Liver Regeneration

George K. Michalopoulos* and Marie C. DeFrances

Liver regeneration after the loss of hepatic tissue is a fundamental parameter of liver response to injury. Recognized as a phenomenon from mythological times, it is now defined as an orchestrated response induced by specific external stimuli and involving sequential changes in gene expression, growth factor production, and morphologic structure. Many growth factors and cytokines, most notably hepatocyte growth factor, epidermal growth factor, transforming growth factor–α, interleukin-6, tumor necrosis factor–α, insulin, and norepinephrine, appear to play important roles in this process. This review attempts to integrate the findings of the last three decades and looks toward clues as to the nature of the causes that trigger this fascinating organ and cellular response.

Liver regeneration, having presumably evolved to protect animals in the wild from the catastrophic results of liver loss caused by food toxins, has been an object of curiosity for many years. The ancient Greeks recognized liver regeneration in the myth of Prometheus. Having stolen the secret of fire from the gods of Olympus, Prometheus was condemned to having a portion of his liver eaten daily by an eagle. His liver regenerated overnight, thus providing the eagle with eternal food and Prometheus with eternal torture. In modern times, the best experimental model for the study of liver regeneration is that introduced by Higgins and Anderson (*1*): a simple operation (partial hepatectomy, PHx) in which two-thirds of the liver of a rat is removed. Specific liver lobes are removed intact, without damage to the lobes left behind. The residual lobes enlarge to make up for the mass of the removed lobes, though the resected lobes never grow back. The whole process lasts 5 to 7 days. Partial hepatectomy is the most often used stimulus to study liver regeneration because, compared with other methods that use hepatic toxins (such as CCl_4), it is not associated with tissue injury and inflammation, and the initiation of the regenerative stimulus is precisely defined (removal of liver lobes).

Studies with hepatic resections in larger animals (dogs and primates) and humans have established that the regenerative response is proportional to the amount of liver removed. Even small resections (<10%) are followed by eventual restoration of the liver to full size. When liver from large dogs is transplanted into small dogs, liver size gradually decreases until the size of the organ becomes proportional to the new body size (*2*). Conversely, in two recent cases of baboon liver transplanted to humans, the transplanted intact liver of the baboon rapidly grew in size (within a week) until it reached the size of human liver (*3*). These studies demonstrate that liver mass is precisely regulated and that signals from the body can have negative as well as positive effects on liver mass until the correct size is reached.

In contrast to other regenerating tissues (bone marrow, skin), liver regeneration is not dependent on a small group of progenitor or stem cells. [Cells with stem cell properties, however, may appear in large numbers when mature hepatocytes are inhibited from proliferation. For a review of this subject, see (*4*).] Liver regeneration after PHx is carried out by proliferation of all the existing mature cellular populations composing the intact organ. These include hepatocytes (the main functional cells of the organ); biliary epithelial cells (lining biliary ducts); fenestrated endothelial cells [a unique type of endothelial cells with large cytoplasmic gaps (fenestrae) that allow maximal contact between circulating blood and hepatocytes]; Kupffer cells (macrophages in hepatic sinusoids); and cells of Ito [stellate cells unique to the liver and located under the sinusoids; they surround hepatocytes with long processes, store vitamin A, synthesize connective tissue proteins, and secrete several growth factors (*5*)]. All of these cells proliferate to rebuild the lost hepatic tissue. Hepatocytes are the first to proliferate. Multiple parameters, including diurnal light stimuli and feeding patterns as well as others, affect the duration of the interval between PHx and the initiation of DNA synthesis in hepatocytes (*6*). Typically this interval is 10 to 12 hours in rats. The kinetics of cell proliferation differ slightly from species to species. The first peak of DNA synthesis in hepatocytes occurs at about 24 hours, with a smaller peak between 36 and 48 hours. Because only two-thirds of the hepatic tissue is removed, restoration of the original number of hepatocytes theoretically requires 1.66 proliferative cycles per residual hepatocyte. Most of the hepatocytes (95% in young and 75% in very old rats) in the residual lobes participate in one or two proliferative events (*7*).

Hepatic parenchyma is organized in units called hepatic lobules, which are built around portal triads and central veins. Portal triads are composed of microscopic branches of three vessels: portal vein (bringing blood to the liver from the intestine), hepatic artery (bringing highly oxygenated blood), and bile ductule (carrying bile away to larger bile ducts). The blood carried by the branches of the portal vein and hepatic artery proceeds through the sinusoids and drains into the central venules located at the center of the lobule. Hepatocyte proliferation starts in the areas of the lobules surrounding the portal triads (periportal) (*8*) and then proceeds to the pericentral areas by 36 to 48 hours. The other cells of the liver enter into DNA synthesis about 24 hours after the hepatocytes, with a peak of DNA synthesis at 48 hours or later (*9*) (Fig. 1). The kinetics of cell proliferation and the growth factors produced by proliferating hepatocytes suggest that hepatocytes provide the mitogenic stimuli leading to proliferation of the other cells. After 2 to 3 days during which all cellular elements of the liver proliferate, liver histology at day 3 to 4 after PHx is characterized by clumps of small hepatocytes surrounding capillaries. Typical hepatic histology is gradually restored through a series of steps (*10*). Ito cells send processes that penetrate the hepatocyte clumps and start producing several types of laminin. Eventually, the small hepatocyte clumps become rearranged into the typical hepatocyte plates seen in the mature liver. The capillaries of the small hepatocyte clumps (surrounded by typical capillary basement membrane) change into true hepatic sinusoids (surrounded by very scant matrix and lined by fenestrated endothelial cells and Kupffer cells). The hepatic matrix composition also changes from one of high laminin content to that typical of mature liver (very scant matrix containing primarily fibronectin, collagen types IV and I, and several other proteins and glycosaminoglycans in smaller amounts). By day 7, hepatic histology consists of lobules larger in size than before regeneration. Hepatocytes become arranged in plates consisting of two cell layers (as opposed to the one cell layer

The authors are in the Department of Pathology, University of Pittsburgh, Pittsburgh, PA 15261, USA.

*To whom correspondence should be addressed.

of the normal liver) (*11*). It is not clear whether there is a net increase in the number of lobules or whether existing lobules merely become larger in size, though evidence suggests that both phenomena occur (*12*).

