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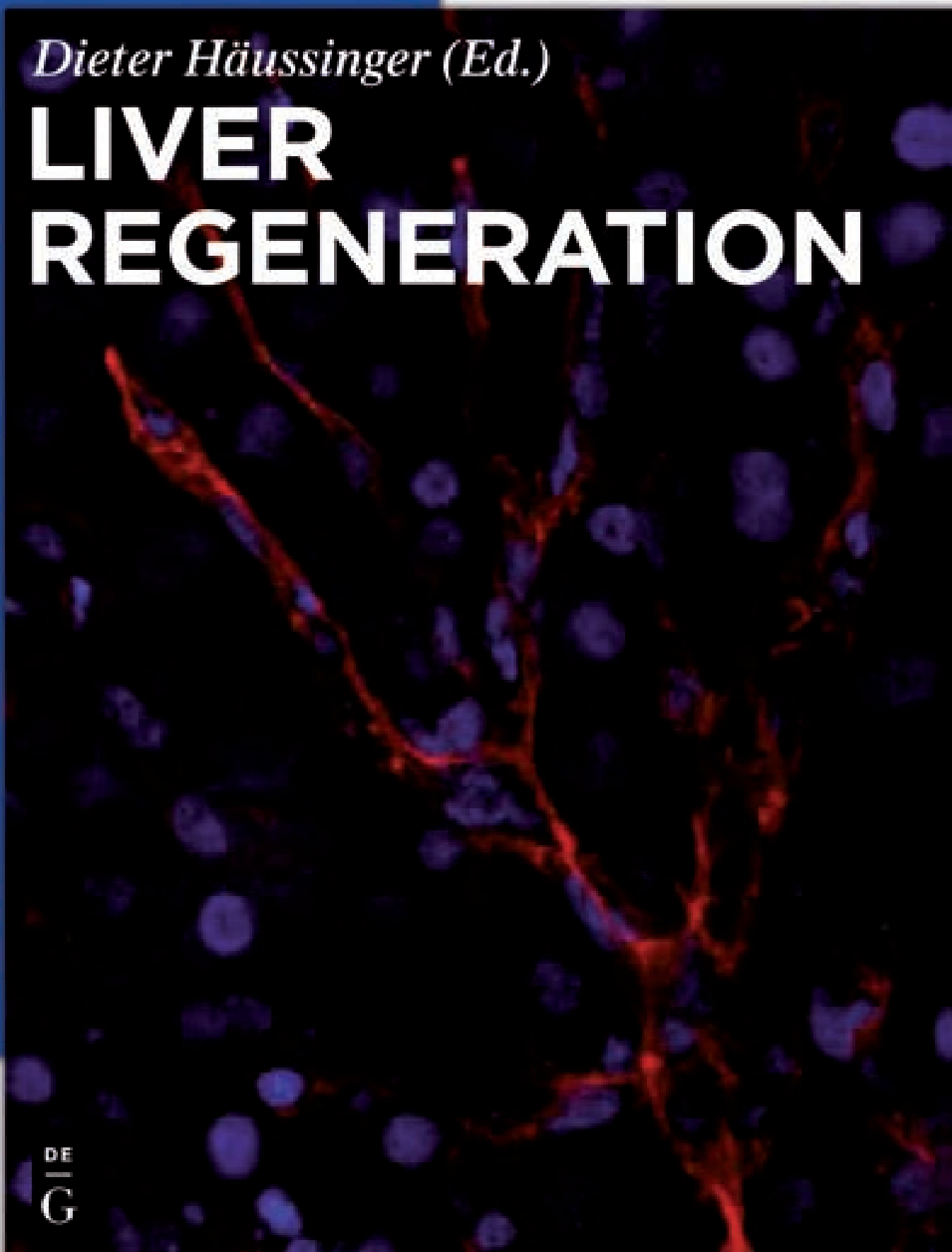

Dieter Häussinger (Ed.)

LIVER REGENERATION

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This book has 46 figures and 7 tables.

The cover image shows a section through a regenerating rat liver 5 days after partial hepatectomy. Sprouting blood vessels are shown in red color, and nuclei in blue color. The image was produced by the Lammert and Häussinger laboratories.

