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Topical Review

Molecular and Cellular Mechanisms Involved in Transepithelial Transport

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Introduction

Transcellular transport or transcytosis is a vesicular transport activity across polarized cell layers of epithelia and endothelia that involves endocytosis and exocytosis at different cell surface domains. Small molecules, macromolecules, or microorganisms are transported either by fluid-phase or by adsorptive transcytosis, depending on whether or not they interact with specific or nonspecific cell surface binding sites.

The structural prerequisite for vectorial transcellular transport is a polarized cell layer. In simple epithelia, the cell surface consists of functionally and structurally distinct plasma membrane domains (Simons & Fuller, 1985; Rodriguez-Boulan & Nelson, 1989). The luminal or apical plasma membrane, often in contact with the environment, is separated from the basolateral domain by a lateral membrane specialization, the junctional complex, with its zonula occludens (tight junction) and zonula adherens (Farquhar & Palade, 1963) (Fig. 1). The tight junctions which seal the apex of the cells provide an efficient diffusion barrier for many ions, small molecules, and all macromolecules. The basolateral cell surface consists of two domains, the lateral membrane involved in cell-cell interactions via cell adhesion molecules (CAMs) and the basal membrane which binds to basal lamina components through receptors such as integrins (Boyer & Thiery, 1989).

Studies on the biogenesis of polarized cell membrane proteins in rat hepatocytes (Feracci et al., 1987), in rabbit jejunal enterocytes (Massey et al., 1987), and in a human colon carcinoma cell line (Caco-2) (Matter et al., 1990*a*) indicate that transcytosis is involved in the maintenance of epithelial cell polarity. Thus, transcytosis plays a dual role in epithelia: maintenance of cell polarity and vectorial delivery of substances across the polarized cell layer.

In epithelia, transcytosis is essential in the passive transfer of antibodies from the mother to the offspring (Kraehenbuhl, Bron & Sordat, 1979; Rodewald, Lewis & Kraehenbuhl, 1983), in providing the newborn with milk growth factors (Thornburg et al., 1984; Siminoski et al., 1986; Gonnella et al., 1987), in sampling antigens (Owen, 1977; Neutra et al., 1987; Weltzin et al., 1989; Kraehenbuhl et al., 1991) and in secretion of protective mucosal antibodies (Kühn & Kraehenbuhl, 1982; Solari & Kraehenbuhl, 1985; Mestecky & McGhee, 1987). While some of these physiologic functions require apical to basolateral transcytosis, others involve transport in the opposite direction. In both cases, certain basic cellular functions are required.

Vesicular Traffic Mediates Transepithelial Transport

Transcytosis is a multistep process which involves common mechanisms shared by all animal cells as well as epithelium-specific mechanisms. In the epithelial cells studied so far, endocytosis proceeds from both cell surface domains with comparable kinetics, and the internalized molecules initially accumulate in distinct apical and basolateral early endosomes. Tracer studies indicate that the content of early endosomes from both cell surfaces can be delivered to common late endosomes (Bomsel et al., 1989; Fujita, Reinhart & Neutra, 1990; Hughson & Hopkins, 1990). How molecules move from one early endocytic pathway to the opposite cell surface domain remains unclear.

Key Words transcytosis · epithelium · membrane traffic · endocytosis · receptors · antibodies

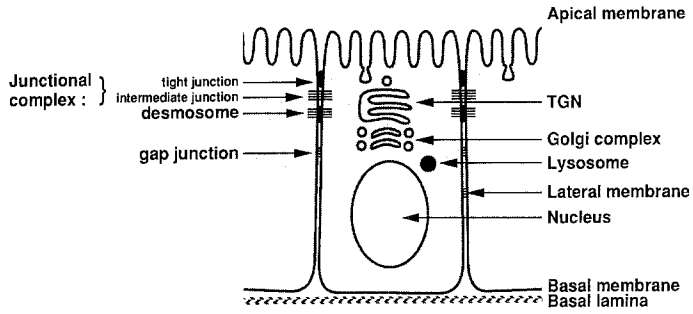


Fig. 1. Schematic representation of a simple epithelium. The cell surface consists of three distinct domains: (i) the apical or luminal plasma membrane usually in contact with the environment; (ii) the lateral membrane interacting with the adjacent cell surface via cell adhesion molecules (CAMS), the junctional complex including tight and intermediate junctions, desmosomes, and possibly gap junctions; (iii) the basal membrane binds to basal lamina components (collagen, glycoproteins, proteoglycans) through receptors such as integrins.

