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Is there a specific role for the plasma membrane Ca^{2+}-ATPase in the hepatocyte?

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Abstract

The plasma membrane Ca^{2+}-ATPase (PMCA) is responsible for the fine, long-term regulation of the cytoplasmic calcium concentration by extrusion of this cation from the cell. Although the general kinetic mechanisms for the action of both, well coordinated hydrolytic activity and calcium transport are reasonably understood in the majority of cell types, due to the complex physiologic and biochemical characteristics shown by the hepatocyte, the study of this enzyme in this cell type has become a real challenge. Here, we review the various molecular aspects known to date to be associated with liver PMCA activity, and outline the strategies to follow for establishing the role of this enzyme in the overall physiology of the hepatocyte. In this way, we first concentrate on the basic biochemical aspects of liver cell PMCA, and place an important emphasis on expression of its molecular forms to finally focus on the critical hormonal regulation of the enzyme. Although these complex aspects have been studied mainly under normal conditions, the significance of PMCA in the calcium homeostasis of an abnormal liver cell is also reviewed. (Mol Cell Biochem **xxx:** 1–15, 2005)

Key words: PMCA, Ca^{2+} homeostasis, hepatocyte, hormone regulation

Introduction

The plasma membrane Ca^{2+}-ATPase (PMCA) pumps cytosolic calcium to the extracellular space and presents a major role in long-term control of intracellular free Ca^{2+} levels in eukaryotic cells [reviewed in 1–3]. The majority of PMCA properties have been determined using human erythrocyte ghosts and later confirmed utilizing the purified enzyme [4–8]. ^{45}Ca-uptake and $(Ca^{2+} + Mg^{2+})$-ATPase kinetic constants as well as the mechanisms by which erythrocyte PMCA activity is controlled, became the reference to compare with the Ca^{2+}-ATPase activity associated with both the microsomal membrane fraction, and ATPases from other cell types. Although some tissues show high Ca^{2+}-ATPase activity similar to the erythrocyte Ca^{2+} pump, other tissues such as liver, evidenced a more complex situation. Liver PMCA kinetics present major differences with respect to the erythrocyte enzyme, the most relevant being regulated by hormones through membrane-associated proteins.

The main goal of this review was to analyze the knowledge generated throughout the years concerning the main properties and regulatory mechanisms that control the plasma membrane Ca^{2+}-ATPase present in the hepatocyte under normal and pathological conditions. Divided in three main sections, i.e., Biochemistry, molecular forms expression, and hormonal regulation; this review attempts to set forth a critical evaluation of the observations made by many scientists over the years to define whether there is a specific role for plasma membrane Ca^{2+}-ATPase in the hepatocyte.

Biochemistry of liver PMCA

Isolation and purification of a plasma membrane fraction free of contaminating Ca^{2+}-ATPases derived from mitochondria

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and endoplasmic reticulum vesicles was the first problem to solve for characterization of liver PMCA. Several strategies have been used to homogenate and fractionate the tissue using hypotonic [9–27], isotonic [13, 28–39], or hypertonic [40–42] solutions, supplemented with calcium [11, 13, 24, 29, 39], with EGTA or in the absence of added calcium [31–33, 35−37]. Surprisingly, addition of protease inhibitors during liver fractionation was not reported in the majority of these studies, with the exception of the work reported by Kessler *et al*. [38]. Plasma membrane vesicles have been purified mainly from nuclear or microsomal fractions by centrifugation using sucrose or Percoll density gradients. However, contamination with ER membranes determined by the 5′-nucleotidase/glucose-6-phosphatase activity ratio was variable [24, 35, 41, 42].

The second problem to solve was separation of the sinusoidal, and canalicular regions of plasma membrane [13, 14, 43, 44] to define whether PMCA is restricted to a specific functional domain of the hepatocyte [24, 26, 38].

Ca^{2+}-ATPase activity in liver plasma membrane vesicles

A high-affinity Ca^{2+}-ATPase has been consistently observed by many authors in liver plasma membrane vesicles; however, the reported Vmax values fluctuate from 33 [17] to 1,300 nmoles Pi mg protein^{-1} min^{-1} [32], and the apparent Ka for Ca^{2+} from 25 nM [24] to 1.6 μM [32]. Ca^{2+}-ATPase activity shows a sigmoid curve when assayed in the presence of different substrate concentrations. An apparent Km of 200 μM was obtained considering free ATP as substrate, but a Km of 4 μM was reported when Mg-ATP was used instead [37]. Endogenous nanomolar levels [32, 37] or exogenous micromolar concentrations of Mg^{2+} stimulates Ca^{2+}-ATPase activity, nevertheless, millimolar Mg^{2+} concentrations inhibit this activity [41, 42].

The kinetic constants reported for ^{45}Ca-uptake also have shown important variations. The apparent affinity for Ca^{2+} fluctuates from 17 nM [17] to 1–6 μM [32, 37], and Vmax values range from 35 pmoles [17] to ca. 4 nanomoles Ca^{2+} mg protein^{-1} min^{-1} [32, 37]. Calcium transport shows two apparent affinities for ATP, where Km for the high-affinity site corresponds to 81 μM and for the low-affinity site, 10 mM [17]. A single Km value of 150 μM for both free ATP and Mg-ATP complex has been also reported [37]. Addition of millimolar concentrations of Mg^{2+} has appeared necessary for ^{45}Ca-uptake to take place [17, 24, 26, 30, 31, 36–39].

The great variability of kinetic data between the papers mentioned previously could be explained considering the different procedures employed to isolate liver plasma membrane vesicles and the methods used to measure both Ca^{2+}-ATPase activity and ^{45}Ca-uptake. Moreover, variables such as age, weight, and sex of experimental animals as well as feeding conditions also could have influenced the results. Whether hepatocyte plasma membrane domains show significant differences in PMCA activity or not remains controversial, because studies using plasma membrane subfractions claiming to be enriched in blood sinusoidal, lateral or canalicular membrane regions show discrepancies attributable to the highly variable methodology conditions employed [24, 26, 38]. It should be pointed out that the canalicular region located in close proximity to the endoplasmic reticulum containing abundant inositol 1,4,5-triphosphate receptors (IP_3R) [43] accounts for ca. 10% of the total hepatocyte plasma membrane. The canalicular domain is free of Na^+-K^+-ATPase and glucagon-stimulated adenylate cyclase activity and shows to be enriched in leucyl-naphthylamidase, γ-glutamyl-transpeptidase, 5′-nucleotidase, alkaline phosphatase, and Mg^{2+}-ATPase activities [13, 14, 44, 45]. Sialic acid, sphingomyelin, and cholesterol content in the bile canalicular domain doubles the levels found in blood sinusoidal and lateral regions [44]. Because cholesterol has been shown to modify the activity of cardiac muscle PMCA [46–48] drastically, it remains to be defined whether this lipid affects the PMCA contained in these regions. On the other hand, blood sinusoidal and lateral membrane domains represent 90% of total plasma membrane area, and contain high Na^+-K^+-ATPase and glucagon-stimulated adenylate cyclase activities [44, 45].

Ca^{2+}-ATPase activity in erythrocyte ghosts demonstrates a Vmax of 33 nmoles Pi mg protein^{-1} min^{-1}, an apparent K_{Ca} of 2 μM, and Km values for ATP of 3.5 and 120 μM for high and low-affinity sites, respectively. Inside-out red blood cell membrane vesicles transport approximately 13 nmoles Ca^{2+} mg protein^{-1} min^{-1}, and requires the addition of millimolar Mg^{2+} [49]. Based on this information, it can be concluded that erythrocyte and liver PMCA show similar properties when assayed in their original membrane microenvironment. An increased sensitivity to Mg^{2+} shown by liver Ca^{2+}-ATPase is the only important difference found to date with respect to the erythrocyte enzyme.

Partially purified liver PMCA

To clearly define its properties, Ca^{2+}-ATPase has been partially purified from liver plasma membrane using gel filtration chromatography [15, 16, 19−21, 23, 25, 42], and incorporated into liposomes [20, 25]. Several experiments using this type of preparations showed a Ka in the nanomolar range [15, 19, 25] and a Vmax that fluctuated within a small range from 0.120 [23] to 0.750 [16] μmoles Pi mg prot^{-1} min^{-1}. Micromolar levels of Mg^{2+} stimulate ATPase activity [15, 20, 42] in contrast to millimolar concentrations, which inhibit the enzyme [15]. These results confirmed previous observations

using plasma membrane vesicles with the enzyme located in its natural microenvironment [32, 37, 40, 42].

The molecular weight of partially purified Ca^{2+}-ATPase shows important variations depending on the method employed. For instance, a molecular weight of 200 kD has been defined by filtration on Sephadex G-200 [19, 41]. Moreover, when stases radius and sedimentation coefficients are considered, the molecular weight of the protein corresponds to 140 kD, and by SDS-PAGE, 120 kD [42] and 70 kD [19]. However, the molecular weight of the Ca^{2+}-dependent vanadate-inhibited phosphorylated protein corresponded to 118 kD [20]. It is important to mention that none of these results were obtained using protease inhibitors during enzyme purification.

On the other hand, presence of an ecto-ATPase stimulated by Ca^{2+} and Mg^{2+} in a crude plasma membrane fraction of the liver [19−21] has originated an intense debate [25, 37, 50]. These ecto-ATPases are neither CaM-activated nor vanadate-inhibited [21, 50]. The plasma membrane ecto-ATPase has been purified [51], and through the production of antibodies, localized for instance in cardiac muscle [52]. These antibodies do not cross-react either with classical PMCA or with sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA). This ecto-ATPase has been also localized in tissues such as kidney, and skeletal muscle, as well as brain. It has been reported to present homology with two membrane glycoproteins: a hepatic cell-adhesion molecule called cell-CAM 105, and with protein pp120/HA4, recognized by the insulin receptor tyrosine kinase [53]. Moreover, presence of extracellular ATP as a substrate for this ecto-ATPase has suggested its involvement with several metabolic pathways such as gluconeogenesis and ureagenesis [54]. Because this ecto-ATPase releases ADP from ATP in the extracellular space, and because ADP is a ligand for the P2Y purinergic family of receptors that interact with G proteins [55], association of these receptors with modulation of PMCA in the hepatocyte remains to be elucidated. In human hepatocytes, it has been reported that P2Y receptors control both glycogen metabolism and several proliferation-associated responses [56].

Affinity purified liver PMCA

Moore and Kraus-Friedmann [30] partially purified a liver Ca^{2+}-ATPase by CaM-affinity chromatography from CaM-depleted, Triton X-100 solubilized microsomal membrane fractions. Ca^{2+}-ATPase activity shown by the purified protein was stimulated by micromolar levels of Ca^{2+}, presented dependency on Mg^{2+} and was stimulated by addition of exogenous CaM; nevertheless, TFP partially inhibited this calmodulin effect exerted upon Ca^{2+}-ATPase activity [30]. Unfortunately, no further purification nor characterization of the isolated enzyme was carried out in this study to determine whether this partially purified Ca^{2+}-ATPase was conclusively the liver PMCA.

Using monoclonal antibody 5F10 directed toward a cytoplasmic sequence present in all PMCA isoforms [57], Kessler *et al*. [38] purified the liver PMCA to homogeneity, reported a molecular weight of 140 kD and for the first time studied its immunolocalization. They showed that blood sinusoidal domain is enriched with PMCA, while lateral and canalicular regions present a lower PMCA density.

Immuno-electron microscopy studies have shown that stellate and sinusoidal endothelial cells express PMCA [58, 59]. In this case, PMCA was localized in caveolae of stellate cells and in fenestrae of endothelial cells.

