic tumors; they express greatly elevated levels of glucose transporters, particularly GLUT1, which span the plasma membrane and drive the high rates of glucose uptake by these cells. Radiologists take advantage of this elevated glucose uptake by injecting into the circulation radiolabeled glucose [2-deoxy-2-(^{18}F)fluoro-D-glucose, FDG] and observing its rapid concentration in tumors (see Figure 2.22C).

In the 1950s, Warburg proposed that this altered energy metabolism was the driving force in the formation of cancer cells, a notion that was discredited in the decades that followed. However, the process of aerobic glycolysis that he discovered was ultimately found to operate in a wide variety of human cancer cells and is now thought to represent one of the many consequences of cell transformation.

Aerobic glycolysis, sometimes called the **Warburg effect**, remains a subject of much contention, as its rationale in cancer cell biology has never been fully resolved: why do as many as 80% of cancer cells metabolize most of their glucose via glycolysis when completion of glucose degradation in mitochondria by the citric acid cycle would afford them vastly more ATP to fuel their own growth and proliferation? Is aerobic glycolysis required for maintenance of the cancer cell phenotype, or does it represent nothing more than a side effect of cell transformation that plays no causal role in cell transformation and tumor growth?

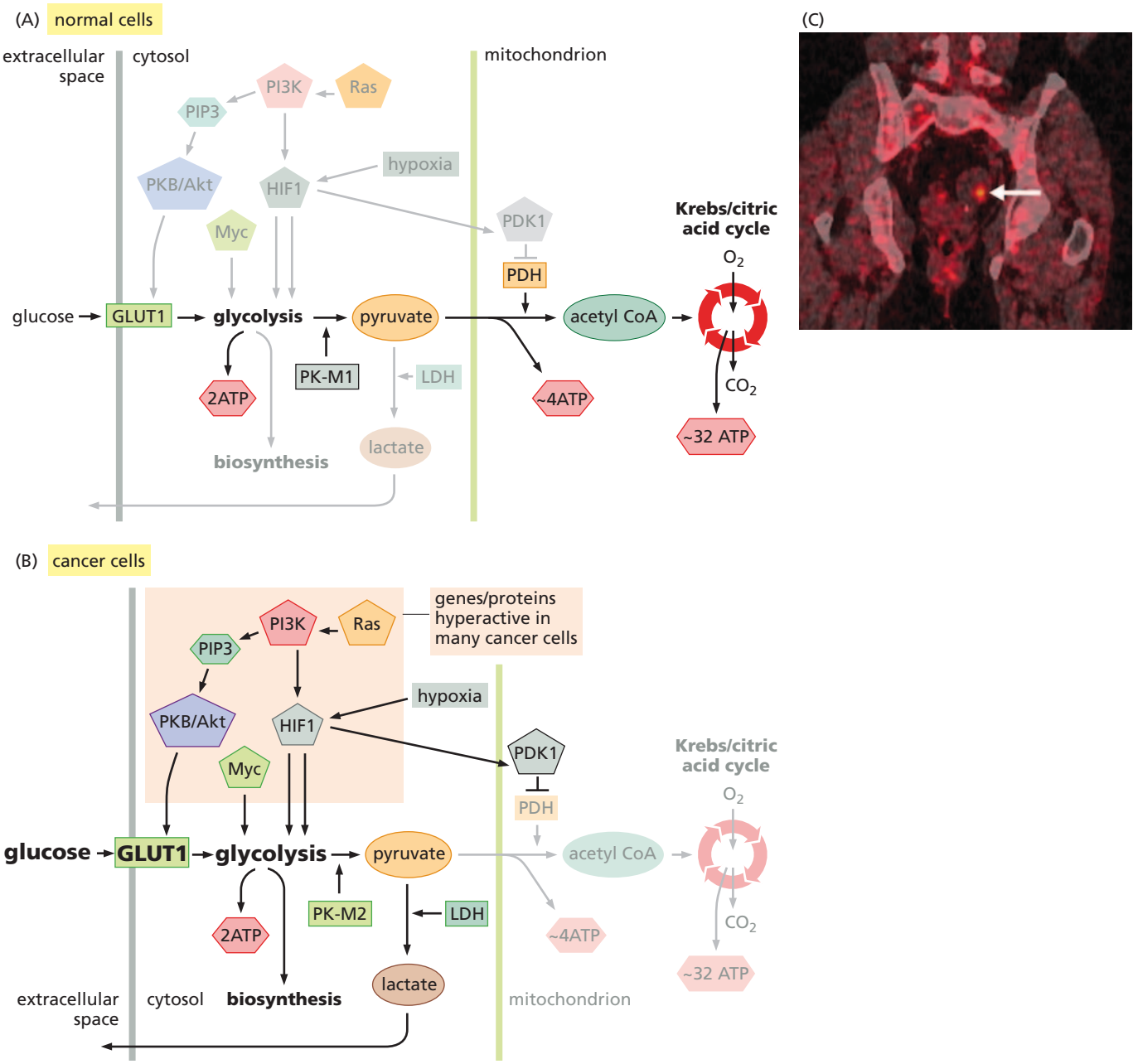


Figure 2.22 Changes in glucose metabolism in cancer cells
(A) In most normal nonproliferating cells having access to adequate oxygen, glucose is imported into the cells by glucose transporters (GLUTs) and then broken down by glycolysis and the citric acid cycle. During the last step of glycolysis, pyruvate kinase form M1 (PK-M1) ensures that its product, pyruvate, is imported into the mitochondria, where it is oxidized by pyruvate dehydrogenase (PDH) into acetyl CoA for processing in the citric acid cycle. Altogether, the mitochondria can generate as much as 36 ATP molecules per glucose molecule. (B) In cancer cells, including those with access to ample oxygen, the GLUT1 glucose transporter imports large amounts of glucose into the cytosol, where it is processed by glycolysis. However, as the last step of glycolysis, pyruvate kinase M2 (PK-M2) causes its pyruvate product to be diverted to lactate dehydrogenase (LDH-A), yielding the lactate that is secreted in abundance by cancer cells. Because relatively little of the initially imported glucose is metabolized by the mitochondria, as few as 2 ATPs are generated per glucose molecule. Moreover, many of the intermediates generated during glycolysis are diverted toward biosynthetic uses. This mode of metabolic regulation resembles the metabolic state of normal, rapidly dividing cells, which also divert a significant portion of their glycolytic intermediates to biosynthetic pathways. Enzymes are in *rectangles*, glucose metabolites are in *ovals*, low–molecular-weight compounds are in *hexagons*, regulatory proteins are in *pentagons*. (C) 2-Deoxy-2-(^{18}F)fluoro-D-glucose positron-emission tomography (FGD-PET) makes it possible to visualize tumors in the body that have concentrated large amounts of glucose because of the hyperactivity of the GLUT1 transporter in the associated cancer cells. In the case shown here, FDG-PET revealed a small tumor (*bright orange; arrow*) in the region near an ovary of a woman who was under treatment for breast cancer but was otherwise without symptoms. X-ray-computed tomography (CT) was used at the same time to image the outlines of the tissues of this patient. This highly sensitive technology provided the first indication of an incipient ovarian cancer in this patient. (C, from R.A. Milam, M.R. Milam and R.B. Iyer, *J. Clin. Oncol.* 25:5657–5658, 2007.)

One explanation of aerobic glycolysis comes from the observation that the cancer cells within a tumor often have inadequate access to oxygen, as we will discuss in detail in Chapter 13. The resulting hypoxic state limits cancer cells to glycolysis and thus to inefficient ATP production—just as normal cells would be limited under these conditions. Because of the Warburg effect, cancer cells would seem to be well adapted to this oxygen starvation, since glycolysis operates normally under hypoxic conditions. Still, this fails to explain why cancer cells, even when provided with abundant oxygen, do not take advantage of this oxygen to generate ATP in far larger quantities.

Another rationale for aerobic glycolysis derives from the fact that glycolysis actually serves a second role independent of ATP generation: the intermediates in the glycolytic pathway function as precursors of many molecules involved in cell growth, including the biosynthesis of nucleotides and lipids. By blocking the last step of glycolysis (see below), cancer cells ensure the accumulation of earlier intermediates via feedback reactions in this pathway. These glycolytic intermediates can then be diverted into critically important biosynthetic reactions. This behavior contrasts with that of normal cells, which are generally not actively proliferating, do not require large-scale biosynthetic reactions, and depend largely on ATP to sustain their metabolic activity. (By some estimates, normal cells use more than 30% of their imported glucose to make ATP, while cancer cells use only ~1% of their glucose for this purpose—a striking contrast in metabolic organization.)

A complete rationale for why cancer cells use aerobic glycolysis is still not in hand. However, independent of how this question is resolved, there is yet another: how do cancer cells actually manage to avoid mitochondrial processing of glucose metabolites? Pyruvate kinase (PK) catalyzes the last step of glycolysis—the conversion of phosphoenolpyruvate (PEP) to pyruvate. As noted earlier, this end product of glycolysis is normally destined for import into the mitochondria, where it is broken down in the citric acid cycle (see Figure 2.22). The M1 isoform of PK typically is expressed in most adult tissues, while the M2 isoform is expressed by early embryonic cells, rapidly growing normal cells, and cancer cells. For reasons that are still poorly understood, the commonly expressed M1 isoform of PK ensures that its product, pyruvate, is dispatched from the cytosol into the mitochondria, while the M2 isoform that is expressed instead in cancer cells causes its pyruvate product to be reduced to lactate in the cytosol. Relative to the M1 form of PK, the M2 enzyme has a very slow **turnover number**, which results in a backup of glycolytic intermediates and their diversion into biosynthetic pathways. Importantly, the relative inactivity of the citric acid cycle in cancer cells is not due to defects in the mitochondria: they are normal and fully capable of receiving pyruvate and processing it in the citric acid cycle.

Experimental evidence indicates that the growth of tumors actually depends on the expression of the M2 form of PK and on the elevated expression of the glucose importer GLUT1 and lactate dehydrogenase-A (LDH-A), the latter being involved in reducing pyruvate to lactate, which is then secreted (see Figure 2.22B). When any one of these is inhibited, tumor growth slows down, sometimes dramatically. Observations like these provide the first indications that the bizarre glucose metabolism of cancer cells creates a physiologic state on which cancer cell growth and proliferation depend.

2.7 Cancers occur with vastly different frequencies in different human populations

The nature of cancer suggests that it is a disease of chaos, a breakdown of existing biological order within the body. More specifically, the disorder seen in cancer appears to derive directly from malfunctioning of the controls that are normally responsible for determining when and where cells throughout the body will multiply. In fact, there is ample opportunity for the disorder of cancer to strike a human body. Most of the more than 10^{13} cells in the body continue to carry the genetic information that previously allowed them to come into existence and might, in the future, allow them to multiply once again. This explains why the risk of uncontrolled cell proliferation in countless sites throughout the body is substantial throughout the lives of mammals like ourselves.

