The Golgi Apparatus: Panel Point of Cytosolic Ca²⁺ Regulation

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Key Words

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Abstract

The Golgi apparatus (GA), an intermediate organelle of the cell inner membrane system, plays a key role in protein glycosylation and secretion. In recent years, this organelle has been found to act as a vital intracellular Ca²⁺ store because different Ca²⁺ regulators, such as the inositol-1,4,5-triphosphate receptor, sarco/endoplasmic reticulum Ca²⁺-ATPase and secretory pathway Ca²⁺-ATPase, were demonstrated to localize on their membrane. The mechanisms involved in Ca²⁺ release and uptake in the GA have now been established. Here, based on careful backward looking on compartments and patterns in GA Ca²⁺ regulation, we review neurological diseases related to GA calcium remodeling and propose a modified cytosolic Ca²⁺ adjustment model, in which GA acts as part of the panel point.

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Introduction

The Golgi apparatus (GA), also known as the Golgi complex, was identified during an investigation of the nervous system by the Italian physician Camillo Golgi in

1898. In the initial 80 years, GA was found to function in cell secretion and protein remodeling, such as glycosylation and phosphorylation for example.

But the problem is whether and how the GA plays a role in cell signaling. In the early 1990s, experiments using electron probe and fluorescence confocal microscopy indicate that high concentrations of Ca²⁺ are present in the GA. Under normal conditions, GA concentrations attain millimolar levels (10⁻⁴ to 10⁻³ M), which are similar to or even higher than those in the endoplasmic reticulum (ER; 10⁻⁴ M) and significantly higher than Ca²⁺ concentrations found in mitochondria (10⁻⁶ M) [1–3]. Intracellular Ca²⁺ stores mainly controlled the variation in cytosolic calcium, which is the vital signal switch that regulates many kinds of physiological and pathological process. Therefore, a high Ca²⁺ concentration in the GA cisternae probably takes part in Ca²⁺ signaling.

It is now widely accepted that GA participates in the regulation of cytosolic Ca²⁺ as an important intracellular Ca²⁺ store. In recent years, inositol-1,4,5-triphosphate receptor Ca²⁺ channel (IP₃R), sarcoplasmic reticulum (SR)/ER Ca²⁺-ATPase (SERCA) and secretory pathway Ca²⁺-ATPase (SPCA) have been demonstrated to sequentially localize on GA membranes, and their operating mecha-

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nism and function on GA Ca^{2+} release and uptake have been clarified [4, 5]. Furthermore, the cooperation of GAs with other intracellular Ca^{2+} stores, such as mitochondria and ER, is recognized much better as well. Meanwhile, various physiological and pathological implications induced by GA Ca^{2+} remodeling have been discovered and explained. Thus, it is necessary to assess the contribution of the GA to cytosolic Ca^{2+} regulation. In other words, what is the exact role of the GA in the Ca^{2+} regulation network should be answered on the ground of all the existing evidence.

GA Ca^{2+} Regulators

In current investigations, GA Ca^{2+} regulators should include calcium channels, which release Ca^{2+} to the cytoplasm; pumps, also named Ca^{2+} -ATPase, which transport Ca^{2+} into the GA, and binding and regulatory proteins, which buffer and adjust activities of the channel or pump.

Ca^{2+} Channel

IP_3R is the principal intracellular Ca^{2+} channel and is mainly localized on the SR/ER, which can recognize IP_3 as a ligand and release Ca^{2+} in the intracellular store. However, Pinton et al. [6] suggested that IP_3R also exists on the GA membrane, because IP_3 can release the free Ca^{2+} ions in the GA cisternae at a high level (0.3 mM). Furthermore, Lin et al. [7] demonstrated IP_3R on the GA membrane using an immunohistochemical test.

Conversely, many experiments have demonstrated that other common intracellular Ca^{2+} ions which release signals could not release Ca^{2+} from the GA. For instance, Surroca and Wolff [8] found that the ryanodine receptor (RyR) Ca^{2+} channel was absent on the GA membrane. Previous results also manifested that normal release factors, such as cyclic adenine dinucleotide phosphateribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), did not open the channel on GA [9]. Consequently, IP_3R should probably be the only GA calcium channel which is able to increase the level of cytosolic calcium.

However, Cifuentes et al. [10] argued that the RyR channel probably takes part in the Golgi Ca^{2+} release in rat sympathetic neurons, and Lissandrona et al. [11] proved that the *trans*-Golgi subcompartment is endowed with functional RyRs in neonatal cardiac myocytes. Therefore, it also can be confirmed that RyRs may locate on the GA; hitherto they have been discovered in *trans*-Golgi subcompartments.

Ca^{2+} -ATPase

Accordingly, two types of Ca^{2+} -ATPase localize on the GA membrane, one is SERCA, which is thapsigargin sensitive and present in the SR/ER membrane, and the other is SPCA, which is thapsigargin insensitive and mainly exists on the membrane of secretory organelles from the GA to secretory vesicles [9].

