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Transport Systems in Cholangiocytes: Their Role in Bile Formation and Cholestasis

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Formation of bile requires the coordinated function of two epithelial cell types: hepatocytes, that are responsible for secretion of the major osmolytes and biliary constituents and cholangiocytes that regulate the fluidity and alkalinity of bile through secretion of osmolytes such as Cl^- and HCO_3^- Studies in isolated cholangiocyte preparations have elucidated the basic transport mechanisms involved in constitutive and stimulated secretory activities in the biliary epithelium. Basolateral Na^+/H^+ exchanger and Na^+:HCO_3^- symporter mediate HCO_3^- uptake, while an apical cAMP-activated Cl^-/HCO_3^- exchanger secretes bicarbonate into the lumen. Cholangiocytes also possess a cAMP-stimulated Cl^- conductance (CFTR) and a Ca-activated Cl^- channel, both likely located at the apical membrane. Cholangiocyte secretory functions are regulated by a complex network of hormones mainly acting via the cAMP system. In addition, recent data indicate that part of the regulation of ductular secretion may take place at the apical membrane of the cholangiocyte through factors present into the bile, such as ATP, bile acids and glutathione.

Primary damage to the biliary epithelium is the cause of several chronic cholestatic disorders (cholangiopathies). From a pathophysiological point of view, common to all cholangiopathies is the coexistance of cholangiocyte death and proliferation and various degrees of portal inflammation and fibrosis. Cholestasis dominates the clinical picture and, pathophysiologically, may initiate or worsen the process. Alterations in biliary electrolyte transport could contribute to the pathogenesis of cholestasis in primary bile duct diseases. Cystic Fibrosis-related liver disease represents an example of biliary cirrhosis secondary to a derangement of cholangiocyte ion transport. Most primary cholangiopathies recognize an immune-mediated pathogenesis. Cytokines, chemokines, and proinflammatory mediators released in the portal spaces or produced by the cholangiocyte itself, likely activate fibrogenesis, stimulate apoptotic and proliferative responses, and alter the transport functions of the epithelium.

The formation of bile requires the coordinated function of two epithelial cell types that should be viewed as forming a functional bile secretory unit. Hepatocytes are responsible for production of the canalicular bile, and for secretion of the major osmolytes and biliary constituents, such as bile acids, lipids, glutathione, organic cations and anions, xenobiotics, proteins and electrolytes. Cholangiocytes, located downstream, determine the fluidity and alkalinity of hepatocellular bile through absorbitive/secretory processes. These functions are regulated by a complex network of hormones and mediators the inter-

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[b] *Abbreviations:* CFTR, cystic fibrosis transmembrane conductance regulator; DIDS, 4,4-diisothiocyanatostibene-2,2-disulfonic acid; NPPB, 5'-nitro-2(2)-phenylpropyl-aminobenzoate; R-PIA, [R]-N6-[1-methyl-2-phenylethyl]adenosine; NECA, 5'-(N-ethylcarboxyamido)-adenosine; RT-PCR, reverse transcription polymerase chain reaction.

action of which results in net secretion or absorbtion. In addition, some of the compounds secreted by hepatocytes are likely able to signal to the biliary epithelium. The intrahepatic biliary tree can thus be considered as a critical site for rapid regulation of bile volume and composition [1-5].

Although cholangiocytes are relatively few in number as compared to hepatocytes, they seem to be very effective secretory cells, since, in humans, are responsible for 30 percent of bile volume, depending on the physiologic conditions [5]. Cholangiocytes perform this task trough secretion of osmolites such as Cl^- and HCO_3^- that establish a transepithelial potential difference and an osmotic gradient that drive into the lumen Na^+ and water through the paracellular pathway and water selective channels [6, 7], respectively. Still in the 1960s, it was demonstrated that secretin hydrocholeresis originated from the bile ducts and was associated with reciprocal changes in Cl^- and HCO_3^- concentration. Indeed, in basal conditions, Cl^- and HCO_3^- concentrations in bile are similar to plasma, but there is a fall in Cl^- concentration and a rise in HCO_3^- concentration, up to 70 mM, consistent with the operation of an apical Cl^-/HCO_3^- exchange [1, 8].

