Minireview

COP-coated vesicles in intracellular protein transport

Cordula Harter*

Institut für Biochemie I, Universität Heidelberg, Im Neuenheimer Feld 328, D-69120 Heidelberg, Germany

Received 18 May 1995

Abstract COP-coated vesicles have originally been implicated in vesicular transport between subcompartments of the Golgi complex in mammals in a *cis* to *trans* direction. More recently, a role for COP-coated vesicles in transport between the endoplasmic reticulum (ER) and Golgi in mammalian cells has been proposed. Under certain conditions COP-coats have been localized to special domains of the transitional ER and to the cis side of the Golgi complex. This led to the assumption that COP-coated vesicles are involved in export of proteins from the ER. In addition, new findings point to a function of COP-coated vesicles in back transport of proteins from the Golgi to the ER. At present it is not known whether COP-coated vesicles move only in one or in both directions between ER and Golgi.

Key words: Golgi membrane; Endoplasmic reticulum; Vesicle coat; Vesicle budding; Anterograde transport; Retrograde transport

1. Introduction

Intracellular transport between membrane-bound organelles of the endocytic and exocytic pathways is mediated by coated vesicular carriers (for reviews see [1–4]). Three types of vesicle coats have been defined on a molecular basis: (i) the clathrin/adaptin coat [5], (ii) the COP-coat [6] and (iii) the COPII-coat [7]. Clathrin-coated vesicles bud from two types of membranes: from the plasma membrane during endocytosis and from the trans-Golgi network in transport to endosomal organelles [5]. COP-coats have been localized to Golgi membranes as well as to special domains of the transitional ER in mammals [8,9]. COPII-coated vesicles can be generated from ER membranes in *S. cerevisiae* [7].

The specificity of clathrin-coated vesicles from the two types of membranes is provided by protein complexes, named adaptors, which seem to be involved in sorting and concentration of membrane proteins [5]. For non-clathrin-coated vesicles a function in sorting is less defined. Biosynthetic protein transport from the ER, through the Golgi complex and to the plasma membrane has been proposed to occur by default, i.e. without sorting and concentration steps [10,11]. Accordingly, residency of a protein in a particular organelle requires some kind of structural information in the polypeptide chain in order to be excluded from a departing transport vesicle. Proteins that do not contain a retention signal are transported in COP-coated vesicles between successive Golgi cisternae in mammals [6,12]. COP-coated vesicles have also been suggested to play a role in ER to Golgi transport both in mammals and in yeast [13,14]. However, their exact role in this transport step is presently unknown. In yeast, COPII-coated vesicles can mediate protein transport between ER and Golgi without need for COP proteins [7]. Homologues to mammalian COP-coat subunits have been identified in yeast [13,15,16]. and homologous subunits of COPII coats have been found in mammals [17,18]. The differences between COP- versus COPII-mediated transport remains to be determined (see also the review by Barlowe in this issue).

2. Golgi-derived COP-coated vesicles

COP-coated vesicles can be formed in vitro by incubation of mammalian Golgi membranes with cytosol and ATP [19,20]. These vesicles, originally termed non-clathrin coated vesicles have been characterized biochemically and by immunocytochemistry [6,21,22]. They are uniform in size, with a diameter of about 75 nm. Their fuzzy coat on the cytoplasmic surface, which is 18 nm thick, is distinct from but resembles the clathrin coat. The coating process is confined to budding regions on Golgi membranes and this was taken as a first indication that vesicle budding might be driven by the assembly of the coat from cytoplasmic precursors [6,12]. Addition of GTPγS, a non-hydrolyzable analogue of GTP, during the generation of Golgi-derived transport vesicles in vitro results in the accumulation of COP-coated vesicles. This has allowed their isolation and identification of the major protein components [21,22].

Recently an integral membrane component of COP-coated vesicles, termed p24, has been characterized [23]. This protein belongs to a family of integral membrane proteins with type I topology. All members of this family contain typical heptad repeats of hydrophobic residues in their lumenal domain that might be part of a coiled-coil structure [23]. Coiled-coil motifs have been found in several proteins involved in membrane fusion reactions [24]. It has been hypothesized that p24 of COP-coated transport vesicles might have a function in vesicle budding [23]. One might also envisage a role for p24 as a protein anchor either for the cytoplasmic coat or cargo proteins or for both. Indeed, there are indications that another member of this family, Emp24p, is involved in sorting of cargo proteins. Emp24p is a component of ER-derived COPII-coated vesicles in *S. cerevisiae* [25].

