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The endoplasmic reticulum–Golgi intermediate compartment

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The recent identification of an endoplasmic reticulum–Golgi intermediate compartment has added to the complexity of the structural and functional organization of the early secretory pathway. Protein sorting along the endoplasmic reticulum–Golgi pathway depends on different signals and mechanisms, some of which guarantee recycling from various levels of the Golgi apparatus to biosynthetically earlier compartments.

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Introduction

The secretory (or exocytic) pathway is subdivided into a series of different membrane compartments of defined molecular composition. Newly synthesized exocytic proteins enter the pathway at the rough endoplasmic reticulum (ER) and are subsequently sorted to different organelles including Golgi apparatus, plasma membrane, endosomes and lysosomes. Recent interest in the protein traffic field concerns the mechanism by which itinerant proteins find their proper place within the cell without compromising the molecular identity of the individual organelles along the pathway [1,2]. The current view is that individual compartments communicate by shuttle vesicles. These vesicles carry soluble and membrane proteins along the secretory pathway by bulk flow unless a specific mechanism arrests the proteins in a given organelle. Some signals for protein retention have indeed been identified. However, the mere fact that no signals for forward transport have yet been found does not necessarily exclude their existence in some proteins. To understand protein traffic fully it is important to establish the boundaries and functions of individual compartments and subcompartments, to determine the mechanism of vesicular transport, and to elucidate protein signals for retention (and perhaps forward transport) and the molecular basis of protein retention.

It should be noted that there is no unambiguous use of the term compartment (or organelle) in the scientific literature. In this review, we define a membrane-bounded compartment of a cell as a physically distinct entity with a characteristic set of stably anchored endogenous (or resident) proteins. Compartments involved in membrane traffic mediate the transport of itinerant proteins via vesicular or dynamic tubular interactions. Thus, we refer to the ER and the Golgi apparatus as compartments or organelles. Rough and smooth ER are considered subcompartments of the ER while, in the absence of clearly defined membrane boundaries [1,2], the *cis*-Golgi network (CGN), *cis*/medial-Golgi, *trans*-Golgi and the *trans*-Golgi reticulum (TGN) are tentatively considered to be subcompartments of the Golgi apparatus. The membrane of a subcompartment may still be heterogenous and exhibit structurally and functionally distinct domains.

In the present article we focus on the ER-to-Golgi pathway. A novel aspect in interpreting the interactions of ER and Golgi is the recent identification of an ER–Golgi intermediate compartment (ERGIC) that is presently defined by a 53 kD transmembrane protein (p53). We discuss the features of the ERGIC and their implications for the structural and functional organization of the early secretory pathway, including problems of protein retention and recycling.

The ER–Golgi intermediate compartment defined by p53

An obvious present limitation in following the movement of proteins within the secretory pathway is the paucity of accepted marker proteins for individual subcompartments, in particular for the exit face of the rough ER, the transitional elements, and the entry face of the Golgi apparatus, the *cis*-Golgi, as well as the complex system of tubules and vesicles in between the ER and the Golgi. Recently a monoclonal antibody was used to identfy an apparently non-glycoslyated, homo-oligomeric transmembrane protein, p53, that is predominantly localized in tubulovesicular membranes near the *cis*-side of the Golgi apparatus, as visualized by immunoelectron microscopy [3]. Such tubulovesicular clusters were also identified at quite some distance from the Golgi apparatus. In some cell types, low levels of p53 were occasionally observed in the first fenestrated *cis*-Golgi cisterna, now often referred

Abbreviations

CGN—*cis*-Golgi network; **ER**—endoplasmic reticulum; **ERGIC**—ER–Golgi intermediate compartment; **MHC**—major histocompatibility complex; **PDI**—protein disulphide isomerase; **TEN**—*trans*-ER reticulum; **TGN**—*trans*-Golgi reticulum.

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to as the CGN [2]. The p53-harboring membranes were shown to represent membranes involved in ER–Golgi transport by studying the transport of the G protein of vesicular stomatitis virus (tsO45 mutant). When the transport from the ER was blocked by incubation at 15°C, the newly synthesized G protein co-localized with p53 [4]. As this temperature is known to arrest the transport of exocytic proteins between ER and Golgi, p53 must be a marker for the ER–Golgi intermediate elements, which are now known as the ERGIC.