Clonogenic Capacity of Hepatocytes: Prometheus Revisited

Modern studies have validated the ancient myth of Prometheus by providing experimental evidence that liver has an almost unlimited capacity to regenerate. Using PHx to its limits, it was shown that rat liver regenerated each time after 12 sequential hepatectomies (*13*). The clonogenic potential of the hepatocyte itself has also been found to be almost unlimited. This was recently shown in two mouse models, in which livers are rendered incapable of sustaining animal life by experimentally induced defects. In one model large amounts of urokinase are expressed in hepatocytes under the influence of the albumin promoter (*14*). The other is an animal model of hereditary tyrosinemia type I, a recessive liver disease caused by a deficiency of fumarylacetoacetate hydrolase (*14*). Precisely determined numbers of normal hepatocytes injected into these livers enter into clonogenic growth, create nodules, restore liver mass, and rescue the animals. In the second model, about 1000 hepatocytes were found to be sufficient to generate nodules of normal cells, colonize and rescue the entire liver, and establish normal architecture (*15*). Cells from the first-generation nodules of normal hepatocytes were isolated and serially transplanted to other mice. These cells (carried so far through four generations of serial transplantation) could also rescue the mice despite having undergone a clonal expansion in a previous host. Mathematical calculations from this model have established that mature hepatocytes could indeed support Prometheus' eagle. A single hepatocyte can expand through a minimal number of 34 cell divisions, giving rise to 1.7×10^{10} cells (*16*). Because a normal rat liver has on average 3×10^8 hepatocytes, one can calculate that a single rat hepatocyte under these conditions has enough clonogenic capacity to generate about 50 rat livers. Studies in culture have also shown that hepatocytes under the influence of hepatocyte growth factor (HGF) and epidermal growth factor (EGF) dedifferentiate, undergo multiple proliferative events, expand in a clonal fashion, and redifferentiate to form mature hepatocytes or even duct-like structures (*17*). All of these findings demonstrate that mature hepatocytes are not terminally differentiated cells. In contrast, they can proliferate almost without limit to secure their own preservation. This was unexpected, given the high ploidy and the complexity of functions of mature hepatocytes.

Proliferation and Differentiation: Hepatocyte as the Phenotypic Acrobat

Perhaps more remarkable than the capacity of hepatocytes to proliferate is that they do so while simultaneously performing all essential functions needed for homeostasis. These functions include glucose regulation, synthesis of many blood proteins (including albumin and coagulation proteins), secretion of bile, biodegradation of toxic compounds, and others. It is indeed remarkable that little if any disturbance is seen in these functions when only 33% of the organ remains and 90% of the cells in the residual organ are undergoing proliferation or mitosis, or both. This remarkable performance is due to a complex array of interactive events involving matrix regulation, dissolution and resynthesis of highly specialized hepatocyte membrane domains, and proper alignment and sorting out of more than 150 chromosomes at any given mitotic event (most mature hepatocytes in the rat and human have 4N ploidy and many have even higher ploidy numbers (*18*)].

Recent studies from several laboratories have focused on events that occur shortly after PHx. Urokinase receptor appears in the hepatocyte plasma membrane, and urokinase activity increases within 1 to 5 min (*19*). The hepatocyte plasma membrane becomes hyperpolarized within 30 min (*20*). Marked morphologic changes are seen within the first 5 hours in the bile canaliculi (*21*) even though little decrease in bile secretion is noted (*22*). Within 30 min after PHx there is induction of several new genes collectively called "immediate early genes" [for a review, see (*23*)]. This induction is independent of new protein synthesis. IGFBP1, a plasma protein that binds insulin-like growth factor I (IGF-I) and IGF-II, increases remarkably to 100-fold of its original expression (*24*). Activation of the transcription factor STAT3 (signal transducer and activator of transcription–3) occurs within 30 min, peaking at 3 hours and persisting beyond 5 hours (*25*). Active nuclear factor kappa B (NF-κB) (p50-p65 complex) appears within minutes after PHx (*26*). NF-κB and STAT3 are rapidly activated after appropriate signals and translocated to the nucleus. Many of the immediate early genes contain in their promoter region sequences reactive to NF-κB and STAT3. AP1 activity also increases rapidly as a result of new synthesis of both c-Fos and c-Jun. LRF-1, another leucine zipper protein, is also induced rapidly after PHx and participates in complex formation with c-Jun (*27*). The different forms of AP1 persist for several hours after PHx (*23*). Activation of STAT3, NF-κB, and AP1 is likely to be a major part of the intracellular signaling cascade leading to DNA synthesis. CCAAT/enhancer-binding protein α (C/EBPα) amounts decrease, whereas C/EBPβ amounts increase. Additional "hepatic-associated" transcription factors such as hepatic nuclear factor 1 (HNF1), HNF4, HNF3, and others remain essentially unchanged.

Similar changes in transcription factors are also seen in hepatocytes in culture undergoing clonal expansion (*17*). Several "fetal" markers appear in regenerating liver, identical to those expressed in fetal liver. These include alpha-fetoprotein, hexokinase (as opposed to glucokinase), and fetal isozymes of aldolase, pyruvate kinase, and others (*28*). [For a thorough review of all literature before 1975, see (*29*).] Other proteins linked to the cell cycle, including enzymes related to DNA synthesis (thymidine kinase), new chromatin synthesis (histone mRNA), cyclin, and cyclin-dependent kinase (CDK) changes, and cell cycle–related gene expression (*30*) undergo changes as in other proliferating cell systems. There

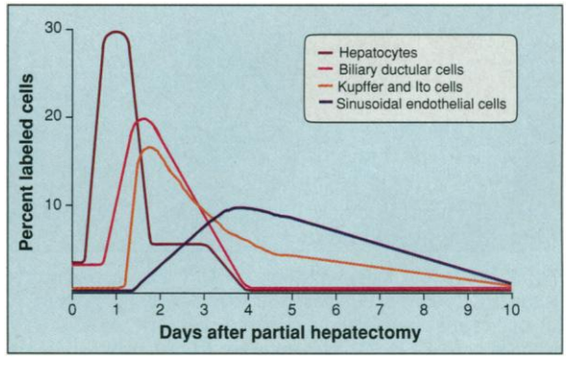

Fig. 1. Time kinetics of DNA synthesis in different liver cell types during liver regeneration after partial hepatectomy. The four major types of liver cells undergo DNA synthesis at different times. Hepatocyte DNA synthesis peaks at 24 hours, whereas the other cell types proliferate later. Regenerating hepatocytes produce growth factors that can function as mitogens for these cells. This has suggested that hepatocytes stimulate proliferation of the other cells by a paracrine mechanism. The figure was generated by graphic adaptation of the data presented in the two publications of (*9*).

is also a rather pronounced accumulation of triglycerides in hepatocytes from 20 to 72 hours after PHx associated with marked induction of lipogenic enzymes (*31*). All of these parameters eventually return to normal by day 5 to 7 after PHx along with cessation of proliferation and restoration of hepatic architecture. Thus, hepatocytes manage to proceed with mitogenesis and provide differentiated functions through subtle changes in liver-associated transcription factors, marked induction of other DNA binding activities associated with mitogenesis, and temporary partial reversion to a quasifetal phenotype.

What Triggers Liver Regeneration After Partial Hepatectomy?

Few aspects of liver regeneration have spawned as much research as the quest to find what triggers the regenerative response. Earlier studies had shown that when hepatic tissue or isolated hepatocytes are transplanted into extrahepatic sites, they also enter into DNA synthesis after PHx of the liver of the host (*32*). When rats are joined in pairs through parabiotic circulation, hepatectomy of one member of the pair causes regeneration of the intact liver of the other member, with the maximum effect seen when the liver of one animal is totally removed (*33*). These studies have provided convincing evidence that a mitogenic signal or signals for hepatocytes appear in the blood during liver regeneration. Any hypotheses made to explain the mechanism of initiation of liver regeneration have to account for the mitogenic stimuli for hepatocytes appearing in circulation during regeneration and for the rapid changes occurring in hepatocytes within 30 min after PHx. Current scenarios under intense study that attempt to explain these findings are discussed below.