SERCAs

There are 3 genes of SERCA in the human genome, ATP2A1, ATP2A2 and ATP2A3, which belong to the P2A ATPase gene family. They encode over 11 different transcripts and result in 9 distinct proteins [12]. In vertebrates, the 3 paralogous SERCA genes generate multiple SERCA isoforms [13]. Invertebrates, however, typically have only 1 single SERCA gene that is orthologous to the vertebrate housekeeping SERCA2 [14]. All SERCA subtypes are a single subunit integral membrane protein with a cytosolic head containing an actuator, which is conserved in P-type ATPase, a nucleotide binding domain, a phosphorylation domain and 10 hydrophobic helices transmembrane domains [12].

SERCAs mainly occur in ER/SR and other inner system organelles, even embedding in the nuclear envelope, and play an important role in Ca^{2+} transportation [15, 16]. Evaluation on separated GA vesicles from epidermal keratinocytes indicated SERCAs located on the *cis*-Golgi compartment close to the ER, and SERCA2b is the principal isoform in this organelle [17].

Being a type of Ca^{2+} -ATPase, SERCA possesses high transforming activities and relatively lower affinity to Ca^{2+} , and hypersensitivity to thapsigargin. SERCA1a, for example, achieves a turnover rate of 130 s^{-1} ; its affinity to calcium is 284 nM ($K_{0.5}$); also, its sensitive concentration to thapsigargin is 0.031 nM [18]. While the SERCA family includes different subtypes, their Ca^{2+} turnover rate, affinity and sensitivities to thapsigargin are slightly different. In addition, studies on SERCA of *Plasmodium falciparum* indicated that artemisinin can also target and block them, and then induce cell death [19].

Although details on the catalytic mechanism of SERCAs have not been elucidated clearly, several possible explanations were raised. Almost all these models are based on the transformation between two major conformational states, designated E1 and E2. In the E1 conformation, the two Ca^{2+} binding sites are of high affinity and face the cytoplasm. In the E2 state, the Ca^{2+} binding sites are of low affinity and face the luminal side, which were summarized in figure 1 [12].

On the physiological role of SERCAs, Periasamy et al. [20] once suggested that deletion of both *Atp2a2* alleles is incompatible with life in mice. However, ablation in one of these alleles does not impact the function of SERCA in the heart. Tavadia et al. [21] confirmed that humans lacking one functional *ATP2A2* allele do not develop cardiomyopathy. Likewise, the effect of heterozygous knockout of *Atp2a2* in mice is also paralleled by compensatory responses, with only a slight impact on cardiac contractility and relaxation without eliciting cardiac disease [20, 22]. However, the effect of reduced Ca^{2+} uptake caused by heterozygous knockout of *Atp2a2* is manifested in keratinocytes and leads to Darier's disease [23]. In spite of this, other studies point to a more critical regulation of the apparent affinity of the Ca^{2+} pump for cytosolic Ca^{2+} ions [24, 25]. Genetic manipulation in the mouse that leads to the expression of the high Ca^{2+} affinity variant SERCA2b in cardiomyocytes instead of the normal SERCA2a triggers cardiac hypertrophy and heart failure [26, 27].

SPCAs

SPCAs, a latest finding of Ca^{2+} pump series, were firstly isolated from yeast and named plasma membrane ATPase related (PMR) or SSC1 [28, 29]. Several years later, PMR1 was found to locate in the GA membrane and act as $\text{Ca}^{2+}/\text{Mn}^{2+}$ transporter [30–32]. The first mammalian homologue was proven in the rat [33] and termed secretory pathway Ca^{2+} -ATPase because of its relationship to mammalian secretion. Up to now, SPCAs have been identified in many other species, such as *Caenorhabditis elegans*, the fruit fly and humans. Based on the phylogenetic tree established by Sorin et al. [34], PMR1 and its putative homologue from the rat, SPCA, fall into a third group on the basis of sequence homology, which is clearly distinct from both SERCA and plasma membrane Ca^{2+} -ATPase pumps. In view of the evolution of P-type ATPase, SPCA/PMR should be the ancient form and SERCA ought to be the developmental form. In the yeast, the ER level of free Ca^{2+} maintains a steady state which reaches only 10 μM and can contribute to the control of *Prm1* [35], that is to say, *Saccharomyces cerevisiae*, an ancient eukaryote, lacks the developed Ca^{2+} pump, SERCA.

Two genes of SPCA, called *ATP2C1* and *ATP2C2* for their evolutionary similarity of SERCA, were identified within the human genome. Moreover, *ATP2C1* genes express 4 distinct transcripts and 3 polypeptides [36]. The structure of SPCAs was similar to that of SERCAs: the sequence of the SPCA protein comprises domains of 10 hydrophobic transmembrane helices (M1–M10), cytosolic actuator, phosphorylation site and nucleotide bind-

Fig. 1. Catalytic cycle of SERCAs. Firstly, 2 Ca^{2+} ions combine to a cytoplasmic high-affinity binding site of E1 to form 2 Ca^{2+} -E1-P, and then a high-energy phosphorylated enzyme in which the bound Ca^{2+} ions become occluded. Consequently, the high-energy 2 Ca^{2+} -E1-P convert to the low-energy intermediate 2 Ca^{2+} -E2-P, whereby the Ca^{2+} binding sites are converted to a low-affinity state and reorient towards the luminal side. Finally, Ca^{2+} is released to the calcium store and E2 is regenerated to the E1 form (Wuytack et al. [12]).

