Temporal Changes in the Expression and Distribution of Adhesion Molecules during Liver Development and Regeneration

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Abstract. We have compared by immunocytochemistry and immunoblotting the expression and distribution of adhesion molecules participating in cell-matrix and cell-cell interactions during embryonic development and regeneration of rat liver. Fibronectin and the fibronectin receptor, integrin $\alpha_5\beta_1$, were distributed pericellularly and expressed at a steady level during development from the 16th day of gestation and in neonate and adult liver. AGp110, a nonintegrin fibronectin receptor was first detected on the 17th day of gestation in a similar, nonpolarized distribution on parenchymal cell surfaces. At that stage of development haemopoiesis is at a peak in rat liver and fibronectin and receptors $\alpha_5\beta_1$ and AGp110 were prominent on the surface of blood cell precursors. During the last 2 d of gestation (20th and 21st day) hepatocytes assembled around lumina. AGp110 was initially depolarized on the surface of these acinar cells but then confined to the lumen and to newly-formed bile canaliculi. At birth, a marked increase occurred in the canalicular expression of AGp110 and in the branching of the canalicular network. Simultaneously, there was enhanced expression of ZO-1, a protein component of tight junctions. On the second day postpartum, presence of AGp110 and of protein constituents of desmosomes and intermediate junctions, DGI and E-cadherin, respectively, was notably enhanced in cellular fractions insoluble in nonionic detergents, presumably signifying linkage of AGp110 with the cytoskeleton and assembly of desmosomal and intermediate junctions. During liver regeneration after partial hepatectomy, AGp110 remained confined to apical surfaces, indicating a preservation of basic polarity in parenchymal cells. A decrease in the extent and continuity of the canalicular network occurred in proliferating parenchyma, starting 24 h after resection in areas close to the terminal afferent blood supply of portal veins and spreading to the rest of the liver within the next 24 h. Distinct acinar structures, similar to the ones in prenatal liver, appeared at 72 h after hepatectomy. Restoration of the normal branching of the biliary tree commenced at 72 h. At 7 d postoperatively acinar formation declined and one-cell-thick hepatic plates, as in normal liver, were observed.

CELL adhesion is of fundamental importance in morphogenesis and cytodifferentiation, and particularly in the establishment and maintenance of cell polarization in epithelia (Edelman, 1986; Ekblom et al., 1986; Trelstad, 1984; Takeichi, 1991; Simons and Fuller, 1985; Wiley et al., 1990). In differentiated simple epithelia intercellular adhesive junctions are found on contiguous (lateral) surfaces and interactions with the extracellular matrix occur along the basal plasmalemma that faces the basement membrane (Simons and Fuller, 1985). The liver is unique among simple epithelia in that fibronectin, a major extracellular matrix protein (Hynes, 1990) appears in nonpolarized fashion at apical (canalicular), lateral and basal (sinusoidal) cell surfaces, as distinct molecular forms (Hughes and Stamatoglou, 1987; Enrich et al., 1988), in contrast to other extracellular matrix components such as collagens, laminin, and proteoglycans which are exclusively located on sinusoidal cell surfaces (Hughes and Stamatoglou, 1987; Stow et al., 1985). There are at least two fibronectin-binding cell surface receptors in adult rat liver. The major receptor is integrin $\alpha_5\beta_1$, that interacts in an RGD-dependent way with the cell-binding domain of fibronectin (Johansson et al., 1987). Recently, we have identified a nonintegrin glycoprotein, AGp110, that interacts in an RGD-independent manner with an as yet unidentified region of fibronectin (Stamatoglou et al., 1990*a*). In previous studies we showed that integrin $\alpha_5\beta_1$, like fibronectin, is distributed along each of the surface domains of the polarized hepatocyte and, by contrast, AGp110 was located exclusively on the apical domain (Stamatoglou et al., 1990*b*). The biological significance of two fibronectin receptors in the liver is currently unknown but in vitro both $\alpha_5\beta_1$ and AGp110 colocalize at focal contacts on the periphery of the ventral surface of substratum-attached hepatocytes and appear to act synergistically in the establishment of a fibro-

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nectin-adherent epithelial layer (Stamatoglou et al., 1990*b*). On the basis of these and other experiments we suggested that AGp110 may be critically involved in transient interactions between hepatocytes and a fibronectin matrix during development of the fully polarized parenchymal cells (Stamatoglou et al., 1990*a*,*b*).

In the present study we have examined developmentally regulated changes in expression of fibronectin, AGp110 and integrin $\alpha_5\beta_1$ in embryonic and neonatal rat liver. As markers for establishment of polarization and morphological differentiation of the hepatic parenchyma, we have studied the expression of the tight junction protein ZO-1 (Stevenson et al., 1986) and the cadherin intercellular adhesion molecules desmoglein (DGI) and E-cadherin (Takeichi, 1991; Magee and Buxton, 1991; Koch et al., 1990; Goodwin et al., 1990; Wheeler et al., 1991), components of desmosomes (Schwarz et al., 1990) and adherens (intermediate) junctions (Boller et al., 1985), respectively. As a model of induced differentiation, we have also investigated the spatial distribution of AGp110 during proliferation and restoration of normal morphology following partial hepatectomy in the adult rat. The results indicate a developmentally regulated expression and distribution of AGp110 and a distinct correlation between the stable canalicular distribution of the receptor and the differentiated state of the parenchymal cell.