Hepatocyte Growth Factor

Hepatocyte growth factor (HGF), also known as scatter factor (SF), was first identified as a blood-derived mitogen for hepatocytes in culture as part of the effort to identify blood-borne hepatic mitogens arising during liver regeneration (*34*). HGF and its receptor c-Met (*34*) are key factors for liver growth and function. Homozygous deletions of the genes of either protein are associated with embryonic lethality due in part to arrested hepatic development (*35*). Several studies have shown that plasma concentrations of HGF rise substantially in humans when hepatic mass is decreased (*36*). In the rat, plasma concentrations of HGF rise more than 20-fold within 1 hour after PHx (*37*). HGF concentrations decline slowly during the first 24 hours but remain elevated for more than 72 hours, eventually returning to normal. This hypothesis (depicted in Fig. 2) assumes that the rapid rise of HGF in the plasma is the mitogenic stimulus leading hepatocytes into DNA synthesis. This scenario is compatible with the time kinetics of the appearance of blood-borne regenerative factors as well as the rapid changes in immediate early gene expression. HGF induces expression of some immediate early genes [LRF-1 and IGFBP1 (*38*)], suggesting that HGF may be one of the stimuli leading to the rapid changes in gene expression after PHx. HGF is a potent mitogen for hepatocytes in culture (*39*). It is reasonable to postulate that the rise in plasma of a potent hepatocyte mitogen 1 hour after PHx is responsible for leading hepatocytes to DNA synthesis 23 hours later. Although this may be the case, the causes for the rise in plasma HGF are not entirely clear. Liver is responsible for clearing most of the circulating HGF, but HGF's rate of elimination does not sufficiently change after PHx to explain the magnitude of the HGF rise in plasma (*40*). Expression of HGF mRNA increases in hepatic Ito cells 3 to 6 hours after PHx and lasts for 24 hours (*41*). This cannot explain a rise in plasma HGF within 1 hour after PHx either, but it may account for the persistence of elevated concentrations of HGF throughout the regenerative process. An increase in HGF mRNA during liver regeneration is also seen in mesenchymal cells of some other tissues [for example, lung and spleen (*42*)]. The mechanism for this diffuse effect is not clear. Recent studies with constructs from promoters of the genes of both HGF and its receptor (c-Met) suggest that interleukins-1 and -6 (IL-1 and IL-6) may be involved (*43*).

If HGF is the initial mitogenic stimulus for hepatocytes in liver regeneration, injection of HGF in normal rats through the portal vein should cause hepatocyte DNA synthesis. This indeed occurs, but the number of hepatocytes entering DNA synthesis is relatively small and limited to the periportal sites. Similar results were obtained with infusion of EGF and transforming growth factor–α (TGF-α). This suggests that hepatocytes in normal liver are not ready to respond to mitogenic signals without a set of "priming" events that switch them into a responsive state (*44*). When HGF injection was preceded by infusion of a small amount of collagenase, the mitogenic effect of HGF was dramatically amplified. DNA synthesis was induced in more than 60% of hepatocytes; collagenase itself had no effect (*45*). Some direct and indirect evidence indicates that matrix degradation occurs shortly after PHx and thus may serve as

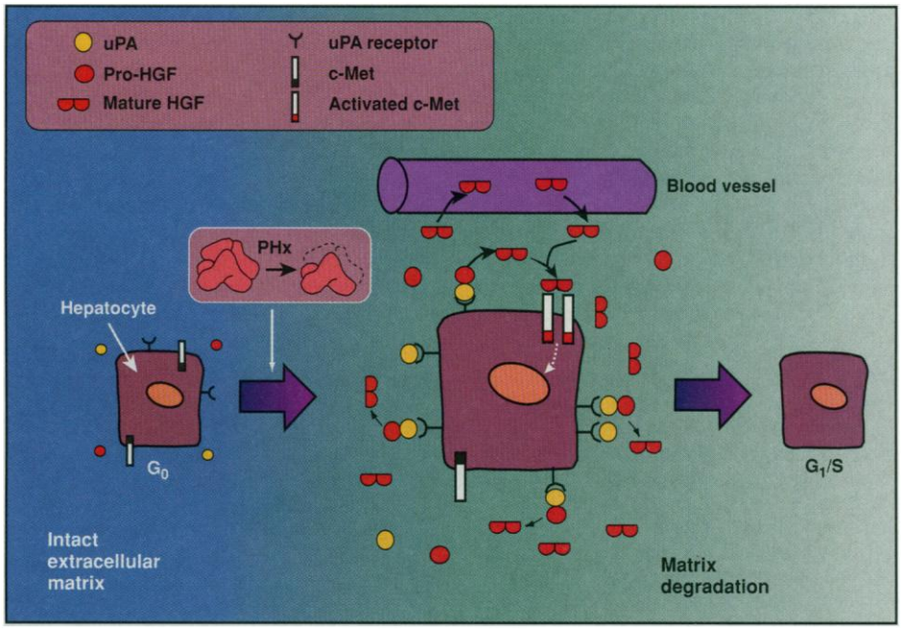

Fig. 2. Proposed model for the role of HGF in liver regeneration. Rapid up-regulation of the uPA receptor leads to activation of uPA within 5 min after PHx. This initiates a protease cascade causing degradation of the scant extracellular matrix surrounding hepatocytes and releasing, among others, matrix-bound inactive pro-HGF. uPA activates pro-HGF into the mature active form. Active HGF is released in the blood and stimulates hepatocyte DNA synthesis by an endocrine or paracrine mechanism by binding to the c-Met receptor.

the in vivo corollary of this collagenase experiment. Urokinase (uPA) is known to be involved in activation of a proteolytic cascade involving conversion of plasminogen to plasmin. The latter activates matrix-degrading metalloproteinases (46). The activity of uPA rises sharply within 5 min after PHx as a result of translocation of the uPA receptor (uPA-R) to the plasma membrane (47). Conversion of plasminogen to plasmin and increased proteolysis of some components of the hepatic biomatrix (namely laminin, entactin, and fibronectin) is seen shortly after PHx (48). Previous studies have shown that the hepatic biomatrix contains large amounts of HGF (49) predominantly around portal triads (50). Matrix breakdown may cause rapid release of HGF, thus accounting for the rapid rise of HGF in plasma. In addition, several studies have shown that uPA converts inactive, matrix-binding, single-chain HGF to its active, receptor-binding, two-chain form (51). A set of events driven by increased uPA activity that leads (through a protease activation cascade) to proteolysis of hepatic biomatrix may not only result in the release but also the activation of hepatic HGF, which then may bind its receptor on hepatocytes directly from the matrix or through the blood. In one study, an increase in two-chain HGF was seen in the liver within 15 min after PHx (47); however in another study active HGF in the liver was seen only after chemical injury but not after PHx (52). There is enhanced overall uptake of injected HGF by regenerating liver (50). Tyrosine phosphorylation of c-Met is seen many hours before initiation of DNA synthesis (53), which also suggests involvement of HGF in the generation of the mitogenic signal. Further studies of the interactive pathways between uPA, HGF, and matrix are needed to precisely define the role of these factors at the early stages of regeneration.

