[Frontiers in Bioscience 14, 1088-1098, January 1, 2009]

Epithelial cell surface polarity: the early steps

Lene N. Nejsum[1], W. James Nelson[1]

[1]Departments of Biology, and Molecular and Cellular Physiology, The James H. Clark Center, Bio-X Program, Stanford University, 318 Campus Drive E200, Stanford, CA 94305-5430

TABLE OF CONTENTS

1. ABSTRACT

Establishment and maintenance of epithelial cell surface polarity is of vital importance for the correct function of transporting epithelia. To maintain normal cell function, the distribution of apical and basal-lateral proteins is highly regulated and defects in expression levels or plasma membrane targeting can have severe consequences. It has been shown recently that initiation of cell-surface polarity occurs immediately upon cell-cell contact, and requires components of the lateral targeting patch, the Exocyst and the lateral SNARE complex to specify delivery of basolateral proteins to the site of cell-cell adhesion. The Exocyst and SNARE complex are present in the cytoplasm in single epithelial cells before adhesion. Upon initial cell-cell adhesion, E-cadherin accumulates at the forming contact between cells. Shortly hereafter, components of the lateral targeting patch, the Exocyst and the lateral SNARE complex, co-localize with E-cadherin at the forming contact, where they function in specifying the delivery of basal-lateral proteins to the forming contact.

2. EPITHELIAL CELL AND TISSUE FUNCTIONS

Polarized transporting epithelia comprise physically and chemically tight adherent cell monolayers that separate two biological compartments. They regulate ionic homeostasis by vectorial transport of ions and solutes through ion channels and transporters located on different plasma membrane domains that face these two biological compartments (Figure 1A). Topologically, the apical surface of the epithelial cells generally faces the outside compartment of the organism, and the basal and lateral (basal-lateral) surfaces, which are attached to the substratum and the neighboring cells, respectively, face the serosa and inside compartment of the organism. The plasma membrane protein contents of these three surfaces are different, comprising a highly regulated combination of receptors, ion channels, and ion exchangers and transporters (Figure 1C), which together mediate ion and solute exchange between the two biological compartments separated by the epithelium.

The protein composition of the apical and basal-lateral plasma membrane domains varies between specialized epithelial cells, and defines the transport function of the epithelium (e.g., absorptive or secretory). The apical and the lateral cell surfaces are separated by the tight junction, which forms a tight, gasket-like seal around the top of each cell. The tight junction controls paracellular transport of ions and solutes between cells, and prevents intermixing of apical and basal-lateral plasma membrane lipids and membrane proteins.

Sorting of plasma membrane proteins to their target plasma membrane domain is crucial for the maintenance of functional transporting epithelia; increased or decreased amounts of, or incorrect sorting of specific plasma membrane proteins are common in human metabolic diseases and cancers. These abnormalities include several disorders associated with defects in water and salt homeostasis including untreated diabetes, acute and chronic renal failure, and urethral obstruction, all of which have altered levels of normal, functional proteins at the plasma membrane (1-7). Gene mutations in transport proteins also cause a wide range of metabolic diseases. For example, mutations in the vasopressin regulated renal water channel aquaporin-2 (AQP2) results in a severe urinary concentration defect with a daily urine production of up to 20 liters (Nephrogenic Diabetes Insipidus); mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) block transcellular Cl^- movement resulting in an abnormally high NaCl concentration in the airway surface liquid and increased bronchial mucus accumulation (8, 9).

Other abnormalities resulting from mis-sorting of polarized proteins involve mis-sorting of proteins to the incorrect plasma membrane domain, such as in familial hypercholesterolemia. For example, a point mutation in the basal-lateral low density lipoprotein (LDL) receptor results in targeting of mutant LDL receptor to the apical plasma membrane (10).

3. REGULATION OF PROTEIN SORTING IN POLARIZED EPITHELIAL CELLS

Correct homeostatic function of transporting epithelia in response to external stimuli such as hormones requires that the plasma membrane distribution and amounts of ion channels and transporters are tightly regulated. The fastest means of regulating protein content on the plasma membrane involves rapid protein insertion by regulated exocytosis, and removing proteins by endocytosis. An example of this regulation is found in the renal collecting duct in which the vasopressin-regulated water channel AQP2 fine-tunes urine concentration in response to vasopressin stimulation. AQP2 is localized in principal cells in intracellular vesicles just beneath the apical plasma membrane (11). Upon vasopressin stimulation, AQP2 localized in intracellular vesicles, is phosphorylated by protein kinase A (PKA), where after AQP2-containing vesicles are inserted into the apical plasma membrane, instantly increasing water reabsorption and thereby increasing urine concentration (Figure 1C) (12). Upon vasopressin removal, AQP2 is rapidly internalized from the apical membrane by endocytosis, thereby lowering collecting duct water permeability (Figure 1C) (12). Shuttling of AQP2 between a vesicle and plasma membrane pool enables rapid regulation of collecting duct water permeability and urine concentration.

A slower means of regulating protein content on the plasma membrane is by adaptation to long-term external stimuli, which alter plasma membrane protein composition through changes in transcription and/or translation of specific proteins. AQP2 is also regulated by these mechanisms. Thirst increases transcription, translation, and targeting of AQP2 to the apical plasma membrane, whereas increased water intake ultimately leads to decreased transcription and translation of AQP2 (13-14).

The spatial regulation of proteins to either the apical or basal-lateral plasma membrane domains is regulated through specific sorting stations in the trans-Golgi Network (TGN) and (recycling) endosomes, and delivery pathways between these stations and target plasma membrane (s). In general, newly synthesized apical and basal-lateral proteins are sorted from each other in the TGN of polarized epithelial cells. Since this also occurs in non-polarized fibroblasts, which do not have cell surface polarity like that of transporting epithelial cells, it is thought that protein sorting in the TGN is a constitutive mechanism in many cell-types (15).

Sorting of apical and basal-lateral proteins is mediated by distinct intrinsic motifs. Protein sorting to the apical plasma membrane is dependant on sorting motifs exposed to the lumen of the TGN that include N- or O-linked carbohydrate moieties on the extracellular domain, and a glycosylphosphoinositol (GPI) lipid anchor (16, 17). It is not known, however, if glycosylation is directly involved in protein sorting *per se* or for correct protein folding or oligomerization which exposes a sorting motif (18). Some newly synthesized apical proteins are transported from the TGN to the apical plasma membrane via lipid rafts, and others may be sorted into the basal-lateral plasma membrane pathway and then re-sorted to the apical plasma membrane following endocytosis from the basal-lateral membrane; it has been suggested, however, that this pathway may be an effect of experimental conditions (18-20).