To be more accurate, the risk of cancer is far greater than the $>10^{13}$ population size would suggest, since this number represents the average, steady-state population of cells in the body at any point in time during adulthood. The aggregate number of cells that are formed during an average human lifetime is about 10^{16}, a number that testifies to the enormous amount of cell turnover—involving cell death and replacement (almost 10^7 events per second)—that occurs continuously in many tissues in the body. As discussed in Chapters 9 and 12, each time a new cell is formed by the complex process of cell growth and division, there are many ways for things to go awry. Hence, the chance for disaster to strike, including the inadvertent formation of cancer cells, is great.

Since a normal biological process (incessant cell division) is likely to create a substantial risk of cancer, it would seem logical that human populations throughout the world would experience similar frequencies of cancer. However, when cancer **incidence** rates (that is, the rates with which the disease is diagnosed) are examined in various countries, we learn that the risks of many types of cancer vary dramatically (**Table 2.5**), while other cancers (not indicated in Table 2.5) do indeed show comparable incidence rates across the globe. So, our speculation that all cancers should strike different human populations at comparable rates is simply wrong. Some do and some don't. This realization forces us to reconsider our thinking about how cancers are formed.

Table 2.5 Geographic variation in cancer incidence and death rates

Countries showing highest and lowest incidence of specific types of cancer[a]			
Cancer site	**Country of highest risk**	**Country of lowest risk**	**Relative risk H/L[b]**
Skin (melanoma)	Australia (Queensland)	Japan	155
Lip	Canada (Newfoundland)	Japan	151
Nasopharynx	Hong Kong	United Kingdom	100
Prostate	U.S. (African American)	China	70
Liver	China (Shanghai)	Canada (Nova Scotia)	49
Penis	Brazil	Israel (Ashkenazic)	42
Cervix (uterus)	Brazil	Israel (non-Jews)	28
Stomach	Japan	Kuwait	22
Lung	U.S. (Louisiana, African American)	India (Madras)	19
Pancreas	U.S. (Los Angeles, Korean American)	India	11
Ovary	New Zealand (Polynesian)	Kuwait	8
Geographic areas showing highest and lowest death rates from specific types of cancer[c]			
Cancer site	**Area of highest risk**	**Area of lowest risk**	**Relative risk H/L[b]**
Lung, male	Eastern Europe	West Africa	33
Esophagus	Southern Africa	West Africa	16
Colon, male	Australia, New Zealand	Middle Africa	15
Breast, female	Northern Europe	China	6

[a]See C. Muir, J. Waterhouse, T. Mack et al., eds., Cancer Incidence in Five Continents, vol. 5. Lyon: International Agency for Research on Cancer, 1987. Excerpted by V.T. DeVita, S. Hellman and S.A. Rosenberg, Cancer: Principles and Practice of Oncology. Philadelphia: Lippincott, 1993.
[b]Relative risk: age-adjusted incidence or death rate in highest country or area (H) divided by age-adjusted incidence or death rate in lowest country or area (L). These numbers refer to age-adjusted rates, for example, the relative risk of a 60-year-old dying from a specific type of tumor in one country compared with a 60-year-old in another country.
[c]See P. Pisani, D.M. Parkin, F. Bray and J. Ferlay, *Int. J. Cancer* 83:18–29, 1999. This survey divided the human population into 23 geographic areas and surveyed the relative mortality rates of various cancer types in each area.

Some of the more than 100 types of human cancers do seem to have a high proportion of tumors that are caused by random, unavoidable accidents of nature and thus occur with comparable frequencies in various human populations. This seems to be true for certain pediatric tumors. In addition to this relatively constant "background rate" of some specific cancers, yet other factors appear to intervene in certain populations to increase dramatically the total number of cancer cases. The two obvious contributory factors here are heredity and environment.

Which of these two alternatives—heredity or environment—is the dominant determinant of the country-to-country variability of cancer incidence? While many types of disease-causing alleles are distributed unequally in the gene pools of different human populations, these alleles do not seem to explain the dramatically different incidence rates of various cancers throughout the world. This point is demonstrated most dramatically by measuring cancer rates in migrant populations. For example, Japanese experience rates of stomach cancer that are 6 to 8 times higher than those of Americans (**Figure 2.23**). However, when Japanese settle in the United States, within a generation their offspring exhibit a stomach cancer rate that is comparable to that of the surrounding population. For the great majority of cancers, disease risk therefore seems to be "environmental," where this term is understood to include both physical environment and lifestyle.

As indicated in Table 2.5, the incidence of some types of cancer may vary enormously from one population to the next. Thus, breast cancer in China is about one-sixth as common as in the United States or Northern Europe. Having excluded genetic contributions to this difference, we might then conclude that as many as 85% of the breast cancers in the United States might in theory be avoidable, if only American women were to experience an environment and lifestyle comparable to those of their Chinese counterparts. Even within the American population, there are vast differences in cancer mortality: the Seventh-Day Adventists, whose religion discourages smoking, heavy drinking, and the consumption of pork, die from cancer at a rate that is only about three-quarters that of the general population.

For those who wish to understand the **etiologic** (causative) mechanisms of cancer, these findings lead to an inescapable conclusion: the great majority of the commonly occurring cancers are caused by factors or agents that are external to the body, enter into the body, and somehow attack and corrupt its tissues. In a minority of cancers, substantial variations in cancer risk may be attributable to differences in reproductive behavior and the resulting dramatic effects on the hormonal environment within the human female body.

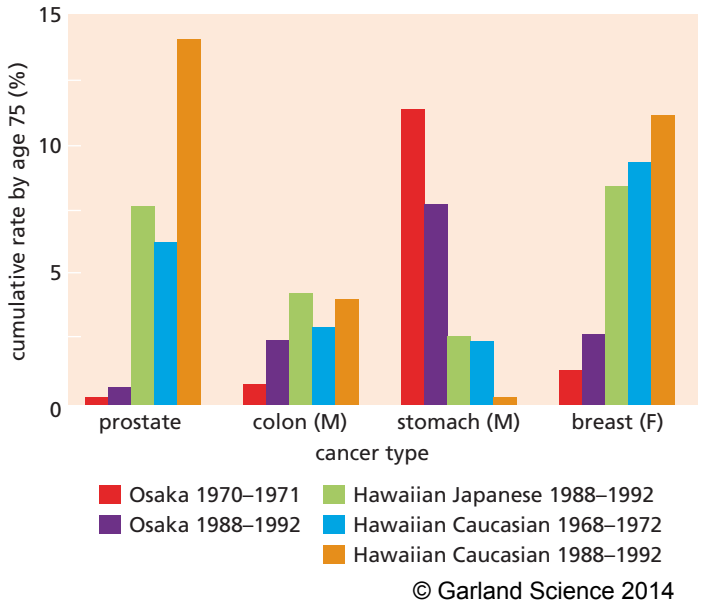


Figure 2.23 Country-to-country comparisons of cancer incidence Public health records reveal dramatic differences in the incidence of certain cancers in different countries. Here, the relative incidences of a group of cancers in Japan and in the American island of Hawaii are presented. Invariably, after Japanese have immigrated to Hawaii, within a generation their cancer rates approach those of the population that settled there before them. This indicates that the differing cancer rates are not due to genetic differences between the Japanese and the American populations. (From J. Peto, *Nature* 411:390–395, 2001.)

Let us imagine, for the sake of argument, that avoidance of certain obvious cancer-causing factors in diet and lifestyle resulted in a 50% reduction in the risk of dying from cancer in the West, leaving the disease of cancer as the cause of about 10% of overall mortality in this population. Under these conditions, given the approximately 10^{16} mitoses occurring in each human body during a normal life span, we calculate that only 1 in 10^{17} cell divisions—the total number of cell divisions occurring in the bodies of 10 individuals during their lifetimes—would lead directly or indirectly to a clinically detectable cancer. Now, we become persuaded that in spite of the enormous intrinsic risk of developing cancer, the body must be able to mount highly effective defenses that usually succeed in holding off the disease for the 70 or 80 years that most of us spend on this planet. These defenses are the subject of many discussions throughout this book.

2.8 The risks of cancers often seem to be increased by assignable influences including lifestyle

Evidence that certain kinds of cancers are associated with specific exposures or lifestyles is actually quite old, predating modern epidemiology by more than a century. The first known report comes from the observations of the English physician John Hill, who in 1761 noted the connection between the development of nasal cancer and the excessive use of tobacco snuff. Fourteen years later, Percivall Pott, a surgeon in London, reported that he had encountered a substantial number of skin cancers of the scrotum in adolescent men who, in their youth, had worked as chimney sweeps. Within three years, the Danish sweepers guild urged its members to take daily baths to remove the apparently cancer-causing material from their skin. This practice was likely the cause of the markedly lower rate of scrotal cancer in continental Europe when compared with Britain even a century later.

Beginning in the mid-sixteenth century, silver was extracted in large quantities from the mines in St. Joachimsthal in Bohemia, today Jáchymov in the Czech Republic. By the first half of the nineteenth century, lung cancer was documented at high rates in the miners, a disease that was otherwise almost unheard of at the time. Once again, an occupational exposure had been correlated with a specific type of cancer.

In 1839, an Italian physician reported that breast cancer was a scourge in the nunneries, being present at rates that were six times higher than among women in the general population who had given birth multiple times. By the end of the nineteenth century, it was clear that occupational exposure and lifestyle were closely connected to and apparently causes of a number of types of cancer.

The range of agents that might trigger cancer was expanded with the discovery in the first decade of the twentieth century that physicians and others who experimented with the then-recently invented X-ray tubes experienced increased rates of cancer, often developing tumors at the site of irradiation. These observations led, many years later, to an understanding of the lung cancer in the St. Joachimsthaler miners: their greatly increased lung cancer incidence could be attributed to the high levels of radioactivity in the ores coming from these mines.

Perhaps the most compelling association between environmental exposure and cancer incidence was forged in 1949 and 1950 when two groups of epidemiologists reported that individuals who were heavy cigarette smokers ran a lifetime risk of lung cancer that was more than twentyfold higher than that of nonsmokers. The initial results of one of these landmark studies are given in **Table 2.6**. These various epidemiologic correlations proved to be critical for subsequent cancer research, since they suggested that cancers often had specific, assignable causes, and that a chain of causality might one day be traced between these ultimate causes and the cancerous changes observed in certain human tissues. Indeed, in the half century that followed the 1949–1950 reports, epidemiologists identified a variety of environmental and lifestyle factors that were strongly correlated with the incidence of certain cancers (**Table 2.7**); in some of these cases, researchers have been able to discover the specific biological mechanisms through which these factors act.

Table 2.6 Relative risk of lung cancer as a function of the number of cigarettes smoked per day[a]

	Lifelong nonsmoker	Smokers			
Most recent number of cigarettes smoked (by subjects) per day before onset of disease	—	≥1, <5	≥5, <15	≥15, <25	≥25
Relative risk	1	8	12	14	27

[a]The relative risk indicates the risk of contracting lung cancer compared with that of a nonsmoker, which is set at 1.
From R. Doll and A.B. Hill, *BMJ* 2:739–748, 1950.