Studies in isolated cholangiocyte preparations [9, 10] have elucidated the basic transport mechanisms involved in constitutive and stimulated secretory activities in the biliary epithelium In rat biliary epithelial cells, a basolateral Na^+/H^+ exchanger [11, 12] (the NHE-I isoform [13], and an electrogenic $Na^+:HCO_3^-$ symporter mediate acid extrusion. In human biliary cholangiocytes, an electroneutral Na^+-dependent Cl^-/HCO_3^- exchanger [14], rather than the electrogenic $Na^+:HCO_3^-$ symporter mediates HCO_3^- influx into the cell. A basolateral Na^+-K^+-ATPase [15] maintains the Na^+ gradient required for operation of the above acid/base transporters. Accumulation of HCO_3^- within the bile duct cell can also occur through carbonic anhydrase catalyzed hydration of CO_2 into the duct cell, followed by backward transport of protons on Na^+/H^+ exchange or H^+ pumps. In both human and rat cholangiocytes [11, 12, 14] once accumulated inside the cell, HCO_3^- is secreted into the biliary lumen by a cAMP-activated Cl^-/HCO_3^- exchanger (the AE-2 isoform [14, 16]) that has been localized to the apical membrane by both morphologic and functional criteria. This is the only acid/base transporter clearly identified in the apical membrane, however, apical Na^+/H^+ exchange isoforms (NHE-2 and NHE-3) are known to be expressed in Na^+ reabsorbing epithelia, including the gallbladder [17, 18]; by RT-PCR, expression of NHE-2 mRNA was reported in freshly isolated cholangiocytes [19]: an apical Na^+-dependent, amiloride inhibitable H^+ efflux mechanism was recorded in microperfused biliary ductules (J.L. Boyer, personal communication).

As in other secretory epithelia, in both human and rat cholangiocytes, basolateral Cl^- uptake involves bumetanide-sensitive $Na^+/K^+/2Cl^-$ cotransport and bumetanide inhibits luminal swelling induced by forskolin in bile duct units [20]. Cholangiocyte Cl^- efflux pathways have been studied by patch clamp techniques. Cholangiocytes possess a cAMP-stimulated Cl^- conductance [21] with electrophysiological features similar to the cystic fibrosis transmembrane conductance regulator (CFTR)[b] [22] and CFTR has been detected at the apical membrane of rat and human bile duct cells also by immunohistochemical techniques[21, 23]. In addition, a Ca^{2+}-activated Cl^- channel [21, 24] is likely located at the apical membrane. Extracellular ATP, that has been shown to increase cytosolic Ca^{2+} levels and to stimulate Cl^- efflux in cholangiocyte cell lines [25], is among its physiologic agonists [24]. Cholangiocytes also possess a Ca^{2+}- and cAMP- insensitive high conductance anion channel, inhibited by pertussis toxin sensitive GTP-binding proteins [26, 27], but its physiologic role and membrane location are presently unknown. Finally, a basolateral barium sensitive K^+ conductance provides a leak pathway for K^+ accumulated and maintains the membrane hyperpolarization needed to provide the driving force for Cl^- exit. It is interesting to note that in bile duct units, administration of barium (that

inhibits K^+ channels) together with bumetanide blocks the luminal expansion induced by forskolin [20].

Secretin receptor is coupled to adenyl cyclase and it is well established that cAMP is the second mediator of secretin [28]. Secretin and cAMP administration to cholangiocyte cell lines activate low conductance Cl^- selective channels [29], likely the CFTR. Although Cl^- is nine times more permeable that HCO_3^- through these channels, ion fluxes will actually depend on the magnitude of the respective electrochemical driving forces [30]. However cAMP did not increase HCO_3^- efflux in cells pretreated with DIDS, an inhibitor of Cl^-/HCO_3^- exchanger, but not of CFTR, suggesting that, contrary to the airways epithelium, no substantial Cl^- conductance is operated by cAMP-activated Cl^- channels in the biliary epithelium [31].

