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Morphogenesis of the Polarized Epithelial Cell Phenotype

ENRIQUE RODRIGUEZ-BOULAN AND W. JAMES NELSON

Polarized epithelial cells play fundamental roles in the ontogeny and function of a variety of tissues and organs in mammals. The morphogenesis of a sheet of polarized epithelial cells (the trophectoderm) is the first overt sign of cellular differentiation in early embryonic development. In the adult, polarized epithelial cells line all body cavities and occur in tissues that carry out specialized vectorial transport functions of absorption and secretion. The generation of this phenotype is a multistage process requiring extracellular cues and the reorganization of proteins in the cytoplasm and on the plasma membrane; once established, the phenotype is maintained by the segregation and retention of specific proteins and lipids in distinct apical and basal-lateral plasma membrane domains.

THE STRUCTURAL AND FUNCTIONAL POLARITY OF EPITHELIAL cells is important in the vectorial function of a variety of mammalian organs and tissues. Studies have been carried out on polarized epithelial cells in vivo (in liver, intestine, kidney, and preimplantation mouse embryos) and in vitro with cultures of kidney [Madin-Darby canine kidney (MDCK), LLC-PK1, and others] and intestinal (HT-29, T-84, and Caco2) cells (*1*). These studies have shown that the polarized epithelial cell phenotype is characterized by (Fig. 1) (i) the distribution of plasma membrane proteins and lipids to three distinct surface domains, apical, lateral, and basal; (ii) tight junctions that separate apical and lateral surface domains and form barriers to the intercellular diffusion of ions and macromolecules; (iii) cohesive cell-cell interactions formed by cell adhesion molecules (CAMs) and a highly developed junctional complex; and (iv) the polarized distribution of cytoplasmic organelles and the cytoplasmic and cortical cytoskeleton.

These structural characteristics are responsible for several biological roles of polarized epithelial cells (*1*) (Fig. 1). (i) Transporting epithelia form selective permeability barriers between the biological compartments (lumen and serosa) of different ionic compositions (*2*). (ii) Transporting and secretory epithelia actively regulate the composition of these biological compartments by carrying out specialized vectorial functions in absorption, transcytosis, and secretion. These vectorial functions depend on the polarized distributions of channels and transport enzymes to the apical and basal-lateral domains of the plasma membrane (*1, 2*). (iii) The cohesive monolayer structure of the epithelium, in which cells are linked together through the junctional complex and the cytoskeletal contractile apparatus, is responsible for the folding of epithelial germ layers during embryo development (for example, during gastrulation and formation of the neural and intestinal tubes) (*3*).

Many of the membrane proteins of polarized epithelial cells are common to nonpolarized cells [for example, the Na^+- and K^+-dependent adenosine triphosphatase (Na^+,K^+-ATPase) and growth factor receptors]. However, their nonrandom distribution on the membrane in polarized epithelial cells is characteristic of the vectorial functions performed by this cell type (*1*). For example, the basal-lateral membrane location of the Na^+,K^+-ATPase and apical membrane location of Na^+ channels results in the generation of a transepithelial gradient of Na^+ that facilitates vectorial uptake and

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transport of ions and solutes (*2*). Some proteins are specific and essential to the polarized epithelial cell phenotype. These proteins are usually localized to the apical membrane domain (for example, hydrolases), although other structures may be localized to the boundary of the apical membrane (for example, the tight junction) (Fig. 1). However, the number of specific proteins that distinguish the polarized epithelial cell phenotype is unknown.

The distributions of cytoplasmic organelles and components of the cytoskeleton are also characteristic of polarized epithelial cells. Microtubules, which may participate in vesicle transport to and from different plasma membrane domains, are oriented along the apico-basal axis. Actin microfilaments and cytokeratin intermediate filaments are associated with different cell-cell and cell-substratum contacts, where they may play a role in maintaining the structural continuum of the epithelium (*4*). Distinct cytocortical cytoskeleton complexes are associated with apical and basal-lateral domains of the plasma membrane. These complexes contain proteins related structurally and functionally to components of the erythrocyte membrane skeleton (*5*) that are bound to specific integral membrane proteins [for example, Na^+,K^+-ATPase (*6*)]. The membrane cytoskeleton may function in the establishment and maintenance of domains of specific proteins on the plasma membrane.

Generation of the Polarized Epithelial Cell Phenotype

An example of the generation of a polarized epithelium in development is the formation of the trophectoderm in preimplantation mammalian embryos (*7*) (Fig. 2). After several rounds of cleavage of the fertilized egg, the blastomeres undergo a morphological change at the morula stage (8 to 16 cells). The outer blastomeres become flattened and polarized and establish intercellular junctions (a process termed compaction). This population of blastomeres gives rise to the trophectoderm, a polarized epithelium surrounding a fluid-filled lumen, the blastocoel, and the inner cell mass. Compaction can be inhibited by antibodies against the CAM uvomorulin (*8, 9*). Uvomorulin is expressed in the fertilized egg and gradually becomes localized to the contact zone between blastomeres in the morula (*9*). Other proteins expressed early in development (two-cell stage) are also gradually redistributed during these series of morphological changes in the morula (*10*). For example, the Na^+,K^+-ATPase is not polarized up to the morula stage but gradually becomes localized to the basal-lateral membrane of the trophectodermal cells during compaction (*11*).

