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Sorting signals

P.P. Breitfeld, J. E. Casanova*, N.E. Simister†, S.A. Ross*, W.C. McKinnon and K.E. Mostov*

Department of Pediatrics (Hematology), University of Massachusetts Medical School, Worcester, Massachusetts, USA, *Department of Anatomy, University of California, San Francisco, California, USA, and †Whitehead Institute, Nine Cambridge Center, Cambridge, Massachusetts, USA.

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Introduction

Eukaryotic cells contain a large variety of membranes and organelles, each of which has a distinct protein composition. Proteins found in many compartments, including the endoplasmic reticulum (ER), the Golgi, lysosomes, and the plasma membrane, as well as secretory proteins, are synthesized by ribosomes on the rough endoplasmic reticulum (RER) and then transported to their appropriate destinations. Some proteins reside in a single location for long periods. Others shuttle between two or more locations, for example, the low-density lipoprotein (LDL) receptor, which recycles hundreds of times between the plasma membrane and endosomes.

How are proteins that are made on the RER targeted to their proper locations? There are several recent reviews of this field (Pfeffer and Rothman, *Annu Rev Biochem* 1987, 56:829–852) [1–5], and we can discuss only a few new developments here. One has been the identification of the structural features of proteins, known as sorting signals, that control their targeting. Presumably these sorting signals interact with other proteins that constitute the cellular sorting 'machinery'. Sorting signals may either act positively, to direct a protein to a compartment, or negatively, to exclude a protein from a pathway.

Not all traffic need be signal-mediated. For example, in endocytosis many surface receptors are selectively endocytosed in vesicles which non-selectively endocytose a small amount of extracellular fluid. Intracellular transport also uses vesicles that 'pinch off' from one compartment and fuse with another. These vesicles may also contain fluid-phase proteins that are non-specifically transported by 'default' (Wieland *et al.*, *Cell* 1987, 50:289–300). It is difficult to exclude rigorously all interactions that might confer selectivity because even a weak interaction can accomplish sorting, especially if repeated. It is not clear whether membrane proteins can also be transported by default.

Identification of sorting signal

A few clear examples of sorting signals have been identified. Three soluble proteins found in the lumen of the RER (grp 78, grp 94 and protein disulfide isomerase) all have the sequence Lys-Asp-Glu-Leu (KDEL, in the single-letter code) at their carboxy terminals. This conservation suggested that KDEL might be a signal for retention in the RER. Deletion of this sequence from grp 78 resulted in its secretion. More dramatically, addition of KDEL to the carboxy terminus of lysozyme caused the lysozyme–KDEL fusion protein to be retained in the RER (Munro and Pelham, *Cell* 1987, 48:899–907).

KDEL proteins are not simply anchored to the RER membrane by a KDEL receptor. Instead, the KDEL proteins reach the nearest compartment of the *cis* Golgi [6] and are then rerouted back into the RER, apparently by a receptor (Fig. 1). In yeast it is HDEL, rather than KDEL, that is the sequence for retention [7]. Three genes have been identified in which mutations interfere with the retention of HDEL proteins [7], one of which might encode the HDEL receptor. Identification of the KDEL signal does not explain how all proteins, especially membrane proteins, are retained in the RER, nor how proteins are retained in other compartments such as the Golgi. Other sequences have been identified in various membrane proteins that cause retention in either the RER or the Golgi (Paabo *et al.*, *Cell* 1987, 50:311–317) [8,9].

Sorting signals need not always be short, contiguous, conserved sequences. They may be composed of non-contiguous sequences that come together only when the protein is folded. Signals may also be so divergent that there is no discernible sequence homology, but they may share some overall structure or property that allows them to bind to the same receptor. Using mutations to map sorting signals also carries the risk that the overall conformation of the protein will be altered; this can non-specifically affect its transport (Doyle *et al.*, *J Cell Biol* 1985,

Abbreviations

ER—endoplasmic reticulum; **HA**—hemagglutinin; **LDL**—low-density lipoprotein; **M-6-P**—mannose-6-phosphate; **RER**—rough ER; **pIg**—polymeric immunoglobulin; **TGN**—*trans* Golgi network.