A 58 kD resident protein (p58) of the *cis*-Golgi cisterna [5•] also appears to be present partly in elements that may represent the ERGIC [6•]. Unlike p53, p58 carries immature N-linked and O-linked glycans [5•]. Another marker for the ERGIC is the small GTP-binding protein, Rab2 [7]. Furthermore, Rab1bp [8•] is also, at least in part, associated with the ERGIC. GTP-binding proteins are thought to act as central regulators of the formation, targeting and fusion of ER-to-Golgi transport vesicles.

Using p53 as a marker protein it was possible to isolate the ERGIC of Vero cells by fractionating a post-nuclear supernatant on Percoll and Metrizamide gradients [9••]. The analysis of various marker proteins for rough ER and of a *bona fide* marker for *cis*-Golgi, GlcNAc-phosphodiester-N-acetylglucosaminidase, demonstrated biochemically that the ERGIC is not related to its neighboring compartments.

Organization of the ER-to-Golgi pathway

How does the existence of an ERGIC influence our thinking of how the ER-to-Golgi pathway is organized? Biochemical uniqueness of a membrane fraction, as shown for the ERGIC, does not necessarily exclude membrane continuities with neighboring organelles, as exemplified by the connections between rough and smooth ER in liver and pancreas. There are four major ways in which the ERGIC could be structurally related to ER and Golgi (Fig. 1). First, the ERGIC may be a physically separated membrane structure, also termed salvage compartment [10], in which case two vesicular steps would be required to transport proteins from the ER to the Golgi, and thus the ERGIC would be a true compartment by our definition. Second, in keeping with the classical model of Palade [11], the ERGIC may not be a compartment, but a pleomorphic transport intermediate. Such a view was supported by Saraste and Svensson [6•], who studied the dynamics of p58. Third, the ERGIC may represent the first CGN tubulo-vesicular cisterna [2,12]. In this model a single round of vesicular transport would transfer the proteins from ER to Golgi and the 15°C block would have to be placed between the CGN and the next *cis*-medial cisterna. Finally, the ERGIC may be a subcompartment of the ER and thus be referred to as the *trans*-ER network (TEN), which would include the transitional elements. In this case, the 15°C would block one single round of vesicular transport between the TEN and the CGN.

One complication is that p53 and p58 appear to cycle via the Golgi apparatus (see below) so that they may not tell us if the ERGIC is a stable membrane structure or a transport intermediate. A panel of monoclonal antibodies produced against the ERGIC isolated from Vero cells stained a reticular structure that extended farther into the cell periphery (A Schweizer, H-P Hauri, unpublished data). On the basis of immunofluorescence and immunoelectron microscopy experiments involving one of these antigens, a 63 kD membrane protein (p63), the reticular structure is stable to temperature shifts and in close apposition to, and possibly continuous with, the rough ER (A Schweizer, G Griffiths, T Bächi, H-P Hauri, abstract 65a, American Society for Cell Biology, Annual Meeting, Boston, MA, December 1991). The distributions of p63, p53 and VSV G-protein (arrested at 15°C) overlap, demonstrating that p63-containing membranes mediate exocytic protein transport. Furthermore, p63 was enriched to a similar degree as p53 in the purified ERGIC fraction. Unlike p53, the distribution of p63 was little affected by low temperatures or brefeldin A. These results suggest that the ERGIC may be a stable reticular compartment (or subcompartment) that is defined by the resident marker protein p63, while p53 may preferentially label the exit sites of this network. The compartment appears to be fragile and therefore difficult to visualize as an intact network by conventional fixation procedures (G Griffiths, personal communication). Based on these findings we presently favor the TEN model (Fig. 1d). This model would also be in accord with the data obtained from semi-permeable cells in which the vesicular transport from ER to Golgi can be reconstituted. It was found that protein transport from, but not into, the 15°C compartment required co-factors that are typical for vesicular transport [13].

Further studies on the characterization of the p63 network are in progress and should help to visualize the proposed connections with the rough ER and clarify the relationship of the ERGIC with other smooth membranes including those referred to as smooth ER, calciosomes [14•] and the s-cyclophilin compartment [15•]. It is worth noting that there is no accepted marker with which to define smooth ER in cells other than in the liver. Thus, smooth membranes that do not have Golgi characteristics are often collectively referred to as smooth ER without any further specification.