CaM-overlay experiments of purified liver PMCA showed that liver Ca^{2+}-ATPase presents a higher affinity for CaM binding than the erythrocyte enzyme. Although a masked CaM binding domain in liver PMCA has been postulated to be accessible under denaturing experimental conditions [38], the high affinity for Ca^{2+} shown by liver PMCA could have been caused by endogenous CaM already bound to liver PMCA. This possibility is supported by the observation that in presence of CaM, the affinity for Ca^{2+} shown by the erythrocyte PMCA changes from the μM to the nM range [3]. PMCA maximal activity can also be stimulated by acidic phospholipids [60, 61], phosphorylation [62, 63], self-association [64], and mild proteolysis [65]. Phospholipids interact with a sequence of PMCA toward the NH_2 end [66]. The phosphorylation site is localized near the carboxy terminal sequence of the enzyme and constitutes part of the PMCA regulatory region. It comprehends ca. 100 residues for the "a" isoforms and 150 aminoacids for the "b" isoforms [reviewed in 67].

On the other hand, CaM-binding domain in hPMCA1, the predominant isoform expressed by liver under normal and pathological conditions [68−70], presents the following sequence: LRRGQILWFRGLNRIQTQIRVVNAFRSS [71]. This sequence allows liver PMCA to bind calmodulin with high affinity [72, 73]. It is possible that CaM bound to PMCA1b could render unavailable the phosphorylation sequence to PKA or PKC [74], dimerization [75], as well as proteolytic activation of PMCA [76, 77].

Phosphatidylserine, a phospholipid able to activate erythrocyte PMCA and capable of preventing further stimulation by CaM [78], is distributed only on the inner side of the plasma membrane of both blood sinusoidal and bile canalicular domains of hepatocytes [45], therefore, liver PMCA localized in these domains could be considered constantly phosphatidylserine-activated. However, canalicular membranes that contain high cholesterol levels might counteract the effects of phosphatidylserine, because high cholesterol/phospholipid ratios have been shown to inhibit PMCA activity [47, 79, 80]. On the other hand, since liver expresses

the splice variant *pmca1* [69, 81], splicing at this site affects both the sequence contiguous to the phospholipid-sensitive region and also the sequence involved in PMCA inhibition [67].

Inhibition of liver PMCA

Taking advantage of the inhibitory effect of vanadate on the *P*-type class of ion motive ATPases [82, 83], this compound has been used to characterize liver PMCA. In presence of millimolar Mg^{2+} and K^+, both Ca^{2+}-ATPase and ^{45}Ca-uptake of liver plasma membranes are shown to be very sensitive to vanadate. Values of vanadate $K_i \leq 1\ \mu M$ for ^{45}Ca-uptake have been reported by several authors [36, 37], whereas 1 μM vanadate induced an inhibition of 95% of Ca^{2+}-ATPase activity [38]. When ^{45}Ca-uptake was assayed in absence of K^+ but using millimolar Mg^{2+}, vanadate K_i increased up to 20 μM [17]. A diminished inhibitory effect of vanadate was observed when Ca^{2+}-ATPase was assayed using micromolar levels of Mg^{2+} [24]. In absence of both Mg^{2+} and K^+, vanadate presents no effects even at 2 mM concentration [41, 42]. Erythrocyte Ca^{2+}-ATPase activity and ^{45}Ca-uptake were maximally inhibited by vanadate when incubated with millimolar Mg^{2+} and K^+ showing a $K_i = 5\ \mu M$ [84, 85]. Therefore, erythrocyte and liver PMCA again showed no differences toward vanadate sensitivity.

Lanthanum (La^{3+}) specifically inhibits turnover of the phosphorylated catalytic intermediate of PMCA, producing a decrease in both Ca^{2+}-ATPase and ^{45}Ca-uptake. Because it has no effect on other P-type ion-transport ATPase, it allows identification of PMCA even in crude membrane fractions [82, 86–89]. Lead (Pb^{2+}) has been also shown to inhibit erythrocyte PMCA interfering with the formation of phosphorylated intermediates [90, 91]. On the other hand, Ca^{2+}-dependent hydroxylamine and La^{3+}-sensitive protein phosphorylation have been assayed by several authors using liver plasma membrane vesicles [17, 24, 26, 38]. Molecular weight of the phosphorylated PMCA intermediate varies from 105 [17] and 200 kD [24], with intermediate values of 135 [36] to 140 kD [38] for the phosphoenzyme from basolateral plasma membrane vesicles. Molecular weight of the phosphorylated intermediate determined in erythrocyte plasma membrane vesicles using La^{3+} corresponds to 140 kD [88]. Again, this value is similar to the apparent molecular weight of the liver phosphoenzyme detected in membrane vesicles from the basolateral domain obtained in presence of protease inhibitors [38]. Because endogenous proteolytic activity was not blocked during isolation and purification of plasma membrane proteins in many of the studies performed to date, it appears very likely that phosphoproteins showing low molecular weight might represent proteolytic products of phosphorylated PMCA.

Effect of exogenous CaM, anti-CaM drugs and novel molecules on PMCA

Because ^{45}Ca-uptake and Ca^{2+}-ATPase activity present in erythrocyte ghosts are stimulated 3-5 fold by exogenous CaM and this stimulation is blocked by several anti-CaM drugs [1, 2], these parameters also have been assayed to characterize liver Ca^{2+}-ATPase. It has been commonly observed that liver Ca^{2+}-ATPase and ^{45}Ca-uptake are not stimulated by addition of CaM in both plasma membrane vesicles [24, 26, 32, 38, 41, 42], nor are they inhibited by anti-CaM drugs such as R24571 [39], trifluoroperazine (TFP) and W7 [26, 39, 41]. These properties were also observed with the partially purified enzyme using gel filtration chromatography [15–19].

Several factors might explain lack of stimulation by exogenous CaM; first, PMCA could have been activated by endogenous phosphatidylserine [13, 14], an acidic phospholipid known to activate erythrocyte PMCA [92]. Second, because no protease inhibitors were used during membrane and protein purification, elimination of the autoinhibitory sequence by endogenous proteases might have activated the PMCA [76, 77, 93]. Although TFP could have blocked activation of PMCA by proteolysis, by phosphatidylserine and by CaM [94], the concentration required to achieve this was higher than the level normally used [26]. The compound R24571 is able to remove the stimulation induced by phosphatidylserine if CaM is absent [94]. Mellitin, the anti-CaM peptide, inhibited Ca^{2+}-ATPase activity up to 80%, suggesting a direct interaction between mellitin and PMCA [24].

V_{max} and Ca^{2+} affinity of PMCA are increased by PKA and PKC phosphorylation [reviewed in 3, 95]. All PMCA isoforms show the sequence for PKC phosphorylation that occurs at a single threonine in the CaM binding domain [96]. Phosphorylation of serine 1178 by PKA has also been shown [97]; nonetheless, this site is only present in the PMCA1 isoform [3]. In different cell types expressing PMCA1 isoform, Ca^{2+}-ATPase activity was stimulated by PKA and PKC [98–100]; however, activation of Ca^{2+}-ATPase activity by PKC or PKA has not been observed in liver PMCA. The membrane microenvironment also has been postulated as a factor involved in the refractory behavior of hepatocytes toward PKC activation [95].

Regucalcin has been suggested as a stimulating protein of liver Ca^{2+}-ATPase [101]. Experimental evidence indicates that this protein interacts with membrane lipids and with sulfhydryl groups contained in Ca^{2+}-ATPase. It potentiates the effects of DTT and protects liver PMCA from N-ethylmaleimide (NEM) inhibition [102–104]. Regucalcin action is also dependent on intact membrane structure, because digitonin-treated membranes do not recover normal activity by incubation with this protein [102–104]. It has also been postulated as a regulatory molecule in the Ca^{2+} signaling pathway in regenerating liver [104, 105] as well

as in hepatoma cells [106, 107]. In regenerating liver, an important increase in Ca^{2+} content and elevated Ca^{2+}-ATPase activity were observed between 12 and 48 h after hepatectomy in comparison with sham-operated animals [104]. These changes appear to be followed concomitantly by an enhanced regucalcin mRNA expression [105]. Regucalcin mRNA and its translated protein have been detected [107] in hepatoma HepG2 cells.

The finding of new molecules able to activate or inhibit liver PMCA, suggests that this enzyme is modulated in a way different from that observed with erythrocyte PMCA [15, 16]. For instance, presence of a temperature-sensitive activator that is not inhibited by TFP has been postulated [15]. Moreover, an inhibitory protein of liver PMCA has been shown to mediate both Mg^{2+} and glucagon effects [16, 23]. These results raise new questions related with regulation of liver PMCA by hormones, a phenomenon that will be discussed later.

On the other hand, the protease known as calpain that activates PMCA by removing the autoinhibitory domain [76, 77], in the erythrocyte promotes cleavage of the enzyme to a 124 kD protein product that becomes maximally activated [77]. This neutral Ca^{2+}-activated protease found in rat and human liver [93] also has been involved in a wide range of pathologies [108].

Molecular forms expression of liver PMCA

PMCA is encoded by four isogenes located in different chromosomes: *pmca1* is located in chromosome 12, *pmca2* in chromosome 3, *pmca3* in chromosome X, and *pmca4* in chromosome 1 [109−112]. All genes code for a protein with a molecular weight between 127 and 135 kD [reviewed in 3, 65, 113] and contains 10 transmembrane domains connected by three cytoplasmic loops [3]. The first loop connects transmembrane domains 2 and 3 and contains the phospholipid interacting sequence. The second cytoplasmic loop connects transmembrane domains 4 and 5 and contains the catalytic site. The third intracytoplasmic loop constitutes the regulatory region of PMCA because it contains the calmodulin-binding domain, the phosphorylation sites, and the C terminus of the protein with the PDZ domain. PMCA variants are basically different in CaM binding-site length and in sequence at the carboxy end [reviewed in 3, 65, 113, 114].

A large diversity of rat and human PMCA isoforms have been described as products of alternative splicing in two main splicing sites, known as sites A and C [115, 116]. Site A is located in the vicinity of the phospholipid-responsive domain toward the 5′-end. The four encoding genes, with the exception of gene 1, produce alternative isoforms at this site. Three exons are involved in alternative splicing of *pmca2* gene, while only one is involved in *pmca3* and *pmca4* genes. Site C is very near the 3′-end of the PMCA molecule in the calmodulin (CaM) binding site. The alternative splicing process at this site is complex due to variants generated by internal donor splice sites [115, 116]. For instance, although only one exon is alternatively processed in the *pmca1* gene, it includes four internal splicing donor sequences. In the case of *pmca2* and *pmca3* two exons are involved, and in the case of *pmca4* a single exon is alternatively spliced. A *pmca4d* variant originated from an internal donor sequence in human and rat cardiac muscle, as well as in mouse C2C12 and rat Sol8 myotubes has been described [117]. Kinetic constants of several PMCA isoforms have been determined in different expression systems showing a great variability depending on the encoding gene (Table 1).

Nearly all tissues expressing PMCA proteins codified by *pmca1* and *pmca4* are considered housekeeping genes, while *pmca2* and *pmca3* distribution follows a tissue-specific pattern [66, 79, 122, 123]. Recent information obtained from null PMCA1 and PMCA4 mutants in mice, indicates a major housekeeping function for PMCA1 in comparison with PMCA4, considering that only homozygous mutants for PMCA1 produce unviable embryos [124, 125]. Housekeeping function is understood as the central and most important function for the PMCA1 form in extrusion of calcium from the cell.

Null mutants for PMCA4 are viable and female $PMCA4^{-/-}$ mice are fertile in contrast with male $PMCA4^{-/-}$, which are infertile due to deficiency in sperm motility [124, 126]. In general, the observation that human PMCA1 is more abundant than PMCA4 according to corresponding mRNAs expression [66, 122, 123] or protein expression patterns [79], supports the possibility that PMCA1 indeed plays a preponderant housekeeping role.

