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Protein Sorting by Directed Maturation of Golgi Compartments

Bernard B. Allan and William E. Balch*

How does the Golgi stack mediate transport of cargo from the endoplasmic reticulum (ER) to the cell surface? A possibility is that cargo-containing vesicles derived from the ER form early Golgi compartments that then mature by retrieval of processing enzymes from later Golgi compartments. Maturation continues at terminal Golgi compartments by retrieval of transport components from the endocytic pathway to promote sorting of cargo to multiple cellular destinations. Hence, retrograde movement may integrate exocytic and endocytic pathways in eukaryotic cells and coordinate membrane flow and cargo transport through the Golgi stack.

The Golgi exists as a stack of functionally polarized cisternae in mammalian cells (Fig. 1). Cargo exported from the ER by vesicle carriers first appears in pre-Golgi intermediates that move on microtubule tracks to the cis Golgi region. As cargo moves through the stack, it is modified by Golgi-associated processing enzymes. Finally, the trans side of the Golgi stack serves as a key sorting station, directing cargo to multiple intracellular and extracellular destinations. The challenge to understanding Golgi function has been relating the morphological organization of the stack to the function of vesicle carriers, whose role in cargo movement has been controversial. We highlight results that suggest that vesicles recycle processing enzymes and post-Golgi sorting determinants to promote progressive maturation of ER-derived intermediates to form functional Golgi compartments. We illustrate how this process, referred to as directed maturation, uses the primary activity of recycling to integrate membrane flow through the exocytic pathway to achieve normal growth in development.

The Transport Machinery

Movement of cargo between exocytic and endocytic compartments requires transiently coated vesicle carriers (*1*). The term "vesicle" encompasses any membrane-enclosed structure into which cargo is segregated by a membrane fission event. Fission establishes a new boundary to distinguish one compartment from the next. Biosynthetic cargo exiting the ER includes newly synthesized proteins and lipids that are moved to distinct cellular and extracellular destinations. Other cargo incorporated into vesicles includes proteins that are continuously recycled between compartments. These components encompass the transport machinery involved in cargo selection, vesicle formation, and targeting and fusion of vesicles.

A fundamental principle of membrane traffic is that vesicle formation is initiated by the selection and concentration of cargo. This occurs through interactions between sorting determinants on the cargo and cytosolic coat components that direct cargo to the forming vesicle (*2*). Soluble cargo (cargo found in the lumen of the compartment) will necessarily require sorting receptors to couple the protein to the cytosolic coat machinery. A variety of coat complexes participate in vesicle formation. These complexes include COPII (coat protein complex II), involved in export of biosynthetic cargo from the ER (*3*); COPI (coat protein complex I), involved in vesicle formation on ER-derived pre-Golgi intermediates, Golgi compartments (*4*, *5*), and endocytic compartments (*6*); retromers involved in endosome-to-Golgi retrograde transport (*7*); and caveolin (*8*) and clathrin and their adaptor proteins (APs) (*1*). Caveolin and clathrin-AP complexes direct cargo selection from the trans face of the Golgi and the cell surface. Coat recruitment, cargo selection, and vesicle fission are coordinated by guanosine triphosphatases (GTPases). These GTPases include Sar1, directing COPII function; ARF1 (ADP-ribosylation factor 1), directing COPI function; and dynamin, which participates in clathrin vesicle formation at the cell surface (*1*).

The recruitment of biosynthetic cargo by vesicle coats is coupled with the acquisition of components that direct the vesicle to its destination (targeting determinants). These components include members of the SNARE (for SNAp-REceptor) family of proteins (*9*). SNARE complexes are required to identify the vesicle and its downstream target. Other proteins that work in conjunction with SNAREs, including giantin (*10*), p115 (*11*), EEA1 (*12*), the exocyst protein complex (*13*), and the nSec-1 family (*14*), mediate the docking and fusion of transport vesicles. The targeting and fusion machinery is likely to be coordinated by the activity of Rab GTPases (*15*). Taken as a whole, coat and targeting components function together at different steps of the exocytic and endocytic pathways to form vesicle "sorting machines" that move specific types of cargo from one destination to the next. Cargo