Epithelial Cells Possess Two Distinct Sets of Early Endosomes

The first cellular event in transcytosis consists of the internalization of molecules by endocytosis, via a selective receptor-mediated process or by bulk uptake at either cell surface domain. The incoming vesicles fuse with the early endocytic compartment, a tubulo-vesicular organelle also termed CURL (*c*ompartment of *u*ncoupling of *r*eceptor from *l*igand) (Geuze et al., 1984; Braulke et al., 1987). In hepatocytes (Geuze et al., 1984; Wall & Hubbard, 1985; Schiff et al., 1986), kidney tubular cells or cell lines (van Deurs & Christensen, 1984; von Bonsdorff, Fuller & Simons, 1985; Parton et al., 1989), and enterocytes (Rodewald, 1980; Fujita et al., 1990) there are distinct sets of early apical and basolateral endosomes. The vesicles associated with early endocytic compartments are generally characterized by an acidic content (pH 6.0–6.5) (Mellman, Fuchs & Helenius 1986). Early apical and basolateral endosomes from epithelial cells are unable to fuse with each other in cell-free systems in vitro, but they can fuse with late endosomes (Bomsel et al., 1990).

The early endocytic compartment of most eukaryotic cells is a sorting organelle where membrane proteins or internalized molecules are directed to different pathways: recycling, transcytosis, or delivery to late endosomes or lysosomes. In some cells, however, recycling of fluid phase or membrane proteins, ligands and their receptors can also occur from late endosomes (Hughson & Hopkins, 1990). The sorting along the endocytic pathway may differ spatially depending on the cell type and on the internalized molecules. How sorting occurs in early endosomes and how transcytotic vesicles are formed remains an enigma.

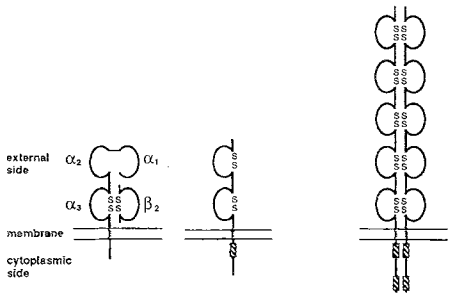
Soluble Molecules Cross Epithelia by Fluid-Phase Transcytosis

Transcytosis of soluble markers has been examined in intact organs and some functional properties of the transcytotic carrier vesicles have been characterized (Hayakawa et al., 1990; Klapper, Graf & Fuchs, 1991). Because access to the two surface domains is restricted in intact organs, the kinetics of fluid internalization, recycling, transcytosis, and intracellular retention has been analyzed mainly in cultured cell lines grown on permeable supports. In intestinal and kidney cell lines, fluid phase markers enter separate apical and basolateral early endosomes, but their fate depends on the cell type. For example, in differentiated HT-29 human colon carcinoma cells, the content of basolateral early endosomes is almost entirely recycled, while less than 10% is transcytosed (Poulain-Godefroy et al., 1990). In MDCK cells, the rates of internalization from the luminal or the basolateral surface are identical, but the fate of molecules internalized from the apical or basolateral domain differs. Using horseradish peroxidase (HRP) as tracer, it was observed that early apical endosomes distributed only 10% of their fluid-phase content to late endosomes, while the other 90% was released from the cells, 45% by transcytosis and 45% by recycling. In contrast, about 75% of the HRP that entered basolateral early endosomes was directed to the late endocytic compartment, and only 15% was transcytosed (Bomsel et al., 1989). Whereas in MDCK cells the apical to basolateral transcytotic pathway is dominant, in Caco-2 cells apical to basolateral transcytosis was detected by some investigators (Hidalgo, Raub & Borchardt, 1989) but not by others (Hughson & Hopkins, 1990). Thus, it appears that transcytosis in different cell types has distinct rates and directionality. Furthermore, rates and directions of transcytosis in cultured "model" cell monolayers such as MDCK, HT-29, and Caco-2 cells may not accurately reflect transport activity in the corresponding cells in vivo.

Transcytosis of Plasma Membrane Proteins Contributes to the Establishment of Epithelial Cell Surface Polarity

Transcytosis of plasma membrane proteins is also used by epithelial cells for the maintenance of distinct cell surface domains. In hepatocytes, all newly

synthesized apical (canalicular) plasma membrane proteins are first inserted in the basolateral (sinusoidal) membrane and then are transcytosed to their final destination (Feracci et al., 1987; Bartles & Hubbard, 1988). In the simpler epithelial cell type MDCK, most plasma membrane proteins are directly routed from the Golgi complex to their final destination (Simons & Wandinger-Ness, 1990) and transcytosis serves mainly to correct missorted proteins (Matlin et al., 1983). The direct and indirect pathways of delivery can also coexist in the same cell. This has been documented for the human intestinal cell line Caco-21, in which some luminal membrane proteins transit through the basolateral membrane while others are directly routed to their final domain (Le Bivic et al., 1990; Matter et al., 1990*b*). Among the membrane proteins which follow the indirect pathway, the rate of appearance in the apical domain differs apparently due to differences in the efficiency with which they are endocytosed and enter the transcytotic pathway (Bartles & Hubbard, 1988; Matter et al., 1990). In MDCK cells, certain membrane glycoproteins have recently been shown to undergo transcytosis in both directions (Brändli, Parton & Simons, 1990). Thus, while some proteins are transported unidirectionally by transcytosis, others are transported bidirectionally across the cell. Whether this bidirectional membrane flow mediates a physiological function is not known.



