The Role of Phosphoinositide 3-Kinase in Taurocholate-induced Trafficking of ATP-dependent Canalicular Transporters in Rat Liver*

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Recent studies indicate that wortmannin, a potent inhibitor of phosphatidylinositol (PI) 3-kinase, interferes with bile acid secretion in rat liver; taurocholate induces recruitment of ATP-dependent transporters to the bile canalicular membrane, and PI 3-kinase products are important in intracellular trafficking.

We investigated the role of PI 3-kinase in bile acid secretion by studying the in vivo effect of taurocholate, colchicine, and wortmannin on bile acid secretion, kinase activity, and protein levels in canalicular membrane vesicle (CMV) and sinusoidal membrane vesicle (SMV) fractions from rat liver. Treatment of rats or perfusion of isolated liver with taurocholate significantly increased PI 3-kinase activity in both membrane fractions. Taurocholate increased protein content of ATP-dependent transporters, which were detected only in CMVs, whereas increased levels of p85 and a cell adhesion molecule, cCAM 105, were observed in both fractions.

Colchicine prevented taurocholate-induced changes in all proteins studied, as well as the increase in PI 3-kinase activity in CMVs, but it resulted in further accumulation of PI 3-kinase activity, p85, and cCAM 105 in SMVs. These results indicate that taurocholate-mediated changes involve a microtubular system.

Wortmannin blocked taurocholate-induced bile acid secretion. The effect was more profound when wortmannin was administered prior to treatment with taurocholate. When wortmannin was given after taurocholate, the protein levels of each ATP-dependent transporter were maintained in CMVs, whereas the levels of p85 and cCAM decreased in both membrane fractions. Perfusion of liver with wortmannin before taurocholate administration blocked accumulation of all proteins studied in CMVs and SMVs.

These results indicate that PI 3-kinase is required for intracellular trafficking of itself, as well as of ATP-dependent canalicular transporters.

Bile acids are the predominant organic solutes in bile. Only 5% of the total bile acid pool is produced daily as a result of 7α hydroxylation of cholesterol in the liver; the remaining 95% of bile acids undergoes enterohepatic circulation and is transferred into the bile from plasma by mechanisms that are only partially understood (1, 2). Three bile acid transporters have been identified in the basolateral plasma membrane of hepatocytes, but none are known to associate with vesicular trafficking of bile acids to the canaliculus (3–5). Although bile acids bind with varied affinity to several hepatocellular cytoplasmic proteins (6), the role of protein binding in intracellular movement is uncertain. Because administration of microtubular inhibitors, such as colchicine, reduces bile acid secretion but not uptake from plasma, a microtubule-based vesicular mechanism of transcellular bile acid transport has been proposed (7, 8).

In recent years, several ATP-dependent transporters have been functionally identified in CMVs for bile acids, organic cations, phosphatidylcholine, and nonbile acid organic anions (9–13). Cloning identified the responsible proteins as spgp, mdr1, mdr2, and mrp2, respectively (14–17). spgp transports taurocholate but not several other bile acids by an ATP-dependent mechanism (17). Additional canalicular bile acid transporters may be present. CMVs also manifest bile acid and nonbile acid organic anion transport, which is driven by a non-ATP-dependent membrane potential (18, 19); whether different transporters are involved in these processes is controversial (19).

While studying the function of the phosphatidylcholine translocator, MDR3 (human/mdr2 (rat) in rat liver, we observed that ATP-dependent phosphatidylcholine translocation from the inner to the outer leaflet of the canalicular membrane is enhanced by addition of taurocholate in vitro (20) and in vivo (21). The latter observation prompted consideration that subcellular localization, trafficking, and regulation of canalicular ATP-dependent transporters may play an important role in normal bile secretion and in cholestasis. Administration of taurocholate in vivo resulted in specific increase in all known ATP-dependent transporters in the canalicular membrane, as well as canalicular ectoenzymes and cCAM 105, a canalicular adhesion molecule; the effects were prevented by pretreatment with colchicine (21). These observations suggested that canalicular ATP-dependent transporters undergo microtubule-dependent trafficking.

