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REVIEW

Beyond the Cell

The cell biology of the hepatocyte: A membrane trafficking machine

Ryan J. Schulze[1*], Micah B. Schott[1*], Carol A. Casey[2,3], Pamela L. Tuma[4], and Mark A. McNiven[1]

The liver performs numerous vital functions, including the detoxification of blood before access to the brain while simultaneously secreting and internalizing scores of proteins and lipids to maintain appropriate blood chemistry. Furthermore, the liver also synthesizes and secretes bile to enable the digestion of food. These diverse attributes are all performed by hepatocytes, the parenchymal cells of the liver. As predicted, these cells possess a remarkably well-developed and complex membrane trafficking machinery that is dedicated to moving specific cargos to their correct cellular locations. Importantly, while most epithelial cells secrete nascent proteins directionally toward a single lumen, the hepatocyte secretes both proteins and bile concomitantly at its basolateral and apical domains, respectively. In this *Beyond the Cell* review, we will detail these central features of the hepatocyte and highlight how membrane transport processes play a key role in healthy liver function and how they are affected by disease.

Introduction

Hepatocytes comprise up to 80% of the total cell population and volume of the human liver and are intimately associated with both arterial and venous blood (Blouin et al., 1977). Remarkably, >12% of our blood volume resides within the liver, flowing past and over long rows, or "cords," of hepatocytes. Thus, each hepatocyte is literally "bathed in blood" along multiple surfaces via a system of highly fenestrated vessels that course through the liver to enable the bidirectional, cell-to-plasma exchange of components. This physical intimacy facilitates two central functions of the liver in its role as a vital hematological filter: the production of blood plasma proteins and the concomitant endocytic uptake of lipids, growth factors, and other trophic agents. While essential, this exceptional purification capacity puts the liver at risk by making it highly susceptible to damage from excessive exposure to fat, alcohol, drugs, and other toxins as well as a host of pathogens, in particular hepatitis viruses. To meet these demands, the hepatocyte has evolved into a supercharged membrane transport device that operates a complex vesicle-based protein sorting machinery superimposed upon an organized cytoskeletal scaffold. So sophisticated is this sorting system that no human-devised artificial apparatus is yet able to provide even a temporary substitute during liver failure.

This review will provide insights into hepatocyte function as an exceptional cellular model to study membrane transport and how this process is used to meet daily physiological demands. We will first provide some background into the cellular organization of the liver, hepatocyte polarity, and cytoskeletal architecture. With this foundation, we can understand how membrane trafficking supports the central tasks of the hepatocyte that translate into basic liver functions. These functions include the secretion of proteins and lipids to both apical and basolateral plasma membrane domains, the formation of bile, endocytosis-based filtering of the blood, and detoxification of substances such as alcohol. Finally, we will review how these processes are used and even "hijacked" by viral pathogens that lead to organ damage.

Liver anatomy and hepatocyte polarity

Liver vascularization is unusual compared with other organs, because it receives a simultaneous mixture of arterial and venous blood. Oxygenated blood arriving directly from the aorta via the hepatic artery represents only 25% of the incoming blood supply. In contrast, the remaining 75% of inbound blood is partially deoxygenated but nutrient rich, originating from various organs in the gastrointestinal system via the portal vein (Fig. 1 A; Vollmar and Menger, 2009). This vascular

[1]Division of Gastroenterology and Hepatology, Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN; [2]Research Service, Department of Veterans Affairs, Nebraska-Western Iowa Health Care System, Omaha, NE; [3]Departments of Internal Medicine and Biochemistry & Molecular Biology, University of Nebraska Medical Center, Omaha, NE; [4]The Catholic University of America, Washington, DC.

*R.J. Schulze and M.B. Schott contributed equally to this paper; Correspondence to Mark McNiven: mcniven.mark@mayo.edu; Pamela L. Tuma: tuma@cua.edu.

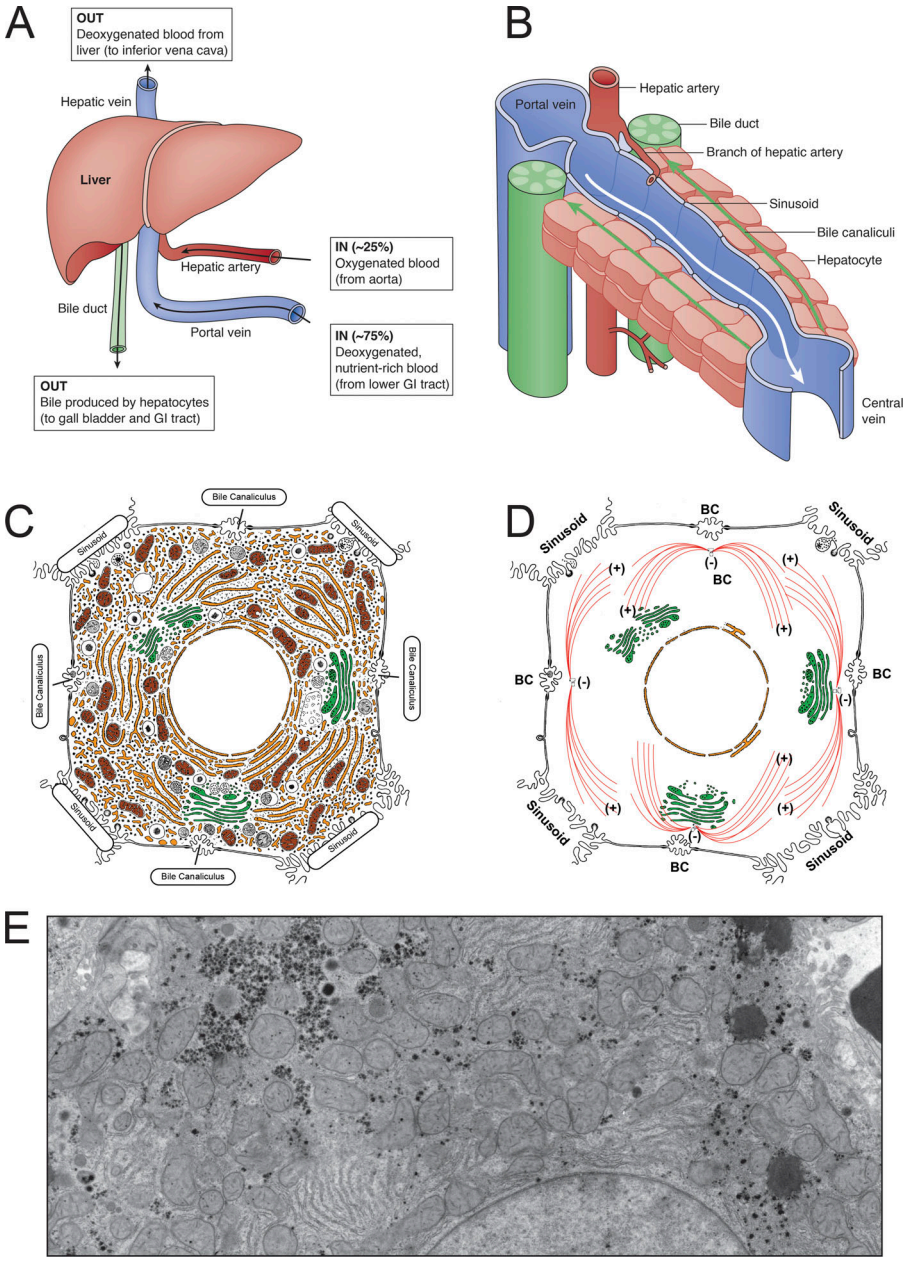

Figure 1. **Liver and hepatocellular architecture. (A)** Organization of the hepatic blood supply. The liver receives a mixture of nutrient-rich blood from the lower gastrointestinal tract via the portal vein (~75%) as well as oxygenated blood from the heart via the hepatic artery (~25%). Deoxygenated blood from the liver is released into the hepatic vein, while bile is released into the common bile duct for delivery to the gall bladder and gastrointestinal tract to aid in digestion. **(B)** Schematic of the hepatic sinusoid. Portal venous and hepatic arterial blood enters the hepatic sinusoid and flows along cords of hepatocytes to the central vein. Bile flows in the opposite direction through bile canaliculi before entering a bile ductule. **(C)** Drawing of the complexity of the crowded cytoplasm of the hepatocyte. This sketch represents a single hepatocyte flanked by four bile canaliculi (BC) and four sinusoids, representing the apical and basolateral membranes of the hepatocyte, respectively. Mitochondria, ER, and Golgi membranes are highlighted in red, orange, and green, respectively. Panel C is reprinted and modified with permission from Porter and Bonneville (1973). **(D)** Multiple microtubule-organizing centers are observed in a single hepatocyte, with the (–) end of microtubules extending outward from periapical domains with (+) ends oriented toward the basolateral domains. **(E)** Electron micrograph of the crowded cytoplasm from a pericentral hepatocyte (courtesy of Keith Porter, personal collection). Magnification of 8,100.

