Mechanisms of Disease

FRANKLIN H. EPTIF, M.D., Editor

MOLECULAR PATHOGENESIS OF CHOLESTASIS

MICHAEL TRAUNER, M.D., PETER J. MEIER, M.D., AND JAMES L. BOYER, M.D.

T HE formation of bile is a vital function, and its impairment by drugs or infectious, autoimmune, metabolic, or genetic disorders results in the syndrome commonly known as cholestasis. The secretion of bile normally depends on the function of a number of membrane transport systems in hepatocytes and bile-duct epithelial cells (cholangiocytes) and on the structural and functional integrity of the bile-secretory apparatus. This review summarizes the molecular defects in hepatocellular membrane transporters that are associated with various forms of cholestatic liver disease in humans.

MOLECULAR MECHANISMS OF BILE FORMATION

Bile formation is an osmotic secretory process that is driven by the active concentration of bile salts and other biliary constituents in the bile canaliculi. The transport of solutes from the blood to the bile is driven by transport systems in the plasma membrane of the basolateral (sinusoidal) and apical (canalicular) surfaces of hepatocytes. The transport systems most relevant to bile formation in the human liver are illustrated in Figure 1 and listed with their corresponding functions in Table 1.

The basolateral plasma membrane contains the Na⁺/K⁺–ATPase that maintains the physiologic extracellular and intracellular ion gradients (more sodium outside the cell than inside; more potassium inside than outside). In addition, Na⁺/K⁺–ATPase, together with a potassium channel, helps to generate a transmembrane electrical potential of approximately −35 mV. These chemical and electrical potentials are used for the maintenance of intracellular ion and pH homeostasis. They provide the driving forces for proton extrusion by a mechanism of sodium–hydrogen exchange and for bicarbonate entry by a mechanism of sodium–bicarbonate symport, as well as for the electrogenic sodium-dependent uptake of conjugated bile salts (or bile acids). Bile salts are the most abundant solutes in bile. Their transport from plasma into hepatocytes is predominantly mediated by the sodium–taurocholate cotransporter (NTCP). In contrast to conjugated bile salts, the unconjugated bile salt cholate, the organic anion sulfobromophthal- ein, and numerous other lipophilic albumin-bound compounds are transported from plasma into hepatocytes by sodium-independent transport systems, including the organic-anion–transporting polypeptide (OATP) (Fig. 1 and Table 1).

Under physiologic conditions, active transport of solutes across the canalicular membrane of hepatocytes represents the rate-limiting step in bile formation. This unidirectional concentrative step is driven by an array of ATP-dependent export pumps that belong to the ATP-binding cassette family of membrane transporters (Fig. 1 and Table 1). The first of these canaliculocytes to be localized and characterized was the multidrug-resistance-1 P-glycoprotein (MDR1), which mediates the canalicular excretion of bulky lipophilic cations (e.g., anticancer drugs, calcium-channel blockers, cyclosporine A, and various other drugs). However, its physiologic role in overall bile formation remains unclear, because its level of expression in liver is relatively low and its level of endogenous substrate is not known. In contrast, a clear and liver-specific function in bile formation could be assigned to the multidrug-resistance-3 P-glycoprotein (MDR3; multidrug-resistance-2 P-glycoprotein in rodent livers) (Fig. 1 and Table 1). As demonstrated in mice in which the gene for multidrug-resistance-2 P-glycoprotein has been deleted and in transfected yeast, this P-glycoprotein is a phospholipid transporter that translocates phosphatidylcholine from the inner to the outer leaflet of the canalicular membrane, where it can be selectively extracted by intracanalicular bile salts and secreted into bile as vesicles and mixed micelles.

Another important hepatobiliary export pump is the canalicular multispecific organic-anion transporter, which is a canaliculosem of the multidrug-resistance–associated protein (MRP2). It mediates ATP-dependent canalicular excretion of a wide range of amphipathic anionic substrates, including leu-
Figure 1. Transport Polarity of Normal Hepatocytes and Bile-Duct Epithelial Cells (Cholangiocytes).

