

Review

The $\text{Ca}^{2+}/\text{Mn}^{2+}$ pumps in the Golgi apparatus

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Abstract

Recent evidence highlights the functional importance of the Golgi apparatus as an agonist-sensitive intracellular Ca^{2+} store. Besides Ca^{2+} -release channels and Ca^{2+} -binding proteins, the Golgi complex contains Ca^{2+} -uptake mechanisms consisting of the well-known sarco/endoplasmic reticulum Ca^{2+} -transport ATPases (SERCA) and the much less characterized secretory-pathway Ca^{2+} -transport ATPases (SPCA). SPCA supplies the Golgi compartments and, possibly, the more distal compartments of the secretory pathway with both Ca^{2+} and Mn^{2+} and, therefore, plays an important role in the cytosolic and intra-Golgi Ca^{2+} and Mn^{2+} homeostasis. Mutations in the human gene encoding the SPCA1 pump (*ATP2C1*) resulting in Hailey–Hailey disease, an autosomal dominant skin disorder, are discussed.

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1. Introduction

Early experiments with electron-probe microanalysis on cryosections of mammalian cells reported a strong and uniform labelling of Ca^{2+} within the Golgi apparatus and secretory vesicles, with weaker signals recorded from the *trans*-Golgi network (TGN) and scattered vesicles [1]. There have been reports that the Golgi complex may store up to 5% of the total cellular Ca^{2+} at significantly higher concentrations (1–2 mM) than any other region of the cell [2]. However, the physiological meaning of these findings were elucidated only recently.

The Ca^{2+} pools represented by the endoplasmic reticulum (ER) and the secretory pathway (Golgi complex and different types of secretory vesicles) are believed to participate in the regulation of a variety of cell functions. On the one hand, a sufficiently high luminal Ca^{2+} concentration in these organelles, ranging from approximately 0.1 to 1 mM, is absolutely required for the normal

synthesis, chaperone-dependent processing, glycosylation, sorting and eventual breakdown of newly formed proteins [3–5]. Additionally, retrograde membrane traffic from the Golgi compartment to the ER [6], intra-Golgi transport [7] and selective aggregation of regulated secretory proteins in the TGN [8] also critically depend on luminal Ca^{2+} . On the other hand, these intracellular stores are potential providers for activator Ca^{2+} ions in the cytosol where it controls a whole range of physiological processes depending on the amplitude, the frequency and the subcellular localization of the cytosolic Ca^{2+} signal [9]. Furthermore, the organelles constituting the ER and the secretory pathway also typically contain several different luminal Ca^{2+} -binding proteins, such as calreticulin [10] and calnexin [11] in the ER and Cab45 [12], CALNUC (nucleobindin) [13], p54/NEFA [14] and calumenin [15] in the Golgi apparatus, and are equipped with Ca^{2+} -release channels such as the inositol 1,4,5-trisphosphate (IP_3) receptor and/or the ryanodine receptor. Hence the ER, but also the Golgi compartments [16] and perhaps secretory vesicles [17,18] can potentially act as dynamic Ca^{2+} stores.

In addition to Ca^{2+} , the secretory pathway requires the presence of a sufficiently high luminal Mn^{2+} concentration.

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Although Mn^{2+} and Ca^{2+} show very similar ionic radii and coordination chemistry, both ions clearly control different functions. Mn^{2+} is an essential cofactor for a number of enzymes in the cytoplasm such as aminopeptidase P [19]. In the Golgi apparatus, a sufficient supply of Mn^{2+} is absolutely required for correct glycosylation of secretory proteins [20]. In addition, Mn^{2+} can replace Ca^{2+} to support growth in yeast [21]. On the other hand, high concentrations of cytoplasmic Mn^{2+} are cytotoxic [22]. Therefore, accumulation of Mn^{2+} in the secretory pathway is an important detoxification pathway for excess Mn^{2+} . Finally, the mitochondria-localized superoxide dismutase is a Mn^{2+} -dependent enzyme [23] that protects these organelles from oxidative damage. Also prokaryotes contain a Mn^{2+} -containing superoxide dismutase, which is used to disarm the reactive oxygen species secreted into the phagosomes of macrophages that engulf the bacterial cells. There exists a sort of competition for Mn^{2+} between macrophages and the intracellular bacterial invaders: macrophages try to starve the bacteria of Mn^{2+} by the use of their natural resistance-associated macrophage protein 1 (Nramp1), which removes Mn^{2+} from their phagosome, while the bacterial invaders also have Mn^{2+} transporters (some of them are similar to Nramp) with which they try to suck up Mn^{2+} from the phagosome they are entrapped in [24,25].

In this review, we will first describe the basic characteristics of the SPCA Ca^{2+}/Mn^{2+} ATPases, which are at least partially responsible for loading the Golgi store with Ca^{2+} (and with Mn^{2+}). In a second and third part, we will respectively highlight the Ca^{2+} -uptake and Ca^{2+} -release mechanisms of the Golgi apparatus. In the final part, we will

review how mutations in the gene encoding the human SPCA1 might lead to Hailey–Hailey disease.