Cyclic AMP and secretin administration increase Cl^-/HCO_3^- exchange activity [12], but these effects are inhibited by the Cl^- channel inhibitor NPPB [12], suggesting that Cl^-/HCO_3^- exchange activation requires functional cAMP-sensitive Cl^- conductances. Contrary to CFTR, stimuli regulating Cl^-/HCO_3^- exchange are not well established. In all systems studied, intracellular alkalinization, increases the activity of the AE-2 isoform of Cl^-/HCO_3^- exchange, a feature that distinguishes it from AE-1 [32]. Cyclic AMP administration to hepatocytes and cholangiocytes increase Cl^-/HCO_3^- exchange activity, however this effect appears to be microtubule-dependent in hepatocytes [33, 34] and Cl^- channel dependent in cholangiocytes [12]. Interestingly, when targeted to the basolateral membrane, i.e., opposite to CFTR, AE-2 appears to lack the ability to be stimulated by cAMP [13] and in cystic fibrosis pancreatic HCO_3^- secretion is impaired [35]. Thus, association of Cl^-/HCO_3^- exchange with CFTR appears mandatory for cAMP-dependent HCO_3^- efflux. Studies in microdissected rat intrahepatic bile ducts and polarized bile duct units have consistently shown that luminal pH is higher than that of the bathing medium and increases following administration of cAMP agonists [36, 37]. The lumen expands following forskolin administration and both luminal alkalinization and expansion are inhibited in the absence of HCO_3^- from the perfusate. In aggregate these findings confirm the role of Cl^- channels and Cl^-/HCO_3^- exchanger as the main mechanisms for ductal biliary fluidification and alkalinization, however they do not establish the mechanistic relationship between Cl^- and HCO_3^- transport.

When cAMP agonists are administered to secretory epithelial cells, a number of events takes place: CFTR opens allowing Cl^- efflux, Cl^-/HCO_3^- exchange is stimulated, K^+ channels maintain the membrane hyperpolarization and exocytotic phenomena are activated [12, 38]. CFTR plays a key role in all these changes [22, 39], in fact these processes are defective in pancreatic duct cells expressing a mutated CFTR, but are restored by complementation with wild-type CFTR. The capability to control other transport proteins is a well recognized features of CFTR [22] and it is also well known that the multiple ionic defects of cystic fibrosis, can not be explained only on the basis of impaired Cl^- transport. It has been suggested that CFTR, may regulate the activity of other ion transporters through an autocrine mechanism involving extracellular ATP [40, 41]. For example, in nasal and bronchial epithelia, extracellular ATP interacting with specific receptors, opens outward rectifying Cl^- channels and inhibits apical Na^+ channels [41]. This hypothesis could provide a link between CFTR activation and apical transport of ions different from Cl^- also in the biliary epithelium.

Extracellular ATP acts as a secretagogue in a number of epithelia and several observations suggest that it may do so also in cholangiocytes. ATP is present into the bile in doses able to activate purinergic receptors [42], and isolated cholangiocytes have been shown to respond to ATP by activating Cl^- channels [24]. In cholangiocyte cell lines extracellular ATP induced a P_{2u} receptor-mediated increase in the activity of the Na^+/H^+ exchanger [43,

44] and shifted to the right Na^+/H^+ exchange set point. Since both hepatocytes [45] and cholangiocytes [42] appear to release purinergic nucleotides, extracellular ATP may act as a paracrine signal promoting cellular HCO_3^- uptake and thus transepithelial HCO_3^- fluxes. Interestingly, Na^+/H^+ exchange activity was increased also by agents raising cellular cAMP levels, and this effect was inhibited by glybenclamide, a CFTR inhibitor that blocks ATP release from the cell [41, 46], and by the specific extracellular ATP scavenger hexokinase. In aggregate, these data suggest that cAMP may increase Na^+/H^+ exchange through ATP release from the cell and that ATP release is somehow regulated by CFTR. It is important to underline that CFTR does not appear to possess the capability to act as an ATP channel [47], and its role in ATP release is likely purely regulatory. Given the direct pH_i dependence of cholangiocyte AE-2 [12], the rise in intracellular pH_i, increases apical Cl^-/HCO_3^- exchange and further amplifies the secretory response that is also facilitated channel-mediated efflux of Cl^-. Consistent with this hypothesis, administration of ATP to cells treated with cAMP further increased Cl^-/HCO_3^- exchange, an effect that was inhibited in the presence of amiloride [44].