Functional compartmentalization of the ER-to-Golgi pathway

Table 1 lists some major functions, their corresponding markers and their presumed compartmentalization within the early secretory pathway. It is important to note that the localization of some markers may vary somewhat among different cell lines, particularly within the Golgi apparatus [16]. The sequential remodelling of N-linked glycan is the classical and best known example of functional compartmentalization within the early secretory pathway. Here, we concentrate on selective examples for which the precise localization is controversial but has recently been investigated in respect to a putative ER–Golgi intermediate localization by subcellular fractionation, by using transport inhibitors or by applying the 15°C criterion. Incubation at exactly 15°C for at least 3 h prevents protein transport into the CGN but allows its exit from the rough ER. It is important at this point to

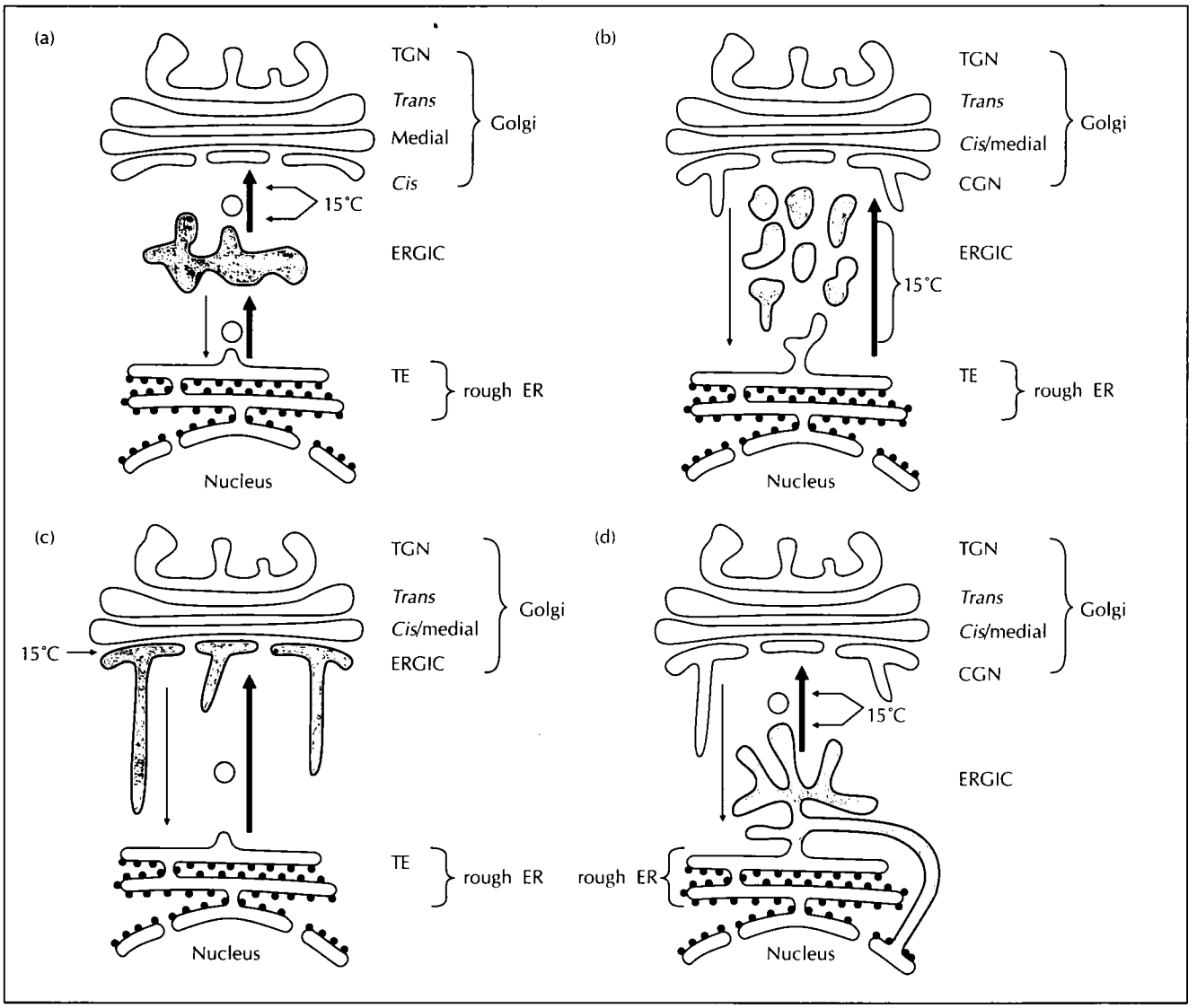

Fig. 1. Four schematic models of the relationship of the endoplasmic reticulum–Golgi intermediate compartment (ERGIC) to rough ER and Golgi. (a) The ERGIC is a physically separated membrane structure, and two vesicular steps are required to transport proteins from the ER to the Golgi [10]. (b) The ERGIC is an intermediate and transports the proteins itself [6•]. (c) The ERGIC is the first tubulo-vesicular cisterna of the *cis*-Golgi and proteins are transferred by a single round of vesicular transport [2,12]. (d) The ERGIC is a subcompartment of the ER, the *trans*-ER network, and it contains the transitional elements itself. The putative localization of the 15°C block is indicated in each model. TGN, *trans*-Golgi reticulum; CGN, *cis*-Golgi network; TE, transitional elements of the rough ER.

emphasize that temperature blocks are kinetic in nature and not absolute. Therefore, slightly higher temperatures, even if raised by only one degree, may lead to significantly different results.