ing site [5]. However, Wei et al. [37] found that the most obvious difference in the two pump series is that SPCA contains only one calcium binding site: site II in transmembrane domains of SERCA. However, site I in SERCA was replaced by other unconserved amino acid residues in SPCA. Furthermore, SPCAs possess other divergences, such as a shorter loop in the nucleotide binding domain and a longer loop at the Golgi lumen. Meanwhile, compared to PMR1, hSPCA1 is even more degenerated on the calcium binding EF-hand motif in the amino terminus; nevertheless, $^{45}\text{Ca}^{2+}$ overlay experiments on the glutathione S-transferase-purified amino terminus of hSPCA1 also indicated the binding of Ca^{2+} [14].

Although SPCA1 is highly expressed in epidermal keratinocytes and nerve tissues, its expression occurs in almost all other human tissues at variable levels, which suggests that *ATP2C1* is a housekeeping gene. In contrast, the expression of *ATP2C2* is found to be more limited and is usually induced by different stimuli. Its mRNA is presented along the gastrointestinal tract and also in the prostate, bone marrow, lung and trachea [36, 38]. Meanwhile, our investigation confirmed its expression and variation during cerebral ischemia and reperfusion injury [39, 40]. Furthermore, the function of SPCA2 should also be cell or tissue specific. Garside et al. [41] found that acinar-specific *Atp2c2* mRNA, which is regulated by MIST1, is a novel transcript consisting of only the 3' end of the gene, and the expression products localize to ER.

Fig. 2. Catalytic cycle of SPCAs. At first, cytoplasmic Ca^{2+} or Mn^{2+} bind with high affinity to the E1 state and then E1 becomes phosphorylated. This phosphorylated enzyme E1-P(Ca^{2+}) or E1-P(Mn^{2+}) consequently undergoes a conformational change to a low-energy E2-P phosphorylated enzyme and Ca^{2+} or Mn^{2+} are released into the GA cisternae. At last, E2-P is hydrolyzed, and hence free E2 enzyme regenerated (Missiaen et al. [5]).

Feng et al. [42] argued that SPCA2 upregulation can increase cytosolic Ca^{2+} levels in breast cancer cells, which is mediated by Orai1, a plasma membrane-located calcium channel. It is interesting that SPCA2 functions independently of its ATPase activity or store-operated Ca^{2+} entry in tumorigenesis.

In contrast to the wider subcellular distribution of SERCA on both the ER and GA, SPCA1 is located specifically on the GA stack and secretory vesicles [32, 43]. These locations were also proven by experiments of *C. elegans*; SPCA1 was heterologously expressed on COS-1 cells [31] and the human SPCA1a on CHO cells [44]. SPCA1 separated from rat liver cosedimented with the Golgi-localized Ca^{2+} -binding protein CALNUP [45]. Fluorescence microscopy of the endogenous expression of SPCA1 in HeLa [46] and PC12 cells [47] also revealed its Golgi-like distribution. Furthermore, SPCA1 was found to be present in the *trans*-Golgi stacks and networks from human keratinocytes using immunocytochemistry and sucrose gradient centrifugation [11, 47].

As a Ca^{2+} -ATPase, SPCA possesses relatively low transforming activities and high affinity to Ca^{2+} , and is not sensitive to thapsigargin. The SPCA turnover rate is about 20 s^{-1} for SPCA1a, 23 s^{-1} for SPCA1b and 27 s^{-1} for SPCA1d, showing no significant difference among SPCA1 subtypes [18]. The highest reported turnover rate for

SPCA is 41 s^{-1} [48]. The average affinity to calcium of the three human SPCA1 subtypes is around 10 nM ($K_{0.5}$); it is over one order lower than that of SERCA [18]. More importantly, sensitivity of SPCA to thapsigargin ($K_d = 28 \mu\text{M}$) is six orders lower than that of SERCA [47]. Furthermore, with regard to other enzymatic inhibitors, SPCA is much more insensitive to orthovanadate and 2,5-di-(tert-butyl)-1,4-benzohydroquinone [49].

In 2005 and 2006, Dode et al. [18, 48] characterized the overall and partial reactions of the catalytic cycle of SPCAs. SPCA activities can also be explained by the reversible cycle between the E1/E2 conformation. First, cytoplasmic Ca^{2+} or Mn^{2+} bind with a high-affinity site to the E1 state, after which E1 becomes phosphorylated. This phosphorylated enzyme, E1-P(Ca^{2+}) or E1-P(Mn^{2+}), consequently undergoes a conformational change to a low-energy E2-P phosphorylated enzyme and Ca^{2+} or Mn^{2+} are released in the cisternae of the GA. Lastly, E2-P is hydrolyzed, and the free E2 enzyme is regenerated [5] (fig. 2).