Receptors	Fcγ (MHC - class I-like)	mFcγ-B_2 hFcγ - C	poly - Ig
Tissue distribution	small intestine yolk sac	placenta	glandular and mucosal epithelia
Endocytosis signal (ss) tyrosine - dependent	-	-	+
tyrosine - independent	-	+	-
Structure	heterodimer	?	homodimer ?
	α - chain : MHC class I-like β chain : β_2 microglobulin	2 Ig - like ectodomains	5 Ig - like ectodomains 2 internalization signals (ss) on cytoplasmic tail

Fig. 2. Receptors mediating transcytosis of immunoglobulins across epithelia.

Immunoglobulins Cross Epithelia by Receptor-Mediated Transcytosis

Receptor-mediated transcytosis is a property of many epithelial cells in mucosal and glandular tissues. Some epithelia, such as the placental syncytial trophoblast in lagomorphs, rodents, and primates (Kraehenbuhl et al., 1979), the rat yolk sac fetal endoderm (Guenthert & Rodewald, 1990), the rodent neonatal small intestine (Rodewald et al., 1983; Rodewald & Kraehenbuhl, 1984), and the mammary glands of ruminants (Larson, Heary & Devery, 1980) express IgG Fc receptors. These epithelial Fc receptors mediate apical to basolateral transcytosis in the placenta, the yolk sac, and the neonatal small intestine, and in the opposite direction in the mammary glands. The rat intestinal and human placental Fc receptors have been isolated and their corresponding cDNAs cloned and sequenced. The intestinal receptor, and probably the yolk sac receptor, is related to MHC class I proteins (Simister & Mostov, 1989), while the human placental receptor is similar to the murine macrophage/lymphocyte Fc receptor (Stuart et al., 1989) (Fig. 2). Binding of IgG to the intestinal receptor is pH-dependent (Rodewald, 1980), which allows uptake in the acidic environment of the intestinal lumen and release in the interstitial space at neutral pH. The same pH-dependence of binding functions differently in the yolk-sac epithelium where both apical and basolateral cell surfaces are exposed to neutral milieux. IgG is internalized by yolk-sac epithelial cells in the fluid phase, and binding to the yolk-sac receptor occurs not at the cell surface but in the early apical endosomes, presumably as a result of acidification in the endocytic compartment (Guenthert & Rodewald, 1990). Much less is known about the properties of the placental receptor (Niezgodka et al., 1981). Since binding to this receptor is pH independent, it is believed that the driving force for vectorial transport is the large concentration gradient of IgG across the syncytiotrophoblast. The mammary gland Fc receptor has not yet been characterized.

The polymeric Ig receptor mediates basolateral to apical transcytosis of polymeric IgA and IgM antibodies across mucosal and glandular epithelial barriers into various secretions, as well as through liver from the blood compartment into bile. The poly-Ig receptor, like the Fc receptors, is a member of the immunoglobulin gene family (Mostov, Friedlander & Blobel, 1984; Banting et al., 1989; Krajci et al., 1989) (Fig. 2). Its complex itinerary has been analyzed in the rat liver (Brown, Russell &

Mestecky, 1982; Geuze et al., 1984; Hoppe, Connolly & Hubbard, 1985; Limet et al., 1985; Sztul, Howell & Palade, 1985*a,b*; Perez et al., 1988; Klapper et al., 1990), the rabbit mammary gland (Solari & Kraehenbuhl, 1984), human intestinal cell lines (Mostov & Blobel, 1982), and cultured hepatocytes (Musil & Baenziger, 1987*a,b*). During transport or at the luminal cell surface, the receptor is cleaved by a thiol-protease (Musil & Baenziger, 1987) and the cleaved portion, termed secretory component (SC), remains bound to its ligand upon release into secretions. SC masks proteolytic cleavage sites on the Fc portions of the IgA molecules (Lindh, 1975). Thus, the receptor has a dual function: it mediates transcytosis of polymeric IgA antibodies and as SC, it protects the secreted antibodies (primarily sIgA) from proteolysis in the mucosal environment.

