Mini-Review

The Sorting of Proteins to the Plasma Membrane in Epithelial Cells

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THE plasma membrane of epithelial cells is polarized by division into apical and basolateral domains of unique protein and lipid composition (reviewed in Simons and Fuller, 1985). Development and maintenance of plasma membrane polarity is achieved by the sorting of membrane constituents. Both membrane and secretory proteins must be recognized as apical or basolateral, segregated from each other, and delivered to their correct final destinations. This brief review will summarize recent investigations into these sorting events and discuss possible mechanisms of recognition and segregation.

Sorting Pathways for Viral and Endogenous Proteins

Experimental analysis of sorting in epithelial cells was advanced significantly in 1978 when Rodriguez-Boulan and Sabatini discovered that spike glycoproteins of enveloped viruses are targeted to particular plasma membrane domains in Madin-Darby canine kidney (MDCK) cells (Rodriguez-Boulan and Sabatini, 1978; Rodriguez-Boulan and Pendergast, 1980). MDCK cells are polarized in culture and are capable of sorting membrane and secretory proteins (Simons and Fuller, 1985; Kondor-Koch et al., 1985; Beaudry et al., 1985; Chang and Berlin, 1985; Gottlieb et al., 1986*a*; Caplan et al., 1986). When the cells are infected with influenza virus, the influenza membrane proteins hemagglutinin and neuraminidase are transported only to the apical plasma membrane. Vesicular stomatitis virus (VSV) G protein is, in contrast, routed to the basolateral domain (Rodriguez-Boulan and Pendergast, 1980). Subsequent studies have shown that other viral membrane proteins are also sorted in MDCK cells. The E2 protein of Semliki Forest virus, for example, is localized basolaterally in infected cells and in cultures transfected with the cloned gene (Roman and Garoff, 1985).

The influenza hemagglutinin and the VSV G protein follow the same pathway through the cell at least as far as the *trans* part of the Golgi complex (Rindler et al., 1984; Fuller et al., 1985). It is probably as they leave the Golgi complex that the apical protein is segregated from the basolateral protein (Matlin and Simons, 1984; Misek et al., 1984; Rindler et al., 1985; Pfeiffer et al., 1985).

Recent experiments indicate that endogenous epithelial cell proteins are also transported along independent, direct pathways from the Golgi complex to both plasma membrane domains. MDCK cells secrete laminin and a proteoglycan basolaterally and other proteins apically (Kondor-Koch et al., 1985; Beaudry et al., 1985; Chang and Berlin, 1985; Gottlieb et al., 1986*a*; Caplan, M. J., personal communication). This fact in itself suggests that independent vesicular pathways to both sides of the cell must exist. In addition, pulse-labeled sodium/potassium ATPase, a basolateral membrane protein, has been observed by Caplan to first reach the cell surface on the basolateral domain, consistent with a direct route from cytoplasmic organelles to the proper part of the plasma membrane (Caplan et al., 1986). In intestinal cells, the apical membrane protein aminopeptidase is also delivered directly to the apical plasma membrane (Sjostrom et al., 1985). For at least these secretory and membrane proteins then, the pathways of intracellular transport and sorting appear to be identical to those of the viral constituents.

In some cases proteins may be transported on a more complex route. The transcytotic poly-Ig receptor (membrane-bound secretory component) is first inserted in the basolateral domain and then transferred to the apical domain, with or without immunoglobulin ligands (Mostov and Deitcher, 1986). In addition, preliminary evidence that other apical membrane proteins traverse a similar route in liver and intestine has been obtained using cell fractionation (Ferraci et al., 1985; Maroux et al., 1985). Because of potential problems with cross-contamination between cell fractions, the latter data should, at present, be viewed with caution (see also Sjostrom et al., 1985).

Sorting by Default

A simple mechanism for sorting transported proteins to the two plasma membrane domains is one in which only one of the two classes of proteins, apical or basolateral, is specifically recognized by the cell. This class would be directed into a vesicular pathway destined for one plasma membrane domain. The other unrecognized class of proteins would enter a different vesicular pathway passively and be sorted by default to the other domain. Free entry of unrecognized membrane or secretory proteins into transport vesicles would be restricted on the pathway requiring active recognition. In contrast, no transported proteins would be excluded from the vesicles on the passive pathway.