Materials and Methods

Partial Hepatectomy

Two-thirds partial hepatectomy was performed on Hypnorm-anaesthetized male Sprague-Dawley rats weighing 200–250 g according to established procedures (Higgins and Anderson, 1931). The operation involves removal of the median and left lateral lobes. Livers were surgically removed from sacrificed animals. Embryos and neonate animals were provided by the NIMR Animal facility.

Antibodies

Antisera against rat AGp110 and fibronectin were raised in rabbits as described (Stamatoglou et al., 1990*a*). Affinity-purified hen antintegrin $\alpha_5\beta_1$ and rabbit anti-α_5 were donated by Dr. S. Johansson (Biomedical Centre, Uppsala, Sweden). Rabbit antisera against DGI and E-cadherin were gifts from Dr. A. I. Magee (NIMR, London, U. K.) and Dr. R. Kemler (Max-Planck-Institut, Freiberg, Germany) and rat mAb against ZO-1 was kindly provided by Dr. B. R. Stevenson (University of Alberta, Edmonton, Canada). ABC reagents were purchased from Vector (Peterborough, U. K.), goat anti-rabbit/alkaline phosphatase, goat anti-rat/alkaline phosphatase and rabbit anti-chicken/alkaline phosphatase from Sigma (Poole, Dorset, U. K.).

Immunocytochemistry

Livers from hepatectomized and control rats were fixed by portal perfusion with 4% paraformaldehyde. Livers of embryos and neonate animals were fixed in ice-cold acetone. All tissues were embedded in paraffin wax. Sections were dewaxed in xylene, equilibrated in alcohols of decreasing concentrations, and hydrated in distilled water. Endogenous alkaline phosphatase was quenched by incubating for 15 min in 15% acetic acid. Washes and antibody dilutions were in PBS, pH 7.5, containing 0.05% Tween 80. Sections were incubated in this buffer for 30 min before immunostaining.

For AGp110 or fibronectin we used ABC reagents from Vector (Peterborough, U. K.) according to the manufacturer's specification. In outline, sections were incubated with primary antisera (1:200), then with anti-rabbit/biotin and finally with ABC reagent (a mixture of biotin and streptavidin/alkaline phosphatase). The sections were developed with BCIP/NBT, counterstained with haematoxylin and mounted in Apathy's mounting medium.

For staining of integrin $\alpha_5\beta_1$ we incubated with anti-$\alpha_5\beta_1$ IgG (15 μg/ml) and then with rabbit anti-chicken/alkaline phosphatase. Developing and counterstaining were as described above. In control experiments primary antisera were omitted, but otherwise the sections were processed as described above. All these controls were negative.

Tissue Extractions

Livers were minced and extracted in Tris-saline, pH 7.4, containing 200 mM octyl glucoside, 2 mM EDTA, 2 mM PMSF, and 2 mM NEM as described (Stamatoglou et al., 1990*a*). Alternatively, the tissue was first extracted in Tris-saline, pH 7.4, 2 mM PMSF, 2 mM NEM containing 15 mM CHAPS, spun at 10,000 *g* for 20 min and the supernatant collected. The pellet was then suspended in electrophoresis sample buffer (Laemmli, 1970) and boiled.

Electrophoresis

SDS-PAGE was carried out according to Laemmli (1970). Western blotting of proteins on nitrocellulose and detection using antibody probes was as previously described (Stamatoglou et al., 1987).

Results

Embryonic Development

At the 18th day of gestation fibronectin was distributed throughout the parenchyma, lining intercellular and open spaces, the latter presumably blood vessels and sinusoidal progenitors (Fig. 1 *a*). At this stage there is active haemopoiesis in the liver and blood cell precursors and megakaryocytes were prominently stained (Fig. 1 *a*). This distribution did not change up to term and postnatally fibronectin was also localized on all surface domains of polarized hepatocytes as previously described (Hughes and Stamatoglou, 1987; Enrich et al., 1988).

Integrin $\alpha_5\beta_1$ was abundant and uniformly distributed on surfaces of parenchymal cells in embryonic livers (Fig. 1 *b*) as in adult, differentiated hepatocytes (Stamatoglou et al., 1990*b*). In haemopoietic fetal liver $\alpha_5\beta_1$ was also present on the surface of blood cell precursors (Fig. 1 *b*).

AGp110 was first detected immunocytochemically in livers of 17-d-old embryos. The antigen was most conspicuous on surfaces of blood cell precursors (Fig. 1, *c* and *d*) and in the cytoplasm of megakaryocytes, which give rise to platelets (Fig. 1 *h*; 20 d embryo). In parenchymal cells, the distribution of AGp110 followed three morphologically distinct, but temporally overlapping, phases: (*a*) on day 17, faint, non-polarized stain was observed on the surfaces of parenchymal cells (Fig. 1, *d–f*). This persisted until the 21st day of gestation. (*b*) On day 20 extensive organization of hepatocytes around AGp110-positive lumina was detected (Fig. 1, *g* and *h*). AGp110 remained on all surfaces of acinar cells (Fig. 1 *h*) for a short time (up to day 21) and was thereafter polarized to apical surfaces (Fig. 1 *i*). Acinar lumina were often stellate in appearance and invariably larger in cross-section than typical bile canaliculi. Formation of acini was detected throughout the liver in late embryos and newborn animals (Fig. 1, *g–j*). (*c*) Eventually, from the 21st day of embryonic development, but mostly after birth, AGp110 was localized in typical bile canaliculi (Fig. 1, *i* and *j*) and overall there appeared to be an increase in the level of AGp110 after birth, though this was not always apparent in immunolocalization experiments (Fig. 1).

