MICROBIOLOGICAL REVIEWS, Dec. 1996, p. 697–721 Vol. 60, No. 4
0146-0749/96/$04.00+0

The Secretory Pathway of Protists: Spatial and Functional Organization and Evolution

BURKHARD BECKER* AND MICHAEL MELKONIAN

Botanisches Institut, Universität zu Köln, D-50931 Cologne, Germany

INTRODUCTION

Cells interact with their environment in various ways. They secrete a great variety of molecules to modify their environment, to protect themselves by some type of extracellular matrix or cell wall, or to interact with other cells. Whereas smaller molecules are usually transported across the plasma membrane by carriers located within the membrane, the majority of secreted proteins and polysaccharides in eukaryotes are released into the medium by exocytosis. In prokaryotes, secretion of proteins is directly mediated by the plasma membrane via either signal-recognition-particle-dependent translocation which is homologous to protein translocation into the endoplasmic reticulum (ER) in eukaryotic cells (331) or the prokaryote-specific *sec* system (277). In contrast, eukaryotic cells have evolved a complex secretory pathway, consisting of several membrane-bound compartments which contain different sets of proteins (endoplasmic reticulum, Golgi apparatus, vacuoles, vesicles, collectively termed the endomembrane system) (Fig. 1). Upon insertion into the endoplasmic reticulum, proteins travel through this membrane system to reach subsequent compartments and their final destination. At various steps, carbohydrates and lipids enter this pathway. The secretory pathway (anterograde or biosynthetic pathway) is countered by an endocytotic pathway (retrograde pathway) originating at the plasma membrane, with the major destination being lysosomes (or vacuoles). The two pathways are interconnected by crossroads at various steps. Thus, eukaryotic cells have evolved a complex intracellular traffic system, in which vesicular shuttles are believed to be the major transport vehicles.

New experimental strategies involving mainly the mammalian or yeast system have led to the discovery of several pro-

* Corresponding author. Mailing address: Botanisches Institut, Lehrstuhl I, Universität zu Köln, D-50923 Cologne, Germany. Phone: 49 221 4703795. Fax: 49 221 4705181. Electronic mail address: bbecker@biolan.uni-koeln.de.

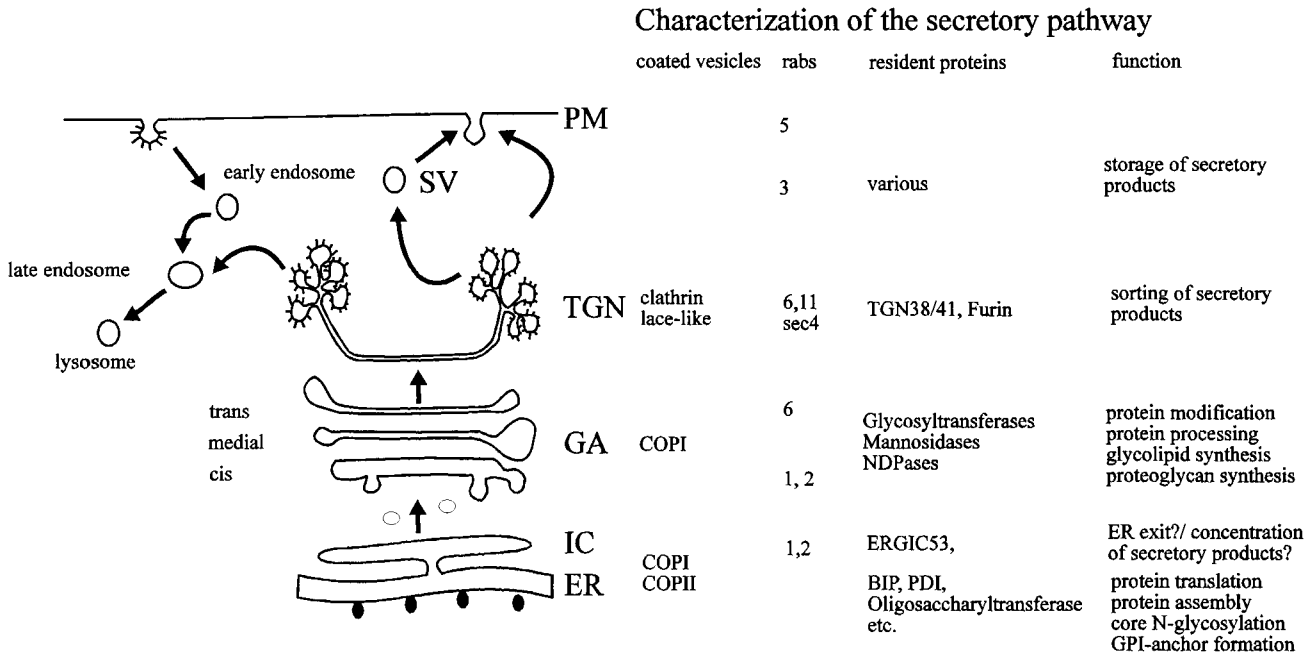


FIG. 1. Schematic representation of the endomembrane system of a mammalian cell. IC, intermediate compartment; NDPases nucleotide diphosphatases; TGN *trans*-Golgi network; SV secretory vesicle; rabs, G-proteins belonging to the ras-superfamily first detected in rat brain; sec, yeast secretion genes; TGN38/41, resident *trans*-Golgi network proteins of 38 and 41 kDa, respectively; ERGIC53, 53-kDa protein of the ER Golgi intermediate compartment; BiP, binding protein (ER protein of the 70-kDa heat shock protein family); PDI, protein disulfide isomerase. See the text for details.

teins involved in the function of this complex traffic system. The use of a large number of mutants with mutations of intracellular traffic in *Saccharomyces cerevisiae*, the development of in vitro assays for individual transport steps, and the isolation of vesicular intermediates have led to the identification of proteins involved in the secretory pathway, and every month new proteins are added (see the *Guidebook to the Secretory Pathway* [267] for the currently most complete list of proteins). Some general principles of the secretory (and endocytotic) pathway have become clear (recently summarized in reference 268). Most components of the secretory pathway are conserved between yeast and mammalian cells, and many reviews have summarized these results (see, e.g., references 9, 67, 157, 169, 227, 267, 268, 300, and 303). However, considering the evolu-

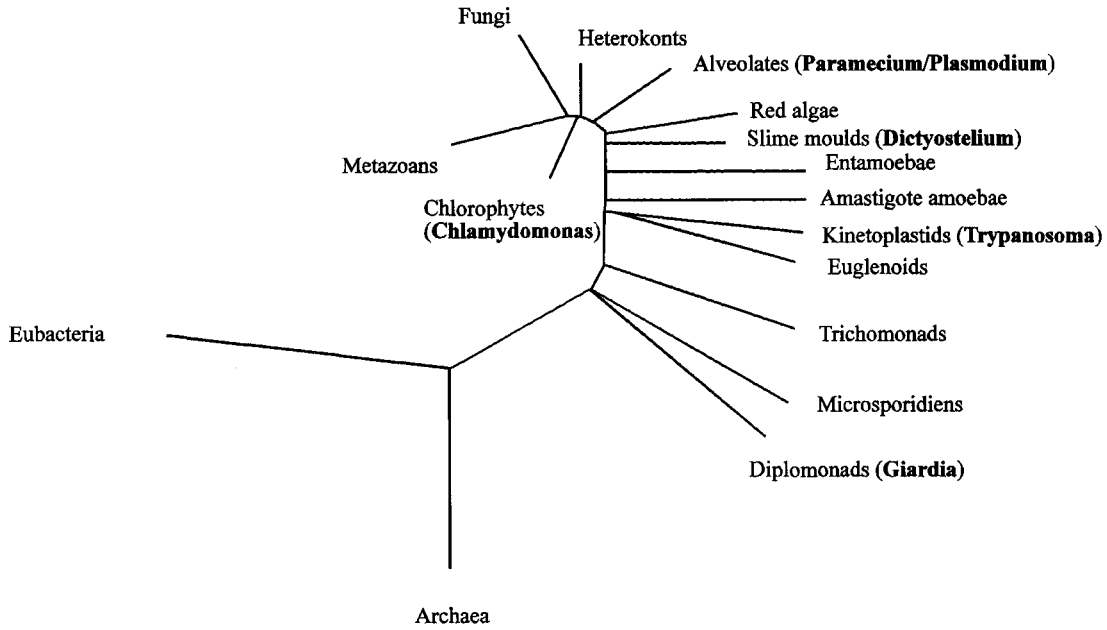


FIG. 2. Evolution of eukaryotes as deduced from complete 18S rRNA sequences. In this unrooted distance matrix tree, the evolutionary distance between the various groups is represented by the length of the branches. Modified from reference 286.

tionary relationships between eukaryotes (Fig. 2), this is not a real surprise. In the phylogenetic tree of life as based on small-subunit rRNA sequence comparisons, fungi and animals evolved late and are presumably sister groups (294). Whether the structure and function of the endomembrane system are conserved in lower eukaryotes (protists) is less well known, although many lower eukaryotes offer unique model systems to study the secretory pathway. Several reviews on specialized aspects of the secretory pathway of protists are available (see, e.g., references 250 and 326), but a comparison of these "simpler" systems with each other and with the mammalian or yeast system is still lacking. Such a comparison should help to identify conserved or derived elements of the secretory pathway and to understand the complex evolutionary history of intracellular traffic.

In this review, we will briefly summarize the principal aspects of the mammalian and yeast secretory system and then concentrate on five groups of protists, which represent major lines of eukaryotic cell evolution. We consider first the diplo- and trichomonads (two of the earliest lines of eukaryotic cell evolution [Fig. 2]), then the kinetoplastids, the slime mold *Dictyostelium*, and finally two lineages within the "crown" of eukaryote cell evolution, alveolates (ciliates and apicomplexans) and green algae. For comparison and as an introduction to the secretory pathway, the mammalian and yeast system will be discussed first. Finally, we present a hypothesis of how such a complex system of intracellular traffic might have evolved.

MAMMALIAN AND YEAST SYSTEM

Spatial Organization

A schematic representation of the spatial organization of the secretory and endocytotic pathway in a mammalian cell is shown in Fig. 1. Proteins are directed into the secretory pathway by an amino-terminal leader sequence (signal peptide) which is recognized by the signal recognition particle. After binding of the signal recognition particle-ribosome complex to the ER membrane, translation continues and the nascent polypeptide is cotranslationally translocated through the membrane (291), a process which we will not discuss further in this review because only few comparative data exist for protists. In the lumen of the ER, a specific endoprotease removes the signal peptide, and protein assembly (folding and formation of disulfide bridges) and core N glycosylation take place (125). After assembly, proteins leave the ER in vesicles and are transported to the Golgi apparatus (GA), where further modification (N-linked glycan processing, O glycosylation, phosphorylation, sulfation, and proteolytic cleavage) and sorting of proteins to their various destinations occur (110). Whether exit from the ER is by simple bulk flow or requires positive sorting information is presently controversial (13, 139, 292). The GA in mammalian cells comprises several stacks of three to five cisternae each, cisternae of different stacks being interconnected by membranous tubules (257). On the basis of immunolocalization of resident Golgi proteins, three different subcompartments (*cis* = forming face, *medial* and *trans* = secretory face) were proposed (94, 266). However, the discovery of the *trans*-Golgi network, the *cis*-Golgi network, which might be identical to the later-proposed intermediate compartment, and the overlapping distribution of enzymes of different subcompartments (see, e.g., references 224, 255, and 321) have caused confusion about the exact number of subcompartments and the boundary between the well-defined ER and the GA (141). Rothman and Orci (269), for example, suggested that the GA comprises all subcompartments in the secretory pathway between the ER and the plasma membrane (PM). Krijnse-Locker et al., however, recently proposed (170) that a true GA starts where the first enzymes of N-glycosidic carbohydrate processing (a typical Golgi event) can be detected. Using a mouse hepatitis virus system, these authors detected such enzymes from the second *cis*-most GA cisterna onwards. Thus, in their opinion, the first cisterna is part of the intermediate compartment and does not belong to the GA.

At the *trans* side of the GA, products are transported in different types of vesicles to the lysosomes (clathrin coated [67, 169]) or to the plasma membrane (probably involving the newly discovered lace-like coated vesicles [178]).

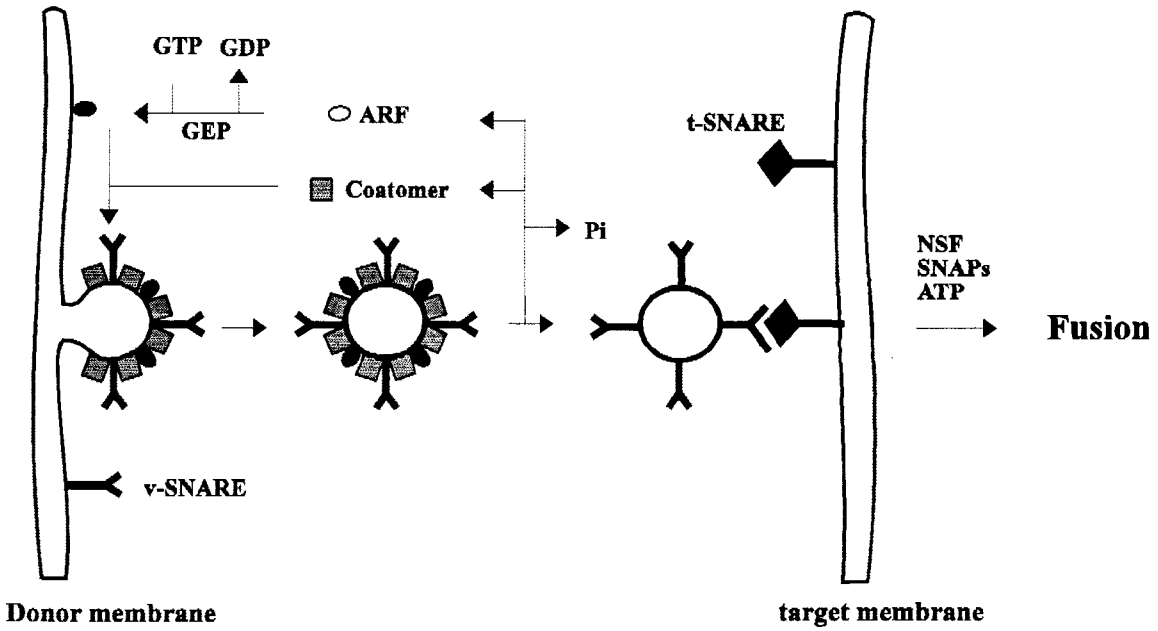
The secretory pathway in yeasts comprises basically the same components as in mammalian cells. However, in *Saccharomyces cerevisiae* (as in other true fungi [158]), no stacks of GA cisternae are observed but, instead, the Golgi apparatus consists of several discrete tubular elements, which are dispersed throughout the cytoplasm (258, 259). Therefore, the number of subcompartments cannot be easily analyzed by immunolocalization and is therefore based mainly on transport assays with mutants (see, e.g., references 136 and 322).

Functional Organization

Vesicles are generally assumed to be the transport vehicles for macromolecules between the different compartments of the endomembrane system. They are formed as coated vesicles, which can fuse with their target membrane after removal of the protein coat (Fig. 3) (summarized in reference 268). The localization of the various types of coats in the secretory pathway is indicated in Fig. 1.

An in vitro intra-Golgi transport assay (12) was used to find the first non-clathrin-coated vesicle type (now called COPI-coated vesicles). Further work suggested that this vesicle type is involved in anterograde and retrograde transport between the ER and Golgi and between different Golgi subcompartments (summarized in reference 268). Recently, a different type of coated vesicle (COPII) which is involved in ER-to-Golgi transport was discovered (summarized in reference 273), and the role of COPI in anterograde transport has been questioned (24; see references 10, 245, and 273 for detailed discussions). Transport from the GA to the various destinations occurs by clathrin-coated vesicles (242) or lace-like-coated vesicles (178).

Formation of vesicles. The formation of Golgi-derived coated vesicles (COPI) in vitro depends on the presence of seven "coat" proteins (which together form a high-molecular-weight complex called coatomer), adenosyl-ribosylation factor (ARF), and ATP (Fig. 3) (232, 233). Sequence information has been obtained for six of the seven different COP proteins (see reference 273 for a summary), and the homologous proteins of *S. cerevisiae* are known for several COP proteins (273). Binding of COP proteins to the membrane and vesicle formation are regulated by the GTP-binding protein ARF (280, 305). Again, ARF-homologous proteins were found in *S. cerevisiae* (166). ARF is present in a membrane-bound form and a cytosolic form. After exchange of GDP with GTP, ARF associates with the membrane and induces vesicle formation (151). The ARF-specific GDP-GTP exchange protein is presumably the target of brefeldin A, a fungal toxin which blocks secretion (91, 152, 234). Vesicle budding is regulated by the modulation of ARF activity, involving trimeric G-proteins (92) and protein kinase C (74). Budding of clathrin- or COPII-coated vesicles is similar to the formation of COPI-coated vesicles (273, 298, 312). A GTP-binding protein is always involved and induces the binding of the protein coat to the membrane.



Transport step	Protein coat	G-protein	GDP/GTP Exchange protein (GEP)	v-SNARE	t-SNARE
ER → Golgi	COPII	SAR	SEC12	BOS1 SEC22 BET1	SED5
intra-Golgi (retrograde)	COPI?	ARF?	?	SFT1	SED5
Golgi → PM	clathrin	ARF	?	SNC1 SNC2	SSO1 SSO2

FIG. 3. Outline of a vesicular transport step between different compartments. Coat assembly is dependent on a small GTP-binding protein like ARF or SAR (secretion-associated and ras-superfamily-related gene 1), which initiates vesicle budding. The assembled coated vesicle includes a compartment-specific v-SNARE (vesicular SNARE). After uncoating, the v-SNARE interacts specifically with a target membrane-specific t-SNARE (target SNARE) and both with additional cytosolic factors to form a fusion complex, which initiates membrane fusion. For references to the various factors, see the text.

Fusion of vesicles. After vesicles have been formed, they are transported to the target membrane, a process which at least in some cases involves active transport on cytoskeletal elements by using motor proteins like kinesin, cytoplasmic dynein, or myosin (summarized in references 5 and 61). Prior to fusion, vesicles are uncoated, which is probably induced by hydrolysis of GTP in ARF. Also, other protein factors, such as hsp70 and auxilin in the case of clathrin-coated vesicles, can be involved (317). After docking of the vesicles at the target membrane, a fusion complex is formed, which initiates membrane fusion by using ATP and acyl coenzyme A (268). The fusion complex consists of the *N*-ethylmaleimide-sensitive fusion (NSF) protein (28, 199, 330), soluble NSF attachment proteins (SNAPs) (132), and membrane proteins called SNAP receptors (SNAREs) (295). According to a widely accepted hypothesis, NSF and SNAPs function as general membrane traffic factors (although their exact role in the fusion process is still unclear [reviewed in reference 328]), whereas the specificity of vesicle targeting depends on the SNAREs (Fig. 3) (the SNARE hypothesis is discussed in reference 295). A central postulate of the SNARE hypothesis is the presence of vesicular membrane proteins (v-SNAREs) that interact with corresponding membrane proteins on the target membrane (t-SNAREs) (30, 268, 295, 300, 303). So far, v- and t-SNAREs have been identified for ER-to-GA transport in yeasts (30), retrograde intra-Golgi transport in yeasts (15) and Golgi-to-plasma membrane transport in yeast and mammalian cells (30, 114) (Fig. 3). The fusion process is regulated by various proteins including small G-proteins of the rab family (30).

Recent results indicate that not all membrane fusion events (e.g., apical transport in mammalian epithelia) depend on rab-NSF-SNAP-SNARE-mediated membrane fusion (162). An NSF-related ATPase (p97) has been shown to be involved in some NSF-independent membrane fusion events (181, 256).

Role of G-proteins. G-proteins are believed to regulate the secretory pathway (210, 247). They include proteins of the ras superfamily (ARF and rab proteins) as well as trimeric G-proteins. The best-characterized family of G-proteins are the rab proteins, with of about 30 members (227). The different rab proteins are specifically associated with certain membranes of the endomembrane system (Fig. 1) (57). Binding of rab proteins to the membrane is mediated by isoprenoids covalently bound to the protein. Isoprenoids are added by specific isoprenyltransferases, and the hypervariable C-terminal domain of the rab proteins is responsible for specific membrane recognition (31, 56). Every G-protein has its own set of activating, inhibiting, or nucleotide exchange-stimulating factors, and the first proteins acting on rab proteins have already been characterized (e.g., rab GDP dissociation inhibitor [100, 316; for reviews, see references 33 and 334). rab proteins cycle between a membrane-attached and a cytosolic form. Detachment of rab proteins from membranes involves binding to a protein called guanosine diphosphate dissociation inhibitor (334). It has been proposed that detachment of rab proteins from the membrane (i.e., cycling between a membrane-bound and cytosolic form) is required for proper function of vesicular protein transport. However, recent results on Ypt1 and Sec4 proteins (rab homologs of *S. cerevisiae*) indicate that detachment of rab proteins is not required for their function (238).

Other components needed for vesicular transport in the endomembrane system include calcium ions and protein phosphatases (27, 71).

Protein targeting. It is generally believed that proteins containing no targeting signal in their sequence follow the default pathway, which in mammalian cells leads to secretion of proteins. Retention in (or targeting to) a certain organelle requires positive sorting information in proteins. The best-analyzed organelle in this respect is the ER. In yeast and in mammalian cells, retention is based mainly on retrieval of resident ER proteins from the GA back to the ER (237). However, for some proteins such as calreticulin, a retention mechanism also contributes to ER localization (296). Several amino acid motifs allowing the retrieval of proteins have been identified (e.g., KDEL, KXKXX, or KKXX in the one-letter code for amino acids, where X is any amino acid [see reference 245 for a summary]). Recently, another tyrosine-containing motif, similar to the clathrin-dependent endocytosis signal, was found (200). ER proteins are retrieved from the GA within COPI-coated vesicles (187). Retention of luminal ER-proteins is mediated by a receptor-based salvage mechanism, whereby KDEL-bearing proteins are retrieved from a post-ER compartment by recycling a membrane-bound receptor (KDEL receptor, *erd2* gene product [245]). Membrane proteins of the ER behave in much the same way, using retrieval signals like a C-terminal KKXX motif, which interacts directly with coat proteins of COPI-coated vesicles (187). In *S. cerevisiae*, a second system, independent of a KDEL receptor, seems to be involved in the retrieval of resident ER proteins (29).