The formation of epithelial structures continues during embryogenesis (*9, 12*). A basic structure in the development of many organs and tissues are branched, hollow linings of epithelial cells that arise from smaller epithelial buds. In most parenchymal tissues, the surrounding mesenchymal cells participate in development of this highly branched morphology (*13*). The number of genes required to generate tissue-specific branching patterns is not known, but it has been suggested that the number could be small (*14*). In the developing kidney, the branching of the epithelial bud (the ureter bud) is accompanied by the conversion of surrounding mesenchymal cells to epithelial cells, which form glomeruli and distal and proximal tubules (*9, 15*). During the aggregation of mesenchymal cells and their conversion to epithelial cells in the developing kidney, two adhesion proteins (*16–18*), uvomorulin (involved in cell-cell contact) and laminin (involved in cell-substratum contact), are expressed. Antibodies raised against these proteins have different effects on the conversion of mesenchymal cells into a polarized kidney epithelium. Antibodies against uvomorulin, which inhibit trophectoderm formation in the morula, do not appear to alter kidney tubule formation (*17*). However, antibodies against the A chain of laminin inhibit the polarization of the mesenchymal cells (*18*).

Formation of basement membrane (Fig. 1) also regulates the morphogenesis and maintenance of the differentiated state of other polarized epithelial cells [for example, mammary epithelium and hepatocytes (*19*)]. Furthermore, there is evidence that the composition of the basement membrane is tissue-specific and influences tissue-specific gene expression (*19*). Taken together, these results suggest that development of the polarized epithelial cell phenotype

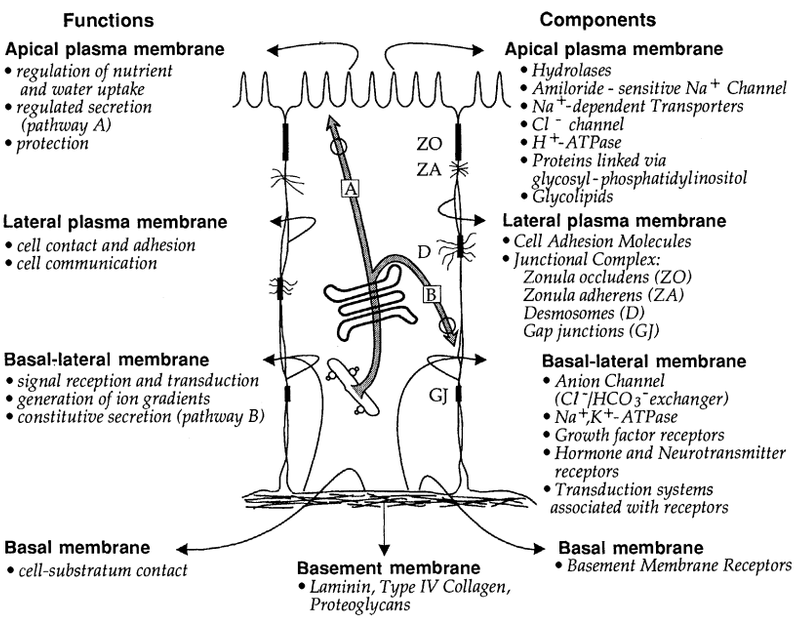

Fig. 1. The polarized epithelial cell phenotype: functionally and biochemically distinct plasma membrane domains. Polarized epithelial cells perform a variety of well-characterized functions that are a reflection of the polarized distribution of enzymes and transport systems to specific domains of the plasma membrane. The apical domain of the plasma membrane faces the lumen and carries out uptake of ions and nutrients from this biological compartment via specific ion channels and transporters. The apical membrane is enriched in glycolipids, which may form a protective barrier against hydrolases and changes in *p*H in the lumenal medium. In secretory epithelial cells, the apical plasma membrane is usually the site of regulated secretion (pathway A). The lateral plasma membrane is the site of cell-cell contact and communication, which are mediated by specialized components of the junctional complex (*78*). Tight junctions [zonula occludens (ZO)] block the paracellular pathway between lumen and serosa (*79*) and form a diffusion barrier to proteins and lipids in the plane of the lipid bilayer. Initial formation of cell-cell contacts appears to be regulated by cell adhesion molecules (*24*) and is subsequently stabilized by belt [zonula adherens (ZA)] and spot desmosomes (D). Cell-cell communication is mediated by gap junctions (GJ), specialized intercellular channels for small solutes. The basal plasma membrane is attached to the basement membrane (basal lamina) through specific receptors for laminin, type IV collagen, and proteoglycans (*19*). Enzymes, transport activities, and receptors localized to the basal-lateral plasma membrane are involved in the generation of ion gradients across the apical plasma membrane, in signal reception and transduction, and in the constitutive secretion (pathway B) (*1*). In some epithelial cells, the Cl^- channel, H^+-ATPase, the Cl^-/HCO_3^- exchanger, and Na^+,K^+-ATPase are localized to the opposite plasma membrane domain than that shown in the figure.

is initiated by cell-cell and cell-substratum contact.