3. Coat proteins

The coat of COP-coated vesicles consists of eight polypeptides: seven of which, termed COPs (for *co*at *p*roteins), are

*Corresponding author. Fax: (49) (6221) 56-4366.
E-mail: harter@novsrv1.pio1.uni-heidelberg.de

SSDI 0014-5793(95)00621-4

pre-assembled in a cytosolic complex, named coatomer. The remainder coat component is ADP ribosylation factor (ARF), a small GTP-binding protein (Table 1) [22,26,27]. Both coatomer and ARF are mainly cytosolic with only minor amounts present on membranes. All subunits of mammalian coatomer (α-COP, β-COP, β′-COP, γ-COP, δ-COP, ε-COP and ζ-COP) have been cloned and sequenced, except α-COP. In *S. cerevisiae*, the homologues of α-, β-, β′- and γ-COP are the products of the RET1, SEC26, SEC27 and SEC21 gene, respectively (Table 1) [16,28,29]. Sequence comparison between mammalian COPs and their yeast homologues reveals a high degree of similarity suggesting a conserved function of COPs in evolution [15,16,28,30]. Mutant yeast cells defective in SEC21, SEC26 and SEC27 are defective in ER to Golgi transport [13,16]. In addition, mutants in SEC21, SEC27 and RET1 are affected in retrieval of proteins back to the ER [29].

β-COP shows some homology to β adaptin of clathrin-coated vesicles [31], while ζ-COP is related to the 17 kDa and 20 kDa subunits of the clathrin/adaptin system [32]. For δ- and ε-COP no homology has been found to any known protein (Auerbach et al., in preparation) [33]. Remarkably, α-COP [29,30] and β′-COP [16,34,35] contain in their N-terminal domains four and five WD-40 repeated motifs, respectively. WD-40 motifs are typically found in β subunits of trimeric G proteins but also in other subunits of hetero-oligomeric protein complexes (for review see [36]). The function of this motif is unknown, however. It might represent an oligomerization motif and/or mediate binding of coatomer to membrane-associated proteins. Possible binding partners of WD-40 motifs present in α- or β′-COP include G_α-subunits or ARF. Both G_α and ARF are able to bind βγ subunits of trimeric G proteins, and both are involved in the recruitment of coatomer to membranes [37,38].

Each individual COP is a constituent of the cytosolic coatomer as well as of transport vesicles as shown by immunolocalization and peptide sequence comparison [22,33,34]. Both membrane-bound and cytosolic coatomer contain each COP in an equimolar stoichiometry. ζ-COP is the only coatomer subunit that, in addition to its coatomer-associated form, is also present as a soluble monomer in the cytosol [32].

ARF was originally discovered as cofactor in Cholera toxin-catalyzed ADP-ribosylation of stimulatory G protein α-subunits [39]. It belongs to the Ras superfamily of GTPases (for review see [40]), but differs from other members of this family by the absence of a carboxyl-terminal prenylation motif and by the presence of a myristylation on an amino-terminal glycine residue, a modification essential for membrane association [41]. Membrane-associated ARF is concentrated in coated buds on Golgi membranes and is a stoichiometric component of COP-coated vesicles [8,26,42]. In yeast as well as in mammals, ARF function is essential for transport in vivo [43–45].

4. Possible functions of COP coats

Coat proteins might have several functions: (i) to act as a mechanical device to force a flat membrane into a bud, (ii) to prevent premature or random fusion and (iii) to sort and concentrate cargo.

4.1. Vesicle formation and consumption

Vesicle formation is initiated by the recruitment of ARF and coatomer from the cytosol to the Golgi membrane (Fig. 1) [46,47]. ARF binding precedes the binding of coatomer. Membrane-bound *N*-myristylated ARF contains GTP, while cytosolic ARF is in the GDP state [41]. Membrane binding is triggered by the exchange of GDP for GTP and is prevented by treatment with brefeldin A [48,49], a fungal metabolite that prevents secretion [50]. Thus, the nucleotide exchange factor is a likely target for brefeldin A. It has been suggested that the nucleotide exchange reaction induces a conformational change in ARF that results in the exposure of the amino-terminal myristic acid residue (for review see [40]). This hydrophobic tail is believed to serve as a membrane anchor of ARF-GTP. A certain population of ARF-GTP has been shown to bind to Golgi membranes in a saturable manner, indicative for the existence of a specific ARF-receptor [51]. The molecular identity of this receptor is unknown. Saturable, specific binding of ARF primes the Golgi membrane to recruit coatomer [47]. Coatomer binding initiates coat assembly thereby triggering the formation of coated buds [8,42]. In order to release a coated vesicle, membrane fission has to occur at the base of the bud. This fission event is dependent on long chain acyl-coenzyme A, such as palmitoyl-CoA [52]. Vesicle formation and transport are blocked by a non-hydrolyzable analogue of palmitoyl-CoA [52]. This might indicate that palmitoylation of a fusion protein is involved in vesicle release. Indeed, palmitoylation of a 62-kDa protein has recently been demonstrated to affect vesicular transport [53]. ARF and coatomer are the only cytosolic protein components that are required for the formation of functionally active transport vesicles from isolated Golgi membranes [54].