In order to elucidate the molecular mechanisms which mediate immunoglobulin receptor membrane trafficking and transcytosis of their corresponding ligands in epithelia, kidney and mammary cell lines were stably transfected with wild-type or mutated receptor cDNAs (Mostov & Deitcher, 1986; Mostov, Breitfeld & Harris, 1987; Breitfeld et al., 1989*b*; Hunziker & Mellman, 1989; Hunziker, Mâle & Mellman, 1990; Schaerer et al., 1990). These studies have shown that most if not all sorting and targeting information resides on the cytoplasmic tail of these receptors (Mostov, de Bruoyn & Deitcher, 1986; Breitfeld et al., 1990; Casanova et al., 1990; Hunziker et al., 1990).

Transcytosis Requires Distinct Carrier Vesicles

As noted above, the two early endocytic compartments of epithelial cells are structurally and functionally separate organelles and are unable to fuse with each other in cell-free systems in vitro (Bomsel et al., 1990). The transepithelial transport of fluid phase markers or membrane proteins from one pole of the cell to the other probably requires sorting of the transcytosed molecules into distinct carrier vesicles derived from the early endosomes (Geuze et al., 1984). Transcytotic carrier vesicles could then follow one of the three pathways depicted in Fig. 3. In the direct pathway, the transcytotic vesicles which bud from an early endocytic compartment would directly recognize and fuse with the opposite plasma membrane. This model implies two distinct transcytotic carriers, one moving from early apical endosomes to the basolateral membrane and one moving in the opposite direction. Recent evidence from transfected MDCK cells, however, indicates that both the epithelial Fc receptor and the poly-Ig receptor recycle at both cell surfaces before transcytosing or releasing their ligand (Breitfeld et al., 1989*b*; Hunziker et al., 1990). These observations favor a shuttle model, in which a transcytotic carrier vesicle moves between the apical and basolateral early endocytic compartments. This could be accomplished by a single type of carrier vesicle with fusion competence for early endosomes at both cell poles. One cannot rule out, however, a third model in which another intermediate organelle, such as the late endosome or the *trans*-Golgi network, is an obligatory stop in the transcytotic pathway. Such an indirect pathway would require multiple sorting events, in the early endosomes and the intermediate organelle.

Morphological studies have so far failed to clearly identify transcytotic vesicles (Geuze et al., 1984; Hoppe et al., 1985; Takahashi, Nakane & Brown, 1982) and results from cell fractionation protocols have been disappointing (Perez et al., 1988; Quintart, Baudhuin & Courtoy, 1989), mainly because of the lack of specific markers. Recently, a population of distinct carrier vesicles involved in basolateral to apical transcytosis of the poly-Ig receptor in rat liver has been isolated by combining differential centrifugation and immunoisolation (Sztul et al., 1991). The fraction immunoisolated with an antibody specific for the cytoplasmic tail of the receptor (Solari, Kühn & Kraehenbuhl, 1985) was used as an immunogen for the generation of polyclonal antibodies. The immune serum recognized a single 108 kDa polypeptide enriched in the immunoisolated fraction that may represent a marker for transcytotic carrier vesicles. Whether several distinct transcytotic vesicular carriers mediate different transport functions in epithelial cells remains to be established.

Microtubules Are Involved in Transcytosis

There is increasing evidence that microtubules play a role in some but not all membrane trafficking activities in the cell (Kelly, 1990). Kinesin- and dynein-like motors have been shown to move vesicles to the plus or the minus ends of microtubules, respectively (Howard, Hudspeth & Vale 1989; Schroer & Sheetz, 1989; Schroer, Steuer & Sheetz, 1989) (Fig. 4). On the secretory pathway of nonpolarized cells, exocytic vesicles move from the Golgi complex to the cell surface along microtubules. Depolymerization of microtubules does not interrupt exocytosis but decreases the efficiency of secretion probably by preventing approximation of the secretory vesicles

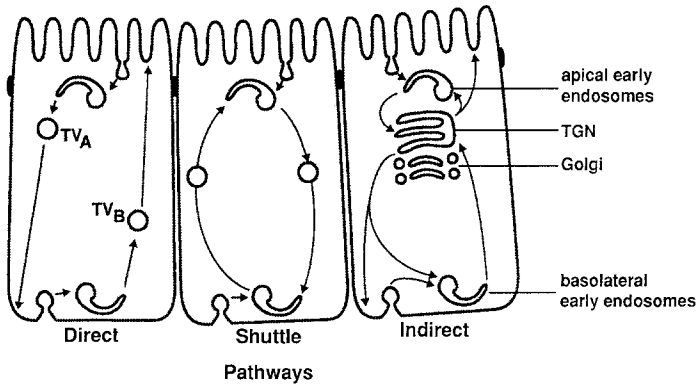


Fig. 3. The transcytotic pathways. Three models that can account for transcytosis across epithelial cells are proposed. In the *direct pathway*, sorting of transcytotic molecules occurs in an early or late endocytic compartment depending on the cell type. The transcytotic vesicles which bud from the early endosomes directly recognize and fuse with the opposite plasma membrane. This model requires distinct transcytotic carrier vesicles (TV_A and TV_B). In the *shuttle pathway*, a single type of transcytotic vesicle buds from and recognizes both early endocytic compartments. In the *indirect* model, an intermediate compartment such as the TGN or late endosomes, is a necessary stop on the transcytotic pathway.

