The Polymeric Immunoglobulin Receptor

A Model Protein to Study Transcytosis

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Introduction

The most basic type of organization of cells into tissues is that of epithelia (1). Epithelial cells line a cavity or cover a surface and can form a selective barrier to the exchange of molecules between the lumen of an organ and an underlying tissue. The apical cell surface faces the lumen and maintains a distinctly different lipid and protein composition from its basolateral counterpart. For decades physiologists have studied the movements of small molecules, such as water, ions, or sugars across epithelia and it is now becoming increasingly clear that large molecules, such as proteins, can also cross an epithelial cell layer. One way this movement could occur is by diffusion between cells, i.e., by a paracellular route. However, in many types of epithelia the extracytoplasmic leaflet of apposing cells is fused together by a tight junction which normally precludes the paracellular transport of macromolecules (2).

Macromolecules can be transported across epithelial cells with tight junctions in a process termed transcytosis (3). The first step in this specialized pathway of intracellular membrane trafficking is endocytosis (reviewed in reference 4). Efficient endocytosis requires that macromolecules bind as ligands to specific high-affinity receptors on the cell surface. The receptors and bound ligands are then concentrated in specialized clathrin-coated pit structures on the cell surface which invaginate, and pinch off to form coated vesicles. These vesicles subsequently lose their coats and fuse with endosomes. Some molecules are endocytosed nonspecifically when a small volume of liquid is trapped in forming endocytic vesicles (5).

A wide variety of macromolecules enter cells by endocytosis, but most of these are not transcytosed. It is in the endosome that macromolecules are sorted to at least three destinations. Many proteins, such as transferrin and its receptor, recycle out of this compartment, back to the original cell surface. Others, such as the epidermal growth factor receptor, are ultimately delivered to lysosomes where they are degraded. Still other molecules are sorted into transcytotic vesicles which travel to the opposite pole of the cell and fuse with the plasma membrane, releasing their contents. It is generally believed that the transported proteins contain specific structural features or sorting signals that contain the information specifying into which pathway the protein will be targeted. A number of such sorting signals have been identified including a signal for transcytosis, which will be described below.

Transcytosis can occur in either direction, from the apical to basolateral cell surface, or from the basolateral to apical cell surface. Examples of transcytosis include the transport of insulin and serum albumin across endothelia (6), epidermal growth factor across kidney epithelia (7), and intestinal epithelia (8), and transferrin across capillaries in the brain (3). The best-studied examples of transcytosis are the transport of immunoglobulins that occurs in at least three situations in mammals: transport of IgG across the intestinal epithelium in newborn rats (9), transport of IgG across the human placenta (10), and transport of IgA and IgM across various mucosae (11).

IgG transcytosis

Many cells in the immune system express receptors that bind the Fc portion of immunoglobulins. These Fc receptors (FcR) have diverse functions, such as signaling the regulation of B-cell development and the release of cytokines and cytotoxins. Related receptors are also involved in the transcytosis of immunoglobulins across epithelial cells. The transcytosis of IgG has been best studied in the intestines of neonatal rats (3, 9). Rat milk contains a high concentration of IgG, which when ingested by the neonate, passes through the stomach intact and then reaches the small intestine. Enterocytes in the proximal small intestines express an FcR on their apical surface (termed with FcRn), which binds IgG at pH 6.0; the pH of the intestinal lumen. The FcRn and ligand are endocytosed and transcytosed to the basolateral cell surface. Here, the IgG dissociates, due to the slightly higher pH (7.4), and is released into the circulation of the animal. The receptor may recycle for multiple rounds of IgG transport, although this has not been directly demonstrated.

The structure of the FcRn has been analyzed by biochemical and recombinant DNA techniques (12, 13). The FcRn contains two polypeptide chains (Fig. 1 A). The smaller subunit, p14, is the well-known β_2-microglobulin. The larger subunit, p51, is 50% identical throughout its length with class I major histocompatibility antigens. A related receptor has recently been found in the fetal yolk sac of the rat (14). The major histocompatibility antigen molecules are primarily involved in

