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Pathways of protein sorting and membrane traffic between the rough endoplasmic reticulum and the Golgi complex

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Recent results have provided increasing evidence for the existence of an intermediate membrane compartment between the rough endoplasmic reticulum and the Golgi complex which seems to function in protein sorting and the regulation of membrane traffic in the early part of the exocytic pathway. Localization of resident marker proteins has shown that this compartment consists of both peripheral and central elements. The aim of the present review is to combine the data on the pre-Golgi compartment with previous ideas of membrane traffic at the ER-Golgi interface. We propose a model which describes how mobile, endosome-like elements of the pre-Golgi compartment function in the generation of the compositional and functional boundary between the widely distributed ER and the more centrally located Golgi stacks.

Key words: endoplasmic reticulum / Golgi complex / pre-Golgi compartment / protein transport / membrane traffic

STUDIES CARRIED out in the 1960s and 70s demonstrated that the endoplasmic reticulum (ER) is the principal site of membrane-associated protein synthesis. Furthermore, examination of regulated secretory cells showed that their exported protein products accumulate in storage granules before being released to the exterior of the cells. Although the general outline of the secretory pathway (ER to Golgi complex to plasma membrane) has been known for some time[1] it has turned out to be difficult to pinpoint the structures and processes that carry out many of the intermediate steps of transport of proteins within the cell. This is true even today when new and powerful methodology has been applied and developed to identify and localize protein molecules that operate in this process.[2] A key to the unraveling of the pathways of protein transport appears to be the understanding of the organization of the Golgi complex.

The early electron microscopic studies showed that in many cell types the Golgi has a polarized structure and the secretory granules are found on one side of the cisternal stack.[3] These observations lead to the suggestion that transport is a flow process which involves the progressive movement of cisternae from the immature (*cis*) to the mature (*trans*) side of the structure.[3,4] The vectorial *cis* to *trans* movement of proteins across the Golgi was first demonstrated in immuno-cytochemical studies of the transport of viral membrane proteins. This was accomplished by using either temperature-sensitive viral mutants to synchronize transport[5,6] or following the vectorial clearance of proteins from the Golgi complex after inhibition of protein synthesis.[7] By this time cytochemical and cell fractionation studies had shown the compositional differences of different Golgi domains.[3] These findings, as well as observations on the recycling of secretory granule membranes from the plasma membrane to the Golgi and the discovery of coated vesicles, influenced new views which replaced the earlier flow concept of Golgi organization.[3,8] According to these, transport across the Golgi is a discontinuous process involving vesicle budding and fusion between stationary Golgi compartments.[9,10] The model has gained further support from observations obtained using *in vitro* assays which reconstitute intra-Golgi protein transport.[2]

The characteristic, ordered architecture of the Golgi complex has largely influenced the ideas of the functional organization of this organelle. This structure stands in clear contrast to the pleiomorphic structure of the elements of the endocytic pathway.[11] In the endocytic pathway the sorting of proteins includes the segregation of proteins within a compartment into vacuolar and tubular domains, followed by their targeted delivery to different destinations.[12,13] In contrast to the subcompartments of the Golgi complex, which are still largely defined through their specific content of marker proteins, an endocytic compartment can be defined by its sorting function.

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If this definition of a compartment as a sorting device is applicable to membrane traffic in general, the understanding of the organization of the secretory pathway requires the description of the sorting processes. Recent studies suggest that the major sites of protein sorting in the exocytic pathway reside at the two sides of the Golgi complex. Initially described as pre- and post-Golgi sites where newly synthesized viral membrane proteins accumulate at reduced temperature, about 15°C and 20°C, respectively[14-16] these compartments can presently be viewed as crossroad stations which regulate membrane traffic along the exocytic pathway.[2,17,18,72] An interesting possibility is that the observed temperature effects are due to specific inhibition of some molecular processes that control protein sorting and transport in these compartments.

Presently, the situation in the study of protein transport is particularly interesting since two lines of the research, i.e. morphological studies of transport pathways and studies of the molecular mechanism of transport, are beginning to converge. The identification of component proteins of the transport machinery became possible through the development of *in vitro* transport assays[19] and studies of yeast secretory mutants (ref 20; see also the review of Franzusoff, pp 309-324, this issue). These studies have emphasized the molecular complexity of the transport process and the need to place newly identified control molecules in the framework of the compartmental organization of membranes. Furthermore, the use of recombinant DNA technology to modify protein structure has made it obvious that transported proteins contain peptide motifs that are involved in their sorting to different cellular locations. In many transport steps this directed delivery of proteins is dependent on cytoskeletal elements, such as microtubules[21] and associated motors,[22] and according to recent evidence is controlled by signalling mechanisms involving GTP-binding proteins.[23]

In this review we summarize recent experimental results on the pathways and mechanisms of protein transport between the rough ER and the Golgi complex and discuss the function of a recently identified pre-Golgi compartment in protein transport and recycling between these two major organelles of the biosynthetic pathway. The present considerations form a basis for a different view of the intracellular pathways of membrane traffic and the biogenesis of the Golgi complex.