Enhanced expression of AGp110 in neonate liver was confirmed by immunoblotting experiments which focused on comparisons with other adhesion molecules. We used anti-

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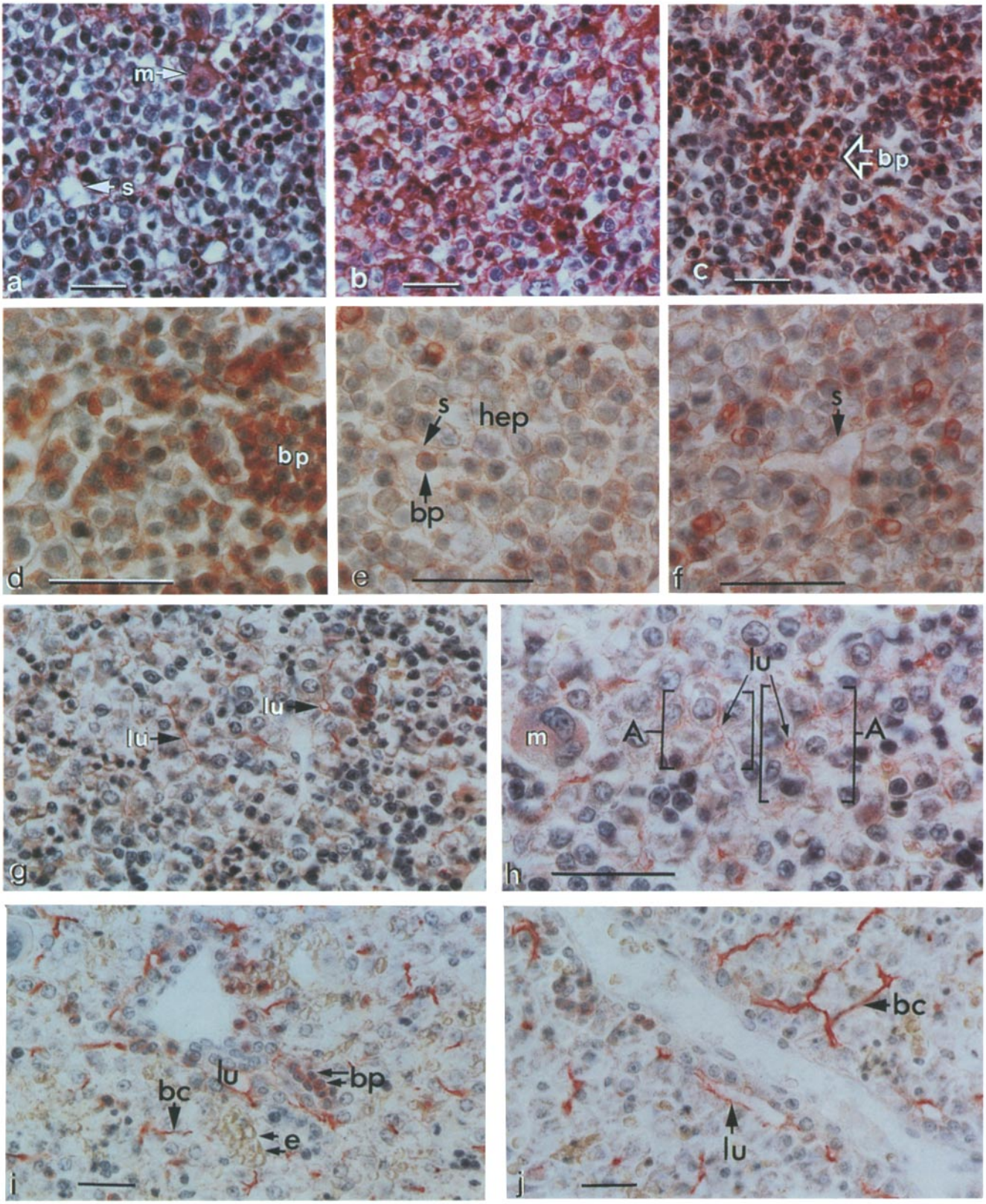

Figure 1. Distribution of fibronectin (*a*), integrin $\alpha_5\beta_1$ (*b*) and AGp110 (*c–j*) in embryonic (*a–i*) and neonatal (*j*) livers. Sections of liver were immunolabeled with specific antisera (see Materials and Methods). Red deposit of alkaline phosphatase chromogenic substrate shows the localization of the antigen. (*a*) Fibronectin in 18-d embryo liver. Pericellular stain. Note fibronectin-positive megakaryocytes (*m*) and blood vessels/sinusoidal (*s*) walls. (*b*) Integrin in 18-d embryonic liver. Strong pericellular stain is visible around all cells. (*c*) AGp110 in 17-d embryonic liver. Groups of blood cell precursors (*bp*) are positive for AGp110. (*d–f*) AGp110 in 17-d embryonic liver. Parenchymal cells (*hep*) exhibit faint pericellular staining and no evidence of polarization. Blood cell precursors (*bp*) show strong surface expression of AGp110. Linings of sinusoidal-like spaces (*s*) are AGp110-positive. (*g–h*) AGp110 in 20-d embryo. Parenchymal cells organize around AGp110-positive lumina (*lu*). At higher magnification (*h*) it is evident that hepatocytes participating in this acinar formation (*A*) still exhibit pericellular nonpolarized staining as in earlier embryos. Megakaryocytes (*m*) show AGp110 expression in cytoplasm. (*i*) AGp110 in 21-d embryo. Protein starts to concentrate in canalicular-like structures (*bc*). Large duct with AGp110-positive lumen (*lu*) could be a bile duct precursor since the whole structure around the large blood vessel resembles a portal space. At this stage there is scant evidence of non-polarized AGp110 distribution. Mature erythrocytes (*e*) are AGp110-negative. (*j*) AGp110 in 1-d neonatal liver. At this stage conspicuous AGp110-positive bile canaliculi (*bc*) containing AGp110 are found. Bar, 50 μm.