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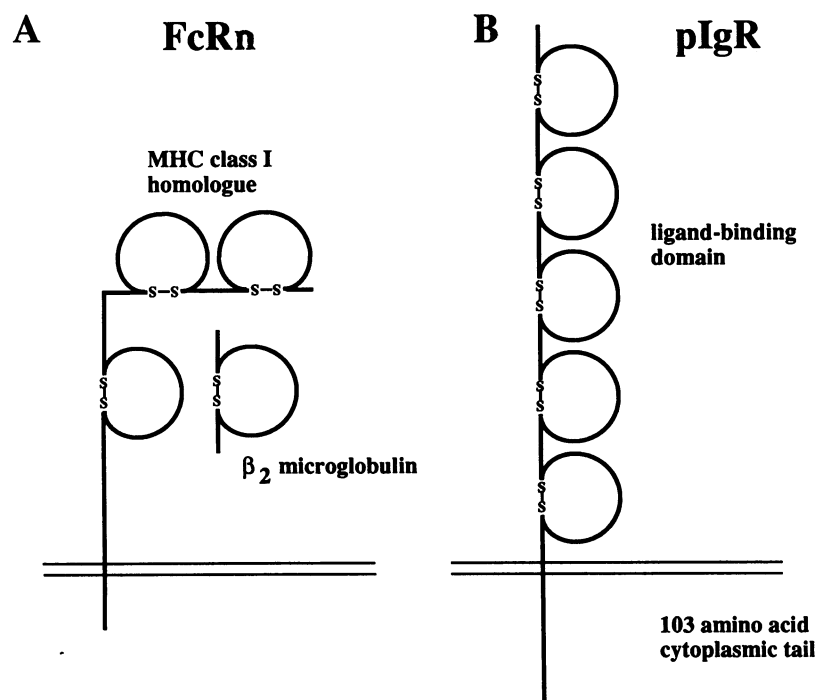
1. *Abbreviations used in this paper:* FcR, Fc receptor; FcRn, intestinal FcR; MDCK, Madin-Darby canine kidney cells; pIgR, polymeric immunoglobulin receptor; SC, secretory component; TGN, *trans*-Golgi network.

A   **FcRn**   **B**   **pIgR**

Figure 1. Structure of transcytosing immunoglobulin receptors. The FcRn is depicted in *A* and the pIgR in *B*. The extracellular portions are on top and the cytoplasmic domains are on the bottom.

presenting peptide antigens to T cells, whereas the FcRn is an evolutionarily related molecule with a completely different immunological function.

There is little direct data concerning the transcytosis of IgG across human placenta. It has recently been found that human trophoblast cells express a receptor closely related to the FcRII class of IgG receptors. This class of receptor was originally found on lymphocytes and macrophages (10). It is not known if this placental receptor functions in transporting IgG across the placenta or if it has a different function, such as protecting the placenta from immune complexes. However, recent observations support the notion that this receptor is indeed a molecule involved in IgG transport. The macrophage and lymphocyte FcRII receptors have been expressed in the Madin-Darby canine kidney (MDCK) cell line (15). This cell line forms a well-polarized epithelial monolayer in culture and has been widely used for studies of protein trafficking in polarized cells. The expressed FcRII receptor transcytoses IgG from the apical to basolateral cell surface in this cell line, which is consistent with the hypothesis that the placental receptor transports IgG.

*Transcytosis of polymeric immunoglobulins*

The major class of immunoglobulin found in a wide variety of mucosal secretions, such as gastrointestinal and respiratory secretions, milk, saliva, tears, and bile is IgA (16–18). IgA is produced by submucosal plasma cells that are often found in gut-associated and bronchus-associated lymphoid tissue (18). After secretion, IgA is taken up by an overlying epithelial cell, transported across the cell, and released into external secretions (17), where the IgA forms the first specific immunologic defense against infection. This system transports only polymeric immunoglobulins (17); dimers or higher oligomers of IgA are transported, as are pentamers of IgM, although transport of the latter is less efficient. The receptor which transports the IgA and IgM is known as the polymeric immunoglobulin receptor (pIgR). This receptor has a ligand-binding domain, a single

membrane-spanning segment, and a cytoplasmic COOH-terminal domain of 103 amino acids (Fig. 1 *B*). The extracellular ligand-binding portion contains five homologous repeating domains of 100–110 residues each. These domains are members of the immunoglobulin superfamily, and most closely resemble immunoglobulin variable regions (19).