In contrast, the transmembrane sequences of several resident Golgi enzymes contain structural motifs which are believed to function as retention signals (134), although some exceptions have been reported (see, e.g., references 195 and 278). The transmembrane domain (all resident Golgi proteins sequenced so far are type II membrane proteins) requires nonspecific short cytoplasmic and luminal sequences for its function (134). Golgi proteins form heterooligomers (223), and it has been proposed that this prevents inclusion in vesicular transport intermediates (225), although this model cannot explain all the observations on Golgi retention (see references 134 and 218 for detailed discussions). However, recent studies indicate that not all proteins are targeted to the GA by a retention mechanism. Two *cis*-Golgi proteins of *S. cerevisiae* are localized by recycling (148, 278). Similar results have also been reported for some mammalian Golgi proteins (60, 154). Golgi localization of yeast Emp47p depends on its C-terminal dilysine motif, and the protein is recycled through the ER (278). Another hypothesis emphasizes the importance of protein-lipid interaction for Golgi retention and protein retrieval (37, 197, 218; see also the section on evolution of the secretory pathway below). Heterologous expression of sialyltransferase in *S. cerevisiae* demonstrated that Golgi retention signals are conserved between yeast and mammalian cells (279).

Residence of proteins specific for the *trans*-Golgi network seems to depend on retrieval from the plasma membrane. Proteins carrying a tyrosine-containing retention motif are retrieved with clathrin-coated vesicles (reviewed in references 196 and 329 for *S. cerevisiae*). Interaction of these retention motifs with the medium chains μ1 and μ2 of the two clathrin-associated protein complexes AP1 and AP2 was recently demonstrated (231).

Proteins targeted for lysosomes are synthesized at the rough ER. As they travel through the secretory pathway, the oligosaccharide side chains are modified by phosphorylation of mannose sugars, and the latter are subsequently recognized by the mannose-6-phosphate receptors (MPR) (169). Binding of lysosomal enzymes by MPR occurs in the *trans*-Golgi network. Analogous to receptor-mediated endocytosis, MPR-lysosomal enzyme complexes associate with clathrin and bud from the membrane as clathrin-coated vesicles (67, 191). In contrast to mammalian cells, *S. cerevisiae* apparently lacks MPRs, and so vacuolar protein targeting proceeds along an MPR-independent pathway (summarized in references 62 and 157). This MPR-independent pathway may additionally exist in mammalian cells (67, 169). Although mammals and *S. cerevisiae* use different receptors for sorting of lysosomal and vacuolar proteins, recent results indicate that the underlying mechanisms are similar (62), in that they both require phosphatidylinositol-3-kinase and clathrin-coated vesicles for delivery of lysosomal and vacuolar proteins (42, 70, 169, 242).

SECRETORY PATHWAY OF PROTISTS

Diplo- and Trichomonads

Diplo- and trichomonads are mainly parasitic flagellates which lack mitochondria and peroxisomes (119). In trichomonads, electron microscopic examination revealed the presence of a well-developed secretory pathway (e.g., GA) (98). In contrast, in the diplomonad *Giardia lamblia* (trophozoites and cysts), no GA, coated vesicles, or typical lysosomes had been observed (111, 119, 286). However, recent studies demonstrated the presence of a secretory pathway (206; see below for details). Peripheral vacuoles underlying the plasma membrane (Fig. 4) were shown to perform some lysosomal functions, since they took up exogenous ferritin and contained acid phosphatase (32, 111), DNase, RNase, and thiol-dependent and -independent proteinases (189).

Cyst formation. *G. lamblia* exhibits a two-stage life history. The flagellate trophozoites colonize the upper small intestine of mammals, where they attach to the intestinal epithelium via a specialized organelle, the adhesive disk (2). Some trophozoites are induced to encyst, and mature cysts are secreted in the feces and can infect new individuals after ingestion and excystment. The cysts are surrounded by a thick cell wall consisting of polysaccharides containing glucose, *N*-acetylgalactosamine,

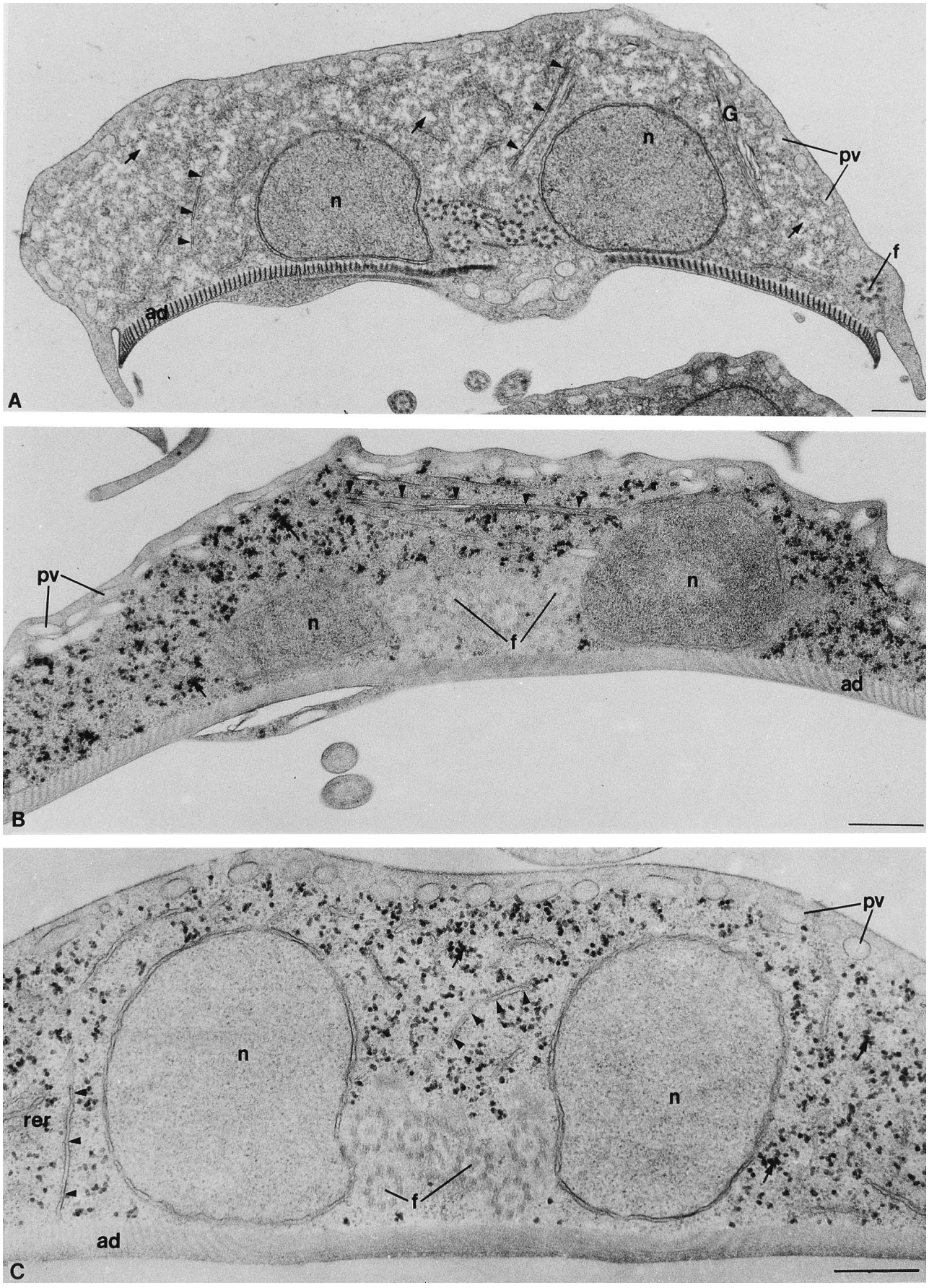

A
n
n
G
pv
f
ad
B
pv
n
n
f
ad
C
pv
n
n
rer
f
ad

FIG. 4. Cross sections through *G. lamblia* trophozoites showing the endomembrane system. (A) Conventional electron micrograph shows cross sections of peripheral vesicles (pv), flagella (f), adhesive disk (ad), and nuclei (n), as well as numerous endomembranes (arrowheads) traversing the cytoplasm. G refers to the Golgi-like perinuclear membrane stack, and the small arrows point to the negative images left by the numerous glycogen granules in the cytoplasm. (B) A similar section stained with KFeCN, showing the same structures, except that the glycogen granules are darkly stained and the major membrane cisternae (arrowheads) are parallel to the dorsal surface of the cell. (C) Similar section stained with $KMnO_4$. Rough ER (rer, arrowheads) can be seen. The glycogen granules are also darkly stained. The peripheral vesicles tend to delineate a cortical area of the cytoplasm that lacks the ribosomes and the glycogen granules of the main part of the cytoplasm. Bars, 0.5 μm. Reproduced from reference 206 with permission of Academic Press.

and a few proteins (201; see reference 2 for a summary of the cyst wall and encystation process). Ultrastructural studies (112, 119) have focused mainly on trophozoites, since experimental induction of encystation has only recently become possible (131). In these ex situ encysting cells, parallel smooth membrane arrays (Golgi-like stacks) which contained acid phosphatase activity were observed (261). These stacks were initially not observed in nonencysting cells (261); however, by using ultrastructural techniques that enhance the preservation of the endomembrane system, these putative Golgi stacks (Fig. 4 and 5), as well as coated pits and 50- to 80-nm vesicles (Fig. 5), were also visualized throughout the cytoplasm in preencysting trophozoites (206). Two enzymes, which are present in the GA of mammalian cells (galactosyltransferase and *N*-acetylgalactosaminyltransferase) were found only during encystation (193). Cyst wall antigens were detected in the Golgi-like stacks during encystation, implicating involvement of the Golgi-like stacks in cyst wall formation (261). Apparently, the cyst wall proteins are not transported to the cell surface in small vesicles which are formed at the rim of the stack. Instead, they are transported by large, electron-dense, encystation-specific vesicles, which presumably originate from enlarged *trans*-Golgi cisternae (261). Interestingly, a major trophozoite surface antigen (130), which is synthesized by encysting and nonencysting *Giardia* cells (205, 261), was shown by immunoelectron microscopy to be present in the ER of both cell types but not in the large encystation-specific vesicles (205, 261). These results indicate that in *G. lamblia*, constitutive secretion (surface antigen) and regulated secretion (cyst wall proteins) occur simultaneously by different vesicular pathways (205).

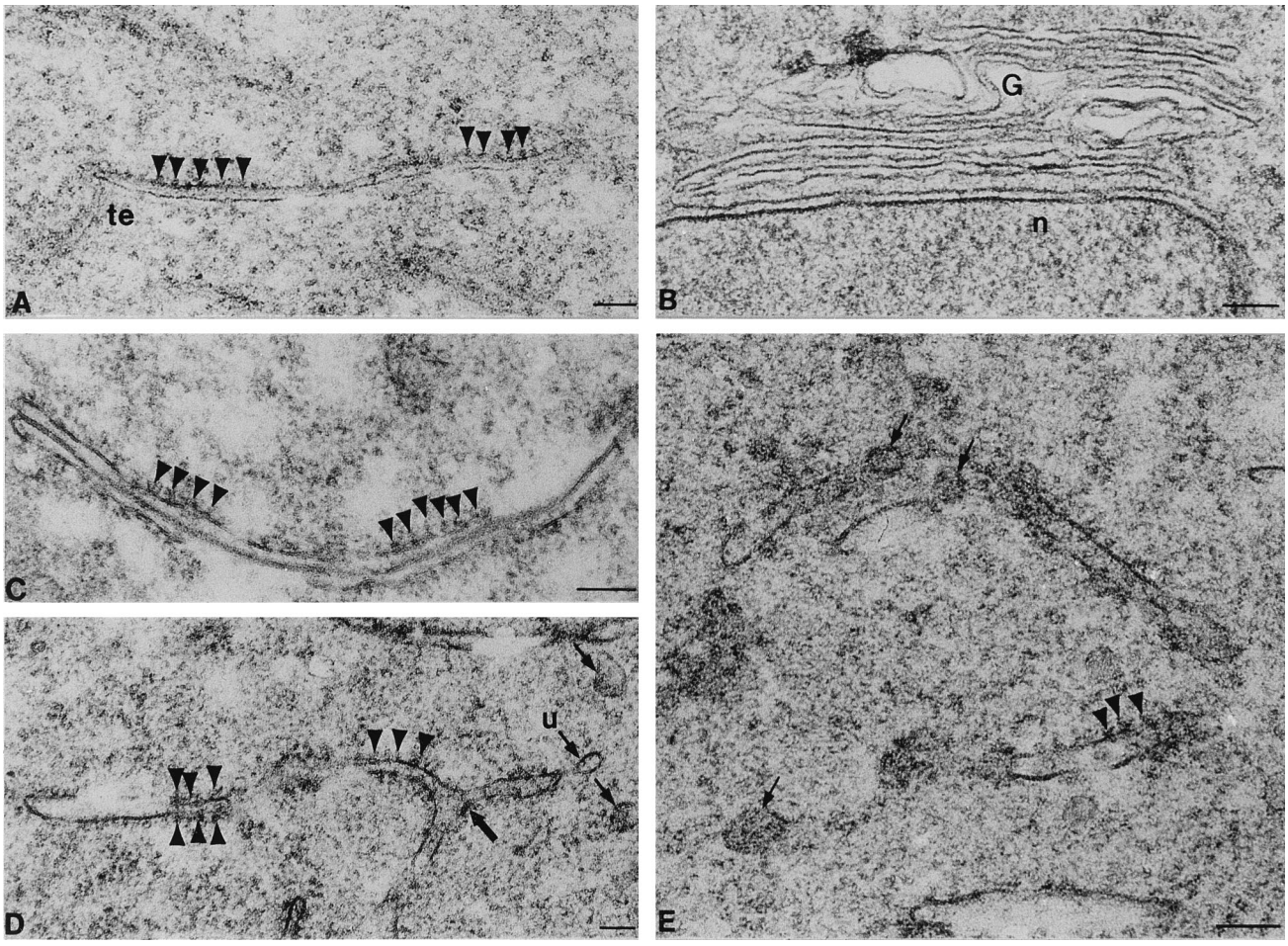


FIG. 5. Higher-magnification transmission electron micrograph sections stained with tannic acid, which enhances coated vesicles. (A and C) Transitional elements (te), in which one face is rough (studded with ribosomes [arrowheads]) and the other is smooth, are thought to be the first post-ER compartment. (B) Golgi-like stack of perinuclear smooth membranes. (D) ER/transitional element showing cisternal branching. Two of the small vesicles are coated, while the third is not. (E) smooth and transitional (arrowheads) membrane cisternae and numerous small coated vesicles (arrows). Bars, 0.1 μm. Reproduced from reference 206 with permission of Academic Press.

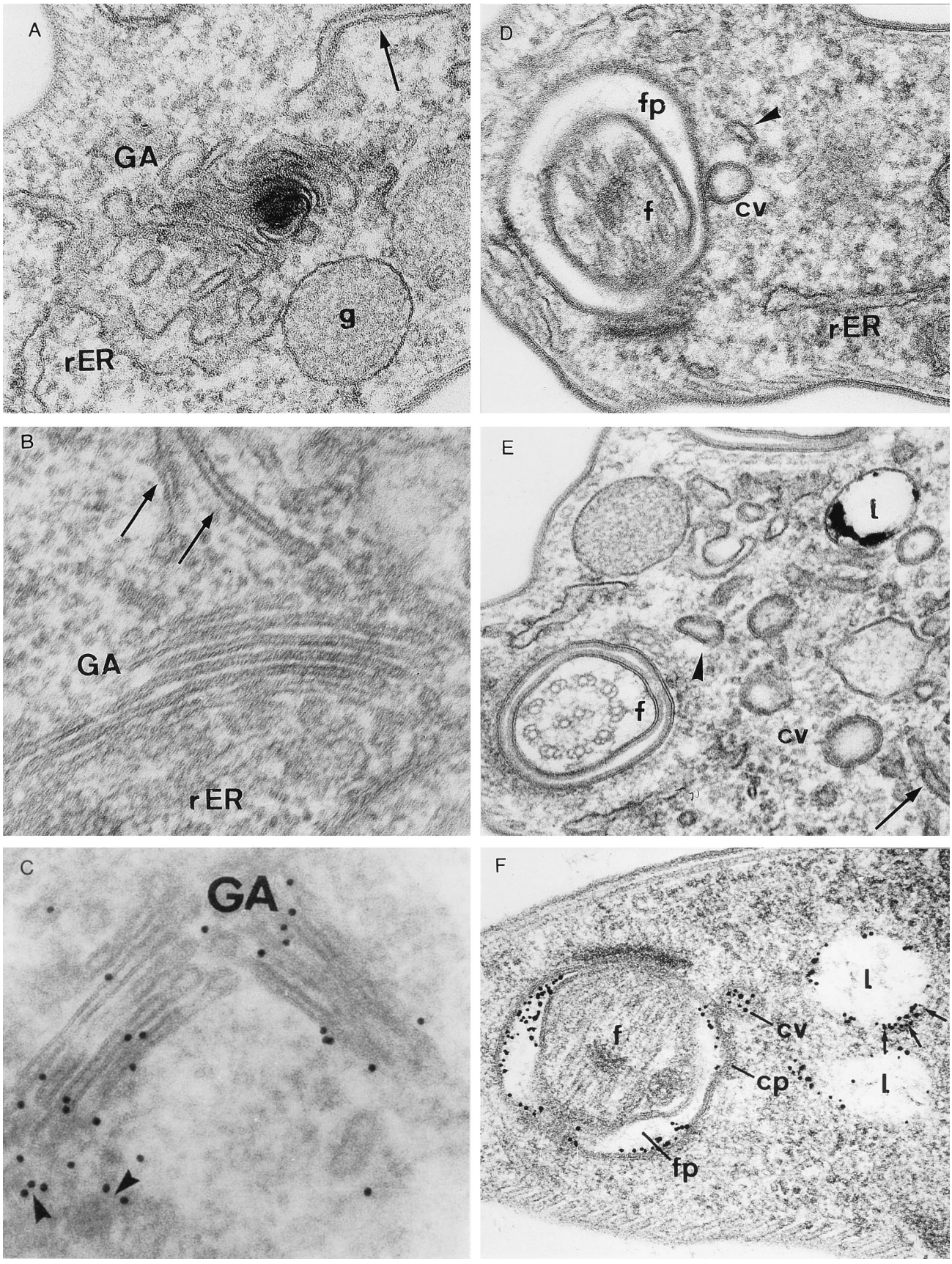

A
GA
g
rER
D
fp
f
cv
rER
B
GA
rER
E
l
f
cv
C
GA
F
l
f
cv
cp
l
fp

FIG. 6. Endomembrane system of *Trypanosoma brucei*. (A to C) Elements of the exocytotic pathway. The secretory pathway consists of rough ER (rER), GA, and a smooth flattened cisterna believed to be the trypanosomal equivalent of the *trans*-Golgi network (arrow). g, glycosome. (C) Post embedding labelling of cryosections with VSG-specific antibodies and a gold-labelled secondary antibody. Arrowheads indicate tubulovesicular elements of the endocytic pathway, which are also labelled. (D to F) Elements of the endocytic pathway. Endocytosis takes place in the flagellar pocket (fp), where coated vesicles (cv) are formed, which contain the surface coat (VSG) and a clathrin analog. f, flagellum; l, lysosome; cp, coated pit. Arrowheads indicate tubulovesicular elements of the endocytic pathway. (F) Preembedding labelling of VSG with VSG-specific antibodies directly labelled with gold particles. Cells were incubated for 2 min in the presence of gold-labelled antibody before being prepared for epon embedding and sectioning. Small arrows, VSG-specific labelling (primarily surface bound in lysosomes). Bar, 0.5 μm Micrographs courtesy of M. Duszenko, Tübingen, Germany.

Molecular components of the secretory pathway in *G. lamblia*. Not much is known about the molecular machinery of the secretory pathway in *G. lamblia*. A gene encoding ARF was found in trophozoites (219), which partially complements ARF function in *S. cerevisiae* (184). In agreement with the presence of ARF in *G. lamblia*, brefeldin A blocks secretion, remarkably in both encysting (cyst wall) and nonencysting (surface antigens) cells (193). A gene for a giardial BiP (binding protein, ER protein member of the HSP70 protein family) homolog, which contains a C-terminal KDEL ER retention signal, was isolated (145). Immunofluorescence studies indicate the presence of a membrane-associated β-COP homolog in both cell types, which disappeared from the membranes in brefeldin A-treated cells (193).

Kinetoplastids

The kinetoplastids form another relatively early line of eukaryotic cell evolution (Fig. 2). Members of the kinetoplastids and the related euglenoids have interested biologists for a long time, since several species are large enough to examine the cell architecture in detail under a light microscope. By using vital dyes such as neutral red or osmium impregnation, a "GA" was first demonstrated by light microscopy in several euglenoids and kinetoplastids (see, e.g., references 137, 147, and 221). However, in this early period, the function of the GA remained obscure. Since no recent investigations of the secretory pathway of euglenoids seem to have been performed with modern tools of cell biology, we shall discuss only recent work on kinetoplastids.

Secretory products in *Trypanosoma* and *Leishmania* species. Most members of the kinetoplastids are obligate parasites, which cause severe diseases in vertebrates. Their cell surface molecules are important for their survival in the host cells. These molecules and other proteins important in host-parasite interactions have been intensively studied as potential sources for vaccine development. They include membrane-bound glycoproteins like gp63 (an endoprotease) (120) and glucose transporters (249) in *Leishmania* spp., the unusual lipophosphoglycan of *Leishmania* promastigotes (reference 222 and references therein), and the variant surface glycoproteins (VSGs) in *Trypanosoma* spp. (65, 315), as well as secreted proteins like acid phosphatase in *Leishmania* spp. (163) and α-mannosidase in *Trypanosoma cruzi* (302). All known surface components are heavily glycosylated. The biosynthesis of lipophosphoglycan is just now being investigated in detail (222), whereas N glycosylation in trypanosomatids is already well characterized (see reference 241 for a review). N glycosylation (241) and the formation of the glycanphosphatidylinositol membrane anchor (66) are comparable to those of other eukaryotic organisms, although some unique features exist (241), and several enzymes involved in the biosynthesis of the dolichol-oligosaccharide precursor and processing in the ER have been studied (241). Less is known about glycan processing in the GA; however, the activities of a few GA enzymes have been measured (GlcNAc-transferase [135, 270] and galactosyltransferases [135, 248]). Since most work on secretion in kinetoplastids (reviewed in reference 59) has been on the VSGs, we will focus on these studies.