The pre-Golgi compartment

The existence of an intermediate compartment between the rough ER and Golgi was demonstrated in studies using low temperature to arrest the transport of newly synthesized Semliki Forest virus (SFV) spike glycoproteins.[15] In cells incubated at 15°C this compartment was visualized by immunolocalization of viral membrane proteins as pleiomorphic vacuolar and tubulo-vesicular structures in the Golgi region as well as in more peripheral locations. Within these pleiomorphic structures the viral proteins were present both in the vacuolar membrane domains as well as in some of the protruding tubular domains. Shortly after the release of the temperature arrest the vacuolar membranes were largely depleted of the viral proteins whereas the proteins were still detected in the tubular structures, suggesting segregation of the proteins between these two membrane domains. These results together with the structural similarity of these elements with earlier described endosomal/CURL (Compartment for Uncoupling of Ligand and Receptor) structures[12] suggested that the pre-Golgi vacuoles have a sorting function.[15]

The elements of the pre-Golgi compartment display a dilated vacuolar morphology at 15°C suggesting that at low temperature accumulation of membranes occurs at these sites.[15] Similarly, the budding of coronaviruses has been described to occur in a dilated, smooth-surfaced, tubulo-vesicular compartment.[24,25] The membranes of this budding compartment were associated with rough ER cisternae, including apparent transitional elements, and also present close to the *cis* face of the Golgi stack.[25] The morphological appearance of this budding compartment and its location with respect to the rough ER and Golgi suggest that it corresponds to the same site where the SFV spike proteins were observed to accumulate at 15°C. One of the processes that determine the intracellular budding of coronaviruses seems to be the specific retention of the viral E1 glycoprotein in the intermediate membranes between the rough ER and Golgi (see the review of Griffiths and Rottier, pp 367-381, this issue).

Immunolocalization of one of the recently identified marker proteins, a 58 kDa membrane protein (p58),[26] has also been used to study the morphology of the pre-Golgi compartment in different cell types. Double-localization of p58 and SFV spike glycoproteins in BHK cells shifted to 15°C has

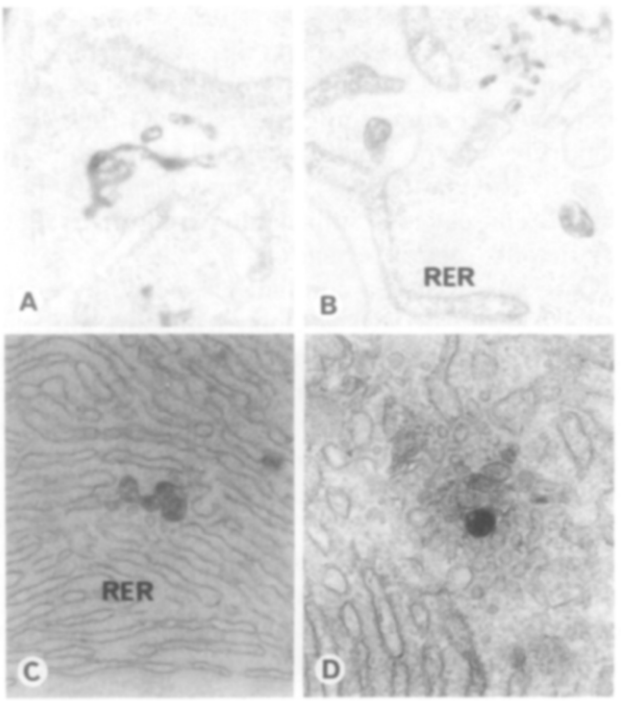

Figure 1. Structure of the peripheral pre-Golgi elements. These elements were visualized in mouse myeloma cells (A, B) and rat pancreatic acinar cells (C, D) by immunoperoxidase electron microscopy using antibodies against the 58 kDa marker protein (p58). Note the pleiomorphic structure of the pre-Golgi elements. In the acinar cells the p58-positive elements are surrounded by numerous p58-negative tubules and vesicles.

demonstrated that these proteins are present in the same membrane structures.[27] In the electron microscope the p58-positive elements appear as pleiomorphic structures, highly heterogenous both in size and shape, and their morphology also varies in different cell types (Figure 1). In the pancreatic exocrine cells the p58-positive elements are found within tubulo-vesicular, smooth membrane clusters in the vicinity of rough ER cisternae (Figure 1).[28] In all cell types studied, p58 is also detected in the fenestrated *cis*-Golgi cisternae.[26-28]

The p58-containing elements can be double-labeled with antibodies against β-COP, one of the subunit proteins of Golgi-associated coat structures,[29,30] indicating that these coats bind to the cytoplasmic surface of the pre-Golgi elements (Figure 2A, B). When cells are treated with the fungal antibiotic Brefeldin A (BFA), these coat structures rapidly dissociate from Golgi membranes[31] and many resident Golgi proteins assume an ER-like distribution with the concomitant disappearance of identifiable Golgi stacks.[32] An interesting observation is that in BFA-treated cells the pre-Golgi compartment, in contrast to the Golgi cisternae, does not fuse with the ER but remains as a distinct, although morphologically altered, structure.[27,33] This would indicate that the formation of pre-Golgi elements is independent of the β-COP-containing coats. Therefore, the function of these coats most likely relates to some process(es) that occur(s) in the pre-Golgi compartment. As suggested in Figure 3, these coats may bind to (some of) the tubular domains of the pre-Golgi elements. In line with the suggestion of Duden *et al*,[34] the function of the coats could be to stabilize these tubular protrusions and inhibit their fusion with the rough ER cisternae. This conclusion is supported by results obtained with BFA-treated cells.[33] It is possible that in the absence of coat association the Golgi-like tubules continuously form but fuse back to the ER.

The diagrams in Figure 3 propose a transport pathway for SFV spike glycoproteins from the rough ER cisternae to the Golgi complex, based on information on the localization of these proteins during the 15°C temperature arrest and shortly after the release of the transport block by shifting cells to physiological temperature.[15,18,27] The exit of the viral proteins from the rough ER occurs by budding of small vesicular or tubular structures (Figure 3A). These primary transport vesicles are likely to have a defined membrane composition and could function in the concentration of the transported viral membrane proteins.[35] However, they may enclose a rather unselected sample of luminal proteins of the ER. It is also suggested that these vesicles fuse together to form the large, pleiomorphic pre-Golgi elements (Figure 3A, B). As discussed further below, the viral proteins are then transported to the *cis*-Golgi along with the microtubule-dependent movement of the pre-Golgi elements (Figure 3B, C).