Additional evidence for recruitment of canalicular bile acid transporters is provided by kinetic studies that suggest that the ATP-dependent transporters for bile acids and nonbile acid organic anions are saturated under basal conditions (12, 13). Therefore, we proposed that the substantial increase in bile

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¶ The abbreviations used are: CMV, canalicular membrane vesicle; SMV, sinusoidal membrane vesicle; mdr, multidrug resistance protein; mrp, multidrug resistance-associated protein; cCAM 105, canalicular cell adhesion molecule; spgp, sister of P-glycoprotein; PI, phosphatidylinositol.
acid secretion after taurocholate administration in vivo could result from either rapid transporter activation or recruitment to the canalicular domain (21). Incubation of CMVs with taurocholate increased ATP-dependent bile acid transport by 15%; however, similar studies in CMVs after administration of taurocholate in vivo resulted in over 100% increase within 10 min, suggesting that transporter mobilization is the likely mechanism (21).

In hepatocytes, newly synthesized canalicular ectoenzymes, such as 5′-nucleotidase and dipeptidyl peptidase IV, and adhesion molecules, such as cCAM 105, are transported from the trans-Golgi network to the basolateral plasma membrane domain, from which they undergo transcytosis to the canalicular plasma membrane domain (22, 23). The intracellular trafficking of canalicular ATP-dependent transporters has not been determined; however, new evidence suggests that these proteins traffic from the trans-Golgi network to subapical endosomes and/or directly to the canalicular plasma membrane.  

Recent studies indicate that PI3-kinase, which is involved in growth factor receptor-mediated signaling cascades leading to mitogenic responses, is also required for membrane trafficking (24, 25). The first indication that PI 3-kinase and its lipid products participate in intracellular membrane trafficking was the observation that Vps34p, which is required for intracellular trafficking in yeast, shares sequence homology with the catalytic subunit (p110) of mammalian type 1 PI 3-kinase (26). Mammalian type 1 PI 3-kinases phosphorylate PI, PI 4-phosphate, and PI 4,5-bisphosphate to produce PI 3-phosphate, PI 3,4-bisphosphate, and PI 3,4,5-trisphosphate, respectively (27). PI 3,4-bisphosphate and PI 3,4,5-trisphosphate are absent in resting cells and rapidly appear after cell activation (28). Yeast cannot produce PI 3,4-bisphosphate or PI 3,4,5-trisphosphate; however, these lipids may play a role in regulating vesicular trafficking in higher eukaryotic cells (27). In mammalian cells, PI 3-kinase is required for platelet-derived growth factor-dependent delivery of the platelet-derived growth factor receptor to lysosomes (29) and for insulin-dependent trafficking of the glucose transporter 4 (GLUT4) to the plasma membrane (30).

The present study was designed to determine whether recruitment of taurocholate-induced ATP-dependent transporters or cCAM 105 to the canalicular and basolateral plasma membrane domains is associated with or results from changes in PI 3-kinase activity. We measured PI 3-kinase activity in rat CMVs and SMVs after treatment in vivo with taurocholate, colchicine, and a PI 3-kinase inhibitor, wortmannin, which, at the doses used, specifically inhibits PI 3-kinase activity (31). Protein levels were measured in CMV and SMV fractions using antibodies against the transporters, cCAM 105, and PI 3-kinase. The results were correlated with bile acid secretion in vivo and protein levels of PI 3-kinase, cCAM 105, and ATP-dependent transporters in SMV and CMV fractions. Our study revealed that PI 3-kinase is required for microtubule-based transcytosis of bile acids and targeting of ATP-dependent transporters to the bile canalicular domain.

**EXPERIMENTAL PROCEDURES**

Male Sprague-Dawley rats weighing 250–300 g were purchased from Charles River Farms (Wilmington, MA). Tris base, EDTA, CaCl₂, sucrose, HEPES, taurocholic acid, colchicine, protein A-Sepharose beads, L-γ-leucine-p-nitroanilide, L-γ-glutamyl-p-nitroanilide, wortmannin, and all other reagents were purchased from Sigma and were of the highest purity. [γ-32P]ATP (6000 Ci/mmol) and [γ-32P]PI-ATP (6000 Ci/mmol) were obtained from NEN Life Science Products. Reagents for SDS-polyacrylamide gel electrophoresis and for protein determination were from Bio-Rad. Enhanced chemiluminescence reagents were from Amersham Pharmacia Biotech. p85 (polyclonal, anti-PI 3-kinase), was generated against a p85 SH2 glutathione S-transferase fusion protein; C219 (monoclonal, anti-mdr1, mdr2, and spp) antibody was from Signet Laboratories; EAG15 (polyclonal, anti-mrp2) was a gift from Dr. D. Kepler, Heidelberg, Germany; K12 (polyclonal, anti-spp) was a gift from Dr. B. Steiger, Zurich, Switzerland, and Ab65 (polyclonal, anti-cCAM 105) was a gift from Dr. S. H. Lin, Houston, TX.

