FEBS Letters 486 (2000) 2–9 FEBS 24259

Hypothesis

Protein sorting in the Golgi apparatus: a consequence of maturation and triggered sorting

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Received 29 June 2000; revised 11 October 2000; accepted 12 October 2000

First published online 6 November 2000

Edited by Veli-Pekka Lehto

Abstract To explain how resident proteins distribute in peak-like patterns at various positions in the secretory pathway, Glick and co-workers postulated that resident proteins comprise different populations (termed kin populations) and that these compete with each other for entering retrograde transport carriers [Glick et al. (1997) FEBS Lett. 414, 177–181]. Using modelling and computer simulation, they could demonstrate that differences in competitiveness sufficed to generate overlapping but distinct peak-like steady state distributions of the different kin populations across the Golgi stack. In this study, we have tested the robustness of the competition model and find that over-expression or changes in the number of kin populations affect their overall steady state distributions. To increase the robustness of the system, we have introduced a milieu-induced trigger for recycling. This allows for a decrease in the coupling between kin populations permitting both over-expression as well as changes in the number of kin populations. We have also extended the model to include a Golgi to endoplasmic reticulum (ER) recycling pathway and find that only a small amount of resident proteins may recycle at any time without upsetting their observed distributions in the Golgi stack. The biological relevance of a trigger-induced sorting mechanism and ER recycling is discussed.

Key words: Golgi apparatus; Recycling; COPI; Maturation; Modelling

1. Introduction

Early morphological studies of the secretory pathway suggested that Golgi cisternae form at the *cis*-Golgi network (CGN) and subsequently disassemble at the *trans*-Golgi network (TGN) giving rise to the idea that cisternae are transient carriers of anterograde cargo and that they mature in a *cis* to *trans* direction. This led to the formulation of the cisternal maturation or progression model which for the past 35 years has stood as a good alternative to the vesicular transport model [2–7]. Following the principles of the cisternal maturation model, anterograde cargo is modified by the numerous enzymes which make up the Golgi apparatus. Such enzymes are delivered to the cisterna via retrograde transport carriers (e.g. COPI vesicles or transient tubular intermediates) and are derived from an adjacent but downstream cisterna (see Fig. 1). At the same time, the receiving cisterna donates enzymes that have performed their function to an adjacent but upstream cisterna. This continuous counter-current flow of enzymes ensures a gradual maturation of each cisterna and its cargo and predicts that enzymes are subjected to a high level of recycling. Consistent with this, most glycosylation enzymes distribute in peak-like patterns, over two or more cisternae [8–11]. Also, Golgi resident glycosylation enzymes have been shown to be sorted and concentrated into COPI-derived vesicles, in vitro [12]. These vehicles serve as functional transport intermediates, in vitro [12,13]. Though it remains to be shown, it is likely that retrograde COPI vesicles will be the major driving force of the cisternal maturation process.

To explain how Golgi resident proteins are distinguished from anterograde cargo, sorting signals have been postulated in Golgi resident proteins. One such signal, the K(X)KXX signal, is present in cytoplasmic domains of several proteins that reside in the early parts of the pathway (e.g. the endoplasmic reticulum (ER) and in the ER to Golgi interface). The K(X)KXX signal binds directly to COPI providing a mechanistic link for signal-mediated sorting [14,15]. In the context of cisternal maturation, sampling in the lateral plane for cytoplasmic motifs by COPI is thought to provide the necessary specificity for sorting of resident proteins away from anterograde cargo [12]. However, a direct link between COPI and resident proteins does not explain how resident proteins exhibit peak-like steady state distributions over the *medial*/*trans* or *trans*/TGN cisternae rather than the ER and the *cis* cisternae. But by ascribing populations of resident proteins with different degrees of competitiveness to enter retrograde carriers, Glick and co-workers could show through modelling and computer simulation that different populations formed distinct but overlapping distributions, at steady state covering the entire range of the pathway [1]. A high degree of competitiveness ensured an early *cis* distribution while poor competitiveness resulted in a more *trans*-like distribution. A drawback with this competition model is, however, that the steady state distribution of a particular population is not just a consequence of its own competitiveness but also, and more importantly, the relative competitiveness of all other kin populations that exist in the pathway. In other words, the distribution of each and every protein is derived from the sum of distributions of all other resident proteins. Such a dependency puts a strain on any system, particularly if chang-

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PII: S0014-5793(00)02155-4

ing one or more parameters. We have here examined how the competition model accommodates changes in the number of resident proteins that comprise a given population or in the overall number of populations used in the simulation. We assume that each population contains multiple copies of the same resident protein or a mixture of resident proteins which exhibit similar sorting properties and term these kin populations [16,17]. We find that the 'competition model' is affected by over-expression as well as alterations in the number of kin populations causing significant changes in steady state distributions.