There are two sinusoidal systems for bile-salt uptake in hepatocytes (upper panel, left-hand side) — a sodium–taurocholate cotransporter (NTCP) and a sodium-independent organic-anion transporter (OATP). Sodium-dependent uptake of bile salts through the NTCP is driven by an inwardly directed sodium gradient generated by Na⁺/K⁺-ATPase and the membrane potential generated in part by a potassium channel. In addition, the basolateral membrane contains a sodium–hydrogen exchanger and a sodium–bicarbonate symporter (upper panel, right-hand side). The canalicular membrane contains several ATP-dependent export pumps: the multidrug-resistance-1 P-glycoprotein (MDR1), the phospholipid transporter multidrug-resistance-3 P-glycoprotein (MDR3), the canalicular multispecific-organic-anion transporter (MRP2 or cMOAT), and the canalicular bile-salt–export pump (BSEP or SPGP). In addition, the canalicular membrane contains several ATP-independent transport systems, including a chloride channel (distinct from the cystic fibrosis transmembrane regulator protein), a chloride–bicarbonate anion exchanger isoform 2 (AE2) for secretion of bicarbonate, and a glutathione (GSH) transporter. Cholangiocytes (lower panel) contain a chloride channel that corresponds to the cystic fibrosis transmembrane regulator (CFTR) and a chloride–bicarbonate anion exchanger isoform 2 (AE2) for secretion of bicarbonate. Furthermore, cholangiocytes have absorptive functions for a variety of bile solutes, including bile salts, amino acids, and glucose. PL denotes phospholipid, OA organic anion, BS β bile salt, OC organic cation, and A anion (possibly GSH).
Table 1. Nomenclature, Location, and Function of Hepatocyte and Cholangiocyte Membrane Transporters Involved in Bile Secretion.

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>Location</th>
<th>Function</th>
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<tbody>
<tr>
<td><strong>Hepatocyte</strong></td>
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<tr>
<td>Ion transporters</td>
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<tr>
<td>Sodium–potassium ATPase</td>
<td>Na+/K+-ATPase</td>
<td>Basolateral (sinusoidal) membrane</td>
<td>Maintains physiologic sodium and potassium gradients (extracellular Na⁺ &gt; intracellular Na⁺; intracellular K⁺ &gt; extracellular K⁺)</td>
</tr>
<tr>
<td>Potassium channel</td>
<td>K⁺ channel</td>
<td>Basolateral (sinusoidal) membrane</td>
<td>Determines membrane potential</td>
</tr>
<tr>
<td>Sodium–proton exchanger isoform 1</td>
<td>NHE1</td>
<td>Basolateral (sinusoidal) membrane</td>
<td>Acid extruder, housekeeping gene for intracellular pH and cell volume</td>
</tr>
<tr>
<td>Sodium–bicarbonate symporter</td>
<td>Na⁺–HCO₃⁻ symporter</td>
<td>Basolateral (sinusoidal) membrane</td>
<td>Acid extruder, facilitates bicarbonate entry into hepatocytes and bile by way of the canalicular chloride–bicarbonate anion exchanger isoform 2</td>
</tr>
<tr>
<td>Chloride–bicarbonate anion exchanger isoform 2</td>
<td>AE2</td>
<td>Canalicular membrane</td>
<td>Acid loader — excretes bicarbonate into bile and stimulates bile flow independent of bile salts</td>
</tr>
<tr>
<td>Chloride channel</td>
<td>Cl⁻ channel</td>
<td>Canalicular membrane</td>
<td>Facilitates chloride entry into bile</td>
</tr>
<tr>
<td><strong>Organic-solute transporters</strong></td>
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<tr>
<td>Sodium–taurocholate cotransporter</td>
<td>NTCP</td>
<td>Basolateral membrane</td>
<td>Primary carrier for conjugated bile-salt uptake from portal blood</td>
</tr>
<tr>
<td>Organic-anion–transporting polypeptide</td>
<td>OATP</td>
<td>Basolateral membrane</td>
<td>Multispecific carriers for sodium-independent uptake of bile salts, organic anions, and other amphipathic organic solutes from portal blood</td>
</tr>
<tr>
<td>Multidrug-resistance-1 P-glycoprotein*</td>
<td>MDR1</td>
<td>Canalicular membrane</td>
<td>ATP-dependent excretion of various organic cations, xenobiotics, and cytoxins into bile</td>
</tr>
<tr>
<td>Multidrug-resistance-3 P-glycoprotein (phospholipid transporter)*</td>
<td>MDR3</td>
<td>Canalicular membrane</td>
<td>ATP-dependent translocation of phosphatidylcholine from inner to outer leaflet of membrane bilayer</td>
</tr>
<tr>
<td>Multidrug-resistance–associated protein (canalicular multispecific organic-anion transporter)*</td>
<td>MRP2 (cMOAT)</td>
<td>Canalicular membrane</td>
<td>Mediates ATP-dependent multispecific organic-anion transport (e.g., bilirubin diglucuronide) into bile; contributes to bile-salt–independent bile flow</td>
</tr>
<tr>
<td>Canalicular bile-salt–export pump* (sister of P-glycoprotein)</td>
<td>BSEP (SPGP)</td>
<td>Canalicular membrane</td>
<td>ATP-dependent bile-salt transport into bile; stimulates bile flow dependent on bile salts; spgp of rat liver mediates ATP-dependent bile-salt transport, indicating that it represents the canalicular bile-salt transporter of the mammalian liver</td>
</tr>
<tr>
<td><strong>Glutathione transporter†</strong></td>
<td>GSH transporter</td>
<td>Canalicular membrane</td>
<td>Glutathione transport into bile; stimulates bile flow independent of bile salts</td>
</tr>
<tr>
<td><strong>Cholangiocyte</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion transporters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystic fibrosis transmembrane regulator</td>
<td>CFTR</td>
<td>Apical (luminal) membrane</td>
<td>Chloride channel; facilitates chloride entry into bile</td>
</tr>
<tr>
<td>Chloride–bicarbonate anion exchanger isoform 2</td>
<td>AE2</td>
<td>Apical (luminal) membrane</td>
<td>Facilitates bicarbonate secretion into bile and contributes to bile flow independent of bile salts</td>
</tr>
<tr>
<td><strong>Organic-solute transporters‡</strong></td>
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*These transporters are members of the ATP-binding cassette family.
†These transporters have been defined on the basis of functional evidence; they have not yet been cloned from the human liver.
‡A number of transport systems have been functionally characterized at the luminal membrane of cholangiocytes for reclaiming substances from bile, including glucose, amino acids, and bile salts.3

