



Published in final edited form as:

Compr Physiol. 2013 January ; 3(1): 515–539. doi:10.1002/cphy.c120013.

Calcium Signaling in the Liver

Maria Jimena Amaya¹ and Michael H. Nathanson^{1,*}

¹Section of Digestive Diseases, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut

Abstract

Intracellular free Ca^{2+} ($[\text{Ca}^{2+}]_i$) is a highly versatile second messenger that regulates a wide range of functions in every type of cell and tissue. To achieve this versatility, the Ca^{2+} signaling system operates in a variety of ways to regulate cellular processes that function over a wide dynamic range. This is particularly well exemplified for Ca^{2+} signals in the liver, which modulate diverse and specialized functions such as bile secretion, glucose metabolism, cell proliferation, and apoptosis. These Ca^{2+} signals are organized to control distinct cellular processes through tight spatial and temporal coordination of $[\text{Ca}^{2+}]_i$ signals, both within and between cells. This article will review the machinery responsible for the formation of Ca^{2+} signals in the liver, the types of subcellular, cellular, and intercellular signals that occur, the physiological role of Ca^{2+} signaling in the liver, and the role of Ca^{2+} signaling in liver disease.

Introduction

Many of the functions of the liver are regulated by increases in intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$; a list of abbreviations used in this article is provided in Table 1). This includes vesicular trafficking and canalicular contraction, which are regulated by cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_c$) and participate in the control of bile secretion (252, 398, 400, 415); glucose and energy metabolism, which occur through the modulation of regulatory enzyme activity (35, 109, 200) and through changes in mitochondrial Ca^{2+} ($[\text{Ca}^{2+}]_m$) (154, 326); and control of the cell cycle, which includes modulation of transcription (157, 314) and anti- and proapoptotic proteins (17, 221, 258) to regulate cell proliferation and death, and is regulated by nucleoplasmic Ca^{2+} ($[\text{Ca}^{2+}]_n$) (332). To control, yet discriminate among so many functions simultaneously, $[\text{Ca}^{2+}]_i$ signals are highly organized in time (e.g., through the amplitude of Ca^{2+} spikes and the frequency of Ca^{2+} oscillations) and space (e.g., through Ca^{2+} gradients and waves). These specialized features of Ca^{2+} signals are mediated by the PLC/InsP3/InsP3R cascade, which may be activated by Ca^{2+} mobilizing hormones or growth factors and leads to release of Ca^{2+} stored in the endoplasmic reticulum (ER) (24, 25, 27, 72, 105, 379, 381). The interplay between different organelles in liver cells also determines the proper spatio-temporal modulation of Ca^{2+} signals and the execution of specific functions (310, 326, 343). In addition, Ca^{2+} signaling patterns are not just organized within individual cells but are coordinated among cells as well, through a complex communication network

dependent on signaling through gap junctions (105, 266, 344) and paracrine messengers (104, 115, 259, 351) that extends across the hepatic lobule. This article describes the basic components required for the formation of Ca^{2+} signals in the liver, the mechanisms of distinct Ca^{2+} signaling pattern formation, and their involvement in processes related to health and disease.

General Molecular and Cellular Mechanisms of Ca^{2+} Signaling

Molecular machinery for Ca^{2+} signal formation in the cytoplasm

The Ca^{2+} signaling system contains a wide molecular set of signaling components that act in synergy to drive Ca^{2+} signals with unique spatial and temporal properties (25, 28). $[\text{Ca}^{2+}]_c$ signals can be derived from the extracellular medium or from internal stores. In the case of the latter, several intracellular messengers bind to and regulate the activity of specific intracellular receptors to promote Ca^{2+} release (25, 41). Inositol-1,4,5-trisphosphate (InsP3) is one such Ca^{2+} -mobilizing messenger and acts through the InsP3 Receptor (InsP3R). Similarly, Ca^{2+} itself and cyclic ADP ribose (cADPR) promote $[\text{Ca}^{2+}]_c$ signals through their interaction with ryanodine receptors (RyRs). Additionally, nicotinic acid adenine dinucleotide phosphate (NAADP) activates two-pore channels (TPCs) (25, 28, 41, 58, 178).

InsP3-mediated Ca^{2+} release is initiated by the binding of Ca^{2+} mobilizing hormones or growth factors to plasma membrane G-protein-coupled receptors (GPCRs) (23, 25, 357) or receptor tyrosine kinases (RTKs) (23, 25, 220), respectively. GPCRs are composed of seven hydrophobic, helical, membrane-spanning domains and a cytosolic G-protein binding site. G-proteins are heterotrimeric and consist of α , β , and γ subunits (357, 414). Each α -subunit has intrinsic GTPase activity at the guanine nucleotide binding site, and specific binding sites for effector proteins such as phospholipase C (PLC), adenylate cyclase, or other hydrolases. Once a ligand binds to its specific GPCR, guanosine diphosphate (GDP) is rapidly exchanged for GTP, which results in G-protein dissociation from the receptor and α -subunit dissociation from the β - γ complex. The α and β - γ complex each may then activate effector proteins in different biochemical pathways. When GTP is hydrolyzed by GTPase, the heterotrimeric G-protein complex reassociates and can be reactivated (188, 357). Several GPCRs are expressed and have been particularly well studied in the liver. These include the V1a vasopressin receptor, the α_{1B} adrenergic receptor, several subtypes of the P2Y class of purinergic receptors, and the angiotensin receptors (129, 337, 338, 381).

RTKs exhibit ligand-activated protein kinase activity and have only one membrane-spanning helix, connecting the extracellular ligand-binding domain to the cytoplasmic protein tyrosine kinase domain. Kinase activity is switched on by receptor dimerization and transphosphorylation of tyrosine residues within the kinase activation loop in the catalytic cytoplasmic domain. This in turn leads to the recruitment of a series of adaptor proteins containing an SH2 (Src homology domain 2), SH3 (Src homology domain 3), or PTB (phosphotyrosine binding) activating signaling pathways that generally regulate cell proliferation, migration, and survival. Protein phosphatases may terminate the signal by dephosphorylating tyrosine residues to disassemble the signaling complexes formed around the activated receptors (25, 220, 350). Several RTKs are expressed in the liver (40, 64, 170, 331, 363, 373) including the hepatocyte growth factor (HGF) receptor c-Met, the epidermal

growth factor (EGF) receptor, and the insulin receptor, and their actions are implicated in glucose and lipid metabolism (254, 358), liver fibrosis (18), regeneration (389), and hepatocellular carcinogenesis (181, 383, 389).

Once Ca^{2+} -mobilizing hormones or growth factors bind to their specific plasma membrane receptors, membrane-associated PLC is activated. There are multiple subtypes of PLC (323), and several isoforms of these subtypes have been identified; in particular, $\text{PLC}\beta 1$ and $\text{PLC}\beta 2$ are activated by GPCRs, while $\text{PLC}\gamma$ is activated by RTKs (25, 323). Activation of PLC by GPCRs hydrolyzes phospholipid phosphatidylinositol-4-5-bisphosphate (PIP₂) in the plasma membrane, resulting in the formation of diacylglycerol (DAG) and InsP₃. DAG remains at the plasma membrane to activate protein kinase C (PKC), while InsP₃ diffuses into the cytosol to bind to the InsP₃R, causing receptor opening and subsequent Ca^{2+} release from intracellular stores (25). Activation of PLC by RTKs was initially thought to similarly act on the plasma membrane pool of PIP₂ (25) but evidence suggests it may alternatively hydrolyze nuclear PIP₂ to generate InsP₃ and Ca^{2+} release within the nucleoplasm (137, 331) (Fig. 1). The InsP₃R is generally found in the membrane of the ER (367) and the nuclear envelope (NE) (176, 249) although InsP₃Rs have been localized to the plasma membrane in certain tissues (92), as well as along the nucleoplasmic reticulum (NR) (see below) (176, 249).

Three isoforms of the InsP₃R have been identified and are denoted types I, II, and III (125, 240, 369). KO mice have been generated for each of the three isoforms (126, 248). InsP₃R I deletion causes death of most of these mice *in utero* and the remaining have ataxia and tonic-clonic seizures. On the other hand, InsP₃R II and InsP₃R III double KO animals only show gross morphological or functional defects after postnatal day 20, when they manifest weight loss due to digestive system dysfunction (126). The InsP₃R sequence contains approximately 2700 amino acids and its structure has six membrane-spanning domains with the large C-terminal end directed toward the cytoplasm and the narrower end facing the ER lumen (180). There are three regions within the InsP₃R: an N-terminal InsP₃-binding domain, a Ca^{2+} channel-forming C-terminal domain, and a regulatory domain flanked by the InsP₃-binding domain and the channel region. Along their amino acid sequence, InsP₃Rs contain several sites for phosphorylation by kinases such as cyclic AMP-dependent protein kinase (PKA), cyclic GMP-dependent kinase, PKC, Ca^{2+} -calmodulin-dependent protein kinase II, and Akt kinase (191). Additionally, several protein partners interact with the regulatory domain to modulate the channel activity and with the C-terminal region to determine the subcellular localization of the receptor (32, 69). Upon InsP₃ binding, the receptor undergoes a conformational change resulting in opening of the Ca^{2+} channel, to allow Ca^{2+} release from the ER into the cytosol. Each InsP₃R isoform acts as an InsP₃-gated Ca^{2+} channel (33, 151, 317). However, their sensitivity to InsP₃ is not uniform, with type II being the most sensitive, followed by type I and lastly type III (277). Ca^{2+} release through the InsP₃R requires InsP₃ but the concentration of Ca^{2+} in the cytosol modulates the open probability of the Ca^{2+} channel (33, 150, 317). The open probability of Type I InsP₃R exhibits a bell-shaped dependence on $[\text{Ca}^{2+}]_i$ concentration. Therefore, incremental increases in $[\text{Ca}^{2+}]_i$ concentration up to 1 to 2 $\mu\text{mol/L}$, sustain and amplify Ca^{2+} release, while higher concentrations become progressively inhibitory. This biphasic $[\text{Ca}^{2+}]_i$

concentration dependence indicates that high concentrations of Ca^{2+} can feedback to turn the channel off, making the activity of the channel self-limiting (33). This property is responsible for Ca^{2+} oscillations (33, 88, 114). In contrast, the type III InsP3R exhibits a sigmoidal dependence on $[\text{Ca}^{2+}]_i$, and thus remains open in the presence of high Ca^{2+} concentrations. In this sense, activation of this isoform results in rapid and complete emptying of Ca^{2+} stores (150, 151). Single-channel studies of the type II InsP3R revealed that, like the type III InsP3R, this isoform stays open in the presence of high Ca^{2+} concentrations (317). Unexpectedly, cells expressing only the type II isoform exhibit sustained $[\text{Ca}^{2+}]_i$ oscillations, similar to what is observed in cells expressing only the type I InsP3R (261). This may reflect the fact that modifier proteins can alter the Ca^{2+} dependence of these isoforms (256).

The sequences of the InsP3R isoforms are considerably homologous but each subtype is expressed and regulated in a distinct fashion. There also are isoform-specific differences in tissue expression and subcellular distribution, suggesting that the various isoforms serve distinct roles in $[\text{Ca}^{2+}]_i$ signaling (257). Particularly, hepatocytes express only type I and type II InsP3R, and type II is the major isoform (165, 409). The type III InsP3R is not detected in hepatocytes, but is the predominant isoform in the bile duct epithelial cells, cholangiocytes (163). Moreover, isoforms may be expressed in distinct sub-cellular locations. The InsP3R II is most concentrated in the apical region of hepatocytes, while InsP3R I is expressed throughout the cell (165, 267). In contrast, the InsP3R III is most concentrated in the apical region of cholangiocytes, while InsP3R I and II are dispersed throughout the cell (163). The apical localization of the type II and III InsP3R in hepatocytes and cholangiocytes, respectively, represents a specialized region for triggering polarized Ca^{2+} waves (see below) (163, 165).

Ca^{2+} can also be released from the ER through RyRs. These channels are activated by Ca^{2+} itself through a mechanism known as Ca^{2+} -induced Ca^{2+} release (CICR), or by interaction with cADPR (25, 251, 253). Although RyRs seem to be absent from hepatocytes (135, 165), there is pharmacological evidence for RyR sensitivity, since RyR inhibition reduces the speed of hormone-induced $[\text{Ca}^{2+}]_i$ waves (267). In addition, ryanodine binding sites have been identified in hepatic microsomes (356), and also a truncated RyR has been identified in hepatocytes, which increases the frequency of InsP3-induced oscillations, although it is not able to promote Ca^{2+} release (303). Moreover, there is evidence that CD38 (ADP-ribosyl cyclase), the enzyme that catalyzes the formation of cADPR from NAD^+ is expressed in the liver (342). In rat hepatocytes, CD38 is mainly expressed in the NE, where it contributes to nuclear Ca^{2+} ($[\text{Ca}^{2+}]_n$) signaling (133, 192). Despite the different observations suggesting the presence of RyRs in the liver, their contribution to Ca^{2+} signaling in hepatocytes is an aspect that needs further investigation.

In addition to InsP3, Ca^{2+} , and cADPR, NAADP can also promote Ca^{2+} release into the cytosol (25, 127). NAADP was originally found in sea urchin eggs (209) and is the most potent Ca^{2+} mobilizing messenger yet described (128). This intracellular messenger was initially shown to interact with lysosome-related acidic compartments (71, 418). Further evidence has revealed TPCs (7, 178) as the NAADP-activated Ca^{2+} release channels, with three differentially distributed isoforms (TPC 1, 2, and 3) (50, 58, 224, 340, 424). TPC2, in

particular, has predominant lysosomal localization and has been identified in most human tissues, but with particularly abundant expression in the liver (58). NAADP interacts with high affinity with TPC2 (58), although recent evidence suggests that NAADP may bind to an accessory protein within a larger TPC complex (224). This interaction is tightly controlled by luminal pH and Ca^{2+} concentration (306). TPC2 mediates $[\text{Ca}^{2+}]_i$ release after activation with low nanomolar concentrations of NAADP while micromolar concentrations desensitize the receptor. Moreover, NAADP-mediated Ca^{2+} release is subsequently amplified by CICR from InsP3Rs (58, 424). Therefore, this Ca^{2+} release from lysosomal stores may play an important role in shaping $[\text{Ca}^{2+}]_c$ signals (60, 294). However, endogenous activators of NAADP and physiological functions of NAADP/TPC2-mediated Ca^{2+} release in the liver still remain elusive.

Mitochondrial Ca^{2+}

In addition to the role that mitochondria play in the control of metabolism and respiration, these specialized organelles also contribute to the tight spatiotemporal control of Ca^{2+} signals by taking up Ca^{2+} from or releasing Ca^{2+} back to the cytosol (310, 343) (Fig. 2). In fact, mitochondria display an enormous capacity to accumulate Ca^{2+} in the very rapid time scale of hundreds of milliseconds (61) thus shaping and maintaining $[\text{Ca}^{2+}]_c$ at resting levels.

Ca^{2+} uptake across the inner mitochondrial membrane (IMM) is mediated by the ruthenium-red-sensitive $[\text{Ca}^{2+}]_m$ uniporter (61, 81, 237, 349) and/or the rapid uptake mode, a transport mechanism that might contribute to $[\text{Ca}^{2+}]_m$ uptake from fast $[\text{Ca}^{2+}]_c$ transients *in vivo* (147). In addition, the outer mitochondrial membrane voltage-dependent anion channel may modulate Ca^{2+} access to the mitochondrial intermembrane space (136). This type of mitochondrial transport is driven by the potential gradient across the mitochondrial membrane (146). Ca^{2+} efflux occurs through the electrogenic Na^+ - Ca^{2+} antiporter and through a Na^+ -insensitive but H^+ -sensitive Ca^{2+} efflux pathway (22, 167). These efflux mechanisms are active transport systems (146). The molecular identity of these transport systems has remained largely elusive. However, recent studies have revealed novel candidate proteins that may mediate Ca^{2+} transport across the IMM (152). Ca^{2+} can also be released from mitochondria through the permeability transition pore (PTP), a voltage-dependent, high conductance channel that requires a permissive load of matrix Ca^{2+} for opening (20, 21, 80). Formation of the PTP results from increases in mitochondrial free Ca^{2+} , membrane depolarization, and alkaline matrix pH (147, 299), and induces the PT, a permeability increase of the IMM to ions and solutes with molecular masses up to 1500 Da (20, 21, 80). Activation of the PTP leads to rapid release of large amounts of $[\text{Ca}^{2+}]_m$ into the cytosol (173, 183). The PTP can be inhibited by antioxidants, reducing agents, and the immunosuppressive peptide cyclosporin A (21, 146, 278). Endogenous inhibitors of PT include adenosine diphosphate (ADP), Mg^{2+} , and increased mitochondrial membrane potential (146). Persistent opening of the PTP can induce irreversible cell injury and death (20, 21, 80, 146, 276). On the other hand, reversible activation of the PTP is observed in normal mitochondrial function and plays a role in $[\text{Ca}^{2+}]_i$ signaling (146, 173, 174) (Fig. 2). This is possible through several signaling steps; the mitochondrial membrane potential gradient allows $[\text{Ca}^{2+}]_m$ uptake through the uniporter; this influx of cations is compensated

by H⁺ efflux resulting in increased mitochondrial pH, which in turn activates formation of the PTP. This alters the membrane potential gradient, allowing Ca²⁺ to be released from the mitochondria, triggering [Ca²⁺]_m-induced Ca²⁺ release (mCICR) (173). mCICR, in regions where mitochondria are densely distributed, allows Ca²⁺ waves to propagate across the cytosol (153, 173). Indeed, mitochondria participate in hepatocyte [Ca²⁺]_i signaling (85, 153). This ability to contribute to the spatiotemporal control of Ca²⁺ signaling is due to the close association between mitochondria and [Ca²⁺]_i release sites. This has been confirmed by the observation that [Ca²⁺]_m can closely parallel the [Ca²⁺]_i increase induced by receptor activation. Furthermore, the increase in [Ca²⁺]_m following release of Ca²⁺ from intracellular stores is faster and larger than the increase that follows influx of extracellular Ca²⁺ (324). There is not only close proximity but also dynamic physical interaction between mitochondria and the ER (84, 348). Additionally, there is functional evidence for microdomains where mitochondria and InsP3Rs are in close apposition, so that mitochondria take up a significant fraction of the Ca²⁺ released by the InsP3R (85, 153, 183, 324). Approximately 30% of the total cellular mitochondrial pool is located in these microdomains of high [Ca²⁺]_i (367). Of note, sub-cellular regions that contain fewer mitochondria show greater sensitivity to InsP3 (153). This property of mitochondria can locally modulate InsP3R sensitivity and thus define the threshold for InsP3 to trigger [Ca²⁺]_i signals in different subcellular regions (153).

[Ca²⁺]_m uptake affects multiple factors in cell metabolism such as mitochondrial proton motive force, electron transport and the activity of dehydrogenases associated with the tricarboxylic acid cycle (154, 326). In isolated hepatocytes, slow or small [Ca²⁺]_i elevations are not transmitted effectively into mitochondria, and therefore, are unable to activate mitochondrial metabolism (326). Conversely, [Ca²⁺]_i oscillations trigger [Ca²⁺]_m oscillations and sustained nicotinamide adenine dinucleotide NAD(P)H formation (311). Therefore, the frequency rather than the amplitude of [Ca²⁺]_i oscillations regulates mitochondrial metabolism (154, 311). Indeed, Ca²⁺ signaling in the cytosol and mitochondria are closely related, allowing the tight regulation of spatiotemporal Ca²⁺ signaling and cell metabolism.

Nuclear Ca²⁺

Most Ca²⁺ signaling machinery found in the cytoplasm is duplicated in the nucleus, and there is evidence that [Ca²⁺]_n signals can occur independent of [Ca²⁺]_c signals (106, 215). The nucleus is bordered by the NE, a specialized region of the ER, which has a double function as it insulates the nucleoplasm from the cytoplasm and also has the ability to store and release Ca²⁺ (207, 249, 279). It is noteworthy that the NE is not simply a smooth-surfaced outer boundary but is interrupted by invaginations that reach deep within the nucleoplasm and could even traverse the nucleus completely. The existence of such a complex branched network of invaginations forming a NR represents an additional regulatory Ca²⁺ domain within the nucleus (106, 234). Phosphoinositides (as well as the enzymes responsible for their synthesis, namely, phosphatidylinositol and phosphatidylinositol 4-phosphate kinases) have long been known to be present in the nucleus (176), although their precise localization within the nucleus remains unknown (177). Furthermore, different PLC isoforms have been identified in the nucleus and may provide *in*

situ production of IP₃ and DAG (74, 394). Both the NE and NR express InsP₃Rs and numerous lines of evidence support a role for nuclear InsP₃Rs in regulating [Ca²⁺]_n release (57, 160, 171, 215, 238, 362). Of note, InsP₃Rs can be found in the outer (ONM) (62, 233, 362) and inner nuclear (241, 249) membranes. Additionally, sarco/ER Ca²⁺ ATPase (SERCA) uptake pumps are found in the ONM and have been shown to be identical to those in the ER (207). Therefore, the nucleus expresses the machinery necessary for local Ca²⁺ release and reuptake. Such machinery may be activated selectively through tyrosine kinase pathways (73). Indeed, IGF-1 and integrins cause PIP₂ breakdown in the nucleus but not at the plasma membrane (73), while activation of GPCRs causes breakdown of PIP₂ in the plasma membrane, but not in the nucleus (307). Similarly, activation of the HGF receptor c-Met or the insulin receptor causes their rapid translocation to the nucleus, to selectively hydrolyze nuclear PIP₂ and generate InsP₃-dependent [Ca²⁺]_n signals (137, 331). It also has been hypothesized that relocation of MAP kinase to the nucleus activates nuclear PLC to generate InsP₃ in the nucleus (347).

[Ca²⁺]_n signaling may also occur by passive transmission of [Ca²⁺]_i signals into the nucleus through the nuclear pore complexes (NPCs), which are involved in transport of macromolecules from the cytoplasm to nucleoplasm and vice versa (366). In fact, this mechanism was originally thought to be the principal one responsible for formation of [Ca²⁺]_n signals. The ion conductance of NPC is very large and this should allow free solute and ion diffusion. Then, [Ca²⁺]_i signals should generate identical [Ca²⁺]_n signals (3). It has been reported, however, that the NPC conductance can be dramatically reduced by accumulation of Ca²⁺ inside nuclear cisterna (gating) or by transport of macromolecules through the NPC (plugging) (365), although this view is controversial (132). Opinions on the Ca²⁺ permeability of NPC are also far from uniform. Both reports of free (51, 355) and restricted (11, 134, 230) permeability of Ca²⁺ through NPC have been shown. Studies monitoring diffusion of photoactivatable GFP across the NE suggest that permeability of the nuclear pore may be regulated by [Ca²⁺]_i rather than by Ca²⁺ stores within the NE (285). In either case, since EF-hand Ca²⁺-binding motifs are present in proteins of the nuclear pore, it is possible that these function as Ca²⁺ gating sensors for the pore (347).

Although [Ca²⁺]_n signals can originate inside the nucleus, and the permeability of nuclear pores to Ca²⁺ is regulated, there is considerable evidence that [Ca²⁺]_n can passively follow [Ca²⁺]_i (160, 225). For example, stimulation of hepatocytes with vasopressin results in a Ca²⁺ wave that appears to spread from the cytosol into the nucleus (223). Moreover, a mathematical analysis of Ca²⁺ waves in hepatocytes stimulated with vasopressin suggests that [Ca²⁺]_n signals can be described simply by diffusion of Ca²⁺ inward from the NE (118).

[Ca²⁺]_n can also contribute to Ca²⁺ signals in the cytosol. For example, Ca²⁺ puffs, which are highly transient and localized [Ca²⁺]_i signals that result from the coordinated opening of small clusters of InsP₃Rs, can spread across the cell by diffusing across the nucleus (421). Puffs can be triggered by a subthreshold concentration of agonist and the resulting [Ca²⁺]_i signal rapidly dissipates by diffusion in the cytosol and sequestration of Ca²⁺ into intracellular stores. However, since the range of diffusion of Ca²⁺ in the nucleus can be much greater than in the cytosol (118), Ca²⁺ puffs generated near the NE can spread into

and across the nucleus to spread to other, more distant regions of the cytosol (225). Thus, the nucleus may function as a path that helps distribute Ca^{2+} to the cytosol.