At present, we do not possess comprehensive knowledge with regard to any pathology related with defects present in one or several PMCA genes. Nevertheless, evolutionary conservation of these Ca^{2+}-ATPases suggests that these are essential in the survival of vertebrate species [127]. Important evidence supporting the hypothesis that there is no redundancy of functions in the different PMCA isoforms has been obtained from knockout mice. It has been reported that these knockout mice, in which PMCA2 mRNA expression has been molecularly cancelled, result with severe hearing defects [128]. This physiologic effect also has been observed in mice experiencing spontaneous mutations in the PMCA2 gene involving a single substitution that exchanges Gly for Ser in the small cytoplasmic loop [129, 130] or Glu for Lys within a conserved transmembrane domain [121, 132].

Table 1. Kinetic parameters of four PMCA isoforms measured in different expression systems

PMCA isoform	**1b**	**2a**	**2b**	**2w**	**2x**	**2z**	**3a**	**4a**	**4a**	**4b**	**4b**	**4b**	**4b**	**4b**
Reference	120	71	71	118	118	118	121	119	70	119	120	70	118	71
Expression system	Sf9	COS-7	COS-7	Sf9	Sf9	Sf9	CHO	Sf9	COS-1	Sf9	Sf9	COS-1	Sf9	COS-7
Kinetic parameter														
Apparent K*m* Ca^{2+} (μM)														
Without CaM	7*			∼0.5*	∼0.5*	∼ 0.5*					7*		∼ 2.0*	
Plus CaM	2*	0.09	0.06	∼0.5*	∼0.5*	∼ 0.5*	0.3		0.54		2*	0.25	∼ 0.5*	0.16
Apparent K*m* ATP (nM)														
Intermediate phosphorylation	53			ND	200	ND					340		800	
Apparent K*m* CaM (nM)	2.5	8.4	2.1	10	10	10	5–10	∼650	126	∼35	5.2	18	50—100	9.8
Apparent CaM binding affinity	3*										3*			
Ca^{2+} ATPase V_{max} (nmoles Pi/mg/min)														
Without CaM		ND	ND	75	80	39	210*		ND				45	
Plus CaM		ND	ND	190	210	71	700*		ND				190	
^{45}Ca-uptake (nmoles Ca^{2+}/mg/min)	ND													
Without CaM		3.67	4.21	ND	ND	ND	ND	ND		ND	ND			1.45
Plus CaM		7.83	5.87	ND	ND	ND	ND	ND		ND	ND			6.15

ND = Data not determined. * Data calculated from graphs. CaM, calmodulin.

Liver pmca gene expression during normal development and pathological conditions

Human liver. Gene expression of *pmca* genes has been studied in human liver at fetal and adult stages. Spliced variants *hpmca1b, hpmca2*, and *hpmca4a* were detected in 20–22 week-old human male fetus by RT-PCR and by Southern blot [122]. In human adult liver, the most abundant site A splice variants correspond to *hpmca1x* (70%) and *hpmca4x* (28%,) followed by *hpmca2x* and *2w*, representing $\leq$ 1%. Isoforms spliced at site C correspond to *hpmca1b*, *hpmca4b*, and *hpmca2b*, representing 70, 28, and <2%, respectively [68] (Table 2). PCR amplification using degenerate mixed primers and Northern blot analysis indicated that *pmca4* was the most abundant isoform in human liver followed by *pmca1*, while the least abundant was *pmca2* [133].

Mouse and rat liver. Using *in situ* hybridization techniques, it has been shown that *pmca1* is widely expressed in mouse embryos at 9.5 days *post coitum (pc)*, while *pmca4* was detected in fetus at 12.5 days *(pc)* with high expression in liver [123]. Recent work employing RT-PCR analysis and exploring simultaneously relative mRNA levels of *pmca1* and *pmca4* in mutants $PMCA4^{-/-}$ showed *pmca1* mRNA only in adult mouse liver [124]. RT-PCR results using fetal (13 and 17 days *pc*), newborn and normal adult rat liver indicated high expression of *pmca* variants spliced at sites A and C [70]. In adult rat liver and in isolated hepatocytes, the most abundant variant was *pmca1*, and *pmca2w* was clearly detected [133]. Interestingly, *pmca4* was not detected in rat liver [131], although variant *pmca4b* has been detected by other groups [70] (Table 2). These results should be taken

Table 2. pmca transcripts detected in rat and human liver by RT-PCR

	pmca1		*pmca2*		*pmca4*	
Species	SITE A	SITE C	SITE A	SITE C	SITE A	SITE C
Rat*	1x	1b	2w	2b	4x	4b
			2x		4z	4a
						4d
Human**	1× (70%)	1b (70%)	2w	2b	4× (28%)	4b (28%)
			2×			4a

*[69, 70, 133].
**[68, 122, 133].
The isoforms spliced in sites A and C are shown in decreasing order of abundance. Percentages show the most abundant isoforms detected in human liver [68].

with caution because it might be that relative amounts are below detection level. Moreover, certain specific conditions not well understood might be required to trigger their expression. These concepts support the relevance of PMCA in liver and open many questions related to the way *pmca* gene expression is controlled.

Kinetic experiments performed with plasma membranes isolated from regenerating liver as well as neoplastic cells of the murine hepatocarcinoma AS-30D, maintain much lower plasma membrane Ca^{2+}-ATPase activity than normal liver, while maintaining similar calcium transport. It was suggested that a more efficient mechanism to regulate calcium transport through modulation of an ATP/Pi exchange process in the catalytic cycle of the plasma membrane Ca^{2+}-ATPase present in AS-30D cells might be operating [27]. Because presence or absence of a specific isoform in the plasma membrane might explain these results, a detailed study of housekeeping the PMCA1 isoform in normal, regenerating and neoplastic liver cells in presence of the other isoforms, becomes of paramount relevance [70].

RT-PCR experiments employing AS-30D cells indicate that *pmca1x* and *pmca1b* were the most abundant variants. These isoforms were also observed in normal hepatocytes from fetal and adult rat as well as during regeneration, results that further support the housekeeping role of *pmca1* gene [68]. *pmca4a* and *pmca4d* were not detected in hepatoma AS-30D cells and *pmca4b* and *pmca2w* showed a decreased expression [70].

Proliferation-induced normal adult hepatocytes did not show alterations in *pmca1x, pmca1b*, *and pmca4b* expression from 2-17 days after surgery. The level of these isoforms was similar to the expression level found in growing fetal rat liver. The *pmca2w* variant as well as *pmca4x* and *pmca4z* showed lower but constant expression levels throughout liver regeneration. It is interesting to note that after 4 days of hepatectomy *pmca4a* and *pmca4d* expression was triggered; however, these splice variants are not expressed during fetal development [70].

Although at present available information as to how liver PMCA1 expression becomes regulated remains fragmented, it has been shown that hormones and agonists can induce PMCA1 expression via multiple second messenger pathways including PKC, cAMP, and Ca^{2+} as well as through participation of the c-fos and jun-proto-oncogenes [98, 99]. The second messenger pathway and sensitivity to hormones appear to be dependent on cell type. Because in the liver *pmca1* corresponds to the predominant isoform, future work is needed to determine how expression of this gene and its splicing pattern is controlled. Nonetheless, it is known that prolonged hormone treatments induce *pmca* mRNA [98] accumulation, where characteristics of the PMCA promoter correspond to a housekeeping gene [134].

At protein level, PMCA1 is the sole isoform detected to date in adult rat liver by Western blot [81]. Why *pmca2* and *pmca4* splice transcripts variants seem not to evolve to mature mRNAs? Which mechanisms control their translation? Are these splice variants expressed by non-parenchymal cells? These are open questions for which answers are still needed.

Hormonal regulation of liver PMCA

PMCA regulation has been studied by different groups based on hormone-dependent free Ca^{2+} concentration changes in liver cells [18, 23, 31, 135−144]. Vasopressin added directly to liver plasma membrane vesicles does not modify Ca^{2+}-ATPase activity [31]. However, when the liver is perfused with vasopressin at different concentrations from 10^{-10} to 10^{-7} M, gradual inhibition of ^{45}Ca-uptake is induced [31]. This effect is also induced by perfusion with other Ca^{2+}-mobilizing hormones such as 10^{-5} M epinephrine and 10^{-7} M angiotensin II, but not by 10^{-7} M glucagon [31]. Isolated hepatocytes incubated with vasopressin at nanomolar concentration also show a decrease in plasma membrane Ca^{2+}-ATPase activity, whereas insulin and 10^{-10} M glucagon do not modify enzyme activity [136]. Later, it was found that the C-proteolytic product of glucagon, glucagon-(19−29) also known as miniglucagon [137], corresponds to a better inhibitor than glucagon itself [138, 139] (Fig. 1). Nanomolar concentrations of miniglucagon inhibit Ca^{2+}-ATPase with a mechanism associated with heterotrimeric G proteins without adenylate cyclase activation [139−141]. A cholera toxin-sensitive $G\alpha_s$ protein has been shown as mediator of the inhibition [142]. Furthermore, a Gs protein also mediates the inhibitory effect of human PTH, calcitonin [143], and endothelin B [144] on rat liver PMCA. These results support early work related with regulation of liver Ca^{2+}-ATPase by membrane-associated proteins [15, 16, 18] (Fig. 1).

Agonist-induced free Ca^{2+} transients and PMCA in hepatocytes

Changes in hepatocyte intracellular Ca^{2+} levels induced by an agonist-receptor complex signaling through the phosphoinositide pathway, together with the spatial and temporal organization of cytoplasmic calcium signals, have been studied in isolated hepatocytes as well as in whole liver [145−147]. Vasopressin, angiotensin II, noradrenaline [148] as well as glucagon, ADP and ATP [149−152] promote a train of spikes from basal 100–150 nM Ca^{2+} to peak 600 nM free Ca^{2+} concentration. Time-course of these Ca^{2+} transients is agonist-specific, i.e, vasopressin induces spikes of 10-sec duration, whereas ATP induces oscillations of up to 90-sec duration using isolated hepatocytes. Recovery rate of