Once the budding is completed, the vesicle coat has to be removed in order to allow the vesicle to fuse with the target

Table 1
Coat proteins of Golgi-derived transport vesicles

Protein	M_r (Da)	Present in		Monomeric in cytosol	Homologue in *S. cerevisiae*/protein features
		Coatomer	Vesicles		
α-COP	~160,000	+	+	−	Ret1p/four WD40 repeats of β-transducin family
β-COP	107,010	+	+	−	Sec26p/homologous to β-adaptin of AP2
β′-COP	102,041	+	+	−	Sec27p/five WD40 repeats
γ-COP	97,385	+	+	−	Sec21p
δ-COP	57,109	+	+	−	−
ε-COP	34,500	+	+	−	−
ζ-COP	20,219	+	+	+	Homologous to AP19/17 of the AP1/AP2 complex
ARF	21,000	−	+	+	ADP-ribosylation factor; small GTP-binding protein

For references see text.

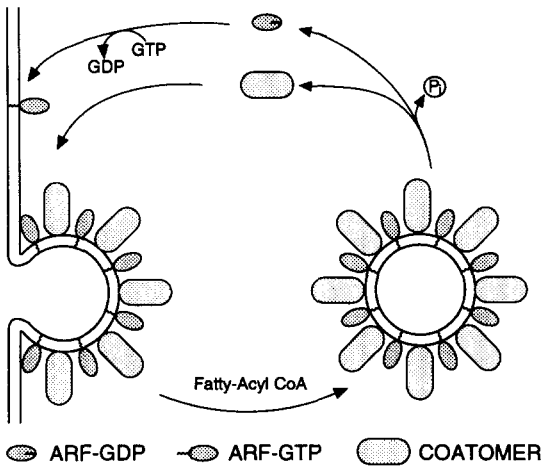

Fig. 1. Steps in the budding of a COP-coated Golgi-derived transport vesicle. Membrane-binding of ARF is triggered by the exchange of GDP for GTP. The myristic acid tail is exposed and serves as a membrane anchor for ARF-GTP. Specific ARF-binding primes the membrane to recruit coatomer. Coatomer binding initiates coat assembly, thus driving the formation of buds. A coated vesicle is released by a process that requires fatty acyl coenzyme A. The coat disassembles following hydrolysis of GTP. Coat subunits can be re-used for another cycle of coated vesicle formation. Model based on [54].

membrane. This uncoating reaction depends on hydrolysis of GTP bound to ARF [55]. Conditions which prevent GTP hydrolysis (either by generating coated vesicles in the presence of GTPγS or in cells that are transfected with a GTPase mutant of ARF) result in the accumulation of coated vesicles that are incompetent for fusion with the acceptor membrane [44,45,55]. Uncoating might be considered as a reversal of coat assembly: when GTP is hydrolyzed, ARF undergoes a conformational change that results in retraction of the myristic acid tail and release of ARF-GDP into the cytosol followed by the release of coatomer (Fig. 1). This cycle allows the protein coat to serve as a device to prevent direct fusion between Golgi subcompartments [56].