Organization of the ER-Golgi interface

According to the prevailing concept of ER to Golgi transport the export of proteins from the rough ER occurs in specialized regions of this compartment termed the transitional elements. Electron microscopy has identified these elements in many secretory cells as partly ribosome-coated, partly smooth-surfaced structures in the vicinity of the Golgi complex.[1] This transport model (Figure 4A) requires that newly synthesized proteins move within the extensive rough ER network and reach the centrally located transitional sites. However, the mechanism

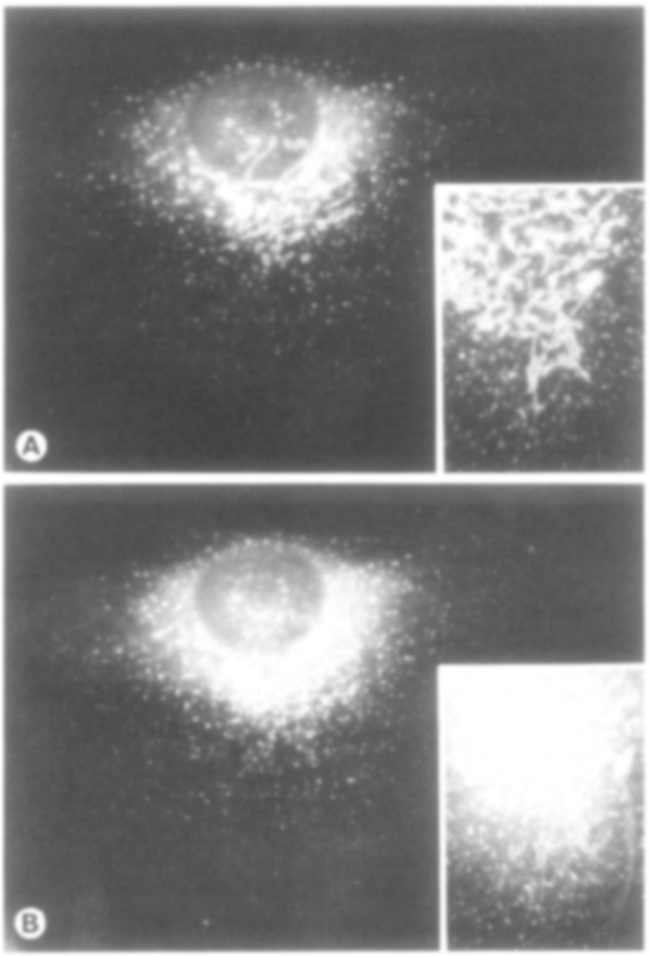

Figure 2. The pre-Golgi elements have a widespread distribution in cells. BHK-21 cells were fixed, permeabilized with Triton-X-100 and stained with antibodies against β-COP (A) (see ref 29) and the 58 kDa pre-Golgi marker protein (B) (see ref 27). Note the co-distribution of the two proteins in the Golgi region (the insets show their co-localization in tubular/cisternal elements) as well as in punctate structures scattered in the cytoplasm.

of such vectorial transfer is not known and, in light of the rapid drainage of many proteins from the rough ER,[35,36] it seems unlikely that it could be due to diffusion of proteins within the membrane or lumen of this compartment.

The vesicle shuttle hypothesis of ER to Golgi transport suggests that proteins are transported from the transitional elements to the Golgi complex in carrier vesicles (Figure 4A).[1] This conclusion is based on the structure of the transitional elements, suggesting vesicle budding or fusion,[37] as well as autoradiographic and cell fractionation studies of protein transport in pancreatic exocrine cells.[38] Furthermore, the participation of small vesicular intermediates in the early part of the secretory pathway is indirectly supported by recent *in vitro* studies which have indicated that ER to Golgi and intra-Golgi transport have similar biochemical requirements.[39-41] However, direct evidence concerning the nature of the ER to Golgi transport intermediates is still lacking and, in spite of various attempts,[42-45] these elements have not yet been isolated in sufficiently pure form to allow their detailed characterization.

There has existed a conflict between the vesicle shuttle concept of ER to Golgi transport and the growing evidence for the existence of an intermediate compartment between the rough ER and the Golgi complex. Namely, it has been difficult to place such a compartment in the known morphological framework of the organization of the ER-Golgi interface. Recent results on the localization of resident marker proteins (rat p58 and human p53) of the intermediate membranes, however, appear to provide an answer to this dilemma. In addition to showing the presence of these proteins in tubulovesicular and cisternal elements in the *cis*-Golgi region[26,46] (a location that the vesicle shuttle model would predict) these studies have also demonstrated their concentration in peripheral smooth membrane structures indicating that the elements of the pre-Golgi compartment have a widespread distribution in cells[26-28] (Figure 2A). Studies of the coronavirus budding compartment have also shown that some of these membranes have a peripheral location.[25]

The diagram in Figure 4B combines the information on the structure and function of the pre-Golgi compartment with previous views of ER to Golgi transport. Based on the observed intracellular distribution of the pre-Golgi elements this model suggests that the exit of proteins from the rough ER occurs throughout the entire surface of this compartment. This conclusion is also supported by results showing the accumulation of viral membrane proteins at 15°C in peripheral structures[15,18,47] where they co-localize with marker proteins of the pre-Golgi compartment, p58 and p53.[27,48] These marker proteins apparently continuously cycle between the rough ER and *cis*-Golgi.[27,48,56] In cells treated with BFA p58 accumulates in dispersed, tubular membrane clusters suggesting that the inhibition of ER to Golgi transport by this drug occurs close to the exit from the rough ER.[27] These structures are continuous with rough ER cisternae[27,49] and with time seem to become centralized and fuse together to form extensive smooth membrane structures, whose morphology can vary in different cell types.[50]