One prediction of the default sorting model is that unrecognized proteins would leave the cell in one direction exclusively via the default pathway. Experimental observations do not support this prediction. Normally, MDCK cells secrete laminin and a proteoglycan basolaterally and do not secrete lysosomal hydrolases (Caplan, M., personal communi-

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cation). In the presence of ammonium chloride, however, laminin, the proteoglycan, and the hydrolases are secreted both apically and basolaterally (Caplan et al., 1985; Caplan, M., personal communication). Ammonium chloride and other weak bases are believed to prevent recognition of lysosomal hydrolases in the Golgi complex by causing saturation of the receptor responsible for transferring the enzymes to the lysosome (Gonzalez-Noriega et al., 1980). This presumably permits the hydrolases to leak out of the cell via the Golgi complex. Ammonium chloride may similarly prevent recognition of laminin and the proteoglycan by the cell's sorting machinery (see below). If so, the experiments with MDCK cells argue against the default model since the proteins are secreted in both directions instead of only in the single default direction. This argument against the default model is not, however, definitive because it is unknown if ammonium chloride completely or only partially abolishes recognition of the proteins. If, for example, ammonium chloride addition caused less efficient recognition of laminin instead of no recognition at all, then residual directed secretion to the basolateral side might be superimposed on secretion by default to the apical side. Experimentally this result would appear to be random, undirected secretion from both domains.

Other experiments on the secretion of exogenous proteins expressed in MDCK cells also fail to support the existence of a default sorting mechanism. Kondor-Koch et al. (1985) as well as Beaudry et al. (1985) and Gottlieb et al. (1986*a*) expressed hen oviduct lysozyme and other foreign secretory proteins in MDCK cells. Many of these proteins are normally released from secretory granules under hormonal regulation. MDCK cells do not have secretory granules and are not known to secrete in response to hormones. For this reason, the expressed proteins were presumed to lack sorting signals recognizable by MDCK cells and to be, therefore, neutral probes of vesicular pathways to the cell surface. The default model predicts that these unrecognized proteins would be secreted in only one direction. Instead, the foreign secretory proteins were released in approximately equal amounts on both sides of the cell (Kondor-Koch et al., 1985; Beaudry et al., 1985; Gottlieb et al., 1986*a*).

Sorting Signals on Transported Proteins

During the sorting of proteins to the apical and basolateral domains, specific structural features of the transported proteins must be recognized by epithelial cells. The precise nature of these structural features remains a mystery. In the case of the influenza virus and VSV membrane glycoproteins, the glycans are not directly involved since addition of tunicamycin to infected cells does not prevent correct targeting (Roth et al., 1979; Green et al., 1981). Instead, for a given protein a defined part of the sequence or conformation of the polypeptide chain itself may determine whether a protein will be delivered to the apical or basolateral plasma membrane through its interaction with specific cellular components. Such a determinant would constitute a sorting signal for the protein (Blobel, 1980). In the simplest case, signals for all apical proteins would be identical, as would signals for basolateral proteins.

Sorting signals may be confined to a specific part of transported proteins. Transmembrane proteins can be subdivided into an extracytoplasmic or luminal segment, a membrane-spanning segment, and the cytoplasmic "tail". If the sorting signal was located on the cytoplasmic tail, it would be accessible to the cytoskeleton and membrane coat proteins such as clathrin, and in membrane vesicles it would be in a position to interact with and recognize the cytoplasmic face of the plasma membrane. In this case, secretory proteins, which are entirely luminal, would have to bind to a membrane-spanning receptor to be sorted. The cytoplasmic part of the receptor would then provide the required sorting signal.

Alternatively, sorting signals localized to the luminal segment might also be advantageous. It is now recognized that the pH of different parts of the exocytic pathway varies considerably (see below). During transport of a protein, the conformation of its luminal segment might be altered by the changing ionic environment, exposing a latent sorting signal and initiating the sorting process.

