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Generation of nonidentical compartments in vesicular transport systems

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How can organelles communicate by bidirectional vesicle transport and yet maintain different protein compositions? We show by mathematical modeling that a minimal system, in which the basic variables are cytosolic coats for vesicle budding and membrane-bound soluble *N*-ethyl-maleimide–sensitive factor attachment protein receptors (SNAREs) for vesicle fusion, is sufficient to generate stable, nonidentical compartments. A requirement for establishing and maintaining distinct compartments is that each coat preferentially packages certain SNAREs during vesicle budding. Vesicles fuse preferentially with the compartment that contains the highest concentration of cognate SNAREs, thus further increasing these SNAREs. The stable steady state is the result of a balance between this autocatalytic SNARE accumulation in a compartment and the distribution of SNAREs between compartments by vesicle budding. The resulting nonhomogeneous SNARE distribution generates coat-specific vesicle fluxes that determine the size of compartments. With nonidentical compartments established in this way, the localization and cellular transport of cargo proteins can be explained simply by their affinity for coats.

Introduction

All eukaryotic cells use vesicular trafficking to transport proteins and lipids (for reviews see Rothman and Wieland, 1996; Schekman and Orci, 1996; Barlowe, 2000). Vesicles bud from one compartment, taking along both soluble and membrane proteins as well as lipids, and fuse with another compartment. Transport in the anterograde direction must be counterbalanced by retrograde traffic to keep the size of compartments constant and reuse components of the transport machinery. The bidirectional traffic would tend to equalize the composition of the compartments, yet most proteins and some lipids are concentrated in one organelle and define its identity. How can such nonuniform distributions be achieved?

Vesicular transport involves budding and fusion. Budding is mediated by coats, which are cytosolic proteins that bind to a membrane, induce curvature, and eventually pinch off a vesicle (for review see Schekman and Orci, 1996). Coats also bind proteins to be packaged into vesicles. The COPI and COPII coats, which are involved in the transport between the Golgi apparatus and the ER, combine both functions. They are recruited to the membrane by the small G proteins Arf1 and Sar1, respectively, which in turn are converted into their active GTP-bound states by membrane-associated nucleotide-exchange factors. Clathrin coats, involved in endocytosis and in transport between the Golgi and endosomes, have two functional components: clathrin itself is responsible for shaping the membrane, whereas various adaptors (e.g., AP1, AP2, GGA) bind cargo during budding (for reviews see Barlowe, 2000; Kirchhausen, 2000; Robinson, 2004). Again, recruitment of these proteins to the membrane requires additional proteins. Fusion follows uncoating of a budded vesicle and is mediated by membrane proteins, called SNAREs (Sollner et al., 1993b). The cytoplasmic domains of one type of SNARE (t-SNARE), consisting of a heavy chain and two light chains, pair with the cytoplasmic domain of another type of SNARE (v-SNARE), consisting of a single chain (Fukuda et al., 2000; Parlati et al., 2000). During fusion, the chains assemble into a parallel four-bundle helix, bringing the two lipid bilayers in close apposition and culminating in membrane fusion (Sutton et al., 1998; Weber et al., 1998). After fusion, the SNARE complexes are dissociated by the ATPase NSF, recycling them for another round of transport (Sollner et al., 1993a; Mayer et al., 1996). For each fusion step in the cell a different combination of t- and v-SNAREs is required (for review see Pelham, 2001). Many additional proteins participate in vesicular transport (e.g., Rab, Sec1, and tethering proteins), and several aspects of the process remain controversial (for reviews see Pelham and Rothman, 2000; Wickner, 2002;

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Abbreviation used in this paper: BFA, brefeldin A.
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Palmer and Stephens, 2004), but coats and SNAREs may constitute the basic components for many transport steps (Rothman and Wieland, 1996). In fact, the addition of purified COPI or COPII coats plus their activated G proteins leads to vesicle budding from liposomes (Matsuoka et al., 1998; Spang et al., 1998), and the incorporation of purified SNAREs into liposomes is sufficient for one round of vesicle fusion (Weber et al., 1998; McNew et al., 2000). Undoubtedly, there are many other factors that increase the efficiency of the system in vivo and allow its regulation, but the in vitro experiments raise the possibility that coats and SNAREs, together with their activators, may be the basic components for establishing nonidentical compartments.

Several explanations have been proposed for how distinct compartments are generated, but they provide only partial answers and raise further questions. The t-SNAREs have been suggested to determine the identity of a compartment by directing the fusion of specific vesicles, but how are the SNAREs themselves localized? It has been proposed that SNAREs may be concentrated according to the cholesterol content of a membrane (Bretscher and Munro, 1993; Rayner and Pelham, 1997), but, in this case, how would cholesterol be localized? The same kind of circularity arises if one assumes the specific binding of a coat to a compartment. How does a coat "know" where to go? The specific coat binding would have to be caused by the localization of certain proteins, and again the question is how these are targeted. Thus, despite enormous insight into the molecular details of vesicular trafficking, the fundamental problem of how compartments are generated is still unanswered. Moreover, an explanation is needed why a nonhomogeneous distribution of protein and lipid is a stable state. The robustness of the system is best illustrated by experiments with the drug brefeldin A (BFA): when added to cells, the Golgi enzymes are redistributed back into the ER, but when the drug is removed, they return to their original position (Lippincott-Schwartz et al., 1990). The experiment also raises the question of why the Golgi grows back to its original size. How is the size of an organelle generally determined?

Here, we demonstrate that a minimal vesicular transport system, with coats and SNAREs as basic components, can generate stable, nonidentical compartments. Even this simple system can only be understood through mathematical models. These models can explain the generation of specific compartments, as well as their size regulation and the transport of cargo proteins to different cellular destinations. Although a mathematical model has been developed previously which generates protein gradients in preexisting Golgi cisternae (Glick et al., 1997; Weiss and Nilsson, 2000), our analysis provides the first self-consistent explanation for the generation of distinct, stable compartments.