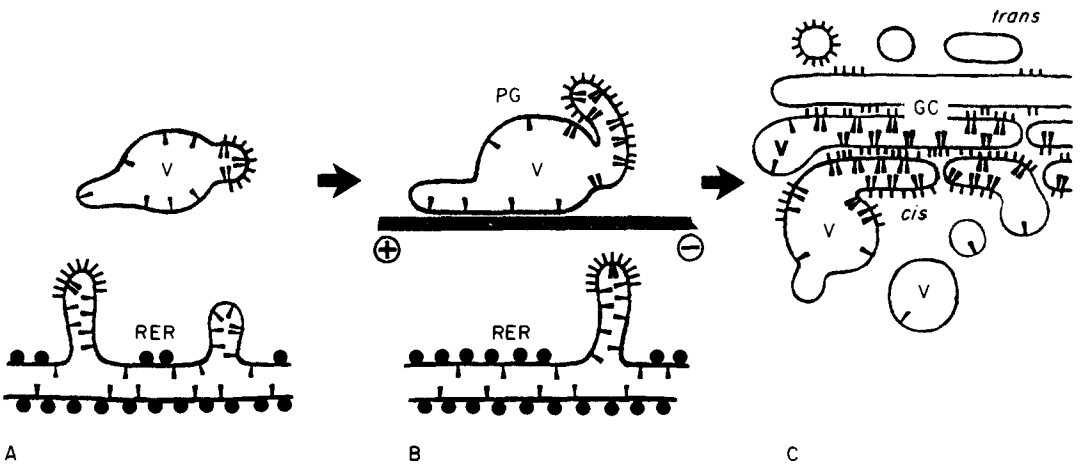

Figure 3. A schematic illustration of the transport pathway of Semliki Forest virus (SFV) spike glycoproteins from the rough endoplasmic reticulum to the entry face of the Golgi complex. See also refs 15 and 27. The single spikes indicate the monomeric p62 (precursor of E2 and E3 glycoproteins) and E1 glycoproteins of SFV and the double spikes p62-E1 heterodimers. (A) The viral membrane proteins exit from the rough ER (RER) in small vesicular or tubular buds (with unknown coat structures) which give rise to primary RER-derived transport elements. These vesicles then form/fuse with pleiomorphic pre-Golgi elements (PG) which consist of vacuolar (v) and tubular domains. (B) Within the pre-Golgi elements the viral proteins are segregated into the tubular domains with which the β-COP coats are suggested to associate. The formation of p62-E1 dimers may coincide with this segregation step. The transport of the pre-Golgi elements from peripheral locations to the Golgi region is shown to occur along microtubules in retrograde (i.e. plus to minus) direction. (C) At the entry (*cis*) face of the Golgi complex (GC) the coated, tubular parts of the pre-Golgi elements participate in the formation of the *cis*-most cisterna.

The model (Figure 4B) further suggests that the pre-Golgi elements are mobile structures which translocate from peripheral sites to the central Golgi region. As well as structurally,[15] they would also in this respect resemble endosomal elements, which have been demonstrated to be centralized along microtubular tracks.[21,51] This conclusion is based on experiments showing that the microtubule-depolymerizing drug nocodazole causes rapid accumulation of p58-containing pre-Golgi elements in the periphery of the cells and that the clustering of these elements to the Golgi region, observed in a translocation assay, is efficiently inhibited by this drug.[27] Also, the pre-Golgi elements, which frequently have an elongated shape,[27] can be seen to coalign with microtubules (J. Saraste, unpublished data). Further support for this point can be obtained from experiments with live cells when appropriate markers for vital staining, e.g. fluorescent lipids,[52] are available and the movement of individual peripheral elements can be followed.

If ER to Golgi transport is considered to be a vesicle-mediated, short distance transport step then, based on the available morphological information, the compensatory recycling of membrane from *cis*-Golgi complex to the ER could also be mediated by vesicular elements (Figure 4A). In the extended transport model (Figure 4B) the peripheral pre-Golgi elements, rather than central *cis*-Golgi structures, represent the major acceptor membranes for rough ER-derived transport vesicles. Accordingly, the recycling of membrane (and components) back to the rough ER is suggested to occur already from the pre-Golgi elements that reside in peripheral locations. Furthermore, the cells contain numerous pre-Golgi structures (Figure 2),[27,28] indicating that a large quantity of membrane is transported to the *cis*-Golgi along with the microtubule-dependent centralization of these elements. Since their sorting activity is also concentrated during this process, it seems likely that a considerable part of the membrane that leaves the ER in the primary transport vesicles is returned to