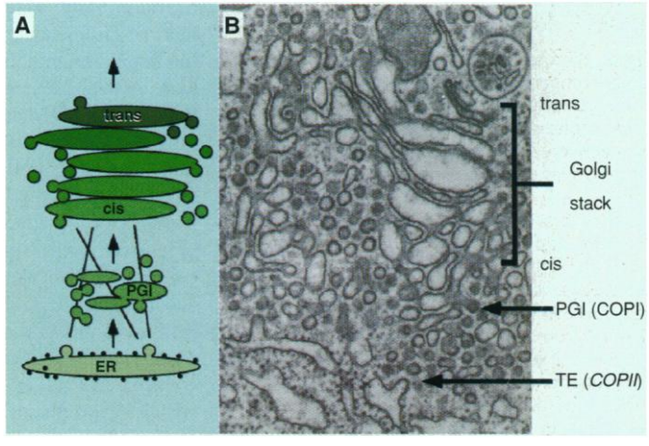

Fig. 1. Morphological structure of the Golgi region. (**A**) Cargo exported from the ER is first found in specialized ER export sites composed of ER-derived COPII vesicles and tubular elements (TE). These form pre-Golgi intermediates (PGI) that recycle proteins by COPI and that deliver cargo to the Golgi complex along microtubule tracks (black lines). The Golgi complex consists of a compositionally polarized stack of compartments arranged in a cis or "entering" face and a trans or "exit" face. The stack is surrounded by numerous vesicles that participate in Golgi traffic. (**B**) An image of the ER-Golgi region in pancreatic acinar cell (kindly provided by G. Palade).

The Scripps Research Institute, Department of Cell Biology, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA.

*To whom correspondence should be addressed. E-mail: webalch@scripps.edu

selection by vesicles emphasizes the need for the selective recycling of membrane-anchored sorting and targeting determinants that will be used in repeated rounds of vesicle formation.

A Changing View of Sorting in the Golgi

How do vesicle-sorting machines direct transport through the Golgi? The biochemical factors that are required for movement in the stack were unknown until the advent of a cell-free assay to study intra-Golgi transport. This assay uses isolated Golgi membranes to measure the sequential processing of cargo molecules by Golgi-associated enzymes (*16*). Here, Rothman and colleagues (*17*) provided evidence for vesicles, COPI coats, and the ARF1 GTPase in COPI coat assembly. These studies also identified NSF (NEM-sensitive factor) (*18*), an adenosine triphosphatase that regulates the SNARE machinery (*19*) in vesicle targeting and fusion. Moreover, they suggested that sequential populations of vesicles were used to promote transport between different cisternae (*17*). These observations led to the model that COPI coats mediate the movement of biosynthetic cargo through sequential Golgi compartments in a cis-to-trans (or anterograde) direction.

The hypothesis that COPI-coated vesicles direct anterograde transport of biosynthetic cargo has been repeatedly challenged (*4*, *5*, *20*–*22*). The first evidence that COPI may really direct retrograde transport came with the finding that it bound the Lys-Lys-X-X motif present in the cytoplasmic tail of several transmembrane proteins (*21*, *23*). This motif directs recycling from pre-Golgi and Golgi compartments to the ER (*24*). The primary role for COPI in retrograde transport has now been verified biochemically, genetically, and morphologically (*4*, *5*, *25*, *26*).