Secretion of variant surface glycoproteins in *Trypanosoma* species. VSGs are synthesized with a transient N-terminal signal peptide (36). The protein is anchored transiently in the ER membrane by its C terminus, which is replaced in the ER by a glycophospholipid consisting of ethanolamine, mannose, glucosamine, and *sn*-1,2-dimyristyl phosphatidylinositol (GPI) (113). In addition, N-linked oligosaccharides are added to some VSGs in the ER, and these are sometimes converted to complex glycans in the GA (315). The secretory pathway in *Trypanosoma brucei* (Fig. 6) consists of ER and Golgi stacks, each of the latter containing four to six well-aligned cisternae in close association with a transitional ER element (Fig. 6). Close to the *trans* side of a GA stack, a prominent system of flattened cisternae and tubulovesicular elements (Fig. 6) (95) is present, which has been suggested to be the homolog of the *trans*-Golgi network (95). Transport of the VSGs to the cell surface is rapid. In pulse-chase experiments with [^{35}S]methionine, 50% of the labelled VSGs are transported to the cell surface within 15 min (17, 113).

Surprisingly, monensin, a ionophore for monovalent cations which in many systems inhibits the transport of glycoproteins from the GA to the plasma membrane (215), had no effect on VSG transport to the cell surface (16, 95, 113), although the drug caused morphological changes (i.e., swelling of *trans*-GA cisternae) typical for monensin (95). In addition, monensin treatment prevented modification of N-linked glycans (16). This was taken as evidence for a novel secretory route to the cell surface that bypassed the GA (113), as also proposed for some other systems (see reference 215 for a detailed discussion). However, immunolocalization of VSGs showed their presence in the ER, the GA, and the *trans*-Golgi network irrespective of whether cells were treated with monensin (95), indicating that VSG also follows the classical route to the cell surface. It was speculated that an *sn*-1,2-dimyristyl phosphatidylinositol (GPI) anchor may confer an intrinsically high diffusion rate to VSGs, thus bypassing the monensin block, which could exist for other proteins in the secretory pathway (96). Much less is known about the biogenesis of lysosomal proteins in *Trypanosoma* species. It has been suggested that transport via the cell surface is the major trafficking pathway for proteins targeted to the lysosome (38).

Molecular components of the secretory pathway in kinetoplastids. Hardly anything is known about the molecular machinery of secretion in *Trypanosoma* species or kinetoplastids in general. A few glycosyltransferases have been characterized biochemically (see above). Advances in the molecular genetics of *Leishmania* species (such as the availability of lipophosphoglycan mutants and genetic complementation) (78, 272) have lead to the isolation of genes involved in the secretory pathway. A galactofuranosyltransferase specific for lipophosphoglycan biosynthesis (272) and a GA protein required for intracellular compartmentalization of lipophosphoglycan biogenesis (78) were cloned and sequenced. The latter protein shows 28% identity (55% similarity) to a yeast protein (Vrg4/Van2) which

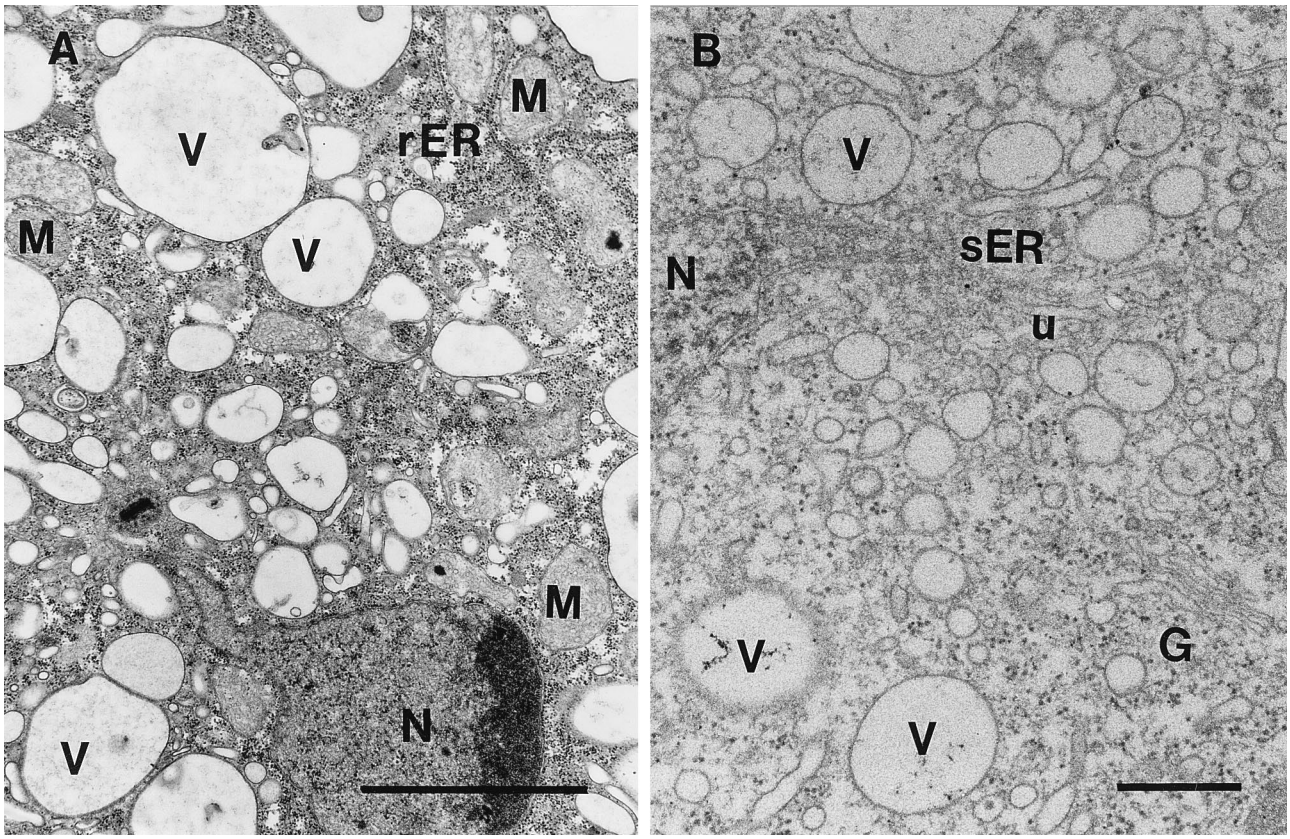


FIG. 7. Standard TEM of vegetative amoebae of *D. discoideum* cultured under axenic conditions. (A) Presence of many vacuoles of the endosomal/lysosomal system (V) in the cytoplasm. In addition, many mitochondria (M), the nucleus (N), and rough ER (rER) can be seen. (B) A small Golgi stack (G) and smooth ER (sER) can be seen. Bar, 1 μm. Micrographs courtesy of U. Roos, Zürich, Switzerland.

is essential for GA function (78). In addition, a homolog of the *N*-acetylglucosaminyl-1-phosphate transferase involved in formation of the dolichol-oligosaccharide precursor (190) and a homolog of mammalian BiP (18) were sequenced. The presence of clathrin was implicated from the presence of a 180-kDa polypeptide in isolated coated vesicles, although no cross-reactivity with antibodies against mammalian clathrin was observed (282). Similar to mammalian cells, *Trypanosoma* cells show transient glycosylation of N-glycosidic carbohydrates in the ER lumen, causing a delay in exit from the ER (176, 313).

Slime Molds

The slime mould *Dictyostelium discoideum* is an intensively studied model system in developmental and cell biology. Upon starvation, the vegetative amoebae aggregate and form a fruiting body with three different types of cells (basal disk cells, stalk cells, and spores). *D. discoideum* cells can be manipulated easily by genetic and biochemical means. The most significant work dealt with differentiation and aggregation of cells, but studies on the biogenesis and turnover of cell surface components and lysosomal hydrolases have also yielded interesting results.

As in yeast cells research on the secretory pathway of *D. discoideum* is based almost exclusively on biochemical studies (summarized in reference 50). There are no recent studies on the secretory pathway that were performed by ultrastructural techniques. Figure 7 shows transmission electron micrographs of vegetative amoebae cultured under axenic conditions. The cytoplasm is dominated by various types of vacuoles of the endosomal/lysosomal system and mitochondria (Fig. 7A). Sometimes rough ER (Fig. 7A) and tiny Golgi stacks consisting of three or four cisternae (Fig. 7B) are seen.

Biogenesis and secretion of lysosomal hydrolases. Lysosomal enzymes are synthesized initially at the rough ER as precursor polypeptides which are transported to the Golgi complex. In a late Golgi or early endosomal compartment, the precursors are cleaved into intermediate forms, which are converted to mature enzymes in the lysosomes (50, 58, 262). Complete proteolytic processing depends on an acidic environment, which is, however, not required for efficient sorting of proteins (51). The proteolytically processed lysosomal enzymes are also secreted into the culture medium by a special pathway (50) which involves a distinct postlysosomal compartment (239).

The lysosomal enzymes of *D. discoideum* are highly acidic glycoproteins modified by the addition of sulfate (58, 116) and methylphosphate (117, 118, 121) to N-glycosidic oligosaccharides. The degree of processing of the N-linked glycans is developmentally regulated (8, 72, 165, 263). In contrast to the MPR-dependent sorting of mammalian lysosomal enzymes, these modifications are not essential for proteolytic processing or targeting of lysosomal hydrolases in *D. discoideum* (47, 49, 99). Instead, mutants with mutations in N glycosylation showed a reduced secretion rate of lysosomal proteins to the culture medium (47, 49, 99). However, treatment of cells with the glycosylation inhibitor tunicamycin, which yields incompletely glycosylated proteins, had no significant effect on the secretion of contact site A protein (an adhesion molecule) to the cell surface (156). Inhibition of the early proteolytic processing of

lysosomal enzymes by antipain or leupeptin caused missorting and oversecretion of precursor forms (262). As in mammalian cells, sorting of lysosomal enzymes is dependent on clathrin-coated vesicles (271).

Spore development. During spore development, cells form secretory granules (prespore vesicles) which contain spore coat proteins (as a preassembled glycoprotein complex precursor [105, 324]) and a polysaccharide consisting of galactose and *N*-acetylgalactosamine (105, 186), as well as lysosomal hydrolases (186). Subsequent work has shown that the prespore vesicles receive hydrolases from lysosomes and newly synthesized hydrolases from the Golgi complex (186). It was suggested that cells either restructure preexisting lysosomes into prespore vesicles or transport proteins between these two organelles.

Molecular components of the secretory pathway in *D. discoideum*. Not much is known about the secretory machinery of *D. discoideum* in molecular terms. Small G-proteins of the ras superfamily were detected by immunoblotting (43), and the sequences of several homologs of the rab/ypt protein family have now also been reported (45, 46, 276). So far, only a rab4 homolog has been localized almost exclusively to the spongiome of the contractile vacuole complex, although some labelling of lysosomal membranes was also observed (46). The gene for the clathrin heavy chain has been cloned and sequenced (57% identity to the mammalian clathrin heavy chain [228]). Some enzymes of N-linked glycan processing have been partially characterized at the biochemical level (see, e.g., references 177, 284, and 285), and mutants with mutations in various N-glycosylation steps have been isolated (35, 117). A cytosolic fraction prepared from *D. discoideum* can replace a similar fraction from mammalian cells in the in vitro intra-Golgi transport assay, indicating that similar cytosolic components operate in *D. discoideum* (240).

As in other systems, secretion depends on calcium ions (63), and an ATP-driven Ca^{2+}/H^{+} antiporter was detected in the endomembrane system of *D. discoideum* (265).

Alveolates (Ciliates and Apicomplexans)

Ciliates. Ciliates are often used as unicellular model systems for secretion. In contrast to yeasts, which have only constitutive secretion, ciliates reveal both constitutive and regulated secretion. Like yeasts, ciliates are genetically amenable and can be grown in defined media. Most studies deal with regulated secretion of either trichocyst formation in *Paramecium* species or mucocyst formation in *Tetrahymena* species. Sometimes constitutive secretion of hydrolases in *Tetrahymena* species or in other ciliates is also studied.

The GA is the central sorting organelle in the secretory pathway. In ciliates, no classical Golgi complex has been observed, and the existence of a GA remained uncertain for some time (106). Only in the early 1970s was the existence of a GA in ciliates finally recognized (106). The GA consists of several hundred tiny stacks which are located predominantly at the periphery of a cell. Most stacks contain only two or three fenestrated GA cisternae (6, 175, 192, 244), often appearing in thin sections as a mass of vesicles (6). The stacks are closely associated with the smooth transitional membrane of the rough ER and with cortical mitochondria (6, 106, 175, 192, 244). Non-clathrin-coated vesicles (similar to the COP-type vesicles of mammalian cells) have been observed between the ER and Golgi stack (6, 244). Enzyme cytochemistry showed that the *trans*-most cisterna contained acid phosphatase and thiamine pyrophosphatase (175, 192). In addition, a single cisterna located near the proximal end of basal bodies was stained. It has been suggested that both represent the equivalent of the *trans*-Golgi network. This is supported by the presence of clathrin-coated buds on the *trans* face of the GA stack (6) and by the labelling of the *trans*-most cisterna with wheat germ agglutinin (7). By using the quick-freeze deep-etch technique, a filamentous network in the ER-Golgi transition zone and in the *trans*-Golgi region was detected (6). It was proposed that these networks are responsible for the close physical association of the ER and Golgi stack in *Paramecium* species (6).

(i) Trichocyst formation. Trichocysts are spindle-shaped extrusive organelles of alveolates, which are discharged upon external stimuli (149). They are specialized secretory vesicles, which fuse upon stimulation with the plasma membrane. The trichocyst matrix expands upon discharge to form a paracrystalline rod three to five times its original length (149). In *Pseudomicrothorax dubius*, early pretrichocysts are formed by fusion of bristle-coated (similar to clathrin) electron-dense vesicles ("dense vesicles") with electron-translucent vesicles ("clear vesicles"), both of which originate from the *trans*-Golgi network (244) (Fig. 8). Similar results were obtained for trichocyst formation in *Paramecium* species (123, 150, 216, 333). The pretrichocysts grow by fusion with additional dense and clear vesicles and other pretrichocysts until they reach a final diameter of 2 μm (Fig. 8C). Dense and clear vesicles deliver different components of the mature trichocyst (tip and shaft precursor, respectively [244]). When pretrichocysts reach their maximum diameter, formation of the paracrystalline shaft starts and is followed by formation of the tip. When the condensation process is terminated, the mature trichocysts are attached with their narrow or pointed ends to the plasma membrane, a process called docking (244). Transport of trichocysts to their docking sites in the cell cortex depends on microtubules (133, 252) and microfilaments (252). The exocytosis of secretory granules in ciliates (trichocysts and mucocysts) upon external stimuli has been extensively reviewed recently (251) and need not be discussed here.

The extrusive organelles of *Tetrahymena* species are the mucocysts (summarized in reference 149). It has been proposed, as for trichocysts, that mucocysts originate from ER-derived vesicles (149). However, since mucocyst formation in *Tetrahymena* species has not been investigated recently, it might well be that as with *Paramecium* trichocysts, mucocysts originate from *trans*-Golgi vesicles.

Mature trichocysts contain a compact, highly ordered array of proteins. About 34 major and 80 minor acidic proteins, which represent four different categories of proteins (287) encoded by multigene families (198), have been identified (310). Trichocyst proteins arise from high-molecular-weight precursors by proteolytic processing (see references 3, 288, and 314 for a discussion of *Tetrahymena* mucocysts). Proteolytic cleavage is required for the assembly of the paracrystalline matrix (3, 288). In the presence of monensin, no proteolytic processing of trichocyst proteins and no formation of mature trichocysts are observed (3). The expansion of trichocyst proteins is calcium dependent (122, 129) and is modulated by pH (122). It has therefore been suggested that *Paramecium* trichocysts are acidic (44, 129) like other late compartments of the secretory pathway in mammalian cells (9) and that sorting of proteins to the regulated secretory pathway (trichocysts) depends on acidification (122, 124) as in mammalian cells. However, analysis of acidity by using acridine orange (194) or the 3-(2,4-dinitroanilino)-3′-amino-*N*-methyldipropylamine (DAMP) technique (see reference 9 for experimental details) (124) showed clearly that *Paramecium* trichocysts are not acidic. It has therefore been suggested that *Paramecium* species use a different mechanism to segregate trichocyst proteins from other secretory

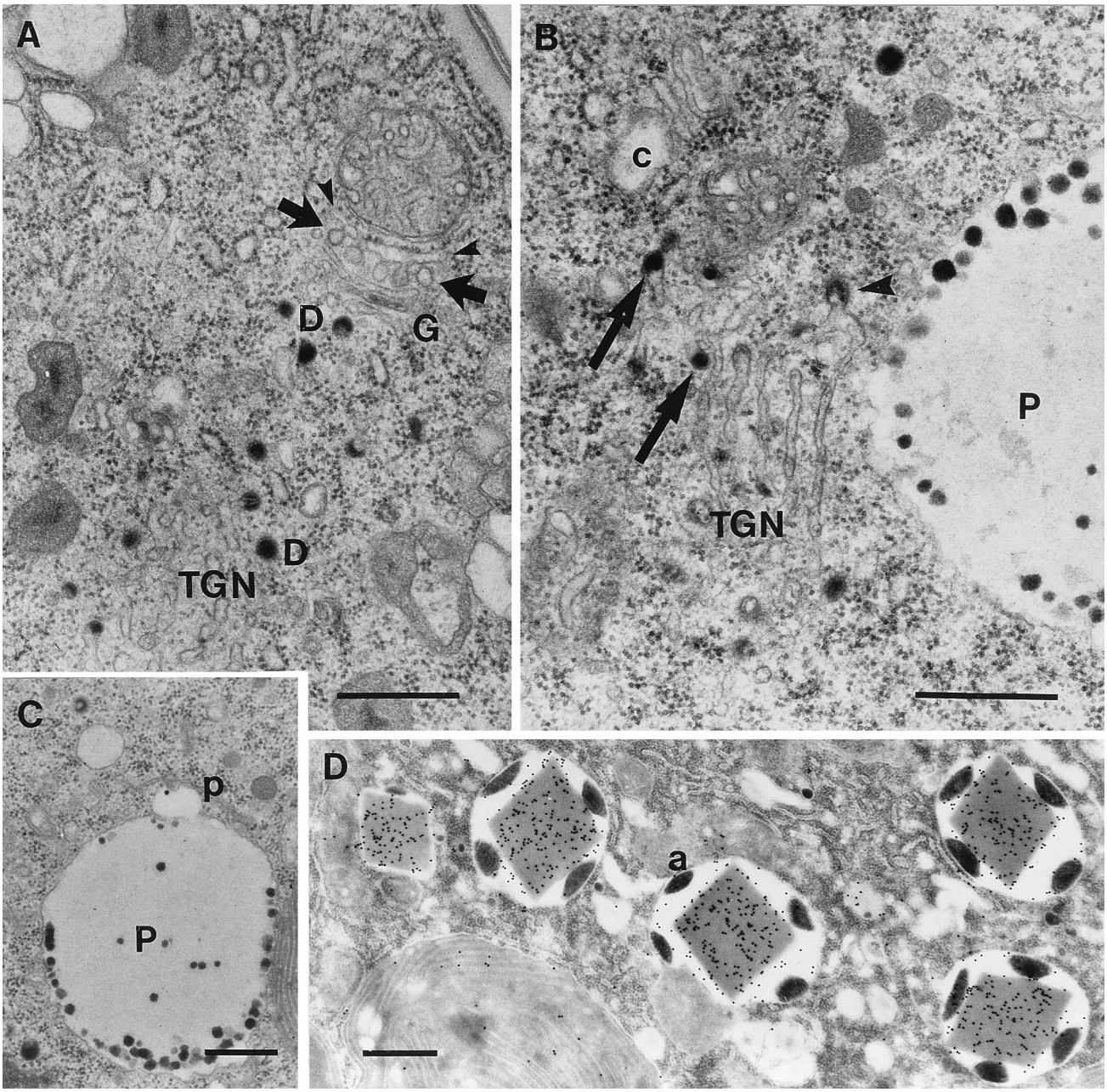


FIG. 8. Formation of trichocysts in *Pseudomicrothorax dubius* as observed in the electron microscope. (A) The Golgi stack (G) is composed of three cisternae. Transition vesicles (small arrows) are observed along the *cis* face of the stack. The stack is always associated with transitional ER (small arrowheads) and a mitochondrion. A well-developed tubular *trans*-Golgi network (TGN) is located far from the Golgi stack. Dense vesicles (D) are observed near both the stack and the *trans*-Golgi network. (B) Section through a *trans*-Golgi network adjacent to a developing pretrichocyst (P). Electron-dense material (arrows) accumulates in the *trans-Golgi* network tubules and budding, coated vesicles (arrowhead). An irregularly shaped, coated, clear vesicle (C) containing flocculent material is also seen. (C) Stage of a developing pretrichocsyst (P). The diameter increases by fusion with clear vesicles (C) and smaller pretrichocysts (p). (D) Fully formed trichocysts observed in cross section showing the four electron-dense pretrichocyst arms (a). Immunogold labelling with a polyclonal antitrichocyst antibody. Bars, 0.5 μm. Reproduced from reference 244 with permission of Elsevier, Paris.

proteins (124). Trichocyst proteins are retained in secretory vesicles (retention model), whereas other proteins (e.g., lysosomal enzymes) are retrieved by a receptor-mediated process (124), similar to the formation of secretory granules in the β cells of the pancreatic islet (172).

(ii) Constitutive secretion. Work related to constitutive secretion has dealt mainly with secretion of lysosomal acid hydrolases in *Tetrahymena* species (reviewed in reference 308). Similar to the formation of mucocysts, secreted β-hexosaminidase is proteolytically processed in two steps (161). Lysosomal proteins contain glucosylated N-glycosidic oligosaccharides of the high-mannose type, indicating that processing of these structures in the GA does not take place (304). Analysis of constitutive secretion in *Tetrahymena* species is difficult since acid hydrolases are in part also secreted during the egestion of food vacuoles or exocytosis of mucocysts (see, e.g., reference

307). With at least three different types of secretory processes and various endocytotic events, the genus *Tetrahymena* has one of the most complex system of membrane traffic in protists (309).

