

Dynamic transport of SNARE proteins in the Golgi apparatus

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Localization of a membrane protein in a subcellular compartment can be achieved by its retention in the compartment or by its continuous transport toward this compartment. Previous results have suggested that specific enzymes are localized in the Golgi apparatus at least in part by selective retention and exclusion from transport vesicles. However, the function of some Golgi SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins is not compatible with their exclusion from transport vesicles. To help understand the mechanism accounting for the localization of SNARE proteins in the Golgi apparatus, we analyzed their lateral distribution in the Golgi cisternae and their incorporation into transport vesicles. According to our results, all SNARE proteins are efficiently incorporated into transport vesicles, indicating that the localization of SNARE proteins in the Golgi apparatus is not based on a static retention mechanism. Detailed analysis suggested that incorporation into transport vesicles was more efficient for SNARE proteins restricted to the cis face of the Golgi as compared with SNAREs present at the trans face. Furthermore, overexpression of a cis-Golgi SNARE protein altered concomitantly its incorporation in transport vesicles and its intra-Golgi localization. These observations suggest that, contrary to resident Golgi enzymes, SNARE proteins are localized in the Golgi apparatus as the result of a dynamic transport equilibrium.

cisternal maturation | glycosyltransferase | secretion | vesicular transport | membrane sorting

Intracellular transport is mostly achieved in eukaryotic cells through vesicular intermediates that shuttle between membrane compartments. To maintain the specific composition of each compartment while allowing constant vesicular transport, the composition of the vesicles is tightly controlled, as well as their individual destinations. SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins are a family of membrane proteins that allow fusion between membranes, while controlling at least in part the specificity of the fusion events. Each SNARE complex is composed of four subunits that can form a tetrameric complex. During the fusion of a transport vesicle with a target membrane compartment, one of the SNAREs is present on the vesicular membrane (v-SNARE), whereas three are present in the target membrane (t-SNAREs). The formation of the tetrameric SNARE complex brings the two membranes together and allows the specific fusion of the vesicle with the compartment exhibiting the appropriate t-SNAREs.

There are many SNARE complexes in cells, and their characterization has been a subject of intense research in recent years. One of the best characterized SNARE complexes is composed of Bet1, ERS24, membrin, and syntaxin 5 (1). It controls the fusion of endoplasmic reticulum (ER)-derived vesicles with the cis-Golgi, and it is generally believed that Bet1 is the v-SNARE subunit in this complex (1), although some have proposed that ERS24 might be the v-SNARE (2, 3). At least one other SNARE complex (GS15-GOS28-syntaxin5-Ykt6) is

present in the Golgi apparatus, but this complex is more concentrated on the trans face and might control intra-Golgi transport (either anterograde or retrograde) (4), or possibly transport between the Golgi apparatus and endosomes (5).

To provide some degree of specificity for intracellular transport, SNARE proteins must be present at their site of action, and absent elsewhere. Each individual SNARE protein is thus both an actor in intracellular transport and a membrane cargo subject to intracellular sorting mechanisms. When considering, for example, Bet1, its function (as a v-SNARE) requires its presence in ER-to-Golgi transport vesicles, and presumably its constant recycling from the Golgi back to the ER. Indeed, Bet1 has been detected in COPII-coated ER-to-Golgi transport vesicles as well as in COPI-coated retrograde transport vesicles. An incubation at 15°C, which inhibits ER-to-Golgi transport, results in an accumulation of Bet1 in ER-derived exit structures, further indicating that Bet1 is constantly cycling between the Golgi apparatus and the ER (6). Bet1 is also largely restricted to the cis face of the Golgi apparatus (C1 and C2 cisternae), but the mechanisms that allow its exclusion or removal from medial and trans cisternae are not clear. Efficient retrograde transport of Bet1 might be sufficient to prevent further transport along the Golgi stack, but the absence of Bet1 in trans-Golgi cisternae might also be caused by its exclusion from anterograde transport vesicles. Interestingly, it seems that the localization of Bet1 in the cis-Golgi may not be determined by its interaction with its cognate SNARE proteins (3).