2.9 Specific chemical agents can induce cancer

Coal tar condensates, much like those implicated in cancer causation by Percivall Pott's work, were used in Japan at the beginning of the twentieth century to induce skin cancers in rabbits. Repeated painting of localized areas of the skin of their ears resulted, after many months, in the outgrowth of carcinomas. This work, first reported by Katsusaburo Yamagiwa in 1915, was little noticed in the international scientific community of the time (**Figure 2.24**). In retrospect, it represented a stunning advance, because it directly implicated chemicals (those in coal tar) in cancer causation. Equally important, Yamagiwa's work, together with that of Peyton Rous (to be described in Chapter 3), demonstrated that cancer could be induced at will in laboratory animals. Before these breakthroughs, researchers had been forced to wait for tumors to appear spontaneously in wild or domesticated animals. Now, cancers could be produced according to a predictable schedule, often involving many months of experimental treatment of animals.

By 1940, British chemists had purified several of the components of coal tar that were particularly **carcinogenic** (that is, cancer-causing), as demonstrated by the ability of these compounds to induce cancers on the skin of laboratory mice. Compounds such as 3-methylcholanthrene, benzo[*a*]pyrene, and 1,2,4,5-dibenz[*a*,*h*]anthracene were common products of combustion, and some of these hydrocarbons, notably benzo[*a*]pyrene, were subsequently found in the condensates of cigarette smoke as well

(A) (B)

Figure 2.24 The first induction of tumors by chemical carcinogens
(A) In 1915, Katsusaburo Yamagiwa reported the first successful induction of cancer by repeated treatment of rabbit ears with a chemical carcinogen, in this case coal tars. (B) The skin carcinomas (*arrows*) that he induced on the ears of these rabbits are preserved to this day in the medical museum of the University of Tokyo. This particular carcinoma was harvested and fixed following 660 days of painting with coal tar. (Courtesy of T. Taniguchi.)

Table 2.7 Known or suspected causes of human cancers

Environmental and lifestyle factors known or suspected to be etiologic for human cancers in the United States[a]	
Type	**% of total cases[b]**
Cancers due to occupational exposures	1–2
Lifestyle cancers	
Tobacco-related (sites: e.g., lung, bladder, kidney)	34
Diet (low in vegetables, high in nitrates, salt) (sites: e.g., stomach, esophagus)	5
Diet (high fat, low fiber, broiled/fried foods) (sites: e.g., bowel, pancreas, prostate, breast)	37
Tobacco plus alcohol (sites: mouth, throat)	2

Specific carcinogenic agents implicated in the causation of certain cancers[c]	
Cancer	**Exposure**
Scrotal carcinomas	chimney smoke condensates
Liver angiosarcoma	vinyl chloride
Acute leukemias	benzene
Nasal adenocarcinoma	hardwood dust
Osteosarcoma	radium
Skin carcinoma	arsenic
Mesothelioma	asbestos
Vaginal carcinoma	diethylstilbestrol
Oral carcinoma	snuff
ER+ breast cancer[d]	hormone replacement therapy (E + P)[e]

[a]Adapted from American Cancer Society. Cancer Facts & Figures 1990. Atlanta: American Cancer Society, Inc.
[b]A large number of cancers are thought to be provoked by a diet high in calories (see Sidebar 9.10) acting in combination with many of these lifestyle factors.
[c]Adapted from S. Wilson, L. Jones, C. Coussens and K. Hanna, eds., Cancer and the Environment: Gene–Environment Interaction. Washington, DC: National Academy Press, 2002.
[d]ER+, estrogen receptor–positive.
[e]E + P, therapy containing both estrogen and progesterone.

(**Figure 2.25**). These findings suggested that certain chemical species that entered into the human body could perturb tissues and cells and ultimately provoke the emergence of a tumor. The same could be said of X-rays, which were also able to produce cancers, ostensibly through a quite different mechanism of action.

While these discoveries were being reported, an independent line of research developed that portrayed cancer as an infectious disease. As described in detail in Chapter 3, researchers in the first decade of the twentieth century found that viruses could cause leukemias and sarcomas in infected chickens. By mid-century, a wide variety of viruses had been found able to induce cancer in rabbits, chickens, mice, and rats. As a consequence, those intent on uncovering the origins of human cancer were pulled in three different directions, since the evidence of cancer causation by chemical, viral, and radioactive agents had become compelling.

2.10 Both physical and chemical carcinogens act as mutagens

The confusion caused by the three competing theories of carcinogenesis was reduced significantly by discoveries made in the field of fruit fly genetics. In 1927, Hermann Muller discovered that he could induce mutations in the genome of *Drosophila*

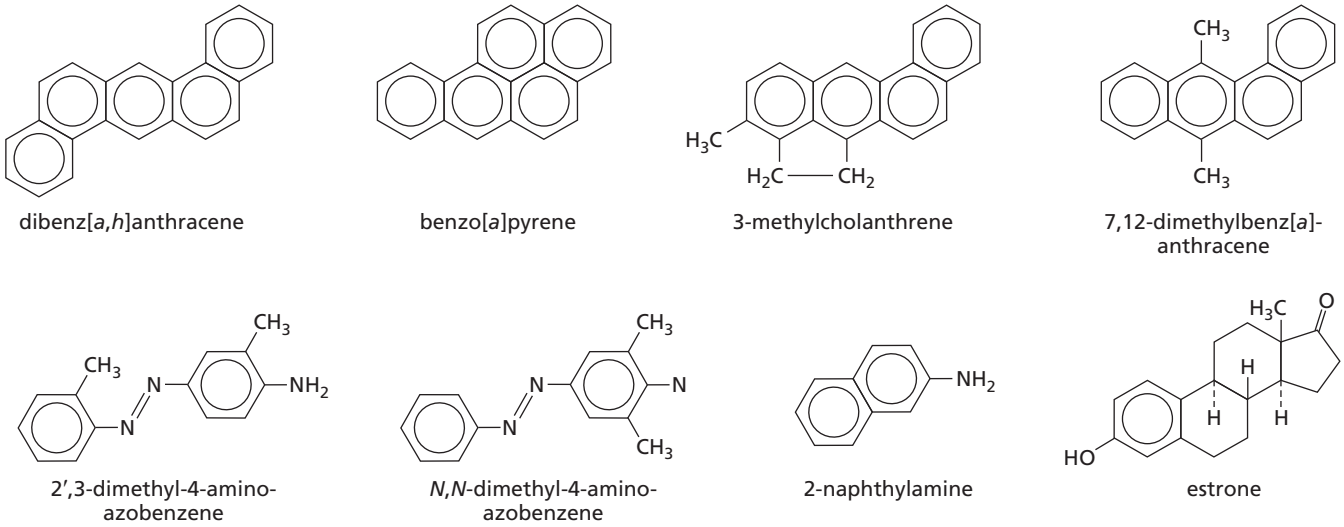


Figure 2.25 Structures of carcinogenic hydrocarbons These chemical species arise from the incomplete combustion of organic (for example, carbon-containing) compounds. Each of the chemical structures shown here, which were already determined before 1940, represents a chemical species that was found, following purification, to be potently carcinogenic. The four compounds shown in the top row are all polycyclic aromatic hydrocarbons (PAHs). (From E.C. Miller, *Cancer Res.* 38: 1479–1496, 1978.)

melanogaster by exposing these flies to X-rays. Most important, this discovery revealed that the genome of an animal was mutable, that is, that its information content could be changed through specific treatments, notably irradiation. At the same time, it suggested at least one mechanism by which X-rays could induce cancer: perhaps radiation was able to mutate the genes of normal cells, thereby creating mutant cells that grew in a malignant fashion.

By the late 1940s, a series of chemicals, many of them alkylating agents of the type that had been used in World War I mustard gas warfare, were also found to be **mutagenic** for fruit flies. Soon thereafter, some of these same compounds were shown to be carcinogenic in laboratory animals. These findings caused several geneticists to speculate that cancer was a disease of mutant genes, and that carcinogenic agents, such as X-rays and certain chemicals, succeeded in inducing cancer through their ability to mutate genes.

These speculations were hardly the first ones of this sort. As early as 1914, the German biologist Theodor Boveri, drawing on yet older observations of others, suggested that chromosomes, which by then had been implicated as carriers of genetic information, were aberrant within cancer cells, and that cancer cells might therefore be mutants. Boveri's notion, along with many other speculations on the origin of cancer, gained few adherents, however, until the discovery in 1960 of an abnormally configured chromosome in a large proportion of cases of chronic myelogenous leukemia (CML). This chromosome, soon called the Philadelphia chromosome after the place of its discovery, was clearly a distinctive characteristic of this type of cancer (**Figure 2.26**). Its reproducible association with this class of tumor cells suggested, but hardly proved, that it played a causal role in tumorigenesis.

In 1975 Bruce Ames, a bacterial geneticist working at the University of California in Berkeley, reported experimental results that lent great weight to the theory that carcinogens can function as mutagens. Decades of experiments with laboratory mice and rats had demonstrated that chemical carcinogens acted with vastly different potencies, differing by as much as 1 million-fold in their ability to induce cancers. Such experiments showed, for example, that one microgram of aflatoxin, a compound produced by molds growing on peanuts and wheat, was as potently carcinogenic as a 10,000 times greater weight of the synthetic compound benzidine. Ames posed the question whether these various compounds were also mutagenic, more specifically, whether compounds that were potent carcinogens also happened to be potent mutagens.

The difficulty was that there were no good ways of measuring the relative mutagenic potencies of various chemical species. So Ames devised his own method. It consisted of applying various carcinogenic chemicals to a population of *Salmonella* bacteria growing in Petri dishes and then scoring for the abilities of these carcinogens to mutate the bacteria. The readout here was the number of colonies of *Salmonella* that grew out following exposure to one or another chemical.