2. Secretory-pathway Ca^{2+} ATPases

2.1. SPCA genes and proteins

At present, three distinct classes of type-II phosphorylation (P)-type Ca^{2+} -transport ATPases have been identified in mammalian cells: plasma membrane (PMCA), sarco/endoplasmic reticulum (SERCA), and Golgi-associated secretory-pathway Ca^{2+} -transport ATPases (SPCA). Most of our knowledge of the SPCA class comes from studies of the yeast *Saccharomyces cerevisiae* homologue, named somewhat confusingly PMR1 for *plasma membrane ATPase-related* [26]. Since then, PMR1 homologues have been found in other fungi, worms, insects, mammals, and even in more distantly related forms including bacteria [27], suggesting that these pumps may represent the most ancient and widespread class of Ca^{2+} pumps [28].

There are at least two human SPCA-encoding genes: *ATP2C1* and *ATP2C2* encoding SPCA1 and SPCA2, respectively. Analysis of the deduced amino acid sequences indicates that SPCA1 (and also yeast PMR1) contains all highly conserved domains characterizing the P-type ATPases [26,29]. All SPCA1 proteins contain 10 hydrophobic segments, presumably forming the transmembrane domain in a SERCA-like manner. Sequence motifs demonstrated to be critical for SERCA-pump function, including the Thr–Gly–Glu loop in the A domain, the phosphate-

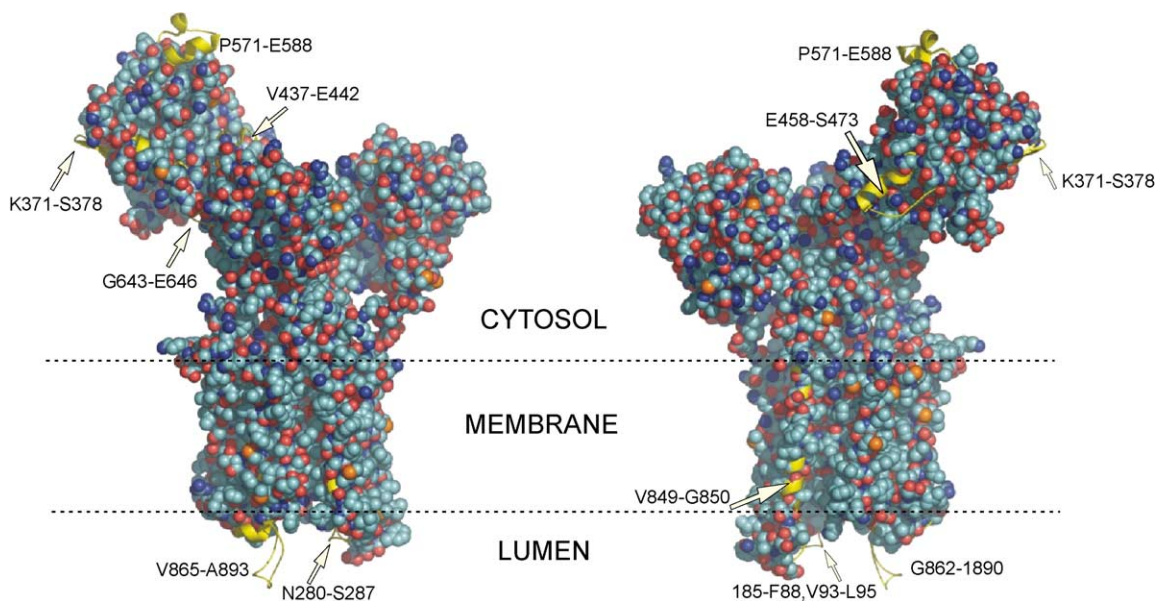


Fig. 1. Superimposed structures of rabbit SERCA1 in the E_1 conformation (PDB database entry 1EUL) and a model of human SPCA1 generated by SWISS-MODEL [100]. The N-terminus and C-terminus were omitted from the model. It includes amino acids 23–905 of human SPCA1. All atoms of the SPCA1 structure are shown as spheres (red, oxygen; blue, nitrogen; grey, carbon). Only the backbone of the SERCA1 structure is shown (yellow ribbons) and is therefore mostly hidden by the SPCA1 atoms, except where the SPCA1 sequence is shorter. The longer loops in SERCA1 are present in the nucleotide-binding domain (N) and in luminal loops connecting the transmembrane helices. The positions of these loops in the rabbit SERCA1 sequence are indicated.

accepting aspartate, the ATP- and FITC-binding regions, and the Asp–Pro–Pro–Arg loop connecting the N and P domain, are also conserved in SPCA pumps. Sequence alignment and comparative modeling of the three-dimensional protein structure of human SPCA1 based on that of rabbit SERCA1, reveals longer loops in SERCA1 in the N domain and longer luminal loops connecting the transmembrane helices than in SPCA1 (Fig. 1). The amino acids that form the binding site for one of the transported Ca^{2+} ions in SERCA, more specifically site II, are conserved in SPCA1/PMR1. The residues belonging to site I of SERCA have been replaced by non-conservative amino acids in SPCA1/PMR1. These data suggest that, like in the PMCA, a single Ca^{2+} -binding site exists in SPCA1/PMR1 [30].

