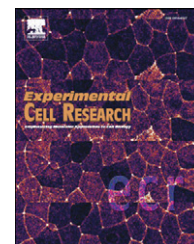


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## Research Article

# Synchronous intra-Golgi transport induces the release of $\text{Ca}^{2+}$ from the Golgi apparatus

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### ABSTRACT

The mechanisms of secretory transport through the Golgi apparatus remain an issue of debate. The precise functional importance of calcium ions ( $\text{Ca}^{2+}$ ) for intra-Golgi transport has also been poorly studied. Here, using different approaches to measure free  $\text{Ca}^{2+}$  concentrations in the cell cytosol ( $[\text{Ca}^{2+}]_{\text{cyt}}$ ) and inside the lumen of the Golgi apparatus ( $[\text{Ca}^{2+}]_{\text{GA}}$ ), we have revealed transient increases in  $[\text{Ca}^{2+}]_{\text{cyt}}$  during the late phase of intra-Golgi transport that are concomitant with a decline in the maximal  $[\text{Ca}^{2+}]_{\text{GA}}$  restoration ability. Thus, this redistribution of  $\text{Ca}^{2+}$  from the Golgi apparatus into the cytosol during the movement of cargo through the Golgi apparatus appears to have a role in intra-Golgi transport, and mainly in the late  $\text{Ca}^{2+}$ -dependent phase of SNARE-regulated fusion of Golgi compartments.

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## Introduction

Modulation of the cytosolic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_{\text{cyt}}$ ) serves as an important signalling system in cell regulation [1]. The

molecular mechanism of membrane fusion that is essential for vital cellular activities, such as intracellular transport, hormone secretion, enzyme release, and neurotransmission, involves the assembly and disassembly of a specialised fusion protein that is

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**Abbreviations:** ARF, ADP-ribosylation factor;  $[\text{Ca}^{2+}]_{\text{cyt}}$ , free calcium ion concentration in the cell cytosol;  $[\text{Ca}^{2+}]_{\text{ER}}$ , free calcium ion concentration in the endoplasmic reticulum;  $[\text{Ca}^{2+}]_{\text{GA}}$ , free calcium ion concentration in the Golgi apparatus; CHX, cycloheximide; COPI, coat protein I; ER, endoplasmic reticulum; Fluo-3-AM, acetoxymethyl ester form of the  $\text{Ca}^{2+}$ -sensitive dye Fluo-3; FRET, fluorescence resonance energy transfer; FURA-2-AM, acetoxymethyl ester form of the  $\text{Ca}^{2+}$ -sensitive dye FURA-2; GA, Golgi apparatus; GT, galactosyl transferase; HBSS, HEPES-buffered salt solution; HFs, human fibroblasts; PC-I, procollagen I; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptors; ST-Aeq, sialyl transferase-aequorin; VSVG, the ts045 temperature-sensitive variant of the G protein of vesicular stomatitis virus

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present in the opposed cell membrane bilayers, the function of which is regulated by  $\text{Ca}^{2+}$ .

Cell fusion events are mediated or regulated by the soluble N-ethylmaleimide-sensitive factor (NSF; a soluble hexameric ATPase) attachment protein receptor (SNARE) proteins [2–4]. These SNAREs are membrane-tethered and they are believed to bring cognate membrane bilayers into very tight apposition, thus promoting their fusion [4,5]. After this fusion, the SNARE complexes are disassembled by NSF, which allows the SNAREs to be recycled for another round of fusion [6]. The SNAREs *per se* cannot induce rapid fusion of membranes [7]. Membrane fusion studies using SNARE-reconstituted liposomes and bilayers have further demonstrated that there is a low fusion rate between SNARE-reconstituted liposomes in the absence of  $\text{Ca}^{2+}$  [7]. Exposure of these SNARE liposomes to  $\text{Ca}^{2+}$  drives vesicle fusion at a near physiological time-scale, demonstrating that  $\text{Ca}^{2+}$  and SNAREs in combination are the minimal fusion machinery in cells [7]. After the approach of the fusing membranes, the SNAREs destabilisation of the membranes within the contact region needs higher  $[\text{Ca}^{2+}]_{\text{cyt}}$  [8].

The best characterised role for  $\text{Ca}^{2+}$  in vesicular transport is in  $\text{Ca}^{2+}$ -triggered fusion of synaptic and secretory vesicles at the plasma membrane [9]. To induce fusion of synaptic vesicles with the presynaptic membrane it is enough to increase the  $[\text{Ca}^{2+}]$  near the synaptic vesicles attached to the presynaptic membrane by some 10–100-fold. However, even if this  $[\text{Ca}^{2+}]$  is increased 1–2-fold, the rate of fusion between the synaptic vesicles and presynaptic membrane can increase by 2-fold [10]. Recent evidence has highlighted a regulatory role for  $\text{Ca}^{2+}$  in fusion reactions in the secretory pathway. The two main organelles of the secretory pathway, the endoplasmic reticulum (ER) and the Golgi apparatus (GA), are both  $\text{Ca}^{2+}$  stores [11]. Two classes of ATPase  $\text{Ca}^{2+}$  pumps maintain the luminal  $[\text{Ca}^{2+}]$  in the ER ( $[\text{Ca}^{2+}]_{\text{ER}}$ ) and the GA ( $[\text{Ca}^{2+}]_{\text{GA}}$ ): the sarco-ER calcium ATPase (SERCA)  $\text{Ca}^{2+}$  pumps are located on the ER and the most *cis* cisternae of the GA [12–14]; and the secretory pathway  $\text{Ca}^{2+}$  ATPase (SPCA)  $\text{Ca}^{2+}$  pumps are located on the medial-*trans* cisternae of the GA [15]. Studies that have analysed the subcellular localisation of  $\text{Ca}^{2+}$  have indicated that the  $[\text{Ca}^{2+}]_{\text{GA}}$  is particularly high, and that there is a  $\text{Ca}^{2+}$  gradient across the organelles of the secretory pathway [11,16]. Both the ER and the GA have also been shown to have inositol 1,4,5-trisphosphate ( $\text{IP}_3$ ) receptors for the triggered release of  $\text{Ca}^{2+}$  during cell signalling [17].