**Treatment of Rats in Vivo**—Rats were anesthetized with ether and sodium pentobarbital (50 mg/kg intraperitoneal). Taurocholate (20 μmole in 1 ml of PBS) was injected intravenously over 2 min. Forty-five min later, rats were killed, and the liver was rapidly perfused at room temperature with 0.25 M sucrose and 10 μM HEPES-Tris containing protease inhibitors (2 μg/ml aprotinin, 2 μg/ml leupeptin, 2 μg/ml pepstatin, 100 μg/ml phenylmethylsulfonyl fluoride, and 5 μg/ml benzamidine), and homogenized in 5 volumes of buffer. Other rats were injected with 2.5 mg of colchicine/kg 2.5 h before taurocholate treatment and killed 45 min later. CMVs were isolated from liver homogenates by nitrogen cavitation and Ca²⁺ precipitation (32), and SMVs were isolated using bovine serum albumin as standard and Bio-Rad dye.

**Isolated Perfused Rat Liver**—Rats were anesthetized with sodium pentobarbital (50 mg/kg), and nonrecirculating single pass liver perfusion was performed according to Hems et al. (33). Briefly, the common bile duct was cannulated with PE-10 tubing; the pancreatic duodenal branch of the portal vein was ligated; and the portal vein and hepatic veins were cannulated with a 14-gauge Teflon catheter. The effect of wortmannin on taurocholate secretion was determined using two protocols. In the first, the liver was constantly perfused at 37 °C with 30 ml/min with CO₂/O₂ (5%/95%) oxygenated Krebs-Ringer bicarbonate buffer containing 5.5 mM glucose, 200 units of heparin/100 ml, 1% bovine serum albumin, and 100 μM taurocholate. [3H]Taurocholate (2 × 10⁶ cpm) was added to the perfusion buffer after 10 min. Bile flow was achieved by maintaining portal pressure at 16 cm of H₂O. O₂ supply, temperature, and buffer pH (7.35–7.40) throughout the perfusion. Bile was collected at 3-min intervals, and samples were weighed to determine volume and secretion of [3H]taurocholate. The effluent perfusion buffer was also collected for measurement of [3H]taurocholate. Wortmannin was dissolved in dimethyl sulfoxide, diluted to 100 nM in buffer immediately prior to use. Taurocholate (100 nM) was infused at 30 ml/min for 60 min, after which wortmannin was added to taurocholate and infused for an additional 30-min period. [3H]Taurocholate secretion was measured in bile collected at 3-min intervals before and after wortmannin treatment. In a second protocol, wortmannin (100 nM) was infused in Krebs-Ringer bicarbonate buffer for 30 min, at which time taurocholate (100 μM), [3H]taurocholate (2 × 10⁶ cpm), and fresh wortmannin (100 nM) were infused for an additional 30 min. In both protocols, the liver was removed after perfusion, and basolateral and canalicular membrane vesicles were prepared and characterized.

**PI 3-Kinase Assay**—Two hundred fifty ng of CMV and SMV protein were used for each assay. Assays were performed in a reaction mixture containing 0.001% Nonidet P-40, 125 μM ATP, 125 mCi MOPS, pH 7.0, 25 mM MgCl₂, 5 mM EGTA, and 0.2 mg/ml sonicated lipids PI-4,5-bisphosphate (1:1) (36, 37) and 107 cpm) was added to the perfusion buffer after 10 min. Choline chloride was achieved by maintaining portal pressure at 16 cm of H₂O. O₂ supply, temperature, and buffer pH (7.35–7.40) throughout the perfusion. Bile was collected at 3-min intervals, and samples were weighed to determine volume and secretion of [3H]taurocholate. The effluent perfusion buffer was also collected for measurement of [3H]taurocholate. Wortmannin was dissolved in dimethyl sulfoxide, diluted to 100 nM in buffer immediately prior to use. Taurocholate (100 nM) was infused at 30 ml/min for 60 min, after which wortmannin was added to taurocholate and infused for an additional 30-min period. [3H]Taurocholate secretion was measured in bile collected at 3-min intervals before and after wortmannin treatment. In a second protocol, wortmannin (100 nM) was infused in Krebs-Ringer bicarbonate buffer for 30 min, at which time taurocholate (100 μM), [3H]taurocholate (2 × 10⁶ cpm), and fresh wortmannin (100 nM) were infused for an additional 30 min. In both protocols, the liver was removed after perfusion, and basolateral and canalicular membrane vesicles were prepared and characterized.