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sera against fibronectin, integrin subunit α_5, AGp110, DGI, E-cadherin, and ZO-1. DGI, E-cadherin and ZO-1 are components of desmosomes (Schwarz et al., 1990), intermediate-type junctions (Boller et al., 1985), and tight junctions (Stevenson et al., 1986), respectively. Fibronectin and the α_5 subunit of integrin were expressed at a steady level from the 16th day of gestation up to adult age (Fig. 2, *a* and *b*, respectively). E-cadherin was also readily detected at all stages, expression of DGI increased noticeably, and AGp110 and ZO-1 dramatically, during embryogenesis (Fig. 2). A two-step extraction procedure was followed for AGp110, DGI, and E-cadherin. Samples were first extracted in the nonionic detergent CHAPS and the insoluble residue was then extracted in SDS buffer. Since DGI and E-cadherin are insoluble in nonionic detergents once incorporated into mature desmosomes and intermediate junctions (Nelson et al., 1990; Takeichi, 1991; Penn et al., 1989; Pasdar et al., 1991) and AGp110 was found to be associated with actin-containing microvilli (Stamatoglou et al., 1990*b*), this sequential extraction permitted us to investigate the assembly of intercellular junctions and the stable association of AGp110 with the actin cytoskeleton. Synthesis of AGp110 in the embryo was low compared with the dramatic increase observed after birth (Fig. 2 *c1*). The amount of AGp110 in the CHAPS-insoluble (presumably actin-associated) fraction grew 2 d postpartum and in adult normal livers more than half of the total detected AGp110 was insoluble in CHAPS (Fig. 2 *c2*). Expression of DGI and E-cadherin in CHAPS-soluble fractions, i.e., in a form not incorporated into junctions was constant and steady from embryo to adult (Fig. 2, *d1* and *e1*), but an increase in the amount of these proteins in CHAPS-insoluble/SDS-soluble fractions of livers of 1–2-d-old animals (Fig. 2, *d2* and *e2*, respectively) indicated active formation of desmosomes and intermediate junctions occurring postnatally and simultaneously. Increased synthesis of ZO-1 was also conspicuous in livers of newborn animals (Fig. 2 *f*). Overall, therefore, although some intermediate and tight junctions probably form in embryonic liver (Fig. 2, *e2* and *f*), full assemblies of intercellular junctions on the same scale as in the adult liver did not apparently materialize until after birth.

Liver Regeneration

Regeneration of the liver was examined in the adult organ after partial hepatectomy. Western blotting experiments of livers at various stages of regeneration (12 h to 8 d) failed to reveal a change in expression of AGp110 apart from a slight reduction 48 h postoperationally (not shown). However, although eventually all hepatocytes are potentially proliferative (Alison, 1986), there is a spatial distribution of proliferation during the initial phase of restorative growth (Fabrikant, 1967; Alison, 1986) and hepatocytes in periportal areas may divide twice, while cells close to the central vein divide only once (Rabes, 1976). Hence, we resorted to immunocytochemistry to study more local changes in the distribution of AGp110 in hepatic areas of high or low proliferating activity.

At 12 h after partial hepatectomy there was no evidence as yet of mitoses nor of an alteration in the pattern of distribution of AGp110 (Fig. 3 *b*) compared with normal adult liver (Fig. 3 *a*).