Studies with polarized epithelial cells grown in culture have shown that under conditions where there is neither cell-cell nor cell-substratum contact, single cells exhibit nonpolarized distributions of marker proteins of apical and basal and lateral membrane domains (*20*). However, cell-cell or cell-substratum contact induces the reorganization of membrane proteins (Fig. 2). Attachment of single MDCK cells to a substratum is sufficient to induce a rapid (12 to 24 hours) polarization of apical membrane marker proteins to the free cell surface (*21, 22*). In addition, a large fraction (60%) of apical surface markers are stored in a novel vacuolar apical compartment (VAC), which also contains numerous microvilli (*22*). Basal-lateral membrane proteins are excluded from the VAC and remain randomly localized over the entire cell surface (*22*). VAC-like structures have been described in a variety of epithelial cell types (*23*).

How does cell-cell or cell-substratum contact at one end of the cell lead to the generation of a stable apical pole at the opposite end of the cell? The pattern of extracellular contact points could cause localized changes in the biophysical properties of the cytocortex and plasma membrane. These changes may be propagated beyond the immediate point of contact through alterations in the organization of the cytoplasmic or cytocortical cytoskeleton.

Induction of cell-cell contact is regulated by Ca^{2+}-dependent CAMs [for example, uvomorulin or E-cadherin (*24*)]. Cell-cell contact results in the formation of the junctional complex on the lateral membrane between adjacent cells (Fig. 1) and the reorganization of proteins of the basal-lateral plasma membrane. However, in contrast to the rapid formation of the apical plasma membrane domain, the generation of the basal-lateral membrane domain occurs gradually [approximately 24 to 36 hours in MDCK cells (*21, 25*)]. This may be a consequence of the requirement not only to accumulate proteins specifically at the basal-lateral plasma membrane domain, but also to remove proteins trapped in the forming apical domain (*26*); the induction of cell-cell contact results in the rapid formation of the tight junction and the demarkation of the forming apical and basolateral domains of the plasma membrane. In addition, on induction of cell-cell contact, the VAC is exocytosed toward the regions of cell-cell contact and contributes significantly to the rapid formation of the apical surface (*22*) (Fig. 3A). The molecular basis for the specificity of VAC fusion with the plasma membrane is not known.

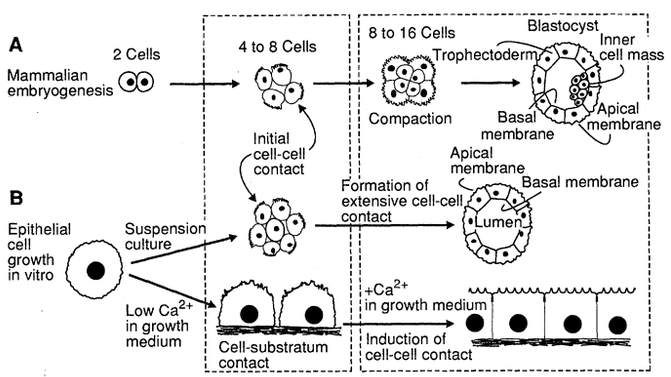

Fig. 2. Model systems for analyzing the generation of the polarized epithelial cell phenotype. Studies of polarized epithelial cell systems in vivo and in vitro have shown that the generation of a polarized epithelial cell phenotype is characterized by two successive events: (i) formation of an apical pole after initial cell-cell contact; and (ii) development of the basal-lateral pole after extensive cell-cell adhesion. (**A**) An example of the sequence of events involved in the generation of polarity in epithelial cells is the formation of the trophectoderm in the preimplantation mammalian embryo. Initially, blastomeres are loosely associated and have microvilli on the outside-facing (apical) region of the plasma membrane, which has been interpreted to be the first sign of cellular polarization. At the morula stage, the blastomeres then begin to flatten on each other (compaction), maximize cell-cell contact, generate a polarized organization of basal-lateral plasma membrane proteins (for example, the Na^+,K^+-ATPase), and form a transporting polarized epithelium (the trophectoderm). (**B**) (Top) Established cell lines derived from kidney epithelium (MDCK, LLC-PK1, and MA-104) or intestinal epithelium (HT-29 and Caco2) and cells isolated from thyroid have been used extensively to analyze the generation of epithelial cell polarity. In suspension culture, aggregated cells rapidly develop an apical pole (apical microvilli and membrane proteins). Subsequently, the cell aggregates form extensive cell-cell contacts and a basal pole and begin to develop a fluid-filled lumen. (Bottom) The roles of cell-substratum and cell-cell contact in the generation of epithelial cell polarity can be uncoupled in vitro. Cells grown on a substratum in growth medium containing low concentrations of Ca^{2+} (5 to 50 μM) attach to the substratum but do not form cell-cell contacts; single cells develop an apical pole but do not form a basal-lateral plasma membrane domain. When cell-cell contact is induced, the cells gradually develop full cellular polarity with apical and basal-lateral plasma membrane domains.

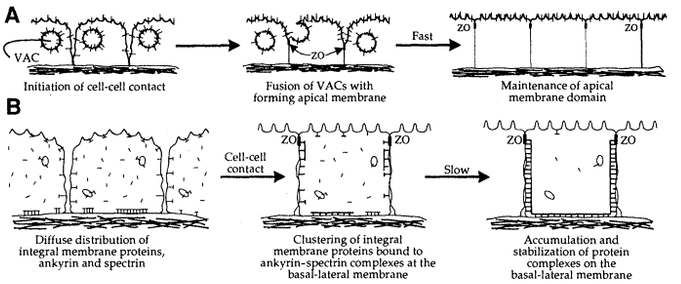

Fig. 3. Mechanisms involved in the generation of (**A**) apical and (**B**) basal-lateral domains of the plasma membrane. See text for further details.

