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From a common progenitor to distinct liver epithelial phenotypes

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The vertebrate liver presents a fascinating case study for how cell form is optimized for function. To execute its duties the liver assembles two distinct lumen-forming epithelial phenotypes: Firstly, cords with a branched, capillary-like luminal network formed between hepatocytes (bile canaliculi); and secondly, tubular ducts formed by biliary epithelial cells arranged around a central cavity and connected to the bile canaliculi. How these remarkably different epithelial polarity phenotypes are generated and joined into a contiguous luminal network are major unresolved questions. Recent studies have characterized the divergence of the two epithelial lineages from common progenitors, described the coordination of bile canaliculi formation with bile duct branching during biliary tree morphogenesis and implicated RhoA-dependent E-cadherin adhesion in the decision to polarize with hepatocytic or biliary phenotype.

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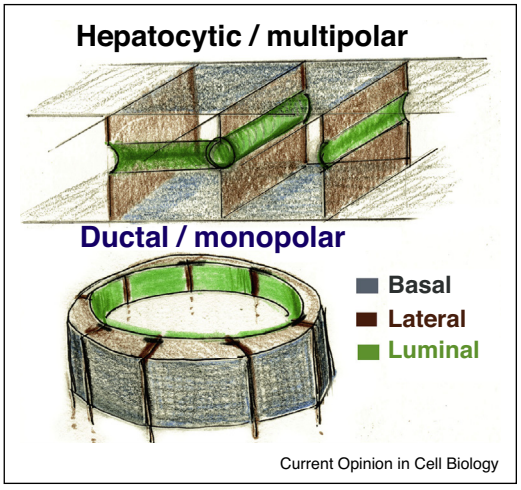
The liver's two epithelial cell types — hepatocytes and biliary cells (also called cholangiocytes) — share all epithelial hallmarks, which include the establishment of distinct apical and basolateral surface domains that are separated by tight junctions. Yet both cell types differ drastically in how they organize these domains to suit their different functions: Hepatocytes arrange in cords that weave through a system of fenestrated blood vessels (the sinusoids). This allows hepatocytes to maximize their basolateral surface area used for molecular exchange with the portal blood supply and hence freely secrete serum proteins while absorbing solutes and xenobiotics. Bile duct cells, by contrast, organize into a closed monolayered tubule that transports bile. On the cellular level, bile duct cells organize in monolayers where each epithelial cell contributes its single apical domain to a central lumen, that is, they are monopolar (Figure 1, 'Ductal'). Hepatocytes, by contrast, are multipolar because they simultaneously form luminal surfaces with multiple neighbors thereby generating bile canaliculi, a branched capillary luminal network (Figure 1, 'Hepatocytic'). While bile duct cell polarity is similar to that of most epithelial tissues, hepatocyte polarity is unique. In the following chapters I will discuss evolving concepts that explain how the different epithelial phenotypes might be generated and how they join to form contiguous luminal tracts.

The polarization sequences in the embryonic and adult liver

The hepatic progeny originates from a monolayered epithelial tube, the foregut (Figure 2a, E8.5). Hepatic specified foregut cells proliferate and invade the surrounding mesenchyme as nonpolar liver epithelial progenitors called hepatoblasts (HBs, Figure 2a, E9.5), which give rise to both hepatocytes and biliary cells [1,2]. The first polarized structure to emerge from the HB mass in mammals is a monolayer of adherent, strongly Ecadherin-positive cells around the portal veins, called the ductal plate at around E15.5 in the mouse. Induced by the periportal mesenchyme, these cells acquire biliary characteristics [3]. Parts of the ductal plate develop into bile ducts, while others have been proposed to become hepatic stem cells [4,5]. HBs outside the vicinity of portal veins develop into hepatocytes. Bile canaluculi become apparent only in late gestation (E18), and continue to elongate postnatally.