Kotheine C4, glutathione-S conjugates, glucuronides (e.g., bilirubin diglucuronide and estradiol-17β-glucuronide), and sulfate conjugates, and is responsible to a large extent for the generation of bile flow independent of bile salts within the bile canaliculi.12 Finally, an ATP-dependent transport process is also involved in the canalicular secretion of bile salts. However, despite the quantitative importance of biliary bile salts, the definite molecular identification of the canalicular bile-salt transporter of the mammalian liver has lagged behind that of the other canalicular transporters mentioned above. Although the canalicular ecto-ATPase has been proposed as a possible candidate,13 other investigators have provided evidence that the canalicular bile-salt transporter may also be a transport protein of the ATPase-binding cassette type.14 This assumption has recently been confirmed by full-length cloning of a P-glycoprotein, known as the “sister of P-glycoprotein” (spgp), from rat livers and its functional expression in the oocytes of Xenopus laevis and in SF9 insect cells demonstrating marked ATP-dependent bile-salt transport.15 Together with the expression of the sister of P-glycoprotein in pig livers,16 these findings strongly indicate that this transporter represents the canalicular bile-salt export pump of the mammalian liver.

Besides primary active-transport processes at the plasma membranes of the canaliculi, the formation and final composition of canalicular bile depend on several other less well defined mechanisms, includ-
ing canalicular exocytosis of transcytotic and sub-
canalicular vesicles, the activities of numerous nu-
cleotidases and peptidases, periodic contractions of
the bile canaliculi, and various activities of elec-
trolyte transporters and ion channels of hepatocytes or
bile-ductule epithelial cells. The last-named are
closely associated with the biliary excretion of chlo-
ride and bicarbonate and include the chloride--bicarbonate anion exchanger isofrom 2 in both hep-
tocytes and bile-duct epithelial cells and the cystic fibrosis transmembrane regulator (CFTR), a chlo-
ride channel on the luminal membrane of bile-duct
epithelial cells (Fig. 1 and Table 1). Regulated ex-
pression of these and other bile-duct transporters
and channels contributes substantially to the daily
output of bile, and their functional deficiency might
be an important cause of cholestatic liver disease.

**EXPERIMENTAL MODELS AND THEIR CLINICAL CORRELATES**

Several animal models of intrahepatic and obstruc-
tive cholestasis simulate human cholestatic diseases. These disorders include sepsis-induced cholestasis (in endotoxin-treated rats), oral-contraceptive–induced cholestasis and cholestasis of pregnancy (in ethinyl estradiol–treated rats), and extrahepatic biliary ob-
struction induced by ligation of the common bile
duct. The cholestatic effects of endotoxin and endo-
toxin-induced cytokines not only have a role in the
pathogenesis of sepsis-induced cholestasis, but also
may explain defects in hepatobiliary excretory func-
tion during total parenteral nutrition and in alcohol-

ic and viral hepatitis. Many drugs (e.g., cyclospor-
ine A and chlorpromazine) also cause intrahepatic
cholestasis at the level of the bile canaliculus in both
humans and animals.