The gradual accumulation of proteins (such as Na^+,K^+-ATPase) on the basal-lateral plasma membrane coincides both temporally and spatially with the gradual reorganization and redistribution of ankyrin and spectrin (*27*) (Fig. 3B). In single MDCK cells, ankyrin and spectrin are distributed randomly on the plasma membrane and in the cytoplasm (*27*). A fraction of ankyrin and spectrin is in a relatively soluble, high molecular weight complex with Na^+,K^+-ATPase (*28*), which is a high-affinity membrane-binding site for ankyrin (*6*). Cell-cell contact initiates the clustering of these preexisting complexes of ankyrin, spectrin, and integral membrane proteins (such as Na^+,K^+-ATPase) into insoluble and metabolically stable polymers in regions of cell contacts (*6, 27, 28*) in a process that may be analogous to ligand-induced patching of membrane and cytoskeletal proteins in other cells (*29*). Clustering of membrane proteins and assembly of the membrane-associated cytoskeleton may be induced by homotypic interaction between uvomorulin molecules on adjacent cell membranes (*24*). Uvomorulin has also been detected in a complex containing ankyrin and spectrin, indicating a direct molecular linkage between CAM and the cortical cytoskeleton (*30*). However, protein complexes containing ankyrin, spectrin, and for instance, the Na^+,K^+-ATPase that are trapped in the apical membrane are not induced to cluster because of the absence of cell-cell contact in this region of the plasma membrane (*28, 30*). These protein complexes remain relatively soluble and metabolically unstable and may be removed from the membrane by internalization and degradation (*31*).

Different forms of ankyrin and spectrin are expressed on the apical membrane of polarized epithelial cells that contain brush borders (*32*) (for example, intestinal epithelium and proximal kidney tubule). These proteins may regulate the organization of specific membrane proteins to the apical membrane.

In summary, the establishment of the polarized epithelial cell phenotype is a multistage process that involves reorganization of the cell surface, cytocortex, and cytoplasm. In developing epithelia, these processes appear to depend on the expression of proteins that

mediate cell-cell and cell-substratum contact. In vitro studies indicate that formation of the apical and basal-lateral plasma membranes can be uncoupled in both time and space. Generation of the apical pole is a rapid process induced by initial cell-cell or cell-substratum contact, and generation of the basal-lateral pole is a more gradual process requiring extensive cell-cell contact. The generation of these membrane domains appears to require remodeling of the plasma membrane by recruitment of proteins from preexisting cytoplasmic and membrane pools of proteins (*27, 28*).

Maintenance of Polarity

The protein composition of apical and basal-lateral plasma membrane domains of native epithelial cells (*33*) and MDCK cell monolayers (*34*) is different; the few proteins shared by both domains may play a role in shuttling vesicles between the membrane domains. Maintenance of these different protein compositions is an active process that depends on coordinate insertion of new proteins and degradation of old proteins in the appropriate surface domain. The initial biosynthetic stages of proteins destined for the membrane domains seem to be shared (*1*). Apical and basal-lateral proteins codistribute during synthesis in the endoplasmic reticulum and subsequently are cotransported through the Golgi complex. Two different scenarios have been proposed for subsequent sorting.

In the first scenario, based on cell fractionation studies of native epithelia (intestine and liver), apical and basal-lateral proteins are transported together from the Golgi apparatus to the basal-lateral surface (Fig. 4A). Here, apical proteins are removed by endocytosis and relocated to the apical surface (*35*); however, an apical glycoprotein has been shown to be directly targeted to the apical surface in native intestinal epithelium (*36*).

The second scenario is based on studies on the expression of exogenous viral glycoproteins in epithelial cell lines. Detailed studies of the budding of enveloped RNA viruses in MDCK cells showed that certain viral envelope glycoproteins are targeted to the apical surface while others are targeted to the basal-lateral surface (*37*). Hemagglutinin (HA), the envelope glycoprotein of influenza virus, is an example of the first group, whereas the envelope glycoprotein of vesicular stomatitis virus (VSV) is an example of the second. In MDCK cells, HA and the VSV envelope glycoproteins are cotransported through the endoplasmic reticulum and Golgi complex (Fig. 4A) but are segregated intracellularly in the trans Golgi complex, in a compartment named GERL or trans Golgi network (TGN) (*38*). In the TGN, HA and the VSV envelope glycoproteins are incorporated into different transport vesicles, which are then targeted to and fuse with the apical or basal-lateral plasma membrane domains, respectively (*39*). These results have been extended to endogenous proteins of MDCK cells, such as the α subunit of Na^+,K^+-ATPase, which is also targeted directly to the basolateral surface of MDCK cells, and several other apical and basal-lateral glycoproteins in this cell line and in an intestinal cell line (*40*).

