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FORUM comment

Golgi retention signals: do membranes hold the key?

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The diverse forms and functions of cellular organelles are, presumably, a consequence of their particular molecular compositions. The generation and maintenance of this diversity is achieved by the targeting of newly synthesized proteins to specific locations and their subsequent retention there. Sequences that retain proteins in the endoplasmic reticulum (ER) have been identified at the C-termini of resident ER proteins, where they are readily accessible to potential receptors. By contrast, recent results have demonstrated that retention of proteins in the Golgi complex involves sequences located within transmembrane domains. This suggests the novel possibility that the membrane composition of the Golgi complex plays a role in retention of resident Golgi proteins.

Newly synthesized proteins destined for insertion into the plasma membrane or for secretion move through the Golgi complex *en route* from the ER to the cell surface. These proteins are thought to move through the exocytic pathway by default with the bulk flow of lipid, thus not requiring signals for transport[1]. This implies that resident proteins of the ER and Golgi complex possess signals that retain them in the appropriate compartment.

Much progress has been made in defining retention signals for resident ER proteins. The best understood signal is the sequence Lys-Asp-Glu-Leu (KDEL) found at the C-termini of soluble, luminal resident ER proteins such as protein disulphide isomerase and GRP78/BiP (Ref. 2). Putative receptors for this sequence have been identified in both animal cells and yeast, and are postulated to retrieve escaped KDEL-containing proteins from a post-ER compartment and return them to the ER[3].

A C-terminal retention signal for membrane proteins of the ER was first defined for the adenovirus E3/19 kDa protein, and is present on some other membrane proteins of the ER as well[4,5]. In this case, the retention sequence (KKXX or KXKXX) of the proteins is found on the cytoplasmic side of the ER membrane, but the mechanism by which it retains proteins is unknown. Sequences at the N-termini of several other ER proteins are required for retention[6,7], but have not yet been demonstrated to be sufficient for retention (i.e. capable of retaining a reporter protein). Thus, although it is likely that other types of ER retention signals exist, only the KDEL and KKXX sequences can be classified as such at the present time.

Golgi complex structure and function

In cells of higher eukaryotes, the Golgi complex has a characteristic morphology consisting of perinuclear stacks of flattened cisternal membranes (see Ref. 8 for a recent review). It functions predominantly in post-translational processing of newly synthesized membrane and secreted proteins, as well as in protein sorting. The Golgi stacks are polarized, and four subcompartments have been defined functionally: *cis*, *medial*, *trans*, and *trans* Golgi network (TGN). Newly synthesized proteins are thought to move vectorially through the stacks, in the *cis* to *trans* direction. Different glycosidases and glycosyltransferases are enriched in specific subcompartments, allowing orderly and sequential processing of oligosaccharides.

Intra-Golgi protein transport has been reconstituted *in vitro* by Rothman and colleagues, and occurs by a vesicular mechanism[9]. Transport of newly synthesized lipids through the exocytic pathway may also be vesicular, since sphingolipid transport and protein transport have similar requirements (see Ref. 10 for a review). The lipid composition across the Golgi stacks may be polarized, like the protein composition. Different sites of lipid synthesis, modification and degradation could produce a gradient of lipids such as cholesterol and sphingolipids in Golgi complex membranes[10,11]. Pagano and colleagues have shown that the fluorescent ceramide analogue C_6-NBD-ceramide selectively partitions into *trans* Golgi membranes in fixed cells[12] (Fig. 1). This localization reflects the interaction of C_6-NBD-ceramide with endogenous Golgi lipids, and may depend on the higher cholesterol content of *trans* Golgi cisternae as well as the (glyco)sphingolipid concentration[12,13]. Finally, asymmetry between the cytoplasmic and luminal leaflets of the lipid bilayer may also vary in different Golgi cisternae[11,14].

