Regulation of Cell Surface Polarity from Bacteria to Mammals

W. James Nelson

The generation of unique domains on the cell, cell surface polarity, is critical for differentiation into the diversity of cell structures and functions found in a wide variety of organisms and cells, including the bacterium *Caulobacter crescentus*, the budding yeast *Saccharomyces cerevisiae*, and mammalian polarized epithelial cells. Comparison of the mechanisms for establishing polarity in these cells indicates that restricted membrane protein distributions are generated by selective protein targeting to, and selective protein retention at, the cell surface. Initiation of these mechanisms involves reorientation of components of the cytoskeleton and protein transport pathways toward restricted sites at the cell surface and formation of a targeting patch at those sites for selective recruitment and retention of proteins.

Regulation of the composition and polarized distribution of proteins at the cell surface are important factors in the generation of structural and functional diversity between cells. In the relatively simple prokaryote *Caulobacter crescentus*, restricted protein distributions in the membrane of the predivisional cell are necessary for the generation of structurally and functionally distinct progeny (a swarmer cell and a stalked cell) (*1*). In the budding yeast *Saccharomyces cerevisiae*, recruitment of vesicles to a restricted area of the cell surface during mating and cell division (budding) gives rise to polarized cell growth (*2*, *3*). In multicellular organisms, generation of restricted distributions of proteins in different domains of the cell surface is critical for the development of a variety of cell type–specific functions; in polarized epithelial cells, for example, restriction of proteins to structurally and functionally distinct apical and basal-lateral membrane domains is fundamental to cellular regulation of ion and solute transport between different biological compartments that are separated by the epithelium (*4*). Different genetic and cell biological approaches are being used to exploit specific properties of C. *crescentus*, S. *cerevisiae*, and mammalian polarized epithelial cells that together may lead to a general understanding of the pathways involved in the biogenesis of cell surface polarity.

To provide a conceptual framework for comparing mechanisms in these diverse cells, we can consider two simple pathways for generating a restricted protein distribution at the cell surface (Fig. 1): (i) a selective targeting pathway, in which proteins of one type are recruited directly to a targeting patch at the cell surface, and (ii) a selective retention pathway, in which proteins of one or more types are randomly delivered to the cell surface, but proteins of only one type are retained in the targeting patch, while other proteins are removed either passively by diffusion away from the targeting patch or actively by internalization. Control points in each pathway regulate protein sorting and intracellular trafficking, recognition and insertion of proteins in the targeting patch, and selective protein retention (Fig. 1). Comparison between C. *crescentus*, S. *cerevisiae*, and mammalian polarized epithelial cells reveals that both selective targeting and selective retention pathways determine protein distributions at the cell surface in these diverse cells. Remarkably, many control points are also conserved. Below, the pathways for generating cell surface polarity in each cell type are compared, and a preliminary synthesis of common regulatory mechanisms is proposed.

The author is in the Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305–5426.

Polarity and Diversity in *C. crescentus*

Caulobacter crescentus is a prokaryote that exhibits a striking degree of cell polarity (*1*). It exists in two cellular forms that are generated in the predivisional cell: a flagellum-bearing swarmer cell that does not replicate DNA and a sessile stalked cell that does (Fig. 2). The swarmer cell is structurally distinguishable from the stalked cell by polar appendages that comprise a flagellum, pili, and chemotaxis and phage receptors. These polar appendages are synthesized toward the end of the DNA replication stage of the stalked cell and become localized in the predivisional cell to the pole opposite the stalk. After cell division, the two cell types undergo distinct patterns of gene expression and development. The progeny stalked cell initiates another round of DNA replication and generates a new swarmer cell. The swarmer cell stage is transient: the polar appendages are lost and replaced by a stalk, the cell initiates DNA replication, and the cycle continues.

The distinctive structural and functional organization of the swarmer and stalked cells is established in the predivisional cell, before cell division, and is regulated in part by gene transcription (*5*). The flagellum is assembled, for example, by the transcription of flagellar genes in the order required for sequential protein assembly (basal body → hook → flagellum) (*6*). After cell division, expression of cell type–specific genes is also transcriptionally regulated, either through differential localization of transcription factors (*5*) or differences in the organization of the chromosomes in the two cell types (*7*).

However, given that the flagellum and the chemotaxis machinery are synthesized in the predivisional cell and localized to the swarmer cell pole before cell division, regulation of protein complex assembly at restricted areas of the predivisional cell membrane must also be post-transcriptional (*1*). Because proteins can insert into bacterial membranes after translation, it is possible that proteins are either selectively recruited to the poles or randomly inserted into the membrane and selectively retained at one of the poles. Analysis of the methyl-accept-

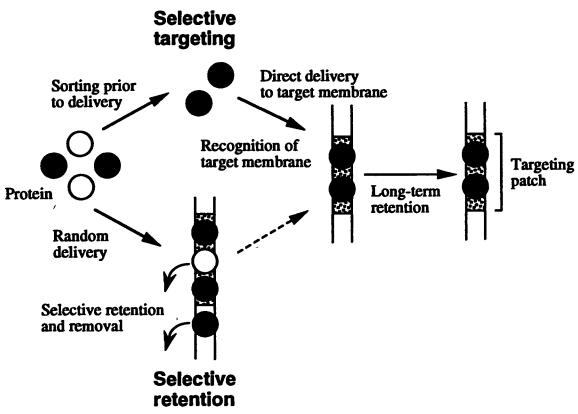

Fig. 1. Selective targeting and retention pathways for generating restricted protein distributions at the cell surface.

ing chemotaxis receptor protein (MCP) has provided insight into the mechanisms involved.