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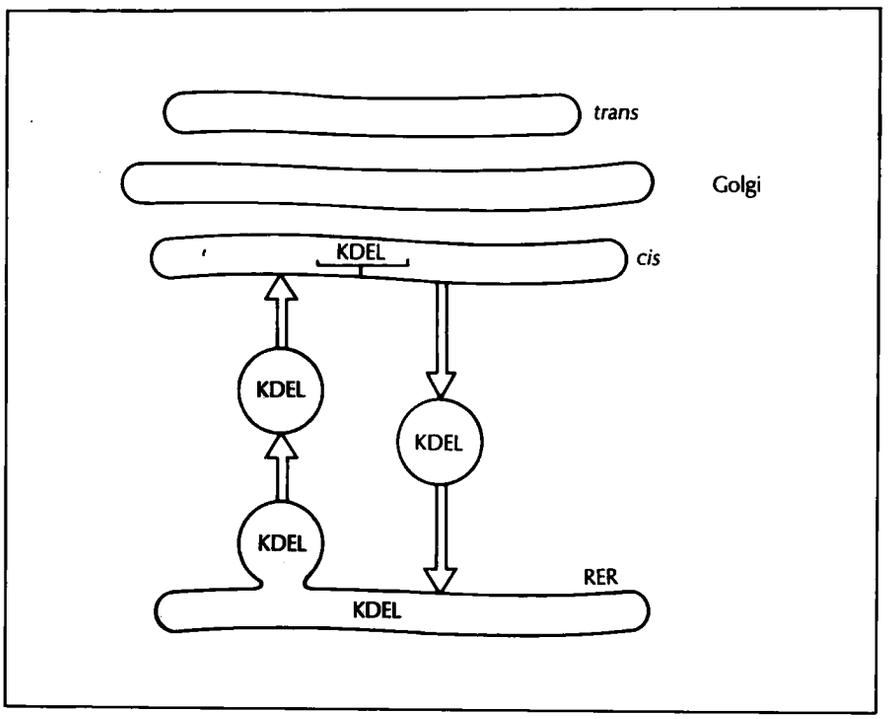

Fig. 1. Recycling of KDEL-containing proteins. Proteins that have the sequence KDEL at their carboxy terminals (depicted here as simply KDEL) are found in the lumen of the rough endoplasmic reticulum (RER). Some are contained in vesicles that bud off from the transitional region of the RER and reach the *cis* cisternae of the Golgi. Here a receptor has been postulated to capture these proteins and direct them into vesicles that recycle back to the RER. It is not known if this receptor itself recycles.

100:704–714). These problems have made the identification of presumptive sorting signals very difficult in many cases.

Sorting signals can also be generated by post-translational modification. An example of animal cells is the addition of the mannose-6-phosphate (M-6-P)) signal, in the *cis* Golgi, to enzymes destined for lysosomes [1]. In the *trans* Golgi or the associated *trans* Golgi network (TGN), receptors bind M-6-P-containing proteins and sort them into a lysosomal pathway. We do not yet know what signal is recognized by the transferase, causing it to add the phosphate [10].

Signals for clathrin-coated vesicles.

At the cell surface, many receptors are endocytosed by clathrin-coated vesicles [5]. As was shown initially for the LDL receptor, mutations in the cytoplasmic domain of the receptor can prevent rapid endocytosis. In particular, mutation of the tyrosine at position 807 to a cysteine slows internalization (Davis *et al.*, *J Biol Chem* 1987, 263:4075–5082). Mutation of tyrosine residues in the cytoplasmic domains of the M-6-P receptor (Lobel and Kornfeld, personal communication) and the polymeric immunoglobulin (pIg) receptor (Breitfeld and Mostov, unpublished observations) also reduces the rate of endocytosis.

The hemagglutinin (HA) of influenza has a cytoplasmic domain of 10 amino acids and is normally not endocytosed. Lazarovits and Roth [11] mutated the third, sixth or ninth residue of the cytoplasmic domain to tyrosine. The mutant with tyrosine at the sixth position was endocytosed, whereas the other mutants were not endocytosed at all. These results suggest that a tyrosine residue can sometimes contain sufficient information to form a minimal signal for endocytosis. The context of neighboring sequences is probably important, but it is not clear how.