Electron microscopy provides the resolution necessary to precisely visualize intracellular transport, in particular intracellular transport vesicles. However, electron microscopy can only be performed on fixed cells, and, in many cases, the dynamics of an individual protein can at best be extrapolated from its observed equilibrium distribution. The presence of a protein in transport vesicles is a good indication that it is actively transported, but intracellular transport vesicles are transient elements and difficult to characterize morphologically (their morphology changes as their cytosolic coat depolymerizes shortly after their formation) and topologically (they can be subject to very rapid transport in the cytosol). It is thus preferable to study the site of formation of vesicles to gain an insight on their specific composition and role in intracellular transport. This strategy has been instrumental in the characterization of membrane sorting during the formation of endocytic clathrin-coated vesicles (7). However, the characterization of budding events at the level of intracellular organelles is often a more difficult task.

Abbreviations: SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; v-SNARE, SNARE present on the vesicular membrane; t-SNARE, SNARE present on the target membrane; ER, endoplasmic reticulum; KDEL, Lys-Asp-Glu-Leu.

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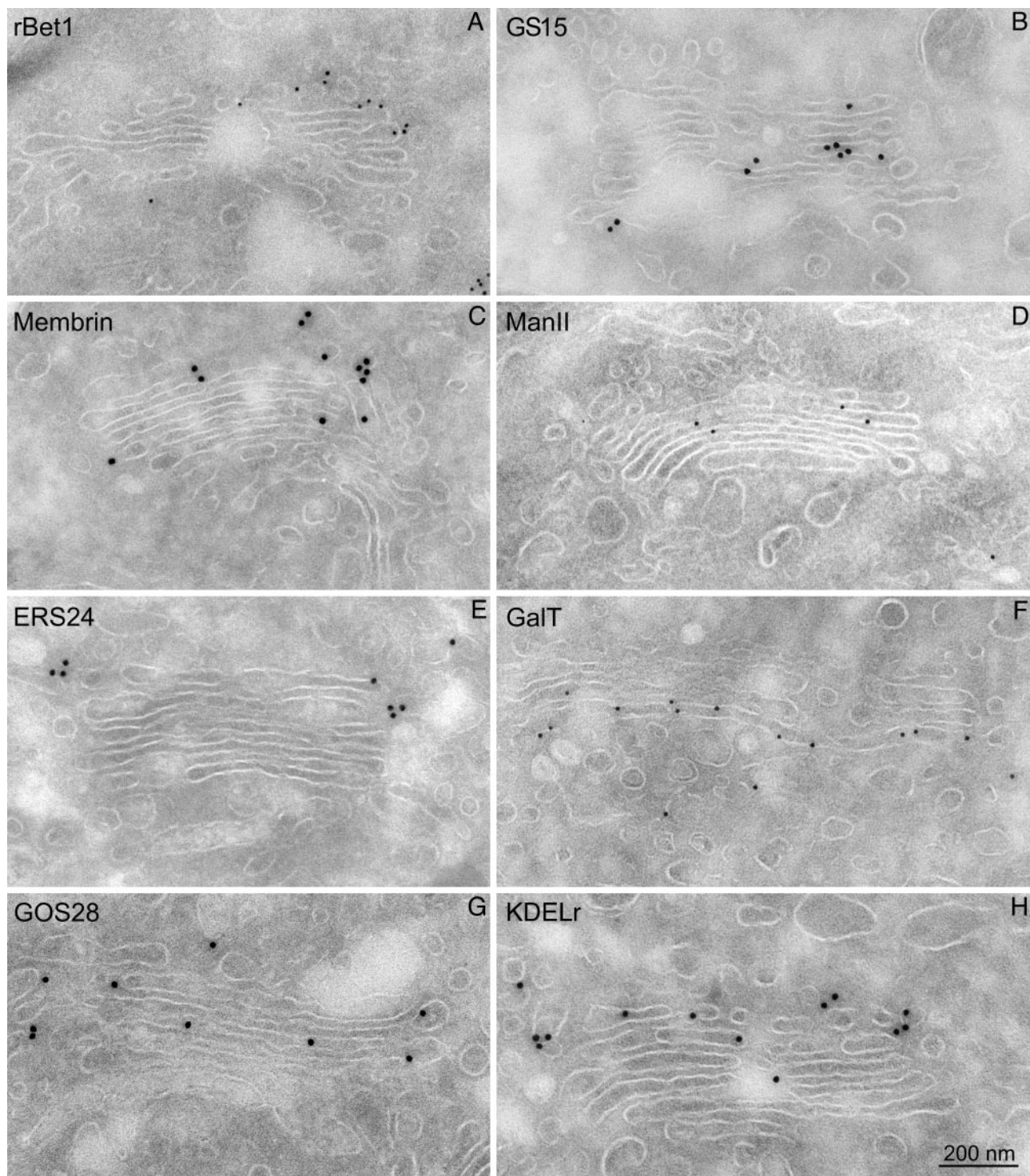