In the predivisional cell, MCP is synthesized and appears at the pole of the forming swarmer cell (*8, 9*). Localization of MCP to this pole is dependent on two factors. First, there is an intrinsic localization signal close to the COOH-terminus of MCP that is required for MCP assembly at the pole; deletion of this signal results in random localization of the protein over the entire membrane (*9*), indicating that it is directly involved in restricting membrane insertion of MCP at the pole. It is possible that targeting of MCP to the swarmer cell pole is determined by a direct interaction between the localization signal and a targeting patch on the membrane that specifies the site for protein insertion (below); alternatively, targeting of MCP may be mediated by a protein chaperone that recognizes the targeting patch. The MCP localization signal appears to be within the same region as the highly conserved domain that is the binding site for CheA and CheW, two proteins known to form a complex with the chemotaxis receptor (*9, 10*). Together, these results suggest that a selective targeting pathway for protein localization to the pole exists in these cells (Fig. 1) and that formation of a multisubunit complex between MCP, CheA, and CheW is critical for targeting the complex to the pole (*11*).

Second, the generation of cell surface polarity requires a targeting patch for selective assembly of the flagellum and the chemotaxis machinery in the swarmer pole of the predivisional cell (*12*). At present, the nature of the targeting patch is unknown. However, assembly of the polar appendages is initiated at the site of the previous cell division, which in enteric bacteria contains a periseptal annulus (*13*). The basal body, or a component of the periseptal annulus, or a structure involved in chromosome segregation may form a targeting patch for the earliest proteins synthesized, thereby nucleating assembly of components of the polar appendages at that site in the predivisional cell (*1, 12*). Subsequent assembly could then be driven by a cascade of specific interactions between those proteins initially localized to the targeting patch and newly synthesized proteins that are produced in their order of assembly.

The swarmer pole targeting patch for the chemotaxis machinery can be saturated; increased expression of the *mcpA* gene results in localization of excess MCP at the stalked pole of the predivisional cell (*11*). Under these conditions, MCP is selectively retained at the swarmer pole and is degraded at the stalked pole; a short sequence at the COOH-terminus, which is outside the localization signal of MCP, is the signal for degradation (*11*). This suggests a selective retention pathway also exists for localization of MCP under these conditions.

In summary, the generation of cell surface polarity in *C. crescentus* appears to be determined in part by selective recruitment of proteins to a targeting patch in the membrane, but also by selective retention of proteins coupled with differential protein degradation. Important control points in these pathways are specific localization and degradation signals encoded within the protein sequence and a saturable targeting patch at the pole that nucleates the assembly of specific protein complexes.

Polarized Growth in Budding Yeast

Saccharomyces cerevisiae is a unicellular eukaryote that exhibits polarized growth in response to either intracellular signals for cell division that lead to bud formation (vegetative cycle) or extracellular signals for mating that lead to the formation of a mating projection (*2, 3, 14*). Both forms of polarized cell growth are initiated by signals from the cell surface that result in realignment of the cytoskeleton and biosynthetic machinery toward a targeting patch at the bud site (Fig. 3). Unlike *C. crescentus*, membrane protein transport to the cell surface in *S. cerevisiae* is mediated by vesicles, which become selectively targeted to the bud site. Genetic approaches have been exploited in *S. cerevisiae* to identify genes that are required for distinct stages in vesicle transport to, and docking with, the site of bud growth at the cell surface.

Bud formation in cells that express **a** or α mating type occurs close to the site of the previous bud (axial budding), whereas bud formation in **a**/α diploid cells occurs at opposite poles of the mother cell (bipolar budding). Genetic and microscopic analyses indicate that budding involves a hierarchy of temporally distinct stages: (i) bud site and pattern selection, (ii) bud site assembly, (iii) cytoskeleton rearrangement, and (iv) vesicle recruitment to the bud site.

Analysis of bud site selection during axial budding has revealed that the new bud nearly always forms close to the site of the previous bud (*2, 14, 15*), indicating retention of a targeting patch for vesicle recruitment at the site of cytokinesis (*16, 17*) (Fig. 3A). Several proteins have been localized to the region of cytokinesis (Cdc3, Cdc10, Cdc11, and Cdc12), but they disappear from the bud site early in the subsequent cell cycle (*18*).

Five genes are required for bud site and pattern selection: *BUD1*, *BUD2*, and *BUD5* are necessary for stabilizing axial or bipolar bud formation and *BUD3* and *BUD4* are necessary for recognition of the axial bud site [(*17, 19*), reviewed in (*3*)]. A mutation in any one of these genes disrupts the polarity of bud formation but does not perturb bud assembly (*17*). Thus, the *BUD* genes are not required, per se, for bud assembly or growth, but are required for recognition of the targeting patch at the cell surface that defines the site for polarized bud formation (*17, 19*). Although direct functional studies of the proteins encoded by the *BUD* genes have not yet been performed, analysis of gene sequences has revealed some interesting functional properties of all of the proteins: Bud1, small Ras-like guanosine triphosphate (GTP)–binding protein (*19*); Bud2, GTP activating protein (GAP) (*20*); and Bud5, guanosine diphosphate (GDP)–GTP exchange protein (*19, 21*). The sequences of *BUD3* and *BUD4* genes are not yet known (*17*).

Assembly and growth of the bud require recruitment and fusion of transport vesicles with the bud site (*3*). Five other genes have been identified that are necessary for bud site assembly (*CDC24*, *CDC42*, *CDC43*, *BEM1*, and *BEM2*). For example, mutations in *CDC24* do not result in bud formation, but in an overall increase in cell diameter (*21, 22*). One of the bud site assembly genes encodes a small GTP-binding protein (*CDC42*) (*21, 22*). Another gene, *CDC43*, encodes a protein required for geranylgeranyltransferase activity in *S. cerevisiae* (*23*) and is homologous to the protein encoded by *DPR1/RAM1* (*24*) that prenylates the COOH-terminus of small GTP-binding proteins. Anchorage of Bud1 and Cdc42 pro-

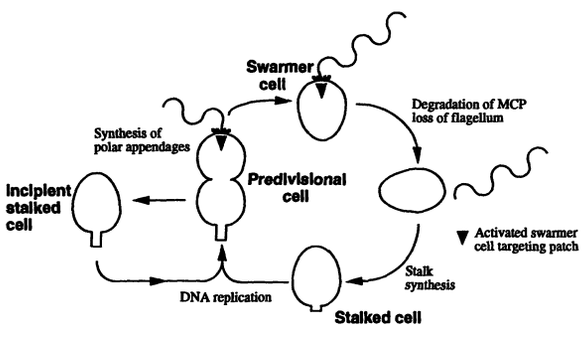

Fig. 2. *Caulobacter crescentus* cell cycle. MCP, methyl-accepting chemotaxis receptor protein.