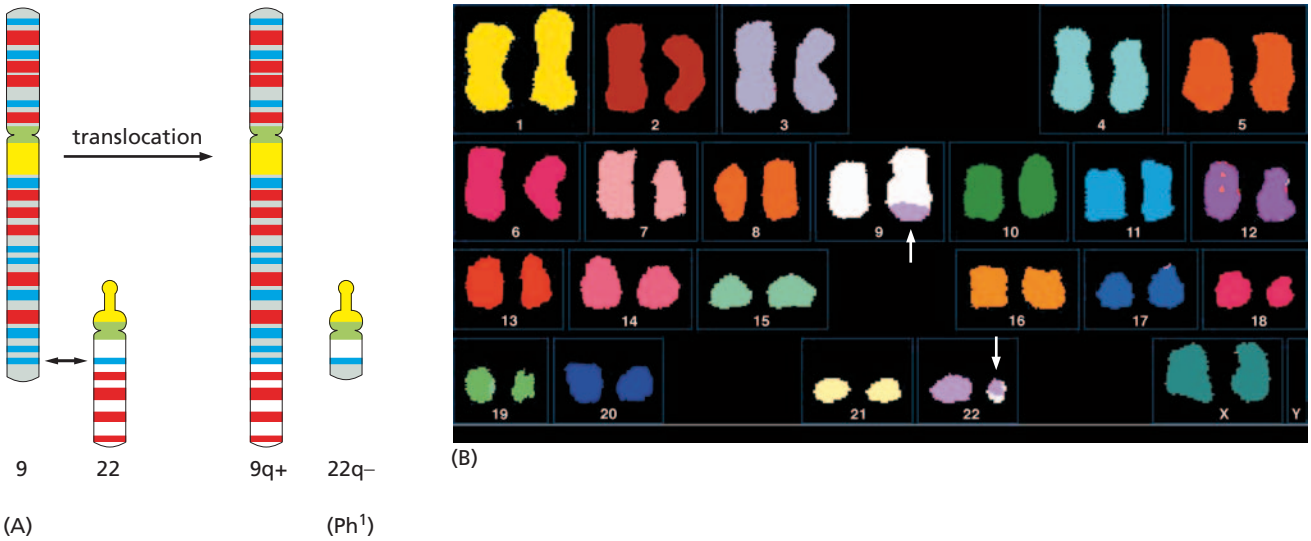


Figure 2.26 Structure of the Philadelphia chromosome
Analyses of the banding patterns of stained metaphase chromosomes of chronic myelogenous leukemia (CML) cells first revealed the characteristic tiny chromosome (called the "Philadelphia chromosome" or Ph^1) that is present in the leukemia cells of most CML patients. (A) This banding pattern, determined through light-microscopic surveys, is illustrated here schematically. While the chromosomal translocation generating the two altered chromosomes (9q+,22q–) is *reciprocal* (for example, involving a loss and a gain by both), the sizes of the exchanged chromosomal arms are unequal, leading to the greatly truncated Chromosome 22 (for example, 22q–). The small arrow indicates the point of crossing over, known as the translocation *breakpoint*. (B) The relatively minor change to the tumor cell karyotype that is created by the CML translocation is apparent in this SKY analysis, in which chromosome-specific probes are used, together with fluorescent dyes and computer-generated coloring, to visualize the entire chromosomal complement of CML cells. As is apparent, one of the two Chromosomes 9 has acquired a *light purple* segment (a color assigned to Chromosome 22) at the end of its long arm. Reciprocally, one of the two Chromosomes 22 has acquired a white region (characteristic of Chromosome 9) at the end of its long arm (*arrows*). (A, from B. Alberts et al., Molecular Biology of the Cell, 5th ed. New York: Garland Science, 2008. B, courtesy of Thomas Ried and Nicole McNeil.)

In detail, Ames used a mutant strain of *Salmonella* that was unable to grow in medium lacking the amino acid histidine. The mutant allele that caused this phenotype was susceptible to back-mutation to a wild-type allele. Once the wild-type allele was formed in response to exposure to a mutagen, a bacterium carrying this allele became capable of growing in Ames's selective medium, multiplying until it formed a colony that could be scored by eye (**Figure 2.27**).

In principle, Ames needed only to introduce a test compound into a Petri dish containing his special *Salmonella* strain and count the bacterial colonies that later appeared. There remained, however, one substantial obstacle to the success of this mutagenesis assay. Detailed studies had shown that after carcinogenic molecules entered the tissues of laboratory animals, they were metabolized into yet other chemical species. In many cases, the resulting products of metabolism, rather than the initially introduced chemicals, seemed to be the agents that were directly responsible for the observed cancer induction. These metabolized compounds were found to be highly reactive chemically and able to form covalent bonds with the various macromolecules known to be present in cells—DNA, RNA, and protein.

The original, unmodified compounds that were introduced into laboratory animals came to be called **procarcinogens** to indicate their ability to become converted into actively carcinogenic compounds, which were labeled **ultimate carcinogens**. This chemical conversion complicated the design of Ames's mutagenesis assay. If many compounds required metabolic activation before their carcinogenicity was apparent, it seemed plausible that their mutagenic powers would also be evident only after such conversion. Given the radically different metabolisms of bacteria and mammalian cells, it was highly unlikely that Ames's *Salmonella* would be able to accomplish the metabolic activation of procarcinogens that occurred in the tissues of laboratory animals.

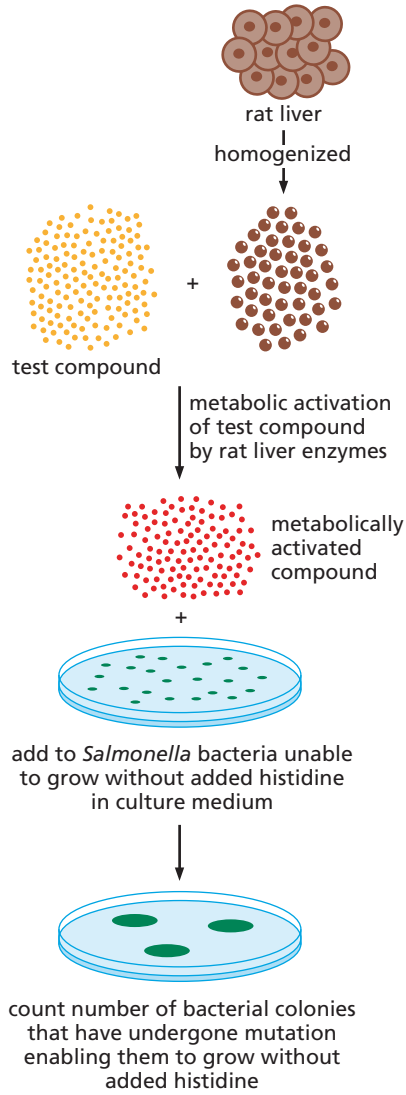


Figure 2.27 The Ames test for gauging mutagenicity The Ames test makes it possible to quantitatively assess the mutagenic potency of a test compound. To begin, the liver of a rat (or other species) is homogenized to release its enzymes. The liver homogenate (*brown dots*) is then mixed with the test compound (*orange*), which often results in the liver enzymes metabolically converting the test compound to a chemically activated, mutagenic state (*red*). This mixture (still containing the liver homogenate, *not shown*) is applied to a dish of mutant *Salmonella* bacteria (*small green dots*) that require the amino acid histidine in their culture medium in order to grow. Since histidine is left out of the medium, only those bacteria that are mutated to a histidine-independent genotype (and phenotype) will be able to grow, and each of these will yield a large colony (*green*) that can be counted with the naked eye, indicating how many mutant bacteria (and thus mutant alleles) were generated by the brief exposure to the activated compound.

Earlier work of others had shown that a great many chemicals introduced into the body undergo metabolic conversion, specifically in the liver. Moreover, many of these conversions could be achieved in the test tube simply by mixing such chemicals with homogenized liver. So Ames mixed rat liver homogenates with his test compounds and then introduced this mixture into the Petri dishes carrying *Salmonella*. (We now know that the metabolic activation of procarcinogens in the liver is often mediated by enzymes that are normally involved, paradoxically, in the **detoxification** of compounds introduced into the body; see Section 12.6.)

With the addition of this extra step, Ames's assay revealed that a number of known carcinogens were also actively mutagenic. Even more important were the correlations that Ames found. Chemicals that were potently mutagenic were also powerful carcinogens. Those that were weakly mutagenic induced cancer poorly. These correlations, as plotted by others, extended over five orders of magnitude of potency (**Figure 2.28**).

As we have read, the notion that carcinogens are mutagens predated Ames's work by a quarter of a century. Nonetheless, his analyses galvanized researchers interested in the origins of cancer, since the results addressed the carcinogen–mutagen relationship so directly. Their reasoning went like this: Ames had demonstrated the mutagenic powers of certain chemical compounds in bacteria. Since the genomes of bacterial and animal cells are both made of the same chemical substance—double-stranded DNA—it was likely that the compounds that induced mutations in the *Salmonella* genome were similarly capable of inducing mutations in the genomes of animal cells. Hence, the "Ames test," as it came to be known, should be able to predict the mutagenicity of these compounds in mammals. And in light of the correlation between mutagenic and carcinogenic potency, the Ames test could be employed to screen various substances for their carcinogenic powers, and thus for their threat to human health. By 1976, Ames and his group reported on the mutagenic potencies of 300 distinct organic compounds. Yet other tests for mutagenic potency were developed in the years that followed (**Sidebar 2.1**).

Ames's results led to the next deduction, really more of a speculation: if, as Ames argued, carcinogens are mutagens, then it followed that the carcinogenic powers of various agents derived directly from their ability to induce mutations in the cells of target tissues. As a further deduction, it seemed inescapable that the cancer cells created by chemical carcinogens carry mutated genes. These mutated genes, whatever their identity, must in some way be responsible for the aberrant growth phenotypes of such cancer cells.

This logic was transferable to X-ray carcinogenesis as well. Since X-rays were mutagens and carcinogens, it followed that they also induced cancer through their ability to mutate genes. This convergence of cancer research with genetics had a profound effect on researchers intent on puzzling out the origins of cancer. Though still unproven, it appeared likely that the disease of cancer could be understood in terms of the mutant genes carried by cancer cells.

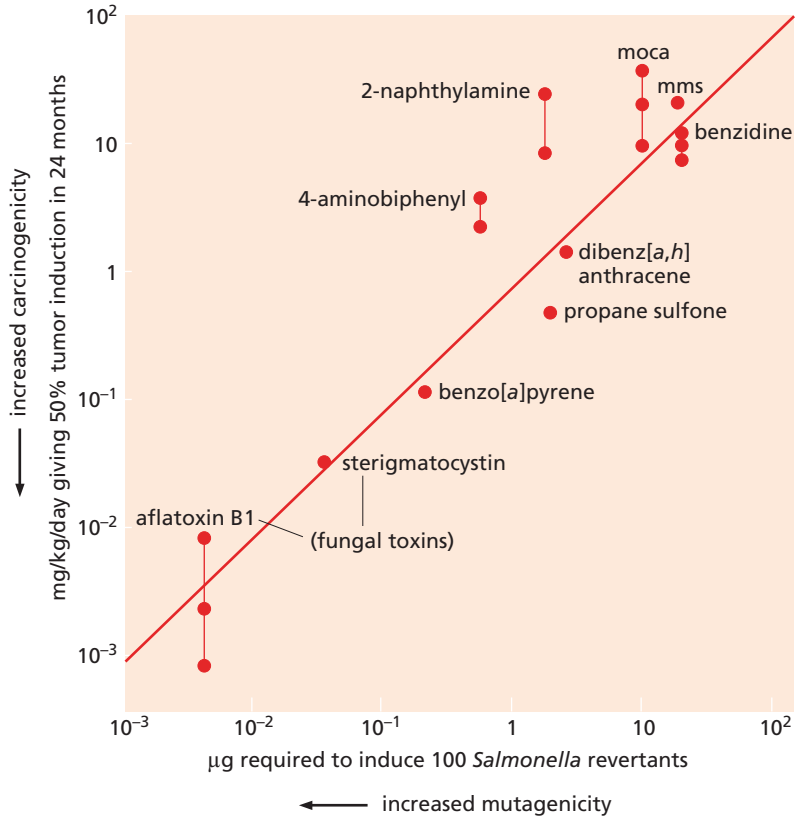


Figure 2.28 Mutagenic versus carcinogenic potency On this log–log plot, the relative carcinogenic potencies of a group of chemicals (*ordinate*) that have been used to treat laboratory animals (rats and mice) are plotted as a function of their mutagenic potencies (*abscissa*) as gauged by the Ames test (see Figure 2.27). Since both the ordinate and abscissa are plotted as the amount of compound required to elicit an observable effect (yielding tumors in 50% of treated animals or 100 colonies of mutant *Salmonella* bacteria, termed here "revertants"), the compounds that are the most potent mutagens and most potent carcinogens appear in the lower left of this graph. Note that both parameters vary by five orders of magnitude. moca—4,4′-methylenebis(2-chloroaniline), used in manufacture of polyurethane; mms—methyl methanesulfonate, an alkylating mutagen. (Adapted from M. Meselson et al., in H.H. Hiatt et al., eds., Origins of Human Cancer, Book C: Human Risk Assessment. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1977.)