The *ATP2C1* gene for SPCA1 is located on human chromosome 3q21 and consists of 28 exons [31,32]. As shown in Fig. 2, alternative processing at the 3'-end of the human *ATP2C1* pre-mRNA produces at least four distinct *ATP2C1* splice variants (*ATP2C1a–d*, corresponding to proteins SPCA1a–d) [33]. *ATP2C1a* (SPCA1a, 919 aa) results from splicing of exons 26 to 27, and its translational

stop codon is located in exon 27. *ATP2C1b* (SPCA1b, 939 aa) arises from splicing of exons 27 to 28 following activation of internal 5' splice donor site 1 (D_1). Splicing of exons 26 to 28 gives rise to *ATP2C1c* (SPCA1c, 888 aa) and splicing of exon 27 to 28 following activation of the second internal 5' splice donor site (D_2) in exon 27 produces *ATP2C1d* (SPCA1d, 949 aa). Mutation analysis in Hailey–Hailey patients suggested that SPCA1c has very limited functional activity [34]. Moreover, due to the exclusion of exon 27 in SPCA1c, transmembrane segment 10 is disrupted, probably resulting in an aberrant Ca^{2+} -pump protein.

The *ATP2C2* gene encoding human SPCA2 is located on chromosome 16q24.1 and consists of 25 exons [29,35]. So far, there are no reports available on the possibility of alternative splicing of *ATP2C2* pre-mRNA transcripts. All the exon–intron boundaries are conserved between *ATP2C1* and *ATP2C2*. Human SPCA2 exhibits about 60% sequence identity with the human SPCA1. Although all the important residues involved in Ca^{2+} and Mn^{2+} pumping are conserved, there are no reports so far demonstrating that SPCA2 actually pumps these ions.

2.2. Expression and localization of SPCAs

SPCA1 is highly expressed in human epidermal keratinocytes and at variable levels in all other human tissues tested [31,36], suggesting that *ATP2C1* is a housekeeping gene. It is presently unknown which SPCA1 isoforms are expressed in the different tissues except for human keratinocytes, which express all four isoforms at the mRNA level [33]. In contrast, expression of *ATP2C2* was found to be more limited (Vanoevelen et al., unpublished data). *ATP2C2* mRNA was present all along the gastrointestinal tract, from the stomach to the rectum with the exception of the oesophagus, and also in prostate, bone marrow, lung and trachea.

Whereas the SERCA pumps are expressed in both the ER and the Golgi complex, the SPCAs appear to be more specifically confined to the latter compartments of the secretory pathway, i.e. the Golgi stacks, the TGN and the secretory vesicles. The PMR1 protein of yeast cells was localized to the medial compartment of the Golgi complex [20,37]. The *Caenorhabditis elegans* SPCA1 heterologously expressed in COS-1 cells [38] and the human SPCA1a in CHO cells [29] were also immunolocalized to the Golgi area. SPCA1 in rat liver co-sedimented with the Golgi localized Ca^{2+} -binding protein CALNUC [39]. Fluorescence microscopy of the endogenously expressed SPCA1 in HeLa cells [40], in PC12 cells [39] and in cryosections of human colon mucosa (Vanoevelen et al., unpublished data) also revealed a Golgi-like distribution. Subcellular fractionation of mouse and rat β -cell-derived lines revealed SPCA1 immunoreactivity in both microsomal and dense-core secretory vesicle-enriched fractions, indicating SPCA1 expression in the more distal parts of the secretory pathway [41]. Furthermore,