4.2. Sorting and concentration

COP-coated vesicles generated from Golgi membranes contain cargo proteins in concentrations similar to those found in the parental Golgi membrane. This was taken as an indication that Golgi-derived COP-coated vesicles are bulk carriers [6,12]. However, for export from the ER the situation is less clear. At least for some proteins a concentration step seems to occur during export from the ER, possibly in COP-coated vesicles [57,58]. A net concentration of cargo could be explained by an interaction of cargo proteins with components of the cytoplasmic coat. In the case of membrane proteins, concentration and sorting via their cytoplasmic tail similar to receptor concentration in endocytosis might be considered [59]. For secretory (lumenal) proteins, concentration and sorting could occur through transmembrane proteins, as has been suggested for Emp24p, a component of COPII-coated vesicles [25]. However, no direct evidence exists for a cargo-coat interaction in COP-coated vesicles. Concentration might also be obtained by the sequestration of proteins to distinct domains of an organelle: proteins to be retained form a matrix in their residential organelle similar to what has been postulated for the Golgi [60], whereas proteins destined for export diffuse to specialized domains that are competent for budding and are trapped by a coated structure (Fig. 2). Specific elements of the transitional ER have been shown to be enriched in coatomer and thus might represent such exit sites [9]. Interestingly, these domains can be distinguished from areas containing a component of COPII coats (Sec23p) [17]. Identification and quantitation of the cargo of COP-coated versus COPII-coated vesicles should provide an answer as to their specificity.

4.3. Retrograde transport

Recent findings suggest a role for COP-coated vesicles in retrograde transport from the Golgi complex to the ER (Fig. 2). First, coatomer has been shown to bind to a di-lysine motif (KKXX) present in ER-resident membrane proteins [61] and known to serve as a retrieval signal [62]. Second, a yeast mutant defective in the gene for α-COP is deficient in the retrieval of proteins containing a di-lysine motif to the ER [29]. Possibly, di-lysine tagged membrane proteins are sorted into retrograde moving COP-coated vesicles. Isolation of those putative retrograde carriers would confirm a role of coatomer in retrograde trafficking.

5. Conclusion

The reconstitution of distinct intracellular transport events in cell free systems allowed the identification of components that are required for specific transport steps. In the case of coated vesicle formation, a general mechanism seems to apply for distinct vesicle types in various cells (from yeast to human): a small GTP-binding protein plays a key role in the recruitment of coat components that exist in the cytosol as a preformed oligomeric complex. Coat assembly then drives bud formation, and finally a coated vesicle is released. Hydrolysis of a guanosine triphosphate bound to the small GTP-binding protein delivers the energy required for uncoating. Coat proteins are released into the cytoplasm and are re-used for another round of vesicle formation. For COP-coated vesicles specific questions

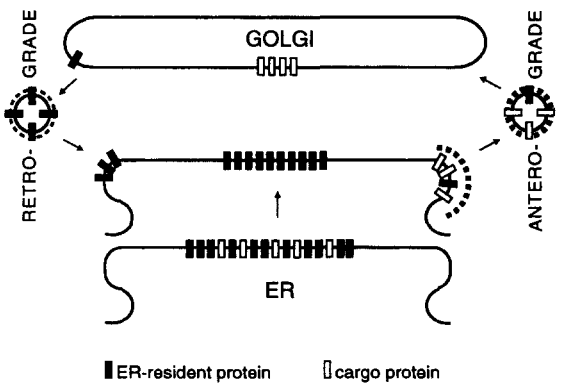

Fig. 2. Model for the anterograde and retrograde ER-Golgi transport of membrane proteins by COP-coated vesicles. In the ER proteins destined for export are sequestrated to budding competent exit sites. Anterograde transport occurs without an active concentration step leaving behind resident proteins of a certain organelle. Proteins that escaped retention are transported in retrograde moving vesicles.

remain to be answered. For example, what is the identity of the nucleotide exchange factor for ARF, the likely target for brefeldin A, and which molecule serves as a putative coatomer receptor? Is coatomer present on retrograde moving transport vesicles and if so, what regulates anterograde versus retrograde transport? A combination of experimental systems – varying from yeast genetics to cell free systems – should allow to generate various COP-coated vesicle populations and to characterize their individual function.

Acknowledgements: I thank Walter Nickel and Bernd Helms for critical comments on the manuscript, Jochen Pavel for help with the figures, and the members of the lab for fruitful discussions. Financial support was obtained from the DFG (SFB 352).