2.11 Mutagens may be responsible for some human cancers

The connection between carcinogenesis and mutagenesis seemed to shed light on how human tumors arise. Perhaps many of these neoplasms were the direct consequence of the mutagenic actions of chemical and physical carcinogens. The mutagenic chemicals, specifically, procarcinogens, need not derive exclusively from the combustion of carbon compounds and the resulting formation of coal tars. It seemed plausible that chemical species present naturally in foodstuffs or generated during cooking could also induce cancer. Even if many foods did not contain ultimate carcinogens, chemical conversions carried out by liver cells or by the abundant bacteria in the colon might well succeed in creating actively mutagenic and thus carcinogenic chemical species.

Sidebar 2.1 Other tests for mutagenicity help assess possible carcinogenicity The Ames test is only one of a number of biological assay systems that can be used to assess the mutagenic potency of suspected carcinogenic chemicals. Many of these other assays depend upon exposing mammalian cells directly to the chemical compounds being tested and the subsequent use of a diverse array of biological readouts. For example, a test for sister chromatid exchange (SCE) measures crossing over between the two paired chromatids that are formed by DNA replication during the S phase and persist in paired form during the late (that is, G_2) phase of a cell's growth-and-division cycle. Many mutagenic agents have been shown to provoke this SCE. Mutagenic agents may also register as being capable of inducing the formation of fragmented cell nuclei, that is, **micronuclei**. Use of genetics has made it possible to select mammalian cells that have lost by mutation their thymidine kinase or HGPRT (hypoxanthine guanine phosphoribosyl transferase) enzymes. The ability to examine under the light microscope the chromosomal array (that is, the karyotype; see Figure 1.11) of cells in metaphase of mitosis makes it possible to screen for chromosomal aberrations inflicted by test compounds. Yet another assay gauges the degree of DNA labeling in those cells that are in the G_1 or G_2 phase of the cell cycle (described in Chapter 8); since cellular DNA synthesis normally occurs in the S phase, such non-S-phase labeling, which is sometimes referred to as "unscheduled DNA synthesis," has also been shown to be a good indicator of the genomic damage that has been inflicted on a cultured cell, since this type of DNA synthesis represents one key step in the process used by cells to repair damaged DNA.

None of these tests has proven to be ideal as a predictor of the carcinogenicity of a test substance. The Ames test, as an example, has been found by some to have a sensitivity (% of established carcinogens identified as mutagens) of about 54% and a specificity (% of noncarcinogens identified as nonmutagens) of 70%.

As this research on the causes of human cancer proceeded, it became apparent that virtually all compounds that are mutagenic in human cells are likely to be carcinogenic as well. However, the converse does not seem to hold: chemical compounds that are carcinogenic are not necessarily mutagenic. Thus, by the 1990s, extensive use of the Ames test showed that as many as 40% of the compounds that were known to be carcinogenic in rodents showed no obvious mutagenicity in the *Salmonella* mutation assay. So, the conclusions drawn from the initial applications of Ames's test required major revision: some carcinogens act through their ability to mutate DNA, while others promote the appearance of tumors through nongenetic mechanisms. We will encounter these nonmutagenic carcinogens, often called tumor **promoters**, again in Chapter 11.

Ames and others eventually used his test to catalog the mutagenic powers of a diverse group of chemicals and natural foodstuffs, including many of the plants that are common and abundant in the Western diet. As Ames argued, the presence of such compounds in foodstuffs derived from plants was hardly surprising, since plants have evolved thousands, possibly millions of distinct toxic chemical compounds in order to defend themselves from predation by insects and larger animals. Some of these naturally toxic compounds, initially developed as anti-predator defenses, might also, as an unintended side effect, be mutagenic (**Table 2.8**).

A diverse set of discoveries led to the model, which remains unproven in many of its aspects to this day, that a significant proportion of human cancer is attributable directly to the consumption of foodstuffs that are mutagenic and hence carcinogenic.

Table 2.8 A sampling of Bruce Ames's roster of carcinogens identified in the normal diet[a]

Foodstuff	Compound	Concentration in foodstuff
Black pepper	piperine	100 mg/g
Common mushroom	agaritine	3 mg/g
Celery	furocoumarins, psoralens[b]	1 μg/g, 0.8 μg/g
Rhubarb	anthraquinones	varies
Cocoa powder	theobromine	20 mg/g
Mustard, horseradish	allyl isothiocyanate	varies
Alfalfa sprouts	canavanine[c]	15 mg/g
Burnt materials[d]	large number	varies
Coffee	caffeic acid	11.6 mg/g

[a]Ames has cited 37 naturally occurring compounds that have registered as carcinogens in laboratory animals; one or more have been found in each of the following foodstuffs: absinthe, allspice, anise, apple, apricot, banana, basil, beet, broccoli, Brussels sprouts, cabbage, cantaloupe, caraway, cardamom, carrot, cauliflower, celery, cherries, chili pepper, chocolate, cinnamon, cloves, coffee, collard greens, comfrey herb tea, coriander, corn, currants, dill, eggplant, endive, fennel, garlic, grapefruit, grapes, guava, honey, honeydew melon, horseradish, kale, lemon, lentils, lettuce, licorice, lime, mace, mango, marjoram, mint, mushrooms, mustard, nutmeg, onion, orange, paprika, parsley, parsnip, peach, pear, peas, pepper (black), pineapple, plum, potato, radish, raspberries, rhubarb, rosemary, rutabaga, sage, savory, sesame seeds, soybean, star anise, tarragon, tea, thyme, tomato, turmeric, and turnip.
[b]The levels of these can increase 100-fold in diseased plants.
[c]Canavanine is indirectly genotoxic because of oxygen radicals that are released, perhaps during the inflammatory reactions associated with elimination of canavanine-containing proteins.
[d]On average, several grams of burnt material are consumed daily in the form of bread crusts, burnt toast, and burnt surfaces of meats cooked at high temperature.

Adapted from B.N. Ames, *Science* 221:1256–1264, 1983; B.N. Ames and L.S. Gold, *Proc. Natl. Acad. Sci. USA* 87:7777–7781, 1990; and L.S. Gold, B.N. Ames and T.H. Slone, Misconceptions about the causes of cancer, in D. Paustenbach, ed., Human and Environmental Risk Assessment: Theory and Practice, New York: John Wiley & Sons, 2002, pp. 1415–1460.

Sidebar 2.2 The search for elusive human carcinogens Ideally, the identification of important human carcinogens should have been aided by the use of *in vitro* assays, such as the Ames test (see Section 2.10), and *in vivo* tests—exposure of laboratory animals to agents suspected of causing cancer (see Section 2.9). In truth, however, these various types of laboratory tests have failed to register important human carcinogens. Instead, we have learned about their carcinogenicity because of various epidemiologic studies. For example, the most important known human carcinogen—tobacco smoke—would likely have escaped detection because it is a relatively weak carcinogen in laboratory rodents; and another known human carcinogen—asbestos—would have eluded detection by both *in vitro* and *in vivo* laboratory tests. Conversely, some frequently used drugs, such as phenobarbital and isoniazid, register positively in the Ames test, and saccharin registers as a carcinogen in male laboratory rats, but epidemiologic evidence indicates conclusively that none of these is actually associated with increased cancer risk in humans who have been exposed to these compounds over long periods of time. Hence, the development of truly useful, predictive tests of human carcinogens still lies in the future.

Included among these foodstuffs is, for example, red meat, which upon cooking at high temperatures generates compounds such as heterocyclic amines, which are potently mutagenic (see Section 12.6).

The difficulties in proving this model derive from several sources. Each of the plant and animal foodstuffs in our diet is composed of thousands of diverse chemical species present in vastly differing concentrations. Almost all of these compounds undergo metabolic conversions once ingested, first in the gastrointestinal tract and often thereafter in the liver. Accordingly, the number of distinct chemical species that are introduced into our bodies is incalculable. Each of these introduced compounds may then be concentrated in some cells or quickly metabolized and excreted, creating a further dimension of complexity.

Moreover, the actual mutagenicity of various compounds in different cell types may vary enormously because of metabolic differences in these cells. For example, some cells, such as **hepatocytes** in the liver, express high levels of biochemical species designed to scavenge and inactivate mutagenic compounds, while others, such as fibroblasts, express far lower levels. In sum, the ability to relate the mutagenicity of foodstuffs to actual rates of mutagenesis and carcinogenesis in the human body is far beyond our reach at present—a problem of intractable complexity (**Sidebar 2.2**).

2.12 Synopsis and prospects

The descriptions of cancer and cancer cells developed during the second half of the nineteenth century and the first half of the twentieth indicated that tumors were nothing more than normal cell populations that had run amok. Moreover, many tumors seemed to be composed largely of the descendants of a single cell that had crossed over the border from normalcy to malignancy and proceeded to spawn the billions of descendant cells constituting these neoplastic masses. This model drew attention to the nature of the cells that founded tumors and to the mechanisms that led to their transformation into cancer cells. If one could understand why a cell multiplied uncontrollably, somehow other pieces of the cancer puzzle were likely to fall into place.

Still, existing observations and experimental techniques offered little prospect of revealing precisely why a cell altered its behavior, transforming itself from a normal into a malignant cell. The carcinogen = mutagen theory seemed to offer some clarification, since it implicated mutant cellular genes as the agents responsible for disease development and, therefore, for the aberrant behavior of cancer cells. Perhaps there were mutant genes operating inside cancer cells that programmed the runaway proliferation of these cells, but the prospects for discovering such genes and understanding their actions seemed remote. No one knew how many genes were present in the human genome and how to analyze them. If mutant genes really did play a major part in cancer causation, they were likely to be small in number and dwarfed by the apparently vast number of genes present in the genome as a whole. They seemed to be the proverbial needles in the haystack, in this case a vast haystack of unknown size.

This theorizing about cancer's origins was further complicated by two other important considerations. First, many apparent carcinogens failed the Ames test, suggesting that they were nonmutagenic. Second, certain viral infections seemed to be closely connected to the incidence of a small but significant subset of human cancer types. Somehow, their carcinogenic powers had to be reconciled with the actions of mutagenic carcinogens and mutant cellular genes.

By the mid-1970s, recombinant DNA technology, including gene cloning, began to influence a wide variety of biomedical research areas. While appreciating the powers of this new technology to isolate and characterize genes, cancer researchers were unable, at least initially, to exploit it to track down the elusive mutant genes that were responsible for cancer. One thing was clear, however. Sooner or later, the process of cancer **pathogenesis** (disease development) needed to be explained and understood in molecular terms. Somehow, the paradigm of DNA, RNA, and proteins, so powerful in elucidating a vast range of biological processes, would need to be brought to bear on the cancer problem.