To improve the robustness of the competition model, we have uncoupled the dependency between each kin population by assigning a 'trigger' value for sorting. We postulate that the sorting ability of resident proteins is triggered through gradual changes in the lumenal milieu of the cisterna. Thus, a given resident protein or kin family is predicted to exist in either of two states, a resident state or a sorting state. We find that the 'trigger' model improves the robustness of the system in that it permits both over-expression as well as changes in the number of kin populations. We include also in our modelling the ER and the recently described COPI-independent pathway which allows membranes and resident proteins to recycle directly from the *trans*/TGN to the ER [18,19].

2. Results

Any model aimed at explaining the dynamic aspects of the Golgi apparatus must take into account the occurrence of asymmetric protein distributions across the stack, the robustness of such distributions to over-expression and the occurrence of glycosyltransferases in transport carriers. Computer simulation is here a powerful tool, particularly if several parameters have to be considered at the same time. A good example of this can be found in work by Leibler and colleagues modelling spindle formation in mitosis or bacterial

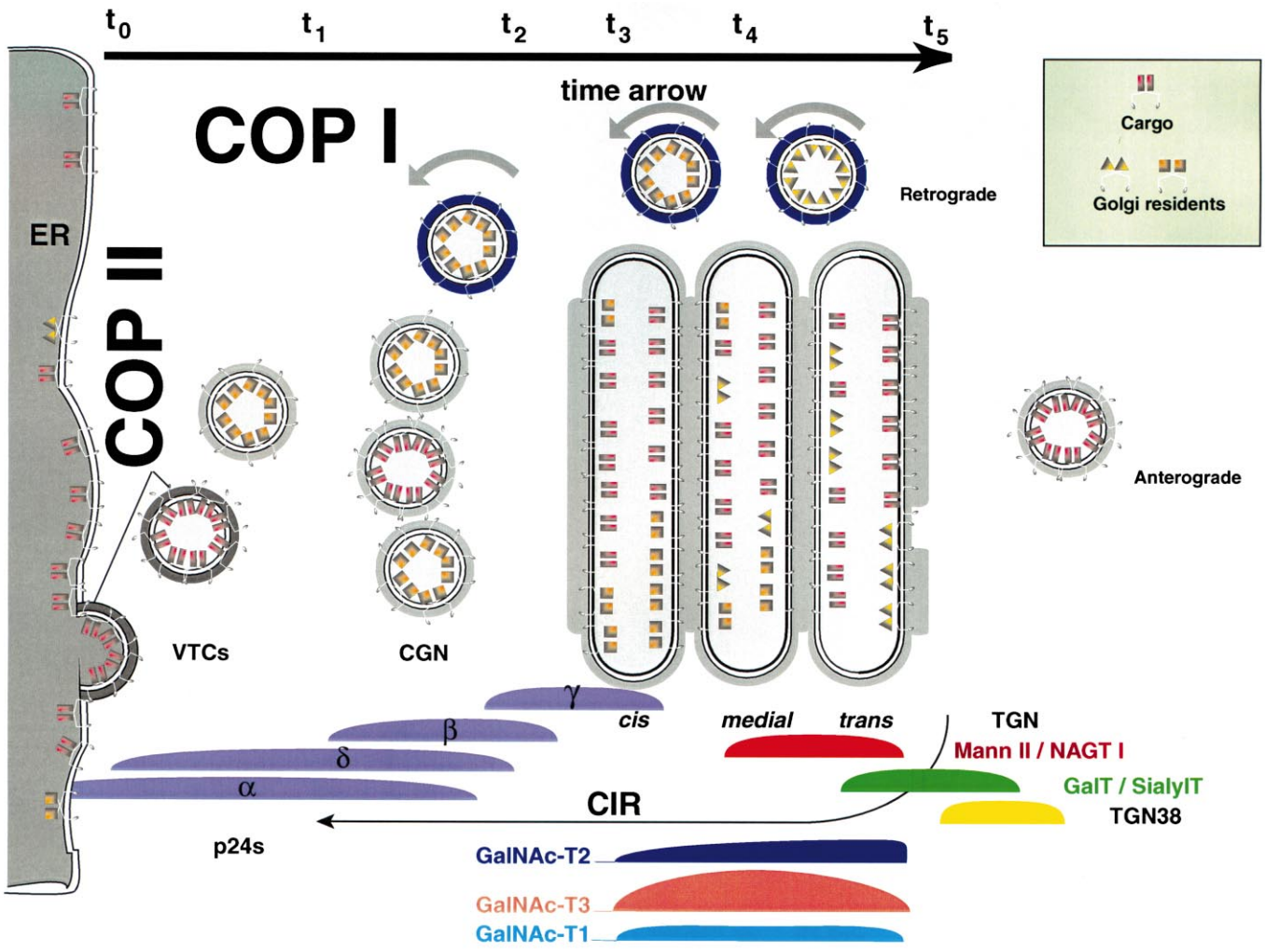

Fig. 1. The cisternal maturation model. Transport between ER and the Golgi is mediated by retrograde COPI and anterograde COPII vesicles, resulting in the de novo formation of new cisternae which move to the *cis* face of the Golgi stack. Cisternae within the stack mature by receiving and sending retrograde vesicles from older to younger cisternae, respectively. Finally, they mature into the TGN from where anterograde cargo is delivered to various destinations in the cell. The TGN is consumed in terms of resident proteins which travel backwards. Remaining lipids are recycled as well (see Section 3). Resident proteins are constantly recycled within the stack by means of the retrograde COPI vesicles or through a COPI-independent route (CIR) while anterograde cargo remains in the cisternae where it is modified by enzymes passing through in the retrograde direction.

