Organelles in focus

Golgi apparatus: Homotypic fusion maintains biochemical gradients within the Golgi and improves the accuracy of protein maturation

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Eukaryotic cells are compartmentalized into organelles which, although constantly exchanging proteins and lipids with their environment, maintain a relatively well-defined biochemical identity. How can such large heterogeneities of chemical composition between (and within) organelles be maintained if different organelles are in constant contact through mass transport? Generic nonlinearities in the transport processes, as would result from specific molecular interactions, can cause the spontaneous chemical differentiation of interacting organelles and compartments within organelles. For the Golgi apparatus, the role of which is to process an incoming flux of lipids and proteins, this spontaneous differentiation decreases inter-cisternal exchange and increases the protein transit time under conditions of high incoming flux. This mechanism enables the Golgi apparatus to spontaneously adjust the protein transit time to the amount of protein requiring processing, thereby improving the processing accuracy of even a limited amount of maturation enzymes.

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1. Introduction

Compartmentalization was a crucial step towards the existence of complex living systems. The cell itself is a compartment, exchanging molecules with its environment, and organelles within the cell also constantly exchange material with one another (Misteli, 2011). They must however retain a specific biochemical and physical identity (in terms of the protein and lipid composition, pH, etc., Wilson and Ragnini-Wilson, 2010; Munro, 2005) necessary for their biological function. At the center of the secretory pathway, the Golgi apparatus is an archetypical example of a dynamical organelle, organized into compartments with well-defined biochemical identities, and constantly exposed to flux of molecules (Lippincott-Schwartz et al., 2000). The Golgi imports immature proteins and lipids from the E.R. and exports them after a succession of chemical maturation steps (Rabouille et al., 1995), for instance, the conversion of ceramides into sphingolipids, or protein glycosylation (Hirschberg and Snider, 1987). Maturation involves successive chemical reactions in the different Golgi compartments, each possessing specific enzymes (Dunphy et al., 1981).

The dominant mechanism for protein transport through the Golgi apparatus is still controversial (Elsner et al., 2003). The vesicular transport model proposes that the different Golgi cisternae (typically five to eight of them, usually regrouped into cis, medial and trans Golgi regions) are fairly static structures between which material is exchanged by vesicles. The cisternal maturation model suggests that the cisternae themselves move through the stack and change their chemical identity with time (Bonfanti et al., 1998). Transport through the Golgi might be a combination of both mechanisms. Here, we focus on vesicular exchange between compartments, a mechanism also relevant to the transport between other cellular organelles; E.R. to Golgi transport for instance, or

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transport from the plasma membrane to endosomes along the endocytic pathway.

If the rates of exchange between the Golgi compartments were constant and uniform, the flux of a given molecular species between two compartments would vary linearly with the concentration difference between the two compartments. This type of transport is equivalent to Fick’s law: $J_f = -D \frac{dc}{dx}$ which governs diffusive processes, and would naturally result in uniform protein and lipid concentrations throughout the Golgi ($J_f$ is the flux, $D \frac{dc}{dx}$ is the local concentration gradient, and $D$ is the diffusion coefficient). Generally, the linear relationship between concentration and fluxes should break down at high protein concentration because of the finite capacity of transport vesicles, or the finite availability of coat proteins. This saturation mechanism (similar to the saturation of enzymatic reaction in the Michaelis–Menten kinetics, Michaelis and Menten, 1913) would however not prevent the homogenization of the Golgi compartments, but would merely slow down the process. There is a growing interest in identifying and modeling the molecular processes that bias transport and permit the existence of stationary concentration gradients within and between cellular organelles (Heinrich and Rapoport, 2005; Binder et al., 2009).

They likely involve specific molecular interactions during vesicle docking and fusion, for instance mediated by matching pairs of SNARE proteins (McNew et al., 2000; Chen and Scheller, 2001). Other types of specific interactions, e.g. involving coat proteins (Bremser et al., 1999) or molecular motors (Jordens et al., 2001), might influence transport and it appears important to go beyond molecular details and to understand the implication of generic transport non-linearities for intra-cellular transport.