This models requires the presence of purinergic receptors at the apical membrane of the cholangiocyte. We have addressed this issue by studying the effects of apical and basolateral addition of ATP on Na^+/H^+ activity in a polarized rat cholangiocyte cell line [45]. In these studies (M. Strazzabosco unpublished results) apical administration of ATP, but not adenosine significantly increased basolateral Na^+/H^+ exchanger activity; the pharmacological profile of the different agonists is consistent with the presence of apical P_{2u} receptors. Interestingly, Na^+/H^+ exchange appeared to respond very little to ATP when administered from the basolateral side, but was clearly activated by adenosine and other P1 receptors agonists such as NECA and R-PIA [49]. More generally, these data demonstrate that part of the regulation of ductular secretion may take place at the apical membrane of the cholangiocyte and that activation of an apical receptor can signal to a carrier located at the opposite membrane and that there is a differential polarity of regulatory signals acting on the same carrier.

Renewed interest in intrahepatic bile duct epithelium physiology and pathobiology has been stimulated by the role played by this epithelium in several chronic cholestatic disorders. Cholangiopathies ranges from conditions in which a normal epithelium is damaged by disordered autoimmunity, infectious agents, toxic compounds or ischemia, to genetically determined disorders arising from an abnormal bile duct biology, such as cystic fibrosis or biliary atresia. From a pathophysiological point of view, common to all cholangiopathies is the coexistance of cholangiocyte death and proliferation and various degrees of portal inflammation and fibrosis [4, 50]. Cholestasis dominates the clinical picture and, pathophysiologically, may initiate or worsen the process [51, 52].

Although the pathophysiological significance of the described ion transport mechanisms is as yet not clear, alterations in biliary electrolyte transport could contribute to the pathogenesis of cholestasis in primary bile duct diseases. From a speculative point of view cystic fibrosis liver disease is a very interesting model in that it represents an example of biliary cirrhosis secondary to a derangement of cholangiocyte ion transport. Low rates of HCO_3^-, Cl^- and fluid secretion from cholangiocytes may predispose the biliary epithelium to toxic damages or to infections [53]. Indeed, CFTR is not expressed in hepatocytes and bile duct cell damage can be demonstrated ultrastructurally well before hepatic parenchymal cell damage appears [54]. However overt liver decompensation is clearly much less frequent than pulmonary and pancreatic disease. The reason behind this difference is unclear, but in the case of the liver, other osmolites and regulatory factors secreted by the hepatocytes, such as bile acids and glutathione and ATP, can probably sustain bile flow and minimize the effects of impaired CFTR function.

Most primary cholangiopathies recognize an immune-mediated pathogenesis, however mechanisms of cholestasis in these diseases are unclear. Cholestasis may be due to obliteration of bile ducts, but it is usually present before the onset of ductopenia and it can be seen even in the absence of canalicular dilatation and bile plugs [4, 50-52]. Cytokines, chemokines, and proinflammatory mediators released in the portal spaces [55] or produced by the cholangiocyte itself, likely activate fibrogenesis, stimulate apoptotic and proliferative responses, and alter the transport functions of the epithelium. For example, the low sensitivity of primary biliary cirrhosis to immune-suppressive therapy, the association with secretory failure in other exocrine glands (sicca syndrome) and the beneficial effect of choleretic bile acids, has been suggested to indicate that, in addition to immune-regulation, biliary electrolyte transport could be impaired as well [56]. A defective expression of the gene coding for the AE-2 apical anion exchanger has been reported in patients with primary biliary cirrhosis [56]. AE-2 protein expression was also decreased both in liver biopsies from primary biliary cirrhosis patients, as assessed by immunohistochemistry [57]. Preliminary experiments have shown a decrease in cAMP-stimulated HCO_3^- efflux in cholangiocytes isolated from patients with primary biliary cirrhosis [58]. These data suggest that alterations in biliary electrolyte transport may contribute to the pathogenesis of cholestasis in primary disorders of bile ducts. Deranged cell function may be virus-induced [59, 60] or be a paracrine/autocrine effect of inflammatory mediators. On the contrary to cholestasis induced by bile duct ligation, where inflammation is minimal, most ductopenic syndromes are associated to portal inflammation and the release of a number of cytokines [55] that may have profound effects on epithelial cell function, altering its transport properties [61-63]. For example, interferon-gamma has been shown to reduce CFTR expression in T-84 colonic cells [61] and IL-6 decreases the expression of the Na^+ dependent taurocholate transporter in hepatocytes [64].