On the physiological and pathological functions of SPCAs, Okunade et al. [50] recognized that homozygous null mutations in the ATP2C1 gene encoding SPCA1 seem to be lethal in mammals, but they are tolerated in lower eukaryotes, including yeast and *C. elegans* [30, 51], which means compensatory mechanisms presumably ensure their survival. For instance, in yeast, the Ca^{2+} transport function of SPCA1 (PMR1) can be compensated by the inductive Pmc1, a $\text{Ca}^{2+}/\text{H}^{+}$ transporter on the vacuolar membrane [52]. Van Baelen et al. [46] used RNA interference to understand the role of SPCA1 in HeLa cells. Luminal [Ca^{2+}] measurements using Golgi-targeted aequorin showed that endogenous SPCA1 was responsible for Ca^{2+} uptake in a Golgi subcompartment, which indicated that in these cells a SPCA1-related Ca^{2+} store may affect cytosolic Ca^{2+} signals. In the same approach of gene knockdown, SPCA1 has also proven to be an important component of Ca^{2+} signaling in insulin-secreting cells [53] and demonstrated to be an essential factor to protein processing and degradation [54] as well as to cause morphological GA alterations [11]. Furthermore, SPCA1 deficit renders cells hypersensitive to ER stress [54]. Additionally, downregulation of SPCA1 affects the differentiation and growth of neurons, and trafficking of proteins through the Golgi complex was also hampered [55].

Ca²⁺ Binding Protein

Ca^{2+} binding proteins are able to serve as signal transducers to regulate many physiological functions by integrating calcium ions; they act as buffers in the cell, which

enable effective cellular calcium control, but their biochemical nature regarding calcium ion accumulation has been less studied. In fact, Ca^{2+} binding protein should be Ca^{2+} buffers in the cell, which ensure an effective and fine mechanism of cellular calcium control [56, 57].

As a calcium store, some Ca^{2+} binding proteins were found to locate in the GA. So far, CALNUC, Cab45 and p54/NEFA have been identified and possibly function in a similar way to calreticulin in ER lumens for binding Ca^{2+} [9]. Kawano et al. [58] located CALNUC in GA lumens of insect cells in 2000. Much earlier research showed that CALNUC could increase Ca^{2+} concentration in the GA cisternae, which was independent of agonist or thapsigargin [7]. Studies on the relationship between CALNUC content and SPCA1 expression indicated that overexpression of SPCA1 raised the level of CALNUC [45]. Associated with Ca^{2+} elevation in GA lumens triggered by its overexpression, CALNUC probably cooperated with SPCA1 to control the agonist-induced calcium signal. Furthermore, Scherer et al. [59] and Morel-Haux et al. [60] found that Cab45 and p54/NEFA were also present in the GA lumen in 1996 and 2002, respectively. However, their physiological mechanism and relationship with other regulators are poorly understood till now.

In addition, some Ca^{2+} binding proteins are also integrated in the GA membrane. They can be divided into two main categories according differences in the membrane binding order following calcium combination: (1) remodeling anchors, such as L-CaBP1, NCS-1 and calpain, and (2) mooring polypeptides, for instance hippocalcin, neurocalcin δ and cPLA2 [9]. Previously, these proteins were investigated and merely regarded as calcium buffers to induce 'off reactions' of the cytosolic Ca^{2+} [56], but, as indicated by recent research, they could also act as inhibitors of calcium channels. Haynes et al. [61] showed that L-CaBP could block the IP_3R calcium channel at high levels of cytosolic Ca^{2+} , thus decreasing the release of calcium. In neurons, anchors allied to mooring polypeptides to impact cytosolic Ca^{2+} , and these combinations include L-CaBP and hippocalcin, and NCS-1 and neurocalcin δ [62]. Therefore, Ca^{2+} binding proteins are also referred to as calcium sensor proteins [63].

Ca^{2+} Regulatory Proteins

A study investigating compartments of apoptotic signal transduction identified another conserved anti-apoptotic protein, besides caspase-2, located on the GA membrane, which is involved in Ca^{2+} signal modification [64]. Gubser et al. [65] separated this Golgi anti-apoptotic pro-

tein (GAAP) from the vaccinia virus and other eukaryotes in 2007. Its alignment of amino acid sequences indicated that the GAAP is widely distributed in humans, the orangutan, dog, mouse, rat, *Xenopus laevis*, zebra fish, fruit fly and even in the model plant *Arabidopsis thaliana*. All of these proteins consist of similar amounts of amino acid residues and 6–7 transmembrane domains are predicted, which resemble the BAX inhibitor-1 (BI-1). However, further studies on anti-apoptotic mechanisms of human GAAP found no interruption of the BAX and cytochrome c combination, but decreased activities of the IP_3R calcium channel to lower cytoplasmic Ca^{2+} , though their structure is similar to BI-1 [66], i.e. human GAAP probably functions in the same way as Bcl- X_L on the ER membrane [67].

GA in Cytosolic Ca^{2+} Regulation

GA Ca^{2+} Release

The release of Ca^{2+} from the GA is similar to that released from the ER, especially in rapid calcium release, which is dependent on an agonist such as IP_3 [68]. Experiments by Missiaen et al. [69] on ATP-induced Ca^{2+} release from ER and GA in HeLa cells indicated that there is almost no distance on initial rates and latencies between the two types of organelles, i.e. both compartments were mobilized during agonist stimulation. However, Ca^{2+} released from the GA was more quickly inactivated than Ca^{2+} released from the ER, thus resulting in less Ca^{2+} release [70]. Detailed time course experiments further showed that a maximal cytosolic Ca^{2+} response only required low levels of ER or GA store depletion [68].

