Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 2279–2283, March 1998
Cell Biology

Vesicles on strings: Morphological evidence for processive transport within the Golgi stack

(freeze-fracture/electron microscopy)

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Contributed by James E. Rothman, December 23, 1997

ABSTRACT ***Cis*-Golgi cisternae have a higher freeze-fracture particle density than *trans*-cisternae. Transport vesicles neighboring *cis* or *trans* positions of the Golgi stack have a particle concentration comparable to that of the adjacent cisterna and the buds emerging from it. This implies that transport vesicles remain locally within the stack during their lifetime, near their origin, favoring a processive pattern of transport in which vesicle transfers occur preferentially between adjacent cisternae in the stack. A "string theory" is proposed to account for processive transport, in which a carpet of fibrous attachment proteins located at the surface of cisternae (the strings) prevent budded vesicles from diffusing away but still allow them to diffuse laterally, effectively limiting transfers to adjoining cisternae in the stack. Fibrous elements that multivalently connect otherwise free COPI-coated vesicles and uncoated transport vesicles to one or two cisternae simultaneously are discerned readily by electron microscopy. It is suggested that long, coiled coil, motif-rich, Golgi-specific proteins including p115, GM130, and possibly giantin, among others, function as the proposed strings.**

The Golgi stack receives vesicles from the endoplasmic reticulum (ER) at its *cis*-face and dispatches vesicles carrying cargo originating in the ER from its *trans*-face to diverse addresses within cells (for review, see ref. 1). Such anterograde transport across the intervening cisternae from the *cis*- to the *trans*-face of the stack likely is mediated by COPI (coatomer)-coated vesicles (2, 3). Coatomer more recently also has been linked to the retrograde transport of escaped ER proteins back to the ER (4, 5), suggesting that two subpopulations of COPI-coated vesicles containing either anterograde-directed or retrograde-directed cargo may bud from Golgi cisternae. That this is the case recently was established directly by immunoelectron microscopic studies of intact cells (6). Together, the two observed populations account for most if not all Golgi-associated vesicles and buds.

In the present study, we use freeze-fracture electron microscopy to assess possible heterogeneity in Golgi-associated buds and vesicles on the basis of their intramembrane particle content. Quantitation of the particle density of fracture faces affords a relative measure of the protein content of a membrane (for review, see refs. 7 and 8). Particles correspond to integral membrane proteins; protein-rich membranes have a higher particle density, and protein-poor membranes have a lower particle density (9). Heterogeneity in protein particle density was observed previously in the Golgi complex of insulin cells *in situ* (10). Specifically, fracture faces of *cis*-Golgi cisternae have approximately three times the particle density found in fracture faces of *trans*-cisternae.

We now report a corresponding variation in particle density among Golgi-associated vesicles that originates from the underlying *cis–trans* cisternal asymmetry: Particle-poor and particle-rich cisternae yield particle-poor and particle-rich transport vesicles, respectively. Moreover, we find that both types of vesicles remain close to their cisterna of origin, implying that there is little randomization of vesicles along the *cis–trans* axis of the stack. We also report that COPI-coated vesicles, and their uncoated products, are tethered to stacks by one or more apparently flexible fibrous elements that are roughly as long as a vesicle is wide. We propose that the tethering of budded transport vesicles by such flexible "strings" allows processive transport by limiting transfers to adjacent cisternae in the stack and can account for the lack of *cis–trans* randomization deduced from freeze-fracture microscopy.

MATERIALS AND METHODS

Golgi fractions purified from Chinese hamster ovary (CHO) cells were pelleted, fixed with glutaraldehyde, dehydrated, and processed, including tannic acid staining for conventional thin section electron microscopy as previously described (11, 12). For freeze-fracture (13), pellets of Golgi fractions fixed in glutaraldehyde were cryoprotected by immersion in a 30% glycerol solution in phosphate buffer, frozen in Freon 22 cooled in liquid nitrogen, and processed in a Balzers 301 freeze-fracture apparatus. Thin sections and freeze-fracture replicas were observed in a Philips LS 420 electron microscope, and suitable areas were photographed at a calibrated magnification. Negatives were printed on photographic paper at a final magnification of ×132,000.

The density of protein particles per unit area of freeze-fractured membranes was determined by applying a test circle of 6-mm diameter on suitable regions of the pictures and counting the particles falling within the circle (14). The test circle was applied on Golgi cisternal elements or centered around buds/vesicles to calculate their respective particle content.