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Preface

The liver has a high capacity to regenerate, which was already known in ancient Greece, as exemplified in the Prometheus saga. Although liver regeneration has been paradigmatic for organ repair and renewal for more than 2,000 years, only during the past decades has much effort been devoted to the understanding of the molecular and cell biological mechanisms underlying liver regeneration. Such knowledge is of crucial importance for clinical medicine not only regarding liver physiology and pathology, but also for the use of stem cells for cell therapy and liver surgery. This graduate-level text book provides an overview of the current state of knowledge about the molecular mechanisms of liver regeneration. The chapters were written by renowned experts and active researchers in the field of liver regeneration; some of them members of the Collaborative Research Center 575 "Experimental Hepatology." Hepatic stem cells are introduced, and the important players involved in regeneration, such as oval cells, bone marrow, and stellate cells, are reviewed. Also, the cell-signaling pathways that initiate liver regeneration and regulate the switch between proliferation and apoptosis are presented. The book also treats the epigenetic regulation of liver stem cells and the roles of inflammation and angiogenesis in liver regeneration. This compact overview of the fascinating regenerative capacity of the liver will be of interest to both, graduate students and postdoctorate scientists in molecular biology, biochemistry, and medicine, and it is hoped that this survey on the various aspects of liver regeneration will stimulate further research in this area and help young scientists develop their research strategies. The topics treated are central to the biomedical curriculum, including stem cell research, cancer biology, cell signaling, and epigenetics.

I would like to express my sincere thanks not only to the authors for their excellent contributions but also to my collaborators, Mrs. Katrin Nagel, editor for science, technology, and medicine, from de Gruyter Publishers for her excellent collaboration and professional help in preparing and producing this book project, and Dr. Martin Lay for the artwork and beautiful illustrations.

Düsseldorf, May 2011

Dieter Häussinger

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Abbreviations

2-AAF	2-acetylaminofluorene
2/3 PHx	two-thirds partial hepatectomy
AFP	alpha-fetoprotein
AP-1	activator protein 1
APAP	acetaminophen
APP	acute phase proteins
ASC	adult stem cell
ASH	alcoholic steatohepatitis
ASM	acidic sphingomyelinase
ATSCs	adipose tissue stromal cells
BLP	basal lamina proteins
BM	bone marrow
Bmp/BMP	bone morphogenetic protein
BMSCs	bone marrow stem cells
BTLA	B and T lymphocyte attenuator
BV	blood vessels
CCC	cell–cell contacts
CCl_4	carbon tetrachloride
ChIP	Chromatin immunoprecipitation
CHX	cycloheximide
CoH	canal of Hering
CR	cysteine rich
CRD	cysteine-rich domain
CT	computed tomography
DcR3	decoy receptor 3
DD	death domains
Dhh	Desert hedgehog
DISC	death-inducing signaling complex
Dpp	Decapentaplegic
DPPIV	dipeptidyl-peptidase-IV-deficient
ECM	extracellular matrix
EGF	epidermal growth factor
EGFP	enhanced green fluorescent protein
EMT	epithelial-to-mesenchymal transition
ENA-78	epithelial neutrophil-activating protein
ER	endoplasmic reticulum
ESC	embryonic stem cell
FADD	Fas-associated death domain
FFA	free fatty acid
FGF	fibroblast growth factors

FLRV	future liver remnant volume
Fsrp	Follistatin related protein
FXR	farnesoid-X-receptor
G-CSF	granulocyte-colony stimulating factor
G-CSFR	G-CSF receptor
GF	growth factor
GFAP	glial fibrillary acidic protein
GS	glutamine synthetase
HB-EGF	heparin-binding EGF
HCC	hepatocellular carcinomas
Hep	hepatocytes
HGF	hepatocyte growth factor
Hh	hedgehog
HHIP	hedgehog interacting protein
HIFs	hypoxia-inducible factors
HPCs	hepatic progenitor cells
HSA	hepatocyte sinusoid alignment
HSCs	hepatic stellate cells
HUVEC	human umbilical vein endothelial cells
HVEM	herpes virus entry mediator
IBD	intralobular bile duct
ICAM	intercellular adhesion molecule
IGFBP1	insulin growth factor binding protein 1
Ihh	Indian hedgehog
iPSs	inducible-pluripotent stem cells
IKK	IKB kinase
ILK	integrin linked kinase
INR	international normalized ratio
JNK	Jun Kinase
KCs	Kupffer cells
LGLs	large granular lymphocytes
L-NAME	N^G-nitro-L-arginine methyl ester
LPS	lipopolysaccharide
LSECs	Liver sinusoidal endothelial cells
LSCs	liver stem cells
LT	liver transplantation
MAP	mitogen-activated protein
MAPCs	multipotent adult progenitor cells
M-CSF	macrophage colony stimulating factor
MELD	model for end-stage liver disease
MIP	macrophage inflammatory protein
MMP	matrix metalloproteinases
MSCs	mesenchymal stem cells
NASH	non-alcoholic steatohepatitis
NC	neighboring cell
NICD	Notch intracellular domain
NIK	NFκB inducing kinase