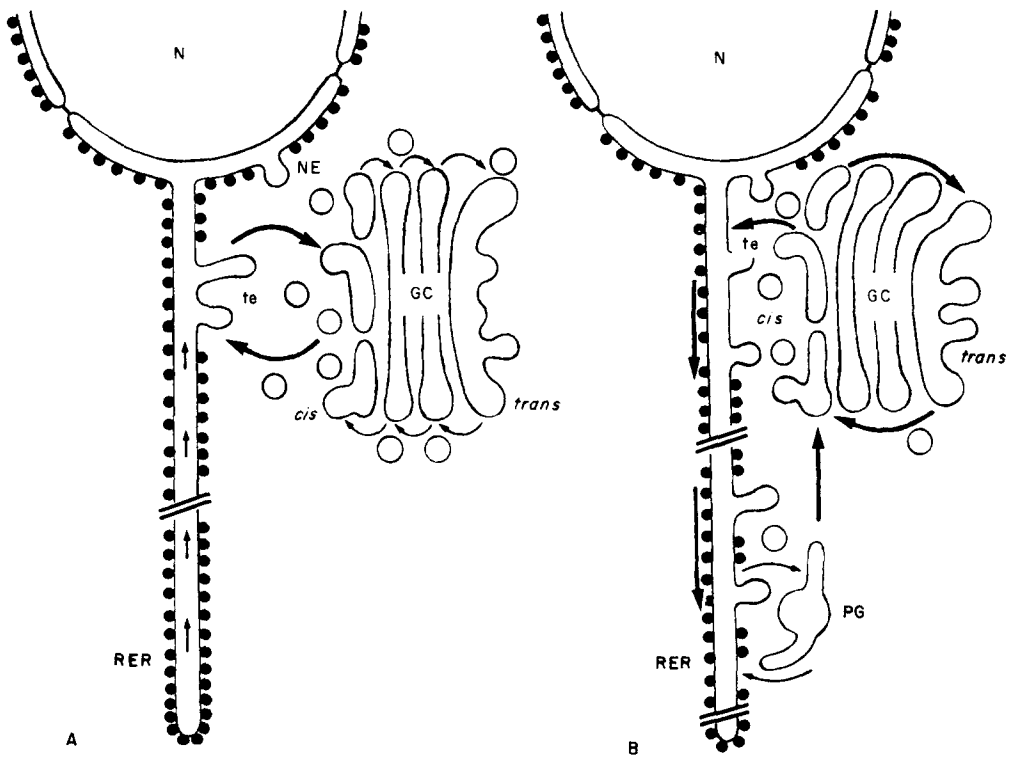

Figure 4. Different models for the organization of membrane traffic between the rough ER and the Golgi complex. (A) Newly synthesized proteins move within the membrane or lumen of the rough (RER) or nuclear envelope (NE) and their exit from this compartment occurs in transitional elements (te), located close to the *cis*-side of the Golgi complex (GC). In this model transport from the transitional elements to the *cis*-Golgi is a short distance transport step which is mediated by small carrier vesicles and vesicles of similar size are also suggested to function in the compensatory recycling of membrane and return of proteins from *cis*-Golgi back to the rough ER. (B) This model is an extension of model A and suggests that a pre-Golgi compartment (PG), analogous to peripheral endosomes, functions as an intermediate in transport between the rough ER and *cis*-Golgi. Proteins are exported from the rough ER in small transport vesicles (or tubules) which can bud throughout the rough ER system. The pre-Golgi elements are widely distributed in cells and act as acceptor membranes for the fusion of the rough-ER derived transport vesicles. They are also suggested to represent sorting organelles from which recycling of membrane and components back to the rough ER can occur. This short cycle of transport is indicated by thin arrows. Like endosomal elements, the pre-Golgi elements are suggested to be concentrated in the central Golgi region by movement along microtubules. As a result of this process, their sorting activity is concentrated in the *cis*-Golgi from which large amount of membrane is recycled back to the ER by vesicular/tubular transport. This local insertion of membrane, together with the known cytoskeleton-dependent movements of the ER, is suggested to provide the mechanism for the compensatory backflow of ER membrane towards the periphery of the cell. This long cycle of transport is indicated in B by thick arrows.

this compartment from *cis*-Golgi. This recycling of membrane over short intracellular distances could be mediated by vesicular or tubular transport. In fact, this central recycling could explain many of the characteristic morphological features of transitional ER and *cis*-Golgi, including the occasionally observed direct, tubular continuities between Golgi elements and the rough ER.[53]

Assuming that a large amount of membrane is inserted into the ER in a central location to compensate for the peripheral budding of vesicles, then one has to consider additional mechanisms which operate in the redistribution of this added membrane. One possible mechanism could be a backflow of membrane towards peripheral parts of the ER network, a process that could be intimately

related to the growth of the ER itself. Recent studies have indicated that ER elements extend outwards (anterogradely) along microtubules,[21,54,55] i.e. in a direction that is opposite to the direction of the movement of pre-Golgi elements.

As indicated in Figure 4B, the recycling of proteins back to the rough ER could either occur from the peripheral pre-Golgi elements or central *cis*-Golgi structures. Different proteins could utilize either one or both of these pathways. For instance, the two known marker proteins of the pre-Golgi compartment are also detected in *cis*-Golgi suggesting that they are recycled to the ER from this central location. The utilization of these pathways may also depend on the physiological state of the cell and vary in different cell types.

Function of the pre-Golgi compartment in ER retention

Studies of the retention of resident ER proteins in mammalian and yeast cells have contributed considerably to our understanding of the sorting of proteins between the rough ER and the Golgi complex and we can now begin to understand the mechanism of the process in molecular terms.[56] Many luminal ER proteins, which contain a carboxyterminal KDEL/HDEL-motif,[57] appear to utilize a receptor-mediated mechanism which returns them to the ER from a post-ER location.[58] Biochemical experiments and the localization of the KDEL-receptor indicate that the compartment from which this recycling occurs corresponds to the 15°C compartment,[59-62] also termed the salvage compartment.[63] The concentration of the human receptor has also been observed in the Golgi complex,[62,64] although its precise location in the Golgi is not yet known.

Morphological studies have not detected the KDEL-proteins in other elements than the rough ER,[60,61,65] suggesting that the visit of these proteins in the post-ER sorting compartment is rapid and transient. Furthermore, the lysosomal enzyme cathepsin D, to which the KDEL-motif has been attached, receives Man-6-P with slow kinetics, although ultimately the bulk of the protein is modified due to the activity of the two enzymes which apparently reside in the post-ER sorting site.[60] The above observations can be explained if the recycling of KDEL-proteins to the rough ER occurs both from the peripheral pre-Golgi elements as well as from central *cis*-Golgi structures (Figures 4B and 5A). The receptor could utilize two different recycling mechanisms and pathways, one dependent on the binding of the KDEL/HDEL-containing ligands and the other ligand-independent. This idea is supported by recent studies of Lewis and Pelham showing that the overexpression of KDEL-ligands results in the redistribution of the receptor from the Golgi region, where it is normally concentrated, to peripheral locations.[64]