Results

The basic two-compartment model

We first test whether two nonidentical compartments, which could represent the ER and Golgi, can be generated with a minimal vesicular transport system comprising only coats and SNAREs as basic variables (Fig. 1). By contrast with previous arguments, we do not a priori assume that the two compartments differ; rather, this should be the outcome of the modeling. Vesicles can therefore bud from both compartments with either coat A or coat B, which may represent COPI and II, respectively. The budding rates, which implicitly depend on the function of G proteins or other factors, may differ for the two coats, but are initially assumed to be constant. In an extended model, we will later include proteins, such as nucleotide exchange factors for G proteins, which stimulate the budding of coated vesicles. We consider two sets of cognate SNAREs that mediate fusion of a vesicle with a compartment by pairing: SNARE X pairs with SNARE U, and SNARE Y pairs with SNARE V. These would correspond to the v- and t-SNARE pairs involved in ER- and Golgi- fusion reactions, respectively. During budding, SNAREs are packaged into a vesicle in amounts that depend on their affinities for a coat. Golgi SNAREs can indeed bind to COPII (Miller et al., 2003; Mossessova et al., 2003), and in our model we assume that SNAREs generally bind to coats. For simplicity, we initially assume that the cognate SNAREs have identical binding constants for the coats (e.g., SNAREs X and U bind equally well to coat A). After budding, the coats are rapidly released, and uncoated vesicles can then fuse with either compartment, depending on the concentrations of cognate SNAREs in a vesicle and in a compartment; the fusion rate will be high if both the vesicle and compartment contain high concentrations of these SNAREs (e.g., of SNAREs X and U; we assume that it does not matter which partner is present in a compartment or a vesicle). The assumption that vesicles can fuse back into the originating compartment is not well supported by experimental data (Kamena and Spang, 2004), but we will later discuss mechanisms that reduce the extent of back fusion. Further assumptions are that vesicles do not fuse with one another, that X-U and Y-V SNARE pairs, generated during fusion, are immediately dissociated by NSF, and that vesicle budding and fusion can occur over the entire surface of each organelle (assuming that they only occur at the rims of the compartments gives similar results; unpublished data).

The system is described by two sets of differential equations, one for the time-dependent changes of the number of vesicles and compartment sizes, the other for the changes of SNARE amounts (see Materials and methods; Supplement 1 contains the full set of equations, which can be directly combined with common integration programs, available at http://www.jcb.org/cgi/content/full/jcb.200409087/DC1).

Generation of nonidentical compartments

An intuitive understanding of the system can be obtained when one considers a steady-state situation. In this case, the fluxes between the compartments of both the SNAREs and membrane lipids must be equal. This can be achieved in a trivial way if vesicles moving in opposite directions contain identical concentrations of the same SNAREs, i.e., if both compartments have the same composition. However, another possibility to obtain a steady state is as follows. When many vesicles with a low content of SNAREs X and U go in one direction, and few

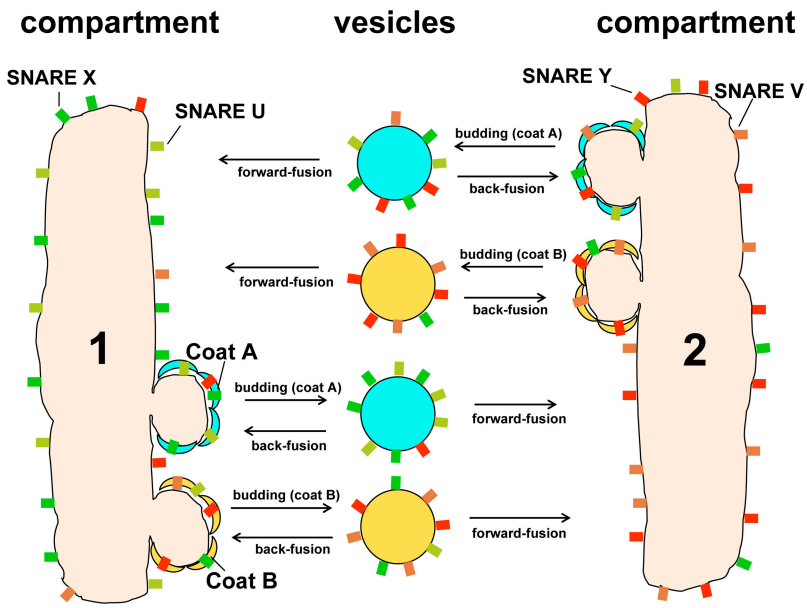

Figure 1. **A simple two-compartment system.** Vesicles bud from compartments 1 and 2, which may represent the ER and Golgi, with either coat A or B, which may represent COPI and COPII, respectively. The different types of vesicles (blue and yellow for vesicles budded with coats A and B, respectively) can fuse with either the compartment they originate from or the other compartment. SNAREs X, U, Y, and V (bars in dark green, light green, red, and brown, respectively) are packaged into the vesicles according to their affinities for a coat (coat A has a high affinity for SNAREs X and U, and coat B for SNAREs Y and V). Fusion of the vesicles with a compartment is mediated by SNARE pairing (X with U and Y with V), with the fusion rates dependent on the concentrations of cognate SNAREs in vesicles and compartments. The distribution of the SNAREs corresponds approximately to the nonuniform steady state (see Fig. 2 c), with SNAREs X and U primarily in compartment 1 and SNAREs Y and V in compartment 2.

vesicles with a high content of SNAREs X and U in the other, the same number of SNARE molecules can be moved in opposite directions and the content of SNAREs X and U in the two compartments can be kept constant. However, the lipid balance is not maintained, because more vesicles move in one direction than the other. To achieve a steady state for lipids, the transport of SNAREs Y and V has to be balanced in a similar way as that of SNAREs X and U, but with the net vesicle transport biased in the reverse direction. The final result is a steady state for both the SNAREs and the lipids. Because the vesicles have different concentrations of cognate SNAREs when they move in opposite directions, this implies that the two compartments differ in their SNARE contents, i.e., are nonidentical.

The mathematical modeling supports these qualitative considerations and clarifies under which conditions the steady states with identical or nonidentical compartments are obtained (Fig. 2). The critical condition for the generation of nonidentical compartments is that SNAREs X and U bind better to coat A than to coat B, and that SNAREs Y and V bind better to coat B than to coat A (the ratios of dissociation constants $q_X = K_X^B/K_X^A$, $q_U = K_U^B/K_U^A$, $q_Y = K_Y^A/K_Y^B$, and $q_V = K_V^A/K_V^B$ must be above a certain threshold value; in the following we set $q = q_X = q_U = q_Y = q_V$). As shown in Fig. 2 a, below the threshold, the trivial steady state is obtained with equal amounts of SNAREs in both compartments ($X_1 = X_2 = U_1 = U_2 = Y_1 = Y_2 = V_1 = V_2$; X_1 is the amount of SNARE X in compartment 1, X_2 the amount of SNARE X in compartment 2, etc.). At a critical q-value (bifurcation point), the steady state with this uniform SNARE distribution becomes unstable (Fig. 2 a, dotted line), and two new stable steady states with nonuniform SNARE distributions appear, such that one type of cognate SNAREs is mostly present in one compartment and the other type in the other: SNAREs X and U are concentrated in compartment 1, and SNAREs Y and V in compartment 2; $X_1 = U_1 = Y_2 = V_2 > X_2 = U_2 = Y_1 = V_1$), or the other way around ($X_2 = U_2 = Y_1 = V_1 > X_1 = U_1 = Y_2 = V_2$; the latter corresponds to an exchange of the numbering of the two compartments). Symmetry breaking requires that all SNAREs have a preference for a coat (see SNARE distributions), although the q's need not be equal. It should be noted that despite their accumulation in one compartment, all SNAREs continuously cycle between the two compartments.