Despite their different causes, each of these dis-
cases results in marked functional impairment of hepatoctelial uptake and canalicular ecretion of
bile salts and various other organic anions. Chole-
cestasis results from impaired transport of these
compounds into bile and the loss of osmotic driving
forces for bile secretion.

**MOLECULAR MECHANISMS OF CHOLESTASIS**

**Hepatocellular Transporters**

Decreased or even absent expression of specific
hepatocellular transport proteins has been found in
several clinical forms (Table 2) and experimental
models of cholestasis. These abnormalities explain
the impairment of transport functions, with a subse-
quent reduction in bile flow and the development of
cholestasis.

The discovery that familial disorders of cholestasis
may be related to mutations in genes controlling
hepatocellular transport systems known to be in-
volved in the formation of bile is rapidly bridging the
gap between basic science and clinical medicine. Pro-
gressive familial intrahepatic cholestasis is a severe
type of cholestatic liver disease that is inherited as an
autosomal recessive trait (Table 2). The disease pre-
sents in infancy and results in progressive cholestasis
and liver failure. Three types of progressive familial

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**Table 2. Molecular Changes of Hepatocellular-Transport Systems in Patients with Cholestatic Disorders.**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Molecular Change</th>
<th>Comments</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Progressive familial intrahepatic</td>
<td></td>
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</tr>
<tr>
<td>cholestasis</td>
<td>Mutation in P-type ATPase</td>
<td>Mapped to chromosome 18q21–22; low serum γ-glutamyltransferase concentrations</td>
<td>Carlton et al.29</td>
</tr>
<tr>
<td>Type 1</td>
<td></td>
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<tr>
<td>Type 2</td>
<td>Absence of sister of P-glycoprotein</td>
<td>Mutation of sister of P-glycoprotein gene (chromosome 2q24); low serum γ-glutamyltransferase concentrations</td>
<td>Strautnieks et al.30</td>
</tr>
<tr>
<td>Type 3</td>
<td>Absence of multidrug-resistance-3 P-glycoprotein RNA and protein</td>
<td>Mutation of multidrug-resistance-3 P-glycoprotein gene (chromosome 7q31); high serum γ-glutamyltransferase concentrations</td>
<td>Deleuze et al.31</td>
</tr>
<tr>
<td>Benign recurrent intrahepatic</td>
<td>Mutation in P-type ATPase</td>
<td>Mapped to progressive familial intrahepatic cholestasis 1 locus (18q21–22)</td>
<td>Bull et al.30</td>
</tr>
<tr>
<td>cholestasis</td>
<td></td>
<td></td>
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<tr>
<td>Extraphatic biliary atresia</td>
<td>Decrease in sodium–taurocholate cotransporter RNA</td>
<td>Inverse correlation with serum bilirubin concentrations; increase after successful portoenterostomy</td>
<td>Shneider et al.32</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>Increase in organic-anion–transporting polypeptide RNA</td>
<td></td>
<td>Kullak-Ublick et al.33</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>Decrease in chloride–bicarbonate anion exchanger, isofrom 2 RNA and protein</td>
<td>Hepatocytes and cholangiocytes affected</td>
<td>Prieto et al.34</td>
</tr>
<tr>
<td>Biliary obstruction</td>
<td>Increase in multidrug-resistance-1 and multidrug-resistance-3 P-glycoprotein RNA</td>
<td>Direct correlation with serum bilirubin concentrations</td>
<td>Nozawa et al.35</td>
</tr>
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</table>
intrahepatic cholestasis are just now being recognized. Type 1, also known as Byler's disease and described originally in an Amish family, is characterized by low serum $\gamma$-glutamyltransferase concentrations, high serum bile-salt concentrations, normal serum cholesterol concentrations, and low biliary phospholipid concentrations. This form of progressive familial intrahepatic cholestasis has been mapped by positional cloning to chromosome 18q21–22.28,40

Benign recurrent intrahepatic cholestasis, a recurrent cholestatic disorder in adults, has also been mapped to the same region of chromosome 18q21–22,33,40 leading to speculation that there may be a familial cholestasis gene that is responsible for both disorders despite their different phenotypes and prognosis. Indeed, recently a gene mutation in patients with progressive familial intrahepatic cholestasis type 1 and benign recurrent intrahepatic cholestasis has been described.29 This gene (called FIC1) normally encodes a P-type ATPase that is expressed predominantly in the small intestine as well as in the liver and is likely to have an important role in the enterohepatic circulation of bile acids. Its known function is to transfer aminophospholipids from the outer to the inner leaflet of the plasma-membrane bilayer. Further studies of the functional role of this P-type ATPase should contribute importantly to the understanding of bile formation and cholestasis.