How the Golgi complex retains its structure and organization in the face of dynamic membrane traffic remains a mystery. Peripheral Golgi coat proteins have been identified; some participate in vesicular transport (non-clathrin-coat proteins) and others may be important for maintaining the structure of the cisternae ('scaffold' proteins)[8]. Recent work with the fungal metabolite brefeldin A has challenged our thinking on Golgi structure and dynamics (see Ref. 8 for a review). Treatment of cells with brefeldin A blocks secretion and causes a rapid disappearance of the Golgi complex with redistribution of resident Golgi proteins into the ER. This may reflect a normal recycling pathway between the Golgi complex and ER. The earliest known effect (after <60 s) of brefeldin A treatment

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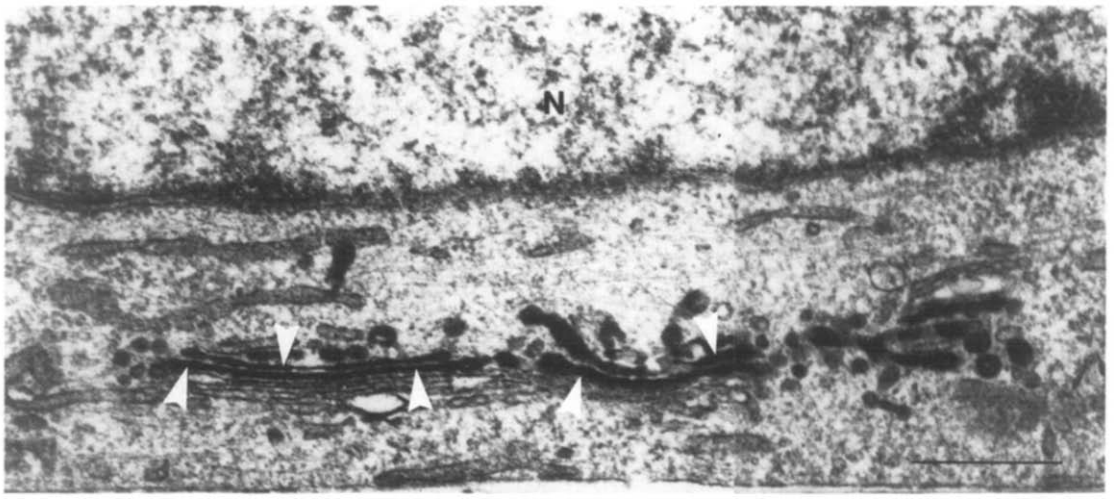

FIGURE 1

A fluorescent ceramide analogue (C_6-NBD-ceramide) selectively partitions into a subset of Golgi membranes (shown to be the *trans* Golgi cisternae by other experiments) in fixed CHO-K1 cells. The probe (indicated by arrowheads) was detected after labelled cells were photobleached in the presence of diaminobenzidine. The selectivity of labelling may reflect the polarity of the lipid composition of Golgi membranes. N, nucleus. Bar, 0.5 μm. Reproduced, with permission, from Ref. 12.

is the dissociation of the coat protein β-COP from Golgi membranes. Thus, the dissociation of the Golgi scaffold could be responsible for all the observed brefeldin A effects.

Retention of resident Golgi proteins

The first clue that retention signals for Golgi proteins might be different from those described above for ER proteins came from studies of the E1 glycoprotein of an avian coronavirus, infectious bronchitis virus (IBV). The IBV E1 protein is a convenient model for endogenous Golgi proteins, since it is retained in *cis* Golgi membranes when expressed from cDNA in animal cells[15]. The IBV E1 protein spans the membrane three times, and deletion analysis suggested that information for retention in the Golgi complex might be contained in the first membrane-spanning domain[16]. Further