architecture results in nearly 1,500 ml of arterial and venous blood uniting every minute while entering the liver. This blood mixture flows into the highly fenestrated liver sinusoids, passing along and over the basolateral surfaces of numerous rows of hepatocytes (Fig. 1 B), and is dispersed over a combined area nearly equivalent to the playing surface of two basketball courts laid end to end (>800 m^2). These hepatocellular cords are organized into hexagonal lobules built around a central vein to facilitate drainage of the filtered and modified blood into the hepatic vein and ultimately the inferior vena cava. Importantly, this organization ensures that the hepatocytes are among the first cells exposed to everything we ingest and absorb via our gut, whether it is nutritious or toxic. In addition to this intricate blood flow anatomy, hepatocytes excrete lipids, salts, and degraded proteins from their apical plasma membranes into small channels or "canaliculi" that feed bile contents through an intricate ductular system called the intrahepatic "biliary tree." Bile is then drained from the liver into the gall bladder for storage and subsequently injected into the intestinal lumen during feeding.

Like all epithelial cells, hepatocytes possess apical (canalicular) and basolateral (sinusoidal) plasma membrane domains composed of distinct surface proteins, channels, and receptors (a small selection of such proteins is shown in Table 1). Within the sinusoidal domain reside a host of different receptor tyrosine kinases such as the EGF receptor, key lipid- and iron-scavenging receptors such as the low-density lipoprotein receptor (LDLR) and transferrin receptor (TfR), as well as numerous bile acid uptake transporters. In the canalicular domain, ATP-binding cassette (ABC) transporters and other bile acid efflux transporters predominate. Hepatocytes are unique in that they may have several basolateral and apical domains in close proximity

Table 1. **Selected secreted and membrane-localized proteins of the hepatocyte**

Gene	Protein	Function
Secreted proteins		
ALB	Human serum albumin	Carrier protein
AFP	α-fetoprotein	Fetal carrier protein
TF	Transferrin	Iron binding/transport
PLG	Plasminogen (Plasmin)	Proteolysis/fibrinolysis
SERPINA1	α-1-antitrypsin	Protease inhibitor
CRP	C-reactive protein	Acute-phase protein
	Various coagulation factors (with the exception of factor VIII)	
Basolateral (sinusoidal) membrane proteins		
LDLR	LDLR	Uptake of LDL particles
Various	Na^+/K^+ ATPase	Sodium/potassium exchange
TFRC/TFR2	TfR	Iron import into hepatocytes
SLC10A1	Sodium/bile acid cotransporter (NTCP)	Sinusoidal bile acid uptake
SLCO1B1/3	Organic anion-transporting peptides	Bile acid transporters
PIGR	Polymeric Ig receptor	IgA transcytosis
ASGR1/2	Asialoglycoprotein receptors	Glycoprotein removal
Apical (canalicular) membrane proteins		
ABCB4	MDR3	Biliary phospholipid secretion
ABCB11	BSEP	Bile salt efflux from hepatocytes
ATP8B1	Aminophospholipid flippase	Lipid asymmetry
ABCG5/8	Sterolin 1 and 2	Sterol absorption/secretion
SLC4A2	Solute carrier family 4 (AE2)	Anion exchange
ALPL	Liver alkaline phosphatase	Alkaline phosphatase
ABCB1	MDR1	Organic cation secretion
ABCC2	MRP2	Biliary transport
ABCG2	BCRP	ABC transporter

(Fig. 1 C). How then is the integrity of each of these secretory, excretory, and endocytic pathways maintained? It appears that the microtubule cytoskeleton plays a key role in organizing these pathways. Contrary to the single central microtubule aster observed in nonpolarized cells, polyploid hepatocytes arising from incomplete cytokinesis have multiple centriole pairs that can lead to the existence of multiple microtubule-organizing centers (Guidotti et al., 2003; Margall-Ducos et al., 2007). These nucleation sites appear near each apical (canalicular) domain and propagate asters of microtubules with plus ends oriented outward toward the basolateral domains (McNiven and Marlowe, 1999; Müsch, 2004). In close proximity to these organizing centers are numerous Golgi stacks, suggesting that newly synthesized proteins are packaged for export at these sites and use plus end–directed motors such as the kinesins for movement toward the blood-facing basolateral (sinusoidal) domains ahead of exit into circulation (Fig. 1 D). Conversely, endocytosed ligands from the sinusoidal domain might use minus end–directed microtubule motors such as cytoplasmic dynein to gain access to the cell interior and the apical (canalicular) membrane. In addition to this microtubule framework, a dense actin cortical web resides at both plasma membrane domains. A thick actin web that surrounds the canalicular domain is exceptionally prominent and believed to provide a scaffold for myosin Vb that is involved in canalicular assembly (Wakabayashi et al., 2005), while conventional myosin II has been implicated in canalicular contraction to push newly secreted bile into the biliary tree (Tsukada and Phillips, 1993; Tsukada et al., 1995; McNiven and Marlowe, 1999). Together, the organization of these cytoskeletal networks is key to the central hepatocellular processes of secretion, endocytosis, and transcytosis.

The hepatocyte as a protein and biliary secretion factory

By EM, the hepatocyte is teeming with membranous secretory organelles and mitochondria (Fig. 1 E). The presence of an extensive ER and Golgi network underscores the role of the hepatocyte as a secretory workhorse. Based on the enormous secretory output of the hepatocyte, it is not surprising that many of our earliest insights into protein secretion are derived from seminal morphological studies performed using rat livers (Palade and Siekevitz, 1956).

As in most cells, the origin of proteins destined for secretion from the hepatocyte begins with ribosomal synthesis and transport of nascent polypeptides through the Sec61 translocon into the lumen of the ER ahead of vesicular trafficking to the Golgi apparatus (Rapoport et al., 2017). It is within the ER and Golgi lumina that chaperone-assisted folding and (in the case of most secretory proteins) glycosylation occur (Braakman and Bulleid, 2011; Moremen et al., 2012). Proteins exported from the hepatocyte are packaged into Golgi-derived secretory vesicles for transport to and exocytosis at the basolateral membrane (Saucan and Palade, 1994).

Key proteins secreted into circulation from hepatocytes include α-fetoprotein, albumin, transferrin, plasminogen, fibrinogen, and clotting factors (Table 1). Synthesized only by hepatocytes, serum albumin is the most highly secreted protein of any cell; produced at an astonishing rate of 1.5×10^7 molecules·min^{-1}, this equates to the human liver releasing on average >10 g of albumin per day into blood circulation, constituting greater than half of circulating plasma protein content (Peters, 1996; Arroyo et al., 2014; Levitt and Levitt, 2016).

In addition to the significant complement of proteins exported from the hepatocyte, hepatocytes must establish a unique polarity via the selective routing of resident membrane proteins to either the sinusoidal or canalicular membranes (Fig. 2). This is especially critical for the production of bile, a complex molecular soap composed mainly of cholesterol, phospholipids (predominantly phosphatidylcholine), electrolytes, conjugated bilirubin, and bile acids (for a detailed review, see Boyer, 2013). Bile aids in the emulsification, digestion, and adsorption of dietary fats within the intestinal lumen, as well as the removal of foreign biological substances (xenobiotics) and endogenous waste products. Bile synthesis begins in the hepatocyte and its components are transported across the apical membrane into the bile canaliculi formed between adjacent hepatocytes (Fig. 1 B). Driven by osmotic gradients, the bile travels through a series of ductules to the hepatic bile duct. As bile transits through these ducts, it is modified (e.g., through alkalinization and dilution) by the cholangiocytes, the ductular epithelial cells (Banales et al., 2019). The hepatic bile duct empties into the gall bladder, where bile is ultimately concentrated and stored until its hormonally stimulated release into the intestine via the common bile duct. Remarkably, this hepatocyte-driven process results in the excretion of up to 800 ml of bile into the gut every day.