Patients with findings typical of progressive familial intrahepatic cholestasis type 1, but unrelated to the original Byler family, have also been described in isolated populations in the Middle East, Greenland, and Sweden and are considered to have Byler’s syndrome. Homozygosity mapping and linkage analysis in a Middle Eastern family resulted in the identification of a gene locus on chromosome 2q24.30 This disorder has been designated progressive intrahepatic cholestasis type 2. The gene locus for the canalicular bile-salt transporter has also been mapped to the same region of chromosome 2,28 and mutations in this transporter gene may be responsible for progressive familial intrahepatic cholestasis type 2 (Thompson RJ: personal communication) (Table 2).

In contrast to types 1 and 2, the third subtype, progressive familial intrahepatic cholestasis type 3, is characterized by high serum $\gamma$-glutamyltransferase concentrations, as well as by bile-duct proliferation and inflammatory infiltrates in the portal areas. The underlying defect is a mutation (a 7-bp deletion or a point mutation) of the multidrug-resistance-3 gene (Table 2), resulting in the complete absence of the multidrug-resistance-3 P-glycoprotein in the liver of these patients and a substantial decrease in biliary phospholipid concentrations with normal canaliculir excretion of bile salts.31,32 Phospholipids in bile normally protect bile-ductule epithelial cells from the toxicity of bile salts by forming mixed micelles; therefore, the marked decrease or absence of biliary phospholipids may explain the presence of bile-duct injury in these patients.

Thus, progressive familial intrahepatic cholestasis type 3 provides an important link between a hepatocellular (canalicular) transport defect and the development of cholangiopathies. Many patients with neonatal cholestasis (e.g., extrahepatic biliary atresia and bile-duct paucity syndromes) and adult cholangiopathies and other cholestatic syndromes (e.g., primary biliary cirrhosis, primary sclerosing cholangitis, and vanishing-bile-duct disorders) now need to be reevaluated for possible defects in the multidrug-resistance-3 gene and its product.9 Patients with primary biliary cirrhosis have normal concentrations of multidrug-resistance-3 P-glycoprotein messenger RNA (mRNA),41 suggesting that decreased expression of this gene is not involved in its pathogenesis.

Although no defects associated with the multidrug-resistance-1 gene have been identified so far, mutations in this gene would be expected to have an indirect role in certain types of drug-induced cholestasis. As a consequence of such a hypothetical defect, hepatocellular accumulation of drugs could result in cholestasis, because substrates of multidrug-resistance-1 P-glycoprotein, such as cyclosporine A, inhibit transport of ATP-dependent canalicular bile salts42,43 and organic anions in rats.42

The Dubin–Johnson syndrome is caused by a point mutation of the gene for the canalicular multispecific-organic-anion transporter (also called multidrug-resistance–associated protein), resulting in the absence of this protein in the livers of affected patients.44,45 Although patients with the Dubin–Johnson syndrome usually have hyperbilirubinemia rather than cholestasis, this syndrome is yet another example of the way in which a mutation of a hepatocellular-transporter gene can impair biliary excretory function. This syndrome is characterized by abnormal biliary excretion of endogenous conjugates (e.g., bilirubin digluconide and coproporphyrin I) and exogenous amphiphilic anionic conjugates (e.g., conjugates of sulfobromophthalein and of indocyanine green and iopanoic acid, an oral cholecystographic agent), which are normally excreted by the canalicular multispecific organic-anion transporter.46,47