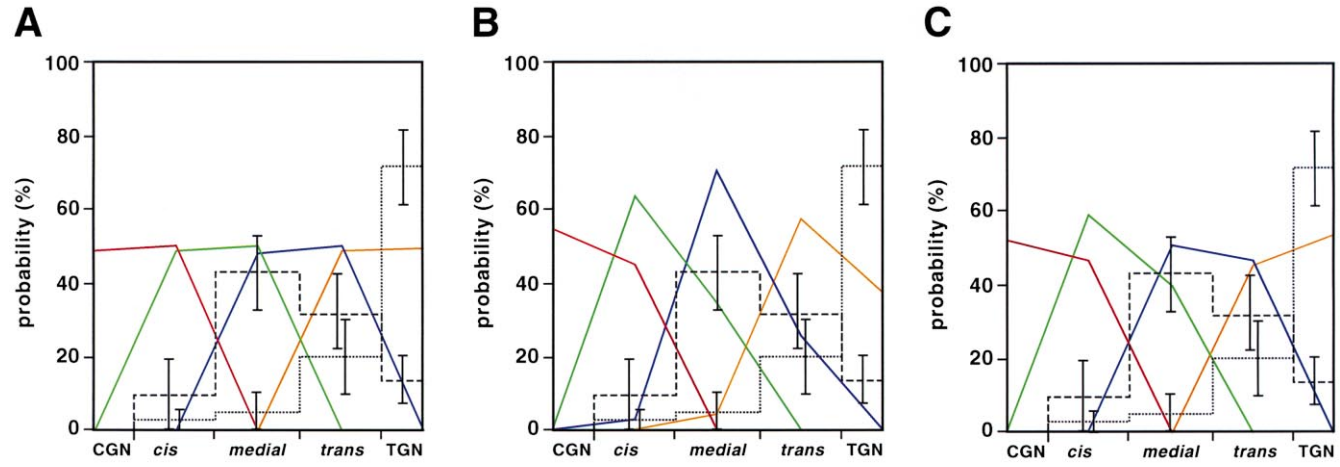

Fig. 2. Steady state distribution of resident proteins in the competition model with four kin populations upon over-expression. A: Without over-expression, B: with over-expression of the *trans*/TGN kin population, and C: with over-expression of the *medial*/*trans* kin population. The sorting abilities for all kin populations are constant throughout the stack and differ by a factor of 100 (see text for details). Dashed and dotted lines with error bars represent the experimental data obtained for Mann II and SialylT, respectively [9]. In the absence of perturbations (A), the blue and the orange curves agree well with the experimental whereas upon over-expression (B and C) considerable deviations become apparent.

chemotaxis [20–22]. Here, modelling was based on detailed knowledge of the two systems allowing for accurate time-resolved simulations. In terms of the Golgi apparatus, our knowledge of the microscopic details is still too small to permit modelling in full detail. Instead, stochastic modelling can be used where average parameters are entered. This results in a more coarse grained stochastic sketch but serves nevertheless an important purpose in defining and testing systems in terms of minimal parameters.

2.1. Sorting by competition

The first stochastic approach to model protein sorting in the Golgi apparatus was performed by Glick and co-workers [1]. A series of assumptions were made as follows: first, protein transport in the anterograde direction is by cisternal maturation. As such, only retrograde carriers are considered, each carrying an amount of N resident proteins. Second, on average the same amount of vesicles is back-transported from each cisterna per time unit. This results in a constant retrograde membrane flux which keeps the structural integrity of the Golgi stack intact. Third, at any given time, there exist a fixed number of cisternae per stack. The first cisterna, *cis*, does not bud any vesicles at all and only receives material from the second cisterna. The *trans*-most cisterna only sends resident proteins backwards but does not receive any. Fourth, since the *trans*-most cisterna scatters by sending cargo in the retrograde direction, it is removed when emptied. All cisternae subsequently move forward one position and a new *cis* cisterna is formed de novo. Fifth, Golgi resident proteins exist in two states, either being bound to COPI or not. Only resident proteins bound to COPI may enter a retrograde transport carrier. Sixth, because of the restricted number of 'seats' in a retrograde transport carrier, all resident proteins bound to COPI compete for retrograde transport. Seventh, anterograde cargo is presumed to follow the cisterna when it is 'hopping' forward until it reaches the TGN from where it is transported out of the Golgi stack. As such, anterograde cargo does not contribute to intra Golgi transport and hence, does not figure in these simulations. Since each vesicle carries only an N amount of proteins, the different kin populations have to compete for retrograde transport in one budding event. The degree of competitiveness is determined by collective phenomena such as affinity for COPI, kin recognition [23], lipid bilayer thickness [24] or unknown factors. We assume that in each cisterna, k, a fraction of all proteins belonging to a kin population (i) compete for free 'seats' in a retrograde transport carrier. The total number of copies which make up this particular fraction is denoted by $n(i,\mathrm{k})$. The parameter, $e(i,\mathrm{k})$, referred to as the 'sorting ability' of each kin population (i) in cisterna k, contains all microscopic details of the competition event. Increasing the value of $e(i,\mathrm{k})$ denotes a higher sorting ability of the particular kin population to enter a retrograde transport carrier. In the original competition model of Glick and co-workers, the sorting ability for each individual kin population (i) was constant across the stack. Using this notation, the total amount of competing proteins in cisterna k is given by Eq. 1 and the probability to take one protein molecule from one kin population out of cisterna k is given by Eq. 2a in Table 1. A typical result from a computer simulation