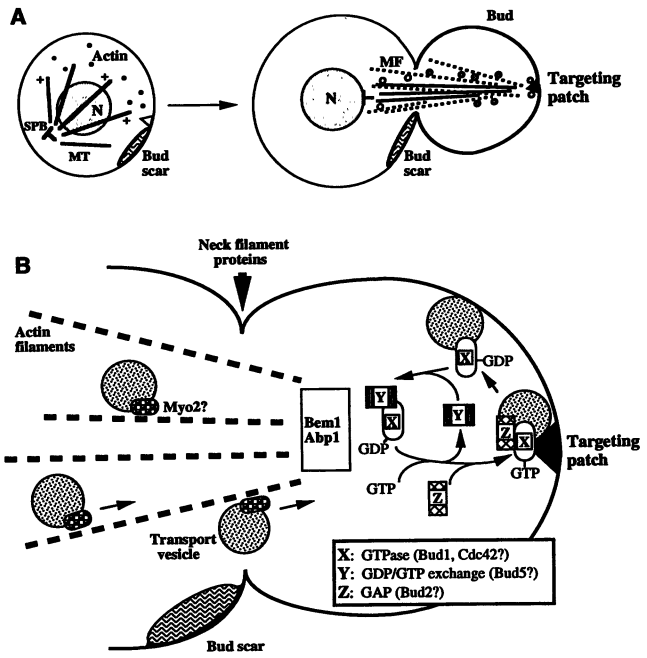

Fig. 3. Bud formation in *S. cerevisiae*. (**A**) Before bud formation, microtubules (MT) are nucleated from the spindle pole body (SPB) and are dispersed in the cytoplasm together with actin. Activation of an inert targeting patch (△), located close to the bud scar, results in polarized membrane growth at that site to form a bud. During this time, microtubules (MT) and actin microfilaments (MF) become oriented toward the site of bud growth. (**B**) Within the growing bud, vesicles are targeted along actin microfilaments, perhaps by Myo2 protein; microtubules are not necessary for bud growth, but analysis of a suppressor of mutations in *MYO2* indicates that microtubules may play a secondary role. Bem1 and Abp1 may be involved in organizing actin filaments toward the bud site. Vesicle docking with the targeting patch may be regulated by a GTPase cycle involving Bud proteins; although interactions between components of the putative GTPase cycle and transport vesicles are depicted at the targeting patch, it is equally possible that interactions occur during transport from the mother cell. Note that the *BUD3* and *BUD4* gene products are not represented here as their functions are unknown at present.

teins to the membrane could be mediated by COOH-terminus prenylation.

The finding that proteins involved in bud site selection and assembly are components of a guanosine triphosphatase (GTPase) cycle is intriguing. Cycles of GTP hydrolysis of small GTP-binding proteins, which are driven by GAP and GTP-GDP exchange proteins, are thought to regulate the accuracy of biochemical reactions by monitoring or promoting interactions between different proteins (*25*). A precedence for the role for GTPases in vesicle trafficking is provided by two well-described GTPases, Ypt1 (*26*) and Sec4 (*27*); inactivating mutations in either gene inhibit vesicle fusion with a target membrane compartment in the secretory pathway and results in vesicle accumulation at either an early (Ypt1) or late stage (Sec4) of the pathway. By analogy, a GTPase cycle involving GTPases (Bud1 and Cdc42), a GAP protein (Bud2), and a GDP-GTP exchange protein (Bud5) could regulate interactions of vesicles with the targeting patch at the bud site (*19*) (Fig. 3B). The identity of the proteins encoded by the *BUD3* and *BUD4* genes will be important in this context, however, since the encoded proteins are good candidates for recognizing or activating the targeting patch for axial bud formation (*17*).

Bud site assembly and growth are also coupled to the reorganization of the actin cytoskeleton (*28*) (Fig. 3A). Disruption of the single actin gene in *S. cerevisiae* results in abnormal cell growth and intracellular accumulation of vesicles (*29*). It is not yet clear how the cytoskeleton is linked to bud assembly at the membrane. However, one of the bud site assembly genes, *BEM1*, encodes a protein that contains two SH3 domains (*30*). Originally detected in Src, the function of SH3 domains is unknown, but they contain a motif of 50 amino acids residues that is in several proteins that are generally associated with the cortical cytoskeleton or localized to the cell surface (*31*). Deletion of *BEM1* results in large, multinucleate yeast with disrupted actin organization (*30*). The Abp1 protein also contains an SH3 domain and is an actin-associated protein that may regulate actin filament assembly at the bud site (*31*); overproduction of Abp1 protein also results in abnormal bud formation. Both the Bem1 and Abp1 proteins may interact with a component of the bud site and, through their SH3 domains, with the actin cytoskeleton. These interactions would link the cytoskeleton to the bud site and, thereby, the transport of intracellular vesicles along microfilaments from the mother cell directly to the site of asymmetric cell growth (*30*) (Fig. 3B).

Vesicle transport along actin filaments may be mediated by motor proteins similar to myosin. Mutations in a novel myosin gene, *MYO2*, result in the accumulation of secretory vesicles and prevent bud growth similar to phenotype produced by mutations in the actin gene (*32*). However, *myo2* mutations do not affect bulk flow of proteins to other areas of the cell surface (*32*), suggesting that vesicles are specifically recruited along actin filaments by the Myo2 protein toward the targeting patch at the bud site (Fig. 3B). A multicopy suppressor that corrects the *myo2* phenotype encodes a protein with sequence homology to kinesin (*33*), a mechanoenzyme that mediates vesicle translocation along microtubules toward the "plus," or growing, end (*34*). This raises the possibility that microtubule systems play some role in vesicle recruitment to the bud site.