Because the apical surface of the hepatocyte also serves as the luminal domain for the anastomosing network of bile canaliculi, it has a unique composition to withstand direct exposure to the harsh bile environment. The distinct configuration of the apical domain also reflects its specialized exocrine functions for the synthesis, transport, and release of bile acids, products of detoxification, phospholipids, and cholesterol. These functions are primarily mediated by different classes of the ATP-dependent ABC transporters (for an extensive list, see Boyer, 2013). Transport of cytotoxins and xenobiotics is accomplished by ABC transporters such as multidrug-resistance protein 1 (MDR1/ABCB1) and multidrug and extrusion protein 1. Other bile contents (such as conjugated bilirubin, sulfate conjugates, protoporphyrins, and other related organic anions) are deposited into the canaliculi via the bile salt export pump (BSEP/ABCB11), multidrug resistance–related protein 2 (MRP2/ABCC2), and breast cancer–resistant protein (BCRP/ABCG2). Water transport into the bile is also critical for bile formation and is accomplished by aquaporins 0 and 8 (AQP0 and AQP8), which shuttle between the canalicular surface and subapical vesicles to transport water into bile upon hormonal stimulation (Marinelli et al., 2003; Yang et al., 2005). As depicted in Fig. 3, the majority of apical membrane proteins arrive from transcytosis (discussed in a later section), while others, including several of the ABC transporters (e.g., BSEP, MRP2, and MDR1/2), are trafficked directly from the Golgi or via subapical endosomes (Kipp and Arias, 2000; Wakabayashi et al., 2005; Zeigerer et al., 2012).

The basolateral membrane forms the sinusoidal surface that directly contacts portal blood. In addition to receptor tyrosine kinases and trophic receptors, the sinusoidal surface contains a subset of ABC transporters and solute transporters that mediate retrieval of bile acids and other biliary components from the circulation. The sodium taurocholate cotransporter (NTCP) is the major mechanism for retrieval of conjugated (water-soluble) bile acids from the portal blood, whereas several members of the organic anion-transporting polypeptides are responsible for retrieving unconjugated (water-insoluble) bile acids. Organic anion transporters facilitate uptake of prostaglandin and drugs from the circulation, while organic cation transporters retrieve small organic cations. MRP3/ABCC3, MRP4/ABCC4, and MRP6/ABCC6 mediate efflux of bile components into the blood, and expression of both MRP3 and MRP4 is enhanced by cholestasis. As with their canalicular counterparts, these newly synthesized transporters are directly delivered from the TGN to the sinusoidal surface; however, little is known about the specific molecules and mechanisms regulating this process (Ihrke and Hubbard, 1995; Tuma and Hubbard, 2001).

The ultimate endocytic blood-filtering machine

In addition to solute transporters used for bile acid transport, proteins present at the sinusoidal membrane are critical for the hepatocyte to internalize factors from the blood. As the first organ exposed to venous blood draining from the gut, the liver has evolved into a biological filter used to remove and process dietary nutrients (e.g., glucose, lipids, and iron) as well as toxins that could damage organs without detoxification capabilities. Hepatocytes internalize many of these extracellular materials via endocytosis (Fig. 3). A variety of endocytic mechanisms have been described in the hepatocyte, including fluid-phase and caveolae-based endocytosis, the most active and well defined being receptor-mediated endocytosis (RME; Mayor et al., 2014; Kaksonen and Roux, 2018). During RME, receptors at the basolateral plasma membrane bind with high specificity to extracellular ligands contained within blood plasma and are incorporated into clathrin-coated pits. Once inside the cell, the endocytosed receptors, ligands, and cargo proteins are sorted and trafficked to a variety of destinations, recycled back to the plasma membrane, or degraded within multivesicular bodies, late endosomes, and lysosomes. Endocytic vesicle trafficking is guided by numerous Rab GTPases that function to control

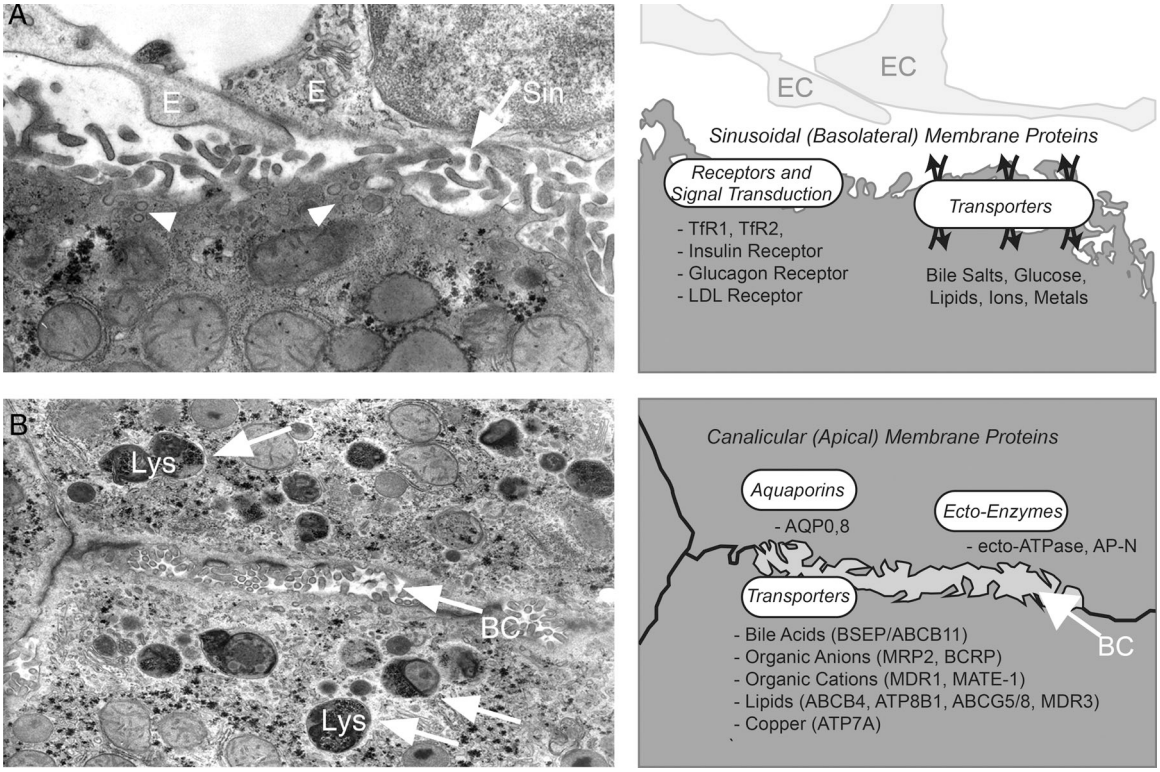

Figure 2. **Distinct proteins of the hepatocyte sinusoidal and canalicular domains. (A)** Left: Electron micrograph reveals the ultrastructure of the sinusoidal plasma membrane with numerous microvilli protruding into the perisinusoidal space (marked "Sin," arrow) adjacent to sinusoidal endothelial cells (marked "E"). The sinusoidal domain contains abundant coated endocytic pits marked by arrowheads, highlighting the striking endocytic capacity of the hepatocyte. The corresponding cartoon (right) illustrates some of the prominent sinusoidal resident proteins that include a wide variety of receptors, signal transduction proteins, and transporters. **(B)** Left: The canalicular domain also contains microvilli that protrude into the bile canaliculus (marked "BC," arrow), where bile transport and lysosomal secretion occurs (arrows denote electron-dense lysosomes). As shown in the cartoon (right), the canalicular domain is dominated by ectoenzymes and numerous transporters that shuttle water, bile acids, lipids, and other organic and inorganic molecules across the membrane and into the bile canaliculus. Electron micrographs (magnification of 9,100) are reprinted with permission from Schroeder and McNiven (2009). AQP, aquaporin; MATE-1, multidrug and extrusion protein 1; AP-N, alanine aminopeptidase.

vesicle fate by binding specific effector proteins (Zhen and Stenmark, 2015; Pfeffer, 2017). These Rab effectors are responsible for an array of important functions, including vesicle fusion, membrane remodeling, and binding to molecular motors that traverse along microtubule tracks.