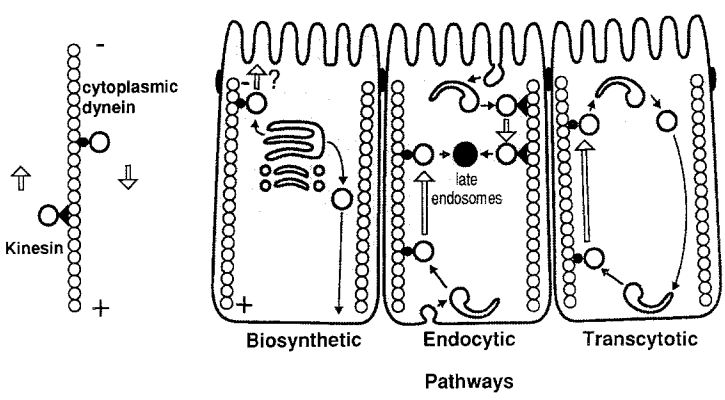


Fig. 4. Microtubule-assisted membrane traffic in epithelial cells. Microtubules run parallel to the lateral membrane with their minus end oriented toward the apical plasma membrane. Microtubule-dependent vesicular movement occurs along the biosynthetic, endocytic, and transcytotic pathways (wide white arrows). Microtubule-independent movements are indicated by the black arrows. Movement requires motors associated with the cytoplasmic face of vesicle membranes. In epithelial cells, kinesin-like motors would move vesicles apically toward the minus end of microtubules. Dynein-like motors in epithelial cells may or may not be involved in movement in the opposite direction.

and the plasma membrane (Kornfeld & Mellman, 1989). In epithelial cells, microtubules also play a role in the polarized delivery of secretory proteins to the apical cell surface. For example, secretory granules in goblet cells move toward the luminal surface along microtubules arranged in a regular vertical pattern parallel to the lateral membrane, and this movement is disturbed by microtubule disruption (Specian & Neutra, 1984). A similar organization of microtubules was documented in confluent cultured epithelial cells (Gorbsky & Borisy, 1985; Achler et al., 1989; Bacallao et al., 1989) where the minus end was found to be oriented toward the apical cell surface (Bacallao et al., 1989). Microtubule-disrupting drugs also alter polarized delivery of membrane proteins (Quaroni, Kirsch & Weiser, 1979; Danielsen, Cowell & Poulsen, 1983; Eilers, Klumperman & Hauri, 1989). In Caco-2 cells, for example, nocadozole inhibits the delivery of proteins to the apical but not the basolateral cell surface (Eilers et al., 1989).

Although endocytosis itself is not microtubule-dependent, vesicle movement along the endocytic pathway is mediated by microtubules. Specifically, translocation of peripheral early endosomes to later, more central endocytic compartments was shown to be microtubule-dependent both in nonpolarized (Hirsch, Fedorko & Cohn, 1968; Herman & Albertini, 1984) and polarized cells (Hughson & Hopkins, 1990; Bomsel et al., 1990; Gruenberg, Griffiths & Howell, 1989). Microtubules are also involved in transcytosis from the basolateral to the apical membrane. For instance, disruption of the microtubules with colchicine in rat liver reduced transcytosis of polymeric IgA from blood to bile with a concomitant accumulation of IgA-containing endosomes at the cell periphery near the sinusoidal surface (Goldman et al., 1983). Low doses of colchicine in perfused rat liver reduced the rate of both receptor-mediated and fluid-phase transcytosis, lead to the progressive accumulation of newly-synthesized apical proteins in the basolateral membrane, and reduced the secretion of plasma proteins into bile (Hubbard & Barr, 1991). In MDCK cells co-expressing the poly-Ig and epithelial Fc receptors, basolateral to apical transcytosis of poly-Ig receptor was found to require an intact microtubule network, while transcytosis of Fc receptor in the opposite direction was microtubule independent (Hunziker et al., 1990).