Ca^{2+} signaling patterns in individual cells

Ca^{2+} signals are highly organized in both space and time, from the subcellular to the cellular to the whole tissue level, to ensure reliability and specificity (24, 72, 105, 381). In the liver, this organization is achieved in the form of single transient or sustained $[\text{Ca}^{2+}]_i$ increases, Ca^{2+} oscillations and $[\text{Ca}^{2+}]_i$ waves (105, 381). In hepatocytes, Ca^{2+} oscillations originate from the periodic opening of Ca^{2+} channels located in the membrane of the ER, following activation of the PLC/InsP3/InsP3R cascade, and their shape and duration depends on the concentration and the type of agonist in use (413). For example, populations of isolated hepatocytes respond to agonists such as vasopressin, phenylephrine, or angiotensin with regular biphasic increases in $[\text{Ca}^{2+}]_i$, composed by two main events. The first is a rapid peak and then fall in $[\text{Ca}^{2+}]_i$, which occurs over a period of seconds. This is the result of Ca^{2+} release from InsP3-sensitive stores and does not depend on extracellular Ca^{2+} , since it can be observed in Ca^{2+} -free medium (189, 337). The second event is the sustained plateau in $[\text{Ca}^{2+}]_i$, which follows the rapid peak. This occurs only in the presence of extracellular Ca^{2+} that is necessary to reload depleted intracellular stores (155). In the case of vasopressin, lower concentrations induce $[\text{Ca}^{2+}]_i$ oscillations, while higher concentrations induce sustained increases in $[\text{Ca}^{2+}]_i$. In contrast, stimulation of hepatocytes with phenylephrine or the bile acid ursodeoxycholic acid (UDCA) typically evoke $[\text{Ca}^{2+}]_i$ oscillations at all concentrations (30, 337). Additionally, ATP stimulation leads to complex oscillations corresponding to a large peak, followed by smaller amplitude oscillations superimposed on a plateau phase (98). In all cases, the information of the agonist-induced Ca^{2+} increase is encoded in the rate of Ca^{2+} events or the amplitude of the Ca^{2+} rise, a phenomenon known as frequency encoding (26, 104, 105, 129). The duration of the $[\text{Ca}^{2+}]_i$ oscillations can also be determined by the agonist (413). For example, phenylephrine induces shorter $[\text{Ca}^{2+}]_i$ spikes than vasopressin or angiotensin (412, 413).

Frequency-encoded Ca^{2+} signals are thought to play an important role in determining the extent and targeting of $[\text{Ca}^{2+}]_i$ signals to downstream Ca^{2+} -sensitive proteins, yielding higher fidelity than other types of $[\text{Ca}^{2+}]_i$ signals (e.g., amplitude modulated) (379, 381). In this sense, the frequency of $[\text{Ca}^{2+}]_i$ spiking has been shown to differentially induce gene expression through the transcription factors, NFAT, NF κ B, and OAP (100, 222, 384), to modulate Ras and the MAP kinase pathway (384) and to regulate mitochondrial metabolism (154) with greater efficacy than a single Ca^{2+} pulse or a persistent increase in $[\text{Ca}^{2+}]_i$. Decoding the information contained in a $[\text{Ca}^{2+}]_i$ spike is essential to ensure the precise processing of Ca^{2+} signals. The activation and inactivation rates of Ca^{2+} /calmodulin-dependent protein kinase II (87) and the Ca^{2+} -dependent translocation of calmodulin (79), PKC (16, 304, 393) or the RAS GTPase activating protein, RASAL (397), suggests that these Ca^{2+} -sensitive proteins can sense the frequency of Ca^{2+} signals and discretely modulate enzymatic activity. Mitochondria sense the frequency of oscillatory $[\text{Ca}^{2+}]_i$ signals as well (325) by increasing the dehydrogenase NAD(P)H production in a time-averaged manner, thus modulating mitochondrial oxidative metabolism (326, 327).

Changes in InsP3 concentration are not the sole determinant of Ca^{2+} oscillations (246). Thapsigargin (117), which releases Ca^{2+} from the ER through an InsP3-independent mechanism, and nonhydrolyzable InsP3 analogs (396) can trigger $[\text{Ca}^{2+}]_i$ oscillations. The bell-shaped dependence of the InsP3R open probability on $[\text{Ca}^{2+}]_i$ concentration is thought to be one of the regulatory mechanisms responsible for oscillatory Ca^{2+} signals (33, 88, 317). Additionally, the maintenance of hormone-induced Ca^{2+} oscillations requires the constant replenishment of the ER with Ca^{2+} entering the cell through Ca^{2+} entry channels in the plasma membrane. Although several types of Ca^{2+} entry channels may be involved in maintaining proper Ca^{2+} concentrations in the ER, store-operated Ca^{2+} channels (SOCs), which are activated by a decrease in Ca^{2+} in the ER lumen, play a major role in liver cells (14, 15, 182). The liver expresses the SOCs Orai1 (a member of the Ca^{2+} release-activated channel modulator family of proteins) and transient receptor potential vanilloid 1 (TRPV1), which are plasma membrane Ca^{2+} channels, and the ER protein stromal interaction factor 1 (STIM1). It has been shown that these SOCs have a high selectivity for Ca^{2+} and that the Orai1 and STIM1 activation mechanism involves Ca^{2+} release from a putative small subregion of the ER likely close to the plasma membrane (15). However, the nature of such an ER subregion, its relationship with the bulk of the ER, and the steps involved in STIM1 interaction with Orai1 and other proteins still need to be determined in liver cells.

Ca^{2+} signals can also encode information spatially, in the form of $[\text{Ca}^{2+}]_i$ waves (105, 381). These are characterized by an initial local increase in Ca^{2+} concentration, which then propagates in the whole cell at an approximately constant rate (104). This spatial organization has been observed in isolated hepatocytes (338, 381) and in individual cells in the perfused liver (328, 382). In nonpolarized preparations of hepatocytes, vasopressin, and phenylephrine induce $[\text{Ca}^{2+}]_i$ waves that begin at a single locus, and this site of origin remains constant regardless of the agonist (338). The subcellular distribution of the InsP3R has been implicated in this spatial organization of Ca^{2+} waves (161). In polarized hepatocytes, the type II InsP3R (the most abundant isoform) is localized in the pericanalicular region (267) and this localized expression establishes a trigger zone from which Ca^{2+} waves originate (165) (Fig. 3). Additionally, the function of this trigger zone as an initiation site for Ca^{2+} waves depends on the integrity of lipid rafts (263). Similarly, the apical localization of InsP3Rs determines the initiation of Ca^{2+} signals in other cell systems (272, 417) including cholangiocytes, which express mostly the type III InsP3R (163). In these systems, InsP3 is generated by PLC activation near plasma membrane hormone receptors, which are often on the basolateral membrane, so it would be counterintuitive that Ca^{2+} signals instead would begin at the apical membrane. Certain biophysical properties of the type II (317) and III (150) InsP3Rs may explain why these receptors initiate Ca^{2+} waves at the trigger zone, but an alternative explanation is that clustering of InsP3Rs in the apical region lowers the threshold for Ca^{2+} release (371, 421). Indeed, the rise time is prolonged and the Ca^{2+} wave speed is reduced in hepatocytes with decreased expression or in which there is redistribution of the type II InsP3R (161).

The mechanism that governs apical-to-basolateral Ca^{2+} waves in hepatocytes remains elusive. In pancreatic acinar cells, the basolateral expression of the RyR Ca^{2+} release channel (213) may be the lead for Ca^{2+} wave propagation. RyR is activated by Ca^{2+} itself, which results in CICR. Therefore, InsP3 in the trigger zone initiates Ca^{2+} waves, which

propagate across the cell by RyR-mediated CICR (172, 212). Cholangiocytes, on the other hand do not express the RyR but low levels of InsP3Rs are found in the basolateral region (163). Since Ca^{2+} can coactivate the InsP3R (33), Ca^{2+} waves in cholangiocytes likely propagate by a modified form of CICR in which Ca^{2+} released from the apical trigger zone sensitizes basolateral InsP3Rs allowing Ca^{2+} waves to form. In hepatocytes, pharmacological evidence using RyR inhibitors suggests that the spread of Ca^{2+} waves depends upon serial activation of apical InsP3Rs, and then basolateral RyRs (267), similar to what occurs in pancreatic acinar cells (212). However, RT-PCR studies suggest that hepatocytes lack RyRs (165). Conversely, a truncated but functional form of the RyR was detected in hepatocytes (303). The subcellular distribution of this novel RyR variant and its role in the formation of Ca^{2+} waves still needs to be determined. Of note, the speed or amplitude of $[\text{Ca}^{2+}]_i$ waves in hepatocytes is not altered in Ca^{2+} -free medium (267), which suggests that their propagation depends only on release of Ca^{2+} from intracellular stores. Furthermore, Ca^{2+} waves can be induced in hepatocytes by direct introduction of a nonmetabolizable analog of InsP3 (105) or by treating these cells with agents such as tert-butyl hydroperoxide or certain bile acids that raise Ca^{2+} in the cytosol independently of InsP3 (76, 336). These observations strongly suggest that Ca^{2+} , and not InsP3, plays the major role in the propagation of the waves. However, a modeling system has suggested instead that InsP3, and not Ca^{2+} , is necessary for InsP3-induced Ca^{2+} waves (179).

Intercellular communication: Spread of Ca^{2+} signals from cell to cell

$[\text{Ca}^{2+}]_i$ waves are not confined to single cells, but rather a Ca^{2+} increase in one cell can be conveyed to neighboring cells giving rise to intercellular Ca^{2+} waves (129, 339). Ca^{2+} waves spread between hepatocytes (266), and this communication depends on gap junctions, which, in fact, are permeable to both Ca^{2+} and InsP3 (344). Either molecule could act, therefore, as the diffusible Ca^{2+} -mobilizing second messenger (344). Additionally, hormone-induced $[\text{Ca}^{2+}]_i$ signaling is highly coordinated in these cells, which depends upon gap junction conductance as well (266). Further evidence for a role of gap junctions has been provided by studies demonstrating blockage of intercellular Ca^{2+} waves by gap junction inhibitors (346, 385) or electroporation of antibodies to the gap junction components, connexins (38). Hepatocytes express two connexin isoforms, connexin 26 (Cx26), and connexin 32 (Cx32) (111), which is the most abundant in the liver (111, 204). Expression of both of these isoforms is dramatically reduced and coordination of $[\text{Ca}^{2+}]_i$ signals is impaired after bile duct ligation (BDL) (111). Moreover, hepatocytes isolated from Cx32 KO mice have impaired intercellular propagation of Ca^{2+} signals (78, 282). Additionally, transmission of Ca^{2+} from cell to cell is possible by the expression of Cx32 or connexin 43 (Cx43) in a liver cell line where intercellular Ca^{2+} signals are not normally observed (214). Such findings show that gap junctions allow second-messenger communication to organize Ca^{2+} signals in adjacent cells. Furthermore, functional gap junction channels are essential for the spread of intralobular Ca^{2+} waves (129).

Other factors are required to support the spread of $[\text{Ca}^{2+}]_i$ waves. Increases in InsP3 are necessary in each cell across which a Ca^{2+} wave spreads (39). Moreover, the presence of both InsP3 and Ca^{2+} is required to control the transmission of a $[\text{Ca}^{2+}]_i$ wave across a hepatocyte (385). Additionally, Ca^{2+} waves only propagate if agonist is binding to its

specific receptor at the surface of each cell. This likely means that the presence of hormone at each cell ensures that a sufficient level of excitability is achieved in the cytosol through the generation of the necessary levels of intracellular messengers, to support the propagation of a $[Ca^{2+}]_i$ wave (328, 385). Intercellular Ca^{2+} waves are further organized by hepatocyte “pacemaker cells” (214). These cells have increased expression of hormone receptor, which makes them more sensitive to hormonal stimulation. Therefore, Ca^{2+} signals occur sooner in pacemaker cells than in other cells stimulated with the same concentration of hormone, due to higher InsP3 production that drives $[Ca^{2+}]_i$ waves to neighboring cells (77). Of note, this increased sensitivity does not result from differences in downstream Ca^{2+} signaling components such as G-proteins or InsP3R (77, 387). This specialized organization might explain why vasopressin-induced waves begin in the region of the central venule, where the vasopressin V1a receptor is most heavily expressed, and then propagate to the portal region, where it is less heavily expressed (271). Additionally, pacemaker regions have been observed for other agonists like ATP and phenylephrine. Collectively, Ca^{2+} wave organization and propagation depends on several key aspects: InsP3 generation in each cell; a subset of cells with relatively increased expression of hormone receptor to initiate Ca^{2+} signals and thus serve as pacemakers for their neighbors; and gap junctions to coordinate the progression of Ca^{2+} signals among cells (56, 214).

Ca^{2+} waves may also be communicated between cells by paracrine signaling, involving the release of a messenger, its diffusion in the extracellular space, binding of the substance to a receptor, and activation of a signaling cascade that ultimately increases $[Ca^{2+}]_c$ (104). One such extracellular messenger in the liver is ATP. This agonist is secreted by both hepatocytes (351) and bile duct cells (115, 259) and stimulates the G-protein-coupled P2Y receptors that are also expressed in both cell types (196, 250, 269). Therefore, secretion of ATP by hepatocytes stimulates InsP3-mediated Ca^{2+} signaling in neighboring hepatocytes, as well as in bile duct cells (351), creating a paracrine signaling system in which signal propagation to adjacent cells does not depend on intercellular communication through gap junctions.

Ca^{2+} signals also regulate specific functions in other liver cell types. For example, $[Ca^{2+}]_i$ mobilization is implicated in the differentiation (activation) of hepatic stellate cells (HSCs) and portal (myo)fibroblasts during liver fibrosis (198, 292). HSC and portal fibroblasts express receptors for several Ca^{2+} -mobilizing agonists, such as endothelin (198, 322), platelet-derived growth factor (PDGF) (86, 110), vasopressin (305) and ATP (102, 316), and upregulation of serotonin (5-HT₂) receptors may be observed after HSC activation (292). Liver myofibroblast activation may exhibit different profiles of Ca^{2+} responses to endothelin and PDGF (198). Furthermore, CD38-mediated Ca^{2+} signals (through production of cADPR and NAADP) contribute to liver fibrosis via HSC activation (194). Moreover, activated fibroblasts and HSC are contractile due to their expression of motor proteins that can be regulated by Ca^{2+} -dependent and Ca^{2+} -sensitization mechanism pathways (175, 330, 420). In addition, InsP3R type 1 is shifted to the nucleus and to cell extensions upon HSC activation and Ca^{2+} release in these extensions promotes localized contractions (201).

Physiological Functions of Ca²⁺ Signals

Bile secretion

Bile secretion depends on the function of a number of membrane transport systems in hepatocytes and bile-duct epithelial cells (cholangiocytes) and on the structural and functional integrity of the bile-secretory apparatus (265). Ca²⁺ has been implicated in the regulation of such functions (Fig. 2). Agents that increase [Ca²⁺]_c from either internal or external sources in isolated hepatocytes inhibit bile secretion in isolated perfused livers, independently of PKC or hemodynamic effects. Furthermore, inhibition of Ca²⁺ signals prevents this inhibition of bile secretion (273). These findings suggest that increased [Ca²⁺]_c may play an inhibitory role in the regulation of hepatocyte bile production (273).

Possibly, Ca²⁺ inhibits bile flow by increasing paracellular permeability, which has been reported in isolated perfused livers and isolated hepatocytes (229), and would allow reflux of biliary constituents into the sinusoidal space, thereby dissipating the osmotic gradient that drives bile flow (274). In contrast to the inhibitory effect on net bile secretion, Ca²⁺ has been shown to mediate organic anion secretion through the insertion of the transporter Multidrug resistance-associated protein 2 (MRP2) into the canalicular membrane (83). Additionally, Ca²⁺ plays an important role in maintaining bile salt secretion through posttranslational regulation of the bile salt export pump (BSEP) (202) (Fig. 2). These effects are associated with pericanalicular Ca²⁺ release through the type II InsP3Rs that localize to this region in hepatocytes (165). These roles of Ca²⁺ in canalicular secretion can be supported by the observations that subplasmalemmal Ca²⁺ signals generally are obligatory for exocytosis (370), and Ca²⁺ acts as a regulator of exocytosis in the liver, in particular (53), suggesting that Ca²⁺ would be necessary for canalicular MRP2 and BSEP insertion. Moreover, the pericanalicular clustering of type II InsP3Rs creates a “trigger zone” that produces apical-to-basolateral Ca²⁺ waves in hepatocytes (165) which might promote bile secretion in a similar fashion to what has been observed in other polarized epithelia, including pancreatic acinar cells (187, 275) and cholangiocytes (116). These apparent discrepancies in the effects of Ca²⁺ on bile secretion in hepatocytes may be explained by differences in the nature of choleric and cholestatic Ca²⁺ signals. Therefore, it may be important to consider that the magnitude, timing, and subcellular localization of Ca²⁺ signals all contribute to their physiological effects (72).

Canalicular contraction is a crucial step in conducting bile flow through the canalicular network and into the bile ducts (290, 399). Ca²⁺ mediates this process, since microinjection of Ca²⁺, or stimulation with vasopressin (266, 400) or ATP (398) promotes canalicular contraction in hepatocytes. Furthermore, [Ca²⁺]_i increases result in the phosphorylation of myosin light-chain kinase which ultimately generates contractions in the pericanalicular actin network (415), which are organized within the hepatic lobule to form peristaltic waves (399). These waves are in turn essential to maintain bile flow (301).

The biliary tree plays an important role in conditioning the canalicular bile that is secreted by hepatocytes (265). Cholangiocytes perform this function through the regulation of biliary bicarbonate secretion (164). The prototypical and most extensively characterized pathway for bicarbonate secretion is initiated by stimulation of the secretin receptor which leads to

formation of cAMP and activation of the cystic fibrosis transmembrane conductance regulator (CFTR). Chloride released into the ductular lumen then is thought to be exchanged for bicarbonate via a chloride-bicarbonate exchanger (186, 208). An alternative or secondary secretory pathway is stimulated by neurotransmitters such as acetylcholine (ACh) and autocrine agents such as ATP, and is mediated by InsP3 and $[Ca^{2+}]_c$ (269). The downstream effect of Ca^{2+} is to activate Ca^{2+} -dependent chloride channels on the apical plasma membrane, which in turn release chloride that is exchanged for bicarbonate via a chloride-bicarbonate exchanger (186, 208). Although these two pathways were initially thought to act separately, a cross-talk has been suggested by the observation that Ca^{2+} potentiates adenylyl cyclase activity and cAMP formation in cholangiocytes via a calcineurin-dependent pathway (6), which may represent an indirect mechanism for Ca^{2+} -stimulated secretion. However, further evidence has suggested that cAMP- and CFTR-mediated secretion may have a more direct association with Ca^{2+} (259). Increases in cAMP result in apical, CFTR-dependent secretion of ATP, leading to stimulation of apical P2Y nucleotide receptors and subsequent activation of apical, type III InsP3Rs. This in turn activates Ca^{2+} -dependent chloride channels and then bicarbonate secretion (101, 259). These observations suggest that cAMP acts through an autocrine loop that involves ATP release, activation of P2Y receptors, and then an increase in $[Ca^{2+}]_c$. Moreover, this might represent a convergence of secretory pathways in the cholangiocyte at the level of InsP3R-mediated Ca^{2+} release (259). Therefore, stimulation of basolateral M3 muscarinic receptors by ACh leads to type I and II InsP3R-mediated Ca^{2+} release. This Ca^{2+} in turn drives apical Ca^{2+} -dependent chloride channels, resulting in biliary bicarbonate secretion. In contrast, stimulation of basolateral secretin receptors results in cAMP formation, subsequent activation of CFTR, and ATP-mediated bicarbonate secretion. Further studies revealed that this purinergic-signaling axis is present in both small and large cholangiocytes. ATP released from “upstream” small cholangiocytes lining the small intrahepatic bile ducts can be secreted into the bile, and can act as a paracrine signal to the large cholangiocytes lining the larger “downstream” bile ducts, stimulating Ca^{2+} -dependent secretion. Moreover, small cholangiocytes, which do not express CFTR (120), respond to extracellular nucleotides through Ca^{2+} -activated chloride efflux, representing an additional driving force for cholangiocyte secretion (411).

Primary cilia, which are sensory organelles expressed in the apical membrane of cholangiocytes, also relate to Ca^{2+} signaling and secretion. Primary cilia sense increases in osmolarity within the bile duct lumen, through the activation of the Ca^{2+} -permeable ion channel TRPV4. This allows entry of extracellular Ca^{2+} into the cytoplasm, increases in bile flow and ATP release and bicarbonate secretion (141). Primary cilia can also sense and transduce mechanical stimuli from within the bile duct lumen, so that increases in bile flow activate both cAMP production and Ca^{2+} release (245).

Glucose metabolism

Glucose metabolism in the liver is controlled through the coordination of glycogen synthesis and breakdown. Ca^{2+} plays an important role in these events, modulating the phosphorylation and dephosphorylation, and hence the activity of the regulatory intracellular enzymes glycogen synthase and glycogen phosphorylase (35, 109, 200). Several Ca^{2+} -mobilizing hormones have been implicated in these events. For example, vasopressin,

angiotensin-II and α -adrenergic agonists stimulate PLC, InsP3 increase, and Ca^{2+} release from the ER, leading to the phosphorylation of glycogen phosphorylase, decrease in glycogen synthase activity, and glycogenolysis (107, 297). Nucleotides promote glycogenolysis in a similar fashion through the activation of P2Y nucleotide receptors in both rat and human hepatocytes (96, 97, 190). The bile acids UDCA, lithocholic acid, and taurolithocholic acid (TLCA) activate phosphorylase in a comparable way to vasopressin (48, 75), although through a Ca^{2+} -dependent but InsP3-independent manner (48). Physiological levels of glucagon can evoke Ca^{2+} increases in the liver (67, 361) by activation of either InsP3-dependent or cAMP-dependent signaling pathways, resulting in glycogen phosphorylase activation (361, 395). The glycogenolytic capacity of the liver is regulated by several factors such as the distribution of gluconeogenic enzymes, which are localized in the periportal region (184). Perhaps for this reason, ATP and glucagon stimulate glucose release mostly in the periportal area. On the other hand, vasopressin and norepinephrine stimulate glucose release in pericentral hepatocytes, which can be explained by the fact that these cells are more sensitive to such agonists than periportal hepatocytes (129, 386). This differential sensitivity to glucose-mobilizing hormones is overcome by intercellular communication, which coordinates the regulation of glucose metabolism across the whole liver through gap junctions. For example, norepinephrine or glucagon-induced glucose release is impaired in isolated perfused livers from Cx32-deficient mice (368). In an analogous way, treatment of isolated perfused livers or isolated rat hepatocyte couplets (107) with a gap junction blocker decreases vasopressin or glucagon-induced glucose release. However, gap junction inhibition does not affect glycogen breakdown induced in a receptor-independent fashion. Moreover, hormone-induced glucose release is also impaired if hepatocytes are dispersed (107). Collectively, these observations demonstrate that gap junctions play a very important role in the control of hormone-induced glucose release in the liver in response to the heterogeneous distribution of hormone receptors. Stress conditions can challenge this coordination of metabolic function; Cx32-deficient mice become hypoglycemic in response to fasting, whereas WT animals do not, and endotoxin-induced hypoglycemia is aggravated in Cx32-deficient mice (78). Because the coordination of glucose storage and release assures balanced glucose metabolism in the liver, the actions of these two pathways are complemented by one another to maintain homeostasis. For example, insulin antagonizes the glycogenolytic activity of glucagon and α 1-adrenergic agonists in the liver (34, 89, 407). It is thought that insulin interferes with glucagon by reducing elevated levels of cAMP (34, 159, 407), whereas insulin inhibits the effects of α 1-agonists by interfering with their ability to elevate cytosolic free Ca^{2+} (380).

Cell proliferation

Ca^{2+} plays an important role in cell proliferation (143, 364) as well and in progression of the cell cycle (185, 308, 318, 334, 390). Temporal and spatial patterning of Ca^{2+} signals determines their specificity, and Ca^{2+} signaling patterns can vary in different parts of the cell (28, 72, 157, 215). For example, oscillation frequency, amplitude, and time delay of cytosolic calcium oscillations are essential kinetic parameters to determine entry into the cell cycle (403, 404). Furthermore, the proliferative ability of calcium is closely related to the subcellular compartments where it is released. The heterologous expression of the Ca^{2+} -binding protein parvalbumin (PV) has been used to study the role of Ca^{2+} signaling in the

regulation of the cell cycle. PV variants targeted to the nucleus or the cytoplasm were used to investigate the relative importance of Ca^{2+} signals in each of these cellular compartments for regulation of the cell cycle in the hepatoma cell lines SkHep1 and HepG2. It was found that nucleoplasmic rather than $[\text{Ca}^{2+}]_c$ is essential for cellular proliferation, and is necessary, in particular, for progression through early prophase (332) (Fig. 2). On the other hand, both cytosolic and nuclear Ca^{2+} signals were important for proliferation of LX-2 immortalized human HSCs and primary rat HSCs through CaMK II-mediated regulation of Cdc25C phosphorylation (359). Additional studies led to the observation that receptors for HGF, EGF, and insulin, three potent growth factors in the liver, translocate to the nucleus to generate InsP3-dependent Ca^{2+} signals (9, 137, 331), suggesting that certain growth factors may stimulate proliferation of hepatocytes by selectively inducing Ca^{2+} signals in the nucleus.

What are the specific targets of Ca^{2+} signals in the nucleus that are responsible for cell proliferation? $[\text{Ca}^{2+}]_n$ signals activate the transcription factors cAMP response element binding (CREB) (157) and Elk1 (314) and stimulate the intranuclear activity of PKC (106) and CaMK-IV (90), which would be expected to stimulate cell proliferation (Fig. 2). A recent study used a rapid subtraction hybridization (RaSH) approach to select genes whose expression was affected by a small alteration in $[\text{Ca}^{2+}]_n$ concentration. Legumain (LGMN), an asparaginyl endopeptidase involved in tumor metastasis was identified in this screen as a novel specific target for $[\text{Ca}^{2+}]_n$ signals in SkHep1 cells. $[\text{Ca}^{2+}]_n$ buffering resulted in decreased LGMN expression. Furthermore, LGMN gene knockdown resulted in decreased cell proliferation, suggesting that expression of this protein is associated with increased $[\text{Ca}^{2+}]_n$ -dependent proliferation. These findings highlight a new role for LGMN and provide evidence that $[\text{Ca}^{2+}]_n$ signals regulate cell proliferation in part through the modulation of LGMN expression (8).