The suggested sorting of the KDEL-receptors into tubular domains of the pre-Golgi elements, induced by the binding of ligands (Figure 5A), finds an analogous situation in the recycling of plasma membrane receptors which occurs in the early part of the endocytic pathway (Figure 5B). In the latter case the uncoupling of ligands from their respective receptors is induced by the luminal pH conditions.[51] This results in the segregation of the receptors into tubular domains of the peripheral endosomes and their recycling back to the cell surface.[12,13,51] Similarly, KDEL-proteins that have left the rough ER in the lumen of the transport vesicles, could bind to their receptors in the peripheral pre-Golgi elements and rapidly be recycled back to the rough ER. Related to the function of the pre-Golgi compartment, it has been suggested that the vacuolar concentration of calcium ions could be a regulating factor in the interaction of the KDEL-containing ligands with their receptors in the post-ER salvage compartment.[61,66,67]

One of the major, luminal ER proteins, the binding protein (BIP), which also contains a KDEL-sequence, interacts with newly synthesized, either incompletely folded or unassembled secretory and membrane proteins.[35] As suggested in Figure 5A, it is possible that the quality control processes which regulate the exit of proteins from rough ER to the Golgi involve a recycling pathway that operates between the rough ER and the pre-Golgi compartment.[68] Like KDEL-proteins, the unmature proteins could maintain an ER-like distribution although they continuously exit from the ER and rapidly transit through the pre-Golgi compartment. An example of such a situation was provided by a recent study demonstrating that MHC Class I molecules, which in certain cell types are not transported to the cell surface, are retained in the ER by a recycling process.[69] In general, the maturation of certain proteins could require that they encounter the two luminal conditions that prevail in the ER and pre-Golgi compartment.

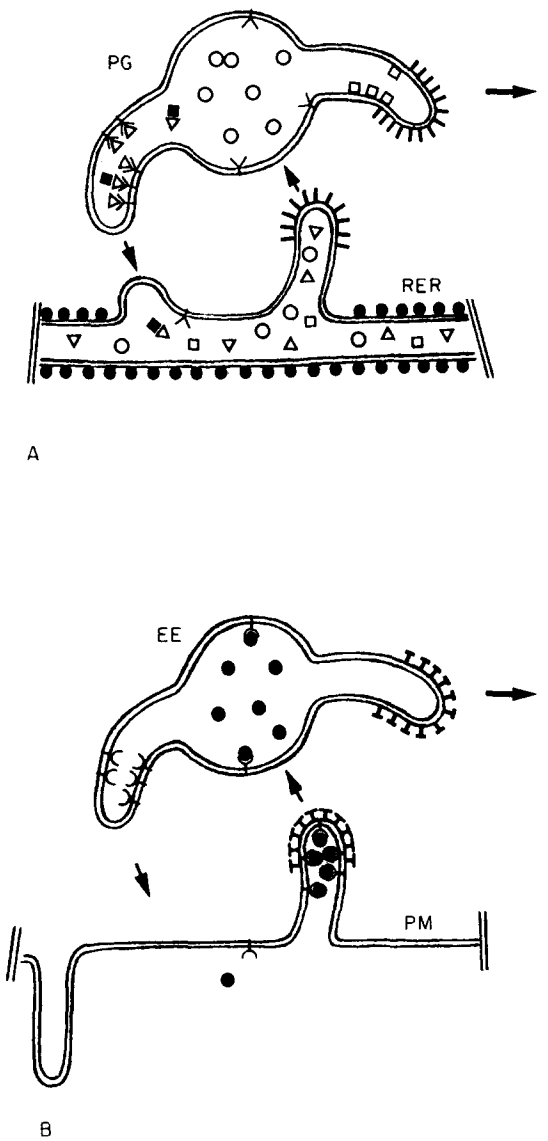

Figure 5. Protein sorting in pre-Golgi elements. A model for the sorting of exported and recycling proteins in peripheral pre-Golgi elements is suggested in diagram A. According to this schema 'escaped', soluble ER proteins, e.g. KDEL-tagged proteins, such as BIP (▵) bind to their receptors(s) (Y), and these complexes are then sorted into tubular domains of the pre-Golgi elements for recycling back to the rough ER. Unoccupied receptors travel with the element until ligand binding induces sorting and recycling. Illustrated is also the possibility that unfolded proteins (■), which bind to BIP until folding or quaternary assembly is completed, are continuously recycled through the pre-Golgi compartment. In comparison, the operation of peripheral endosomes in the recycling of plasma membrane receptors is shown in diagram B. The receptor (e.g. asialoglycoprotein receptor) and the corresponding ligand are dissociated in endosomes and the unoccupied receptor then segregates into endosomal tubules and is returned to the cell surface.

Transport across the Golgi stack

The above view on the structure and function of the pre-Golgi elements is also partly based on observations which suggested that these elements operate in the biogenesis of the Golgi cisternae. Immunoelectron microscopic localization of SFV spike glycoproteins in cells shortly after the reversion of the 15°C transport arrest showed that tubular parts of the pre-Golgi elements (containing viral membrane proteins) were seen in parallel with the cisternal elements at the entry face of the Golgi stack[15,18] (Figure 3C). These results suggest that the pre-Golgi tubules provide a pathway for the entry of the viral membrane proteins to the Golgi stack and at the same time function in the generation of the cisternal structure of this organelle (Figure 6).

This structural transformation clearly requires extensive membrane fusion. If the role of the cytoplasmic coats is to stabilize the tubular protrusions and inhibit membrane fusion, then controlled disassembly of these coats would be required in *cis*-Golgi so that the formation of lamellar structure can take place. In the central *cis*-Golgi the β-COP-containing coatamers may also interact with other cytoskeletal proteins and together with these form a protein scaffold which may function in the formation and maintenance of the cisternal structure as well as in the control of membrane traffic within the Golgi complex.[34]

If the Golgi cisternae are formed by the tubular extensions of the pre-Golgi compartment, then the vectorial transport of proteins through the Golgi stack is likely to be mediated by the cisternae themselves.[15] Such a view (Figure 6) is in contrast to the vesicle shuttle hypothesis, which considers the Golgi cisternae as more stationary entities between which transport occurs by small vesicles.[70] The strongest evidence that supports the function of vesicular transport intermediates derives from studies of *in vitro* systems in which distinct steps of intra-Golgi transport have been reconstituted.[40] In these studies Rothman and co-workers have provided morphological evidence for the function of both coated and non-coated vesicular intermediates in the transport of the VSV G-protein between Golgi subcompartments *in vitro*.[71]

