Hepatic Stem Cell Compartment: Activation and Lineage Commitment (43661)

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There is increasingly robust experimental evidence in support of the presence of a pluripotent cell compartment in the liver (1–7). This compartment can under certain conditions function as a stem cell compartment and provide the needed progeny for regeneration of the hepatic parenchyma (8, 9). In the adult rat, specific conditions can be utilized to induce proliferation of a distinct population of small epithelial cells in the ductal structures of the liver (10, 11). These cells, conventionally described as oval cells, are characterized by ovoid nuclei and basophilic cytoplasma (10), and display features of both bile duct cells and fetal hepatocytes (11–13). There are three experimental systems, two in the rat and one in the mouse, in which it has been conclusively demonstrated that oval cells are capable of differentiation into hepatocytes (8, 11, 14). The developmental potential of oval cells is, however, not restricted to hepatic lineages. Oval cells can differentiate into intestinal-type epithelia, and have been implicated in the development of pancreatic tissues (8, 11, 15–17; Fig. 1). The observations that subpopulations of proliferating oval cells phenotypically similar to early hepatoblasts, and that oval cells originate in or around the ductular structures in the portal area, strongly support the notion that the hepatic stem cell compartment resides in these structures (2, 7, 9). Furthermore, present evidence clearly indicates that the hepatic stem cell compartment functions as a facultative stem cell compartment that is activated when the parenchymal cells are unable to proliferate in response to growth stimuli (2, 8, 18, 19).

In this paper, we will review our recent results on the localization and growth factor involvement in the activation of hepatic stem cells as well as the lineage commitment of these cells in the rat liver.

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Results and Discussion

Localization of Hepatic Stem Cells. The experimental system used to initiate proliferation and differentiation of oval cells in rat liver involves the administration of acetylaminofluorene (AAF) to male Fischer 344 rats (approximately 150 g) by gavage five times over a 1-week period, at the end of which a two-third partial hepatectomy (PH) is performed (8). After 1-day recovery, AAF administration is continued for 4 days, resulting in a total dose of 9 mg AAF/rat. Animals are then sacrificed at specified times after the operation. In this experimental system, the AAF/PH model, a rapid and extensive proliferation of oval cells takes place after the PH, first in the portal area and later these cells expand into the liver acinus and differentiate into small basophilic hepatocytes (Fig. 2; 8). The powerful activation of the stem cell compartment seen in the AAF/PH model is a consequence of a close to complete mitoinhibitory effect of AAF upon the adult hepatocytes that prevents the regeneration from the remaining liver tissue (8, 20). Similarly, following liver injury induced by D-galactosamine, another experimental system used to activate the hepatic stem cell, liver parenchyma are replaced by oval cells that differentiate into hepatocytes (11).

In the adult liver, the lining cells of the canals of Hering are thought to represent a pluripotent cell compartment (1–7). A stem cell nature of undefined periductal cells has also been proposed (7). Furthermore, the origin of oval cells from any component of the biliary tree has been suggested (21). We have observed recently that proliferation of desmin-positive Ito cells is closely associated with the early stages of oval cell proliferation in the AAF/PH model (Fig. 3, A and B; 22). Since we can identify the early population of oval cells by use of the monoclonal antibody OV-6 (22) and thereby discriminate between replicating oval cells and desmin-positive Ito cells, we have attempted to identify and localize the cell population that first responds to the growth stimulus provided by PH in the AAF/PH model (23). Results from a combination of immunohistochemistry with OV-6 and desmin antibodies and autoradiography following [^{3}H]thymidine administra-