Protein folding, a classical function of the rough ER, is mediated by protein disulfide isomerase (PDI) and perhaps related proteins, as well as chaperone proteins such as the immunoglobulin binding protein, BiP [17]. It is likely that the ERGIC contributes to protein folding as a soluble calcium-binding protein with a PDI active site motif, CaBP1, co-purifies with, and is highly enriched in, the ERGIC (A Schweizer, F Peter, P Van Nguen, H-D Söling, H-P Hauri, unpublished data), while PDI is not appreciably enriched in the isolated ERGIC fraction [9••].

Many proteins that are normally transported from ER to Golgi, when improperly folded or incompletely assembled, undergo degradation at a pre-Golgi site. It has become clear now that pre-Golgi degradation starts in the ER itself but continues, at least for some proteins, in post-ER compartments, most probably in the ERGIC, as exemplified by the unassembled H2a subunit of the asialoglycoprotein receptor [18•] and truncated ribophorin I [19•]. H2a and its intermediate 35 kD degradation product carry pre-Golgi-type glycans and cofractionate with rough ER. As further degradation of the 35-kD intermediate is blocked when the cells are depleted of ATP, the second phase of degradation may occur at a post ER-site. ATP is required for protein exit from the ER. Clear evidence for two degradative compartments was obtained

Table 1. Compartmentalization of some cellular functions of the early secretory pathway and their corresponding markers.

Organelle	Function	Marker	Method of localization
Rough ER	Protein folding and disulfide-mediated oligomerization	PDI and related proteins, BiP	EM, SF
	Protein degradation	T-cell receptor	I
		Asialoglycoprotein receptor	SF
		Ribophorin	I
ERGIC	?	p53, p58 (in part)	EM, SF
	Palmitoylation (reversible)	p62, p63	SF, EM
	Vesicular transport	rab2p	EM
	Protein folding	CaBP1	IF, SF
	Protein degradation	Ribophorin I	I
Golgi			
CGN	?	p58	EM*
	Generation of Man6-phosphate lysosomal signal (1st step)	GlcNAc-phospho-transferase	SF*
	Initial O-glycosylation	Peptide-N-acetylgalactos-aminyltransferase	EM, SF*
	Palmitoylation (irreversible)	Addition of palmitic acid	IF, SF*
cis/medial	Uncovering of Man6-phosphate signal	GlcNAc-phosphodiester-N-acetylglucosaminidase	SF*
	Sphingomyelin synthesis	Sphingomyelin synthase	SF*

