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Targeting and retention of Golgi membrane proteins

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Recent cloning of genes encoding membrane proteins of the Golgi complex has allowed investigation of protein targeting to this organelle. Targeting signals have been identified in three glycosyltransferases, a viral envelope protein and several proteins of the *trans*-Golgi network. Interestingly, the targeting signals for membrane proteins of the Golgi stacks seem to be contained in transmembrane domains. Information in the cytoplasmic tails is required for the targeting of *trans*-Golgi network proteins. Mechanisms involving both retention and retrieval have been invoked.

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Introduction

Plasma membrane and secreted proteins are synthesized on ribosomes bound to the endoplasmic reticulum (ER), translocated into the ER and transported through the Golgi complex en route to the plasma membrane [1]. Proteins destined for lysosomes or regulated secretory granules follow the same pathway but are diverted after moving through the Golgi complex. Multiple rounds of vesicular budding and fusion are believed to transport proteins and lipids through the exocytic pathway [2]. Organelle identity is maintained in the face of extensive membrane flow.

Transport through the secretory pathway to the plasma membrane is believed to occur by default [3]. This means that once a protein has been translocated into the ER, it needs no further signals for transport to the cell surface. Thus, resident proteins of the ER and Golgi complex must possess specific localization signals that prevent further transport in the pathway. Several classes of ER localization signals have been identified [4,5]. This review will focus on the recent identification of localization signals for resident proteins of the Golgi complex.

Golgi complex structure and function

The unusual structure of the Golgi complex has intrigued cell biologists since the advent of the electron microscope. In higher eukaryotes, the organelle consists of flattened cisternal membranes with dilated rims, arranged in polarized stacks near the microtubule organizing center [6]. The number of cisternae per stack and the number of stacks per cell vary widely in different cell types. Protein traffic moves vectorially through the Golgi complex, from the entry or *cis*-face to the exit or *trans*-face.

The Golgi complex plays a central role in post-translational processing and sorting of protein and lipid traffic. The traditional view of the Golgi complex invokes at least four functional subcompartments: *cis*-, medial-, *trans*- and *trans*-Golgi network (TGN) [6,7]. The TGN consists of the last cisterna and its associated tubular network, and is the sorting site for lysosomal and regulated secretory proteins [7]. The recently identified 'ER–Golgi intermediate compartment' [8] may perform additional sorting functions on the *cis*-side of the Golgi complex, and will be referred to here as the *cis*-Golgi network (CGN) [4]. Glycosyltransferases and glycosidases involved in oligosaccharide processing are believed to be enriched in specific Golgi subcompartments in the order in which they act [9].

This traditional view of the Golgi complex has recently been challenged. Mellman and Simons [10•] suggest that there are only three Golgi compartments: CGN, Golgi stacks and TGN. In this model, oligosaccharide processing would occur only in the stacks, and sorting would occur in the CGN and TGN. Glycosyltransferases and glycosidases would reside throughout the stacks. As their specificity determines the order in which they act, there should be no need to segregate them in different subcompartments. Mellman and Simons [10•] also point out that transport through the Golgi stacks may normally occur through transient tubular connections rather than discrete vesicle budding and fusion events. In another challenge of current Golgi models, Saraste and Kuismanen [11•] revive the idea of cisternal progression as a possibility for movement of membrane traffic through the Golgi complex. In this model, resident enzymes must be continuously recycled as cisternae 'mature' to the next stage.

Abbreviations

CGN—*cis*-Golgi network; **DPAP A**—dipeptidyl aminopeptidase A; **ER**—endoplasmic reticulum; **IBV**—infectious bronchitis virus; **GnTI**—N-acetylglucosaminyltransferase I; **GT**—β1,4-galactosyltransferase; **IBV**—infectious bronchitis virus; **ST**—α2,6-sialyltransferase; **TGN**—*trans*-Golgi network; **TMD**—transmembrane domain.

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The first immunolocalization of two different glycosyltransferases in the same cell has been recently reported. Nilsson *et al.* [12•] co-localized an endogenous *trans*-Golgi enzyme, β1,4-galatosyltransferase (GT), and a transfected version of a medial-Golgi enzyme, N-acetylglucosaminyltransferase I (GnTI), in HeLa cells. Each enzyme was concentrated in neighboring sets of two cisternae, with a substantial amount of both in the *trans*-cisterna. In spite of the overlapping distributions, the authors suggested that each cisterna was unique as it contained a unique mixture of these transferases. It is possible that a similar situation exists for other Golgi enzymes. This would imply that the Golgi stacks are indeed subcompartmentalized, although perhaps not as stringently as previously proposed. It is likely that subcompartmentalization varies between cell types as well.

