# Retention and retrieval in the endoplasmic reticulum and the Golgi apparatus

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Resident proteins of the exocytic pathway contain at least two types of information in their primary sequence for determining their subcellular location. The first type of information is found at the carboxyl terminus of soluble proteins of the endoplasmic reticulum (ER) and in the cytoplasmic domain of some ER and Golgi membrane proteins. It acts as a retrieval signal, returning proteins that have left the compartment in which they reside. The second type of information has been found in the membrane-spanning domain of several ER and Golgi proteins and, though the mechanism by which it operates is still unclear, it acts as a retention signal, keeping the protein at a particular location within the organelle. The presence of both a retrieval signal and a retention signal in a trans-Golgi network resident protein suggests that more than one mechanism operates to ensure correct localization of resident proteins along the exocytic pathway.

## Introduction

Current opinion favours the idea that the vast majority of proteins synthesized in the ER leave this organelle by default [1]. This unrestricted transport, often referred to as the bulk flow, continues along the exocytic pathway until the proteins reach the plasma membrane. A subset of molecules, however, resists bulk flow. These include enzymes involved in post-translational modifications such as protein folding, glycosylation, sulphation and phosphorylation. Each of these enzymes needs to be maintained at particular points along the exocytic pathway. At least two types of signal would ensure such compartmental localization. The first, termed a retention signal, would permit movement along the bulk-flow pathway until the correct site had been reached. Forward movement would then be prevented by denying the protein access to budding transport vesicles of the anterograde pathway. The second type of signal, termed a retrieval signal, would only act once the protein had left the compartment in which it resides. This signal would depend on specific binding to components involved in retrograde transport. Resident proteins that contain only retrieval signals would be expected to cycle rapidly between two or more compartments. The presence of a retention signal would reduce the need for recycling in direct proportion to the strength of the retention signal. The balance between the strengths of retention and retrieval signals would be determined by whether a protein functions by remaining exclusively in a compartment (e.g. Golgi enzymes) or by cycling between adjacent compartments on a pathway (e.g. TGN38; see below). Below is a detailed description of examples of both retention and retrieval signals found in resident proteins of the exocytic pathway and the mechanisms by which they operate. Examples of less well characterized signals can be found in the reference list [2•,3,4•,5•,6,7,8•,9•].

## The KDEL retrieval signal

The carboxy-terminal tetrapeptide KDEL (single-letter code for amino acids) is found in many proteins resident in the lumenal ER. When transplanted into various reporter molecules, it localizes them to the ER, showing that it is both necessary and sufficient for this process [10]. However, these reporter molecules display post-translational modifications that are carried out by enzymes in the Golgi apparatus, showing that they have left the ER at least once during their lifetime. This finding has revived an earlier hypothesis that proteins resident in the ER escape it and enter the Golgi apparatus, where they are sorted and returned to the ER [11]. A sorting receptor was postulated and later identified both in yeast [12,13] and in mammals [14,15]. At steady state, the receptor, termed erd 2, localizes to the cis side of the Golgi apparatus and upon ligand binding redistributes to the ER [16]. Biochemical characterization of the receptor showed that it specifically binds the ligand and does so in a pH-dependent manner, with an optimum around pH 5.0 [17•]. It has been observed that

the pH along the exocytic pathway becomes increasingly acidic towards the *trans*-Golgi network (TGN), and it is likely that this allows erd 2 to bind its ligand with high affinity. The binding would then signal the receptor and its ligand to be returned to the ER, where a more neutral pH would release the ligand into the lumen. Erd 2 would then return to its original location, where it would await another round of ligand delivery. The cycling of the receptor raises the question as to whether the receptor itself has a retrieval signal. Extensive mutational analysis of its cytoplasmic domain has failed to reveal any mutants deficient in retrograde transport, arguing that this domain of erd 2 does not contain a retrieval signal. Instead, it was shown that an aspartic acid residue in one of the seven membrane-spanning domains was crucial for its retrograde movement [18•]. This was a rather surprising finding, but helical-wheel representation suggested that this aspartic acid residue was in a position to interact with the membrane-spanning domain of another protein. Upon binding to the ligand, the receptor would be induced to oligomerize, forming patches which would trigger retrograde movement. Whether the formation of such patches is sufficient for recycling or additional signals are needed remains to be established.

