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Roles of Ca^{2+} and secretory pathway Ca^{2+}-ATPase pump type 1 (SPCA1) in intra-Golgi transport

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Abbreviations: $[Ca^{2+}]_{cyt}$, cytosolic Ca^{2+} concentration; $[Ca^{2+}]_{GA}$, Golgi Ca^{2+} concentration; $[Ca^{2+}]_{ER}$, sarco-(endo)plasmic Ca^{2+} concentration; COPI, coat protein type I; ER, endoplasmic reticulum; GA, Golgi apparatus; IP_3R, inositol-1,4,5-trisphosphate receptor; PM, plasma membrane; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; SERCA, sarco-(endo)plasmic reticulum Ca^{2+}-ATPase; SPCA, secretory pathway Ca^{2+}-ATPase

Mechanisms for intra-Golgi transport remain a hotly debated topic. Recently, we published data illuminating a new aspect involved in intra-Golgi transport, namely a release of free cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) from the lumen of Golgi cisternae that is fundamental for the secretion and the progression of newly synthesized proteins through the Golgi apparatus (GA). This increase in $[Ca^{2+}]_{cyt}$ during the late stage of synchronous intra-Golgi transport stimulates the fusion of membranes containing cargo proteins and Golgi cisternae, allowing the progression of proteins through the GA. Subsequent restoration of the basal $[Ca^{2+}]_{cyt}$ is also important for the delivery of cargo to the proper final destination. Additionally, the secretory pathway Ca^{2+}-ATPase Ca^{2+} pump (SPCA1) plays an essential role at this stage. The fine regulation of membrane fusion is also important for the formation and the maintenance of the Golgi ribbon and SPCA1, which regulates $[Ca^{2+}]_{cyt}$ levels, can be considered a controller of trafficking. This evidence contradicts a model of intra-Golgi transport in which permanent membrane continuity allows cargo diffusion and progression.

The plasma membrane (PM) and the membranes of many intracellular organelles separate the cytosol from environments with different free Ca^{2+} concentrations ($[Ca^{2+}]$) and electrical potentials.[1] The cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_{cyt}$) is around 20,000-fold lower than in the mammalian extracellular fluids, and varies between 50–100 nM, whereas in the external fluids the $[Ca^{2+}]$ is about 2 mM. In addition, the lumens of several cellular organelles, like the endoplasmic reticulum (ER) and the Golgi apparatus (GA), store high amount of free Ca^{2+}, in the order of 1.0 mM and 0.3–0.4 mM, respectively,[2] comparable with those in the external fluids. The $[Ca^{2+}]$ in all these compartments is finely regulated and the correct Ca^{2+} homeostasis is important for the majority of intracellular pathways, including the protein trafficking and secretion.

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Membrane fusion is considered to be essential for intracellular transport. Fusion between membranes within the secretory and endocytic pathways is mediated by SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins.[3,4] The SNAREs cannot induce rapid fusion of membranes, but proper level of $[Ca^{2+}]_{cyt}$ is necessary.[5] Recently, we reported that a transient increase in $[Ca^{2+}]_{cyt}$ during the late phase of intra-Golgi transport was simultaneous with a decreases in Ca^{2+} stored in the lumen of the Golgi cisternae ($[Ca^{2+}]_{GA}$) and maximal $[Ca^{2+}]_{GA}$ restoration ability.[6] $[Ca^{2+}]_{GA}$ started to decrease 7 min after the restoration of intra-Golgi transport.[6] However, we do not exclude the ER as a possible source of the increased $[Ca^{2+}]_{cyt}$, mainly from the trans-ER, strictly associated with the GA.[7] The release of Ca^{2+} from the GA rapidly flows through opened Ca^{2+} channels (IP_3R; inositol-1,4,5-trisphosphate receptor), creating a local 'cloud' with an increase of $[Ca^{2+}]_{cyt}$ surrounding the GA and generating a local Ca^{2+} signal.[8] The $[Ca^{2+}]_{cyt}$ can also rapidly stimulate IP_3Rs.[1,9] Indeed, it is also possible that the ER has a role in restoring the $[Ca^{2+}]_{GA}$ that is crucial for cargo progression,[10] because there are direct interactions between the ER and the GA.[7] Therefore, focusing attention on the role that the GA have in regulating the Ca^{2+} during intra-Golgi trafficking, we can assume that the decreased $[Ca^{2+}]_{GA}$ could be due to (1) an impaired pumping of Ca^{2+} into the GA, (2) an increased opening of the IP_3R channels or (3) a combination of both of these two. Of these, we believe the third possibility to be the most plausible.