Table 1
Equations used

$$c(\mathrm{k}) = \Sigma_i e(i,\mathrm{k}) n(i,\mathrm{k}) \quad (1)$$

$$p(i,\mathrm{k}) = e(i,\mathrm{k}) n(i,\mathrm{k}) / c(\mathrm{k}) \quad (2a)$$

$$P(m) = m!/((N-m)!N!) p(i,\mathrm{k})^m (1-p(i,\mathrm{k}))^{N-m} \quad (2b)$$

$$e(i,\mathrm{cisterna}) = e(i) \times (\tanh(\mathrm{H}^{\mathrm{cisterna}} - \mathrm{H}^{\mathrm{ER}} + \mathrm{Hi}) + 1)/2 \quad (3)$$

The probability to draw m copies of kin population (i) in N trials (note that N seats in the retrograde transport carrier have to be occupied) is given by Eq. 2b. Hence, the set of all probabilities $P(m)$ for $m < N$ becomes a binominal distribution. To simplify the numerics, we have substituted the evaluation of this probability distribution by taking only average values, $N \times p(i,\mathrm{k})$, as was also done in the study by Glick and co-workers [1]. As such, in one budding event, $N \times p(i,\mathrm{k})$ particles of kin i from cisterna k are back-transported.

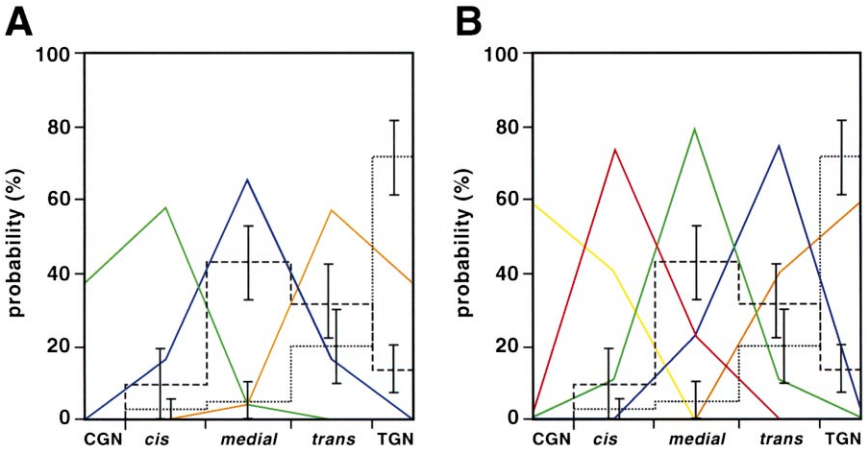

Fig. 3. Steady state distribution of resident proteins in the competition model upon changes in the number of kin populations. A: After deleting or B: adding one kin population. These perturbations resulted in strong deviations from the original steady state (see Fig. 2A). This is due to that the constant sorting abilities throughout the stack lead to a strong coupling of the kin populations. As in Fig. 2, the dashed and dotted lines denote experimental data.

based on the above competition scheme is shown in Fig. 2A. For comparison, the co-distribution of β1,2-*N*-acetylglucosaminyltransferase I (GlcNAc-T1) and α1,3-1,6-mannosidase II (Mann II) as determined in HeLa cells is depicted with a dashed line whereas the co-distribution of β1,4-galactosyltransferase (GalT) and α2,6-sialyltransferase (SialylT), also determined in HeLa cells, is shown as a dotted line [9]. Five cisternae, CGN, *cis*, *medial*, *trans* and the TGN and four kin populations, CGN/*cis* (red), *cis*/*medial* (green), *medial*/*trans* (blue) and *trans*/TGN (orange) were used. The number of protein copies per kin population was set to 2×10^5 and their respective sorting abilities were set to be constant across the stack. The competition values $e(i)$ for the different kin populations were then set to drop towards the *trans* face. The following values were ascribed for the different kin population: $e=1$ (CGN/*cis*); 10^{-2} (*cis*/*medial*); 10^{-4} (*medial*/*trans*); 10^{-6} (*trans*/TGN). As can be seen by inspecting Eq. 2a, the absolute values of $e(i)$ are not important but rather the ratio between the different $e(i)$ values which in this model needs to be 100. This simulation does not take into account what happens to proteins which reside exclusively in the TGN such as TGN38 [25]. This is due to the fact that the TGN has to empty and that this cisterna is then removed in order for all cisternae to move forward one step. To include TGN38, one would have to relax the boundary conditions and incorporate more information about how the TGN is organised (e.g. subdomains) and what happens to the TGN during the scattering process, both which were outside the scope of these simulations. As shown in Fig. 2A, the two middle kin populations (green and blue) yielded peak-like distributions with distinct but overlapping peaks, each distributing over two cisternae upon reaching steady state. The blue curve, denoting the *medial*/*trans* kin population, can be compared with the experimental data for GlcNAc-T1 and Mann II (dashed line) and the *trans*/TGN localising kin population with the observed distribution of GalT and SialylT (dotted line). Taking into account the simplicity of the model, the numerical data show quite nice agreement with experimental curves.