(iii) Molecular components of the secretory pathway in ciliates. Analysis of the molecular components involved in secretion in ciliates is just starting. Small GTP-binding proteins associated with secretory vesicles have been detected (246). Several members of the rab/ypt and rho subfamily of low-molecular-weight G-proteins have been partially sequenced by PCR techniques (115). A homolog of the liver-specific f-antigen (57% identity) of unknown function was found to be associated with the Golgi complex and with mucocysts in *Tetrahymena* species (159). A 63-kDa phosphoprotein (parafusin) which appears to function in exocytosis of trichocysts was identified in *Paramecium* species and biochemically characterized (128), and the amino acid sequence was determined from its cloned cDNA (299). By using polyclonal antibodies (274, 332) and PCR techniques (332), this protein was also found in *D. discoideum* (274) and in mammalian cells (274, 332). Recent work involves the isolation and characterization of mutants blocked in either constitutive (160) or regulated (126, 235, 253, 275) secretion. A few *Paramecium* mutants have been analyzed in some detail (126). For example the "trichless" mutant was shown to have a defect in the processing of matrix precursors of trichocysts (126). In other mutants, proteolytic processing appears to be normal, but intracellular transport between the ER and Golgi is impaired (126). Molecular analysis of these mutants will greatly facilitate the identification of proteins functioning in the secretory pathway of ciliates.

Apicomplexans. *Plasmodium* species are protozoan parasites which infect erythrocytes and are the causative agents of malaria. The parasite shows a complex life history. During its asexual life history, merozoites infect erythrocytes, where, after several developmental stages, 16 progeny merozoites are formed. Mature erythrocytes have no intracellular organelles and few biosynthetic activities. *Plasmodium* species as actively growing organisms have certain requirements for the uptake of nutrients and expulsion of waste. Thus, they must extensively remodel the erythrocyte by exporting numerous proteins and must establish the specialized machinery for their trafficking (summarized in reference 102). Because of the importance of this stage for malaria infections, some work on the secretion of proteins from the parasite to the host cell has been undertaken.

During the intraerythrocyte stage, *Plasmodium* proteins are transported to various compartments in the erythrocyte (e.g., intraerythrocytic tubulovesicular membranes induced by the parasite [19, 102]). Proteins are translocated into the ER. The amino acid sequences for five different secretory proteins are known. All have typical N-terminal signal sequences and are transported in heterologous translation/translocation systems into microsomes (102). Many small vesicles are associated with the ER. No typical Golgi stacks have been observed in the parasite during the intraerythrocyte stage; therefore, these vesicles have been described as the GA of the parasites (179). However, the Golgi function of these vesicles has been questioned (19). Instead, a transport function between the ER and plasma membrane has been suggested (19). In agreement with the absence of a Golgi stack, *Plasmodium* species lack N glycosylation of proteins during the intraerythrocytic stage (80). However, phosphorylation and proteolytic processing of secreted proteins in post-ER compartments have been observed (69). On the other hand, several conserved characteristics of the secretory pathway were detected. Homologous genes to the mammalian BiP gene (*pfgrp* [173, 174]) and KDEL receptor gene (*pferd2* [103]) have been cloned and sequenced from *Plasmodium falciparum*. The pfgrp protein was localized to the ER by immunogold techniques (173), whereas the pferd2 protein was localized to a different compartment by indirect immunofluorescence. pferd2 showed redistribution to the ER upon brefeldin A treatment (103). Brefeldin A, as in other systems, inhibited protein secretion at various stages of the cell cycle (64, 69, 101, 153, 229), although it did not block the secretion of some proteins (101). Another typical Golgi marker (sphingomyelin synthase) was detected in the parasite as well as in the erythrocyte host (tubulovesicular membranes [104]). Recently, another protein of the tubulovesicular membranes was shown to be immunological related to a mammalian Golgi protein (188), indicating that these tubulovesicular membranes may play a role similar to the GA in *Plasmodium*-infected erythrocytes (188).

Green Algae

The green algae differ in several aspects from the other organisms discussed so far. They are photosynthetic and compose unicellular as well as multicellular taxa, and the protoplasts are almost always enclosed in an elaborate extracellular matrix, which is the major secretory product of the protoplast. The diversity of types of extracellular coverings in the green algae is enormous, and includes single or multiple layers of scales (reviewed in reference 23), cell walls derived by fusion of scales (theca [for a review, see reference 23]), crystalline glycoprotein-type cell walls (for a review, see reference 1), mucilaginous layers, and cellulose/pectin-based cell walls. Cell wall glycoproteins of *Chlamydomonas* and related taxa have been characterized in great detail (for a review, see reference 1). They contain O-linked heteroglycans consisting of arabinose, galactose, mannose, and glucose. Much less is known about N glycosylation in green algae. The presence of N-linked glycans has been reported for various taxa (14, 25, 26, 142, 254). Tunicamycin, an inhibitor of N glycosylation, affected cell growth and mating in *Chlamydomonas reinhardtii* (204, 260), and tunicamycin-resistant mutants have been described (97). However, only preliminary data (formation of dolichol-sugars in a crude microsomal fraction from *Chlorella* species) regarding the biogenesis of N-linked glycans are available (254).

The GA and other components of the secretory pathway are well developed in green algae and in algae in general. The ultrastructure of the endomembrane system in green algae has been studied in great detail and was recently summarized (86). Therefore, we will focus our discussion here on functional aspects of secretion in three well-studied model systems.

Scale formation in prasinophyte algae. Scales may be defined as structures of distinct size and shape which are located on the cell body and flagellar surfaces. Many taxa possess more than one scale type, and up to seven morphologically different scale types have been reported in a single taxon. Prasinophyte scales are nonmineralized, and carbohydrates are their major constituents (see reference 23 for a recent review of scale structure and composition). Various studies (summarized in reference 23) have shown that scales can be detected by electron microscopy in the GA, in secretory vesicles, and, in some taxa, in a membraneous reticulum anterior to and physically separated from the Golgi stacks (e.g., *Scherffelia dubia* [Fig. 9A] [207]). Since for many scale types a maturation process from the proximal (*cis*) to the distal (*trans*) face of the Golgi stack can be observed (90, 207, 214), it is widely accepted that scales are assembled in the Golgi stacks, transported to the cell surface by secretory vesicles, and released to the cell surface by exocytosis (Fig. 9B). Cells often have more than one Golgi

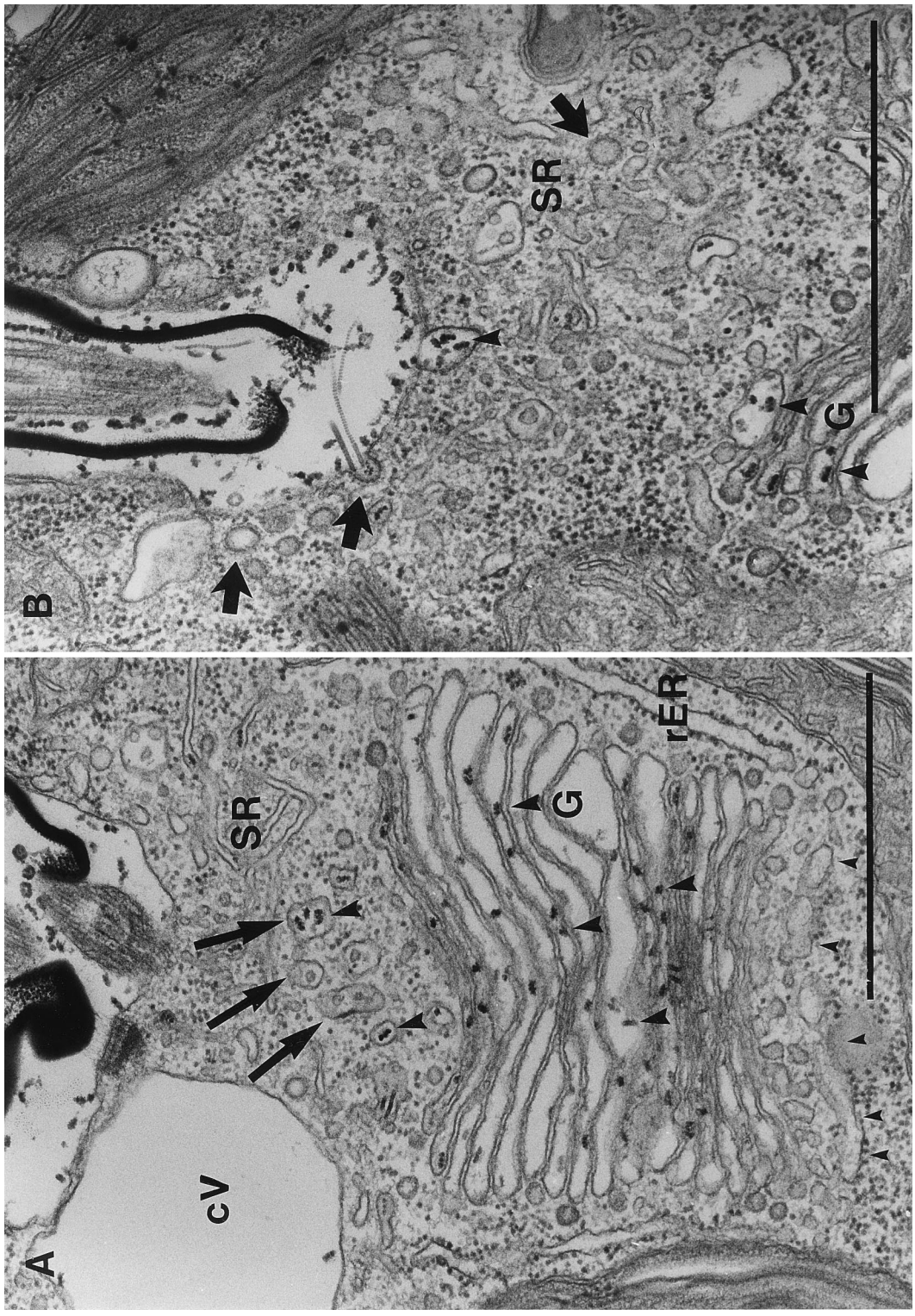


FIG. 9. Conventional TEM of cells of *Scherffelia dubia* during flagellar regeneration. (A) Longitudinal section showing a prominent Golgi stack (G) associated with rough ER (rER), transitional elements (small arrowheads), closely associated with the *cis* face of the Golgi stack, a membranous reticulum (scale reticulum [SR]), the contractile vacuole (cV), and numerous secretory vesicles (arrows). (B) In addition, differents stages of exo- and endocytosis can be seen. Coated buds and vesicles are indicated with small arrows. Note the presence of scales (arrowheads) in the Golgi stack and secretory vesicles but not in the transitional vesicles at the rims of the Golgi stack. Bars, 1 μm. Micrographs courtesy of B. Bölinger, Cologne, Germany.

stack, and all stacks are equivalent in their capacity to synthesize all types of scales of a taxon. The Golgi stack of prasinophyte algae contains about 20 cisternae (86). Each cisterna is capable of producing different scale types simultaneously (190, 202, 207, 213, 214). Intracisternal differentiation has often been observed; that is, specific scale types are formed within certain areas of a GA cisterna. For example, the pentagonal scales and flagellar hairs of *S. dubia* are found at the rims of a cisterna whereas the man scales are found exclusively in the central part of the cisterna (207).

A recent investigation of GA activity during the first minutes of experimentally induced flagellar-scale biogenesis in *S. dubia* revealed that structurally complete scales can be recognized 5 min after deflagellation in the third to sixth cisternae from the *cis* face (22). Between 5 and 20 min, scale-bearing cisternae successively extend to the *trans* face of the GA. Interestingly, scales are never seen inside the numerous transition vesicles at the rim of the Golgi stack (Fig. 9A) (22, 207). Thus, during scale biogenesis, the GA clearly follows the classical membrane flow, or "cisternal progression," model (217).

Similar results have previously been obtained for the secretion of scales in the prymnesiophyte alga *Pleurochrysis scherffelii* (for reviews, see references 40 and 211) and will be therefore be only briefly discussed. The scales of *P. scherffelii* are ~1.5 to 2 μm in diameter and consist of two types of fibrils (radial and spiral fibrils) and additional amorphous material (41). Polysaccharides (including cellulose) and proteins are the major constituents of these scales (90% of dry weight). Each scale is synthesized by a single Golgi cisterna. The Golgi stack comprises 20 to 30 cisternae, and enzyme histochemistry demonstrated that it is subcompartmentalized (NDPase, *cis*; arylsulfatase, *medial*; acid phosphatase, *trans* [41]). Assembly of the radial fibrils of the scale starts at the 10th to 12th cisternae from the *cis* face (40). Other parts of a scale are assembled in a more *trans* position in the stack. Once scale assembly is completed, the *trans*-most cisterna leaves the Golgi stack. Fusion of the entire *trans*-Golgi cisterna with the plasma membrane was directly visualized by video microscopy (39). In this example, the scales are far too large to be transported in vesicles. Therefore, intra-Golgi transport of prymnesiophyte scales by cisternal progression has usually been considered a "rare formula connected with the unusual geometry and size of the product" (110). The formation of coccoliths in prymesiophytes has recently been reviewed (107, 182). By using immunogold techniques, acidic polysaccharides of the coccoliths were shown to be synthesized in medial Golgi cisternae (203). These polysaccharides cosegregate with calcium into vesicles, which are assumed to transport the polysaccharides to the coccolith-forming saccule (203).

Application of monensin to scale-producing prasinophytes (83, 88) yields *trans* face curling, swelling, and production of multivesicular elements similar to the effects reported for monensin in mammalian cells (215). The ultrastructure of scales synthesized in the presence of monensin was altered, and the secretion rate was reduced (88). Other cell poisons interfering with the intracellular regulation of the potassium or calcium ion concentration (82, 83) altered the structure of the endomembrane system and the secretion of scales as in other systems.

***C. reinhardtii* and related organisms.** The ultrastructure of the endomembrane system of *C. reinhardtii* and related taxa has been investigated by several authors (see, e.g., references 84, 89, and 140; a detailed description is given in reference 86). However, although *C. reinhardtii* has been called the green yeast because of to its great potential for genetic analysis (143), and although the structure of the crystalline glycoprotein cell wall is well studied (for a review, see reference 1), less work has been done on the secretory pathway. O glycosylation of cell wall proteins of *C. reinhardtii* was investigated in some detail. Monoclonal antibodies against O-glycosidic carbohydrate epitopes have been used to determine the intracellular localization of O glycosylation (138, 337). Two antibodies known to recognize different carbohydrate epitopes were found to label two distinct regions of the Golgi stack, indicating a subcompartmentalization of the stack (138).

An analysis of the endomembrane system was performed with the large chlamydomonad *Gloeomonas kupfferi* (85). The Golgi stacks of *G. kupfferi* contain 16 cisternae (84). Cytochemical localization of acid phosphatase and thiamine pyrophosphatase showed the presence of these activities in the *trans* cisternae of the GA stack (85). As in other eukaryotes, brefeldin A disrupts Golgi integrity and converts the stacks into a network of swollen tubules (68). The cell wall of *G. kupfferi* consists of glycoproteins (probably O glycosidic, since no *N*-acetylglucosamine is present), which are synthesized in the GA (87). At the *trans* side of the stack, the glycoproteins are packaged into vesicles and transported to the contractile vacuole (87), from which the material is released.

The desmids. The secretion of cell wall material in relation to morphogenesis has been studied in the desmid *Micrasterias denticulata* (reviewed in reference 208). The *Micrasterias* cell is flat and leaf- or plate-like, consisting of several lobes (Fig. 10A). The two halves of a *Micrasterias* cell are arranged like mirror images. Each semicell has one polar lobe and four lateral lobes. After cell division, the two semicells separate and each forms a new semicell (Fig. 10A). Morphogenesis depends on the localized secretion of cell wall material at the areas of forming cell lobes and reduced secretion rates at zones of indentions (209). Three different types of vesicles are derived from a Golgi stack/*trans*-Golgi network unit (Fig. 10B) (209). Each Golgi stack contains 11 cisternae (209). "Dark" vesicles with an electron-dense content (cell wall material) and large vesicles containing mucilage are formed at the *trans* cisterna (Fig. 10B). Biogenesis of the dark vesicles starts at the fifth to sixth cisterna, where bulging of the margin can be observed. These dilations are filled with electron-dense material (Fig. 10A). The third type consists of vesicles of 70 nm in diameter, which are often clathrin coated and formed at the *trans*-Golgi network. The large and dark vesicles are present in the whole cell peripheries. However, dark vesicles are much more concentrated in the growing lobes than in zones of indentions (209). Prior to fusion, dark vesicles associate with specific fusion sites (Fig. 10C) (209). Microfilaments but not microtubules seem to play a role in vesicle transport (185, 311, 318). Secretion and wall morphogenesis are inhibited by tunicamycin, an inhibitor of N glycosylation, and cyclopiazonic acid, an inhibitor of the Ca^{2+}-dependent ER-ATPase (155).

Transport of vesicles from the ER to the Golgi was reconstituted in a cell-free system (226). The in vitro system shows the same characteristics (vesicle size and structure, ATP dependence, and 16°C block) as reconstituted yeast or mammalian systems. The presence of an intermediate compartment (here called the low-temperature compartment) in *Micrasterias americana* has been proposed (226).

Molecular components of the secretory pathway in green algae. Little is known about the molecular components of the secretory pathway in green algae. Small G-proteins of the rab/ypt subfamily have been identified in *Dunaliella* (264), *Volvox* (108, 109), and *Chlamydomonas* (81, 212) species. A few glycoslytransferases have been partially characterized (arabinose-hydroxyproline-*O*-glycosyltransferase from *Volvox* and *Chlamydomonas* [144, 336] species and galactose-hydroxypro-

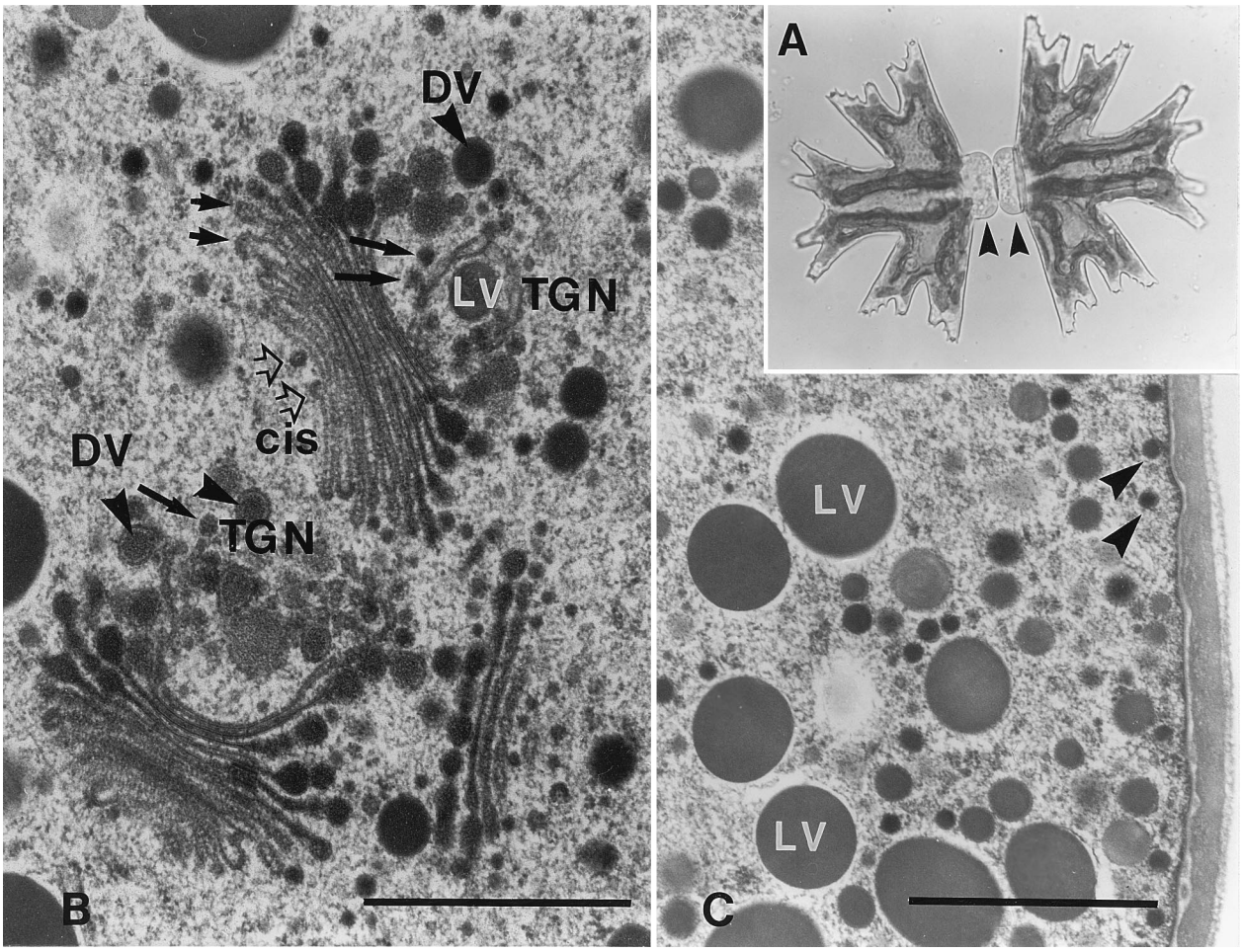


FIG. 10. *Micrasterias* secretory pathway. (A) Cell of *M. americana* as observed in the light microscope. Micrograph courtesy of B. Surek, Cologne, Germany. (B) The Golgi apparatus of *M. denticulata* during cell morphogenesis. Three types of vesicles are formed (DV, dark vesicles containing primary cell wall material; SV, small vesicles containing unknown material; LV, large vesicles containing mucilage). Open arrows indicate transition vesicles probably fusing with cisternae at the *cis* face of the stack. Small arrows indicate bulges of the cisternal margin, which expand to mature dark vesicles. (C) The cortical cytoplasm of a growing lobe. Dark vesicles are regularly spaced with respect to each other and in contact with the plasma membrane. LV, large vesicles. Bar, 1 μm. Microcraphs courtesy of U. Meindl, Salzburg, Austria.

line-O-glycosyltransferase from *Chlamydomonas* species [336]). Recently, the first mutants affecting O glycosylation have been described (319). Clathrin-coated vesicles associated with the contractile vacuole and the Golgi complex were observed in various green algae, including *Chlamydomonas* species (325) and have been characterized biochemically from *Chlamydomonas* (77) and *Dunaliella* (335) species. Antibodies against bovine clathrin heavy chain cross-reacted with a 180-kDa protein of isolated coated vesicles of *Chlamydomonas* species (77).

