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Origins of Cell Polarity

Review

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Cell polarity is the ultimate reflection of complex mechanisms that establish and maintain functionally specialized domains in the plasma membrane and cytoplasm. The spatial arrangement and protein composition of these domains facilitate cellular processes as diverse as differentiation, localized membrane growth, activation of the immune response, directional cell migration, and vectorial transport of molecules across cell layers.

In this review, two phylogenetically distant eukaryotic cells, budding yeast and mammalian epithelial cells, are examined to highlight advances in our understanding of how cell polarity is established. Both of these cells are characterized by a high degree of cellular asymmetry (see Figure 1) and have been used extensively to study how cell polarity is developed. The specific focus here is on the molecular nature of the intrinsic and extrinsic spatial cues that establish structural and molecular asymmetry at the cell surface, the mechanisms that interpret signals from these cues to generate new membrane domains, and the reorganization of the cell around these spatially defined sites (see Figure 2). The evidence supports a model in which a hierarchy of three sequential stages is required to establish cell polarity from a spatial cue (see Figure 3). This hierarchy consists of the following: marking a site and decoding the cue; reinforcing the cue; propagating the cue. Feedback regulation at each stage coordinates and reinforces the proper ordering of these events, resulting in the maintenance of cell polarity. Each level of this hierarchy is color coded in the figures to facilitate comparisons between yeast and epithelial cells. Because these two cell types are separated by a large phylogenetic distance, the similarity of stages suggests that mechanisms used for imposing cell polarity might be quite general.

Budding Yeast

Saccharomyces cerevisiae displays pronounced cellular asymmetry during its normal growth and division (budding) and as the result of its response to mating pheromone (projection formation) (Figure 1A). Localized plasma membrane growth and cell wall remodeling underlie the formation of buds and mating projections. As a yeast cell initiates a cell cycle, or responds to mating pheromone, its secretory apparatus, its cytoskeleton, and numerous other proteins and organelles become organized anisotropically to facilitate polarized cell growth. The pathway for development of cell polarity during bud formation starts with an intrinsic spatial cue, set up during the previous cell cycle by cortical actin or septin cytoskeletal proteins (Figure 2A). A signaling complex containing at least two RAS-related GTP-binding proteins assembles at the site marked by the cue, then a polarized actin and septin cytoskeleton assembles, and the secretory apparatus becomes oriented toward the spatial cue. Considerable information about bud formation has been obtained during the past several years, and some advances in our understanding of mating projection formation and orientation have also been made recently.

Bud Site Selection Occurs in Response to Intrinsic Spatial Cues

Both genotype and nutritional conditions determine which of three budding patterns yeast cells will adopt. *MAT***a** and *MAT*α cells construct bud sites adjacent to the previous bud site (axial budding pattern), while *MAT***a**/*MAT*α cells bud from sites that are either near the previous bud site or at the opposite end of the cell (bipolar budding pattern) (Chant and Pringle, 1995, and references therein). A yeast cell undergoing pseudohyphal growth always buds from the same pole, namely the pole opposite the original junction with its mother cell (unipolar budding pattern) (Kron et al., 1994). These three budding patterns presumably optimize the evolutionary fitness of yeast growing in the haploid and diploid states, depending on the supply of nutrients (Gimeno and Fink, 1992). Placement of cortical cues for each budding pattern is dependent upon one of two cytoskeletal proteins, septins and actin.

Partial loss-of-function mutations in septins, cytoskeletal proteins that are arranged in a ring at the bud neck and are required for cytokinesis (Sanders and Field, 1994), can cause defects in the axial budding of **a** or α cells (Flescher et al., 1993). This result suggests that septins assembled during the previous cell cycle are a component of the spatial cue for selection of an axial bud site during one cell cycle. Two novel proteins required for axial budding, Bud3 and Bud4, colocalize with the septins, but are lost from the bud neck in septin mutants (Chant et al., 1995; Herskowitz et al., 1995). These observations support the view that septins are required for selection of axial bud sites and suggest further that Bud3 and Bud4 might mark or translate, or do both to, the septin spatial cue. An additional protein required for axial budding, Axl1 (Fujita et al., 1994), has homology to a protease and has features that suggest a role distinct from Bud3 and Bud4. While Bud3 and Bud4 are normally expressed in cells with all budding patterns, Axl1 is expressed only in cells that bud axially. Ectopic *AXL1* expression in **a**/α cells changes budding to the axial pattern (Fujita et al., 1994). Mutations in *AXL1*, *BUD3*, or *BUD4* result in bipolar, not random, budding. The above observations suggest that there is a hierarchy in the response to intrinsic positional cues for bud site selection and that Axl1 is required to recognize the axial cue.

For bipolar budding, positional cues mark the bud tip and mother–daughter neck (Chant and Pringle, 1995). Actin, instead of septins, is important for generation of the bipolar budding pattern. The actin cytoskeleton, a component of the cortical growth apparatus, is concentrated at the bud tip and septum area at different cell