## The K(X)KXX and the RR retrieval signals

Several ER-resident membrane proteins have now been shown to contain signals similar to that of the KDEL motif in their cytoplasmic domains [19,20,21•]. In resident proteins with a type I topology (amino terminus is in the lumen), the signal has been shown to consist of two critical lysines, which have to be in a −3 and a −4/−5 position relative to the carboxyl terminus [-K(X)KXX, where X is any amino acid]; in type II proteins (carboxyl terminus is in the lumen), the signal consists of two critical arginines (RR), which have to be within the first five amino-terminal residues of the protein. When transplanted into reporter molecules, these motifs are both necessary and sufficient for ER localization, yet allow the reporter molecule to acquire Golgi modifications [21•,22•]. They are, therefore, similar to the KDEL motif in that they act as retrieval signals, returning lost ER proteins from as far away as the *trans*-Golgi cisternae. It is not clear, though, whether these motifs are recognized by receptors similar to the one identified for the KDEL motif or whether they interact directly with components of a retrograde transport machinery. Microtubules play an intrinsic role in retrograde transport, and the K(X)KXX motif can drive polymerization of microtubules *in vitro* [23]. This motif has recently been shown to bind specifically to coatomer [24•], a component involved in vesicle-mediated transport. Whether or not binding to coatomer and microtubules negates the need for a receptor remains to be seen.

It is becoming increasingly clearer that the sole determinants for correct localization cannot be the double lysine or the double arginine, nor can it be the KDEL retrieval motif, as these motifs can be found in proteins that are not residents of the ER. For example, ERGIC 53, a type I transmembrane protein, and p63, a type II transmembrane protein, contain the double lysine and the double arginine motif, respectively [25,26]. Yet both these molecules localize to the intermediate compartment, as does the soluble KDEL-containing CaBP1 (calcium binding protein 1) [27]. Although both morphological and biochemical evidence shows that the intermediate compartment is physically connected to the ER [28], this connection does not explain how these molecules may localize at steady state to this 'sub' compartment. Furthermore, removal of the K(X)KXX motif from an endogenous ER enzyme, UDP glucuronosyltransferase (UDP-GT), does not result in loss of ER retention [22•] and oligosaccharide analysis of endogenous ER proteins has failed to reveal any Golgi modifications [29], suggesting that endogenous ER proteins hardly ever leave this organelle. Retention signals must, therefore, exist in proteins endogenous to the ER as well as to the intermediate compartment, and must operate independently of the ER retrieval signals described above. The latter would then correct for those rare mistakes that result in loss of residency. A clue as to where such retention signals may reside comes from studies carried out on resident enzymes of the Golgi apparatus.

## The membrane-spanning domain as a retention signal

So far, all characterized Golgi glycosylation enzymes have been shown to have a type II topology and to be anchored in the membrane by their uncleaved signal peptide. It is this domain that has received a lot of attention during the past three years as several groups, including ours, have shown that the membrane-spanning domain (the signal anchor) and part of its flanking regions contain sufficient information for Golgi localization (see [30,31•] for references). This was shown by replacing the membrane-spanning domain and part of the flanking regions of various reporter molecules with corresponding regions of different Golgi-resident enzymes. These reporter molecules were found to be retained in the Golgi apparatus, and in those studies in which immuno-electron microscopy was used, were even retained in the correct cisternae (see [30,31•] for references). Similarly, the membrane-spanning domains of a resident protein of the nuclear membrane, which is continuous with the ER, were found to be sufficient for correct localization [32]. The mechanism for Golgi retention could not be saturated by overexpression, suggesting that retention is not a receptor-mediated event. This led to the conclusion that the membrane-spanning domain is a retention rather than a retrieval signal, and

two types of model have been put forward to explain how a membrane-spanning domain could mediate retention. The first model is based on retention through oligomerization, and the second model postulates retention through differences in membrane thickness along the exocytic pathway.

## Retention through oligomerization

We and others noted that overexpression of β1,4-galactosyltransferse (GalT) and α2,6-sialyltransferse (SialylT) did not lead to cell-surface expression [33,34]. Instead, these *trans*-Golgi enzymes backed up into the ER, an observation consistent with premature oligomerization caused by high levels of expression. Because the membrane-spanning domain and part of the flanking regions óf both GalT and SialylT were sufficient to mediate this effect, it was postulated that these domains would aid in the formation of oligomeric structures that are large enough to ensure exclusion from anterograde transport vesicles [31•,33,35].

Evidence exists for higher order structures consistent with such oligomers. Machamer and co-workers [36] showed that the first membrane-spanning domain of the E1 protein of IBV (avian coronavirus infectious bronchitis virus) mediates oligomerization of a reporter molecule, the G protein of VSV (vesicular stomatitis virus), in the Golgi apparatus. Extensive mutational analysis of the IBV E1 membrane-spanning domain showed that polar residues, lining one face of a predicted α-helix, are important both for the retention and oligomerization of this molecule [37•]. This type of sidedness of polar residues is also found in the membrane-spanning domains of other resident Golgi enzymes, suggesting a role for these residues in Golgi retention.