Clearly, the field of immune regulation of cholangiocyte function will prove to be very rewarding in the future [65]. Understanding the basics of cholangiocyte transport will soon lead to the development of cell-specific pharmacological or genetic agents [66-68] that, by targeting specific cyto- or chemokines or transport systems involved in the different steps of the inflammatory/fibrotic/cholestatic response, will provide effective and safe treatment of cholestatic diseases.

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REFERENCES

1. Tavoloni, N. The intrahepatic biliary epithelium: an area of growing interest in hepatology. Sem. Liv. Dis. 7:280-292, 1987.
2. Strazzabosco, M., Zsembery, A., and Fabris, L. Electrolyte transport in bile ductular epithelial cells. J. Hepatol. 24 (suppl. 1):78-87, 1996.
3. Strazzabosco, M. and Boyer, J.L. Ion transporters that regulate intracellular pH and secretion in bile duct epithelial cells. In A.E. Sirica and D.S. Longnecker, eds. *Pathology of Intrahepatic Biliary and Pancreatic Duct Cells.* Marcel Dekker Inc., New York, 1996. pp. 113 -118.
4. Strazzabosco, M., Spirlì, C., Zsembery, A., Granato, A., Fabris, L., Cavestro, G.M., Iemmolo, R.M., Okolicsanyi, L., and Crepaldi, G. Pathophysiology of the biliary epithelium. In: D. Alvaro, A. Benedetti and M. Strazzabosco, eds. *Vanishing Bile Duct Syndrome: Pathophystology and Treatment.* Kluwer Academic Publishers. 1997. pp. 117-127.
5. Nathanson, M.H. and Boyer, J.L. Mechanisms and regulation of bile secretion. Hepatol. 14:551-566, 1991.