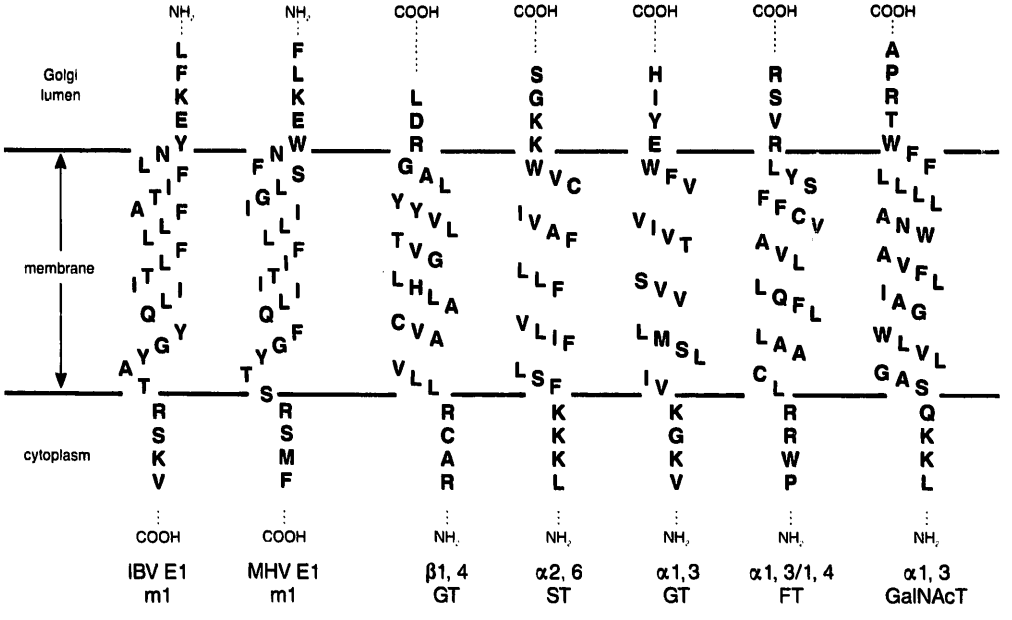

FIGURE 2

Transmembrane domains of Golgi proteins. The amino acid sequences are shown in single-letter code, and displayed as α-helices with the appropriate topology in the membrane. IBV E1 m1, the first membrane-spanning domain of the E1 protein from the avian infectious bronchitis virus; MHV E1 m1, the first membrane-spanning domain from the mouse hepatitis virus E1 protein; β 1,4 GT, bovine and human β-1,4-galactosyltransferase; α 2,6 ST, rat α-2,6-sialyltransferase; α 1,3 GT, bovine α-1,3-galactosyltransferase; α 1,3/1,4 FT, human α-1,3/1,4-fucosyltransferase; α 1,3 GlcNAcT, human α-1,3-*N*-acetylglucosaminyltransferase.

work has now shown that this transmembrane domain does indeed contain a Golgi retention signal, since it is sufficient to retain two proteins that are normally transported to the plasma membrane. Uncharged polar residues (Asn, Thr and Gln) that line one face of a predicted α-helix seem to be the important feature of the retention signal[17].

A number of groups have begun to analyse the targeting signals for endogenous Golgi proteins. cDNA clones encoding several glycosyltransferases and one glycosidase have recently been obtained[18,19]. Interestingly, all are type II membrane proteins that have their N-terminus in the cytoplasm and have an uncleaved signal sequence that serves as the membrane-spanning domain. Colley *et al.* first showed that α-2,6-sialyltransferase (ST), a transferase enriched in *trans* Golgi membranes in most cell types, was efficiently secreted from transfected cells if a cleavable signal sequence replaced the normal N-terminus[20]. This localized the retention signal to a region of the protein that included the cytoplasmic tail, transmembrane domain, and a short portion of the luminal domain called the stem. Recently, Munro found that the only portion of ST required to retain a type II plasma membrane protein in the Golgi complex was its membrane-spanning domain, although retention was enhanced when a portion of the stem was also included[21].

