The trans-golgi compartment

A new distinct intracellular Ca^{2+} store

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The Golgi apparatus (GA) is an intracellular organelle that plays a central role in lipid and protein post-translational modification and sorting. In addition, the GA has been also shown to be involved in Ca^{2+} signalling, as: (i) it accumulates Ca^{2+} within its lumen in an ATP-dependent process catalyzed by two enzymes, the sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) and the secretory pathway Ca^{2+} ATPase1 (SPCA1), and (ii) it releases Ca^{2+} during cell stimulation in response to inositol 1,4,5-trisphosphate (IP_3) receptor activation. Therefore, on this aspect, the GA appears to behave similarly to the major intracellular Ca^{2+} store, the endoplasmic reticulum (ER). By using a new FRET-based Ca^{2+} probe, specifically targeted to the trans-compartment of the GA, we demonstrate that the organelle is heterogeneous in terms of Ca^{2+} handling, the trans-Golgi being insensitive to IP_3 and capable of accumulating Ca^{2+} solely through the activity of SPCA1. The SERCA and the IP_3 receptor appear to be restricted to the cis- and intermediate GA compartments. Moreover, selective reduction of Ca^{2+} concentration within the trans-Golgi, obtained by reducing the level of SPCA1 by RNAi, results in major alterations of protein trafficking within the secretory pathway and induces the collapse of the entire GA morphology.

The Golgi apparatus (GA) is a specialized membranous organelle involved in lipids and proteins modification during transport from their site of synthesis in the endoplasmic reticulum (ER) to other subcellular compartments, such as lysosomes, secretory vesicles and plasma membrane.[1] Morphologically it is quite heterogeneous and, by EM analysis, it is possible to distinguish stacks of flat cysternae (cis- and medial Golgi), tubular-reticular networks and vesicles (trans-Golgi).[2-4] These morphological differences parallel a distinct functionality: for example, glycosyl-transferase enzymes, acting on newly synthesized proteins, have distinct distribution and complementary role in the various GA compartments: mannosidase I is primarily located and active in the cis- and medial Golgi, while sialyl-transferase, fucosyl-transferase or sulphatases are found within the trans-Golgi cisternae and its more distal tubular reticular membrane network (the trans-Golgi network, TGN).[5]

In the last decade, it became clear that the GA also plays a key role as intracellular Ca^{2+} store: using the aequorin Ca^{2+} probe targeted to the organelle, it has been demonstrated that the compartment behaves similarly to the main intracellular Ca^{2+} store of non-excitable cells, the ER. It is indeed endowed, for Ca^{2+} uptake, with the sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase, SERCA (together with the secretory pathway Ca^{2+} ATPase1, SPCA1,[6]) and with inositol-trisphosphate receptors, IP_3Rs, as Ca^{2+} release channels.[7,8] The GA, therefore, has been considered as another important dynamic Ca^{2+} store that participates in determining the spatio-temporal complexity of the Ca^{2+} signal within the cell (reviewed in ref. 9). A number of indirect evidence suggests that the lumenal Ca^{2+} within the GA is fundamental in controlling some key processes occurring in the organelle (post-translational