RESULTS AND DISCUSSION

Freeze-fracture replicas of intracellular membranes delimiting cisternal-like or vesicular-like compartments were interpreted with the following rules (15): (*i*) The fracture plane splits the membranes along their hydrophobic interior and exposes the inner aspect of either the cytosolic leaflet (=protoplasmic P-face) or the lumenal leaflet (=exoplasmic E-face); (*ii*) the fracture plane of P-faces usually contains two to three times more morphologically detectable particles than the complementary E-faces (16); (*iii*) for vesicular/cisternal intracellular compartments, P-faces always appear as concave-looking structures, and E-faces always appear as convex-looking structures.

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Abbreviations: COPI, coatomer; ER, endoplasmic reticulum; CHO, Chinese hamster ovary.

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Fig. 1 *A* and *B* show a pair of thin section and freeze-fracture views of an isolated Golgi cisterna from a CHO cell after incubation with cytosol, ATP, and GTPγS to initiate transport and to accumulate COPI-coated vesicles (11, 17); the cisternal membrane displays several budding profiles (Fig. 1*B*, arrows). On thin section, buds were enveloped by their characteristic COPI (coatomer) coat (Fig. 1*A*, arrows). The freeze-fracture process (Fig. 1*B*) has exposed the convex, poorly particulated E-face of the cisterna from which buds emerge toward the observer as convex circular protrusions of the convex cisternal membrane (the cytosolic COPI coat associated with the buds that can be seen in thin sections was undetectable by freeze-fracture electron microscopy).

When the cytoplasmic leaflet (P-face) of a cisterna was exposed (Fig. 1*C*), the cisternal membrane appeared concave and had a higher particle content than the E-face. The buds now appear as small circular depressions (dimples) pitting the cisternal P-face. Budding events occurring *in vitro* can therefore be identified on both P- and E-faces of a Golgi cisterna. Fig. 2*A* shows a Golgi stack *in situ* in a CHO cell revealed by freeze-fracture. The stacked cisternae show either their concave P faces or convex E faces. The *cis*-most and *trans*-most cisternae, both exposed as P faces, clearly differed in their particle density, the former showing more particles per unit area than the latter. Thus, the *cis–trans* gradient of protein particle content in Golgi cisternae originally described in studies of insulin cells (10) also occurred in CHO cells.

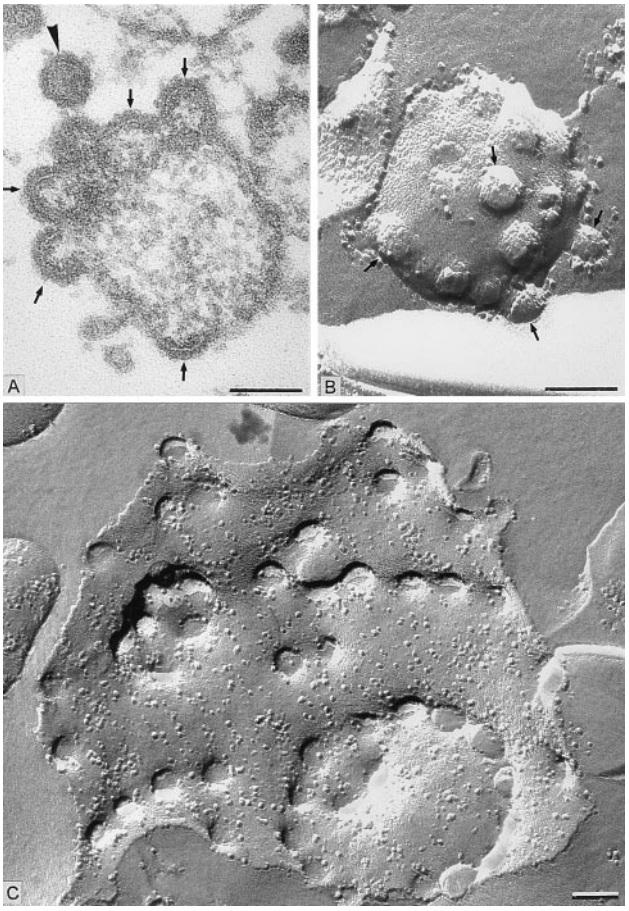

FIG. 1. Thin section and freeze-fracture views of Golgi elements. (*A*) Thin section of an isolated Golgi cisterna after cell-free incubation in the presence of cytosol, ATP, and GTPγS (17). Several COPI-coated buds are visible (arrows). The arrowhead shows a free coated vesicle. (*B*) Freeze-fracture view of a similar Golgi cisterna. The convex E-face of the cisterna is exposed and is deformed by several convex circular protuberances (arrows); each represents a coated bud emerging toward the observer. (*C*) Freeze-fracture view of another such Golgi cisterna. The concave, particle-rich P-face is interrupted by numerous concave circular depressions (dimples) that represent the necks of the coated buds budding away from the observer (compare with *B*, E-face). (Magnifications: *A*, ×90,000; *B*, ×91,000; *C*, ×62,000; Bar = 100 nm.)