*Not yet localized by immunoelectron microscopy, so localization is tentative. EM, immunoelectron microscopy; SF, subcellular fractionation; I, transport inhibitors; IF, immunofluorescence; PDI, protein disulfide isomerase; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment; CGN, *cis*-Golgi network.

in studies on an anchorless form of ribophorin I that is degraded with biphasic kinetics [19•]. The first phase occurs in the ER, while the second phase can be inhibited by carboxyl cyanide m-chlorophenyl hydrazone, which blocks protein exit from the ER and is thus associated with a later compartment. Based on these studies we postulate that the ERGIC contributes to pre-Golgi degradation of proteins. In some cases degradation may already be completed in the ER. For instance, in permeabilized cells chimeric proteins containing T-cell receptor transmembrane sequences are rapidly degraded without production of detectable intermediates in the absence of factors required for protein export from the ER [20•].

Another point of interest is the site at which the lysosomal targeting signal, mannose 6-phosphate, is generated. Incubation of a lymphoma cell line at 15°C does not prevent the initial phosphotransferase step but does block the subsequent action of GlcNAc-phosphodiester-N-acetylglucosaminidase, which suggests a pre-Golgi localization of phosphotransferase [21]. In Vero cells, however, neither enzyme appreciably co-distributes on density gradients with p53 [9••], suggesting that in this cell line both enzymes are localized primarily in the Golgi apparatus rather than the ERGIC. It is not clear if the discrepancies are due to cell type variations.

The site where O-glycosylation starts has not been firmly identified either. In Vero cells the first enzyme of this process, peptide-N-acetyl-galactosaminyltransferase, co-purifies by cell fractionation with Golgi markers and not with the ERGIC (A Schweizer, H Clausen, H-P Hauri, unpublished data). Thus, in Vero cells, peptide O-glycosylation is primarily a Golgi event, perhaps associated with the CGN. Earlier lectin studies have indeed pointed to the *cis*-most Golgi cisterna as the site of N-acetyl-galactosamine addition in intestinal cells [16].

Sphingomyelin synthesis in the liver was recently established as a *cis*/medial-Golgi event by cell fractionation [22], but a possible contribution of the ERGIC was not investigated. Cell fractionation experiments with Vero cells now show that sphingomyelin synthase co-distributes with the Golgi region but not with p53 (A Schweizer, G van Meer, H-P Hauri, unpublished data). In addition, the sphingomyelin content of the isolated ERGIC fraction is extremely low. These results are in full agreement with a *cis*/medial-Golgi localization of sphingomyelin synthesis.

It is likely that the major functions of the ERGIC have not yet been revealed. However, the isolation of this compartment from Vero cells is a first promising step

toward the elucidation of these functions. Candiate functions that have not been analyzed in respect to the ERGIC include calcium storage [14•] and the initial step of major histocompatibility complex (MHC) class I antigen presentation, i.e. the transport of cystosolically processed peptides into the endomembrane system. Another function may be palmitoylation. Mundy and Warren [23•] have described a major, reversibly palmitoylated protein, p62, that cofractionates with p58 and whose acylation dramatically increases both during mitosis and when intracellular protein transport is blocked by various agents. They suggest that p62 may play an important role in vesicular transport and may function as an acylating enzyme. Reversible acylation is thought to be catalyzed by an enzyme other than that responsible for irreversible acylation, which is a Golgi function [2]. Interestingly, the ERGIC protein p63 is also palmitoylated when protein traffic between ER and Golgi is blocked by brefeldin A (A Schweizer, H-P Hauri, unpublished data), suggesting that p63 might be related to p62.

Protein retention signals

Three types of signals have been identified that are necessary and sufficient to retain proteins in the early secretory pathway (Table 2): a carboxyl-terminal tetrapeptide lysine-aspartic acid-glutamic acid-leucine (KDEL) or related sequence of soluble proteins within the endomembrane system; a carboxyl-terminal KKXX or KXKXX motif (where K is lysine and X is almost any amino acid) exposed on the cytosolic side of type I transmembrane proteins; and transmembrane domains that often include the flanking regions on both sides of the membranes.