Ca^{2+} also plays an important role in the proliferation of bile duct cells. The proliferative activity of large cholangiocytes is regulated by the activation of cAMP-dependent mechanisms (4, 121, 219). On the other hand, small cholangiocyte proliferation is regulated by the activation of α 1-adrenergic receptors and occurs through Ca^{2+} /calcineurin-dependent activation of nuclear factor of activated T cells 2 and specificity protein 1. Furthermore, histamine stimulates small cholangiocyte proliferation through the activation of the H1HR receptor and InsP3/ Ca^{2+} signaling (120, 122). These findings implicate α 1-adrenergic and histamine receptors (in particular, H1HR) as key factors in the regulation of normal cholangiocyte proliferation and function. This is also important in pathological ductopenic conditions associated with damage of large ducts in which Ca^{2+} -dependent small cholangiocyte proliferation may be a key compensatory mechanism for maintaining homeostasis and overall bile duct function (219, 239).

Apoptosis

Apoptosis can result from a variety of extracellular or intracellular stimuli, which converge with activation of caspases to lead directly to cell death (227). Apoptosis generally occurs through one of two pathways. The extrinsic pathway is initiated by death receptors of the tumor necrosis factor receptor superfamily (108). The intrinsic pathway involves the

convergence of intracellular stress signals on mitochondria, resulting in the formation of the PTP (10, 63). In many cells, the death receptor pathway may act through mitochondria as well by inducing the cleavage of the proapoptotic molecule Bid and subsequent mitochondrial permeabilization (231). Therefore, mitochondrial permeabilization is key to both pathways. Apoptosis is modulated by a number of proteins in part through the InsP3R or InsP3R-induced Ca^{2+} release mechanisms (Fig. 2). These in turn mediate effects in $[\text{Ca}^{2+}]_m$, since a subset of mitochondria are in close proximity to InsP3Rs (324), and mitochondrial and $[\text{Ca}^{2+}]_c$ signals are interrelated (154, 183). In particular, the mitochondrial apoptotic pathway is inhibited by members of the Bcl-2 family of proteins, including Bcl-2, Bcl-xL, and Mcl-1 (1, 144, 243, 321). Bcl-2 inhibits apoptosis in part by decreasing the size of ER Ca^{2+} stores (17), whereas Bcl-xL acts in part by inhibiting expression of the InsP3R (221, 405). Collectively, this reduces Ca^{2+} release from the ER into the cytosol and consequently decreases the amount of Ca^{2+} transmitted to surrounding mitochondria. Since mitochondria are less likely to become overloaded with Ca^{2+} , the formation of the PTP is prevented and the overall result is a diminished cellular sensitivity to apoptotic stimuli (17, 221) (Fig. 2). In fact, overexpression of Bcl-xL provides protection against apoptotic cell death in rat hepatoma cells (124). On the other hand, neither InsP3R expression nor ER Ca^{2+} stores are affected by Mcl-1. Rather, Mcl-1 inhibits Ca^{2+} signaling directly within mitochondria (258). In contrast, the proapoptotic Bcl-2 family members Bax and Bak promote ER Ca^{2+} overload through the control of InsP3R phosphorylation (287), which leads to sensitization of mitochondria to calcium-mediated fluxes (284). These alterations serve as important upstream signals for cytochrome c release (283) (Fig. 2). Additionally, cytochrome c that has leaked from mitochondria via the PTP binds to the InsP3R, which facilitates release of toxic amounts of Ca^{2+} from the ER (36). This is thought to result in a positive feedback loop that causes further Ca^{2+} overload of mitochondria and then further leakage of cytochrome c (36). Inhibition of the interaction between cytochrome c and the InsP3R inhibits development of apoptosis by blocking this positive feedback loop (37). Therefore, Bcl-2 family members regulate their pro- or antiapoptotic functions at least in part through a range of complementary effects on Ca^{2+} signaling pathways.

Mitochondrial permeabilization and cell death can also be induced by dysregulation of redox transition, namely, the uncontrolled generation of reactive oxygen species (ROS) (216, 298). Oxidants increase $[\text{Ca}^{2+}]_i$ load by stimulating IP3Rs in the ER, while inhibiting SERCA pumps and Ca^{2+} extrusion via plasma membrane channels (59). In turn, $[\text{Ca}^{2+}]_m$ uptake stimulates oxidative metabolism, resulting in enhanced ATP production, and hence ROS generation as a byproduct of oxidative phosphorylation (320). Above a certain degree of $[\text{Ca}^{2+}]_m$ and ROS rise, a progressive Ca^{2+} surge prompts a feed-forward loop that induces prolonged PTP opening and commits cells to death (298) by the combination of a set of events: IMM depolarization, which causes cessation of oxidative phosphorylation and ROS production; matrix swelling and cristae unfolding, and the ensuing breaches in the OMM with release of stored Ca^{2+} and of apoptogenic proteins (20, 80, 142, 319). In fact, Ca^{2+} -induced production of ROS has been shown to promote cytochrome c release in rat liver mitochondria through mitochondrial permeability transition-dependent mechanisms (300). Furthermore, an analogue of the lipid peroxides formed during oxidative stress, tertbutyl-

hydroperoxide, prompts NAD(P)H oxidation, increasing mitochondrial free Ca^{2+} and ROS, eventually leading to PTP opening and cell death (216).

It is important to note that when PTP induction is transient or limited to a fraction of mitochondria in a cell, a short burst of ROS production could occur. These ROS could serve as signaling components to propagate waves of short PTP openings in the surrounding mitochondria, producing additional ROS with possible functions in the regulation of chaperones, kinases, and gene expression. Hence, PTP could use Ca^{2+} -dependent and independent ROS generation to tune many cellular routines unrelated to death induction (195).

Ca^{2+} Signals in Liver in Health and Disease

Liver growth: Regeneration versus hepatocellular carcinoma

The role of Ca^{2+} in cell proliferation assumes special importance in the process of liver regeneration. Liver regeneration after the loss of hepatic tissue is a fundamental parameter of the response to liver injury (255). It is induced by acute damage to the organ and is regulated in a well-orchestrated manner by cytokines, growth factors, hormones, and neurotransmitters that help promote massive proliferation of hepatocytes, which switch from a quiescent to a proliferative phenotype. This results in the restoration of liver mass and function within days after partial tissue loss (112, 377). A number of Ca^{2+} mobilizing hormones and growth factors are involved in this process of proliferative signaling. These include adenosine triphosphate (ATP) (138), arginine vasopressin (AVP) (281), noradrenaline (82), HGF (12, 260), EGF (260, 373), and insulin (331).

Alterations in components of the Ca^{2+} signaling machinery, namely, the different InsP3R isoforms and SERCA pumps, have been reported to occur during liver regeneration (95, 113, 232). Other reports have suggested that the sensitivity of liver cells to Ca^{2+} mobilizing agonists (169), as well as agonist-induced calcium signals are modified after partial hepatectomy (PH) in the rat (197). Another study suggested that these modifications reflect the remodeling of Ca^{2+} signals, which is thought to be essential for the initial regenerative response (280). Thus, Ca^{2+} signals are thought to adapt to the context of regulated proliferation induced by PH. Is there a fine regulation mechanism for proliferation during liver regeneration and does Ca^{2+} signal compartmentalization influence this process? Lagoudakis et al. investigated the role of $[\text{Ca}^{2+}]_c$ during liver regeneration in the rat, targeting PV to this compartment (206). The study demonstrated *in vivo*, at the organ level that hepatocytes require unaltered cytosolic calcium signaling to progress through the cell cycle after PH, even though liver cell lines do not share this requirement, as discussed above (332). This may reflect the fact that primary hepatocytes are more sensitive than liver cell lines to Ca^{2+} buffering, since buffering of Ca^{2+} in the nucleus of primary hepatocytes *in vivo* leads to widespread apoptosis, liver failure, and death (unpublished observation). Alternatively, perhaps this is due to the fact that primary hepatocytes and liver cell lines do not express the same repertoire of intracellular calcium channels in the different cell compartments (215). Progression through the cell cycle from G_0 to G_1 and S phases is triggered presumably by Ca^{2+} mobilizing agonists that are known to be released in the early stages of liver regeneration after PH, such as HGF and EGF (260), ATP (138), AVP (281),

and noradrenaline (82). Furthermore, $[Ca^{2+}]_c$ signals may be important for other early events after PH, such as gene transcription and phosphorylation of ERK and CREB, which are crucial for hepatocyte progression after PH (341, 352, 372), and which are dependent on cytosolic and/or nuclear Ca^{2+} signaling (91, 99, 157, 205). Specific targets of $[Ca^{2+}]_c$ signals during liver regeneration remain unknown.

A recent report has shown that $[Ca^{2+}]_m$ signals also play an important role in the progression of liver regeneration after PH (145). Experiments in SkHep1 cells expressing PV in the mitochondria revealed that buffering of $[Ca^{2+}]_m$ leads to decreased apoptosis through altered expression of proapoptotic and antiapoptotic proteins. *In vivo* studies showed that $[Ca^{2+}]_m$ buffering accelerates liver regeneration after PH. Therefore, the regulation of apoptosis by $[Ca^{2+}]_m$ signals is an additional mechanism by which hepatocyte proliferation and liver regeneration may be modulated. Future advances in this field should lead to a better understanding of how these various Ca^{2+} compartments act in an integrated manner to regulate liver regeneration.

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy in adults and the third most common cause of cancer death worldwide (228). Its complex molecular pathogenesis is regulated by several signaling pathways (391, 392, 406). In particular, the role of growth factors, which act in part through $[Ca^{2+}]_i$ release (28), has been highlighted in various studies and their signaling pathways are known targets for the discovery of new therapeutic approaches (391, 392, 406). In particular, HGF and its receptor c-met have been implicated in tumor invasion in HCC (247). The versatility of MET-mediated biological responses is modulated by subcellular compartmentalization of MET signaling pathways (389). In fact, c-met translocates to the nucleus upon HGF stimulation to generate InsP3-dependent Ca^{2+} signals there, and this process is thought to regulate cell proliferation (137). The invasive potential of HCC cells has also been linked to altered store-operated Ca^{2+} entry (148), through regulation by β ig-h3, an extracellular matrix protein that is implicated in tumorigenesis and metastasis (374, 378). Selective buffering of nuclear but not $[Ca^{2+}]_c$ also impairs growth of liver tumors *in vivo* (332) (Fig. 2). Additionally, $[Ca^{2+}]_n$ signals mediate cell proliferation through the regulation of the asparaginyl endopeptidase LGMN (8). The observations that HGF stimulates increased expression of this protein, and expression also is increased in tumor cells compared to normal cells in liver specimens from patients with hepatocellular carcinoma (HCC), suggests that LGMN's positive effects in cell proliferation may promote carcinogenesis.

Two therapeutic agents, interferon-alpha (IFN α) and 5-fluorouracil (5-FU) are used experimentally in combination against HCC (264, 295, 345). A recent study revealed that $[Ca^{2+}]_i$ might be a potent upstream proapoptotic messenger acting at the early stage of IFN- α /5-FU-induced apoptosis through the mitochondrial pathway (422). Furthermore, the novel squamosamide derivative and potent antioxidant *N*-(2-(4-hydroxy-phenyl)-ethyl)-2-(2,5-dimethoxy-phenyl)-3-(3-methoxy-4-hydroxy-phenyl)-acrylamide also known as FLZ, has been shown to have antitumor activity in HCC cells via modulation of the expression or activation of cell-cycle regulatory proteins, which are associated with decreased Ca^{2+} /ROS levels. Therefore, FLZ represents a potential therapeutic agent for the treatment of HCC (315). Understanding the interplay between Ca^{2+} signaling and other signaling pathways,

and how compartmentalized Ca^{2+} signals may control cell proliferation will reveal new clues of HCC pathogenesis, with the potential to lead to the discovery of more specific and effective therapeutic approaches.

Cholestasis

Bile secretion is one of the primary functions of the liver and defects in this process result in cholestasis. Chronic cholestatic conditions of the liver are among the most serious forms of liver disease. These conditions often result from diseases of the bile ducts (329, 388). Secretion in cholangiocytes can be mediated in part by Ca^{2+} (101, 164). More specifically, InsP3-mediated Ca^{2+} signals are important for normal bile secretion as discussed above, and loss of InsP3R is a common event in cholestasis (312). In particular, apical type III InsP3R expression is greatly decreased or absent in the bile ducts of patients with a range of cholestatic disorders including bile duct obstruction resulting from stone disease or malignancy, biliary atresia, primary biliary cirrhosis and sclerosing cholangitis (354). In accordance with these observations, animals subjected to common BDL or lipopolysaccharide (LPS) injection, which are accepted models of cholestasis (329, 354), had selective loss of InsP3R expression in biliary epithelia (354) (Fig. 4). As a result, Ca^{2+} signaling and Ca^{2+} -mediated bicarbonate secretion is impaired. However, upstream components of the Ca^{2+} signaling pathway are preserved, which suggests that the selective decrease in InsPRs is the cause of defective Ca^{2+} signaling. Furthermore, this decrease in InsP3Rs is thought to interfere also with cAMP-mediated ductular secretion (218, 259). The loss of InsP3R from bile duct epithelia has been observed in other experimental animal models of cholestasis (5, 203, 217, 262, 360, 388). These findings may be explained by the fact that type III is the most abundant InsP3R isoform in cholangiocytes (163), and contribute to the formation of apical-to-basolateral Ca^{2+} waves (163, 166, 270, 409), which are critical for secretion in bile duct cells, due to their role in the transport of fluid and electrolytes across the apical membrane (116).

Although cholestasis is often associated with defects in the bile ducts (329, 388), genetic or acquired disorders of hepatocyte transporters can also account for this condition (388). Recent studies have demonstrated that insertion of the transporter MRP2 in the plasma membrane is Ca^{2+} dependent and loss of type II InsP3R results in impaired canalicular organic anion secretion in hepatocytes (83) (Fig. 2). Moreover, loss of this isoform impairs the choleric effect of tau-roursodeoxycholic acid (TUDCA) in cells treated with the cholestatic bile acid TLCA (83). Similarly, loss of type II InsP3R expression or loss of pericanalicular expression of the receptor leads to impaired BSEP-dependent secretion in hepatocytes (Fig. 2), and is observed in animal models of estrogen and endotoxin-induced cholestasis (202). Indeed, decreased type II InsP3R expression in the liver had been observed in an earlier study in animals subjected to BDL (103) and mistargeting of hepatocellular transporters is a common feature of cholestatic disorders (333). Being the most expressed isoform in this cell type, and localized preferentially to the canalicular region (267), the type II InsP3R regulates polarized Ca^{2+} waves (165), which in turn may regulate secretion in an analogous manner to what takes place in cholangiocytes (259, 354).

The mechanisms by which the decrease in InsP3R expression occurs remain unknown. Certain hepatic proteins can have decreased expression in BDL and other cholestatic conditions through the activation of nuclear hormone receptors like the farnesoid X receptor (FXR) (94). However, it is not known whether the promoter regions of any of the InsP3R isoforms contain response elements for FXR. Proinflammatory cytokines released during cholestasis are known to inhibit fluid secretion in cholangiocytes, so one could question their contribution to decreased expression of InsP3R. However, it has been shown that proinflammatory cytokines do not impair Ca^{2+} -mediated secretion in these cells (360). Consistent with this observation, interleukin 1 increases rather than decreases transcription of the type I *InsP3R* gene (49). Perhaps increased degradation rather than decreased production could cause the observed loss of InsP3R expression. Indeed, InsP3R ubiquitination and proteasomal degradation are triggered upon secretagogue stimulation in pancreatic acinar cells (410).

These findings suggest that in canalicular cholestasis, decreased expression and localization of canalicular transporters results in part from loss of pericanalicular Ca^{2+} signaling. This has implications for the treatment of cholestatic disorders. UDCA, the only therapy of proven benefit for certain types of chronic cholestatic liver disease (296, 309), ameliorates liver function abnormalities in part by stimulating biliary exocytosis in a Ca^{2+} -dependent fashion (30, 31). Moreover, these effects are partially mediated by the cooperative action of Ca^{2+} -dependent conventional PKC alpha (cPKC α), which promotes transporter insertion into the canalicular membrane (29), and PKA (408), which phosphorylates the type II InsP3R potentiating InsP3-evoked Ca^{2+} release (52, 55). Furthermore, the cytoprotective effects of UDCA and its taurine conjugate TUDCA are associated with enhancement of Ca^{2+} signaling, inhibition of which blocks the activation of key survival pathways (244). In fact, UDCA stimulates secretion of the Ca^{2+} -mobilizing agonist ATP from hepatocytes into bile, which may promote bile flow and act on downstream bile-duct epithelia by stimulating fluid and electrolyte secretion (268). Additionally, UDCA can locally stimulate cholangiocytes to release ATP and initiate P2Y receptor-mediated Ca^{2+} -dependent fluid secretion (115). These findings are consistent with the hypothesis that stimulation of certain types of Ca^{2+} signaling in bile ducts and hepatocytes may provide a strategy for the treatment of cholestatic diseases. Taken together, loss of apical InsP3Rs in cholangiocytes and hepatocytes appear to be a common basis for impaired secretion and the development of cholestasis. These findings suggest a choleric effect for Ca^{2+} through localized Ca^{2+} release in the pericanalicular region. In contrast, earlier studies had suggested a cholestatic effect, in which global Ca^{2+} signals induced by ionophores may promote cholestasis (273). Understanding the localized nature of these, Ca^{2+} signaling microdomains may provide insight into mechanisms of cholestasis and lead to the discovery of new alternatives to treat this condition.

The role of gap junctions in cholestasis has also been reported. Correa et al. investigated the involvement of Cx32, the major gap junction protein in the liver (111, 204), in the hepatic response to cholestasis (78). This study demonstrated that Cx32 deficiency impairs intercellular communication and the transmission of Ca^{2+} signals between hepatocytes after treatment with endotoxin. This finding is consistent with previous observations that Ca^{2+}

waves and Ca^{2+} oscillations depend upon gap junctions in hepatocyte couplets (266), and that decrease in Cx32 protein levels after BDL is associated with impaired coordination of hormone-induced Ca^{2+} signals (111). Furthermore, Cx32 deficiency in mice leads to prolonged cholestasis when compared to wild type animals, suggesting that Cx32 expression is important for recovery from cholestasis after treatment with endotoxin (78). This report proposed an important role of gap junctions in the liver, to assure integrated and uniform tissue response in times of stress (78). Similarly, a subsequent study showed that cholestatic bile acids inhibit gap junction permeability and prevent the coordination of Ca^{2+} oscillations in hepatocytes and cholangiocytes (47). These findings suggest that this inhibitory effect might contribute to the cholestatic effect of these bile acids and may be an early event before the downregulation of Cx32 that has been observed previously in other models of cholestasis (111, 139). In contrast, opposing results were shown subsequently, in which TLCA-induced cholestasis was partially reversed by TUDCA in both wild type and Cx32-deficient mice, suggesting that gap junctional intercellular communication is neither essential for the cholestatic effect of TLCA nor needed for the choleric effect of TUDCA (313). In aggregate, these observations are consistent with the idea that altered Ca^{2+} signaling is involved in the pathogenesis of cholestasis and can be modulated for therapeutic benefit, but careful examination of the response of patients to cholestasis may ultimately be necessary to establish a common basis for this disorder and the relevance of these observations for human disease.

Viral hepatitis

Viruses encode proteins and utilize host cellular proteins and signaling pathways to enhance viral replication, so regulating cellular Ca^{2+} signals is an ideal strategy for altering the intracellular environment to favor viral replication (66). Hepatitis B virus (HBV) is a pararetrovirus that affects more than 350 million people worldwide and chronic infection with HBV is the major cause for the development of HCC (158). The multifunctional HBV protein HBx is essential for replication (68, 425) and is thought to make significant contributions to the development of HBV-associated HCC (44). Elevation of $[\text{Ca}^{2+}]_c$ signals as a result of HBx has been reported in several studies. HBx regulation of $[\text{Ca}^{2+}]_c$ was shown to be essential for HBV replication in HepG2 cells and cultured primary rat hepatocytes (46, 130); calcium signals also promoted HBV capsid assembly in HepG2 cells (70). HBx elevation of $[\text{Ca}^{2+}]_c$ signals is required for other activities of HBx, such as regulation of cell proliferation, activation of Pyk2/FAK-Src kinases, and activation of the transcription factor AP-1 (43, 45, 46, 131, 288). A very recent study added to these findings proposing that HBx stimulates HBV replication through increases in $[\text{Ca}^{2+}]_m$ uptake, which promotes ER Ca^{2+} depletion and increased store-operated Ca^{2+} entry, causing an increased plateau level of $[\text{Ca}^{2+}]_c$ spikes (419). Indeed, continuing studies geared toward the discovery of the precise molecular basis of HBx regulation of Ca^{2+} signaling may help identify specific targets that regulate HBV replication and HCC development.

Hepatitis C Virus (HCV) also alters Ca^{2+} signals upon infection. HCV infection is a major cause of chronic hepatitis, liver cirrhosis, and HCC, affecting more than 170 million people worldwide (353). Several studies have suggested that HCV promotes alterations in mitochondrial oxidative metabolism (289, 376). Piccoli et al. provided insight into the

mechanism by which this may occur, demonstrating that HCV protein expression results in Ca^{2+} overload in the mitochondrial compartment, which triggers alterations in the bioenergetic balance and nitro-oxidative stress (302). This study proposes that these effects are primed by an HCV protein pool localized on ER-associated mitochondrial membranes. In this model, HCV-induced ER stress, which has been documented previously (19, 375, 376), may provoke depletion of ER Ca^{2+} , which in turn may be captured by the mitochondria to result in the described alterations (302). Cumulatively, these results suggest that restoring $[\text{Ca}^{2+}]_m$ homeostasis might prevent or even reverse the effects of HCV.

ER stress

Effects of ER stress in the liver are important for the pathogenesis of a number of metabolic and inflammatory conditions. The ER represents a central regulator of protein folding, quality control, trafficking, and targeting. Therefore, the ability to adapt its capacity to manage synthetic, metabolic, and other unfavorable conditions is extremely important to maintain homeostasis in the cell. Disequilibrium between ER load and folding capacity establishes ER stress, and the ability to adapt to physiological levels of ER stress is important to all cells, but especially to professional secretory cells (156), like hepatocytes (Fig. 2). This adaptation is achieved by the activation of the unfolded protein response (UPR) which consists of the complex coordinated action of three distinct signal transduction pathways mediated by inositol requiring enzyme (IRE) 1 α , protein kinase RNA-like ER kinase (PERK), and activating transcription factor 6 α (ATF6 α) (168, 236). Following activation, UPR signaling pathways act synergistically to encode functions that ameliorate the stressed state of the ER. If ER stress persists, the cell is functionally compromised and signal alarm pathways are activated to lead to apoptosis (168, 236, 335).

UPR activation has been reported in several physiological responses, including glucose and lipid metabolism, inflammation, autophagy, and apoptosis (168). The role of Ca^{2+} has been particularly noted in the process of ER stress-induced apoptosis. Disequilibrium in ER Ca^{2+} levels can activate the UPR, and constant or pronounced ER stress leads to apoptosis (168). ER stress-associated cell death results from the action of several apoptosis mediators. Some of these are activated by the UPR sensors, whereas others are related to Ca^{2+} and redox homeostasis (236). The proapoptotic proteins Bax and Bak as well as the antiapoptotic Bcl-2 that reside in the ER membrane and are known to regulate Ca^{2+} homeostasis, interact with members of the UPR cascades like IRE1 α and c-jun-N-terminal kinase to mediate ER stress-induced apoptosis (162, 210, 401, 416). Furthermore, ER stress-inducing agents lead to sustained Ca^{2+} release from the ER, accumulation of Ca^{2+} in the mitochondria and subsequent mitochondrial permeabilization, resulting in release of apoptotic effectors from mitochondria into the cytosol (93). When the antiapoptotic protein Bcl-2 is over expressed, resting free ER Ca^{2+} and cell death are decreased (119). Moreover, deficiency of the proapoptotic proteins Bak and Bax results in lower free ER Ca^{2+} content, and reduced sensitivity to apoptotic agents (286). Furthermore, Bax Inhibitor-1 induces Ca^{2+} release under acidic conditions leading to increased cell death (193). In some cases during UPR activation, translation is resumed prematurely before ER stress is completely resolved. Consequently, newly synthesized proteins can undergo oxidative protein folding in the ER generating ROS (242). Additionally, protein folding in the ER can lead to activation of

InsP3Rs and release of $[Ca^{2+}]_i$ from the ER, which in turn is transmitted to the mitochondria to produce ROS (298). In both cases, the generation of ROS results in apoptosis. Thus, current evidence suggests that Ca^{2+} and Ca^{2+} -sensitive proteins play a key role in the development of ER stress-induced apoptosis. However, the exact signaling pathways that mediate this process in hepatocytes and whether ER stress affects the Ca^{2+} signaling machinery and/or downstream functions of Ca^{2+} are subjects that need further investigation.