The hepatocyte has a remarkable capacity for RME. For example, within 1 min of ligand stimulation, half of all insulin receptors or EGF receptors are internalized via clathrin-coated pits from the hepatocellular sinusoidal membrane (Burgess et al., 1992; Di Guglielmo et al., 1994). Although clathrin-coated pits are very small in diameter (~50 nm), these structures occupy 2% of the entire hepatocyte surface (Carpentier et al., 1985). Based on this calculation and the density of these structures by EM (Fig. 2), one could conservatively estimate that the adult liver contains ~10^{14} clathrin-coated pits in contact with the bloodstream. Using existing morphometry of the plasma membrane for hepatocytes (Hubbard et al., 1983), these tiny pits would collectively occupy a surface area roughly the size of an average parking space (~15 m^2) that turns over and then reassembles within a matter of minutes. With such a high capacity for rapid endocytosis, hepatocytes are well suited to effectively filter blood contents from the hepatic portal system.

Regulation of systemic insulin and glucose

As nicely described by Tokarz et al. (2018) as part of this *Journal of Cell Biology* review series, insulin is secreted into the blood in concentrated pulses by pancreatic β-cells following a meal. Insulin binds to insulin receptors to signal the storage of dietary nutrients such as glucose and fatty acids within the liver, adipose, muscle, and other tissues. Glucose storage by the liver is particularly important to glucose homeostasis, and hepatocytes can store ~100 g (or 400 kilocalories) within branched chains of glycogen polysaccharides (Wasserman, 2009). Whereas postprandial insulin causes a decrease in blood glucose levels, this is counterbalanced by the catabolic hormone glucagon secreted from pancreatic α cells to stimulate glucose and lipid utilization in hepatocytes via the cAMP–PKA pathway (Authier and Desbuquois, 2008; Habegger et al., 2010).

The liver is among the first organs in contact with newly secreted insulin, with hepatocellular RME clearing as much as 50–80% of blood insulin before it accesses peripheral tissues (Meier et al., 2005; Tokarz et al., 2018). Endocytic uptake of the insulin receptor is critical for activation of the PI3K–AKT pathway, which signals from the plasma membrane and early endosomes to promote lipid and glucose storage, respectively (Braccini et al., 2015). Endocytosis of the insulin receptor

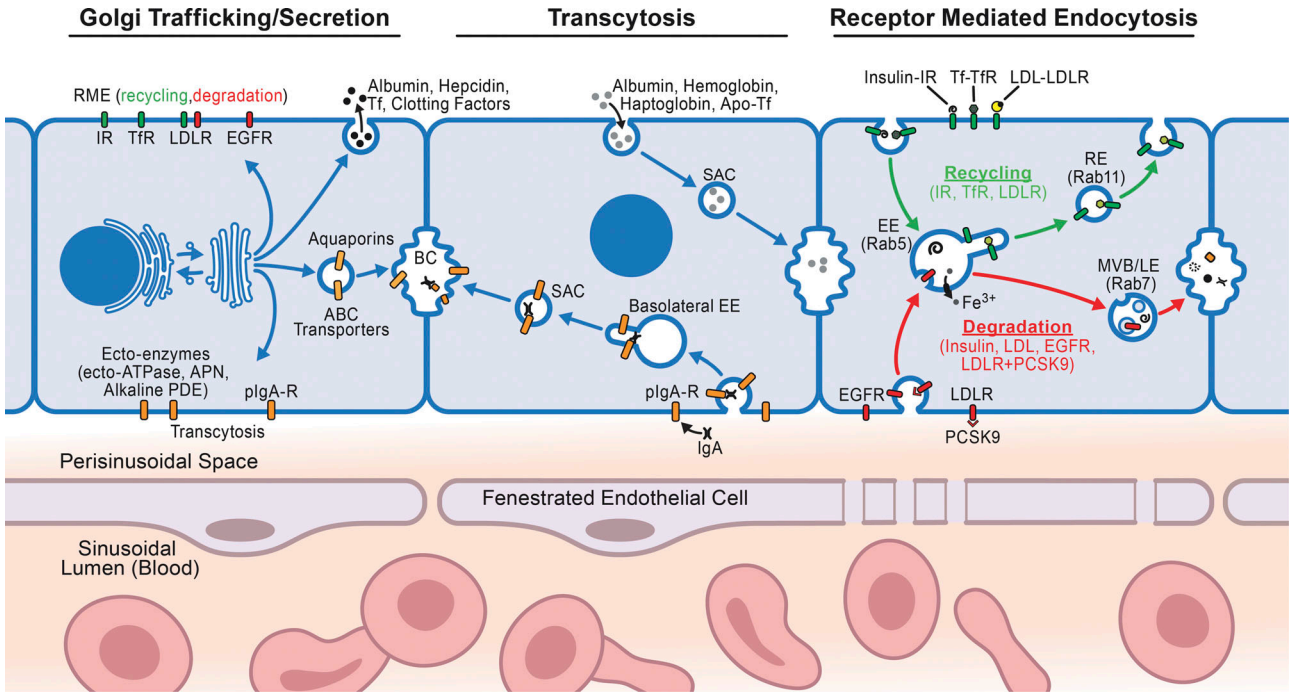

Figure 3. **Vesicle trafficking pathways prominent to the hepatocyte.** Left: Hepatocyte ER–Golgi trafficking pathways deliver newly synthesized secreted soluble factors into the perisinusoidal space and deliver nascent transmembrane cargo directly to the canalicular or sinusoidal membrane. Some of the transmembrane cargoes at the sinusoidal plasma membrane may be destined for endocytosis into recycling or degradative pathways or by transcytosis for delivery to the bile canaliculus lumen. Middle: Transcytosis occurs by endocytosis of soluble factors or transmembrane cargo that are delivered either directly to the subapical compartment or indirectly via basolateral early endosomes before deposition into the bile canaliculus (BC). Right: During RME, receptor–ligand complexes are imported into early endosomes and sorted into recycling or degradative pathways. Recycling endosomes deliver receptors back to the plasma membrane, whereas degradation occurs within multivesicular bodies and late endosomes that may secrete their contents directly into the bile canaliculus for export. APN, aminopeptidase N; EE, early endosome; EGFR, EGF receptor; IR, insulin receptor; LE, late endosome; MVB, multivesicular body; PDE, phosphodiesterase; pIgA-R, polymeric IgA receptor; RE, recycling endosome; SAC, subapical compartment; Tf, transferrin.

causes its dissociation from the insulin ligand within the early endosome, where the receptor is then sorted into recycling pathways that traffic back to the plasma membrane (Fehlmann et al., 1982). Typical "recycling endosomes" are decorated in Rab8 or Rab11 and are linked to specific effector proteins, some of which tether the vesicle to cytoskeletal tracks for routing back to the cell surface (Naslavsky and Caplan, 2018). In contrast to insulin receptor recycling, the insulin ligand remains in the endosomal lumen and is degraded within bodies and late endosomes that associate with Rab7. These acidic vesicles contain numerous acid hydrolases that function to degrade proteins, lipids, and other materials (reviewed in Berg et al., 1995; Huotari and Helenius, 2011; Scott et al., 2014).

It is noteworthy that endocytic vesicles themselves may also control glucose homeostasis independent of receptor trafficking. For example, knockdown of the early endosomal trafficking protein Rab5 in mouse liver causes hypoglycemia with no change in serum insulin and glucagon levels but functions to reduce glucose-6-phosphatase, a critical enzyme in glucose production (Zeigerer et al., 2015). Loss of Rab5 also leads to the massive accumulation of stored glycogen and fat within the liver (Zeigerer et al., 2012), highlighting the functional relationship between metabolism and endocytic vesicles in hepatocytes.

Endocytosis of two types of TfRs is key for iron homeostasis

An additional major function of the liver is the systemic homeostasis of iron, an essential cofactor for many cellular functions, including mitochondrial respiration, gene transcription, and DNA replication and repair (Ganz, 2013; Bogdan et al., 2016). Dysregulation of iron homeostasis has severe consequences to human health, as shown by genetic disorders of iron storage and other metabolic disease states (Pantopoulos, 2018). Iron cannot be synthesized de novo; therefore, the body relies on dietary iron that is absorbed and stored within liver hepatocytes and macrophages (Rishi and Subramaniam, 2017). Interestingly, distinct mechanisms of endocytosis are responsible for both the storage and release of iron by the hepatocyte.