Ca^{2+} release should be different in diverse GA compartments. Vanoevelen et al. [70] reported the phenomenon that agonists did not stimulate Ca^{2+} release when thapsigargin was injected into cells, and SPCA located in GA lumen was insensitive to agonist triggering. These results suggest either that ER and immature GA cisternae are involved in Ca^{2+} release or that the SPCA1 located in the *trans*-Golgi cisternae does not contribute to cytosolic Ca^{2+} signaling. It is reasonable to assume that a constant luminal Ca^{2+} concentration may be needed for proper execution of luminal functions. Other research, however, shows that SPCA can perhaps enhance Ca^{2+} release by reacting to CALNUC [45].

GA Ca^{2+} release is dependent on activities of IP_3R and RyR, the two main Ca^{2+} channels. Immuno-coprecipitation of GAAP and IP_3R illustrated that GAAP is able to act as a downregulator of calcium signals [66], and that

Bcl-X_L and other members of the Bcl-2 family, which can impact the Ca²⁺ channel, might be involved in GA Ca²⁺ release.

GA Ca²⁺ Uptake

Ca²⁺ uptake by the GA is quite different from that by the ER. Vanoevelen et al. [70] found that ER Ca²⁺ uptake can be absolutely blocked by thapsigargin, which means ER Ca²⁺ uptake depends only on SERCA. In contrast, both SERCA and SPCA1 Ca²⁺-ATPase contribute to Ca²⁺ uptake in the GA lumen. Although a significant portion of Ca²⁺ uptake into GA compartments depends on SERCA, it has been shown that Ca²⁺ uptake by the GA is reduced but not completely diminished in the presence of thapsigargin [6, 8, 46, 70–73]. Thus, contribution of the two pump types to GA Ca²⁺ uptake should be evaluated carefully.

The relative ratio of SPCA1/SERCA pumps to the total Ca²⁺ uptake into GA is tissue and cell type specific, and results differ between studies. Based on the report of Rojas et al. [72], Ca²⁺ uptake in a Golgi-enriched fraction of rat liver was completely dependent on SERCA, since the uptake was almost completely inhibited by thapsigargin. However, Taylor et al. [73] indicated that thapsigargin inhibited Ca²⁺ uptake in the GA stack of rat liver cells by only 50%. Pinton et al. [6] and Van Baelen et al. [46] observed that SERCAs were responsible for 50–85% of the Ca²⁺ uptake in the GA of HeLa cells by aequorin-marked determination. Lin et al. [7] showed that about 70% Golgi Ca²⁺ uptake relied on SERCA in HeLa and CHO cells which overexpressed the Ca²⁺-binding protein CAL-NUC. These varying results indicate that GA Ca²⁺ uptake is dependent on both SERCA and SPCA. However, SERCA, which possesses a higher Ca²⁺ turnover rate, probably contributes to a major proportion of the uptake. Interestingly, human keratinocytes mainly used the SPCA1 Ca²⁺ pump (67%) to load the Golgi complex with Ca²⁺, and this relatively large contribution of the SPCA1 pumps for loading the GA with Ca²⁺ in keratinocytes may be relevant for Hailey-Hailey disease [71].

Since SERCA and SPCA are located on different GA compartments, Ca²⁺ uptake should be distinct and dependent on the respective GA compartment. Accordingly, high concentrations of calcium in milk derived from secretory pathways, especially from the *trans*-Golgi compartment, depend mainly on SPCA due to its limited location. In galactosis, SERCA and SPCA of GA up-regulate early, but SPCA increased its expression early, i.e. 1 week before parturition [74, 75]. Results of Reinhardt and Horst [74] and Reinhardt et al. [75], however,

indicated that SPCA1 overexpression dramatically decreased expression of SERCA, plasma membrane Ca²⁺-ATPase and the ER calcium binding protein calreticulin [45], suggesting that SPCA exerts a reciprocal effect on SERCA in the GA membrane. In contrast, Vanoevelen et al. [70] and Callewaert et al. [71] consider that overexpression of SPCA has no effect on SERCA or IP₃R due to their different locations within GA compartments [70, 71].

Spatial Heterogeneity of the GA in Ca²⁺ Level and Regulation

As mentioned above, Ca²⁺ release should be different in diverse GA compartments. Vanoevelen et al. [70] argued that GA compartments that contain SERCA are the responding units of the agonist; nevertheless, SPCA located in GA lumens were insensitive to agonist. Therefore, both ER and *cis*-GA cisternae are involved in Ca²⁺ release, and, conversely, *trans*-Golgi cisternae on which SPCA1 is located do not contribute to cytosolic Ca²⁺ signaling. Further evidence indicates that a constant luminal Ca²⁺ concentration should be required for proper execution of functions. For example, when Breton et al. [76] described the whole glycosylation procedure of the secretory pathway of the cell, and the heterogeneity of glycosyltransferase distribution in GA was raised; this distribution parallels the difference in GA morphology.