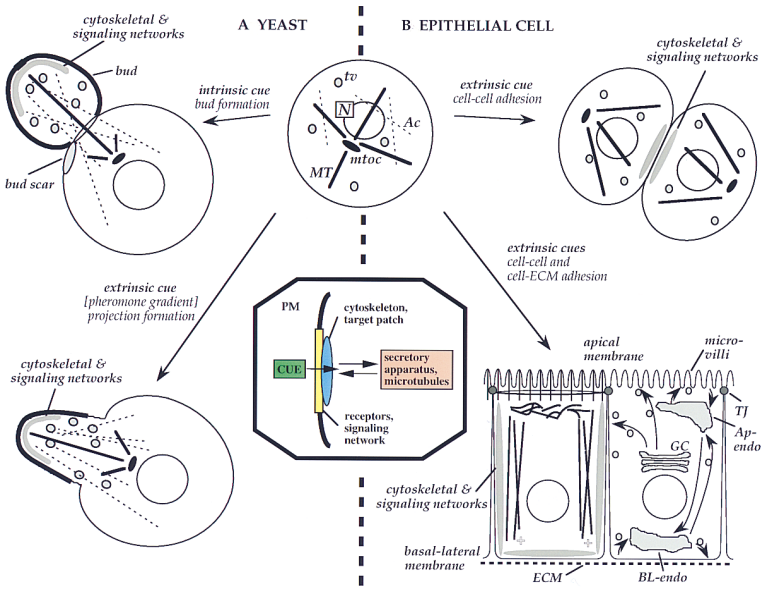

Figure 1. Schematic Representation of the Structural Organization of Budding Yeast and Polarized Epithelial Cells

Cell polarity of both cell types is initiated from a "nonpolarized cell" (top, center) that has a relatively isotropic organization of actin filaments (Ac, dotted lines), microtubules (MT, solid bold lines), microtubule organizing center (mtoc), transport vesicles (tv), and nucleus (N). Establishment of cell polarity requires a hierarchy of stages (middle, center), including the following: a spatial cue; membrane-associated receptors and signaling networks, which mark and interpret the cue; localized assembly of the cytoskeleton and targeting patch, which reinforce the cue; changes in the distribution of microtubules and the secretory apparatus, which propagates the cue to the cell interior (each stage is color coded for comparison with other figures). Cell polarity is manifested somewhat differently in yeast and epithelial cells.

In yeast (A), an intrinsic cue induces assembly of a cytoskeletal and signaling network (shaded) in close proximity to the bud scar (in **a** or α cells, see text), which results in reorganization of the cytoskeleton and the secretory apparatus, leading to bud formation (top, left). Extrinsic cues generated by pheromone gradients result in a similar reorganization of the yeast cell leading to formation of a mating projection (bottom, left).

In epithelial cells (B), extrinsic cues from cell–cell adhesion result in the formation of cytoskeletal and signaling networks at cell contacts resulting in partial reorganization of the cells (top, right). However, full establishment of epithelial cell polarity requires both cell–cell and cell–ECM adhesion (bottom, right). Under these conditions, cells establish structurally and functionally distinct apical and basal–lateral membrane domains.

(Left Cell) Microtubules (solid bold lines) are reorganized into a mat of short filaments under the apical membrane and bundles of long filaments parallel to the lateral membrane with the same polarity (plus ends at the base of the cell); actin filaments (solid lines) form the core of each microvillus in the apical membrane, and insert into the terminal web that is associated with a circumferential band of actin filaments near the tight junction (TJ); the membrane skeleton forms along the lateral membrane (not shown).

(Right Cell) Components of the secretory apparatus become restricted to different regions of the cytoplasm, including the Golgi complex (GC) and apical (Ap-endo) and basal (BL-endo) endosomes. Protein trafficking between these compartments (arrows) may be specified by targeting patches localized to each membrane (see text for details).

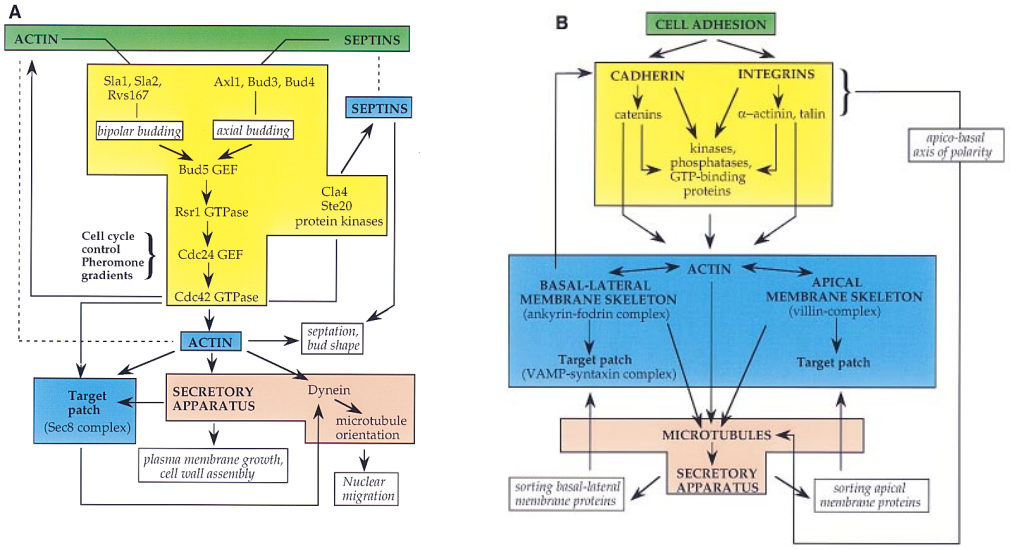

Figure 2. Molecular Pathways for Cell Polarity Development in Budding Yeast and Mammalian Epithelial Cells

The stages are color coded to facilitate direct comparisons between budding yeast (A) and mammilian epithelial cells (B) and with Figures 1 and 3. Note that in (A) the actin and septin cytoskeleton proteins are shown in both green and blue to signify dual roles as spatial cues and as proteins required to reinforce and propagate the cues (see text).

cycle stages, and actin and associated proteins such as Rvs167p are important for bipolar but not axial budding (Bauer et al., 1993; Drubin et al., 1993). For axial and bipolar budding, when a new cell cycle begins, a signaling apparatus containing at least two small GTP-binding proteins assembles at a site marked by the cytoskeleton during the preceding cell cycle.