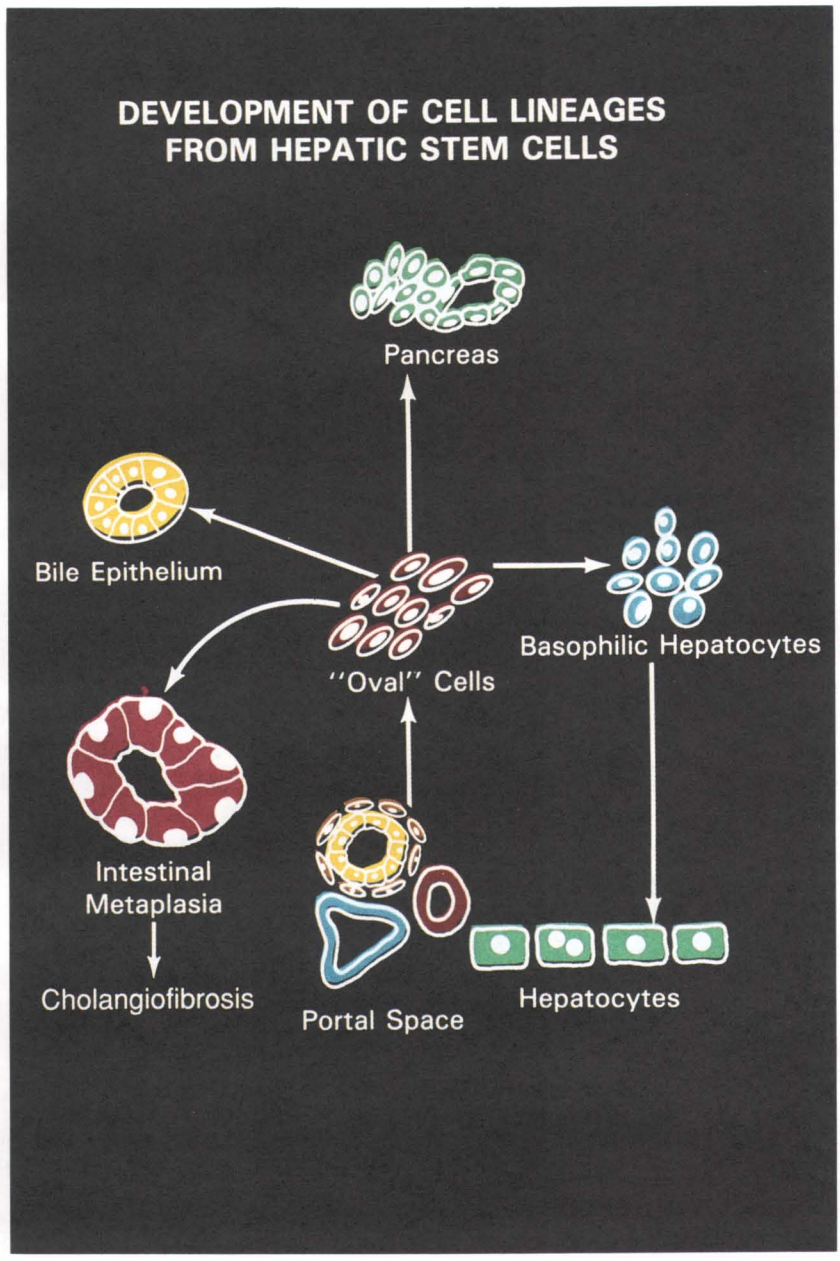

Figure 1. Schematic representation of the development of cell lineages from hepatic stem cells.

tion are shown in Figure 4. Both OV-6 and desmin-positive cells were labeled with [^{3}H]thymidine already at 4 hr after PH. The thymidine-labeled cells were present either as individual cells embedded in the periportal matrix or as a part of ductules in close proximity to the portal vein. The large ducts in the periportal space remained unlabeled 12 hr after the PH, but increased numbers of labeled OV-6-positive ductular cells as well as desmin-positive periportal cells were observed (Fig. 4; 23). By 72 hr, the majority of the cells in the periportal area were labeled, including approximately one half of the cells in the large duct, but the hepatocytes remained unlabeled (Fig. 4). In addition to the thymidine-labeled cells in the periportal area, we also observed both OV-6- and desmin-positive thymidine-labeled cells in the Glisson capsule at this early time point (23).

The present results are in agreement with data obtained in other models showing proliferation of ductular and periductular cells at early stages of stem cell activation (7, 21, 24). The observation that both OV-6- and desmin-positive thymidine-labeled cells are also seen in the Glisson capsule shortly after PH in the AAF/PH model may suggest that these cells could be part of the pluripotent cell population activated in this experimental system. However, no infiltration of OV-6-positive cells into the liver acinus is observed in the vicinity of the Glisson capsule in the AAF/PH model