In the last decades, when GA has been considered as another important dynamic Ca²⁺ store that participates in determining the spatiotemporal complexity of the Ca²⁺ signal within the cell [77], the relationship between the heterogeneity of GA and cytoplasmic Ca²⁺ regulation has been elucidated much more clearly. In 2010, Pizzo et al. [78] raised that the *trans*-Golgi compartment is a new distinct intracellular Ca²⁺ store and displays unique characteristics of Ca²⁺ homeostasis [11]. In these two important contributions, spatial heterogeneity of the GA regarding the Ca²⁺ level and regulation were well addressed. As shown in the figure 3, they insist that the GA can be divided into three distinct subcompartments: (i) the *cis*-Golgi, with a luminal [Ca²⁺] around 250 μM expressing mainly SERCA and IP₃Rs; (ii) the medial-Golgi with SERCA and SPCA1 but not with IP₃Rs, and (iii) the *trans*-Golgi with SPCA1 and RyRs (but not IP₃Rs) and a luminal [Ca²⁺] of 130 μM [78], and the different subcompartments should display its distinct role in Ca²⁺ release and uptake. Differences in the distribution of calcium channels and pumps certainly cause different actions of release and uptake, which is worth in-depth investigation in the future.

Association of the GA with Other Organelles on Cytosolic Ca^{2+} Regulation

In the pancreatic acinar cell, the GA cooperated with mitochondria to establish a cytosolic calcium concentration gradient. In this cell, mitochondrial distribution is heterogeneous, exhibiting obviously higher amounts in three different sites, perinuclear, perigranular and sub-plasmalemmal sites, than the rest of the cell. The highest density of mitochondria is found around the granular region [79] where they restrict agonist-evoked calcium signals to the apical granular pole [80, 81]. Dolman et al. [82] reported the GA location in relation to that of the perigranular mitochondrial belt and the potential significance of this localization for secretory trafficking in this classic secretory cell type. Research on Mist1, a transcription factor, indicated that interference on its expression affected both the pattern of the Ca^{2+} signal and the distribution of GA and mitochondria [83], and even the expression of the ATP2C2 gene [41]. Staining freshly isolated live pancreatic acinar cells for GA and mitochondria also revealed that the GA is closely associated with a subset of mitochondria concentrated around the granular area, which indicates that a specific protein links the two organelles. The GA was found to be tightly 'linked' to the mitochondria in osmstress within the granular region [79]. This linkage is probably similar to the association between ER and mitochondria with a special protein molecule reported by Rizzuto et al. [84] in HeLa cells, which is related to the Ca^{2+} signal effect. Based on the above and the results of Johnson et al. [79], it can be concluded that the association of ER, GA and mitochondria may be locally regulated by cytosolic Ca^{2+} signals, and that the GA should be the intermediate link in the association [9].

Effect of the GA on the Cytosolic Ca^{2+} Signals

So far, we have described activities of the GA as an intracellular calcium pool. The GA is able to participate in the establishment of cytosolic Ca^{2+} signals via multiple approaches, such as Ca^{2+} release by IP_3R and RyR, Ca^{2+} uptake by SERCA and SPCA, and buffering and inhibitory actions by calcium binding protein and anti-apoptotic protein. It can even establish the calcium gradient in the cytoplasm by cooperating with ER and mitochondria (summarized in fig. 4).

Much more importantly, as a vital Ca^{2+} signal in the cell, Ca^{2+} oscillation established by GA regulators mentioned in figure 4 is required for further research. Missiaen et al. [85, 86] argued that the GA established baseline Ca^{2+} oscillation independently of ER participation. Certainly, other studies also indicated that the GA is not es-

Fig. 3. Spatial heterogeneity of GA in Ca^{2+} level and regulation. The GA can be divided into three distinct subcompartments. Part I is the *cis*-Golgi which maintains a luminal $[\text{Ca}^{2+}]$ around 250 μM and mainly contains SERCA and IP_3Rs . Part II is the medial-Golgi in which SERCA and SPCA1 are embedded and IP_3Rs are excluded. Part III is the *trans*-Golgi with SPCA1 and RyRs (but not IP_3Rs) and a luminal $[\text{Ca}^{2+}]$ of about 130 μM (Pizzo et al. [78]).

sential to set up baseline oscillation [45], but that it does play an essential role in the frequency, mode, time course and period of oscillation in MIN6 pancreatic gland cells [53] and spermatozoa [87, 98].

Figure 4 depicts the GA-associated regulation of cytosolic Ca^{2+} . As an intracellular calcium store, the GA is able to participate in the establishment of cytosolic Ca^{2+} signals via multiple approaches, such as Ca^{2+} release by Ca channels, including IP_3R and RyR (red circles and squares), Ca^{2+} uptake by pumps including SERCA and SPCA (green quadrilateral and diamond), and buffering and inhibitory actions by calcium binding protein (blue pentagon, hexagon and octagon) and anti-apoptotic protein (pink triangle). Arrows indicate ion turnover or combinations, and dashed lines indicate inhibition.