A Signaling Apparatus Assembles at the Bud Site to Interpret the Cue

Genetic approaches led to the discovery of one GTP-binding protein (Rsr1p, sometimes called Bud1p) involved in bud site selection (Bender and Pringle, 1989), another that is required for bud site assembly (Cdc42p) (Adams et al., 1990), and a guanylyl nucleotide exchange factor (GEF) (Cdc24p) for Cdc42p that appears to link the two GTP-binding proteins together functionally and physically (Bender and Pringle, 1989; Zheng et al., 1995).

Rsr1 is essential for recognizing intrinsic spatial cues that underlie both axial and bipolar budding patterns (Bender and Pringle, 1989; Herskowitz et al., 1995; Pringle et al., 1995). In an *rsr1* null mutant, buds are formed but they are positioned randomly. Cdc42, on the other hand, appears to be essential for organizing all cellular constituents toward the bud site; *cdc42* mutants grow isotropically and lack detectable polarity (Adams et al., 1990).

How are Rsr1 and Cdc42 controlled and how are their activities linked together to select bud sites and form buds? Mutations in the genes that encode guanylyl nucleotide exchange factors (GEF) and GTPase-activating proteins (GAPs) for Rsr1 (Bud5 GEF, Bud2 GAP) and for Cdc42 (Cdc24 GEF) cause phenotypes resembling those of *rsr1* and *cdc42* mutants (Bender, 1993; Chant et al., 1991; Park et al., 1993). Also, mutations in *RSR1* and *CDC42* that block nucleotide hydrolysis or exchange cause a loss of function in bud site selection and polarity development, respectively (Ruggieri et al., 1992; Ziman et al., 1991). Therefore, these GTP-binding proteins must cycle between the GTP and GDP states to function, like the Rab proteins, but in contrast with RAS. One simple model (for additional models, see Herskowitz et al., 1995; Pringle et al., 1995) is that Bud5 GEF activates Rsr1 at a cortical site marked by the actin or septin cytoskeleton during the preceding cell cycle. Cdc24 GEF, which has been shown to bind specifically to activated (GTP-bound) Rsr1, is recruited to the site of Rsr1 activation, resulting in recruitment and local activation of Cdc42.

There is growing evidence that a large complex of signaling proteins is assembled at the cortical site of bud formation. A protein called Bem1, for example, is localized at bud tips (Pringle et al., 1995) and binds to both Cdc24 and Rsr1 (Zheng et al., 1995), and probably to other proteins, and might serve as a scaffold to bring together proteins in a complex at the growth site. Cdc42, in turn, binds to a pair of homologous protein kinases, Ste20 and Cla4 (see below). Other signaling proteins implicated in polarity establishment and bud growth, such as Rho small GTP-binding proteins (see Yamochi et al., 1994, and references therein) and MAP kinases (Mazzoni et al., 1993) might interact with the Cdc42 complex and respond to Cdc42 activation. Rho1, like Cdc42 and Bem1, is concentrated at bud tips (Yamochi et al., 1994). The cytoskeleton is a target of these various signaling proteins.

Actin and Septin Assembly Reinforce the Spatial Cue

Completion of the pathway depicted in Figure 2A involves stimulation of cytoskeleton assembly by the Cdc42 GTPase after it is activated by Cdc24. Cell polarity development during bud formation can be described as a cyclic process in which cortical actin and septin cytoskeleton assembly are linked from one cell cycle to the next by Rsr1 and Cdc42 GTPases. A major role of Cdc42 is to regulate actin and septin assembly. It appears that GTP-bound Cdc42 binds and activates Cla4 and Ste20 (Cvrćková et al., 1995; Simon et al., 1995a), protein kinases with mammalian homologs of unknown function. Cla4 and Ste20 carry out redundant functions during mitotic growth and are required for maintenance of septins at the bud site (Cvrćková et al., 1995). Identification of the targets of these kinases and determination of whether there are other effectors for Cdc42 besides Ste20 and Cla4, such as additional PAK-related protein kinases and GAPs, are now important. A protein phosphatase (Cdc55) (Healy et al., 1991) and two casein kinases (Yck1 and Yck2) (Robinson et al., 1993) may also regulate neck filament assembly.

Components of the yeast actin cytoskeleton, which include highly conserved actin-binding proteins such as profilin, cofilin, tropomyosin, capping protein, and fimbrin, as well as novel proteins such as Sla1, Abp1, Sla2, and Rvs167 (reviewed by Welch et al., 1994), are potential targets of regulation by Cdc42. Significantly, purified Cdc42 in the activated (GTP-bound) state will stimulate cortical actin assembly in permeabilized yeast cells (Li et al., 1995). Because many soluble proteins have presumably been extracted during the preparation of these permeabilized cells, this result suggests that Cdc42 and associated effector molecules might act directly on actin nucleation sites. In support of this possibility, Cdc42 is concentrated in regions of the cell cortex rich in filamentous actin and actin-associated proteins (Ziman et al., 1993). The continued application of genetic and biochemical analysis should now facilitate elucidation of the full pathway leading from Cdc42 to cytoskeleton assembly. It will also be important to determine whether four yeast proteins of the Rho family, a family implicated in actin assembly regulation in mammalian cells, play a role in yeast cytoskeleton assembly. Genetic interactions between Bem2, a Rho GAP, and components of the actin cytoskeleton, the phenotypes of *bem2* mutants (see Wang and Bretscher, 1995, and references therein), and localization of Rho1 to actin-rich regions of the cell cortex (Yamochi et al., 1994) suggest a role for yeast Rho proteins in actin assembly.

