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Localization of proteins to the Golgi apparatus

Sean Munro

The Golgi apparatus has two main roles. The first of these is the modification of newly synthesized proteins and lipids as they pass through the organelle. The second is to serve as a major sorting point in the secretory pathway, with proteins and lipids being selectively targeted to several different organelles. These two functions are performed by a population of Golgi resident proteins. The modification enzymes of the Golgi include the glycosidases and glycosyltransferases responsible for synthesizing the huge diversity of complex oligosaccharides that are attached to both glycoproteins, on *N*-linked and *O*-linked glycan branches, and glycolipids. Indeed, the Golgi is the site of synthesis of many sphingolipids, including sphingomyelin, and glucosylceramide, the precursor of many other glycolipids. Other Golgi enzymes sulfate tyrosines, attach palmitoyl groups and proteolytically cleave specific proteins. These enzymes have been most extensively characterized in mammalian cells, and in other organisms further enzymes must be present, such as those responsible for the synthesis of cell wall polysaccharides in plants and fungi, and scales in algae.

Once the Golgi enzymes have completed their actions, the resulting modified proteins and lipids are transported to their final locations, and it is this process that requires the second class of Golgi residents. These Golgi-localized sorting proteins recruit vesicle coats, collect particular sets of proteins and lipids into transport vesicles and then pinch off and target these vesicles to their correct destination. Finally, the Golgi itself must be sorted within the cell, with motors and structural components attached to maintain its intracellular location, which, in mammalian cells, is usually around the microtubule-organizing centre.

The population of resident enzymes and sorting components must be maintained despite the large flow of proteins and lipids through the Golgi to other organelles in the cell. Therefore, mechanisms must exist to distinguish between Golgi residents and Golgi transients so that the two populations of proteins are sorted differently. The situation is complicated by the fact that the Golgi consists of several discrete cisternae, and individual Golgi residents are often found restricted to a subset of these. Vesicular trafficking components such as t-SNARES are restricted to one end of the Golgi or the other, and the modification enzymes are not distributed evenly between the cisternae but rather are usually found in the order in which they act on substrates. For the enzymes, this intra-Golgi segregation is often not precise, with proteins found overlapping and spread over two or more cisternae in a graded fashion, which itself can vary between cell types for a given protein¹. Nonetheless, there must be mechanisms to account for the different distributions of particular proteins between the cisternae.

Mapping signals for Golgi localization

The main approach to understanding Golgi protein retention has been to try and identify the parts of Golgi proteins that are responsible for their localization. Unlike the endoplasmic reticulum (ER), there is at present no evidence for a population of

For the Golgi apparatus to perform its various unique roles it must maintain a population of resident proteins. These residents include the enzymes that modify the proteins and lipids passing through the Golgi, as well as the proteins involved in vesicle formation and protein sorting. For several of these residents, it has been possible to identify regions that are crucial for specifying a Golgi localization. Consideration of how these targeting domains could function has provided insights into the organization of the Golgi and its protein and lipid content.

soluble residents in the Golgi lumen, but instead all the proteins are either integral membrane proteins or peripheral membrane proteins on the cytoplasmic face of the Golgi. The integral membrane proteins, and in particular the glycosyltransferases, have been studied most extensively, by making chimeras between Golgi proteins and plasma membrane proteins, and examining the location of these proteins in the cell.

In both yeast and mammals, the glycosyltransferases have a common structure – a single transmembrane domain (TMD) with a short N-terminal cytoplasmic portion (Fig. 1). Work on several such enzymes has revealed that the TMD of the proteins is a key determinant of their localization, and in many cases this domain is sufficient to confer Golgi localization when transplanted into another protein (for review, see Ref. 2). In addition, it appears that, for some enzymes, the sequences flanking the TMD, or the luminal portion of the protein, contribute to localization as well. The importance of the TMD for Golgi localization has also been seen with other Golgi proteins such as the SNAREs Sed5p and Sft1p^{3,4} and with particular viral proteins targeted to the Golgi (see below).