The current understanding of the general pathway taken by the pIgR is summarized in Fig. 2, where an epithelial cell is depicted with the apical surface at the top and the basolateral surface at the bottom. The ligand-binding portion of the pIgR is depicted by an open circle and the cytoplasmic tail by a closed one. The receptor is synthesized in the endoplasmic reticulum (step 1) and is then transported to the Golgi apparatus (step 2). It is in the *trans*-most cisternae of this organelle, the *trans*-Golgi network (TGN), that the pIgR is sorted into vesicles that are targeted directly to the basolateral cell surface (step 3). At this surface the receptor binds IgA (step 4) and is subsequently endocytosed (step 5). Once packaged into transcytotic vesicles (step 6) the pIgR is targeted for delivery to the apical cell surface (step 7) where the extracellular, ligand-binding portion of the pIgR is cleaved and released (step 8). This cleaved fragment is known as secretory component (SC) and remains associated with the IgA in the extracellular secretions. It has the additional function of stabilizing the IgA against denaturation or proteolysis in the harsh external environment.

Expression of the pIgR in MDCK cells

This review will focus on the cellular and molecular mechanisms of the membrane trafficking of the pIgR. Two related processes will be discussed: the sequential targeting of the pIgR from the basolateral cell surface to the apical one, and its post-endocytotic sorting into the transcytotic pathway. To study the pIgR pathway, the cloned rabbit pIgR cDNA has been expressed in MDCK cells which do not express an endogenous receptor for immunoglobulin transport. When grown on porous filter supports these cells form a well-polarized epithelial
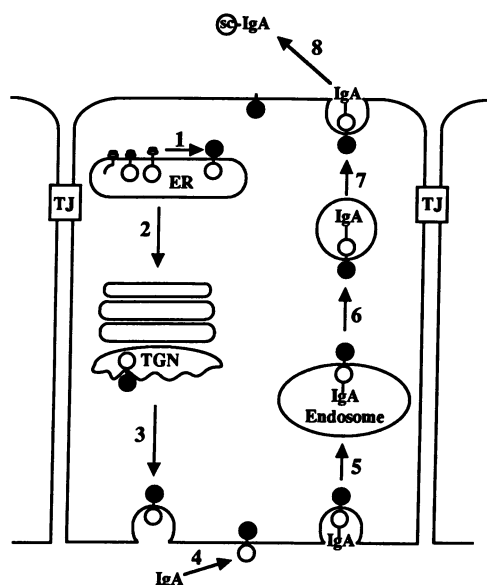
Figure 2. The general intracellular pathway taken by the pIgR. An epithelial cell with tight junctions (TJ) is depicted with the apical surface at the top and the basolateral surface at the bottom. The receptor is synthesized in the endoplasmic reticulum (step 1) and is then transported to the Golgi apparatus (step 2). From the TGN the pIgR is delivered to the basolateral surface (step 3) where it can bind IgA (step 4) and can be subsequently endocytosed (step 5). The receptor is packaged into transcytotic vesicles (step 6) and transported to the apical cell surface (step 7) where the extracellular, ligand-binding portion of the pIgR is cleaved off and released (step 8). This cleaved fragment is known as secretory component (SC) and remains associated with the IgA in the extracellular secretions.

monolayer (1), with tight junctions separating the apical from the basolateral surface. In effect, a simple epithelial tissue is reconstituted in culture. The monolayer is impermeable, especially to macromolecules; hence, one can experimentally access either the apical surface or, through the filter, the basolateral surface. In these cells, the pIgR is synthesized as a 90-kD precursor and then processed to a doublet of 100 and 105 kD due to heterogeneous carbohydrate modifications. Proteolytic cleavage also occurs in these cells, and the free SC is released almost exclusively into the apical medium. This mimics the situation in vivo; SC is released at the lumenal surface and not into the bloodstream. [^{125}I]-labeled IgA is specifically taken up by the cells and transported into the apical medium. This transport is unidirectional, occurring only in the basolateral to apical direction and with a half-time of $\sim$ 30 min (20).