These two different scenarios have not been reconciled. They may represent actual differences between epithelia, or differences in experimental procedures used for the studies in native epithelia (cell fractionation) and cultured cell lines (cell surface labeling) (*41*). The pathway from the Golgi complex to the basal-lateral surface to the apical surface has been demonstrated in MDCK cells by analysis of the polymeric immunoglobulin (poly-Ig) receptor expressed from transfected cDNA (*42*). In intestinal, liver, and mammary epithelia, poly-Igs (IgA and IgM) are transported from the basal-lateral compartment (where the Igs are produced by lymphoid cells) to the luminal compartment (where the Igs are used to block infectious agents). Because endogenous MDCK cell proteins are targeted vectorially (*40*), the poly-Ig receptor pathway does not appear to be a generalized mechanism for protein sorting in these kidney cells but is representative of a specialized protein designed to shuttle between the two surface domains.

After proteins reach their respective cell surface domain, additional sorting mechanisms operate. Polarized epithelial cells have distinct endosomal populations associated with the apical and basal-lateral domains of the plasma membrane (*43*) (Fig. 4B). Endogenous transferrin receptors and transfected asialoglycoprotein receptors recycle between endosomes and the basal-lateral surface of MDCK cells (*44*); other receptors such as those for poly-Ig, epidermal growth factor, Fc, and nerve growth factor may be transported by the endosomal pathway across the cell (transcytosis) (*42, 45*) (Fig. 4B). Transfection experiments, similar to those carried out with the poly-Ig receptor (*42*), may help to elucidate the mechanisms that direct recycling and transcytosing receptors to different pathways.

Epithelial cells, such as the zymogen-producing cells of the exocrine pancreas, are also capable of polarized secretion (*46*). On stimulation of hormone and neurotransmitter receptors on the basal-lateral membrane, large zymogen granules fuse with the apical surface (regulated secretion, Fig. 1). Hormones and proenzymes that accumulate in regulated secretory granules possess specific signals that target the transport of the secretory protein from the TGN into the granule (*47*). Secretory activity is performed in a continuous fashion by most epithelial cells (constitutive secretion, Fig. 1) (*46*). In MDCK cells, basement membrane components are secreted constitutively into the basal medium, and a 30- to 40-kD protein is secreted into the apical medium (*48*). Secretory proteins released in a polar fashion may carry "signal" information that allows them to interact with apical or basal-lateral membrane sorting machinery. The polarized secretion of basal lamina components is dependent on an acid environment because it is disrupted by incubation in the presence of weak bases (*48, 49*). Exogenous secretory proteins (introduced by transfection) are secreted by MDCK cells in almost equal proportion from both surfaces (*50*). A possible interpretation of these findings is that most secretory proteins in MDCK cells have no "signals for transport" and are then carried along by default with the "bulk flow" of fluid to both apical and basal-lateral domains of the plasma membrane. The default secretory pathway may differ between epithelial cell types. For example, in liver and intestinal cell lines it is almost exclusively directed toward the basal-lateral membrane (*41*).

Sorting Signals on Apical and Basal-Lateral Membrane Proteins

Intrinsic sorting signals in membrane glycoproteins may consist of carbohydrate groups or amino acid sequences. Phosphorylated mannose side chains on lysosomal hydrolases are recognized in the Golgi complex by specific receptors that target the hydrolases to lysosomes (*51*). However, N-linked carbohydrates do not appear to mediate the targeting of epithelial surface proteins. The abolition or modification of N-linked glycosylation (by tunicamycin or lectin-resistance mutation) does not alter the correct sorting of HA, VSV envelope glycoprotein, or endogenous surface glycoproteins in MDCK cells (*52*).

Expression studies of chimeras of apical and basal-lateral membrane–targeted viral glycoproteins, and of truncated proteins in which cytoplasmic or transmembrane domains have been deleted, suggest that sorting signals reside in the ectodomain of the protein (*53*). Some discrepant results (*54*) are probably attributable to loss of the quaternary structure of the proteins, which may affect their targeting properties (*55*). The transmembrane and cytoplasmic

domains of VSV envelope glycoprotein seem to target a secretory protein (human chorionic gonadotropin) to the basal membrane (*56*), suggesting that more than one sorting signal may exist in a given protein. The nature of the sorting signals in the poly-Ig receptor is also complex. Removal of the cytoplasmic or transmembrane domains results in direct targeting of the receptor to the apical domain of the plasma membrane. This is consistent with the notion that the apical sorting information also resides in the amino acid sequence of the ectodomain (*42*). It is not known whether a basal-lateral sorting signal is present in the cytoplasmic amino acid sequence of the poly-Ig receptor. Phosphorylation of this region of the protein may modify the expression of apical and basolateral sorting signals in the ectodomain of the protein (*57*).

Polarized epithelial cells not only sort plasma membrane and secretory proteins, but also segregate lipids to different plasma membrane domains. The apical membrane is enriched in glycosphingolipids and depleted in phosphatidylcholine relative to the basal-lateral membrane (Fig. 1) (*58*). Two glycolipids, glucosylceramide and sphingomyelin, are transported vectorially from their site of synthesis in the Golgi complex to the apical membrane of MDCK cells. The concentration per unit surface area of these glycolipids in the apical membrane is two- to tenfold that in the basal membrane (*58*). Certain glycolipids, particularly glucosylceramide, can form clusters (presumably through hydrogen bonds) in the plane of the bilayer (*59*). Clustering of the glycolipids, after exceeding critical glycolipid to phospholipid ratios at the TGN, may cause their preferential accumulation into transport vesicles destined for the apical membrane (*58*).