Resident Golgi membrane proteins

Enzymes involved in glycosylation

A number of Golgi glycosyltransferases and one glycosidase have been cloned [13,14]. Interestingly, they all share the same type II membrane topology, with an uncleaved signal-anchor sequence near the amino terminus, and the catalytic carboxyl-terminal domain in the lumen. These enzymes remodel the core N-linked oligosaccharides added to newly synthesized proteins in the ER, add sugars to serine and threonine residues to produce O-linked oligosaccharides, and terminally glycosylate glycolipids [13]. A different transferase is responsible for each specific sugar linkage. Additional membrane-bound Golgi enzymes involved in glycosylation (but not yet cloned) include a specific phosphotransferase and glycosidase that produce the mannose-6-phosphate marker on lysosomal hydrolases [15], and sugar–nucleotide transporters that import sugar substrates into the Golgi lumen [16].

TGN proteins

Several prohormone-processing proteases that probably reside in the TGN have been cloned. In yeast, Kex1p and Kex2p have a type I membrane topology, with a cleaved amino-terminal signal sequence and a single membrane-spanning domain. Dipeptidyl aminopeptidase A (DPAP A) has a type II membrane topology [17]. These enzymes are believed to reside in the last Golgi subcompartment (possibly the TGN equivalent), but this classification is tentative due to the difficulty in distinguishing yeast Golgi subcompartments morphologically. The mammalian furin/PACE protease shares homology with Kex2p and has the same membrane topology [18]. TGN38, a protein of unknown function in mammalian cells, has a type I membrane topology as well [19]. In the examples noted, protein topology in the membrane correlates with residence in either the Golgi stacks (type II) or the TGN (type I). However, there are viral type I proteins that are targeted to the Golgi stacks [20], and more exceptions are bound to emerge as more cloned cDNAs are obtained.

Viral proteins

Some enveloped viruses assemble at Golgi membranes instead of the plasma membrane (reviewed in [20]). These viruses encode one or more membrane proteins that are specifically targeted to the Golgi complex, and direct viral assembly. After budding into the lumen, the virions are thought to traverse the secretory pathway in transport vesicles. Viral membrane proteins have proven to be valuable models for protein targeting in the exocytic pathway. The study of membrane protein targeting to the Golgi complex is no exception.

Targeting signals of resident Golgi proteins

Golgi stack proteins

The first Golgi localization signal was identified on a viral membrane protein. The avian coronavirus infectious bronchitis virus (IBV) M protein (formerly called E1) is targeted to the CGN/*cis*-region of the Golgi complex when expressed from cDNA in mammalian cells [21]. The first of the three transmembrane domains (TMDs) of M protein was found to be necessary and sufficient for Golgi localization, as it could retain several reporter proteins normally transported to the plasma membrane [22,23•]. Uncharged polar residues (asparagine, threonine and glutamine) that line one face of a predicted α-helix constitute the important feature of the retention signal. When any of these key residues was mutated, the reporter protein was transported to the cell surface [23•,24].

In contrast to the avian IBV M protein, the related M protein from a murine coronavirus is targeted to the *trans*-Golgi/TGN when expressed from cDNA [25]. This TGN localization may explain why Armstrong and Patel [26] found that unlike the avian M protein, the murine M protein required the carboxyl-terminal 18 amino acids for Golgi localization (although this sequence was not sufficient for retention of a reporter protein). The first TMD of murine coronavirus M protein has a polar face similar to that of the IBV M protein, but it fails to retain a reporter protein in the Golgi stacks or TGN [24]. Presumably, the amino acid differences in the first TMD of the murine M protein prevent its recognition in the *cis*-Golgi, and allow signals in other domains of the molecule to retain the protein in a later Golgi compartment.

Several investigators have recently examined targeting of endogenous Golgi glycosyltransferases (reviewed in [27•]). Studies with GT, GnTI and α2,6-sialyltransferase (ST) have confirmed the finding that TMDs are critical for Golgi retention [28•–36•]. Using reporter molecules, Golgi targeting was reconstituted when only the TMD from the transferase was present (Table 1). In some cases, correct subcompartmentalization of the chimeric proteins was demonstrated by immunoelectron microscopy [28•,30•,36•]. Although the TMDs of these enzymes possess Golgi targeting information, flanking sequences were often required for most efficient retention [28•,32•,33•,35•]. These flanking sequences may actually contain targeting information, or they may serve to posi-

tion the TMD in the membrane properly. ST may have redundant targeting information in the part of the molecule called the stem, a sequence near the TMD on the lumenal side of the membrane. A reporter construct with the ST stem and cytoplasmic tail but with 17 leucine residues replacing the TMD was still retained in the Golgi [32•]. A soluble, truncated form of ST lacking the cytoplasmic tail and TMD, but possessing the stem was also retained in the Golgi complex [33•].