In the late phase of intra-Golgi transport, 5–7 min after the release of the temperature block in our experimental asay,[6,11] the increase of $[Ca^{2+}]_{cyt}$ is critical for cargo progression. One critical step is the remodelling of the more rigid trans-GA membranes, which contain more cholesterol and glycolipids,[12] operated by Ca^{2+}-sensitive and locally recruited Golgi enzymes.[13] At the same stage, after tethering of fusing compartments operated by SNAREs,[3,4] the destabilization of membranes within the contact region needs increased $[Ca^{2+}]_{cyt}$.[14,15] An increase in $[Ca^{2+}]_{cyt}$ leads to the recruitment of the cytosolic phospholipase A(2) α (hereafter $cPLA_2$) on the membranes of the GA.[14,16]

$cPLA_2$ is regulated by phosphorylation and Ca^{2+}, whereby Ca^{2+} binds the C2 domain of the enzyme, inducing the translocation of the $cPLA_2$ to the GA and vesicles.[13,16] Indeed, the $cPLA_2$-C2 domain itself moves to the GA during the passage of vesicular stomatitis virus G (VSVG) through the trans-Golgi.[13,16] The enzymatic activity of $cPLA_2$ or its binding to the membranes, could be responsible for the increased sensitivity to $[Ca^{2+}]_{cyt}$ of the secretory pathway membranes that are tightly attached one to each other by SNAREs. An increase in $[Ca^{2+}]_{cyt}$ is also required during the release of the trans-SNARE complex,[5] which allows formation of the intercisternal connections.[17] However, the $[Ca^{2+}]_{cyt}$ increase observed during the late stage of intra-Golgi transport has to quickly return to its basal levels of 50–100 nM, allowing the exit of cargo from the GA.[7,18-20] All of the cellular Ca^{2+}-ATPase pumps, including the secretory-pathway Ca^{2+}-transport ATPase pump type 1 (SPCA1), which probably have a main role in the GA, act to reduce this otherwise 'toxic' level of Ca^{2+} from the cytosol.

The mammalian Ca^{2+}-ATPase pumps accumulate Ca^{2+} into different compartments against the Ca^{2+} gradient, using ATP as the source of energy. This group of pumps includes four isoforms of the plasma-membrane Ca^{2+}-ATPases (PMCA),[21] three sarco-(endo)plasmic reticulum Ca^{2+}-ATPases (SERCA),[22] and two located on the GA and the secretory granules Ca^{2+}-ATPases (SPCAs).[23] SERCAs are specifically located on the ER and the most cis-GA;[19,24,25] SPCAs are located mostly on the GA and are excluded from the ER.[26,27] The two SPCA isoforms, SPCA1 and SPCA2,[28-30] are differently distributed; in mammals, SPCA1 is expressed in all tissues,[28] whereas SPCA2 is expressed in only a limited set of tissues.[30] We recently characterized the (sub) compartmental distribution of SPCA1 along the secretory pathway.[27] SPCA1 is mostly excluded from the cores of the Golgi cisternae and is mainly distributed on the lateral rims of Golgi stacks, in the non-compact zones that interconnect different Golgi stacks and in the tubular cluster of the trans-Golgi network.[27] This localization suggest SPCA1 has a role regulating the local $[Ca^{2+}]_{cyt}$ in (sub)compartments surrounding the GA where membrane fusions occur during protein trafficking. A schematic of SPCA1 redistribution along the secretory pathway is provided in **Figure 1**.

ATP2C1 encodes for the human SPCA1 protein, with four known mRNA splice variants.[31] Gene silencing of *ATP2C1* inhibits the correct organization of the GA[32] and affects the subsequent development of the affected tissue.[33] SPCA1 depletion inhibits the exit of VSVG from the GA and delays retrograde redistribution of the GA glycosylation enzymes into the ER caused by brefeldin A (BFA);[27] however, the exit of these enzymes from the ER is not affected and the decreased sensitivity of SPCA1 depleted cells to BFA is not related to GA fragmentation.[27] Additionally, SPCA1 depletion by RNA interference induces GA fragmentation; these fragments lack the cis-most and trans-most cisternae and remain within the perinuclear region.[27]This suggests that correct SPCA1 functioning is crucial to intra-Golgi transport and for the maintenance of the Golgi ribbon.