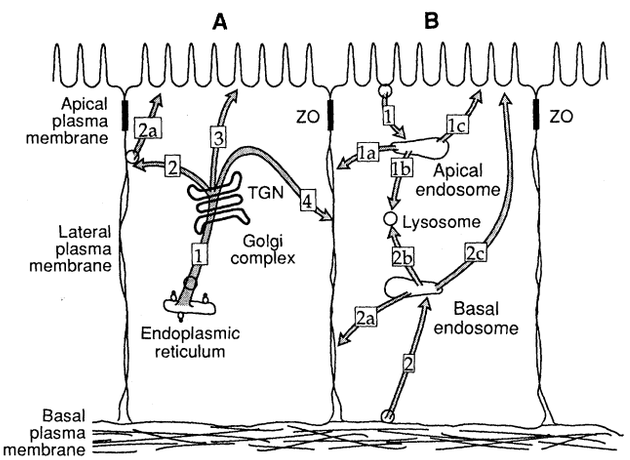

Fig. 4. Protein transporting pathways in epithelial cells. (**A**) Biosynthetic pathway. After synthesis in the endoplasmic reticulum, apical and basal proteins are transported together to the Golgi complex (pathway 1). In intestinal and liver cells, there is evidence for initial delivery of both groups of proteins to the basal-lateral membrane (pathway 2); basal proteins are retained, whereas apical proteins are endocytosed and delivered to the apical membrane (pathway 2a). A different scenario has emerged from studies of the intracellular transport of apical and basolateral viral glycoproteins by the polarized kidney cell line MDCK. Influenza virus HA and VSV envelope glycoprotein are transported together from the endoplasmic reticulum to the Golgi complex (pathway 1). In a distal Golgi compartment, the TGN, HA and VSV envelope glycoprotein are sorted and incorporated into different transport vesicles, which transport them vectorially to the apical (HA, pathway 3) or basolateral (VSV envelope glycoprotein, pathway 4) plasma membrane. An endogenous protein, Na^+,K^+-ATPase, is delivered to the basal-lateral membrane by pathway 4 in MDCK cells. Pathways 2 and 2a exist in MDCK cells and are used by the receptors for poly-Ig and epidermal growth factor. (**B**) Recycling pathways. Ligands endocytosed via the apical membrane receptors are delivered to apical endosomes (pathway 1), whereas ligands endocytosed via basal membrane receptors are delivered to a different endosome population, the basal endosomes (pathway 2). From either set of endosomes, receptors may recycle back to the same surface (pathways 1c and 2a), transcytose to the opposite surface (pathways 1a and 2c), or be transferred to lysosomes and degraded (pathways 1b and 2b).

The targeting of glycolipids to the apical membrane may be linked to the sorting of apical membrane proteins (*58*). This hypothesis is supported by studies on a novel group of glycoproteins anchored to the membrane through covalent linkage to a glycolipid, glycosylphosphatidylinositol (GPI) (*60*). Six endogenous GPI-anchored MDCK glycoproteins were found to be localized preferentially on the apical surface; similar results were obtained in LLC-PK1 (pig kidney) and two human intestinal cell lines (*61*). Furthermore, exogenous GPI-anchored proteins introduced by transfection into MDCK cells are also targeted to the apical surface, and addition of GPI by recombinant DNA procedures to exogenous secretory proteins or to the ectodomain of a basal-lateral viral glycoprotein results in the targeting of these proteins to the apical surface (*62*). These experiments suggest that GPI may behave as an apical targeting signal in epithelial cells, perhaps through clustering with glycolipids in the TGN. Clustering of GPI-anchored proteins is suggested by high immobile fractions detected by fluorescence recovery after photobleaching (FRAP) in fibroblasts (*63*).

A model attempting to organize information on the sorting of proteins and lipids in MDCK cells is shown in Fig. 5. According to this model the pathways of bulk flow of proteins and lipids to the apical and basal-lateral membranes are different: the vesicles of one pathway are glycolipid-rich and go to the apical surface, and the vesicles of the other pathway are glycolipid-poor and go to the basolateral membrane (*58*). Positive sorting information may be present in the ectodomain of both apically and basolaterally targeted glycoproteins. Alternatively, positive information may be present in one group of proteins (for example, apical), and the other group reaches the plasma membrane by default. The sorting information is recognized by specific receptors in the TGN. For apical proteins, the receptor is either an integral membrane protein with affinity for glycolipid patches in the TGN (perhaps a GPI-anchored protein), or the glycolipids themselves. GPI-anchored proteins are sorted to the apical pathway by clustering of the GPI moieties or by receptors for their ectodomain. Interaction of basolateral proteins with cytosolic components of the cortical cytoskeleton may contribute to their sorting at the level of the TGN or at the cell surface. In this case the sorting signal would be located on the cytoplasmic domain of the membrane protein. These complexes of membrane proteins and ankyrin would associate specifically with the basal-lateral membrane through affinity to the preexisting membrane cytoskeleton.

Vesicle Transport to Plasma Membrane Domains

Uncoated vesicles (with a diameter of 200 nm) transport HA and VSV envelope glycoprotein from the Golgi complex to the cell surface; clathrin does not seem to participate in the budding of these vesicles from the TGN (*39*). Elements of the cytoplasmic cytoskeleton may provide specific "tracks" for the movement of transport vesicles. Tracks from the TGN to the apical or basal-lateral membrane may be different, and these differences may be recognized by domain-specific receptors in the cytoplasmic aspect of the vesicles. Alternatively, the vectorial delivery of vesicles to specific membrane domains may be accounted for by a recognition event between the vesicle and the corresponding surface domain. Guanosine triphosphate–binding proteins may play a role in such recognition events (*64*).