Sustained ER stress and activation of the UPR have also been observed in several liver diseases, and Ca^{2+} signaling-associated defects have been implicated, in particular, in the progression of obesity and the obesity-related disorder Nonalcoholic fatty liver disease (NAFLD). Indeed, incorporation of saturated fatty acids into the ER disrupt its structure and function (42, 226), affecting the folding capacity and chaperone function by altering ER Ca^{2+} homeostasis (54, 140, 149, 402). Several lines of evidence suggest that members of the Bcl-2 family of proteins, which directly alter ER Ca^{2+} , may play a direct role in saturated fatty acid-induced hepatocyte cell death and the progression of NAFLD (2, 13, 65, 235, 291). Moreover, palmitic acid requires the flux of Ca^{2+} and cytochrome C release to mediate ER stress-induced apoptosis (423). Furthermore, a new link has been established between ER stress and obesity, in which protein and mRNA levels of SERCA2b are markedly reduced in the livers of obese mice and recovery of SERCA2b significantly ameliorates ER stress, increases glucose tolerance and achieves euglycemia in severely obese and diabetic mice (293) (Fig. 2). Subsequent studies demonstrated that alterations in the ER fatty acid and lipid composition result in the inhibition of SERCA activity and ER stress. Hence, abnormal lipid and Ca^{2+} metabolism are important contributors to hepatic ER stress in obesity (123).

Therapeutic agents that affect Ca^{2+} signaling are currently being used for conditions like spasticity, and other compounds have been tested for specific UPR proteins in the liver (199). However, further investigation is needed to better understand the molecular basis of ER stress-induced liver damage and the precise involvement of Ca^{2+} signaling in this process to develop targeted therapies for these conditions.

Conclusion

Ca^{2+} signaling in the liver results from the orchestrated action of multiple members of the Ca^{2+} signaling machinery in the different cells that form this highly specialized organ. Spatial and temporal Ca^{2+} signaling patterns should be considered not only at the subcellular and cellular level, but also in the context of the whole organ, in which cells maintain constant communication to achieve the proper performance of liver function. We furthermore are now beginning to understand how defects in Ca^{2+} signaling at these various levels play a role in the pathogenesis of a wide range of liver diseases. The understanding of how defects in the Ca^{2+} signaling machinery and in Ca^{2+} signaling patterns may lead to pathological conditions, may in turn result in the rational design of novel, more specific, and more effective therapeutic approaches.

Acknowledgments

This study was supported by the following NIH Grants: DK57751, DK34989, DK 45710, and DK61747.

References

1. Adams JM, Cory S. The Bcl-2 protein family: Arbiters of cell survival. *Science*. 1998; 281:1322–1326. [PubMed: 9735050]
2. Akazawa Y, Cazanave S, Mott JL, Elmi N, Bronk SF, Kohno S, Charlton MR, Gores GJ. Palmitoleate attenuates palmitate-induced Bim and PUMA up-regulation and hepatocyte lipoapoptosis. *J Hepatol*. 2010; 52:586–593. [PubMed: 20206402]
3. Alonso MT, Garcia-Sancho J. Nuclear Ca(2+) signalling. *Cell Calcium*. 2011; 49:280–289. [PubMed: 21146212]
4. Alpini G, Glaser SS, Ueno Y, Pham L, Podila PV, Caligiuri A, Lesage G, Larusso NF. Heterogeneity of the proliferative capacity of rat cholangiocytes after bile duct ligation. *Am J Physiol*. 1998; 274:G767–G775. [PubMed: 9575860]
5. Alpini G, Lenzi R, Zhai WR, Slott PA, Liu MH, Sarkozi L, Tavoloni N. Bile secretory function of intrahepatic biliary epithelium in the rat. *Am J Physiol*. 1989; 257:G124–G133. [PubMed: 2750903]
6. Alvaro D, Alpini G, Jezequel AM, Bassotti C, Francia C, Fraioli F, Romeo R, Marucci L, Le Sage G, Glaser SS, Benedetti A. Role and mechanisms of action of acetylcholine in the regulation of rat cholangiocyte secretory functions. *J Clin Invest*. 1997; 100:1349–1362. [PubMed: 9294100]
7. Anderson PA, Greenberg RM. Phylogeny of ion channels: Clues to structure and function. *Comp Biochem Physiol B Biochem Mol Biol*. 2001; 129:17–28. [PubMed: 11337248]
8. Andrade V, Guerra M, Jardim C, Melo F, Silva W, Ortega JM, Robert M, Nathanson MH, Leite F. Nucleoplasmic calcium regulates cell proliferation through legumain. *J Hepatol*. 2011; 55:626–635. [PubMed: 21237226]
9. Angelis Campos AC, Rodrigues MA, de Andrade C, de Goes AM, Nathanson MH, Gomes DA. Epidermal growth factor receptors destined for the nucleus are internalized via a clathrin-dependent pathway. *Biochem Biophys Res Commun*. 2011; 412:341–346. [PubMed: 21821003]
10. Ashkenazi A, Dixit VM. Death receptors: Signaling and modulation. *Science*. 1998; 281:1305–1308. [PubMed: 9721089]
11. Badminton MN, Campbell AK, Rembold CM. Differential regulation of nuclear and cytosolic Ca₂₊ in HeLa cells. *J Biol Chem*. 1996; 271:31210–31214. [PubMed: 8940122]
12. Baffy G, Yang LJ, Michalopoulos GK, Williamson JR. Hepatocyte growth-factor induces calcium mobilization and inositol phosphate production in rat hepatocytes. *J Cell Physiol*. 1992; 153:332–339. [PubMed: 1429853]
13. Barreiro FJ, Kobayashi S, Bronk SF, Werneburg NW, Malhi H, Gores GJ. Transcriptional regulation of Bim by FoxO3A mediates hepatocyte lipoapoptosis. *J Biol Chem*. 2007; 282:27141–27154. [PubMed: 17626006]
14. Barritt GJ, Chen J, Rychkov GY. Ca(2+)-permeable channels in the hepatocyte plasma membrane and their roles in hepatocyte physiology. *Biochim Biophys Acta*. 2008; 1783:651–672. [PubMed: 18291110]
15. Barritt GJ, Litjens TL, Castro J, Aromataris E, Rychkov GY. Store-operated Ca₂₊ channels and microdomains of Ca₂₊ in liver cells. *Clin Exp Pharmacol Physiol*. 2009; 36:77–83. [PubMed: 19196257]
16. Bartlett PJ, Young KW, Nahorski SR, Challiss RA. Single cell analysis and temporal profiling of agonist-mediated inositol 1,4,5-trisphosphate, Ca₂₊, diacylglycerol, and protein kinase C signaling using fluorescent biosensors. *J Biol Chem*. 2005; 280:21837–21846. [PubMed: 15788407]
17. Bassik MC, Scorrano L, Oakes SA, Pozzan T, Korsmeyer SJ. Phosphorylation of BCL-2 regulates ER Ca₂₊ homeostasis and apoptosis. *EMBO J*. 2004; 23:1207–1216. [PubMed: 15010700]
18. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest*. 2005; 115:209–218. [PubMed: 15690074]
19. Benali-Furet NL, Chami M, Houel L, De Giorgi F, Vernejoul F, Lagorce D, Buscail L, Bartenschlager R, Ichas F, Rizzuto R, Paterlini-Brechot P. Hepatitis C virus core triggers apoptosis in liver cells by inducing ER stress and ER calcium depletion. *Oncogene*. 2005; 24:4921–4933. [PubMed: 15897896]
20. Bernardi P. Mitochondrial transport of cations: Channels, exchangers, and permeability transition. *Physiol Rev*. 1999; 79:1127–1155. [PubMed: 10508231]

21. Bernardi P, Krauskopf A, Basso E, Petronilli V, Blachly-Dyson E, Di Lisa F, Forte MA. The mitochondrial permeability transition from in vitro artifact to disease target. *FEBS J.* 2006; 273:2077–2099. [PubMed: 16649987]
22. Bernardi P, Rasola A. Calcium and cell death: The mitochondrial connection. *Subcell Biochem.* 2007; 45:481–506. [PubMed: 18193649]
23. Berridge MJ. Inositol trisphosphate and calcium signaling. *Ann N Y Acad Sci.* 1995; 766:31–43. [PubMed: 7486679]
24. Berridge MJ. Elementary and global aspects of calcium signalling. *J Physiol.* 1997; 499 (Pt 2): 291–306. [PubMed: 9080360]
25. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: Dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol.* 2003; 4:517–529. [PubMed: 12838335]
26. Berridge MJ, Galione A. Cytosolic calcium oscillators. *FASEB J.* 1988; 2:3074–3082. [PubMed: 2847949]
27. Berridge MJ, Irvine RF. Inositol phosphates and cell signalling. *Nature.* 1989; 341:197–205. [PubMed: 2550825]
28. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol.* 2000; 1:11–21. [PubMed: 11413485]
29. Beuers U, Bilzer M, Chittattu A, Kullak-Ublick GA, Keppler D, Paumgartner G, Dombrowski F. Tauroursodeoxycholic acid inserts the apical conjugate export pump, Mrp2, into canalicular membranes and stimulates organic anion secretion by protein kinase C-dependent mechanisms in cholestatic rat liver. *Hepatology.* 2001; 33:1206–1216. [PubMed: 11343250]
30. Beuers U, Nathanson MH, Boyer JL. Effects of tauroursodeoxycholic acid on cytosolic Ca²⁺ signals in isolated rat hepatocytes. *Gastroenterology.* 1993; 104:604–612. [PubMed: 8425704]
31. Beuers U, Nathanson MH, Isales CM, Boyer JL. Tauroursodeoxycholic acid stimulates hepatocellular exocytosis and mobilizes extracellular Ca⁺⁺ mechanisms defective in cholestasis. *J Clin Invest.* 1993; 92:2984–2993. [PubMed: 8254052]
32. Bezprozvanny I. The inositol 1,4,5-trisphosphate receptors. *Cell Calcium.* 2005; 38:261–272. [PubMed: 16102823]
33. Bezprozvanny I, Watras J, Ehrlich BE. Bell-shaped calcium-response curves of Ins(1,4,5)P₃- and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature.* 1991; 351:751–754. [PubMed: 1648178]
34. Blackmore PF, Assimacopoulos-Jeannet F, Chan TM, Exton JH. Studies on alpha-adrenergic activation of hepatic glucose output. Insulin inhibition of alpha-adrenergic and glucagon actions in normal and calcium-depleted hepatocytes. *J Biol Chem.* 1979; 254:2828–2834. [PubMed: 218955]
35. Blackmore PF, Strickland WG, Bocckino SB, Exton JH. Mechanism of hepatic glycogen synthase inactivation induced by Ca²⁺-mobilizing hormones. Studies using phospholipase C and phorbol myristate acetate. *Biochem J.* 1986; 237:235–242. [PubMed: 3099747]
36. Boehning D, Patterson RL, Sedaghat L, Glebova NO, Kurosaki T, Snyder SH. Cytochrome c binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis. *Nat Cell Biol.* 2003; 5:1051–1061. [PubMed: 14608362]
37. Boehning D, van Rossum DB, Patterson RL, Snyder SH. A peptide inhibitor of cytochrome c/inositol 1,4,5-trisphosphate receptor binding blocks intrinsic and extrinsic cell death pathways. *Proc Natl Acad Sci U S A.* 2005; 102:1466–1471. [PubMed: 15665074]
38. Boitano S, Dirksen ER, Evans WH. Sequence-specific antibodies to connexins block intercellular calcium signaling through gap junctions. *Cell Calcium.* 1998; 23:1–9. [PubMed: 9570005]
39. Boitano S, Dirksen ER, Sanderson MJ. Intercellular propagation of calcium waves mediated by inositol trisphosphate. *Science.* 1992; 258:292–295. [PubMed: 1411526]
40. Bonner JC. Regulation of PDGF and its receptors in fibrotic diseases. *Cytokine Growth Factor Rev.* 2004; 15:255–273. [PubMed: 15207816]
41. Bootman MD, Berridge MJ, Roderick HL. Calcium signalling: More messengers, more channels, more complexity. *Curr Biol.* 2002; 12:R563–R565. [PubMed: 12194839]
42. Borradaile NM, Han X, Harp JD, Gale SE, Ory DS, Schaffer JE. Disruption of endoplasmic reticulum structure and integrity in lipotoxic cell death. *J Lipid Res.* 2006; 47:2726–2737. [PubMed: 16960261]

43. Bouchard MJ, Puro RJ, Wang L, Schneider RJ. Activation and inhibition of cellular calcium and tyrosine kinase signaling pathways identify targets of the HBx protein involved in hepatitis B virus replication. *J Virol.* 2003; 77:7713–7719. [PubMed: 12829810]
44. Bouchard MJ, Schneider RJ. The enigmatic X gene of hepatitis B virus. *J Virol.* 2004; 78:12725–12734. [PubMed: 15542625]
45. Bouchard MJ, Wang L, Schneider RJ. Activation of focal adhesion kinase by hepatitis B virus HBx protein: Multiple functions in viral replication. *J Virol.* 2006; 80:4406–4414. [PubMed: 16611900]
46. Bouchard MJ, Wang LH, Schneider RJ. Calcium signaling by HBx protein in hepatitis B virus DNA replication. *Science.* 2001; 294:2376–2378. [PubMed: 11743208]
47. Boucherie S, Koukoui O, Nicolas V, Combettes L. Cholestatic bile acids inhibit gap junction permeability in rat hepatocyte couplets and normal rat cholangiocytes. *J Hepatol.* 2005; 42:244–251. [PubMed: 15664251]
48. Bouscarel B, Fromm H, Nussbaum R. Ursodeoxycholate mobilizes intracellular Ca²⁺ and activates phosphorylase a in isolated hepatocytes. *Am J Physiol.* 1993; 264:G243–G251. [PubMed: 8447407]
49. Bradford PG, Maglich JM, Kirkwood KL. IL-1 beta increases type 1 inositol trisphosphate receptor expression and IL-6 secretory capacity in osteoblastic cell cultures. *Mol Cell Biol Res Commun.* 2000; 3:73–75. [PubMed: 10775502]
50. Brailoiu E, Churamani D, Cai X, Schrlau MG, Brailoiu GC, Gao X, Hooper R, Boulware MJ, Dun NJ, Marchant JS, Patel S. Essential requirement for two-pore channel 1 in NAADP-mediated calcium signaling. *J Cell Biol.* 2009; 186:201–209. [PubMed: 19620632]
51. Brini M, Murgia M, Pasti L, Picard D, Pozzan T, Rizzuto R. Nuclear Ca²⁺ concentration measured with specifically targeted recombinant aequorin. *EMBO J.* 1993; 12:4813–4819. [PubMed: 8223490]
52. Bruce JI, Straub SV, Yule DI. Crosstalk between cAMP and Ca²⁺ signaling in non-excitabile cells. *Cell Calcium.* 2003; 34:431–444. [PubMed: 14572802]
53. Bruck R, Nathanson MH, Roelofsen H, Boyer JL. Effects of protein kinase C and cytosolic Ca²⁺ on exocytosis in the isolated perfused rat liver. *Hepatology.* 1994; 20:1032–1040. [PubMed: 7927205]
54. Burdakov D, Petersen OH, Verkhratsky A. Intraluminal calcium as a primary regulator of endoplasmic reticulum function. *Cell Calcium.* 2005; 38:303–310. [PubMed: 16076486]
55. Burgess GM, Bird GS, Obie JF, Putney JW Jr. The mechanism for synergism between phospholipase C- and adenylylcyclase-linked hormones in liver. Cyclic AMP-dependent kinase augments inositol trisphosphate-mediated Ca²⁺ mobilization without increasing the cellular levels of inositol polyphosphates. *J Biol Chem.* 1991; 266:4772–4781. [PubMed: 1848225]
56. Burgstahler AD, Nathanson MH. Coordination of calcium waves among hepatocytes: Teamwork gets the job done. *Hepatology.* 1998; 27:634–635. [PubMed: 9462668]
57. Bustamante JO, Michelette ER, Geibel JP, Dean DA, Hanover JA, McDonnell TJ. Calcium, ATP and nuclear pore channel gating. *Pflugers Arch.* 2000; 439:433–444. [PubMed: 10678739]
58. Calcraft PJ, Ruas M, Pan Z, Cheng X, Arredouani A, Hao X, Tang J, Rietdorf K, Teboul L, Chuang KT, Lin P, Xiao R, Wang C, Zhu Y, Lin Y, Wyatt CN, Parrington J, Ma J, Evans AM, Galione A, Zhu MX. NAADP mobilizes calcium from acidic organelles through two-pore channels. *Nature.* 2009; 459:596–600. [PubMed: 19387438]
59. Camello-Almaraz C, Gomez-Pinilla PJ, Pozo MJ, Camello PJ. Mitochondrial reactive oxygen species and Ca²⁺ signaling. *Am J Physiol Cell Physiol.* 2006; 291:C1082–C1088. [PubMed: 16760264]
60. Cancela JM, Churchill GC, Galione A. Coordination of agonist-induced Ca²⁺-signalling patterns by NAADP in pancreatic acinar cells. *Nature.* 1999; 398:74–76. [PubMed: 10078532]
61. Carafoli E. The fateful encounter of mitochondria with calcium: How did it happen? *Biochim Biophys Acta.* 2010; 1797:595–606. [PubMed: 20385096]
62. Cardenas C, Escobar M, Garcia A, Osorio-Reich M, Hartel S, Foskett JK, Franzini-Armstrong C. Visualization of inositol 1,4,5-trisphosphate receptors on the nuclear envelope outer membrane by freeze-drying and rotary shadowing for electron microscopy. *J Struct Biol.* 2010; 171:372–381. [PubMed: 20457258]

63. Cardone MH, Roy N, Stennicke HR, Salvesen GS, Franke TF, Stanbridge E, Frisch S, Reed JC. Regulation of cell death protease caspase-9 by phosphorylation. *Science*. 1998; 282:1318–1321. [PubMed: 9812896]
64. Carver RS, Stevenson MC, Scheving LA, Russell WE. Diverse expression of ErbB receptor proteins during rat liver development and regeneration. *Gastroenterology*. 2002; 123:2017–2027. [PubMed: 12454858]
65. Cazanave SC, Mott JL, Elmi NA, Bronk SF, Werneburg NW, Akazawa Y, Kahraman A, Garrison SP, Zambetti GP, Charlton MR, Gores GJ. JNK1-dependent PUMA expression contributes to hepatocyte lipoapoptosis. *J Biol Chem*. 2009; 284:26591–26602. [PubMed: 19638343]
66. Chami M, Oules B, Paterlini-Brechot P. Cytobiological consequences of calcium-signaling alterations induced by human viral proteins. *Biochim Biophys Acta*. 2006; 1763:1344–1362. [PubMed: 17059849]
67. Charest R, Blackmore PF, Berthon B, Exton JH. Changes in free cytosolic Ca²⁺ in hepatocytes following alpha 1-adrenergic stimulation. Studies on Quin-2-loaded hepatocytes. *J Biol Chem*. 1983; 258:8769–8773. [PubMed: 6134732]
68. Chen HS, Kaneko S, Girones R, Anderson RW, Hornbuckle WE, Tennant BC, Cote PJ, Gerin JL, Purcell RH, Miller RH. The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks. *J Virol*. 1993; 67:1218–1226. [PubMed: 8437213]
69. Choe CU, Ehrlich BE. The inositol 1,4,5-trisphosphate receptor (IP3R) and its regulators: Sometimes good and sometimes bad teamwork. *Sci STKE*. 2006; (363):re15. [PubMed: 17132820]
70. Choi Y, Gyoo PS, Yoo JH, Jung G. Calcium ions affect the hepatitis B virus core assembly. *Virology*. 2005; 332:454–463. [PubMed: 15661175]
71. Churchill GC, Okada Y, Thomas JM, Genazzani AA, Patel S, Galione A. NAADP mobilizes Ca(2+) from reserve granules, lysosome-related organelles, in sea urchin eggs. *Cell*. 2002; 111:703–708. [PubMed: 12464181]
72. Clapham DE. Calcium signaling. *Cell*. 1995; 80:259–268. [PubMed: 7834745]
73. Clark EA, Brugge JS. Integrins and signal transduction pathways: The road taken. *Science*. 1995; 268:233–239. [PubMed: 7716514]
74. Cocco L, Faenza I, Fiume R, Maria BA, Gilmour RS, Manzoli FA. Phosphoinositide-specific phospholipase C (PI-PLC) beta1 and nuclear lipid-dependent signaling. *Biochim Biophys Acta*. 2006; 1761:509–521. [PubMed: 16624616]
75. Combettes L, Berthon B, Doucet E, Erlinger S, Claret M. Bile acids mobilise internal Ca²⁺ independently of external Ca²⁺ in rat hepatocytes. *Eur J Biochem*. 1990; 190:619–623. [PubMed: 2373086]
76. Combettes L, Dumont M, Berthon B, Erlinger S, Claret M. Release of calcium from the endoplasmic reticulum by bile acids in rat liver cells. *J Biol Chem*. 1988; 263:2299–2303. [PubMed: 3257491]
77. Combettes L, Tran D, Tordjmann T, Laurent M, Berthon B, Claret M. Ca(2+)-mobilizing hormones induce sequentially ordered Ca²⁺ signals in multicellular systems of rat hepatocytes. *Biochem J*. 1994; 304 (Pt 2):585–594. [PubMed: 7998996]
78. Correa PR, Guerra MT, Leite MF, Spray DC, Nathanson MH. Endotoxin unmasks the role of gap junctions in the liver. *Biochem Biophys Res Commun*. 2004; 322:718–726. [PubMed: 15336523]
79. Craske M, Takeo T, Gerasimenko O, Vaillant C, Torok K, Petersen OH, Tepikin AV. Hormone-induced secretory and nuclear translocation of calmodulin: Oscillations of calmodulin concentration with the nucleus as an integrator. *Proc Natl Acad Sci U S A*. 1999; 96:4426–4431. [PubMed: 10200278]
80. Crompton M. The mitochondrial permeability transition pore and its role in cell death. *Biochem J*. 1999; 341 (Pt 2):233–249. [PubMed: 10393078]
81. Crompton M, Kunzi M, Carafoli E. The calcium-induced and sodium-induced effluxes of calcium from heart mitochondria. Evidence for a sodium-calcium carrier. *Eur J Biochem*. 1977; 79:549–558. [PubMed: 923566]

82. Cruise JL, Muga SJ, Lee YS, Michalopoulos GK. Regulation of hepatocyte growth - alpha-1 adrenergic-receptor and Ras P21 changes in liver-regeneration. *J Cell Physiol.* 1989; 140:195–201. [PubMed: 2545731]
83. Cruz LN, Guerra MT, Kruglov E, Mennone A, Garcia CR, Chen J, Nathanson MH. Regulation of multidrug resistance-associated protein 2 by calcium signaling in mouse liver. *Hepatology.* 2010; 52:327–337. [PubMed: 20578149]
84. Csordas G, Renken C, Varnai P, Walter L, Weaver D, Buttle KF, Balla T, Mannella CA, Hajnoczky G. Structural and functional features and significance of the physical linkage between ER and mitochondria. *J Cell Biol.* 2006; 174:915–921. [PubMed: 16982799]
85. Csordas G, Thomas AP, Hajnoczky G. Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. *EMBO J.* 1999; 18:96–108. [PubMed: 9878054]
86. Czochra P, Klopocz B, Meyer E, Herkel J, Garcia-Lazaro JF, Thieringer F, Schirmacher P, Biesterfeld S, Galle PR, Lohse AW, Kanzler S. Liver fibrosis induced by hepatic overexpression of PDGF-B in transgenic mice. *J Hepatol.* 2006; 45:419–428. [PubMed: 16842882]
87. De Koninck P, Schulman H. Sensitivity of CaM kinase II to the frequency of Ca²⁺ oscillations. *Science.* 1998; 279:227–230. [PubMed: 9422695]
88. De Young GW, Keizer J. A single-pool inositol 1,4,5-trisphosphate-receptor-based model for agonist-stimulated oscillations in Ca²⁺ concentration. *Proc Natl Acad Sci U S A.* 1992; 89:9895–9899. [PubMed: 1329108]
89. Dehaye JP, Hughes BP, Blackmore PF, Exton JH. Insulin inhibition of alpha-adrenergic actions in liver. *Biochem J.* 1981; 194:949–956. [PubMed: 7030320]
90. Deisseroth K, Heist EK, Tsien RW. Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. *Nature.* 1998; 392:198–202. [PubMed: 9515967]
91. Deisseroth K, Tsien RW. Dynamic multiphosphorylation passwords for activity-dependent gene expression. *Neuron.* 2002; 34:179–182. [PubMed: 11970860]
92. Dellis O, Dedos SG, Tovey SC, Taufiq UR, Dubel SJ, Taylor CW. Ca²⁺ entry through plasma membrane IP₃ receptors. *Science.* 2006; 313:229–233. [PubMed: 16840702]
93. Deniaud A, Sharaf el dein O, Maillier E, Poncet D, Kroemer G, Lemaire C, Brenner C. Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis. *Oncogene.* 2008; 27:285–299. [PubMed: 17700538]
94. Denson LA, Sturm E, Echevarria W, Zimmerman TL, Makishima M, Mangelsdorf DJ, Karpen SJ. The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. *Gastroenterology.* 2001; 121:140–147. [PubMed: 11438503]
95. Diaz-Munoz M, Canedo-Merino R, Gutierrez-Salinas J, Hernandez-Munoz R. Modifications of intracellular calcium release channels and calcium mobilization following 70% hepatectomy. *Arch Biochem Biophys.* 1998; 349:105–112. [PubMed: 9439588]
96. Dixon CJ, Hall JF, Webb TE, Boarder MR. Regulation of rat hepatocyte function by P2Y receptors: Focus on control of glycogen phosphorylase and cyclic AMP by 2-methylthioadenosine 5'-diphosphate. *J Pharmacol Exp Ther.* 2004; 311:334–341. [PubMed: 15152027]
97. Dixon CJ, White PJ, Hall JF, Kingston S, Boarder MR. Regulation of human hepatocytes by P2Y receptors: Control of glycogen phosphorylase, Ca²⁺, and mitogen-activated protein kinases. *J Pharmacol Exp Ther.* 2005; 313:1305–1313. [PubMed: 15764738]
98. Dixon CJ, Woods NM, Webb TE, Green AK. Evidence that rat hepatocytes co-express functional P2Y1 and P2Y2 receptors. *Br J Pharmacol.* 2000; 129:764–770. [PubMed: 10683201]
99. Dolmetsch RE, Lewis RS, Goodnow CC, Healy JI. Differential activation of transcription factors induced by Ca²⁺ response amplitude and duration. *Nature.* 1997; 386:855–858. [PubMed: 9126747]
100. Dolmetsch RE, Xu K, Lewis RS. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature.* 1998; 392:933–936. [PubMed: 9582075]
101. Dranoff JA, Masyuk AI, Kruglov EA, Larusso NF, Nathanson MH. Polarized expression and function of P2Y ATP receptors in rat bile duct epithelia. *Am J Physiol Gastrointest Liver Physiol.* 2001; 281:G1059–G1067. [PubMed: 11557527]