TNF-α and IL-6

Several converging lines of evidence from recent work have established that tumor necrosis factor–α (TNF-α) and IL-6 are important components of the early signaling pathways leading to regeneration. Previous studies suggested that endotoxin, one of the key stimulants leading to TNF-α production by Kupffer cells, may be involved in liver regeneration (54). In more recent studies, treatment with antibodies to TNF-α before PHx resulted in decreased DNA synthesis and abrogation of increases in Jun kinase, c-jun mRNA, and nuclear AP1 activity (55). Compounds containing the element gadolinium (which paradoxically increase TNF-α mRNA in Kupffer cells) enhanced the induction of IL-6 as well as c-jun, C/EBPβ, and C/EBPΔ, and they increased nuclear amounts of AP1 (56). These events occur very early during the regenerative response, suggesting that TNF-α has a role to play in the early signaling pathways of liver regeneration. Of interest is the fact that during the increase in liver weight induced by a variety of xenobiotics (see below), there is no increase in mRNA for any growth factor or cytokine except TNF-α mRNA (57). DNA synthesis after PHx is severely impaired in mice with TNF-α type I receptor deficiency, and the normally expected increases in STAT3 and NF-κB do not occur (58). These defects were corrected after injection of IL-6, suggesting that the role of TNF-α is to regulate secretion of IL-6.

IL-6 is secreted by Kupffer cells, and this secretion is stimulated by TNF-α. IL-6 is a major signal for the stimulation of acute phase protein synthesis by hepatocytes as part of the overall inflammatory response (59). The plasma IL-6 concentration increases after PHx, reaching high levels by 24 hours (56, 60). IL-6 has been variably reported to be mitogenic or mitoinhibitory for hepatocytes in primary culture, though other studies did not confirm such effects (61). IL-6 is a mitogen in cultures of bile duct epithelial cells (62). In a recent study, hepatocyte DNA synthesis during liver regeneration was found to be suppressed in mice carrying a homozygous deletion of the IL-6 gene (63). STAT3 activation, a known function of EGF and IL-6, was markedly reduced. Similar reductions were noted with AP1, Myc, and cyclin D1. Changes both in DNA synthesis and in cell cycle gene expression were corrected by injection of IL-6.

The above studies with TNF-α and IL-6 clearly document that the early signaling mechanisms that trigger liver regeneration do not proceed normally without these cytokines. The data are interesting, especially in relation to IL-6. If EGF (also known to activate STAT3) were to compensate for IL-6, then liver regeneration in IL-6–deficient mice would not be defective. The findings imply that IL-6 is essential and irreplaceable by other cytokines that use part of its signaling pathways. This also includes other gp130 receptor–sharing cytokines, such as oncostatin M, leukemia inhibitory factor (LIF), and ciliary neurotrophic factor (CNTF). Liver regeneration in the IL-6–deficient mice, however, does eventually proceed to completion (63). Though these studies document a requirement for IL-6 (and TNF-α), they do not necessarily distinguish between a facultative role, in which the factor must be present for regeneration to proceed, versus a triggering role, in which changes in the factor or its receptor initiate new signaling that leads to mitogenesis.

EGF and TGF-α

Both EGF and TGF-α are primary mitogens for hepatocytes in culture. In rats, sialadenectomy, which causes a major reduction in plasma EGF, also decreases the hepatic regenerative response. On the other hand, plasma EGF concentrations rise only very slightly (less than 30%) after PHx (64). EGF, however, may play a mitogenic role in liver regeneration by abruptly becoming more available to hepatocytes after PHx. EGF is continually made available to the liver by the Brunner's glands of the duodenum, through portal circulation (65). EGF is taken up by liver in one pass and, as with HGF, it deposits itself in the periportal matrix (66). A decrease of hepatic mass to one-third by PHx increases the concentration of EGF (available through the portal circulation) per unit liver weight by a factor of 3. In addition norepinephrine, a substance that also increases dramatically after PHx (see below), stimulates secretion of EGF by the Brunner's glands (67), which may further increase the amount of EGF entering the liver after PHx. Rapid tyrosine phosphorylation and down-regulation of the EGF receptor occur shortly after PHx (68), suggesting that EGF may indeed play a mitogenic role early in the process.

Though EGF may be involved at the earliest stages, TGF-α appears to play a role at later times. TGF-α mRNA is induced in hepatocytes within 2 to 3 hours after PHx, rises to a peak between 12 and 24 hours, and remains elevated for at least 48 hours after PHx (69). TGF-α produced by hepatocytes may be a mitogenic stimulus to hepatocytes through an autocrine mechanism. Enhanced expression of TGF-α in hepatocytes under the influence of the albumin promoter leads to sustained high levels of hepatocyte DNA synthesis and eventually to tumor formation (70). Whether these findings apply to regeneration, however, is not entirely clear. In mice carrying a homozygous deletion of the TGF-α gene, liver regeneration proceeds normally (71). This, however, may be due to a compensatory increase in other members of the EGF receptor family of ligands. TGF-α protein in the plasma shows only a small rise after PHx (72). Despite a large increase in TGF-α mRNA, TGF-α protein amounts in regenerating liver increase only twofold (73). This is much less than the rise in TGF-α that induces hepatocyte proliferation in transgenic models.

Other Growth Factors with Paracrine Effects

In addition to potential effects on hepatocytes, TGF-α may be part of the mitogenic signals synthesized by hepatocytes leading adjacent stromal cells into proliferation about 24 hours after proliferation of hepatocytes. TGF-α is a mitogen for endothelial cells. In addition to TGF-α, other such growth factors that may have paracrine effects on endothelial cells are also produced by regenerating hepatocytes, such as acidic fibroblast growth factor (FGF) (*74*) and vascular endothelial growth factor (VEGF) (*75*). Production of these growth factors may be part of a programmed set of events that aim to restore normal histology. Regenerating liver, in analogy to rapidly growing tumors, must synthesize new stroma and blood vessels. Not surprisingly, this is achieved by using the same angiogenic signals used by tumors, many of which also secrete TGF-α, acidic FGF, and VEGF. This may also be the role for another as yet not fully characterized growth factor known as hepatic stimulatory substance (HSS) (*76*). A factor similar to HSS [described as augmentor of liver regeneration (ALR)] was recently cloned and sequenced. It is not clear, however, whether the sequenced molecule plays a role in liver regeneration (*77*).