Clathrin-coated vesicles contain proteins, called adaptors, that link the cytoplasmic domains of receptors to clathrin [12]. Adaptors bind to the purified cytoplasmic domains of the LDL receptor, M-6-P receptor, pIg receptor and the endocytosed mutant HA [13]. This tyrosine-containing HA tail interacted weakly, while the pIg receptor interacted more strongly. This may indicate that the pIg receptor cytoplasmic domain contains information for optimal endocytosis whereas the tyrosine-containing HA domain has only the minimal tyrosine.

Trans Golgi sorting

In the *trans* Golgi network or TGN, proteins can be sorted into two special pathways leading either to lysosomes or to regulated secretory vesicles. The M-6-P receptor and its ligand bud off the TGN in clathrin-coated vesicles and are delivered to a pre-lysosomal compartment [14,15]. The M-6-P receptor can also endocytose ligand from the cell surface and deliver it to the same compartment. The large cation-independent form of the M-6-P receptor has a 163-amino acid carboxy-terminal cytoplasmic domain, which has recently been dissected into two sorting signals, one for budding from the TGN and the other for endocytosis from the plasma membrane

(Lobel and Kornfeld, personal communication). Truncating 89 residues from the carboxy terminus produced a receptor that was defective in the sorting of newly made lysosomal enzymes from the TGN to lysosomes, but could still endocytose ligand from the cell surface. Truncation leaving only the 20 residues nearest the membrane produced a receptor that was defective in both TGN sorting and endocytosis. Mutating the tyrosines that are 24 and 26 residues away from the membrane produced a receptor that was impaired in endocytosis but only partially defective in TGN sorting. It appears that the membrane-distal, carboxy-terminal half of the cytoplasmic domain is needed for TGN sorting, whereas the membrane-proximal half is needed for endocytosis.

Certain cells secrete proteins by two separate pathways: constitutive and regulated [3]. Proteins in the regulated pathway are stored in secretory vesicles and released in response to an external stimulus. These proteins are segregated from the constitutive pathway via clathrin-coated membranes in the TGN [16]. Often, the proteins in this pathway self-aggregate, and this may be part of the sorting mechanism. Sorting signals do seem to be involved, although they have not been identified (Moore and Kelly, *Nature* 1986, 321:443–436) [17,18]. Recently, a group of 25 kD proteins have been isolated by their ability to bind to several regulated secretory proteins (growth hormone, prolactin and insulin) but not to various constitutively secreted proteins [19]. These 25 kD proteins are localized to the Golgi region and may be the receptors that segregate proteins into the regulated pathway.

Sorting signals in polarized cells

Many cells are highly asymmetric and have specialized regions of the plasma membrane. One of the simplest examples is the polarized epithelial cell whose plasma membrane is divided into two regions: an apical surface that faces the lumen of a cavity and a basolateral surface that contacts adjacent cells and the basement membrane [4]. These surfaces have very different protein and lipid compositions, which are maintained by at least four processes: initial biosynthetic targeting; anchoring to the submembranous cytoskeleton; intercellular junctions that restrict diffusion between the two surfaces, and endocytosis followed by recycling to the original surface, transcytosis to the opposite surface, or degradation. Polarized cells can also constitutively secrete soluble proteins from one surface or the other (Gottlieb *et al.*, *Proc Natl Acad Sci USA* 1986, 83:2100–2104) [20,21].

To study these processes in cell culture, many investigators have used MDCK cells (Matlin, *J Cell Biol* 1986, 103:2565–2568) [4]. When grown on permeable filter supports, they form a tight polarized monolayer, which allows separate biochemical access to the two surfaces. Newly made proteins destined for the two surfaces travel together up to the TGN (Rindler *et al.*, *J Cell Biol* 1984, 98:1304–1319). At this point they are sorted into separate, non-clathrin-coated, vesicles, which deliver the proteins to the proper surface [22]. In contrast, all newly made plasma membrane proteins in hepatocytes are delivered first to the basolateral (sinusoidal) surface and selected proteins are then transcytosed to the apical (bile canalicular) surface [2]. Apparently, no protein is delivered directly from the Golgi to the apical surface and it will be interesting to discover if this is also true for lipids. Protein sorting in intestinal cells may be somewhere between these two extremes. In the highly differentiated intestinal cell line, Caco2, most soluble proteins are secreted exclusively basolaterally, whereas apical membrane proteins and some soluble proteins are delivered directly to the apical surface [23,24]. In intestines, however, there is evidence that apical proteins are first delivered to the basolateral membrane and then transcytosed (Maggey *et al.*, *J Membr Biol* 1987, 86:19–25). *In vivo*, enterocytes represent a gradient of differentiation states along the crypt–villus axis. Cells in the crypt may initially deliver apical proteins to the basolateral surface, whereas Caco2 cells may resemble villus cells, which use a direct pathway.