We next examined whether simulated distributions could resist over-expression. We have previously estimated the level of over-expression in stable cell-lines expressing different epitope-tagged glycosylation enzymes in HeLa cells. It appeared that SialylT (determined by enzymatic activity) and Mann II (determined by comparative gold labelling) could be stably over-expressed about 50–100 or 5–6 times, respectively, without affecting their steady state distributions or that of endogenous GalT [9]. To test the competition model in terms of over-expression, we changed the amount of resident proteins ascribed to the *medial*/*trans* or the *trans*/TGN kin population by adding an extra 6.5×10^4 (6% of the *medial*/*trans* kin population was calculated to consist of Mann II) or 9.9×10^4 molecules (1% of the *trans*/TGN kin population was calculated to consist of SialylT), respectively. Over-expression of the *trans*/TGN kin population (Fig. 2B) resulted in a shift of all kin populations towards the *cis* side, all displaying somewhat sharper peaks. Although the gross features of the steady state remained similar, a visible change in shape occurred and the absolute values of the *medial*/*trans* distribution (blue) changed by a factor of two, while the *trans*/TGN curve (orange) changed somewhat less. This contradicts experimental observations, where little or no change was observed in terms of the position of two markers for the *trans*/TGN kin population, GalT and SialylT [9]. Similarly, over-expression of the *medial*/*trans* kin population (Fig. 2C) affected the *cis*/*medial* kin population so that it now distributed more over the *cis* cisterna. In summary, the peak distributions of each kin-population were altered in terms of their shapes, in effect pushed in the *cis* direction as a result of over-expression. We next examined whether the competition model was affected by changes in the number of kin populations. First, we deleted the *cis*/CGN kin population, simulating only three kin populations but keeping all other parameters (e.g. number of cisternae) fixed. The resulting steady state is shown in Fig. 3A. As can be seen, the result revealed a big deviation from the distributions obtained simulating four kin populations (Fig. 2A). The distributions were more peaked and their centres shifted towards the *cis* side. In Fig. 3B we simulated steady state distributions upon adding a fifth kin population (yellow). The newly introduced kin population competes strongest and consequently, localised over the CGN/*cis* cisternae. The distributions of all other kin populations were now shifted to the *trans* face and were more narrow compared to