Many effects of Cdc42 are likely to be mediated through actin and septins, since these cytoskeletal elements are not properly organized in *cdc42* mutants and since mutations in actin, actin-associated proteins, and septins can cause severe defects in morphogenesis (reviewed by Welch et al., 1994). The cytoskeleton, in turn, appears to control where the plasma membrane and cell wall grow. Little is known about the spatial control of cell wall growth and remodeling, but a number of clues as to how exocytosis might be restricted to one plasma membrane compartment (the bud) exist.

Spatial Control of the Secretory Pathway, Actin Cables, and Microtubules Propagates the Cortical Spatial Cue to the Cytoplasm

Membrane components of the secretory pathway in budding yeast are polarized toward the bud where rapid plasma membrane growth occurs. Golgi structures cluster near mating projections, near the site of bud emergence, and in growing buds (Preuss et al., 1992, and references therein). Vesicles are concentrated at growth sites, and a Sec6–Sec8–Sec15 protein complex is associated with the plasma membrane at the bud tip where it might regulate docking of exocytic transport vesicles (TerBush and Novick, 1995). These observations suggest that spatial control of vesicle targeting might operate at two levels, formation of vesicles near the growth site and localization of a vesicle targeting patch at the site of vesicle fusion. As depicted in Figure 2A, the actin cytoskeleton might control vesicle targeting by organizing the secretory apparatus, the targeting patch, or both. Vesicle targeting might promote further polarization of the cytoskeleton through the delivery of cytoskeleton-anchoring proteins to the plasma membrane at the growth site so that the secretory apparatus and cytoskeleton each reinforce localization of the other. As further evidence for a linkage between actin and the secretory apparatus, late in the cell cycle both undergo a 180° reorientation away from the bud tip toward the mother–bud neck where they, together with the septins, facilitate septation. The identity of the spatial cue for orientation toward the septum is not known.

How the cytoskeleton polarizes the secretory pathway toward the bud site to propagate the cue into the cytoplasm is not known but could be through cytoplasmic actin cables oriented along the mother–daughter axis (Figure 1A) and myosins. Actin cables and myosin organize mitochondria in the cytoplasm and, in doing so, might insure their inheritance (Drubin et al., 1993; Simon et al., 1995b). The myosin heavy chain Myo2 is a candidate to mediate the interaction of actin with the secretory pathway, as this protein is important for polarized growth and for organization of actin structures and is concentrated at sites of cortical growth (Lillie and Brown, 1994, and references therein). Furthermore, *myo2* mutants rapidly accumulate vesicles, although the origin of these vesicles remains uncertain (Govindan et al., 1995). It is also important to determine whether polarized assembly of the cytoskeleton, or delivery of vesicles to a specific site on the cell surface, or both play a role in establishing polarity of other cellular components. Orientation of microtubules during formation of mating projections (Read et al., 1992) and buds (Palmer et al., 1992) depends on actin function and appears to be mediated by a cortically localized dynein (Yeh et al., 1995), suggesting an interaction between microtubule components and components of the actin cytoskeleton, the secretory pathway, or both.

Control of Yeast Polarity Development during the Cell Cycle and by Pheromone

Different programs of cell polarity are tightly coupled to cell cycle stages. In late G1, activation of Cdc28 protein kinase by G1 cyclins (START) results in polarization of cellular constituents toward the presumptive bud site. Actin polarization upon Cdc28 activation can occur in the absence of protein synthesis, suggesting a relatively direct link of the cyclin-dependent protein kinase to cytoskeleton assembly (see Lew and Reed, 1995). Though neither Cdc24 nor Cdc42 contains Cdc28 phosphorylation consensus sites, they are potential targets for this regulation. Zheng et al. (1995) suggested that Cdc24 might be the regulatory target because activated (GTP-bound) Rsr1 that binds to Cdc24 is lethal in combination with reduced levels of G1 cyclins (Zheng et al., 1995, and references therein). This observation could be explained if Cdc28 (or a Cdc28 target) interacts with Cdc24 in a manner mutually exclusive with binding of Rsr1–GTP to Cdc24.

In G2, the B cyclins induce a switch from polarized to isotropic surface growth. This effect might be mediated by down-regulation of Cdc42 resulting from interaction of B cyclins with a recently identified protein Nap1 (Kellogg and Murray, 1995). The timing of the apical–isotropic growth switch controls bud shape and might determine whether a bud is round as in haploid cells, ovoid as in diploid cells, or elongated as in psuedohyphal cells. Near the end of the cell cycle, the decline in B cyclin levels triggers repolarization of the yeast cell toward the mother–bud neck for septation (Lew and Reed, 1995). It is now important to identify the targets of the Cdc28 kinase–cyclin regulation that mediate changes in cell polarity.

While cyclin–cyclin-dependent protein kinase complexes control the timing of polarity development, a feedback mechanism, or checkpoint, linking polarity development back to cell cycle progression has recently been revealed. This mechanism would insure that the nucleus only divides after a bud has formed. Thus, *cdc42*, *cdc24*, and *myo2* mutants, each of which causes defects in bud formation, individually exhibit a cell cycle delay in G2 (see Lew and Reed, 1995).