Much more is known about the molecular components of the secretory pathway in higher plants (reviewed in references 21 and 297; a discussion of the spatial organization of the secretory pathway of higher plants is in included in the excellent review by Staehelin and Moore [297]), the descendants of green algae. Several components of the secretory pathway have been found, including ARF (see, e.g., references 168 and 183), COPII (20, 76), BiP (75), KDEL receptor (20), protein disulfide isomerase (289, 290), and various rab proteins (see, e.g., references 146 and 306). N glycosylation of proteins is well characterized, and the presence of N-glycosylation-processing enzymes of the GA (including Golgi mannosidases and *N*-acetylglucosaminyl transferase I) is well established (167). On the basis of their biochemical properties, these proteins seem to be homologous to their mammalian counterparts (167). In contrast to mammalian cells, protein targeting to the vacuole depends on sorting information, present in at least three different parts of the polypeptides (N-terminal and C-terminal propeptides and sequences within the mature protein) (220).

COMPARISON: SIMILARITIES AND DIFFERENCES

For comparison, selected elements of the secretory pathway of the protists discussed in this review are presented in Table 1. Although our knowledge for any protist is far from complete, some interesting conclusions can be drawn. All elements are present in at least two or three groups of protists, and most of them can even be detected in the two most "primitive" eukaryote phyla discussed, the diplomonads and the kinetoplastids. For example, homologous proteins to the mammalian BiP have been found in *Giardia* and *Plasmodium* species and higher plants (Table 1), and similar effects of the inhibitor monensin on Golgi structure were observed in kinetoplastids, ciliates, and green algae (Table 1). Taken together, these data

TABLE 1. Comparison of the secretory pathway of *G. lamblia*, kinetoplastids, *D. discoideum*, alveolates, green algae, and higher plants with the mammalian and yeast system

Pathway component	Comment	Situation in[a]:					
		G. lamblia	Kinetoplastids	*D. discoideum*	Alveolates[b]	Green algae and higher plants[c]	Mammals and yeasts
ER proteins	e.g., BiP, PDI	BiP	BiP		BiP*	BiP*	+ B, S
	Glycosylation enzymes		+ B, S	+ B		+ B	+ B, S
ER retrieval system	KDEL receptor				+ S*	+ S*	+ S
	Brefeldin A effect	+			+*	+	+
Golgi stack	No. of cisternae	+ ca. 6	+ 4–6 (15–30 for *Euglena* spp.)	+ 3–4	+ 2–3	+ 5–20	+ 3–5 (mammals)
Golgi proteins	Glycosyltransferases	+ B	+ B, S	+ B		+ B*	+ B, S
	Other		+ S				+ B, S
	NBD-ceramide labelling	+			+*		+
trans-Golgi network	Ultrastructure		+		+	+	+
	Monensin effect		+		+	+	+
Coated vesicles	COPI	ARF	−	−	+ U	ARF*	ARF, coatomer
	COPII					SAR*, sec12*	SAR, sec12, sec13, sec31, etc.
	Clathrin	+ U	+ B, U	Clathrin (h)	+ U	+ B, U	Clathrin (1+h), adaptins
rab proteins	No. of different rab proteins		+ 9	+	+ 5	+ 6	+ ca. 30
Fusion machinery	Characterized components			+ B		+ B*	SNAREs, SNAPs, NSF

[a] B, biochemical evidence; S, structural evidence; U, ultrastructural evidence.
[b] *, found in *Plasmodium* species.
[c] *, found in higher plants.

imply that the spatial organization and the molecular machinery is well preserved in eukaryotes, suggesting that a similar secretory pathway was already present in the "ur-eukaryote." However, there is one element which shows considerable variation within eukaryotes: the presence of a Golgi stack and the number of GA cisternae within a stack. A Golgi stack is absent in budding yeast cells and in two presumably ancient eukaryotic phyla, the microsporidia (48) and the archamoebae (e.g., *Pelomyxa* species) (327). However, as in *G. lamblia*, it cannot be excluded that a reinvestigation of the ultrastructure of these taxa by enhanced fixation techniques will demonstrate a Golgi complex. It is important to note that the absence of a Golgi stack does not imply that these cells possess no functional equivalent of a GA. The cytoplasm of microporidia and archamoebae is filled with various vesicles (48, 327), which might be the Golgi equivalent as in budding yeast cells. The situation is further complicated by the fact that all microsporidia are obligatory parasites. Whether these organisms have suffered extreme parasitic reduction or have retained their primitive characters is undecided (reviewed in reference 54).

The number of cisternae per Golgi stack shows considerable variation within eukaryotes. Golgi stacks can be small, as in ciliates, in which only 2 or 3 cisternae per stack are present, or can contain up to 30 cisternae (e.g., in certain euglenoids). The reason for this variability is not known; however, organisms like ciliates, which have little protein glycosylation (lysosomal proteins of *Tetrahymena* species contain only high-mannose glycans [304], as do the trichocyst proteins of *Pseudomicrothorax* species [243]) and do not synthesize complex polysaccharides, generally possess small stacks. In contrast, various algal phyla synthesize large amounts of complex carbohydrates (e.g., prasinophyte scales [23]) and exhibit Golgi stacks with a large number of cisternae. Therefore, it is tempting to speculate that GA structure is defined by the amount of complex carbohydrates synthesized by a taxon. In this respect, it is important to stress that the production of large amounts of carbohydrates alone does not imply that a Golgi stack of a taxon consists of a large number of cisternae. For instance, higher plants secreting large amounts of carbohydrates for their cell wall possess only three to five cisternae per Golgi stack (297). However, the chemical structures of the secretory products of only few taxa have been analyzed so far. Clearly, more information is needed before the relationship between quantity and complexity of secretory products and the number of Golgi stacks per cell and Golgi cisternae in a stack can be established.

EVOLUTION OF THE SECRETORY PATHWAY

The evolution of eukaryotes represents the largest discontinuity in the history of life since it originated on earth about 3.5 to 3.8 billion years ago. Because of the basic similarities between prokaryotes and eukaryotes, it is well accepted that both arose from a common ancestor. However, the origin and early evolution of the eukaryotic cell are not understood (although detailed scenarios of eukaryotic cell evolution have been published [52, 53, 73]). A major difference between the two types of cells is the different organization of cellular membranes. In most prokaryotes, membrane-associated processes, like the

biosynthesis of membrane proteins and membrane lipids, and attachment of the single chromosome to a membrane, are associated with the plasma membrane. In contrast, in eukaryotes, these processes are associated with the nuclear/ER membrane system, whereas the plasma membrane serves other functions (e.g., exo- and endocytosis).

The nuclear/ER membrane system and the eukaryotic plasma membrane have different compositions in terms of their protein and lipid constituents. The nuclear/ER membrane system is composed mainly of phospholipids (as is the plasma membrane of eubacteria), whereas the plasma membrane of eukaryotes contains large amounts of sterols and glycolipids (180, 320). These differences probably reflect adaptations of the lipid composition of membranes for their different functions and may be important in relation to the evolution of the endomembrane system.

The following scenario can be envisaged. Removal of certain functions from the plasma membrane during evolution facilitated adaptation of this membrane to other processes (e.g., exo- and endocytosis). Since biogenesis of membrane proteins and lipids is restricted to the ER-type membrane, separation of these membrane domains required the evolution of a transfer system for proteins and membrane lipids between the nuclear/ER system and the plasma membrane. A two-step model for the evolution of the endomembrane system is presented in Fig. 11.

Extant eukaryotic cells use mainly retrieval systems for targeting proteins within the secretory pathway (see the section on protein targeting), which involves cycling of proteins between two adjacent membrane compartments. This might be a key to understanding the evolution of a Golgi compartment. Complete biochemical individuality between two membrane systems (i.e., nuclear/ER and plasma membrane) is not possible when direct vesicular transport between them is occurring and when protein retrieval is the major protein-targeting mechanism. An additional compartment intercalated between the ER and the plasma membrane would also help to maintain different lipid compositions. Whether the lipid composition of the plasma membrane is defined in the secretory pathway (with a gradient from ER to the plasma membrane) or in the endocytotic pathway is currently debated (e.g., transport of glucosylceramide to the plasma membrane from the GA [11] or through a non-GA pathway [323] (for reviews, see references 4, 37, and 320). However, recent results indicate that at least the ratio between phosphatidylinositol and phosphatidylcholine is altered in the outer membrane leaflet of a Golgi cisterna by cytosolic phosphatidylinositol transfer protein (yeast Sec14 [79, 230]). This process is essential for Golgi function (see the detailed discussion on the role of Sec14 in reference 293). In addition, ARF stimulates phospholipase D (34, 171), which hydrolyzes phosphatidylcholine and might help to alter the lipid composition in the GA. The current models of endomembrane evolution (Fig. 11) (52, 53, 73) assume a sequential evolution of vesicular transport steps. Therefore, it is most likely that all vesicular transport systems had a common origin. This is most obvious for COPI and clathrin-coated vesicles: β-COP and β-adaptin are homologous proteins (93, 273, 281), as are ζ-COP and AP17/AP19 (273). The binding of coatamer and AP1 depend on ARF (232, 298, 312). In addition, an ER retrieval signal related to the tyrosine containing endocytosis signal (200) and an endocytosis signal related to the ER retrieval signal (KKXX) (164) were recently found. In contrast, no obvious homologies between COPII and any other coated-vesicle type was found. However, the formation of vesicles is similar: as for clathrin and COPI, a small ARF-like GTP-binding protein must be activated prior to binding of the coat proteins of COPII to the membrane. In addition, two of the coat proteins of COPII show the WD amino acid sequence motifs, which are also present in COPI proteins (273). Thus, it seems most likely that all characterized coated vesicle types evolved from a common ancestor.

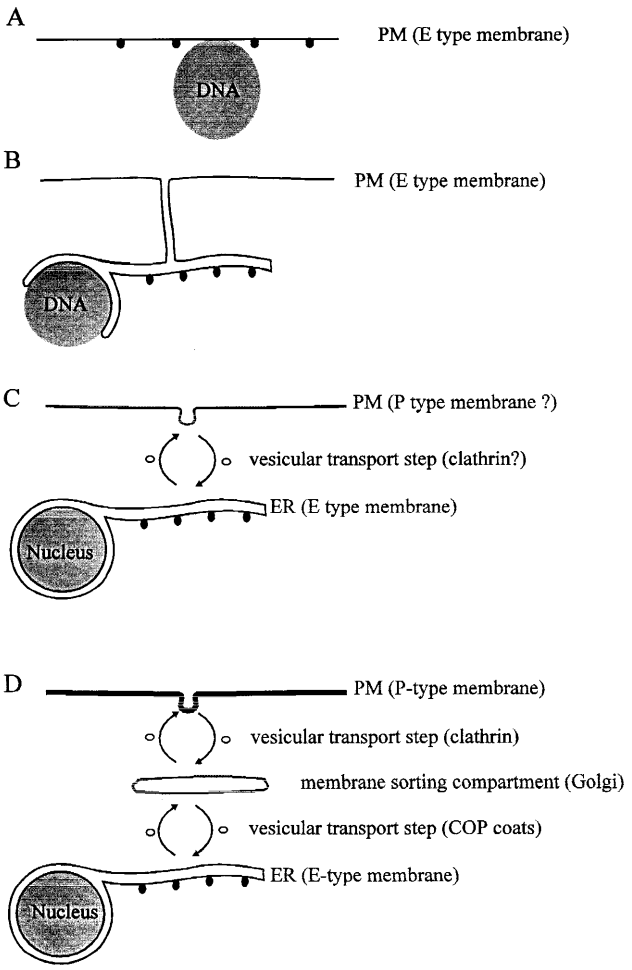


FIG. 11. Evolution of the secretory pathway showing hypothetical steps in the evolution of a primitive endomembrane system. E type membrane, membrane consisting of phospholipids; P type membrane, membrane consisting of phospholipids and large amounts of sterols and glycolipids.

Once a Golgi-like compartment was formed, the various protist groups and their descendants optimized this basic system for their own requirements. This would also explain the variability of the GA observed. The GA can be adapted to very different cellular environments. A interesting example is the transport of nucleus-encoded plastidic proteins to the plastids by using the secretory pathway. The plastids of euglenoids possess three envelope membranes (127). Nucleus-encoded plastidic proteins carry unusual N-terminal extensions, with homology to the signal sequences of proteins targeted to the ER (55, 283). The 26.5-kDa LHCP II apoprotein of *Euglena gracilis* was localized to the GA by immunogold techniques (236, 237). Recently, protein transport to the plastids via the ER and Golgi was also demonstrated in a biochemical transport assay (301), indicating that the secretory pathway in *E. gracilis* is involved in protein transport into plastids.

ACKNOWLEDGMENTS

We thank B. Bölinger, M. Duszenko, F. Gillin, U. Meindl, R. Peck, U. P. Roos, and B. Surek for supplying micrographs and for helpful discussions, and we thank K. Fischer for technical help in preparing the manuscript.

Work in our laboratory was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

1. **Adair, W. S., and W. J. Snell.** 1990. The *Chlamydomonas reinhardtii* cell wall: structure, biochemistry and molecular biology, p. 15–84. *In* W. S. Adair and M. Mecham (ed.), Organization and assembly of plant and animal extracellular matrix. Academic Press, Inc., San Diego, Calif.
2. **Adam, R. D.** 1991. The biology of *Giardia* spp. Microbiol. Rev. **55:**706–732.
3. **Adoutte, A., N. Gareau de Loubresse, and J. Beisson.** 1984. Proteolytic cleavage and maturation of the crystalline secretion products of *Paramecium*. J. Mol. Biol. **180:**1065–1082.
4. **Allan, D., and K.-J. Kallen.** 1994. Is plasma membrane lipid composition defined in the exocytic or the endocytic pathway? Trends Cell Biol. **4:**350–353.
5. **Allan, V.** 1995. Membrane traffic motors. FEBS Lett. **369:**101–106.
6. **Allen, R. D., and A. K. Fok.** 1993. Nonclathrin vesicle coats and filament networks in the transition zone and *trans*-Golgi region of the Golgi complex of *Paramecium*. J. Struct. Biol. **110:**215–226.
7. **Allen, R. D., C. C. Schroeder, and A. K. Fok.** 1989. Intracellular binding of wheat germ agglutinin by Golgi complexes, phagosomes, and lysosomes of *Paramecium multimicronucleatum*. J. Histochem. Cytochem. **37:**195–202.
8. **Amatayakul-Chantler, S., M. A. J. Ferguson, R. A. Dwek, T. W. Rademacher, R. B. Parekh, I. E. Crandall, and P. C. Newell.** 1991. Cell surface oligosaccharides on *Dictyostelium* during development. J. Cell Sci. **99:**485–495.
9. **Anderson, R. G., and L. Orci.** 1988. A view of acidic intracellular compartments. J. Cell Biol. **106:**539–543.
10. **Aridor, M., S. I. Bannykh, T. Rowe, and W. E. Balch.** 1995. Sequential coupling between COPII and COPI vesicle coats in endoplasmic reticulum to Golgi transport. J. Cell Biol. **131:**875–893.
11. **Babia, T., J. W. Kok, M. Van der Haar, R. Kalicharan, and D. Hoekstra.** 1994. Transport of biosynthetic sphingolipids from Golgi to plasma membrane in HT29 cells: involvement of different carrier vesicle populations. Eur. J. Cell Biol. **63:**172–181.
12. **Balch, W. E., W. G. Dunphy, W. A. Braell, and J. E. Rothman.** 1984. Reconstitution of the transport of protein between successive compartments of the Golgi measured by the coupled incorporation of N-acetylglucosamine. Cell **39:**405–416.
13. **Balch, W. E., and M. G. Farquhar.** 1995. Beyond bulk flow. Trends Cell Biol. **5:**16–19.
14. **Balshüsemann, D., and L. Jaenicke.** 1990. The oligosaccharides of the glycoprotein pheromone of *Volvox carteri* f. *nagariensis* Iyengar (Chlorophyceae). Eur. J. Biochem. **192:**231–237.
15. **Banfield, D. K., M. J. Lewis, and H. R. B. Pelham.** 1995. A SNARE-like protein required for traffic through the Golgi complex. Nature (London) **375:**806–809.
16. **Bangs, J. D., N. W. Andrews, G. W. Hart, and P. T. Englund.** 1986. Post-translational modification and intracellular transport of a trypanosome variant surface glycoprotein. J. Cell Biol. **103:**255–263.
17. **Bangs, J. D., D. Hereld, L. Krakow, G. W. Hart, and P. T. Englund.** 1985. Rapid processing of the carboxy terminus of a trypanosome variant surface glycoprotein. Proc. Natl. Acad. Sci. USA **182:**3207–3211.
18. **Bangs, J. D., L. Uyetake, M. J. Brinckman, A. E. Balber, and J. C. Boothroyd.** 1993. Molecular cloning and cellular localization of a BiP homologue in *Trypanosoma brucei*. Divergent ER retention signals in a lower eukaryote. J. Cell Sci. **105:**1101–1113.
19. **Banting, G., J. Benting, and K. Lingelbach.** 1995. A minimalist view of the secretory pathway in *Plasmodium falciparum*. Trends Cell Biol. **5:**340–343.
20. **Bar-Peled, M., A. Da Silva Conceiçao, L. Frigerio, and N. V. Raikhel.** 1995. Expression and regulation of *aERD2*, a gene encoding the KDEL receptor homolog in plants, and other genes encoding proteins involved in ER-Golgi vesicular trafficking. Plant Cell **7:**667–676.
21. **Bassham, D. C., and N. V. Raikhel.** 1996. Transport proteins in the plasma membrane and the secretory system. Trends Plant Sci. **1:**15–20.
22. **Becker, B., B. Bölinger, and M. Melkonian.** 1995. Anterograde transport of algal scales through the Golgi complex is not mediated by vesicles. Trends Cell Biol. **5:**305–307.
23. **Becker, B., B. Marin, and M. Melkonian.** 1994. Structure, composition and biogenesis of prasinophyte cell coverings. Protoplasma **181:**233–244.
24. **Becker, B., and M. Melkonian.** 1995. Intra-Golgi transport mediated by vesicles? Bot. Acta **108:**172–173.
25. **Becker, B., L. Perasso, A. Kammann, M. Salzburg, and M. Melkonian.** 1996. Scale-associated glycoproteins of *Scherffelia dubia* (Chlorophyta) form high-molecular-weight complexes between the scale layers and the flagellar membrane. Planta **191:**503–510.
26. **Becker, D., and M. Melkonian.** 1992. N-linked glycoproteins associated with flagellar scales in a flagellate green alga: characterization of interactions. Eur. J. Cell Biol. **57:**109–116.
27. **Beckers, C. J. M., and W. E. Balch.** 1989. Calcium and GTP: essential components in vesicular trafficking between the endoplasmic reticulum and Golgi apparatus. J. Cell Biol. **108:**1245–1256.
28. **Beckers, C. J. M., M. R. Block, B. S. Glick, J. E. Rothman, and W. E. Balch.** 1989. Vesicular transport between the endoplasmic reticulum and the Golgi stack requires the NEM-sensitive fusion protein. Nature (London) **339:**397–398.
29. **Beh, C. T., and M. D. Rose.** 1995. Two redundant systems maintain levels of resident proteins within the yeast endoplasmic reticulum. Proc. Natl. Acad. Sci. USA **92:**9820–9823.
30. **Bennett, M. K.** 1995. SNAREs and the specificity of transport vesicle targeting. Curr. Opin. Cell Biol. **7:**581–586.
31. **Beranger, F., H. Paterson, S. Powers, J. De Gunzburg, and J. F. Hancock.** 1994. The effector domain of Rab6, plus a highly hydrophobic C terminus, is required for Golgi apparatus localization. Mol. Cell. Biol. **14:**744–758.
32. **Bockman, D. E., and W. B. Winborn.** 1968. Electron microscopic localization of exogenous ferritin within vacuoles of *Giardia muris*. J. Protozool. **15:**26–30.
33. **Boguski, M. S., and F. McCormick.** 1993. Proteins regulating ras and its relatives. Nature (London) **366:**643–654.
34. **Boman, A. L., and R. A. Kahn.** 1995. Arf proteins: the membrane traffic police. Trends Biochem. Sci. **20:**147–150.
35. **Boose, J. A., and E. J. Henderson.** 1991. Conditional intercellular cohesion in a *Dictyostelium discoideum* mutant which is temperature sensitive for correct processing of asparagine-linked oligosaccharides. Glycobiology **1:**295–305.
36. **Boothroyd, J. C., C. A. Paynter, G. A. M. Cross, A. Bernards, and P. Borst.** 1981. Variant surface glycoproteins of *Trypanosoma brucei* synthesized with cleavable hydrophobic sequences at the carboxy and amino termini. Nucleic Acids Res. **9:**4735–4743.
37. **Bretscher, M. S., and S. Munro.** 1993. Cholesterol and the Golgi apparatus. Science **261:**1280–1281.
38. **Brickman, M. J., and A. E. Balber.** 1994. Transport of a lysosomal membrane glycoprotein from the Golgi to endosomes and lysosomes via the cell surface in African trypanosomes. J. Cell Sci. **107:**3191–3200.
39. **Brown, R. M., Jr.** 1971. Movement of the protoplast and function of the Golgi-apparatus in the algae. Publ. Wiss. Film Sekt. Biol. **7:**361.
40. **Brown, R. M., Jr.** 1974. The Golgi apparatus and endomembrane system: its role in the biosynthesis, transport and secretion of wall constituents in *Pleurochrysis*. Port. Acta Biol. **14:**369–384.
41. **Brown, R. M., Jr., and D. K. Romanovicz.** 1976. Biogenesis and structure of Golgi-derived cellulosic scales in *Pleurochrysis*. I. Role of the endomembrane system in scale assembly and exocytosis. Appl. Polymer Symp. **28:**537–585.
42. **Brown, W. J., D. B. DeWald, S. D. Emr, H. Plutner, and W. E. Balch.** 1995. Role of phosphatidylinositol 3-kinase in the sorting and transport of newly synthesized lysosomal enzymes in mammalian cells. J. Cell Biol. **130:**781–796.
43. **Browning, D. D., M. B. Poludnikiewicz, G. A. Proteau, and D. H. O'Day.** 1993. The regulation of GTP-binding proteins during fertilization and zygote differentiation in *Dictyostelium discoideum*. Exp. Cell Res. **205:**240–245.
44. **Busch, G. R., and B. D. Satir.** 1989. The secretory vesicle in living *Paramecium* is acidic. J. Cell Sci. **92:**197–204.
45. **Bush, J., K. Franek, J. Daniel, G. B. Spiegelman, G. Weeks, and J. Cardelli.** 1993. Cloning and characterization of five novel *Dictyostelium discoideum rab*-related genes. Gene **136:**55–60.
46. **Bush, J., K. Nolta, J. Rodriguez-Paris, N. Kaufmann, T. O'Halloran, T. Ruscetti, L. Temesvari, T. Steck, and J. Cardelli.** 1994. A Rab4-like GTPase in *Dictyostelium discoideum* colocalizes with V-H^+-ATPases in reticular membranes of the contractile vacuole complex and in lysosomes. J. Cell Sci. **107:**2801–2812.
47. **Bush, J. M., D. L. Ebert, and J. A. Cardelli.** 1990. Alterations to N-linked oligosaccharides which affect intracellular transport rates and regulated secretion but not sorting of lysosomal acid phosphatase in *Dictyostelium discoideum*. Arch. Biochem. Biophys. **283:**158–166.
48. **Canning, E. U.** 1990. Phylum Microspora, p. 53–72. *In* L. Margulis, J. O. Corliss, M. Melkonian, and D. Chapman (ed.), Handbook of protoctista. Jones & Bartlett Publishers, Boston.
49. **Cardelli, J. A., J. M. Bush, D. Ebert, and H. H. Freeze.** 1990. Sulfated *N*-linked oligosaccharides affect secretion but are not essential for the transport, proteolytic processing, and sorting of lysosomal enzymes in *Dictyostelium discoideum*. J. Biol. Chem. **265:**8847–8853.
50. **Cardelli, J. A., and R. L. Dimond.** 1988. Transport and targeting of lysosomal enzymes in *Dictyostelium discoideum*, p. 363–399. *In* R. Das and P. Robbins (ed.), Protein transfer and organelle biosynthesis. Academic Press, Inc., Orlando, Fla.
51. **Cardelli, J. A., J. M. Richardson, and D. Miears.** 1989. Role of acidic intracellular compartments in the biosynthesis of *Dictyostelium* lysosomal