The net vesicle fluxes between the compartments behave as predicted from the qualitative considerations given above. For q below the bifurcation point, the net fluxes from compartment 1 to 2 mediated by coat A and coat B vesicles are equal $\left(F_1^A = F_1^B\right)$ (Fig. 2 b). Above the bifurcation point, the forward vesicle flux with coat B becomes much larger than that with coat A $\left(F_1^B > F_1^A\right)$ (Fig. 2 b). For retrograde transport from compartment 2 to 1 the situation is simply reversed: coat A vesicles carry most of the vesicle flux $\left(F_2^A > F_2^B\right)$. If compartments 1 and 2 represent ER and Golgi, respectively, coat B (COPII) vesicles are largely responsible for anterograde transport, and coat A (COPI) vesicles for retrograde transport. Thus, even though we did not a priori assume that the coats differentiate between the two compartments, they carry the net fluxes in different directions, as experimentally observed.

The analysis also shows that the coat B–mediated vesicle flux from compartment 1 to 2 and the coat A–mediated vesicle flux in the reverse direction have a maximum when plotted against q (Fig. 2 b): if the coats have an extreme preference for their preferred SNAREs, the SNARE distribution becomes very asymmetrical (essentially no Y and V in compartment 1 and no X and U in compartment 2; Fig. 2 a). Coat B vesicles budding from compartment 1 and coat A vesicles budding from compartment 2 then contain essentially no SNAREs at all and therefore cannot fuse with any compartment, resulting in disruption of compartment communication. Thus, although the SNAREs need to bind preferentially to a coat to generate nonidentical compartments, the binding should not be too strong.

A more detailed explanation of the steady-state vesicle fluxes is given in Fig. 2 c ($q = 100$). Coat A vesicles budding

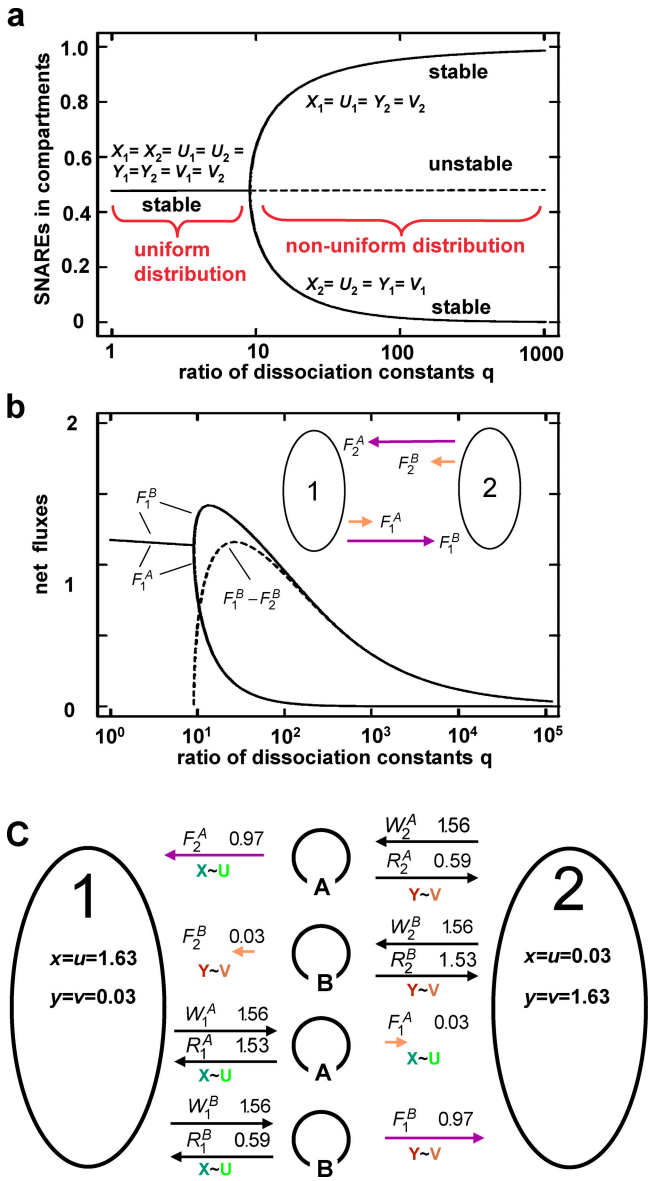

Figure 2. **Generation of nonidentical compartments.** (a) The amounts of SNAREs in the compartments are plotted against the ratio of the dissociation constants q for the binding of SNAREs to the two coats, $q = q_X = q_U = q_Y = q_V$ with the following:

$$q_X = K_X^B / K_X^A, q_U = K_U^B / K_U^A, q_Y = K_Y^A / K_Y^B,$$
$$q_V = K_V^A / K_V^B, \text{ and } K_X^A = K_U^A = K_Y^B = K_V^B = 1.$$

The SNARE amounts were normalized (X_i/X; U_i/U; Y_i/Y; V_i/V; $i = 1, 2$; X_i, U_i, Y_i, and V_i are the amounts of SNAREs in compartments 1 or 2, and X, U, Y, and V are the total amounts of SNAREs, $X = U = Y = V = 0.5$; note that we use capital letters for amounts and lower case letters for concentrations). Other parameters have the following values: $\kappa = 40$, $w^A = w^B = 1.0$. The bifurcation point is at $q = 9.04$. Below this point, the SNARE distribution is uniform, above it there are two stable states in which the two types of cognate SNAREs are concentrated in different compartments. (b) The net vesicle fluxes between the two compartments are plotted versus q (the fluxes were normalized such that the sum of net forward fluxes equals unity). Below the bifurcation point, the fluxes with coat A and B vesicles are equal, above it, the flux from compartment 1 to 2 with coat B vesicles is larger than that with coat A ($F_1^B > F_1^A$). The reverse relationship holds for transport from compartment 2 to 1 ($F_1^B = F_2^A; F_1^A = F_2^B$). The dotted line shows the difference between the forward and backward fluxes of coat B vesicles ($F_1^B - F_2^B$) and of coat A vesicles ($F_2^A - F_1^A$), which can be used to localize cargo with affinity for coat B and coat A, respectively (see Fig. 4). (c) The steady-state concentrations of SNAREs X, U, Y, and V (x, u, y, and v), and the vesicle fluxes (normalized so that the sum of net forward fluxes equals unity) were calculated for $q = 100$ (all other parameters as in panel a). W_i^A and W_i^B are the fluxes with which vesicles bud from the two compartments. R_i^A and R_i^B are the fluxes with which budded vesicles fuse back into the originating compartment, and F_i^A and F_i^B are the fluxes with which they fuse with the other compartment. The lengths of the arrows correspond approximately to the flux sizes, and the preferential SNARE pairing mediating fusion is indicated with colored letters. The normalized sizes of compartments (S_i/S; $i = 1, 2$), of the vesicle population that budded from compartment 1 with coat A (N_1^A/S)($= N_2^B/S$), and of the vesicle population that budded from compartment 1 with coat B (N_1^B/S)($= N_2^A/S$) are 0.3, 0.02, and 0.18, respectively ($S = S_1 + S_2 + N_1^A + N_1^B + N_2^A + N_2^B$).