As described above, during bud formation, yeast can establish an axis of cell polarity in response to an intrinsic cortical cue. However, haploid cells can also establish an axis of cell polarity along a gradient of mating pheromone (Segall, 1993). The pheromone response pathway appears to intersect with the polarity pathway used for bud formation. This conclusion was first supported by the identification of special mutant alleles of Cdc24 and the Cdc24-binding protein Bem1 that are defective in formation of mating projections but not buds (Chenevert et al., 1994). Recently, the Cdc24–Cdc42–Ste20 protein complex has been shown to be an essential component of the signaling pathway leading from the pheromone receptor to transcription and cell cycle control (Simon et al., 1995a; Zhao et al., 1995). In cells responding to pheromone gradients, this complex is required for both polarity development and the pheromone signaling pathway and thus links the two. Significantly, both pheromone receptors and Cdc42 are concentrated at mating projection tips (Jackson et al., 1991; Ziman et al., 1993). Thus, it now appears that the signaling complex containing Bem1, Cdc24, Cdc42, and Ste20/Cla4, which marks and interprets cortical spatial cues, can either be linked to Rsr1 for bud formation in response to intrinsic cues, or to the pheromone receptor and its associated heterotrimeric GTP-binding protein complex for mating projection formation in response to

an extrinsic cue (a pheromone gradient). In support of this conclusion, Rsr1 and other proteins required for bud site selection are not involved in projection formation (Chenevert et al., 1994).

Far1, a protein involved in pheromone-induced cell cycle arrest, is also required for mating projection orientation and appears to mask a default spatial cue for projection formation (Chang, 1991; Dorer et al., 1995; Valtz et al., 1995) so that haploid yeast can assemble the Bem1–Cdc24–Cdc42–Ste20/Cla4 signaling apparatus at the cortical site with the highest pheromone receptor occupancy. When the pheromone signal pathway is saturated, or when Far1 is defective, a projection is formed proximal to the bud site from the previous cell cycle (Dorer et al., 1995; Madden and Snyder, 1992). This suggests a hierarchy for activation of positional cues for sites of mating projection formation. The bud site from the previous cell cycle is used as a positional cue for both default mating projection sites and axial bud site selection. Whether the same proteins mark and interpret the cue for both pathways is not known.

Polarized Epithelial Cells

Our discussion of yeast bud formation shows that a cascade of molecular events, initiated by spatial cues at the cell surface, results in establishment of cell polarity. These spatial cues induce the localized assembly of specialized cytoskeletal and signaling networks, which subsequently direct the repositioning of the cytoskeleton and secretory apparatus toward the cue leading to the formation of a new membrane domain.

Next, we examine how polarity is established in simple epithelial cells. The structural asymmetry of these cells is distinctive (Figure 1B). Subsets of membrane and cytoskeletal proteins localize to functionally and structurally distinct membrane domains, termed apical and basal–lateral, and microtubules and sorting compartments of the secretory apparatus are asymmetrically distributed in the cytoplasm. This polarized organization is the basis for the function of these cells in vectorial transport of ions and solutes across the epithelium. As in budding yeast, the pathway for polarity development in epithelial cell starts with a cortical spatial cue. In epithelial cells, the cue is cell adhesion. The site of the cue on the cell surface is marked by adhesion receptor proteins. Specialized cytoskeletal and signaling networks assemble around these receptors and position other cytoskeletal complexes and protein-sorting compartments relative to the spatial cue. Subsequently, protein sorting from these compartments to the cell surface and retention in the membrane reinforce and maintain the structural and functional specializations of these membrane domains that were initiated by cell adhesion (Figure 2B).

Spatial Cues for Epithelial Cell Polarity: Cell Adhesion Generates Asymmetry at the Cell Surface and the Axis of Cell Polarity

In the absence of extracellular contacts, single epithelial cells exhibit very few of the structural characteristics of polarized cells (Figure 1B). Extracellular contacts between single cells and extracellular matrix (ECM), or between cells in the absence of ECM, are sufficient to initiate segregation of membrane and cytoskeletal proteins between contacting and noncontacting surfaces of cells (Figure 1B).

Interactions between epithelial cells and extracellular contacts are specified by adhesion receptor proteins. Epithelial cell–cell adhesion is mediated principally by E-cadherin, a member of the Ca^{2+}-dependent cadherin superfamily of adhesion receptors (reviewed by Kemler, 1992). E-cadherin-mediated adhesion is sufficient to start the segregation of apical membrane proteins into the noncontacting (free) membrane and basal–lateral membrane proteins into the contacting membrane (Vega Salas et al., 1987; Wang et al., 1990a) (Figure 1B). Significantly, ectopic expression of E-cadherin in nonpolarized fibroblasts induces Ca^{2+}-dependent cell–cell adhesion and accumulation of some membrane proteins (e.g., Na/K-ATPase) at sites of cell–cell contacts similar to that in polarized epithelial cells (McNeill et al., 1990).

Cell adhesion to ECM is mediated by the integrin superfamily of adhesion receptors (reviewed by Hynes, 1992; Clark and Brugge, 1995). These interactions generate differences in protein distributions between contacting and noncontacting surfaces (Vega Salas et al., 1987) and, in addition, refine the apical–basal axis of polarity; a useful marker of this axis is the location of the tight junction at the apical–lateral membrane boundary (Figure 1B). Although cadherin-mediated adhesion generates differences in apical and basal–lateral proteins between noncontacting and contacting cell surfaces (see above), the absence of ECM results in localization of tight junction proteins (e.g., ZO-1) all along cell–cell contacts (Wang et al., 1990a). However, subsequent accumulation of endogenous ECM within cell aggregates results in localization of tight junction proteins to the apical–lateral membrane boundary and formation of a fluid-filled lumen containing ECM (Wang et al., 1990a). When these aggregates are surrounded by ECM, the apical membrane reforms on the lumenal surface and the tight junction relocates to the new apical–lateral membrane boundary, resulting in reversal of the axis of cell polarity (Wang et al., 1990b).