6. Nielsen, S., Smith, B.L., Christensen, E.I., and Agre, P. Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. Proc. Natl. Acad. Sci. USA 90:7275-7279, 1993.
7. Roberts, S.K., Yano, M., Ueno, Y., Pham, L., Alpini, G., Agre, P., and LaRusso, N.F. Cholangiocytes express the aquaporin CHIP and transport water via a channel-mediated mechanism. Proc. Natl. Acad. Sci. USA 91:13009-13013, 1994.
8. Wheeler, H.O. and Ramos, O.L. Determinants of the flow and composition of bile in the unanesthetized dog during constant infusion of sodium taurocholate. J. Clin. Invest. 39:161-170, 1960.
9. Strazzabosco, M. and Boyer, J.L. Use of isolated bile ductular epithelial cells in biliary physiology. In: *Methods in Biliary Research.* M. Muraca, ed. CRC Press, Inc. Boca Raton, Florida, 1995. pp. 279-291.
10. Alpini, G., Phillips, J.O., Vroman, B., and LaRusso, N.F. Recent advances in the isolation of liver cells. Hepatol. 20:494-454, 1994.
11. Strazzabosco, M., Mennone, A., and Boyer, J.L. Intracellular pH_i regulation in isolated rat bile duct epithelial cells. J. Clin. Invest. 87:1503-1512, 1991.
12. Alvaro, A., Cho, W.K., Mennone, A., and Boyer, J.L. Effect of secretin on pH_i regulation in isolated rat bile duct epithelial cells. J. Clin. Invest. 92:1314-1325, 1993.
13. Spirlì, C., Granato, A., Zsembery, A., Okolicsanyi, L., LaRusso, N.F., and Strazzabosco, M. Gene expression and polarized distribution of membrane acid/base carriers in a differentiated rat cholangiocyte cell line (NRC-1). J. Hepatol. 26 (suppl. 1):63, 1997. (Abs)
14. Strazzabosco, M., Joplin, R., Zsembery, A., Wallace, L., Spirlì, C., Fabris, L., Granato, A., Rossanese, A., Poci, C., Neuberger, J.M., Okolicsanyi, L., and Crepaldi, G. Na-dependent and -independent Cl^-/HCO_3^- exchange mediate cellular HCO_3^- transport in cultured human intrahepatic bile duct cells. Hepatol. 25:976-985, 1997.
15. Scoazec, I.Y., Fléjou, I.F., Feldmann, G., and Housset, C. In situ phenotypic characterization of cells of bile duct lineage. Hepatol. 20:251A, 1994. (Abs)
16. Martinez-Ansò, E., Castillo, J.E., Diaz, J., Medina, J.E., and Prieto, J. Immunohistochemical detection of Cl^-/HCO_3^- anion exchanger in human liver. Hepatol. 19:1400-1406, 1994.
17. Weinman, S.A. and Reuss, L. Na^+/H^+ exchanger and Na^+ entry across the apical membrane of *Necturus* gallbladder. J. Gen. Physiol. 83:57-74, 1984.
18. Weinmann, S.A. and Reuss, L. Na^+/H^+ exchange at the apical membrane of *Necturus* gallbladder. J. Gen. Physiol. 80:299-321, 1982.
19. Marti, U., Elsing, C., Renner, E.L., Liechti-Gallati, S., and Reichen, J. Differential expression of Na^+/H^+ antiporter mRNA in biliary epithelial cells and Hepatocytes. J. Hepatol. 24:498-502, 1996.
20. Singh, S.K., Mennone, A., and Boyer, J.L. Na-K-2Cl cotransport participates in stimulated fluid secretion in isolated polarized bile duct units from rat liver. Hepatol. 24:149A, 1996. (abs)
21. Fitz, J.G., Basavappa, S., McGill, J., Melhus, O., and Cohn, J.A. Regulation of membrane chloride currents in rat bile duct epithelial cells. J. Clin. Invest. 91:319-328, 1993.
22. Fuller, C.M. and Benos, D.J. CFTR! Am. J. Physiol. 263:C267-C286, 1992.
23. Cohn, J.A., Strong, T.V., Picciotto, M.R., Nairn, A.C., Collins, F.S., and Fitz, J.G. Localization of the Cystic Fibrosis Transmembrane Conductance Regulator in human bile duct epithelial cells. Gastroenterol. 105:1857-1864, 1993.
24. McGill, J., Basavappa, S., Shimohura, G.H., Middleton, J.P., and Fitz, J.G. ATP activates ion permeabilities in biliary epithelial cells. Gastroenterol. 107:236-243, 1994.
25. Basavappa, S., Middleton, J., Mangel, A.W., McGill, J.M., Cohn, J.A., and Fitz, J.G. Cl^- and K^+ transport in human biliary cell lines. Gastroenterol. 104:1796-1805, 1993.
26. McGill, J.M., Basavappa, S., and Fitz, J.G. Characterization of high conductance anion channels in rat bile duct epithelial cells. Am. J. Physiol. 262:G703-710, 1992.
27. McGill, J.M., Gettys, T.W., Basavappa, S., and Fitz, J.G. GTP-binding proteins regulate high conductance anion channels in rat bile duct epithelial cells. J. Memb. Biol. 133:253-261, 1993.
28. Lenzen, R., Alpini, G., and Tavoloni, N. Secretin stimulates bile ductular secretary activity through the cAMP system. Am. J. Physiol. 263:G527-G532, 1992.
29. McGill, J.M., Basavappa, S., Gettys, T.W., and Fitz, J.G. Secretin activates Cl^- channels in bile duct epithelial cells through a cAMP-dependent mechanism. Am. J. Physiol. 266:G731-G736, 1994.
30 Gray, M.A., Pollard, C.E., Coleman, H.L., Greenwell, J.R., and Argent, B.E. Anion selectivity and block of the small conductance chloride channel on pancreatic duct cells. Am. J. Physiol. 259:C752-C761, 1990.