References

[1] Pearse, B.M.F. and Bretscher, M.S. (1981) Annu. Rev. Biochem. 50, 85–101.
[2] Pryer, N.K., Wuestehube, L.J. and Schekman, R. (1992) Annu. Rev. Biochem. 61, 471–516.
[3] Rothman, J.E. and Orci, L. (1992) Nature 355, 409–415.
[4] Rothman, J.E. (1994) Nature 372, 55–63.
[5] Pearse, M.F. and Robinson, M.S. (1990) Annu. Rev. Cell Biol. 6, 151–171.
[6] Orci, L., Glick, B.S. and Rothman, J.E. (1986) Cell 46, 171–184.
[7] Barlowe, C., Orci, L., Yeung, T., Hosobuchi, M., Hamamoto, S., Salama, N., Rexach, M.F., Ravazzola, M., Amherdt, M. and Schekman, R. (1994) Cell 77, 895–908.
[8] Orci, L., Palmer, D.J., Ravazzola, M., Perrelet, A., Amherdt, M. and Rothman, J.E. (1993a) Nature 362, 648–652.
[9] Orci, L., Perrelet, A., Ravazzola, M., Amherdt, M., Rothman, J.E. and Schekman, R. (1994) Proc. Natl. Acad. Sci. USA 91, 11924–11928.
[10] Pfeffer, S.R. and Rothman, J.E. (1987) Annu. Rev. Biochem. 56, 829–852.
[11] Wieland, F.T., Gleason, M.L., Serafini, T.A. and Rothman, J.E. (1987) Cell 50, 289–300.
[12] Orci, L., Malhotra, V., Amherdt, M., Serafini, T. and Rothman, J.E. (1989) Cell 56, 357–368.
[13] Hosobuchi, M., Kreis, T. and Schekman, R. (1992) Nature 360, 603–605.
[14] Pepperkok, R., Scheel, J., Horstmann, H., Hauri, H.P., Griffiths, G. and Kreis, T.E. (1993) Cell 74, 71–82.
[15] Harter, C., Draken, E., Lottspeich, F. and Wieland, F.T. (1993) FEBS Lett. 332, 71–73.
[16] Duden, R., Hosobuchi, M., Hamamoto, S., Winey, M., Byers, B. and Schekman, R. (1994) J. Biol. Chem. 269, 24486–24495.
[17] Orci, L., Ravazzola, M., Meda, P., Holcomb, C., Moore, H.P., Hicke, L. and Schekman, R. (1991) Proc. Natl. Acad. Sci. USA 88, 8611–8615.
[18] Shaywitz, D.A., Orci, L., Ravazzola, M., Swaroop, A. and Kaiser, C. A. (1995) J. Cell Biol. 128, 769–778.
[19] Balch, W.E., Dunphy, W.G., Braell, W.A. and Rothman, J.E. (1984) Cell 39, 405–416.
[20] Balch, W.E., Glick, B.S. and Rothman, J.E. (1984) Cell 39, 525–536.
[21] Malhotra, V., Serafini, T., Orci, L., Shepherd, J.C. and Rothman, J. E. (1989) Cell 58, 329–336.
[22] Serafini, T., Stenbeck, G., Brecht, A., Lottspeich, F., Orci, L., Rothman, J.E. and Wieland, F.T. (1991) Nature 349, 215–220.
[23] Stamnes, M.A., Craighead, M.W., Hoe, M.H., Lampen, N., Geromanos, S., Tempst, P. and Rothman, J.E. (1995) J. Cell Biol., submitted.
[24] Oas, T.G. and Endow, S.A. (1994) Trends Biochem. Sci. 19, 51–54.
[25] Schimmöller, F., Singer-Krüger, B., Schröder, S., Krüger, U.C.B. and Riezman, H. (1995) EMBO J. 14, 1329–1339.
[26] Serafini, T., Orci, L., Amherdt, M., Brunner, M., Kahn, R.A. and Rothman, J.E. (1991b) Cell 67, 239–253.
[27] Waters, M.G., Serafini, T. and Rothman, J.E. (1991) Nature 349, 248–251.
[28] Stenbeck, G., Schreiner, R., Herrmann, D., Auerbach, S., Lottspeich, F., Rothman, J.E. and Wieland, F.T. (1992) FEBS Lett. 314, 195–198.
[29] Letourneur, F., Gaynor, E.C., Hennecke, S., Demolliere, C., Duden, R., Emr, S.D., Riezman, H. and Cosson, P. (1994) Cell 79, 1199–1207.
[30] Gerich, B., Orci, L., Tschochner, H., Lottspeich, F., Ravazzola, M., Amherdt, M., Wieland, F. and Harter, C. (1995) Proc. Natl. Acad. Sci. USA 92, 3229–3233.