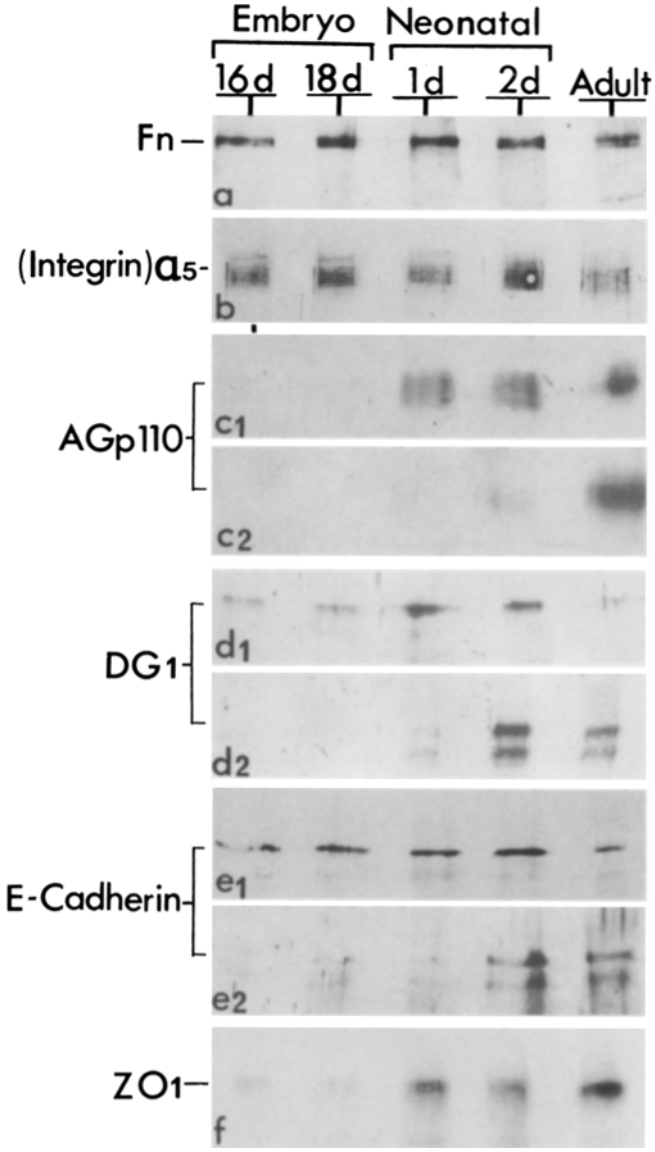

Figure 2. Relative amounts of fibronectin (*a*), integrin α_5 subunit (*b*), AGp110 (*c*), DGI (*d*), E-cadherin (*e*), and ZO-1 (*f*) during development. Livers were extracted either with 200 mM octyl glucoside (*a*, *b*, and *f*), or subjected to sequential extraction (*c–e*) with 10 mM CHAPS (*c1*, *d1*, and *e1*) followed with 5% SDS (*c2*, *d2*, and *e2*). Equal amounts of protein from each extract were run in SDS-PAGE under reducing conditions, blotted onto nitrocellulose and probed with specific antisera. (*a*) Fibronectin expression appears constant during development, in the neonate and adult livers. (*b*) Expression of the α_5 subunit of integrin remains approximately constant, as fibronectin. (*c*) Expression of AGp110 is kept at a low level during development and increases markedly after birth. Amount of AGp110 in CHAPS-insoluble fraction (*c2*) increased from the second day post-partum and in the adult more than half of total AGp110 is associated with that fraction. (*d*) DGI in CHAPS-soluble fraction remains constant (*d1*), but expression in insoluble fraction (*d2*) increases after birth. (*e*) E-cadherin, like DGI, is shown in constant amounts in CHAPS extracts (*e1*), but an increase in the insoluble residue (*e2*) occurs after birth. (*f*) Expression of ZO-1 increases after birth.

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At 24 h a distinct change in the overall distribution of AGp110 became obvious: staining remained conspicuous in the vicinity of central veins but was markedly reduced in interportal and periportal areas (Fig. 3 *c*), that is in parts of liver close to the afferent portal blood supply. Most of the dividing cells were found there (Fig. 3, *d* and *e*), as expected (Fabrikant, 1967; Alison, 1986). The reduction in staining observed at low magnification views (Fig. 3 *c*) was due to a less extensive, apparently discontinuous, canalicular network (Fig. 3, *d* and *e*). At the cellular level, no evidence of depolarized localization of AGp110 could be detected, even in mitotic cells (Fig. 3, *d* and *e*). In the majority of these cells the cleavage furrow was vertical to the axis of distally apposed bile canaliculi (Fig. 3, *d* and *e*) as previously described (Bartles and Hubbard, 1986). Close to the central veins, virtually no divisions were observed and the AGp110-positive canalicular network appeared mostly normal (Fig. 3 *f*). Between 24 and 48 h, proliferating activity spreads throughout the liver (Alison, 1986), and at 48 h we observed dividing cells randomly distributed in the liver parenchyma. AGp110 was still localized in bile canaliculi but staining and extent of canalicular branching appeared reduced (Fig. 3 *g*). The number of mitoses within lobules appeared to have declined at 48 h after hepatectomy in comparison to the number of cell divisions detected at 24 h, in accordance with other studies (Alison, 1986).

At 72 h after resection an overt tendency of hepatocytes to organize around stellate, AGp110-positive lumina was manifested (Fig. 3, *h* and *i*). A distinct spatial gradation was apparent in the occurrence of this phenomenon: most acinar formations were observed in periportal areas (Fig. 3, *h* and *i*), where proliferating activity had been high, and the least were seen in the central area of the lobule (not shown). In transverse view the lumen of acini occasionally appeared to split apart two-cell thick hepatic plates (Fig. 3 *i*).

At 7 d postoperatively there were few acini and the typical AGp110-positive canalicular network and one-cell thick hepatic plate structure were almost restored, though some two-cell-thick plates were still seen (Fig. 3 *j*).

Discussion

This study has demonstrated a distinct correlation of the distribution of AGp110, a putative fibronectin receptor (Stamatoglou et al., 1990*a*) with the state of parenchymal differentiation and restorative cell proliferation during rat liver development and regeneration. During developmental, restorative, or neoplastic growth (Ogawa et al., 1979; Stamatoglou et al., 1991), the liver parenchyma may exhibit a certain degree of apparent structural heterogeneity, but basically three types of morphology could be detected: unpolarized or partially polarized cells, acinar formation and fully differentiated, polarized hepatocytes. The first type of morphology was detected in embryos and poorly differentiated carcinomas, plate formation occurs in differentiated adult liver and acini are observed in certain types of carcinomas, in late embryonic livers and at the postreplicative stage during regeneration.

Hepatocytes appeared essentially nonpolarized at the 16–20th day of gestation, as is evident by the pericellular distribution of AGp110, the disorganized state of the parenchyma and the low expression of the tight junction component ZO-1 and intermediate junction protein E-cadherin. Tight junctions occlude and designate parts of apposed contiguous surfaces to the biliary tree, thus being a prerequisite of polarization. E-cadherin has been directly implicated in epithelial polarization (Gumbiner and Simons, 1986). Canalicular structures that have been observed in livers of early embryos (Luzzatto, 1981) may not be functionally differentiated, since tight junctions do not mature morphologically before the end of gestation (Montesano et al., 1975). In common with AGp110, other canalicular plasma membrane antigens exhibit a depolarized distribution on the surface of embryonic hepatocytes (Moreau et al., 1988) including the intercellular adhesion molecule C-CAM (Odin and Öbrink, 1988).