Are proteins that lack sorting signals sent by default to one surface or the other? In MDCK cells, many exogenously expressed secretory proteins (e.g. lysozyme; Kondor-Koch *et al.*, *Cell* 1985, 43:297–306) are released from both surfaces. It is difficult to be sure that these exogenous proteins have no sorting signals. It seems likely that a truly unsorted soluble molecule would randomly enter vesicles leaving the TGN and be secreted at the two surfaces in proportions that reflect the relative flux of TGN-derived vesicular volume reaching these surfaces. It is not clear what the default pathway for membrane protein is, or even if one exists.

Attempts to map sorting signals on membrane proteins by expressing genetically altered proteins in polarized cell shave not yielded a clear picture. In some cases, altering or deleting the cytoplasmic domain or deleting the membrane anchor produces proteins that are transported with the same polarity as the intact molecules (Roman and Garoff, *J Cell Biol* 1986, 106:2607–2618; Roth *et al.*, *J Cell Biol* 1987, 104:709–782). These results suggest that sorting signals reside in the luminal domain of the molecule. However, there are counter examples where the mutant molecules have altered polarity (Gonzalez *et al.*, *Proc Natl Acad Sci USA* 1987, 84:3734–3742; Stephens and Compans, *Cell* 1986, 47:1053–1059; Puddington *et al.*, *Proc Natl Acad Sci* 1987, 84:2756–2760). Pieces of various proteins have also been fused, producing chimeras. These fusion proteins have yielded confusing results, and have the potential complication that the two fused pieces may contain competing signals.

Several pitfalls may account for some of these discrepancies. Firstly, the cells actually producing the protein must be highly polarized. This problem is especially important in transient expression systems where a few cells make most of the protein, and in viral expression systems, when the cells are dying. Secondly, it is important to distinguish between steady-state levels, as opposed to initial delivery or post-endocytotic redistribution. Thirdly, perturbations in folding can affect sorting [25,26].

Membrane proteins and soluble proteins may be targeted to the proper surface by the same mechanism. For example, two membrane proteins, HA and the tail-minus mutant form of the pIg receptor, are both targeted to the apical surface in MDCK cells (Roth *et al.*, *J Cell Biol* 1987, 104:769–782) [27]. If these proteins have their anchors removed, the now soluble proteins are still apically targeted, suggesting that the same signal and cognate receptor mechanisms can be used to target membrane and soluble proteins to the apical surface [27]. Glycolipid-anchored proteins and certain glycolipids are also apically sorted [4,28]. Certain lipids and lipid anchors may contain their own sorting signals, which might operate on different principles to those of transmembrane proteins.

The sorting of proteins after endocytosis is a major process in the maintenance of cell polarity. In MDCK cells, roughly 50% of each surface is endocytosed per hour [4]. In hepatocytes, endocytosis followed by specific post-endocytotic sorting is the only way to reach the apical surface. A particulary useful system for the study of polarity and post-endocytotic sorting is the pIg receptor (Mostov and Simister, *Cell* 1985, 43:389–390; Mostov and Deitcher, *Cell* 1986, 46:613–621). This receptor is delivered from