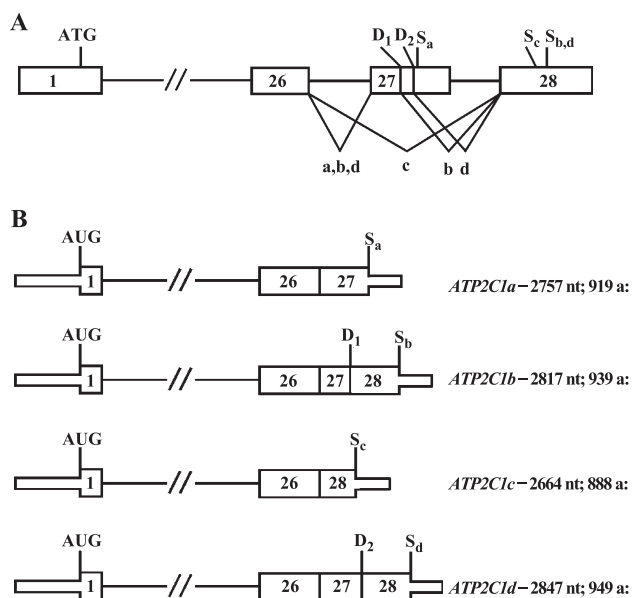


Fig. 2. *ATP2C1* gene structure and 3' alternative transcript processing. Panel A shows the 3' organization of the *ATP2C1* gene and provides an overview of alternative splicing events. Exons are represented by boxes, with wide boxes depicting the open reading frame. The thin horizontal line represents the position of introns. The horizontal line with breaks represents the gene elements positioned between exon 1 and exon 26. ATG denotes the translation start codon. $S_{a,b,c,d}$ represent the positions of the translation stop codon for each splice variant. The internal 5' splice sites, D_1 and D_2 , are represented. Alternative splicing patterns generating isoforms *ATP2C1a–d* are depicted. Panel B describes the four *ATP2C1* splice variants. *ATP2C1a* results from the splicing of exons 26 to 27 and its translation stop codon is located in exon 27. *ATP2C1b* arises from splicing of exons 27 to 28 following activation of internal 5' splice donor site D_1 . Splicing of exons 26 to 28 gives rise to *ATP2C1c* and activation of splicing at internal site D_2 in exons 27 to 28 results in the production of *ATP2C1d*. The relative sizes of each splice variant are as given in the figure. Figure adapted from Fairclough et al. [33].

SPCA1 was found to be present in the trans-Golgi of human keratinocytes by means of both sucrose-gradient centrifugation and immunocytochemistry [42]. In the latter study, the ER fraction had a lower density than the Golgi fraction, which is difficult to reconcile with the generally accepted respective differences in density of these subcellular compartments.

The subcellular localization of SPCA2 has not been reported yet. Our recent immunohistochemical analysis using cryosections of human colon tissue showed colocalization of SPCA2 and Golgi markers such as TGN46 and 58K (Vanoevelen et al., unpublished data).

2.3. Role of SPCAs as Ca^{2+} pumps

Until recently, PMR1 was partially characterized only in the yeast *S. cerevisiae*, an organism that lacks SERCA pumps and IP_3R channels. It is presently not entirely clear which pumps are responsible for Ca^{2+} accumulation into its ER. Both PMR1 [43] and COD1/Spf1 [44] are possible candidates. On the contrary, the role of the PMR1 pump in Golgi Ca^{2+} accumulation in yeast is well characterized. Yeast PMR1 transports one Ca^{2+} ion per ATP molecule and displays a K_m for Ca^{2+} of 0.07 μM for Ca^{2+} -dependent ATP hydrolysis [45], identical to the K_m for $^{45}\text{Ca}^{2+}$ transport reported by Wei et al. [46]. PMR1 is insensitive to the classical SERCA inhibitors thapsigargin, cyclopiazonic acid and 2,5-di-(*tert*-butyl)-1,4-benzohydroquinone [28]. Unfortunately, as yet, there is no selective inhibitor for PMR1.

Null yeast strains defective in PMR1 illustrated a pleiotropic effect on Golgi function including impaired proteolytic processing, incomplete glycosylation, and abnormal pre-, post- and intra-Golgi translocation of secreted proteins [20,26,37]. Further, yeast PMR1 deletion mutants cannot grow on low- Ca^{2+} media.

Human SPCA1 has been shown to transport Ca^{2+} with a slightly lower affinity than yeast PMR1 and comparable to that of the ER-based SERCA pump. Half-maximal activation of human SPCA1d heterologously expressed in COS-1 cells occurred at 0.20 μM free Ca^{2+} [33], which is equivalent to the 0.26 μM Ca^{2+} reported for the human SPCA1a isoform following expression in yeast [29] and the 0.25 μM Ca^{2+} reported for the orthologue from *C. elegans* expressed in COS-1 cells [38]. Also the two other human SPCA1 isoforms (SPCA1b and SPCA1c) have been tested for their Ca^{2+} -transport activity (Van Baelen et al., unpublished data). Human SPCA1b was shown to transport Ca^{2+} with the same apparent affinity as the other human SPCA1 isoforms. SPCA1c was non-functional, which was expected since the protein lacks part of the 10th transmembrane segment. Like yeast PMR1, animal SPCA1 pumps are not inhibited by thapsigargin and are at least two orders of magnitude less sensitive than SERCA to inhibition by cyclopiazonic acid or 2,5-di-(*tert*-butyl)-1,4-benzohydroquinone [47].

Ca^{2+} in the lumen of the Golgi apparatus controls a variety of important functions, including luminal and membrane protein traffic [3], cargo condensation, and precursor processing [8]. Further, the SPCA1 pump may play a central role in the secretion of milk. During lactation large movements of Ca^{2+} occur in the mammary gland. Rat and cow milk contain 60 and 30 mM Ca^{2+} , respectively [48]. Two-thirds of this Ca^{2+} comes in the milk as part of casein micelles via the secretory pathway. Therefore, a mechanism must be in place to support a high flux of Ca^{2+} into the Golgi of these cells. Interestingly, of all Ca^{2+} -transport ATPases that are upregulated in the lactating mammary gland (SERCA, PMCA, SPCA), only the SPCA1 pump is already upregulated 1 week before parturition [49].