Recent results do not favor the cytoplasmic tail as the site for the sorting signal. Some viral membrane glycoproteins do not require their cytoplasmic segments for initial delivery to the correct plasma membrane domain. Roth and colleagues produced a chimera between the luminal portion of an apical protein, the influenza hemagglutinin, and the transmembrane segment and cytoplasmic tail of a basolateral protein, the VSV G protein. When expressed in a polar epithelial cell line, the chimera was delivered only to the apical domain (Roth et al., 1987). Hemagglutinin converted to a secretory protein by deletion of the cytoplasmic tail and the transmembrane segment was also transported exclusively to the apical pole, suggesting that the sorting signal for this protein might reside on the luminal segment (Roth et al., 1987). Alteration of the cytoplasmic tail from influenza neuraminidase, another apical protein, also failed to disrupt its sorting (Jones et al., 1985). Similarly, truncation of the cytoplasmic tail of Semliki Forest virus E2, leaving mainly the membrane spanning and luminal segments, did not alter localization of the mutated protein to the basolateral surface of MDCK cells (Roman and Garoff, 1985).

Despite these findings, the localization and nature of sorting signals remain very complex and ill-defined problems. Recent observations suggest that two different segments of the same protein might interact to yield a pathway involving both domains of the plasma membrane. Normally, the transcytotic Ig receptor is first transported to the basolateral domain and then to the apical domain (Mostov and Deitcher, 1986). When the protein is expressed in MDCK cells without a cytoplasmic tail, it is transported directly to the apical domain (Mostov, K., personal communication).

Acidic pH and Sorting

Although the mechanisms for sorting of epithelial proteins to the cell surface are unknown, one important factor is the existence of acidic compartments on the exocytic pathway. Several recent reports suggest that the Golgi complex, like elements of the endocytic pathway, may be acidified (Glickman et al., 1983; Robbins et al., 1984; Anderson and Pathak, 1985; Schwartz et al., 1985). Cytochemical observations from Anderson, in particular (Anderson and Pathak, 1985), indicate that the *trans*-most cisterna may be the most acidic, although the actual pH has not been estimated.

Acidic intracellular compartments have commonly been investigated by the use of weak bases such as ammonium chloride and chloroquine. Weak bases raise the pH of acidic compartments such as the endosome and the lysosome (Maxfield, 1982; Poole and Ohkuma, 1981). They also cause swelling of cytoplasmic vesicles (Ohkuma and Poole, 1981), possibly by inducing a redistribution of membrane from the cell surface and other parts of the cell (Schwartz, A., personal communication). The latter may occur because the pH and ionic alterations interfere with the balanced regulation of membrane movement, or as an indirect osmotic response to the accumulation of the base.

Weak bases inhibit receptor recycling and ligand degradation during endocytosis by causing receptor–ligand complexes and unloaded receptors to accumulate intracellularly (Gonzalez-Noriega et al., 1980; Strous et al., 1985). This effect is not limited to bona fide receptors since VSV G protein is also trapped in cytoplasmic vesicles in the presence of weak bases (Pesonen and Simons, 1983; Rizzolo et al., 1985; Gottlieb et al., 1986*b*).

The effects of weak bases on the endocytic pathway are at least partially due to alterations in endosomal pH. Many ligands are known to dissociate from their receptors at the mild acidic pH of the endosome (Helenius et al., 1983); when this pH is raised by weak bases, receptor–ligand dissociation is blocked, the ligand is unable to go to the lysosome for degradation, and the receptor does not recycle. Although there is no evidence, acidic pH might also be required for receptors to cluster in forming vesicles for the return trip to the plasma membrane (Geuze et al., 1983). When weak bases raise the endosomal pH, clustering might be inhibited and receptors without ligands and other internalized membrane proteins like VSV G protein would accumulate. It is also possible, however, that ionic changes caused by the weak bases other than pH alterations trap recycling proteins intracellularly by inducing a nonspecific shift of membrane from the cell surface to the inside of the cell.