In several cell systems microtubules appear to mediate directed vesicle and organelle movement (*65*). The role of microtubules in

the sorting and targeting of viral glycoproteins to different membrane domains in polarized MDCK cells has been investigated. Although microtubule inhibitors did not disrupt the polarized delivery of VSV envelope glycoprotein to the basal-lateral membrane, discrepant results have been obtained on the delivery of influenza HA to the apical surface (*66*). Studies of endogenous membrane protein targeting in polarized Caco2 cells have shown that the delivery of an apical protein is altered in the presence of a microtubule disrupting agent, nocodazole (*67*). The disruption of microtubules by Colcemid also leads to the accumulation of apical markers and microvilli in the basal-lateral membrane of intestinal cells, which eventually pinch off to form vesicles containing brush borders in the cytoplasm (*67*). These results indicate that microtubules are important in the delivery of proteins to the apical membrane in polarized cells. However in the mouse preimplantation embryo, the cytoskeleton, and in particular the microtubule array, is involved in the spatial organization of the protein biosynthetic apparatus and endocytic system but does not affect the acquisition of surface polarity (*7*). Whether the disruption in the delivery of kidney and intestinal epithelial surface proteins in the presence of microtubule inhibitors reflects the profound disorganization of the Golgi complex caused by these drugs (*68*) or the disappearance of a directed microtubule-based track to the apical surface remains to be seen.

Analysis of the delivery of proteins and lipids to the cell surface ultimately requires the development of in vitro reconstitution systems of protein transport from the Golgi complex to the plasma membrane, similar to those developed to study vesicular transport between the endoplasmic reticulum and Golgi complex and between the Golgi cisternae (*69*). Procedures to prepare mechanically "perforated" cells have been successfully applied to reconstitute transport to the cell surface of a viral glycoprotein in fibroblasts (*70*). Extension of these procedures to polarized cells will facilitate the study of the factors involved in polarized intracellular transport. Methods to purify donor TGN fractions, transport vesicles, and apical plasma membrane fractions with the cytoplasmic aspect exposed (*71*) may also be useful in reconstituting protein transport.

After proteins arrive at the appropriate plasma membrane domain their subsequent distribution is restricted. Detergent extraction and FRAP indicate that there are mobile and immobile protein fractions in both the apical and basal-lateral membranes (*72*). Linkage to the membrane cytoskeleton may be responsible for the maintenance of epithelial cell surface polarity; there are domain-specific cytoskeletons, and some polarized plasma membrane proteins have high affinity for cytoskeletal elements (*27*). Another mechanism for restricting protein diffusion in the membrane may be the interaction of the ectodomain of the protein with other proteins in the lipid bilayer or with peripheral factors such as basal lamina components (*73*).

Mobile protein fractions may be restricted to specific membrane domains by the tight junction (Fig. 1). Breakdown of the tight junction, after dispersal of cells with proteases or disruption of cell-cell contacts with chelating agents, is followed by a gradual loss of cell surface polarity as apical and basal-lateral membrane proteins mix over the surface of the cell (*74*). However, these experimental strategies also result in the loss of all lateral membrane contact and disrupt the organization of the cytocortex, effects that may equally be responsible for the randomization of surface components. Perhaps the clearest evidence of the barrier role of tight junctions is the demonstration that lipids incorporated into the outer leaflet of the apical surface in MDCK cells cannot diffuse to the lateral membrane, unless they have the ability to flip-flop to the inner leaflet (*58*). This finding suggests that the tight junction acts as a barrier only on the exoplasmic leaflet of the bilayer, which is where the major differences in lipid composition between the two surfaces are expected to be found.

The Polarized Epithelial Cell Phenotype and Human Disease

Regulation of the ionic environment between external and internal compartments of an organism is fundamental to homeostasis. Polarized epithelial cells play a critical role in this regulation in a variety of tissues and organs. Because the generation and maintenance of the polarized epithelial cell phenotype is a multistage process, alterations by environmental factors or genetic mutation at any stage in the development of this phenotype may have dire consequences on tissue function and organism survival. Approximately 85% of human tumors are carcinomas that are derived from epithelia, and many carcinomas are characterized by morphological changes in cell polarity (*75*). In some nonmalignant diseases, alterations in the polarity of specific proteins have been found; for example, polycystic kidney disease and renal ischemia are characterized by a loss of Na^+,K^+-ATPase from the basal-lateral plasma membrane and appearance of the enzyme at the apical membrane (*76*). In other diseases, loss or disorganization of whole membrane domains has been found; for example, some forms of familial enteropathy of the small intestine (Davidson's disease or microvillar inclusion disease) are characterized by the loss of the apical membrane brush border and appearance of large intracellular vesicles containing brush borders that are reminiscent of VACS (*77*). It is