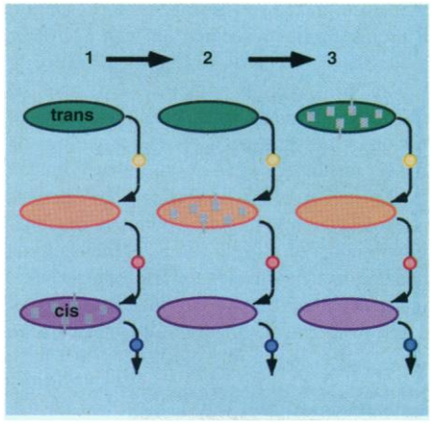

Fig. 2. Directed maturation of cargo-containing Golgi compartments. Step 1: An ER-derived cis compartment (purple) containing a pulse of cargo molecules (gray) changes composition as it selectively sheds recycling components (blue) and receives vesicles containing processing enzymes (red) from a more mature cisternae (step 2) (orange). This compartment undergoes a maturation to form the trans compartment (step 3) (green). The primary colors of the retrograde vesicles illustrate that they contain selected compositions of recycling components derived from different levels of the stack. The gradient of processing enzymes and targeting components across the stack is shown by the mixture of primary colors defining each compartment. In this way, the Golgi maintains its overall morphological structure, while cargo is posttranslationally modified as the compartment migrates in the cis-to-trans direction (steps 1 to 3).

How can the proposed role of COPI coats in anterograde cargo transport be reconciled with its clear role in retrograde transport? One possibility is that COPI-mediated transport is bidirectional based on the observation that the Golgi may contain two classes of COPI vesicles in vivo (*25*). One class contained recycling components, whereas a second class contained biosynthetic cargo. One issue to be resolved with this model is the direction of movement of the second class of vesicles containing biosynthetic cargo. A second concern is that the concentration of biosynthetic cargo in vesicles is identical to that of the stack. This lack of concentration, an activity important in COPII and clathrin function, raises the question of how these vesicles [or even the recently suggested role of transient tubular connections between adjacent cisternae of the stack (*27*)] could contribute to vectorial transport through the Golgi stack (*28*). A second possibility is that COPI coats, instead of moving biosynthetic cargo forward, retrieve transport machinery components and processing enzymes to the cargo-containing compartment. Indeed, the original cell-free Golgi biochemical assay cannot distinguish between anterograde cargo movement and retrograde processing enzyme movement. In this model, COPI-mediated retrograde, but not anterograde, transport is the driving force for Golgi function.

The potential importance of retrograde traffic in Golgi function revived an old idea that the Golgi stack is a collection of maturing compartments. In this "cisternal progression" model (*29*), compartments containing newly synthesized cargo derived from the ER migrated (in an unknown fashion) in a cis-to-trans direction through the stack. This model arose from morphological observations that large cargo complexes such as procollagen precursors were simply too big to be incorporated into the small vesicle carriers surrounding the Golgi stack (*30*, *31*). A recent reexamination of procollagen movement through the Golgi stack established that procollagen does not enter Golgi-associated COPI vesicles but remains within the compartment formed after its export from the ER (*32*). Moreover, this compartment underwent a change in its biochemical composition as it occupied different positions in the cis-to-trans orientation of the Golgi stack. This is consistent with the fact that newly synthesized proteins entering on the cis face encounter Golgi processing enzymes that are organized in the cis-to-trans direction across the stack. These processing enzymes are not restricted to a single cisterna in the stack but spill over into adjacent cisternae (*33*). Therefore, although the boundaries of the cisternae are distinct morphologically and only rarely, if at all, connected (*28*, *34*), the biochemical boundaries are less precisely defined, possibly because of the dynamic movement of processing enzymes between sequential layers of the stack. The lack of a specific selective mechanism for the movement of procollagen from the cis to the trans side of the stack supports observations that biosynthetic cargo that is concentrated during exit from the ER is not further concentrated in Golgi cisternae (*35*).

Movement of Golgi Processing Enzymes by COPI

The mobility of processing enzymes within the stack supports a role for COPI retrograde vesicles in Golgi maturation. By tagging resident Golgi processing enzymes with green fluorescent protein, their movements have been followed with fluorescence video microscopy. These proteins have diffusion coefficients consistent with their unrestrained mobility within a Golgi cisterna (*36*). Moreover, COPI vesicles have now been shown to concentrate Golgi processing enzymes (*37*) and to serve as transport intermediates in vitro (*22*). These results are consistent with the fact that the recycling of processing enzymes is required for normal Golgi function in vivo (*38*, *39*). Use of recycling vesicles suggests that retrograde retrieval of a defined population of processing enzymes can, in principle, catalytically process all cargo proteins found within a maturing compartment.