Norepinephrine

In hepatocyte primary cultures, norepinephrine amplifies the mitogenic signals of both EGF and HGF by acting on the α1 adrenergic receptor (*78*). Norepinephrine rises rapidly in the plasma within 1 hour after PHx (*79*). It also induces secretion of EGF from the Brunner's glands of the duodenum, thus potentially making more EGF available for mitogenic stimulation of hepatocytes (*67*). Norepinephrine also offsets the mitoinhibitory effects of TGF-β1 on cultured hepatocytes isolated from the early stages of regeneration (*80*). Prazosin (a specific blocker of α1 adrenergic receptor) as well as sympathetic denervation greatly decrease DNA synthesis at 24 hours after PHx, though DNA synthesis eventually returns to normal by 48 to 72 hours (*79*). The dependence of liver regeneration on norepinephrine may be due to all of these reasons.

Insulin

Pancreatic islets supply insulin to the liver continually through the portal vein. If the amount of portal circulation to the liver is decreased (by portacaval shunt, which diverts portal vein flow to the inferior vena cava), the liver atrophies (*81*). Injection of insulin prevents or reverses this atrophy by a process involving hepatocyte replication (*82*). Insulin, however, does not have mitogenic effects on hepatocytes when injected into normal animals. Liver regeneration itself is blunted after portacaval shunt and this is corrected by insulin infusion. Hepatocyte proliferation in culture is enhanced by insulin in the presence of growth factors. However, insulin by itself is not a primary mitogen for hepatocytes. These findings, as with IL-6 discussed above, document that the signaling pathways generated by insulin and its receptor must be present for the mitogenic signal to proceed normally. This should not imply, however, that changes in insulin concentration per se trigger the events leading to mitogenesis. Insulin concentration in plasma decreases rapidly after PHx, whereas glucagon concentration increases. This is probably part of the homeostatic response by which glucose concentrations are maintained at a steady state in the blood during regeneration.

Nuclear Hormone Receptor Ligands and Xenobiotics

Triiodothyronine (*83*) and retinoic acid derivatives (*84*) stimulate hepatocyte DNA synthesis in vivo but are not effective in hepatocyte cultures. This is similar to the effects of lead nitrate, barbiturates, anti-epileptics (dilantin), diazepam, and hypolipidemic agents known as peroxisome proliferators that act as ligands of the steroid hormone family of peroxisome proliferator–activated receptors (PPARs) (*85*). These chemicals do not cause tissue injury; rather, they induce an above-normal increase in hepatic weight (typically by 180 to 250%) through a combination of events that include hepatocyte DNA synthesis and cellular hypertrophy (*86*). The levels of TGF-α or HGF mRNA do not change, but induction of TNF-α mRNA has been noted during this process (*57*, *84*). The increase in liver weight is associated with down-regulation of insulin and EGF receptors, and eventually hepatocytes become resistant to the mitogenic effects of HGF and EGF (*87*). Most of the xenobiotics inducing these effects promote hepatic neoplasia in rodents. It is not clear whether all of these chemicals act similarly. The discovery of the PPAR family of receptors has shed light on the role of nuclear receptor ligands as regulators of hepatocyte growth and differentiation. The recent findings with triiodothyronine and retinoic acid also suggest that this family of ligands needs to be further evaluated for a role in hepatic growth regulation.

Regeneration Triggers: All of the Above? A Universalist Approach

The scenarios discussed above for HGF, EGF, TGF-α, IL-6, TNF-α, norepinephrine, and insulin are by no means mutually exclusive (Fig. 3). What is in fact remarkable is that solid experimental evidence suggests that they all contribute in some way to the events leading hepatocytes from the G_0 phase to the G_1 and S phases of the cell cycle. Undoubtedly HGF plays a definitive role in this process. It is capable of

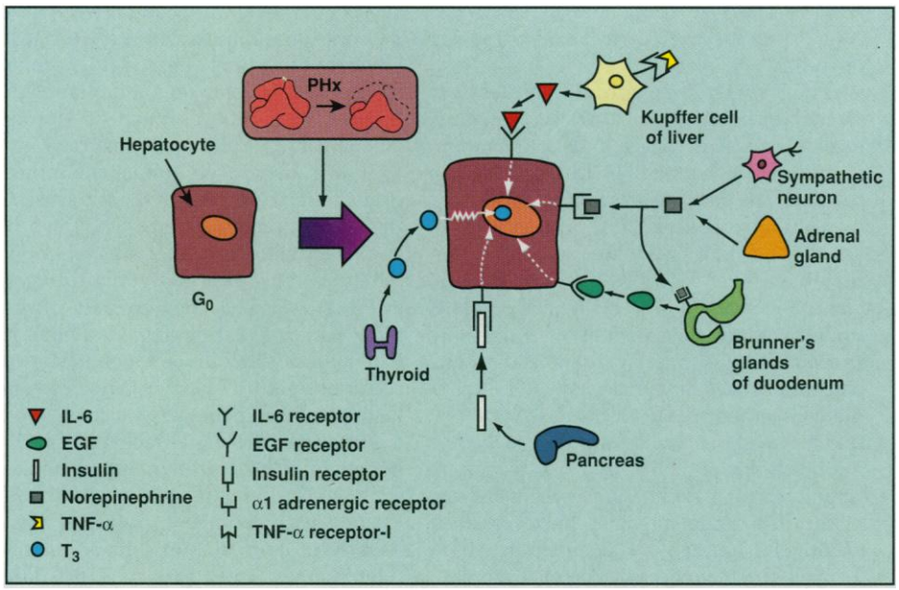

Fig. 3. Generation of the mitogenic stimuli for hepatocytes depends on the integrity of many signaling pathways, including IL-6, thyroid hormones, insulin, norepinephrine, and EGF (from Brunner's glands of the duodenum). These signals and the tissue sources of origin are shown diagrammatically.

generating a complete mitogenic signal in hepatocyte cultures in the absence of any other signaling cytokines. The increase of HGF in the plasma makes it a reasonable candidate as the main overall trigger of the regenerative process. EGF is also a hepatocyte mitogen that is continually available to liver. The changes in EGF receptor concentrations before an increase in TGF-α suggest that EGF also plays a role in the set of events immediately after PHx. Other cytokines may also be essential, but perhaps in a facultative role. There is strong experimental evidence that signaling pathways generated by insulin, IL-6, and norepinephrine (α1 adrenergic receptor) must be intact for regeneration to proceed normally, but none of these substances is a primary mitogen for hepatocytes. An increase in IL-6 is known to cause rapid synthesis of acute phase proteins but not DNA synthesis. Insulin and norepinephrine are not mitogens on their own, though they amplify the mitogenic response of EGF and HGF. Regeneration, albeit slower, is eventually completed in IL-6 deficiency, low concentrations of insulin, complete α1 adrenergic blockade, infusion of TGF-β1 (see below), and even after localized radiation with 15 grays (1 gray = 100 rads) (*88*). In fact, there is nothing known that completely prevents liver regeneration. This suggests that there is a redundancy of the early "priming" signals. Obviously, the fundamental change induced by PHx is a threefold increase in relative blood flow (and all the normal blood constituents) per unit liver weight. It is not entirely clear how this eventually triggers events as rapid as the increase in uPA activity within 5 min or activation of transcription factors and induction of immediate early genes within 30 min. It is quite likely that many factors are involved in this process. Eventually the rise within 30 to 60 min of HGF in the plasma adds a strong endocrine mitogenic signal to hepatocytes already "primed" by EGF, IL-6, insulin, matrix remodeling, and others, which leads hepatocytes into DNA synthesis. In analogy to studies of the big-bang theory of the universe, research in liver regeneration still needs to sort out the earliest signals associated with triggering the origin of the regenerative response.