Table 1. Targeting signals in Golgi proteins.

Protein	Localization within Golgi	Domain(s) with targeting signal†	Reference
IBV M (E1)	*cis* (CGN?)	1st TMD (Asn, Thr, Gln)	[23•,24]
GnT I	medial	TMD	[35•,36•]
GT	*trans*	TMD	[28•–31•]
ST	*trans*/TGN	TMD + stem	[32•–34•]
TGN38	TGN	cytoplasmic tail (tyrosine)	[40•]
Kex2p	TGN?‡	cytoplasmic tail (tyrosine)	[43•]
Kex1p	TGN?‡	cytoplasmic tail	[44•]
DPAP A	TGN?‡	cytoplasmic tail	[45•]

†Key residues are shown in parentheses if known. ‡It is unclear if the last Golgi compartment defined genetically in yeast is equivalent to the TGN.

There are two isoforms of GT produced by initiation at different sites, resulting in a 13 amino acid extension of the amino-terminal cytoplasmic tail in one isoform. Lopez *et al.* [37] suggested that the isoform with the longer tail was preferentially targeted to the plasma membrane [37]. This has not been observed by other investigators [30•,31•]. A recent study on transcriptional and translational control of isoform expression [38] suggests that long GT is expressed in all cells at a basal level, while expression of short GT is specifically turned on when higher activities are required (e.g. in lactating mammary tissue). This observation predicts that the two isoforms are functionally equivalent *trans*-Golgi resident enzymes, and supports the idea that the GT TMD is critical for Golgi localization of both isoforms.

The residues within the TMDs of GT, ST and GnTI that are required for Golgi retention have not yet been identified. Aoki *et al.* [29•] reported that a cysteine and a histidine residue in the cytoplasmic half of the TMD of GT were essential for Golgi retention of their reporter molecule. However, Nilsson *et al.* [28•] found that the lumenal half of this TMD (which does not contain the cysteine or histidine) was sufficient for Golgi retention of the reporter protein used in their study. An important point is that there is no primary sequence homology within TMDs of the Golgi proteins that have been cloned [27•,39]. This is true even for enzymes believed to be enriched in the same Golgi subcompartment. Thus, the targeting mechanism may involve a degenerate signal like the signal sequence for ER translocation, or a signal present in a three-dimensional structure or signal patch.

TGN proteins

Another type of targeting signal has been identified for residents of the TGN (Table 1). TGN38 is transported to the plasma membrane when its cytoplasmic tail is deleted [19], and a recent report identifies a tyrosine-containing signal in the tail that is required for TGN localization [40•]. This tyrosine signal (Tyr-Gln-Arg-Leu) is similar to internalization signals on receptors that are endocytozed from the plasma membrane [41]. Although the TGN38 sequence can function as an internalization signal, one mutation in this sequence prevented TGN localization without blocking internalization from the plasma membrane [40•]. This suggests that internalization from the plasma membrane is not the sole function of the signal in TGN targeting. It is not known if internalization from the plasma membrane is a normal part of the targeting pathway for TGN38. Interestingly, similar 'internalization-like' signals have been identified in the cytoplasmic tails of lysosomal membrane proteins [42]. It is also not clear whether these molecules travel through the plasma membrane to lysosomes.

Like TGN38, processing proteases in yeast (Kex1p, Kex2p and DPAP-A) all require their cytoplasmic tails for correct intracellular targeting [43•–45•]. A specific tyrosine residue in the cytoplasmic tail of Kex2p is critical for Golgi localization [43•]. Interestingly, clathrin is required for Golgi retention of these late Golgi enzymes, as a temperature-sensitive mutation in clathrin heavy chain caused mis-sorting to the cell surface at the non-permissive temperature [46•]. Importantly, a marker for an earlier Golgi subcompartment (guanosine diphosphatase), was not affected [46•]. This implies that the cytoplasmic tails of these yeast TGN residents may interact directly (or indirectly through adaptins) with clathrin, and that this interaction is required for efficient retention.