Genetic analysis, however, has shown that microtubules are generally not necessary for bud site selection or assembly but are required for karyokinesis (*28, 35*). During bud formation, microtubules are reorganized (Fig. 3A). This is preceded by movement of the spindle pole body (SPB) to a site adjacent to the forming bud (*16*). Microtubules span the cytoplasm from the SPB to the tip of the forming bud. The Spa2 protein (*36*) is localized to the bud tip, but it is not known whether it is involved in microtubule capture and reorientation of the SPB and microtubule array (*16*); an alternative possibility is that other, as yet unidentified, proteins within the targeting patch form the site for microtubule capture.

Saccharomyces cerevisiae also become polarized during mating between **a** and α haploid cells (*37*). Cell type–specific pheromones are released that act on the cell of the opposite mating type through an interaction between the pheromone and a G protein–coupled receptor (*37*). This signal arrests the vegetative cell cycle (*38*) and overrides the bud site selection and pattern program of the *BUD* genes (*3*). The cells form projections at the cell surface by asymmetric (polarized) growth at the site of highest concentration of pheromone receptors (*39*). Several of the proteins involved in bud assembly and growth also participate in generating the mating projection. Mutations in *BEM1* result in abnormal deposition of the cell wall and abnormal organization of actin filaments in the mating projection (*30*). Mutation of the actin gene, but not the tubulin genes, also results in abnormal growth of the projection (*40*); mutations in the tubulin genes lead to abnormalities in both nuclear distribution and karyokinesis (*35*). The process that initiates asymmetric cell growth and the generation of the mating projection is unknown (*37*). *STE2* mutations that involve deletion of the COOH-terminus of the pheromone receptor have

no effect on vegetative cell morphology (*41*), but cells increase in size uniformly (without a mating projection) in response to mating factor (*39*). Mutations in special alleles of *BEM1* (*30*) have a similar phenotype to that of *STE2* mutations, indicating a link between pheromone receptors and proteins involved in mating projection assembly. Perhaps clustering of pheromone receptors at the cell surface induces the reorganization of the cytoskeleton through linkages established by the Bem1 protein and creates a targeting patch that selectively recruits vesicles to the site of the mating projection (*30*).

In summary, polarized cell growth of *S. cerevisiae* is generated by selective targeting of proteins to a restricted region of the cell surface (the bud site). The recruitment of vesicles to the bud site requires activation of a targeting patch at the site of cytokinesis or mating hormone–receptor interaction and realignment of the cytoskeleton. Protein delivery is mediated by translocation of vesicles along cytoskeletal filaments to the bud site and may be regulated by a GTPase cycle that specifies docking of vesicles with the targeting patch.

Surface Domains in Mammalian Epithelial Cells

Polarized epithelial cells regulate ion and solute transport between two biological compartments that are separated by the epithelium (*4*). This requires that restricted protein distributions are generated and maintained in structurally and functionally distinct domains of the cell surface, termed apical and basal-lateral, that face different compartments (*4*). The development of cell lines that maintain this differentiated phenotype in tissue culture (*4*) and of sophisticated cell biological methods to analyze protein trafficking and sorting to different membrane domains (*42*) has shown that there are two types of pathways for protein delivery to the cell surface.

In Madin-Darby canine kidney (MDCK) cells, a cell line derived from polarized renal epithelia, proteins are generally sorted into different vesicle populations in the trans Golgi network (TGN) and recruited directly to either the apical or basal-lateral membranes (a selective targeting pathway) (Fig. 1) (*4*). It should be noted, however, that during the generation of MDCK cell surface polarity, some proteins are delivered to both membrane domains and sorted subsequently by differential retention and turnover (a selective retention pathway) (Fig. 1) (*43*). In hepatocytes in situ (*44*), and to a lesser extent in the intestinal cell line Caco-2 (*45*), proteins are first delivered to the basal-lateral membrane; basal-lateral proteins are selectively retained, while apical proteins are resorted and selectively targeted to the apical membrane. Differences in protein sorting in these cells may reflect the presence of cell type–specific machinery for protein sorting or localization of the same sorting machinery to different membrane compartments. An apical membrane protein, dipeptidyl peptidase IV, is sorted to the apical membrane in both hepatocytes and MDCK cells (*46*), suggesting that the same signal is recognized by the sorting machinery in both cell types.

These observations have raised four questions: What are the structural features of proteins that lead to sorting of apical and basal-lateral proteins into different vesicle populations; how are these structural features recognized by the sorting machinery; how are different populations of vesicles recruited to specific membrane domains; and what is the mechanism of protein retention at the cell surface?

Sorting of one class of proteins to the apical membrane is determined by a glycosyl phosphatidylinositol (GPI) membrane anchor (*47*); the GPI membrane anchor is transferrable to different proteins indicating that the signal is the anchor itself, and not the polypeptide backbone of the protein (*47*). Glycosphingolipids are also sorted to and concentrated in the exoplasmic leaflet of the apical membrane of MDCK and intestinal cells (*48*) (Fig. 4). Based on these observations, it has been proposed that sorting of GPI-anchored proteins may be mediated by interactions with glycosphingolipids in the TGN (*49*); GPI-anchored proteins and other apical membrane proteins cocluster into glycosphingolipid patches that are formed by hydrogen bonding (*50*), which could result in vesicle formation and budding from the TGN (*49*) (Fig. 4). This model is supported by two observations: first, GPI-anchored proteins have reduced lateral mobility immediately after being delivered from the TGN to the apical membrane (*51*); second, hemagglutinin, a viral transmembrane protein that is selectively targeted to the apical membrane, can be extracted from MDCK cells in a detergent-insoluble fraction that contains a high concentration of glycosphingolipids (*52*). Hemagglutinin, and perhaps endogenous transmembrane proteins, may be sorted through either a direct affinity for glycosphingolipids or by binding to an "apical sorting receptor" that, in turn, binds to glycosphingolipids (*49*). Characterization of resident proteins of vesicles containing glycosphingolipids (*53*) and GPI-anchored proteins (*54*) may provide insight into the identity of this "apical sorting receptor." The role of glycosphingolipids and GPI-anchorage in protein sorting in hepatocytes is less well understood; a GPI-anchored protein is delivered to basal-lateral membranes, together with all other proteins, and then slowly resorted to the apical membrane (*55*). Although these results emphasize the role of glycosphingolipids in apical protein sorting in the TGN of MDCK cells, it is likely that other mechanisms of sorting apical proteins remain to be identified.