31. Zsembery, A., Spirlì, C., Poci, C., Okolicsanyi, L., Crepaldi, G., and Strazzabosco, M. Cyclic-AMP stimulates bicarbonate efflux in bile duct epithelial cells through Cl^-/HCO_3^- exchange independently of Cl^- conductance activation. J. Hepatol. 25:(suppl. 1): 132, 1996.
32. Humphreys, B.D., Jiang, L., Chernova, M.N., and Alper, S.L. Hypertonic activation of AE-2 anion exchanger in *Xenopus* oocytes via NHE-mediated intracellular alkalinization. Am. J. Physiol. 268:C201-C209, 1995.
33. Benedetti, A., Strazzabosco, M., Ng, O.C., and Boyer, J.L. Regulation of activity and apical targeting of the Cl^-/HCO_3^- exchanger in rat hepatocytes. Proc. Natl. Acad. Sci. USA 91:792-796, 1994.
34. Bruck, R., Benedetti, A., Strazzabosco, M., and Boyer, J.L. Intracellular alkalinization induces choleresis and stimulate HCO_3^- and horseradish peroxidase excretion in isolated perfused rat liver by vescicular mediated exocytosis. Am. J. Physiol. 265:G347-G353, 1993.
35. Kopelman, H., Corey, M., Gaskin, K., Durie, P., Weizman, Z., and Forstner, G. Impaired chloride secretion, as well as bicarbonate secretion underlies the fluid secretary defect in the cystic fibrosis pancreas. Gastroenterol. 95:349-355, 1988.
36. Roberts, S.K., Kuntz, S.M., Gores, G.J., and LaRusso, N.F. Regulation of bicarbonate-dependent ductular bile secretion assessed by lumenal micropuncture of isolated rodent intrahepatic bile ducts. Proc. Natl. Acad. Sci. USA 90:9080-9084, 1993.
37. Mennone, A., Alvaro, D., Cho, W., and Boyer, J.L. Isolation of small polarized bile duct units. Proc. Natl. Acad. Sci. USA 92:6527-6531, 1995.
38. Argent, B.E. and Gray, M.A. Regulation and formation of fluid and electrolyte secretions by pancreatic duct epithelium. In A.E. Sirica and D.S. Longnecker eds. *Pathology of Intrahepatic Biliary and Pancreatic Duct Cells.* Marcel Dekker Inc., New York, 1996. pp. 349-377.
39. Marino, C.R. CFTR and the pathophysiology of pancreatic disease in cystic In A.E. Sirica and D.S. Longnecker eds. *Pathology of Intrahepatic Biliary and Pancreatic Duct Cells.*. Marcel Dekker Inc., New York, 1996. pp. 379-407.
40. Reisin, I.L., Prat, A.G., Abraham, E.H., Amara, J.F., Gregory, R.J., Ausiello, D.A., and Cantiello, B.Y. The cystic fibrosis transmembrane conductance regulator is a dual ATP and chloride channel. J. Biol. Chem. 32:20584-20591, 1994.
41. Schwiebert, E.M., Egan, W., Hwang, T.-H., Fuhner, S.B., Allen, S.S., Cutting, G.R., and Guggino, W.B. CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. Cell 81:1063-1073, 1995.
42. Chari, R.S., Schutz, S.M., Haebig, J.E., Shimokura, G.H., Cotton, P.B., Fitz, J.G., Meyers, W.C. Adenosine nucleotides in bile. Am. J. Physiol. 270:G246-G252, 1996.
43. Elsing, C., Kassner, A., and Stremmel, W. Sodium, Hydrogen antiporter activation by extracellular adenosine triphosphate in biliary epithelial cells. Gastroenterol. 111:1321-1332, 1996.
44. Zembery, A., Spirlì, C., Granato, A., Okolicsanyi, L., Crepaldi, G., and Strazzabosco, M. Autocrine regulation of transepithelial H^+/HCO_3^- transport through CFTR-mediated ATP release in bile duct cells. Hepatol. 24:255A, 1996. (Abs)
45. Schlosser, S.F., Burgstahler, A.D., and Nathanson, M.H. Isolated rat hepatocytes can signal to other hepatocytes and bile duct cells by release of nucleotides. Proc. Natl. Acad. Sci. USA 93:9948-9953, 1996.
46. Sheppard, D.N. and Welsh, M.J. Inhibition of the cystic fibrosis transmembrane conductance regulator by ATP-sensitive K^+ channel regulators. Ann. N. Y. Acad. Sci. 707:275-284, 1993.
47. Reddy, M.M., Quinton, P.M., Haws, C., Wine, J.J., Grygorczyk, R., Tabcharani, J.A., Hanrahan, J.W., Gunderson, K.L., and Kopito, R.R. Failure of the cystic fibrosis transmembrane conductance regulator to conduct ATP. Science 271:1876-1879, 1996.
48. Vroman, B. and LaRusso, N.F. development and characterization of polarized primary cultures of rat intrahepatic bile duct epithelial cells. Lab. Invest. 74:303-313, 1996.
49. Olah, M.E. and Stiles, G.L. Adenosine receptor subtypes: Characterization and therapeutic regulation. Ann. Rev. Pharmacol. Toxicol. 35:581-606, 1995.
50. Roberts, S.K., Ludwig, J., LaRusso, N.F. The pathobiology of biliary epithelia. Gastroenterol. 112:269-279, 1997.
51. Woolf, G,M. and Vierling, J.M. Disappearing intrahepatic bile ducts: the syndromes and their mechanisms. Sem. Liv. Dis. 13:261-275, 1993.
52. Popper, H. General pathology of the liver: light microscopic aspects serving diagnosis and interpretation. Sem. Liv. Dis. 6:175-184, 1986.
53. Colombo, C., Battezzati, P.M., and Podda, M. Hepatobiliary disease in cystic fibrosis. Sem. Liv. Dis. 14:259-269, 1994.
54. Lindblad, A., Hultcrantz, R., and Strandvik, B. Bile duct destruction and collagen deposition: a prominent ultrastructural feature of the liver in cystic fibrosis. Hepatol. 16:372-381, 1992.