Basal-lateral sorting motifs are located in the cytoplasmic domain of proteins and frequently contain either a tyrosine residue in the amino acid sequence NPXY or YXXØ (X is any amino acid, and Ø is an amino acid with bulky hydrophobic group), or a dihydrophobic di-leucine motif (21). Basal-lateral sorting signals interact with clathrin adaptor complexes AP1, AP2, AP3 or AQP4. AP1B, which is localized in the recycling endosome, recognizes tyrosine-based basal-lateral sorting motifs through its µ1B subunit, and absence of APµ1B in LLC-PK1 cells results in mis-localization of the basal-lateral LDL-receptor to the apical plasma membrane (22, 23). Following exit from the TGN, some proteins such as E-cadherin, vesicular stomatitis virus glycoprotein (VSV-G), CD147 and Transferrin Receptor (TfR) pass through the recycling endosome *en route* to the plasma membrane in a

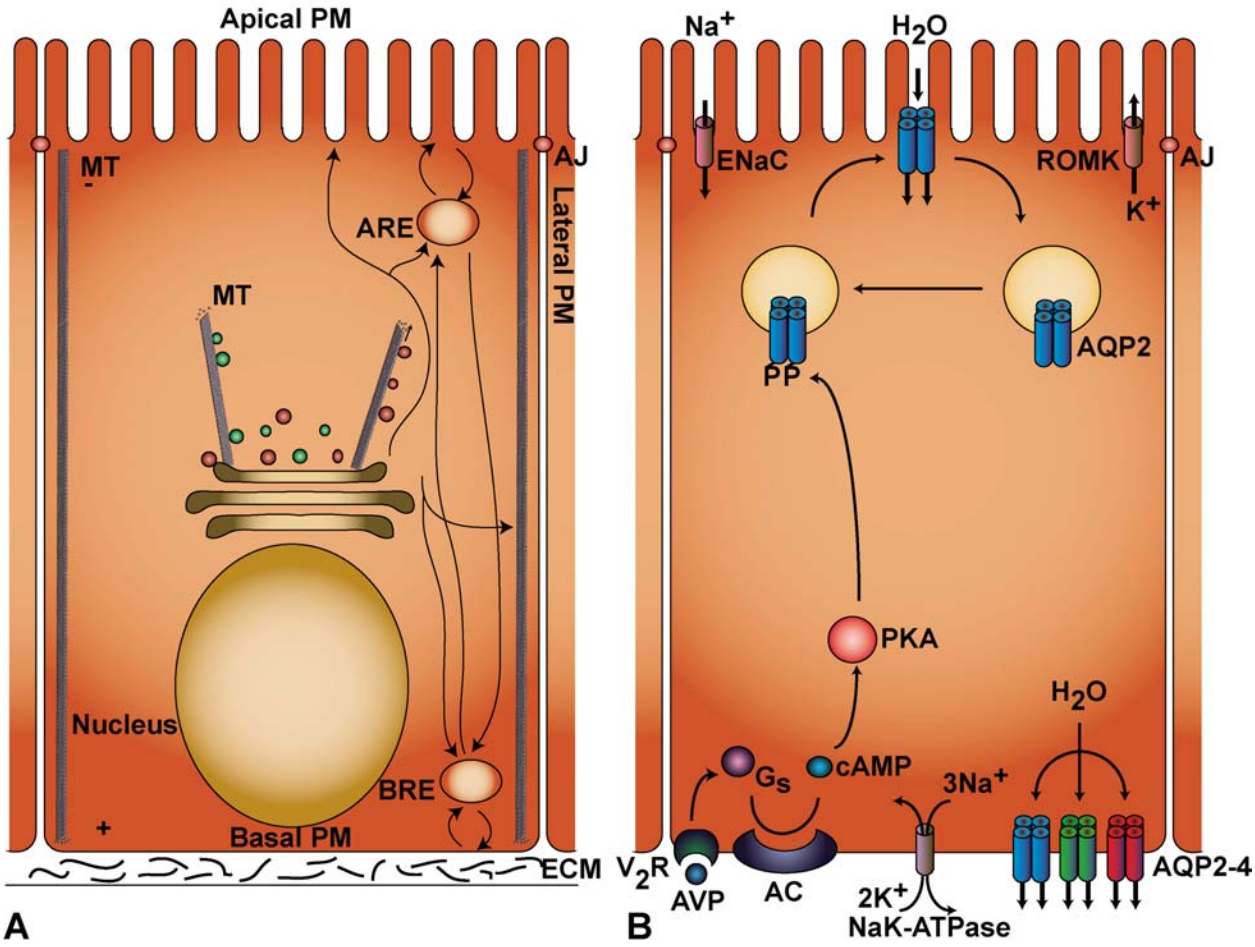

Figure 1. (A) Schematic representation of a polarized epithelial cell. The adherens junction (AJ). is localized at the boundary between the apical and basal-lateral plasma membranes (PM). The apical PM faces the lumen, the lateral PM adheres to the adjacent cell and the basal PM adheres to the extracellular matrix (ECM). The schematic shows microtubule (MT) organization and regulation of vesicle transport for apical and basal-lateral proteins, either along microtubules from the Golgi or via endocytic pathways to the apical recycling endosome (ARE) or basolateral recycling endosome (BRE). (B) Schematic example of polarized epithelial cell; a cortical collecting duct principal cell. NaK-ATPase, which is the driving force for the transepithelial salt and water movement, is localized on the basal-lateral plasma membrane, where it pumps out Na^+ that enter the cell through apical Na channels (ENaC). K^+, which are pumped into the cell by NaK-ATPase activity, exit the cell via the apical ROMK channel. This creates an osmotic gradient, which facilitates the transepithelial movement of water. Water enters the collecting duct principal cells via apical AQP2 and exits via basal-lateral AQP2, AQP3 and AQP4. Without the stimulation of the antidiuretic hormone vasopressin (AVP), the majority of AQP2 is localized to intracellular vesicles, and hence the collecting duct water permeability is low. Water permeability is increased by binding of vasopressin to the vasopressin receptor type 2 (V_2R) on the basal-lateral plasma membrane, which activate G_s proteins, which in turn activate adenylate cyclase leading to increased levels of cyclic AMP and activation of protein kinase A (PKA). PKA phosphorylates AQP2 at the C-terminal thereby inducing regulated exocytosis of AQP2 containing vesicles, insertion of AQP2 into the apical plasma membrane, resulting in instantaneous increased water reabsorption and urine concentration. Upon AVP removal, AQP2 is endocytosis, resulting in decreased water reabsorption. Note: this is not a complete schematic of all receptors and transporters of a collecting duct principal cell.

μ1B dependent manner (24-27). On the other hand, LDL-R is delivered from the TGN directly to the plasma membrane, but is dependent on APμ1B in the recycling endosome for recycling (25). In contrast to these basal-lateral membrane proteins, Na/K-ATPase accumulates in the basal-lateral membrane independently of AP1, and may be regulated by interactions with the membrane-cytoskeleton (23, 28). Hence, there appears to be multiple routes and regulatory pathways from the TGN to the basal-lateral plasma membrane.