Fig. 2 *B* and *C*, representing Golgi membranes incubated *in vitro* to reconstitute transport, show cisternae with a high particle density in the process of budding vesicles. The concave P-faces of the emerging buds have a density of particles comparable to that of the parental cisterna, and the high particle density indicates that they derive from the *cis*-Golgi region of the stack. Notably, fully formed vesicles that are free of the cisternal membrane also have a particle density comparable to the nearby buds and cisternae. By contrast, Fig. 2 *D* and *E*, from the same sample, show that the buds and fully formed vesicles associated with cisternae that have a much lower particle density (and therefore derive from the *trans* portion of the Golgi) also have a low particle density.

To put this on a quantitative basis, quantitation of the protein particle density on cisternal and bud or vesicle P-faces was carried out. (Because of their overall very low number of particles, E-faces were not suitable for reliable measurement.) To facilitate statistical analysis, each cisternal membrane was categorized as being in either of two discrete classes based on its particle density: a "low" particle density class (deriving from the *trans* half of the stack) having zero to three particles (within an arbitrary but fixed circular area; see *Materials and Methods*) and a "high" density class (deriving from the *cis* half of the stack) having between four and eight particles (eight being the highest value observed).

If free vesicles randomize, then both high and low particle density vesicles should be associated with both high (*cis*) and low (*trans*) particle density cisternae. But if vesicles do not randomize, the particle density of associated vesicles should closely reflect that of the most proximal cisterna and its buds. Therefore, to assess the degree of randomization of free vesicles along the *cis–trans* axis, we quantitated the particle density of free vesicles in relation to that of neighboring cisternae. Cisternae in each class were chosen from random micrographs when they had one or more free vesicles within 50 nm (approximately half of a vesicle diameter) (*cf.* Fig. 2 *B–E*). The particle density of the P-faces of the cisternae and of the associated vesicles then were scored. In this analysis, free vesicles easily could be distinguished from buds because only the former are separated by a continuous band of cytoplasm from the cisternal membrane. After the experiment, the particle density of cisternae assigned to each class also was determined.

The results (Table 1) establish that the particle density of vesicles is not statistically different from that of the associated cisternae, whether the cisterna is from the *cis* (high density class) or *trans* (low density class) region of the stack, confirming our qualitative impression. These results indicate that cisternae and vesicles on the *cis* half of the Golgi stack are characterized by a higher particle density than those on the *trans* half and that a budding vesicle takes with it a particle density comparable to that of its parental cisterna.

Although it is not known precisely what sort of proteins these particles represent, particle density nonetheless can serve to mark the level in the stack at which a vesicle originates. This simple principle, together with the observation that the particle density in vesicles closely matches that in proximal cisternae (Table 1), allows us to deduce that vesicles do not migrate far from their site of emergence during their lifetime in the Golgi, i.e., before fusing within the Golgi or departing from the Golgi area (for example, to fuse with the ER). This restriction in turn implies that any transfers within the stack must be done in an approximately serial fashion (whether anterograde- or retrograde-directed).

Our morphological observations therefore support a simple model in which vesicle transport within the Golgi stack uses a