Golgi retention mechanisms

Two general mechanisms by which targeting signals could direct Golgi localization have been reviewed [39], and are outlined in Fig. 1. The retrieval mechanism [Fig. 1(a)] invokes a receptor that recognizes a signal on Golgi proteins that have escaped from their correct subcompartment and returns them (either directly to the appropriate subcompartment, or to the ER for another round of transport and retention). Retrieval of soluble ER residents with the carboxyl-terminal Lys-Asp-Glu-Leu (KDEL) type targeting signal from a post-ER compartment has been documented [4]. The existence of a 'retrograde' membrane traffic pathway that retrieval receptors could follow has been inferred from studies with the drug brefeldin A [47]. A retrieval receptor could constitutively recycle or be induced to recycle after ligand binding.

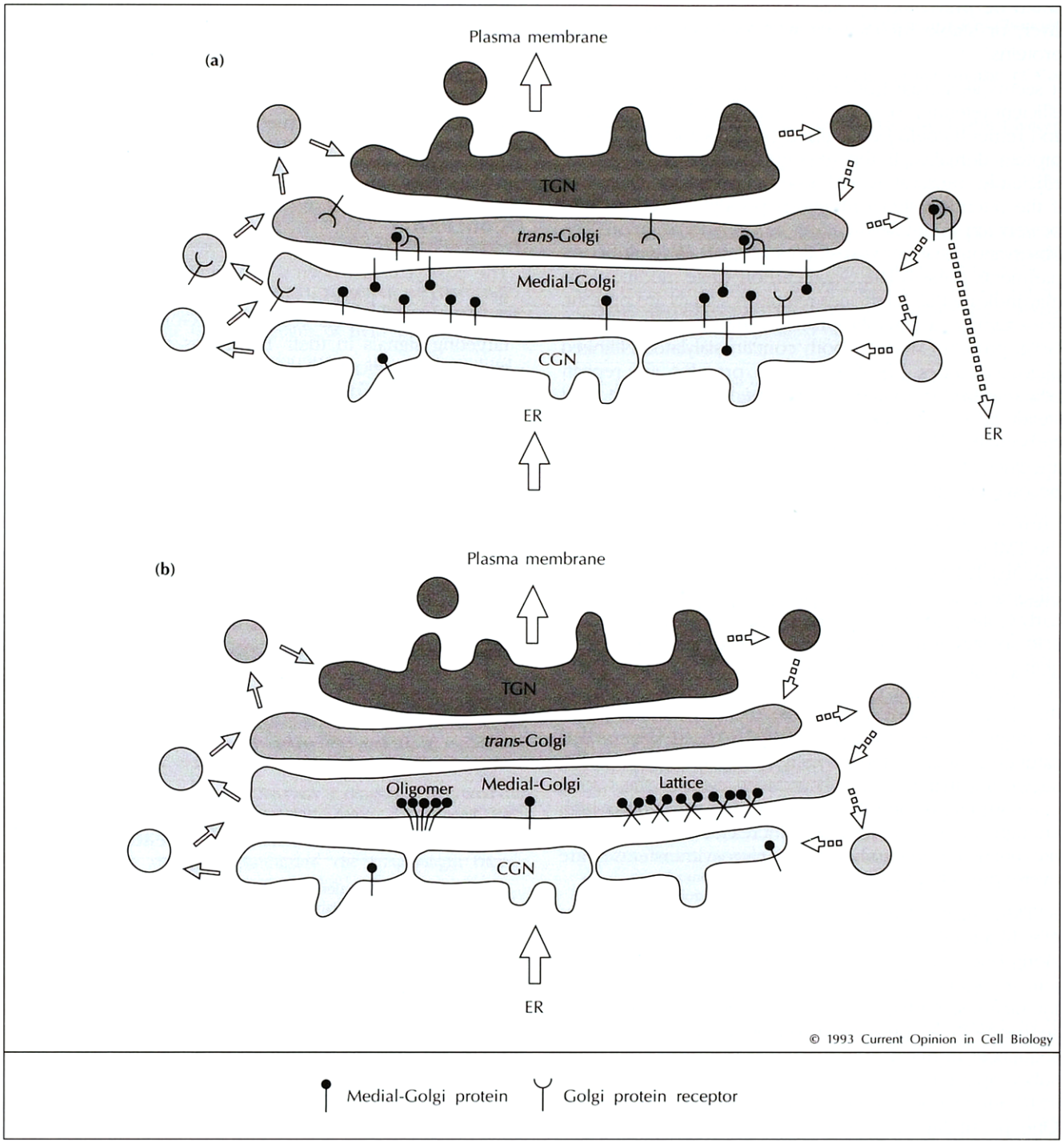

Fig. 1. Models for Golgi membrane protein targeting. A hypothetical medial-Golgi protein is shown. Hypothesized 'retrograde' pathways are shown by broken arrows. **(a)** Retrieval mechanism, in which a recycling receptor binds escaped proteins in a later subcompartment and returns them directly to the appropriate subcompartment or the ER for another round of transport and chance to be retained. **(b)** Retention mechanism, where a structure incompatible with transport (e.g. oligomer or lattice) forms in the appropriate subcompartment preventing further transport. The oligomer or lattice could contain different molecules that are retained in the same subcompartment, and could be stabilized by interactions on the cytoplasmic and/or lumenal side of the membrane. Efficient targeting may require both mechanisms and involve recognition of signals on the same or different domains of the protein.

The other possibility is a true 'retention' mechanism [Fig. 1(b)]. In this case, Golgi proteins would form a structure that is incompatible with transport (such as an oligomer or lattice). This structure would be specified by the retention signal and perhaps induced by micro-environmental changes in a given Golgi subcompartment. Transport

could be prevented by size, immobility in the lipid bilayer, or stable interactions with cytosolic or lumenal proteins.

It seems likely that both mechanisms might operate for efficient retention of Golgi proteins. Signals for retention and retrieval could be localized on the same or different protein domains. If the retention mechanism operates efficiently, retrieval would not be necessary. However if the retention signal functions poorly, retrieval would be very important for steady-state Golgi localization. The observation that some residents of early Golgi subcompartments possess late Golgi carbohydrate modifications may indicate that inefficient retention signals are present. For example, a *cis*-Golgi protein (GIMPc) and a medial-Golgi protein (MG160) both contain sialylated N-linked oligosaccharides [48,49]. If these proteins are repeatedly retrieved from later compartments to maintain their steady state localizations, they could acquire late Golgi carbohydrate modifications. The idea that these proteins are efficiently retrieved can be tested when cloned cDNAs become available.