Recycling endosomes contain both apical and basal-lateral membrane proteins but are able to separate proteins into different sub-domains, presumably based on differences in their sorting motifs, to ensure delivery to the correct plasma membrane domain (29). Interestingly, the biosynthetic route of the μ1B dependent basal-lateral

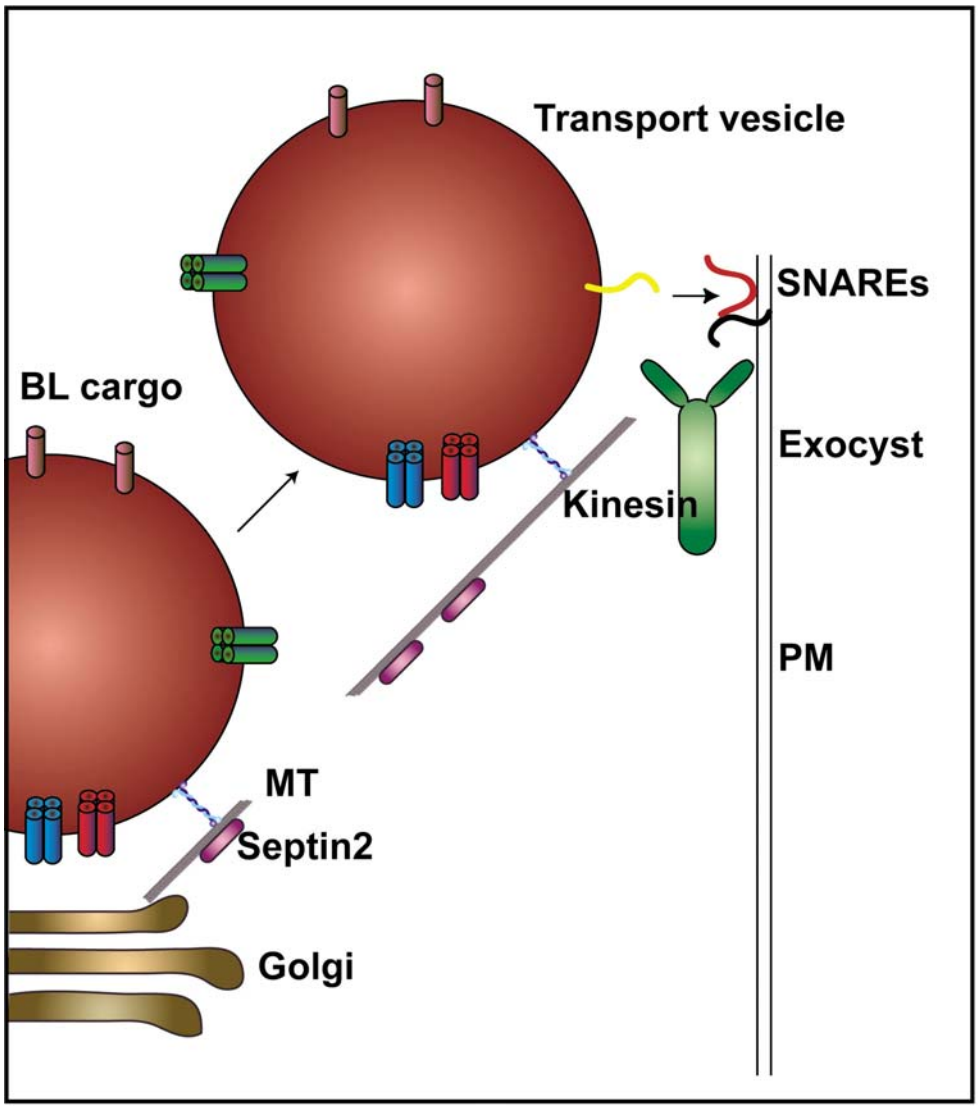

Figure 2. Schematic representation of the biosynthetic route of basal-lateral proteins from the Golgi to the lateral PM. Post-Golgi carriers containing basal-lateral cargo leave the trans Golgi network and travel via MTs to the lateral PM just beneath the AJ. Kinesins are thought to facilitate vesicle movement along polyglutaminated microtubules which bind to Septin2. At the lateral PM, vesicles are tethered by the Exocyst complex, followed by subsequent docking and fusion through the SNARE complex.

proteins changes upon cell polarization. Newly synthesized VSVG and TfR pass through the recycling endosomes in a μ1B dependent manner in non-polarized cells, but seem to be independent of μ1B containing recycling endosomes in polarized cell monolayers (26). Hence, μ1B containing recycling endosomes may be less important in the biosynthetic pathway in polarized epithelia, and more important in recycling.

Vesicle delivery to different membrane domains appears to involve microtubule-based trafficking. Vesicles carrying apical and basal-lateral cargo appear to exit the TGN along microtubule tracks composed of polyglutamylated tubulin (30). Septin 2 binding to polyglutaminated tubulin inhibits binding of the microtubule-associated protein 4 to those microtubules, thereby generating fast tracks for vesicle delivery to the cell surface (Figure 2). In the absence of Septin 2, vesicle delivery to the cell surface is decreased which results in a loss of cell surface polarity, but not tight junction function, and inhibits morphogenesis of a columnar epithelium (30). Microtubule plus ends have been shown to extend towards adherens junctions in a dynein-dependent manner, where they appear to function as tracks for vesicles carrying newly synthesized basal-lateral cargo from the TGN to the plasma membrane (Figure 2) (31, 32). Microtubule plus ends have also been shown to extend into the apical plasma membrane domain, and the kinesin KIF5B is involved in transport of the apical protein p75 to the apical plasma membrane domain in polarized MDCK cells (33). Interestingly, apical transport of p75 in non-polarized MDCK cells is KIF5B independent indicating that cell

polarization induces changes in mechanisms involved in both apical and basal-lateral membrane protein trafficking in the biosynthetic pathway (33).

At the plasma membrane, vesicles dock and fuse through specific apical and basal-lateral complexes of soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) proteins (Figure 2) (for review see (34)). Prior to fusion with the plasma membrane, basal-lateral cargo vesicles are thought to be initially tethered to the lateral plasma membrane by a multiprotein complex termed the Exocyst complex, also named the Sec6/8 complex (Figure 2). Different t-SNAREs have been localized to the apical (syntaxin-3) and basal-lateral membranes (syntaxin-4) (35). Significantly, mis-targeting of syntaxin-3 to the lateral plasma membrane leads to compensatory mis-targeting of apical proteins to that surface, disruption of tight junction formation and inability to form a polarized monolayer (36). It is unclear how the t-SNARE complex is localized to the basal-lateral membrane domain. The mammalian homolog of lethal giant larvae (Mlgl) has been shown to interact with both syntaxin-4 and SNAP23 in contact naive MDCK cells (37). In yeast, the Mlgl homolog is essential for the fusion of exocytic vesicles with the plasma membrane (38). Moreover, the yeast homologs of Lgl, Scro7p and Scro77p, directly interact with the yeast Exocyst component Exo84, and therefore Mlgl may serve as a link between the Exocyst and the SNARE complex (39).

In polarized cells, the Exocyst is localized to the apex of the lateral plasma membrane in the region of the adherens junction, and in an intracellular compartment which could be recycling endosomes (40-42). In non-polarized single epithelial cells, the Exocyst is localized in the cytoplasm, and to the leading edge of migrating cells (43, 44). The Exocyst complex is an eight subunit complex (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84) that is highly conserved between different organisms (45). Quick-freeze/deep-etch electron microscopy of the purified rat brain Exocyst complex showed that it comprises two "arms" approximately 6 nm in width and 15 nm in length that radiate from a more globular body that is 30 nm long and 13 nm wide; the "arm" morphology and the large size of the complex suggest that it may form a bridge between the plasma membrane or cytoskeleton and transport vesicles, which have an average diameter of 84nm (VSV-G); interestingly, Exo70 is an extended rod, 155 Å, 35 Å wide, principally comprised of α-helices (45-47). Inhibition of the Exocyst with function-blocking antibodies resulted in a decrease in basal-lateral, but not apical protein delivery to the plasma membrane in permeabilized polarized MDCK cells; this result indicates that the Exocyst is specific for basal-lateral cargo vesicles, and that it is up-stream of the SNARE complex in vesicle docking and fusion with the plasma membrane (40).