Another example of higher order structures comes from work on purifed rat liver Golgi membranes, from which large structures can be isolated after detergent extraction. These structures consist mainly of *medial*-Golgi enzymes and can be reversibly disassembled by the addition of salt [38]. It is not yet clear what type of interactions maintain these structures under detergent conditions, but it is clear that *medial*-Golgi enzymes have the capacity to form hetero-oligomers. This was demonstrated by selectively localizing N-acetylglucosaminyltransferase 1 (NAGT I) to the ER [39•]. The cytoplasmic domain of this enzyme was replaced with that of an ER-resident protein, the human invariant chain, p33, which harbours a double arginine retrieval signal at its amino terminus [21•]. When this p33/NAGT I hybrid protein was expressed in the ER, another *medial* enzyme, mannosidase II, accumulated in this compartment also. It seems likely that these were not the only *medial* enzymes to accumulate in the ER, as increasing levels of p33/NAGT I resulted in a complete disappearance of recognizable Golgi

stacks. This not only argues for extensive oligomers, but also suggests that *medial* enzymes play a structural role in the organization of the Golgi stack. We also showed that the membrane-spanning domain and part of the stalk region of NAGT I were necessary but not sufficient for these effects, linking these parts of the molecule to the formation of hetero-oligomers [39•].

## Retention through membrane thickness

The striking observation that the membrane-spanning domain of SialylT could be replaced with polyleucines without loss of Golgi localization suggested that the primary sequence of this domain was not required for Golgi localization [34], a conclusion supported by the recent work of Dahdal and Colley [40]. Furthermore, Masibay *et al.* [41•] observed that the hydrophobic parts of membrane-spanning domains of Golgi residents are, on average, shorter than those of plasma membrane proteins. This is due, in part, to the presence of more polar residues in the membrane-spanning domains of Golgi residents. This, coupled with the observation that length of the membrane-spanning domain was important for retention [34,41•], led Bretscher and Munro [42] to suggest that the thickness of the membrane determines the point at which resident Golgi enzymes are retained. This model relies on the observation that the ER has a low cholesterol content as compared with the plasma membrane. Early work by Orci *et al.* [43] suggested that a gradient of cholesterol exists across the Golgi stack. This gradient would be maintained by budding vesicles selectively enriching their membrane with cholesterol. These vesicles would, therefore, have a thicker membrane than the compartment from which they bud. The distribution of Golgi enzymes across the Golgi stack would thus reflect the different lengths of their membrane-spanning domains.

There is insufficient evidence to decide which of these two mechanisms is correct or whether the truth lies somewhere in between. However, it is worth pointing out that retention itself must be a regulated process. For example, resident enzymes of the Golgi apparatus enter transport vesicles during mitosis [44,45]. This is so that the Golgi apparatus can be broken down into vesicles, a process that appears to ensure equal partitioning of the organelle between the two daughter cells [46]. One possible mechanism would involve changing the phosphorylation state of the cytoplasmic domains of each resident enzyme. As the cytoplasmic domains of Golgi residents are known to play an accessory role in retention [33], this might be expected to relax retention, thereby allowing residents to enter budding vesicles. Because resident enzymes play a significant role in maintaining organelle structure [39•], an understanding of the mechanisms that regulate retention will ultimately lead to the rules that govern organelle structure.

## Retention and retrieval

So far, we have described two different signals for compartmentalization along the exocytic pathway and suggested that both might be present in some proteins. Direct evidence that this is the case has been provided by work on TGN38, a resident of the *trans* Golgi network. TGN38 [47•–49•] and other proteins of the TGN, in both mammals (furin; [50]) and yeast (dipeptidyl aminopeptidase A; [51]), have been shown to have a localization signal in their cytoplasmic tail. In the case of TGN38, this tyrosine-based signal (-YQRL-) can be transplanted into various reporter molecules which then localize to the TGN, showing that it is both necessary and sufficient for localization. Deletion or point mutation of this signal leads to the accumulation of TGN38 on the cell surface, suggesting that it normally acts as a retrieval signal. This conclusion is borne out by the observation that TGN38 is known to cycle between the TGN and the plasma membrane and by the resemblance of the YQRL motif to other internalization signals found in plasma membrane receptors (e.g. the transferrin receptor). Although the YQRL signal by itself can localize TGN38, an additional signal exists in the membrane-spanning domain which is also sufficient for localization to the TGN [52•]. This signal is similar to those retention signals just described for Golgi-resident enzymes. It now remains to be seen whether retention and retrieval signals can co-exist in other resident proteins along the exocytic pathway.

## Acknowledgements

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