Diseases Related to GA Ca^{2+} Regulation

Dermatoses

Hailey-Hailey disease, also named familial benign chronic pemphigus (OMIM 16960), was first described by the Hailey brothers [89]. It is now proven that this autosomal dominant skin disease is caused by the loss of one functional copy of ATP2C1, which encodes the Golgi Ca^{2+} pump SPCA1 [38]. SPCA1 is highly expressed in hu-

Fig. 4. Regulation of cytosolic Ca^{2+} associated with GA. The GA, an intracellular calcium store, is able to participate in the establishment of cytosolic Ca^{2+} signals via multiple approaches, such as calcium release by Ca^{2+} channels (IP₃R and RyR), Ca^{2+} uptake via pumps (SERCA and SPCA), buffering and inhibition by calcium binding protein (blue symbols) and GAAP. Arrows indicate ion turnover or combination, and dotted lines with a hyphen at the end of the line indicate inhibition. GV = Golgi vesicle.

man epidermal keratinocytes and contributed more (67%) to total GA Ca^{2+} turnover to maintain the homeostasis of cytoplasmic Ca^{2+} . Its mutation causes impaired homeostasis of intracellular and extracellular Ca^{2+} . Intracellular calcium disorders cause damage to and death of epidermal keratinocytes, and extracellular calcium disorders lead to the loss of calcium gradient and impaired cell adhesion. Physiological and pathological characteristics of the disease are the most typical evidence for GA Ca^{2+} regulation [13, 17, 36, 38, 44].

Darier's disease (OMIM 108740) is a rare autosomal dominant skin disorder characterized by loss of adhesion between epidermal cells and abnormal keratinization [90], which is caused by a variety of mutations in the ATP2A2 gene. These mutations decrease the expression of the SERCA2b pump, which attaches to ER/SR and GA, and affects Ca^{2+} uptake and storage. Consequently, keratinocytes from Darier's disease patients have lower ER Ca^{2+} and higher extracellular Ca^{2+} concentrations [23]. Interestingly, the calcium store still shows a normal Ca^{2+} response to raised extracellular Ca^{2+} concentrations in these mutant keratinocytes, which has been attributed to the compensatory overexpression of the SPCA pump [91]. In fact, the disease manifests clinical features similar to the Hailey-Hailey disease. In the molecular pathology of these two diseases, the epidermal Ca^{2+} gradient is often confused regarding the similar distribution and role of SERCA and SPCA [13]. However, because SERCA2b is expressed at relatively high levels in neurons in the brain [92], the incidence of neuropsychiatric disorders is increased in some Darier's patients [93].

Neurodegenerative Diseases

In our earlier review, we revisited the relationship between GA Ca^{2+} signaling and neurodegenerative diseases, including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) [94].

AD is a common and devastating neurodegenerative disease, which is characterized by cerebral accumulation of amyloid protein plaques and neurofibrillary tangles. It is caused by amyloid deposition, which is induced by pro-protein convertases and secretase in abnormal calcium homeostasis (OMIM 104300) [95–98]. Dysregulation of Ca^{2+} signaling can also be conferred by presenilin (PS) mutations. For example, PS/g-secretase activity could directly alter gene expression of IP₃R through cleavage of the amyloid precursor protein (APP) into the APP intracellular domain, which has been shown to be transcriptionally active [99]. Using aequorins, either targeted to the ER or the GA, the familial AD-linked PS mutants were found to lower the Ca^{2+} content of intracellular stores, and the phenomenon was most prominent in cells expressing PS2 mutants [100]. In addition, CALNUC was found to bind to APP in the GA, and its overexpression in neuroblastoma cells can significantly reduce the level of endogenous APP [101]. Moreover, Ca^{2+} signaling may be highly relevant for the pathogenesis of AD, and its dysregulation may contribute to several key features of AD, including increased amyloid β production [102, 103], hyperphosphorylation of tau [104], enhanced vulnerability to cell death and even memory-related deficits [105, 106]. However, further studies have to elucidate why and how GA calcium signals participate in AD pathogenesis.

ALS is an idiopathic human degenerative and, eventually, lethal disorder causing progressive degeneration of cortical and spinal motoneurons and interwoven networks to cytosolic calcium [107]. Antibodies to Ca^{2+} channels in ALS patients' IgG directly lead to motoneuron cell death by a mechanism requiring extracellular calcium, which is mediated by neuronal-type calcium channels [108]. ALS IgG may be cytotoxic [109] because of its function on presynaptic calcium regulation. It enhances transmitter release, induces apoptotic death of cultured motoneurons and elicits a distinctive cytopathology with raised Ca^{2+} in the GA, ER and presynaptic boutons of mouse motoneurons, causing extensive GA, ER and mitochondria swelling [110]. Grosskreutz et al. [111] reviewed calcium dysfunction in ALS, and they considered cytosolic and then intracellular calcium overload to be the primary cause of motoneuron cell death. This indicates that GAs in presynaptic boutons of neurons are probably associated with ALS pathogenesis.

HD is caused by polyglutamine expansion in huntingtin (Htt). Htt-associated protein-1 (HAP1) was the first identified Htt binding molecule, and its carboxyl terminus sequence is similar to IP_3R [112, 113]. As an intracellular Ca^{2+} release channel, release activities of IP_3R on ER or GA can be promoted by Htt, i.e. Htt can raise the cytosolic calcium concentration to establish calcium overload by IP_3R control [94].