102. Dranoff JA, Ogawa M, Kruglov EA, Gaca MD, Sevigny J, Robson SC, Wells RG. Expression of P2Y nucleotide receptors and ectonucleotidases in quiescent and activated rat hepatic stellate cells. *Am J Physiol Gastrointest Liver Physiol.* 2004; 287:G417–G424. [PubMed: 14764443]
103. Dufour JF, Luthi M, Forestier M, Magnino F. Expression of inositol 1,4,5-trisphosphate receptor isoforms in rat cirrhosis. *Hepatology.* 1999; 30:1018–1026. [PubMed: 10498655]
104. DuPont G, Combettes L, Leybaert L. Calcium dynamics: Spatio-temporal organization from the subcellular to the organ level. *Int Rev Cytol.* 2007; 261:193–245. [PubMed: 17560283]
105. DuPont G, Swillens S, Clair C, Tordjmann T, Combettes L. Hierarchical organization of calcium signals in hepatocytes: From experiments to models. *Biochim Biophys Acta.* 2000; 1498:134–152. [PubMed: 11108957]
106. Echevarria W, Leite MF, Guerra MT, Zipfel WR, Nathanson MH. Regulation of calcium signals in the nucleus by a nucleoplasmic reticulum. *Nat Cell Biol.* 2003; 5:440–446. [PubMed: 12717445]
107. Eugenin EA, Gonzalez H, Saez CG, Saez JC. Gap junctional communication coordinates vasopressin-induced glycogenolysis in rat hepatocytes. *Am J Physiol.* 1998; 274:G1109–G1116. [PubMed: 9696712]
108. Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. *Nature.* 2001; 411:342–348. [PubMed: 11357141]
109. Exton JH. Mechanisms of hormonal regulation of hepatic glucose metabolism. *Diabetes Metab Rev.* 1987; 3:163–183. [PubMed: 3032541]
110. Failli P, Ruocco C, De Franco R, Caligiuri A, Gentilini A, Giotti A, Gentilini P, Pinzani M. The mitogenic effect of platelet-derived growth factor in human hepatic stellate cells requires calcium influx. *Am J Physiol.* 1995; 269:C1133–C1139. [PubMed: 7491901]
111. Fallon MB, Nathanson MH, Mennone A, Saez JC, Burgstahler AD, Anderson JM. Altered expression and function of hepatocyte gap junctions after common bile duct ligation in the rat. *Am J Physiol.* 1995; 268:C1186–C1194. [PubMed: 7762611]
112. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *Hepatology.* 2006; 43:S45–S53. [PubMed: 16447274]
113. Feng L, Krausfriedmann N. Changes in 1,4,5-inositol trisphosphate binding following partial-hepatectomy. *Biochem Biophys Res Commun.* 1994; 205:291–297. [PubMed: 7999038]
114. Finch EA, Turner TJ, Goldin SM. Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release. *Science.* 1991; 252:443–446. [PubMed: 2017683]
115. Fiorotto R, Spirli C, Fabris L, Cadamuro M, Okolicsanyi L, Strazzabosco M. Ursodeoxycholic acid stimulates cholangiocyte fluid secretion in mice via CFTR-dependent ATP secretion. *Gastroenterology.* 2007; 133:1603–1613. [PubMed: 17983806]
116. Fitz JG, Basavappa S, McGill J, Melhus O, Cohn JA. Regulation of membrane chloride currents in rat bile duct epithelial cells. *J Clin Invest.* 1993; 91:319–328. [PubMed: 7678606]
117. Foskett JK, Roifman CM, Wong D. Activation of calcium oscillations by thapsigargin in parotid acinar cells. *J Biol Chem.* 1991; 266:2778–2782. [PubMed: 1847134]
118. Fox JL, Burgstahler AD, Nathanson MH. Mechanism of long-range Ca²⁺ signalling in the nucleus of isolated rat hepatocytes. *Biochem J.* 1997; 326 (Pt 2):491–495. [PubMed: 9291123]
119. Foyouzi-Youssefi R, Arnaudeau S, Borner C, Kelley WL, Tschopp J, Lew DP, Demaurex N, Krause KH. Bcl-2 decreases the free Ca²⁺ concentration within the endoplasmic reticulum. *Proc Natl Acad Sci U S A.* 2000; 97:5723–5728. [PubMed: 10823933]
120. Francis H, Glaser S, DeMorrow S, Gaudio E, Ueno Y, Venter J, Dostal D, Onori P, Franchitto A, Marzioni M, Vaculin S, Vaculin B, Katki K, Stutes M, Savage J, Alpini G. Small mouse cholangiocytes proliferate in response to H1 histamine receptor stimulation by activation of the IP3/CaMK I/CREB pathway. *Am J Physiol Cell Physiol.* 2008; 295:C499–C513. [PubMed: 18508907]
121. Francis H, Glaser S, Ueno Y, Lesage G, Marucci L, Benedetti A, Taffetani S, Marzioni M, Alvaro D, Venter J, Reichenbach R, Fava G, Phinizy JL, Alpini G. cAMP stimulates the secretory and proliferative capacity of the rat intrahepatic biliary epithelium through changes in the PKA/Src/MEK/ERK1/2 pathway. *J Hepatol.* 2004; 41:528–537. [PubMed: 15464232]

122. Francis HL, DeMorrow S, Franchitto A, Venter JK, Mancinelli RA, White MA, Meng F, Ueno Y, Carpino G, Renzi A, Baker KK, Shine HE, Francis TC, Gaudio E, Alpini GD, Onori P. Histamine stimulates the proliferation of small and large cholangiocytes by activation of both IP(3)/Ca(2+) and cAMP-dependent signaling mechanisms. *Lab Invest.* 2012; 92:282–294. [PubMed: 22064319]
123. Fu S, Yang L, Li P, Hofmann O, Dicker L, Hide W, Lin X, Watkins SM, Ivanov AR, Hotamisligil GS. Aberrant lipid metabolism disrupts calcium homeostasis causing liver endoplasmic reticulum stress in obesity. *Nature.* 2011; 473:528–531. [PubMed: 21532591]
124. Fukuda K, Yamamoto M. Acquisition of resistance to apoptosis and necrosis by Bcl-xL over-expression in rat hepatoma McA-RH8994 cells. *J Gastroenterol Hepatol.* 1999; 14:682–690. [PubMed: 10440213]
125. Furuichi T, Yoshikawa S, Miyawaki A, Wada K, Maeda N, Mikoshiba K. Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P400. *Nature.* 1989; 342:32–38. [PubMed: 2554142]
126. Futatsugi A, Nakamura T, Yamada MK, Ebisui E, Nakamura K, Uchida K, Kitaguchi T, Takahashi-Iwanaga H, Noda T, Aruga J, Mikoshiba K. IP3 receptor types 2 and 3 mediate exocrine secretion underlying energy metabolism. *Science.* 2005; 309:2232–2234. [PubMed: 16195467]
127. Galione A, Churchill GC. Interactions between calcium release pathways: Multiple messengers and multiple stores. *Cell Calcium.* 2002; 32:343–354. [PubMed: 12543094]
128. Galione A, Petersen OH. The NAADP receptor: New receptors or new regulation? *Mol Interv.* 2005; 5:73–79. [PubMed: 15821155]
129. Gaspers LD, Thomas AP. Calcium signaling in liver. *Cell Calcium.* 2005; 38:329–342. [PubMed: 16139354]
130. Gearhart TL, Bouchard MJ. Replication of the hepatitis B virus requires a calcium-dependent HBx-induced G1 phase arrest of hepatocytes. *Virology.* 2010a; 407:14–25. [PubMed: 20719353]
131. Gearhart TL, Bouchard MJ. The hepatitis B virus X protein modulates hepatocyte proliferation pathways to stimulate viral replication. *J Virol.* 2010b; 84:2675–2686. [PubMed: 20053744]
132. Gerasimenko O, Gerasimenko J. New aspects of nuclear calcium signalling. *J Cell Sci.* 2004; 117:3087–3094. [PubMed: 15226390]
133. Gerasimenko OV, Gerasimenko JV, Tepikin AV, Petersen OH. ATP-dependent accumulation and inositol trisphosphate- or cyclic ADP-ribose-mediated release of Ca²⁺ from the nuclear envelope. *Cell.* 1995; 80:439–444. [PubMed: 7859285]
134. Gerasimenko OV, Gerasimenko JV, Tepikin AV, Petersen OH. Calcium transport pathways in the nucleus. *Pflugers Arch.* 1996; 432:1–6. [PubMed: 8662261]
135. Giannini G, Conti A, Mammarella S, Scrobogna M, Sorrentino V. The ryanodine receptor/calcium channel genes are widely and differentially expressed in murine brain and peripheral tissues. *J Cell Biol.* 1995; 128:893–904. [PubMed: 7876312]
136. Gincel D, Zaid H, Shoshan-Barmatz V. Calcium binding and translocation by the voltage-dependent anion channel: A possible regulatory mechanism in mitochondrial function. *Biochem J.* 2001; 358:147–155. [PubMed: 11485562]
137. Gomes DA, Rodrigues MA, Leite MF, Gomez MV, Varnai P, Balla T, Bennett AM, Nathanson MH. c-Met must translocate to the nucleus to initiate calcium signals. *J Biol Chem.* 2008; 283:4344–4351. [PubMed: 18073207]
138. Gonzales E, Julien B, Serriere-Lanneau V, Nicou A, Doignon I, Lagoudakis L, Garcin I, Azoulay D, Duclos-Vallee JC, Castaing D, Samuel D, Hernandez-Garcia A, Awad SS, Combettes L, Thevananther S, Tordjmann T. ATP release after partial hepatectomy regulates liver regeneration in the rat. *J Hepatol.* 2010; 52:54–62. [PubMed: 19914731]
139. Gonzalez HE, Eugenin EA, Garces G, Solis N, Pizarro M, Accatino L, Saez JC. Regulation of hepatic connexins in cholestasis: Possible involvement of Kupffer cells and inflammatory mediators. *Am J Physiol Gastrointest Liver Physiol.* 2002; 282:G991–G1001. [PubMed: 12016124]
140. Grolach A, Klappa P, Kietzmann T. The endoplasmic reticulum: Folding, calcium homeostasis, signaling, and redox control. *Antioxid Redox Signal.* 2006; 8:1391–1418. [PubMed: 16986999]

141. Gradilone SA, Masyuk AI, Splinter PL, Banales JM, Huang BQ, Tietz PS, Masyuk TV, Larusso NF. Cholangiocyte cilia express TRPV4 and detect changes in luminal tonicity inducing bicarbonate secretion. *Proc Natl Acad Sci U S A*. 2007; 104:19138–19143. [PubMed: 18024594]
142. Grimm S, Brdiczka D. The permeability transition pore in cell death. *Apoptosis*. 2007; 12:841–855. [PubMed: 17453156]
143. Groigno L, Whitaker M. An anaphase calcium signal controls chromosome disjunction in early sea urchin embryos. *Cell*. 1998; 92:193–204. [PubMed: 9458044]
144. Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. *Genes Dev*. 1999; 13:1899–1911. [PubMed: 10444588]
145. Guerra MT, Fonseca EA, Melo FM, Andrade VA, Aguiar CJ, Andrade LM, Pinheiro ACN, Casteluber MCF, Resende RR, Pinto MCX, Fernandes SOA, Cardoso VN, Souza-Fagundes EM, Menezes GB, de Paula AM, Nathanson MH, Leite MD. Mitochondrial calcium regulates rat liver regeneration through the modulation of apoptosis. *Hepatology*. 2011; 54:296–306. [PubMed: 21503946]
146. Gunter TE, Gunter KK, Sheu SS, Gavin CE. Mitochondrial calcium transport: Physiological and pathological relevance. *Am J Physiol*. 1994; 267:C313–C339. [PubMed: 8074170]
147. Gunter TE, Yule DI, Gunter KK, Eliseev RA, Salter JD. Calcium and mitochondria. *FEBS Lett*. 2004; 567:96–102. [PubMed: 15165900]
148. Guo YS, Tang J, Chen B, Huang W, Li Y, Cui HY, Zhang X, Wang SJ, Chen ZN, Jiang JL. ssig-h3 regulates store-operated Ca(2+) entry and promotes the invasion of human hepatocellular carcinoma cells. *Cell Biol Int*. 2011; 35:811–817. [PubMed: 21395558]
149. Gwiazda KS, Yang TL, Lin Y, Johnson JD. Effects of palmitate on ER and cytosolic Ca²⁺ homeostasis in beta-cells. *Am J Physiol Endocrinol Metab*. 2009; 296:E690–E701. [PubMed: 19141690]
150. Hagar RE, Burgstahler AD, Nathanson MH, Ehrlich BE. Type III InsP3 receptor channel stays open in the presence of increased calcium. *Nature*. 1998; 396:81–84. [PubMed: 9817204]
151. Hagar RE, Ehrlich BE. Regulation of the type III InsP(3) receptor by InsP(3) and ATP. *Biophys J*. 2000; 79:271–278. [PubMed: 10866953]
152. Hajnoczky G, Csordas G. Calcium signalling: Fishing out molecules of mitochondrial calcium transport. *Curr Biol*. 2010; 20:R888–R891. [PubMed: 20971432]
153. Hajnoczky G, Hager R, Thomas AP. Mitochondria suppress local feedback activation of inositol 1,4, 5-trisphosphate receptors by Ca²⁺ *J Biol Chem*. 1999; 274:14157–14162. [PubMed: 10318833]
154. Hajnoczky G, Robb-Gaspers LD, Seitz MB, Thomas AP. Decoding of cytosolic calcium oscillations in the mitochondria. *Cell*. 1995; 82:415–424. [PubMed: 7634331]
155. Hansen CA, Yang LJ, Williamson JR. Mechanisms of receptor-mediated Ca²⁺ signaling in rat hepatocytes. *J Biol Chem*. 1991; 266:18573–18579. [PubMed: 1655756]
156. Harding HP, Ron D. Endoplasmic reticulum stress and the development of diabetes: A review. *Diabetes*. 2002; 51 (Suppl 3):S455–S461. [PubMed: 12475790]
157. Hardingham GE, Chawla S, Johnson CM, Bading H. Distinct functions of nuclear and cytoplasmic calcium in the control of gene expression. *Nature*. 1997; 385:260–265. [PubMed: 9000075]
158. Hayashi PH, Di Bisceglie AM. The progression of hepatitis B- and C-infections to chronic liver disease and hepatocellular carcinoma: Epidemiology and pathogenesis. *Med Clin North Am*. 2005; 89:371–389. [PubMed: 15656931]
159. Hems DA, Whitton PD. Control of hepatic glycogenolysis. *Physiol Rev*. 1980; 60:1–50. [PubMed: 6243781]
160. Hennager DJ, Welsh MJ, DeLisle S. Changes in either cytosolic or nucleoplasmic inositol 1,4,5-trisphosphate levels can control nuclear Ca²⁺ concentration. *J Biol Chem*. 1995; 270:4959–4962. [PubMed: 7890598]
161. Hernandez E, Leite MF, Guerra MT, Bruna-Romero O, Rodrigues MA, Gomes DA, Giordano FJ, Dranoff JA, Nathanson MH. The spatial distribution of inositol 1,4,5-trisphosphate receptor isoforms shapes Ca²⁺ waves. *J Biol Chem*. 2007; 282:10057–10067. [PubMed: 17284437]

162. Hetz C, Bernasconi P, Fisher J, Lee AH, Bassik MC, Antonsson B, Brandt GS, Iwakoshi NN, Schinzel A, Glimcher LH, Korsmeyer SJ. Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1alpha. *Science*. 2006; 312:572–576. [PubMed: 16645094]
163. Hirata K, Dufour JF, Shibao K, Knickelbein R, O'Neill AF, Bode HP, Cassio D, St Pierre MV, Larusso NF, Leite MF, Nathanson MH. Regulation of Ca²⁺ signaling in rat bile duct epithelia by inositol 1,4,5-trisphosphate receptor isoforms. *Hepatology*. 2002; 36:284–296. [PubMed: 12143036]
164. Hirata K, Nathanson MH. Bile duct epithelia regulate biliary bicarbonate excretion in normal rat liver. *Gastroenterology*. 2001; 121:396–406. [PubMed: 11487549]
165. Hirata K, Pustl T, O'Neill AF, Dranoff JA, Nathanson MH. The type II inositol 1,4,5-trisphosphate receptor can trigger Ca²⁺ waves in rat hepatocytes. *Gastroenterology*. 2002; 122:1088–1100. [PubMed: 11910359]
166. Holzinger F, Schteingart CD, Ton-Nu HT, Eming SA, Monte MJ, Hagey LR, Hofmann AF. Fluorescent bile acid derivatives: Relationship between chemical structure and hepatic and intestinal transport in the rat. *Hepatology*. 1997; 26:1263–1271. [PubMed: 9362371]
167. Hoppe UC. Mitochondrial calcium channels. *FEBS Lett*. 2010; 584:1975–1981. [PubMed: 20388514]
168. Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell*. 2010; 140:900–917. [PubMed: 20303879]
169. Huertabahena J, Villalobosmolina R, Corvera S, Garciasainz JA. Sensitivity of liver-cells formed after partial-hepatectomy to glucagon, vasopressin and angiotensin-II. *Biochim Biophys Acta*. 1983; 763:120–124. [PubMed: 6311282]
170. Huh CG, Factor VM, Sanchez A, Uchida K, Conner EA, Thorgeirsson SS. Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. *Proc Natl Acad Sci U S A*. 2004; 101:4477–4482. [PubMed: 15070743]
171. Humbert JP, Matter N, Artault JC, Koppler P, Malviya AN. Inositol 1,4,5-trisphosphate receptor is located to the inner nuclear membrane vindicating regulation of nuclear calcium signaling by inositol 1,4,5-trisphosphate. Discrete distribution of inositol phosphate receptors to inner and outer nuclear membranes. *J Biol Chem*. 1996; 271:478–485. [PubMed: 8550605]
172. Husain SZ, Prasad P, Grant WM, Kolodecik TR, Nathanson MH, Gorelick FS. The ryanodine receptor mediates early zymogen activation in pancreatitis. *Proc Natl Acad Sci U S A*. 2005; 102:14386–14391. [PubMed: 16186498]
173. Ichas F, Jouaville LS, Mazat JP. Mitochondria are excitable organelles capable of generating and conveying electrical and calcium signals. *Cell*. 1997; 89:1145–1153. [PubMed: 9215636]
174. Ichas F, Jouaville LS, Sidash SS, Mazat JP, Holmuhamedov EL. Mitochondrial calcium spiking: A transduction mechanism based on calcium-induced permeability transition involved in cell calcium signalling. *FEBS Lett*. 1994; 348:211–215. [PubMed: 8034044]
175. Iizuka M, Murata T, Hori M, Ozaki H. Increased contractility of hepatic stellate cells in cirrhosis is mediated by enhanced Ca²⁺-dependent and Ca²⁺-sensitization pathways. *Am J Physiol Gastrointest Liver Physiol*. 2011; 300:G1010–G1021. [PubMed: 21393429]
176. Irvine RF. Nuclear lipid signalling. *Nat Rev Mol Cell Biol*. 2003; 4:349–360. [PubMed: 12728269]
177. Irvine RF. Nuclear inositide signalling – expansion, structures and clarification. *Biochim Biophys Acta*. 2006; 1761:505–508. [PubMed: 16574480]
178. Ishibashi K, Suzuki M, Imai M. Molecular cloning of a novel form (two-repeat) protein related to voltage-gated sodium and calcium channels. *Biochem Biophys Res Commun*. 2000; 270:370–376. [PubMed: 10753632]
179. Jafri MS, Keizer J. Diffusion of inositol 1,4,5-trisphosphate but not Ca²⁺ is necessary for a class of inositol 1,4,5-trisphosphate-induced Ca²⁺ waves. *Proc Natl Acad Sci U S A*. 1994; 91:9485–9489. [PubMed: 7937794]
180. Jiang QX, Thrower EC, Chester DW, Ehrlich BE, Sigworth FJ. Three-dimensional structure of the type 1 inositol 1,4,5-trisphosphate receptor at 2.4 Å resolution. *EMBO J*. 2002; 21:3575–3581. [PubMed: 12110570]

181. Joffre C, Barrow R, Menard L, Calleja V, Hart IR, Kermorgant S. A direct role for Met endocytosis in tumorigenesis. *Nat Cell Biol.* 2011; 13:827–837. [PubMed: 21642981]
182. Jones BF, Boyles RR, Hwang SY, Bird GS, Putney JW. Calcium influx mechanisms underlying calcium oscillations in rat hepatocytes. *Hepatology.* 2008; 48:1273–1281. [PubMed: 18802964]
183. Jouaville LS, Ichas F, Holmuhamedov EL, Camacho P, Lechleiter JD. Synchronization of calcium waves by mitochondrial substrates in *Xenopus laevis* oocytes. *Nature.* 1995; 377:438–441. [PubMed: 7566122]
184. Jungermann K, Katz N. Functional specialization of different hepatocyte populations. *Physiol Rev.* 1989; 69:708–764. [PubMed: 2664826]
185. Kahl CR, Means AR. Regulation of cell cycle progression by calcium/calmodulin-dependent pathways. *Endocr Rev.* 2003; 24:719–736. [PubMed: 14671000]
186. Kanno N, Lesage G, Glaser S, Alpini G. Regulation of cholangiocyte bicarbonate secretion. *Am J Physiol Gastrointest Liver Physiol.* 2001; 281:G612–G625. [PubMed: 11518673]
187. Kasai H, Augustine GJ. Cytosolic Ca²⁺ gradients triggering unidirectional fluid secretion from exocrine pancreas. *Nature.* 1990; 348:735–738. [PubMed: 1701852]
188. Katz A, Wu D, Simon MI. Subunits beta gamma of heterotrimeric G protein activate beta 2 isoform of phospholipase C. *Nature.* 1992; 360:686–689. [PubMed: 1465134]
189. Kawanishi T, Blank LM, Harootunian AT, Smith MT, Tsien RY. Ca²⁺ oscillations induced by hormonal stimulation of individual fura-2-loaded hepatocytes. *J Biol Chem.* 1989; 264:12859–12866. [PubMed: 2473983]
190. Keppens S, De Wulf H. Characterization of the liver P2-purinoceptor involved in the activation of glycogen phosphorylase. *Biochem J.* 1986; 240:367–371. [PubMed: 3814090]
191. Khan MT, Wagner L, Yule DI, Bhanumathy C, Joseph SK. Akt kinase phosphorylation of inositol 1,4,5-trisphosphate receptors. *J Biol Chem.* 2006; 281:3731–3737. [PubMed: 16332683]
192. Khoo KM, Han MK, Park JB, Chae SW, Kim UH, Lee HC, Bay BH, Chang CF. Localization of the cyclic ADP-ribose-dependent calcium signaling pathway in hepatocyte nucleus. *J Biol Chem.* 2000; 275:24807–24817. [PubMed: 10818108]
193. Kim HR, Lee GH, Ha KC, Ahn T, Moon JY, Lee BJ, Cho SG, Kim S, Seo YR, Shin YJ, Chae SW, Reed JC, Chae HJ. Bax Inhibitor-1 Is a pH-dependent regulator of Ca²⁺ channel activity in the endoplasmic reticulum. *J Biol Chem.* 2008; 283:15946–15955. [PubMed: 18378668]
194. Kim SY, Cho BH, Kim UH. CD38-mediated Ca²⁺ signaling contributes to angiotensin II-induced activation of hepatic stellate cells: Attenuation of hepatic fibrosis by CD38 ablation. *J Biol Chem.* 2010; 285:576–582. [PubMed: 19910464]
195. Kinnally KW, Peixoto PM, Ryu SY, Dejean LM. Is mPTP the gatekeeper for necrosis, apoptosis, or both? *Biochim Biophys Acta.* 2011; 1813:616–622. [PubMed: 20888866]
196. Kitamura T, Brauneis U, Gatmaitan Z, Arias IM. Extracellular ATP, intracellular calcium and canalicular contraction in rat hepatocyte doublets. *Hepatology.* 1991; 14:640–647. [PubMed: 1916664]
197. Kitamura T, Watanabe S, Ikejima KI, Hirose M, Miyazaki A, Yumoto A, Suzuki S, Yamada T, Kitami N, Sato N. Different features of Ca²⁺ oscillations in differentiated and undifferentiated hepatocyte doublets. *Hepatology.* 1995; 21:1395–1404. [PubMed: 7737647]
198. Kojima N, Hori M, Murata T, Morizane Y, Ozaki H. Different profiles of Ca²⁺ responses to endothelin-1 and PDGF in liver myofibroblasts during the process of cell differentiation. *Br J Pharmacol.* 2007; 151:816–827. [PubMed: 17533428]
199. Kraskiewicz H, FitzGerald U. InterfERing with endoplasmic reticulum stress. *Trends Pharmacol Sci.* 2012; 33:53–63. [PubMed: 22112465]
200. Krebs EG. The Albert Lasker Medical Awards. Role of the cyclic AMP-dependent protein kinase in signal transduction. *JAMA.* 1989; 262:1815–1818. [PubMed: 2550680]
201. Kruglov EA, Correa PR, Arora G, Yu J, Nathanson MH, Dranoff JA. Molecular basis for calcium signaling in hepatic stellate cells. *Am J Physiol Gastrointest Liver Physiol.* 2007; 292:G975–G982. [PubMed: 17204544]
202. Kruglov EA, Gautam S, Guerra MT, Nathanson MH. Type 2 inositol 1,4,5-trisphosphate receptor modulates bile salt export pump activity in rat hepatocytes. *Hepatology.* 2011; 54:1790–1799. [PubMed: 21748767]