Within the blood, iron is bound by the protein transferrin, a ligand for the TfR. Two forms of the TfR (TfR1 and TfR2) differ in their tissue distributions and play distinct roles in iron homeostasis (Ganz, 2013; Bogdan et al., 2016; Kawabata, 2019). Tfr1 is ubiquitously expressed and is responsible for the constitutive uptake of iron into cells. TfR2 is restricted to hepatocytes and erythroid cells and is important for iron sensing and homeostasis despite having a 25-fold lower affinity for transferrin (West et al., 2000). Iron-associated transferrin binds to these receptors and is internalized by clathrin-mediated endocytosis. Following its internalization, iron dissociates from transferrin inside the endosome, leaving transferrin-bound TfR to be

recycled back to the plasma membrane (reviewed in Schroeder and McNiven, 2014). Iron within the endosomal lumen is transferred to the cytoplasm by iron transporters such as DMT1, where it is quickly bound by ferritin, a large 24-subunit protein complex assembled into hollow spherical shape. Ferritin is largely retained within the hepatocyte, where it can sequester up to 4,500 iron atoms within a bioavailable, soluble form and regulate iron availability at subcellular structures such as DNA (Theil, 1987).

In addition to iron uptake, hepatocytes also govern the release of iron into the bloodstream by signaling to Kupffer cells and other circulating macrophages that are rich in stored iron. The TfR2 on the hepatocyte sinusoidal surface is critical for this iron-sensing mechanism (Wallace et al., 2007). When circulating iron levels are too high, TfR2 signals the production and secretion of the peptide hepcidin from hepatocytes, which binds directly to the iron export channel ferroportin on the surface of macrophages and, to a lesser extent, other hepatocytes (Nemeth et al., 2004; Ramey et al., 2010). Hepcidin stimulates the rapid endocytosis and degradation of ferroportin within late endosomes, thereby reducing iron export and promoting storage (Anderson and Frazer, 2017). Thus, unlike other epithelial cells, the hepatocyte supports two distinct iron transport processes: (1) the constitutive TfR1-centric pathway, which internalizes iron largely used by the hepatocyte itself; and (2) the regulated TfR2 pathway, which helps to mediate iron homeostasis for the body as a whole.

Transcytosis as the nexus between hepatocellular secretion and endocytosis

The hepatocyte is masterful in its ability to secrete numerous different proteins while simultaneously filtering the blood via a ramped-up endocytic system. An important component linking these pathways is that of transcytosis, a mechanism whereby macromolecular cargo is transported from one plasma membrane domain of a cell to the other within a membrane-bound vesicle (Fig. 3). All epithelial cells use transcytosis as a delivery system for fluid or protein cargo transport in both the basolateral-to-apical and apical-to-basolateral directions. However, the latter pathway has yet to be described for hepatocytes. In general, basolateral-to-apical (sinusoidal-to-canalicular) transcytosis in hepatocytes serves to retrieve macromolecules (e.g., albumin, hemoglobin/haptoglobin complexes, IgA, and apotransferrin) from blood sinusoids for release into the bile. Additionally, only hepatocytes (exclusively) and enterocytes (in part) use an "indirect" pathway, whereby newly synthesized proteins are first delivered from the Golgi to the sinusoidal membrane before being transcytosed to the canalicular membrane (Massey-Harroche, 2000; Treyer and Müsch, 2013). Thus, hepatocytes provide an especially unique system in which to study transcytosis.

Early studies in hepatocytes revealed a robust rate of indirect "sinusoidal-to-canalicular" transcytosis. Studies on fluid phase transcytosis alone suggest that a remarkable ~600–850 vesicles (100 nm in diameter) fuse with the canaliculus every minute (Crawford, 1996). That means that the apical canalicular membrane surface area would double or triple every 20 min if there were no compensatory mechanisms for relief of this membrane buildup. Transcytosis requires the hepatocyte to recognize, sort, and traffic specific membrane cargoes to the canalicular membrane. While the underlying mechanisms remain elusive, earlier studies in polarized WIF-B cells have established that lipid species such as cholesterol and glycosphingolipids appear to be essential for transcytotic efflux from basolateral early endosomes (Nyasae et al., 2003). Because lipid depletion impairs the transcytosis of a wide range of canalicular residents, it is likely that cholesterol and glycosphingolipids do not directly facilitate sorting but alter the activity of a general transcytotic regulator. A likely candidate for such a regulator has emerged in the 20-kD membrane protein myelin and lymphocyte protein 2 (MAL2), the subcellular distribution of which is regulated by cholesterol (Ramnarayanan and Tuma, 2011). Knockdown studies in HepG2 cells and WIF-B cells show that delivery of the polymeric IgA receptor and newly synthesized canalicular residents requires MAL2 for transport from basolateral endosomes to the subapical compartment, a precursor vesicular compartment to the canalicular surface (de Marco et al., 2002; In and Tuma, 2010). However, the specific mechanisms by which MAL2 mediates basolateral endosome sorting and/or efflux have not been determined.

Lipoproteins, lipid droplets (LDs), lipolysis, and lipophagy

The liver is a critical depot for lipid uptake, storage, breakdown, and release. The hepatocyte takes up dietary lipid (in the form of chylomicron remnants) and releases a substantial amount of lipid back into the circulation as very low-density lipoprotein (VLDL) particles. These 30- to 80-nm structures are repositories of neutral lipid (primarily cholesteryl esters [CEs] and triacylglycerols [TAGs]) enclosed within a phospholipid monolayer and studded with various amphipathic apolipoproteins (usually apoB100 but also apoCI, CII, and CIII and/or apoE; Scorletti and Byrne, 2013). The fatty acids present in hepatocellular VLDL particles are sourced largely from four pools: (1) circulating albumin-bound fatty acids released from the adipose tissue during the process of lipolysis, (2) internalized remnant lipoproteins and chylomicrons from the diet, (3) high-density lipoprotein–derived CEs that enter the hepatocyte via class B scavenger receptor-mediated uptake at the plasma membrane during the process of reverse cholesterol transport (see below), and (4) TAGs synthesized by the process of de novo lipogenesis (Choi and Ginsberg, 2011; Shen et al., 2018).

Initiation of hepatic VLDL assembly is coupled to the translation and translocation of apoB100 into the lumen of the rough ER, where it is then cotranslationally bound together with various neutral lipids (primarily CE and TAG). VLDL assembly is also mediated in part by the microsomal triglyceride transfer protein (Hussain et al., 2003; Fig. 4). Nascent VLDL particles bud from a single leaflet of the ER bilayer into the lumen of the smooth ER. These precursor particles mature as they flux through the ER and Golgi (Fig. 4), where in both compartments additional lipidation can occur via the action of proteins such as TM6SF2 (Smagris et al., 2016), in addition to phosphorylation or glycosylation posttranslational modifications of apoB100 itself (Yang et al., 1989; Swift, 1996). Surprisingly, despite the fact that