In mammalian cells, the endoproteolytic proprotein convertases [50–52] and the secretases [53] found in the Golgi or secretory vesicles are dependent on Ca^{2+} . Ca^{2+} dyshomeostasis in these compartments could contribute to various amyloidoses including Alzheimer's disease.

Besides being implicated in luminal Golgi processes, SPCA1 may also be involved in setting up cytosolic Ca^{2+} signals. COS-1 cells overexpressing the *C. elegans* SPCA1, but not SERCA, showed typical baseline Ca^{2+} oscillations in response to extracellular ATP, both in the absence [54] and in the presence of a functional ER [55]. Studies with exogenous expression may however face the problem that the protein is targeted to a wrong compartment or that overexpression of a Ca^{2+} transporter in cells may affect the expression levels of other Ca^{2+} transporters or Ca^{2+} -binding proteins. A significant part of the *C. elegans* SPCA1 pumps indeed overflowed into the ER in COS-1 cells overexpressing these Ca^{2+} pumps [56,57]. Expression of SERCA, PMCA and the ER Ca^{2+} -binding protein calreticulin was dramatically diminished in COS-7 cells overexpressing rat SPCA1 [39]. On the contrary, in our studies, expression levels of endogenous IP_3R and SERCA2b were unaffected upon overexpression of *C. elegans* SPCA1 in COS-1 cells [54].

An alternative approach to explore the contribution of SPCA1 to cytosolic Ca^{2+} signaling is to disrupt its expression by RNA-mediated interference. HeLa cells lacking SPCA1 could still set up baseline Ca^{2+} spiking when stimulated with histamine, indicating that the SPCA1-containing Ca^{2+} store was not absolutely needed to trigger these oscillations [40]. However, baseline Ca^{2+} oscillations occurred less frequently than in control cells. SPCA1 has also been depleted from MIN6 pancreatic β -cells by the use of small interfering RNAs [41]. The shape, duration, and decay rate of the Ca^{2+} oscillations in response to glucose plus tetraethyl ammonium were modified in SPCA1-depleted cells. These observations point at least to some contribution of SPCA1 in the shaping of the intracellular Ca^{2+} signal in HeLa and MIN6 β -cells.

The functional importance of SPCA1 in human keratinocytes will be discussed in the section about Hailey–Hailey disease later in this review.

Although all the essential residues involved in Ca^{2+} pumping are conserved, it is currently far from clear whether SPCA2 is able to transport Ca^{2+} , and what actually its biological function might be.

2.4. Role of SPCAs as Mn^{2+} pumps

Yeast PMR1 [20,58], *C. elegans* SPCA1 [38] and human SPCA1a [29], SPCA1b (Van Baelen et al., unpublished data), SPCA1d [33] but not SPCA1c (Van Baelen et al., unpublished data) were shown to transport Mn^{2+} with high affinity. Hence, SPCAs clearly differ from SERCAs since SPCAs translocate very efficiently either Ca^{2+} or Mn^{2+} . Although SERCA1a can also catalyse the transport of Mn^{2+} instead of Ca^{2+} , it does this at a very slow rate [59,60]. It is at present not clear why SPCA1 can transport both Ca^{2+} and Mn^{2+} , whereas SERCA pumps have a strong preference for Ca^{2+} over Mn^{2+} . Studies in which the species-invariant Gln-783 in transmembrane region 6 and Val-335 in transmembrane region 4 were mutated, revealed that these residues were critical for Mn^{2+} transport by the yeast PMR1 [45,61]. Most likely, these mutations can selectively alter the access of Mn^{2+} to its binding site, indicating that these residues presumably form a gateway for Mn^{2+} entry in SPCAs.

It is presently unknown whether SPCA2 is able to transport Mn^{2+} , despite the fact that all important residues involved in Mn^{2+} pumping are conserved between SPCA1 and SPCA2.

Yeast PMR1 null mutants show defects in protein glycosylation that are unrelated to Ca^{2+} but can instead be specifically alleviated by an increased supply of Mn^{2+} to the medium. This seems to be the case for both N-linked saccharides such as in invertase as well as for O-linked glycosylated proteins such as chitinase [20]. Heterologous expression of SERCA1a in yeast could complement the Ca^{2+} -related defects, but not the Mn^{2+} -related defective phenotypes [29]. Enhanced secretion of proteins [62,63] and impaired sorting [20] represent additional biochemical defects detected in PMR1-mutant strains.