A second class of Golgi membrane proteins for which a localization determinant has been identified includes the proteases of the *trans* Golgi network (TGN; furin in mammalian cells, and DPAP-A, Kex1p and Kex2p in yeast) and TGN38, a protein of unknown function. For all of these proteins, short sequences in the cytoplasmic tail have been shown to be crucial for specifying TGN localization⁵⁻⁸. In the mammalian proteins furin and TGN38, these sequences are short, tyrosine-containing motifs similar to those required for endocytosis.

Sean Munro is in the MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK CB2 2QH. E-mail: sean@mrc-lmb.cam.ac.uk

Mechanisms for localization

Sorting signals used to locate proteins to specific organelles within the secretory pathway can act in one of two ways – either as a retention signal that anchors the protein in the correct compartment or as a retrieval signal used to capture the protein when it is in the wrong place and return it to the organelle it escaped from. This latter type of mechanism applies to the localization signals in the cytoplasmic tails of TGN proteins. These proteins recycle continuously through the cell surface or endosomes, and the signal specifies return of the protein to the TGN^{7,9}. In mammalian cells, this TGN retrieval signal initially directs endocytosis from the cell surface and then somewhere in the endosomal system causes the protein to be diverted away from the normal endocytic fates of lysosomal delivery or recycling back to the cell surface and instead directs passage into vesicles destined for the TGN. The vesicle coats, and other machinery, involved in this endosome-to-TGN step have yet to be identified, but further understanding of this pathway might well derive from the recent identification of a set of genes that are required for this step in yeast^{10,11}. However, this retrieval of TGN proteins will not be discussed further here as it primarily reflects sorting events in the endocytic pathway whose only connection with the Golgi is that this is the destination of the transport structures formed.

The sorting of Golgi enzymes retained by their TMDs appears to involve a different mechanism. The late-acting Golgi enzymes such as sialyltransferase and galactosyltransferase are found in the *trans* Golgi and TGN and yet they do not appear to be cycling through the cell surface^{12,13}. This implies that they are prevented from entering the transport vesicles leaving the TGN. Two models have been proposed for how the TMDs of Golgi enzymes could serve to mediate this retention. These are the oligomerization or 'kin-recognition' model and the lipid-sorting, or 'bilayer-thickness', model (Fig. 2).

The oligomerization model proposes that the enzymes in a particular cisterna interact to form structures too large to enter transport vesicles. This model arose initially from work on a viral Golgi protein that forms homo-oligomers¹⁴ and was extended by the observation that two enzymes of the *medial* Golgi, N-acetylglucosaminyltransferase I (NAGT I) and mannosidase II, are tightly associated *in vivo*¹⁵. It has also been suggested that the immobility of these enzyme oligomers could be augmented by their binding to a putative Golgi matrix located between the cisternae¹⁶. The lipid-sorting model envisages that the bilayer of the Golgi cisternae is not homogeneous but contains distinct lipid domains between which the Golgi enzymes partition differentially¹⁷. This idea arose from the fact that Golgi TMDs do not contain any obvious distinguishing sequence motif, and attempts to find key residues by mutagenesis have not been successful. Instead, the TMDs of mammalian Golgi enzymes are on average five residues shorter than those of plasma membrane proteins and contain more of the bulky residue phenylalanine^{17,18}. A similar difference is also observed for proteins of the yeast secretory pathway (S. Munro, unpublished). The

relevance of this difference in TMD length to retention is supported by mutagenesis studies, which have shown that lengthening the TMD of sialyltransferase or galactosyltransferase results in reduced retention and that a synthetic TMD of 17 leucines gives Golgi retention, whereas one of 23 leucines does not^{19–21}. Not all mutagenesis studies have been interpreted as being consistent with TMD length being the key signal for retention^{2,22}. The localization of different Golgi enzymes to different cisternae within the stack could well reflect a role for further sorting signals in addition to TMD length (see below). However, it is important to stress that results with chimeric membrane proteins must always be interpreted with caution as placing a TMD from a multimeric protein in a heterologous context could expose hydrophilic residues normally buried in the intact protein.