In addition to genetic defects of the hepatocellular transport systems (see above), exposure to cholestatic injury may also result in molecular changes in basolateral and canalicular transport systems in patients with acquired forms of cholestasis. The potential mechanisms for these forms of cholestatic liver disease include changes in the rate of gene transcription; post-transcriptional changes in mRNA processing, stability, and translational efficiency; impaired intracellular sorting or targeting; impaired protein activation (e.g., by phosphorylation or dephosphorylation); and increased protein degradation.48
Molecular alterations of basolateral transport proteins may contribute to the functional impairment of bile formation by diminishing the hepatocellular uptake of biliary constituents.\(^{24,26}\) In addition, these alterations may serve to prevent further accumulation of toxic biliary constituents (especially bile salts) within hepatocytes. For example, concentrations of basolateral sodium–taurocholate cotransporter polypeptide mRNA are decreased in patients with extrahepatic biliary atresia, and the concentrations increase if biliary drainage is restored by a portoenterostomy (Kasai procedure).\(^{34}\) The mRNA concentrations are inversely related to the serum total bilirubin concentrations,\(^{34}\) suggesting that the retention of biliary constituents may lead to down-regulation of the sodium–taurocholate cotransporter. The promoter of the sodium–taurocholate cotransporter gene in rats contains sequences for several transcriptional regulatory elements involved in cytokine signaling, as well as for elements responsive to steroids and bile salts, and bile salts can suppress the promoter activity of this gene in vitro.\(^{49}\) Furthermore, the administration of endotoxin in rats inhibits the activity of critical transcription factors (e.g., hepatocyte nuclear factor 1) that normally regulate this promoter, resulting in decreased gene transcription in sepsis-induced cholestasis.\(^{50}\)

Gene expression of the organic-anion–transporting polypeptide, in contrast, appears to be up-regulated in humans with cholestatic liver disease (primary sclerosing cholangitis) in which concentrations of organic-anion–transporting polypeptide mRNA are increased and up-regulated when transfected hepatocytes are exposed to bile salts in cells transfected in vitro. This up-regulation might minimize the accumulation of potentially toxic compounds by transporting these substances out of the hepatocytes into portal venous plasma.\(^{55}\)

Since the transport of biliary constituents across the canalicular membrane is the rate-limiting step in bile formation, the impairment of canalicular transport systems should have a major role in the pathogenesis of acquired forms of intrahepatic cholestasis, similar to its role in hereditary defects (see above). Decreased canalicular secretion of bile salts and a broad range of anionic conjugates (e.g., bilirubin diglucuronide) is a fundamental pathophysiologic defect in all forms of cholestasis.\(^{23,25,27}\) Indeed, the molecular expression of the corresponding transport systems, such as the bile-salt export pump and the canalicular multispecific organic-anion transporter, is decreased in experimental models of cholestasis, findings that may provide the molecular basis for impaired excretion of bile salts and the development of jaundice in humans with cholestasis.\(^{51,52}\)

Expression of the chloride–bicarbonate exchange isoform 2 is reduced in the livers of patients with primary biliary cirrhosis.\(^{36,37}\) Since chloride–bicarbonate exchange activity contributes to the secretion of both canalicular and ductular bile (Table 1), decreased hepatic expression of this transporter could lead to impaired bile flow. Diminished expression of anion exchanger isoform 2 has also been reported in the salivary glands of patients with primary biliary cirrhosis and sicca syndrome,\(^{53}\) which may indicate a more generalized epithelial failure of bicarbonate secretion. In contrast, concentrations of hepatic MDR1 and MDR3 mRNA are increased in patients with obstructive cholestasis,\(^{38}\) in whom such increases are strongly correlated with increases in serum bilirubin and alkaline phosphatase concentrations.

In summary, the altered expression of hepatocellular transport systems in human cholestatic liver disease and experimental models of cholestasis\(^{24,26,51,52,54-58}\) may provide a molecular correlate for the functional changes that occur in cholestasis. Some of these changes contribute to cholestasis, whereas others may limit the accumulation of toxic biliary constituents in hepatocytes.

**Cholangiocyte Transporters**

Knowledge about cholangiocyte transport function at the molecular level in cholestasis is more limited.\(^3\) Mutations of the CFTR,\(^{59}\) which is located on the luminal membrane of cholangiocytes, but not hepatocytes,\(^{21}\) result in impairment of ductal secretion of chloride and water. This defect is associated with mucosal obstruction of the intrahepatic bile ducts, leading to focal areas of biliary fibrosis and cirrhosis in patients with cystic fibrosis.\(^{60}\)

**OTHER STRUCTURAL AND FUNCTIONAL DEFECTS: ALTERED CYTOSKELETON, TIGHT JUNCTIONS, VESICULAR TRANSPORT, AND SIGNAL TRANSDUCTION**