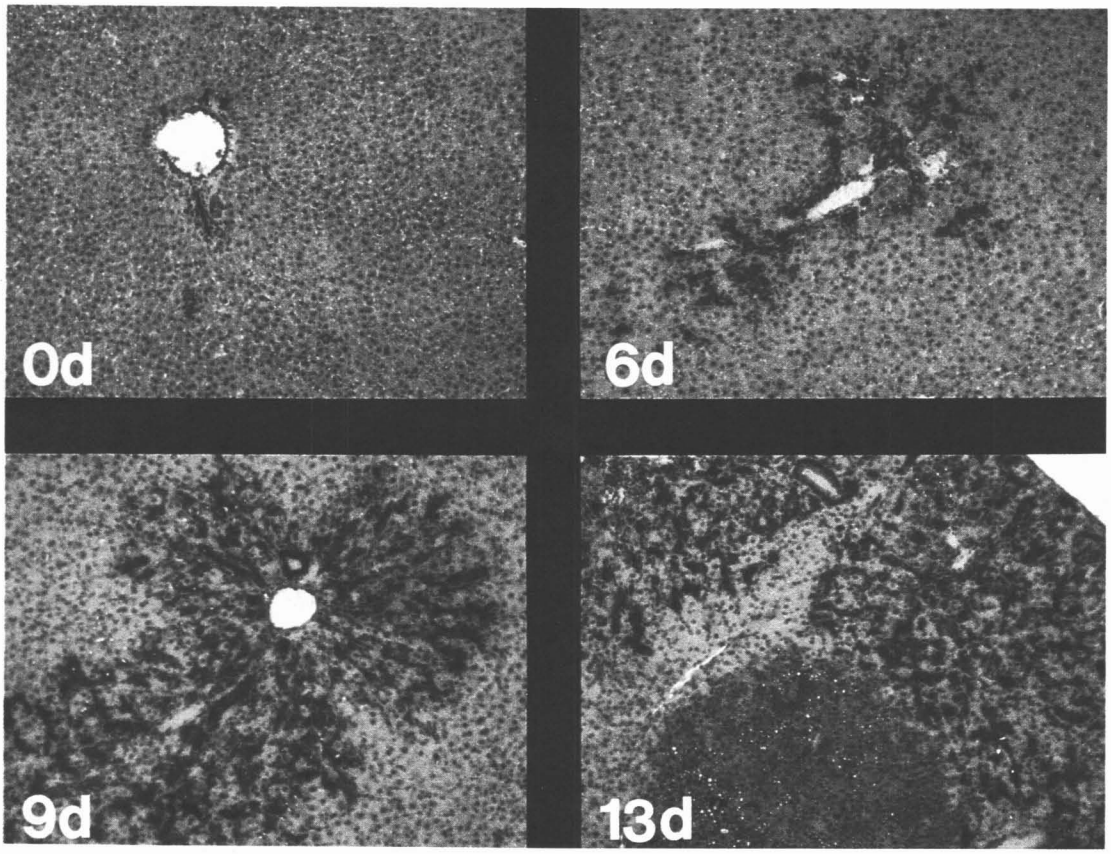

Figure 2. Development and expansion of the oval cell population in the AAF/PH model. Oval cells are identified by γ-glutamyltransferase staining. The time of partial hepatectomy is Day 0 (0d), and 6d, 9d, and 13d are days following the operation.

(23). Therefore, it seems unlikely that the cells of the Glisson capsule can contribute progenitors for the expanding oval cell population in this model.

Although the data from the detailed time course of hepatic stem cell activation in the AAF/PH model has given us new insights into the close association between a mesenchymal cell population (i.e., Ito cells) and the emerging oval cells (vide infra), we still have not resolved whether the hepatic stem cell compartment comprises only the bile ductular cells or includes a nondescript periductular cell population. Nevertheless, our data clearly show that the majority of thymidine-labeled OV-6-positive cells first observed after PH in the AAF/PH model reside in the bile ductules (23). Moreover, at the time when few of the OV-6-positive cells in the large bile ducts become labeled with thymidine, the ductular-derived OV-6-positive and thymidine-labeled "oval" cells have already started to infiltrate into the liver acinus (23). We conclude that the major source of oval cells, at least in the AAF/PH model, is derived from the lining cells of the biliary ductules, and that these cells constitute the hepatic stem cell compartment.

Growth Factors Involved in Hepatic Stem Cell Activation. During normal hepatic regeneration as well as during renewal from the stem cell compartment, several growth factors appear to affect the proliferation and differentiation of hepatic cells (22, 25, 26). We have, therefore, addressed the question of whether the same growth factors known to be involved in normal hepatic regeneration are also involved in the regeneration from the stem cell compartment.

There are three "primary" growth factors associated with normal liver regeneration, namely, transforming growth factor-α (TGF-α), hepatocyte growth factor (HGF), and acidic fibroblast growth factor (aFGF) (27). Each one of these growth factors is also capable of inducing replication of primary hepatocytes *in vitro* (27). In addition, transforming growth factor-β1 (TGF-β1) is also expressed during hepatic regeneration, and it has been proposed that TGF-β1 may provide at least part of the negative growth signals controlling the liver size following the compensatory hyperplasia that occurs after loss of liver mass (28).