A positive sorting signal for one class of basal-lateral membrane proteins in MDCK cells and hepatocytes is characterized by a short amino acid sequence (~14 residues) located close to the transmembrane domain (*56*). Mutational analysis of the low-density lipoprotein (LDL) and polyimmunoglobulin A (poly IgA) receptors suggests a consensus sequence for this sorting signal: ArgAsn-XAspXX$\overset{\text{Ser}}{\underset{\text{Thr}}{}}$XX Ser, in which X is any amino acid (*56*). In some proteins, a basal-lateral sorting signal includes a tyrosine residue that confers an increased rate of endocytosis from the cell surface (*57*). However, in the LDL and poly IgA receptors, signals for basal-lateral sorting and endocytosis are distinct (*58*).

The structural characteristics of this basal-lateral sorting signal suggest that it may be recognized by cytosolic proteins related to the family of adaptor proteins that regulate signal-mediated endocytosis of receptor proteins at the cell surface (*56, 59*) (Fig. 4). The endocytosis sorting signal comprises a spacer sequence of seven or more residues that separates the transmembrane domain from a structurally conserved tetrapeptide that forms a tight β turn (*60*), which is recognized by cytosolic adaptor proteins (components of the clathrin coated pit assembly) (*61*). Binding of proteins through the basal-lateral signal to adaptor proteins could induce clustering of proteins in the TGN membrane and initiate vesicle formation and budding (*56–59*) (Fig. 4). At present, however, it is not known whether the consensus signal for basal-lateral membrane protein sorting in the TGN forms a tight β turn or whether a binding site forms that is recognized by a specific class of adaptor proteins (AP1) that is localized to the Golgi complex (*61*).

It is probable that more than one signal-mediated pathway exists for sorting basal-lateral proteins in the TGN. For example, integrins and laminin appear to be transported from the TGN to the basal-lateral membrane in separate vesicle populations (*62*); some proteins contain a cytoplasmic domain that is probably too short (*63*) to be recognized by cytosolic adaptor proteins; and proteins that span the membrane multiple times (for example, ion transporter and channel proteins) have not yet been studied in detail. The nature of other basal-lateral sorting signals is unknown. In addition, it is likely that some proteins are delivered to the cell surface by bulk flow

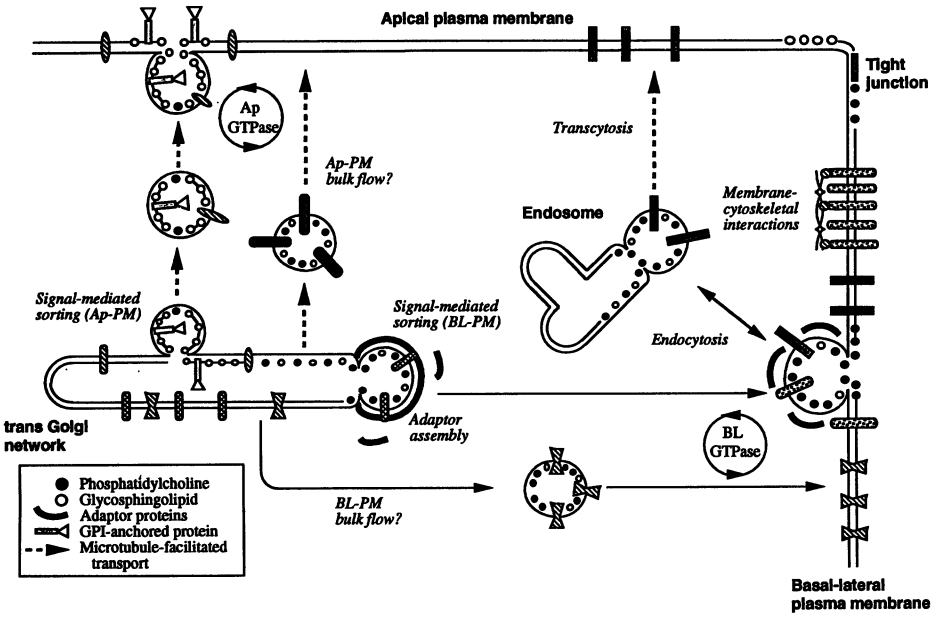

Fig. 4. Mammalian polarized epithelial cell organization of protein trafficking pathways. Protein sorting and transport between the trans Golgi network (TGN) and different cell surface domains are regulated. In the TGN, proteins can be sorted by signal-mediated or bulk flow pathways into different vesicles (see text). Signal-mediated sorting of proteins to the apical plasma membrane (Ap-PM) is regulated by glycosphingolipid patching, whereas signal-mediated sorting of protein to the basal-lateral plasma membrane (BL-PM) is regulated by protein clustering by adaptor proteins; other signal-mediated pathways may also exist. The bulk flow pathway may comprise a population of vesicles different from those of the signal-mediated pathways. Vesicle transport to the cell surface is facilitated by components of the cytoskeleton; evidence indicates a predominant role for microtubules, but actin filaments may also be involved. Docking with targeting patches in each membrane is regulated by domain-specific GTPase cycles (Ap-GTPase, apical membrane GTPase; BL-GTPase, basal-lateral membrane GTPase). At the cell surface, proteins may diffuse in the plane of the lipid bilayer; global diffusion between domains is constrained by the tight junction located at the boundary of the apical and basal-lateral membranes, whereas local diffusion is constrained by protein binding to the membrane cytoskeleton.