The Exocyst co-immunoprecipitates with microtubules, and may contribute to regulating microtubule organization at the adherens junctions. Also, the Exocyst co-immunoprecipitates with four different Septins, including Septin 2 (also known as Nedd5) (45). Septin 2 organization at the adherens junction is regulated by IQGAP, which also co-localizes with the Exocyst and Cdc42 (48, 49).

The Exocyst may have additional functions in epithelial cells. Exocyst subunits are localized to early endosomes, transferrin-positive common recycling endosomes, and Rab11a-positive apical recycling endosomes of polarized epithelial cells (42, 50). Evidence suggests a role in the recycling endosome, since expression of APμ1B enhances recruitment of the two subunits Sec8 and Exo70 to the recycling endosome (41). The Exocyst also appears to be required for apical and basal-lateral protein recycling, and basal-lateral-to-apical transcytosis (50). Finally, the Exocyst subunit Exo70 also interacts with the Arp2/3 complex, a key regulator of actin dynamics, at the leading edge of migrating cells; here, the Exocyst and Arp2/3 may be involved in coordinating membrane traffic and cytoskeleton dynamics during cell migration (44).

4. INITIAL ESTABLISHMENT OF CELL POLARITY

What are the spatial cues required to establish epithelial cell polarity? Single MDCK cells in suspension culture, in which there is neither contact with the extracellular matrix or other cells, have little evidence of cell surface polarity (51). However, when these cells are allowed to form cell-cell contacts, apical and basal-lateral membrane proteins accumulate correctly on the free (apical) and bounded (lateral) cell surfaces. Significantly, the tight junction protein ZO-1 localizes along the lateral plasma membrane not at a point between the "apical" and "lateral" plasma membrane. It seems therefore that an external cue associated with cell-cell interaction is needed for cells to generate initial apical-basal polarity.

Contact between two single epithelial cells is initiated by lamellipodia that extend from the cells and involves opposing cell surfaces interactions between the Ca++-dependent cell-cell adhesion protein, E-cadherin (Figure 3B; (52)). In fully polarized cells, E-cadherin is localized on the lateral plasma membrane, and in some cell-types is concentrated at the apex of the lateral membrane below the tight junction (adherens junction). E-cadherin mediated cell-cell adhesion is essential for normal development of epithelia. Genetic deletion of E-cadherin inhibits formation of the trophectoderm, a transporting epithelium that surrounds the blastocoel and inner cell mass, and results in preimplantation death (53).

Upon initial cell-cell contact formation, E-cadherin rapidly accumulates at contact points (52). As cell-cell adhesion progresses, E-cadherin localize along the contact with the highest concentrations at the edges (52). Although the cortical actin bundle is disrupted and reorganized underneath cell-cell contacts, the remainder of the bundle around the non-contacting region of the plasma membrane appears to extend into the edges of the contacts; acto-myosin contraction drives the completion of contact formation and the highest levels of activated myosin II localize to the edges of the expanding cell-cell contact (54).

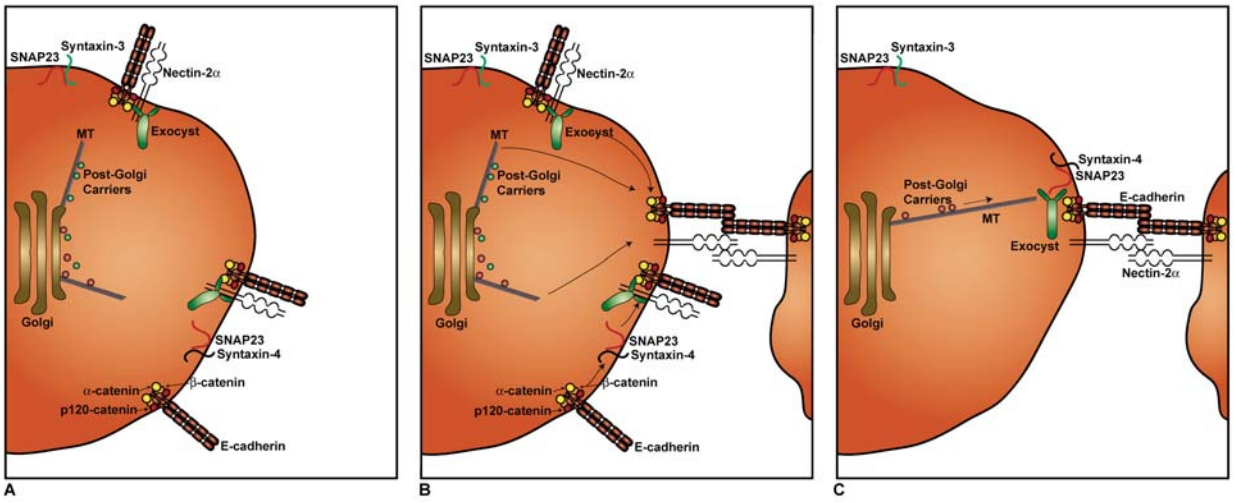

Figure 3. (A) Schematic representation of a single non-polarized epithelial cell. In a single cell, a subset of E-cadherin is found in a complex with Nectin-2α and components of the Exocyst complex. It is unknown if the Exocyst complex is fully assembled and functional in single cells. β-catenin binds to E-cadherin and α-catenin to β-catenin. P120-catenin binds to the juxtamembrane domain of E-cadherin. Apical (syntaxin-3 and SNAP23) and basal-lateral (syntaxin-4 and SNAP23) SNARE complexes are present in separate microclusters at the plasma membrane. Post-Golgi carriers leave the Golgi via microtubules (MT), and basal-lateral carriers predominantly fuse and dock with the "basal" plasma membrane. (B) Upon cell-cell adhesion, E-cadherin and Nectin-2α homodimers form trans-interactions with E-cadherin and Nectin-2α homodimers from the opposing cell, respectively. This interaction initiates the recruitment of microtubules, the Exocyst complex and the basal-lateral SNARE complex to the forming cell-cell contact. The localization of the apical SNARE complex during initial cell-cell adhesion is unknown, but as the apilca SNARE complex localizes to the apical, unbound plasma membrane in polarized cells (35), we hypothesize that the apical SNARE complex is not involved in initial cell-cell adhesion (C) Following cell-cell contact formation, microtubules extend into the contact, and post-Golgi carriers carrying basal-lateral cargo travel via microtubules to the forming contact. At the forming contact, the Exocyst and SNARE complexes are fully functional in mediating docking and fusion of basal-lateral post-Golgi carriers.