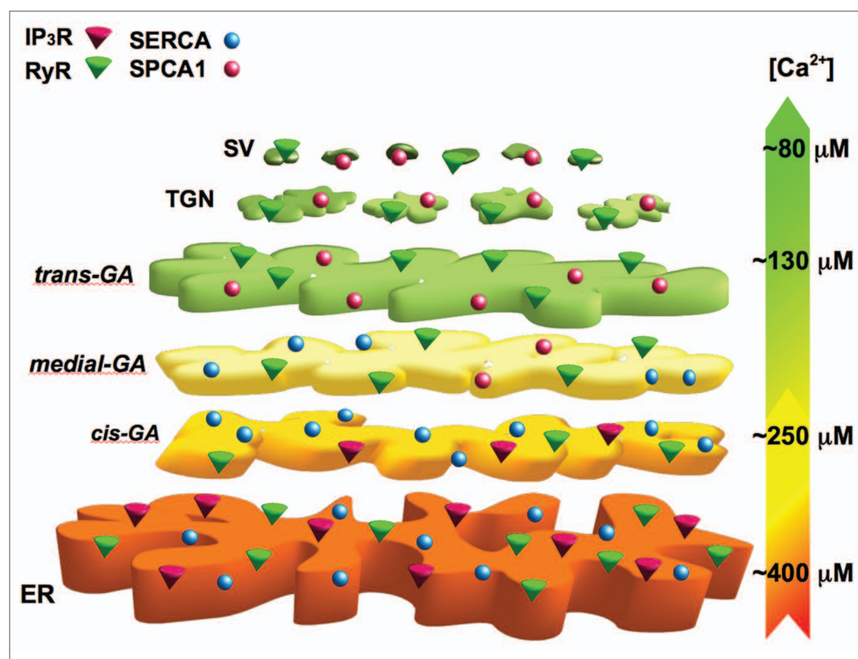

Figure 1. Ca^{2+} concentration and molecular tool-kit gradient through the secretory pathway. The endoplasmic reticulum (ER) is endowed with SERCA, IP_3Rs and, in some cells, RyRs and its luminal $[Ca^{2+}]$ is estimated to be around 400 μM. The Golgi apparatus (GA) can be divided in three distinct sub-compartments: the cis-Golgi, with a luminal $[Ca^{2+}]$ around 250 μM and expressing mainly SERCA and IP_3Rs; the medial-Golgi with SERCA and SPCA1, but not with IP_3Rs; the trans-Golgi with SPCA1 and RyRs (but not IP_3Rs) and a luminal $[Ca^{2+}]$ of ~130 μM. Finally, secretory vesicles (SV) are endowed with SPCA1 and RyRs and show a $[Ca^{2+}]$ around 80 μM. TGN, trans-Golgi network. This model is based on quantitative data for the ER and GA obtained in HeLa cells,[7,14,15] while the data on the secretory vesicles have been extrapolated from experiments carried out in insulin secreting cells.[17]

modifications, protein sorting and trafficking, etc.;[10-12]), and thus dynamic variations of the $[Ca^{2+}]$ within the Golgi could affect cell functions in a variety of ways.

Whether the GA is homogeneous in term of Ca^{2+} handling or whether it can be divided in different sub-compartments, as its morphology and functionality suggest, remained obscure,[13] due to lack of tools to directly investigate this question. In a very recent paper, we have addressed this problem by developing a new, genetically encoded fluorescent Ca^{2+} indicator specifically targeted to the trans-Golgi that allows the quantitative and dynamic measurement of luminal $[Ca^{2+}]$ in this compartment at the single cell level. This probe has unexpectedly revealed that the trans-Golgi compartment behaves differently from the overall GA: it takes up Ca^{2+} almost exclusively via SPCA1 (and not by SERCA); it does not release Ca^{2+} in response to IP_3 generation (but rather accumulates the cation as a consequence of the cytoplasmic Ca^{2+} rises); it is endowed (in some cells) with functional ryanodine receptors, RyRs, thus representing a potential store responding to local Ca^{2+}-induced Ca^{2+} release or to second messengers such as cADPR and NAADP that activate the RyRs.[14]

The Ca^{2+} concentration within the trans-Golgi (~130 μM)[14] appears to be significantly different than that measured in overall GA (~2–300 μM)[7] and ER (~3–400 μM)[15,16] of the same cells or in secretory granules (~80 μM) as measured in other cell types.[17] Taken together, the data from different laboratories, cells and probes suggest that there is a decrease in the lumenal Ca^{2+} concentration down the secretory pathway, ER > cis-Golgi > trans-Golgi > secretory vesicles (**Fig. 1**). Worth noting, this decrease in the free Ca^{2+} within the lumena of these compartments is not paralleled by a reduction in total Ca^{2+} content, rather the opposite, indicating that the Ca^{2+} buffering capacity increases drastically from the ER to the secretory compartment.