Sorting of the pIgR to the basolateral cell surface

The complexity of the cellular itinerary of the pIgR suggests that it may contain multiple sorting signals that act in a temporal and hierarchical fashion. One location for such signals is the 103 amino acid, COOH-terminal cytoplasmic domain. Being in the cytoplasm, this receptor "tail" would be accessible to interact with cytoplasmic proteins that presumably constitute the cellular sorting machinery. To address this issue a mutant pIgR was constructed that lacked the 101 COOH-terminal amino acids of the cytoplasmic domain (21). When expressed in MDCK cells, this tail-minus pIgR does not appear at the basolateral surface, rather it is sent directly to the apical surface from the Golgi and is cleaved to SC. In a separate construction, the receptor was further truncated by deleting both the trans-

membrane and cytoplasmic domains, producing a soluble receptor (22). This "anchor-minus" receptor is secreted predominantly from the apical pole of MDCK cells, which suggests that the extracellular (or lumenal) portion of the pIgR may contain an apical sorting signal, and that the cytoplasmic domain contains one or more signals that specify basolateral sorting.

To test this hypothesis several deletions were made in the cytoplasmic domain of the pIgR and it has now been demonstrated that only the 17 amino acids closest to the membrane are required for basolateral targeting (Casanova, J., G. Apodaca, and K. Mostov, submitted for publication). A truncated receptor containing only these residues in the cytoplasmic domain is basolaterally targeted, whereas deletion of these residues, leaving the remainder of the tail intact, produces a receptor that is targeted directly to the apical surface. Moreover, transplantation of this 17–amino acid signal to a heterologous, normally apical protein (placental alkaline phosphatase) redirects it to the basolateral surface. This signal ensures that the majority of the pIgR is directed to the basolateral cell surface where its ligand is found.

Endocytosis of the pIgR

The next step in the transcytosis of the receptor is its endocytosis and delivery to the endosome. The signal for endocytosis of the receptor lies in the 30 COOH-terminal amino acids of the cytoplasmic tail. Deletion of these 30 amino acids produces a receptor that follows the pathway of the wild-type receptor, except that the rate of endocytosis from the basolateral surface is decreased by $\sim$ 60% (23). Exactly the same phenotype is produced by mutation of a tyrosine residue in this segment to a serine. This result is consistent with observations in other systems, which have shown that tyrosine residues are important for rapid endocytosis in coated pits (24, 25) and demonstrates a similar role for tyrosine in the pIgR. A second tyrosine residue is located elsewhere in the pIgR tail, yet mutation of this tyrosine alone reduces the endocytotic rate by only 5–10%. However, mutation of both tyrosines together virtually eliminates endocytosis, suggesting that both residues may play a role in this process (Okamoto, C., and K. Mostov, unpublished results).

As described above, when a ligand molecule is endocytosed from the basolateral surface it enters the endosome, and it has three possible fates: transcytosis to the apical surface, recycling to the basolateral surface, or degradation. An assay has recently been developed that allows one to examine the fate of ligand endocytosed at the basolateral surface (26). In this assay [^{125}I]labeled monovalent Fab fragments, derived from antibodies against SC, are added to the basolateral surface of cells for a short 10-min pulse at 37°C, and then the cells are washed extensively. Cells are then incubated in fresh medium over a 2-h period at 37°C. 55% of the internalized ligand is transcytosed and delivered to the apical medium, whereas $\sim$ 20–25% recycles and appears in the basolateral medium. Very little (3–5%) is degraded, as assayed by conversion to acid-soluble products. The recycling of receptor to the basolateral surface provides a further opportunity for it to be reendocytosed and subsequently transcytosed. Ligand can also be endocytosed from the apical plasma membrane (26), but this pool of internalized ligand mostly recycles back to the apical surface. It appears that once the pIgR reaches the apical plasma membrane, it is essentially "trapped" and can only be recycled back to the apical surface.

Phosphorylation: a signal for transcytosis

Phosphorylation is one signal that can direct the segregation of receptor into the transcytotic pathway. The pIgR has been shown to be phosphorylated on a serine residue in its cytoplasmic domain (27), and phosphorylation is thought to occur at the basolateral surface and/or shortly after endocytosis. Mutation of this serine to an alanine, which cannot be phosphorylated, produces a receptor that is not efficiently transcytosed, but rather recycles at the basolateral surface (28). In contrast, mutation of this serine to an aspartic acid, whose negative charge may mimic that of the phosphate group, produces a receptor that is targeted initially basally and is subsequently transcytosed more efficiently than the wild-type pIgR.