The transient depolarized state of AGp110 in embryonic hepatocytes contributes to the maximizing of cell–fibronectin interactions with both available receptors since $\alpha_5\beta_1$ and fibronectin display a pericellular distribution too. During that time AGp110 has a wider distribution but much lower expression than in adult tissue. In earlier embryos AGp110 could not be detected (our unpublished results). Cellular interactions with fibronectin could provide intercellular cohesion in the liver at that stage of development. Proper intercellular adhesion seems to be lacking since pericellular distribution of C-CAM (Öbrink, 1991) peaks at the end of gestation (Odin and Öbrink, 1988) and limited formation of intermediate junctions and desmosomes occurs prepartum. In the adult organ AGp110 is located exclusively in bile canaliculi and the liver is perhaps the only simple epithelium where fibronectin is also located in this apical domain, in addition to the basolateral surface (Hughes and Stamatoglou, 1987; Enrich et al., 1988). AGp110 could therefore interact with fibronectin at that site or perhaps be involved in sorting out and transport of fibronectin isoforms (Enrich et al., 1988) as discussed before (Stamatoglou et al., 1990*a*).

Of interest was the high level of expression of AGp110 on blood cells. Most of these are likely to have been precursors of mature cells with a high proportion of erythroblastoid cells. However, mature erythrocytes did not have any AGp110. Interestingly, the integrin fibronectin receptor is also lost during erythroid differentiation (Patel and Lodish, 1986). Similarly, megakaryocytes were positive, but after a blood fractionation we could not detect the protein in platelets. Mature mononuclear cells are AGp110-positive (our unpublished results).

The formation of structurally differentiated liver after birth coincided with a considerable increase in the level of expression of AGp110, of the tight junction protein ZO-1, of the desmosomal component DGI and the intermediate junction protein E-cadherin. Sequential extractions with nonionic detergent and then with SDS established that the increased expression of cadherins was due to protein incorporated into structures insoluble in nonionic detergents. This is known to signify linkage of junctional molecules to insoluble cytoskeletal elements and assembly of mature junctions (Nelson et al., 1990; Takeichi, 1991; Penn et al., 1989; Pasdar et al., 1991). Labile E-cadherin and DGI detected in embryonic livers might be non-functional or form "immature" junctions (Nagafuchi and Takeichi, 1988; Takeichi, 1991; Garrod and Fleming, 1990). Detection of AGp110 in the insoluble residue, from the 2nd day postpartum, was most likely due to an association with actin fibers of canalicular microvilli,

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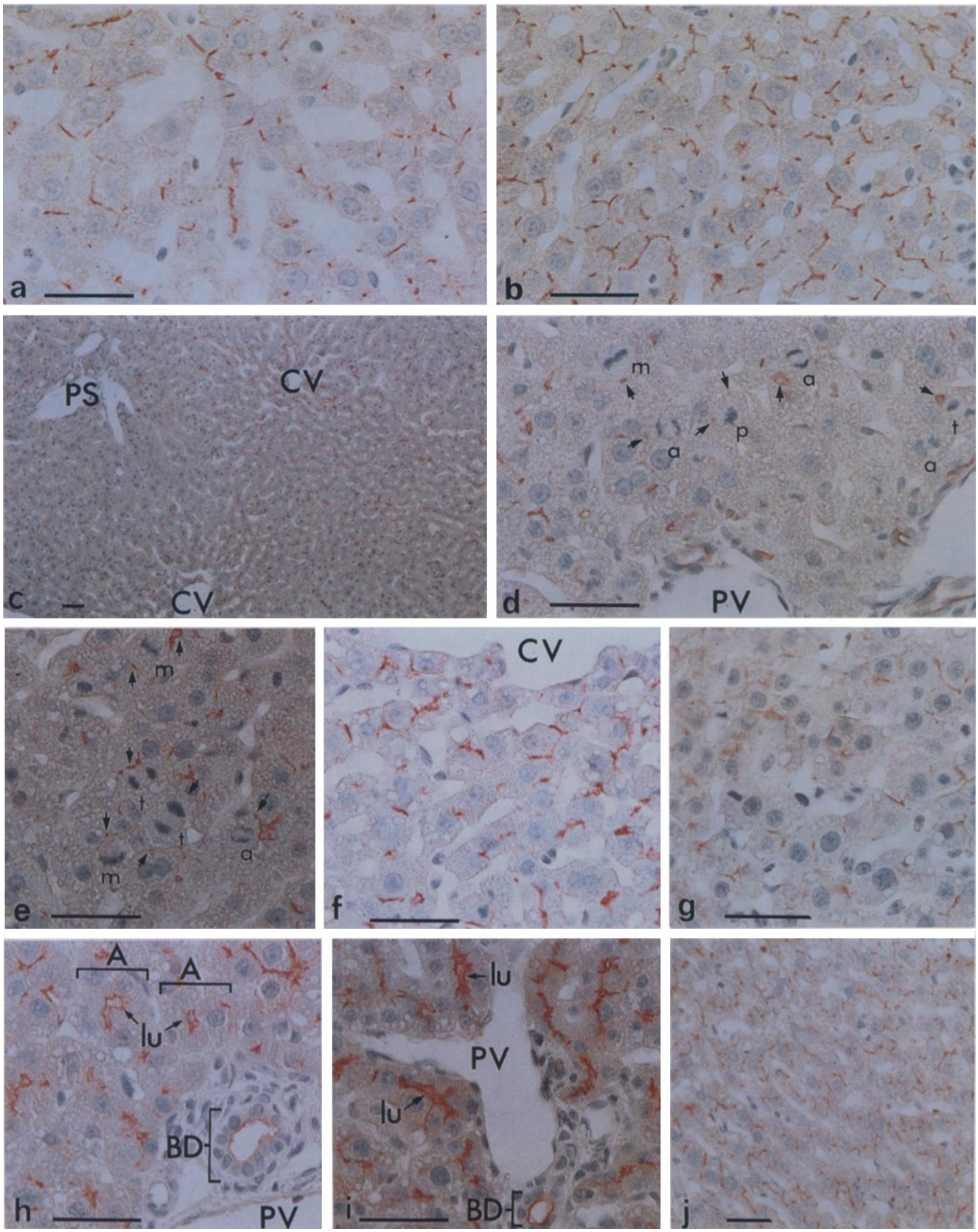