The first cells entering DNA synthesis after PH in the AAF/PH model are the OV-6- and desmin-positive cells in the periportal area (Fig. 4). Coincident with the appearance of these cells, an increase in the expression of TGF-α, HGF, and TGF-β1 is observed, whereas increased expression of aFGF is first seen 24 hr later

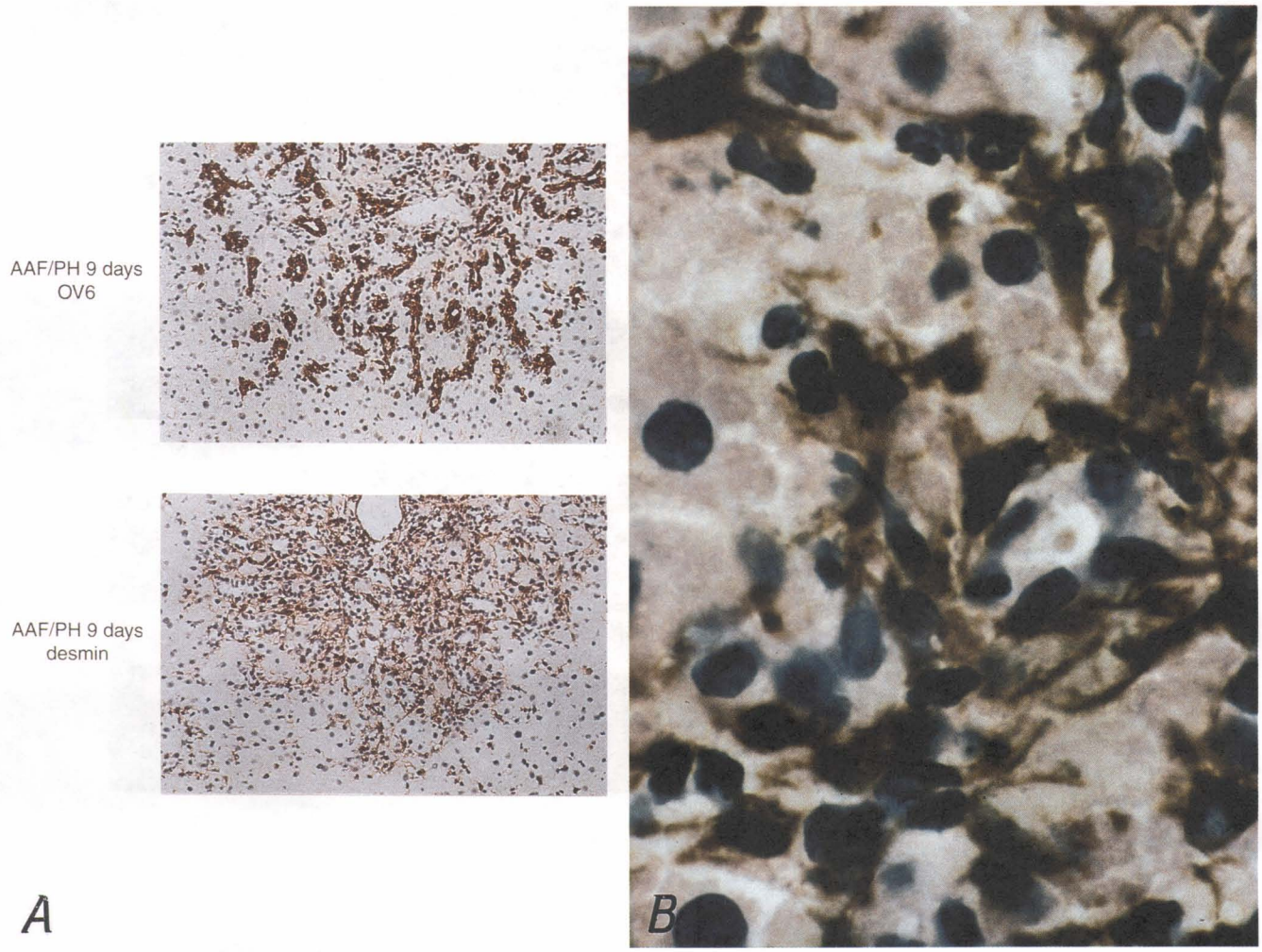

Figure 3. Relationship between OV-6-positive oval cells and desmin-positive Ito cells in the AAF/PH model. (A) Low power view of the oval and Ito cell distribution at Day 9 after partial hepatectomy in the AAF/PH model. (B) High power view illustrating the close association of desmin-positive Ito cells with oval cells in the same model.