55. Martinez, O.M., Villanueva, J.C., Gershwin, M.E., and Krams, S.M. Cytokine patterns and cytotoxic mediators in primary biliary cirrhosis. Hepatol. 21:113-119, 1995.
56. Prieto, J., Qian, C., Garcia, N., Diez, J., and Medina, J.F. Abnormal expression of anion exchanger genes in primary biliary cirrhosis. Gastroenterol. 105:572-578, 1993.
57. Medina, J.F., martinez-Ansó, E., Vasquez, J., and Prieto, J. Decreased anion exchanger immunoreactivity in the liver of patients with primary biliary cirrhosis. Hepatol. 25:1217, 1997.
58. Strazzabosco, M., Joplin, R., Zsembery, A., Granato, A., Spirlì, C., Poci, C., and Crepaldi, G. Bicarbonate transport in cultured human intrahepatic bile duct epithelial cells from normal subjects and patients with primary biliary cirrhosis. Hepatol. 20:405A, 1994. (Absract)
59. Xu, L., Guo, L., Joplin, R., Neuberger, J., Rixon, F., Aitken, J., Carman, W., Perrillo, R., and Mason, A. Viral induction of primary biliary cirrhosis. J. Hepatol. 26(suppl. 1):61, 1997.
60. Zhou, Y.F., Guetta, E., Yu, Z.X., Finkel, T., and Epstein, S.E. Human cytomegalovirus increases modified low density lipoprotein uptake and scavenger mRNA expression in vascular smooth muscle cells. J. Clin. Invest. 98:2129-2138, 1996.
61. Besançon, F., Przewlocki, G., Baró, I., Hongre, A.-S., Escande, D., and Edelman, A. Interferon-γ downregulates CFTR gene expression in epithelial cells. Am. J. Physiol. 267:C1398-C1404, 1994.
62. Balfour Sartor, R. Cytokines in intestinal inflammation: pathophysiological and clinical considerations. Gastroenterol. 106:533-539, 1994.
63. Perdue, M.H. and McKay, D.M. Integrative immunophysiology in the intestinal mucosa. Am. J. Physiol. 267:G151-G165, 1994.
64. Green, R.M., Whiting, J.F., Rosenbluth, A.B., Beier, D., and Gollan, J.L. Interleukin-6 inhibits hepatocyte taurocholate uptake and sodium-potassium-adenosine triphosphatase activity. Am. J. Physiol. 267:G1094-G1100, 1994.
65. Adams, D.H. Biliary epithelial cells: innocent victims or active participants in immunemediated liver disease? J. Lab. Clin. Med. 128:528-530, 1996.
66. Grubman, S.H., Fang, S.L., Mulberg, A.E., Perrone, R.D., Rogers, L.C., Lee, D.W., Artnentano, D., Murray, S.L., Dorkin, H.L., Cheng, S.H., Smith, A.E., and Jefferson, D.M. Correction of the cystic fibrosis defect by gene complementation in human intrahepatic biliary epithelial cell lines. Gastroenterol. 108:584-592, 1995.
67. Yang, Y., Raper, E.S., Cohn, J.A., Engelhardt, J.F., and Wilson, J.M. An approach for treating the hepatobiliary disease of cystic fibrosis by somatic gene transfer. Proc. Natl. Acad. Sci. USA 90:4601-4605, 1993.
68. Wagner, J.A., Chao, A.C., and Gardner, P. Molecular strategies for therapy of cystic fibrosis. Ann. Rev. Pharmacol. Toxicol. 35:257-276, 1995.