Figure 3. Immunolocalization of AGp110 in regenerating rat livers. Tissues were removed at intervals after partial hepatectomy and processed for immunocytochemistry as described in Materials and Methods. (*a*) Control adult liver. AGp110 is localized in bile canaliculi. (*b*) 12 h after partial hepatectomy. No detectable change from control liver in distribution of AGp110. (*c*) 24 h postoperatively. Canalicular stain appears weak around portal spaces (*PS*) but stronger in the vicinity of central veins. (*CV*). (*d* and *e*) 24 h postoperatively. Detail of proliferating periportal parenchyma. Canalicular network is diminished and discontinuous. Arrows point at discernible canaliculi of

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where AGp110 is located in differentiated hepatocytes (Stamatoglou et al., 1990*b*). The experiments therefore indicate that full junctional assembly occurred concurrently with an increase in AGp110 expression and association of this fibronectin receptor with the actin cytoskeleton. This observation may relate to "colony formation" of hepatocytes in primary culture (Stamatoglou et al., 1990*b*) during which cell-substratum focal contacts containing receptors integrin $\alpha_5\beta_1$ and AGp110 are redistributed from the ventral periphery of adjacent hepatocytes to the periphery of the whole colony and actin fibers in these cells become interlinked and continuous, presumably interconnected by E-cadherin-containing intermediate junctions. Colony formation in vitro strongly resembles embryonic compaction, a cadherin-dependent event (Adamson et al., 1990), and indicates an integration of cell-matrix and cell-cell interactions, analogous to the developmentally regulated, simultaneous association of cadherin molecules and AGp110 with junctions and the actin cytoskeleton.

Overt depolarization was not detected during regeneration either in the replicative or post-replicative stage. Although a less extensive canalicular network was observed in the most actively proliferating area of the parenchyma (zone 1 of the liver "acinus" as defined by Rappaport, 1980), AGp110-positive bile canaliculi were distinguished even in mitotic cells. However, during division, the rounding up of the cell, formation of the cleavage furrow and the ensuing dichotomy would inevitably expose part of the canalicular channels that surround each and every hepatocyte, thus instating a "partial" polarization. This would occur if the plane of division involves either basolateral surfaces only, as described herein and elsewhere (Bartles and Hubbart, 1986) or includes the canalicular domain too, a phenomenon we and others (Bartles and Hubbart, 1986) observed occasionally. To avert constant mixing of bile and blood we postulate that the part of the canalicular network that intersects the plane of the division furrow is sealed off prior to cytokinesis by formation of "belt" tight junctions (zonulae occludens) between adjoining cells facing the cell in mitosis (Fig. 4). The canaliculi which are located at the poles remain connected to the rest of the biliary tree. This would explain the reduced and discontinuous canalicular network we observed at 24 h after hepatectomy at sites close to portal spaces, and then, at 48 h, in the whole liver when the proliferating activity had spread throughout the parenchyma. Such tight junction formation is known to occur in follicular epithelial cells (Zeligs and Wollman, 1979) during cytokinesis. Furthermore, this obliteration of part of the apical surface may necessitate a suitable adaptation of protein transport (endosomal) mechanisms within the cell and such changes have been detected (Enrich et al., 1991). Finally, the restoration of the AGp110-positive canalicular network commences between 48 and 72 h and peaks soon after that. This scheme accommodates the observations reported here and falls in between predictions reported elsewhere, according to which hepatocytes preserve all normal contacts (Bartles and Hubbard, 1986) or loose polarity (Odin and Öbrink, 1988): the hepatocyte in mitosis does retain some, but not all, normal contacts and apical domains and is therefore essentially polarized during division (Fig. 4). Our present results, in agreement with Odin and Öbrink (1988), suggest that daughter cells move out of the hepatic plate and tight junctions do not form de novo immediately after division. Furthermore, during and after cell division, the canalicular remnants linking the mi-