Transcytosis is Mediated by a Complex Molecular Machinery

Transport of proteins between the various compartments of the secretory, endocytic, and transcytotic pathways requires sequential formation and fusion of carrier vesicles. Presumably, common mechanisms are involved in the budding of vesicles from donor compartments and fusion with acceptor compartments. In order to provide directional and specific transport processes, however, the various vesicles must also possess distinct components which allow them to specifically recognize their target. If several different sorting decisions are made in different cell compartments along the secretory, endocytic, and transcytotic pathways, this would require that specific sets of proteins and lipids are recruited into distinct carrier vesicles and that several signals function sequentially. In addition, specialized sorting mechanisms would have to operate so that the polarity of the epithelial surfaces is preserved. Recent reviews on the basic properties of vesicular transport (Wilschut, 1989; Balch, 1990), endocytosis (Gruenberg & Howell, 1989; Hubbard, 1989; Rodman, Mercer & Stahl, 1990), and transcytosis (Breitfeld et al., 1989*a*) are available for additional information.

In this review, we briefly describe recent advances in our understanding of the general molecular mechanisms involved in endocytosis, addressing of membrane proteins, and membrane fusion. We then reexamine the role of these phenomena in transcytosis.

Receptor-Mediated Endocytosis Requires Signals

A tyrosine internalization signal has recently been identified on the cytoplasmic tail of several endocytosed membrane proteins. Mutation of a single tyrosine residue at a defined position on the cytoplasmic tail of the LDL-receptor (Davis et al., 1987), the cation-independent mannose (6-phosphate-receptor (Lobel et al., 1989), and the transferrin receptor (Jing et al., 1990; McGraw & Maxfield, 1990) resulted in impaired endocytosis. The importance of tyrosine in initiating clustering and internalization of plasma membrane proteins was demonstrated by introducing a single tyrosine in the cytoplasmic tail of resident cell surface proteins that are normally not endocytosed, including influenza virus hemagglutinin (Lazarovits & Roth, 1988) and glycophorin (Ktistakis et al., 1990); such mutations resulted in endocytosis. There is no amino acid consensus sequence surrounding the critical tyrosine on the cytoplasmic tails of endocytosed proteins. This suggests that the signal consists of a degenerate primary sequence, as reported for the sequences involved in targeting proteins into the endoplasmic reticulum and the mitochondria. Two motifs have been proposed: in one model a turn is present on the side of the tyrosine closest to the transmembrane domain (Ktistakis, Thomas & Roth, 1990), while in the other two aromatic amino acids separated by two residues from a stable surface loop (Jing et al., 1990). In the two models, the predicted structures are stabilized by hydrogen bonds.

Some membrane proteins, such as the poly-Ig receptor, contain two tyrosine internalization signals. Other membrane proteins, however, such as the macrophage Fc receptor, undergo coated pit clustering and internalization that is tyrosine independent, since mutation of the tyrosine residue in the motifs described above does not inhibit endocytosis (Mellman, 1991). This observation is consistent with two independent internalization signals, one mediated by a tyrosine motif and another of unknown structure. It is not known whether both signals interact with the same cellular machinery.

Coated Vesicles are Involved in Sorting

It is generally accepted that selective clustering of specific membrane proteins occurs in coated pits both at the cell surface and in the Golgi complex. The cytoplasmic clathrin coat is composed of clathrin triskelions and accessary proteins, the adaptors, which appear to link clathrin to the cytoplasmic tails of transmembrane proteins (Pearse & Robinson, 1990). Two types of adaptor molecules have been identified: type 1, which is localized in Golgi-coated pits, and type 2, which is restricted to plasma membrane coated pits (Pearse, 1987). Both adaptors consist of heterodimers of 100 kDa proteins called adaptins associated with two smaller proteins. The plasma membrane adaptor (HA 2) consist of α and β adaptins, while the Golgi adaptor (HA 1) contains γ and β' adaptins (Kirchhausen et al., 1989; Robinson, 1989; Ponnambalam et al., 1990). It is likely that more members in this family will be identified.

It is not known whether the coated pits seen on endocytic compartments contain a unique adaptor, but adaptors presumably play an important role in clustering membrane proteins in the organelles where sorting decisions have to be made. Recently, it has been shown that the cytoplasmic tails of the cation-independent mannose-6-phosphate receptor, the LDL receptor, and the poly-Ig receptor specifically bind to plasma membrane adaptors (Pearse, 1988; Glickman, Conibear & Pearse, 1989). The fea-

tures on the cytoplasmic domains of membrane proteins recognized by adaptors have not yet been characterized, although some clustering and internalization signals have been identified as described above. These signals, probably recognizing adaptins, may consist of discrete linear amino acid sequences or conformations shared by various membrane proteins. Whether recruitment of membrane proteins into nonclathrin coated pits requires similar adaptors and signals has not been documented. Poly-Ig and Fc receptors have been shown to cluster in the tubular extensions of early endosomes (Geuze et al., 1984; Rodewald & Kraehenbuhl, 1984), but whether transcytotic vesicles are actually formed by clathrin-mediated withdrawal of these membrane microdomains remains to be established.