Less is known about the role of SPCAs in Mn^{2+} detoxification. High cytosolic concentrations of Mn^{2+} are cytotoxic because they interfere with Mg^{2+} -binding sites on proteins, compromise the fidelity of DNA polymerases [64] and lead to a disruption of the normal membrane traffic along the secretory pathway most likely by interference with the motor proteins linking the membranes to the cytoskeleton [65]. Mn^{2+} overload can lead to neurological disorders similar to Parkinson's disease by inhibiting tyrosine hydroxylation, a rate-limiting step in the synthesis of dopamine [66]. Mn^{2+} can also induce apoptosis in PC12 cells [22]. In yeast, PMR1 appears to be a principal component of the route involved in removing excess Mn^{2+} from the cytoplasm. Mn^{2+} removal from the cytosol via its accumulation in the Golgi apparatus and exit via exocytosis appears to be the main system for preventing Mn^{2+} toxicity in yeast.

Remarkably, cytosolic Mn^{2+} , which accumulates in PMR1 mutants, can act as a scavenger of superoxide radicals explaining why a mutation of PMR1 can bypass the requirement for cytosolic superoxide dismutase in aerobic growth [58,67]. Another remarkable phenotype of yeast PMR1 mutants defective in Mn^{2+} transport, but with normal Ca^{2+} transport, is their dramatically decreased Ty1 retrotransposition, an effect ascribed to the inhibitory effect of increased cytosolic Mn^{2+} on reverse transcriptase [68].

Also in mammalian cells Mn^{2+} ions in the lumen of the Golgi complex are required for the addition of complex carbohydrates onto N- and O-glycosylated proteins [69,70]. The Golgi-based galactosyl transferase, which functions as the catalytic component of lactose synthase and which is involved in the glycosylation of glycoproteins, has an absolute requirement for Mn^{2+} [71]. Mn^{2+} is also needed for optimal activity of the Golgi-localized casein kinase in the lactating mammary gland [72]. It is therefore not surprising that SPCA1 is significantly upregulated before parturition in mammary tissue of rats [49].

3. Ca^{2+} uptake in the Golgi

It is now generally accepted that SPCA1 is present in the Golgi membranes, but it is not the only pump in this compartment. A significant part of the Ca^{2+} uptake into the Golgi compartment depends on SERCA Ca^{2+} pumps since it has been shown that Ca^{2+} uptake by the Golgi apparatus is reduced in the presence of thapsigargin [13,16,40,56,57,73,74]. The thapsigargin-independent Ca^{2+} uptake has been ascribed to PMCA Ca^{2+} pumps in transit through the Golgi apparatus to the plasma membrane [74]. However, it is more likely that it depends on SPCA1 since the thapsigargin-resistant Ca^{2+} uptake disappears when SPCA1 expression is disrupted using RNA interference [40]. It can therefore be concluded that both SERCA and SPCA1 Ca^{2+} pumps contribute to the Ca^{2+} uptake in the Golgi apparatus.

The relative contribution of the SPCA1 and SERCA Ca^{2+} pumps to the total Ca^{2+} uptake in the Golgi apparatus is cell-type dependent and the percentages vary between different reports. According to Rojas et al. [73], Ca^{2+} uptake in a Golgi-enriched fraction of rat liver totally depended on a SERCA Ca^{2+} pump, since the uptake was almost completely inhibited by thapsigargin. In contrast, Taylor et al. [74] showed that thapsigargin inhibited Ca^{2+} uptake into a stacked Golgi fraction of rat liver by only 50%. Using aequorin measurements, it was observed that SERCA Ca^{2+} pumps were responsible for 50% [16] till 85% of the Ca^{2+} uptake in the Golgi compartment of HeLa cells [40]. Golgi Ca^{2+} uptake in HeLa and CHO cells overexpressing the Ca^{2+} -binding protein CALNUP depended for about 70% on SERCA pumps [13]. Very interestingly, human keratinocytes mainly used the SPCA1 Ca^{2+} pump (67%) to load the Golgi complex with Ca^{2+} [56]. This relatively large

contribution of the SPCA1 pumps for loading the Golgi stores with Ca^{2+} in keratinocytes may be relevant for the observation that mutations in the *ATP2C1* gene preferentially affect the skin in Hailey–Hailey patients.

Ca^{2+} uptake into the ER of HeLa cells, keratinocytes, 16HBE14o- cells and COS-1 cells was almost completely inhibited in the presence of thapsigargin, indicating that all the Ca^{2+} uptake by the ER was mediated by SERCAs [57].

4. Ca^{2+} release from the Golgi

The Golgi apparatus shares with the ER the property of rapidly releasing Ca^{2+} upon agonist-dependent IP_3 generation. Pinton et al. [16] showed that the free Ca^{2+} concentration inside the Golgi lumen is ~ 0.3 mM and this Ca^{2+} can be released upon stimulation with agonists coupled to IP_3 production. Moreover, IP_3 receptors have been immunolocalized in the Golgi apparatus [13].