[31] Duden, R., Griffiths, G., Frank, R., Argos, P. and Kreis, T.E. (1991) Cell 64, 649–665.
[32] Kuge, O., Hara, K.S., Orci, L., Ravazzola, M., Amherdt, M., Tanigawa, G., Wieland, F.T. and Rothman, J.E. (1993) J. Cell Biol. 123, 1727–1734.
[33] Hara-Kuge, S., Kuge, O., Orci, L., Amherdt, M., Ravazzola, M., Wieland, F.T. and Rothman, J.E. (1994) J. Cell Biol. 124, 883–892.
[34] Stenbeck, G., Harter, C., Brecht, A., Herrmann, D., Lottspeich, F., Orci, L. and Wieland, F.T. (1993) EMBO J. 12, 2841–2845.
[35] Harrison-Lavoie, K.J., Lewis, V.A., Hynes, G.M., Collison, K.S., Nutland, E. and Willison, K.R. (1993) EMBO J. 12, 2847–2853.
[36] Neer, E.J., Schmidt, C.J., Nambudripad, R. and Smith, T.F. (1994) Nature 371, 297–300.
[37] Franco, M., Paris, S. and Chabre, M. (1995) FEBS Lett. 362, 286–290.
[38] Donaldson, J.G., Kahn, R.A., Lippincott, S.J. and Klausner, R.D. (1991) Science 254, 1197–1199.
[39] Kahn, R.A. and Gilman, A.G. (1986) J. Biol. Chem. 261, 7906–7911.
[40] Nuoffer, C. and Balch, W.E. (1994) Annu. Rev. Biochem. 63, 949–990.
[41] Kahn, R.A., Goddard, C. and Newkirk, M. (1988) J. Biol. Chem. 263, 8282–8287.
[42] Orci, L., Palmer, D.J., Amherdt, M. and Rothman, J.E. (1993) Nature 364, 732–734.
[43] Stearns, T., Willingham, M.C., Botstein, D. and Kahn, R.A. (1990) Proc. Natl. Acad. Sci. USA 87, 1238–1242.
[44] Dascher, C. and Balch, W.E. (1994) J. Biol. Chem. 269, 1437–1448.
[45] Zhang, C.J., Rosenwald, A.G., Willingham, M.C., Skuntz, S., Clark, J. and Kahn, R.A. (1994) J. Cell Biol. 124, 289–300.
[46] Donaldson, J.G., Cassel, D., Kahn, R.A. and Klausner, R.D. (1992) Proc. Natl. Acad. Sci. USA 89, 6408–6412.
[47] Palmer, D.J., Helms, J.B., Beckers, C.J., Orci, L. and Rothman, J. E. (1993) J. Biol. Chem. 268, 12083–12089.
[48] Donaldson, J.G., Finazzi, D. and Klausner, R.D. (1992) Nature 360, 350–352.
[49] Helms, J.B. and Rothman, J.E. (1992) Nature 360, 352–354.
[50] Misumi, Y., Miki, A., Takatsuki, A., Tamura, G. and Ikehara, Y. (1986) J. Biol. Chem. 261, 11398–11403.
[51] Helms, J.B., Palmer, D.J. and Rothman, J.E. (1993) J. Cell Biol. 121, 751–760.
[52] Pfanner, N., Orci, L., Glick, B.S., Amherdt, M., Arden, S.R., Malhotra, V. and Rothman, J.E. (1989) Cell 59, 95–102.
[53] Mundy, D.I. and Warren, G. (1992) J. Cell Biol. 116, 135–146.
[54] Ostermann, J., Orci, L., Tani, K., Amherdt, M., Ravazzola, M., Elazar, Z. and Rothman, J.E. (1993) Cell 75, 1015–1025.
[55] Tanigawa, G., Orci, L., Amherdt, M., Ravazzola, M., Helms, J.B. and Rothman, J.E. (1993) J. Cell Biol. 123, 1365–1371.
[56] Elazar, Z., Orci, L., Ostermann, J., Amherdt, M., Tanigawa, G. and Rothman, J.E. (1994) J. Cell Biol. 124, 415–424.
[57] Balch, W.E., McCaffery, J.M., Plutner, H. and Farquhar, M.G. (1994) Cell 76, 841–852.
[58] Balch, W.E. and Farquhar, M.G. (1995) Trends Cell Biol. 5, 16–19.
[59] Trowbridge, I.S., Collawn, J.F. and Hopkins, C.R. (1993) Annu. Rev. Cell. Biol. 9, 129–161.
[60] Nilsson, T. and Warren, G. (1994) Curr. Opin. Cell Biol. 6, 517–521.
[61] Cosson, P. and Letourneur, F. (1994) Science 263, 1629–1631.
[62] Nilsson, T., Jackson, M. and Peterson, P.A. (1989) Cell 58, 707–718.