The pivotal role of transmembrane domains in signals for retention in the *trans* Golgi has been confirmed by studying β-1,4-galactosyltransferase (GT). Russo *et al.* showed that the cytoplasmic tail and transmembrane domain of this protein could retain the marker protein pyruvate kinase in the *trans* Golgi (R. N. Russo, N. L. Shaper, D. Taatjes and J. H. Shaper, pers. commun.). Deletion of most of the cytoplasmic tail of GT did not affect its retention, but incorporation of a cleavable signal sequence resulted in rapid secretion (R. D. Teasdale, G. D'Agostaro and P. A. Gleeson, pers. commun.). Nilsson *et al.* produced chimeras of GT and a type II protein normally found at the plasma membrane and in endosomes. As few as 11 residues from the luminal side of the transmembrane domain of GT were sufficient to retain the chimera in the Golgi complex of transfected cells[22]. Aoki *et al.* replaced blocks of the membrane-spanning domain of GT with the analogous portions of the transferrin receptor. One replacement eliminated retention of GT, and two of the four amino acids in this sequence (His and Cys) were required (D. Aoki, N. Lee, C. Dubois and M. N. Fukuda, pers. commun.). Thus, retention signals for two *trans* Golgi proteins (ST and GT) and one *cis* Golgi protein (IBV E1) are found within membrane-spanning domains.

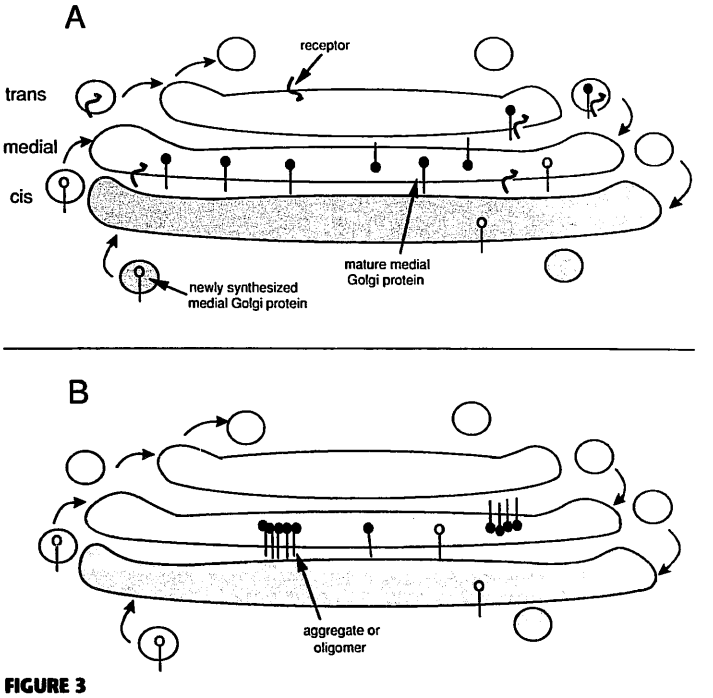

FIGURE 3

Models for retention of Golgi proteins by signals in membrane-spanning domains. A hypothetical *medial* Golgi protein is shown. (A) Receptor-mediated model, where a constitutively recycling receptor protein retrieves any proteins that have escaped from the correct subcompartment. The site of receptor recognition is within the membrane-spanning domain. (B) Aggregation model, where a change in the microenvironment induces the aggregation or oligomerization of the Golgi protein as it reaches the correct subcompartment. This aggregate would not be capable of entering budding transport vesicles. These two models are not mutually exclusive.

Is the TGN different?

By contrast to the results described above, retention of TGN proteins may not involve membrane-spanning domains. Although signals for retention of proteins in the TGN have not yet been identified, there is one report that retention of a TGN protein called TGN38 requires the cytoplasmic tail (in this case, the C-terminus)[23]. KEX2, a membrane-bound protease in yeast that resides in a late Golgi compartment (the TGN equivalent?), requires its cytoplasmic tail for proper localization[24]. Clathrin is also required for retention of KEX2, since the protease is mislocalized to the cell surface in cells lacking the gene for clathrin heavy chain[25].