This difference in TMD length is certainly consistent with what is known about the lipid composition of the different membranes of the cell. The bilayer of the ER consists mainly of phospholipids, whereas the plasma membrane is rich in sphingolipids and sterols, which order and thicken the bilayer²³, thereby increasing its impermeability. Thus, a change in bilayer thickness is likely to occur in the Golgi apparatus where sphingolipids are synthesized. Indeed, it has been proposed that the multicisternal nature of the Golgi could reflect the fact that its original function is to sort these lipids¹⁷. To account for Golgi enzyme retention, the lipid-sorting model proposes that either the vesicles leaving the TGN would bud from thicker sphingolipid/sterol-rich domains or the process of budding would specifically form such domains, which would exclude the shorter TMDs of the Golgi enzymes. The relative contributions of sterols and sphingolipids to this thickening might vary between species. Cholesterol is abundant in mammalian plasma membranes, but, in insects and nematodes, which are sterol auxotrophs and hence have less sterol, the sphingolipids have longer acyl chains than those of mammalian cells^{24,25}.

Since these two models were proposed, there has been no definitive proof that either, or indeed any other model, is correct. However, two recent sets of observations are more consistent with the lipid-based model. First, the association between NAGT I and mannosidase II mentioned above has been shown to be mediated by the lumenal domains of these proteins rather than the TMDs that are responsible for their retention^{21,26}. This raises the possibility that this interaction does not reflect a general and broad association of all the enzymes in a particular compartment but, rather, reflects a specific association to form a multi-enzyme complex. Indeed, two such multi-enzyme complexes of Golgi transferases have recently been found in the *cis* Golgi of yeast [J. Jungmann and S. Munro, *EMBO J.* (in press)]. Second, the mobility of Golgi enzymes within the bilayer of living cells has recently been examined by fusing green fluorescent protein (GFP) to Golgi proteins and following the diffusion of these Golgi-retained chimeras after photobleaching of living cells²⁷. This revealed that both *medial* and *trans* enzymes are highly mobile, with diffusion coefficients similar to the most mobile

proteins of the plasma membrane, such as rhodopsin or glycosylphosphatidylinositol (GPI)-anchored proteins. However, it should be noted that the extent of protein oligomerization is predicted to have only a small effect on the lateral mobility of membrane proteins [as the size of a complex increases, the diffusion coefficient (D) is predicted to fall in proportion to the log of the radius of the complex²⁸]. Thus, while these GFP results are difficult to reconcile with very large complexes, or with the transferases being anchored to an intracisternal matrix, they cannot exclude smaller oligomers of a few hundred proteins.

Although more work will clearly be required to understand TMD-mediated retention, an additional issue must also be considered. This arises from the recent revival of interest in the idea that specific signals in proteins can specify efficient forward movement in the secretory pathway. Although this has been studied most extensively in ER-to-Golgi transport, it is already well established that signals on proteins leaving the TGN can target them to lysosomes and to the apical and basolateral surfaces of polarized cells. It seems likely that there will also be signals acting to concentrate proteins into exocytic vesicles in nonpolarized cells²⁹. This implies that part of the reason that the Golgi enzymes in the TGN do not move forward in the pathway is simply that they lack the signals to do so.

Retention of proteins in the medial cisternae

The arguments above apply to the question of how Golgi proteins avoid getting into forward-moving