Fig. 1. Lateral distribution of endogenous Golgi markers. The distribution in the Golgi apparatus of several SNARE proteins (Bet1, membrin, ERS24, GOS28, and GS15), two resident enzymes (ManII and GalT), and the KDEL receptor (KDELr) was determined. With the exception of GalT, which could be detected only in HeLa cells, all images were obtained in normal rat kidney (NRK) cells. The corresponding quantification is presented in Table 1.

One of the key questions regarding the dynamics of the Golgi apparatus is whether Golgi-specific glycosylation enzymes are present in transport vesicles, because the answer could help differentiate whether Golgi cisternae are stable structures traversed by a flow of forward-moving secreted proteins, or maturing transport elements where the presence of glycosylation enzymes is only transient. However, due to the limita-

tions outlined above, this point is difficult to establish and has led to recent controversy (8–11). In an attempt to extrapolate the dynamics of individual markers from their equilibrium distribution, we hypothesized recently that the proteins actively transported in and out of the Golgi apparatus should be concentrated at the rims of the Golgi cisternae, where transport vesicles form. Accordingly, we observed that the KDEL

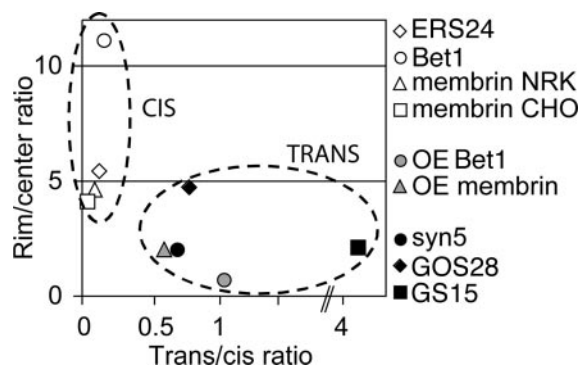


Fig. 4. The lateral distribution of Golgi SNAREs is correlated to their cis-trans localization within the Golgi apparatus. For each marker analyzed, the lateral distribution (rim/center ratio), as determined in this study, is indicated. The labeling in the trans cisternae (C4 and C5) was divided by the labeling in the cis cisternae (C1 and C2) to indicate the cis-trans localization of each marker. The determination of the cis-trans distribution of endogenous Golgi SNAREs is described in ref. 4. For endogenous markers, cis-Golgi SNAREs exhibit a high rim/center ratio and trans-Golgi SNAREs a lower rim/center ratio. Overexpressed (OE) Bet1 and membrin gain access to the trans-Golgi, and their rim/center ratio is concomitantly decreased.

Overexpression of Tagged SNARE Proteins Affects both Their Lateral Distribution and Their Localization Within the Golgi Stack. The results described above suggest that Golgi-resident enzymes are localized in individual Golgi stacks by exclusion from transport vesicles, whereas the distribution of SNARE proteins is the result of a much more dynamic transport process. To establish a causal link between the lateral distribution of SNARE proteins and their transport within the Golgi stack, we attempted to perturb their dynamics by overexpressing individual SNARE proteins in transfected cells.

We first analyzed the distribution of Bet1 in CHO cells overexpressing Bet1 fused to a protein A tag (20). In these conditions, the localization of the tagged Bet1 was shifted toward the trans face of the Golgi stack (Fig. 2), presumably due to its overexpression. This shift probably results from saturation of the sorting mechanism ensuring proper localization of Bet1. Alternatively the presence of a protein A tag might perturb sorting of tagged SNAREs. Irrespective of the precise mechanism accounting for this alteration of sorting, this situation gave us an opportunity to study the relationship between the dynamics of Bet1 and its intracellular distribution. The shift in the cis-trans localization of Bet1 was accompanied by a change in its lateral distribution, with a strong decrease in Bet1 concentration at the rims of the Golgi (Table 2). This change could be observed in all of the cisternae of the stack, because Bet1 was only modestly concentrated in the rims of the most cis cisterna of the Golgi, and depleted from the rims in other cisternae. This finding suggests that the efficient incorporation of endogenous Bet1 in transport vesicles is necessary to ensure its localization in the cis-Golgi.