from compartment 1 with the budding rate W_1^A have a high content of SNAREs X and U, both because X and U are high in compartment 1 and because coat A has a preference for these SNAREs. On the other hand, these vesicles have very little SNAREs Y and V, because these are low in compartment 1 and have a weak affinity for coat A. These coat A–budded vesicles will thus fuse back with compartment 1 by X-U pairing (high back fusion flux R_1^A; Fig. 2 c), and only few vesicles fuse with compartment 2 where X and U are present in low amounts (low forward fusion flux F_1^A). Coat B vesicles budding from compartment 1 with the rate W_1^B have an intermediate content of both SNARE X and U, as well as Y and V: X and U are high in compartment 1, but do not bind well to coat B, and Y and V are low, but bind well to this coat. These vesicles will either fuse with compartment 2, using Y-V pairing (high forward fusion flux F_1^B), or fuse back with compartment 1, using X-U pairing (relatively high back fusion flux R_1^B; Fig. 2 c). For compartment 2, the situation is reversed.

The analysis shows that the net vesicle flux into each compartment occurs mostly by vesicles that have budded with the coat that preferentially binds the SNAREs accumulating in this compartment (e.g., coat A vesicles go to compartment 1 where SNAREs X and U accumulate). The nonuniform stable steady state can be understood as a balance between two opposing processes: (1) vesicle fusion, which increases the content of cognate SNAREs in a compartment and thus the potency with which it can attract vesicles containing these SNAREs, resulting in an autocatalytic effect, and (2) vesicle budding, which distributes the SNAREs between the compartments.

The model gives robust nonuniform solutions over a wide range of parameter values, the only critical parameter being q, but, of course, the concentrations of the SNAREs and the sizes of compartments and vesicle population may vary. The simple model thus explains the generation of nonidentical compartments, but one unsatisfactory aspect is that there is a high rate of futile cycling of coat A vesicles at compartment 1 (high rates of budding and back fusion) and of coat B vesicles at compartment 2 (Fig. 2 c). This can be avoided by relatively small modifications of the model (see Cargo-dependent vesicle transport).

Stability and compartment sizes

To illustrate the stability of the nonidentical compartment system, we have modeled the time course of recovery of cells from

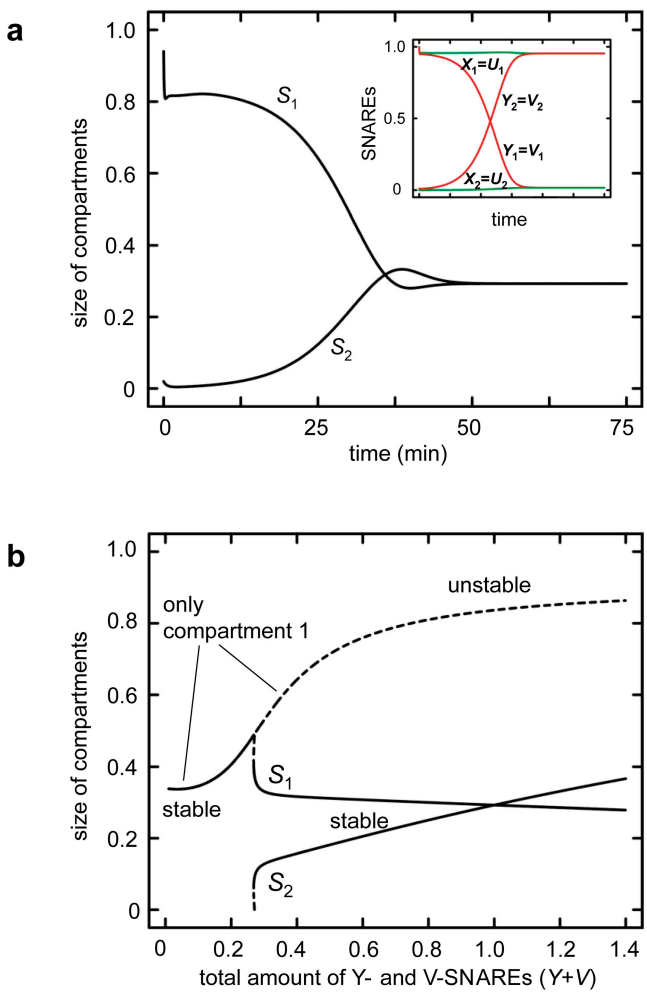

Figure 3. **Stability of the system and compartment size regulation.** (a) The time-dependent recovery of the system, starting with a negligible size of compartment 2 and essentially all the SNAREs in compartment 1 was modeled. This is similar to the reformation of the Golgi from the ER after BFA treatment. Plotted are the normalized compartment sizes (S_i/S; $i = 1, 2$) and, in the inset, the normalized amounts of SNAREs (X_i/X; U_i/U; Y_i/Y; V_i/V; $i = 1, 2$) in the two compartments. (b) The normalized compartment sizes are plotted versus the total amounts of SNARE Y and V. Below a critical point compartment 2 disappears and the residual SNAREs Y and V are found in the only remaining compartment. The dotted line corresponds to the single compartment situation, which becomes unstable if the total SNARE concentration exceeds the critical value. The calculations were done with the same parameter values as in Fig. 2 with $q = 100$.

BFA treatment (Lippincott-Schwartz et al., 1990), where the Golgi (compartment 2) has initially a negligible size, and essentially all its enzymes and SNAREs are present in the ER (Fig. 3 a). The system returns to the steady state by moving SNAREs Y and V into compartment 2, and by increasing the size of compartment 2 at the expense of compartment 1. Although compartment 2 can be regenerated, it must have initially the size of at least one vesicle.