Interpreting the Cue: Assembly of Cytoskeletal and Signaling Networks at Sites of Interactions between Cell Adhesion Receptors and Extracellular Contacts

Integrin- and cadherin-mediated adhesions induce localized assembly of specialized cytoskeletal and signaling networks at the contacting cell surface(s). Binding of the cytoskeleton to adhesion receptors strengthens cell adhesion and maintains signaling from the cues. In addition, assembly of cytoskeletal and signaling networks may direct both local and global changes in the spatial organization of cell surface proteins, microtubules, and the secretory apparatus.

Integrins bind directly to two cytoskeletal proteins, α-actinin and talin, which in turn link integrins to a cytoskeletal matrix comprising actin filaments and other actin-associated proteins, including vinculin, zyxin, and paxillin (Hynes, 1992; Clark and Brugge, 1995; Figure 2B). This matrix both strengthens the ECM–integrin interaction and forms a protein scaffold for assembly of a signaling network comprising focal adhesion kinase,

components of the RAS pathway (e.g., SOS and Grb2), and GTP-binding proteins (reviewed by Clark and Brugge, 1995; Figure 2B). Targets of this signaling network are components of the actin cytoskeleton (see below) and, perhaps, pathways regulating gene expression (see Clark and Brugge, 1995).

Cadherin-mediated cell adhesion also results in localized assembly of cytoskeletal and signaling networks (Figure 2B). A family of related cytoplasmic proteins (β-catenin, plakoglobin, and p120) bind tightly to the cytoplasmic domain of cadherins. These proteins bind α-catenin, which has some homology to vinculin, indicating that α-catenin may link the cadherin–catenin complex to the cytoskeleton (reviewed by Gumbiner, 1993). In vitro studies show that α-catenin binds to actin (Rimm et al., 1995) and fodrin (Lombardo et al., 1994). α-Actinin is also a component of the cadherin–catenin complex and may provide an additional link between the cadherin–catenin complex and actin (Knudsen et al., 1995). Formation of complexes between cadherin–catenin and the cytoskeleton strengthens cell adhesion and provides a protein scaffold for a signaling network (Figure 2B) comprising kinases (Src and Yes) (Tsukita et al., 1991) and a protein-tyrosine phosphatase (PTPμ) (Brady-Kalnay et al., 1995). This signaling network may regulate cadherin–catenin interactions with the actin cytoskeleton (Behrens et al., 1993) and signaling through β-catenin (see Gumbiner, 1996 [this issue of *Cell*]). Components of the RAS signaling pathway and small GTP-binding proteins may also associate with the cadherin–catenin complex. Activation of protein kinase C appears to accelerate cell–cell adhesion (Lewis et al., 1994), and small GTP-binding proteins (e.g., Rho) localize to cell–cell contacts in some epithelial cells (Adamson et al., 1992). Together, these observations indicate that phosphorylation may be a positive or negative regulator of functions of the cadherin–catenin complex in cell adhesion, assembly of the cytoskeleton, and signal transduction (Figure 2B).

Propagating Signals from Cell Adhesion Receptors to Assembly of Domain-Specific Cytoskeletal Structures and Repositioning Microtubules and Secretory Apparatus

Interactions between adhesion receptors and cytoskeletal and signaling networks may form a template for propagating signals for cellular reorganization from the spatial cue (Figure 2B). Activation of both integrins and cadherins by extracellular contacts leads to the assembly of an actin-based cytoskeleton at sites of cell adhesion (reviewed by Clark and Brugge, 1995; Gumbiner, 1993). Assembly may be mediated by mass action through clustering of adhesion receptors, or posttranslational modifications of proteins by kinases or phosphatases resulting in changes in protein–protein affinities, or activation of the small GTP-binding proteins Rho and Rac that rapidly change the status of actin polymerization and distribution (see above). Locally, cell adhesion also leads to the assembly of the fodrin-based membrane skeleton. Fodrin is a long, rod-shaped protein that binds actin, protein 4.1, adducin, and other proteins to form a protein skeleton on the cytoplasmic face of the plasma membrane (reviewed by Bennett, 1990). Linkage of the membrane skeleton to the cadherin–catenin complex may be mediated by binding between either fodrin or actin to α-catenin (see above). Fodrin also binds to ankyrin, which in turn binds with high affinity to integral membrane proteins, including Na/K-ATPase and Cl^-/HCO_3^- exchanger (reviewed by Bennett, 1990).

Assembly of the membrane skeleton may play a direct role in the early formation of a membrane domain at sites of cell adhesion by directing the retention and accumulation of specific proteins. Although studies have been limited to a few proteins, the available evidence indicates that interactions between the cadherin–catenin complex and membrane skeleton are required to localize specific proteins such as Na/K-ATPase to sites of cell adhesion (Hammerton et al, 1991). First, induction of membrane skeleton assembly at cell–cell contacts in retinal pigmented epithelial cells by ectopic expression of E-cadherin results in restriction of Na/K-ATPase distribution to sites of E-cadherin-mediated adhesion (Marrs et al., 1995). Second, expression of E-cadherin lacking the catenin-binding domain does not result in localization of either Na/K-ATPase or fodrin to cell–cell contacts (McNeill et al., 1990). Third, overexpression of the actin-binding domain of fodrin appears to competitively inhibit the association of actin with the membrane and results in both the disruption of membrane skeleton organization and the relocalization of Na/K-ATPase into cytoplasmic vesicles (Hu et al., 1995). Incorporation of specific membrane proteins, such as Na/K-ATPase, into the membrane skeleton may preferentially exclude them from endocytic pathways, resulting in their accumulation at sites of cell adhesion (Hammerton et al, 1991). In contrast, membrane proteins that are not directly linked to the membrane skeleton may be internalized more rapidly. Differences in rates of internalization result in the accumulation of membrane proteins linked to the membrane skeleton at sites of cell adhesion, leading to the formation of a specialized membrane domain.