Current evidence suggests that the signals identified in the TMDs of Golgi proteins act as true retention signals. The first TMD of IBV M protein causes formation of large oligomers of the reporter protein when it reaches the early Golgi [23•,50]. Reporter proteins with single amino acid substitutions within the TMD that inactivate the targeting signal do not form these oligomers. Even with very high levels of expression, the reporter containing this TMD does not leak out to the cell surface [23•], as might be predicted if a receptor-based system was saturated. Instead, the protein 'backs up' into the ER, suggesting that retention is induced even in the wrong compartment if the concentration of the protein is high enough. Consistent with this, increased ER localization has been observed when Golgi glycosyltransferases are overexpressed [28•,30•,32•,33•].

If TMD signals are involved in retention, how might they function? The finding that uncharged polar residues along one face of a putative α-helix in the IBV M protein are required for retention implicates protein–protein interactions. Perhaps this face interacts with the same sequence in an identical molecule, or with compatible sequences in different molecules that reside in the same subcompartment. However, it is difficult to imagine how TMD interactions alone could create large oligomers. One possibility (suggested by Nilsson *et al.* [28•]) is that lumenal and/or cytoplasmic domains interact to form small clusters or oligomers, which are organized into larger arrays or lattices by TMD interactions between neighboring clusters. TMD interactions between different Golgi proteins that reside in the same subcompartment could create hetero-oligomers that dictate the precise enzymatic composition of that subcompartment.

By contrast to TMD retention signals, it is tempting to speculate that the signals identified in the cytoplasmic tails of TGN residents are retrieval signals. Clearly, the tyrosine-based signal on TGN38 can function in internalization from the plasma membrane [40•]. In addition, the targeting mechanism for TGN38 localization can apparently be saturated. Plasma membrane staining was readily detected in cells expressing high levels of TGN38 [40•]. However, the tyrosine-containing sequence in the cytoplasmic tail may also be capable of directly retaining the protein in the TGN (possibly by interacting with clathrin), and thus function as a true retention signal [46•].

Conclusion

The past year has been a productive one for identification of Golgi protein targeting signals. The surprising finding that proteins residing in Golgi stacks possess targeting signals in their TMDs has provoked speculation about oligomer-based retention mechanisms and Golgi subcompartmentalization. The search for receptor-based retrieval mechanisms has been extended to TGN proteins with the discovery of targeting signals in their cytoplasmic tails. Elucidation of the mechanisms by which resident proteins are retained is the first step in understanding the complicated structure and function of the Golgi complex.

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