Just how the Golgi maintains its organization of processing enzymes through the activity of recycling vesicles has been the topic of speculation (*27*, *30*, *31*). This may occur by a graded interaction of COPI (or other unknown coats) with sorting signals found on recycling proteins (Fig. 2). Those proteins with high-affinity sorting signals may be recycled more rapidly and efficiently than those with lower affinity. Thus, the biochemical composition of intermediates derived from the ER would change continuously as they mature in a cis-to-trans direction through the stack, while the Golgi maintains its overall morphological appearance. The selection of enzymes into retrograde vesicles would ensure progressive specialization of compartments in the stack because sorting of processing enzymes would be coupled to the machinery that directs vesicles to their target compartments. Such coupling occurs in recy-

cling to the ER from pre-Golgi intermediates (*40*). Here, the disassembly of the COPII coat required for export of anterograde cargo from the ER is coupled with the recruitment of the COPI recycling machinery necessary for the retrograde pathway. Thus, COPI coats are likely to play a key role in maintaining the proper steady-state distribution of Golgi processing enzymes within the Golgi stack.

Important insight into Golgi maturation has come from studies with brefeldin A (BFA), a drug that prevents COPI recruitment to Golgi membranes and triggers delivery of all Golgi processing enzymes to the ER (*41*). BFA blocks the activation of ARF1 (*42*), a step necessary for the recruitment of COPI coats and vesicle formation. In the absence of COPI, Golgi cisternae form elongated tubules (*43*). These tubules may represent vesicle precursors that have the full potential to fuse. Under normal conditions, such "uncoupled" fusion, where retrograde trafficking is uncontrolled (*44*) and accelerated over normal steady state (*45*), would not be expected because COPI-mediated sorting and fission would precede the fusion event. If this were not the case, the entire organization of the early secretory pathway would be compromised. The aberrant collapse of Golgi membranes to the ER after inhibition of COPI assembly highlights the presence of a dynamic and tightly regulated vesicle retrograde pathway regulating the function of the entire stack (*42*, *46*). COPI-mediated recycling could provide a mechanistic framework for the morphological process of cisternal progression.

Role of SNAREs in Directed Maturation

Is the activity of SNARE components consistent with a major role for retrograde vesicles in Golgi function? The best understood Golgi SNAREs are yeast Sed5p and its mammalian homolog syntaxin 5 (Syn5) (*47*). These proteins are required for ER-to-Golgi transport and recycle between the ER and the cis face of the Golgi stack (*39*, *48*, *49*). Functionally interfering with Syn5 blocks ER-to-Golgi transport, suggesting that Syn5 or associated factors participate in pre-Golgi intermediate function (*49*). Here, Syn5 may mark pre-Golgi compartments as targets for fusion with retrograde, Golgi-derived COPI vesicles that contain processing enzymes, to form the cis-Golgi compartment (*49*). In yeast, when movement of Sed5p out of the ER is blocked, Golgi structures dissipate (*39*). Under these conditions, the late Golgi SNARE Sft2p, a protein that targets vesicles to Sed5p-containing Golgi compartments, is redistributed to multiple vesicular structures (*39*), probably because these trans-Golgi cisternae-derived vesicles have no place to go. Because the normal recycling of Syn5 and Sed5p is essential for maintaining Golgi structure and function, the maturation of both yeast and mammalian Golgi compartments is evolutionarily conserved and may rely on COPI-mediated recycling.

The results discussed above combined with other lines of morphological and biochemical evidence (*4*, *30*, *31*, *47*, *50*) alter our traditional perception (*17*) of how the Golgi functions to sort proteins. We now envisage that retrograde, not anterograde, vesicles play a prominent role by functionally modifying cargo-containing compartments generated by the ER (Fig. 3).

Protein Sorting at the TGN by Directed Maturation

Does maturation continue at the trans face of the Golgi (also referred to as the trans-Golgi network or TGN) where vesicle traffic moves cargo to multiple destinations (*51*)? Given the necessity to retrieve sorting and targeting components that direct vesicles to their correct post-TGN destinations, a number of lines of evidence suggest that it does. In this way, the TGN sits at the crossroads of the endocytic and exocytic pathways, enabling the cell to balance membrane flow between the two pathways to maintain the proper composition of intracellular organelles and cell surfaces.