So how is SPCA1 involved in maintaining the Golgi ribbon? The organization of the GA depends on many factors, such as the ability of cells to maintain their Golgi stacks within a restricted space, and the normal functioning of the golgins and the SNARE/Rab machineries.[34] There is a need for correct positioning and normal functioning of the centrosome, the polymerization and growing of microtubules, the presence of ER-to-GA transport and regulated levels of $[Ca^{2+}]$ in both cytosol and secretory compartments.[34] If any of these machineries are blocked, the stacks will not fuse with each other and the Golgi ribbon will not form.[35] There is evidence that SPCA1 is crucial for the maintenance of the structure of the GA and that its inactivation affects intracellular transport. Inactivation of the *Hansenula polymorpha PMR1* gene (the yeast homologue of *ATP2C1*) reduces cell viability and functionality of the secretory pathway.[36] Recently, a very similar result to ours on GA fragmentation in SPCA1-interfered HeLa cells was reported.[37] All these observations support our data, confirming that SPCA1 inactivation inhibits the correct organization and functioning of the GA.[37]

However, our observations contradict some other previous reports describing SPCA1-depleted cells in which GA appears not to be fragmented[33,38] and protein trafficking is unaffected,[33] although the exit of GPI-anchored proteins and VSVG from the ER was inhibited.[38] Okunade et al.[33] used several criteria to determine there were no effects to transport in SPCA1-depleted mice, including observations made of clathrin-coated buds, complexes of cell junctions, desmosomes and the development of the basement membrane. However, these parameters have low dependency on intracellular transport, because during the long life of epithelial cells they synthesize only very small amounts of the matrix proteins involved in the formation of the basement membrane, while the cell junctional complexes are relatively inert. Similarly, clathrin-coated buds are poorly involved in anterograde intracellular transport, but mostly in endocytosis. In addition, different cellular models and species were used: in our work we used human cells[27] whereas Okanude et al. used a murine model.[33] SPCA1 depletion seems also to have different effects in different cellular phenotypes; for instance, the lost of one allele of the *ATP2C1* gene induces Hailey-Hailey disease in humans, demonstrating that SPCA1 has a crucial role in the regulation of $[Ca^{2+}]$ in keratinocytes, whereas in the murine model this appears not to be the case.[39]

A potential explanation of how SPCA1 affects the formation of the Golgi ribbon is that the generation and maintenance of the Golgi ribbon is a membrane fusion-dependent process that is a Ca^{2+}-mediated.[2] The transient increase in $[Ca^{2+}]_{cyt}$ detected during the late phase of intra-Golgi transport was co-incident with a Ca^{2+} efflux from the GA itself.[6] Simultaneously, there was a decline in the maximal $[Ca^{2+}]_{GA}$ restoration capacity, which is operated by the Golgi Ca^{2+} pumps. [6] This temporary redistribution of Ca^{2+} from the GA into the cytosol during cargo movement through the GA appears to have a crucial role in intra-Golgi transport and mainly in the late Ca^{2+}-dependent phase of SNARE-regulated fusion between the different Golgi subcompartments.[6]

Similarly, these $[Ca^{2+}]_{cyt}$ changes will also regulate SNARE cofactors that are involved in the release of the trans-SNARE complex that is formed between two distinct compartments during cargo progression through the GA.[14,40-42] However, it appears