The KDEL retention mechanism has been elucidated in detail [10,24]. The retention of most luminal ER proteins, and one transmembrane protein [25•], depends on the presence of the KDEL motif. It is not a retention signal in the true sense, but instead allows retrieval of the proteins from a post-ER site, initially termed salvage compartment, by means of KDEL receptors. In the past year it has become apparent that some KDEL proteins reside in membranes other than the rough ER. When postmitochondrial membranes of hepatocytes were fractionated by density gradient centrifugation and probed with a KDEL-specific antibody, some KDEL proteins, including grp94 and another calcium-binding protein, CaBP1, did not co-fractionate with rough ER [26••]. Whereas grp94 co-distributed with marker enzymes that are more typical for smooth ER [26••], CaBP1 was resident in the ERGIC in Vero cells (see above). This suggests that KDEL guarantees retention at a pre-Golgi site but the final location of individual KDEL proteins within the early secretory pathway requires an additional anchoring mechanism that is still unidentified. Additional retention mechanisms might explain why, after removal of the KDEL signal, luminal proteins are secreted at vastly different rates [27]. Furthermore, it is not clear if this KDEL retention system is used by all luminal proteins. A case in point is the secretory form of cyclophilin that is retained by a non-KDEL-like carboxyl-terminal segment [15•].

The KKXX or KXKXX motif, here termed double lysine motif, is found on the cytosolic side of a group of type 1 transmembrane proteins [28]. The lysine in position −5 can be replaced by arginine without disruption of its retention function [29•]. The motif was initially discovered in the adenovirus E3/19K protein that is trapped in the ER of virus-infected cells. Although one report proposes another type of signal for the retention of E3/19K [30], there is little doubt that the double lysine motif indeed acts as a retention signal. This motif was initially considered a signal for retention in the rough ER, but subsequently has been found in proteins associated with other membranes. Rough ER proteins carrying the motif include the β-subunit of the signal sequence receptor [31•] and an essential 45 kD yeast protein [32•]. However, smooth ER proteins such as 3-hydroxy-3-methylglutaryl CoA-reductase and UDP-glucuronosyl transferase also carry this motif [28,29•]. Surprisingly, a double lysine motif is also present in the ERGIC marker p53 (R Schindler, M Zerial, F Lottspeich, H-P Hauri, unpublished data). Overall, the subcellular distribution of this motif in the rough ER, smooth ER and ERGIC is analogous to that of KDEL, and argues for a mechanism of receptor-mediated retrieval of the proteins from a post-ERGIC compartment. The presence of the double lysine motif proteins in yeast might facilitate the identification of such a presumed receptor by using the strategy of Pelham [10] that was so successful for finding KDEL

Table 2. Retention signals and mechanisms of protein retention along the ER-to-Golgi pathway.

Signal	Site of retention	Mechanism of retention
Carboxyl-terminal KDEL or similar sequence (luminal)	rough ER, ERGIC	Receptor-mediated retrieval
Carboxyl-terminal KKXX or KXKXX* (cytosolic)	rough ER, smooth ER, ERGIC	Receptor-mediated retrieval?
Transmembrane domain	ER, Golgi	Network formation by homotypic and heterotypic oligomerization?
Cytosolic domain	TGN	Interaction with cytosolic Golgi matrix?

*K is lysine and X is almost any amino acid. ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment; TGN, *trans*-Golgi network; KDEL, lysine–aspartic acid–glutamic acid–leucine.

receptors. Interestingly, a double lysine motif was also found in a soluble cytosolic protein, Bet2p, that mediates membrane attachment of the small GTP-binding protein, Ypt1p, which in turn is required for vesicular transport from ER to Golgi [33•]. It is not known, however, if the motif is important for the function of Bet2p.

Most transmembrane proteins of pre-Golgi membranes do not carry a double lysine motif, and are thus retained by a different mechanism that is largely unknown. An exception is the rotavirus protein, VP7, which is retained by a transmembrane segment, and the signal sequence is also of importance [34]. The three Golgi proteins whose anchoring has been studied in detail are also retained by means of their transmembrane anchors and the flanking regions play an additional role. These proteins are the E1 glycoprotein of avian coronavirus [35•] that accumulates preferentially in the *cis*-Golgi in transfected cells and the *trans*-Golgi enzymes galactosyltransferase [36•] and sialyltransferase [37•]. It has been suggested that these proteins oligomerize upon recognition of identical or related proteins in the Golgi, forming a complex of sufficient size to prevent entry into transport vesicles [36•]. Another mechanism of retention has to be postulated for the TGN marker protein, TGN38 [38], which is not retained in the Golgi complex when its cytoplasmic segment is deleted. Similarly, but surprisingly if compared with the avian E1 [35•], a short cytosolic deletion of the mouse corona virus E1 glycoprotein results in its transport to the plasma membrane [39•]. However, this cytosolic segment alone is not sufficient for Golgi retention. Most of the protein sequence seems to be required. The diversity of retention signals strongly suggests that several fundamentally different mechanisms can retain proteins within the secretory pathway.