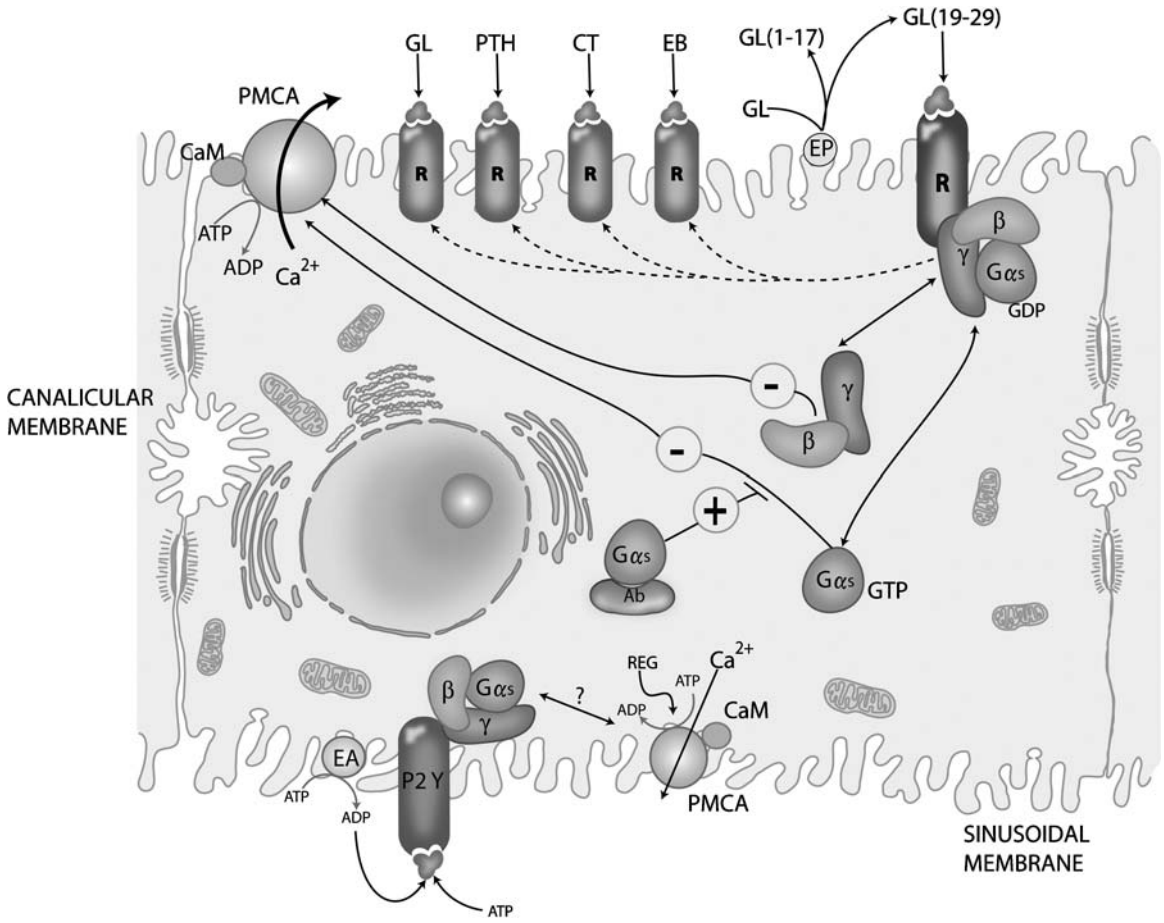

Fig. 1. Regulation of plasma membrane Ca^{2+}-ATPase (PMCA) activity in the hepatocyte. Glucagon (GL) and its product miniglucagon (GL 19–29) generated by an intramembrane endonucleopeptidase (EP) that cleaves specifically between Arg^{17} and Arg^{18}, have been involved in regulation of hepatocyte PMCA. Glucagon, miniglucagon, parathyroid hormone (PTH), endothelin B (EB), and calcitonin (CT), have been shown to inhibit liver PMCA activity through G protein action, independently of adenyl cyclase activation. GTP promotes dissociation of the heterotrimeric G protein, releasing subunits α and $\beta\gamma$ that inhibit PMCA activity. Antibodies anti$G\alpha_s$ ($G\alpha_s$Ab) restore PMCA activity. The presence of an ecto-ATPase (EA) in the plasma membrane of the sinusoidal domain, releases ADP from ATP and becomes a ligand for P2Y purinergic receptors known to interact with G proteins, that in turn modulate PMCA activity. The role of G proteins upon cytosolic Ca^{2+}-binding proteins, such as calmodulin (CaM) and regucalcin (REG) known to modulate PMCA activity, represent also an important regulatory mechanism of PMCA.

individual Ca^{2+} transients is clearly different for each agonist but transient frequency is dependent on agonist concentration as well as on extracellular calcium concentration [145, 146]. Heterotrimeric G proteins of the Gαq class couple the plasma membrane agonist-receptor complex to isoforms of phospholipase C (PLC) to activate production of inositol-1, 4,5-triphosphate (IP3) and diacylglycerol (DAG) through hydrolysis of phosphatidylinositol 4,5-biphosphate. IP3 binds to its receptor in the endoplasmic reticulum membrane and activates release of calcium to the cytosol [153] (Fig. 1). This flux together with Ca^{2+}entry through the plasma membrane [154] explain the first phase of Ca^{2+} transients. The falling phase of the spike involves re-uptake of calcium by SERCA and/or by Ca^{2+} being pumped out of the hepatocyte by PMCA [155]. It is noteworthy that the Na^{+}/Ca^{2+} (NCX) exchanger appears to play only a minor role in regulation of Ca^{2+} in rat hepatocytes [156,157]; however, some evidence points to a possible role for this exchanger under oxidative stress in human hepatoma cells (HepG2) [158].

A 50% PMCA inhibition carried out by miniglucagon in rat hepatocytes [159], shows Ca^{2+} transients elicited by ATP or by phenylephrine. The recovery phase of these agonist-induced Ca^{2+}-transients was not affected by PMCA activity inhibition. Moreover, PMCA inhibition supported prolonged generation of Ca^{2+}oscillations in absence of extracellular calcium [159]. These results suggest that hormone-induced PMCA inhibition could increase Ca^{2+} mobilization elicited by different agonists in hepatocytes

[31]. Because frequency of Ca^{2+} oscillations also depends on extracellular level of calcium and on rate of Ca^{2+} influx [160, 161], an interaction appears possible between hepatocyte extracellular calcium-sensing receptor [162] and proteins controlling PMCA activity.

Glycogen metabolism and bile secretion appear to be under control of these Ca^{2+} oscillations [135, 162]. Agonist-induced Ca^{2+} waves originate from a specific site in the apical region, where IP_3R is localized [43], and propagate to basolateral region and nucleus of hepatocytes [163−167]. Ca^{2+} oscillations are propagated to neighbor hepatocytes and eventually to whole liver by diffusion of IP_3 and/or Ca^{2+} itself through gap-junctions and also to the nucleus of hepatocytes [164, 168, 169]. Activation of IP_3R and ryanodine receptors (RyR) mediates propagation of the Ca^{2+} signal (Fig. 2). Based on pharmacologic and functional evidence

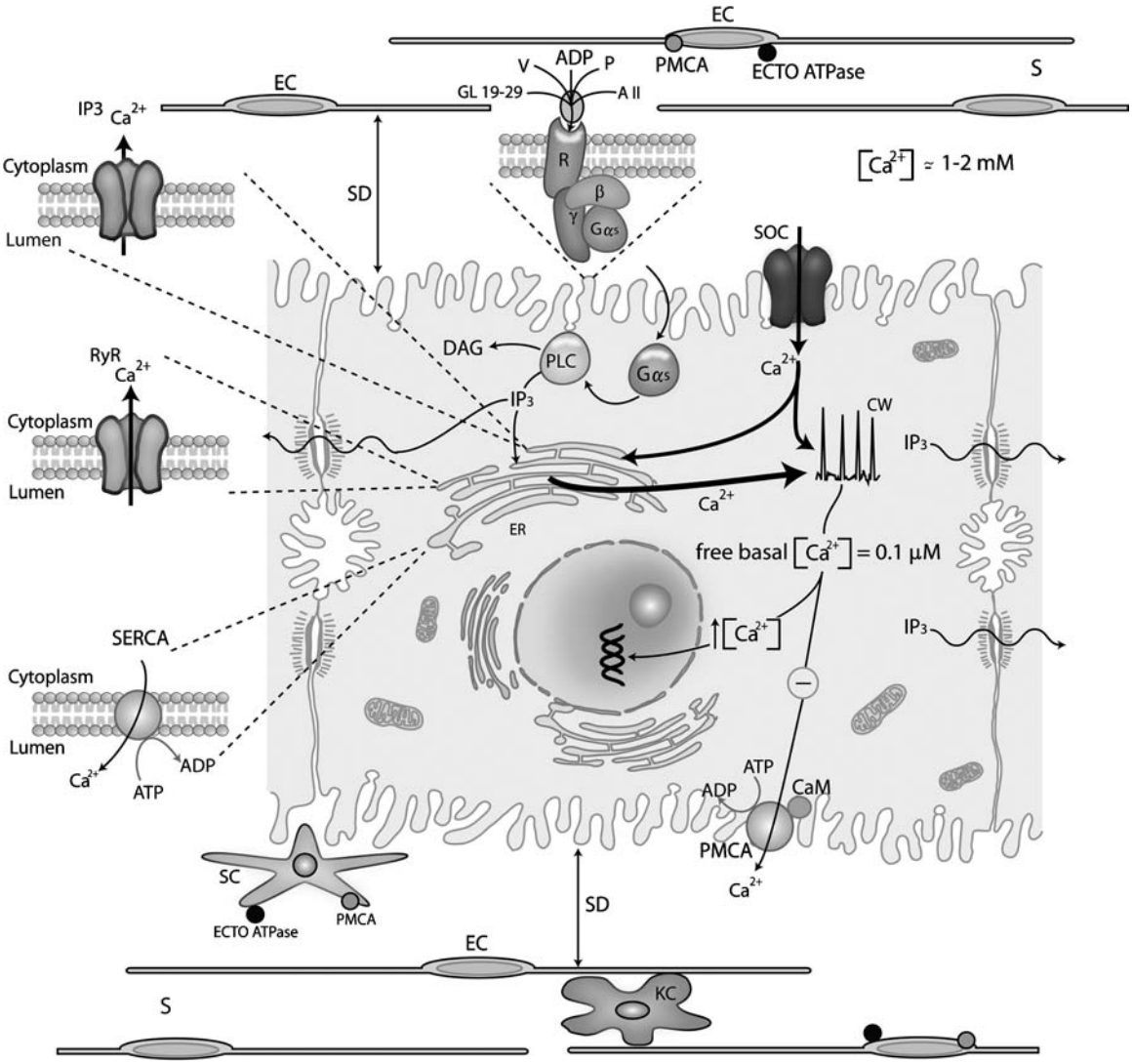

Fig. 2. Calcium homeostasis in the hepatocyte. Vasopressin (V), angiotensin II (AII), phenylephrine (P), miniglucagon (GL 19-29), and ADP induce Ca^{2+} transients by a common mechanism involving inositol-1,4,5-triphosphate (IP3). The basal Ca^{2+} level $\approx$ 100 nM, increases up to 700 nM after release of Ca^{2+} from the endoplasmic reticulum (ER). Focal activation of IP3 receptors (IP_3R) localized in the ER induces efflux of Ca^{2+} from ER stores. Ryanodine receptors (RyR) localized in ER membranes also release Ca^{2+} to the cytosol. A Ca^{2+} concentration decrease in the ER activates plasma membrane store-operated Ca^{2+} channels (SOCs), that in turn activates the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA). Propagation of the Ca^{2+} signal within a single hepatocyte and towards neighbor hepatocytes through gap junctions, is mainly mediated by diffusion of IP_3. An increase in free basal cytoplasmic Ca^{2+}-concentration stimulates a Ca^{2+} flux into the nucleus, inducing changes in gene expression and alternative splicing. Kupffer cells (KC) releasing several important growth factors together with stellate cells (SC), play also an important role in hepatocyte calcium homeostasis. Endothelial cells (EC), Space of Disse (SD), Sinusoids (S), and Ca^{2+}-waves (CW).

it seems that non-muscle tissues such as liver might present RyR [170, 171]. Heterogeneity in agonist receptor density as well as in hepatocyte sensitivity (pericentral hepatocytes are more sensitive to vasopressin than periportal hepatocytes, but the opposite is true for ATP), leads to directional activation of Ca^{2+}-dependent processes [165−167]. Because PMCA plays a major role in regulation of cytosolic Ca^{2+} levels, a gradient of PMCA activity could be associated with the direction of Ca^{2+}-waves. Whether these Ca^{2+} waves regulate PMCA gene expression or splice variant isoforms formation deserves future work [169, 172] (Fig. 2).