Recently, the question has been raised on the role of the morphological pathway of intra-Golgi transport, suggested by the *in vitro* studies, in intact cells.[32,72] Alternative pathways, e.g. those based on tubular interconnections between cisternae of different

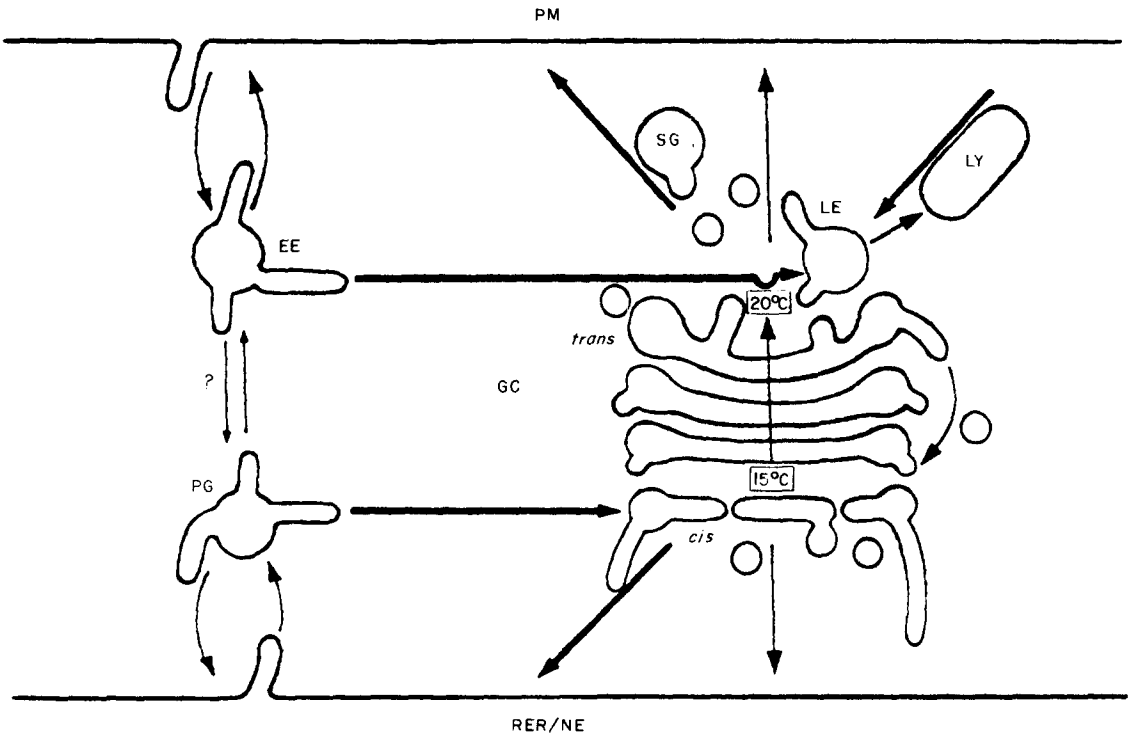

Figure 6. The function of pre-Golgi and endosomal elements in the generation of central Golgi organization. The thick arrows indicate long distance transport steps which are dependent on microtubules and thin arrows steps that are short in distance and, although may be facilitated by microtubules, can also occur (by vesicular transport) in the absence of microtubules. Both pre-Golgi elements (PG) and endosomes (EE, early endosome; LE, late endosome) move from the periphery of the cells to the central Golgi region along microtubular tracks. At the *cis* face of the Golgi complex (GC) tubular parts of the pre-Golgi elements function in the biogenesis of Golgi cisternae. Forward traffic across the Golgi is suggested to occur by cisternal movement which may be facilitated by microtubules as well as involve other cytoskeletal elements. At the *trans* side of the Golgi the cisternae fuse with tubular elements of the endocytic pathway. The compensatory backflow of membranes (and recycling of components) in *trans* to *cis* direction is suggested to involve vesicular (or tubular) transport. This diagram also suggests that there exist direct pathways from peripheral pre-Golgi elements to the plasma membrane (PM) and from peripheral endosomes to the rough ER/nuclear envelope (RER/NE) which bypass the central Golgi structures. Like Golgi elements, lysosomes (LY) also appear to be maintained in the central Golgi region by microtubule-dependent movement. SG, secretory granule.

stacks, have been suggested.[72] One possibility is that in the *in vitro* system the VSV G-protein is recruited to a vesicle-mediated pathway which remains operational under conditions in which the normal pathway is inhibited due to the absence of functional cytoskeletal elements. In intact cells the observed vesicular carriers could function in membrane recycling pathways within the Golgi (Figure 6), for which there is increasing evidence.[52,59,64,70,73] Furthermore, the efficient protein transport carried out by the dispersed Golgi elements in cells treated with microtubule-depolymerizing drugs could involve a vesicular pathway, similar to the one observed in the *in vitro* studies.

In intact cells transport across the Golgi could occur by a cisternal shuttle mechanism where the cisternae act as transient carrier elements. Accordingly, after being released from the *cis*-compartment

the cisternae could be transported to the *trans*-side, where there is a membrane fusion step. Alternatively, and in analogy to the above view of ER to Golgi transport (Figure 4B), the stacked cisternae may functionally belong to the *trans*-Golgi membrane system and could be viewed as pre-*trans* elements. In this case transport across the Golgi stack would represent a maturation sequence which leads to the formation of the *trans*-Golgi sorting compartment (TGN) (Figure 6). These views suggest that the Golgi complex is composed of two successive membrane systems, the pre/*cis*-Golgi membranes and the stack/*trans*-Golgi system. This idea shares common features with the Golgi model presented by Rothman.[74] It is also supported by the observed effects of temperature (Figure 6) and BFA on membrane traffic,[32] and recent data on the localization of β-COP[29] and small GTP-binding proteins (see the review of Goud, pp 301-307, this issue) in the Golgi complex.