The intracellular domain of E-cadherin binds to β-catenin and p120-catenin. β-catenin binds dynamically to monomeric α-catenin; in contrast, α-catenin dimers bind poorly to the E-cadherin-β-catenin complex, but strongly to the actin cytoskeleton (55, 56). It is thought that E-cadherin accumulation at the cell-cell contact increases the local concentration of α-catenin such that α-catenin monomers that dissociate from the E-cadherin/β-catenin complex dimerize in the cytoplasm and bind actin filaments, thereby stabilizing actin at the lateral plasma membrane (55, 56). p120-catenin is involved in regulating E-cadherin endocytosis and stability (for review see (57)).

Recently, it was directly tested if cell surface polarity is established during initial cell-cell contact between two single MDCK cells (43). The distributions of two structurally and functionally homologous proteins, AQP5 and AQP3 which localize to the apical and basal-lateral membrane, respectively, were examined during initial cell-cell adhesion. AQP3 accumulated at the cell-cell contact within minutes following the initiation of E-cadherin mediated cell-cell adhesion, and thereafter co-accumulated and co-localized with E-cadherin throughout the process of cell-cell adhesion (43). In striking contrast, the apical AQP5 did not accumulate or localize with E-cadherin during cell-cell adhesion, indicating that recruitment of AQP3 to cell-cell contacts was specific for basal-lateral, but not the apical membrane AQP5 (43). Significantly, a pool of newly-synthesized AQP3 tagged with photo-activated GFP and activated in the Golgi was directly inserted into the plasma membrane at initial cell-cell contacts, indicating that AQP3 accumulation was due to recruitment of exocytic vesicles to the plasma membrane (43).

5. ESTABLISHMENT OF THE LATERAL TARGETING PATCH UPON INITIAL CELL-CELL ADHESION

The spatio-temporally coordinated re-organization of AQP3 and E-cadherin during initial cell-cell adhesion appears to be due to the delivery of newly synthesized AQP3 to the forming cell-cell contact. This mechanism implies that components involved in basal-lateral sorting in fully polarized cells, including microtubules, the Exocyst and SNARE complexes (collectively termed "the lateral targeting patch") also become oriented towards, and organized at initial cell-cell contacts. Since it has not been possible to GFP-tag mammalian Exocyst complex or syntaxin-4 so that they retain function and localization, the localization of components of the lateral targeting patch upon initial cell-cell adhesion has been examined in fixed cells. The Exocyst complex localizes in the cytoplasm in single epithelial cells (40, 43). As soon as the cell-cell contact was initiated, Exocyst components were observed on the plasma

membrane within the contact formed by E-cadherin (Figure 3B). As the contact expanded, increasing amounts of the Exocyst localized to the plasma membrane at the forming contact, and when the contact was complete the Exocyst was exclusively localized to the contact with E-cadherin (43). The Exocyst was fully functional at the initial cell-cell contact, as injection of function-blocking Sec8 antibodies abolished insertion of newly synthesized AQP3 into the plasma membrane at the forming contact (43).

It is unknown how the Exocyst is recruited to the initial contacts between two single cells, or localized to the apical junctional complex in polarized cells (40). In MDCK cell monolayers, Exocyst components are recruited to cell-cell contacts with a mixture of junctional proteins; E-cadherin and nectin-2α, a member of the Ig-superfamily of adhesion proteins, are in a complex with the Exocyst in polarized cells and may be directly involved in the recruitment of the Exocyst to the plasma membrane (58). Following maturation of cell-cell adhesions, the Exocyst may be sorted to the apex of the lateral membrane with components of tight junction and nectin complexes (58). In single cells prior to cell-cell contact, the Exocyst component Sec6 and ZO-1 co-localize in the cytosol (LN Nejsum, unpublished results) (Figure 3A). Moreover, Sec6 and ZO-1 co-localize at all stages of initial cell-cell adhesion between two single cells (LN Nejsum, unpublished results). Hence, Exocyst components and components of the tight junction complex may travel to the plasma membrane by the same transport mechanism. Studies have shown that the Exo70 subunit of the Exocyst is involved in this recruitment and anchoring of the Exocyst to the plasma membrane. The Rho protein TC10 promotes the recruitment of Exo70 to the plasma membrane in 3T3 adipocytes, and may involve a direct interaction of the Exo70 subunit with phosphatidylinositol 4,5-biphosphates mediated by the positively charged C-terminus of Exo70 (59, 60).

Even less is known about how the Exocyst is anchored to the plasma membrane. The Exo70 subunit of the Exocyst may be involved as expression of a dominant-negative form of Exo70 mislocalized Sec8 from the plasma membrane localization to an intracellular localization in HeLa cells (60). Alternatively, an Arf-like3 GTPase (dnd) may play a role as expression of a dnd mutant resulted in mislocalization of Sec5 in Drosophila embryonic fusion cells (61). In yeast, the Rab GTPase Sec4 is involved in recruitment of the Exocyst to the plasma membrane through interaction with the Sec15 subunit, and GTP-bound forms of Rho1 and Cdc42 directly interact with, and are essential for correct localization of the Exocyst (62-64). Also in yeast, Sec3 directly interacts with the GTP-bound form of Cdc42 and phosphatidylinositol 4,5-biphosphate, and this is essential for exocytosis and Exocyst localization (65). It is unknown whether these mechanisms are important in regulating Exocyst distribution in mammalian epithelial cells.

In single MDCK cells, both the apical (syntaxin-3) and basal-lateral (syntaxin-4) t-SNAREs localize to the plasma membrane on both the free (apical) surface and plasma membrane adhering to the extracellular matrix (Figure 3A) (66). Syntaxin-3 and syntaxin-4 assemble into separate microdomains distributed over the whole plasma membrane, and SNAP-23 (a component of both the apical and basal-lateral SNARE complexes) localized to these microclusters (66). It is unknown how and when SNARE complexes are transported to and maintained at their respective plasma membrane domain upon initial cell-cell adhesion. Syntaxin- 4 co-localized with E-cadherin at initial cell-cell contacts (Figure 3C), and this localization appeared spatio-temporally similar to that of E-cadherin at different stages of cell-cell adhesion (43). Moreover, disruption of the SNARE complex by injection of tetanus toxin, which cleaves the vesicular SNARE VAMP3/Cellubrevin, abolished AQP3 insertion into the plasma membrane at the forming contact, showing that the lateral SNARE complex, like the Exocyst, is functional at the forming contact (43). That the Exocyst complex and syntaxin 4 have different distributions in single cells and differ in their arrival at the initial cell-cell contact, indicates that these two components of the lateral targeting patch have different mechanisms for localization to the site of initial cell-cell contact. This was confirmed in studies in which one of the two complexes was disrupted and the effect on the other monitored: syntaxin-4 was recruited to the lateral plasma membrane in cells in which Exocyst distribution had been disrupted by micro-injection of a function-blocking antibody against the Exocyst subunit Sec8, and disruption of the basal-lateral SNARE complex by injection of tetanus toxin, which cleaves the v-SNARE cellubrevin/VAMP3, did not affect localization of Exocyst components to the lateral plasma membrane (43). In addition, both the Exocyst and SNARE complexes localized normally to the lateral plasma membrane in the presence of nocodazole, which disrupts microtubules (43). Taken together, these results show that components of the lateral targeting patch localize independently of each other to the lateral plasma membrane, but are required to function sequentially as a 'holo-complex' to mediated AQP3 (basal-lateral) vesicle delivery, tethering and fusion with the plasma membrane at cell-cell contacts (Figure 1C).