(Fig. 5, A and B). All the growth factors are then expressed at high levels throughout the period of expansion and differentiation of the oval cells and return to levels seen in normal liver at the end of the regeneration process (Fig. 5B). The cellular distribution of the growth factor transcripts differs; TGF-α and aFGF transcripts are found in both Ito cells and oval cells (22, 26), whereas the HGF transcripts are found only in Ito cells (25). The TGF-β1 transcripts are located mainly in Ito cells, but the early population of oval cells also contain the TGF-β1 transcripts (29). The data on cellular distribution of all the receptors corresponding to the growth factors have revealed that all are located on oval cells (21, 24, 30).

The most straightforward interpretation of these data is that the same primary growth factors that are involved in liver regeneration from existing differentiated parenchyma also are involved in regeneration from the stem cell compartment. In fact, a slight and transient increase in the expression of the 2.1-kb transcript of α-fetoprotein (AFP), an indicator of liver stem cell activation (vide infra), is observed following PH of normal rat liver (H. Nakatsukasa, R. P. Evarts, S. S. Thorgeirsson, unpublished results). These observations support the role and possible importance of growth factors in stem cell activation. The data further suggest that the stem cell compartment may be transiently activated during regeneration after PH of a normal healthy liver.

We have discovered recently a novel ligand/receptor system, the stem cell factor (SCF)/c-*kit* system, that may be uniquely involved in the earliest stages of hepatic stem cell activation (31). In the AAF/PH model, the expression of both SCF and c-*kit* is seen before the expression of AFP (Fig. 6), and the levels of both the SCF and the c-*kit* transcripts decline before those of TGF-α, aFGF, HGF, and TGF-β1 (Fig. 5). We have also shown that in contrast to TGF-α, HGF, aFGF, and TGF-β1, the SCF/c-*kit* system is only slightly and transiently activated in regeneration after