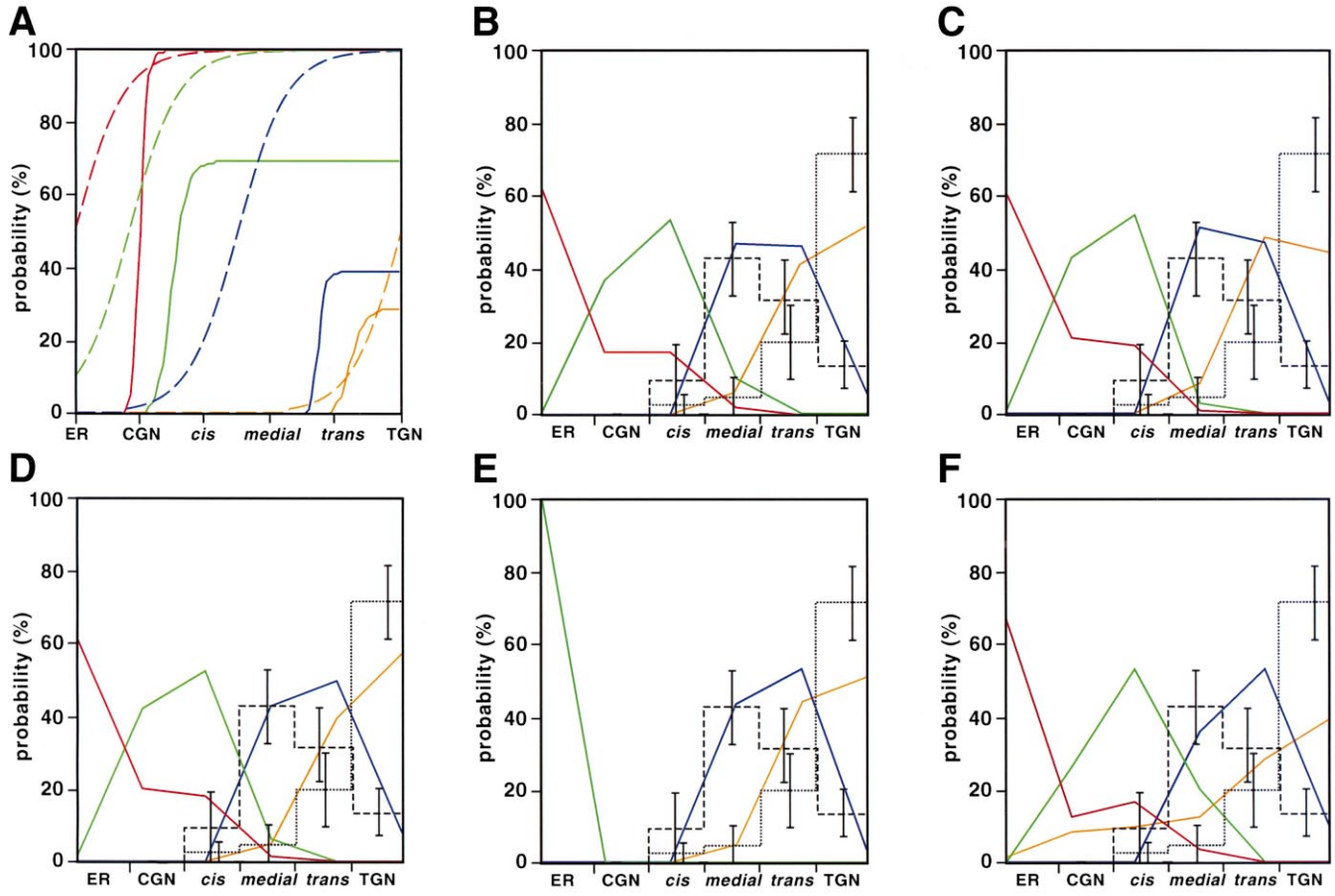

Fig. 4. Trigger-induced sorting. A: The rising sorting abilities for the trigger model according to Eq. 3. Full and dashed lines denote sorting abilities with varying steepness. B–D: Steady state distribution of resident proteins in the trigger model with ER and four kin populations without over-expression (B), with over-expression of the *trans*/TGN kin population (C), and with over-expression of the *medial*/*trans* kin population (D). E: Steady state distribution of resident proteins in the trigger model with ER after deleting one kin population. As can be seen, the blue and orange curves agree well with the experimental data, which was not the case for the old competition model (Fig. 3A). The clear deviations of the *cis*-most localising kin populations are due to the simplified transport scheme used in the simulations and therefore, an artefact of the specific nature of the numerical model. F: Redistribution of proteins belonging to the *trans*/TGN kin population, when the TGN is nearly empty (see text for details) resulted in a significant increase of these proteins in *cis* and *medial* cisternae, which is inconsistent with experimental findings. As in Fig. 2, the dashed and dotted lines denote experimental data.

the peaks depicted in Fig. 2A. Taken together, the results obtained from over-expression and alterations in the number of kin populations suggest a lack of robustness in the competition model. These findings were not altered upon introducing an ER into the competition model (data not shown). The ER was assumed to send on average as much protein (picked by chance) to the CGN as the CGN returns protein back to the ER. If the TGN is empty, all cisternae but the ER move one step ahead and a new empty CGN is inserted. In all other details, the numerics remained unchanged. The introduction of such an ER-like compartment is similar to the introduction of an additional 'passive' cisterna.

2.2. Trigger-induced sorting

To improve the robustness of the competition model, we have introduced a new competition scheme where each kin population is assigned a sorting ability that changes from cisterna to cisterna in a steep sigmoid fashion (Fig. 4A). By this assignment, retrograde transport occurs in a triggered fashion when a given kin population reaches the 'right' position in the stack. We term this dynamically modified competition model the 'trigger' model. The reason for introducing a trigger is based on an observed lumenal pH gradient across the Golgi stack, which is maintained by cisternal proton pumps [26,27]. The pH gradient is suggested to contribute to conformational changes of the resident proteins giving rise to, for example, an increased ability to recruit COPI [28]. Alternatively, as the lipid bilayer thickness and its lipids change in a *cis* to *trans* direction, this may contribute to the switch of the resident protein into a 'sorting state' [24]. For simplicity, we assume a linearly decreasing trigger, e.g. the appropriately rescaled difference in concentration of H^+ in the particular cisterna ($H^{cisterna}$) and that of the ER (H^{ER}). Then the sorting ability, according to Eq. 3 (Table 1), depends in a sigmoid fashion on the increase in concentration of H^+ across the stack, where Hi fixes the point of maximum steepness for a certain kin population. A protein belonging to a certain kin population stays in its resident state in the maturing cisterna until a critical pH is reached which causes a conformational change switching the protein to its sorting state with a higher sorting ability. Through this mechanism, the protein stays on average at the same spatial position and only cycles erratically to neighbouring cisternae. Only proteins which are in the sorting state within the same cisterna com-