Most human and animal cholestatic liver disorders are associated with profound changes in the cytoskeleton of the hepatocytes, including disruption of microtubules, increases in intermediate filaments, and accumulation of disorganized bundles of actin microfilaments in the pericanalicular domain (Fig. 2).\(^{18}\) These cytoskeletal changes result in the loss of apical microvilli and diminished contractility of the canalicular membrane and may also contribute to the leakiness of tight junctions between cells.\(^{61}\) Some forms of severe familial cholestasis, resulting in cirrhosis in children in Canada (North American Indian childhood cirrhosis), are associated with large increases in pericanalicular microfilaments reminiscent of but not identical to toxicity from phalloidin.\(^{62}\)

Cholestasis induced experimentally in rats is also associated with the disruption of the structural and functional integrity of the hepatocellular tight junc-
This abnormality results in increases in paracellular permeability, regurgitation of biliary constituents into plasma, and reduction of the osmotic gradients in the bile canaliculi that normally constitute the driving force for bile secretion. The hepatic localization and expression of the tight-junction proteins that normally form the junctional barrier, such as zona occludens 1 and occludin, are altered in rats by ligation of the common bile duct and treatment with ethinyl estradiol.64-66 Aggregates of zona occludens 1 along the borders of the canaliculi become distorted and accumulate in the cytoplasm, and occludin no longer localizes with zona occludens 1, resulting in “leaky junctions.”66

Targeting of membrane components, transcytosis, and canalicular exocytosis of vesicles are also disrupted during cholestasis, resulting in the retention of apical (canalicular) transporters on the basolateral surface of hepatocytes and a delay in vesicle transport to the bile canaliculi.67-69 Accumulation of vesicles within the pericanalicular region of hepatocytes is a characteristic morphologic finding in many forms of cholestatic liver injury (Fig. 2).70,71 High concentrations of bile salts, such as chenodeoxycholic acid, inhibit the function of molecular motors, such as kinesin and dynein, that move vesicles along microtubules.72 Impairment of the movement of vesicles during cholestasis results in a decreased number of functional transporters in the canalicular membrane and thus contributes to cholestasis.73

Calcium signaling within and between hepatocytes is impaired in cholestasis.74,75 Gap-junction proteins (connexins 32 and 26) disappear within 24 hours after ligation of the common bile duct in rats, which results in a decline in the migration of calcium waves between individual hepatocytes (Fig. 2).76 This decline may diminish orderly contractions of the bile canaliculi and impair the process of microperistalsis that normally facilitates the movement of bile from the terminal canaliculi toward the bile ductules in the portal tracts toward to the direction of blood flow.76

Cyclic-AMP–mediated intracellular signaling in hepatocytes is also impaired after ligation of the common bile duct, as a consequence of altered expression and subcellular localization of heterotrimetric G proteins. These changes, together with changes in the composition of liver-membrane lipids and the detergent effect of bile salts, may contribute to the impairment of adenylate cyclase activity77,78 and decrease the stimulatory effects of glucagon and vasoactive intestinal peptide on bile secretion.77,78 In contrast, in cholangiocytes, secretin-receptor gene expression is up-regulated after ligation of the common bile duct79 and may contribute to the increased choleretic effect of secretin that follows the proliferation of bile ductules in rats. Up-regulation of bicarbonate secretion by cholangiocytes, together with down-regulation of the canalicular multispecific organic-anion transporter that excretes bilirubin, may account for the well-known clinical complication of “white bile” that occurs during prolonged obstruction of the bile ducts.

THERAPEUTIC IMPLICATIONS AND FUTURE PERSPECTIVES

Pharmacologic Therapy

Ursodiol (ursodeoxycholic acid) is currently the accepted therapy for patients with primary biliary cirrhosis, and it may extend life by slowing the progression of disease, according to the results of three trials.80 It may also have beneficial effects in certain other cholestatic liver disorders, including primary sclerosing cholangitis, intrahepatic cholestasis of pregnancy, and cystic fibrosis.81 However, large trials have not been conducted among patients with these diseases, and a recent small, randomized study of primary sclerosing cholangitis showed no benefit of ursodiol with regard to survival.82