203. Kullak-Ublick GA, Meier PJ. Mechanisms of cholestasis. *Clin Liver Dis.* 2000; 4:357–385. [PubMed: 11232196]
204. Kumar NM, Gilula NB. Cloning and characterization of human and rat liver cDNAs coding for a gap junction protein. *J Cell Biol.* 1986; 103:767–776. [PubMed: 2875078]
205. Kupzig S, Deaconescu D, Bouyoucef D, Walker SA, Liu Q, Polte CL, Daumke O, Ishizaki T, Lockyer PJ, Wittinghofer A, Cullen PJ. GAP1 family members constitute bifunctional Ras and Rap GTPase-activating proteins. *J Biol Chem.* 2006; 281:9891–9900. [PubMed: 16431904]
206. Lagoudakis L, Garcin I, Julien B, Nahum K, Gomes DA, Combettes L, Nathanson MH, Tordjmann T. Cytosolic calcium regulates liver regeneration in the rat. *Hepatology.* 2010; 52:602–611. [PubMed: 20683958]
207. Lanini L, Bachs O, Carafoli E. The calcium pump of the liver nuclear membrane is identical to that of endoplasmic reticulum. *J Biol Chem.* 1992; 267:11548–11552. [PubMed: 1317870]
208. Lazaridis KN, Strazzabosco M, Larusso NF. The cholangiopathies: Disorders of biliary epithelia. *Gastroenterology.* 2004; 127:1565–1577. [PubMed: 15521023]
209. Lee HC, Aarhus R. A derivative of NADP mobilizes calcium stores insensitive to inositol trisphosphate and cyclic ADP-ribose. *J Biol Chem.* 1995; 270:2152–2157. [PubMed: 7836444]
210. Lei K, Davis RJ. JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. *Proc Natl Acad Sci U S A.* 2003; 100:2432–2437. [PubMed: 12591950]
211. Leite FM.; Guerra, MT.; Nathanson, MH. Ca²⁺ Signaling in the Liver. In: Arias, I.; Wolkoff, A.; Boyer, J.; Shafritz, D.; Fausto, N.; Alter, H.; Cohen, D., editors. *The Liver: Biology and Pathobiology.* John Wiley & Sons, Ltd; 2009. p. 485-510.
212. Leite MF, Burgstahler AD, Nathanson MH. Ca²⁺ waves require sequential activation of inositol trisphosphate receptors and ryanodine receptors in pancreatic acini. *Gastroenterology.* 2002; 122:415–427. [PubMed: 11832456]
213. Leite MF, Dranoff JA, Gao L, Nathanson MH. Expression and subcellular localization of the ryanodine receptor in rat pancreatic acinar cells. *Biochem J.* 1999; 337 (Pt 2):305–309. [PubMed: 9882629]
214. Leite MF, Hirata K, Puhl T, Burgstahler AD, Okazaki K, Ortega JM, Goes AM, Prado MA, Spray DC, Nathanson MH. Molecular basis for pacemaker cells in epithelia. *J Biol Chem.* 2002; 277:16313–16323. [PubMed: 11850419]
215. Leite MF, Thrower EC, Echevarria W, Koulen P, Hirata K, Bennett AM, Ehrlich BE, Nathanson MH. Nuclear and cytosolic calcium are regulated independently. *Proc Natl Acad Sci U S A.* 2003; 100:2975–2980. [PubMed: 12606721]
216. Lemasters JJ, Theruvath TP, Zhong Z, Nieminen AL. Mitochondrial calcium and the permeability transition in cell death. *Biochim Biophys Acta.* 2009; 1787:1395–1401. [PubMed: 19576166]
217. Lesage G, Glaser S, Ueno Y, Alvaro D, Baiocchi L, Kanno N, Phinizy JL, Francis H, Alpini G. Regression of cholangiocyte proliferation after cessation of ANIT feeding is coupled with increased apoptosis. *Am J Physiol Gastrointest Liver Physiol.* 2001; 281:G182–G190. [PubMed: 11408271]
218. LeSage GD, Alvaro D, Glaser S, Francis H, Marucci L, Roskams T, Phinizy JL, Marzioni M, Benedetti A, Taffetani S, Barbaro B, Fava G, Ueno Y, Alpini G. Alpha-1 adrenergic receptor agonists modulate ductal secretion of BDL rats via Ca²⁺- and PKC-dependent stimulation of cAMP. *Hepatology.* 2004; 40:1116–1127. [PubMed: 15486932]
219. LeSage GD, Glaser SS, Marucci L, Benedetti A, Phinizy JL, Rodgers R, Caligiuri A, Papa E, Tretjak Z, Jezequel AM, Holcomb LA, Alpini G. Acute carbon tetrachloride feeding induces damage of large but not small cholangiocytes from BDL rat liver. *Am J Physiol.* 1999; 276:G1289–G1301. [PubMed: 10330021]
220. Lev S, Moreno H, Martinez R, Canoll P, Peles E, Musacchio JM, Plowman GD, Rudy B, Schlessinger J. Protein tyrosine kinase PYK2 involved in Ca²⁺-induced regulation of ion channel and MAP kinase functions. *Nature.* 1995; 376:737–745. [PubMed: 7544443]
221. Li C, Fox CJ, Master SR, Bindokas VP, Chodosh LA, Thompson CB. Bcl-X(L) affects Ca²⁺ homeostasis by altering expression of inositol 1,4,5-trisphosphate receptors. *Proc Natl Acad Sci U S A.* 2002; 99:9830–9835. [PubMed: 12118121]

222. Li W, Llopis J, Whitney M, Zlokarnik G, Tsien RY. Cell-permeant caged InsP3 ester shows that Ca²⁺ spike frequency can optimize gene expression. *Nature*. 1998; 392:936–941. [PubMed: 9582076]
223. Lin C, Hajnoczky G, Thomas AP. Propagation of cytosolic calcium waves into the nuclei of hepatocytes. *Cell Calcium*. 1994; 16:247–258. [PubMed: 7820844]
224. Lin-Moshier Y, Walseth TF, Churamani D, Davidson SM, Slama JT, Hooper R, Brailoiu E, Patel S, Marchant JS. Photoaffinity labeling of nicotinic acid adenine dinucleotide phosphate (NAADP) targets in mammalian cells. *J Biol Chem*. 2012; 287:2296–2307. [PubMed: 22117075]
225. Lipp P, Thomas D, Berridge MJ, Bootman MD. Nuclear calcium signalling by individual cytoplasmic calcium puffs. *EMBO J*. 1997; 16:7166–7173. [PubMed: 9384593]
226. Listenberger LL, Han X, Lewis SE, Cases S, Farese RV Jr, Ory DS, Schaffer JE. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc Natl Acad Sci U S A*. 2003; 100:3077–3082. [PubMed: 12629214]
227. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: Requirement for dATP and cytochrome c. *Cell*. 1996; 86:147–157. [PubMed: 8689682]
228. Llovet JM, Beaugrand M. Hepatocellular carcinoma: Present status and future prospects. *J Hepatol*. 2003; 38:S136–S149. [PubMed: 12591191]
229. Lowe PJ, Miyai K, Steinbach JH, Hardison WG. Hormonal regulation of hepatocyte tight junctional permeability. *Am J Physiol*. 1988; 255:G454–G461. [PubMed: 2845804]
230. Lui PP, Kong SK, Fung KP, Lee CY. The rise of nuclear and cytosolic Ca²⁺ can be uncoupled in HeLa cells. *Pflugers Arch*. 1998; 436:371–376. [PubMed: 9644218]
231. Luo X, Budihardjo I, Zou H, Slaughter C, Wang X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell*. 1998; 94:481–490. [PubMed: 9727491]
232. Magnino F, Luthi M, Schmidt K, Dufour JF. The calcium signaling machinery during hepatic regeneration. *J Hepatol*. 2000; 32:78. [PubMed: 10673070]
233. Mak DO, Foskett JK. Single-channel inositol 1,4,5-trisphosphate receptor currents revealed by patch clamp of isolated *Xenopus* oocyte nuclei. *J Biol Chem*. 1994; 269:29375–29378. [PubMed: 7961913]
234. Malhas A, Goulbourne C, Vaux DJ. The nucleoplasmic reticulum: Form and function. *Trends Cell Biol*. 2011; 21:362–373. [PubMed: 21514163]
235. Malhi H, Bronk SF, Werneburg NW, Gores GJ. Free fatty acids induce JNK-dependent hepatocyte lipoapoptosis. *J Biol Chem*. 2006; 281:12093–12101. [PubMed: 16505490]
236. Malhi H, Kaufman RJ. Endoplasmic reticulum stress in liver disease. *J Hepatol*. 2011; 54:795–809. [PubMed: 21145844]
237. Malli R, Graier WF. Mitochondrial Ca²⁺ channels: Great unknowns with important functions. *FEBS Lett*. 2010; 584:1942–1947. [PubMed: 20074570]
238. Malviya AN, Klein C. Mechanism regulating nuclear calcium signaling. *Can J Physiol Pharmacol*. 2006; 84:403–422. [PubMed: 16902586]
239. Mancinelli R, Franchitto A, Gaudio E, Onori P, Glaser S, Francis H, Venter J, DeMorrow S, Carpino G, Kopriva S, White M, Fava G, Alvaro D, Alpini G. After damage of large bile ducts by gamma-aminobutyric acid, small ducts replenish the biliary tree by amplification of calcium-dependent signaling and de novo acquisition of large cholangiocyte phenotypes. *Am J Pathol*. 2010; 176:1790–1800. [PubMed: 20185575]
240. Maranto AR. Primary structure, ligand binding, and localization of the human type 3 inositol 1,4,5-trisphosphate receptor expressed in intestinal epithelium. *J Biol Chem*. 1994; 269:1222–1230. [PubMed: 8288584]
241. Marchenko SM, Yarotsky VV, Kovalenko TN, Kostyuk PG, Thomas RC. Spontaneously active and InsP3-activated ion channels in cell nuclei from rat cerebellar Purkinje and granule neurones. *J Physiol*. 2005; 565:897–910. [PubMed: 15774532]
242. Marciniak SJ, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, Nagata K, Harding HP, Ron D. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev*. 2004; 18:3066–3077. [PubMed: 15601821]

243. Martinou JC, Youle RJ. Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. *Dev Cell*. 2011; 21:92–101. [PubMed: 21763611]
244. Marzioni M, Francis H, Benedetti A, Ueno Y, Fava G, Venter J, Reichenbach R, Mancino MG, Summers R, Alpini G, Glaser S. Ca²⁺-dependent cytoprotective effects of ursodeoxycholic and tau-roursodeoxycholic acid on the biliary epithelium in a rat model of cholestasis and loss of bile ducts. *Am J Pathol*. 2006; 168:398–409. [PubMed: 16436655]
245. Masyuk AI, Masyuk TV, Splinter PL, Huang BQ, Stroope AJ, Larusso NF. Cholangiocyte cilia detect changes in luminal fluid flow and transmit them into intracellular Ca²⁺ and cAMP signaling. *Gastroenterology*. 2006; 131:911–920. [PubMed: 16952559]
246. Matsu-ura T, Michikawa T, Inoue T, Miyawaki A, Yoshida M, Mikoshiba K. Cytosolic inositol 1,4,5-trisphosphate dynamics during intracellular calcium oscillations in living cells. *J Cell Biol*. 2006; 173:755–765. [PubMed: 16754959]
247. Matsumoto K, Nakamura T. Emerging multipotent aspects of hepatocyte growth factor. *J Biochem*. 1996; 119:591–600. [PubMed: 8743556]
248. Matsumoto M, Nakagawa T, Inoue T, Nagata E, Tanaka K, Takano H, Minowa O, Kuno J, Sakakibara S, Yamada M, Yoneshima H, Miyawaki A, Fukuuchi Y, Furuichi T, Okano H, Mikoshiba K, Noda T. Ataxia and epileptic seizures in mice lacking type 1 inositol 1,4,5-trisphosphate receptor. *Nature*. 1996; 379:168–171. [PubMed: 8538767]
249. Mauger JP. Role of the nuclear envelope in calcium signalling. *Biol Cell*. 2012; 104:70–83. [PubMed: 22188206]
250. McGill JM, Basavappa S, Mangel AW, Shimokura GH, Middleton JP, Fitz JG. Adenosine triphosphate activates ion permeabilities in biliary epithelial cells. *Gastroenterology*. 1994; 107:236–243. [PubMed: 8020667]
251. McPherson PS, Campbell KP. The ryanodine receptor/Ca²⁺ release channel. *J Biol Chem*. 1993; 268:13765–13768. [PubMed: 8390976]
252. Meldolesi J, Pozzan T. The endoplasmic reticulum Ca²⁺ store: A view from the lumen. *Trends Biochem Sci*. 1998; 23:10–14. [PubMed: 9478128]
253. Meszaros LG, Bak J, Chu A. Cyclic ADP-ribose as an endogenous regulator of the non-skeletal type ryanodine receptor Ca²⁺ channel. *Nature*. 1993; 364:76–79. [PubMed: 8391127]
254. Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol Cell*. 2000; 6:87–97. [PubMed: 10949030]
255. Michalopoulos GK, DeFrances MC. Liver regeneration. *Science*. 1997; 276:60–66. [PubMed: 9082986]
256. Michikawa T, Hirota J, Kawano S, Hiraoka M, Yamada M, Furuichi T, Mikoshiba K. Calmodulin mediates calcium-dependent inactivation of the cerebellar type 1 inositol 1,4,5-trisphosphate receptor. *Neuron*. 1999; 23:799–808. [PubMed: 10482245]
257. Mikoshiba K. The InsP₃ receptor and intracellular Ca²⁺ signaling. *Curr Opin Neurobiol*. 1997; 7:339–345. [PubMed: 9232803]
258. Minagawa N, Kruglov EA, Dranoff JA, Robert ME, Gores GJ, Nathanson MH. The anti-apoptotic protein Mcl-1 inhibits mitochondrial Ca²⁺ signals. *J Biol Chem*. 2005; 280:33637–33644. [PubMed: 16027162]
259. Minagawa N, Nagata J, Shibao K, Masyuk AI, Gomes DA, Rodrigues MA, Lesage G, Akiba Y, Kaunitz JD, Ehrlich BE, Larusso NF, Nathanson MH. Cyclic AMP regulates bicarbonate secretion in cholangiocytes through release of ATP into bile. *Gastroenterology*. 2007; 133:1592–1602. [PubMed: 17916355]
260. Mine T, Kojima I, Ogata E, Nakamura T. Comparison of effects of Hgf and Egf on cellular calcium in rat hepatocytes. *Biochem Biophys Res Commun*. 1991; 181:1173–1180. [PubMed: 1837217]
261. Miyakawa T, Maeda A, Yamazawa T, Hirose K, Kurosaki T, Iino M. Encoding of Ca²⁺ signals by differential expression of IP₃ receptor subtypes. *EMBO J*. 1999; 18:1303–1308. [PubMed: 10064596]

262. Moseley RH, Wang W, Takeda H, Lown K, Shick L, Ananthanarayanan M, Suchy FJ. Effect of endotoxin on bile acid transport in rat liver: A potential model for sepsis-associated cholestasis. *Am J Physiol.* 1996; 271:G137–G146. [PubMed: 8760117]
263. Nagata J, Guerra MT, Shugrue CA, Gomes DA, Nagata N, Nathanson MH. Lipid rafts establish calcium waves in hepatocytes. *Gastroenterology.* 2007; 133:256–267. [PubMed: 17631147]
264. Nakamura M, Nagano H, Sakon M, Yamamoto T, Ota H, Wada H, Damdinsuren B, Noda T, Marubashi S, Miyamoto A, Takeda Y, Umeshita K, Nakamori S, Dono K, Monden M. Role of the Fas/FasL pathway in combination therapy with interferon-alpha and fluorouracil against hepatocellular carcinoma in vitro. *J Hepatol.* 2007; 46:77–88. [PubMed: 17045692]
265. Nathanson MH, Boyer JL. Mechanisms and regulation of bile secretion. *Hepatology.* 1991; 14:551–566. [PubMed: 1874500]
266. Nathanson MH, Burgstahler AD. Coordination of hormone-induced calcium signals in isolated rat hepatocyte couplets: Demonstration with confocal microscopy. *Mol Biol Cell.* 1992; 3:113–121. [PubMed: 1550953]
267. Nathanson MH, Burgstahler AD, Fallon MB. Multistep mechanism of polarized Ca²⁺ wave patterns in hepatocytes. *Am J Physiol.* 1994; 267:G338–G349. [PubMed: 7943230]
268. Nathanson MH, Burgstahler AD, Masyuk A, Larusso NF. Stimulation of ATP secretion in the liver by therapeutic bile acids. *Biochem J.* 2001; 358:1–5. [PubMed: 11485545]
269. Nathanson MH, Burgstahler AD, Mennone A, Boyer JL. Characterization of cytosolic Ca²⁺ signaling in rat bile duct epithelia. *Am J Physiol.* 1996; 271:G86–G96. [PubMed: 8760111]
270. Nathanson MH, Burgstahler AD, Mennone A, Dranoff JA, Rios-Velez L. Stimulation of bile duct epithelial secretion by glybenclamide in normal and cholestatic rat liver. *J Clin Invest.* 1998; 101:2665–2676. [PubMed: 9637700]
271. Nathanson MH, Burgstahler AD, Mennone A, Fallon MB, Gonzalez CB, Saez JC. Ca²⁺ waves are organized among hepatocytes in the intact organ. *Am J Physiol.* 1995; 269:G167–G171. [PubMed: 7631796]
272. Nathanson MH, Fallon MB, Padfield PJ, Maranto AR. Localization of the type 3 inositol 1,4,5-trisphosphate receptor in the Ca²⁺ wave trigger zone of pancreatic acinar cells. *J Biol Chem.* 1994; 269:4693–4696. [PubMed: 7508924]
273. Nathanson MH, Gautam A, Bruck R, Isaacs CM, Boyer JL. Effects of Ca²⁺ agonists on cytosolic Ca²⁺ in isolated hepatocytes and on bile secretion in the isolated perfused rat liver. *Hepatology.* 1992; 15:107–116. [PubMed: 1727785]
274. Nathanson MH, Gautam A, Ng OC, Bruck R, Boyer JL. Hormonal regulation of paracellular permeability in isolated rat hepatocyte couplets. *Am J Physiol.* 1992; 262:G1079–G1086. [PubMed: 1616038]
275. Nathanson MH, Padfield PJ, O'Sullivan AJ, Burgstahler AD, Jamieson JD. Mechanism of Ca²⁺ wave propagation in pancreatic acinar cells. *J Biol Chem.* 1992; 267:18118–18121. [PubMed: 1517244]
276. Nazareth W, Yafei N, Crompton M. Inhibition of anoxia-induced injury in heart myocytes by cyclosporin A. *J Mol Cell Cardiol.* 1991; 23:1351–1354. [PubMed: 1811053]
277. Newton CL, Mignery GA, Sudhof TC. Co-expression in vertebrate tissues and cell lines of multiple inositol 1,4,5-trisphosphate (InsP₃) receptors with distinct affinities for InsP₃. *J Biol Chem.* 1994; 269:28613–28619. [PubMed: 7961809]
278. Nicolli A, Basso E, Petronilli V, Wenger RM, Bernardi P. Interactions of cyclophilin with the mitochondrial inner membrane and regulation of the permeability transition pore, and cyclosporin A-sensitive channel. *J Biol Chem.* 1996; 271:2185–2192. [PubMed: 8567677]
279. Nicotera P, McConkey DJ, Jones DP, Orrenius S. ATP stimulates Ca²⁺ uptake and increases the free Ca²⁺ concentration in isolated rat liver nuclei. *Proc Natl Acad Sci U S A.* 1989; 86:453–457. [PubMed: 2911591]
280. Nicou A, Serriere V, Hilly M, Prigent S, Combettes L, Guillon G, Tordjmann T. Remodelling of calcium signalling during liver regeneration in the rat. *J Hepatol.* 2007; 46:247–256. [PubMed: 17125880]

281. Nicou A, Serriere V, Prigent S, Boucherie S, Combettes L, Guillon G, Alonso G, Tordjmann T. Hypothalamic vasopressin release and hepatocyte Ca²⁺ signaling during liver regeneration: An interplay stimulating liver growth and bile flow. *Hepatology*. 2003; 38:564A–565A.
282. Niessen H, Willecke K. Strongly decreased gap junctional permeability to inositol 1,4, 5-trisphosphate in connexin32 deficient hepatocytes. *FEBS Lett*. 2000; 466:112–114. [PubMed: 10648823]
283. Nutt LK, Chandra J, Pataer A, Fang B, Roth JA, Swisher SG, O'Neil RG, McConkey DJ. Bax-mediated Ca²⁺ mobilization promotes cytochrome c release during apoptosis. *J Biol Chem*. 2002; 277:20301–20308. [PubMed: 11909872]
284. Nutt LK, Pataer A, Pahler J, Fang B, Roth J, McConkey DJ, Swisher SG. Bax and Bak promote apoptosis by modulating endoplasmic reticular and mitochondrial Ca²⁺ stores. *J Biol Chem*. 2002; 277:9219–9225. [PubMed: 11741880]
285. O'Brien EM, Gomes DA, Sehgal S, Nathanson MH. Hormonal regulation of nuclear permeability. *J Biol Chem*. 2007; 282:4210–4217. [PubMed: 17158097]
286. Oakes SA, Opferman JT, Pozzan T, Korsmeyer SJ, Scorrano L. Regulation of endoplasmic reticulum Ca²⁺ dynamics by proapoptotic BCL-2 family members. *Biochem Pharmacol*. 2003; 66:1335–1340. [PubMed: 14555206]
287. Oakes SA, Scorrano L, Opferman JT, Bassik MC, Nishino M, Pozzan T, Korsmeyer SJ. Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum. *Proc Natl Acad Sci U S A*. 2005; 102:105–110. [PubMed: 15613488]
288. Oh JC, Jeong DL, Kim IK, Oh SH. Activation of calcium signaling by hepatitis B virus-X protein in liver cells. *Exp Mol Med*. 2003; 35:301–309. [PubMed: 14508071]
289. Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, Weinman SA. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology*. 2002; 122:366–375. [PubMed: 11832451]
290. Oshio C, Phillips MJ. Contractility of bile canaliculi: Implications for liver function. *Science*. 1981; 212:1041–1042. [PubMed: 7015506]
291. Panasiuk A, Dzieciol J, Panasiuk B, Prokopowicz D. Expression of p53, Bax and Bcl-2 proteins in hepatocytes in non-alcoholic fatty liver disease. *World J Gastroenterol*. 2006; 12:6198–6202. [PubMed: 17036395]
292. Park KS, Sin PJ, Lee DH, Cha SK, Kim MJ, Kim NH, Baik SK, Jeong SW, Kong ID. Switching-on of serotonergic calcium signaling in activated hepatic stellate cells. *World J Gastroenterol*. 2011; 17:164–173. [PubMed: 21245988]
293. Park SW, Zhou Y, Lee J, Lee J, Ozcan U. Sarco(endo)plasmic reticulum Ca²⁺-ATPase 2b is a major regulator of endoplasmic reticulum stress and glucose homeostasis in obesity. *Proc Natl Acad Sci U S A*. 2010; 107:19320–19325. [PubMed: 20974941]
294. Patel S, Churchill GC, Galione A. Coordination of Ca²⁺ signalling by NAADP. *Trends Biochem Sci*. 2001; 26:482–489. [PubMed: 11504624]
295. Patt YZ, Hassan MM, Lozano RD, Brown TD, Vauthey JN, Curley SA, Ellis LM. Phase II trial of systemic continuous fluorouracil and subcutaneous recombinant interferon Alfa-2b for treatment of hepatocellular carcinoma. *J Clin Oncol*. 2003; 21:421–427. [PubMed: 12560429]
296. Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: Mechanisms of action and therapeutic use revisited. *Hepatology*. 2002; 36:525–531. [PubMed: 12198643]
297. Peach MJ. Molecular actions of angiotensin. *Biochem Pharmacol*. 1981; 30:2745–2751. [PubMed: 6274350]
298. Peng TI, Jou MJ. Oxidative stress caused by mitochondrial calcium overload. *Ann N Y Acad Sci*. 2010; 1201:183–188. [PubMed: 20649555]
299. Petronilli V, Cola C, Massari S, Colonna R, Bernardi P. Physiological effectors modify voltage sensing by the cyclosporin A-sensitive permeability transition pore of mitochondria. *J Biol Chem*. 1993; 268:21939–21945. [PubMed: 8408050]
300. Petrosillo G, Ruggiero FM, Pistolese M, Paradies G. Ca²⁺-induced reactive oxygen species production promotes cytochrome c release from rat liver mitochondria via mitochondrial