pete according to the old competition scheme. In this way the strong coupling of the original competition model has been eliminated. As a consequence, the trigger model introduces a new set of dynamics compared to the competition model. We like to emphasise that only the spatially varying sorting abilities are entered into the numerics. Thus, the assumption of a pre-existing decreasing proton concentration is not crucial (see also Section 3). Indeed, the observed proton gradient, a direct consequence of the action of proton pumps, is self-generated assuming that proton pumps themselves are subjected to triggered recycling (Weiss and Nilsson, in preparation). Also, the proton gradient does not need to be linear as long as it is monotonously increasing over time.

In Fig. 4B, the steady state distributions using the trigger model with four kin populations is shown followed by the same over-expression conditions (Fig. 4C,D) as those used in simulations presented in Fig. 2. To be closer to the real biological system, we included the ER (see description above). This did not affect the results as such as simulations where the ER was omitted gave similar results to those shown in Fig. 4. The *medial*/*trans* and *trans*/TGN kin populations (blue and orange) agree well with the experimental data and moreover, show only slight perturbations upon over-expression (compare with Fig. 2B,C). Also, the trigger model appears robust with respect to variations in the number of kin populations. Upon deletion of one kin population, the *medial*/*trans* (blue) and *trans*/TGN (orange) kin populations remained nearly unchanged (Fig. 4E). There were, however, deviations in the *cis*/*medial* kin population (green). This is due to the fact that retrograde transport in the Golgi stack is required and that the least competing protein of the whole stack has to maintain this. In the case of deleting the *cis*-most population (red), retrograde transport must now be supported by the *cis*/*medial* population. These residents, therefore, cycle constantly between *cis*/CGN and the ER. When introducing a new kin population, the numerics reacted quite sensitive to which parameters were assigned to the new population. This reflects that for proteins being simultaneously in the sorting state, the situation of the old competition model now applies, resulting in a strong coupling and enhanced sensitivity. However, one can still find parameters such that the blue and orange curve remain nearly unchanged (data not shown). Since this could never be achieved within the original competition model (see Fig. 3), the trigger model behaves in a more robust manner also after these perturbations.

Having shown that the trigger model is more robust, we investigated if it would hold up to an introduction of a second recycling pathway directly from the TGN back to the ER. This ER recycling pathway was discovered recently by trapping proteins in the ER that had recycled from the Golgi [29,30]. This recycling pathway is mechanistically distinct from the COPI signal-mediated recycling of Golgi resident proteins in that it is independent of known coat proteins and the small GTPase, arf-1 [18,19]. To test the trigger model, we assume that after 90% of the *trans*/TGN kin population has recycled via COPI-derived retrograde transport carriers, the remaining proteins scatter via 40–50 COPI-independent transport carriers each carrying about 500 proteins back to the ER. By taking this kind of partially periodic boundary condition, a significant increase in *trans*/TGN residents in early Golgi compartments is observed (Fig. 4F). Also, the *cis*/*medial* kin population changes significantly under these conditions showing that a direct recycling pathway from the TGN back to the ER greatly upsets the steady state distributions of both the *trans*/TGN and the *cis*/*medial* kin populations. Although one might argue that simulation of such a particular point on the basis of the trigger model seems inappropriate, we think that having shown the robustness of the trigger model, it is important to demonstrate that a direct recycling route from the TGN back to the ER cannot be extensive (not exceeding 5%, data not shown) without upsetting the overall system. Significant perturbations were also observed with the original competition model using 10% recycling of the *trans*/TGN kin population back to the ER (data not shown).

3. Discussion

The competition model was proposed by Glick and coworkers to explain how resident proteins distribute and maintain their positions throughout the Golgi stack in the context of cisternal maturation. We have in this study investigated the robustness of this model and find that it is not very resistant to alterations in expression levels or numbers of kin populations used in each simulation. This is mainly due to a strong coupling between different kin populations, i.e. that each and every protein distribution depends on the distribution of all other proteins in the Golgi stack. This is reflected by the fact that sorting abilities entered into this model must differ by orders of magnitude to generate steady state distributions resembling those found experimentally. To overcome this, we have uncoupled the strong dependency between kin populations by introducing a trigger for the sorting event. This modified scheme for sorting abilities now shows a spatial increase, e.g. due to a pH gradient across the stack of cisternae, and they differ in their maximum values at most, only by a factor two. Here, the degree of steepness of each curve as well as its maximum value can be modified (see Fig. 4A, dashed and full lines) without affecting the overall results. We postulate in this study that resident proteins exist in two states, a resident state and a sorting state, and that resident proteins switch between these two states depending on the lumenal milieu of the cisterna in which they reside. As the milieu gradually changes as the cisterna matures (e.g. pH and lipid composition) in a *cis* to *trans* direction, a resident protein will in a sigmoid fashion switch to its sorting state (Fig. 4A). This results in the incorporation into retrograde transport carriers which ferry the resident protein to an adjacent and earlier cisterna. Upon reaching this, the resident protein is exposed to a different milieu (e.g. a higher pH) and consequently switches back to its resident state with a high probability. As the milieu of this newer cisterna will change as well, the resident protein will after a short while switch back to its sorting state and the cycle is then repeated. Such a trigger-induced sorting scheme assumes that each resident protein can precisely sample the lumenal milieu and this results in a high evolutionary pressure to maintain the overall structure of each resident protein. In support of this, all Golgi resident proteins examined so far show a remarkable level of conservation across species at the primary sequence level throughout the entire protein. Although we have assumed a linear changing milieu, which underlies the sigmoid varying sorting abilities, the specific nature of the trigger poses no restriction on the model. Assuming that proton pumps in the Golgi stack themselves are sub-