NK	natural killer
NKT	natural killer T
NO	nitric oxide
NOS-2	nitric oxide synthase 2
OCs	oval cells
OSM	oncostatin M
PCI	Protein C inhibitor
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PHx	partial hepatectomy
PI3K	phosphatidylinositol 3-kinase
PRR	pathogen recognition receptors
PUMA	p53 up-regulated modulator of apoptosis
PVE	portal venous embolization
PVP	portal venous pressure
RLGS	restriction landmark genomic scanning
ROS	reactive oxygen species
SC	stem cell
SCF	stem cell factor
SECs	sinusoidal endothelial cells
SERPIN	serine protease inhibitor
sFRP	soluble frizzled related peptide
Shh	Sonic hedgehog
SNS	sympathetic nervous system
SUMO	small ubiquitin-related modifier
tBDL	Total bile duct ligation
TGFalpha	transforming growth factor alpha
TGFbeta	transforming growth factor beta 1
TGFBRI / TGFBRII	TGFbeta receptor I / TGFbeta receptor II
TIMs	TRAF-interacting molecules
TLR	Toll like receptor
TLV	total liver volume
TNF	tumor necrosis factor
TNFalpha	tumor necrosis factor alpha
TNFRI	TNF receptor I
TRADD	TNF-receptor associated death domain
TRAF	TNF-receptor associated factor
TRE	tetracycline response element
TUDC	tauroursodeoxycholate
TWEAK	transforming growth factor like weak inhibitor of apoptosis
Tx	transcription
uPA	urokinase plasminogen activator
uPAR	urokinase plasminogen activator receptor
Wnt	wingless type
YAP	Yes-associated protein

1 Liver Regeneration and Partial Hepatectomy: Process and Prototype

Marie C. DeFrances and George K. Michalopoulos

Learning Targets

1. Recognize the three phases of liver regeneration after partial hepatectomy: initiation/priming, proliferation, and termination
2. Understand the utility and drawbacks of the partial hepatectomy technique to study the process of liver regeneration
3. Describe the major cellular and molecular events that characterize each phase of liver regeneration

1.1 Introduction

The liver is characterized by a unique and extraordinary capacity for self-renewal; it is the only internal solid organ in the mammal to fully regenerate after injury or loss. This occurs through organized proliferation of all resident cell types resulting in restored function. Other organs, such as cardiac muscle (Bergmann et al., 2009) or central nervous system (Brill et al., 2009), may demonstrate some endogenous propensity for regeneration, particularly after an insult, but complete organ restoration and functional recovery (as seen with the liver) are not the norm. In fact, liver tissue deficits are readily and rapidly replenished (in just a matter of 1 or 2 weeks in rodents), even following extensive loss of up to ~75% of liver mass. Such a remarkable competence for renewal has been capitalized upon by surgeons to cure patients of resectable hepatic tumors and cysts as well as to safely and effectively provide a source of transplantable tissue in the case of living related liver donation.

1.2 Liver Regeneration: Historical Perspective

Although a fairly clear understanding of what drives hepatic cells to regenerate has been established during the past several decades, the concept of liver regeneration may have originated thousands of years earlier. Of all internal organs, the liver appears to be the most revered by ancient civilizations who bestowed upon it mystical properties. Among them, the liver was believed to house the soul of the individual (Chen and Chen, 1994), and by virtue of its subcapsular scars and other peculiarities, to harbor insights into the future that could be divined by soothsayers (i.e., hepatoscopy) (Power and Rasko, 2008).

It also figured prominently in Hesiod's myth of Prometheus—a Greek god who, having stolen fire from Zeus to give as a gift to humans, was punished by daily consumption of his liver (which regrew overnight) by Zeus's eagle—the tale embodies the phenomenon of endless hepatic renewal that we embrace today. Some argue, however, that the reference to "liver regeneration" in the story of Prometheus is not one based on the ancient Greeks having any direct knowledge of the process, per se, but merely reflects their assignation of immortality to the gods, and by extension, to their gods' livers (Power and Rasko, 2008)! Regardless of which of these possibilities is true, the mere mention of liver renewal in a work of classical literature familiar to so many over the centuries may have been sufficient enough to prompt early researchers to test its scientific merit.