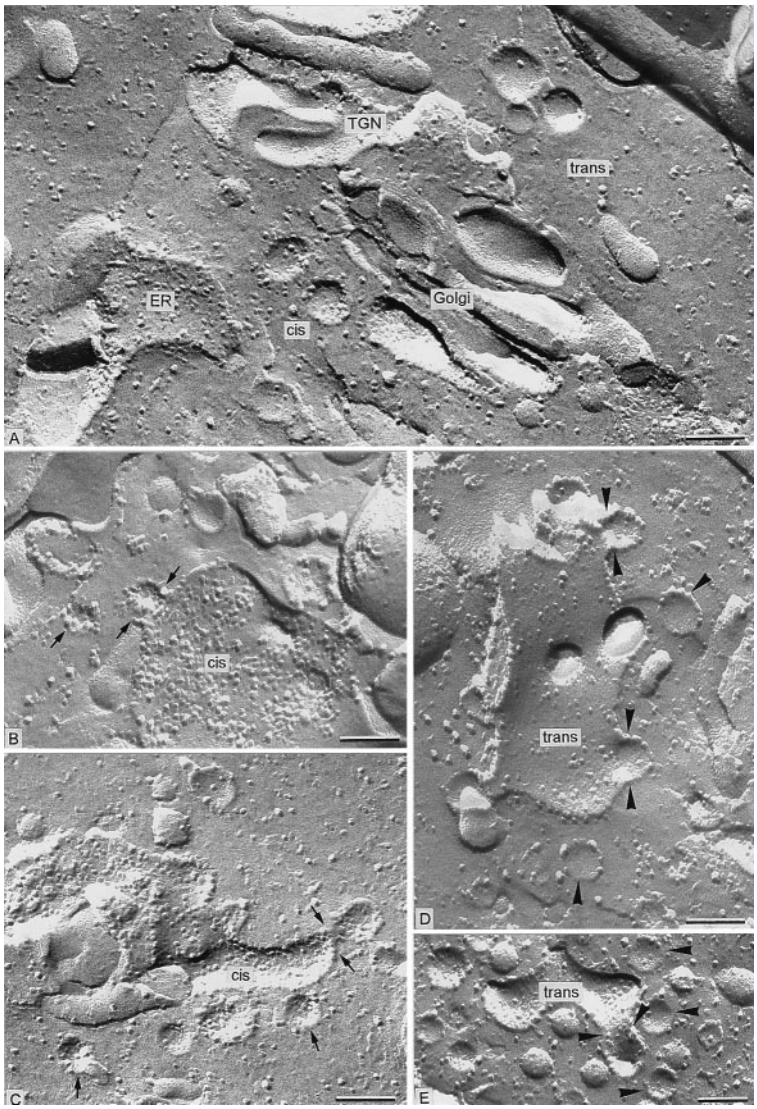

FIG. 2. Freeze-fracture views of Golgi cisternae and transport vesicles. (*A*) Golgi area of a CHO cell *in situ*. Concave P-faces mostly have been exposed, revealing a relatively high particle density on cisternal and vesicle membranes at one pole and a relatively low particle density on cisternae and vesicles at the other pole. The particle-rich Golgi pole is the *cis*-pole; the particle-poor pole is the *trans*-pole. P-faces of a particle-rich endoplasmic reticulum cisternae (ER) and of a tortuous, particle-poor *trans*-Golgi network (TGN) can also be observed. (*B*) *B–E* are from isolated Golgi incubated to reconstitute transport in the presence of cytosol, ATP, and GTPγS (17). Particle-rich cisternal P-face (= *cis*-pole) showing an emerging particle-rich bud (facing arrows). A free vesicle and a budding vesicle (concave P-face) are observed in the vicinity of the cisternal membrane (arrows), each with a particle density comparable to that of the cisterna. (*C*) Particle-rich cisternal membrane (= *cis*-pole) with a particle-rich bud emerging (facing arrows) and two particle-rich free vesicles (arrows). All are exposed as concave P-faces. (*D*) Particle-poor cisternal P-face (= *trans*-pole) with two particle-poor buds in the process of emerging from the parental membrane (facing arrowheads). Two particle-poor vesicles (P-faces) are seen neighboring the cisterna (arrowheads). (*E*) Particle-poor cisternal P-face with a particle-poor bud (facing arrowheads) and three particle-poor vesicles, all exposed as P-faces. See Table 1 for the quantitation of the respective particle densities. (Magnifications: *A–E*, ×79,000; Bar = 100 nm.)

processive mechanism in which vesicles do not dissociate from the stack as they transfer between adjacent cisternae. An alternative, distributive mechanism would have been that transport vesicles dissociate from the stack as they bud off and then later rejoin the stack at random.

A processive mechanism of transport is consistent with the possibility that the Golgi stack operates as distillation tower (18). In the distillation hypothesis, the cisternae of the Golgi stack would serve as the plates of a distillation tower, and anterograde and retrograde cargo- selective transport vesicles [corresponding to the two subpopulations of COPI-coated vesicles recently described (6)] would serve as the two necessary differently directed mobile phases. By virtue of differing in their selectivity for components being separated and their overall directionality, two such mobile phases would allow the stack to separate a complex mixture of proteins much more efficiently than in a single stage of sorting.

Table 1. Quantitation of the freeze-fracture particle density in membranes of free transport vesicles and associated cisternae of Golgi membranes incubated *in vitro* to reconstitute transport

	Particles/μm^2 in:	
	Free vesicles	Cisternae
High particle density class (4-8 particles per probe circle)	2302 ± 654	2706 ± 607
Low particle density class (0-3 particles per probe circle)	1074 ± 275	1349 ± 328

Number of particles per square micrometer of membrane (plus or minus the SD) assessed with a 6-mm probe circle (see *Materials and Methods*). The particle density in free vesicles and that in associated cisternae were not significantly different, whereas the differences between high and low particle density classes (for both free vesicles and cisternae) were significantly different $P < 0.05$).