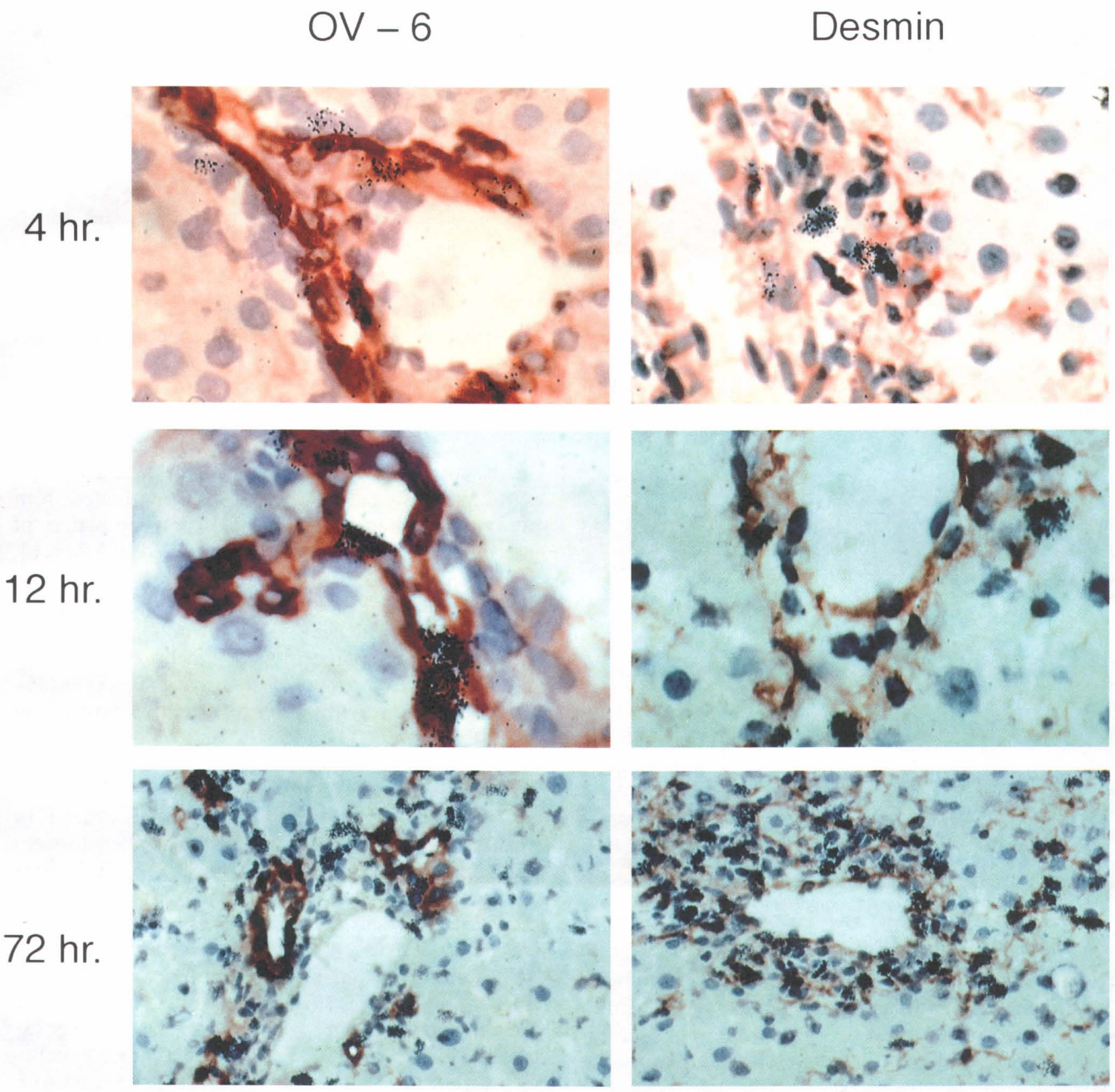

Figure 4. [^{3}H]Thymidine-labeled OV-6- and desmin-positive cells at early time points in the AAF/PH model. [^{3}H]Thymidine was administered intraperitoneally (1 μCi/b body wt) to the animals 2 hr before sacrifice.

PH in normal liver (31). The SCF/c-*kit* signal transduction system is believed to play a fundamental role in the survival, proliferation, and migration of stem cells in hematopoiesis, melanogenesis, and gametogenesis (32). It appears that in all cases, SCF and c-*kit* are involved in the early stages of stem cell activation. In the hemopoietic stem cell system it has also been demonstrated that SCF in combination with selective multipotential colony-stimulating factors can influence the relative frequency of progenitor cells committed to various lineages (33). Whether the SCF/c-*kit* system in the early hepatic stem cell population interacts with

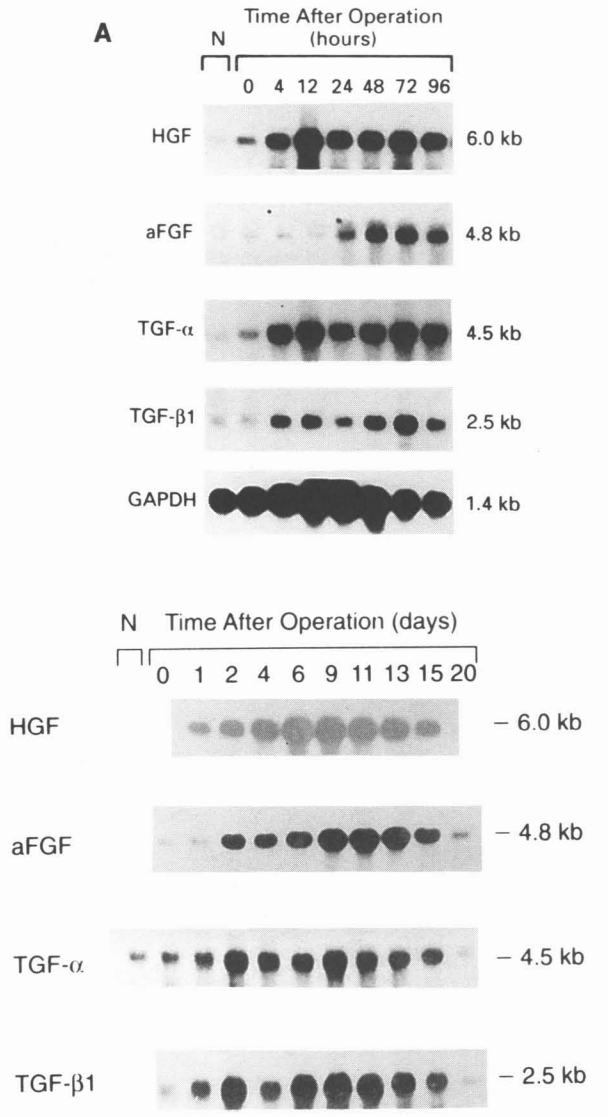

Figure 5. Northern blot analysis of HGF, aFGF, TGF-α, and TGF-β1 expression during activation of the hepatic stem cell compartment. Poly(A)$^+$ RNA was isolated at indicated time points and analyzed by Northern blot analysis (5 μg mRNA) and hybridized with ^{32}P-labeled riboprobes. Glyceraldehyde phosphatedehydrogenase (GAPDH) was used as control. N = normal liver. (A) Times in hours after the operation. (B) Times in days after the operation.

other hepatic growth factors in such a way as to influence the frequency of lineage commitment of progenitor cells is presently not known. However, this exciting possibility can now be tested experimentally.