1.3 Partial Hepatectomy as a Means to Study Liver Regeneration

It is only in the relatively recent past that liver regeneration has become the focus of systematic, rigorous scientific investigation. As surgical techniques underwent refinement and survival following surgery improved in the late 1800s, surgeons and scientists alike began to experiment with hepatic resections in animals (Power and Rasko, 2008). By 1931, Higgins and Anderson (1931) had devised the classic surgical model that is still widely in use today. It is referred to as **two-thirds partial hepatectomy** (2/3 PHx) and was first performed on the rat. Following lapartomy, the anterior lobes (i.e., the large medial lobe and the left lateral lobe) of the rat liver—consisting of approximately 68% of the liver mass (i.e., 2/3)—are ligated at the hilus and resected. As the animal recovers, the excised anterior lobes of the liver do not regrow; rather, the remaining lobes undergo **compensatory hyperplasia** via replication of the cells, therein restoring the liver to its original mass in about one to two weeks (Higgins and Anderson, 1931) (▶Figure 1.1).

The liver is mainly composed of **hepatocytes**, which account for approximately 60% of the cellular constituents (Daoust and Cantero, 1959) (but roughly 80%–90% of liver mass, underscoring the fact that hepatocytes are rather large cells [about 30 uM in diameter]). **Stellate cells** (hepatic stromal cells that produce and secrete growth factors and extracellular matrix and store lipids and fat-soluble vitamins), **Kupffer cells** (resident hepatic macrophages), **sinusoidal endothelial cells** (SECs—specialized endothelia that display punctate membrane conduits, or *fenestrae,* which permit certain blood-borne nutrients, metabolites, and toxins direct access to hepatocytes) and **cholangiocytes** (biliary epithelial cells) contribute the remaining hepatic cell numbers and add to tissue mass.

In response to partial hepatectomy in mammals, an orderly progression in DNA synthetic activity and replication is observed among the different hepatic cell types. In the rat, for example, hepatocytes begin to enter DNA synthesis at about 12 hours post-PHx with a robust peak observed at 24 hours after surgery. (For mice, the pinnacle of DNA synthetic activity is slightly later at 36–44 hours post-PHx.) A second smaller surge of hepatocyte DNA synthetic activity typically occurs about 48 hours later (at 60–72 hours postsurgery). The remaining hepatic cells types replicate subsequently: DNA synthesis in Kupffer cells, stellate cells, and cholangiocytes reaches a maximum at about 48–72 hours post-PHx, while SEC DNA replication peaks at 3–4 days after surgery (Michalopoulos and DeFrances, 1997).