(64) and not by a signal-mediated pathway. It is not clear at present whether the bulk flow pathway comprises a population of vesicles that are distinct from apical and basal-lateral transport vesicles (Fig. 4). A recent study demonstrated that the volume of bulk flow to the apical and basal-lateral membranes in MDCK and Caco-2 cells is different in each cell type; the apical:basal-lateral bulk flow ratio was 67:33 in MDCK cells and 30:70 in Caco-2 cells (65). These differences raise the possibility that bulk flow may constitute a secondary pathway for "signal-independent" sorting of proteins by preferential delivery of vesicles from the TGN to one of the membrane domains.

Since the fidelity of signal-mediated protein sorting and delivery to different membrane domains is very high (4), vesicle trafficking between the TGN and cell surface, and recognition between vesicles and different membrane domains, must be regulated. Similar to *S. cerevisiae*, vesicle trafficking and subsequent docking with the membrane in polarized epithelial cells appear to be regulated by the cytoskeleton and GTP-binding proteins, respectively.

Vesicle traffic between the TGN and different cell surface domains in polarized epithelial cells seems to be predominantly regulated by microtubules (66). Disruption of microfilaments with cytochalasin D does not affect either the kinetics or fidelity of vesicular traffic between the TGN and different cell surface domains in MDCK or Caco-2 cells (67–69). However, Golgi-derived vesicles (70) and the apical membrane of polarized epithelial cells (71) contain the mechanoenzyme myosin I, which translocates vesicles along actin filaments (71). Thus, it remains possible that actin filaments are involved in vesicle transport between the TGN and membrane in these cells, and either some filaments (for example, those at the apical pole of the cell) are resistant to disruption with cytochalasin D (70) or vesicles can reach the apical membrane by a secondary transport pathway (for example, along microtubules or by direct affinity for the apical membrane).

In both MDCK (72) and Caco-2 cells (68, 73), microtubules are organized in a dense mat of short filaments that are randomly oriented beneath the apical membrane and above the nucleus. Long bundles of microtubules also extend down the length of the cell, parallel to cell-cell contacts; the "plus" ends of these microtubules are located in the basal portion of the cell. Depolymerization of microtubules slows vesicle trafficking between the TGN and apical membrane (69, 74) and decreases transcytosis from the basal-lateral to the apical membrane (73, 75) (Fig. 4). In most cases, the fidelity of vesicle docking with the correct target membrane is not affected by microtubule depolymerization, indicating that docking is regulated directly by binding of the vesicle to the cytoplasmic face of the plasma membrane (below). Transport of vesicles from the TGN to the basal-lateral membrane is unaffected by microtubule disruption (66, 69, 73–75). However, both purified apical and basal-lateral transport vesicles bind directly to microtubules in vitro (76). It remains possible that vesicles are normally delivered along microtubules to the basal-lateral membrane but that after microtubule disruption an alternative mechanism directs vesicle traffic to the membrane (above).

The polarized orientation of microtubules suggests that a "plus" end–directed, kinesin-like motor protein could participate in movement of vesicles from the region of the Golgi complex toward the basal-lateral pole of the cell and that a "minus" end–directed, dynein-like motor protein could be involved in vesicle traffic toward the apical pole (34, 66). Although a role for these mechanoenzymes in vesicle trafficking between the TGN and different membrane domains has not yet been demonstrated, vesicle transport between apical and basal-lateral endosome compartments isolated from MDCK cells requires microtubules, dynein, and kinesin (77).

Although much has been learned about protein sorting in the TGN and trafficking of vesicles in polarized epithelial cells, little is known about how each membrane domain forms a distinct targeting patch that specifies recognition, docking, and fusion of a subset of transport vesicles. Studies of how polarized epithelial cells arise from nonpolarized precursor cells have provided insight into this problem by showing that the generation of different cell surface domains requires formation of cell-cell and cell-substratum contacts (4, 78). These extracellular contacts may initiate specific signals at the bounded cell surface (the forming basal-lateral membrane) that generate a targeting patch for recruitment of a subset of vesicles. This is supported by the observation that cell polarity at the first cleavage of a fertilized *Xenopus* egg is generated in part by specific recruitment of a subset of stored vesicles to the cleavage membranes (79). In addition, under certain conditions, formation of the apical membrane in MDCK cells may be facilitated by

direct fusion of storage vesicles containing apical marker proteins with the free cell surface (*4*).

There are several possible mechanisms for transducing signals from extracellular contacts that result in the formation of a targeting patch for vesicle recruitment. For example, occupation of cell adhesion proteins induces a classical second messenger response involving an increase of inositol trisphosphate (IP_3) and activation of Ca^{2+} channels, which lead to an elevation of intracellular Ca^{2+} (*80*) that could trigger localized vesicle fusion with the membrane (*81*). Also, activity of tyrosine kinases (*82*) and phosphatases (*83*) is known to modulate protein interactions involved in assembly of junctional complexes at the cell surface (*84*) and interactions between adhesion receptors and the cytoskeleton (*82*). Finally, cell-cell adhesion induces the assembly of the actin-based membrane cytoskeleton at sites of cell-cell contacts (*85*) and realignment of the microtubule cytoskeleton (*72*), both of which contribute to the generation of cell surface polarity by selective recruitment and retention of proteins to the forming basal-lateral membrane domain.