What molecular mechanism(s) might ensure processive transport? One simple hypothesis (which we term the "string theory") is as follows: A carpet of fibrous proteins (the "strings") could emanate from the surface of Golgi cisternae to form a protein matrix (perhaps with other proteins) that would attach to vesicles and thereby make intercisternal transfers occur locally. Multivalent attachment to strings or to a more complex matrix additionally would allow vesicles to be passed from string to string without leaving the surface of the stack, thereby allowing diffusion around the surface of a given cisterna (in an essentially two-dimensional random walk) until an adjoining cisterna is encountered. In this way, transfers could only occur between cisternae in direct apposition in the stack. The strings could attach to a coat subunit or to a protein emanating from the vesicle membrane or to both. If the strings were long enough (approximately one vesicle diameter), a budding vesicle could be passed between neighboring cisternae without ever dissociating from the stack. Provided only that the strings are inherently flexible, budding vesicles automatically would become attached to strings before they pinch off.

Flexible strings also would permit tethered vesicles to bob up and down as would be necessary for close apposition needed for SNAP receptor (SNARE) complex assembly and fusion.

A fine balance between binding and dissociation would be needed for transport by tethered vesicles to occur at a useful rate. As the simplest possibility, the strings would function best when they are poised individually on the cusp of dissociation because of a rapid, dynamic thermodynamic equilibrium between bound and unbound states: If the attachment of vesicles to the proposed strings were too strong or too long-lived, vesicles would effectively be stuck in place as a fly is stuck to fly paper; if attachment were too weak, the vesicles would fly away and processivity would be lost. But when a vesicle is bound by multiple strings each of which dissociates and can be replaced by a new string in the time frame of inter-cisternal transport (approximately 1 min), then can vesicles effectively diffuse while remaining attached to the surface of the stack, just as a fly walks. Alternatively, a nonequilibrium association–dissociation cycle, driven by the input of energy (such as an ATPase or a GTPase), could assure vesicle mobility.

Fibrous elements linking vesicles to cisternae are indeed observed by electron microscopy of thin sections. Both coated and uncoated vesicles are commonly seen to be attached by several fibers (which are typically approximately one vesicle diameter in maximal length) (Fig. 3 *A–F*). They are often bent, suggesting flexibility. Vesicles can be linked either to a single cisterna (Fig. 3 *B*, *C*, and *E*) or to two cisternae simultaneously, as if in transit between them (Fig. 3 *A* and *F*). Similar fibers interconnecting vesicles can sometimes also be seen (Fig. 3*C*). Fibrous connections between Golgi cisternae *in situ* (19) and *in vitro* (20) have been noted before, as have fibers associated with transport vesicles (17, 21, 22).

What could these fibers be made of? Among a number of possibilities, the Golgi-specific proteins p115 (23) and GM130 (24) are excellent candidates to contribute many of the fibers throughout the stack. The former is a rod of 45 nm, and the latter also is predicted to be a rod on the basis of coiled coil motifs (24–27). p115 is needed for ER-derived, COPII-coated vesicles to bind to the Golgi acting before SNARE complex assembly (28, 29) and also is needed for Golgi-derived, COPI-