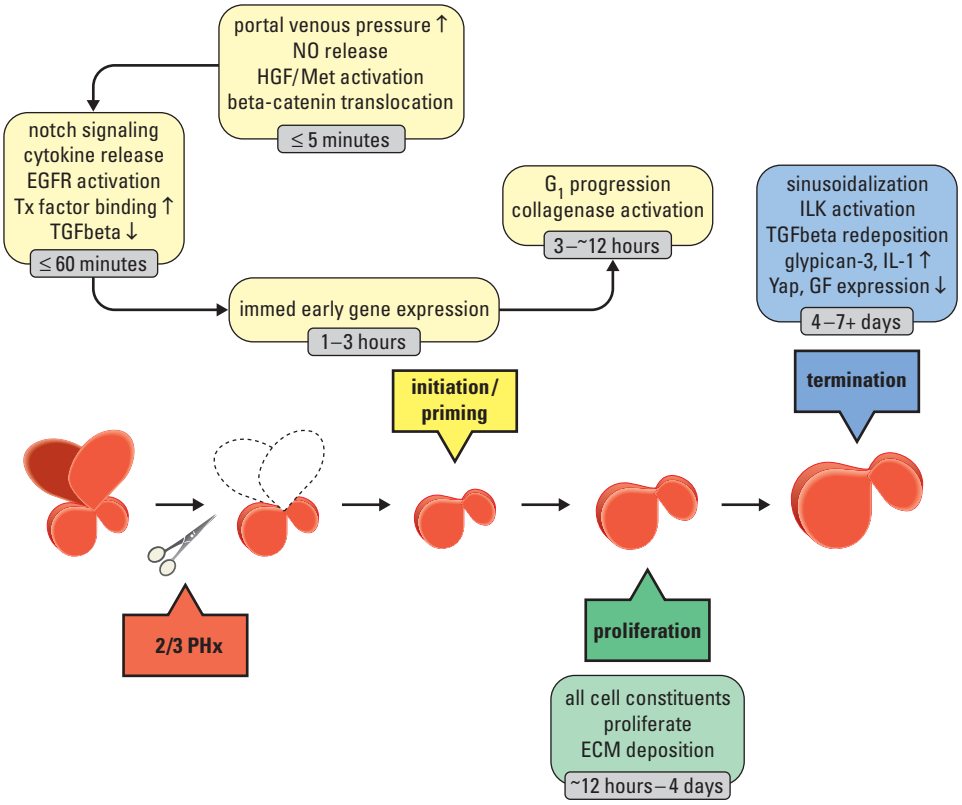

Figure 1.1 **Liver Regeneration after 2/3 Partial Hepatectomy**

Notes: Following surgical resection of the two anterior hepatic lobes of rodents accounting for ~68% (2/3) of liver tissue, the remaining lobes undergo compensatory hyperplasia restoring the liver to its original presurgical mass. Liver regeneration, which reaches completion in about 7–14 days, can be divided into three phases: **Initiation/priming** (which lasts ~12 hours after surgery), **proliferation** (extending from ~12 hours to 4 days post-PHx), and **termination** (accounting for the remainder of the time). Each phase is characterized by specific events as indicated. NO = nitric oxide, Tx = transcription, ECM = extracellular matrix, PHx = partial hepatectomy.

Historically, 2/3 PHx in rodents has been a heavily utilized method to study liver regeneration. It is rather simple to perform with a fairly high survival rate (Palmes and Spiegel, 2004). The procedure can be easily modified so that more or less tissue (than ~70%) is excised, although surgically removing greater than ~75% of hepatic mass compromises survival of the animal due to, among other reasons, hepatic hyperperfusion associated with ischemia/reperfusion injury and acute liver failure. Otherwise, it has been shown that the degree of ensuing hepatic cell replication is proportional to the amount of liver mass excised (Bucher and Swaffield, 1964). It seems that a hepatic rheostat (or *hepatostat*), the exact nature of which remains to be resolved, is at play to delicately regulate initiation and termination of the regenerative response, thus ensuring that it is wholly adequate and appropriate.

Other compensatory hyperplasia models have been developed to study the process of liver regeneration. For example, toxins (such as carbon tetrachloride [CCl_4]) that cause hepatocyte necrosis, inflammation, cytokine release, and liver regeneration can be administered to rodents (Palmes and Spiegel, 2004). Another method induces bipotential liver stem cells (oval cells) to replicate and differentiate into hepatocytes; in one version of this model, rodents are treated with the chemical 2-acetylaminofluorene (2-AAF) to inhibit hepatocyte proliferation and then subjected to partial hepatectomy to stimulate oval cell replication, differentiation, and, ultimately, liver repair (Evarts et al., 1987).

A downside of the PHx model may be that it lacks direct applicability to most common clinical scenarios. For example, patients who must regenerate liver mass after hepatic surgery often have cirrhosis, hepatic viral infection, steatosis, or liver metastases, or are liver transplant recipients. The standard PHx model does not recapitulate the physiologic complexity of these types of cases. In addition, wild animals that undergo *endogenous* liver regeneration do so because of exposure to environmental hepatotoxins or suffer from hepatic infections (i.e., woodchuck hepatitis virus in the case of the groundhog; Snyder et al., 1982), not as a result of a sterile and precise excision of pristine hepatic tissue. Despite these acknowledged drawbacks, the 2/3 PHx model remains a uniquely valuable system to delineate the mechanisms underlying liver regeneration: its relative simplicity, its reproducibility among different laboratories, the fact that hazardous chemicals need not be handled nor administered to animals, and a relative lack of tissue inflammation or necrosis (as seen in some other models, the extent of which can be variable among animals impacting the regenerative response and thus muddling data interpretation) make its use compelling.