On the exocytic pathway in both polar and nonpolar cells, weak bases reduce the transport rate of both secretory and membrane proteins (Strous et al., 1985; Matlin, 1986) and perturb the sorting of secretory proteins. As mentioned before, in MDCK cells proteins of the extracellular matrix normally secreted basolaterally are secreted from both domains in the presence of ammonium chloride (Caplan, M., personal communication). In AtT-20 cells, a nonpolar pituitary tumor cell line which exhibits hormonally regulated secretion, the weak base chloroquine diverts secretory proteins from regulated to constitutive pathways (Moore et al., 1983). In addition, Wagner et al. (1986) observed in human endothelial cells that ammonium chloride and chloroquine prevent storage of von Willebrand factor (a secretory protein) in Weibel–Palade bodies, and cause its immediate secretion.

In contrast to their disruption of secretory protein sorting, weak bases have not been shown to have significant effects on the sorting of membrane proteins. In influenza virus-infected MDCK cells, the hemagglutinin is still delivered apically in the presence of ammonium chloride (Matlin, 1986). Similarly, ammonium chloride has no effect on the localization of sodium/potassium ATPase to the basolateral domain in MDCK cells (Caplan et al., 1986).

During the sorting of proteins on the exocytic pathway, the steps of recognition and segregation may resemble analogous events occurring at the cell surface and in the endosome during endocytosis. In particular, recognition of proteins as apical or basolateral may require specific receptors, with the transported proteins acting as the ligands. Segregation might depend on the clustering of the transported proteins in specific vesicles, much as receptor–ligand complexes cluster in coated pits at the cell surface and as unloaded receptors cluster for recycling from endocytic organelles (Geuze et al., 1983).

Although the precise mechanisms by which weak bases affect exocytic transport and sorting of proteins in epithelial cells are unknown, it is possible that an increase in Golgi complex pH caused by weak bases is of fundamental significance. The affinity of putative sorting receptors for both membrane and secretory proteins might, for example, be higher at acid pH (instead of lower as on the endocytic pathway). Weak bases, then, would inhibit recognition by raising the pH of the Golgi complex and decreasing the efficiency of interaction between proteins and receptors. In addition, the ability of the recognized proteins to collect in transport vesicles might also depend on the acidic conditions. At the normal low pH, clustering might be initiated in the Golgi complex; at the more alkaline pH induced by weak bases, clustering would not occur to the same extent. While the results of experiments with weak bases in no way prove this hypothetical scheme, many of the observations are consistent with these ideas.

Precedent for receptors that bind their ligands better at acidic pH exists. In the neonatal rat, the transcytotic IgG receptor picks up its ligand in the lumen of the acidic small intestine and releases it in the neutral blood (Rodewald, 1976). Transferrin receptor binds transferrin at acid pH but releases it (in the absence of iron) at the neutral pH of the cell surface (Dautry-Varsat et al., 1983). Recent studies of antigen processing have also suggested that Class II histocompatibility proteins may bind processed antigens in an acidic compartment to present them on the cell surface (Goldstein et al., 1985).

Precedent also exists for an effect of weak bases (and therefore possibly pH) on the self-association (clustering ?) of transported molecules. In endothelial cells, ammonium chloride prevents the formation of von Willebrand factor multimers and, as mentioned above, sorting of the protein to specific granules (Wagner et al., 1986). Although the multimers are stabilized by interchain disulfide bonds, association between subunits should precede disulfide bond formation.

The idea that acidity is important for the correct sorting of epithelial proteins might have broader implications. Regulation of specific protein transport through the exocytic and endocytic pathways, in polar and nonpolar cells alike, may be vested in control of compartment pH. Studies of endocytosis over the last few years were initiated with a focus on the hydrolytic lysosome. This skewed our conception of intracompartmental acidity toward one of dissociation and degradation. Ligands bound to cell surface receptors were said to pass an "acid bath" in the endosome (Helenius et al., 1983) to strip off the ligand for transit to the lysosome. An alternative notion is that acidic pH may play a more general role in allowing the cell to distinguish inside from outside in

compartments which are topologically identical to the cell surface and, in this way, permit control of protein movement into, out of, and through the cell.

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