The contribution of recycling to TGN function is exemplified by the lysosomal transport pathway (*52*). In the TGN, mannose-6-phosphate receptors (MPRs) that bind lysosomal hydrolases and deliver these precursors to the lysosome are returned to the TGN through acidic endosomal compartments. Export of MPRs from the TGN uses tyrosine- and di-leucine–based sorting motifs that recognize clathrin and AP adaptor complexes (*53*). These coat machineries are found at the cell surface, but not on the ER or early Golgi compartments. Their presence on the TGN illustrates that ER-derived intermediates and early Golgi compartments (that lack the capability to recruit these components) become modified to use vesicle-mediated pathways common to the endocytic compartments and the cell surface. Indeed, the role of endosomal recycling in TGN function is evident from the fact that mutations in the MPR sorting motif that block recycling or alter the amount of MPR in the cell can change the amount of clathrin coat recruitment to the TGN and interfere with the sorting of lysosomal enzymes (*54*). The existence of two different MPRs, cation dependent and cation independent with different trafficking itineraries (*55*), suggests a close relation between the transport of lysosomal enzymes and the level of expression of these receptors in different cell types to direct flow of cargo from the TGN to the lysosome and the cell surface. Moreover, the importance of endosomal recycling to TGN function is strongly supported by genetic and biochemical studies on cargo traffic from the TGN to the vacuole, the lysosome equivalent in yeast (*56*). Recycling to the TGN through endosomal compartments observed in lysosomal/vacuolar pathways is also found during secretory granule biogenesis (*57*). Here, retrograde traffic is believed to coordinate granule content with sorting and targeting components that are required for fusion of the mature granule to the cell surface (*58*).

Although the major role of the endosome is to deliver proteins from the surface to the lysosome and recycle plasma membrane proteins back to the surface, it also must provide the essential sorting and targeting components to direct biosynthetic cargo from the TGN to the surface (Fig. 3). This is particularly important in polarized cells where the basolateral and apical surfaces have unique functions. Although generation of cell polarity was previously described only in terms of Golgi function (*58*), it is now evident that traffic from the TGN is responsive to a hierarchy of events initiated by extracellular signaling pathways (*59*). Cell-cell (cadherins) and cell-extracellular matrix (ECM) (integrins) contacts generate structural asymmetry at the membrane cell surface. This spatial information, augmented by the localized assembly of a cytoskeleton, creates a "targeting patch" for exocytic transport vesicles formed from the TGN. In Madin-Darby canine kid-

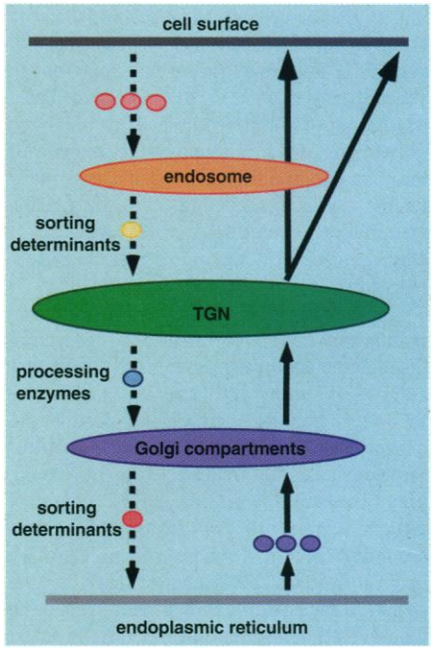

Fig. 3. Directed maturation involves coupling to endocytic recycling at the TGN. The function of the TGN requires recycling of the sorting and targeting determinants retrieved through the endocytic pathway. The continuum of recycling vesicles from endocytic compartments to the ER (dashed lines) provides an opportunity to balance exocytic (solid lines) and endocytic membrane flow required for normal cell growth.