In the end, the breakthrough came from study of the tumor viruses, which by most accounts were minor players in human cancer development. Tumor viruses were genetically simple, and yet they possessed potent carcinogenic powers. To understand these viruses and their import, we need to move back, once again, to the beginning of the twentieth century and confront another of the ancient roots of modern cancer research. This is the subject of Chapter 3.

A major challenge for the future is to understand how various biological and environmental factors, the latter including lifestyle, contribute to the incidence of cancers, many of them quite common ones. For example, as indicated in part in Table 2.5, the incidence of cancers, such as those of the colon, breast, and prostate, shows enormous geographic variation—dramatic differences that cannot be ascribed to differing genetic susceptibilities. In fact, epidemiologists have uncovered many correlations between the frequencies of these and other cancer types and various lifestyle factors (for example, those listed in **Table 2.9**). However, with rare exception, our understanding of the biological and biochemical mechanisms by which these factors increase (or reduce) disease incidence is either incomplete or nonexistent. Indeed, these correlations represent one of the major unsolved mysteries confronting contemporary cancer researchers.

Until we understand how various biological and lifestyle factors succeed in triggering or preventing tumor development, our ability to prevent new cancers (which is usually far more effective than trying to cure them after they have been diagnosed) will be limited. Many of the chapters that follow provide critical information that may ultimately help to unravel these mysteries of cancer etiology.

Table 2.9 Examples of etiologic mysteries: epidemiologic correlations between environmental/lifestyle factors and cancer incidence that lack a clear explanation of causal mechanism[a]

Lifestyle, dietary factor, or medical condition	Altered cancer risk
High birth weight	premenopausal breast cancer ↑ infant acute leukemia ↑
Processed red meat[b]	ER+ breast cancer ↑ squamous cell and adenocarcinoma of lung ↑
Childhood soy consumption	breast cancer ↓
Well-done red meat	prostate cancer ↑
Western diet—high in fat, high in red meat	colorectal, esophageal, liver, and lung cancer ↑
Exercise	hormone-responsive breast cancer ↓
Diet with cruciferous vegetables	prostate cancer ↓
High body-mass index (BMI)	multiple cancer types ↑
Higher ratio of number of daughters to number of sons born to a woman	ovarian carcinoma ↑
Parkinson's disease	melanoma ↑
Low circulating vitamin D	breast cancer incidence, CRC mortality ↑
Periodontal disease	esophageal carcinoma ↑
Coffee consumption	hepatocellular carcinoma ↓

[a]Relative risk (RR) is not given, because not all studies used the same criteria to gauge RR.
↑ = increased risk; ↓ = decreased risk.
[b]Processed red meat generally refers to meat that has been preserved by smoking, curing, salting or adding chemical preservatives.
Abbreviations: ER+ = estrogen receptor–positive; CRC = colorectal cancer.

Key concepts

- The nineteenth-century discovery that all cells of an organism descend from the fertilized egg led to the realization that tumors are not foreign bodies but growths derived from normal tissues. The comparatively disorganized tissue architecture of tumors pointed toward cancer as being a disease of malfunctioning cells.
- Tumors can be either benign (localized, noninvasive) or malignant (invasive, metastatic). The metastases spawned by malignant tumors are responsible for almost all deaths from cancer.
- With some exceptions, most tumors are classified into four major groups according to their origin (epithelial, mesenchymal, hematopoietic, and neuroectodermal).
- Virtually all cell types can give rise to cancer, but the most common human cancers are of epithelial origin—the carcinomas. Most carcinomas fall into two categories: squamous cell carcinomas arise from epithelia that form protective cell layers, while adenocarcinomas arise from secretory epithelia.
- Nonepithelial malignant tumors include (1) sarcomas, which originate from mesenchymal cells; (2) hematopoietic cancers, which arise from the precursors of blood cells; and (3) neuroectodermal tumors, which originate from components of the nervous system.
- If a tumor's cells have dedifferentiated (lost all tissue-specific traits), its origin cannot be readily identified; such tumors are said to be anaplastic.
- Cancers seem to develop progressively, with tumors demonstrating different gradations of abnormality along the way from benign to metastatic.
- Benign tumors may be hyperplastic or metaplastic. Hyperplastic tissues appear normal except for an excessive number of cells, whereas metaplastic tissues show displacement of normal cells by normal cell types not usually encountered at that site. Metaplasia is most frequent in epithelial transition zones.
- Dysplastic tumors contain cells that are cytologically abnormal. Dysplasia is a transitional state between completely benign and premalignant. Adenomatous growths (adenomas, polyps, papillomas, and warts) are dysplastic epithelial tumors that are considered to be benign because they respect the boundary created by the basement membrane.
- Tumors that breach the basement membrane and invade underlying tissue are malignant. An even further degree of abnormality is metastasis, the seeding of tumor colonies to other sites in the body. Metastasis requires not only invasiveness but also such newly acquired traits as motility and adaptation to foreign tissue environments.
- Biochemical and genetic markers seem to indicate that human tumors are monoclonal (descended from one ancestral cell) rather than polyclonal (descended from multiple ancestral cells, each of which independently spawned a population of cancer cells).
- Most normal cells start metabolizing glucose through glycolysis, and then transfer pyruvate (the product of glycolysis) into the mitochondria, where it is further processed to yield 36 ATPs and CO_2. Most cancer cells rely largely on glycolysis alone, which yields lactate and only 2 ATPs.
- The incidence of many (but not all) cancers varies dramatically by country, an indication that they cannot be due simply to a normal biologic process gone awry by chance. While differences in either heredity or environment could explain these variations, epidemiologic studies show that environment (including lifestyle factors) is the dominant determinant of the country-by-country variations in cancer incidence.

- Laboratory research supported the epidemiologic studies by directly implicating chemical and physical agents (tobacco, coal dust, X-rays) as causes of cancers. However, the possibility of cancer as an infectious disease arose when viruses were found to cause leukemias and sarcomas in chickens.
- A possible mechanism that supported carcinogenesis by physical and chemical agents surfaced when mutations were induced in fruit flies by exposing them to either X-rays or chemicals, indicating that they were mutagenic. Since these agents were also known to be carcinogenic in laboratory animals, this led to the speculation that cancer was a disease of mutant genes and that carcinogenic agents induced cancer through their ability to mutate genes.
- In 1975 the Ames test provided support for this idea by showing that many carcinogens can act as mutagens. Additional research showed that although almost all compounds that are mutagenic are likely to be carcinogens, the converse does not hold true. So, some carcinogens act through their ability to mutate DNA, while others promote tumorigenesis through nongenetic mechanisms. Such nonmutagenic carcinogens are called tumor promoters.
- The Ames test combined with other discoveries led to the model, still unproven, that a significant portion of human cancers are attributable to the consumption of foodstuffs that are directly or indirectly mutagenic and hence carcinogenic.

Thought questions

1. What types of observation allow a pathologist to identify the tissue of origin of a tumor? And why are certain tumors extremely difficult to assign to a specific tissue of origin?
2. Under certain circumstances, all tumors of a class can be traced to a specific embryonic cell layer, while in other classes of tumors, no such association can be made. What tumors would fit into each of these two groupings?
3. What evidence persuades us that a cancer arises from the native tissues of an individual rather than invading the body from outside and thus being of foreign origin?
4. How compelling are the arguments for the monoclonality of tumor cell populations, and what logic and observations undermine the conclusion of monoclonality?
5. How can we estimate what percentage of cancers in a population are avoidable (through virtuous lifestyles) and what percentage occur independently of lifestyle?
6. What limitations does the Ames test have in predicting the carcinogenicity of various agents?
7. In the absence of being able to directly detect mutant genes within cancer cells, what types of observation allow one to infer that cancer is a disease of mutant cells?

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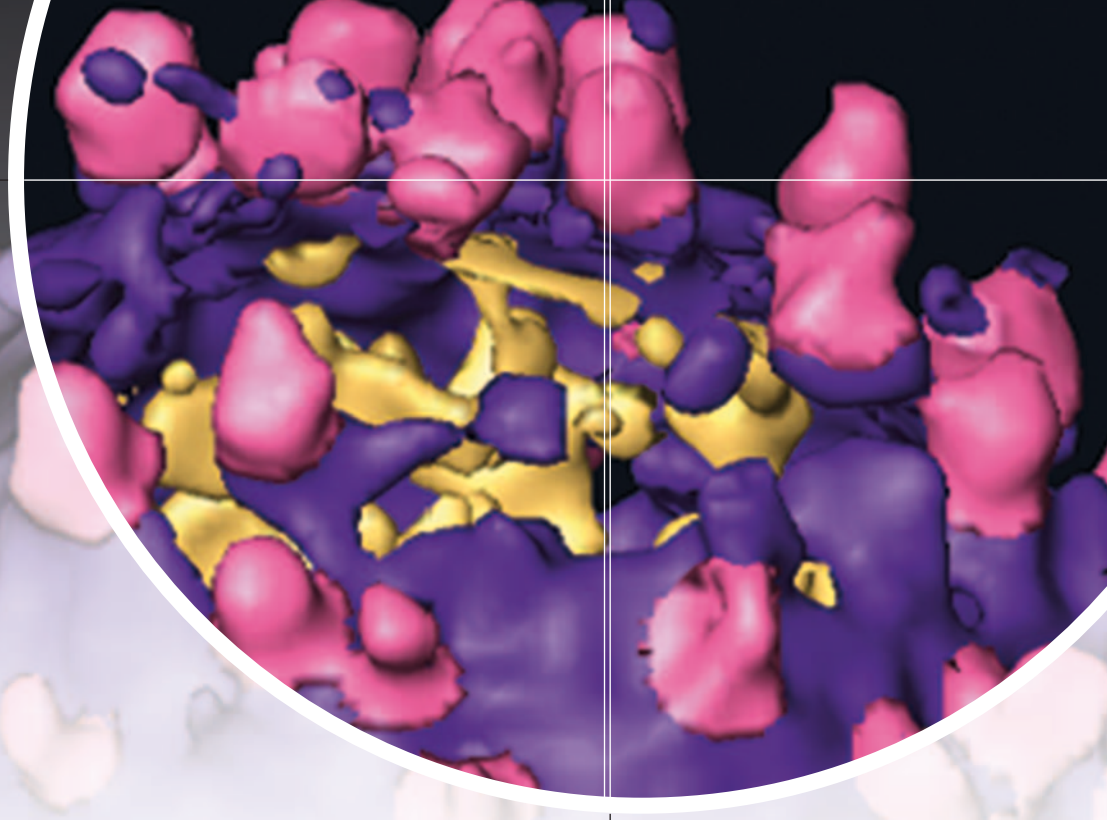

Chapter 3

Tumor Viruses

> A tumor of the chicken ... has been propagated in this laboratory since October, 1909. The behavior of this new growth has been throughout that of a true neoplasm, for which reason the fact of its transmission by means of a cell-free filtrate assumes exceptional importance.
>
> Francis Peyton Rous, cancer biologist, 1911

Viruses are capable of causing a wide variety of human diseases, ranging from rabies to smallpox to the common cold. The great majority of these infectious agents do harm through their ability to multiply inside infected host cells, to kill these cells, and to release progeny virus particles that proceed to infect other hosts nearby. The **cytopathic** (cell-killing) effects of viruses, together with their ability to spread rapidly throughout a tissue, enable these agents to leave a wide swath of destruction in their wake.