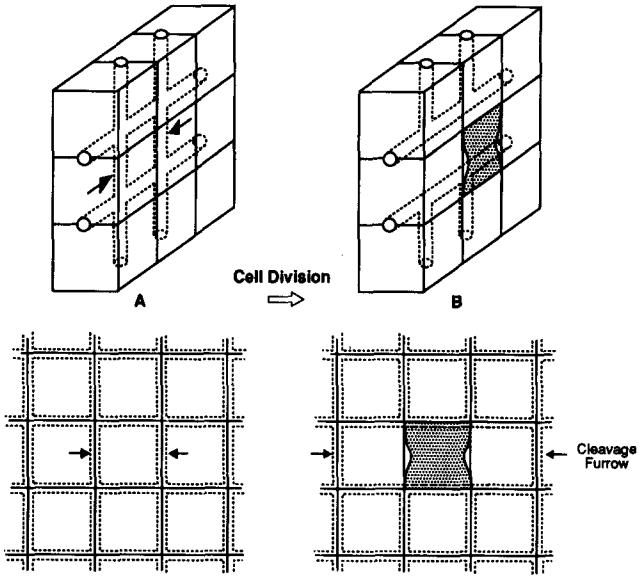

Figure 4. Schematic illustration of canalicular alterations during cell division in regenerating liver (see Discussion), presented in three-dimensional (*top*) and transverse view (*bottom*) of a liver plate. For simplicity, cells are portrayed in cubic form, but a more irregular polyhedral shape is likely. Dotted lines demarcate the canalicular network and solid lines cell borders. Stippled cell in *B* undergoes cell division. The plane of division is vertical to the hepatic plane (*B*) as frequently observed (see Results). Canaliculi that lie adjacent to the constriction caused by the cleavage furrow (indicated by *arrows* in *A*) are obliterated by tight junction formation that seals off the rest of the network to prevent constant mixing of blood and bile. Extensive rounding-up of mitotic cells could result in short, anastomosed segments of canaliculi (not shown). The scheme accommodates the observed reduction in the extent of branching of the canalicular network in proliferating parenchyma at 24 and 48 h postoperatively.

dividing cells. Axis of canaliculi is usually vertical to the plane of division. The stage of mitosis is indicated below each dividing cell. *p*, prophase; *m*, metaphase; *a*, anaphase; *t*, telophase. *PV*, portal vein. (*f*) 24 h postoperatively. Detail of nonproliferating parenchyma close to central vein (*CV*). AGp110-positive canalicular network appears as extensive as in control liver (*a*). Occasionally, dilated canaliculi were observed in these areas. *CV*, central vein. (*g*) 48 h after hepatectomy. AGp110-positive canalicular network appears less extensive throughout the lobule than in control livers (*a*) and similar to proliferating parenchyma at 24 h (*d* and *e*). (*h* and *i*) 72 h after partial hepatectomy. Numerous acinar formations (*A*) with AGp110-positive lumina (*lu*) were detected. The highest concentration of these acini was seen in periportal areas. Occasionally (*i*), lumina appear to separate double-cell plates. The structure of acini (*A*) is distinct from bile ducts (*BD*). *PV*, portal vein. (*j*) 7 d postoperatively. Normal one-cell-thick hepatic plates, typical sinusoids and AGp110-positive canalicular network are observed by that time. Bar, 50 μm.

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totic or postmitotic cell(s) to the hepatic plate could serve as reinforcements of intercellular adhesion by virtue of fibronectin–AGp110 interactions, fibronectin in this case being the link securing adhesive bonds between apposing cells. Overall, proliferation after partial hepatectomy results in a discontinuous and less extensive canalicular network, in multi-cell-thick plates and then in acinar formation before one-cell-thick plates with normal biliary channels are seen. The observed depolarization of distribution of the intercellular adhesion molecule C-CAM (Odin and Öbrink, 1988; Öbrink, 1991) during liver regeneration may reflect a relocation of the protein on existing separate domains of the cell surface and not, necessarily, complete structural depolarization as that seen in embryonic liver.

This study and other investigations (Ogawa et al., 1979; Stamatoglou et al., 1991) have indicated that acinar organization of hepatocytes is a common feature of developmental, restorative and neoplastic growth. These structures always appear in hyperplastic sites during or at the end of a proliferation cycle and degenerate when proper plate formation is established (in neonate liver or after regeneration) or persist, in neoplasia, probably giving rise to adenomas (Stamatoglou et al., 1991). The presence of the apical glycoprotein AGp-110 in the lumen of these acini suggests a canalicular origin for the acinar lumina and indeed they appear as stellate, dilated canaliculi. In transverse views of two-cell-thick plates of regenerating liver at 72 h after hepatectomy, lumina appear to form in between parallel rows of hepatocytes thus indicating a plane of shear to form one-cell-thick plates. In regenerating liver acinar structures could be clearly differentiated from bile duct epithelium, but in embryos some AGp110-positive lumina could indicate bile duct formation (e.g., Fig. 1, *i* and *j*). We would like to emphasize that the acinar structures described herein and elsewhere (Ogawa et al., 1979; Stamatoglou et al., 1991) refer to ductal cell formations and are not related to the so-called liver "acinus," as defined by Rappaport (1980), that describes a parenchymal area in between central veins and portal spaces of adjoining lobules.

In conclusion, we have attempted to rationalize our observations on adhesion molecules during hepatic growth in the context of previous observations. AGp110 is developmentally regulated: the protein appears first in a nonpolarized fashion but is later confined to the apical surface domain of the differentiated hepatocyte. Stable linkage of AGp110 to cytoskeletal elements coincides temporally with intercellular junctional assembly. Finally, we claim that although a complicated structural heterogeneity may apparently be manifest during hepatic growth, there is a small number of cardinal features in all morphological adaptations during either developmental, restorative or neoplastic growth. Identification of adhesive mechanisms in a simpler, controlled process, such as restorative growth, might therefore offer clues to more fundamental issues on morphogenetic development or neoplastic growth.

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