The integrity of the apical and basal-lateral SNARE clusters seems to depend on different mechanisms, as syntaxin-3 clusters are microtubule-dependent whereas syntaxin-4 clusters are dependent on intact actin filaments (66). Microtubule disruption also interferes with syntaxin-3 localization in polarized cells, but disruption of microtubules did not interfere with syntaxin-4 localization to the forming cell-cell contact (43, 67). Since syntaxin-4 interacts with actin filaments *in vitro*, the finding that syntaxin-4 clustering in single non-polarized cells is dependent on intact actin filaments may indicate that F-actin is involved in the anchoring of syntaxin-4 to the lateral plasma membrane in polarized epithelia and to the initial cell-cell contact of adhering cells. However, it remains unknown how syntaxin-4 is targeted to the site of initial cell-cell adhesion (68).

While post-translational mechanisms appear to play a significant role in early stages involved in cell polarization upon initial cell-cell adhesion, transcriptional

regulation of gene expression patterns may be involved in subsequent stages of cell polarization. Gene array analysis at different time points following initial cell-cell adhesion of Caco-2 epithelial cells grown on filter supports showed major transcriptional changes in genes that encode proteins involved in many structural and functional characteristics of epithelial cell polarization that resembled those of differentiating intestinal enterocytes (69). Following initiation of cell polarization, the expression of genes involved in cell proliferation was down-regulated, whereas genes involved in cell polarization including components of the apical junctional complex and tight junctions increased. Significantly, components of the apical trafficking machinery were increased indicating a change in the apical transport pathway upon polarization. However, expression of components of the basolateral trafficking machinery remained relatively constant, which is consistent with the finding that these components are already present in a single epithelial cell before cell-cell adhesion (43, 69).

6. CONCLUSIONS

Establishment and maintenance of epithelial cell polarity is essential for tissue development and correct function of transporting epithelia. Initial cell-cell adhesion between epithelial cells appears to be the spatial cue that initiates epithelial cell-surface polarity, both at the post-translational and transcriptional levels. Initial cell-cell adhesion marks the site of adhesion for establishment of the "lateral targeting patch" that specifies the delivery of basal-lateral vesicles. The lateral targeting patch consists of the Exocyst and the t-SNARE complexes comprising syntaxin-4, which together immediately function at the forming cell-cell contact to dock and fuse basal-lateral vesicles at the plasma membrane, but also to exclude delivery of apical vesicles to those sites.

The subsequent maintenance of correct membrane protein distributions in fully polarized cells is essential for correct epithelial function, and defects in protein regulation and localization are the cause of metabolic diseases. However, it is largely unknown how complexes such as the lateral targeting patch regulate the organization of ion transporters, channels and pumps to the plasma membrane. It is important to gain a better understanding of the sorting pathways and molecular mechanisms responsible for correct sorting and organization of these membrane proteins. A key question is how these pathways regulate the number and composition of ion channels and transporters on the plasma membrane in order to maintain proper ionic homeostasis.

7. ACKNOWLEDGEMENTS

Work in the Nelson laboratory is supported by funds from the NIH (GM35527), and LN Nejsum was additionally supported by post doctoral fellowships from the Danish Medical Research Council and the Lundbeckfoundation.

8. REFERENCES

1. Nejsum, L. N., T. H. Kwon, D. Marples, A. Flyvbjerg, M. A. Knepper, J. Frokiaer & S. Nielsen: Compensatory increase in AQP2, p-AQP2, and AQP3 expression in rats with diabetes mellitus. *Am J Physiol Renal Physiol*, 280, F715-F726 (2001)

2. Kwon, T. H., J. Frokiaer, P. Fernandez-Llama, M. A. Knepper & S. Nielsen: Reduced abundance of aquaporins in rats with bilateral ischemia-induced acute renal failure: prevention by alpha-MSH. *Am J Physiol*, 277, F413-27 (1999)

3. Kwon, T. H., J. Frokiaer, M. A. Knepper & S. Nielsen: Reduced AQP1, -2, and -3 levels in kidneys of rats with CRF induced by surgical reduction in renal mass. *Am.J.Physiol*, 275, F724-F741 (1998)

4. Kwon, T. H., J. Frokiaer, P. Fernandez-Llama, A. B. Maunsbach, M. A. Knepper & S. Nielsen: Altered expression of Na transporters NHE-3, NaPi-II, Na-K-ATPase, BSC-1, and TSC in CRF rat kidneys. *Am.J.Physiol*, 277, F257-F270 (1999)

5. Li, C., J. D. Klein, W. Wang, M. A. Knepper, S. Nielsen, J. M. Sands & J. Frokiaer: Altered expression of urea transporters in response to ureteral obstruction. *Am J Physiol Renal Physiol*, 286, F1154-62 (2004)

6. Li, C., W. Wang, M. A. Knepper, S. Nielsen & J. Frokiaer: Downregulation of renal aquaporins in response to unilateral ureteral obstruction. *Am J Physiol Renal Physiol*, 284, F1066-79 (2003)

7. Li, C., W. Wang, T. H. Kwon, M. A. Knepper, S. Nielsen & J. Frokiaer: Altered expression of major renal Na transporters in rats with unilateral ureteral obstruction. *Am J Physiol Renal Physiol*, 284, F155-66 (2003)

8. Deen, P. M., M. A. Verdijk, N. V. Knoers, B. Wieringa, L. A. Monnens, C. H. van-Os & B. A. van-Oost: Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science*, 264, 92-95 (1994)

9. Zabner, J., J. J. Smith, P. H. Karp, J. H. Widdicombe & M. J. Welsh: Loss of CFTR chloride channels alters salt absorption by cystic fibrosis airway epithelia *in vitro*. *Mol Cell*, 2, 397-403 (1998)

10. Koivisto, U. M., A. L. Hubbard & I. Mellman: A novel cellular phenotype for familial hypercholesterolemia due to a defect in polarized targeting of LDL receptor. *Cell*, 105, 575-85 (2001)

11. Nielsen, S., S. R. DiGiovanni, E. I. Christensen, M. A. Knepper & H. W. Harris: Cellular and subcellular immunolocalization of vasopressin- regulated water channel in rat kidney. *Proc.Natl.Acad.Sci.U.S.A.*, 90, 11663-11667 (1993)

12. Nielsen, S., C. L. Chou, D. Marples, E. I. Christensen, B. K. Kishore & M. A. Knepper: Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin-CD water channels to plasma membrane. *Proc.Natl.Acad.Sci.U.S.A.*, 92, 1013-1017 (1995)

13. Terris, J., C. A. Ecelbarger, S. Nielsen & M. A. Knepper: Long-term regulation of four renal aquaporins in rats. *Am.J.Physiol*, 271, F414-F422 (1996)

14. Yamamoto, T., S. Sasaki, K. Fushimi, K. Kawasaki, E. Yaoita, K. Oota, Y. Hirata, F. Marumo & I. Kihara: Localization and expression of a collecting duct water channel, aquaporin, in hydrated and dehydrated rats. *Journal of experimental nephrology*, 3, 193-201 (1995)