But the peculiarities of certain viral replication cycles may on occasion yield quite another outcome. Rather than killing infected cells, some viruses may, quite paradoxically, force their hosts to thrive, indeed, to proliferate uncontrollably. In so doing, such viruses—often called tumor viruses—can create cancer.

At one time, beginning in the early 1970s, tumor viruses were studied intensively because they were suspected to be the cause of many common human cancers. This notion was eventually rejected based on the evidence subsequently gathered during that decade, which indicated that virus-induced cancers represent only a minority of the cancer types afflicting humans. Nonetheless, this line of research proved to be invaluable for cancer biologists: study of various tumor viruses provided the key for opening many of the long-hidden secrets of human cancers, including the great majority of cancers that have no connection with tumor virus infections.

As we will see, tumor virus research had a highly variable history over the course of the last century. These infectious agents were discovered in the first decade of the twentieth century and then retreated from the center stage of science. Half a century

Movie in this chapter

3.1 Contact Inhibition

later, interest in these agents revived, culminating in the frenetic pace of tumor virus research during the 1970s.

The cancer-causing powers of tumor viruses drove many researchers to ask precisely how they succeed in creating disease. Most of these viruses possess relatively simple genomes containing only a few genes, yet some were found able to overwhelm an infected cell and its vastly more complex genome and to redirect cell growth. Such behavior indicated that tumor viruses have developed extremely potent genes to perturb the complex regulatory circuitry of the host cells that they infect.

By studying tumor viruses and their mechanisms of action, researchers changed the entire mindset of cancer research. Cancer became a disease of genes and thus a condition that was susceptible to analysis by the tools of molecular biology and genetics. When this story began, no one anticipated how obscure tumor viruses would one day revolutionize the study of human cancer pathogenesis.

3.1 Peyton Rous discovers a chicken sarcoma virus

In the last two decades of the nineteenth century, the research of Louis Pasteur and Robert Koch uncovered the infectious agents that were responsible for dysentery, cholera, rabies, and a number of other diseases. By the end of the century, these agents had been placed into two distinct categories, depending on their behavior upon filtration. Solutions of infectious agents that were trapped in the pores of filters were considered to contain bacteria. The other agents, which were small enough to pass through the filters, were classified as viruses. On the basis of this criterion, the agents for rabies, foot-and-mouth disease, and smallpox were categorized as viruses.

Cancer, too, was considered a candidate infectious disease. As early as 1876, a researcher in Russia reported the transmission of a tumor from one dog to another: chunks of tumor tissue from the first dog were implanted into the second, whereupon a tumor appeared several weeks later. This success was followed by many others using rat and mouse tumors.

The significance of these early experiments remained controversial. Some researchers interpreted these outcomes as proof that cancer was a transmissible disease. Yet others dismissed these transplantation experiments, since in their eyes, such work showed only that tumors, like normal tissues, could be excised from one animal and forced to grow as a graft in the body of a second animal.

In 1908, two researchers in Copenhagen reported extracting a filterable agent from chicken leukemia cells and transmitting this agent to other birds, which then contracted the disease. The two Danes did not follow up on their initial discovery, and it remained for Peyton Rous, working at the Rockefeller Institute in New York, to found the discipline of tumor virology (**Figure 3.1**).

In 1909, Rous began his study of a sarcoma that had appeared in the breast muscle of a hen. In initial experiments, Rous succeeded in transmitting the tumor by implanting small fragments of it into other birds of the same breed. Later, as a variation of this experiment, he ground up a sarcoma fragment in sand and filtered the resulting homogenate (**Figure 3.2**). When he injected the resulting filtrate into young birds, they too developed tumors, sometimes within several weeks. He subsequently found that these induced tumors could also be homogenized to yield, once again, an infectious agent that could be transmitted to yet other birds, which also developed sarcomas at the sites of injection.

These serial passages of the sarcoma-inducing agent from one animal to another yielded a number of conclusions that are obvious to us now but at the time were nothing less than revolutionary. The carcinogenic agent, whatever its nature, was clearly very small, since it could pass through a filter. Hence, it was a virus (**Sidebar 3.1**). This virus could cause the appearance of a sarcoma in an injected chicken, doing so on a predictable timetable. Such an infectious agent offered researchers the unique opportunity to induce cancers at will rather than relying on the spontaneous and unpredictable appearance of tumors in animals or humans. In addition to its ability to induce

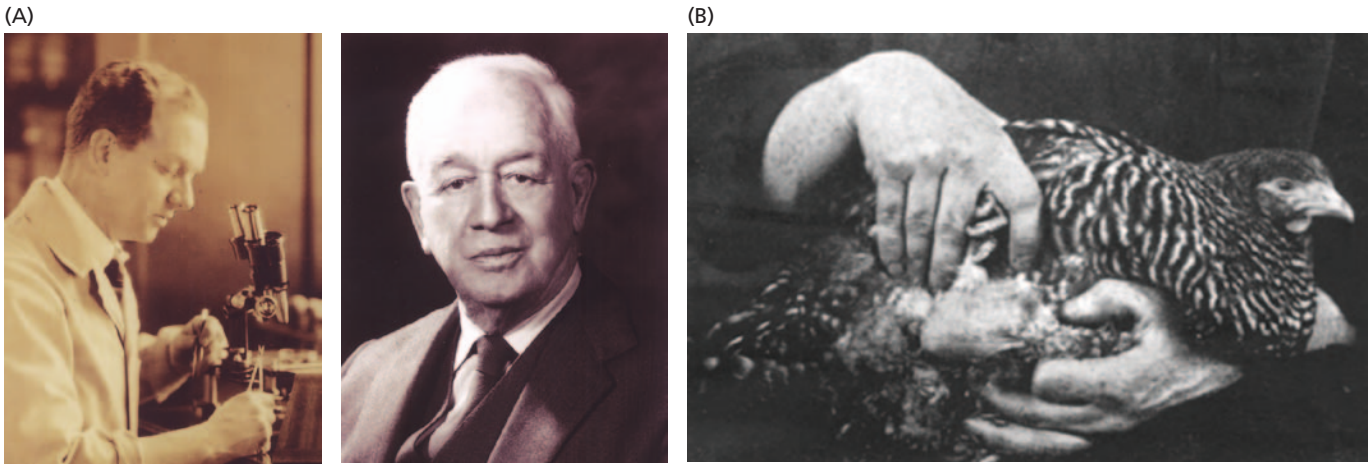

Figure 3.1 Peyton Rous and the hen that launched modern cancer research (A) Francis Peyton Rous began his work in 1910 that led to the discovery of Rous sarcoma virus (RSV) *(left)*. More than 50 years later (1966), he received the Nobel Prize in Medicine and Physiology for this seminal work—a tribute to his persistence and longevity *(right)*. (B) His good fortune began when a Long Island, NY chicken farmer brought Rous, then working at the Rockefeller Institute in New York, a prized barred Plymouth Rock hen. The farmer wanted Rous to treat the large tumor growing in its chest muscle; Rous saw experimental opportunity and dispatched the hen, extracting the tumor. The arthritic hands are likely those of the chicken farmer. (A, courtesy of the Rockefeller University Archives. B, from P. Rous, *J. Exp. Med.* 12:696–705, 1910.)

cancer, this agent, which came to be called Rous sarcoma virus (RSV), was capable of multiplying within the tissues of the chicken; far more virus could be recovered from an infected tumor tissue than was originally injected.

In 1911, when Rous finally published his work, yet another report appeared on a transmissible virus of rabbit tumors, called myxomas. Soon thereafter, Rous and his collaborators found two other chicken viruses, and yet another chicken sarcoma virus was reported by others in Japan. Then, there was only silence for two decades until other novel tumor viruses were discovered. The molecular nature of viruses and the means by which they multiplied would remain mysteries for more than half a century after Rous's initial discovery.

Still, his finding of a sarcoma virus reinforced the convictions of those who believed that virtually all human diseases were provoked by infectious agents. In their eyes, cancer could be added to the lengthening list of diseases, such as cholera, tuberculosis, rabies, and sepsis, whose causes could be associated with a specific microbial agent. By 1913, the Dane Johannes Grib Fibiger reported that stomach cancers in rats could be traced to spiroptera worms that they harbored. His work, for which he received the

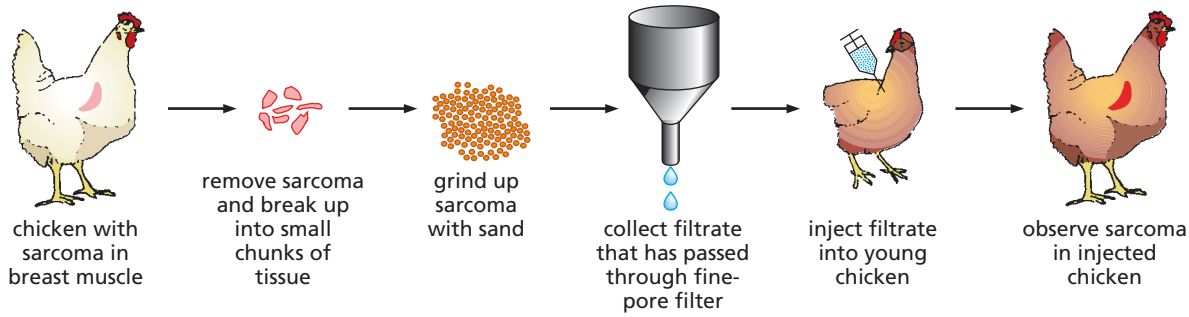

Figure 3.2 Rous's protocol for inducing sarcomas in chickens Rous removed a sarcoma from the breast muscle of a chicken, ground it with sand, and passed the resulting homogenate through a fine-pore filter. He then injected the filtrate (the liquid that passed through the filter) into the wing web of a young chicken and observed the development of a sarcoma many weeks later. He then ground up this new sarcoma and repeated the cycle of homogenization, filtration, and injection, once again observing a tumor in another young chicken. These cycles could be repeated indefinitely; after repeated serial passaging, the virus produced sarcomas far more rapidly than the original viral isolate.

Sidebar 3.1 Viruses have simple life cycles The term "virus" refers to a diverse array of particles that infect and multiply within a wide variety of cells, ranging from bacteria to the cells of plants and metazoa. Relative to the cells that they infect, individual virus particles, often termed **virions**, are tiny. Virions are generally simple in structure, with a nucleic acid (DNA or RNA) genome wrapped in a protein coat (a **capsid**) and, in some cases, a lipid membrane surrounding the capsid. In isolation, viruses are metabolically inert. They can multiply only by infecting and parasitizing a suitable host cell. The viral genome, once introduced into the cell, provides instructions for the synthesis of progeny virus particles. The host cell, for its part, provides the low–molecular-weight precursors needed for the synthesis of viral proteins and nucleic acids, the protein-synthetic machinery, and, in many cases, the polymerases required for replicating and transcribing the viral genome.