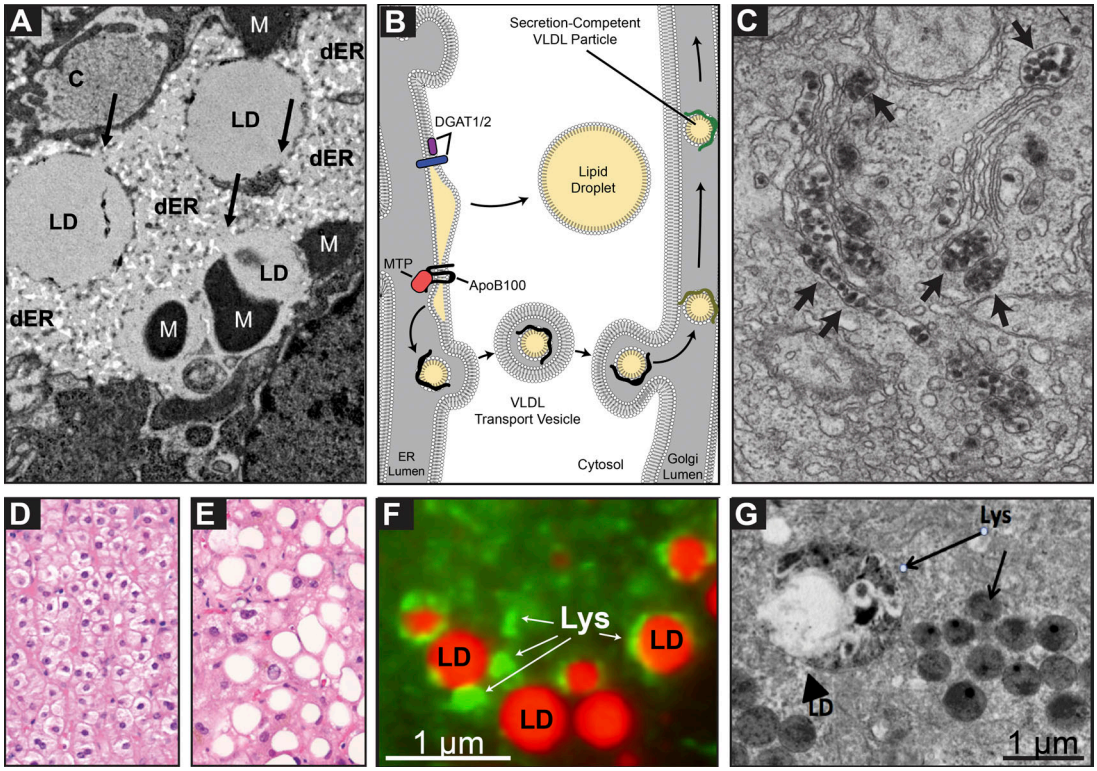

Figure 4. **Lipid accumulation and catabolism in hepatic disease. (A)** Ultrastructure of a "ballooned" human hepatocyte, a hallmark of lipid-induced inflammatory liver diseases such as nonalcoholic steatohepatitis, showing the dilated lumen of the ER (labeled dER) and its intimate connection with LDs, as indicated by arrows, and mitochondria (M). Panel A is reprinted with permission from Caldwell et al. (2010). **(B)** Cartoon illustrates the synthesis of cytosolic LDs and lipoprotein particles and trafficking stages between the ER, cytosol, and Golgi apparatus. DGAT, diacylglycerol acyltransferase; MTP, microsomal triglyceride transfer protein. **(C)** Diseased rat hepatocyte showing the accumulation of lipoprotein particles within the secretory Golgi compartment (arrows) following alcohol-induced liver damage. Fig. 4 C was modified with permission from Ehrenreich et al. (1973). Magnification of 50,000. **(D and E)** Histology of human liver tissue shows the morphology of normal hepatocytes (D) versus the dramatic accumulation of LDs within steatotic hepatocytes (E). At the cellular level, LDs are catabolized in part by lysosomal digestion via lipophagy as shown in F and G. The fluorescence micrograph (F) displays BODIPY-stained LDs in red that intimately associate with lysosomes stained positive by LAMP1 immunofluorescence in green. The electron micrograph (G) illustrates the ultrastructure of this interaction and close proximity of lysosomes to LDs in hepatocytes.

these pathways have been studied for nearly half a century, the trafficking of VLDL particles from the ER and TGN and eventual secretion from the hepatocyte into the bloodstream remain largely undefined. While there is evidence for the existence of distinct vesicular carriers that might participate in these processes, this may be controversial (Hossain et al., 2014). In contrast to vesicular transport, recent intriguing evidence suggests that TANGO1 and related proteins (e.g., TALI), known to play a role in the secretion of bulky cargoes such as procollagen, may collaborate to form "mega carriers" or even physical "tunnels" between compartments that are large enough to assist in the transport of VLDL particles during secretion (Santos et al., 2016; Raote and Malhotra, 2019). Further work in this area will be required to elucidate the mechanisms underlying this important aspect of hepatocellular lipid homeostasis.

Secreted lipoproteins deliver lipids to peripheral tissues such as adipose and muscle for storage and energy utilization. However, hepatocytes are also responsible for the terminal clearance of 80–90% of these particles from blood circulation (Spady, 1992). Hepatocytes internalize plasma lipoproteins through RME into clathrin-coated vesicles (Zanoni et al., 2018). The LDLR assists in the recognition of circulating lipoproteins and is perhaps the best understood route of lipoprotein clearance. LDLR recognizes ApoB100 and ApoE proteins on the surface of low-density lipoproteins (LDLs) and serves as a docking station for the retrieval of these lipid particles from the bloodstream (Goldstein and Brown, 2009). Once internalized, LDLR dissociates from its LDL ligand within the early endosome, where LDLR interacts with a complex receptor-sorting machinery that aids in its recycling back to the plasma membrane for reuse (Fedoseienko et al., 2018). LDLR can also be targeted for degradation in the presence of extracellular cofactors such as proprotein convertase subtilisin/kexin type-9 (PCSK9), which binds directly to the LDLR and targets it for lysosomal degradation. In this manner, PCSK9 thus inhibits the interaction of circulating LDL with its receptor extracellularly (Lagace, 2014). Diseases affecting lipoprotein endocytosis have profound effects on cholesterol levels within the bloodstream, leading to heart disease, atherosclerosis, and other disorders of the vasculature (Defesche et al., 2017). Mutations in LDLR are common causes of familial hypercholesterolemia, and PCSK9 gain-of-function mutations have also been described to reduce LDLR levels, leading to elevated serum cholesterol (Lagace, 2014). Because of this, therapeutic strategies that inhibit PCSK9 function have been

successful in lowering plasma cholesterol levels in combination with statins that inhibit cholesterol synthesis (Dullaart, 2017; Burnett and Hooper, 2018).

Cholesterol can also be trafficked from peripheral tissues and arterial macrophages back to the liver in a process referred to as reverse cholesterol transport, primarily in the form of CE-enriched high-density lipoprotein particles. In a process that is still incompletely understood, receptors on the surface of the hepatocyte (predominantly the class B scavenger receptor SR-B1) can selectively funnel cholesterol into the cell, possibly in a nonendocytic fashion (Pittman et al., 1987; Rosenson et al., 2012; Neculai et al., 2013; Shen et al., 2018). Internalized CEs can subsequently be hydrolyzed within late endosomes/lysosomes via the action of lysosomal acid lipase. Cholesterol is then trafficked out of the endosomal lumen via the action of Niemann–Pick type C proteins 1 and 2 for processing by the ER (Stone et al., 1987; Infante et al., 2008) or transported across the apical membrane into bile canaliculi for excretion (Dikkers and Tietge, 2010).

In addition to CEs and TAGs, enterocyte-derived chylomicrons taken up by the hepatocyte also contain dietary retinyl esters (REs). Here too, the hepatocyte plays a central role in lipid metabolism. The RME-mediated uptake of RE-containing chylomicron remnants into the hepatocyte leads to subsequent processing of the lipids within the endocytic pathway ahead of repackaging in the ER for export back out of the hepatocyte. Very little RE is actually stored in the hepatocyte itself; rather, a substantial amount is subsequently delivered from the hepatocyte via an unknown mechanism to hepatic stellate cells, where >80% of liver-localized REs are stored (Friedman, 2008; Blaner et al., 2009; Blaner, 2019).

Stimulated in part by the insulin pathway, the liver can also package significant quantities of triglycerides in the form of cytosolic LDs, a unique fat-storage organelle that can be harvested to supplement energy reserves during times of fasting or nutrient deprivation (Olzmann and Carvalho, 2019). While this has provided a significant survival advantage over the millennia, it has become a significant liability with sedentary lifestyles and a Western diet rich in both carbohydrates and fat. Indeed, it has been estimated that approximately one third of the global population suffers from nonalcoholic fatty liver disease, which can result in inflammation or liver fibrosis/scarring (nonalcoholic steatohepatitis and cirrhosis), with a certain percentage of patients developing hepatocellular carcinoma. Together, these diseases impose a health care cost burden of over $100 billion annually in the United States alone (Younossi et al., 2016a,b). For these reasons, a better understanding of the cell biology and genetics of how our liver stores and utilizes fat is of great interest moving forward.

As with lipoproteins, the biosynthesis of LDs occurs within the ER but instead results in a membrane distention of neutral lipid toward the cytoplasm (Walther et al., 2017; Fig. 4). The resulting structure comprises a neutral lipid core surrounded by the ER-derived phospholipid monolayer displaying on its surface a plethora of unique structural proteins as well as other proteins, many of which have clear roles in membrane trafficking processes (i.e., members of the Ras-related Rab family of small GTPases, small G proteins, and SNAREs; Bersuker and Olzmann, 2017; Bersuker et al., 2018; Krahmer et al., 2018). These organelles thus represent a dedicated cytoplasmic repository for the sequestration of toxic free fatty acids via esterification into TAG. While not as prodigious at fat storage as the adipocyte (Rutkowski et al., 2015), the hepatocyte is unquestionably adept at retaining significant quantities of LDs.