What Stops Liver Regeneration?

After a spectacular phase of hepatic growth and restructuring, liver regeneration eventually stops. DNA synthesis is mostly complete by 72 hours. Changes in histology follow (*10*). Much more research has focused on the initiation of liver regeneration as compared with its termination. Most of the studies have focused on TGF-β1, a known inhibitor of proliferation in hepatocyte cultures (*89*). TGF-β1, normally synthesized by Ito cells, is also associated with synthesis of hepatic biomatrix in normal and diseased liver (*90*). Immunoreactive TGF-β1 disappears as a wave from the periportal to pericentral regions of the lobule (*91*). The gradual loss of TGF-β1 is followed by a wave of hepatocytes in mitosis, suggesting that removal of TGF-β1 from the environment of hepatocytes is required for normal completion of their cell cycle. The IGF-II/mannose-6-phosphate receptor co-localizes with TGF-β1, and it probably plays a role in binding TGF-β1 to hepatocytes (*91*). TGF-β1 increases in the plasma very shortly after PHx with the same time kinetics as HGF (*92*). This may also be due to release of bound TGF-β1 along with HGF from matrix sites. TGF-β1 released in the plasma is probably inactivated by binding to α2 macroglobulin (*93*).

In the liver, TGF-β1 mRNA increases within 3 to 4 hours after PHx, reaching plateau amounts at 48 to 72 hours (*94*). Because DNA synthesis in hepatocytes eventually stops at that time, it is reasonable to postulate that this may be mediated by a paracrine mito-inhibitory effect of TGF-β1. Infusion of TGF-β1 after PHx suppresses the hepatocyte DNA synthesis peak at 24 hours, though DNA synthesis returns by 72 hours (*95*). Hepatocytes isolated from regenerating liver from 12 to 48 hours after PHx are resistant to TGF-β1 mitoinhibitory effects (*80*). TGF-β1 receptors on hepatocytes also decrease during the same time frame (*96*). Sensitivity to TGF-β1 returns by 96 hours; however, hepatocyte proliferation stops between 48 and 72 hours, a time when they are still resistant to TGF-β1. Norepinephrine decreases the hepatocyte sensitivity to TGF-β1 between 12 and 18 hours after PHx (*80*). Resistance to TGF-β1 by regenerating hepatocytes is an important phenomenon because it may allow hepatocytes to proliferate even though concentrations of TGF-β1 are increasing. Overall, the role that TGF-β1 plays during liver regeneration is not clear. Obviously hepatocytes proceed through regeneration despite the TGF-β1 increase. On the other hand, TGF-β1 is a mito-inhibitor and thus a logical candidate to cause the end of regeneration. It should be pointed out, however, that liver regeneration proceeds to completion (although slowed down) in transgenic mice in which there is enhanced expression of TGF-β1 in the liver (*97*). This raises doubts as to whether TGF-β1 alone is sufficient to act as the terminating signal for liver regeneration. No other specific candidates are known at this point, though potential signals of key metabolites, growth factors, cytokines, or restored normal matrix, for example, may deliver in aggregate a set of signals leading to termination of regeneration.

Aside from its mito-inhibitory effects, TGF-β1 is a strong mitogen for mouse and rat hepatocytes and it may stimulate early changes in hepatocyte motility (*98*). In addition, TGF-β1 may be involved in the regulation of new matrix synthesis as hepatic histology becomes rearranged during and after regeneration.

Conclusions

The last two decades have brought a better understanding of the molecular mechanisms of hepatic growth control. Many questions, however, still remain unanswered. It is likely that liver regeneration will continue to provide a very useful model to study the integration of signaling pathways during organogenesis.

REFERENCES AND NOTES

1. G. M. Higgins and R. M. Anderson, *Arch. Pathol.* **12**, 186 (1931).
2. A. Francavilla *et al.*, *Transplant. Proc.* **20** 494, (1988); S. Kawasaki *et al.*, *Lancet* **339**, 580 (1992).
3. T. E. Starzl *et al.*, *Lancet* **341**, 8853 (1993).
4. S. Sell, *Mod. Pathol.* **7**, 105 (1994); S. S. Thorgeirsson, *FASEB J.* **10**, 1249 (1996); N. Fausto, J. M. Lemire, N. Shiojiri, *Proc. Soc. Exp. Biol. Med.* **204**, 237 (1993).
5. A. M. Gressner, *J. Hepatol.* **22**, 28 (1995); C. N. Housset, D. C. Rockey, S. L. Friedman, D. M. Bissell, *ibid.*, p. 55.
6. S. Yanagi and V. R. Potter, *Life Sci.* **20**, 1509 (1977); E. L. Krawitt, I. Betel, V. R. Potter, *Biochim. Biophys. Acta* **174**, 763 (1969).
7. E. Stöcker and W. D. Heine, *Beitr. Pathol.* **144**, 400 (1971).
8. H. M. Rabes, R. Wirsching, H. V. Tuczek, G. Iseler, *Cell Tissue Kinet.* **9**, 517 (1976).
9. J. W. Grisham, *Cancer Res.* **22**, 842, (1962); J. J. Widmann and H. D. Fahimi, in *Liver Regeneration after Experimental Injury*, R. Lesch and W. Reutter, Eds. (Stratton Intercontinental Medical Book Corp., New York, 1975), pp. 89–98.
10. A. Martinez-Hernandez and P. S. Amenta, *FASEB J.* **9**, 1401 (1995).
11. K. Ogawa, A. Medline, E. Farber, *Br. J. Cancer* **40**, 782 (1979).
12. I. N. Iashina, *Biul. Eksp. Biol. Med.* **70**, 95 (1970).
13. E. Stocker, H. K. Wullstein, G. Brau, *Virchows Arch. B Cell Pathol.* **14**, 93 (1973).
14. J. A. Rhim, E. P. Sandgren, J. L. Degen, R. D. Palmiter, R. L. Brinster, *Science* **263**, 1149 (1994).
15. K. Overturf *et al.*, *Nature Genet.* **12**, 266 (1996).
16. M. Grompe, K. Overturf, M. Al-Dhalimy, M. Finegold, *Hepatology* **24**, 256A (1996).
17. G. D. Block *et al.*, *J. Cell Biol.* **132**, 1133 (1996).
18. C. Nadal and F. Zajdela, *Exp. Cell Res.* **42**, 99 (1966); P. E. Schwarze, E. O. Pettersen, M. C. Shoaib, P. O. Seglen, *Carcinogenesis* **10**, 1267 (1984); L. M. Sargent, N. D. Sanderson, S. S. Thorgeirsson, *Cancer Res.* **56**, 2137 (1996).
19. W. M. Mars, T. H. Kim, D. B. Stolz, M. L. Liu, G. K. Michalopoulos, *Cancer Res.* **56**, 2837, (1996).
20. B. de Hemptinne, F. Lorge, P. J. Kestens, L. Lambotte, *Acta Gastroenterol. Belg.* **48**, 424 (1985).
21. A. G. Yee and J. P. Revel, *J. Cell Biol.* **66**, 200 (1975).
22. H. S. Xu, T. L. Pruett, R. S. Jones, *Ann. Surg.* **218**, 176 (1993).
23. R. Taub, *FASEB J.* **10**, 413 (1996).
24. K. L. Mohn, T. M. Laz, A. E. Melby, R. Taub, *J. Biol. Chem.* **265**, 21914 (1990).
25. D. E. Cressman, R. H. Diamond, R. Taub, *Hepatology* **21**, 1443 (1995).