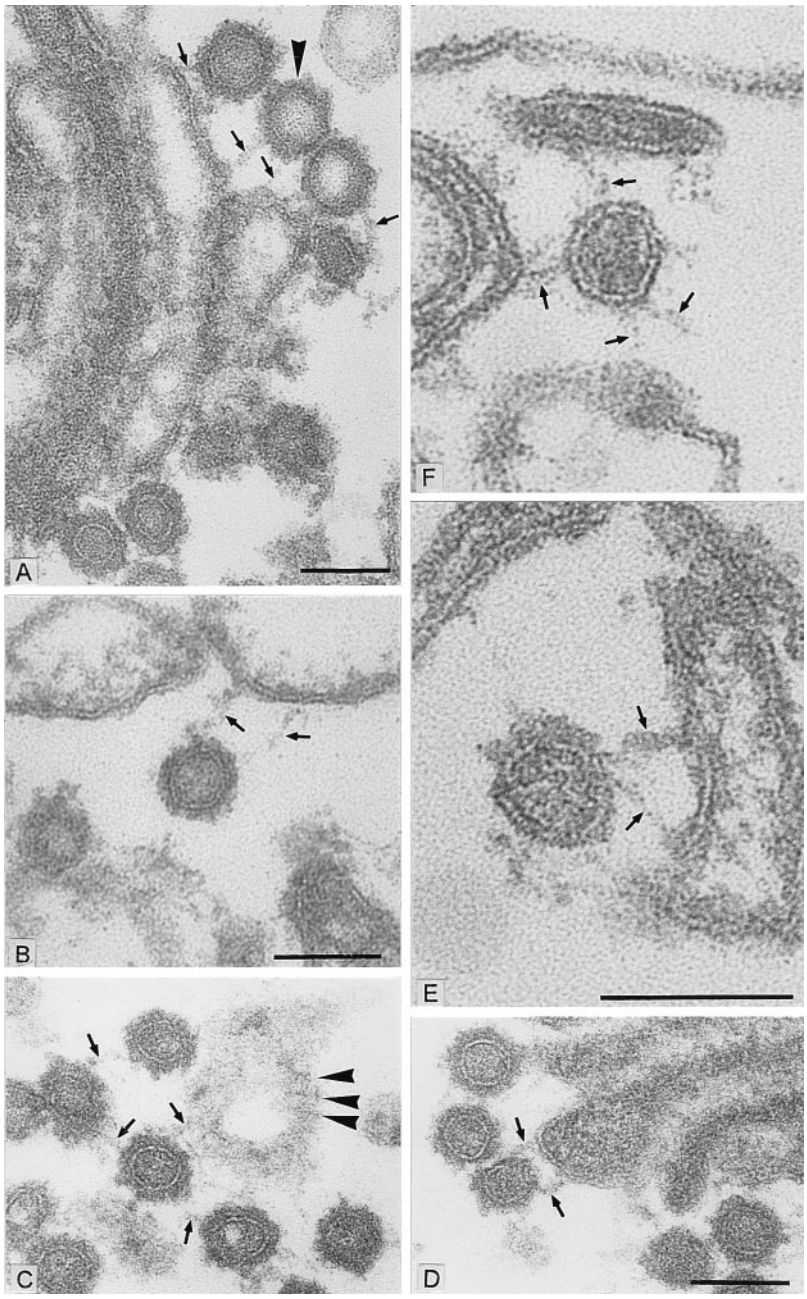

FIG. 3. Thin section images of proteinaceous-like "strings" that link transport vesicles to cisternal elements. (*A*) Coated vesicles with fibrous connections (arrows) connecting them to neighboring Golgi cisternae. The vesicle indicated by the arrowhead is attached simultaneously to two adjoining cisternae. (*B*) A coated vesicle is linked to a cisterna by two apparently flexible fibers (arrows) in the same plane of section. (*C*) A group of coated vesicles showing various filamentous extensions (arrows) making vesicle–vesicle or vesicle–cisterna connections. In grazing section, this cisternal element is seen to have linear features on its surface (indicated by arrowheads) that could correspond to portions of the filaments that link vesicles to cisternae. (*D*) A cisternal tip with a free coated vesicle linked to the tip by two filaments (arrows). (*E*) A partially coated Golgi vesicle tethered with two connections to a single cisternal membrane (arrows). (*F*) An uncoated vesicle attached by at least four fibers (arrows) to at least two distinct cisternal elements. (*A–D*) Golgi incubated with cytosol and ATP in the presence of GTPγS. (*E* and *F*) Golgi incubated with cytosol and ATP without GTPγS. Comparable fibrous attachments connecting vesicles to each other and to cisternae also can be observed in suitable thin sections of Golgi stacks of animal and plant cells but are more difficult to demonstrate because of the high electron density of cytoplasmic matrix, which is absent in cell-free preparations. (Magnifications: *A*, ×123,000; *B*, ×145,000; *C* and *D*, ×138,000; *E* and *F*, ×264,000; Bar = 100 nm.)

coated vesicles to fuse with Golgi cisternae (23, 30). p115 and GM130 bind end-to-end, suggesting a rod with flexible joints (31). GM130 and p115 are most concentrated at the *cis* face of the stack but are present in all cisternae (24, 32). This polarity could ensure that COPI or COPII vesicles from the ER enter the Golgi stack at the *cis* face. A carpet comprised of other fibrous proteins [such as p230 (33)] concentrated at the opposite, *trans* face of the stack could likewise bias the return of vesicles from diverse post- *trans*-Golgi network targets (endosomes, plasma membranes, etc.) to the end of the stack. Other interesting possibilities include the potentially fibrous protein giantin (34–36).

Consistent with the observed tethering (Fig. 3 *A–F*), COPI-coated vesicles remain associated with Golgi stacks at a physiological (0.15 M) salt concentration. The vesicles dissociate at higher than physiological values and are completely dissociated by 0.25 M KCl (37). The fact that the bound COPI-coated vesicles are on the cusp of dissociation under physiological conditions is consistent with a simple physicochemical model for vesicle diffusion on the Golgi surface, as outlined above. Continuing attachment to donor membranes at physiological salt is a singular feature. By contrast, very similar COPI vesicles budding from the ER (38) as well as COPII-coated vesicles budding from ER (39), and various species of transport vesicles budding from TGN (21, 40, 41) efficiently dissociate from their parental membrane. This pattern fits nicely with the need for transport vesicles leaving the ER or the TGN to travel to a distant target membrane, in contrast with processive transport within the Golgi stack.