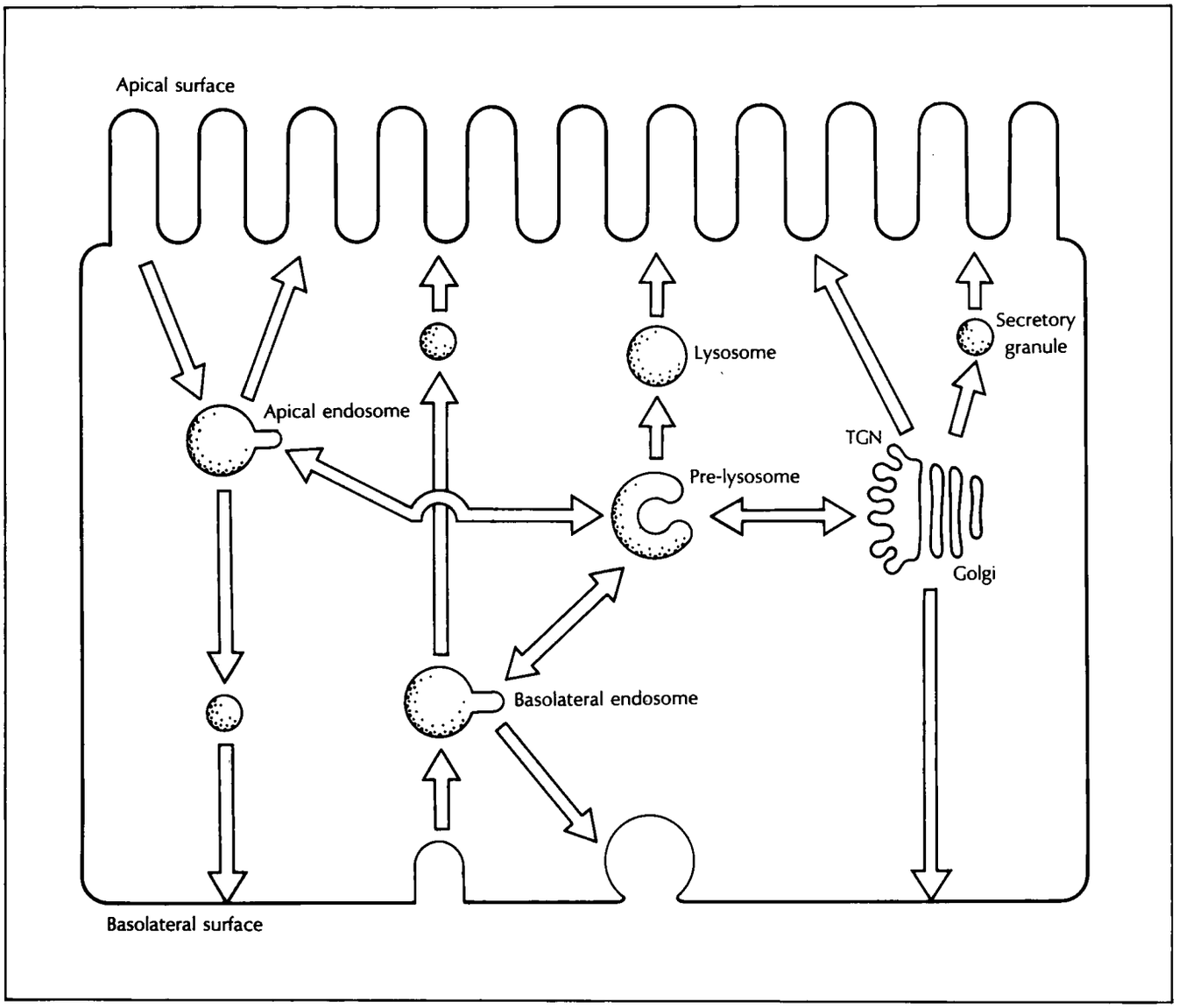

Fig. 2. Pathways of protein delivery in polarized epithelial cells. Proteins leaving either the *trans* Golgi network (TGN) or endosomes have three common destinations: basolateral surface, apical surface, or pre-lysosomes, which lead to lysosomes. Proteins endocytosed from the apical surface apparantly enter a different set of endosomes from proteins endocytosed at the basolateral surface (von Bonsdorf *et al.*, *EMBO J* 1985, 4:2781–2792). The apical to basolateral transcytotic route is involved in receptor-mediated transport of IgG across the small intestines of newborn rats [29], and perhaps in the human placenta. Proteins targeted to the apical surface, for instance, may come from the TGN or may have been endocytosed at either the apical or the basolateral surface. We suggest that in some cases the same signals and sorting machinery for apical targeting may be used in the TGN and endosomes.

the Golgi to the basolateral surface, where it can bind pIg. After endocytosis at the basolateral surface, approximately 50% (per round) is transcytosed to the apical surface, where it is proteolytically cleaved. If either the cytoplasmic domain or the cytoplasmic domain and membrane anchor are deleted, these truncated proteins are delivered directly from the Golgi to the apical surface (Mostov *et al.*, *Cell* 1986, 47:359–364) [27]. These results suggest that the cytoplasmic domain contains a basolateral targeting signal and that the lumenal domain contains an apical targeting signal. Somehow these signals must act sequentially, first directing the pIg receptor basolaterally, and then directing it apically.