Recognition and fusion of vesicles with the basal-lateral membrane in an in vitro reconstituted system were found to be dependent on temperature, energy, and cytosol (*86*). Vesicle fusion was inhibited by a nonhydrolyzable analog of GTP (GTPγS), indicating that both GTP-binding proteins and GTP hydrolysis are required at some stage of the binding and fusion reaction (*86*). GTP-binding proteins of the Rab family are thought to participate in the regulation of the interactions of vesicles with different target membrane compartments in MDCK and other mammalian cells (*25, 87*), and initial studies indicate that Rab8 is localized to basal-lateral transport vesicles derived from MDCK cells (*88*). On the basis of the Ypt1 and Sec4 paradigm for regulation of vesicle trafficking by GTP-binding proteins in *S. cerevisiae* (above), it is anticipated that GTP-binding proteins will be identified that regulate docking of vesicles with either the apical or basal-lateral membrane domains in polarized epithelial cells (*87*) (Fig. 4). It is not known whether these vesicle-bound GTP-binding proteins are recognized by cell surface receptors in the targeting patch or whether a hierarchy of steps is involved in selection of the targeting patch on the membrane, similar to that required for bud growth in *S. cerevisiae* (above) (*3, 17*).

After delivery to the cell surface, proteins have different fates that affect how cell surface polarity is generated and maintained: proteins may diffuse away from the site of delivery; proteins may be selectively retained in the target membrane; or proteins may be internalized from the cell surface and either cycled back to the same membrane (retrieval), delivered to another membrane domain (transcytosis), or degraded. Although proteins are capable of long-range diffusion in the plane of the lipid bilayer (*89*), it appears that protein diffusion within different cell surface domains of polarized epithelial cells is constrained (*90*). Global diffusion of proteins between the apical and basal-lateral membrane domains may be physically inhibited by the tight junction (*91*), which is located at the boundary between these membrane domains (Fig. 4). Local diffusion of some membrane proteins appears to be constrained by binding to the cortical membrane cytoskeleton (Fig. 4); the Na^+,K^+-ATPase (*92, 93*), the Na^+ channel (*94*), and the Cl^-/HCO_3^- exchanger (*93*) bind with high affinity and specificity to ankyrin-fodrin complexes in the membrane cytoskeleton. Domain-specific assembly of the membrane cytoskeleton may participate in generating restricted cell surface distributions of these proteins (*95*) by inhibiting protein diffusion away from the site of vesicle delivery and by excluding proteins from internalization at the cell surface (*96*).

Receptor proteins that are internalized from the cell surface may be recycled from the endosome back to the original membrane domain (*59*) (Fig. 4). Alternatively, proteins can be selectively targeted from the endosome to a different membrane domain (transcytosis) (Fig. 4). For example, the poly IgA receptor is delivered to the basal-lateral membrane from the TGN, internalized, and then sorted in the basal-lateral endosome into transcytotic vesicles for delivery to the apical membrane (*59, 97*). The transcytotic pathway is important for generating cell surface polarity in hepatocytes (*44*) and, to a lesser extent, in the intestinal cell line Caco-2 (*45*). In these cells, protein sorting does not occur in the TGN but after delivery of proteins to the basal-lateral membrane. The mechanism involved in sorting apical and basal-lateral proteins at this location is also unknown. One possibility is that protein sorting occurs directly at the cell surface by selective internalization of apical membrane proteins (basal-lateral membrane proteins are selectively retained) and subsequent delivery to the apical membrane. Alternatively, membrane proteins may be internalized randomly from the cell surface and delivered to the basal-lateral endosome, where they are either sorted into the recycling (resident basal-lateral membrane proteins) or transcytotic pathways (apical membrane proteins) (Fig. 4).

In summary, the generation of cell surface domains in polarized epithelial cells is regulated by both selective targeting and selective retention pathways. Protein sorting occurs in the TGN and at the cell surface. Sorting signals that distinguish apical and basal-lateral membrane proteins have been identified and have provided new insights into the nature of the sorting machinery. Vesicle delivery between the TGN and different cell surface domains is mediated in part by the cytoskeleton and by regulated recruitment to different membrane domains through activity of the Rab family of small GTP-binding proteins. Selective protein retention at the membrane is regulated by the tight junction and the membrane cytoskeleton. The establishment of targeting patches for vesicle recruitment to the basal-lateral membrane may be initiated by signals transduced from extracellular contacts.

Synthesis

Although the analysis of mechanisms involved in the generation of cell surface polarity in *C. crescentus*, *S. cerevisiae*, and mammalian polarized epithelial cells is at an early stage, and focus has been placed on different aspects of the process in each cell type, there are many remarkable similarities. On a general level, the different systems utilize both selective targeting and selective retention pathways for establishing polarized distributions of proteins at the cell surface (Fig. 1). The selective targeting pathways, for example, in *C. crescentus* and mammalian polarized epithelial cells both utilize intrinsic protein signals to regulate protein sorting; the selective retention pathways in both cell types require formation of specific protein complexes at the membrane for distinguishing protein retention from differential protein turnover.

Polarized insertion of proteins at the cell surface is regulated at several control points that also appear similar in these diverse cells. In *C. crescentus*, newly synthesized proteins are recruited directly to the poles of the cell, indicating direct recognition between protein localization signals and targeting patches that are restricted to the poles (below). In *S. cerevisiae* and mammalian polarized epithelial cells, protein delivery is mediated by vesicles that are also recruited to, and recognized by, targeting patches at the cell surface.

Vesicle trafficking in the cytoplasm is controlled in part by mechanoenzymes (for example, myosin, dynein, and kinesin) that translocate vesicles along cytoskeletal filaments oriented toward targeting patches at the cell surface; although results indicate that actin filaments are primarily used in *S. cerevisiae*, and that microtubules are used in mammalian polarized epithelial cells, there is evidence that both filament systems may

participate in each cell type.

Recognition and docking of vesicles with the appropriate targeting patch at the cell surface appear to be regulated by GTP-binding proteins and cycles of GTP hydrolysis; specific GTPases, a GAP protein, and a GDP-GTP exchange protein have been identified in *S. cerevisiae* that are required for vesicle recruitment to the bud site targeting patch, and there is increasing evidence for a similar role of the Rab family of small GTP-binding proteins in regulating vesicle docking with different membrane domains in mammalian polarized epithelial cells.