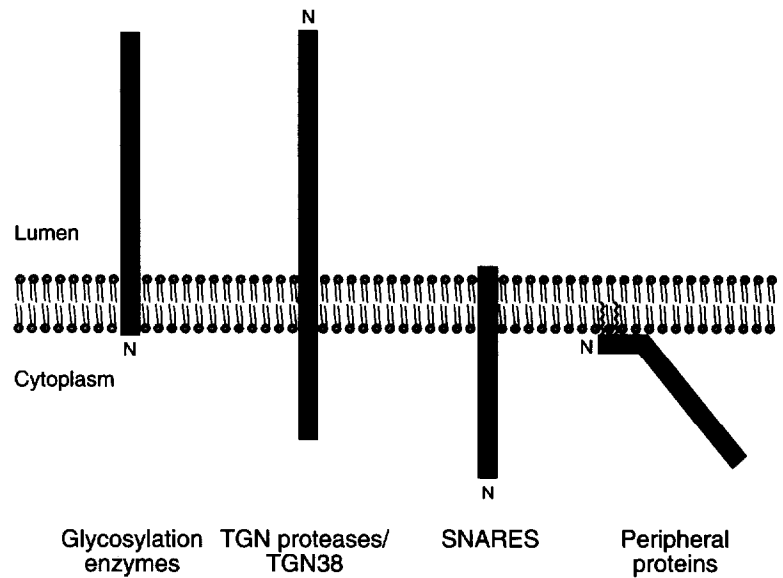
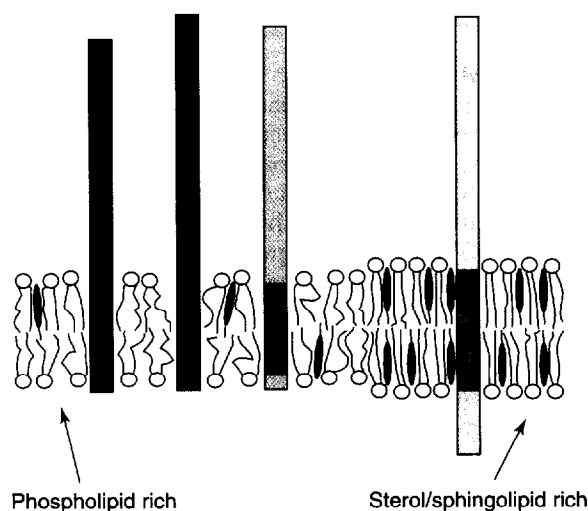


FIGURE 1

Localization domains in Golgi proteins. For several classes of Golgi proteins, it has been possible to identify regions that are sufficient to target a heterologous protein to the Golgi. These domains, shown here in red, vary depending on the type of protein investigated. Thus, for the glycosyltransferases and t-SNAREs, this region is the transmembrane domain (TMD) of the protein, whereas, for the *trans* Golgi network (TGN) proteases, a short motif in the cytoplasmic tail is necessary and sufficient for localization. Finally, for several enzymes peripherally associated with Golgi membranes, the targeting region is a sequence at the N-terminus that includes sites for lipid modification. This diversity reflects different mechanisms acting via the different signals. In several studies, it has also been found that regions outside of the primary sufficient signal also contribute to retention, suggesting that some proteins could contain more than one localization motif.

(a) Segregation by bilayer thickness



(b) Segregation by oligomerization

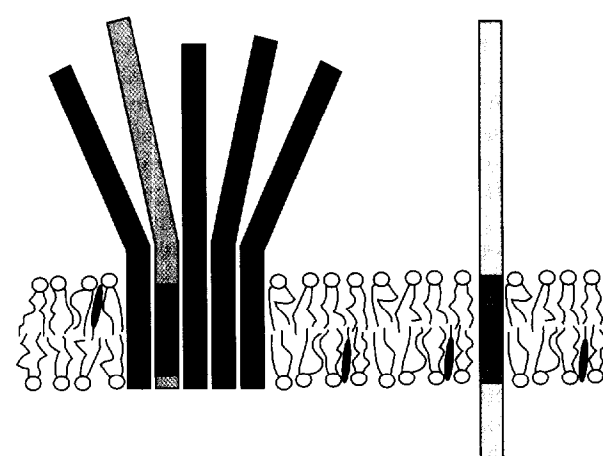


FIGURE 2

Proposed mechanisms for transmembrane domains (TMDs) to segregate enzymes in the membranes of the Golgi. The notion that the TMDs of Golgi enzymes prevent them from entering forward-moving transport vesicles led to two models being proposed for how the proteins could be segregated in the bilayer such that they were excluded from budding vesicles. One proposes that the sterols and sphingolipids destined for post-Golgi membranes form discrete domains whose greater bilayer thickness would selectively exclude the shorter transmembrane domains of the Golgi resident proteins (blue) but allow entry of the transient proteins (yellow). The second model suggests that the residents of a Golgi cisterna oligomerize to form large structures that cannot join the transient proteins in entering anterograde vesicles. The two mechanisms are not mutually exclusive. Moreover, since these mechanisms simply account for clustering of components in the bilayer, they could alternatively be used to collect Golgi enzymes into retrograde vesicles, leaving behind the forward-moving proteins.