On the other hand, cytoplasmic Ca^{2+} concentration is increased by 2,5 di-(tertbutyl)-1,4-benzohydroquinone (tBuBHQ) inducing release of Ca^{2+} from ER without IP3R participation [173]. Hepatocytes exposed to vasopressin, angiotensin II or ATP, show increase in efflux of Ca^{2+} and a rapid return to basal Ca^{2+} levels takes place [173−175]. This effect is also induced by NaF and $AlCl_3$, indicating participation of G-proteins in calcium efflux pathway stimulation [176, 177]. Whether adenosine [178] and bile acids [179, 180], which induce mobilization of Ca^{2+} from ER without IP_3R participation stimulates PMCA activity, remains to be defined.

The results presented previously further confirm the role of PMCA in control of basal free Ca^{2+}concentration and also demonstrate PMCA interaction with G protein-coupled receptors. Since hepatocyte PMCA activity is negatively regulated by G proteins, it appears very likely that specific G protein signaling regulators involved in control of signal termination and in the delay observed between Ca^{2+} oscillations [181] are also involved in PMCA regulation. Subtype-selective G protein coupling to different effectors [182, 183] including PMCA, may also provide new clues to understanding PMCA regulation.

Perspectives

Many functions in liver are under regulation through calcium-mediated processes. For instance, glucose production responds to a Ca^{2+} increase induced by glucagon, as well as processes involved in bile secretion, such as vesicular trafficking and canalicular exocytosis. Likewise, Ca^{2+} presents an important role in cell growth regulation and consequently in events related with apoptotic and necrotic death [184]. Due to the importance of calcium homeostasis in liver physiology, precise control of its intracellular concentration is critical for survival and normal functioning of the hepatocyte [185] (Fig. 2). To achieve normal calcium homeostasis, PMCA actively works in coordination with the NCX located in plasma membrane as well as intracellular systems located in sarcoendoplasmic reticulum, mitochondria, and nucleus. Among these systems, PMCA presents the highest affinity for Ca^{2+} and therefore the best capacity to respond to critical cytosolic Ca^{2+} fluctuations. It is interesting to note that recent information emphasizes that PMCA activity is sufficient to maintain Ca^{2+}-homeostasis in cardiac specific NCX knockout mice [186]. In plasma membranes, the role of the store-operated Ca^{2+} channels (SOCs) is also important to restore the intracellular pool after massive release of calcium from the endoplasmic reticulum [147, 187, 188]. Non-parenchymal cells such as endothelial cells, Kupffer cells, lymphocytes, and stellate cells should also be taken into consideration, since they most probably contribute importantly to maintenance of calcium homeostasis in liver. Since the reason for the existence of such a large number of PMCA isoforms is still not well-understood, identification of isoform-specific interactions with partner proteins might provide a clue [189].

A question that still remains to be answered is related with the fact that we do not know whether *pmca*2 and *pmca*4 transcripts are efficiently translated into proteins in liver, or whether the amount of protein is so low that these transcripts can not be quantified and are therefore difficult to measure. The signaling cascade controlling these genes and the detailed study of their promoter regions also remain to be defined. Moreover, the design of specific inhibitors of Ca^{2+}-ATPase will aid in extending our understanding of the properties of these P-type ATPases. In this respect, the peptide caloxin 3A1 was described recently as an efficient inhibitor of plasma membrane Ca^{2+}-ATPase by means of interaction with the third extracellular region of PMCA (PED_3) [190].

Although based on evidence, the answer for our initial question might be yes, a systematic study of the different PMCA isoforms expressed in normal liver as well as in regenerating and neoplastic liver cells, will undoubtedly help to reveal hormone-related control mechanisms associated to calcium homeostasis in this tissue. New strategies directed toward definition of the correlation between isoform-structure and function, will also contribute to solve the mystery within which for many years the liver Ca^{2+}-ATPase has been shrouded.

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References

1. Schatzmann HJ: The plasma membrane calcium pump of erythrocytes and other animal cells. In: Carafoli E (ed). Membrane

Transport of Calcium. Academic Press, London, 1982, pp. 41–108
2. Rega AF: From the discovery of the Ca^{2+}-pump in plasma membranes to the demonstration of its ubiquitousness. In: The Ca^{2+} pump of plasma membranes. CRC Press, Inc. Boca Raton, FL, 1986, pp 45–58
3. Carafoli E: Biogenesis: Plasma membrane calcium ATPase: 15 years of work on the purified enzyme. FASEB J 8: 993–1002, 1994
4. Wolf HU: Purification of the Ca^{2+}-dependent ATPase of human erythrocyte membranes. Biochim Biophys Acta 219: 521–524, 1970
5. Wolf HU, Dieckvoss G, Lichtner R: Purification and properties of high-affinity Ca^{2+}-ATPase of human erythrocyte membranes. Acta Biol Med Ger 36: 847–858, 1977
6. Haaker H, Racker E: Purification and reconstitution of the Ca^{2+} -ATPase from plasma membranes of pig erythrocytes. J Biol Chem 254: 6598–6602, 1979
7. Niggli V, Penniston JT, Carafoli E: Purification of the $(Ca^{2+}-Mg^{2+})$-ATPase from human erythrocyte membranes using a calmodulin affinity column. J Biol Chem 254: 9955–9958, 1979
8. Gietzen K, Tjcka M, Wolf HU: Calmodulin affinity chromatography yields a functional purified erythrocyte $(Ca^{2+}+Mg^{2+})$-dependent adenosine triphosphatase. Biochem J 189: 81–88, 1980
9. Neville DM: The isolation of a cell membrane fraction from rat liver. J Biophys Biochem Cytol 8: 413–422, 1960
10. Neville DM: Isolation of an organ specific protein antigen from cell-surface membrane of rat liver. Biochim Biophys Acta 154: 540–552, 1968
11. Ray TK: A modified method for the isolation of the plasma membrane from rat liver. Biochim Biophys Acta 196: 1–9, 1970
12. Pilkis SJ, Exton JH, Johnson RA, Park CR: Effects of glucagon on cyclic AMP and carbohydrate metabolism in liver from diabetic rats. Biochim Biophys Acta 343: 250–267, 1974
13. Wisher MH, Evans HW: Functional polarity of rat hepatocyte surface membrane. Isolation and characterization of plasma-membrane subfractions from the blood-sinusoidal, bile-canalicular and contiguous surfaces of the hepatocyte. Biochem J 146: 375–388, 1975
14. Meier PJ, Sztul ES, Reuben A, Boyer JL: Structural and functional polarity of canalicular and basolateral plasma membrane vesicles isolated in high yield from rat liver. J Cell Biol 98: 991–1000, 1984
15. Lotersztajn S, Hanoune J, Pecker F: A high affinity calcium-stimulated magnesium-dependent ATPase in rat liver plasma membranes. Dependence on an endogenous protein activator distinct from calmodulin. J Biol Chem 256: 11209–11215, 1981
16. Lotersztajn S, Pecker F: A membrane-bound protein inhibitor of the high affinity Ca ATPase in rat liver plasma membranes. J Biol Chem 257: 6638–6641, 1982
17. Chan KM, Junger KD: Calcium transport and phosphorylated intermediate of $(Ca^{2+}$-$Mg^{2+})$-ATPase in plasma membranes of rat liver. J Biol Chem 258: 4404–4410, 1983
18. Lotersztajn S, Epand RM, Mallat A, Pecker F: Inhibition by glucagon of the calcium pump in liver plasma membranes. J Biol Chem 259: 8195–8201, 1984.
19. Lin S-H, Fain JN: Purification of $(Ca^{2+}$-$Mg^{2+})$-ATPase from rat liver plasma membranes. J Biol Chem 259: 3016–3020, 1984
20. Lin S-H: Novel ATP-dependent calcium transport component from rat liver plasma membranes. The transporter and the previously reported $(Ca^{2+}$- $Mg^{2+})$-ATPase are different proteins. J Biol Chem 260: 7850–7856, 1985
21. Lin S-H: The rat liver plasma membrane high affinity $(Ca^{2+}$- $Mg^{2+})$-ATPase is not a calcium pump. J Biol Chem 260: 10976–10980, 1985
22. Pecker F, Lotersztajn S: Fe^{2+} and other divalent metal ions uncouple Ca^{2+} transport from $(Ca^{2+}$- $Mg^{2+})$-ATPase in rat liver plasma membranes. J Biol Chem 260: 731–735, 1985
23. Lotersztajn S, Mallat A, Pavoine C, Pecker, F: The inhibitor of liver plasma membrane $(Ca^{2+}$- $Mg^{2+})$-ATPase. Purification and identification as a mediator of glucagon action. J Biol Chem 260: 9692–9698, 1985
24. Bachs O, Famulski KS, Mirabelli F, Carafoli E: ATP-dependent Ca^{2+} transport in vesicles isolated from the bile canalicular region of the hepatocyte plasma membrane. Eur J Biochem 147: 1–7, 1985
25. Pavoine C, Lotersztajn S, Mallat A, Pecker F: The high affinity $(Ca^{2+}$-$Mg^{2+})$-ATPase in liver plasma membrane is a Ca^{2+} pump. Reconstitution of the purified enzyme into phospholipid vesicles. J Biol Chem 262: 5113–5117, 1987
26. Evers C, Hugentobler G, Lester R, Gmaj P, Meier P, Murer H: ATP-dependent Ca^{2+} uptake and Ca^{2+}–dependent protein phosphorylation in basolateral liver plasma membranes. Biochim Biophys Acta 939: 542–550, 1988
27. Mas-Oliva J, Pérez-Montfort R, Cárdenas-García M, Rivas-Duro M: Altered coupling states between calcium transport and (Ca^{2+}, Mg^{2+})-ATPase in the AS-30D ascites hepatocarcinoma plasma membrane. Mol Cell Biochem 100: 39–50, 1991
28. Aronson NN Jr, Touster O: Isolation of rat liver plasma membrane fragments in isotonic sucrose. Methods Enzymol 31: 90–102, 1975
29. Van Amelsvoort JMM, Sips HJ, Van Dam K: Sodium-dependent alanine transport in plasma–membrane vesicles from rat liver. Biochem J 174: 1083–1086, 1978
30. Moore PB, Kraus-Friedmann N: Hepatic microsomal Ca^{2+}–dependent ATPase. Calmodulin-dependence and partial purification. Biochem J 214: 69–75, 1983
31. Prpić V, Green KC, Blackmore PF, Exton JH: Vasopressin-, angiotensin II-, and α_1-adrenergic-induced inhibition of Ca^{2+} transport by rat liver plasma membrane vesicles. J Biol Chem 259: 1382–1385, 1984
32. Epping RJ, Bygrave FL: A procedure for the rapid isolation from rat liver of plasma membrane vesicles exhibiting Ca^{2+}-transport and Ca^{2+}-ATPase activities. Biochem J 223: 733–745, 1984
33. Epping RJ, Bygrave FL: Inhibition by orthovanadate of ATP-dependent Ca^{2+} transport in microsomes isolated from rat liver. Membr Biochem 5: 167–180, 1984
34. Loten EG, Redshaw-Loten JC: Preparation of rat liver plasma membranes in high yield. Anal Biochem 154: 183–185, 1986
35. Birch-Machin MA, Dawson AP: Effects of chelating agents on the Ca^{2+}-stimulated ATPase of rat liver plasma membranes. Biochim Biophys Acta 855: 277–285, 1986
36. Schane FAX, Moore L: Liver plasma membrane calcium transport. Evidence for a Na^{+}-dependent Ca^{2+} flux. J Biol Chem 261: 9886–9889, 1986
37. Birch-Machin MA, Dawson AP: Ca^{2+} transport by rat liver plasma membranes: the transporter and the previously reported Ca^{2+}–ATPase are different enzymes. Biochim Biophys Acta 944: 308–314, 1988
38. Kessler F, Bennardini F, Bachs O, Serratosa J, James P, Caride AJ, Gazzotti P, Penniston JT, Carafoli E: Partial purification and characterization of the Ca^{2+}–pumping ATPase of the liver plasma membrane. J Biol Chem 265: 16012–16019, 1990
39. Kraus-Friedmann N, Biber J, Murer H, Carafoli E: Calcium uptake in isolated hepatic plasma-membrane vesicles. Eur J Biochem 129: 7–12, 1982
40. Coleman R, Michell RH, Finean JB, Hawthorne JN: A purified plasma membrane fraction isolated from rat liver under isotonic conditions. Biochim Biophys Acta 135: 573–579, 1967
41. Iwasa Y, Iwasa T, Higashi K, Matsui K, Miyamoto E: Demonstration of a high affinity Ca^{2+}-ATPase in rat liver plasma membranes. Biochem Biophys Res Commun 105: 488–494, 1982
42. Iwasa T, Iwasa Y, Krishnaraj R: Comparison of the high affinity Ca^{2+}-ATPase and low affinity Ca^{2+}-ATPase in rat liver plasma membranes. Arch Int Pharmacodyn 264: 40–58, 1983