ject to pH-triggered recycling, a self-organising non-linear pH gradient across the stack evolves within 3–4 cycles, giving rise to similar steady state distributions as were found here (Weiss and Nilsson, in preparation).

3.1. Trigger-induced sorting and ER recycling

The notion that anterograde cargo is subjected to a 10–1000-fold concentration throughout the pathway [31–33] leaves open the question how the overall membrane balance of the pathway is maintained. As Golgi resident proteins are sorted and concentrated into COPI-derived vesicles [12], it does not suffice to recycle bulk lipids via COPI vesicles [34]. This effect is most noticeable at the ER to Golgi interface where concentration of cargo has been observed for several anterograde marker proteins and at the TGN where the cisterna is thought to be consumed via forward moving carriers or recycling COPI vesicles. It follows therefore that lipids will remain behind and that these must be recycled in order to keep the overall balance of the pathway. We recently proposed that a COPI-independent recycling pathway performs this function [18,34]. The extent of recycling permitted through this pathway is a matter of debate. Our simulation puts an upper limit to the extent of recycling from the TGN back to the ER via a direct route. If allowing for 10% of recycling, we find great perturbations in the distribution of both the *trans*/TGN and the *cis*/*medial* kin populations. In our recent study on ER recycling of GalNAc-T2, we estimated that the linear density of ER labelling was 150-fold lower than that observed in the Golgi stack [30]. As the surface area of the ER is roughly 10 times larger than the Golgi, this results in a steady state population of 6.3% of GalNAc-T2 in the ER at any given time, a value close to the 5% limit set by the trigger model.

3.2. Future predictions and perspectives

A trigger-induced sorting scheme extends the previous simple but elegant competition model proposed by Glick and coworkers by uncoupling a strong dependency between different kin populations. Several testable predictions are born out from the extended trigger model. First, the localisation of individual resident proteins should be sensitive to perturbations of the lumenal milieu. Inhibition of proton pumps should cause loss of resident proteins to the cell surface if the pH gradient is a contributing factor for inducing the trigger state. Second, over-expression of *medial*/*trans* or *trans*/TGN populations should not alter the distribution of a CGN/*cis* or *cis*/*medial* kin population. Candidate marker proteins belonging to these populations are the members of the p24 family which reside at the *cis* face of the Golgi stack [35–37]. Third, only a low level of recycling back to the ER from the TGN is allowed. As such, increased recycling should result in changes in steady state distributions of kin populations.

The total number of kin populations is arbitrary and can be altered without affecting steady state providing that the number of proteins remains the same. For example, the *medial*/*trans* kin population can be broken up into several sub-populations, each with an individual trigger value. This as long as their combined total number of molecules remains similar to the original kin population or within the boundaries set by the robustness of the system (some over-expression is permitted). As such, although the trigger model builds on and extends the concept of kin recognition [23], the size of individual kin complexes, be it multi enzyme complexes [16,17] or larger oligomeric complexes does not affect the behaviour of the trigger model.

In moving from a model based solely on competition to a trigger-induced sorting model, have we now lost the self-organisational principles of the original model? In the competition model, only relative competitiveness between kin populations was of importance. In the trigger model, we have linked the position of a resident protein to the particular milieu of the cisterna, for example the pH. But modification of the milieu depends on the action of resident proteins (e.g. proton pumps) which themselves are subjected to recycling giving rise to a self-organising system (Weiss and Nilsson, in preparation). This holds true also when considering peripheral proteins such as tethering molecules, GTPases, ATPases, motor proteins and coat proteins provided that these bind to and follow transmembrane proteins. In conclusion, we have shown that a trigger-induced sorting model, building on the previous competition model, suffices to explain how resident proteins maintain their distinct peak-like distributions at various places in the pathway. We hope that by doing so, this will stimulate discussion and prompt experiments to test its validity in the future.

Acknowledgements: We wish to thank Professor T. Geisel (MPI for Fluid Dynamics, Göttingen, Germany) for kind support and Drs Brian Storrie (Blacksburg, VA, USA) and Thomas Surrey (EMBL) for helpful suggestions and discussions.

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