transport vesicles. Although this is clearly relevant to enzymes such as sialyltransferases in the TGN, it is less clear what Golgi retention involves for those enzymes found in earlier cisternae of the stack. This is because at present it is not certain how secreted proteins move forward through the Golgi – that is, what is the process that the Golgi enzymes have to be prevented from following. The debate about whether proteins move from *cis* to *trans* by cisternal maturation or by vesicles is covered elsewhere in this issue, but, until it is resolved, any model for *medial* Golgi enzyme sorting has to be proposed in two versions, depending on how transport works. If there is actually forward vesicular transport between cisternae, then localization of *medial* enzymes could be achieved either by retention mechanisms preventing the proteins ever entering retrograde or anterograde vesicles, or by the proteins being continuously retrieved from later cisternae. However, if proteins move from *medial* to *trans* by maturation, then retention mechanisms cannot apply, and *medial* enzymes must enter retrograde vesicles to maintain their distribution. In yeast, there is evidence for an early Golgi enzyme Och1p cycling rapidly through a later compartment³⁰. Although this observation is compatible with either model of Golgi transport, it does emphasize the need to consider how Golgi localization signals could act to direct recruitment into retrograde vesicles. The localization signal in Och1p has not been mapped, but, for other *medial* enzymes from yeast and mammals, the TMDs have a role in Golgi targeting^{31,32}. Sorting by TMDs into retrograde vesicles could be lipid based if retrograde vesicles select thinner, phospholipid-rich, membrane. Indeed, in electron-micrographs, the bilayer of COPI-coated vesicles appears thinner than that of the cisternae they are budding from³³. Of course, the TMD length need not be the only factor mediating retrograde recycling, and in both yeast and mammalian cells the luminal domain of *medial* enzymes contributes to their retention^{31,32}. This might reflect a requirement for recruitment into a multi-enzyme complex, with more TMDs perhaps allowing better partitioning between lipid domains. Alternatively, there could be specific retrograde receptors to recycle the *medial* enzymes, recognizing their luminal domains, or even their TMDs, analogous to the proposed function of Rer1p in mediating the retrieval of some ER residents³⁴. There is certainly no shortage of Golgi membrane proteins for which functions have yet to be found – for example, MG160, a protein of repeated domain structure conserved between mammals and *Caenorhabditis elegans*³⁵.

Localization to the *cis* cisternae

Consideration of proteins localized to the *cis* Golgi is also hampered by uncertainty as to whether there are anterograde vesicles leaving this compartment, but at least in this case there is good evidence that a protein can achieve a predominately *cis* Golgi distribution by recycling through an earlier compartment, in this case the ER. The best evidence is for proteins involved in trafficking between the ER and Golgi. The putative yeast cargo receptor Emp47p

maintains a predominantly *cis* Golgi distribution by being continuously incorporated into COPI-coated retrograde vesicles and recycled to the ER, where it is then presumably efficiently recruited into vesicles returning to the *cis* Golgi³⁶. Two other receptors in this circuit, the KDEL receptor Erd2p and the Sec12p receptor Rer1p, have a *cis* Golgi distribution in both mammalian cells and yeast and also recycle continuously through the ER^{34,37}. As yet, no *cis* Golgi enzyme has been shown to be retained by such recycling, but a dynamic mechanism would be compatible with the localization of mannosidase I varying between ER and *cis* Golgi in different species³⁸.