The compartment sizes are determined by vesicle fluxes, which are dependent on several parameters, including the budding rate and the total amounts of SNAREs. The different SNAREs must be present at about equal levels to generate compartments of comparable size. For example, when the total amounts of SNAREs Y and V are reduced, compartment 2 shrinks (Fig. 3 b). Below a critical level (bifurcation point), it can no longer exist and the residual SNAREs Y and V are localized to the single, remaining compartment. The compartment sizes can also vary relative to the vesicle population. With the parameters used in Fig. 2 c, ~60% of the total membrane is present in compartments and 40% in vesicles.

SNARE distributions

Up to this point the cognate SNAREs (e.g., X and U) had the same distributions, because we assumed that they have the same preference for a coat (e.g., X and U bind equally strongly to coat A and equally weakly to coat B). Now we consider the possibility that SNAREs X and Y have pronounced preferences for one of the coats (by a factor of 100), whereas SNAREs U and V show less preference (by a factor of 10). Regardless of whether SNAREs U and V bind less well to their preferred coat or more strongly to the wrong coat, they still accumulate to almost the same extent as their SNARE partner in one compartment (Table I, compare rows 2 and 4 with row 1). Thus, the SNARE partner that shows less preference for a coat can be dragged by the more strongly binding partner into a compartment, a fact that is explained by the autocatalytic mechanism of SNARE accumulation. However, if SNAREs U and V have no preference for a coat at all, a nonuniform distribution is no longer achieved (Table I, rows 3 and 5). Coat A must therefore bind both X and U significantly stronger than Y and V, and coat B must have the reverse preference. Contrary to the conventional view, in which the t-SNARE is concentrated in a compartment and the v-SNARE in vesicles, both cognate SNAREs accumulate in the same compartment, as observed experimentally (Volchuk et al., 2004). Again, it should be noted that all SNAREs continuously cycle between the two compartments.

Recent experiments indicate the existence of inhibitory-SNAREs that associate with other SNAREs into inactive complexes (Varlamov et al., 2004). When inhibitory-SNAREs are included in the modeling, the SNARE gradients are steeper (Supplement 2, available at http://www.jcb.org/cgi/content/full/jcb.200409087/DC1), as predicted (Varlamov et al., 2004). In addition, the bifurcation occurs at lower q-values (unpublished data), indicating that the generation of nonidentical compartments becomes more favorable.

Localization and fluxes of cargo

Next, we consider the transport of other proteins (cargo), which, in the simplest case, does not affect vesicle transport. When cargo binds coat A (COPI) better than coat B (COPII), i.e., the ratio of dissociation constants $\kappa^B_{\text{cargo}}/\kappa^A_{\text{cargo}}$ is > 1, cargo will accumulate in compartment 1 (ER), because coat A mediates most of the vesicle flux from compartment 2 to 1 and coat B does not package it well for transport in the reverse direction (Fig. 4 a; cargo 1). This would correspond to the retrieval of an ER protein from the Golgi by binding of coat A (COPI) to a COOH-terminal KKXX sequence (Letourneur et al., 1994; Fiedler et al., 1996) or to the KDEL receptor interacting with the COOH-terminal KDEL sequence of a luminal ER protein (Lewis and Pelham, 1990). When cargo binds coat B

Table I. **Steady-state distributions of SNAREs with different coat preference**

	SNARE levels in compartment 1				SNARE levels in compartment 2			
	X	U	Y	V	X	U	Y	V
All SNAREs have high coat preference	0.954	0.954	0.016	0.016	0.016	0.016	0.954	0.954
X, Y have high preference, U,V bind less well to preferred coat	0.823	0.808	0.114	0.182	0.114	0.182	0.823	0.808
X, Y have high preference, U, V have no preference (weak binding to both coats)	0.461	0.499	0.461	0.499	0.461	0.499	0.461	0.499
X, Y have high preference, U, V bind more strongly to wrong coat	0.879	0.800	0.087	0.150	0.087	0.150	0.879	0.800
X, Y have high preference, U, V have no preference (strong binding to both coats)	0.485	0.471	0.485	0.471	0.485	0.471	0.485	0.471

The steady-state levels of SNAREs in the two compartments were calculated for different cases. Strong coat preference of all SNAREs means that they all bind by a factor of 100 better to the preferred coat ($K_X^A = 1$, $K_U^A = 1$, $K_X^B = 100$, $K_U^B = 100$, $K_Y^A = 100$, $K_V^A = 100$, $K_Y^B = 1$, $K_V^B = 1$). In the case that SNAREs U and V bind less well to the preferred coat, the dissociation constants were increased to either $K_U^A = 10$ and $K_V^B = 10$ or to $K_U^A = 100$ and $K_V^A = 100$ (equally weak binding to both coats). In the case that SNAREs U and V bind more strongly to the wrong coat, the dissociation constants were decreased to either $K_U^B = 10$ and $K_V^A = 10$ or to $K_U^B = 1$ and $K_V^B = 1$ (equally strong binding to both coats). Other parameter values: $w^A = w^B = 1$, and $\kappa = 40$. Amounts of SNAREs are normalized with respect their total amounts X = U = Y = V = 0.5.

better than coat A, it will be concentrated in compartment 2, as expected for a Golgi enzyme (Fig. 4 a; cargo 2). Interestingly, a slight preference of cargo for one of the coats and a weak absolute affinity are sufficient to cause its strong accumulation in one compartment (Fig. 4 b, shown for cargo 1), consistent with the experimental observation that retrieval signals are often short and degenerate amino acid segments that interact only weakly with coat proteins. Cargo is not concentrated by static retention; rather, it cycles with a rate that depends on its affinity for a coat and, when escaped from its home compartment, is retrieved to it. When bulk transport is assumed (cargo can also be packaged into vesicles without affinity for a coat), cargo gradients are less pronounced (unpublished data).

The considerations so far ignore that all cargo that cycles between the ER (compartment 1) and Golgi (compartment 2) enters the ER during its biosynthesis and that many of these proteins are transported from the Golgi to later compartments of the secretory pathway. As a result, there is a net flux of cargo from compartment 1 to compartment 2, and the cargo concentration in the two compartments depends on both coat affinities and overall cargo flux. When cargo has equal affinities for the coats, its concentration is higher in compartment 1 than in compartment 2 and a large fraction cycles back from compartment 2 to 1 (Supplement 3, available at http://www.jcb.org/cgi/content/full/jcb.200409087/DC1). When cargo binds more strongly to coat A, it will be even more concentrated in compartment 1, whereas in the reverse situation it is concentrated in compartment 2 and back cycling is reduced (Supplement 3, Table, available at http://www.jcb.org/cgi/content/org/jcb.200409087/DC1). Cargo net flux thus makes it easier to accumulate ER proteins and harder to concentrate Golgi proteins.