Polarized Delivery of Newly Synthesized Immunoglobulin Receptors Requires Specific Signals on their Cytoplasmic Tail

Signals for basolateral delivery of newly synthesized proteins in epithelial cells have also been mapped to their cytoplasmic tails. Two Fc receptor isoforms, which differ only in that one isoform contains a 47-amino acid insert on the cytoplasmic tail, showed remarkably different cell surface distributions and endocytic patterns when expressed in MDCK cells. The receptor with the insert (which was not internalized in lymphocytes) was delivered to the apical membrane, while the shorter form (which was endocytosed in macrophages) accumulated at the basolateral surface (Hunziker & Mellman, 1989). This raises the possibility that clathrin-mediated clustering in the Golgi complex or the *trans*-Golgi network (TGN) plays a role in basolateral but not apical targeting. In addition, the apical resident membrane protein influenza hemagglutinin was directed to the basolateral cell surface in MDCK cells when an endocytosis signal was introduced on the cytoplasmic tail (Lazarovits & Roth, 1988). Recently, it was shown that a 19-amino acid stretch on the cytoplasmic tail of the poly-Ig receptor tail, containing a tyrosine internalization signal, was sufficient for basolateral delivery in MDCK cells (Breitfeld et al., 1990). If recruitment of membrane proteins into coated pits in the TGN is a prerequisite for basolateral delivery, this implies that a dominant signal, rather than a default pathway, controls basolateral delivery. There is recent data, however, indicating that sorting of a single membrane protein in different polarized epithelia is cell type and tissue specific. The human LDL receptor expressed in transgenic mice accumulated in the basolateral membrane of hepatocytes and enterocytes, but in the apical membrane of kidney tubular cells (Pathak et al., 1990). All of the above shows that the rules that govern sorting of membrane proteins in epithelia are not yet clearly established.

Vesicular Trafficking Requires a Membrane Fusion Machinery

Several components of a system that probably represents a general mechanism for fusion of intracellular vesicles and membrane compartments have been isolated and characterized (Rothman & Orci, 1990). Four components appear to constitute a "fusion particle" which binds to an integral membrane protein on the acceptor compartment, the donor, or both (Weidman et al., 1989). This fusion complex consists of (i) the N-ethylmaleimide sensitive factor (NSF), the functional equivalent of the yeast SEC 18 gene product (Wilson et al., 1989), (ii) a set of three polypeptides called SNAPs, responsible for the attachment of NSF to membranes and homologous to the SEC 17 gene product (Weidman et al., 1989), (iii) "factor B" which requires fatty acylation for activity (Pfanner et al., 1990), and (iv) a 25-kDa protein which acts at a pre-fusion step (Wattenberg et al., 1990). Studies in cell-free systems indicate that some of these proteins function both in the secretory and the endocytic pathways. Fusion of transcytotic vesicles derived from rat liver with canalicular plasma membrane in a reconstituted cell-free system required NSF and a 108-kDa cytosolic protein shown to be associated with transcytotic vesicles in intact hepatocytes (Sztul, Kaplin & Samanta, 1990).

Directionality and specificity in vesicular membrane trafficking probably require an additional organelle-specific system that is likely to involve small GTP binding proteins (small GTPases) with primary sequence homologies to YPT1 and SEC4 gene products in yeast (Bourne et al., 1988; Goud et al., 1988; Walworth et al., 1989; Bourne, Sanders & McCormick, 1990). This family includes the rab proteins (Plutner et al., 1990), the SAR1 protein (Nakano & Muramatsu, 1989), and the ADP-ribosylation factor (ARF) (Sewell & Kahn, 1988) which seem specific for transport between different compartments. The rab protein family consists of at least eight members and they have been found associated with different organelles (Chavrier et al., 1990; Goud et al., 1990). In MDCK cells, the complexity of the small GTPase family is such that it includes at least 30 different isoforms (Chavrier et al., 1990). The recent biochemical isolation of basolateral and apical exocytic vesicles from MDCK cells (Wandinger-Ness et al., 1990), as well as rat liver transcytotic vesicles (Sztul et al., 1991), and their use in cell-free systems, should facilitate elucidation of the roles of various