ney cells, the localization of plasma membrane proteins exported from the TGN through the function of these targeting patches can be modulated by expression of different cadherin cell adhesion molecules and is sensitive to contact with extracellular ECM (*60*). Similarly, in retinal pigmented epithelial cells, developmental switches control the trafficking to apical or basal-lateral surfaces of a number of proteins (*61*). Traffic is dependent on contact with photoreceptor cells (*62*) and, in some cases, is initiated by cues provided by apically localized $\alpha v\beta 5$ integrin receptors (*63*). Because events at the plasma membrane affect intracellular sorting, we conclude that the endocytic and exocytic pathways may be coupled at the TGN (Fig. 3). It is not surprising that bacterial pathogens usurp this pathway to deliver exotoxins through the TGN to the ER for translocation into the cytoplasm to promote their own survival (*64*).

The molecular mechanisms that are involved in coupling exocytic and endocytic pathways at the TGN are beginning to emerge. In addition to SNARE interactions, targeting of TGN-derived vesicles to the cell surface involves a protein complex referred to as the exocyst (*13*). In yeast, the exocyst marks the site of polarized bud growth. In its absence, vesicles target randomly to the surface. In mammalian cells, the homologous exocyst complex is recruited to cell-cell contacts laid down by extracellular spatial cues for targeting to the basal-lateral surface (*65*). Recycling of SNAREs through these targeting patches is important for normal function. In yeast, the syntaxin Tlg1p, which mediates trafficking of chitin synthase III to polarized growth sites (*66*), is found at the junction of the exocytic and endocytic pathways. Interference with its recycling from the plasma membrane prevents the proper targeting of chitin synthase III containing post-Golgi vesicles to the bud site (*66*).

In general, these examples illustrate that maturation of TGN function by recycling through the endosome provides the necessary balance of sorting and targeting machineries to direct cargo to their correct destination. The process of directed maturation simplifies movement of biosynthetic cargo by having only two sorting steps: one at the ER to select protein that is properly folded (*67*) and a second at the TGN, where a flexible sorting decision directs biosynthetic cargo to the proper downstream compartment reflecting the developmental state of the cell.

A Contemporary Paradigm for Golgi Function

The discovery of recycling between the ER and the Golgi and between compartments of the Golgi stack, along with recycling through the endocytic pathway, demonstrates that the entire exocytic pathway requires the process of directed maturation for normal function. In this view (Fig. 3), a continuum is generated in which pre-Golgi intermediates derived from the ER initiate the first step in maturation by returning sorting and targeting components back to the ER. This step is followed by a second step in which acquisition of Golgi processing enzymes, by recycling, leads to the formation of the TGN, which, in the third step, becomes integrated with the endocytic pathway. Although previous models of Golgi function (*17*) offered key insights into the biochemical factors required for vesicle formation and targeting, directed maturation provides a broader foundation for understanding issues regarding the directionality and continuity of membrane flow in the cell during growth and development. By focusing on the role of retrograde trafficking from the cell surface, recycling provides a link between cell surface events and exocytic compartments, enabling the cell to balance and regulate outward membrane flow (Fig. 3). Undoubtedly, many surprises in the future regarding Golgi function will come from questions that address how cargo movement is coupled to the recycling machinery (*47*) or how the coupling of membrane flow with the cytoskeleton enhances the operation of the exocytic and endocytic pathways (*68*). Insight into these issues will play a major role in the evolution of our understanding of directed maturation of Golgi compartments as a mechanism for protein sorting.