The effect of phosphorylation on receptor sorting has also been assessed in a permeabilized cell system that reconstitutes the budding of transcytotic vesicles from MDCK basolateral endosomes (Bomsel, M., and K. Mostov, unpublished results). In this assay, [^{125}I]-labeled Fab fragments of antibodies directed against SC are allowed to bind to the pIgR and are internalized at 18°C. At this temperature, internalization can occur, but the endocytosed proteins are blocked in the endosome and are not transcytosed. The cells are then mechanically perforated by placing a nitrocellulose filter on their apical surfaces and peeling it off. This procedure generates large holes in the plasma membrane which allows cytosolic macromolecules to leak out. The cells are subsequently incubated at 37°C with ATP and cytosol, and transcytotic vesicles, containing the [^{125}I]-labeled Fab marker, are released into the apical medium. When assayed in an identical manner, a marker for recycling proteins, [^{125}I]transferrin, is recycled back to the basolateral cell surface. The majority of the pIgR containing the serine to alanine mutation is found with the pool of transferrin recycling back to the basolateral cell surface. In contrast, the pIgR containing the serine to aspartate mutation is found predominantly in the budding transcytotic vesicles. The budding of transcytotic vesicles requires ATP and cytosolic components. It is also stimulated by GTPγS, a nonhydrolyzable analogue of GTP, suggesting that a GTPase is involved in this process, as has been found in many other membrane trafficking events (29). This system should allow for the dissection of components necessary for the sorting and subsequent packaging of proteins into transcytotic vesicles.

Targeting of transcytotic vesicles

Once the pIgR is packaged into transcytotic vesicles it is transported to the apical cell surface. These vesicles do not randomly find this surface but are thought to be guided there by microtubules. If MDCK cells are treated with the microtubule-depolymerizing drug nocadazole, the rate of transcytosis is slowed by 60–70%. The drug does not affect the overall accuracy of delivery (30, 31). The microtubule-dependent delivery of transcytotic proteins is not confined to MDCK cells. The transport of proteins transcytosed from the basolateral to apical cell surface in Caco-2 cells are similarly affected by nocadazole (32). This suggests that apically-targeted transcytotic vesicles interact with microtubule-dependent motors such as dynein. However, neither the transport of the FcRII receptor from the apical to the basolateral cell surface (31), nor transport of newly synthesized pIgR from the Golgi to the basolateral membrane are affected by nocodazole treatment, suggesting that delivery to the basolateral surface may not require microtubules.

Implications and future studies

Transcytosis allows for the transport and delivery of molecules from one surface to the other while maintaining the integrity of the epithelial monolayer. This process presents the cell with the problem of maintaining the compositional asymmetry of the apical and basolateral surfaces in the face of a constant exchange of membranes and proteins between one surface and the other. For example, in MDCK cells one-half of the cell surface membrane is endocytosed per hour (33). For fluid phase markers 45% of the apically endocytosed marker is transcytosed and 13% of basally endocytosed marker is transcytosed yet the composition of the membrane remains essentially constant (5).

The cell has devised two basic mechanisms for establishing and maintaining the different protein and lipid composition of the apical and basal plasma membranes. The first mechanism allows newly synthesized plasma membrane proteins and lipids to be targeted directly to the appropriate membrane domain from the TGN. However, in certain cell types (e.g., hepatocytes) proteins are only delivered to the basolateral cell surface from this organelle. The second mechanism, and possibly the more important one, is the resorting of membrane proteins after endocytosis from either cell surface (34). In hepatocytes, transcytosis is the only way for membrane proteins to reach the apical surface. In the intestinal cell line, Caco-2, a number of apical proteins either are targeted directly to the apical surface from the TGN, or indirectly by way of the transcytotic pathway (35, 36). The selectivity of the endosome provides the cell with a way to prevent scrambling of the cell surface by allowing only a few select proteins to be transcytosed; many proteins are recycled back to the cell surface of origin.