The presence of  $\text{Ca}^{2+}$  stores and specific regulators of the relative luminal  $[\text{Ca}^{2+}]$  in the secretory pathway support the concept that  $\text{Ca}^{2+}$  or  $\text{Ca}^{2+}$  gradients are important in the regulation of protein trafficking. Several lines of evidence show that  $\text{Ca}^{2+}$  is important for the correct functioning of the GA; recently, it was reported that leakage of  $[\text{Ca}^{2+}]$  from Golgi membranes could be necessary for fusion of Golgi compartments and  $\text{Ca}^{2+}$  chelators have been shown to block intra-Golgi protein transport *in vitro* [18]. Retrograde transport from the GA to the ER is also affected by  $\text{Ca}^{2+}$  chelation [18,19]. All of these observations support the concept that  $[\text{Ca}^{2+}]$  transients or gradients, rather than steady-state  $[\text{Ca}^{2+}]$ , regulate the membrane fusion events that occur during protein trafficking along the secretory pathway. There is also indirect evidence suggesting that during the synchronous passage of cargo across the GA, the local  $[\text{Ca}^{2+}]_{\text{cyt}}$  near the GA can increase; indeed, during synchronous intra-Golgi transport, when a cargo arrives at the GA, the C-terminus of  $\text{PLA}_2\alpha$  (used as a sensor for increases in  $[\text{Ca}^{2+}]_{\text{cyt}}$ ) is redistributed to the Golgi membranes [20] and this event is  $\text{Ca}^{2+}$ -dependent [21].

Here, we have used several different approaches to measure  $[\text{Ca}^{2+}]_{\text{cyt}}$  and  $[\text{Ca}^{2+}]_{\text{GA}}$  during the synchronous passage of cargo through the GA, and we demonstrate that the arrival of cargo at the GA induces an increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$ , especially in the GA area; this is concomitant with a transient decrease in  $[\text{Ca}^{2+}]_{\text{GA}}$ . These changes do not, however, occur immediately after the arrival of cargo, but instead after a few minutes delay, when the cargo has reached the *trans*-side of the GA.

## Materials and methods

### Reagents

All chemical reagents were of analytical grade or higher, and were purchased from Sigma-Aldrich (Saint Louis, MS, USA), unless otherwise specified.

### Constructs

The sialyl transferase-aequorin (ST-Aeq) was kindly provided by Dr. R. Rizzuto (University of Ferrara, Ferrara, Italy), [11], the

**Fig. 1 – Fluo-3 analysis of  $[\text{Ca}^{2+}]_{\text{cyt}}$  during passage of cargo through the Golgi apparatus using the miniwave and maxiwave protocols. (A–C) Miniwave (left) and maxiwave (right) protocols and corresponding representative datasets (as normalised relative counts). VSV-infected HeLa cells and HFs were incubated at 32 °C for 1 h and then shifted to 40 °C for 3 h, with Fluo-3-AM loading during the last 30 min. (A) Miniwave: the temperature change back to 32 °C releases the VSVG/PC-I from the ER, with  $[\text{Ca}^{2+}]_{\text{cyt}}$  monitored during passage of cargo through the GA. Maxiwave: following an additional 15 °C temperature block for 2 h (VSVG/PC-I released from the ER to accumulate in intermediate compartment), the temperature change to 40 °C releases the VSVG/PC-I, with  $[\text{Ca}^{2+}]_{\text{cyt}}$  monitored during passage of cargo through the GA. (B, C) Miniwave: after the temperature-block release, representative data shows maximum Fluo-3 intensity (black arrowheads) in HeLa cells (B, left) seen at ~6 min (duration, 3–4 min); and in HFs (C, left) at ~9 min (maintained for the duration of the experiment; 14 min). Maxiwave: after the temperature-block release, representative data shows maximum Fluo-3 intensities (black arrowheads) in both HeLa cells (B, right) and HFs (C, right) seen at ~5 min. (D) Quantification of  $[\text{Ca}^{2+}]_{\text{cyt}}$  under the miniwave protocol, as illustrated in (B, left) and (C, left), for HeLa cells (left) and HFs (right) at indicated times after the temperature-block release to 32 °C. Data are means ( $\pm$  s.d.) from five cells for each protocol and cell type, with each experiment repeated three times. (E) In control experiments, the temperature shift protocol was carried out in cells incubated at 37 °C for 2 h in the presence of CHX (to inhibit new protein synthesis), then kept at 40 °C for 1 h (still with CHX), and finally shifted to the permissive temperature of 32 °C. (F, G) Over the time recording from 4 to 14 min after the temperature shift, there were no significant changes in Fluo-3 signals in HeLa cells (F), with a slight decrease in the signal seen for HFs (G). Thus the temperature shift protocols do not *per se* alter the Fluo-3 analysis. \*\*\* $P < 0.001$ , in pairwise comparisons (unpaired Student *t*-test).**

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