If intra-Golgi transport is normally processive, how can the phenomenon of transfer of proteins between Golgi stacks after fusion of vesicular stomatitis virus-infected cells (42) be explained? In retrospect, it seems likely that vesicle attachment may have been perturbed in some way as a result of cytopathic effects of the viral infection, possibly because of changes in membrane permeability with resulting changes in salt concentrations, or as a result of the pH 5 treatment needed for fusion, which also alters the permeability of virus-infected cells (43). Because the coated vesicles are on the cusp of dissociating under physiological condition, any significant perturbation might well dislodge vesicles to allow transfers between stacks. Alternatively, collisions between stacks originally present in different cells may occur as cytoplasms forcefully mix during the act of fusion, collisions that normally would not occur at any significant frequency. This could enable stacks to transiently exchange bound vesicles.

Although we originally did not interpret cell-free transport this way (44), it now seems likely that here, too, transport is caused by bound vesicles exchanging between stacks during collisions, rather than by free vesicles, under the salt conditions we have used routinely. It is well established that COPI-coated vesicles containing anterograde-directed cargo transfer between stacks in the cell-free system, and this process has been shown directly both morphologically and biochemically (12, 45). For example, although the COPI-coated vesicles containing vesicular stomatitis virus G protein remain attached to stacks, they nonetheless succeed in distributing equally between donor and acceptor populations of stacks (12). Vesicles also can be isolated from donor stacks by salt extraction and then added back to acceptor stacks, to which they bind and fuse (45). Transfer of vesicles between colliding stacks would result when a vesicle initially tethered to one stack becomes tethered to another stack (possibly as in Fig. 3*F*). Indeed, when stacks are held fixed to a surface—so collisions between them cannot occur—vesicles no longer transfer between stacks (22).

In summary, the biochemical and morphological characteristics of the association of Golgi-derived vesicles with cisternal membranes suggests that transport within the stack may occur according to a simple processive mechanism in which transfers are restricted to apposing cisternae because of dynamic multivalent tethering of transport vesicles. This simple restriction is all that is needed to allow a stack of functionally equivalent cisternae to function in distillation as efficiently as if each were a distinct compartment with its own set of v-SNAREs and t-SNAREs (compare the model in figure 7*d* with that in figure 7*b* in ref. 18).

This work was supported by a grant from the Swiss National Science Foundation (L.O.), a grant from the National Institutes of Health (J.R.), and a grant from the Human Frontier Science Program (to L.O. and J.R.).