26. D. E. Cressman, L. E. Greenbaum, B. A. Haber, R. Taub, *J. Biol. Chem.* **269**, 30429 (1994).
27. J. C. Hsu, T. Laz, K. L. Mohn, R. Taub, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3511 (1991).
28. R. J. Bonney, H. A. Hopkins, P. R. Walker, V. R. Potter, *Biochem. J.* **136**, 115 (1973); P. R. Walker, V. R. Potter, *Adv. Enzyme Regul.* **10**, 339 (1972).
29. R. Tsanev, in *Results and Problems in Cell Differentiation*, J. Reiner and H. Holzer, Eds. (Springer-Verlag, Berlin, 1975), p. 197.
30. P. Loyer *et al.*, *J. Biol. Chem.* **269**, 2491, (1994); N. Fausto *et al.*, *Symp. Fundam. Cancer Res.* **39**, 69 (1986).
31. T. J. Delahunty and D. Rubinstein, *J. Lipid Res.* **11**, 536, (1970).
32. J. W. Grisham, G. F. Leong, B. V. Hole, *Cancer Res.* **24**, 1474 (1964); R. L. Jirtle and G. K. Michalopoulos, *Cancer Res.* **42**, 3000 (1982).
33. F. L. Moolten and N. L. R. Bucher, *Science* **158**, 272 (1967); B. Fisher, P. Szuch, M. Levine, E. R. Fisher, *ibid.* **171**, 575 (1971).
34. G. Michalopoulos, K. A. Houck, M. L. Dolan, N. C. Luetteke, *Cancer Res.* **44**, 4414 (1984); T. Nakamura, K. Nawa, A. Ichihara, *Biochem. Biophys. Res. Commun.* **122**(3) 1450 (1984); E. Gherardi and M. Stoker, *Nature* **346**, 228, (1990); L. Naldini *et al.*, *Oncogene* **6**, 501 (1991).
35. C. Schmidt *et al.*, *Nature* **373**, 699 (1995); Y. Uehara *et al.*, *ibid.*, p. 702.
36. T. Tomiya *et al.*, *Gastroenterology* **103**, 1621 (1992).
37. P. M. Lindroos, R. Zarnegar, G. K. Michalopoulos, *Hepatology* **13**, 743 (1991).
38. E. Weir *et al.*, *ibid.* **20**, 955 (1994)
39. T. Nakamura *et al.*, *Nature* **342**, 440 (1989).
40. R Appasamy *et al.*, *Lab. Invest.* **68**, 270 (1993); K. X. Liu, *Am. J. Phys.* **263**, G642 (1992).
41. R. Zarnegar, M. C. DeFrances, D. P. Kost, P. Lindroos, G. K. Michalopoulos, *Biochem. Biophys. Res. Commun.* **177**, 559 (1991); P. Schirmacher, A. Geerts, A. Pietrangelo, H. P. Dienes, C. E Rogler, *Hepatology* **15**, 5 (1992).
42. K. Yanagita, *Biochem. Biophys. Res. Commun.* **182**, 802 (1992).
43. K. Matsumoto, H. Okazaki, T. Nakamura, *ibid.* **188**, 235 (1992); A. Moghul *et al.*, *Oncogene* **9**, 2045, (1994); N. Kitamura *et al.*, *Experientia* **65**, 49 (1993).
44. E. M. Webber, P. J. Godowski, N. Fausto, *Hepatology* **19**, 489 (1994); F. Roos, A. M. Ryan, S. M. Chamow, G. L. Bennett, R. H. Schwall, *Am. J. Physiol.* **268**, G380 (1995).
45. M. L. Liu, W. M. Mars, R. Zarnegar, G. K. Michalopoulos, *Hepatology* **19**, 1521 (1994).
46. F. Blasi, *Bioessays* **15**, 105 (1993); D. B. Rifkin, *Matrix Suppl.* **1**, 20 (1992).
47. W. M. Mars *et al.*, *Hepatology* **21**, 1695 (1995).
48. T.-H. Kim, W. M. Mars, G. K. Michalopoulos, *ibid.* **24**, 134A (1996).
49. A. Masumoto and N. Yamamoto, *Biochem. Biophys. Res. Commun.* **15**, 90 (1991).
50. M. L. Liu, W. M. Mars, R. Zarnega, G. K. Michalopoulos, *Am. J. Pathol.* **144**, 129 (1994).
51. W. M. Mars, R. Zarnegar, G. K. Michalopoulos, *ibid.* **143**, 949 (1993); L. Naldini *et al.*, *J. Biol. Chem.* **13**, 603 (1995).
52. K. Miyazawa, T. Shimomura, N. Kitamura, *J. Biol. Chem.* **271**, 3615 (1996).
53. M. Horimoto, *J. Hepatol.* **23**, 174, (1995).
54. R. P. Cornell, *Am. J. Physiol.* **241**, E428 (1981); R. P. Cornell *et al.*, *Hepatology* **11**, 916, (1990).
55. A. M. Diehl *et al.*, *Am. J. Physiol.* **267**, G552 (1994).
56. R. M. Rai, *ibid.* **270**, G909 (1996).
57. H. Shinozuka *et al.*, *Lab. Invest.* **71**, 35 (1994).
58. Y. Yamada, I. Kirillova, J. J. Peschon, N. Fausto, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1441 (1997).
59. J. Gauldie, C. Richards, H. Baumann, *Res. Immunol.* **143**, 755 (1992); P. B. Sehgal, *ibid.*, p. 724.
60. H. Matsunami *et al.*, *Transplant. Proc.* **24**, 1971 (1992).
61. T. Kordula *et al.*, *Lymphokine Cytokine Res.* **10**, 23, (1991); S. Kuma *et al.*, *Immunobiology* **180**, 235 (1990).
62. K. Matsumoto, H. Fujii, G. K. Michalopoulos, J. J. Fung, A. J. Demetris, *Hepatology* **20**, 376 (1994).
63. D. E. Cressman *et al.*, *Science* **274**, 1379 (1996).
64. D. E. Jones *et al.*, *Am. J. Physiol.* **268**, G872 (1995); S. Noguchi, Y. Ohba, T. Oka, *J. Endocrinol.* **128**, 425 (1991).
65. P. Skov Olsen *et al.*, *Hepatology* **8**, 992 (1988).
66. R. J. St. Hilaire, G. T. Hradek, A. L. Jones, *Proc. Natl. Acad. Sci. U.S.A.* **80** 3797, (1983)
67. P. Skov Olsen, S. S. Poulsen, P. Kirkegaard, *Gut* **26**, 920 (1985).
68. R. A. Rubin, E. J. O'Keefe, H. S. Earp, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 776 (1982).
69. J. E. Mead and N. Fausto, *ibid.* **86**, 1558 (1989).
70. E. M. Webber, J. C. Wu, L. Wang, G. Merlino, N. Fausto, *Am. J. Pathol.* **145**, 398 (1994); G. H. Lee, G. Merlino, N. Fausto, *Cancer Res.* **52**, 5162 (1992).
71. W. E. Russell, *Mol. Carcinog.* **15**, 183 (1996).
72. T. Tomiya and K. Fujiwara, *Cancer* **77**, 1056 (1996); *Hepatology* **23**, 253 (1996).