We have shown (Dmitrieff and Sens, 2011) that the conjunction of two effects; (i) saturation of the vesicular carriers and (ii) specific molecular interactions during vesicle transport or fusion, creates a dynamical switch beyond a critical concentration, which promotes the retrofusion of secreted vesicles with their compartment of origin. This apparently wasteful phenomenon permits the existence of chemical gradients within the organelle. It also slows down the transit of molecules through the Golgi apparatus, and can strongly increase the organelle efficiency at processing a large influx of proteins.

2. Organelle function

Our simplified model system only includes two compartments (compartments 1 and 2). The first compartment, the “cis-Golgi”, receives an incoming flux of material (e.g. secreted by the E.R.). This material undergoes chemical maturation and is transported to the second compartment, the “trans-Golgi”, before being exported to the rest of the cell. In our model, the chemical identity of a compartment is characterized by the concentration $C$ of a single species ($C_1$ in compartment 1 and in compartment 2, $C_{tot} = C_1 + C_2$ the total concentration in the system). Inter-compartment exchange yields a set of kinetic equations for the two concentrations:

$$
\begin{align*}
\frac{dC_1}{dt} &= J_{influx} - (k_{1-2} C_1 - k_{2-1} C_2) \\
\frac{dC_2}{dt} &= (k_{1-2} C_1 - k_{2-1} C_2) - k_{outflux} C_2
\end{align*}
$$

where $\frac{dC}{dt}$ represents the time derivative, $J_{influx}$ and $k_{outflux}$ are respectively the incoming flux (in compartment 1) and the rate of exit (from compartment 2), $k_{1-2}$ and $k_{2-1}$ are the rates of transport (in s$^{-1}$) from compartment 1 to 2, and compartment 2 to 1, respectively. These rates include multiple steps, which can broadly be grouped into a rate of secretion and a rate of fusion, as sketched in Fig. 1. Linear transport corresponds to constant rates, insensitive to the concentration in the emitting and receiving compartments. Two generic types of non-linearities are introduced in the model (Fig. 1b); (i) vesicular carriers have limited capacity so that the flux of proteins secreted by a compartment saturates at high protein content; $C_1$ is the characteristic concentration for saturation, and (ii) vesicle fusion involves specific molecular interactions, so that the probability of retro and forward fusion depends on the concentrations in the two compartments; $C_2$ is the concentration beyond which specific molecular interactions has a sizable influence on vesicle fusion (see Dmitrieff and Sens, 2011, for a mathematically precise definition of $C_1$ and $C_2$).

For a closed system (without incoming and outgoing fluxes, $J_{in} = k_{out} = 0$), we asked whether compartments following non-linear exchange rules could spontaneously differentiate into different biochemical entities. The results are illustrated in Fig. 1. At low concentration ($C_{tot} \ll C_1$, $C_2$) transport is nearly linear. Heterogeneities between the two compartments relax (diffusively) with time and the two compartments tend to be chemically identical. At higher concentration ($C_{tot}$ larger than a critical concentration $C^*$ depending upon $C_1$ and $C_2$), compartments spontaneously differentiate and adopt distinct chemical identities (Fig. 1c). This critical behavior is due to the fact that a vesicle is more likely to undergo fusion with a compartment of similar composition, a mechanism called homotypic fusion. The composition of a vesicle reflects the composition of the emitting compartment, and it has the tendency to undergo retrofusion with this compartment. Beyond the critical concentration $C^*$, retrofusion becomes dominant, the symmetric state becomes unstable and one compartment becomes enriched in the chemical species of interest (Fig. 1d). In this regime, the flux of material exchanged between the two compartments actually decreases with an increasing total concentration.

For an open system, in which material is imported in compartment 1 (with a flux $J_{in}$) and exported from compartment 2, at a rate $k_{out}$ (Fig. 2), the total concentration in the system results from the balance between the incoming and outgoing fluxes ($C_{tot} = J_{in}/k_{out}$). If the exchange between the two compartments follows the rules outlined above, two qualitatively different regimes can be expected with varying influx: (i) at low influx, the total concentration in the system is smaller than $C^*$, and exchange between compartments is nearly linear, and (ii) at high influx, the concentration is above critical and the exchange between compartments is severely reduced. This can be seen from the evolution of a short pulse of incoming material (pulse-chase procedure, Fig. 2b). In the linear (low pulse) regime, the initiation of the pulse leads to a steady increase of the concentrations $C_1$ and $C_2$ in both compartments, and when the pulse stops, both concentrations eventually decrease because of material export. In the non-linear (high pulse) regime, the critical concentration $C^*$ is reached, and exchange between compartments is impaired. Consequently, the concentration $C_2$ remains low at all times, and the system takes a longer time to export all the imported material.