Missiaen et al. [75] explored the kinetics of ATP-induced Ca^{2+} release from the ER and the Golgi apparatus in HeLa cells. The latencies and initial rates of Ca^{2+} release from the Golgi apparatus and from the ER were similar, indicating that both compartments were mobilized during agonist stimulation. However, Ca^{2+} release from the Golgi apparatus inactivated faster than that from the ER, thus resulting in a smaller extent of Ca^{2+} release. Detailed time-course experiments further showed that a maximal cytosolic Ca^{2+} response only required low levels of ER or Golgi store depletion. In addition, Vanoevelen et al. [57] investigated whether the agonist-induced Ca^{2+} release originated in the SERCA- or in the SPCA1-containing part of the Golgi apparatus by application of extracellular agonists in the presence or absence of thapsigargin. This study showed that agonists failed to release Ca^{2+} in the presence of thapsigargin, i.e. under conditions that only the SPCA1-containing Ca^{2+} store was loaded. The SPCA1-expressing subcompartments of the Golgi complex are therefore insensitive to agonist stimulation. In conclusion, these two very recent reports [57,75] clearly indicate that both the ER and the SERCA-expressing part of the Golgi apparatus are involved in the setting up of cytosolic Ca^{2+} signals. In contrast, the SPCA1-based part of the Golgi complex does not contribute to cytosolic Ca^{2+} signaling. It is conceivable that its fairly constant luminal Ca^{2+} concentration may be needed for proper execution of luminal functions.

There is as yet no evidence for the presence of any of the other known release mechanisms (e.g. cADPR or NAADP) in this compartment.

5. Hailey–Hailey disease

Hailey–Hailey disease (OMIM 16960) or familial benign chronic pemphigus, is an autosomal dominant blistering skin disorder that typically manifests as uncomfortable

erosions or lesions in flexures or at sites of trauma. The disorder with an onset in the third or fourth decade is histologically characterized by the loss of adhesion between suprabasal keratinocytes (acantholysis) and dyskeratosis of the epidermis [31,32]. Ultrastructural studies have revealed the breakdown of desmosome-keratin filament complexes with a perinuclear aggregation of keratin intermediate filaments that have retracted from desmosomal plaques [76–78]. Hailey–Hailey disease displays both clinical and histological similarity to Darier's disease, another dominantly inherited dermatosis in which a related Ca^{2+} pump (SERCA2b) is mutated [79].

Hailey–Hailey disease is caused by mutations inactivating one allele of *ATP2C1* [31,32,34,80–84]. At least 87 different mutations have already been described. They are scattered throughout the *ATP2C1* gene, indicating no hotspots or clustering of mutations in the gene.

Several consequences of SPCA1 mutations have been described. Keratinocytes from Hailey–Hailey disease patients showed a markedly reduced SPCA1 expression at the protein level [42]. Using site-directed mutagenesis, disease-causing point mutations were introduced into the cDNA of the human SPCA1d isoform and expressed in COS-1 cells [33,81]. These two studies indicate that more than half of the investigated mutants displayed low levels of protein expression, despite normal levels of mRNA and correct localization to the Golgi compartment. Other mutants were characterized by lack of ion transport caused by specific alterations to the partial reactions of the catalytic cycle, such as defects in Ca^{2+} and Mn^{2+} binding and inability of the phosphoenzyme intermediate to undergo the energy-transducing $E_1\sim\text{P}\rightarrow E_2\sim\text{P}$ conformational transition [33]. These results further support the theory of haploinsufficiency as a mechanism for the dominant inheritance of Hailey–Hailey disease, by suggesting that epidermal cells are sensitive to changes in the level of functional SPCA1. However, the possibility that some mutations cause Hailey–Hailey disease through a dominant negative mechanism cannot be discounted [34].

In order to understand the link between SPCA1 mutations and Hailey–Hailey disease, one should consider the critical role played by Ca^{2+} in the skin. There exists an epidermal Ca^{2+} gradient in the normal skin: 4-fold higher total Ca^{2+} levels are found in superficial layers relative to the basal epidermis. High extracellular Ca^{2+} concentrations affect essential processes such as keratinocyte differentiation [85,86], adhesion [87], motility [88] and lipid secretion [89]. Extracellular Ca^{2+} uses a well-defined cascade of signaling events, including binding to the PMCA receptor, generation of IP_3 , release of Ca^{2+} from intracellular stores, and subsequent influx of extracellular Ca^{2+} through PMCA channels. Cytosolic Ca^{2+} is needed for the assembly of functional desmosomes [87]. Adhesion is mediated by Ca^{2+} -dependent interactions between the extracellular domains of desmosomal cadherins. The formation and stability of desmosomes further depend on

interactions between transmembrane desmosomal cadherins (desmogleins, desmocollins), submembranous plaque proteins (plakoglobin, plakophilin, desmoplakins) and the cytokeratin filaments [90,91].

A dysregulation of intracellular Ca^{2+} and decreased ATP levels have been reported for keratinocytes from Hailey–Hailey disease patients. Whereas normal keratinocytes displayed Golgi Ca^{2+} levels comparable to other epithelial cells, Golgi Ca^{2+} uptake in Hailey–Hailey disease keratinocytes was slower, and the maximum Ca^{2+} load was significantly lower [42]. Furthermore, keratinocytes from Hailey–Hailey disease patients displayed a higher resting free cytoplasmic Ca^{2+} concentration compared with normal cells and showed a smaller cytosolic Ca^{2+} response to increases in extracellular Ca^{2+} levels [31]. Total Ca^{2+} concentrations were substantially decreased in the superficial layers of the epidermis but not in the basal layer of the epidermis and the dermis. Hence, the epidermal Ca^{2+} gradient in the skin was clearly diminished in patients with Hailey–Hailey disease.