These results also suggest that the sorting processes in endosomes and the TGN may be fundamentally similar. Proteins leaving both compartments can be sorted to either the apical or basolateral surface, to lysosomes, or perhaps to regulated secretion granules. Normally, the pIg receptor is targeted from the endosomes to the apical surface, whereas the mutant pIg receptor is targeted from TGN to the apical surface. We suggest that the same apical targeting signals and sorting mechanisms are involved in both these processes, and that this may be a general phenomenon. Normally the vesicular stomatitis virus G-protein is targeted directly from the TGN to the basolateral surface. If the protein is artificially implanted in the apical surface it is endocytosed. The basolateral targeting signal that normally operates in the TGN apparently also operates in the endosomes, because the vesicular statitis virus G-protein is directed from endosomes to the basolateral surface (Pesonen *et al.*, *J Cell Biol* 1984, 99:7696–802).

The cytoplasmic domain of the pIg receptor consists of 103 amino acids at the carboxy terminus and we have recently dissected it into several independent sorting signals (Casanova *et al.*, unpublished observations).

(1) Altering a tyrosine residue that is 21 amino acids from the carboxy terminus, or deleting 30 residues from this terminus, generates a receptor that is endocytosed slowly from the basolateral surface. All other sorting steps are normal.
(2) Deleting 38 residues from the middle of tail produces a receptor which is endocytosed normally, but only 5% is transcytosed after endocytosis, and 45–50% is degraded (compared with 5% degraded in wild-type). The deletion may disrupt a signal that normally prevents the wild-type receptor form entering a lysosomal pathway.
(3) In MDCK cells, the cytoplasmic domain of the pIg receptor is phosphorylated on a serine. When this is mutated to alanine, the receptor is endocytosed normally, but post-endocytotic sorting is dramatically changed, although in a different way. Recycling is increased to 65%, transcytosis decreased to 15–20% (compared with 40 and 50%, respectively, in wild-type), whereas degradation remains at 5%.
(4) Deleting the entire cytoplasmic domain yields a receptor that is targeted directly from the TGN to the apical surface, whereas all the other mutants described above reach the basolateral surface normally.

Taking the mutants as a group, any residue except the 17 closest to the membrane can be deleted without altering basolaterally targeting, suggesting that these 17 residues (which include the phosphorylated serine) may contain a basolateral targeting signal. We suggest that the basolateral targeting signal in the cytoplasmic tail is 'dominant' initially. After the pIg receptor reaches the basolateral surface, phosphorylation of the serine inactivates this signal, and allows the apical targeting signal to function. Thus, phosphorylation could be the switch that controls sequential targeting of the receptor to the basolateral, and then to the apical, surfaces.

The demonstration that deletion of large segments of the pIg receptor cytoplasmic domain selectively alters some functions, but not others, suggests that this domain is not rigidly folded. Instead, it may have a flexible structure, allowing different sorting signals to interact with various other proteins.

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Annotated references and recommended reading

- • Of interest
- •• Of outstanding interest

1. • KORNFELD S: **Trafficking of lysomal enzymes.** *FASEB J* 1987, 1:462–468.
Very readable review of the M-6-P signal and its receptor, which target enzymes to lysosomes. The authors' laboratory has been the source of much of our knowledge in this field.

2. • BARTLES JR, HUBBARD AL: **Plasma membrane protein sorting in epithelial cells: do secretory pathways hold the key?** *Trends Biochem Sci* 1988, 13:181–184.
Compares data on secretion and membrane traffic in the cannonical MDCK system with the authors' observations on hepatocytes. Raises several useful questions about the relationship between traffic of membrane and soluble proteins.

3. • KELLY RB: **The cell biology of the nerve terminal — review.** *Neuron* 1988, 1:431–437.
Although this review is officially about neuronal cells, it is from a strong cell biological perspective. It synthesizes a wealth of information on targeting, exocytosis and endocytosis, especially in cells with a regulated pathway.

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They also argue that the default pathway for membrane proteins is generally to the basolateral surface.

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