3. Cell physiology

The increased transit time apparent in Fig. 2b may seem to be a functional drawback for a transport organelle such as the Golgi apparatus. However, transport through the Golgi apparatus is typically quite slow considering the small – micrometric – size of the organelle (the typical protein transit time is of order 20 min in mammalian cells, Bonfanti et al., 1998). Furthermore, longer transit times under high pulse conditions seem to be observed experimentally (Trucco et al., 2004), although very few data are available at this time. The role of the Golgi is to expose transit proteins to specific enzymes for chemical maturation. We argue that in this case, the dynamical switch from fast to slow transport under conditions...
of high incoming flux offers a definite functional advantage. One could expect that a high influx of immature proteins would saturate the available maturation enzymes, letting some immature proteins go through the Golgi unprocessed. It was shown mathematically in Dmitrieff and Sens, 2011 that the dynamical switch discussed here can prevent this from happening. Indeed, although the maturation enzymes are saturated with substrate under conditions of high incoming flux (for instance, following Michaelis–Menten kinetics), inter-compartment exchange is yet more reduced. Maturation can take place during a much longer time, leading to a comparatively higher processing efficiency (the fraction of processed to unprocessed proteins that leave the system increases with the total protein concentration). Remarkably, homotypic fusion, the mechanism that permits the maintenance of cisternal identity, also improves maturation quality control.

4. Organelle pathology

Dysfunction of the glycosylation machinery, caused for instance by mutations of the conserved oligomeric Golgi (COG) complex, has been shown to have dramatic consequences, including lethal phenotypes (Wu et al., 2004). Some cancer cells have a fragmented Golgi and exhibit defects in glycosylation (Kolokumphu et al., 2002). The phenomenon described in this article could constitute an important step for glycosylation quality control. A crucial test of its influence would be to monitor the residency time of sensitive proteins through the secretory pathway in general, and through the Golgi in particular, to test whether, in certain pathologies, a higher production of immature protein might be correlated with faster protein transit through the secretory pathway. More generally, there is a strong correlation between dysfunctional transport in the secretory pathway and defects in protein folding and glycosylation, though the causal chain remains controversial (Gonatas et al., 2006; Cooper et al., 2006). The generality of the phenomenon described here suggests that it could play an important role throughout the secretory pathway.

5. Future outlook

Because identity maintenance imposes constraints on transport, it plays a role in the function of trafficking organelles. We showed that an apparent functional drawback (slowdown of intra-organelle transport) can be beneficial (it increases maturation quality control). The interplay between identity maintenance, transport and quality control have not been intensively studied as of yet. We believe that studying this interplay, both experimentally and conceptually, can yield relevant information both in the understanding of intra-organelle transport and of organelle dysfunction.

In our computations, we used a very simplified model with only two compartments and one relevant chemical species, and we made precise assumptions on the mathematical form of the exchanged fluxes (Dmitrieff and Sens, 2011). However, our conclusions are very general: similar behaviors can be found for systems with several compartments and several species, and with various choices of transport functions. The critical fact is that transport actually decreases when the total concentration in the system increases, which is a consequence of the saturation of transport and the increased probability of vesicle retrofusion at high concentration.

Transport through the Golgi remains controversial. Here, we chose to focus on the so-called vesicular transport model, where proteins are carried from one cisterna to the next by transport vesicles. In the cisternal maturation model, newly synthesized proteins are transported through the Golgi without leaving a given
cisterna, which itself undergoes chemical maturation and physical transport. However, this model must involve some kind of recycling, and hence some vesicular transport, and the consequences of specific molecular interactions in this transport should be of interest. For instance, it has been shown that misfolded proteins could undergo retrograde transport toward the E.R. for proper refolding (Park et al., 2001). Specific fusion could also play a crucial role in this process.

References