Several potential molecular mechanisms may account for the beneficial actions of ursodiol. It replaces toxic hydrophobic bile salts in serum, liver, and bile.81,83 Conjugated ursodiol is adsorbed to the interface of the plasma membrane at the extracellular space, where it may prevent the extraction of membrane lipids by more hydrophobic bile salts.84 In patients with defective biliary phospholipid secretion (progressive familial intrahepatic cholestasis type 3), the beneficial effect of ursodiol may be related to its enrichment in bile and the modulation of biliary bile-salt composition in favor of hydrophilic bile salts, which are not toxic to the biliary epithelium, despite the absence of phospholipids in bile.81,85 Ursodiol also down-regulates expression of abnormal major histocompatibility complex (MHC) class I molecules in periportal hepatocytes of patients with primary biliary cirrhosis, whereas expression of abnormal MHC class II molecules on bile-duct epithelial cells does not change.86 However, it is unclear whether these effects represent specific immunomodulatory properties of ursodiol85 or are due to improvement of the cholestatic liver injury, which also increases expression of MHC class I molecules on hepatocytes.86

Experiments in rats indicate that the taurine conjugate of ursodeoxycholic acid, the principal form of ursodeoxycholic acid in the body, may increase the excretory capacity of cholestatic liver cells by stimulating apical exocytosis.74,89,90 This increase, in turn, should induce targeting and insertion of transport proteins into the canalicular membrane and increase the capacity to excrete more hydrophobic bile salts into bile, thereby reducing liver-cell injury.90 The results of kinetic analysis of biliary excretion of synthetic derivatives of bile salts in patients with primary
**Normal Hepatocyte**

1. Loss of gap junctions
2. Leaky junctions
3. Disorganized actin–myosin bundles
4. Disruption of transcytotic vesicular pathway

**Cholestatic Hepatocyte**

1. Loss of gap junctions
2. Leaky junctions
3. Disorganized actin–myosin bundles
4. Disruption of transcytotic vesicular pathway
Figure 2. Cell Contacts, Cytoskeleton, and Vesicular Targeting in Hepatocytes.

Normal hepatocytes (Panel A) have gap junctions (1) that facilitate intercellular communication (e.g., through diffusion of second messengers and propagation of calcium waves; blue arrows) and tight junctions (2) that seal off the canalicular lumen and prevent the regurgitation of biliary constituents into plasma. Canalicular bile is formed by osmotic filtration of water and small electrolytes (red arrows) through the hepatocyte and tight junctions in response to the osmotic gradients generated by the active transport systems of the hepatocyte. A pericanalicular actin–myosin network (3) causes canalicular contractions, which facilitate the flow of bile from pericentral to periportal lobular regions. A microtubule–dependent, transcytotic vesicular pathway (4) mediates the transfer of solutes and macromolecules (e.g., IgA) (black arrow). In addition, this pathway targets canalicular transport systems to the bile canaliculus.

Cholestatic hepatocytes (Panel B) are characterized by the loss of gap-junction proteins (connexins) (1), which results in impaired intercellular communication. Dissociation of the normal localization of proteins associated with tight junctions results in leaky junctions (2) and the collapse of the osmotic gradients that normally form the driving force for bile flow (red arrows). Depolymerization of actin–myosin bundles (3) results in the loss of canalicular-membrane tone and failure to contract, with consequent canalicular paralysis and distention and the formation of bile plugs in the canalicular lumen. Disruption of the transcytotic vesicular pathway (4) results in decreased movement across membranes, with the subsequent accumulation of vesicles within the pericanalicular region of hepatocytes. Impaired vesicle movement and targeting may be due in part to inhibition of microtubular motors, such as kinesin and dynein.

biliary cirrhosis and primary sclerosing cholangitis support the view that therapy with ursodiol increases the capacity of the liver to excrete bile salts.91 Up-regulation of the expression of the chloride–bicarbonate anion exchanger isoform 2 in patients with primary biliary cirrhosis who were treated with ursodiol has also been reported.28,37 It also directly stimulates chloride secretion in human gallbladder cells by activating a calcium-sensitive chloride channel, an effect that might be of benefit in patients with cystic fibrosis.92

Gene Therapy

The retrograde infusion of an adenovirus encoding the human CFTR into the common bile duct of rats resulted in the temporary expression of CFTR protein.93 In addition, this defect has been temporarily corrected in vivo in isolated bile-duct cells from two patients with cystic fibrosis, again using an adenovirus-based vector.94 These approaches open new horizons in the treatment of patients with cystic fibrosis by delivering encoding vectors for the CFTR through endoscopic retrograde cholangiography.95

CONCLUSIONS

The rapid advances in the understanding of the cellular and molecular physiology of bile secretion have led to a better grasp of the pathophysiology of and structural cell damage caused by various hereditary and acquired cholestatic disorders. These advances should lead to the development of more effective preventive measures and new therapeutic strategies for a whole variety of currently untreatable cholestatic liver diseases.

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