15. Yoshimori, T., P. Keller, M. G. Roth & K. Simons: Different biosynthetic transport routes to the plasma membrane in BHK and CHO cells. *J.Cell Biol.*, 133, 247-256 (1996)

16. Rodriguez-Boulan, E., G. Kreitzer & A. Musch: Organization of vesicular trafficking in epithelia. *Nat.Rev.Mol.Cell Biol.*, 6, 233-247 (2005)

17. Lisanti, M. P., I. W. Caras, M. A. Davitz & E. Rodriguez-Boulan: A glycophospholipid membrane anchor acts as an apical targeting signal in polarized epithelial cells. *J.Cell Biol.*, 109, 2145-2156 (1989)

18. Paladino, S., D. Sarnataro, R. Pillich, S. Tivodar, L. Nitsch & C. Zurzolo: Protein oligomerization modulates raft partitioning and apical sorting of GPI-anchored proteins. *J.Cell Biol.*, 167, 699-709 (2004)

19. Polishchuk, R., A. Di Pentima & J. Lippincott-Schwartz: Delivery of raft-associated, GPI-anchored proteins to the apical surface of polarized MDCK cells by a transcytotic pathway. *Nat.Cell Biol.*, 6, 297-307 (2004)

20. Paladino, S., T. Pocard, M. A. Catino & C. Zurzolo: GPI-anchored proteins are directly targeted to the apical surface in fully polarized MDCK cells. *J.Cell Biol.*, 172, 1023-1034 (2006)

21. Le Bivic, A., Y. Sambuy, A. Patzak, N. Patil, M. Chao & E. Rodriguez-Boulan: An internal deletion in the cytoplasmic tail reverses the apical localization of human NGF receptor in transfected MDCK cells. *J.Cell Biol.*, 115, 607-618 (1991)

22. Gan, Y., T. E. McGraw & E. Rodriguez-Boulan: The epithelial-specific adaptor AP1B mediates post-endocytic recycling to the basolateral membrane. *Nat.Cell Biol.*, 4, 605-609 (2002)

23. Folsch, H., H. Ohno, J. S. Bonifacino & I. Mellman: A novel clathrin adaptor complex mediates basolateral targeting in polarized epithelial cells. *Cell*, 99, 189-198 (1999)

24. Ang, A. L., T. Taguchi, S. Francis, H. Folsch, L. J. Murrells, M. Pypaert, G. Warren & I. Mellman: Recycling endosomes can serve as intermediates during transport from the Golgi to the plasma membrane of MDCK cells. *J.Cell Biol.*, 167, 531-543 (2004)

25. Cancino, J., C. Torrealba, A. Soza, M. I. Yuseff, D. Gravotta, P. Henklein, E. Rodriguez-Boulan & A. Gonzalez: Antibody to AP1B adaptor blocks biosynthetic and recycling routes of basolateral proteins at recycling endosomes. *Mol Biol Cell*, 18, 4872-84 (2007)

26. Gravotta, D., A. Deora, E. Perret, C. Oyanadel, A. Soza, R. Schreiner, A. Gonzalez & E. Rodriguez-Boulan: AP1B sorts basolateral proteins in recycling and biosynthetic routes of MDCK cells. *Proc Natl Acad Sci U S A*, 104, 1564-9 (2007)

27. Deora, A. A., D. Gravotta, G. Kreitzer, J. Hu, D. Bok & E. Rodriguez-Boulan: The basolateral targeting signal of CD147 (EMMPRIN) consists of a single leucine and is not recognized by retinal pigment epithelium. *Mol Biol Cell*, 15, 4148-65 (2004)

28. Nelson, W. J. & P. J. Veshnock: Ankyrin binding to (Na+ + K+) ATPase and implications for the organization of membrane domains in polarized cells. *Nature*, 328, 533-6 (1987)

29. Thompson, A., R. Nessler, D. Wisco, E. Anderson, B. Winckler & D. Sheff: Recycling endosomes of polarized epithelial cells actively sort apical and basolateral cargos into separate subdomains. *Mol Biol Cell*, 18, 2687-97 (2007)

30. Spiliotis, E. T., S. J. Hunt, Q. Hu, M. Kinoshita & W. J. Nelson: Epithelial polarity requires septin coupling of vesicle transport to polyglutamylated microtubules. *J Cell Biol*, 180, 295-303 (2008)

31. Ligon, L. A. & E. L. Holzbaur: Microtubules tethered at epithelial cell junctions by dynein facilitate efficient junction assembly. *Traffic*, 8, 808-19 (2007)

32. Shaw, R. M., A. J. Fay, M. A. Puthenveedu, M. von Zastrow, Y. N. Jan & L. Y. Jan: Microtubule plus-end-tracking proteins target gap junctions directly from the cell interior to adherens junctions. *Cell*, 128, 547-60 (2007)

33. Jaulin, F., X. Xue, E. Rodriguez-Boulan & G. Kreitzer: Polarization-dependent selective transport to the apical membrane by KIF5B in MDCK cells. *Dev Cell*, 13, 511-22 (2007)

34. Jahn, R. & R. H. Scheller: SNAREs--engines for membrane fusion. *Nat Rev Mol Cell Biol*, 7, 631-43 (2006)

35. Li, X., S. H. Low, M. miura & T. Weimbs: SNARE expression and localization in renal epithelial cells suggest mechanism for variability of trafficking phenotypes. *Am.J.Physiol Renal Physiol*, 283, F1111-F1122 (2002)

36. Sharma, N., S. H. Low, S. Misra, B. Pallavi & T. Weimbs: Apical targeting of syntaxin 3 is essential for epithelial cell polarity. *J Cell Biol*, 173, 937-48 (2006)

37. Musch, A., D. Cohen, C. Yeaman, W. J. Nelson, E. Rodriguez-Boulan & P. J. Brennwald: Mammalian homolog of Drosophila tumor suppressor lethal (2) giant larvae interacts with basolateral exocytic machinery in Madin-Darby canine kidney cells. *Mol Biol Cell*, 13, 158-68 (2002)

38. Lehman, K., G. Rossi, J. E. Adamo & P. Brennwald: Yeast homologues of tomosyn and lethal giant larvae function in exocytosis and are associated with the plasma membrane SNARE, Sec9. *J Cell Biol*, 146, 125-40 (1999)

39. Zhang, X., P. Wang, A. Gangar, J. Zhang, P. Brennwald, D. TerBush & W. Guo: Lethal giant larvae proteins interact with the exocyst complex and are involved in polarized exocytosis. *J Cell Biol*, 170, 273-83 (2005)

40. Grindstaff, K. K., C. Yeaman, N. Anandasabapathy, S. C. Hsu, E. Rodriguez-Boulan, R. H. Scheller & W. J. Nelson: Sec6/8 complex is recruited to cell-cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell*, 93, 731-740 (1998)

41. Folsch, H., M. Pypaert, S. Maday, L. Pelletier & I. Mellman: The AP-1A and AP-1B clathrin adaptor complexes define biochemically and functionally distinct membrane domains. *J.Cell Biol.*, 163, 351-362 (2003)