2 A. Nies, Y. Sai, and H. Kipp, personal communication.
albumin, and 0.1% Tween 20. Membranes were washed and probed with one of the following antibodies: p85, C219, spgp, EAG15, or Ab65. The second antibodies used were anti-rabbit IgG (New England Biolabs) and goat anti-mouse IgG (Bio-Rad), which were conjugated with horseradish peroxidase. Immune complexes were detected by enhanced chemiluminescence, and the size of proteins was estimated by prestained molecular weight standards. Immunoreactive bands obtained by enhanced chemiluminescence were quantified by densitome-
try. Membranes were reused for blotting after neutralization with 15% H$_2$O$_2$ or stripping at 50 °C (according to procedure from Amersham, Pharmacia Biotech).

RESULTS

We examined the effect of taurocholate, colchicine, and wortmannin on the trafficking of intrinsic canalicular membrane proteins, including ATP-dependent transporters (mdr, spgp, and mrp2) and a nontransporter adhesion molecule (cCAM 105).

Antibodies to mdr1, mdr2, and spgp (C219); spgp (K12); mrp2 (EAG15); and cCAM 105 (Ab65) were used to determine the specific content of the respective proteins in SMVs and CMVs. Anti-p85 antibody was used to quantitate distribution of the regulatory subunit of PI 3-kinase. As shown in Fig. 1, in control animals, mdr, spgp, and mrp2 were expressed exclusively in CMVs; p85 and cCAM 105 were present in both fractions. Intravenous injection of taurocholate produced maximal secretion of taurocholate in bile and significantly increased the level of each protein in its respective plasma membrane fraction. To determine whether accumulation of these proteins in vesicular fractions requires microtubule-mediated transport, we repeated the studies in rats that were pretreated with colchicine. Colchicine decreased taurocholate-induced accumulation of all proteins examined in CMVs; however, p85 and cCAM 105 levels remained elevated in SMVs. These results indicate that intracellular trafficking and recruitment of canalicular ATP-dependent transporters and cCAM 105 differ in response to taurocholate administration.

Because taurocholate administration resulted in accumulation of PI 3-kinase p85 in both vesicular preparations, we determined whether these results correlated with enzymatic activities. The basal activity of PI 3-kinase was greater in SMV than in CMV fractions (3.6 ± 1.3 versus 2.2 ± 0.4 pmol PIP$_3$/mg/min). Taurocholate administration increased PI 3-kinase activity in SMVs and CMVs (Fig. 2), similar to its effects on protein levels (Fig. 1). A direct effect on PI 3-kinase was excluded by observing that enzyme activity in whole cell lysates and anti-p85 immunoprecipitates was unaffected by incubation in 10–100 μM taurocholate or colchicine (data not shown). Pretreatment with colchicine blocked taurocholate-induced increased activity of PI 3-kinase in CMVs (Fig. 2). In SMVs, colchicine increased PI 3-kinase activity 6.4-fold above basal values and 2-fold above taurocholate-induced activity (Fig. 2).

Whether or not taurocholate-induced activation of PI 3-kinase has physiological significance was studied using wortmannin, which, at the dose used, is a specific inhibitor of PI 3-kinase. The effect of wortmannin on taurocholate uptake and bile acid secretion was determined in isolated perfused rat liver. Taurocholate administration stimulated bile acid secretion, which was maximal after 20 min of perfusion and maintained for up to 60 min. Addition of wortmannin after taurocholate reduced bile acid secretion by 50% within 5 min (Fig. 3A). Plasma levels of taurocholate were unaffected (data not shown), indicating that wortmannin did not alter taurocholate uptake but rapidly reduced bile acid secretion. When the sequence of administration was reversed (i.e., wortmannin was administered before taurocholate), bile acid secretion did not increase as much in response to taurocholate (Fig. 3B), which indicates that PI 3-kinase activity is required for taurocholate-induced bile acid secretion.