A central component of these regulatory mechanisms in these cells is the targeting patch that restricts protein and vesicle recruitment and protein retention to specific regions of the cell surface (Fig. 1): the cell poles in *C. crescentus*, the bud site in *S. cerevisiae*, and apical and basal-lateral membrane domains in mammalian polarized epithelial cells. The targeting patch may comprise cell surface receptors for intrinsic protein localization signals, vesicle-bound GTP-binding protein, and sites for cytoskeleton orientation and assembly.

In addition to recruiting vesicles and proteins to the cell surface, the targeting patch appears to play a broader role in the induction of the cellular response to cues to develop cell surface polarity. These cues originate at the cell surface: for example, interaction between pheromones and G protein–coupled receptors during mating in *S. cerevisiae* (formation of the mating projection), cell-cell and cell-substratum contacts in polarized epithelial cells, the periseptal annulus in *C. crescentus*, and the bud scar and site of cytokinesis in *S. cerevisiae*. These sites coincide with the region of polarized cell growth and formation of structurally and functionally distinct membrane domains. It is possible, therefore, that these cues directly activate or generate the targeting patch at the cell surface. Activation of the targeting patch may be mediated by G proteins, increases in intracellular Ca^{2+}, tyrosine kinases, or phosphatases. In addition, these signals may contribute directly to the realignment of components of the cytoskeleton and the biosynthetic machinery of the cell toward the targeting patch, resulting in the recruitment and retention of proteins at specific sites at the cell surface.

Defining the molecular nature of these targeting patches may provide insight into how proteins and vesicles are recruited to restricted sites on the cell surface and how cellular programs for generating cell surface polarity are initiated and propagated. Given the similarities in the pathways and control points in these diverse cells, it is possible that future studies with these systems will complement each other and, thereby, lay a general framework for a detailed understanding of biochemical pathways involved in the generation of cell surface polarity.

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Cytoskeleton–Plasma Membrane Interactions

Elizabeth J. Luna* and Anne L. Hitt

Proteins at the boundary between the cytoskeleton and the plasma membrane control cell shape, delimit specialized membrane domains, and stabilize attachments to other cells and to the substrate. These proteins also regulate cell locomotion and cytoplasmic responses to growth factors and other external stimuli. This diversity of cellular functions is matched by the large number of biochemical mechanisms that mediate the connections between membrane proteins and the underlying cytoskeleton, the so-called membrane skeleton. General organizational themes are beginning to emerge from examination of this biochemical diversity.

The first definition of a "membrane skeleton" (*1*) originated with the observation that the nonionic detergent, Triton X-100, disrupts hydrophobic, but not polar, protein-protein and protein-lipid interactions in the membrane of the human red blood cell (*2*). Interconnected cytoskeletal proteins, including actin and spectrin, and tightly associated integral membrane proteins co-pellet as Triton-insoluble structures with the approximate size and shape of the unsolubilized cells. The possible generality of this approach was suggested by the observation that most of the surface-labeled proteins in tissue culture cells also remain associated with a thin cytoplasmic layer after Triton extraction (*3*). However, Triton insolubility is an insufficient criterion for cytoskeletal attachment because other interactions also confer resistance to nonionic detergents (*4*). Thus, an association with cytoskeletal elements must now be directly demonstrated in order to conclusively identify a protein as a component of the membrane skeleton.

In this review, we will focus on systems in which molecular information is available on the attachments between membrane and cytoskeletal proteins. Because this is a large and fast-moving field summarized previously (*5–7*), we will emphasize recent advances in understanding how the membrane skeleton governs basic cell processes. Thus, we primarily describe specialized membrane domains that have been isolated and dissected biochemically. Most cells coordinate the functions of these domains so that they act in concert with each other and with other membrane and cytoskeletal proteins.

E. J. Luna and A. L. Hitt are with the Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

*To whom correspondence should be addressed.

Control of Cell Shape, Membrane Stability, and Domain Organization

Erythrocytes. Because of its comparative simplicity and the relative ease with which large amounts of homogeneous membrane can be obtained, the best understood membrane skeleton is that of the human red blood cell (*8*). These highly specialized cells are biconcave disks that lack internal organelles and transcellular filament systems. The membrane stability and deformability required during the erythrocyte's 120-day, tortuous journey through the bloodstream are maintained solely by the underlying meshwork of spectrin, actin, and associated proteins (Fig. 1). This meshwork is attached to the membrane by ankyrin and protein 4.1. Ankyrin links band 3 (the anion exchanger) to the β subunit of spectrin near the middle of the extended spectrin tetramer. Protein 4.1 binds to both spectrin subunits near the ends of the tetramer, enhancing the affinity of spectrin for actin. Protein 4.1 also binds in vitro to the transmembrane proteins, band 3 and glycophorin C. Binding between protein 4.1 and band 3 may involve an interaction between the amino acid sequence, LEEDY, near the NH_2-terminus of protein 4.1 and an oppositely charged motif (IRRRY or LRRRY) in the cytoplasmic domain of band 3, although other sites on band 3 also have been implicated (*9*). A sequence (YRHKG) present in glycophorin C contains the same charge distribution and hydrophobicity as the motif in band 3. Finally, spectrin and band 4.1 bind with low affinity to negatively charged phospholipids (*10*), interactions that may help to stabilize the lipid bilayer.

Proteins not bound directly to the membrane also add to the stability of the membrane skeleton (*11*). For instance, a heterodimeric calmodulin-associated protein, adducin, enhances spectrin binding to actin. Tropomyosin and dematin (protein 4.9) bind along the sides of actin filaments, and the tropomyosin-binding protein, tropomodulin, may control the lengths of the short actin filaments bound to the spectrin tetramers.

Studies of mice and humans with hereditary defects in erythrocyte shape and stability confirm the role of the membrane