Cargo-dependent vesicle transport

As mentioned before, vesicle budding is actually dependent on certain additional proteins. For example, COPII is recruited by the GTPase Sar1, which is activated by the nucleotide exchange factor Sec12, an integral ER protein (Barlowe and Schekman, 1993). Likewise, the budding of COPI vesicles requires Golgi-bound nucleotide-exchange factors for the Arf1 GTPase (Peyroche et al., 1996; Chantalat et al., 2003) and is stimulated by the binding of p24 proteins (Reinhard et al., 1999; Goldberg, 2000). In an improved model we therefore include two cargo proteins (cargo 1 and 2). Cargo 1 binds to coat A, which results in its accumulation in compartment 1 (Fig. 4 a). It is required for the budding of coat B vesicles, but is not packaged into these vesicles. Similarly, cargo 2 binds to coat B, resulting in its accumulation in compartment 2, and is required for the budding of coat A vesicles. For example, cargo 1 may correspond to Sec12, which accumulates in the ER (compartment 1) because it has an affinity for COPI (coat A) vesicles, and is needed for the budding of COPII (coat B) vesicles through its effect on Sar1. The result of this situation is that budding from compartment 1 is now mostly with coat B vesicles, because the high concentration of cargo 1 in this compartment promotes coat B–, but not coat A–vesicle budding (Fig. 4 c). Similarly, the high concentration of cargo 2 in compartment 2 promotes coat A–, but not coat B–vesicle budding. Thus, budding from each compartment with the wrong coat is suppressed and futile cycling is reduced ($w_1^A = 0.20$, $\kappa_1^A = 0.19$ in Fig. 4 c vs. $w_1^A = 1.56$, $\kappa_1^A = 1.53$ in Fig. 2 c). This model corresponds better to the experimental observation that vesicle budding from the ER and Golgi is mainly with one coat, with COPII and COPI, respectively. Cargo-stimulated budding stabilizes the coat-SNARE system, as the nonhomogeneous SNARE distribution occurs at lower q-values (unpublished data).

Cargo may also inhibit the back fusion of vesicles, as recently suggested for the ER protein Tip20 and COPII vesicles (Kamena and Spang, 2004). We have modeled this effect by assuming that two cargo proteins, accumulating in compartments 1 and 2, inhibit the fusion of coat A and coat B vesicles, respectively (Supplement 4, available at http://www.jcb.org/cgi/content/full/jcb.200409087/DC1). Although, in principle, fusion is inhibited in both directions, the accumulation of each inhibitor in one compartment results in the preferential inhibition of back fusion (Supplement 4, Figure).

Three-compartment system

The model can be extended to include more compartments, for example, a linear arrangement of three compartments (1–3),

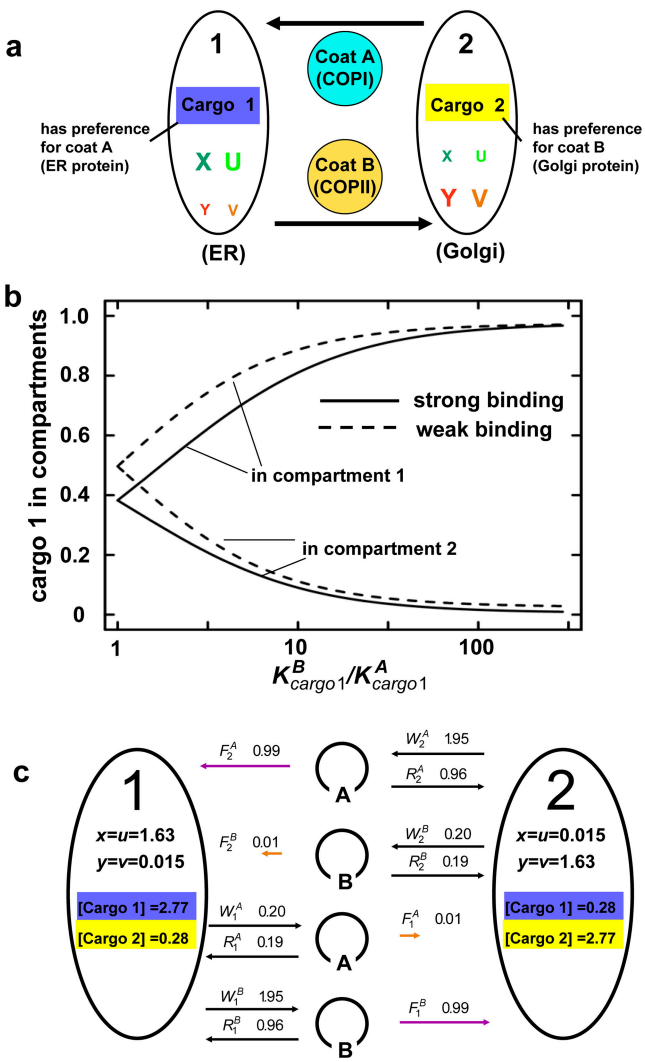

Figure 4. **Cargo is distributed according to its affinity for coats.** (a) Scheme illustrating that cargo with affinity for coat A accumulates in compartment 1, and cargo with affinity for coat B in compartment 2. These situations correspond to the localization of ER and Golgi proteins, respectively. (b) The amounts of cargo 1 in the two compartments are plotted versus the ratio $K^B_{\text{cargo 1}}/K^A_{\text{cargo 2}}$ of dissociation constants for the two coats. The solid lines correspond to $K^A_{\text{cargo 1}} = 1$ (strong binding), the dotted line to $K^A_{\text{cargo 1}} = 100$ (weak binding). With a relatively small preference of cargo for one of the coats it can be greatly concentrated in one compartment, even when the absolute binding constant is low. (c) Steady-state concentrations and fluxes for a model in which cargo is required for vesicle budding (cargo 1 for the budding of coat B vesicles, and cargo 2 for the budding of coat A vesicles). The steady-state concentrations of SNAREs X, U, Y, and V (*x*, *u*, *y*, and *v*), of cargo 1 and 2 (in blue and yellow, respectively), and the vesicle fluxes (normalized so that the sum of net forward fluxes equals unity) were calculated for $q = 100$ as in Fig. 2 c. The different fluxes are defined as in Fig. 2 c. Note that the rates of futile budding and fusion of coat A vesicles from compartment 1 and of coat B vesicles from compartment 2 are significantly reduced compared with those in Fig. 2 c. Other parameters were as follows:

$$K^A_{\text{cargo 1}} = K^B_{\text{cargo 2}} = 1,\ K^B_{\text{cargo 1}} = K^A_{\text{cargo 2}} = 10,$$

$$w^A = w^B = 0.3,\ \kappa = 40.$$

The normalized sizes of compartments (Si/S; $i = 1, 2$), and of the vesicle populations $(N^A_1/S)(= N^B_2/S)$, and $(N^B_1/S)(= N^A_2/S)$ were 0.30, 0.002, and 0.20, respectively.