Recycling of proteins between the ER and the Golgi apparatus

Studies of the Golgi-disrupting drug brefeldin A [40] and of the KDEL retention/recycling system have largely contributed to the view that some proteins can recycle back to the ER from more distal elements of the secretory pathway, along a retrograde pathway. Unfortunately, so far there is little conclusive evidence that observations concerning membrane traffic in brefeldin A-treated cells can readily be extrapolated to normal cells, because of the apparent fusogenic and ionophore [41•] properties of this drug, the extent of which varies among cell type. For instance, it is unclear if cells that vesiculate their Golgi upon brefeldin A treatment but do not translocate Golgi resident proteins back to the ER have a less developed retrograde pathway or respond differently to brefeldin A. Perhaps this problem will be solved once the molecular mechanism of action of brefeldin A is known. On the other hand, brefeldin A is an interesting organelle perturbant that is useful for the study of subcellular distribution of organelle-associated proteins.

Good evidence for protein recycling via the Golgi originates from recent studies on the human KDEL receptor hERD2 that, from immunofluorescence analysis, is known to largely concentrate in the Golgi apparatus of transfected COS cells [42••]. Overexpression of KDEL ligands caused a redistribution of hERD2 from the Golgi apparatus to the ER implying ligand control of receptor recycling. The co-localization of hERD2 with the *trans*-Golgi marker, galactosyltransferase, was unexpected, as previous biochemical experiments with KDEL-tagged cathepsin suggested a retrieval of KDEL proteins from a pre-Golgi salvage compartment. On the other hand, recent studies with calreticulin in hepatocytes showed that the retrieval of some KDEL proteins can in fact occur from the *trans*-Golgi [26••]. This protein of the KDEL family is a resident of the rough ER but possesses carbohydrate side chains of the complex type with terminal galactose residues. All of the molecules carry both KDEL and terminal galactose. This feature, as well as the observation that addition of galactose is blocked in cells cultured at 15°C, indicates recycling via the *trans*-Golgi. Most likely, the retrieval of KDEL proteins can occur from *cis*-, medial- and *trans*-elements of the Golgi but perhaps not from the TGN, as it has been argued that there is no recycling from the TGN to earlier compartments of the secretory pathway [2].

Protein recycling via the CGN has been described for unassembled MHC class I molecules [43••]. In the absence of an accepted marker we operationally define the CGN as the first subcompartment at the *cis*-side of the Golgi apparatus in which sorting into forward (through the Golgi) and backward (to the ER) directions can occur. This definition is analogous to that of the TGN. It differs somewhat, however, from the CGN definition given by Mellman and Simons [2] as we do not consider the ERGIC to be part of the CGN (see above and Fig. 1d). Morphologically, the CGN appears to correspond to the first osmophilic tubular *cis*-element. Cells transfected with an MHC class I (H-2Kd) cDNA accumulated MHC class I molecules primarily in what was described as smooth ER, which may be equivalent to the ERGIC, and in the rough ER including the nuclear membrane [43••]. After incubating the cells at 16°C followed by a 5 min warm-up to 37°C, class I molecules were concentrated in the CGN. After longer times of rewarming the protein assumed its original steady state distribution. The molecular mechanism of MHC class I recycling is unknown, but is probably different from that of the KDEL receptor because recycling of MHC class I molecules is not ligand-dependent and appears to occur preferentially from the CGN.