If not all proteins are transcytosed, then how does the cell recognize those proteins that are, and how are they then sorted away from proteins destined to be recycled or degraded? The answers to these questions are not known at present, but the answer has implications for all processes that involve a step requiring sorting. There is evidence that the signal(s) that specifies if a protein will be transcytosed is contained within the protein itself and is not specific to a particular epithelial cell type. The pIgR, aminopeptidase N, and dipeptidylpeptidase IV are examples of proteins that are transcytosed to various degrees in all cell lines tested (35, 37, 38; Casanova, J., and K. Mostov, unpublished results). The identification of these sorting signals by in vitro mutagenesis may allow for the identification of a putative receptor(s). This receptor would recognize transcytotic proteins and mark them for inclusion in transcytotic vesicles in a fashion analogous to the recognition of lysosomal hydrolases by the mannose-6-phosphate receptor. It is possible that sorting of transcytotic proteins occurs in a morphologically distinct compartment of the endosome. In liver endosomes, the pIgR is found segregated into the tubular extensions of this organelle (39).

One such signal that can regulate the rate of transcytosis, phosphorylation, has been identified. It is not known whether this serine phosphorylation acts as a signal which is recognized directly by a specific receptor protein, or if phosphorylation results in a conformational change that induces the formation of a positive signal for transcytosis. If the function of the pIgR were simply to maximally transcytose IgA, why would the cell use phosphorylation, rather than simply having an aspartate at this site? The most likely explanation is that phosphorylation is

used to regulate transcytosis, perhaps in response to external cues. Are all transcytosed proteins phosphorylated? Probably not. Many of these transcytosed proteins do not contain potential sites for phosphorylation in their cytoplasmic tails, and may instead use a signal analogous to the negative charge of an aspartate residue.

Why is the pathway for transcytosis of pIgR unidirectional? One possibility is that unidirectionality is conferred by the protease that cleaves the pIgR to SC at the apical surface. Once the pIgR reaches the apical surface, it is cleaved to SC and therefore cannot be transcytosed in the opposite direction. The microbial thiol protease inhibitor, leupeptin, inhibits the cleavage of the pIgR to SC (40). In its presence, cleavage to SC is inhibited, but transcytosis of ligand to the apical surface and release into the apical medium is unaffected (26). Apical to basolateral transcytosis is not observed. It may be that the apical and basolateral endosomes "read" the signals present in the pIgR in a different fashion; the signal for pIgR transcytosis is only deciphered by the basolateral endosome. Alternatively, the apical signal hypothesized to be present in the extracytoplasmic domain may be dominant in the endosome; when the receptor arrives in the apical endosome this apical signal remains dominant and the basolateral signal described above cannot operate. The unidirectional transcytosis of the pIgR is not common to all transcytotic proteins. The FcRII can be transcytosed in either direction (15) and MDCK cells express a variety of endogenous glycoproteins that are transcytosed, including several that are transcytosed in both directions (41).

Presently, little is understood about the "sorting machinery" that recognizes these proteins. It must be plastic enough to recognize proteins with diverse functions and no apparent homologies. A more direct analysis of what components are necessary for the sorting and packaging of proteins into transcytotic vesicles and their subsequent targeting to the cell surface are presently underway, and may eventually lead to the identification and purification of this machinery. Sztul and her coworkers have purified putative transcytotic vesicles from rat liver and have identified a 108-kD marker for these vesicles (42). Bomsel and Mostov have now reconstituted the budding of transcytotic vesicles from the basolateral endosomes of MDCK cells (unpublished results). Other strategies to identify important molecules involved in the recognition, sorting, and targeting of transcytotic proteins include binding the cytoplasmic tail of the pIgR to a solid phase support. Affinity chromatography, using this matrix, has been used to identify proteins that specifically bind the wild-type and mutant tails described. These proteins, (Aroeti, B., and K. Mostov, unpublished results) must now be purified and their role in the transcytosis of the pIgR and other sorting steps assessed in the cell-free and permeable-cell systems that have been developed.
have been developed.

Conclusions

Transcytosis allows the epithelial cell to transport molecules from one cell surface to the opposite one while maintaining the epithelial cells function as a selective barrier to molecules entering the underlying tissues. This is not a random process but rather a selective one in which proteins to be transcytosed are sorted in endosomes away from other proteins that will be directed to lysosomes or recycled back to the cell surface. Although we know one mechanism the cell may use to regulate

transcytosis, phosphorylation, we still do not understand how proteins are recognized and sorted into the transcytotic pathway. Mutational analysis, coupled with analysis of the in vitro systems described in this review, may eventually provide us with clues to the general principles that govern protein sorting.

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