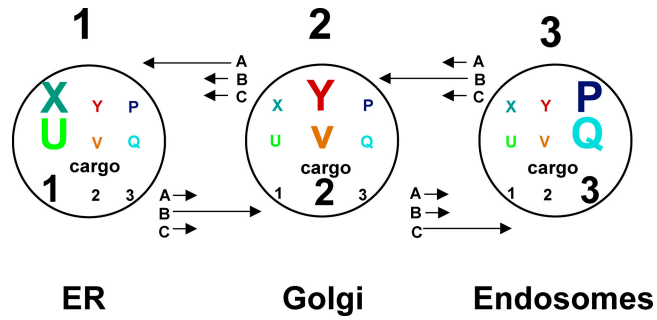

Figure 5. **A linear three-compartment model.** The model comprises three different coats (A–C), each with an affinity for SNAREs (coat A for SNAREs X and U, coat B for SNAREs Y and V, and coat C for SNAREs P and Q), which mediate fusion by pairing (X with U, Y with V, and P with Q). The scheme illustrates the concentration of the three SNAREs in different compartments, which could represent the ER, the Golgi, and endosomes, and the net vesicle fluxes between them. The lengths of the arrows correspond approximately to the magnitude of the net fluxes with the three different coats. Note that each compartment attracts from the other compartments those vesicles that have budded with the coat that has a preference for the SNARE accumulating in it (e.g., compartment 2 attracts coat B vesicles and accumulates SNAREs Y and V). Three cargo proteins (cargos 1, 2, 3) with preferential affinities for coats A, B, and C, respectively, are concentrated in the different compartments. Calculated concentrations corresponding to the scheme are given in Supplement 5, Table, a.

which may represent the ER, the Golgi, and endosomes (Fig. 5). We assume the existence of three coats (A, B, and C), each with a preference for cognate SNAREs (coat A for X and U, coat B for Y and V, and coat C for P and Q), which mediate fusion by pairing. In this case, the system is not symmetrical (compartment 2 differs from compartments 1 and 3 in that it communicates with two, rather than one compartment), and the steady state depends on the initial conditions. For example, when all SNAREs are initially concentrated in compartment 1, the system degenerates into a two-compartment system (Supplement 5, Table, b, available at http://www.jcb.org/cgi/content/full/jcb.200409087/DC1). However, with less extreme initial conditions, including those after BFA treatment (SNAREs Y and V in compartment 1 and negligible size of compartment 2; Supplement 5, Table, a), each of the SNAREs is concentrated in one compartment (Fig. 5): compartment 1 accumulates SNAREs X and U and attracts coat A vesicles originating from compartment 2, compartment 2 accumulates SNAREs Y and V and attracts coat B vesicles originating from compartments 1 and 3, and compartment 3 accumulates SNAREs P and Q and attracts coat C vesicles originating from compartment 2 (Fig. 5). Cargo with affinities for coats A, B, or C (cargos 1–3) will be concentrated in compartments 1, 2, or 3, respectively, by linkage with coat-specific vesicle fluxes (Fig. 5).

Discussion

Here, we provide an explanation for the generation of stable, nonidentical compartments and their size regulation, based on a minimal vesicular transport system comprising coats and SNAREs as major variables. The principle is simple: each compartment accumulates certain SNAREs and attracts from other compartments those vesicles that have budded with the

coat that preferentially binds those SNAREs; the SNAREs go to the compartment that already contains SNAREs with which they can pair, an autocatalytic effect that stabilizes each compartment. The nonuniform stable steady state is the result of a balance between autocatalytic vesicle fusion, which leads to the accumulation of cognate SNAREs in one compartment, and the distribution of SNAREs among the compartments by vesicle budding. Other mechanisms could also contribute to differences among compartments, such as the retention of newly synthesized proteins in the ER, the continuous flow of proteins and lipids through the secretory pathway, or the inability of certain lipids to be packaged into vesicles (Brugger et al., 2000). However, inhibition of protein synthesis does not lead to the rapid homogenization of compartments, and models based on these mechanisms alone do not lead to a stable steady state with nonidentical compartments (unpublished data). The minimal coat-SNARE system with its remarkable robustness could therefore be the basic principle behind the generation of compartments, perhaps the evolutionary precursor of the present complex systems in eukaryotic cells. The coat-SNARE system implicitly includes G proteins and NSF as kinetically nonlimiting factors, but nucleotide hydrolysis by them provides the energy for establishing nonidentical compartments.

The coat-SNARE system only generates nonidentical compartments if the coats preferentially bind certain SNAREs. COPII indeed binds Golgi SNAREs (Miller et al., 2003; Mossessova et al., 2003). There is also some evidence that COPI binds ER SNAREs, as predicted from our model, although the interaction may be mediated by other proteins (Frigerio, 1998; Andag et al., 2001). It seems possible that for each compartment in the cell there may not only be a specific SNARE pair, but also a coat with binding preference for it (although some transport steps, such as the one from the Golgi to the plasma membrane, do not use a known coat). According to our model, the coats must sufficiently discriminate between the SNAREs, but when the discrimination is too extreme, this leads to the budding of vesicles that can no longer fuse with any compartment. We estimate that a coat should optimally bind its preferred SNAREs by a factor of 50–100 better than the other SNAREs. Alternatively, there may be an as yet unknown mechanism that prevents the budding of vesicles lacking SNAREs.

The specific localization of proteins other than SNAREs (cargo) could result from their binding to coats; they would simply follow coat-specific vesicle fluxes. Certain lipids, such as phosphoinositides, could be localized because they are generated by cargo kinases and phosphatases. Although the localization of cargo by coat-specific vesicle fluxes is not a new concept, we show that even a weak cargo–coat interaction and a moderate preference of cargo for a coat are sufficient for its specific localization. Static retention is not required to explain cargo localization.