enzymes: the weak bases ammonium chloride and chloroquine differentially affect proteolytic processing and sorting. J. Biol. Chem. **254:**3454–3463.
52. **Cavalier-Smith, T.** 1988. Eukaryote cell evolution, p. 203–223. *In* W. Greuter and B. Zimmer (ed.), Proceedings of the XIV International Botanical Congress. Koeltz Scientific Books, Koenigstein, Germany.
53. **Cavalier-Smith, T.** 1991. The evolution of cells, p. 271–304. *In* S. Osawa and T. Honjo (ed.), Evolution of life—fossils, molecules, and culture. Springer-Verlag, Tokyo.
54. **Cavalier-Smith, T.** 1993. Kingdom Protozoa and its 18 phyla. Microbiol. Rev. **57:**953–994.
55. **Chan, R. L., M. Keller, J. Canaday, J.-H. Weil, and P. Imbault.** 1990. Eight small subunits of *Euglena* ribulose 1-5 bisphosphate carboxylase/oxygenase are translated from a large mRNA as a polyprotein. EMBO J. **9:**333–338.
56. **Chavrier, P., J. P. Gorvel, E. Stelzer, K. Simons, J. Gruenberg, and M. Zerial.** 1991. Hypervariable C-terminal domain of rab proteins acts as a targeting signal. Nature (London) **353:**769–772.
57. **Chavrier, P., R. G. Parton, H. P. Hauri, K. Simons, and M. Zerial.** 1990. Localization of low molecular weight GTP binding proteins to exocytic and endocytic compartments. Cell **62:**317–329.
58. **Cladaras, M. H., and A. Kaplan.** 1984. Maturation of α-mannosidase in *Dictyostelium discoideum*. J. Biol. Chem. **259:**14165–14169.
59. **Clayton, C., T. Häusler, and J. Blattner.** 1995. Protein trafficking in kinetoplastid protozoa. Microbiol. Rev. **59:**324–344.
60. **Cole, N. B., J. Ellenberg, J. Song, and J. Lippincott-Schwartz.** 1995. Evidence for recycling of Golgi resident proteins through the ER. Mol. Biol. Cell **6**(Suppl.)**:**329. (Abstract.)
61. **Cole, N. B., and J. Lippincott-Schwartz.** 1995. Organization of organelles and membrane traffic by microtubules. Curr. Opin. Cell Biol. **7:**55–64.
62. **Conibear, E., and T. H. Stevens.** 1995. Vacuolar biogenesis in yeast: sorting out the sorting proteins. Cell **83:**513–516.
63. **Coukell, M. B., A. M. Cameron, and N. R. Adames.** 1992. Involvement of intracellular calcium in protein secretion in *Dictyostelium discoideum*. J. Cell Sci. **103:**371–380.
64. **Crary, J. L., and K. Haldar.** 1992. Brefeldin A inhibits protein secretion and parasite maturation in the ring stage of *Plasmodium falciparum*. Mol. Biochem. Parasitol. **53:**185–192.
65. **Cross, G. A. M.** 1984. Structure of the variant glycoproteins and surface coat of *Trypanosoma brucei*. Philos. Trans. R. Soc. London Ser. B **307:**3–12.
66. **Cross, G. A. M.** 1987. Eukaryotic protein modification and membrane attachment via phosphatidylinositol. Cell **48:**179–181.
67. **Dahms, N. M., P. Lobel, and S. Kornfeld.** 1989. Mannose 6-phosphate receptors and lysosomal protein targeting. J. Biol. Chem. **264:**12115–12118.
68. **Dairman, M., N. Donofrio, and D. S. Domozych.** 1995. The effects of brefeldin A upon the Golgi apparatus of the green algal flagellate, *Gloeomonas kupfferi*. J. Exp. Bot. **46:**181–186.
69. **Das, A., H. G. Elmendorf, W. I. Li, and K. Haldar.** 1994. Biosynthesis, export and processing of a 45 kDa protein detected in membrane clefts of erythrocytes infected with *Plasmodium falciparum*. Biochem. J. **302:**487–496.
70. **Davidson, H. W.** 1995. Wortmannin causes mistargeting of procathepsin D. Evidence for the involvement of a phosphatidylinositol 3-kinase in vesicular transport to lysosomes. J. Cell Biol. **130:**797–805.
71. **Davidson, H. W., C. H. McGowan, and W. E. Balch.** 1992. Evidence for the regulation of exocytic transport by protein phosphorylation. J. Cell Biol. **116:**1343–1355.
72. **Davis, S. J.** 1988. Oligosaccharide heterogeneity of glycoproteins sulfated during the vegetative growth of *Dictyostelium discoideum*. J. Cell. Biochem. **38:**77–86.
73. **de Duve, C.** 1990. The primitive phagocyte, p. 511–514. *In* P. Nardon, V. Gianinazzi-Pearson, A. M. Grenier, L. Margulis, and D. C. Smith (ed.), Endocytobiology IV. Institut National de la Recherche Agronomique, Paris.
74. **De Mattels, M. A., G. Santini, R. A. Kahn, G. Di Tullio, and A. Luini.** 1993. Receptor and protein kinase C-mediated regulation of ARF binding to the Golgi complex. Nature (London) **364:**818–821.
75. **Denecke, J., M. H. S. Goldman, J. Demoler, J. Seurinck, and J. Botterman.** 1991. The tobacco luminal binding protein is encoded by a a multigene family. Plant Cell **3:**1025–1035.
76. **D'Enfert, C., M. Gensse, and C. Gaillardin.** 1992. Fission yeast and a plant have functional homologues of the Sar1 and Sec12 proteins involved in ER to Golgi traffic in budding yeast. EMBO J. **11:**4205–4211.
77. **Denning, G. M., and A. B. Fulton.** 1989. Purification and characterization of clathrin-coated vesicles from *Chlamydomonas*. J. Protozool. **36:**334–340.
78. **Descoteaux, A., Y. Luo, S. J. Turco, and S. M. Beverley.** 1995. A specialized pathway affecting virulence glycoconjugates of *Leishmania*. Science **269:**1869–1872.
79. **de Vries, K. J., A. A. J. Heinrichs, E. Cunningham, F. Brunink, J. Westerman, P. J. Somerharju, S. Cockcroft, K. W. A. Wirtz, and G. T. Snoek.** 1995. An isoform of the phosphatidylinositol-transfer protein transfers sphingomyelin and is associated with the Golgi system. Biochem. J. **310:**643–649.
80. **Dieckmann-Schuppert, A., S. Bender, M. Odenthal-Schnittler, E. Bause, and R. T. Schwarz.** 1992. Apparent lack of *N*-glycosylation in the asexual intraerythrocytic stage of *Plasmodium falciparum*. Eur. J. Biochem. **205:**815–825.
81. **Dietmaier, W., S. Fabry, H. Huber, and R. Schmitt.** 1995. Analysis of a family of *ypt* genes and their products from *Chlamydomonas reinhardtii*. Gene **158:**41–50.
82. **Domozych, D. S.** 1986. The role of cations in dictyosomal integrity, processing and secretion in the scale-producing alga, *Pyramimonas*. J. Submicrosc. Cytol. **18:**567–576.
83. **Domozych, D. S.** 1987. An experimental analysis of the dictyosome in the green alga, *Tetraselmis convolutae*. J. Exp. Bot. **38:**1399–1411.
84. **Domozych, D. S.** 1989. The endomembrane system and mechanisms of membrane flow in the green alga, *Gloeomonas kupfferi* (Volvocales, Chlorophyta). I. An ultrastructural analysis. Protoplasma **149:**95–107.
85. **Domozych, D. S.** 1989. The endomembrane system and mechanisms of membrane flow in the green alga, *Gloeomonas kupfferi* (Volvocales, Chlorophyta). II. A cytochemical analysis. Protoplasma **149:**108–119.
86. **Domozych, D. S.** 1991. The Golgi apparatus and membrane trafficking in green algae. Int. Rev. Cytol. **131:**213–253.
87. **Domozych, D. S., and M. Dairman.** 1993. Synthesis of the inner cell wall layer of the chlamydomonad flagellate, *Gloeomonas kupfferi*. Protoplasma **176:**1–13.
88. **Domozych, D. S., and T. J. Korbusieski.** 1985. The disruption of dictyosome structure, polarity and secretory activity in the scale-producing green alga, *Pyramimonas inconstans*. J. Exp. Bot. **36:**1304–1312.
89. **Domozych, D. S., K. R. Mattox, and K. D. Stewart.** 1981. In vivo cell wall ontogenesis in unicellular chlorophycean flagellates. 2. Golgi apparatus activation, extracellular assembly and internal cellular controls. Cytobios **32:**167–178.
90. **Domozych, D. S., B. Wells, and P. J. Shaw.** 1992. Scale biogenesis in the green alga, *Mesostigma viride*. Protoplasma **167:**19–32.
91. **Donaldson, J. G., D. Finazzi, and R. D. Klausner.** 1993. Brefeldin A inhibits Golgi membrane-catalyzed exchange of guanine nucleotide onto ARF protein. Nature (London) **360:**350–352.
92. **Donaldson, J. G., R. A. Kahn, J. Lippincott-Schwartz, and R. D. Klausner.** 1991. Binding of ARF and β-COP to Golgi membranes: possible regulation by a trimeric G protein. Science **254:**1197–1199.
93. **Duden, R., G. Griffiths, R. Frank, P. Argos, and T. E. Kreis.** 1991. β-COP, a 110 kD protein associated with non-clathrin-coated vesicles and the Golgi complex, shows homology to β-adaptin. Cell **64:**649–665.
94. **Dunphy, W. G., R. Brands, and J. E. Rothman.** 1985. Attachment of terminal n-acetylglucosamine to asparagine-linked oligosaccharides occurs in central cisternae of the Golgi stack. Cell **40:**463–472.
95. **Duszenko, M., I. E. Ivanov, M. A. J. Ferguson, H. Plesken, and G. A. M. Cross.** 1988. Intracellular transport of a variant surface glycoprotein in *Trypanosoma brucei*. J. Cell Biol. **106:**77–86.
96. **Duszenko, M., and A. Seyfang.** 1993. Intracellular transport of variant surface glycoproteins in trypanosomes. Adv. Cell Mol. Biol. Membr. **2:**227–258.
97. **Dutcher, S. K., and W. Gibbons.** 1988. Isolation and characterization of dominant tunicamycin resistance mutations in *Chlamydomonas reinhardtii* (Chlorophyceae). J. Phycol. **24:**230–236.
98. **Dyer, D.** 1990. Phylum Zoomastigina, class Parabasalia, p. 252–257. *In* L. Margulis, J. O. Corliss, M. Melkonian, and D. J. Chapman (ed.), Handbook of protoctista. Jones & Bartlett Publishers, Boston.
99. **Ebert, D. L., J. M. Bush, R. L. Dimond, and J. A. Cardelli.** 1989. Biogenesis of lysosomal enzymes in the α-glucosidase II-deficient *modA* mutant of *Dictyostelium discoideum*: retention of α-1,3-linked glucose on N-linked oligosaccharides delays intracellular transport but does not alter sorting of α-mannosidase or β-glucosidase. Arch. Biochem. Biophys. **273:**479–490.
100. **Elazar, Z., T. Mayer, and J. E. Rothman.** 1994. Removal of Rab GTP-binding proteins from Golgi membranes by GDP dissociation inhibitor inhibits inter-cisternal transport in the Golgi stacks. J. Biol. Chem. **269:**794–797.
101. **Elmendorf, H. G., J. D. Bangs, and K. Haldar.** 1992. Synthesis and secretion of proteins by released malarial parasites. Mol. Biochem. Parasitol. **52:**215–230.
102. **Elmendorf, H. G., and K. Haldar.** 1993. Secretory transport in *Plasmodium*. Parasitol. Today **9:**98–102.
103. **Elmendorf, H. G., and K. Haldar.** 1993. Identification and localization of ERD2 in the malaria parasite *Plasmodium falciparum*: separation from sites of sphingomyelin synthesis and implications for organization of the Golgi. EMBO J. **12:**4763–4773.
104. **Elmendorf, H. G., and K. Haldar.** 1994. *Plasmodium falciparum* exports the Golgi marker sphingomyelin synthase into a tubovesicular network in the cytoplasm of mature erythrocytes. J. Cell Biol. **124:**449–462.
105. **Erdos, G. W., and C. M. West.** 1989. Formation and organization of the spore coat of *Dictyostelium discoideum*. Exp. Mycol. **13:**169–182.
106. **Esteve, J.-C.** 1972. L'appareil de Golgi des ciliés. Ultrastructure, particulièrement chez *Paramecium*. J. Protozool. **19:**609–618.
107. **Faber, W. W., Jr., and H. R. Preisig.** 1994. Calcified structures and calcification in protists. Protoplasma **181:**78–105.
108. **Fabry, S., A. Jacobsen, H. Huber, K. Palme, and R. Schmitt.** 1993. Struc-

ture, expression, and phylogenetic relationships of a family of *ypt* genes encoding small G-proteins in the green alga *Volvox carteri*. Curr. Genet. **24:**229–240.
109. **Fabry, S., N. Nass, H. Huber, K. Palme, L. Jaenicke, and R. Schmitt.** 1992. The *yptV1* gene encodes a small G-protein in the green alga *Volvox carteri*: gene structure and properties of the gene product. Gene **118:**153–162.
110. **Farquhar, M. G., and G. E. Palade.** 1981. The Golgi-apparatus (complex) (1954–1981)—from artefact to center stage. J. Cell Biol. **91:**77s–103s.
111. **Feely, D. E., and J. K. Dyer.** 1987. Localization of acid phosphatase activity in *Giardia lamblia* and *Giardia muris* trophozoites. J. Protozool. **34:**80–83.
112. **Feely, D. E., S. L. Larson, and D. G. Chase.** 1984. Structure of the trophozoite and the cyst, p. 3–31. *In* S. L. Erlandsen and E. A. Meyer (ed.), *Giardia* and giardiasis: biology, pathogenesis and epidemiology. Plenum Press, New York.
113. **Ferguson, M. A. J., M. Duszenko, G. S. Lamont, P. Overath, and G. A. M. Cross.** 1985. Biosynthesis of *Trypanosoma brucei* variant surface glycoproteins: N-glycolysation and addition of a novel glycan-phosphoinositol membrane anchor. J. Biol. Chem. **261:**356–362.
114. **Ferro-Novick, S., and R. Jahn.** 1994. Vesicle fusion from yeast to man. Nature (London) **370:**191–193.
115. **Fraga, D., and R. D. Hinrichsen.** 1994. The identification of a complex family of low-molecular-weight GTP binding protein homologues from *Paramecium tetraurelia* by PCR cloning. Gene **147:**145–148.
116. **Freeze, H. H.** 1985. Mannose 6-sulfate is present in the N-linked oligosaccharides of lysosomal enzymes of *Dictyostelium*. Arch. Biochem. Biophys. **243:**690–693.
117. **Freeze, H. H.** 1986. Modifications of lysosomal enzymes in *Dictyostelium discoideum*. Mol. Cell. Biol. **72:**47–65.
118. **Freeze, H. H., and D. Wolgast.** 1986. Biosynthesis of methylphospho-mannosyl residues of *Dictyostelium discoideum* glycoproteins. J. Biol. Chem. **261:**135–141.
119. **Friend, D. S.** 1966. The fine structure of *Giardia muris*. J. Cell Biol. **29:** 317–332.
120. **Funk, V. A., A. Jardim, and R. W. Olafson.** 1994. An investigation into the significance of the N-linked oligosaccharides of *Leishmania* gp63. Mol. Biochem. Parasitol. **63:**23–35.
121. **Gabel, C. A., C. E. Costello, V. N. Reinhold, L. Kurtz, and S. Kornfeld.** 1984. Identification of methylphosphomannosyl residues as components of the high mannose oligosaccharides of *Dictyostelium discoideum* glycoproteins. J. Biol. Chem. **259:**13762–13769.
122. **Garofalo, R. S., and B. H. Satir.** 1984. *Paramecium* secretory content: quantitative studies on in vitro expansion and its regulation by calcium and pH. J. Cell Biol. **99:**224–234.
123. **Gareau de Loubresse, N.** 1993. Early steps of the secretory pathway in *Paramecium*: ultrastructural, immunocytochemical and genetic analysis of trichocyst biogenesis. Adv. Cell Mol. Biol. Membr. **2:**27–59.
124. **Gareau de Loubresse, N., M.-C. Gautier, and L. Sperling.** 1994. Immature secretory granules are not acidic in *Paramecium*: implications for sorting to the regulated pathway. Biol. Cell. **82:**139–147.
125. **Gaut, J. R., and L. M. Hendershot.** 1993. The modification and assembly of proteins in the endoplasmic reticulum. Curr. Opin. Cell Biol. **5:**589–595.
126. **Gautier, M.-C., N. G. de Loubresse, L. Madeddu, and L. Sperling.** 1994. Evidence for defects in membrane traffic in *Paramecium* secretory mutants unable to produce functional storage granules. J. Cell Biol. **124:**893–902.
127. **Gibbs, S. P.** 1978. The chloroplasts of *Euglena* may have evolved from symbiotic green algae. Can. J. Bot. **56:**2883–2889.
128. **Gilligan, D. M., and B. H. Satir.** 1982. Protein phosphorylation/dephosphorylation and stimulus-secretion coupling in wild type and mutant *Paramecium*. J. Biol. Chem. **257:**13903–13906.
129. **Gilligan, D. M., and B. H. Satir.** 1983. Stimulation and inhibition of secretion in *Paramecium*: role of divalent cations. J. Cell Biol. **97:**224–234.
130. **Gillin, F. D., P. Hagblom, J. Harwood, S. B. Aley, D. S. Reiner, M. McCaffrey, M. So, and D. G. Guiney.** 1990. Isolation and expression of the gene for a major surface protein of *Giardia lamblia*. Proc. Natl. Acad. Sci. USA **87:**4463–4467.
131. **Gillin, F. D., D. S. Reiner, M. Gault, H. Douglas, S. Das, A. Wunderlich, and J. Sauch.** 1987. Encystation and expression of cyst antigens by *Giardia lamblia* in vitro. Science **235:**1040–1043.
132. **Glary, D. O., I. C. Griff, and J. E. Rothman.** 1990. SNAPs, a family of NSF attachment proteins involved in intracellular membrane fusion in animals and yeast. Cell **61:**709–721.
133. **Glas-Albrecht, R., B. Kaesberg, G. Knoll, K. Allmann, R. Pape, and H. Plattner.** 1991. Synchronised secretory organelle docking in *Paramecium*. Saltatory movement along microtubules transiently formed from ciliary basal bodies and selective exclusion of microinjected heterologous organelles. J. Cell Sci. **100:**45–54.
134. **Gleeson, P. A., R. D. Teasdale, and J. Burke.** 1994. Targeting of proteins to the Golgi apparatus. Glycoconjugate J. **11:**381–394.
135. **Grab, D. J., S. Ito, U. A. K. Kara, and L. Rovis.** 1984. Glycosyltransferase activities in Golgi complex and endoplasmic reticulum fractions isolated from African trypanosomes. J. Cell Biol. **99:**569–577.
136. **Graham, T. R., and S. D. Emr.** 1991. Compartmental organization of Golgi-specific protein modification and vacuolar protein sorting events defined in a yeast sec18 (NSF) mutant. J. Cell Biol. **114:**207–218.
137. **Grassé, P. P.** 1925. Vacuome et appareil de Golgi des Euglènes. C. R. Acad. Sci. Paris **181:**482–484.
138. **Grief, C., and P. J. Shaw.** 1987. Assembly of cell-wall glycoproteins of *Chlamydomonas reinhardtii*: oligosaccharides are added in medial and trans Golgi compartments. Planta **171:**302–312.
139. **Griffiths, G., R. W. Doms, T. Mayhew, and J. Lucocq.** 1995. The bulk-flow hypothesis: not quite the end. Trends Cell Biol. **5:**9–19.
140. **Gruber, H. E., and B. Rosario.** 1979. Ultrastructure of the Golgi apparatus and contractile vacuole in *Chlamydomonas reinhardtii*. Cytologia **44:**505–526.
141. **Gruenberg, J., and T. E. Kreis.** 1995. Membranes and sorting. Curr. Opin. Cell Biol. **7:**519–522.
142. **Grunow, A., B. Becker, and M. Melkonian.** 1993. Isolation and characterization of the Golgi apparatus from a flagellate scaly green alga. Eur. J. Cell Biol. **61:**10–20.
143. **Gumpel, N. J., and S. Purton.** 1994. Playing tag with *Chlamydomonas*. Trends Cell Biol. **4:**299–301.
144. **Günther, R., E. Bause, and L. Jaenicke.** 1987. UDP-L-arabinose-hydroxyproline-O-glycosyltransferases in *Volvox carteri*. FEBS Lett. **221:**293–298.
145. **Gupta, R. S., K. Aitken, M. Falah, and B. Singh.** 1994. Cloning of the *Giardia lamblia* heat shock protein HSP70 homologs: implications regarding origin of eucaryotic cells and of endoplasmic reticulum. Proc. Natl. Acad. Sci. USA **91:**2895–2899.
146. **Haizel, T., T. Merkle, F. Turck, and F. Nagy.** 1995. Characterization of membrane-bound small GTP-binding proteins from *Nicotiana tabacum*. Plant Physiol. **108:**59–67.
147. **Hall, R. P.** 1930. Osmiophilic inclusions similar to Golgi apparatus in the flagellates *Chromulina*, *Chilomonas* and *Astasia*. Arch. Protistenkd. **69:**7–22.
148. **Harris, S., and M. G. Waters.** 1995. A yeast early Golgi protein is localized via recycling. Mol. Biol. Cell **6**(Suppl.)**:**123. (Abstract.)
149. **Hausmann, K.** 1978. Extrusive organelles in protists. Int. Rev. Cytol. **52:** 197–276.
150. **Hausmann, K., A. K. Fok, and R. D. Allen.** 1988. Immunocytochemical analysis of trichocyst structure in *Paramecium*. J. Ultrastruct. Mol. Struct. Res. **99:**213–225.
151. **Helms, J. B., D. J. Palmer, and J. E. Rothman.** 1993. Two distinct populations of ARF bound to Golgi membranes. J. Cell Biol. **121:**751–760.
152. **Helms, J. B., and J. E. Rothman.** 1993. Inhibition by brefeldin A of a Golgi membrane enzyme that catalyses exchange of guanine nucleotide bound to ARF. Nature (London) **360:**352–354.
153. **Hinterberg, K., A. Scherf, J. Gysin, T. Toyoshima, M. Aikawa, J.-C. Mazie, L. P. da Silva, and D. Mattei.** 1994. *Plasmodium falciparum*: the Pf332 antigen is secreted from the parasite by a brefeldin A-dependent pathway and is translocated to the erythrocyte membrane via the Maurer's clefts. Exp. Parasitol. **79:**279–291.
154. **Hoe, M. H., P. Slusarewicz, T. Misteli, R. Watson, and G. Warren.** 1995. Evidence for recycling of the resident *medial/trans* Golgi enzyme, *N*-acetylglucosaminyltransferase I, in ldlD cells. J. Biol. Chem. **270:**25057–25063.
155. **Höftberger, M., T. Url, and U. Meindl.** 1995. Disturbance of the secretory pathway in *Micrasterias denticulata* by tunicamycin and cyclopiazonic acid. Protoplasma **189:**173–179.
156. **Hohmann, H. P., S. Bozzaro, M. Yoshida, R. Merkl, and G. Gerisch.** 1987. Two-step glycosylation of the contact site A protein of *Dictyostelium discoideum* and transport of an incompletely glycosylated form to the cell surface. J. Biol. Chem. **262:**16618–16624.
157. **Horazdovsky, B. G., D. DeWald, and S. D. Emr.** 1995. Protein transport to the yeast vacuole. Curr. Opin. Cell Biol. **7:**544–551.
158. **Howard, R. J.** 1981. Ultrastructural analysis of hyphal tip cell growth in fungi: Spitzenkörner, cytoskeleton and endomembranes after freeze-substitution. J. Cell Sci. **48:**223–229.
159. **Hummel, R., P. Norgaard, P. H. Andreasen, S. Neve, K. Skjodt, D. Tornehave, and K. Kristiansen.** 1992. *Tetrahymena* gene encodes a protein that is homologous with the liver-specific F-antigen and associated with membranes of the Golgi apparatus and transport vesicles. J. Mol. Biol. **228:**850–861.
160. **Hünseler, P., G. Schneidgen-Kleyboldt, and A. Tiedtke.** 1987. Isolation and characterization of a mutant of *Tetrahymena thermophila* blocked in secretion of lysosomal enzymes. J. Cell Sci. **88:**47–55.
161. **Hünseler, P., A. Tiedtke, and K. von Figura.** 1988. Biosynthesis of secreted β-hexosaminidase in *Tetrahymena thermophila*. Biochem. J. **252:**837–842.
162. **Ikonen, E., M. Tagaya, O. Ullrich, C. Montecucco, and K. Simons.** 1995. Different requirements for NSF, SNAP, and Rab proteins in apical and basolateral transport in MDCK cells. Cell **81:**571–580.
163. **Ilg, T., P. Overath, M. A. J. Ferguson, T. Rutherford, D. G. Campbell, and M. J. McConville.** 1994. *O*- and *N*-glycosylation of the *Leishmania mexicana*-secreted acid phosphatase. Characterization of a new class of phosphoserine-linked glycans. J. Biol. Chem. **269:**24073–24081.
164. **Itin, C., F. Kappeler, A. D. Linstedt, and H.-P. Hauri.** 1995. A novel