Lineage Commitment of Hepatic Stem Cells. The developmental potential of oval cells, as indicated earlier, is not confined to the hepatic lineages, since they have been shown to differentiate into intestinal epithelia, and have been implicated in the development of pancreatic tissues (8, 11, 15–17). These and other data not reviewed here led us to hypothesize that a subpopulation of the early oval cells comprised a pluripotent stem cell population capable of differentiating into diverse lineages including hepatocytic, biliary, intestinal, and pancreatic progeny (Fig. 1). In a recent study on the ultrastructural and biochemical characteristics of nonparenchymal epithelial cell lines isolated from rat liver, we observed an unusual expression pattern of intermediate filament proteins composed of K8, K14, and vimentin (34–36). Although the *in vivo* counterpart of these cells have not been established conclusively, several lines of evidence support the notion that at least some of these nonparenchymal epithelial cell lines represent the *in vivo* progeny of the hepatic stem cells (36–40). It has also recently been reported that expression of K14 protein can be detected in a small population of epithelial cells located in the biliary ductules and in the mesothelial cells of the Glisson capsule (41).

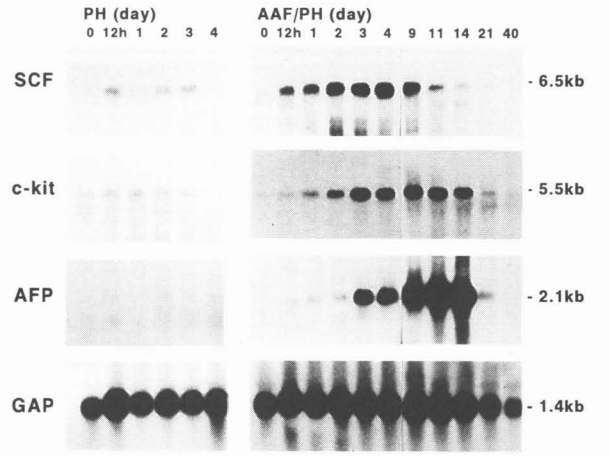

Figure 6. Northern blot analysis of SCF, c-*kit*, and AFP expression during liver regeneration. Rats were subjected to simple partial hepatectomy and AAF/PH treatment and sacrificed as indicated after PH. Poly(A)$^+$ RNA (5 μg mRNA) was analyzed by Northern blot analysis.

We have tested recently the hypothesis that K14 might be an early marker for the emerging hepatic stem cell population in the AAF/PH model.[2] When the time course of K14 and AFP expression is compared in the model, it is evident that K14 expression precedes that of AFP by at least 24 hr, but is then coexpressed with AFP during oval cell proliferation and differentiation (Fig. 7, A and B). These results suggested the possibility that subpopulations of oval cells might selectively express K14 and/or AFP. We have tested this possibility by use of *in situ* hybridization analysis of K14 and AFP expression in serial liver section from the AAF/PH model.[2] The results showed that K14 transcripts were initially detected in epithelial cells located in a subset of the ductular structures in the portal area. These K14-expressing cells were distinctly different from a second population of epithelial cells in the portal area express-

[2] Bisgaard HC, Nagy P, Ton A, Hu Z, Thorgeirsson SS. Differential expression of keratin 14 and alpha-fetoprotein during hepatic oval cell proliferation and differentiation. Submitted for publication.