To determine the role of PI 3-kinase in the regulation of
taurocholate-induced bile acid secretion, we quantified the effect of wortmannin in isolated perfused rat liver on PI 3-kinase activity in SMVs and CMVs and on trafficking of the bile canalicular ATP-dependent transporters and cCAM 105. PI 3-kinase activity in CMVs and SMVs from control and treated perfused liver is presented in Fig. 4. Taurocholate administration increased PI 3-kinase activity 2- and 4-fold in CMVs and SMVs, respectively, which was similar to effects observed in the same fractions from rats treated in vivo (Fig. 2). Wortmannin administration after pretreatment with taurocholate inhibited PI 3-kinase activity in both vesicular fractions. Similar data were obtained when wortmannin administration preceded taurocholate (data not shown). These studies reveal that wortmannin inhibits taurocholate-induced PI 3-kinase activity in SMVs and CMVs obtained from isolated perfused rat liver.

As shown in Fig. 5, similar to effects observed in experiments in which taurocholate was administered intravenously, taurocholate-perfused liver showed an increase in p85, cCAM 105, spgp, mdr, and mrp2 protein levels in CMVs and increase in p85 and cCAM 105 in SMVs. Administration of wortmannin 30 min after the initial treatment with taurocholate reduced taurocholate-induced accumulation of p85 and cCAM 105 proteins in CMVs and SMVs. Taurocholate-induced protein levels of ATP-dependent transporters in CMVs were decreased by wortmannin only when it was administered before taurocholate. However, protein levels of the transporters were not altered if wortmannin was infused after taurocholate (Fig. 5).

**DISCUSSION**

The objective of the present study was to determine the role of PI 3-kinase in bile acid transport and secretion and in intracellular trafficking of bile canalicular ATP-dependent membrane transporters and cCAM 105, a membrane-associated protein that, under basal conditions, undergoes microtubule-dependent transcytosis. PI 3-kinase activity, canalicular ATP-dependent transporters, and cCAM 105 protein levels were analyzed in various experimental conditions. This is the first study that examines these processes in basolateral and canalicular membrane domains of the hepatocyte.

Several isomers of type 1 PI 3-kinase (p110α, β, and δ) have been identified and are regulated by interaction with p85 α or β regulatory subunits (38). The p110γ PI 3-kinase activation is mediated by direct interaction with G-type receptors and does not require p85 (38). Because diverse mechanisms of activation of PI 3-kinase isoforms produce higher phosphorylated forms of 3’-polyphosphoinositides, we measured total PI 3-kinase activity in vesicular fractions and compared these results with the content of p85 regulatory subunit in the same fractions. Although PI 3-kinase activity and p85 are mainly cytosolic, a fraction of the protein and activity are membrane-associated (37) and were detected in CMV and SMV fractions. In contrast, ATP-dependent transporters were found only in CMVs and were never detected in SMVs.

After administration of taurocholate, the levels of all proteins studied and PI 3-kinase activity were increased in the respective plasma membrane fractions. Because the increased activity of PI 3-kinase paralleled accumulation of p85, cCAM 105, and ATP-dependent transporter proteins, the results suggest that taurocholate enhances mobilization of each of these proteins to their respective membrane domains. As shown in Fig. 1B, taurocholate administration increased spgp to a degree greater than the increase in mdr. Because C219 recognizes spgp, mdr1, and mdr2, one might consider that spgp accounts for the C219 results without any effect on the translocation of mdr1 and mdr2; however, this is not the case. Previously, we demonstrated that ATP-dependent canalicular transport of daunomycin (an mdr1 function) and translocation of phosphatidylcholine (an mdr2 function) were similarly increased after taurocholate administration (21). Taurocholate increased the canalicular content and function of each of the mdr protein family members (mdr1, mdr2, and spgp) (21).