42. Yeaman, C., K. K. Grindstaff, J. R. Wright & W. J. Nelson: Sec6/8 complexes on trans-Golgi network and plasma membrane regulate late stages of exocytosis in mammalian cells. *J.Cell Biol.*, 155, 593-604 (2001)

43. Nejsum, L. N. & W. J. Nelson: A molecular mechanism directly linking E-cadherin adhesion to initiation of epithelial cell surface polarity. *J Cell Biol*, 178, 323-35 (2007)

44. Zuo, X., J. Zhang, Y. Zhang, S. C. Hsu, D. Zhou & W. Guo: Exo70 interacts with the Arp2/3 complex and regulates cell migration. *Nat Cell Biol*, 8, 1383-8 (2006)

45. Hsu, S. C., A. E. Ting, C. D. Hazuka, S. Davanger, J. W. Kenny, Y. Kee & R. H. Scheller: The mammalian brain rsec6/8 complex. *Neuron*, 17, 1209-19 (1996)

46. Wandinger Ness, A., M. K. Bennett, C. Antony & K. Simons: Distinct transport vesicles mediate the delivery of plasma membrane proteins to the apical and basolateral domains of MDCK cells. *J.Cell Biol.*, 111, 987-1000 (1990)

47. Hamburger, Z. A., A. E. Hamburger, A. P. West, Jr. & W. I. Weis: Crystal structure of the S.cerevisiae exocyst component Exo70p. *J Mol Biol*, 356, 9-21 (2006)

48. Vega, I. E. & S. C. Hsu: The exocyst complex associates with microtubules to mediate vesicle targeting and neurite outgrowth. *J Neurosci*, 21, 3839-48 (2001)

49. Rittmeyer, E. N., S. Daniel, S. C. Hsu & M. A. Osman: A dual role for IQGAP1 in regulating exocytosis. *J Cell Sci*, 121, 391-403 (2008)

50. Oztan, A., M. Silvis, O. A. Weisz, N. A. Bradbury, S. C. Hsu, J. R. Goldenring, C. Yeaman & G. Apodaca: Exocyst requirement for endocytic traffic directed toward the apical and basolateral poles of polarized MDCK cells. *Mol Biol Cell*, 18, 3978-92 (2007)

51. Wang, A. Z., G. K. Ojakian & W. J. Nelson: Steps in the morphogenesis of a polarized epithelium. I. Uncoupling the roles of cell-cell and cell-substratum contact in establishing plasma membrane polarity in multicellular epithelial (MDCK) cysts. *J.Cell Sci.*, 95 (Pt 1), 137-151 (1990)

52. Adams, C. L., Y. T. Chen, S. J. Smith & W. J. Nelson: Mechanisms of epithelial cell-cell adhesion and cell compaction revealed by high-resolution tracking of E-cadherin-green fluorescent protein. *J.Cell Biol.*, 142, 1105-1119 (1998)

53. Larue, L., M. Ohsugi, J. Hirchenhain & R. Kemler: E-cadherin null mutant embryos fail to form a trophectoderm epithelium. *Proc.Natl.Acad.Sci.U.S.A*, 91, 8263-8267 (1994)

54. Yamada, S. & W. J. Nelson: Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell-cell adhesion. *J Cell Biol*, 178, 517-27 (2007)

55. Drees, F., S. Pokutta, S. Yamada, W. J. Nelson & W. I. Weis: Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. *Cell*, 123, 903-915 (2005)

56. Yamada, S., S. Pokutta, F. Drees, W. I. Weis & W. J. Nelson: Deconstructing the cadherin-catenin-actin complex. *Cell*, 123, 889-901 (2005)

57. Reynolds, A. B.: p120-catenin: Past and present. *Biochim Biophys Acta*, 1773, 2-7 (2007)

58. Yeaman, C., K. K. Grindstaff & W. J. Nelson: Mechanism of recruiting Sec6/8 (exocyst) complex to the apical junctional complex during polarization of epithelial cells. *J.Cell Sci.*, 117, 559-570 (2004)

59. Inoue, M., L. Chang, J. Hwang, S. H. Chiang & A. R. Saltiel: The exocyst complex is required for targeting of Glut4 to the plasma membrane by insulin. *Nature*, 422, 629-33 (2003)

60. Liu, J., X. Zuo, P. Yue & W. Guo: Phosphatidylinositol 4,5-bisphosphate mediates the targeting of the exocyst to the plasma membrane for exocytosis in mammalian cells. *Mol Biol Cell*, 18, 4483-92 (2007)

61. Jiang, L., S. L. Rogers & S. T. Crews: The Drosophila Dead end Arf-like3 GTPase controls vesicle trafficking during tracheal fusion cell morphogenesis. *Dev Biol*, 311, 487-99 (2007)

62. Guo, W., D. Roth, C. Walch-Solimena & P. Novick: The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. *Embo J*, 18, 1071-80 (1999)

63. Guo, W., F. Tamanoi & P. Novick: Spatial regulation of the exocyst complex by Rho1 GTPase. *Nat Cell Biol*, 3, 353-60 (2001)

64. Zhang, X., E. Bi, P. Novick, L. Du, K. G. Kozminski, J. H. Lipschutz & W. Guo: Cdc42 interacts with the exocyst and regulates polarized secretion. *J Biol Chem*, 276, 46745-50 (2001)

65. Zhang, X., K. Orlando, B. He, F. Xi, J. Zhang, A. Zajac & W. Guo: Membrane association and functional regulation of Sec3 by phospholipids and Cdc42. *J Cell Biol*, 180, 145-58 (2008)

66. Low, S. H., A. Vasanji, J. Nanduri, M. He, N. Sharma, M. Koo, J. Drazba & T. Weimbs: Syntaxins 3 and 4 are concentrated in separate clusters on the plasma membrane before the establishment of cell polarity. *Mol Biol Cell*, 17, 977-89 (2006)

67. Kreitzer, G., J. Schmoranzer, S. H. Low, X. Li, Y. Gan, T. Weimbs, S. M. Simon & E. Rodriguez-Boulan: Three-dimensional analysis of post-Golgi carrier exocytosis in epithelial cells. *Nat.Cell Biol.*, 5, 126-136 (2003)

68. Band, A. M., H. Ali, M. K. Vartiainen, S. Welti, P. Lappalainen, V. M. Olkkonen & E. Kuismanen: Endogenous plasma membrane t-SNARE syntaxin 4 is present in rab11 positive endosomal membranes and associates with cortical actin cytoskeleton. *FEBS Lett*, 531, 513-9 (2002)

69. Halbleib, J. M., A. M. Saaf, P. O. Brown & W. J. Nelson: Transcriptional modulation of genes encoding structural characteristics of differentiating enterocytes during development of a polarized epithelium *in vitro*. *Mol Biol Cell*, 18, 4261-78 (2007)

Key Words: Polarity, SNARE, Exocyst, Epithelia, Review

Send correspondence to: Lene N. Nejsum, Department of Biology, The James H. Clark Center, The Bio-X Program, 318 Campus Drive (E200-B), Stanford University, Stanford, CA 94305-5430, Tel: 650-725-7553, Fax: 650-498-5286, E-mail: nejsum@stanford.edu