To check whether the alteration of Bet1 distribution reflected a general defect in the organization of the Golgi apparatus, we tested the localization of the KDEL receptor, another protein localized in the cis-Golgi cisternae and engaged in continuous transport to the Golgi apparatus. Overexpression of Bet1 did not affect the distribution of the KDEL receptor (Fig. 2A), indicating that the general structure of the Golgi apparatus was preserved in these cells.

As observed for Bet1, overexpression of protein A-tagged membrin shifted its localization toward the trans side of the Golgi stack (Fig. 3). This shift was also accompanied by a drop in its concentration at the Golgi rim (Table 3). Thus, overexpression of tagged Bet1 and membrin caused concomitantly a shift of their localization to the more trans cisternae of the Golgi

stack, and a decrease in their concentration at the rims of the Golgi cisternae.

Discussion

From first principles, the concentration of cargo molecules that are actively transported should be greater at rims than centers of Golgi cisternae, reflecting their concentration in departing and fusing transport vesicles. In this study, we have exploited this principle to indirectly establish the dynamics of Golgi SNAREs. The Bet1 protein, which has previously been shown to be actively transported between the ER and the Golgi apparatus, is highly concentrated at the rims of Golgi cisternae. Bet1 was more concentrated at the rims than the cognate t-SNAREs membrin or ERS24. Interestingly, subcellular fractionation experiments also suggested that, in yeast, all four subunits of this SNARE complex cycle continuously between the ER and the Golgi, and that Bet1 is incorporated even more efficiently than other subunits in transport vesicles (22). In addition, quantitative analysis revealed that the concentration of membrin that we detected at the rims of Golgi cisternae was also reflected in adjacent Golgi-derived transport vesicles. These results validate further the concept that a high concentration at the rims of Golgi cisternae reflects an efficient incorporation in intracellular transport vesicles.

All SNARE proteins analyzed in this study were found to be concentrated at the rims of the Golgi compared with its center. This finding suggests that both v- and t-SNARE proteins are continuously retrieved from and returned to the Golgi apparatus, and that this retrieval is essential to determine their precise localization in the Golgi stack. In general terms, our observations on SNAREs yield results opposite to what has been observed for resident Golgi enzymes: mannosidase II was shown to be depleted both at the rims of Golgi cisternae and in transport vesicles (12). Here, we report a similar observation for galactosyl transferase. Thus, localization in the Golgi can be achieved by two different means: either a retention within individual cisternae (for resident Golgi enzymes) or a dynamic transport equilibrium (for SNARE proteins). The two mechanisms are not mutually exclusive and might both contribute to efficient sorting of proteins within the Golgi stack.

Overall, SNARE proteins present in the trans side of the Golgi apparatus exhibit smaller relative concentrations at the rims of the Golgi (Fig. 4). This difference could conceivably be due to the fact that the whole dynamics of the trans cisternae are different from that of the most cis cisternae. However, it is noteworthy that, for all trans-SNAREs analyzed, similar relative concentrations at the rims were observed throughout the Golgi stack and even in cis-Golgi cisternae (data not shown), suggesting that the smaller degree of concentration of trans-SNAREs at the rims reflects the fact that they are less actively transported than cis-SNAREs. A similar observation was made for overexpressed cis-SNAREs Bet1 or membrin: in both cases, the relative concentration at the rims is weaker than for endogenous cis-SNAREs, and this decrease is accompanied by a more trans localization. As illustrated in Fig. 4, a high relative concentration at the rims of the Golgi seems predictive of a cis localization, whereas a weaker concentration in the rims is observed for markers reaching trans-Golgi compartments. It is tempting to speculate that the Golgi distribution of each SNARE protein is controlled mainly by the efficiency with which it is packaged in transport vesicles. Accordingly, trans-SNAREs and overexpressed cis-SNAREs would gain access to trans cisternae by virtue of their ability to escape fast retrograde transport back to the ER. Because our analysis does not distinguish forward transport vesicles from retrograde transport vesicles, this point cannot be addressed unambiguously here and will require the development of new experimental approaches.

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