In addition to deciphering the molecular basis for LD accumulation, significant effort has also been directed toward understanding how the hepatocyte uses this stored fat. In times of nutrient scarcity, LDs can be catabolized by various mechanisms for the release of free fatty acids and cholesterol into the cell for bioenergetic and anabolic processes (Reid et al., 2008; Singh et al., 2009; Ong et al., 2011). As in the adipose tissue, a wide variety of lipolytic enzymes are present in the liver. These include certain members of the patatin-like phospholipase domain containing family members such as PNPLA2, better known as adipose triglyceride lipase (ATGL), which catalyzes the rate-limiting step in triglyceride lipolysis to allow for the rapid catabolism of cytoplasmic LDs (Smirnova et al., 2006; Kienesberger et al., 2009). In addition to cytoplasmic ATGL, carboxylesterases (e.g., CES1 and CES2) comprise a family of luminal ER-localized lipases critical for the mobilization of hepatic TAG; these are thought to be especially critical in mediating the redistribution of fatty acids from storage within cytoplasmic LDs into nascent VLDL particles formed within the ER lumen (Lehner and Verger, 1997; Gilham et al., 2005; Ruby et al., 2017; Lian et al., 2018). The regulation of these lipolytic enzymes is complex and requires the participation of numerous hormones and growth factors among varied signal transduction pathways (Duncan et al., 2007; Zechner et al., 2012, 2017; Schott et al., 2017).

The process of autophagy also appears critical in the turnover of LDs, as evidenced by knockdown studies of key proteins involved in autophagosome biogenesis (Singh et al., 2009; Jaber et al., 2012; Moretti et al., 2018; Morita et al., 2018). These studies indicate a novel pathway for lipid metabolism known as lipophagy, a selective form of autophagy, whereby LDs can be selectively surrounded by autophagic membranes, enclosing them within the interior of an autophagosome. These lipoautophagosomes can then undergo fusion with the endolysosomal system to generate autolysosomes, within which the LDs are broken down via acidic lipases and hydrolases in a similar fashion to lipoprotein particles (Schulze et al., 2017b). The regulation of lipophagy in the hepatocyte remains unclear but is thought to be tightly linked to upstream activity by both the lipolytic machinery (especially ATGL) as well as clearance of proteins from the surface of the LD by chaperone-mediated autophagy (Kaushik and Cuervo, 2015, 2018; Martinez-Lopez and Singh, 2015; Sathyanarayan et al., 2017). Furthermore, recently uncovered roles for key LD-resident proteins (i.e., Rab7 and Rab10) in mediating the envelopment of hepatocellular LDs by autophagic membranes and downstream fusion with degradative organelles suggests that further work in characterization of the LD–autophagosome–lysosome contact site is warranted to better understand hepatic lipid catabolism (Schroeder et al., 2015; Li et al., 2016; Schulze et al., 2017a).

Detoxification of the blood and alcoholic liver injury

By virtue of its extensive vascularization, everything we eat, drink, and breathe passes through the liver. This positions the organ to detoxify the multitude of endotoxins and exotoxins we encounter on a daily basis. In general, fat-soluble toxins enter the liver and are metabolized by over 50 different cytochrome P450 enzymes embedded in the ER membrane (Zanger and Schwab, 2013). These enzymes allow large quantities of toxins to be metabolized by the liver into water-soluble waste products that pass through the bile, urine, or sweat. Complications arise when toxin demands are greater than the clearance mechanisms can handle, leading to the accumulation of protein adducts and highly reactive intermediary metabolites (Jaeschke et al., 2002; French, 2013; Ramachandran and Jaeschke, 2019). Below, we use chronic alcohol consumption as an example of one such toxin overload that leads to hepatocellular injury, emphasizing the detrimental impact of ethanol metabolism on perturbations to vesicle trafficking in the hepatocyte.

By itself, alcohol is not hepatotoxic; rather, its metabolites and byproducts of its metabolism promote injury. Thus, as the major site of alcohol metabolism, the hepatocyte is the most susceptible to chronic consumption. Early (and reversible) stages of alcoholic liver disease are characterized by a fatty liver (steatosis) where hepatocytes accumulate large, cytoplasmic LDs. Acute or chronic exposure leads to triglyceride accumulation by enhancing the expression of several lipogenic enzymes, along with the down-regulation of enzymes responsible for fatty acid oxidation (reviewed in Donohue, 2007). Concomitantly, LD catabolism is compromised in ethanol-exposed hepatocytes due to impaired lipophagy (Rasineni et al., 2014; Schulze et al., 2017a) and impaired lipolysis via cytoplasmic lipases (Schott et al., 2017). Later stages of alcoholic liver disease are characterized by fibrosis and cirrhosis, both of which are largely irreversible. Hepatic fibrosis includes the deposition of extracellular matrix proteins that promote scarring similar to that of cutaneous wounds (for reviews on fibrogenesis, see Kocabayoglu and Friedman, 2013; Seki and Schwabe, 2015; Tsuchida and Friedman, 2017).

Mechanisms of hepatocyte injury by excess alcohol consumption

Alcohol is first converted to acetaldehyde within the hepatocyte by the cytosolic enzyme alcohol dehydrogenase. In the mitochondria, acetaldehyde dehydrogenase converts acetaldehyde to acetate, which is fed into the citric acid cycle as acetyl-CoA. As alcohol metabolism requires large amounts of NAD^+, this cofactor becomes depleted and alters the redox state of ethanol-exposed hepatocytes. This not only dysregulates lipid and carbohydrate metabolism (Weiner et al., 1994; Tsukamoto and Lu, 2001) but also inhibits other NAD^+-requiring enzymes that would otherwise prevent hepatocellular injury.

In addition to alcohol dehydrogenase, alcohol is metabolized by cytochrome P450 2E1 (CYP2E1) to produce acetaldehyde as well as highly reactive oxygen and hydroxyethyl radicals. These metabolites have the capacity to form stable, covalent modifications on proteins, lipids, and DNA (Ristow and Obe, 1978; Kenney, 1982, 1984; Fraenkel-Conrat and Singer, 1988; Wehr et al., 1993; Brooks, 1997; Tuma and Casey, 2003). CYP2E1 activity also results in oxidative stress, a major source of alcohol-induced hepatic dysfunction. This oxidative damage can subsequently result in ER stress and an up-regulated unfolded protein response, ultimately leading to hepatocellular apoptosis, inflammation, and steatosis (Ji, 2015). Likewise, alcohol-induced mitochondrial permeability and decreased membrane potential result in altered cellular energy homeostasis, enhanced apoptosis, and the formation of reactive oxygen species (Das and Vasudevan, 2007). Together with acetaldehyde and the oxygen radicals described above, these highly reactive species can form covalent modifications on cellular proteins, lipids, and DNA. The adducted macromolecules not only lead to hepatic dysfunction but also provoke inflammatory responses that lead to further liver injury (Tuma, 2002; Tsuchida and Friedman, 2017). This vicious cycle is reinforced by the alcohol–induced overexpression of CYP2E1 and down-regulation of protective antioxidant enzymes.

Vesicle trafficking and posttranslational modifications in alcoholic liver injury

Alcohol exposure induces posttranslational protein modifications that are part of the normal repertoire, including methylation, phosphorylation, and acetylation (Park et al., 2003; Kannarkat et al., 2006; Lee and Shukla, 2007; Pal-Bhadra et al., 2007; Lieber et al., 2008; Picklo, 2008; You et al., 2008; Shepard and Tuma, 2009). In particular, numerous proteins have been identified that are lysine hyperacetylated upon ethanol exposure (Shepard and Tuma, 2009).

The modification of proteins by exposure to alcohol has been studied extensively, and it is now apparent that alcohol can induce posttranslational tubulin modifications such as acetylation (Kannarkat et al., 2006; Groebner and Tuma, 2015). These modifications have been linked to alcohol-induced defects in microtubule-dependent protein trafficking, including post-Golgi delivery to the cell surface of secretory and membrane-associated cargo (Tuma et al., 1990, 1991b; Joseph et al., 2008; Shepard et al., 2010a). These microtubule modifications can also explain defects in basolateral-to-apical transcytosis (Groebner et al., 2014), nuclear translocation of a subset of transcription factors (e.g., STATs, but not Smads; Fernandez et al., 2012), and impaired microtubule-based motor translocation and processivity along the filamentous tracks (Groebner et al., 2014).