References and Notes

1. R. Schekman and L. Orci, *Science* **271**, 1526 (1996); S. L. Schmid, *Annu. Rev. Biochem.* **66**, 511 (1997).
2. M. Aridor and W. E. Balch, *Trends Cell Biol.* **6**, 315 (1996); M. Aridor *et al.*, *J. Biol. Chem.* **274**, 4389 (1999).
3. C. Barlowe, *Biochim. Biophys. Acta* **1404**, 67 (1998).
4. E. C. Gaynor, T. R. Graham, S. D. Emr, *ibid.*, p. 33.
5. M. Lowe and T. E. Kreis, *ibid.*, p. 53.
6. F. Gu *et al.*, *J. Cell Biol.* **139**, 1183 (1997).
7. M. N. Seaman *et al.*, *ibid.* **142**, 665 (1998).
8. R. G. Anderson, *Annu. Rev. Biochem.* **67**, 199 (1998).
9. T. Sollner *et al.*, *Nature* **362**, 318 (1993).
10. B. Sonnichsen *et al.*, *J. Cell Biol.* **140**, 1013 (1998).
11. N. Nakamura *et al.*, *Cell* **89**, 445 (1997).
12. S. Christoforidis, H. M. McBride, R. D. Burgoyne, M. Zerial, *Nature* **397**, 621 (1999).
13. D. R. TerBush *et al.*, *EMBO J.* **15**, 6483 (1996); W. Guo *et al.*, *ibid.* **18**, 1071 (1999).
14. J. C. Hay and R. H. Scheller, *Curr. Opin. Cell Biol.* **9**, 505 (1997).
15. O. Martinez and B. Goud, *Biochim. Biophys. Acta* **1404**, 101 (1998).
16. W. E. Balch *et al.*, *Cell* **39**, 405 (1984).
17. J. E. Rothman, *Nature* **372**, 55 (1994); J. E. Rothman and F. T. Wieland, *Science* **272**, 227 (1996).
18. D. W. Wilson *et al.*, *Nature* **339**, 355 (1989).
19. C. Ungermann, B. J. Nichols, H. R. Pelham, W. Wickner, *J. Cell Biol.* **140**, 61 (1998).
20. H. R. B. Pelham, *Cell* **79**, 1125 (1994).
21. P. Cosson and F. Letourneur, *Curr. Opin. Cell Biol.* **9**, 484 (1997).
22. H. D. Love *et al.*, *J. Cell Biol.* **140**, 541 (1998).
23. P. Cosson and F. Letourner, *Science* **263**, 1629 (1994); F. Letourneur *et al.*, *Cell* **79**, 1199 (1994).
24. M. R. Jackson, T. Nilsson, P. A. Peterson, *EMBO J.* **9**, 3153 (1990).
25. L. Orci *et al.*, *Cell* **90**, 335 (1997).
26. J. Klumperman *et al.*, *J. Cell Sci.* **111**, 3411 (1998).
27. A. A. Mironov, P. Weidman, A. Luini, *J. Cell Biol.* **138**, 481 (1997).
28. M. S. Ladinsky *et al.*, *ibid.* **144**, 1135 (1999).
29. M. P. P. Grasse, *C. R. Acad. Sci. Paris* **245**, 1278 (1957); D. J. Morre, T. W. Keenan, C. N. Huang, in *Cytopharmacology of Secretion*, B. Ceccarelli, F. Clementi, J. Meldolesi, Eds. (Raven, New York, 1974), pp. 107–126.
30. S. Bannykh and W. E. Balch, *J. Cell Biol.* **138**, 1 (1997).
31. B. S. Glick, T. Elston, G. Oster, *FEBS Lett.* **414**, 177 (1997).
32. L. Bonfanti *et al.*, *Cell* **95**, 993 (1998).
33. M. G. Farquhar and G. E. Palade, *Trends Cell Biol.* **8**, 2 (1998).
34. A. Rambourg and Y. Clermont, *Eur. J. Cell Biol.* **51**, 189 (1990).
35. W. E. Balch *et al.*, *Cell* **76**, 841 (1994).
36. N. B. Cole *et al.*, *Science* **273**, 797 (1996).
37. T. Nilsson, personal communication.
38. M. H. Hoe *et al.*, *J. Biol. Chem.* **270**, 25057 (1995); S. L. Harris and G. Waters, *J. Cell Biol.* **132**, 985 (1996).