Viral membrane proteins

Some viruses use the Golgi as their site of assembly, either by budding through the Golgi membrane (e.g. coronaviruses), or by directly wrapping their cores in Golgi membranes (poxviruses³⁹). This means that viral membrane proteins destined for the virion are first targeted to the Golgi apparatus. Several such proteins have been examined for Golgi-targeting signals, and in some cases it appears that similar mechanisms are involved to those described above for the endogenous proteins. These include TMD-mediated retention, and cytoplasmic retrieval signals specifying TGN localization^{40–43}. However, in both the multispinning M-protein of coronaviruses, and a single-spanning protein from Uukuniemi virus, a different feature has been found, a cytoplasmic signal that can specify Golgi localization without recycling through the cell surface^{44,45}. Investigating how these signals are recognized could reveal new components of the Golgi structural or trafficking machinery, but, as they are viral proteins, they might involve mechanisms distinct from those used by host proteins, especially if they cause long-term detriment to the cell.

Peripheral Golgi proteins

A large and diverse population of proteins is localized to the cytoplasmic face of Golgi membranes. Obviously, vesicular coat components must be transiently recruited to Golgi membranes, and many of the proteins involved in this recruitment, such as the ARF1 GTPase and its regulatory proteins, are also peripherally attached to the Golgi^{46,47}. In addition, for several families of signal-transduction proteins, at least one isoform is on the Golgi. The precise role of these signalling proteins on the Golgi is unclear, but presumably they are either responsible for regulating events in the Golgi itself or are stored on the Golgi for release to sites elsewhere in the cell. Furthermore, there are isoforms of spectrin and ankyrin proposed to be involved in tethering the Golgi to microtubules, and a family of large coiled-coil proteins of unknown function that often appear as Golgi autoantigens in autoimmune responses^{48,49}. All of these peripheral proteins must distinguish the membranes of the Golgi from those of other organelles. How this is achieved is an intriguing question, especially since these other organelles all appear to have analogous, but distinct, populations of 'hangers-on'. Obviously, for some proteins, the answer could simply be that they bind specifically to

the cytoplasmic tail of a Golgi-localized membrane protein. However, it is also possible that the proteins are recognizing a more general feature of Golgi membranes. For some peripheral Golgi proteins, it has been possible to identify regions that are necessary and sufficient for localization. For three such proteins, endothelial nitric oxide synthase, glutamate decarboxylase and SCG10, this region comprises the first 30–35 N-terminal residues^{50–52}. None of these three regions are related by sequence, but all three are fatty acylated, and this acylation appears to contribute to membrane association. However, acylation is also found on proteins attached to many other membranes, and so the reason for the Golgi specificity of these associations is unclear. A similar situation is seen with protein kinase C ϵ , where the zinc-finger domain is sufficient for Golgi targeting⁵³. Although a related motif is also found in the Golgi-associated ARF GAPs⁴⁷, it is also found in many proteins, including all other protein kinases C, that are targeted to other membranes. At present, it is unclear what protein or lipid receptors these targeting domains are recognizing on Golgi membranes and indeed if any of them share a common receptor. One clue is given by the observation that many peripherally associated proteins, including the vesicle coats, rapidly dissociate when the GTP loading of ARF is inhibited with the drug brefeldin A. This suggests that these proteins recognize either ARF itself, a product of an enzyme activated by ARF, or another protein whose association is mediated in these ways.

In prospective

Studies of Golgi protein targeting have highlighted a possible role for lipids in Golgi function and indicated a potential importance for recycling of enzymes within the Golgi stack. Future progress will depend on understanding more about intra-Golgi transport mechanisms in general, and at present the results of studies on Golgi protein localization are compatible with both the vesicular and maturation models. However, what has already been learned about Golgi enzyme localization means that determining which proteins and lipids are entering vesicles budding from the Golgi stack could help indicate the purpose and destination of these vesicles. It is also clear that we need to understand more about the recruitment of the peripheral proteins involved in the organization and stacking of the cisternae, and in the process of departure from the TGN. It seems likely that investigating the mechanisms by which Golgi residents are localized will continue to reveal more of the hidden workings of this fascinating organelle.

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