43. Nathanson MH, Burthaler AD, Fallon MB: Multistep mechanism of polarized Ca^{2+} wave patterns in hepatocytes. Am J Physiol 267 G338–G349, 1994
44. Evans WH: Resolving biochemically the hepatocyte's multifunctional pericellular membrane. Trends Biochem Sci 2: 169–171, 1977
45. Evans WH: A biochemical dissection of the functional polarity of the plasma membrane of the hepatocyte. Biochim Biophys Acta 604: 27–64, 1980
46. Yeagle PL: Modulation of membrane function by cholesterol. Biochimie 73: 1303–1310, 1991
47. Ortega A, Santiago-García J, Mas-Oliva J, Lepock JR: Cholesterol increases the thermal stability of the Ca^{2+}/Mg^{2+} ATPase of cardiac microsomes. Biochim Biophys Acta 1283: 45–50, 1996
48. Santiago-García J, Delgado-Coello B, Mas-Oliva J: Thermal analysis of the plasma membrane Ca^{2+}-ATPase. Mol Cell Biochem 209: 105–112, 2000
49. Rega AF: Transport of Ca^{2+} and ATP hydrolysis by the Ca^{2+} pump. In: The Ca^{2+} pump of plasma membranes. CRC Press, 1986, pp. 67–90
50. Lin S-H, Russel WE: Two Ca^{2+}-dependent ATPases in rat liver plasma membrane. The previously purified (Ca^{2+}-Mg^{2+})-ATPase is not a Ca^{2+} pump but an ecto-ATPase. J Biol Chem 263: 12253–12258, 1988
51. Tuana BS, Dhalla NS: Purification and characterization of a Ca^{2+}/Mg^{2+} ecto-ATPase from rat heart sarcolemma. Mol Cell Biochem 81 75–88, 1988
52. Kannan S, Elimban V, Fandrich RR, Kardami E, Dhalla NS: Immunolocalization of the sarcolemmal Ca^{2+}/Mg^{2+} ecto-ATPase (myoglein) in rat myocardium. Mol Cell Biochem 197 187–194, 1999
53. Adachi T, Arai, K, Ohkuma S: A comparative study of (Ca^{2+}-Mg^{2+})-ATPase on the lysosomal membrane and ecto-ATPase on the plasma membrane from rat liver. Biol Pharm Bull 19: 1291–1297, 1996
54. Koike M, Kashiwagura T, Takeguchi N: Gluconeogenesis stimulated by extracellular ATP is triggered by the initial increase in the intracellular Ca^{2+} concentration of the periphery of hepatocytes. Biochem J 283: 265–272, 1992
55. Burnstock G: The past, present and future of purine nucleotides as signaling molecules. Neuropharmacology 36: 1127–1139, 1997
56. Dixon CJ, White PJ, Hall JF, Kingston S, Boarder MR: Regulation of human hepatocytes by P2Y receptors: control of glycogen phosphorylase, Ca^{2+} and mitogen-activated protein kinases. J Pharmacol Exp Ther 313: 1305–1313, 2005
57. Adamo HP, Caride AJ, Penniston JT: Use of expression mutants and monoclonal antibodies to map the erythrocyte Ca^{2+} pump. J Biol Chem 267: 14244–14249, 1992
58. Yokomori H, Oda M, Ogi M, Kamegaya Y, Tsukada N, Nakamura M, Ishii H: Hepatic sinusoidal endothelial fenestrae express plasma membrane Ca^{++} pump and $Ca^{++}Mg^{++}$-ATPase. Liver 20: 458–464, 2000
59. Ogi M, Yokomori H, Inao M, Oda M, Ishii H: Hepatic stellate cells express Ca^{2+} pump-ATPase and Ca^{2+}-Mg^{2+}-ATPase in plasma membrane caveolae. J Gastroenterol 35: 912–918, 2000
60. Ronner P, Gazzotti P, Carafoli E: A lipid requirement for the (Ca^{2+}-Mg^{2+})-activated ATPase of erythrocyte membranes. Arch Biochem Biophys 179: 578–583, 1977
61. Niggli V, Adunyah ES, Carafoli E: Acidic phospholipids, unsaturated fatty acids, and limited proteolysis mimic the effect of calmodulin on the purified erythrocyte Ca^{2+}-ATPase. J Biol Chem 256: 8588–8592, 1981
62. Caroni P, Carafoli E: Regulation of Ca^{2+} pumping ATPase of heart sarcolemma by a phosphorylation/dephosphorylation process. J Biol Chem 256: 9371–9373, 1981
63. Wright LC, Chen S, Roufogalis BD: Regulation of the activity and phosphorylation of the plasma membrane Ca^{2+}-ATPase by protein kinase C in intact human erythrocytes. Arch Biochem Biophys 306: 277–284, 1993
64. Kosk-Kosicka D, Bzdega T: Activation of the erythrocyte Ca^{2+}-ATPase by either self-association or interaction with calmodulin. J Biol Chem 263: 18184–18189, 1988
65. Enyedi A, Sarkadi B, Szasz I, Bot G, Gardos G: Molecular properties of the red cell calcium pump. II. Effect of calmodulin, proteolytic digestion and drugs on the calcium-induced membrane phosphorylation by ATP in inside/out red cell membrane vesicles. Cell Calcium 1: 299–310, 1980
66. Zvaritch E, James P, Vorherr T, Falchetto R, Modyanov N, Carafoli E: Mapping of functional domains in the plasma membrane Ca^{2+} pump using trypsin proteolysis. Biochemistry 29: 8070–8076, 1990
67. Strehler EE, Zacharias DA: Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. Physiol Rev 81: 21–50, 2001
68. Stauffer TP, Hilfiker H, Carafoli E, Strehler EE: Quantitative analysis of alternative splicing options of human plasma membrane calcium pump genes. J Biol Chem 268: 25993–26003, 1993
69. Keeton TP, Burk SE, Shull GE: Alternative splicing of exons encoding the calmodulin-binding domains and C termini of plasma membrane Ca^{2+}-ATPase isoforms 1, 2, 3, and 4. J Biol Chem 268: 2740–2748, 1993
70. Delgado-Coello B, Santiago-García J, Zarain-Herzberg A, Mas-Oliva J: Plasma membrane Ca^{2+}-ATPase mRNA expression in murine hepatocarcinoma and regenerating liver cells. Mol Cell Biochem 247: 177–184, 2003
71. Kessler F, Falchetto R, Heim R, Meili R, Vorherr T, Strehler EE, Carafoli E: Study of calmodulin binding to the alternatively spliced C-terminal domain of the plasma membrane Ca^{2+} pump. Biochemistry 31: 11785–11792, 1992
72. Enyedi A, Verma AK, Heim R, Adamo HP, Filoteo AG, Strehler EE, Penniston JT: The Ca^{2+} affinity of the plasma membrane Ca^{2+} pump is controlled by alternative splicing. J Biol Chem 269: 41–43, 1994
73. Elwess NL, Filoteo AG, Enyedi A, Penniston JT: Plasma membrane Ca^{2+} pump isoforms 2a and 2b are unusually responsive to calmodulin and Ca^{2+}. J Biol Chem 272: 17981–17986, 1997
74. Enyedi A, Elwess NL, Filoteo AG, Verma AK, Paszty K, Penniston JT: Protein kinase C phosphorylates the "a" forms of plasma membrane Ca^{2+} pump isoforms 2 and 3 and prevents binding of calmodulin. J Biol Chem 272: 27525–27528, 1997
75. Vorherr T, Kessler T, Hofmann F, Carafoli E: The calmodulin-binding domain mediates the self-association of the plasma membrane Ca^{2+} pump. J Biol Chem 266: 22–27, 1991
76. Wang KKW, Villalobo A, Roufogalis BD: Activation of the Ca^{2+} -ATPase of human erythrocyte membrane by an endogenous Ca^{2+} -dependent neutral protease. Arch Biochem Biophys 260: 696–704, 1988
77. James PH, Vorherr T, Krebs J, Morelli A, Castello G, McCormick DJ, Penniston JT, De Flora A, Carafoli E: Modulation of erythrocyte Ca^{2+}-ATPase by selective calpain cleavage of the calmodulin binding domain. J Biol Chem 264: 8289–8296, 1989
78. Verma N, Adunyah ES, Penniston JT, Carafoli E: Purified (Ca^{2+}-Mg^{2+})-ATPase of the erythrocyte membrane. Reconstitution and effect of calmodulin and phospholipids. J Biol Chem 256: 395–401, 1981
79. Ortega A, Mas-Oliva J: Cholesterol effect on enzyme activity of the sarcolemmal (Ca^{2+} + Mg^{2+})-ATPase from cardiac muscle. Biochim Biophys Acta 773: 231–236, 1984
80. Ortega A, Mas-Oliva J: Direct regulatory effect of cholesterol on the calmodulin stimulated calcium pump of cardiac sarcolemma. Biochem Biophys Res Commun 139: 868–874, 1986