73. W. E. Russell, P. J. Dempsey, S. Sitaric, A. J. Peck, R. J. Coffey, *Endocrinology* **133**, 1731 (1993).
74. M. Kan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7432 (1989).
75. S. Mochida, K. Ishikawa, M. Inao, M. Shibuya, K. Fujiwara, *Biochem. Biophys. Res. Commun.* **226**, 176 (1996).
76. D. R. LaBrecque, G. Steele, S. Fogerty, M. Wilson, J. Barton, *Hepatology* **7**, 100 (1987); W. E. Fleig, *ibid.* **9**, 240 (1989).
77. M. Hagiya *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **16**, 8142 (1994).
78. J. L. Cruise, K. A. Houck, G. K. Michalopoulos, *Science* **227**, 749 (1985); J. L. Cruise and G. K. Michalopoulos, *J. Cell. Physiol.* **125**, 45 (1985).
79. J. L. Cruise, S. J. Knechtle, R. R. Bollinger, C. Kuhn, G. Michalopoulos, *Hepatology* **7**, 1189 (1987).
80. K. A. Houck and G. K. Michalopoulos, *J. Cell. Physiol.* **141**, 503 (1989).
81. T. E. Starzl *et al.*, *Surg. Gynecol. Obstet.* **137**, 179 (1973); P. J. Flynn, *Arch. Pathol.* **85**, 138 (1968).
82. T. E. Starzl, A. Francavilla, K. A. Porter, J. Benichou, A. F. Jones, *Surg. Gynecol. Obstet.* **147**, 193 (1978); T. E. Starzl, K. Watanabe, K. A. Porter, C. W. Putnam, *Lancet* **i**, 821 (1976).
83. J. Short, R. Wedmore, L. Kibert, R. Zemel, *Cytobios* **28**, 165 (1980); A. Francavilla *et al.*, *Hepatology* **20**, 1237, (1994).
84. T. Ohmura *et al.*, *Life Sci.* **58**, PL211 (1996); A. Columbano and H. Shinozuka, *FASEB J.* **10**, 1118 (1996).
85. R. Chu, Y. Lin, M. S. Rao, J. K. Reddy, *J. Biol. Chem.* **270**, 29636, (1995); Y. Zhu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 7921 (1995); S. Green and W. Wahli, *Mol. Cell. Endocrinol.* **100**, 149 (1994); J. K. Reddy, M. K. Reddy, M. I. Usman, N. D. Lalwani, M. S. Rao, *Environ. Health Perspect.* **65**, 317 (1986).
86. L. H. Augenlicht and T. S. Argyris, *Exp. Mol. Pathol.* **22**, 1 (1975).
87. C. Gupta, A. Hattori, J. M. Betschart, M. A. Virji, H. Shinozuka, *Cancer Res.* **48**, 1162 (1988); R. L. Jirtle and S. A. Meyer, *Dig. Dis. Sci.* **36**, 659 (1991); P. Lindroos and G. K. Michalopoulos, *Carcinogenesis* **14**, 731 (1993).
88. R. L. Jirtle and G. K. Michalopoulos, unpublished observations. Very little DNA synthesis was noted, but the hepatic mass was restored nonetheless primarily by hepatocyte enlargement.
89. B. I. Carr, I. Hayashi, E. L. Branum, H. L. Moses, *Cancer Res.* **46**, 2330 (1986).
90. N. Fausto, J. E. Mead, P. A. Gruppuso, A. Castilla, S. B. Jakowlew, *Ciba Found. Symp.* **157**, 165 (1991).
91. R. L. Jirtle, B. I. Carr, C. D. Scott, *J. Biol. Chem.* **266**, 22444 (1991).
92. G. K. Michalopoulos, in *Liver Regeneration and Carcinogenesis*, R. L. Jirtle, Ed. (Academic Press, San Diego, 1995), pp. 27–50.
93. J. LaMarre *et al.*, *J. Clin. Invest.* **87**, 39 (1991).
94. L. Braun *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1539 (1988).
95. W. E. Russell, R. J. Coffey Jr., A. J. Ouellette, H. L. Moses, *ibid.*, p. 5126.
96. R. S. Chari, D. T. Price, S. R. Sue, W. C. Meyers, R. L. Jirtle, *Am. J. Surg.* **169**, 126 (1995).
97. J. B. Kopp *et al.*, *Lab. Invest.* **74**, 991 (1996); N. Sanderson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 2572 (1995).
98. B. Petersen, C. J. Yee, W. Bowen, R. Zarnegar, G. K. Michalopoulos, *Cell Biol. Toxicol.* **10**, 219 (1994); D. Beer-Stolz and G. K. Michalopoulos, *J. Cell. Phys.* **170**, 57 (1997).

99. Supported by NIH grants CA30241 and CA35373.

Stem Cells in the Central Nervous System

Ronald McKay

In the vertebrate central nervous system, multipotential cells have been identified in vitro and in vivo. Defined mitogens cause the proliferation of multipotential cells in vitro, the magnitude of which is sufficient to account for the number of cells in the brain. Factors that control the differentiation of fetal stem cells to neurons and glia have been defined in vitro, and multipotential cells with similar signaling logic can be cultured from the adult central nervous system. Transplanting cells to new sites emphasizes that neuroepithelial cells have the potential to integrate into many brain regions. These results focus attention on how information in external stimuli is translated into the number and types of differentiated cells in the brain. The development of therapies for the reconstruction of the diseased or injured brain will be guided by our understanding of the origin and stability of cell type in the central nervous system.

Definition of the processes that shape the cellular makeup of the central nervous system (CNS) has relied heavily on three distinct procedures: fate mapping, tissue culture, and transplantation. These traditional tools of embryologists have been significantly improved by the recent incorporation of advanced molecular methods. Fate mapping of neuronal precursors in vertebrates points to the existence of multipotential cells that are precursors to both neu-

The author is in the Laboratory of Molecular Biology, National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892, USA.