In addition, using brefeldin A to block the forward, but not the backward, flow of vesicles in the GA[18] and so inducing the back flow of most trans-Golgi membrane and luminal content (including the Ca^{2+} probe) into the medial- and cis-Golgi and eventually into the ER, we obtained indications for the presence of a Ca^{2+} toolkit protein gradient within the GA: the SERCA and IP_3Rs are excluded from the trans-Golgi; the sensitivity to SERCA inhibitors appears in a compartment still devoid of IP_3 sensitivity (medial-Golgi?); eventually, a compartment (presumably the cis-Golgi), can be revealed where both IP_3Rs and SERCA are highly expressed (**Fig. 1**).

Since the new trans-Golgi Ca^{2+} probe utilized for this study[14] has been constructed by including the trans-Golgi targeting sequence of the resident enzyme sialyl-transferase (the same used by Pinton et al. to targeted the aequorin Ca^{2+} sensor to the GA, Go-Aeq)[7] at the N-terminus of a low Ca^{2+} affinity, FRET based indicator (Go-D1cpv),[19] the question that arises is why Go-Aeq is retained in a different GA sub-compartment (not only in the trans-Golgi, but also in the cis/medial-GA) and why the signal of Go-Aeq is so dramatically biased towards reporting the Ca^{2+} changes from the compartment with the high sensitivity to IP_3. As to the first question, the simplest explanation is that, because Go-Aeq is expressed at much higher levels than Go-D1cpv, its targeting is less accurate than that of the novel probe and, therefore, Go-Aeq is easily mis-targeted to the whole GA. Indeed, we found that the distribution of Go-Aeq in the Golgi overlaps not only with that of canonical trans-Golgi markers, but also with proteins typically located in the cis-GA compartment.[14] In addition we often found cells with strong expression in which Go-Aeq was substantially retained also in the ER, while no mis-targeting of the Go-D1cpv in this compartment was ever observed. As to the second question, not only the signal of Go-Aeq is the mean of thousands of cells and of the different Golgi compartments, but, given the non-linear dependence of luminescence on the $[Ca^{2+}]$, the overall signal of this probe is intrinsically dominated by the compartments with highest Ca^{2+} concentration.[20]

A simple numerical example may explain this concept. Let's assume for simplicity that the Golgi is composed of two compartments, each trapping the same amount of aequorin, one with a [Ca^{2+}] of 450 μM and the other of 150 μM. The normalized rates of photon emission (counts/s, cps) from the two compartments would be ~l00 cps from the first compartment and ~15 cps from the second. The mean luminescent signal would thus be dominated by the first compartment (average 55 cps). Most important, if only the first compartment is sensitive to IP_3, the average response would be again biased towards reporting this event and not the small increase of the second compartment (the first would drop from 100 to ~10 cps and the second would rise from ~10 to ~20 cps, on average a mean drop from 55 to 15 cps).

As to the importance of Ca^{2+} within the trans-Golgi, where the only Ca^{2+} uptake mechanism is based on SPCA1 activity, several authors showed that SPCA1 downregulation affects a number of cellular and Golgi specific functions.[21,22] A SPCA1 knockout mouse is also available;[23] in homozygote animals, the loss of the pump causes Golgi stress, expansion of the apparatus, increased apoptosis and embryonic lethality. Moreover, SPCA1 haploinsufficiency causes a genetic predisposition to cancer.[23] The best example of SPCA1-dependent cellular defect comes from keratinocytes of patients with Hailey Hailey disease (with mutations in one allele for SPCA1).[24,25] These cells have been thoroughly investigated for their defects in protein sorting and other specific cell functions.[26-28] On this aspect, we found that reduction of SPCA1 protein level, by impairing trans-Golgi Ca^{2+} homeostasis, resulted in disturbed trafficking of different classes of proteins as well as in marked morphological alterations of the entire Golgi structure.[14] Thus, maintaining the correct luminal [Ca^{2+}] within the trans-Golgi compartment is essential not only for its specific functions, but also for the entire GA architecture.

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