81. Stauffer TP, Guerini D, Carafoli E: Tissue distribution of the four gene products of the plasma membrane Ca^{2+} pump. A study using specific antibodies. J Biol Chem 270: 12184–12190, 1995
82. Pedersen P, Carafoli E: Ion motive ATPases I: ubiquity, properties, and significance to cell function. Trends Biochem Sci 12:146–150, 1987
83. Simons T JB: Vanadate- a new tool for biologists. Nature 281: 337–338, 1979
84. Barrabin H, Garrahan PJ, Rega AF: Vanadate inhibition of the Ca^{2+}-ATPase from human red cell membranes. Biochim Biophys Acta 600: 796–804, 1980
85. Rossi JPFC, Garrahan PJ, Rega AF: Vanadate inhibition of active Ca^{2+} transport across human red cell membranes. Biochim Biophys Acta 648: 145–150, 1981
86. Knauf P, Proverbio F, Hoffman JF: Electrophoretic separation of different phosphoproteins associated with Ca-ATPase and Na, K-ATPase in human red cell ghosts. J Gen Physiol 63: 324–336, 1974
87. Katz S, Blonstein R: Ca^{2+}-stimulated membrane phosphorylation and ATPase activiy of the human erythrocyte. Biochim Biophys Acta 389: 314–324, 1975
88. Lichtner R, Wolf HU: Phosphorylation of the isolated high-affinity (Ca^{2+}- Mg^{2+})-ATPase of the human erythrocyte membrane. Biochim Biophys Acta 598: 472–485, 1980
89. Luterbacher S, Schatzmann HJ: The site of action of La^{3+} in the reaction cycle of the human red cell membrane Ca^{2+}- pump ATPase. Experientia 39: 311–312, 1983
90. Mas-Oliva J: Effect of lead on the erythrocyte (Ca^{2+},Mg^{2+})-ATPase activity. Mol Cell Biochem 89: 87–93, 1989
91. Delgado-Coello B, Mas-Oliva J: Lead affects the formation of the phosphorylated intermediate on the (Ca^{2+},Mg^{2+})-ATPase from human erythrocytes. Med Sci Res 24: 159–161, 1996
92. Niggli V, Adunyah ES, Penniston JT, Carafoli E: Purified (Ca^{2+}-Mg^{2+})-ATPase of the erythrocyte membrane. Reconstitution and effect of calmodulin and phospholipids. J Biol Chem 256: 395–401, 1981
93. Murachi T: Calpain and calpastatin. Trends Biochem Sci 8: 167–169, 1983
94. Adunyah ES, Niggli V, Carafoli E: The anticalmodulin drugs trifluoroperazine and R24571 remove the activation of the purified erythrocyte Ca^{2+}-ATPase by acidic phospholipids and by controlled proteolysis. FEBS Lett 143: 65–68, 1982
95. Monteith GR, Roufogalis BD: The plasma membrane calcium-pump-a physiological perspective on its regulation. Cell Calcium 118: 459–470, 1995
96. Wang, KKW, Wright, LC, Madian, CL, Allen, BG, Conigrave, AD, Roufogalis, BD: Protein kinase C phosphorylates the carboxyl terminus of the plasma membrane Ca^{2+}-ATPase from human erythrocytes. J Biol Chem 266: 9078–9085, 1991
97. James PH, Pruschy M, Vorherr T, Penniston JT, Carafoli E: Primary structure of the cAMP-dependent phosphorylation site of the plasma membrane calcium pump. Biochemistry 28: 4253–4258, 1989
98. Kuo TH, Wang KKW, Carlock L, Diglio C, Tsang W: Phorbol ester induces both gene expression and phosphorylation of the plasma membrane Ca^{2+} pump. J Biol Chem 266: 2520–2525, 1991
99. Kuo TH, Liu B-F, Diglio C, Tsang W: Regulation of the plasma membrane calcium pump gene expression by two signal transduction pathways. Arch Biochem Biophys 305: 428–433, 1993
100. Wang KKW, Du YS, Diglio C, Tsang W, Kuo TH: Hormone-induced phosphorylation of the plasma membrane calcium pump in cultured aortic endothelial cells. Arch Biochem Biophys 289: 103–108, 1991
101. Yamaguchi M: Role of regucalcin in calcium signaling. Life Sci 66: 1769–1780, 2000
102. Takahashi H, Yamaguchi M: Activating effect of regucalcin on (Ca^{2+}-Mg^{2+})-ATPase in rat liver plasma membranes: relation to sulfhydryl group. Mol Cell Biochem 136: 71–76, 1994
103. Takahashi H, Yamaguchi M: Increase of (Ca^{2+}-Mg^{2+})-ATPase activity in hepatic plasma membranes of rats administered orally calcium: the endogenous role of regucalcin. Mol Cell Biochem 144: 1–6, 1995
104. Takahashi H, Yamaguchi M: Enhancement of plasma membrane (Ca^{2+}-Mg^{2+})-ATPase activity in regenerating rat liver: involvement of endogenous activating protein regucalcin. Mol Cell Biochem 162: 133–138, 1996
105. Yamaguchi M, Kanayama Y: Enhanced expression of calcium binding protein regucalcin mRNA in regenerating liver. J Cell Biochem 57: 185–190, 1995
106. Makino R, Yamaguchi M: Expression of calcium-binding protein regucalcin mRNA in hepatoma cells. Mol Cell Biochem 155: 85–90, 1996
107. Murata T, Shinya N, Yamaguchi M: Expression of calcium-binding protein regucalcin mRNA in the cloned human hepatoma cells (HepG2): stimulation by insulin. Mol Cell Biochem 175: 163–168, 1997
108. Mehendale HM, Limaye PB: Calpain: a death protein that mediates progression of liver injury. Trends Pharmacol Sci 26:232–236, 2005
109. Brandt P, Ibrahim E, Bruns GAP, Neve RL: Determination of the nucleotide sequence and chromosomal localization of the ATP2B2 gene encoding human Ca^{2+}-pumping ATPase isoform PMCA2. Genomics 14: 484–487, 1992
110. Latif F, Duh FM, Gnarra J, Tory K, Kuzmin I, Yao M, Stackhouse T, Modi W, Geil L, Schmidt L, LI H, Orcutt ML, Maher E, Richards F, Phipps M, Ferguson-Smith M, Le Paslier D, Linehan WM, Zbar B, Lerman MI: Von Hipperl-Lindau syndrome: cloning and identification of the plasma membrane Ca^{2+}-transporting ATPase isoform 2 gene that resides in the Von Hippel-Lindau gene region. Cancer Res 53: 861–867, 1993
111. Olson S, Wang MG, Carafoli E, Strehler EE, McBride OW: Localization of two genes encoding plasma membrane Ca^{2+}-transporting ATPases to human chromosomes 1q25-32 and 12q21-23. Genomics 9: 629–641, 1991
112. Wang MG, Yi H, Hilfiker H, Carafoli E, Strehler EE, McBride OW: Localization of two genes encoding plasma membrane Ca^{2+}-ATPases isoform 2 (ATP2B2) and 3 (ATP2B3) to human chromosomes 3p26–>p25 and Xq28, respectively. Cytogenet Cell Genet 67: 41–45, 1994
113. Zylinska L, Soszyński M: Plasma membrane Ca^{2+}-ATPase in excitable and nonexcitable cells. Acta Biochim Pol 47: 529–539, 2000
114. Penniston JT, Enyedi A: Modulation of the Plasma Membrane Ca^{2+} Pump. J Membrane Biol 165: 101–109, 1998
115. Shull GE, Greeb J: Molecular cloning of two isoforms of the plasma membrane Ca^{2+}transporting ATPase from rat brain. J Biol Chem 263:8646–8657, 1988
116. Strehler EE, Strehler-Page MA, Vogel G, Carafoli E: mRNAs from plasma membrane calcium pump isoforms differing in their regulatory domain are generated by alternative splicing involving two internal donor sites in a single exon. Proc Natl Acad Sci USA 86: 6908–6912, 1989
117. Santiago-García J, Mas-Oliva J, Saavedra D, Zarain-Herzberg A: Analysis of mRNA expression and cloning of a novel plasma membrane Ca^{2+}-ATPase splice variant in human heart. Mol Cell Biochem 155: 173–182, 1996
118. Hilfiker H, Guerini D, Carafoli E: Cloning and expression of isoform 2 of the human plasma membrane Ca^{2+} ATPase. J Biol Chem 269: 26178–26183, 1994
119. Preiano BS, Guerini D, Carafoli E: Expression and functional characterization of isoform 4 of the plasma membrane calcium pump. Biochemistry 35: 7946–7953, 1996
120. Guerini D, Pan B, Carafoli E: Expression, purification, and characterization of isoform, 1 of the plasma membrane Ca^{2+} pump. J Biol Chem 278: 38141–38148, 2003

121. Brini M, Coletto L, Pierobon N, Kraev N, Guerini D, Carafoli E: A comparative functional analysis of plasma membrane Ca^{2+} pump isoforms in intact cells. J Biol Chem 278: 24500–24508, 2003
122. Brandt P, Neve RL, Kammesheidt A, Rhoads RE, Vanaman TC: Analysis of the tissue-specific distribution of mRNAs encoding the plasma membrane calcium-pumping ATPases and characterization of an alternately spliced form of PMCA4 at the cDNA and genomic levels. J Biol Chem 267: 4376–4385, 1992
123. Zacharias DA, Kappen C: Developmental expression of the four plasma membrane calcium ATPase (Pmca) genes in the mouse. Biochim Biophys Acta 1428: 397–405, 1999
124. Okunade GW, Miler ML, Pyne GJ, Sutliff RL, O'Connor KY, Neumann JC, Andringa A, Miller DA, Prasad V, Doetschman T, Paul RJ, Shull GE: Targeted ablation of plasma membrane Ca^{2+}-ATPase isoforms 1 and 4 indicates a major housekeeping function for PMCA1 and a critical role in hyperactivated sperm motility and male fertility for PMCA4. J Biol Chem 279: 33742–33750, 2004
125. Prasad V, Okunade GW, Miller ML, Shull GE: Phenotypes of SERCA and PMCA knockout mice. Biochem Biophys Res Commun 322: 1192–1203, 2004
126. Schuh K, Cartwright EJ, Jankevics E, Bundschu K, Lieberman J, Williams JC, Armesilla AL, Emerson M, Oceandy D, Knobeloch KP, Neyses L: Plasma membrane Ca^{2+}-ATPase 4 is required for sperm motility and male fertility. J Biol Chem 279: 28220–28226, 2004
127. Lehotsky J, Kaplán P, Murín R, Raeymaekers L: The role of plasma membrane Ca^{2+} pumps (PMCAs) in pathologies of mammalian cells. Front Biosci 7: 53–84, 2002
128. Kozel PJ, Friedman RA, Erway LC, Yamoah EN, Liu LH, Riddle T, Duffy JJ, Doetschman T, Miller ML, Cardell EL, Shull GE: Balance and hearing deficits in mice with a null mutation in the gene encoding plasma membrane Ca^{2+}-ATPase isoform 2. J Biol Chem 273: 18693–18696, 1998
129. Street, VA, Mc-Kee JW, Fonseca RC, Tempel BL, Noben-Trauth K: Mutations in a plasma membrane Ca^{2+}-ATPase gene cause deafness in deafwaddler mice. Nat Genet 19: 390–394, 1998
130. Penheiter AR, Filoteo AG, Croy CL, Penniston JT: Characterization of the deafwaddler mutant of the rat plasma membrane calcium-ATPase 2. Hear Res 162: 19–28, 2001
131. Takahashi K, Kitamura K: A point mutation in a plasma membrane Ca^{2+}-ATPase gene causes deafness in a Wriggle Mouse Sagami. Biochem Biophys Res Commun 261: 773–778, 1999
132. Ueno T, Kameyama K, Hirata M, Ogawa M, Hatsuse H, Takagaki Y, Ohmura M, Osawa N, Kudo Y: A mouse with a point mutation in plasma membrane Ca^{2+}-ATPase isoform 2 gene showed the reduced Ca^{2+} influx in cerebellar neurons. Neurosci Res 42: 287–297, 2002
133. Howard A, Barley NF, Legon S, Walters JRF: Plasma membrane calcium-pump isoforms in human and rat liver. Biochem J 303: 275–279, 1994
134. Du Y, Carlock L, Kuo TH: The mouse plasma membrane Ca^{2+} pump isoforms 1 promoter: cloning and characterization. Arch Biochem Biophys 316: 302–310, 1995
135. Altin JG, Bygrave FL: Second messengers and the regulation of Ca^{2+} fluxes by Ca^{2+}-mobilizing agonists in rat liver. Biol Rev Camb Philos Soc 63: 551–611, 1988
136. Lin S-H, Wallace MA, Fain JN: Regulation of Ca^{2+}-Mg^{2+}-ATPase activity in hepatocyte plasma membranes by vasopressin and phenylephrine. Endocrinology 113: 2268–2275, 1983
137. Mallat A, Pavoine C, Dufour M, Lotersztajn S, Bataille D, Pecker F: A glucagon fragment is responsible for the inhibition of the liver Ca^{2+} pump by glucagon. Nature 325: 620–622, 1987
138. Blache P, Kervran A, Dufour M, Martínez J, Le-Nguyen D, Lotersztajn S, Pavoine C, Pecker F, Bataille D: Glucagon-(19–29), a Ca^{2+} pump inhibitory peptide, is processed from glucagon in the rat liver plasma membrane by a thiol endopeptidase. J Biol Chem 265: 21514–21519, 1990
139. Lotersztajn S, Pavoine C, Deterre P, Capeau J, Mallat A, Le-Nguyen D, Dufour M, Rouot B, Bataille D, Pecker F: Glucagon-(19–29) exerts a biphasic action on the liver plasma membrane Ca^{2+} pump which is mediated by G proteins. J Biol Chem 265: 9876–9880, 1990
140. Jouneaux C, Audigier Y, Goldsmith P, Pecker F, Lotersztajn S: Gs mediates hormonal inhibition of the calcium pump in liver plasma membranes. J Biol Chem 208: 2368–2372, 1993
141. Lotersztajn S, Pavoine C, Deterre P, Capeau J, Mallat A, Le-Nguyen D, Dufour M, Rouot B, Bataille D, Pecker F: Role of G protein $\beta\gamma$ subunits in the regulation of the plasma membrane Ca^{2+} pump. J Biol Chem 267: 2375–2379, 1992
142. Lotersztajn S, Pavoine C, Mallat A, Stengel D, Insel PA, Pecker F: Cholera toxin blocks glucagon-mediated inhibition of the liver plasma membrane (Ca^{2+}-Mg^{2+})-ATPase. J Biol Chem 262: 3114–3117, 1987
143. McKenzie RC, Lotersztajn S, Pavoine C, Pecker F, Epand RM, Orlowski RC: Inhibition of the calcium pump by human parathyroid hormone-(1–34) and human calcitonin in liver plasma membrane. Biochem J 266: 817–822, 1990
144. Jouneaux C, Mallat A, Serradeil-LeGal C, Goldsmith P, Hanoune J, Lotersztajn S: Coupling endothelin B receptors to the calcium pump and phospholipase C via Gs and Gq in rat liver. J Biol Chem 269: 1845–1851, 1994
145. Cobbold PH, Sánchez-Bueno A, Dixon CJ: The hepatocyte calcium oscillator. Cell Calcium 12: 87–95, 1991
146. Thomas AP, Bird GSJ, Hajnóczky G, Robb-Gaspers LD, Putney JW Jr: Spatial and temporal aspects of cellular signaling. FASEB J 10: 1505–1517, 1996
147. Chan C, Harland ML, Webb SE, Chen J, Miller AL, Barritt GJ: Evaluation, using targeted aequorins, of the roles of the endoplasmic reticulum and its (Ca^{2+}+Mg^{2+}) ATPases in the activation of store-operated Ca^{2+} channels in liver cells. Cell Calcium 35: 317–331, 2004
148. Woods NM, Cuthbertson KS, Cobbold PH: Repetitive transients rises in cytoplasmic free calcium in hormone-stimulated hepatocytes. Nature 319: 600–602, 1986
149. Sistare FD, Picking RA, Haynes RC Jr: Sensitivity of the response of cytosolic calcium in quins-2-loaded rat hepatocytes to glucagon, adenine nucleosides, and adenine nucleotides. J Biol Chem 260: 12744–12747, 1985
150. Charest R, Blackmore PF, Exton JH: Characterization of responses of isolated rat hepatocytes to ATP and ADP. J Biol Chem 260: 15789–15794, 1985
151. Dixon CJ, Woods NM, Cuthbertson KSR, Cobbold PH: Evidence for two Ca^{2+} -mobilizing purinoceptors on rat hepatocytes. Biochem J 269: 499–502, 1990
152. Dixon CJ, Cobbold PH, Gren AK: Oscillations in cytosolic free Ca^{2+} induced by ADP and ATP in single rat hepatocytes display differential sensitivity to application of phorbol ester. Biochem J 309: 145–149, 1995
153. Berridge MJ: Inositol trisphosphate and calcium signaling. Nature 361: 315–325, 1993
154. Putney JW Jr, McKay RR: Capacitative calcium entry channels. Bioessays 21: 38–46, 1999
155. Muallem S: The ins and outs of Ca^{2+} in exocrine cells. In: J.W. Putney (ed). Advances in Second Messenger and Phosphoprotein Research. Inositol Phosphates and Calcium Signaling. New York Raven Press. Vol. 26, 1992, pp 351–368
156. Savage AL, Biffen M, Martin BR: Vasopressin-stimulated Ca^{2+} influx in rat hepatocytes is inhibited in high-K^+ medium. Biochem J 260: 821–827, 1989

