The trans-golgi compartment
A new distinct intracellular Ca\textsuperscript{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 shown to be involved in Ca\textsuperscript{2+} signalling, as: (1) it accumulates Ca\textsuperscript{2+} within its lumen in an ATP-dependent process catalyzed by two enzymes, the sarco-endoplasmic reticulum Ca\textsuperscript{2+} ATPase (SERCA) and the secretory pathway Ca\textsuperscript{2+} ATPase (SPCA1), and (2) it releases Ca\textsuperscript{2+} during cell stimulation in response to inositol 1,4,5-trisphosphate (IP\textsubscript{3}) receptor activation. Therefore, on this aspect, the GA appears to behave similarly to the major intracellular Ca\textsuperscript{2+} store, the endoplasmic reticulum (ER). By using a new FRET-based Ca\textsuperscript{2+} probe, specifically targeted to the trans-compartment of the GA, we demonstrate that the organelle is heterogeneous in terms of Ca\textsuperscript{2+} handling, the trans-Golgi being insensitive to IP\textsubscript{3} and capable of accumulating Ca\textsuperscript{2+} solely through the activity of SPCA1. The SERCA and the IP\textsubscript{3} receptor appear to be restricted to the cis- and intermediate GA compartments. Moreover, selective reduction of Ca\textsuperscript{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. Morphologically it is quite heterogeneous and, by EM analysis, it is possible to distinguish stacks of flat cisternae (cis- and medial Golgi), tubular-reticular networks and vesicles (trans-Golgi). These morphological differences parallel a distinct functionality: for example, glycosyltransferases 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).

In the last decade, it became clear that the GA also plays a key role as intracellular Ca\textsuperscript{2+} store: using the aequorin Ca\textsuperscript{2+} probe targeted to the organelle, it has been demonstrated that the compartment behaves similarly to the main intracellular Ca\textsuperscript{2+} store of non-excitable cells, the ER. It is indeed endowed, for Ca\textsuperscript{2+} uptake, with the sarcoplasmic-endoplasmic reticulum Ca\textsuperscript{2+} ATPase, SERCA (together with the secretory pathway Ca\textsuperscript{2+} ATPase, SPCA1) and with inositol-trisphosphate receptors, IP\textsubscript{3}Rs, as Ca\textsuperscript{2+} release channels. The GA, therefore, has been considered as another important dynamic Ca\textsuperscript{2+} store that participates in determining the spatio-temporal complexity of the Ca\textsuperscript{2+} signal within the cell (reviewed in ref. 9). A number of indirect evidence suggests that the luminal Ca\textsuperscript{2+} within the GA is fundamental in controlling some key processes occurring in the organelle (post-translational...
In addition, using brefeldin A to block the forward, but not the backward, flow of vesicles in the GA and so inducing the back flow of most trans-Golgi membrane and luminal content (including the \( \text{Ca}^{2+} \) probe) into the medial- and cis-Golgi and eventually into the ER, we obtained indications for the presence of a \( \text{Ca}^{2+} \) toolkit protein gradient within the GA: the SERCA and IP, Rs are excluded from the trans-Golgi; the sensitivity to SERCA inhibitors appears in a compartment still devoid of IP, sensitivity (medial-Golgi?); eventually, a compartment (presumably the cis-Golgi?), can be revealed where both IP, Rs and SERCA are highly expressed (Fig. 1).

Since the new trans-Golgi \( \text{Ca}^{2+} \) probe utilized for this study 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 \( \text{Ca}^{2+} \) sensor to the GA, Go-Aeq) at the N-terminus of a low \( \text{Ca}^{2+} \) affinity, FRET based indicator (Go-D1cpv), 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 \( \text{Ca}^{2+} \) changes from the compartment with the high sensitivity to IP, . 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. 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 \( \text{Ca}^{2+} \), the overall signal of this probe is intrinsically dominated by the compartments with highest \( \text{Ca}^{2+} \) concentration.
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 $[\text{Ca}^{2+}]$ of 450 $\mu\text{M}$ and the other of 150 $\mu\text{M}$. The normalized rates of photon emission (counts/s, cps) from the two compartments would be $\sim100$ cps from the first compartment and $\sim15$ 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 $\sim10$ cps and the second would rise from $\sim10$ to $\sim20$ cps, on average a mean drop from 55 to 15 cps).

As to the importance of $\text{Ca}^{2+}$ within the trans-Golgi, where the only $\text{Ca}^{2+}$ 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 SPCA1 knockout mouse is also available; in homozygote animals, the loss of SPCA1-dependent cellular defect comes about.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 $\text{Ca}^{2+}$ 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 $[\text{Ca}^{2+}]$ within the trans-Golgi compartment is essential not only for its specific functions, but also for the entire GA architecture.

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