Recycling via the CGN has also been observed for p53 and p58. At steady state, p53 is largely present in the ERGIC. Upon lowering the temperature to 15°C, most of the p53 molecules concentrate closely to the Golgi apparatus [4,44], presumably in the exit sites of the ERGIC and in transport vesicles. Upon brief rewarming to 37°C, long p53-positive tubular processes emanate from the Golgi (A Schweizer, H-P Hauri, unpublished data) similar to those observed for the Golgi marker mannosidase II after a short exposure to brefeldin A [44]. After a warm-up period of 60 min, p53 displays an ER-like pattern before the typical steady state distribution of p53 is established. These experiments suggest that the retrograde pathway from the Golgi is mediated by long membrane tubules that appear to fuse with the rough ER

and/or ERGIC. The p58 protein may recycle along the same pathway, although the interpretation of the experimental data is more complex because at steady state this protein is present mostly in the CGN [5,6].

Based on the experiments with p53 we conclude that the Golgi to ER backward pathway is tubular in nature. It is not known if membrane traffic by dynamic tubules fundamentally differs from a vesicular mechanism. Tubules may be considered as vesicles that pinch off very slowly and thereby form long necks, as has been observed for coated pits in some cell lines. If this is the case, tubule formation could be driven by the coat. Alternatively tubules may form in areas that lack a coat [40], in which case tubule formation would be a consequence of loss of tone by the regional absence of a coat and interactions with microtubules may also be important [44].

A tubular transport mechanism is advantageous over a vesicular one in situations where much membrane but little luminal content has to be transported. This is exactly what is required for the Golgi to ER recycling pathway, which should retrieve lipids with high capacity but exclude secretory proteins destined to be transported through the Golgi to the cell surface [45]. How are exocytic membrane proteins excluded from the retrograde pathway? Assuming that the bulk flow model of protein transport is correct, one would have to postulate that exocytic membrane proteins are passively excluded from entering the retrograde tubules. In contrast, proteins that recycle, such as p53 and p58, would be actively recruited into nascent tubules. Protein sorting into the retrograde pathway would thus be analogous to the sorting of receptors into coated pits at the plasma membrane. The retrieval of p53 may be mediated by its cytosolic KKXX motif, while the recycling of hERD2 receptor appears to depend on a different, ligand-induced conformational change [42••]. The low amount of sphingomyelin in pre-Golgi membranes suggests that lipids are also sorted in the CGN. Sphingomyelin molecules may form hydrogen bond-mediated microdomains with glycosphingolipids and thereby be excluded from the retrograde pathway.

As only a limited set of proteins undergoes recycling, and p53 is a major protein in this pathway, it is not unreasonable to assume that p53 plays an important role in the recycling process itself. As the cDNA sequence of p53 has not pointed to its possible function, verification of this speculation requires more sophisticated analysis including functional inactivation.

Conclusion

Research on the cell biology of the early secretory pathway has entered a new phase with the realization that a complex interposed membrane system, the ERGIC, participates in the dynamic interactions of rough ER and Golgi apparatus. Although important questions remain, the study of novel protein markers and the analysis of the isolated ERGIC have already provided important information concerning the structural and functional organization of the ER–Golgi pathway. Furthermore, the sorting of proteins to individual compartments is known to depend on different signals and mechanisms. Most remarkable is the mechanism(s) of protein retention by recycling via the Golgi apparatus. It has now become evident that protein recycling occurs mainly from the CGN but also from later Golgi subcompartments, as far as the *trans*-Golgi.

Despite these discoveries, many questions remain unanswered. Are all the smooth pre-Golgi membranes involved in forward or backward protein transport, or are there additional separate compartments fulfilling specific functions? Is the ERGIC indeed connected to the rough ER and what are its major functions? What determines slow transit through pre Golgi compartments of proteins that can be recovered by a recycling mechanism? How are proteins and lipids sorted into forward and backward traffic in the early secretory pathway? Answers to these questions will depend on the identification of new marker proteins for the different membranes interposed in between ER and Golgi and for the CGN, which are required for further structural and functional dissection of the pathways. Biochemical and genetic approaches in combination with semipermeable cells and cell-free systems (see reviews in this issue by Schekman pp 587–592 and Gruenberg and Clague, pp 593–599) will continue to be important in identifying the molecular machineries responsible for protein targeting and retention. A complete picture of the secretory pathway will only emerge if both structure and function can be integrated as a whole.

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