Discussion

The term *cis*-Golgi network (CGN) has recently been increasingly used to describe a distinct compartment (composed of the intermediate compartment and the *cis*-Golgi cisterna) at the *cis* face of the Golgi complex which is structurally similar, and presumed to be functionally analogous, to the *trans*-Golgi network (TGN).[2,34,56,69,72,75] The original definition of the TGN as a sorting compartment in the biosynthetic/secretory pathway was largely based on experiments showing the effect of 20°C temperature on protein transport.[76] In these studies immunocytochemical localization of newly synthesized viral spike glycoproteins showed the concentration of these proteins at 20°C in cisternal, vacuolar and tubulovesicular elements at the *trans* side of the Golgi complex.[15,16] Other markers that have been shown to reside in these membranes include galactosyltransferase,[77] sialyltransferase,[78] and C5- and C6-NBD-ceramide.[52] They also appear to correspond to the vacuolar and tubular elements at the *trans*-Golgi which contain a slightly acidic pH.[79]

Both these terms, CGN and TGN, are basically morphological terms which largely relate to the three-dimensional organization of membranes at the entry (*cis*) and exit (*trans*) sides of the Golgi complex and imply that these structures constitute continuous membrane networks. However, by placing the focus on centrally located, Golgi stack-related structures these concepts may only partly describe the organization of the Golgi complex at the crossroads of intracellular membrane traffic pathways. According to a different view, discussed in the case of ER to Golgi transport above and summarized in Figure 6, the complex membrane organization seen at the two sides of the Golgi stack may be a result of the centralization and concentration of the peripheral organelles that operate between the rough ER and *cis*-Golgi and the plasma membrane and *trans*-Golgi, respectively. The sorting and transport activities of these pre-Golgi and endosomal/prelysosomal elements may already take place at the periphery of the cells but increase as these elements move along microtubules towards the central Golgi region.

The complication in defining the organization of the Golgi based largely on morphological criteria is exemplified by recent observations concerning the effect of BFA on the redistribution of cellular endomembranes.[32] Several studies have indicated that in BFA-treated cells the *trans*-Golgi membranes that correspond to the site where proteins accumulate at 20°C predominantly redistribute to the ER.[80-83] In contrast, membranes containing the mannose-6-phosphate receptor, which is thought to recycle between the TGN and late endosomes,[84] and a TGN marker protein (TGN-38),[85] are redistributed from the Golgi region to the periphery of the cells where they appear to establish a communication with early endosomes. These results with BFA suggest that the membranous structures, that are now collectively called the TGN (to describe a compartment of the secretory pathway), represent a heterogenous population of membranes in the *trans*-Golgi crossroads site where the exocytic and endocytic pathways meet (Figure 6).

An interesting possibility is that the redistribution of membranes from the central Golgi region to the periphery of the cells, seen in cells treated with BFA,[32] is due to a change in the direction of their movement along microtubules.[27] This is supported by the findings showing that tubular processes which apparently extend along microtubules, act as intermediates in these drug-induced membrane redistribution events.[33,84,85] However, the BFA-induced backflow of membranes may only partly be due to the observed, tubular transport. Some of the redistribution of Golgi membranes may also occur via vesicular transport over shorter intracellular distances. The existence of such microtubule-independent, vesicular recycling routes from *cis*- and

trans-Golgi to the ER and plasma membrane, respectively (Figure 6), could also explain the redistribution of Golgi elements in cells treated with microtubulus-depolymerizing agents, such as nocodazole,[86] in which the centralization of the peripheral organelles is blocked. Similarly, these pathways might also participate in the formation of the Golgi clusters, observed in mitotic cells.[87,88] Although the redistribution of endomembranes in all the above mentioned cases may share similar pathways, the functional state of the membranes is differentially affected. Different steps of protein transport are inhibited in mitotic and BFA-treated cells.[32,89] In contrast, when microtubules are specifically depolymerized, the peripheral Golgi structures are still capable of apparently normal protein transport and processing. This indicates that the exocytic and endocytic pathways in these cells remain functional and can interact. In fact, the peripheral Golgi complexes in the drug-treated cells may represent a paradigm of an early form of this organelle, which preceeded the development of central membrane organization.

Under normal conditions both endosomal and pre-Golgi elements translocate from the cell periphery to the Golgi region along microtubules (Figure 6). They move in the same (retrograde) direction along microtubules and may even utilize common microtubules as tracks. It is therefore possible that connecting transport pathways exist between these elements and the exocytic and endocytic pathways interact already at the periphery of the cells (Figure 6). The existence of such pathways between peripheral pre-Golgi and endosomal elements, which bypass the biosynthetic apparatus residing in the central Golgi complex, could possibly explain a number of observations. For example, the entry of certain viruses from the cell surface to smooth ER locations[90] and the transport of hormone-receptor complexes to the nuclear envelope.[91] Some cells could also use a route from peripheral endosomes to pre-Golgi membranes for the recycling of transport receptors, such as the mannose-6-phosphate receptor, to the *cis*-Golgi.[70,92] Regarding the opposite direction, the efficient secretion of incompletely glycosylated proteins in certain mutant yeast strains has been suggested to involve a pathway that bypasses the Golgi complex (ref 93; see also review by Franzusoff, pp 309-324, this issue). Also the unusually rapid externalization of a marker peptide, which becomes core glycosylated in the ER,[94] could possibly be explained by assuming a direct route from the pre-Golgi elements to the cell surface. Furthermore, the cellular processes of protein degradation[95] and antigen presentation[96] may involve pathways that link the early steps of the exocytic and endocytic route.

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