1.4 Three Phases of Liver Regeneration after Partial Hepatectomy

An obvious question to ask is, "Why *does* the liver regenerate so rapidly and efficiently after partial hepatectomy?" The answer is understandably complex. The entire process can be roughly divided into three phases:

1) **initiation/priming**—the majority of hepatocytes exit a quiescent state (G_0), enter the cell cycle (G_1), and cross the G_1/S checkpoint. Dissolution of the extracellular matrix (ECM) begins. In the rat, this phase lasts about 12–18 hours. Although it is the shortest of the three phases, it has been perhaps the most intensely analyzed in order to identify *the primary event* that triggers liver regeneration. Studies reveal that rapid and pronounced alterations in a multitude of signaling pathways and other tissue functions occur simultaneously and no single alteration likely predominates (Michalopoulos, 2010).
2) **proliferation**—hepatocytes synthesize DNA, complete the remainder of the cell cycle, and reenter G_0; a small proportion of hepatocytes engage in a subsequent round of mitosis. Remodeling of the ECM proceeds. Other hepatic cell types such as cholangiocytes and SECs divide. This phase extends from 12–18 hours to about 4 days after PHx in rodents.
3) **termination**—the remainder of the regenerative period (day 4 to day 7+) is devoted to diminishment of progrowth cues, recommencement of inhibitory signaling, replenishment of liver mass, and return of hepatic homeostasis (▶Figure 1.1).

1.4.1 Phase One: Initiation/Priming

During the initiation/priming phase of liver regeneration after PHx, the very first event to transpire following excision of liver tissue is an immediate induction of sheer stress in the portal circulation reflected by an increase in portal venous pressure (PVP) (Schoen et al., 2001). The liver is fed by two blood supplies: (1) the portal vein (which provides the liver about 75% of its blood) carries to the liver nutrients, toxins, bile acids, and other substances absorbed or produced by the gastrointestinal tract for further metabolism, if necessary; and (2) the hepatic artery, although contributing less blood by volume, supplies the liver with, among other things, a necessary source of oxygen, hormones, cytokines, and immune surveillants (lymphocytes, monocytes, etc.). Increased PVP is accompanied by release of nitric oxide (NO) in the liver, likely by endothelial cells (Schoen et al., 2001). Blocking NO synthase by N^G-nitro-L-arginine methyl ester (L-NAME) administration inhibits *c-fos* mRNA expression typically induced 15 minutes after PHx (Schoen et al., 2001) and prevents liver enlargement at 48 hours after surgery (Wang and Lautt, 1998). NO may also be produced later in regeneration by Kupffer cells, hepatocytes, or other liver constituents through induction of nitric oxide synthase 2 (NOS-2, also referred to as inducible NOS—iNOS) (Hortelano et al., 2007). Animals engineered to lack NOS-2 show reduced liver mass beginning at 36–48 hours after PHx (Kumamoto et al., 2008; Rai et al., 1998) (although liver mass of mice in one of the studies reached control levels by day 7; Kumamoto et al., 2008).

SECs react to changes in PVP by increasing the diameter of fenestrae and overall porosity at 5 minutes post-PHx (Wack et al., 2001). At the same time (5 min. after surgery), the hepatocyte plasma membrane depolarizes (Zhang et al., 1996), but preventing depolarization does not diminish the gene expression signature usually observed within 1–1.5 hours after surgery, suggesting that depolarization has little impact on the early stages of regeneration (Minuk et al., 1997). Beta-catenin, a transcriptional regulator normally bound to E-cadherin at the hepatocyte plasma membrane, migrates to the hepatocyte nucleus to activate target genes within 5 minutes of resection. This is accompanied by E-cadherin downregulation, which may account in part for beta-catenin's rapid subcellular redistribution (Monga et al., 2001). Proper hepatic development is regulated by the Notch/Jagged signaling system; mutation of either the Jagged-1 or Notch-2 gene is associated with a paucity of intrahepatic bile ducts (referred to as Alagille Syndrome) in humans. Jagged is a cell surface ligand that binds and activates Notch, its transmembrane receptor expressed on adjacent cells. Following interaction, Notch undergoes enzymatic cleavage, and its intracellular domain (NICD) moves to the nucleus to regulate gene transcription. Fifteen minutes after PHx, NICD appears in the nuclei of hepatoctyes (and possibly other hepatic constituents such as endothelial cells) peaking at 15 minutes postsurgery. Injection of Jagged-1 siRNA to rats prior to PHx blunts DNA synthesis particularly at the day 2 post-PHx time point, suggesting that the Jagged/Notch paradigm is active during hepatic repair in addition to development (Köhler et al., 2004).

Within 1 minute after PHx, interaction of the urokinase plasminogen activator (uPA) and its cell surface receptor (uPAR) expressed by hepatocytes promotes increased uPA activity (Mars et al., 1995), which is a significant event because uPA is a serine protease responsible for cleaving and activating a variety of proteins. For example, uPA converts