157. Lidofsky SD, Xie MH, Scharschmidt BF: Na^+-Ca^{2+} exchange in cultured rat hepatocytes: evidence against a role in cytosolic Ca^{2+} regulation or signaling. Am J Physiol 259: G56–61, 1990
158. Kim JA, Kang YS, Lee SH, Lee YS: Inhibitors of Na^+/Ca^{2+} exchanger prevent oxidant-induced intracellular Ca^{2+} increase and apoptosis in a human hepatoma cell line. Free Radic Res 33: 267–277, 2000
159. Green AK, Cobbold PH, Dixon CJ: Effects on the hepatocyte $[Ca^{2+}]_i$ oscillator of inhibition of the plasma membrane Ca^{2+} pump by carboxyeosin or glucagon-(19–29). Cell Calcium 22: 99–109, 1997
160. Hansen CA, Yang L, Williamson JR: Mechanisms of receptor-mediated Ca^{2+} signaling in rat hepatocytes. J Biol Chem 266: 18573–18579, 1991
161. Kawanishi T, Blank LM, Harootunian AT, Smith MT, Tsien RY: Ca^{2+} oscillations induced by hormonal stimulation of individual fura-2-loaded hepatocytes. J Biol Chem 264: 12859–12866, 1989
162. Canaff L, Petit J-L, Kisiel M, Watson PH, Gascon-Barrè M, Jendy GN: Extracellular calcium-sensing receptor is expressed in rat hepatocytes. Coupling to intracellular calcium mobilization and stimulation of bile flow. J Biol Chem 276: 4070–4079, 2001
163. Nathanson MN, Burgstahler AD, Mennone A, Fallon MB, González CB, Saez JC: Ca^{2+} waves are organized among the intact hepatocytes in the intact organ. Am J Physiol 269: G167-G171, 1995
164. Lin C, Hajnóczky G, Thomas AP: Propagation of cytosolic calcium waves into the nuclei of hepatocytes. Cell Calcium 16: 247–258, 1994
165. Robb-Gaspers LD, Thomas AP: Coordination of Ca^{2+} signaling by intracellular propagation of Ca^{2+} waves in the intact liver. J Biol Chem 270: 8102–8107, 1995
166. 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 17: 4695–4703, 1998
167. Dupont G, Tordjman T, Clair C, Swillens S, Claret M, Combettes L: Mechanism of receptor-oriented intercellular calcium wave propagation in hepatocytes. FASEB J 14: 279–289, 2000
168. Clapham DE: Calcium signaling. Cell 80: 259–268, 1995
169. Carafoli E, Nicotera P, Satella L: Calcium signaling in the cell nucleus. Cell Calcium 22: 313–319, 1997
170. Martinez-Merlos T, Cañedo-Merino R, Díaz-Muñoz M: Ryanodine receptor binding constants in skeletal muscle, heart, brain and liver of the Mexican volcano mouse (*Neotomodon alstoni alstoni*; Rodentia: Cricetidae). Comparison with five other rodent species. Int J Biochem Cell Biol 29: 529–539, 1997
171. Lee BS, Sessanna S, Laychock SG, Rubin RP: Expression and cellular localization of a modified type 1 ryanodine receptor and L-type channel proteins in non muscle cells. J Membrane Biol 189: 181–190, 2002
172. Zacharias DA, Strehler E: Changes in plasma membrane Ca^{2+}-ATPase splice-variant expression in response to a rise in intracellular Ca^{2+}. Current Biol 6: 1642–1652, 1996
173. Moore GA, McConkey DJ, Kass GEN, O'Brien PJ, Orrenius S: 2,5-Di(*tert*-butyl)-1,4-benzohydroquinone a novel inhibitor of liver microsomal Ca^{2+} sequestration. FEBS Lett 224: 331–336, 1987
174. Kass GEN, Duddy SK, Moore GA, Orrenius S: 2,5-di-(*tert*-butyl)-1,4-benzohydroquinone rapidly elevates cytosolic Ca^{2+} concentration by mobilizing the inositol 1,4,5-trisphsophate-sensitive Ca^{2+} pool. J Biol Chem 264: 15192–15198, 1989
175. Duddy SK, Kass GEN, Orrenius S: Ca^{2+} -mobilizing hormones stimulate Ca^{2+} efflux from hepatocytes. J Biol Chem 265: 20863–20866, 1989
176. Sternweis PC, Gilman AG: Aluminum: a requirement for activation of the regulatory component of adenylate cyclase by fluoride. Proc Nat Acad Sci USA 79: 4888–4891, 1982
177. Blackmore PF, Exton JH: Studies on the hepatic calcium-mobilizing activity of aluminum fluoride and glucagon. Modulation by cAMP and phorbol myristate acetate. J Biol Chem 261: 11056–11063, 1986
178. Tinton SA, Chow SC, Buc-Calderon PM, Kass GEN: Adenosine stimulates calcium influx in isolated rat hepatocytes. Eur J Biochem 238: 576–581, 1996
179. Capiod T, Combettes L, Noel J, Claret M: Evidence for bile acid-evoked oscillations of Ca^{2+}-dependent K^+ permeability unrelated to a D-*myo*-inositol 1,4,5-trisphosphate effect in isolated guinea pig liver cells. J Biol Chem 266: 268–273, 1991
180. 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 263: 2299–2303, 1988
181. Luo X, Popov S, Bera AK, Wilkie TM, Muallem S: RGS proteins provide biochemical control of agonist-evoked $[Ca^{2+}]_i$ oscillations. Mol Cell 7: 651–660, 2001
182. Bünemann M, Frank M, Lohse MJ: Gi protein activation in intact cells involves subunit rearrangement rather than dissociation. Proc Natl Acad Sci USA 100: 16077–16082, 2003
183. Frank M, Thümer L, Lohse MJ, Bünemann M: G protein activation without subunit dissociation depends on a $G\alpha_i$-specific region. J Biol Chem 280: 24584–24590, 2005
184. Dupont G, Swillens S, Clair C, Tordjmann T, Combettes L: Hierarchical organization of calcium signals in hepatocytes: from experiments to models. Biochim Biophys Acta 1498: 134–152, 2000
185. Murphy E, Cool K, Rich T, Williamson JR: Hormonal effects on calcium homeostasis in isolated hepatocytes. J Biol Chem 255: 6600–6608, 1980
186. Pott C, Goldhaber JI, Philipson KD: Genetic manipulation of cardiac Na^+/Ca^{2+} exchange expression. Biochem Biophys Res Commun 322: 1336–1340, 2004
187. Gregory RB, Barritt GJ: Evidence that Ca^{2+}-release-activated Ca^{2+} channels in rat hepatocytes are required for the maintenance of hormone-induced Ca^{2+} oscillations. Biochem J 370: 695–702, 2003
188. Gregory RB, Sykiotis D, Barritt GJ: Evidence that store-operated Ca^{2+} channels are more effective than intracellular messenger-activated non-selective cation channels in refilling rat hepatocyte intracellular Ca^{2+} stores. Cell Calcium 34: 241–251, 2003
189. Rimessi A, Coletto L, Pinton P, Rizzuto R, Carafoli E: Inhibitory interaction of protein 14–3-3ε with isoform 4 of the plasma membrane Ca^{2+} pump. J Biol Chem 280: 37195–37203, 2005
190. Pande J, Mallhi KK, Grover AK: Role of third extracellular domain of plasma membrane Ca^{2+}-Mg^{2+}-ATPase based on the novel inhibitor caloxin 3A1. Cell Calcium 37: 245–250, 2005