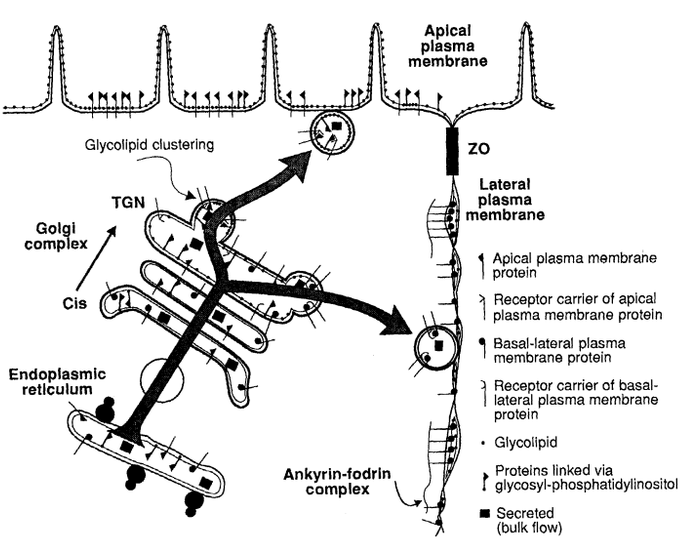

Fig. 5. A model for the sorting of plasma membrane proteins and lipids in the TGN of MDCK cells. In the TGN, apical and basal-lateral proteins are incorporated into different transport vesicles, which are targeted to the apical or the basolateral surface. Specific receptors in the TGN might carry out this function. An alternative mechanism for the sorting of apical and basolateral proteins may involve lipids. Apically targeted vesicles are enriched in glycolipids in the exoplasmic leaflet, whereas basolaterally targeted vesicles are relatively depleted of glycolipids. Some glycolipids are synthesized in the TGN and have the capacity to form hydrogen-bonded clusters; this property is perhaps important for their preferred incorporation into apical vesicles. Proteins anchored via the glycolipid GPI are preferentially targeted to the apical membrane. Thus, sorting to the apical surface might be facilitated by covalent or noncovalent interaction with glycolipids. After reaching the respective membrane, proteins might be stabilized through specific interactions with a domain-specific submembrane cytoskeleton (such as ankyrin-fodrin complexes in the basolateral surface).

not known whether these alterations in epithelial cell polarity represent the causes or effects of these diseases. However, a detailed understanding of how the polarized epithelial cell phenotype is generated and maintained may lead to important insights into their etiology and treatment.

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Inhibition of DNA Binding Proteins by Oligonucleotide-Directed Triple Helix Formation

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Oligonucleotides that bind to duplex DNA in a sequence-specific manner by triple helix formation offer an approach to the experimental manipulation of sequence-specific protein binding. Micromolar concentrations of pyrimidine oligodeoxyribonucleotides are shown to block recognition of double helical DNA by prokaryotic modifying enzymes and a eukaryotic transcription factor at a homopurine target site. Inhibition is sequence-specific. Oligonucleotides containing 5-methylcytosine provide substantially more efficient inhibition than oligonucleotides containing cytosine. The results have implications for gene-specific repression by oligonucleotides or their analogs.

EUKARYOTIC TRANSCRIPTION IS REGULATED BY THE INTERplay of various protein factors at promoters (*1*). Although transcriptional regulation in prokaryotes may be less complex, prokaryotic repressors can function as negative regulators when bound to heterologous operators in eukaryotic promoters (*2*). This observation suggests that displacement of activating proteins might provide a general strategy for gene-specific repression in eukaryotes. Pyrimidine oligonucleotides bind with sequence-specific dependence to homopurine sites in duplex DNA by triple helix formation and could have sufficient specificity and affinity to compete with site-specific DNA binding proteins for occupancy of overlapping target sites (*3*).

RNA and DNA polymer triple helices were first recognized more than 30 years ago. The structure and sequence requirements for triple helix formation have been investigated in vitro under both physiological and nonphysiological conditions. Poly(U) and poly(A) (polyuridylate and polyadenylate) form a stable 2:1 complex, as do poly(dT-dC) and poly(dG-dA) (polydeoxythymidylate, polydeoxycytidylate, and polydeoxyguanylate-polydeoxyadenylate) (*4*). Specificity arises from base triplets (T-A-T and C+G-C) formed by Hoogsteen base pairing of the second pyrimidine strand with the purine strand of the double helix (*5, 6*). Each base pair (bp) in a homopurine double helical DNA sequence affords two sequence-specific hydrogen bonds for triple helix formation. X-ray diffraction patterns of triple-stranded fibers [poly(A)·2 poly(U) and poly(dA)·2 poly(dT)] suggested an A form RNA-like conformation of the two Watson-Crick base-paired strands, with the third strand bound parallel to the purine strand of the duplex by Hoogsteen hydrogen bonds (*6, 7*). Mixed sequence pyrimidine oligonucleotides equipped with EDTA·Fe(II), the ethylenediamine tetraacetic acid chelate of Fe(II), selectively cleave homopurine sites in large DNA molecules (*3*). These studies confirm that, in the local triple helical complex, the pyrimidine (Hoogsteen) oligonucleotide is bound in the major groove, parallel to the Watson-Crick purine strand (*3*). Oligonucleotide-directed triple helix formation has also been reported in several other contexts (*8–10*). One of the most powerful aspects of the oligonucleotide approach to sequence-specific recognition of double helical DNA is the simplicity of the Hoogsteen hydrogen bonding mode. In a formal sense, a site size of more than 15 bp affords an ensemble of more than 30 discrete hydrogen bonds for sequence-specific recognition of DNA. This specificity is theoretically

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