39. S. Wooding and H. R. B. Pelham, *Mol. Biol. Cell.* **9**, 2667 (1998).
40. M. Aridor *et al.*, *J. Cell Biol.* **131**, 875 (1995); T. Rowe *et al.*, *ibid.* **135**, 895 (1996); S. J. Scales, R. Pepperkok, T. E. Kreis, *Cell* **90**, 1137 (1997).
41. R. D. Klausner, J. G. Donaldson, J. Lippincott-Schwartz, *J. Cell Biol.* **116**, 1071 (1992).
42. J. B. Helms and J. E. Rothman, *Nature* **360**, 352 (1992); J. G. Donaldson, D. Finazzi, R. D. Klausner, *ibid.*, p. 350.
43. N. B. Cole *et al.*, *J. Cell Biol.* **140**, 1 (1998).
44. Z. Elazar *et al.*, *ibid.* **124**, 415 (1994).
45. J. Lippincott-Schwartz *et al.*, *Cell* **54**, 209 (1989).
46. C. Dascher and W. E. Balch, *J. Biol. Chem.* **269**, 1437 (1994).
47. B. J. Nichols and H. R. B. Pelham, *Biochim. Biophys. Acta* **1404**, 9 (1998).
48. D. K. Banfield *et al.*, *Nature* **375**, 806 (1995); J. C. Hay *et al.*, *Cell* **89**, 149 (1997).
49. T. Rowe *et al.*, *Science* **279**, 696 (1998).
50. H. R. Pelham, *Trends Cell Biol.* **8**, 45 (1998); B. S. Glick and V. Malhotra, *Cell* **95**, 883 (1998).
51. A. Wandinger-Ness, M. K. Bennett, C. Antony, K. Simons, *J. Cell Biol.* **111**, 987 (1990); E. Ikonen and K. Simons, *Semin. Cell Dev. Biol.* **9**, 503 (1998).
52. R. Le Borgne and B. Hoflack, *Curr. Opin. Cell. Biol.* **10**, 499 (1998).
53. J. S. Bonifacino *et al.*, *Proc. Assoc. Am. Physicians* **4**, 285 (1996); J. Hirst and M. S. Robinson, *Biochim. Biophys. Acta* **1404**, 173 (1998).
54. R. Le Borgne and B. Hoflack, *J. Cell Biol.* **137**, 335 (1997).
55. ———, *Biochim. Biophys. Acta* **1404**, 195 (1998).
56. E. Conibear and T. H. Stevens, *ibid.*, p. 211.
57. S. A. Tooze, *ibid.*, p. 231.
58. P. Keller and K. Simons, *J. Cell Sci.* **110**, 3001 (1997).
59. C. Yeaman, K. K. Grindstaff, W. J. Nelson, *Physiol. Rev.* **79**, 73 (1999).
60. P. A. Piepenhagen and W. J. Nelson, *Mol. Biol. Cell.* **9**, 3161 (1998).
61. J. A. Marrs *et al.*, *J. Cell Biol.* **129**, 507 (1995).
62. D. Gundersen, S. K. Powell, E. Rodriguez-Boulan, *ibid.* **121**, 335 (1993); A. D. Marmorstein *et al.*, *ibid.* **142**, 697 (1998); A. D. Marmorstein *et al.*, *Ann N.Y. Acad. Sci.* **857**, 1 (1998).
63. S. C. Finnemann *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 12932 (1997).
64. J. C. Simpson, C. Dascher, L. M. Roberts, J. M. Lord, W. E. Balch, *J. Biol. Chem.* **270**, 20078 (1995); J. M. Lord and L. M. Roberts, *J. Cell Biol.* **140**, 733 (1998).
65. K. K. Grindstaff *et al.*, *Cell* **93**, 731 (1998); S.-C. Hsu *et al.*, *Trends Cell Biol.* **9**, 150 (1999).
66. J. C. M. Holthuis, B. J. Nichols, H. R. B. Pelham, *Mol. Biol. Cell.* **9**, 3383 (1998).
67. S. M. Hurtley and A. Helenius, *Annu. Rev. Cell Biol.* **5**, 277 (1989); C. Hammond and A. Helenius, *Curr. Opin. Cell Biol.* **7**, 523 (1995).
68. M. A. De Matteis and J. S. Morrow, *Curr. Opin. Cell Biol.* **10**, 542 (1998).

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