1. Farquhar, M. & Palade, G. E. (1981) *J. Cell Biol.* **91,** 77s–103s.
2. Rothman, J. E. (1994) *Nature (London)* **372,** 55–63.
3. Schekman, R. & Orci, L. (1996) *Science* **271,** 1526–1533.
4. Cosson, P. & Letourneur, F. (1994) *Science* **263,** 1629–1631.
5. Letourneur, F., Gaynor, E. C., Hennecke, S., Demollière, C., Duden, R., Emr, S., Riezman, H. & Cosson, P. (1994) *Cell* **79,** 1199–1207.
6. Orci, L., Stamnes, M., Ravazzola, M., Amherdt, M., Perrelet, A., Söllner, T. H. & Rothman, J. E. (1997) *Cell* **90,** 335–349.
7. Branton, D. (1966) *Proc. Natl. Acad. Sci. USA* **55,** 1048–1056.
8. Branton, D. (1969) *Annu. Rev. Plant Physiol.* **20,** 209–238.
9. Yu, J. & Branton, D. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3891–3895.
10. Orci, L. (1982) *Diabetes* **31,** 538–565.
11. Orci, L., Glick, B. S. & Rothman, J. E. (1986) *Cell* **46,** 171–184.
12. Orci, L., Malhotra, V., Amherdt, M., Serafini, T. & Rothman, J. E. (1989) *Cell* **56,** 357–368.
13. Moor, H. & Mühlethaler, K. (1963) *J. Cell Biol.* **17,** 609–628.
14. Losa, G. A., Weibel, E. R. & Bolender, R. P. (1978) *J. Cell Biol.* **78,** 289–307.
15. Branton, D., Bullivant, S., Gilula, N. B., Karnovsky, M. J., Moor, H., Mühlethaler, K., Northcote, D. H., Packer, L., Satir, B., Satir, P., *et al.* (1975) *Science* **190,** 54–56.
16. Satir, P. & Satir, B. (1974) *Exp. Cell Res.* **89,** 404–407.
17. Melançon, P., Glick, B. S., Malhotra, V., Weidman, P. J., Serafini, T., Gleason, M. L., Orci, L. & Rothman, J. E. (1987) *Cell* **51,** 1053–1062.
18. Rothman, J. E. (1981) *Science* **213,** 1212–1219.
19. Franke, W. W., Kartenbeck, J., VanderWoude, W. J., Scheer, U. & Morré, D. J. (1972) *Z. Zellforsch.* **132,** 365–380.
20. Cluett, E. B. & Brown, M. J. (1992) *J. Cell Sci.* **103,** 773–784.
21. Simon, J. P., Ivanov, I. E., Adesnik, M. & Sabatini, D. D. (1996) *J. Cell Biol.* **135,** 355–370.
22. Weidman, P., Roth, R. & Heuser J. (1993) *Cell* **75,** 123–133.
23. Waters, M. G., Clary, D. O. & Rothman, J. E. (1992) *J. Cell Biol.* **118,** 1015–1026.
24. Nakamura, N., Rabouille, C., Watson, R., Nilsson, T., Hui, N., Slusarewicz, P., Kreis, T. E. & Warren, G. (1995) *J. Cell Biol.* **131,** 1715–1726.
25. Sapperstein, S. K., Walter, D. M., Grosvenor, A. R., Heuser, J. E. & Waters, M. G. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 522–526.
26. Barroso, M, Nelson, D. S. & Sztul, E. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 527–531.
27. Yamakawa, H., Soeg, D. H., Yoda, K., Yamasaki, M. & Wakabayashi, T. (1996) *J. Struct. Biol.* **116,** 356–365.
28. Sapperstein, S. K., Lupashin, V. V., Schmitt, H. D. & Waters, M. G. (1996) *J. Cell. Biol.* **132,** 755–767.
29. Barlowe, C. (1997) *J. Cell Biol.* **139,** 1097–1108.
30. Elazar, Z., Orci, L., Ostermann, J., Amherdt, M., Tanigawa, G. & Rothman, J. E. (1994) *J. Cell Biol.* **124,** 415–424.
31. Nakamura, N., Lowe, M., Levine, T. P., Rabouille, C. & Warren, G. (1997) *Cell* **89,** 445–455.
32. Shima, D. T., Haldar, K., Pepperkok, R., Watson, R. & Warren, G. (1997) *J. Cell Biol.* **137,** 1211–1228.
33. Erlich, R., Gleeson, P. A., Campbell, P., Dietzsch, E. & Toh, B.-H. (1996) *J. Biol. Chem.* **271,** 8328–8337.
34. Linstedt, A. D. & Hauri, H. P. (1993) *Mol. Biol. Cell* **4,** 679–693.
35. Seelig, H. P., Schranz, P., Schroter, H., Wiemann, C., Griffiths, G. & Renz, M. (1994) *Mol. Cell. Biol.* **14,** 2564–2576.
36. Fritzler, M. J., Hamel, J. C., Ochs, R. L. & Chan, E. K. L. (1993) *J. Exp. Med.* **178,** 49–62.
37. Malhotra, V., Serafini, T., Orci, L., Shepherd, J. & Rothman, J. E. (1989) *Cell* **58,** 329–336.
38. Bednarek, S. Y., Ravazzola, M., Hosobuchi, M., Amherdt, M., Perrelet, A., Schekman, R. & Orci, L. (1995) *Cell* **83,** 1183–1196.
39. Barlowe, C., Orci, L., Yeung, T., Hosobuchi, M., Hamamoto, S., Salama, N., Rexach, M. F., Ravazzola, M., Amherdt, M. & Schekman, R. (1994) *Cell* **77,** 895–907.
40. Tooze, S. A. & Huttner W. B. (1990) *Cell* **60,** 837–847.
41. Wandinger-Ness, A., Bennett, M. K., Antony, C. & Simons, K. (1990) *J. Cell Biol.* **111,** 987–1000.
42. Rothman, J. E., Miller, R. L. & Urbani, L. J. (1984) *J. Cell Biol.* **99,** 260–271.
43. Carrasco, L., Otero, M. J. & Castrillo, J. L. (1989) *Pharmacol. Ther.* **40,** 171–212.
44. Balch, W. E., Dunphy W. G., Braell W. A. & Rothman J. E. (1984) *Cell* **39,** 405–416.
45. Ostermann, J., Orci, L., Tani, K., Amherdt, M., Ravazzola, M., Elazar, Z. & Rothman, J. E. (1993) *Cell* **75,** 1015–1025.