The decreased Ca^{2+} (and probably also Mn^{2+}) concentration in the lumen of the Golgi complex could lead to impaired glycosylation, proteolytic processing, folding, trafficking, or sorting of key molecules involved in cell-to-cell adhesion, such as the desmosomal glycoproteins. This may cause an inability to maintain structurally intact desmosomes, leading to the cleavage of the epidermal cells. The increased resting cytosolic Ca^{2+} concentration and the decreased cytoplasmic Ca^{2+} response to stimuli could, amongst others, lead to changes in the expression of important genes or to post-translational modifications of proteins [31]. As an example, activation of protein kinase C could phosphorylate desmoplakin and disrupt desmosomes [92].

Besides desmosomes, adherens junctions are also important for the structural and functional integrity of the epidermis. The formation of adherens junctions depends both on adherens junction proteins and on remodeling of the actin cytoskeleton [87]. Whereas adherens junction protein assembly is impaired in Hailey–Hailey disease, actin reorganization was also reported to be downregulated in Hailey–Hailey disease keratinocytes [93] with formation of abnormal stress fibers [78] and an abnormal localization of actin filaments [94]. The inability of Hailey–Hailey disease keratinocytes to reorganize cellular actin in response to raised extracellular Ca^{2+} has been explained by the decreased concentrations of intracellular ATP. It was hypothesized that abnormally high cytoplasmic Ca^{2+} concentration in the keratinocytes may overload the mitochondria, thereby causing an uncoupling of oxidative phosphorylation and a decreased ATP synthesis [95]. Interestingly, ageing results in an increased mitochondrial sensitivity to Ca^{2+} overload [96], consistent with the onset of Hailey–Hailey disease in adulthood. ATP depletion may also result from increased consumption of ATP by cellular Ca^{2+} ATPases in an

attempt to clear excess cytoplasmic Ca^{2+} [97]. Finally, decreased ATP may further exacerbate cellular Ca^{2+} overload by inhibiting ATP-requiring Ca^{2+} ATPases such as SERCA and PMCA, which could normally compensate for the decreased SPCA1 function.

SPCA1 also transports Mn^{2+} into the Golgi lumen. It is less likely that most of the Hailey–Hailey phenotype is related to impaired Mn^{2+} transport, since mutations in SERCA2, which does not transport Mn^{2+} , cause Darier's disease, a similar blistering skin disease [79,98]. There are however clear differences between Hailey–Hailey and Darier's disease since mutations in SPCA1 cause acantholysis, whereas mutations in SERCA2 cause both acantholysis and apoptosis. Some of these more subtle differences may be related to the disturbed Mn^{2+} homeostasis or may reflect the diverse functions of the keratinocyte SPCA1- versus SERCA2-controlled Ca^{2+} stores.

Why do mutations in the *ATP2C1* gene, which is expressed at various levels in all human tissues, cause a disease that is limited to the skin? One possibility is that, while both the SERCA and SPCA1 pumps are involved in Ca^{2+} uptake into the Golgi apparatus, human keratinocytes rely predominantly on SPCA1 pumps for loading the Golgi stores with Ca^{2+} [56]. There is even evidence that SPCA1 is more important in the superficial layers of the skin than in the basal layer [42]. Furthermore, it is known that there are cell-type dependent differences in the extent of glycosylation of desmosomal glycoproteins [99]. As a consequence, the particular glycosylation state of the desmosomal glycoproteins in the epidermis could make them more vulnerable to subtle Ca^{2+} changes caused by haploinsufficiency of *ATP2C1*. Alternatively, noncutaneous tissues may possess compensatory systems that are lacking in the skin. This could also clarify why lesions are exacerbated in stress conditions caused by external factors such as sweating, friction and cutaneous infection. The cell could compensate for the haploinsufficiency of *ATP2C1* by other Ca^{2+} -regulatory mechanisms, but when it is placed under stress, the subtle deficiency becomes exposed.

6. Conclusions

We reviewed the properties of the novel family of Golgi-associated secretory pathway Ca^{2+} -transport ATPases. The identification of *ATP2C1*, encoding the SPCA1 Ca^{2+} pump, as the defective gene in Hailey–Hailey disease proved its important role in proper cell functioning. However, many questions still remain unanswered. How do mutations in the *ATP2C1* gene lead to the clinical phenotype of Hailey–Hailey disease? Why do Hailey–Hailey disease patients not show extracutaneous symptoms, despite the fact that SPCA1 is a housekeeping enzyme present in every cell? Further research is absolutely required to establish the role of the SPCA family in Ca^{2+} signaling and Ca^{2+} and Mn^{2+} homeostasis.

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