Recently, the morphological description of ductal and hepatocyte specification has been underpinned by RNA sequencing based transcriptome analysis of individual HBs taken at different points between HB emergence and late gestation [6••]. Guided by established markers for bipotential and lineage committed HBs, the authors performed RNA sequencing on sorted cells yielding two principle components that corresponded to the biliary and hepatocytic lineages. Their 'pseudotime' transcriptome analysis of these components led them to several profound conclusions: Firstly, Hepatocyte specification begins shortly upon emergence of the liver bud from the foregut and HBs gradually move toward the hepatocytic fate in a synchronous manner. Secondly, HB-to-biliary specification represents a sharp branching from the default hepatocytic differentiation program during a

Figure 1



The organization of polarized surface domains in hepatocytes and bile duct cells: Hepatocytes are multipolar; they form multiple luminal surfaces that interrupt their cell–cell contacting domains, and they have two basal domains. Bile duct cells are monopolar; they establish one luminal and one basal surface opposite from each other and perpendicular to their cell–cell contacting domains.

limited developmental time window (between E11.5 and E14.5). This branching is associated with the silencing of HB and hepatocyte markers and the induction of biliary-specific markers. Chief among induced cellular processes/pathways were those related to cell adhesion, ERK1/2 signaling and tube morphogenesis.

Turnover of mature liver cells is slow, in the order of several months; however, upon hepatectomy differentiated hepatocytes re-enter the cell cycle to replace the lost liver mass. These observations prompted the view that liver homeostasis is maintained by proliferation of mature epithelial cells. Whether the adult liver, like other organs, also possesses bona fide stem cells that contribute to normal cell turnover or injury response is a still ongoing debate. While genetic lineage tracing has yielded conflicting conclusions (see [7]), isolated putative adult liver stem cell populations from normal liver are biliary in origin and can give rise to bipotential adult HBs [4,8,9•].

Taken together, current evidence thus indicates that a common 'polarization sequence' might operate in both the embryonic and in the adult liver (Figure 2): it starts with columnar epithelial cells (hepatic specified foregut/biliary hepatic stem cells) that become nonpolar cells (embryonic and adult HBs), and that repolarize with hepatocytic polarity by default unless induced to adopt ductal polarity. Remarkably, the hepatocytic model cell line WIF-B, one of the few polarized hepatocytic cell lines, spontaneously recapitulates this polarization sequence [10].

What causes branching into hepatocytic versus ductal polarity phenotypes?

No precise mechanisms have been elucidated to date, but current evidence points to the importance of two cellular processes in the polarity decision: cell–matrix-adhesion and cell–cell adhesion.

Bile ducts, like all other monolayered epithelial tissues, are surrounded by a basal lamina composed of collagen IV and laminin. By contrast, the space of Disse that separates hepatocytes from endothelial cells is devoid of a basement membrane [11] as it lacks laminin and the laminin-collagen IV crosslinker nidogen [12]. During ductal plate formation laminin (specifically, a laminin with an a1 chain) is initially provided by the portal mesenchyme, which also triggers laminin expression in the future biliary cells (specifically a5 chain laminin), most likely via TGFβ signaling [13]. Tanimizu *et al.* showed that the ability of HBs to form monolayered cysts *in vitro* depended on the presence of laminin in the 3D culture matrix [14] and on the activity of β1-integrin, a constituent of laminin and collagen receptors [13]. Biliary atresia, an early childhood liver disease characterized by bile duct malformation, has recently been associated with decreased levels of β1-integrin, laminin b1 and nidogen around diseased bile ducts [15]. This is consistent with an essential role of β1-integrin/laminin signaling in bile duct morphogenesis, which might be a general requirement for the establishment of monopolar tubular epithelial structures [16]. Our group established an experimental system that mimics the bipotency of HB polarization to address whether extracellular matrix (ECM)-signaling differences regulate the polarity phenotypes. In our model inducible overexpression of the AMPK-related kinase Par1b causes a switch from monopolar to hepatocyte-like polarization in kidney-derived MDCK cells. The polarity change is accompanied by reduced basement membrane deposition and focal adhesion formation and can be reversed by plating the cells on collagen IV substrates [17,18]. ECM-mediated integrin activation frequently leads to RhoA activation [19,20]. Indeed we measured lower RhoA activity in hepatocytic compared to monopolar MDCK-Par1b cells. Remarkably, RhoA depletion was sufficient to induce heptocytic polarity in MDCK cells. Conversely, pharmacological Rho activation in the hepatocytic cell line WIF-B promoted their monopolar organization [18,21•]. These findings point to RhoA signaling downstream of cell adhesion signaling as a putative key regulator of the polarity phenotypes.

E-cadherin is present at the cell surface of both biliary cells and periportal hepatocytes (hepatocytes outside the periportal zone express N-cadherin instead). Nonetheless, hepatocytes initiate lumen formation at the very cell–cell contacting domains that in tubule-forming epithelia are reserved for the establishment of E-cadherin-based adherens junctions. This makes it likely that