Results obtained from studies of an E1 protein from the coronavirus mouse hepatitis virus (MHV) can be explained with these findings in mind. The MHV E1 protein and the IBV E1 protein (described above) are predicted to have the same topology in the membrane, but they share only limited sequence homology. Armstrong and colleagues found that the first membrane-spanning domain of MHV E1 as well as the C-terminal 18 amino acids were required for retention in the Golgi complex[26,27]. By contrast, the cytoplasmic tail plays no role in retention of the IBV E1 protein (Ref. 28; Machamer, unpublished). Unlike the avian E1 protein, which is retained in *cis* Golgi membranes in transfected cells, the mouse E1 protein reaches the late Golgi complex, and at least some of the protein can be detected in the TGN (P. Rottier and G. Griffiths, pers. commun.). Chimeric avian–murine E1 proteins will be useful in defining the different targeting signals for these proteins.

Mechanism of retention

The finding that Golgi proteins (other than those of the TGN) seem to be retained by sequences buried in the lipid bilayer suggests the novel possibility that the membrane composition of the Golgi complex may specify retention. Interestingly, no luminal (i.e. soluble) resident Golgi proteins have been identified. There is no primary sequence homology in the membrane-spanning domains of the proteins for which Golgi retention signals have been identified. In fact, there are no sequence similarities in this region between any of the Golgi complex glycosyltransferases cloned to date, even those thought to be enriched in the same subcompartment (Fig. 2). In the two cases that have been analysed, the key residues for retention are polar amino acids within transmembrane domains (Ref. 17; Aoki *et al.*, pers. commun.). However, polar amino acids in membrane-spanning domains are not unique to Golgi proteins, so much remains to be learned about the exact requirements for retention and maintenance of resident proteins in distinct Golgi subcompartments.

Two general retention mechanisms can be envisaged that would act through membrane-spanning domains: receptor-mediated and non-receptor-mediated mechanisms. A receptor would itself have to be a membrane protein, with the ligand-binding site within its own membrane-spanning domain. Such a receptor would also require a mechanism for retention in the appropriate Golgi subcompartment, or would need to be constitutively recycled as it retrieved resident proteins that had escaped (Fig. 3A). The latter idea is similar to the mechanism proposed for retrieval of ER proteins by the KDEL receptor[3]. The alternative possibility is that oligomerization or aggregation of the protein is induced via its transmembrane domain when the protein reaches the appropriate subcompartment, thereby preventing incorporation into budding transport vesicles (Fig. 3B).

Both mechanisms require the microenvironments of successive Golgi subcompartments to be different, for differential receptor binding/release or aggregation. Distinct lipid compositions of Golgi subcompartments could theoretically provide such microenvironment differences. This possibility is particularly attractive since the Golgi retention signals that have been identified are buried in the lipid bilayer. The two mechanisms proposed in Fig. 3 are not mutually exclusive. For example, aggregation might retain the majority of resident proteins, while any escaped molecules could be retrieved by a recycling receptor. However, one requirement of any proposed retention mechanism for the Golgi complex is that it must account for the rapid and efficient relocalization of resident Golgi proteins after the removal of brefeldin A.

The role of the Golgi scaffold proteins in the retention process will be important to examine. A sequence on the cytoplasmic side of the membrane could help anchor the protein securely in the lipid bilayer, and perhaps stabilize its retention via interaction with the scaffold. Nilsson *et al.*[22] found that efficient retention in the Golgi complex of a chimeric protein containing the transmembrane domain of GT required a sequence on the cytoplasmic side of the membrane, but the cytoplasmic tail of the reporter protein worked as well as that of GT. Thus, sequences on the cytoplasmic side of the membrane might stabilize retention of Golgi residents, but it is unlikely that they contribute to specific localization in the Golgi complex. In conclusion, the models shown in Fig. 3 are under active investigation. Given the number of investigators now studying Golgi retention signals, we are likely to have some answers soon.

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