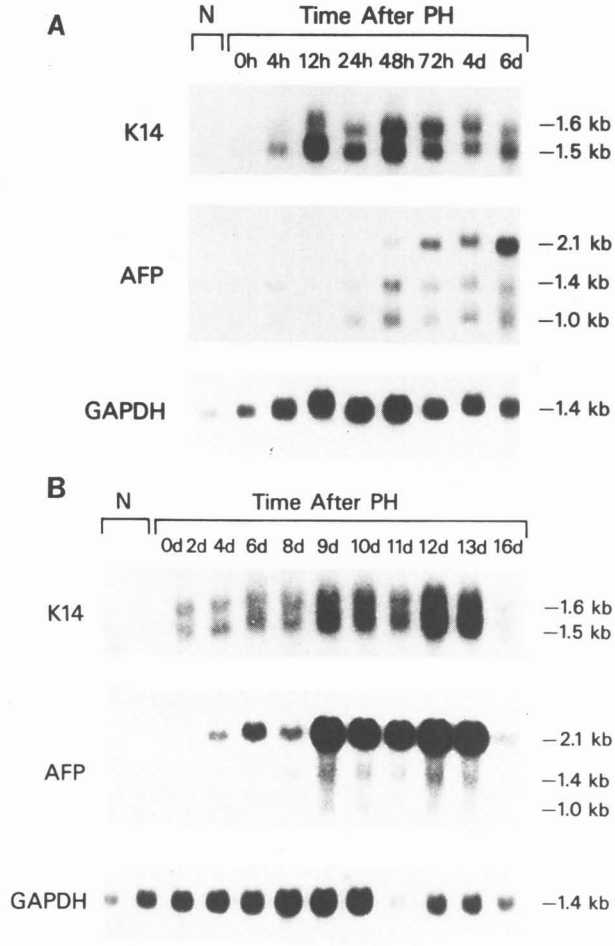

Figure 7. Northern blot analysis of k14 and AFP expression in the AAF/PH model. Poly(A)$^+$ RNA was isolated at indicated time points after the operation (PH), and analyzed by Northern blot analysis (10 μg mRNA) and hybridized with ^{32}P-labeled cDNA probes. (A) Time in hours after the operation. (B) Time in days after the operation.

ing high levels of AFP.[2] As the oval cell population expanded into the liver acini, both K14 and AFP continued to be expressed in a subpopulation of the oval cells. When the oval cells differentiated, K14 transcripts were found in both foci of small hepatocytes and in glandular forms of intestinal-type epithelia, whereas the AFP transcripts were detected only in the basophilic foci of hepatocytes.[2] Based on these data, we have proposed a hypothetical scheme for the development of hepatic stem cell lineages (Fig. 8).[2] We are suggesting that the earliest progeny from the stem cell has a K14$^+$/AFP$^-$ phenotype that can differentiate into two progenitors with K14^{2+}/AFP$^-$ and K14$^{(+)}$/AFP^{2+} phenotypes, respectively. The progenitor with the K14$^{(+)}$/AFP^{2+} phenotype may represent the committed bipotential progenitor destined to give rise to hepatocytic and bile epithelial lineages. This proposition is supported, at least in the AAF/PH model, by the observation that the K13$^{(+)}$/AFP^{2+} phenotype is by far the most numerous and the differentiation of the oval cell population

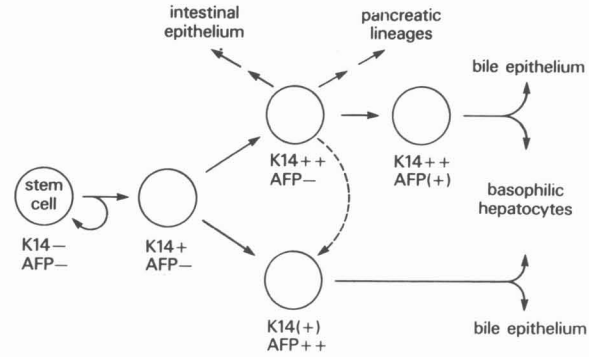

Figure 8. Hypothetical scheme for development of hepatic stem cell lineages.

is directed primarily toward the hepatic parenchyma. We are speculating that the progenitor with the K14^{2+}/AFP$^-$ phenotype is a multipotential progenitor capable of differentiating into hepatocytic as well as other lineages, such as intestinal and pancreatic epithelia. We have never, as noted above, observed expression of AFP in the glandular form of intestinal epithelia that is sometimes formed in the AAF/PH model, whereas the K14 transcripts are consistently present in these structures.[2] These data may lend some support to the notion that the progenitor with the K14^{2+}/AFP$^-$ phenotype may be the precursor of the hepatic intestinal type epithelia.

Further studies are clearly needed to define both the cellular and molecular biology of the hepatic stem cell compartment. However, using lineage markers such as K14 and AFP in combination with activation of the growth factor/receptor systems discussed above may provide a fruitful approach to study the mechanisms involved in the activation and differentiation of the hepatic stem cell and oval cell compartments.

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