Disruption of the microtubular apparatus by colchicine had differential effects on taurocholate-induced accumulation of PI 3-kinase activity and protein levels in CMVs and SMVs. In CMVs, colchicine inhibited taurocholate-induced increase in PI 3-kinase activity, which suggests that taurocholate increases microtubule-dependent trafficking of PI 3-kinase to the canalicular membrane. All taurocholate-induced accumulation of transporter proteins was down-regulated after colchicine treatment in CMVs, whereas in SMVs, cCAM 105 and p85 levels remained elevated. In addition, taurocholate-induced PI 3-kinase activity in SMVs was further increased after colchicine administration, indicating that PI 3-kinase participates in transcytotic vesicular transport of taurocholate and that taurocholate-mediated accumulation of ATP-dependent transporters and PI 3-kinase in CMVs requires the transcytotic pathway.

These results are consistent with the hypothesis that PI 3-kinase is associated with intracellular vesicles that are transcytosed from the basolateral to the canalicular plasma membrane together with other membrane-associated molecules, such as cCAM 105 (23). This is the first evidence that PI 3-kinase is transported by a microtubular apparatus, although association of PI 3-kinase with microtubular filaments has been previously reported in platelet-derived growth factor-stimulated fibroblasts (39). Colchicine-induced microtubular disruption retards the transcytotic vesicular pathway and transfer of eot-enzymes and cCAM 105 to the canalicular membrane, resulting in their accumulation in the basolateral domain. Although the level of p85 was unchanged in SMVs, PI 3-kinase activity significantly increased in SMVs after colchicine treatment. Other isoforms of PI 3-kinase, which are not detectable by the used antibodies, may also accumulate in sinusoidal membranes and contribute to the observed increase in enzymatic activities.

Because systemic administration of wortmannin, a specific PI 3-kinase inhibitor at 50–100 nM could affect other organs,
FIG. 5. Studies in isolated perfused rat liver. The effect of wortmannin (Wm) when administered prior to or following taurocholate (TC) administration on the protein content of PI3K, spgp, mdr (mdr1, mdr2, and spgp), mrp2, and cCAM in CMVs and SMVs. Results are representative of three experiments. A, immunoblotting. B, densitometric quantitation of the effect of wortmannin on TC-induced protein levels in CMVs. C, densitometric quantitation of the effect of wortmannin on TC-induced protein levels in SMVs. Open bars represent results when TC was perfused for 60 min and wortmannin was perfused for the last 30 min. Solid bars indicate the response when Wm was perfused for 60 min and TC was perfused for the last 30 min. Results in B and C are expressed as a percentage of individual protein levels observed after administration of TC and are representative of three experiments.
we used the isolated perfused liver technique for subsequent studies. Administration of taurocholate had similar effects on accumulation of proteins and PI 3-kinase in vesicular fractions whether it was given systemically or by perfusion of an isolated liver. As expected, taurocholate-enhanced bile acid secretion was reduced to 50% by subsequent perfusion with 100 nM wortmannin; however, taurocholate-induced accumulation in CMVs of canalicular ATP-dependent transporters was unaffected, indicating that transporter recruitment to the canalicular membrane is not affected if the inhibitor is administered after taurocholate. In contrast, when wortmannin was administered prior to taurocholate infusion, bile acid secretion and canalicular accumulation of ATP-dependent transporters were profoundly reduced. These results suggest that a major component of taurocholate-induced bile acid secretion and recruitment of transporters to the canalicular membrane are PI 3-kinase-dependent. In addition, other isoforms of PI 3-kinase, which are resistant to low concentrations of wortmannin, may also participate in the regulation of bile acid secretion. Our results are consistent with previous reports suggesting that PI 3-kinase is involved in vesicular trafficking and bile formation (40, 41).

Despite significant taurocholate-induced accumulation of ATP-dependent transporters in CMVs, we did not detect these proteins in SMVs at any time, which suggests that transporter-containing vesicles associate with the bile canalicular membrane without undergoing transcytosis from the basolateral plasma membrane. How and where this association occurs remains to be determined. Both nontranscytotic delivery of ATP-dependent transporters to the canalicular membrane and transcytotic trafficking of cCAM 105 require PI 3-kinase activity Wortmannin also inhibited accumulation of PI 3-kinase protein, which suggests that PI 3-kinase is required for trafficking of itself.

These studies prompt revision of current hypotheses regarding mechanisms of bile acid transport in the hepatocyte, recruitment of canalicular ATP-dependent transporters, and intracellular trafficking patterns for canalicular intrinsic membrane proteins. The dependence of these processes on specific downstream products of PI 3-kinase is the subject of our further studies.

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