In addition to the induction of assembly of cytoskeleton and signaling networks on the contacting membrane, cell adhesion also results in formation of a noncontacting, apical membrane domain (see above). Morphogenesis of the apical membrane coincides with the assembly of an actin-based cytoskeleton that has a protein composition different than that of the basal–lateral membrane skeleton (reviewed by Heintzelman and Mooseker, 1992; Figure 1B). A bundle of actin filaments, cross-linked by villin and fimbrin and linked laterally to the membrane by myosin I, forms the core of long membrane protrusions, termed microvilli, that are characteristic of the apical membrane of absorptive epithelia. These actin filament bundles are linked together by a fodrin lattice in the cytoplasm (terminal web; see Figure 1B). Although mechanisms that initiate assembly of apical microvilli are poorly understood, villin is thought to play a central role; it is possible that villin is initially localized in the vicinity of the apical membrane via attachments to cortical actin at the adherens junction (reviewed by Heintzelman and Mooseker, 1992; see Figure 1B). Significantly, ectopic expression of villin in fibroblasts, which do not normally form apical microvilli, results in the elaboration of long membrane protrusions on the apical membrane that contain bundles of actin filaments cross-linked by villin (Friederich et al., 1989).

Induction of cell adhesion also results in the redistribution of microtubules (Figure 1B). A dense mat of short, randomly oriented microtubules forms in the apical cytoplasm. Long bundles of microtubules, with their plus ends in the basal cytoplasm, form parallel to the lateral membrane along the apical–basal axis of the cell (Bacallao et al., 1989; see Figure 1B). Mechanisms that regulate microtubule reorganization after cell adhesion are not known. It is possible that the orientation of microtubule assembly in the apical–basal axis is determined by linkage of microtubules to cytoskeletal complexes on lateral and basal membranes. For example, ankyrin, a component of the lateral membrane skeleton (see above), binds to and bundles microtubules (reviewed by Bennett, 1992) and a microtubule-binding protein associates with desmosomes (Wacker et al., 1992).

As microtubules reorganize, protein-sorting compartments of the secretory apparatus become localized to the apical and basal cytoplasms (Figure 1B). Microtubule motor proteins, such as dynein or kinesin (reviewed by Walker and Scheetz, 1993), may translocate these compartments toward the minus or plus ends of polarized microtubules, resulting in their distribution in the apical and basal cytoplasm, respectively (Figure 1B). Polarized microtubule organization and restriction of sorting compartments to different regions of the cell may be important in facilitating protein sorting to specific membrane domains (see below).

Reinforcing and Maintaining Cell Polarity by Sorting Proteins to Apical and Basal–Lateral Membrane Domains

To compensate for protein turnover at the cell surface and to maintain polarized distributions of proteins, newly synthesized proteins are sorted in the *trans*-Golgi network (TGN) and endosomes and then delivered to either the apical or basal–lateral membrane (reviewed by Rodriguez-Boulan and Powell, 1992). Sorting mechanisms are poorly understood, but the basic principle is likely to involve localized protein clustering in the plane of the lipid bilayer (reviewed by Rodriguez-Boulan and Powell, 1992). In the TGN, some apical membrane proteins (e.g., GPI-anchored proteins) may be clustered into lipid rafts by hydrogen bonding between glycosphingolipids (reviewed by Lisanti and Rodriguez-Boulan, 1990). Other classes of apical membrane proteins may be sorted through recognition of carbohydrate moieties by a sorting receptor (Fiedler and Simons, 1995). Clustering of basal–lateral membrane proteins may be mediated by a cytosolic protein coat structure, perhaps structurally similar to clathrin, that recognizes a sorting signal in the cytoplasmic domain of proteins (Matter et al., 1994; Aroeti et al., 1993; reviewed by Rodriguez-Boulan and Powell, 1992). However, these mechanisms do not appear to explain sorting pathways for all membrane proteins, indicating that other mechanisms are yet to be uncovered (Mays et al., 1995).

Structural similarities in signals for sorting basal–lateral membrane proteins in the TGN and the ubiquitous endocytic pathway (see above), and the fact that apical and basal–lateral sorting signals are not restricted to proteins in polarized cells, indicate that TGN sorting of apical and basal–lateral proteins into separate transport vesicles may occur in "nonpolarized" cells. Thus, vesicle delivery to the correct membrane domain in polarized epithelial cells may depend on the juxtaposition of sorting compartments and domain-specific cytoskeletal complexes to regulate the direction of vesicle trafficking and specific targeting patches on different membrane domains to specify vesicle docking.