- permeability transition (MPT)-dependent and MPT-independent mechanisms: Role of cardiolipin. *J Biol Chem.* 2004; 279:53103–53108. [PubMed: 15475362]
301. Phillips MJ, Poucell S, Oda M. Mechanisms of cholestasis. *Lab Invest.* 1986; 54:593–608. [PubMed: 2423777]
 302. Piccoli C, Scrima R, Quarato G, D'Aprile A, Ripoli M, Lecce L, Boffoli D, Moradpour D, Capitanio N. Hepatitis C virus protein expression causes calcium-mediated mitochondrial bioenergetic dysfunction and nitro-oxidative stress. *Hepatology.* 2007; 46:58–65. [PubMed: 17567832]
 303. Pierobon N, Renard-Rooney DC, Gaspers LD, Thomas AP. Ryanodine receptors in liver. *J Biol Chem.* 2006; 281:34086–34095. [PubMed: 16973607]
 304. Pinton P, Tsuboi T, Ainscow EK, Pozzan T, Rizzuto R, Rutter GA. Dynamics of glucose-induced membrane recruitment of protein kinase C beta II in living pancreatic islet beta-cells. *J Biol Chem.* 2002; 277:37702–37710. [PubMed: 12149258]
 305. Pinzani M, Marra F. Cytokine receptors and signaling in hepatic stellate cells. *Semin Liver Dis.* 2001; 21:397–416. [PubMed: 11586468]
 306. Pitt SJ, Funnell TM, Sitsapesan M, Venturi E, Rietdorf K, Ruas M, Ganesan A, Gosain R, Churchill GC, Zhu MX, Parrington J, Galione A, Sitsapesan R. TPC2 is a novel NAADP-sensitive Ca²⁺ release channel, operating as a dual sensor of luminal pH and Ca²⁺. *J Biol Chem.* 2010; 285:35039–35046. [PubMed: 20720007]
 307. Plevin R, Palmer S, Gardner SD, Wakelam MJ. Regulation of bombesin-stimulated inositol 1,4,5-trisphosphate generation in Swiss 3T3 fibroblasts by a guanine-nucleotide-binding protein. *Biochem J.* 1990; 268:605–610. [PubMed: 2114096]
 308. Poenie M, Alderton J, Steinhardt R, Tsien R. Calcium rises abruptly and briefly throughout the cell at the onset of anaphase. *Science.* 1986; 233:886–889. [PubMed: 3755550]
 309. Poupon RE, Poupon R, Balkau B. Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. *N Engl J Med.* 1994; 330:1342–1347. [PubMed: 8152446]
 310. Pozzan T, Rizzuto R. High tide of calcium in mitochondria. *Nat Cell Biol.* 2000; 2:E25–E27. [PubMed: 10655598]
 311. Pralong WF, Spat A, Wollheim CB. Dynamic pacing of cell metabolism by intracellular Ca²⁺ transients. *J Biol Chem.* 1994; 269:27310–27314. [PubMed: 7961642]
 312. Pusl T, Nathanson MH. The role of inositol 1,4,5-trisphosphate receptors in the regulation of bile secretion in health and disease. *Biochem Biophys Res Commun.* 2004; 322:1318–1325. [PubMed: 15336978]
 313. Pusl T, Rhode F, Ott T, Drabent B, Willecke K, Beuers U. Gap junctional intercellular communication is not needed for the anticholestatic effect of tauroursodeoxycholic acid in mouse liver. *J Hepatol.* 2005; 42:604–605. [PubMed: 15763348]
 314. Pusl T, Wu JJ, Zimmerman TL, Zhang L, Ehrlich BE, Berchtold MW, Hoek JB, Karpen SJ, Nathanson MH, Bennett AM. Epidermal growth factor-mediated activation of the ETS domain transcription factor Elk-1 requires nuclear calcium. *J Biol Chem.* 2002; 277:27517–27527. [PubMed: 11971908]
 315. Qin Y, Pan X, Tang TT, Zhou L, Gong XG. Anti-proliferative effects of the novel squamosamide derivative (FLZ) on HepG2 human hepatoma cells by regulating the cell cycle-related proteins are associated with decreased Ca(2+)/ROS levels. *Chem Biol Interact.* 2011; 193:246–253. [PubMed: 21835169]
 316. Ralevic V, Burnstock G. Receptors for purines and pyrimidines. *Pharmacol Rev.* 1998; 50:413–492. [PubMed: 9755289]
 317. Ramos-Franco J, Fill M, Mignery GA. Isoform-specific function of single inositol 1,4,5-trisphosphate receptor channels. *Biophys J.* 1998; 75:834–839. [PubMed: 9675184]
 318. Rasmussen CD, Means AR. The presence of parvalbumin in a nonmuscle cell-line attenuates progression through mitosis. *Mol Endocrinol.* 1989; 3:588–596. [PubMed: 2747661]
 319. Rasola A, Bernardi P. The mitochondrial permeability transition pore and its involvement in cell death and in disease pathogenesis. *Apoptosis.* 2007; 12:815–833. [PubMed: 17294078]

320. Rasola A, Bernardi P. Mitochondrial permeability transition in Ca(2+)-dependent apoptosis and necrosis. *Cell Calcium*. 2011; 50:222–233. [PubMed: 21601280]
321. Reed JC. Bcl-2 family proteins. *Oncogene*. 1998; 17:3225–3236. [PubMed: 9916985]
322. Reinehr RM, Kubitz R, Peters-Regehr T, Bode JG, Haussinger D. Activation of rat hepatic stellate cells in culture is associated with increased sensitivity to endothelin 1. *Hepatology*. 1998; 28:1566–1577. [PubMed: 9828221]
323. Rhee SG, Choi KD. Regulation of inositol phospholipid-specific phospholipase C isozymes. *J Biol Chem*. 1992; 267:12393–12396. [PubMed: 1319994]
324. Rizzuto R, Brini M, Murgia M, Pozzan T. Microdomains with high Ca²⁺ close to IP₃-sensitive channels that are sensed by neighboring mitochondria. *Science*. 1993; 262:744–747. [PubMed: 8235595]
325. Rizzuto R, Pozzan T. Microdomains of intracellular Ca²⁺: molecular determinants and functional consequences. *Physiol Rev*. 2006; 86:369–408. [PubMed: 16371601]
326. Robb-Gaspers LD, Burnett P, Rutter GA, Denton RM, Rizzuto R, Thomas AP. Integrating cytosolic calcium signals into mitochondrial metabolic responses. *EMBO J*. 1998; 17:4987–5000. [PubMed: 9724635]
327. Robb-Gaspers LD, Rutter GA, Burnett P, Hajnoczky G, Denton RM, Thomas AP. Coupling between cytosolic and mitochondrial calcium oscillations: Role in the regulation of hepatic metabolism. *Biochim Biophys Acta*. 1998; 1366:17–32. [PubMed: 9714714]
328. Robb-Gaspers LD, Thomas AP. Coordination of Ca²⁺ signaling by intercellular propagation of Ca²⁺ waves in the intact liver. *J Biol Chem*. 1995; 270:8102–8107. [PubMed: 7713913]
329. Roberts SK, Ludwig J, LaRusso NF. The pathobiology of biliary epithelia. *Gastroenterology*. 1997; 112:269–279. [PubMed: 8978368]
330. Rockey DC. Hepatic blood flow regulation by stellate cells in normal and injured liver. *Semin Liver Dis*. 2001; 21:337–349. [PubMed: 11586464]
331. Rodrigues MA, Gomes DA, Andrade VA, Leite MF, Nathanson MH. Insulin induces calcium signals in the nucleus of rat hepatocytes. *Hepatology*. 2008; 48:1621–1631. [PubMed: 18798337]
332. Rodrigues MA, Gomes DA, Leite MF, Grant W, Zhang L, Lam W, Cheng YC, Bennett AM, Nathanson MH. Nucleoplasmic calcium is required for cell proliferation. *J Biol Chem*. 2007; 282:17061–17068. [PubMed: 17420246]
333. Roma MG, Crocenzi FA, Mottino AD. Dynamic localization of hepatocellular transporters in health and disease. *World J Gastroenterol*. 2008; 14:6786–6801. [PubMed: 19058304]
334. Roman RM, Bodily KO, Wang Y, Raymond JR, Fitz JG. Activation of protein kinase C alpha couples cell volume to membrane Cl⁻ permeability in HTC hepatoma and Mz-ChA-1 cholangiocarcinoma cells. *Hepatology*. 1998; 28:1073–1080. [PubMed: 9755245]
335. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol*. 2007; 8:519–529. [PubMed: 17565364]
336. Rooney TA, Renard DC, Sass EJ, Thomas AP. Oscillatory cytosolic calcium waves independent of stimulated inositol 1,4,5-trisphosphate formation in hepatocytes. *J Biol Chem*. 1991; 266:12272–12282. [PubMed: 2061312]
337. Rooney TA, Sass EJ, Thomas AP. Characterization of cytosolic calcium oscillations induced by phenylephrine and vasopressin in single fura-2-loaded hepatocytes. *J Biol Chem*. 1989; 264:17131–17141. [PubMed: 2793847]
338. Rooney TA, Sass EJ, Thomas AP. Agonist-induced cytosolic calcium oscillations originate from a specific locus in single hepatocytes. *J Biol Chem*. 1990; 265:10792–10796. [PubMed: 2113061]
339. Rottingen J, Iversen JG. Ruled by waves? Intracellular and intercellular calcium signalling. *Acta Physiol Scand*. 2000; 169:203–219. [PubMed: 10886035]
340. Ruas M, Rietdorf K, Arredouani A, Davis LC, Lloyd-Evans E, Koegel H, Funnell TM, Morgan AJ, Ward JA, Watanabe K, Cheng X, Churchill GC, Zhu MX, Platt FM, Wessel GM, Parrington J, Galione A. Purified TPC isoforms form NAADP receptors with distinct roles for Ca(2+) signaling and endolysosomal trafficking. *Curr Biol*. 2010; 20:703–709. [PubMed: 20346675]

341. Rudnick DA, Perlmutter DH, Muglia LJ. Prostaglandins are required for CREB activation and cellular proliferation during liver regeneration. *Proc Natl Acad Sci U S A*. 2001; 98:8885–8890. [PubMed: 11447268]
342. Rusinko N, Lee HC. Widespread occurrence in animal tissues of an enzyme catalyzing the conversion of NAD⁺ into a cyclic metabolite with intracellular Ca²⁺-mobilizing activity. *J Biol Chem*. 1989; 264:11725–11731. [PubMed: 2745413]
343. Rutter GA, Rizzuto R. Regulation of mitochondrial metabolism by ER Ca²⁺ release: An intimate connection. *Trends Biochem Sci*. 2000; 25:215–221. [PubMed: 10782088]
344. Saez JC, Connor JA, Spray DC, Bennett MV. Hepatocyte gap junctions are permeable to the second messenger, inositol 1,4,5-trisphosphate, and to calcium ions. *Proc Natl Acad Sci U S A*. 1989; 86:2708–2712. [PubMed: 2784857]
345. Sakon M, Nagano H, Dono K, Nakamori S, Umeshita K, Yamada A, Kawata S, Imai Y, Iijima S, Monden M. Combined intraarterial 5-fluorouracil and subcutaneous interferon-therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. *Cancer*. 2002; 95:2581.
346. Sanderson MJ, Charles AC, Boitano S, Dirksen ER. Mechanisms and function of intercellular calcium signaling. *Mol Cell Endocrinol*. 1994; 98:173–187. [PubMed: 8143927]
347. Santella L, Carafoli E. Calcium signaling in the cell nucleus. *FASEB J*. 1997; 11:1091–1109. [PubMed: 9367344]
348. Satoh T, Ross CA, Villa A, Supattapone S, Pozzan T, Snyder SH, Meldolesi J. The inositol 1,4,5,-trisphosphate receptor in cerebellar Purkinje cells: Quantitative immunogold labeling reveals concentration in an ER subcompartment. *J Cell Biol*. 1990; 111:615–624. [PubMed: 2166053]
349. Scarpa A, Azzone GF. The mechanism of ion translocation in mito-chondria. 4. Coupling of K⁺ efflux with Ca²⁺ uptake. *Eur J Biochem*. 1970; 12:328–335. [PubMed: 5459571]
350. Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell*. 2000; 103:211–225. [PubMed: 11057895]
351. Schlosser SF, Burgstahler AD, Nathanson MH. Isolated rat hepatocytes can signal to other hepatocytes and bile duct cells by release of nucleotides. *Proc Natl Acad Sci U S A*. 1996; 93:9948–9953. [PubMed: 8790437]
352. Servillo G, Della Fazia MA, Sassone-Corsi P. Coupling cAMP signaling to transcription in the liver: Pivotal role of CREB and CREM. *Exp Cell Res*. 2002; 275:143–154. [PubMed: 11969286]
353. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis*. 2005; 5:558–567. [PubMed: 16122679]
354. Shibao K, Hirata K, Robert ME, Nathanson MH. Loss of inositol 1,4,5-trisphosphate receptors from bile duct epithelia is a common event in cholestasis. *Gastroenterology*. 2003; 125:1175–1187. [PubMed: 14517800]
355. Shirakawa H, Miyazaki S. Spatiotemporal analysis of calcium dynamics in the nucleus of hamster oocytes. *J Physiol*. 1996; 494 (Pt 1):29–40. [PubMed: 8814604]
356. Shoshan-Barmatz V. High affinity ryanodine binding sites in rat liver endoplasmic reticulum. *FEBS Lett*. 1990; 263:317–320. [PubMed: 2335233]
357. Simon MI, Strathmann MP, Gautam N. Diversity of G proteins in signal transduction. *Science*. 1991; 252:802–808. [PubMed: 1902986]
358. Sjogren K, Liu JL, Blad K, Skrtic S, Vidal O, Wallenius V, LeRoith D, Tornell J, Isaksson OG, Jansson JO, Ohlsson C. Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. *Proc Natl Acad Sci U S A*. 1999; 96:7088–7092. [PubMed: 10359843]
359. Soliman EM, Rodrigues MA, Gomes DA, Sheung N, Yu J, Amaya MJ, Nathanson MH, Dranoff JA. Intracellular calcium signals regulate growth of hepatic stellate cells via specific effects on cell cycle progression. *Cell Calcium*. 2009; 45:284–292. [PubMed: 19131107]
360. Spirli C, Nathanson MH, Fiorotto R, Duner E, Denson LA, Sanz JM, Di Virgilio F, Okolicsanyi L, Casagrande F, Strazzabosco M. Proinflammatory cytokines inhibit secretion in rat bile duct epithelium. *Gastroenterology*. 2001; 121:156–169. [PubMed: 11438505]

361. Staddon JM, Hansford RG. Evidence indicating that the glucagon-induced increase in cytoplasmic free Ca²⁺ concentration in hepatocytes is mediated by an increase in cyclic AMP concentration. *Eur J Biochem.* 1989; 179:47–52. [PubMed: 2537201]
362. Stehno-Bittel L, Luckhoff A, Clapham DE. Calcium release from the nucleus by InsP₃ receptor channels. *Neuron.* 1995; 14:163–167. [PubMed: 7530018]
363. Steiling H, Wustefeld T, Bugnon P, Brauchle M, Fassler R, Teupser D, Thiery J, Gordon JI, Trautwein C, Werner S. Fibroblast growth factor receptor signalling is crucial for liver homeostasis and regeneration. *Oncogene.* 2003; 22:4380–4388. [PubMed: 12853974]
364. Steinhart RA, Alderton J. Intracellular free calcium rise triggers nuclear-envelope breakdown in the sea-urchin embryo. *Nature.* 1988; 332:364–366. [PubMed: 3127727]
365. Stoffler D, Goldie KN, Feja B, Aebi U. Calcium-mediated structural changes of native nuclear pore complexes monitored by time-lapse atomic force microscopy. *J Mol Biol.* 1999; 287:741–752. [PubMed: 10191142]
366. Strambio-De-Castillia C, Niepel M, Rout MP. The nuclear pore complex: Bridging nuclear transport and gene regulation. *Nat Rev Mol Cell Biol.* 2010; 11:490–501. [PubMed: 20571586]
367. Streb H, Irvine RF, Berridge MJ, Schulz I. Release of Ca²⁺ from a non-mitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature.* 1983; 306:67–69. [PubMed: 6605482]
368. Stumpel F, Ott T, Willecke K, Jungermann K. Connexin 32 gap junctions enhance stimulation of glucose output by glucagon and noradrenaline in mouse liver. *Hepatology.* 1998; 28:1616–1620. [PubMed: 9828226]
369. Sudhof TC, Newton CL, Archer BT III, Ushkaryov YA, Mignery GA. Structure of a novel InsP₃ receptor. *EMBO J.* 1991; 10:3199–3206. [PubMed: 1655411]
370. Sudhof TC, Rothman JE. Membrane fusion: Grappling with SNARE and SM proteins. *Science.* 2009; 323:474–477. [PubMed: 19164740]
371. Swillens S, DuPont G, Combettes L, Champeil P. From calcium blips to calcium puffs: Theoretical analysis of the requirements for interchannel communication. *Proc Natl Acad Sci U S A.* 1999; 96:13750–13755. [PubMed: 10570144]
372. Talarmin H, Rescan C, Cariou S, Glaise D, Zanninelli G, Bilodeau M, Loyer P, Guguen-Guillouzo C, Baffet G. The mitogen-activated protein kinase/extracellular signal-regulated kinase cascade activation is a key signalling pathway involved in the regulation of G(1) phase progression in proliferating hepatocytes. *Mol Cell Biol.* 1999; 19:6003–6011. [PubMed: 10454547]
373. Tanaka Y, Hayashi N, Kaneko A, Ito T, Miyoshi E, Sasaki Y, Fusamoto H, Kamada T. Epidermal growth-factor induces dose-dependent calcium oscillations in single fura-2 loaded hepatocytes. *Hepatology.* 1992; 16:479–486. [PubMed: 1322351]
374. Tang J, Wu YM, Zhao P, Jiang JL, Chen ZN. beta ig-h3 Interacts with alpha 3 beta 1 integrin to promote adhesion and migration of human hepatoma cells. *Exp Biol Med.* 2009; 234:35–39.
375. Tardif KD, Mori K, Kaufman RJ, Siddiqui A. Hepatitis C virus suppresses the IRE1-XBP1 pathway of the unfolded protein response. *J Biol Chem.* 2004; 279:17158–17164. [PubMed: 14960590]
376. Tardif KD, Waris G, Siddiqui A. Hepatitis C virus, ER stress, and oxidative stress. *Trends Microbiol.* 2005; 13:159–163. [PubMed: 15817385]
377. Taub R. Liver regeneration: From myth to mechanism. *Nat Rev Mol Cell Biol.* 2004; 5:836–847. [PubMed: 15459664]
378. Thapa N, Lee BH, Kim IS. TGFBIp/beta ig-h3 protein: A versatile matrix molecule induced by TGF-beta. *Int J Biochem Cell Biol.* 2007; 39:2183–2194. [PubMed: 17659994]
379. Thomas AP, Bird GS, Hajnoczky G, Robb-Gaspers LD, Putney JW Jr. Spatial and temporal aspects of cellular calcium signaling. *FASEB J.* 1996; 10:1505–1517. [PubMed: 8940296]
380. Thomas AP, Martin-Requero A, Williamson JR. Interactions between insulin and alpha 1-adrenergic agents in the regulation of glycogen metabolism in isolated hepatocytes. *J Biol Chem.* 1985; 260:5963–5973. [PubMed: 2860104]
381. Thomas AP, Renard DC, Rooney TA. Spatial and temporal organization of calcium signalling in hepatocytes. *Cell Calcium.* 1991; 12:111–126. [PubMed: 1647873]

382. Thomas AP, Robb-Gaspers LD, Rooney TA, Hajnoczky G, Renard-Rooney DC, Lin C. Spatial organization of oscillating calcium signals in liver. *Biochem Soc Trans.* 1995; 23:642–648. [PubMed: 8566434]
383. Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. *Nat Genet.* 2002; 31:339–346. [PubMed: 12149612]
384. Tomida T, Hirose K, Takizawa A, Shibasaki F, Iino M. NFAT functions as a working memory of Ca²⁺ signals in decoding Ca²⁺ oscillation. *EMBO J.* 2003; 22:3825–3832. [PubMed: 12881417]
385. Tordjmann T, Berthon B, Claret M, Combettes L. Coordinated intercellular calcium waves induced by noradrenaline in rat hepatocytes: Dual control by gap junction permeability and agonist. *EMBO J.* 1997; 16:5398–5407. [PubMed: 9311999]
386. Tordjmann T, Berthon B, Combettes L, Claret M. The location of hepatocytes in the rat liver acinus determines their sensitivity to calcium-mobilizing hormones. *Gastroenterology.* 1996; 111:1343–1352. [PubMed: 8898649]
387. Tordjmann T, Berthon B, Jacquemin E, Clair C, Stelly N, Guillon G, Claret M, Combettes L. Receptor-oriented intercellular calcium waves evoked by vasopressin in rat hepatocytes. *EMBO J.* 1998; 17:4695–4703. [PubMed: 9707428]
388. Trauner M, Meier PJ, Boyer JL. Molecular pathogenesis of cholestasis. *N Engl J Med.* 1998; 339:1217–1227. [PubMed: 9780343]
389. Trusolino L, Bertotti A, Comoglio PM. MET signalling: Principles and functions in development, organ regeneration and cancer. *Nat Rev Mol Cell Biol.* 2010; 11:834–848. [PubMed: 21102609]
390. Twigg J, Patel R, Whitaker M. Translational Control of InsP₃-induced chromatin condensation during the early cell-cycle of sea-urchin embryos. *Nature.* 1988; 332:366–369. [PubMed: 3127728]
391. Villanueva A, Llovet JM. Targeted therapies for hepatocellular carcinoma. *Gastroenterology.* 2011; 140:1410–1426. [PubMed: 21406195]
392. Villanueva A, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. *Semin Liver Dis.* 2007; 27:55–76. [PubMed: 17295177]
393. Violin JD, Zhang J, Tsien RY, Newton AC. A genetically encoded fluorescent reporter reveals oscillatory phosphorylation by protein kinase C. *J Cell Biol.* 2003; 161:899–909. [PubMed: 12782683]
394. Visnjic D, Banfic H. Nuclear phospholipid signaling: Phosphatidylinositol-specific phospholipase C and phosphoinositide 3-kinase. *Pflugers Arch.* 2007; 455:19–30. [PubMed: 17558519]
395. Wakelam MJ, Murphy GJ, Hruby VJ, Houslay MD. Activation of two signal-transduction systems in hepatocytes by glucagon. *Nature.* 1986; 323:68–71. [PubMed: 3018586]
396. Wakui M, Potter BV, Petersen OH. Pulsatile intracellular calcium release does not depend on fluctuations in inositol trisphosphate concentration. *Nature.* 1989; 339:317–320. [PubMed: 2498663]
397. Walker SA, Kupzig S, Bouyoucef D, Davies LC, Tsuboi T, Bivona TG, Cozier GE, Lockyer PJ, Buckler A, Rutter GA, Allen MJ, Phillips MR, Cullen PJ. Identification of a Ras GTPase-activating protein regulated by receptor-mediated Ca²⁺ oscillations. *EMBO J.* 2004; 23:1749–1760. [PubMed: 15057271]
398. Watanabe N, Tsukada N, Smith CR, Edwards V, Phillips MJ. Permeabilized hepatocyte couplets. Adenosine triphosphate-dependent bile canalicular contractions and a circumferential pericanalicular microfilament belt demonstrated. *Lab Invest.* 1991; 65:203–213. [PubMed: 1881122]
399. Watanabe N, Tsukada N, Smith CR, Phillips MJ. Motility of bile canaliculi in the living animal: Implications for bile flow. *J Cell Biol.* 1991; 113:1069–1080. [PubMed: 2040644]
400. Watanabe S, Smith CR, Phillips MJ. Coordination of the contractile activity of bile canaliculi. Evidence from calcium microinjection of triplet hepatocytes. *Lab Invest.* 1985; 53:275–279. [PubMed: 4033067]
401. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, Roth KA, MacGregor GR, Thompson CB, Korsmeyer SJ. Proapoptotic BAX and BAK: A requisite gateway to mitochondrial dysfunction and death. *Science.* 2001; 292:727–730. [PubMed: 11326099]