Cardiac and Cerebrovascular Diseases

Recently, comparative studies on the normal and failing heart indicated that calcium pumps, such as SERCA located on the ER and GA, play a key role in cardiac disease. Raeymaekers et al. [114] argued that modeling Ca^{2+} dynamics of cardiac cells depends on the affinity of SERCA for calcium, and SERCA2a might be a better compromise in normal function and during adrenergic stress. Vandecastels et al. [24] recognized that cardiomyocyte relaxation and contraction are tightly controlled by the activity of SERCA, and aberrantly high or low Ca^{2+} affinity is often observed in cardiac failure and may even trigger it. Jiao et al. [115] proposed that age-related cardiac dysfunction is due to a decline in SERCA2a expression and activity.

Our research on cerebral ischemia and reperfusion injuries indicated that GA stress in morphology, such as fragmentation, and calcium concentration regulation, for example, were found in the Mongolian gerbil brain [116]. In addition, we also discovered that Ca^{2+} concentration in separated GA vesicles showed an opposite trend of cytosolic Ca^{2+} during the process of global cerebral ischemia and reperfusion [unpubl. data]. This means, the GA

Fig. 5. A modified model of cytosolic Ca^{2+} dynamics and homeostasis with GA as panel point. Actions from resting to active can be nominated as 'on' reactions (indicated by red arrows) that are promoted by regulators such as calcium channels and other stimulus receptors (yellow triangles). These reactions include that (1) stimuli induce the entry of external Ca^{2+} and the production of second messengers, and (2) release internal Ca^{2+} that is stored within the ER/SR, GA and mitochondria via IP_3R , RyR and permeability transition pore (PTP). Though most of these Ca^{2+} ions are bound to buffers (yellow circles), a small proportion binds to the effectors that activate various cellular processes that operate over a wide temporal spectrum. In contrast, actions from active to resting can be termed as 'off' reactions (indicated by blue arrows), which are enhanced by various pumps and exchangers (green triangles). These reactions include (1) Ca^{2+} exudation by $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) and the plasma membrane Ca^{2+} -ATPase (PMCA), and (2) Ca^{2+} back transport into intracellular calcium stores such as ER/SR, GA and mitochondria by SERCA, SPCA on ER and GA, and uniporter on mitochondria, and both 'on' and 'off' reactions are probably not associated with a single organelle; in fact, intracellular stores can be combined to impact or establish different calcium states in the cell (their combinations are indicated by a dashed line), in which the GA may act as an intermediate panel point.

is involved in calcium overload via the release of Ca^{2+} and decreased the overload via the uptake of Ca^{2+} during ischemia and reperfusion. Pavlíková et al. [117] established that SPCA1 expression can respond to ischemia and ischemic pre-/postconditioning using real-time PCR and Western blotting. Our studies on SPCA expression in PC12 cells and rats also demonstrated that SPCA, a Ca^{2+} pump located on the GA, responded to ischemia and reperfusion extremely quickly and that these expression responses were related to the Ca^{2+} concentration in the GA vesicle [unpubl. data]. Neural functions associated with GA Ca^{2+} regulation, especially by SPCAs, were reviewed in our previous paper [118].

Other Diseases

Brody myopathy (OMIM 601003), an autosomal recessive disorder, is caused by mutations in the ATP2A1 gene encoding SERCA1 in fast-twitch skeletal muscles. As a result, calcium ions are slow to enter the calcium store (including ER and GA) and therefore muscle relaxation is delayed. Patients suffer from painless muscle cramping and exercise-induced impairment in muscle relaxation [119–121].

Conclusions and Prospects

Ca^{2+} is a highly versatile intracellular signal that can regulate many different cellular functions [122]. The Ca^{2+} signaling system operates in many different ways

to regulate cellular function: for example, Ca^{2+} triggers exocytosis within microseconds, controls muscle contraction within milliseconds, operates over minutes to hours to drive events such as gene transcription and cell proliferation, and these contribute to calcium regulators including organelles, factors and effectors (summarized in fig. 4) [123]. Homeostasis and versatility of Ca^{2+} signal networks are essential for cells to execute functions, and constant intracellular and extracellular calcium stores are required [56]. Previously, only ER and mitochondria were referred to as intracellular calcium stores, but it is now widely accepted that the GA also acts as an important intracellular Ca^{2+} store by regulating cytosolic Ca^{2+} [5]. Based on its calcium regulators and location in intermediated sites of the inner membrane system, and its cooperation with other organelles, it can be concluded that the GA executes a role of panel point in cytosolic Ca^{2+} regulation and calcium signal networks [9].

In 2003, Berridge et al. [56] proposed a model of Ca^{2+} signal dynamics and homeostasis in their review. However, this model ignored the role of the GA on calcium regulation, and, in view of this, we have proposed a modified model (fig. 5). In the modified model, the GA is one of the major intracellular calcium stores and the panel point in cytosolic Ca^{2+} regulation. In the future, studies on the effect of impaired GA signaling on cytosolic calcium regulation are needed to be able to explore all kinds of diseases and pathophysiological events in much wider detail.

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