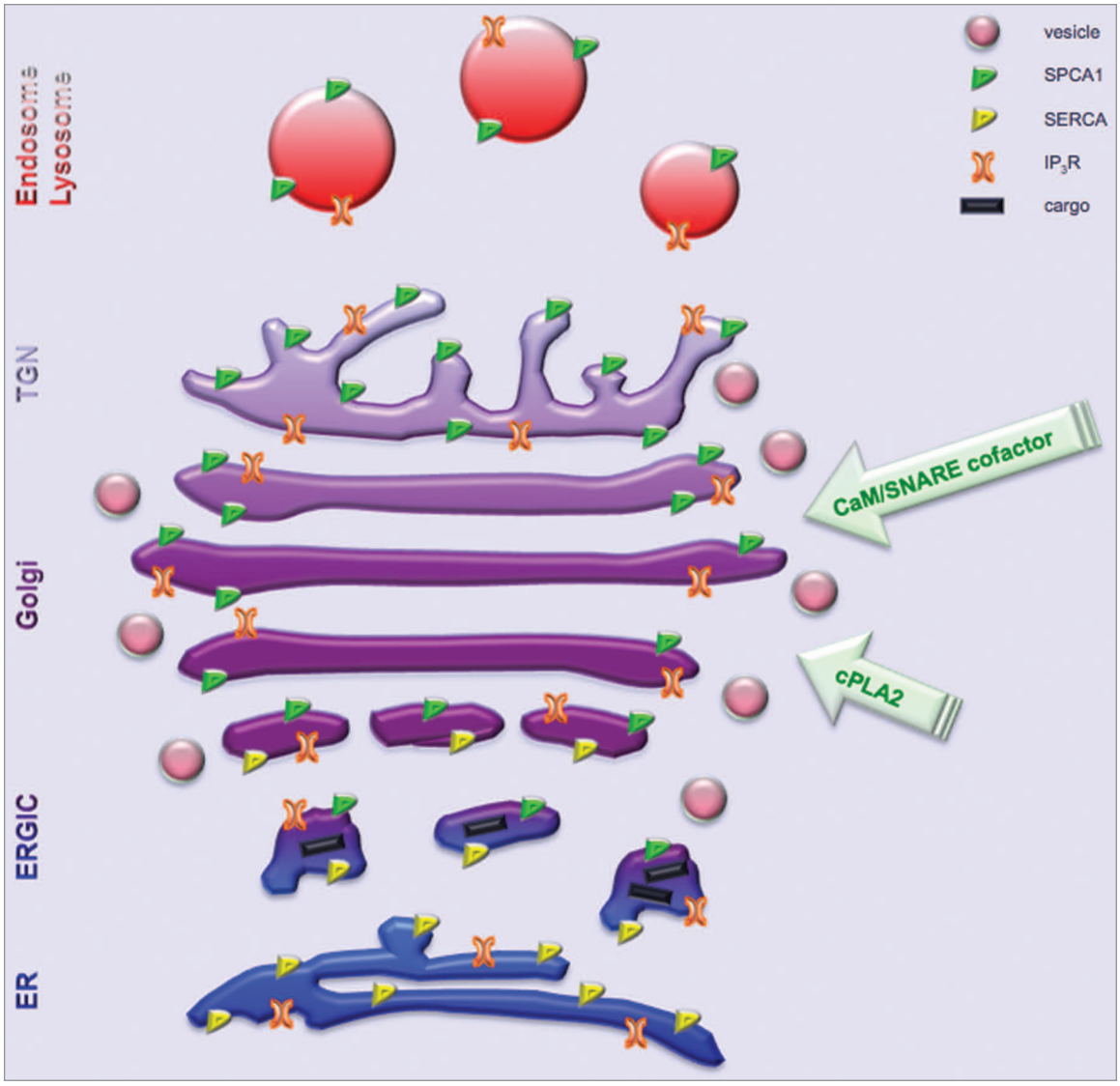

Figure 1. Schematic representation of the (sub)compartmental distribution of SPCA1 along the secretory pathway. SPCA1 is prevalent on the lateral rims of the Golgi cisternae, in the most cis- and trans-Golgi and on the endo/lysosomal compartment, but not on ER membranes. SERCA is typically present on ER membranes and overlapping with SPCA1 distribution only in the most cis-Golgi (ERGIC and cis-Golgi). The arrival of cargo to the GA induces the release of Ca^{2+} from the IP_3R which induces a relocation on the GA of membrane remodeling enzymes (i.e., $cPLA_2$) as well as calmodulin (CaM) and SNARE cofactors Ca^{2+}-sensitive; this redistribution is crucial to orchestrate the SNARE fusion machinery which coordinates the fusion events necessary for the protein trafficking through the GA. Subsequently, the restoration of the basal $[Ca^{2+}]_{cyt}$ requires the activation of the SPCA1, that transfers the increased $[Ca^{2+}]_{cyt}$ into the GA lumen. The diluted colors from the darker ER to the clearest TGN indicates decreasing lumenal $[Ca^{2+}]$.

that at steady state, even a low $[Ca^{2+}]_{cyt}$ is sufficient to promote many constitutive membrane-fusion interactions along the secretory pathway.[7,43-47] For instance, COPI vesicles can fuse with Golgi cisternae at steady-state[48] without any apparent changes in $[Ca^{2+}]_{cyt}$. The presence of SPCA1 at the lateral rims of the GA and in the non compact zones of the Golgi ribbon, where membrane fusion occurs during cargo progression, supports the involvement of SPCA1 not only in GA Ca^{2+} homeostasis, but also triggering (highly localized) increases in $[Ca^{2+}]_{cyt}$. It has also recently been shown that in SPCA1 depleted cells, the basal $[Ca^{2+}]_{cyt}$ is about 1.5-fold lower control cells.[38] If we consider that this basal $[Ca^{2+}]_{cyt}$ is about 50–100 nM, in these SPCA1 depleted cells this $[Ca^{2+}]_{cyt}$ could not be sufficient to drive the fusion of COPI vesicles with membranes of the GA. This is consistent with our observation that SPCA1 depleted cells accumulate COPI vesicles as a result of decreased membrane fusion events. This results in an inhibition of Golgi ribbon formation.

It is now clear that a local release of Ca^{2+} from the lumen of the GA is necessary for intra-Golgi transport;[7] this supports a model of intra-Golgi transport where the movement of protein through the GA requires temporary membrane fusion. In fact, if all of the Golgi cisternae are already interconnected, there is no requirement for SNARE-mediated membrane fusion modulated by the $[Ca^{2+}]_{cyt}$ increase during the late phase of intra-Golgi transport.

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