402. Wei Y, Wang D, Gentile CL, Pagliassotti MJ. Reduced endoplasmic reticulum luminal calcium links saturated fatty acid-mediated endoplasmic reticulum stress and cell death in liver cells. *Mol Cell Biochem.* 2009; 331:31–40. [PubMed: 19444596]
403. Whitaker M. Calcium at fertilization and in early development. *Physiol Rev.* 2006a; 86:25–88. [PubMed: 16371595]
404. Whitaker M. Calcium microdomains and cell cycle control. *Cell Calcium.* 2006b; 40:585–592. [PubMed: 17045645]
405. White C, Li C, Yang J, Petrenko NB, Madesh M, Thompson CB, Foskett JK. The endoplasmic reticulum gateway to apoptosis by Bcl-X-L modulation of the InsP(3)R. *Nat Cell Biol.* 2005; 7:1021–U135. [PubMed: 16179951]
406. Whittaker S, Marais R, Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. *Oncogene.* 2010; 29:4989–5005. [PubMed: 20639898]
407. Williams TF, Exton JH, Friedmann N, Park CR. Effects of insulin and adenosine 3',5'-monophosphate on K⁺ flux and glucose output in perfused rat liver. *Am J Physiol.* 1971; 221:1645–1651. [PubMed: 4330901]
408. Wimmer R, Hohenester S, Pusl T, Denk GU, Rust C, Beuers U. Taurour-sodeoxycholic acid exerts anticholestatic effects by a cooperative cPKC alpha-/PKA-dependent mechanism in rat liver. *Gut.* 2008; 57:1448–1454. [PubMed: 18583398]
409. Wojcikiewicz RJ. Type I, II, and III inositol 1,4,5-trisphosphate receptors are unequally susceptible to down-regulation and are expressed in markedly different proportions in different cell types. *J Biol Chem.* 1995; 270:11678–11683. [PubMed: 7744807]
410. Wojcikiewicz RJ, Ernst SA, Yule DI. Secretagogues cause ubiquitination and down-regulation of inositol 1, 4,5-trisphosphate receptors in rat pancreatic acinar cells. *Gastroenterology.* 1999; 116:1194–1201. [PubMed: 10220512]
411. Woo K, Sathe M, Kresge C, Esser V, Ueno Y, Venter J, Glaser SS, Alpini G, Feranchak AP. Adenosine triphosphate release and purinergic (P2) receptor-mediated secretion in small and large mouse cholangiocytes. *Hepatology.* 2010; 52:1819–1828. [PubMed: 20827720]
412. Woods NM, Cuthbertson KS, Cobbold PH. Repetitive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes. *Nature.* 1986; 319:600–602. [PubMed: 3945348]
413. Woods NM, Cuthbertson KS, Cobbold PH. Agonist-induced oscillations in cytoplasmic free calcium concentration in single rat hepatocytes. *Cell Calcium.* 1987; 8:79–100. [PubMed: 3829123]
414. Wu DQ, Lee CH, Rhee SG, Simon MI. Activation of phospholipase C by the alpha subunits of the Gq and G11 proteins in transfected Cos-7 cells. *J Biol Chem.* 1992; 267:1811–1817. [PubMed: 1309799]
415. Yamaguchi Y, Dalle-Molle E, Hardison WG. Vasopressin and A23187 stimulate phosphorylation of myosin light chain-1 in isolated rat hepatocytes. *Am J Physiol.* 1991; 261:G312–G319. [PubMed: 1872400]
416. Yamamoto K, Ichijo H, Korsmeyer SJ. BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. *Mol Cell Biol.* 1999; 19:8469–8478. [PubMed: 10567572]
417. Yamamoto-Hino M, Miyawaki A, Segawa A, Adachi E, Yamashina S, Fujimoto T, Sugiyama T, Furuichi T, Hasegawa M, Mikoshiba K. Apical vesicles bearing inositol 1,4,5-trisphosphate receptors in the Ca²⁺ initiation site of ductal epithelium of submandibular gland. *J Cell Biol.* 1998; 141:135–142. [PubMed: 9531553]
418. Yamasaki M, Masgrau R, Morgan AJ, Churchill GC, Patel S, Ashcroft SJ, Galione A. Organelle selection determines agonist-specific Ca²⁺ signals in pancreatic acinar and beta cells. *J Biol Chem.* 2004; 279:7234–7240. [PubMed: 14660554]
419. Yang B, Bouchard MJ. The hepatitis B virus X protein elevates cytosolic calcium signals by modulating mitochondrial calcium uptake. *J Virol.* 2012; 86:313–327. [PubMed: 22031934]
420. Yang S, Huang XY. Ca²⁺ influx through L-type Ca²⁺ channels controls the trailing tail contraction in growth factor-induced fibroblast cell migration. *J Biol Chem.* 2005; 280:27130–27137. [PubMed: 15911622]

421. Yao Y, Choi J, Parker I. Quantal puffs of intracellular Ca²⁺ evoked by inositol trisphosphate in *Xenopus* oocytes. *J Physiol.* 1995; 482 (Pt 3):533–553. [PubMed: 7738847]
422. Yin H, Xie F, Zhang J, Yang Y, Deng B, Sun J, Wang Q, Qu X, Mao H. Combination of interferon-alpha and 5-fluorouracil induces apoptosis through mitochondrial pathway in hepatocellular carcinoma in vitro. *Cancer Lett.* 2011; 306:34–42. [PubMed: 21474236]
423. Zhang Y, Xue R, Zhang Z, Yang X, Shi H. Palmitic and linoleic acids induce ER stress and apoptosis in hepatoma cells. *Lipids Health Dis.* 2012; 11:1. [PubMed: 22221411]
424. Zong X, Schieder M, Cuny H, Fenske S, Gruner C, Rotzer K, Griesbeck O, Harz H, Biel M, Wahl-Schott C. The two-pore channel TPCN2 mediates NAADP-dependent Ca(2+)-release from lysosomal stores. *Pflugers Arch.* 2009; 458:891–899. [PubMed: 19557428]
425. Zoulim F, Saputelli J, Seeger C. Woodchuck hepatitis virus X protein is required for viral infection in vivo. *J Virol.* 1994; 68:2026–2030. [PubMed: 8107266]

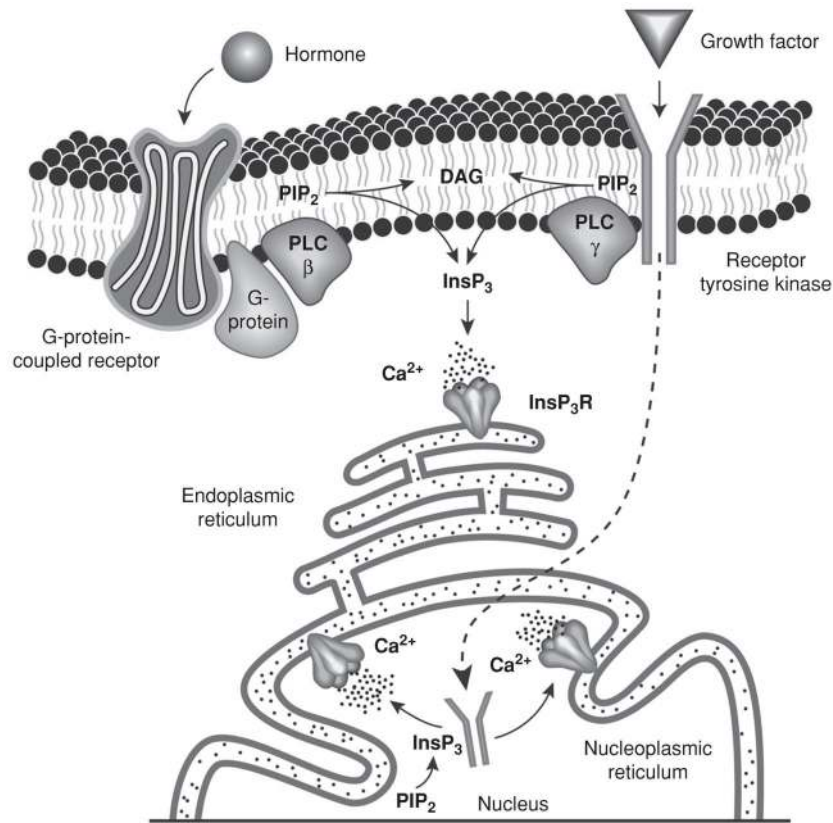


Figure 1. Molecular machinery for Ca^{2+} signal formation in hepatocytes. Ca^{2+} signals may be generated by Ca^{2+} -mobilizing hormones (through activation of G-protein-coupled receptors) or growth factors (through receptor tyrosine kinases). Binding of a ligand to its specific receptor leads to the activation of phospholipase C (PLC), which hydrolyzes Phosphatidylinositol-4-5-bisphosphate (PIP₂) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (InsP₃). DAG remains in the plasma membrane and InsP₃ diffuses throughout the cytosol and binds to the InsP₃ receptor (InsP₃R) in the endoplasmic reticulum to allow Ca^{2+} release to the cytosol. Alternatively, receptor tyrosine kinases may translocate to the nucleus to locally activate PLC and induce Ca^{2+} release from the nucleoplasmic reticulum into the nucleoplasm. Nuclear PIP₂ is depicted. However, its exact localization within the nucleus remains unknown [modified from references (211) and (137), with permission].

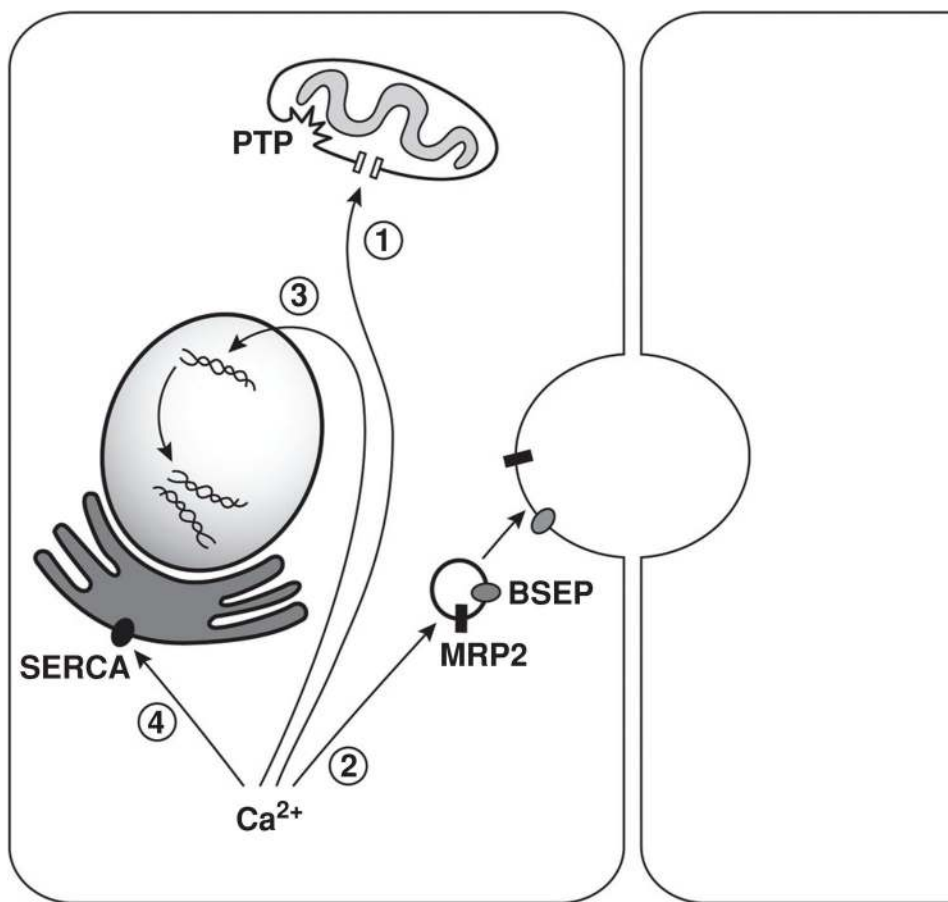


Figure 2.

Physiological and pathophysiological actions of Ca^{2+} in hepatocytes. 1. Cytosolic and mitochondrial Ca^{2+} signals are closely interrelated. Ca^{2+} is transmitted from inositol 1,4,5-trisphosphate receptors (InsP3Rs) to mitochondria, leading to an increase in mitochondrial free Ca^{2+} and the formation of the permeability transition pore (PTP). Reversible opening of the PTP is observed in normal mitochondrial function and shapes Ca^{2+} signals, but persistent opening of the PTP leads to leakage of cytochrome c, augmenting the cellular sensitivity to apoptotic stimuli. 2. Type II InsP3R-mediated Ca^{2+} release regulates organic anion and bile salt secretion through the insertion of the transporters multidrug resistance-associated protein 2 (MRP2) and bile salt export pump (BSEP), respectively, into the canalicular membrane. Loss of this isoform results in impaired transporter activity and contributes to the pathophysiology of intrahepatic cholestasis. 3. Nuclear Ca^{2+} is essential for cell-cycle progression through the control of gene transcription and expression of proliferative proteins. Furthermore, nuclear Ca^{2+} may be associated with liver tumor growth. 4. The endoplasmic reticulum (ER) regulates protein folding, quality control, trafficking, and targeting, and these depend on adequate amounts of Ca^{2+} in the ER lumen. Unbalance between ER load and protein-folding capacity leads to ER stress. In obesity, liver expression and activity of SERCA are impaired, leading to ER stress and defective glucose metabolism.

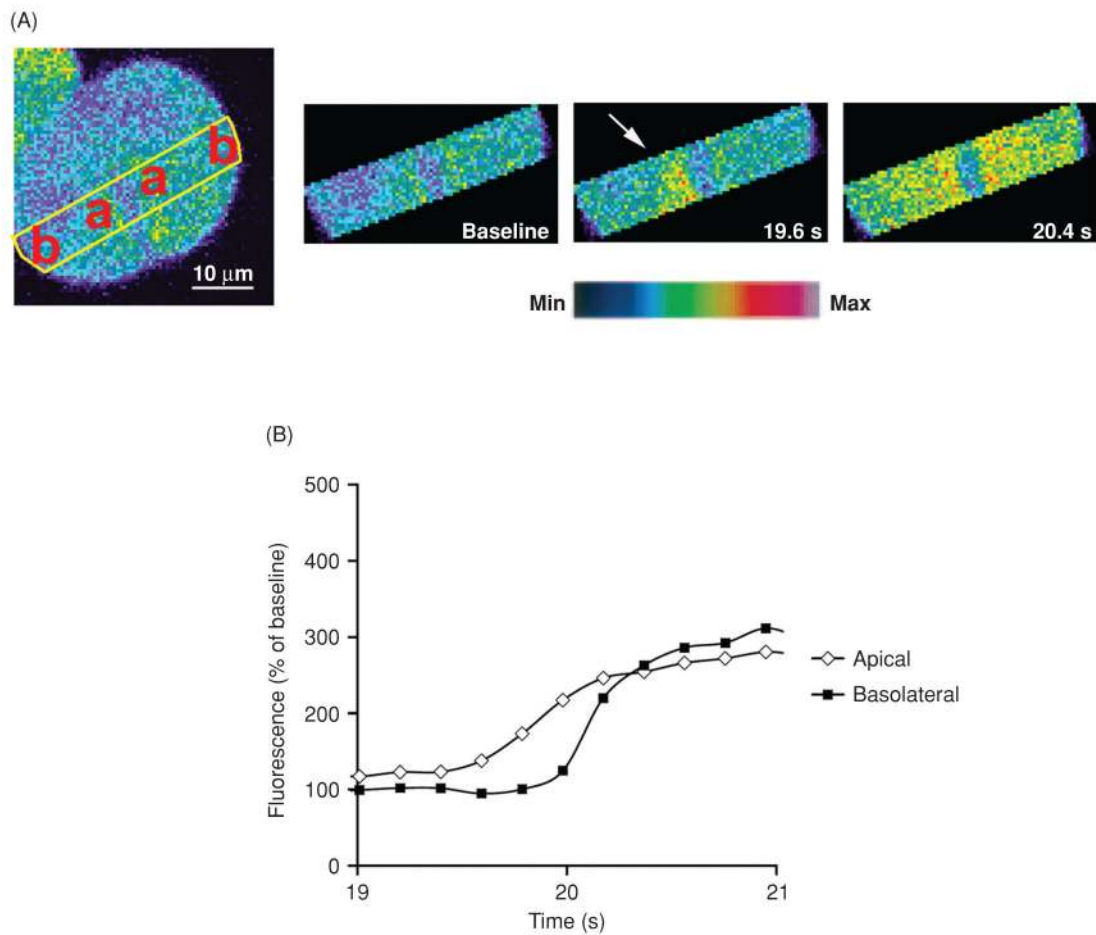


Figure 3.

Ca^{2+} waves begin in the apical region of hepatocytes. (A) Confocal images of an isolated rat hepatocyte couplet loaded with the Ca^{2+} sensitive dye Fluo-4/AM and stimulated with vasopressin. Serial images of the region of interest outlined in yellow show that a Ca^{2+} wave starts in the apical (a) region of the cell and spreads to the basolateral (b) region. Images were pseudocolored according to the scale shown at the bottom. (B) Graphical representation of the fluorescence increase in the apical and basolateral region shows that the apical Ca^{2+} signal precedes the basolateral Ca^{2+} signal [modified from reference (263), with permission].

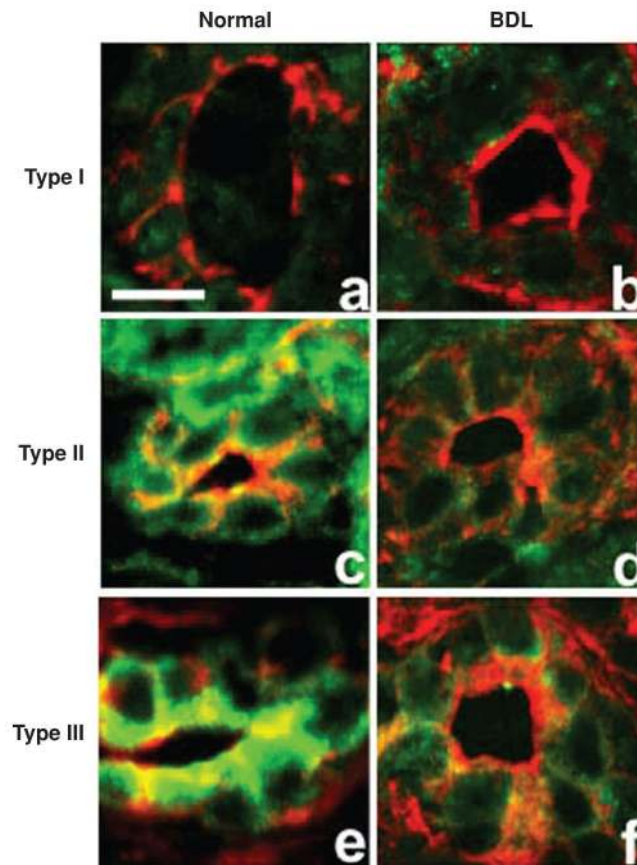


Figure 4. Inositol 1,4,5-trisphosphate receptors (InsP3R) expression is lost in bile duct epithelia after bile duct ligation. Confocal immunofluorescence of liver sections from normal rats and rats subjected to bile duct ligation (BDL) labeled with isoform-specific InsP3R antibodies (green) and rhodamine phalloidin (red). Type 1 InsP3R labeling in normal bile duct cells (A) is found throughout each cell although is expressed at low levels, similar to that observed 2 weeks after BDL (B). Type 2 InsP3R labeling is seen throughout each cell in normal liver sections (C) and it is nearly absent 2 weeks after BDL (D). Type 3 InsP3R labeling is found predominantly in the apical region of bile duct cells in normal liver sections (E) and is also markedly reduced 2 weeks after BDL (F) [reprinted from reference (354), with permission].

Table 1

List of Abbreviations

[Ca ²⁺] _c	Cytosolic calcium
[Ca ²⁺] _i	Intracellular calcium
[Ca ²⁺] _m	Mitochondrial calcium
[Ca ²⁺] _n	Nucleoplasmic calcium
5-FU	5-fluorouracil
Ach	Acetylcholine
ADP	Adenosine diphosphate
AP-1	Activator protein 1
ATP	Adenosine triphosphate
ATF6α	Activating transcription factor 6α
AVP	Arginine vasopressin
BDL	Bile duct ligation
BSEP	Bile salt export pump
cADPR	Cyclic adenosine diphosphate ribose
CaMK	Calcium-calmodulin-dependent protein kinase
cAMP	Cyclic adenosine monophosphate
CD38	Adenosine diphosphate-ribosyl cyclase
CFTR	Cystic fibrosis transmembrane conductance regulator
CGamF	Cholylglycylamido-fluorescein
CICR	Calcium-induced calcium release
c-Met	Hepatocyte growth factor receptor
CMFDA	5-chloromethylfluorescein diacetate
cPKCα	Ca ²⁺ -dependent conventional protein kinase C alpha
CRAC	Calcium release activated channel
CREB	Cyclic adenosine monophosphate response element binding
CsA	Cyclosporin A
Cx26	Connexin 26
Cx32	Connexin 32
DAG	Diacylglycerol
DMSO	Dimethylsulfoxide
EGF	Epidermal growth factor
Elk1	E twenty-six-like transcription factor 1
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
FLZ	N-(2-(4-hydroxy-phenyl)-ethyl)-2-(2,5-dimethoxy-phenyl)-3-(3-methoxy-4-hydroxy-phenyl)-acrylamide
FXR	Farnesoid X receptor
GDP	Guanosine diphosphate
GFP	Green fluorescent protein
GPCR	G-protein-coupled receptor
HBV	Hepatitis B virus

HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HGF	Hepatocyte growth factor
HSC	Hepatic stellate cells
IFN α	Interferon-alpha
IGF-1	Insulin-like growth factor 1
IMM	Inner mitochondrial membrane
INM	Inner nuclear membrane
InsP3	Inositol-1,4,5-trisphosphate
InsP3R	Inositol-1,4,5-trisphosphate receptor
IRE1 α	Inositol requiring enzyme 1 α
JNK	c-jun-N-terminal kinase
LCA	Lithocholic acid
LGMN	Legumain
LPS	Lipopolysaccharide
mCICR	Mitochondrial calcium induced calcium release
MCU	Mitochondrial calcium uniporter
MRP2	Multidrug resistance associated protein 2
NAADP	Nicotinic acid adenine dinucleotide phosphate
NAD(P)H	Nicotinamide adenine dinucleotide phosphate
NAFLD	Non-alcoholic fatty liver disease
NE	Nuclear envelope
NFAT	Nuclear factor of activated T-cells
NFKB	Nuclear factor kappa B
NPC	Nuclear pore complex
NR	Nucleoplasmic reticulum
OAP	Oct-1 associated protein
OMM	Outer mitochondrial membrane
ONM	Outer nuclear membrane
PDGF	Platelet-derived growth factor
PERK	Protein kinase ribonucleic acid-like endoplasmic reticulum kinase
PH	Partial hepatectomy
PIP2	Phosphatidylinositol-4-5-bisphosphate
PKA	Cyclic adenosine monophosphate-dependent protein kinase
PKC	Protein kinase C
PKG	Cyclic guanosine monophosphate-dependent protein kinase
PLC	Phospholipase C
PT	Permeability transition
PTB	Phosphotyrosine binding
PTP	Permeability transition pore
PV	Parvalbumin
RaM	Rapid uptake mode
RASAL	Ras guanosine triphosphatase activating protein

RaSH	Rapid Subtraction Hybridization
ROS	Reactive oxygen species
RTK	Receptor Tyrosine Kinase
RyR	Ryanodine receptor
SERCA	Sarco/endoplasmic reticulum calcium adenosine triphosphatase
SH	Src homology domain
SOC	Store-operated calcium channel
STIM1	Stromal interaction factor 1
TBH	Tertbutylhydroperoxide
tBHP	Tert-butyl hydroperoxide
TUDCA	Tauroursodeoxycholic acid
TLCA	Taurolithocholic acid
TPC	Two-pore channel
TRPV	Transient receptor potential vanilloid
UDCA	Ursodeoxycholic acid
UPR	Unfolded protein response
VDAC	Voltage-dependent anion channel