Vesicle transport between sorting compartments and different plasma membrane domains is facilitated by microtubules. Depolymerization of microtubules slows delivery of basal–lateral and apical membrane proteins from the TGN and disrupts protein trafficking between endosomes and the cell surface (reviewed by Mays et al., 1993). Microtubule motor proteins may be involved in regulating the direction of vesicle transport toward either the minus or plus ends of microtubules. Depletion of kinesin, or kinesin and dynein, disrupts basal–lateral and apical membrane protein delivery, respectively (Lafont et al., 1994). In addition, dynein and the dynactin complex have been identified in post-Golgi transport vesicles from intestinal epithelial cells (Fath et al., 1994). It has been suggested that apical transport vesicles may require a microtubule motor to traverse the distance between the TGN and microvillar terminal web and then an actin motor, such as myosin I (a component of the microvillar cytoskeleton; see above), for transport from the terminal web along actin bundles to microvilli (Fath et al., 1994). Significantly, disruption of microvillar organization following loss of villin expression results in the accumulation of transport vesicles in the apical cytoplasm (Costa de Beauregard et al., 1995), indicating that vesicle transport, docking, or both are inhibited when the apical cytoskeleton is disassembled.

Although disruption of microtubules slows protein delivery to the cell surface, there is little missorting of proteins to the incorrect membrane domain (reviewed by Mays et al., 1993). This indicates that each membrane domain has a targeting patch that recognizes only those transport vesicles that contain the correct docking motif. Mechanisms that specify vesicle/membrane recognition and docking are poorly understood. In vitro reconstitution of vesicle transport from the TGN to plasma membrane in MDCK cells indicates that GTP-binding proteins and GTP hydrolysis are required at some stage of vesicle docking and fusion (Gravotta et al., 1990). Significantly, several GTP-binding proteins localized to basal–lateral transport vesicles and the basal–lateral membrane domain of MDCK cells (Huber et al., 1993). In addition, disruption of the VAMP/syntaxin machinery for docking vesicles on membranes (Warren and Wickner, 1996 [this issue of *Cell*]) partially inhibits the delivery of transport vesicles to the basal–lateral membrane, but not the apical membrane (Ikonen et al., 1995). Whether a molecularly different machinery specifies recognition/docking of specific transport vesicles at the apical membrane is not known.

Mechanisms regulating the assembly of targeting patches for transport vesicles on different membrane domains are poorly understood. However, it is possible that membrane-associated cytoskeletal networks provide binding sites for the vesicle docking machinery. Significantly, disruption of fodrin organization on the basal–lateral membrane (Hu et al., 1995) and villin/microvillar organization in the apical membrane (Costa de

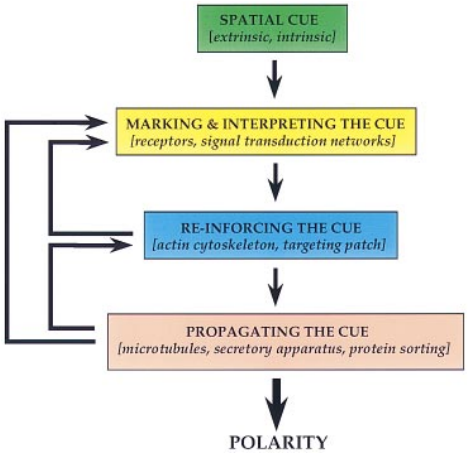

Figure 3. A Common Pathway for Establishment of Cell Polarity Based on Comparisons between Budding Yeast and Mammalian Epithelial Cells

The color scheme is the same as in Figures 1 and 2.

Beauregard et al., 1995) result in the accumulation in cytoplasmic vesicles of apical and basal–lateral membrane proteins, respectively. Thus, protein delivery from sorting compartments to the cell surface may be regulated by specialized cytoskeletal complexes and targeting patches that became localized to different membrane domains in response to spatial cues from cell adhesion (see above). In turn, protein delivery to these membrane domains reinforces and maintains structural and functional differences at the cell surface that were initiated by the spatial cues.

Synopsis: A Heirarchy of Stages for Establishing Cell Polarity

Clearly, there are differences in the structural organization of budding yeast and epithelial cells and in the consequences of polarity establishment on cell function. In particular, development of polarity in yeast is tightly coupled to the cell cycle and cell division, whereas polarity in epithelial cells is coupled to vectorial ion and solute transport in nondividing cells. However, comparison of general mechanisms involved in setting up polarity in these phylogenetically distant cells indicates a common hierarchy of stages (Figure 3). In both cells, polarity is initiated by the establishment of structural and molecular asymmetry at the cell surface by extrinsic and intrinsic spatial cues. These cues require specific membrane receptors or cytoskeletal structures that mark the site of the cue on the cell surface, resulting in restriction of subsequent signaling to the immediate vicinity of the cue. These interactions define a molecular and structural asymmetry in the membrane between the site of the spatial cue and the rest of the cell surface. The spatial cue induces localized assembly of a signaling network. This network comprises small GTP-binding proteins, kinases, phosphatases, and structural/cytoskeletal proteins. Activation of this signaling network results in localized assembly of an actin cytoskeleton matrix and global changes in cell organization.

Spatial information from the cue is reinforced by localized assembly of the cytoskeleton and a targeting patch for transport vesicles and is propagated along the membrane and into the cell interior by changes in the organization of actin and microtubules. Concomitantly, sorting compartments of the secretory apparatus reorientate in the cytoplasm along an axis of polarity relative to position of the cue(s). Delivery of newly synthesized proteins to targeting patches at the cell surface reinforces and stabilizes the molecular and structural asymmetry of the cell surface that was started by the spatial cue. Feedback regulation between stages of the hierarchy consolidates earlier stages, resulting in the maintenance of cell polarity.

As noted at the beginning, the establishment of cell polarity is fundamental to differentiation and a diversity of functions in most, if not all cells. Ordering of the stages of cell polarity establishment into a hierarchy should help in applying the knowledge of molecular mechanisms uncovered in one cell type to other cell types, thereby leading to a more complete understanding of the origins of cell polarity.

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