Alcohol-induced global protein acetylation is correlated with the specific impairment of clathrin-mediated endocytosis (Tuma and Sorrell, 1988; Tuma et al., 1991a; McVicker et al., 2002; Fernandez et al., 2009; Shepard et al., 2010b, 2012). In this case, enhanced protein acetylation impairs dynamin recruitment to the necks of endocytic pits, which compromises vesicle scission and endocytosis (Shepard et al., 2012). The disruption of the endocytic process is a critical feature of alcoholic liver disease pathogenesis and has broad physiological effects on the fluid-phase uptake of macromolecules and trafficking of receptor–ligand complexes that mediate metabolic homeostasis (Tuma et al., 1991a; Camacho et al., 1993; McVicker and Casey, 1999; McNiven and Casey, 2011).

The hepatocyte as a viral incubator

This review has attempted to convey only a fraction of the diverse liver-centric membrane trafficking processes that result from the hepatocyte's intimate association with the blood circulation. As would be expected for a highly vascularized organ that receives and filters our blood, the liver also represents a prime site of exposure to (and, consequently, a first line of defense against) a huge variety of viral, bacterial, and fungal pathogens. In response to pathogenic and inflammatory signals, hepatocytes secrete numerous acute phase proteins (i.e., complement proteins, opsonins, and regulators of the inflammatory response) to stimulate an innate immune response (Zhou et al., 2016). Nonetheless, some viruses have evolved elaborate mechanisms for securing their entry into the hepatocyte. Two prominent viral pathogens, hepatitis B virus (HBV) and hepatitis C virus C (HCV), represent particularly pressing challenges to the liver; complications arising from chronic infection with either HBV or HCV result in severe liver disease and consequently lie at the forefront of global prevention strategies (Stanaway et al., 2016). Estimates from the 2017 *Global Hepatitis Report* (World Health Organization, 2017) suggest that 257 million (3.5% of the global population) or 71 million (1% of the global population) individuals are chronically infected with HBV or HCV, respectively. These staggering statistics underlie an urgent need for understanding the cell biology and hepatocellular life cycles of these and other hepatotropic viruses (Inoue et al., 2018).

For HBV, a DNA virus of the *Hepadnaviridae* family, the major route of access to the hepatocyte is via its receptor at the sinusoidal plasma membrane, the NTCP (Yan et al., 2012). Like many viruses, HBV is taken up into the hepatocyte via endocytosis and escapes though an as-yet poorly understood molecular mechanism that may be pH independent (Rigg and Schaller, 1992; Yuen et al., 2018). Later studies showed that HBV hijacks vesicular compartments such as autophagosomes and multivesicular endosomes to facilitate replication and release (Kian Chua et al., 2006; Watanabe et al., 2007; Sir et al., 2010), which may also involve the regulation of Rab7 activity (Inoue et al., 2015, 2018). The viral genome is transported to the nucleus for transcription, where pregenomic RNA is subsequently released back into the cytosol for reverse transcription and packaging of the resultant DNA into an icosahedral nucleocapsid (Venkatakrishnan and Zlotnick, 2016). HBV then takes full advantage of the secretory prowess of the hepatocyte, using the general secretory pathway outlined above to release prodigious copies of mature enveloped virus into the bloodstream (Yuen et al., 2018).

HCV, an RNA virus of the *Flaviviridae* family, gains entry into the hepatocyte by posing as a circulating lipoprotein particle. Mature virions display two apolipoproteins (E1 and E2) on their surface that sequentially promote interactions with both the LDLR and CD81 at the hepatocellular sinusoidal membrane to facilitate internalization and entry of the virus via RME (Manns et al., 2017). The virus subsequently escapes from the endocytic pathway by envelope fusion with the endosome (Takikawa et al., 2000). The RNA genome is then free to be used as a template for synthesis of HCV structural and nonstructural proteins. Interestingly, the core capsid and nonstructural NS5A proteins appear to be recruited to cytoplasmic LDs in the hepatocyte to promote the assembly of nascent HCV particles in close proximity to the ER (Miyanari et al., 2007). After assembly, these mature particles are then trafficked out of the hepatocyte in a manner largely reminiscent of the VLDL secretion pathway outlined above (Jones and McLauchlan, 2010).

Conclusion and future perspectives

As we have detailed in this review, the hepatocyte truly represents the embodiment of a cellular "jack of all trades." This flexibility is of necessity; by virtue of being uniquely situated at the interface of both blood and bile, the hepatocyte is challenged to efficiently and accurately orchestrate nutrient uptake and blood detoxification while simultaneously managing the packaging and secretion of proteins, lipids, and bile. Indeed, if any of the membrane trafficking events supporting these functions become compromised, the liver can quickly succumb to advanced disease, including hepatitis, cirrhosis, or even hepatocellular carcinoma. It is important to note here that both the hepatocytes and the epithelial cells of the biliary tree (cholangiocytes) are particularly susceptible to neoplastic transformation leading to hepatocellular carcinoma or cholangiocarcinoma, respectively. Both are exceptionally lethal cancers that make liver cancer a leading cause of cancer death worldwide (Bray et al., 2018). As a consequence, an increased appreciation of the cell biology of hepatocellular vesicle trafficking and its relationship with complex liver functions (i.e., tissue regeneration and hepatic lipid metabolism), as well as in the pathogenesis of viral infection and cancer progression, will be vitally important moving forward.

In future research, it will be critical to define how the hepatocyte maintains the fidelity of membrane transport to multiple apical and basolateral domains and how this might be compromised in various disease states. For instance, >60 small GTPases of the Rab family of membrane trafficking proteins are known to exist in mammalian cells, most of which show expression in liver tissue (Stenmark, 2009). There remains an active effort underway toward assigning functions for many of the poorly studied members of this family of small GTPases (Homma et al., 2019). Interestingly, many of these Rabs routinely appear in proteomic analyses of hepatocellular LDs (Khan et al., 2015; Bersuker et al., 2018; Kramer et al., 2018); perhaps a greater understanding of their individual or collaborative functions will prove essential toward understanding not only hepatic steatosis but also varied processes ranging from bile formation and cholesterol metabolism to viral replication and protein secretion. Importantly, a higher-level view of the cell is also required; characterizing the biology of individual organelles within the hepatocyte (i.e., mitochondria and LDs), as well as how cross talk between these organelles contributes to liver function, will also be necessary (Valm et al., 2017; Cohen et al., 2018; Henne, 2019).

Furthering our understanding of the continual communication between the hepatocyte and nonparenchymal cells of the liver is also of intense interest. For example, an active area of study lies in the field of extracellular vesicles, which may allow

for intracellular signaling and delivery of specific proteins/nucleic acids between the hepatocyte and stellate cells or cholangiocytes (Raposo and Stoorvogel, 2013; Hirsova et al., 2016; van Niel et al., 2018; Eguchi et al., 2019). Additionally, more insights are required in our elucidation of the role of RE (vitamin A) trafficking through the hepatocyte and its interrelationship with the cell biology of the hepatic stellate cell, which plays a critical role in extracellular matrix deposition (and hence an important role in liver fibrosis; Tsuchida and Friedman, 2017).

Increased knowledge of how toxic substances (i.e., alcohol, over-the-counter and prescription medications, and certain industrial chemicals) disrupt hepatocellular vesicular trafficking may provide key insights into their role in liver damage. Moreover, further research is needed into how recently identified genetic mutations (i.e., PNPLA3 I148M or a splice variant of HSD17β13) ultimately relate to pathogenic or protective effects with regard to chronic liver disease (Abul-Husn et al., 2018; BasuRay et al., 2019; Ma et al., 2019; Yang et al., 2019). It is only very recently that we have learned how these proteins might affect the hepatocyte at the cellular/organelle level. Finally, novel therapeutic strategies in the treatment of liver diseases may arise if we learn how to better manipulate vesicular trafficking and protective pathways (i.e., autophagy) to reduce hepatic steatosis and prevent complications such as inflammation or fibrosis (Galluzzi et al., 2017). As transplantation is one of the few remaining treatment options for many of these advanced liver diseases, the continued investigation of basic hepatic cellular biology functions is critical if we are to develop more effective (and less invasive) patient interventions moving into the next decade.

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