endocytosis signal related to the KKXX ER-retrieval signal. EMBO J. **14:**2250–2256.
165. **Ivatt, R. L., O. P. Das, E. J. Henderson, and P. W. Robbins.** 1984. Glycoprotein biosynthesis in *Dictyostelium discoideum*: developmental regulation of the protein-linked glycans. Cell **38:**561–567.
166. **Kahn, R. A., F. G. Kern, J. Clark, E. P. Gelmann, and C. Rulka.** 1991. Human ADP-ribosylation factors. J. Biol. Chem. **266:**2606–2614.
167. **Kaushal, G. P., and A. D. Elbein.** 1989. Glycoprotein processing enzymes of plants. Methods Enzymol. **179:**452–478.
168. **Kiyosue, T., and K. Shinozaki.** 1995. Cloning of a carrot cDNA for a member of the family of ADP-ribosylation factors (ARFs) and characterization of the bindings of nucleotides by its product after expression in *E. coli*. Plant Cell Physiol. **36:**849–856.
169. **Kornfeld, S., and I. Mellman.** 1989. The biogenesis of the lysosome. Annu. Rev. Cell Biol. **5:**483–525.
170. **Krijnse-Locker, J., M. Ericsson, P. J. M. Rottier, and G. Griffiths.** 1994. Characterization of the budding compartment of mouse hepatitis virus: evidence that transport from the RER to the Golgi complex requires only one vesicular transport step. J. Cell Biol. **124:**55–70.
171. **Ktistakis, N. T., H. A. Brown, P. C. Sternweis, and M. G. Roth.** 1995. Phospholipase D is present on Golgi-enriched membranes and its activation by ADP ribosylation factor is sensitive to brefeldin A. Proc. Natl. Acad. Sci. USA **92:**4952–4956.
172. **Kuliawat, R., and P. Arvan.** 1994. Distinct molecular mechanisms for protein sorting within immature secretory granules of pancreatic beta-cells. J. Cell Biol. **126:**77–86.
173. **Kumar, N., G. Koski, M. Harada, M. Aikawa, and H. Zheng.** 1991. Induction and localization of *Plasmodium falciparum* stress proteins related to the heat shock protein 70 family. Mol. Biochem. Parasitol. **48:**47–58.
174. **Kumar, N., and H. Zheng.** 1992. Nucleotide sequence of a *Plasmodium falciparum* stress protein with similarity to mammalian 78-kDa glucose-regulated protein. Mol. Biochem. Parasitol. **56:**353–356.
175. **Kurz, S., and A. Tiedtke.** 1993. The Golgi apparatus of *Tetrahymena thermophila*. J. Protozool. **40:**10–13.
176. **Labriola, C., J. J. Cazzulo, and A. J. Parodi.** 1995. Retention of glucose units added by the UDP-GLC:glycoprotein glycosyltransferase delays exit of glycoproteins from the endoplasmic reticulum. J. Cell Biol. **130:**771–779.
177. **Lacoste, C. H., H. H. Freeze, J. A. Jones, and A. Kaplan.** 1989. Characteristics of the sulfation of N-linked oligosaccharides in vesicles from *Dictyostelium discoideum*: *in vitro* sulfation of lysosomal enzymes. Arch. Biochem. Biophys. **273:**505–515.
178. **Ladinsky, M. S., J. R. Kremer, P. S. Furcinitti, J. R. McIntosh, and K. E. Howell.** 1994. HVEM tomography of the *trans*-Golgi network: structural insights and identification of a lace-like vesicle coat. J. Cell Biol. **127:**29–38.
179. **Langreth, S. G., J. B. Jensen, R. T. Reese, and W. Trager.** 1978. Fine structure of human malaria in vitro. J. Protozool. **25:**443–452.
180. **Larsson, C., I. M. Moller, and S. Widell.** 1990. Introduction to the plant plasma membrane—Its molecular composition and organization, p. 1–13. *In* C. Larsson and I. M. Moller (ed.), The plant plasma membrane. Springer-Verlag, KG, Berlin.
181. **Latterich, M., K.-U. Fröhlich, and R. Schekman.** 1995. Membrane fusion and the cell cycle: Cdc48p participates in the fusion of ER membranes. Cell **82:**885–893.
182. **Leadbeater, B. S. C.** 1994. Cell coverings, p. 23–46. *In* J. C. Green and B. S. C. Leadbeater (ed.), The haptophyte algae. Clarendon Press, Oxford.
183. **Lebas, M., and M. Axelos.** 1994. A cDNA encoding a new GTP-binding protein of the ADP-ribosylation factor family from *Arabidopsis*. Plant Physiol. **106:**809–810.
184. **Lee, F.-J. S., J. Moss, and M. Vaughan.** 1992. Human and *Giardia* ADP-ribosylation factors (ARFs) complete ARF function in *Saccharomyces cerevisiae*. J. Biol. Chem. **267:**24441–24445.
185. **Lehtonen, J.** 1983. Action of cytochalasin B on cytoplasmic streaming and morphogenesis in *Micrasterias torreyi* (Conjugatophyceae). Nord. J. Bot. **3:**521–531.
186. **Lenhard, J. M., A. Siegel, and S. J. Free.** 1989. Developing *Dictyostelium* cells contain the lysosomal enzyme α-mannosidase in a secretory granule. J. Cell Biol. **109:**2761–2769.
187. **Letourneur, F., E. C. Gaynor, S. Hennecke, C. Démollière, R. Duden, S. D. Emr, H. Riezman, and P. Cosson.** 1994. Coatamer is essential for retrieval of dilysine-tagged proteins to the endoplasmic reticulum. Cell **79:**1199–1207.
188. **Li, W., G.-A. Keller, and K. Haldar.** 1995. Recognition of a 170 kD protein in mammalian Golgi complexes by an antibody against malarial intraerythrocytic lamellae. Tissue Cell **27:**355–367.
189. **Lindmark, D. G.** 1988. *Giardia lamblia*: localization of hydrolase activities in lysosome-like organelles of trophozoites. Exp. Parasitol. **65:**141–147.
190. **Liu, X., and K.-P. Chang.** 1992. The 63-kilobase circular amplicon of tunicamycin-resistant *Leishmania amazonensis* contains a functional N-acetylglucosamine-1-phosphate transferase gene that can be used as a dominant selectable marker in transfection. Mol. Cell. Biol. **12:**4112–4122.
191. **Lobel, P., K. Fujimoto, R. D. Ye, G. Griffiths, and S. Kornfeld.** 1989. Mutations in the cytoplasmic domain of the 275 kD mannose 6-phosphate receptor differentially alter lysosomal enzyme sorting and endocytosis. Cell **57:**787–796.
192. **Lobo-Da-Cunha, A., and C. Azevedo.** 1993. The Golgi apparatus of the ciliated protozoon *Ichtyophthirius multifiliis*. Eur. J. Protistol. **30:**97–103.
193. **Luján, H. D., A. Marotta, M. R. Mowatt, A. Sciaky, J. Lippincott-Schwartz, and T. E. Nash.** 1995. Developmental induction of Golgi structure and function in the primitive eukaryote *Giardia lamblia*. J. Biol. Chem. **270:**4612–4618.
194. **Lumpert, C. J., R. Glas-Albrecht, E. Eisenmann, and H. Plattner.** 1992. Secretory organelles of *Paramecium* cells (trichocysts) are not remarkably acidic compartments. J. Histochem. Cytochem. **40:**153–160.
195. **Lussier, M., A. M. Sdicu, T. Ketela, and H. Bussey.** 1995. Localization and targeting of the *Saccharomyces cerevisiae* Kre2p/Mnt1p α1,2-mannosyltransferase to a *medial*-Golgi compartment. J. Cell Biol. **131:**913–927.
196. **Luzio, J. P., and G. Banting.** 1993. Eukaryotic membrane traffic: retrieval and retention mechanisms to achieve organelle residence. Trends Biochem. Sci. **18:**395–398.
197. **Machamer, C. E.** 1991. Golgi retention signals: do membranes hold the key? Trends Cell Biol. **1:**141–144.
198. **Madeddu, L., M.-C. Gautier, L. Vayssié, A. Houari, and L. Sperling.** 1995. A large multigene family codes for the polypeptides of the crystalline trichocyst matrix in *Paramecium*. Mol. Biol. Cell **6:**649–659.
199. **Malhotra, V., L. Orci, B. S. Glick, M. R. Block, and J. E. Rothman.** 1988. Role of an N-ethylmaleimide-sensitive transport component in promoting fusion of transport vesicles with cisternae of the Golgi stack. Cell **54:**221–227.
200. **Mallabiabarrena, A., M. A. Jiménez, M. Rico, and B. Alarcón.** 1995. A tyrosine-containing motif mediates ER retention of CD3-E and adopts a helix-turn structure. EMBO J. **14:**2257–2268.
201. **Manning, P., S. L. Erlandsen, and E. L. Jarroll.** 1992. Carbohydrate and amino acid analysis of *Giardia muris* cysts. J. Protozool. **39:**290–296.
202. **Manton, I.** 1966. Observations on scale production in *Pyramimonas amylifera* Conrad. J. Cell Sci. **1:**429–438.
203. **Marsh, M. E.** 1994. Polyanion-mediated mineralization—assembly and reorganization of acidic polysaccharides in the Golgi system of a coccilithophorid alga during mineral deposition. Protoplasma **177:**108–122.
204. **Matsuda, Y., K. Sakamoto, T. Mizuochi, A. Kobata, G. Tamura, and Y. Tsubo.** 1981. Mating type specific inhibition of gametic differentiation of *Chlamydomonas reinhardtii* by tunicamycin. Plant Cell Physiol. **22:**1607–1611.
205. **McCaffery, J. M., G. M. Faubert, and F. D. Gillin.** 1994. *Giardia lamblia*: traffic of a trophozoite variant surface protein and a major cyst wall epitope during growth, encystation and antigenic switching. Exp. Parasitol. **79:**236–249.
206. **McCaffery, J. M., and F. D. Gillin.** 1994. *Giardia lamblia*: ultrastructural basis of protein transport during growth and encystation. Exp. Parasitol. **79:**220–235.
207. **McFadden, G. I., and M. Melkonian.** 1986. Golgi apparatus activity and membrane flow during scale biogenesis in the green flagellate *Scherffelia dubia* (Prasinophyceae). I. Flagellar regeneration. Protoplasma **130:**186–198.
208. **Meindl, U.** 1993. *Micrasterias* cells as a model system for research on morphogenesis. Microbiol. Rev. **57:**415–433.
209. **Meindl, U., S. Lancelle, and P. K. Hepler.** 1992. Vesicle production and fusion during lobe formation in *Micrasterias* visualized by high-pressure freeze fixation. Protoplasma **170:**104–114.
210. **Melancon, P., B. S. Glick, V. Malhotra, P. J. Weidman, T. Serafini, M. L. Gleason, L. Orci, and J. E. Rothman.** 1987. Involvement of GTP-binding "G" proteins in transport through the Golgi stack. Cell **51:**1053–1062.
211. **Melkonian, M., B. Becker, and D. Becker.** 1991. Scale formation in algae. J. Electron Microsc. Techn. **17:**165–178.
212. **Memon, A. R., R. Kawazoe, X. Zhang, D. L. Herrin, and G. A. Thompson, Jr.** 1995. Low molecular mass GTP-binding proteins in *Chlamydomonas reinhardtii* wild type and a wall-less strain. Characterization and comparison with GTP-binding proteins of *Dunaliella salina*. Plant Physiol. Biochem. **33:**225–234.
213. **Moestrup, Ø., and H. A. Thomsen.** 1974. An ultrastructural study of the flagellate *Pyramimonas orientalis* with particular emphasis on Golgi apparatus activity and the flagellar apparatus. Protoplasma **81:**247–269.
214. **Moestrup, Ø., and P. L. Walne.** 1979. Studies on scale morphogenesis in the Golgi apparatus of *Pyramimonas tetrarhynchus* (Prasinophyceae). J. Cell Sci. **36:**437–459.
215. **Mollenhauer, H. H., D. J. Morré, and L. D. Rowe.** 1990. Alteration of intracellular traffic by monensin: mechanism, specificity and relationship to toxicity. Biochim. Biophys. Acta Rev. Biomembr. **1031:**225–246.
216. **Momayezi, M., A. W. Habermann, J. J. Sokolova, R. Kissmehl, and H. Plattner.** 1993. Ultrastructural and antigenic preservation of a delicate structure by cryopreparation: identification and immunogold localization during biogenesis of a secretory component (membrane-matrix connection) in *Paramecium* trichocysts. J. Histochem. Cytochem. **41:**1669–1677.
217. **Morré, D. J., and H. H. Mollenhauer.** 1974. The endomembrane concept: a functional integration of endoplasmic reticulum and Golgi apparatus, p.