The endpoint of the resulting infectious cycle is the production of hundreds, even thousands of progeny virus particles that can then leave the infected cell and proceed to infect other susceptible cells. The interaction of the virus with the host cell can be either a **virulent** one, in which the host cell is destroyed during the infectious cycle, or a **temperate** one, in which the host cell survives for extended periods, all the while harboring the viral genome and releasing progeny virus particles.

Many viruses carrying double-stranded DNA (dsDNA) replicate in a fashion that closely parallels the macromolecular metabolism of the host cell (Figure 3.3). This allows them to use host-cell DNA polymerases to replicate their DNA, host-cell RNA polymerases to transcribe the viral mRNAs from double-stranded viral DNA templates, and host ribosomes to translate the viral mRNAs. Once synthesized, viral proteins are used to coat (**encapsidate**) the newly synthesized viral genomes, resulting in the assembly of complete progeny virions, which then are released from the infected cell.

Since cells do not express enzymes that can replicate RNA molecules, the genomes of many RNA-containing virus particles encode their own RNA-dependent RNA polymerases to replicate their genomes. Poliovirus, as an example, makes such an enzyme, as does rabies virus. RNA tumor viruses like Rous sarcoma virus, as we will learn later in this chapter, follow a much more circuitous route for replicating their viral RNA.

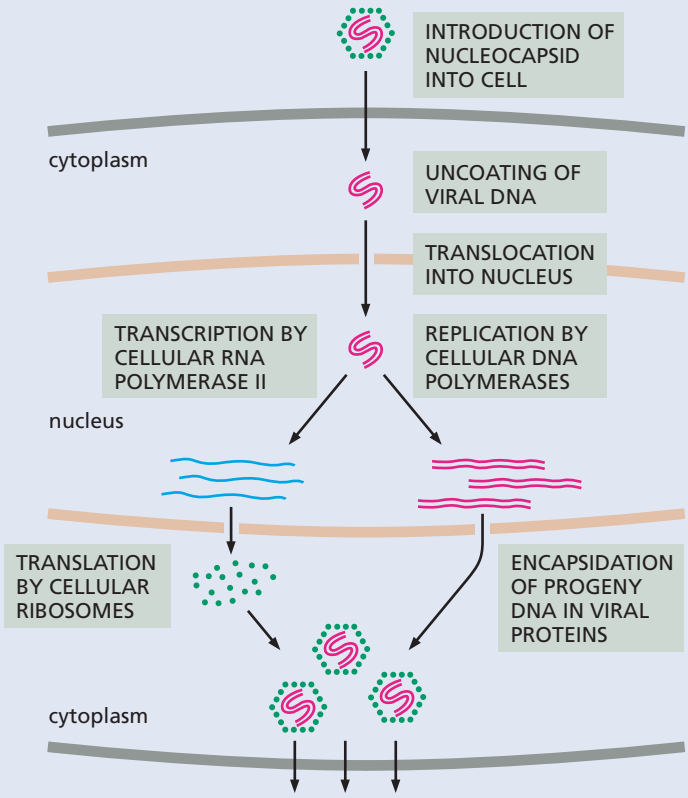

Figure 3.3 Life cycle of viruses with dsDNA genomes The life cycle of viruses with double-stranded DNA (dsDNA) genomes closely parallels that of the host cell. Almost all of the steps leading to the synthesis of viral DNA, RNA, and proteins can be achieved by using the synthetic machinery provided by the infected host cell. Not shown here is the release of progeny virus particles from the infected cell. (Adapted from B. Alberts et al., Molecular Biology of the Cell, 5th ed. New York: Garland Science, 2008.)

1926 Nobel Prize in Physiology or Medicine, represented direct and strong validation of the idea, first indicated by Rous's work, that cancer was yet another example of an infectious disease.

A year after Fibiger's 1926 Nobel award, he passed away and his scientific opus began to disintegrate. The stomach tumors that he had described were not tumors at all. Instead, they were found to be metaplastic stomach epithelia that were the result of the profound vitamin deficiencies suffered by these rats; they lived in sugar refineries and ate sugar cane almost exclusively. Fibiger's Nobel Prize became an embarrassment to the still-small community of cancer researchers. They threw the proverbial baby out with the bathwater, discrediting both his work and the notion that cancer could ever be caused by infectious agents.

Interest in the origins of cancer shifted almost totally to chemically induced cancers. Chemicals had been discovered in the early twentieth century that were clearly carcinogenic (see Section 2.9). Study of Rous sarcoma virus and the other tumor viruses languished and entered into a deep sleep for several decades.

3.2 Rous sarcoma virus is discovered to transform infected cells in culture

The rebirth of Rous sarcoma virus research began largely at the California Institute of Technology in Pasadena, in the laboratory of Renato Dulbecco. Dulbecco's post-doctoral fellow Harry Rubin found that when stocks of RSV were introduced into Petri dishes carrying cultures of chicken embryo fibroblasts, the RSV-infected cells survived, apparently indefinitely. It seemed that RSV parasitized these cells, forcing them to produce a steady stream of progeny virus particles for many days, weeks, even months (**Figure 3.4**). Most other viruses, in contrast, were known to enter into host cells, multiply, and quickly kill their hosts; the multitude of progeny virus particles

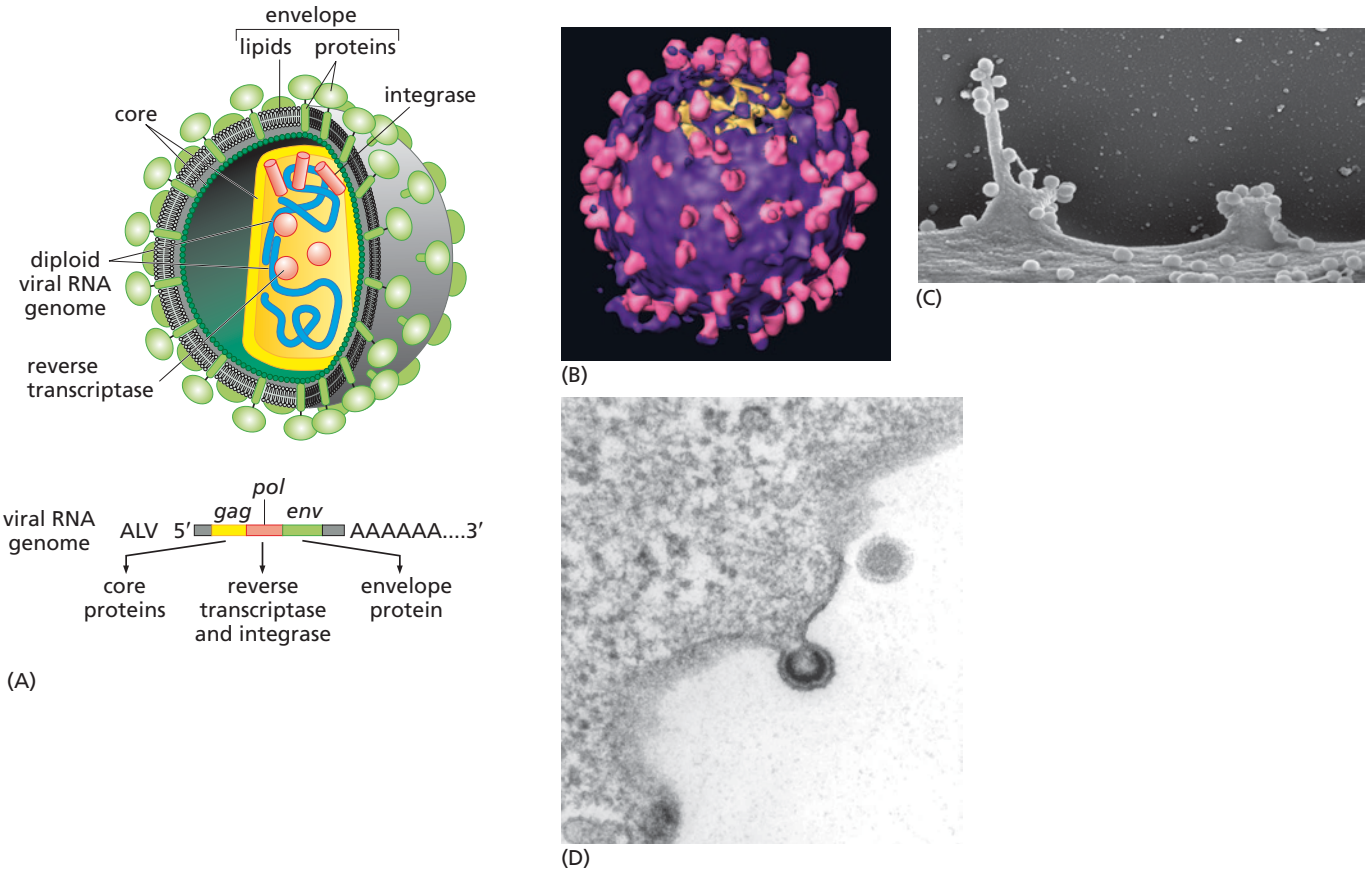

Figure 3.4 The virion of RSV and related viruses (A) An artist's reconstruction of the structure of a *retrovirus* virion, such as that of Rous sarcoma virus, which has four major types of viral proteins. The glycoprotein spikes (encoded by the viral *env* gene) that protrude from the lipid bilayer enable the virion to *adsorb* to the surface of a cell and to introduce its contents into the cytoplasm. Beneath this envelope lies a protein shell formed by the several core proteins encoded by the viral *gag* gene. Within this protein shell are two identical copies of the viral genomic RNA and a number of reverse transcriptase and integrase molecules specified by the viral *pol* gene. (B) Cryoelectron microscopy and complex image-processing algorithms have produced the first high-resolution reconstructed image of a murine leukemia virus (MLV) virion; MLV is related to RSV, and its virion has a structure similar to that of RSV. The glycoprotein spikes are seen here in *magenta*, while the lipid bilayer of the virion is shown in *purple*; parts of the underlying nucleocapsid, revealed at lower resolution, are shown in *yellow*. (C) Scanning electron micrograph of human immunodeficiency virus (HIV) nucleocapsid cores budding from the surface of baby hamster kidney (BHK) cells; HIV-infected lymphocytes generate similar images. HIV is a member of the same family of viruses as RSV and MLV. (D) Transmission electron micrograph showing murine leukemia virus (MLV) particles budding from the surface of an infected cell. As the nucleocapsid cores (containing the gag proteins, the virion RNA, and the reverse transcriptase and integrase enzymes) leave the cell, they wrap themselves with a patch of lipid bilayer taken from the plasma membrane of the infected cell. (A, adapted from H. Fan et al., The Biology of AIDS. Boston, MA: Jones and Bartlett Publishers, 1989. B, from F. Förster et al., *Proc. Natl. Acad. Sci. USA* 102:4729–4734, 2005. C, courtesy of P. Roingeard. D, courtesy of Laboratoire de Biologie Moleculaire.)