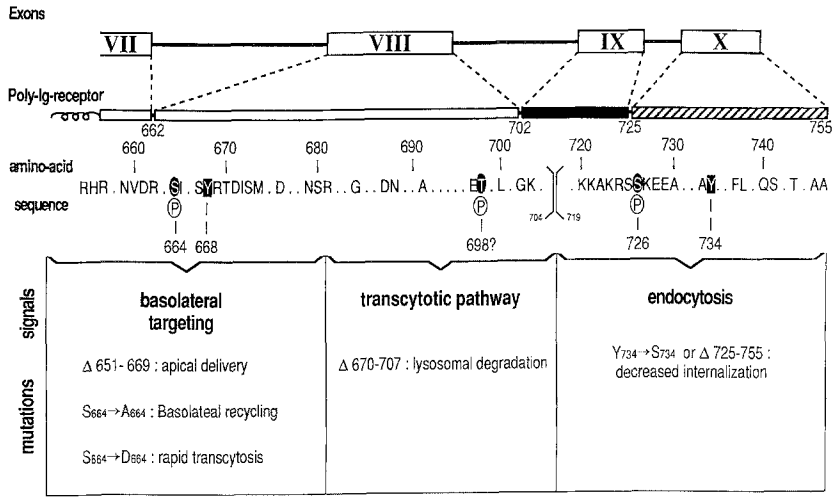


Fig. 5. Sorting and addressing signals on the poly-Ig receptor. The cytoplasmic tail is encoded by 3 exons (C. Hanly, *personal communication*). The amino acids given with a one-letter code represent residues conserved among two rabbit isoforms, the rat and the human sequences. Two tyrosine-dependent internalization signals (Tyr 668 and 734) mediate clusting and endocytosis. Each tyrosine is flanked by a serine residue (Ser 664 and 726) which undergo phosphorylation.

small GTPases in the selective membrane fusion event involved in transcytosis.

Transcytosed Membrane Proteins Possess Sorting Signals on their Cytoplasmic Tail

Most if not all information that directs membrane protein or receptor traffic in the transcytotic pathway appears restricted to the cytoplasmic domain of the membrane proteins or receptors, as documented by deletion mutation analysis (Mostov et al., 1986; Hunziker & Mellman, 1989). Signals for basolateral delivery, endocytosis, or withdrawal from the lysosomal pathway have been found associated with the cytoplasmic tail of several proteins, and have been particularly well defined on the transcytotic poly-Ig receptor and the placental Fc receptor, using mutated receptors expressed in epithelial cell lines (Mostov & Simister, 1985; Breitfeld et al., 1990) (Fig. 5).

In the case of the poly-Ig receptor a stretch of 34 amino acids situated between the two internalization signals on the cytoplasmic tail seems to direct the protein into transcytotic vesicles after endocytosis from the basolateral side. Deletion of this stretch resulted in entry of the receptor into the degradative lysosomal pathway (Breitfeld et al., 1990). Half of the residues in this sequence are conserved among the rat (Banting et al., 1989), human (Krajci et al., 1989), and two alleles of the rabbit poly-Ig receptor (Mostov et al., 1984; Schaerer et al., 1990). The degree of homology, however, is much less than that of the internalization signals. Whether sorting of membrane proteins into transcytotic pathways always requires recruitment into coated pits of early endosomes is not established, although clustering of IgA in hepatocyte endosomes suggests this possibility (Geuze et al., 1984).

Phosphorylation Regulates Membrane Protein Trafficking and Transcytosis

Many membrane proteins, including the poly-Ig receptor, are phosphorylated in the course of their movement through membrane compartments. The role of phosphorylation in trafficking of the poly-Ig receptor in polarized cells and the possible role(s) of phosphorylation in transcytosis is under intensive investigation (Larkin et al., 1986; Solari & Kraehenbuhl, 1987). The receptor is phosphorylated on its cytoplasmic tail. Serine 664 represents the major phosphorylated residue (Casanova et al., 1990), while serine 726 accounts for 10% of phosphorylation (R. Hirt et al., *manuscript in preparation*). These two serines and their surrounding residues which are conserved among species flank the two tyrosine internalization signals, suggesting that they may play a role in modulating interaction of the receptors with the sorting machinery. Mutation of Ser 664 to Ala converted the transcytotic receptor into a recycling receptor, while replacement of the Ser by an Asp residue increased the rate of receptor transcytosis (Casanova et al., 1990). Phosphorylation might serve other functions, however, such as oligomerization of the receptor or enhancement of receptor cleavage at the luminal surface.

Conclusions

Our current understanding of vesicular transport across polarized epithelial cells is largely derived from studies of various cell lines in vitro and rat liver

in vivo. It may be assumed that the basic mechanisms and cellular machineries which control membrane protein sorting, coated pit-mediated internalization, membrane fusion and fission, play important roles in the phenomenon of selective transcytosis. At the present, however, no general rules have been established that explain the traffic of different membrane proteins and ligands across specific epithelial cell types. For example, the pattern of protein movement that seems to represent a default pathway in certain cell types appears to be signal-mediated in others.

The dissection at the molecular level of the components involved in transepithelial traffic of membrane proteins will require complementary experimental approaches, including the isolation of specific transcytotic carrier vesicles, their biochemical characterization, the reconstitution of the various steps in cell-free systems, and analysis of the traffic patterns of transcytotic proteins in different cell types after transfection and in transgenic animals.

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