84–137. *In* A. W. Robards (ed.), Dynamic aspects of plant ultrastructure. McGraw-Hill Book Co., London.
218. **Munro, S.** 1995. An investigation of the role of transmembrane domains in Golgi protein retention. EMBO J. **14:**4695–4704.
219. **Murtagh, J. J., Jr., M. R. Mowatt, C.-M. Lee, F.-J. Scott Lee, K. Mishima, T. E. Nash, J. Moss, and M. Vaughan.** 1992. Guanine nucleotide-binding proteins in the intestinal parasite *Giardia lamblia*. Isolation of a gene encoding an ~20-kDa ADP-ribosylation factor. J. Biol. Chem. **267:**9654–9662.
220. **Nakamura, K., and K. Matsuoka.** 1993. Protein targeting to the vacuole in plant cells. Plant Physiol. **101:**1–5.
221. **Nath, V., and G. P. Dutta.** 1962. Cytochemistry of protozoa, with particular reference to the Golgi apparatus and the mitochondria. Int. Rev. Cytol. **13:**323–355.
222. **Ng, K., E. Handman, and A. Bacic.** 1994. Biosynthesis of lipophosphoglycan from *Leishmania major*: characterization of (β1-3)-galactosyltransferase(s). Glycobiology **4:**845–853.
223. **Nilsson, T., M. H. Hoe, P. Slusarewicz, C. Rabouille, R. Watson, F. Hunte, G. Watzele, E. G. Berger, and G. Warren.** 1994. Kin recognition between *medial* Golgi enzymes in HeLa cells. EMBO J. **13:**562–574.
224. **Nilsson, T., M. Pypaert, M. H. Hoe, P. Slusarewicz, E. G. Berger, and G. Warren.** 1993. Overlapping distribution of two glycosyltransferases in the Golgi apparatus of HeLa cells. J. Cell Biol. **120:**5–13.
225. **Nilsson, T., P. Slusarewicz, M. H. Hoe, and G. Warren.** 1993. Kin recognition: a model for the retention of Golgi enzymes. FEBS Lett. **330:**1–4.
226. **Noguchi, T., and D. J. Morré.** 1991. Vesicular membrane transfer between endoplasmic reticulum and Golgi apparatus of a green alga, *Micrasterias americana*. A 16°C block and reconstitution in a cell-free system. Protoplasma **162:**128–139.
227. **Novick, P., and P. Brennwald.** 1993. Friends and family: the role of the Rab GTPases in vesicular traffic. Cell **75:**597–601.
228. **O'Halloran, T. J., and R. G. W. Anderson.** 1992. Characterization of the clathrin heavy chain from *Dictyostelium discoideum*. DNA Cell Biol. **11:** 321–330.
229. **Ogun, S. A., and A. A. Holder.** 1994. *Plasmodium yoelii*: brefeldin A-sensitive processing of proteins targeted to the rhoptries. Exp. Parasitol. **79:**270–278.
230. **Ohashi, M., K. J. de Vries, R. Frank, G. Snoek, V. Bankaitis, K. Wirtz, and W. B. Huttner.** 1995. A role for phosphatidylinositol transfer protein in secretory vesicle formation. Nature (London) **377:**544–547.
231. **Ohno, H., J. Stewart, M.-C. Furnier, H. Bosshart, I. Rhee, S. Miyatake, T. Saito, A. Gallusser, T. Kirchhausen, and J. S. Bonifacino.** 1995. Interaction of the tyrosine-based signals with clathrin-associated proteins. Science **269:** 1872–1874.
232. **Orci, L., D. J. Palmer, M. Amherdt, and J. E. Rothman.** 1993. Coated vesicle assembly in the Golgi requires only coatomer and ARF proteins from the cytosol. Nature (London) **364:**732–734.
233. **Orci, L., D. J. Palmer, M. Ravazzola, A. Perrelet, M. Amherdt, and J. E. Rothman.** 1993. Budding from Golgi membranes requires the coatomer complex of non-clathrin coat proteins. Nature (London) **362:**648–652.
234. **Orci, L., M. Tagaya, M. Amherdt, A. Perrelet, J. G. Donaldson, J. Lippincott-Schwartz, R. D. Klausner, and J. E. Rothman.** 1991. Brefeldin A, a drug that blocks secretion, prevents the assembly of non-clathrin-coated buds on Golgi cisternae. Cell **64:**1183–1195.
235. **Orias, E., M. Flacks, and B. H. Satir.** 1983. Isolation and ultrastructural characterization of secretory mutants of *Tetrahymena*. J. Cell Sci. **64:**49–67.
236. **Osafune, T., J. A. Schiff, and E. Hase.** 1990. Immunogold localization of LHCP II apoprotein in the Golgi of *Euglena*. Cell Struct. Funct. **15:**99–105.
237. **Osafune, T., J. A. Schiff, and E. Hase.** 1991. Stage-dependent localization of LHCP II apoprotein in the Golgi of synchronized cells of *Euglena gracilis* by immunogold electron microscopy. Exp. Cell Res. **193:**320–330.
238. **Ossig, R., W. Laufer, H. D. Schmitt, and D. Gallwitz.** 1995. Functionality and specific membrane localization of transport GTPases carrying C-terminal membrane anchors of synaptobrevin-like proteins. EMBO J. **14:** 3645–3653.
239. **Padh, H., J. Ha, M. Lavasa, and T. L. Steck.** 1993. A post-lysosomal compartment in *Dictyostelium discoideum*. J. Biol. Chem. **268:**6742–6747.
240. **Paquet, M. R., S. R. Pfeffer, J. D. Burczak, B. S. Glick, and J. E. Rothman.** 1986. Components responsible for transport between successive Golgi cisternae are highly conserved in evolution. J. Biol. Chem. **261:**4367–4370.
241. **Parodi, A. J.** 1993. *N*-Glycosylation in trypanosomatid protozoa. Glycobiology **3:**193–199.
242. **Pearse, B. M. F., and M. S. Robinson.** 1990. Clathrin, adaptors, and sorting. Annu. Rev. Cell Biol. **6:**151–171.
243. **Peck, R. K.** Personal communication.
244. **Peck, R. K., B. Swiderski, and A.-M. Tourmel.** 1993. Secretory organelle biogenesis: trichocyst formation in a ciliated protozoan. Biol. Cell **77:**277–287.
245. **Pelham, H. R. B.** 1995. Sorting and retrieval between the endoplasmic reticulum and the Golgi apparatus. Curr. Opin. Cell Biol. **7:**530–535.
246. **Peterson, J. B.** 1991. Small GTP-binding proteins associated with secretory vesicles of *Paramecium*. J. Protozool. **38:**495–501.
247. **Pfeffer, S. R.** 1994. Rab GTPases: master regulators of membrane trafficking. Curr. Opin. Cell Biol. **6:**522–526.
248. **Pingel, S., and M. Duszenko.** 1992. Identification of two distinct galactosyltransferase activities acting on the variant surface glycoprotein of *Trypanosoma brucei*. Biochem. J. **283:**479–485.
249. **Piper, R. C., X. Xu, D. G. Russell, B. M. Little, and S. M. Landfear.** 1995. Differential targeting of two glucose transporters from *Leishmania enriettii* is mediated by an NH2-terminal domain. J. Cell Biol. **128:**499–508.
250. **Plattner, H.** 1993. Membrane traffic in protozoa. Adv. Cell Mol. Biol. Membr. **2:**1.
251. **Plattner, H., C. J. Lumpert, G. Knoll, R. Kissmehl, B. Höhne, M. Momayezi, and R. Glas-Albrecht.** 1991. Stimulus-secretion coupling in *Paramecium* cells. Eur. J. Cell Biol. **55:**3–16.
252. **Plattner, H., C. Westphal, and R. Tiggemann.** 1982. Cytoskeleton-secretory vesicle interactions during the docking of secretory vesicles at the cell membrane in Paramecium tetraurelia cells. J. Cell Biol. **92:**368–377.
253. **Pollack, S.** 1974. Mutations affecting the trichocysts in *Paramecium aurelia*. I. Morphology and description of the mutants. J. Protozool. **21:**352–362.
254. **Quigley, F., M. Rickauer, and W. Tanner.** 1984. Protein N-glycosylation and its possible role in the cell cycle of *Chlorella*. Plant Sci. Lett. **35:**43–49.
255. **Rabouille, C., N. Hui, F. Hunte, R. Kleckbusch, E. G. Berger, G. Warren, and T. Nilsson.** 1995. Mapping the distribution of Golgi enzymes involved in the construction of complex oligosaccharides. J. Cell Sci. **108:**1617–1627.
256. **Rabouille, C., T. P. Levine, J.-M. Peters, and G. Warren.** 1995. An NSF-like ATPase, p97, and NSF mediate cisternal regrowth from mitotic Golgi fragments. Cell **82:**904–914.
257. **Rambourg, A., Y. Clermont, L. Hermo, and D. Segretain.** 1987. Tridimensional structure of the Golgi apparatus of non-ciliated epithelial cells of the ductuli efferentes in rat: an electron microscopical stereoscopic study. Biol. Cell **60:**103–116.
258. **Rambourg, A., Y. Clermont, and F. Képès.** 1993. Modulation of the Golgi apparatus in Saccharomyces cerevisiae sec7 mutants as seen by three-dimensional electron microscopy. Anat. Rec. **237:**441–452.
259. **Rambourg, A., Y. Clermont, L. Ovtracht, and F. Képès.** 1995. Three-dimensional structure of tubular networks, presumably Golgi in nature, in various yeast strains: a comparative study. Anat. Rec. **243:**283–293.
260. **Ray, D. A., and A. Gibor.** 1982. Tunicamycin-sensitive glycoproteins involved in the mating of *Chlamydomonas reinhardtii*. Exp. Cell Res. **141:**245–252.
261. **Reiner, D. S., M. McCaffery, and F. D. Gillin.** 1990. Sorting of cyst wall proteins to a regulated secretory pathway during differentiation of the primitive eukaryote, *Giardia lamblia*. Eur. J. Cell Biol. **53:**142–153.
262. **Richardson, J. M., N. A. Woychik, D. L. Ebert, R. L. Dimond, and J. A. Cardelli.** 1988. Inhibition of early but not late proteolytic processing events leads to the missorting and oversecretion of precursor forms of lysosomal enzymes in *Dictyostelium discoideum*. J. Cell Biol. **107:**2097–2107.
263. **Riley, G. R., C. M. West, and E. J. Henderson.** 1993. Cell differentiation in *Dictyostelium discoideum* controls assembly of protein-linked glycans. Glycobiology **3:**165–177.
264. **Rodríguez-Rosales, M. P., D. L. Herrin, and G. A. Thompson, Jr.** 1992. Identification of low molecular mass GTP-binding proteins in membranes of the halotolerant alga *Dunaliella salina*. Plant Physiol. **98:**446–451.
265. **Rooney, E. K., and J. D. Gross.** 1992. ATP-driven Ca/H antiport in acid vesicles from *Dictyostelium*. Proc. Natl. Acad. Sci. USA **89:**8025–8029.
266. **Roth, J., and E. G. Berger.** 1982. Immunocytochemical localization of galactosyltransferase in HeLa cells: codistribution with thiamine pyrophosphatase in trans-Golgi cisternae. J. Cell Biol. **92:**223–229.
267. **Rothblatt, J., P. Novick, and T. H. Stevens.** 1994. Guidebook to the secretory pathway. Oxford University Press, Oxford.
268. **Rothman, J. E.** 1994. Mechanisms of intracellular protein transport. Nature (London) **372:**55–63.
269. **Rothman, J. E., and L. Orci.** 1992. Molecular dissection of the secretory pathway. Nature (London) **355:**409–415.
270. **Rovis, L., and S. Dube.** 1982. Identification and characteristics of two N-acetylglucosaminyltransferases associated with *Trypanosomas brucei* microsomes. Mol. Biochem. Parasitol. **5:**173–187.
271. **Ruscetti, T., J. A. Cardelli, M. L. Niswonger, and T. J. O'Halloran.** 1994. Clathrin heavy chain functions in sorting and secretion of lysosomal enzymes in *Dictyostelium discoideum*. J. Cell Biol. **126:**343–352.
272. **Ryan, K. A., L. A. Garraway, A. Descoteaux, S. J. Turco, and S. M. Beverley.** 1993. Isolation of virulence genes directing surface glycosyl-phosphatidylinositol synthesis by functional complementation of *Leishmania*. Proc. Natl. Acad. Sci. USA **90:**8609–8613.
273. **Salama, N. R., and R. W. Schekman.** 1995. The role of coat proteins in the biosynthesis of secretory proteins. Curr. Opin. Cell Biol. **7:**536–543.
274. **Satir, B. H., T. Hamasaki, M. Reichman, and T. J. Murtaugh.** 1989. Species distribution of a phosphoprotein (parafusin) involved in exocytosis. Proc. Natl. Acad. Sci. USA **86:**930–932.
275. **Sauer, M. K., and R. B. Kelly.** 1995. Conjugation rescue of exocytosis mutants in *Tetrahymena thermophila* indicates the presence of functional intermediates in the regulated secretory pathway. J. Eukaryot. Microbiol. **42:**173–183.
276. **Saxe, S. A., and A. R. Kimmel.** 1990. SAS1 and SAS2, GTP-binding protein

genes in *Dictyostelium discoideum* with sequence similarities to essential genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**2367–2378.
277. **Schekman, R.** 1994. Translocation gets a push. Cell **78:**911–913.
278. **Schröder, S., F. Schimmöller, B. Singer-Krüger, and H. Riezman.** 1995. The Golgi-localization of yeast Emp47p depends on its di-lysine motif but is not affected by the *ret1-1* mutation in α-COP. J. Cell Biol. **131:**895–912.
279. **Schwientek, T., C. Lorenz, and J. F. Ernst.** 1995. Golgi localization in yeast is mediated by the membrane anchor region of rat liver sialyltransferase. J. Biol. Chem. **270:**5483–5489.
280. **Serafini, T., L. Orci, M. Amherdt, M. Brunner, R. A. Kahn, and J. E. Rothman.** 1991. ADP-ribosylation factor is a subunit of the coat of Golgi-derived COP-coated vesicles: a novel role for a GTP-binding protein. Cell **67:**239–253.
281. **Serafini, T., G. Stenbeck, A. Brecht, F. Lottspeich, L. Orci, J. E. Rothman, and F. T. Wieland.** 1991. A coat subunit of Golgi-derived non-clathrin-coated vesicles with homology to the clathrin-coated vesicle coat protein β-adaptin. Nature (London) **349:**215–220.
282. **Shapiro, S. Z., and P. Webster.** 1989. Coated vesicles from the protozoan parasite *Trypanosoma brucei*: purification and characterization. J. Protozool. **36:**344–349.
283. **Sharif, A. L., A. G. Smith, and C. Abell.** 1989. Isolation and characterization of a cDNA clone for a chlorophyll synthesis enzyme from Euglena gracilis—the chloroplast enzyme hydroxymethylbilane synthase (porphobilinogen deaminase) is synthesised with a very long transit peptide in *Euglena*. Eur. J. Biochem. **184:**353–359.
284. **Sharkey, D. J., and R. Kornfeld.** 1989. Identification of an *N*-acetylglucosaminyltransferase in *Dictyostelium discoideum* that transfers an "intersecting" *N*-acetylglucosamine residue to high mannose oligosaccharides. J. Biol. Chem. **264:**10411–10419.
285. **Sharkey, D. J., and R. Kornfeld.** 1991. Developmental regulation of processing α-mannosidases and "intersecting" *N*-acetylglucosaminyltransferase in *Dictyostelium discoideum*. J. Biol. Chem. **266:**18477–18484.
286. **Sheffield, H. G., and B. Bjorvatin.** 1977. Ultrastructure of the cyst of *Giardia lamblia*. Am. J. Trop. Med. Hyg. **26:**23–30.
287. **Shigh, S.-J., and D. L. Nelson.** 1991. Multiple families of proteins in the secretory granules of *Paramecium tetraurelia*: immunological characterization and immunocytochemical localization of trichocyst proteins. J. Cell Sci. **100:**85–97.
288. **Shigh, S.-J., and D. L. Nelson.** 1992. Proteolytic processing of secretory proteins in *Paramecium*: immunological and biochemical characterization of the precursors of trichocyst matrix proteins. J. Cell Sci. **103:**349–361.
289. **Shimoni, Y., G. Segal, X.-Z. Zhu, and G. Galili.** 1995. Nucleotide sequence of a wheat cDNA encoding protein disulfide isomerase. Plant Physiol. **107:**281.
290. **Shimoni, Y., X.-Z. Zhu, H. Levanony, G. Segal, and G. Galili.** 1995. Purification, characterization and intracellular localization of glycosylated protein disulfide isomerase from wheat grains. Plant Physiol. **108:**327–335.
291. **Simon, S.** 1993. Translocation of proteins across the endoplasmic reticulum. Curr. Opin. Cell Biol. **5:**581–588.
292. **Singer, S. J.** 1995. It's important to concentrate. Trends Cell Biol. **5:**14–15.
293. **Singer, S. J., G. F. Oster, T. P. McGee, E. A. Whitters, M. K. Y. Fung, J. E. Vance, Z. Cui, and V. A. Bankaitis.** 1992. Debate: phospholipids and the Golgi complex. Trends Cell Biol. **2:**69–71.
294. **Sogin, M. L.** 1991. The phylogenetic significance of sequence diversity and length variations in eukaryotic small subunit ribosomal RNA coding regions, p. 175–188. *In* L. Warren and M. Koprowski (ed.), New perspectives on evolution. Wiley-Liss Inc., New York.
295. **Söllner, T., S. W. Whiteheart, M. Brunner, H. Erdjument-Bromage, S. Geromanos, P. Tempst, and J. E. Rothman.** 1993. SNAP receptors implicated in vesicle targeting and fusion. Nature (London) **362:**318–324.
296. **Sönnichsen, B., J. Füllekrug, P. Nguyen Van, W. Diekmann, D. G. Robinson, and G. Mieskes.** 1994. Retention and retrieval: both mechanisms cooperate to maintain calreticulin in the endoplasmic reticulum. J. Cell Sci. **107:**2705–2717.
297. **Staehelin, L. A., and I. Moore.** 1995. The plant Golgi apparatus: structure, functional organization and trafficking mechanisms. Annu. Rev. Plant Physiol. Plant Mol. Biol. **46:**261–288.
298. **Stamnes, M. A., and J. E. Rothman.** 1993. The binding of AP-1 clathrin adaptor particles to Golgi membranes requires ADP-ribosylation factor, a small GTP-binding protein. Cell **73:**999–1005.
299. **Subramanian, S. V., E. Wyroba, A. P. Andersen, and B. H. Satir.** 1994. Cloning and sequencing of parafusin, a calcium-dependent exocytosis-related phosphoglycoprotein. Proc. Natl. Acad. Sci. USA **91:**9832–9836.
300. **Südhof, T. C., P. De Camilli, H. Niemann, and R. Jahn.** 1993. Membrane fusion machinery: insights from synaptic proteins. Cell **75:**1–4.
301. **Sulli, C., and S. D. Schwartzbach.** 1995. The polyprotein precursor to the *Euglena* light-harvesting chlorophyll a/b-binding protein is transported to the Golgi apparatus prior to chloroplast import and polyprotein processing. J. Biol. Chem. **270:**13084–13090.
302. **Swanson, P. M., C. E. Carter, C. Hager, W. J. Kim, S. Obermeier, and T. N. Oeltmann.** 1992. Identification and characterization of an α-mannosidase from *Trypanosoma cruzi*. Glycobiology **2:**563–569.
303. **Takizawa, P. A., and V. Malhotra.** 1993. Coatomers and SNAREs in promoting membrane traffic. Cell **75:**593–596.
304. **Taniguchi, T., T. Mizuochi, Y. Banno, Y. Nozawa, and A. Kobata.** 1985. Carbohydrates of lysosomal enzymes secreted by *Tetrahymena pyriformis*. J. Biol. Chem. **260:**13941–13946.
305. **Taylor, T. C., R. A. Kahn, and P. Melançon.** 1992. Two distinct members of the ADP-ribosylation factor family of GTP-binding proteins regulate cell-free intra-Golgi transport. Cell **70:**69–79.
306. **Terryn, N., M. Van Montagu, and D. Inzé.** 1993. GTP-binding proteins in plants. Plant Mol. Biol. **22:**143–152.
307. **Tiedtke, A., and H. D. Görtz.** 1983. Acid phosphatase associated with discharging secretory vesicles (mucocysts) of *Tetrahymena thermophila*. Eur. J. Cell Biol. **30:**244–253.
308. **Tiedtke, A., T. Kiy, C. Vosskühler, and L. Rasmussen.** 1993. Pathways of lysosomal enzyme secretion in *Tetrahymena*. Adv. Cell Mol. Biol. Membr. **2:**99–122.
309. **Tiedtke, A., and L. Rasmussen.** 1989. Constitutive secretion of acid hydrolases in *Tetrahymena thermophila*. J. Protozool. **36:**378–382.
310. **Tindall, S. H., L. D. Devito, and D. L. Nelson.** 1989. Biochemical characterization of the proteins of Paramecium secretory granules. J. Cell Sci. **92:**441–448.
311. **Tippit, D. H., and J. D. Pickett-Heaps.** 1974. Experimental investigations into morphogenesis in *Micrasterias*. Protoplasma **81:**271–296.
312. **Traub, L. M., J. A. Ostrom, and S. Kornfeld.** 1993. Biochemical dissection of AP-1 recruitment onto Golgi membranes. J. Cell Biol. **123:**561–573.
313. **Trombetta, S. E., M. Bosch, and A. J. Parodi.** 1989. Glucosylation of glycoproteins by mammalian, plant, fungal, and trypanosomatid protozoa microsomal membranes. Biochemistry **28:**8108–8116.
314. **Turkewitz, A. P., L. Madeddu, and R. B. Kelly.** 1991. Maturation of dense core granules in wild type and mutant *Tetrahymena thermophila*. EMBO J. **10:**1979–1987.
315. **Turner, M. J.** 1982. Biochemistry of the variant surface glycoproteins and surface coat of *Trypanosoma brucei*. Adv. Parasitol. **21:**69–153.
316. **Ullrich, O., H. Horiuchi, C. Bucci, and M. Zerial.** 1994. Membrane association of Rab 5 mediated by GDP-dissociation inhibitor and accompanied by GDP/GTP exchange. Nature (London) **368:**157–160.
317. **Ungewickell, E., H. Ungewickell, S. E. H. Holstein, R. Lindner, K. Prasad, W. Barouch, B. Martin, L. E. Greene, and E. Eisenberg.** 1995. Role of auxilin in uncoating clathrin-coated vesicles. Nature (London) **378:**632–635.
318. **Url, T., M. Höftberger, and U. Meindl.** 1993. Cytochalasin B influences dictyosomal vesicle production and morphogenesis in the desmid *Euastrum*. J. Phycol. **29:**667–674.
319. **Vallon, O., and F.-A. Wollmam.** 1995. Mutations affecting O-glycosylation in *Chlamydomonas reinhardtii* cause delayed cell wall degradation and sex-limited sterility. Plant Physiol. **108:**703–712.
320. **van Meer, G.** 1989. Lipid traffic in animal cells. Annu. Rev. Cell Biol. **5:**247–275.
321. **Velasco, A., L. Hendricks, K. W. Moremen, D. R. P. Tulsiani, O. Touster, and M. G. Farquhar.** 1993. Cell type-dependent variations in the subcellular distribution of α-mannosidase I and II. J. Cell Biol. **122:**39–51.
322. **Vida, T. A., T. R. Graham, and S. D. Emr.** 1990. *In vitro* reconstitution of intercompartmental protein transport to the yeast vacuole. J. Cell Biol. **111:**2871–2884.
323. **Warnock, D. E., M. S. Lutz, W. A. Blackburn, W. W. Young, Jr., and J. U. Baenziger.** 1994. Transport of newly synthesized glucosylceramide to the plasma membrane by a non-Golgi pathway. Proc. Natl. Acad. Sci. USA **91:**2708–2712.
324. **Watson, N., V. McGuire, and S. Alexander.** 1994. The PsB glycoprotein complex is secreted as a preassembled precursor of the spore coat in *Dictyostelium discoideum*. J. Cell Sci. **107:**2567–2579.
325. **Weiss, R. L.** 1983. Coated vesicles in the contractile vacuole/mating structure region of *Chlamydomonas*. J. Ultrastruct. Res. **85:**33–44.
326. **Wetherbee, R., R. A. Andersen, and J. Pickett-Heaps (ed.).** 1994. The protistan cell surface. Springer-Verlag KG, Vienna.
327. **Whatley, J. M., and C. Chapman-Andresen.** 1990. Phylum Karyoblastea, p. 167–185. *In* L. Margulis, J. O. Corliss, M. Melkonian, and D. J. Chapman (ed.), Handbook of protoctista. Jones & Bartlett Publishers, Boston.
328. **Whiteheart, S. W., and E. W. Kubalek.** 1995. SNAPs and NSF: general members of the fusion apparatus. Trends Cell Biol. **5:**64–68.
329. **Wilsbach, K., and G. S. Payne.** 1993. Dynamic retention of TGN membrane proteins in *Saccharomyces cerevisiae*. Trends Cell Biol. **3:**426–432.
330. **Wilson, D. W., C. A. Wilcox, G. C. Flynn, E. Chen, W.-J. Kuang, W. J. Henzel, M. R. Block, A. Ullrich, and J. E. Rothman.** 1989. A fusion protein required for vesicle-mediated transport in both mammalian cells and yeast. Nature (London) **339:**355–359.
331. **Wolin, S. L.** 1994. From the elephant to E. coli: SRP-dependent protein targeting. Cell **77:**787–790.
332. **Wyroba, E., A. Widding Hoyer, P. Storgaard, and B. H. Satir.** 1995. Mammalian homologue of the calcium-sensitive phosphoglycoprotein, parafusin. Eur. J. Cell Biol. **68:**419–426.
333. **Yusa, A.** 1963. An electron microscopical study on regeneration of trichocysts in *Paramecium caudatum*. J. Protozool. **10:**253–262.

334. **Zerial, M., and H. Stenmark.** 1993. Rab GTPases in vesicular transport. Curr. Opin. Cell Biol. **5:**613–620.
335. **Zhang, X. Q., J.-P. Dubacq, and A. Alfsen.** 1993. Biochemical and cytological evidence for the stimulation of clathrin-coated pit (vesicle) formation by exogenous folic acid in *Dunaliella salina* (Chlorophyta). J. Phycol. **29:** 203–209.
336. **Zhang, Y.-H., W. C. Lang, and D. G. Robinson.** 1989. In vitro localization of hydroxyproline O-glycosyl transferases in *Chlamydomonas reinhardtii*. Plant Cell Physiol. **30:**617–622.
337. **Zhang, Y.-H., and D. G. Robinson.** 1990. Cell-wall synthesis in *Chlamydomonas reinhardtii*: an immunological study on the wild type and wall-less mutants *cw2* and *cw15*. Planta **180:**229–236.