Our models are conceptual and qualitative, rather than quantitative, because many of the parameters, such as the binding and rate constants, the SNARE concentrations, and the organelle sizes are only known very approximately. Although a quantitative fit with experimental data is therefore not yet possible, the models still allow insight that is impossible to gain otherwise, particularly because the complex relationship between SNAREs directing vesicle fluxes and being themselves localized by vesicle fluxes is not intuitive. Despite their semiquantitative nature, the models make testable predictions, such as the one concerning the magnitude of preference of a coat for certain SNAREs, or that the size of an organelle should depend on the total number of SNARE molecules. Our models may also serve as a starting point to theoretically analyze other aspects of vesicular transport, such as the generation of Golgi cisternae, their cisternal maturation, or the packaging of cargo into large membrane-bound carriers (Pelham and Rothman, 2000; Palmer and Stephens, 2004).

Materials and methods

The simplest two-compartment system is described by two sets of differential equations. One applies to the time-dependent changes of the numbers of vesicles N_i^A and N_i^B (where *A* and *B* indicate the coat, and $i = 1$ or 2 the compartment they originate from) and of the sizes S_i of the compartments. The other set of equations describes the time-dependent changes of the content of SNAREs X and U as well as Y and V in vesicles (denoted by $n_{X,i}^A, n_{X,i}^B, n_{U,i}^A, n_{U,i}^B$, etc.) and in compartments (X_i, U_i, etc.).

The differential equations for the number of vesicles have the form

$$\frac{dN}{dt} = W - R - F, \tag{1}$$

where *W* denotes the flux of vesicle budding with a certain coat from a compartment, *R* denotes the flux of back fusion into that compartment, and *F* denotes the flux of forward fusion with the other compartment. Each of these terms needs a specification for a coat and compartment. The differential equations for the compartment sizes have the form

$$\frac{dS}{dt} = \underbrace{(-W^A - W^B)}_{\text{budding}} + \underbrace{(R^A + R^B)}_{\text{back fusion}} + \underbrace{(F^A + F^B)}_{\text{forward fusion}}. \tag{2}$$

In the simplest model, we assume that budding rates are given by $W = wS$, with appropriate specification of the type of coat and budding compartment (for example, $W_1^A = w^A S_1$, where w^A denotes a rate constant).

The fusion rates are assumed to be proportional to the number of vesicles and the sizes of the target compartments, i.e., $R = rNS$, and $F = fNS$, again with appropriate specification of coat and compartment (for example, $R_1^A = r_1^A N_1^A S_1$). The frequencies of fusion, *r* and *f*, depend on the concentrations of cognate SNAREs in the vesicles and the target compartment. For example, for the back fusion of coat A vesicles originating from compartment 1, one obtains

$$r_1^A = \kappa(x_1^A u_1 + u_1^A x_1 + y_1^A v_1 + v_1^A y_1), \tag{3}$$

where κ is a rate constant, $x_1^A = n_{X,1}^A / N_1^A$, $u_1^A = n_{U,1}^A / N_1^A$, $y_1^A = n_{Y,1}^A / N_1^A$, and $v_1^A = n_{V,1}^A / N_1^A$ the concentrations of SNAREs in vesicles budded with coat A, and $x_1 = X_1/S_1$, $u_1 = U_1/S_1$, $y_1 = Y_1/S_1$, and $v_1 = V_1/S_1$ the concentrations of SNAREs in compartment 1. The corresponding equation for forward fusion (from compartment 2) is

$$f_2^A = \kappa(x_2^A u_1 + u_2^A x_1 + y_2^A v_1 + v_2^A y_1). \tag{4}$$

For the equations describing SNARE fluxes, the terms *W*, *V*, and *F* in Eqs. 1 and 2 are replaced by *I*, *J*, and *M*, describing their movement by budding, back fusion, and forward fusion, respectively. We assume that during budding the coats bind the SNAREs in a competitive manner with different affinities. For example, the export of SNARE X from compartment 1 by coat A vesicles is given by

$$I_{X,1}^A = W_1^A L^A \frac{x_1/K_X^A}{1 + x_1/K_X^A + u_1/K_U^A + y_1/K_Y^A + v_1/K_V^A}, \tag{5}$$

where L^A denotes the total number of SNARE binding sites in vesicles with coat A, and K_X^A, K_U^A, K_Y^A, and K_V^A are dissociation constants. Eq. 5

assumes competition between the SNAREs for coat binding sites, but the model gives similar results if they do not compete with one another.

The SNARE fluxes during fusion are given by the product of vesicle flux and the concentration of SNAREs within the vesicles. For example, the movement of SNAREs X from coat A vesicles to compartment 1 is described by $J^A_{X,1} = R^A_1 x^A_1$ for back fusion and $M^A_{X,2} = F^A_2 x^A_2$ for forward fusion (from compartment 2).

The full set of equations, which can be simply copied into a common integration program, is given in Supplement 1.

When budding is assumed to be dependent on cargo, the following rate laws are used for coat A and coat B vesicles, respectively: $W^A_i = w^A[\text{cargo } 2]_i S_i$ and $W^B_i = w^B[\text{cargo } 1]_i S_i$ ([cargo1]$_i$ are the concentrations of cargo with affinity for coat A, and [cargo 2]$_i$ the concentrations of cargo with affinity for coat B).

Cargo fluxes are introduced in a similar way as SNARE fluxes, but the fusion frequencies *r* and *f* are considered to be independent of cargo concentrations within the compartments and vesicles.

The model with three compartments is a straightforward extension of the two-compartment model, and even more compartments could easily be introduced.

Online supplemental material

Supplement 1 is a computer program containing the differential equations that describe a two-compartment system with two SNARE pairs, two coats, and one cargo. It can be combined with common integration programs. Supplement 2 gives the results of a two-compartment model in which inhibitory-SNAREs are included (noncognate SNAREs can form inactive complexes). In supplement 3, a model is considered in which cargo enters compartment 1 (ER) by de novo synthesis and exits compartment 2 (Golgi) for later compartments of the secretory pathway. The distribution of cargo depends on both its affinity for coats and on its net flux through the system. Supplement 4 describes a model in which the back fusion of budded vesicles into the originating compartment is inhibited. This is achieved by two cargo molecules that inhibit the fusion of coat A and coat B vesicles, respectively, and accumulate in the appropriate compartment. Supplement 5 gives the calculated amounts of SNAREs and cargos in a three-compartment system (Fig. 5). Two cases of initial conditions are considered. One results in the accumulation of the three different SNARE pairs and cargos in different compartments, as shown in Fig. 5. The other leads to the disappearance of a compartment. Online supplemental materials is available at http://www.jcb.org/cgi/content/full/jcb.200409087/DC1.

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