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Adaptation of core mechanisms to generate cell polarity

W. James Nelson

Department of Molecular and Cellular Physiology, Beckman Center for Molecular and Genetic Medicine, Stanford University School of Medicine, Stanford, California 94305-5435, USA
(wjnelson@stanford.edu)

Abstract

Cell polarity is defined as asymmetry in cell shape, protein distributions and cell functions. It is characteristic of single-cell organisms, including yeast and bacteria, and cells in tissues of multi-cell organisms such as epithelia in worms, flies and mammals. This diversity raises several questions: do different cell types use different mechanisms to generate polarity, how is polarity signalled, how do cells react to that signal, and how is structural polarity translated into specialized functions? Analysis of evolutionarily diverse cell types reveals that cell-surface landmarks adapt core pathways for cytoskeleton assembly and protein transport to generate cell polarity.

Most cells are polarized, including simple single-cell organisms (for example, budding yeast *Saccharomyces cerevisiae* and fission yeast *Schizosaccharomyces pombe*) and cells in multicellular invertebrates (the nematode *Caenorhabditis elegans* or fruitfly *Drosophila*) and vertebrates (mammals); even bacteria are polarized (Fig. 1). There is also an extraordinary diversity in the shapes of polarized cell. Consider the extremely attenuated shape of a mammalian neuron, which can be many metres long, the short rectangular shapes (5–30 μm long) of the single-cell *S. pombe* and cells in epithelial tissues in multicellular organisms, and the asymmetric shape of *S. cerevisiae* or *Drosophila* neuroblasts preparing to divide (Fig. 1). Different cell shapes are not just a quirk of nature, but are coupled to specialized cell functions, for example, to communicate between tissues over long distances (neurons), to provide barriers that regulate ionic homeostasis between different biological compartments (epithelia), and to disperse cellular components to daughter cells upon cell division.

At first glance, this diversity of cell shapes and functions suggests that each cell type must have evolved completely different ways to generate cell polarity that distinguishes, for example, a budding yeast from a multi-cell epithelium. But although the final structure is quite different, the basic toolbox of core mechanisms used to organize the cytoskeleton and deliver membrane proteins is common to all eukaryotic cell types. This review focuses on how these mechanisms are used to generate polarity in evolutionarily diverse cell types with different shapes and functions.

Generating polarity in single cells for mitotic division

S. cerevisiae and *S. pombe* are simple, well-understood examples of how cells generate polarity and couple it to a specific function; simple because they are single-cell organisms that generate cell polarity in order to divide, and well understood because they are genetically tractable. Genetic and cell biological studies point to the importance of localized assembly of actin patches and regulatory proteins to mark one site on the mother cell for membrane growth, and the attachment of actin cables and microtubules to those sites for vesicle delivery and spindle orientation, respectively.

During vegetative growth, *S. cerevisiae* adopt genotype-dependent axial (haploid **a** or α cells) or bipolar (diploid **a**/ α cells) growth patterns to produce a daughter cell bud¹ (Fig. 2a). Two classes of genes prescribe 'landmarks' for axial (*BUD3*, *BUD4* and *BUD10/Axl2*) and bipolar (*BUD8* and *BUD9*) budding of the daughter cell^{2,3}, and a third class is required for both budding patterns (*BUD1/RSR1*, *BUD2* and *BUD5*)^{2,4}. The landmark is retained on the plasma membrane close to (axial or bipolar pattern) or opposite (bipolar pattern) the site (bud scar) of the preceding cytokinesis (Fig. 2a). Membrane growth is anisotropic, occurring only in the new bud (Fig. 2b).

Members of the Ras superfamily of small GTPases (Bud1p/Rsr1p, Cdc42p, and Rho1p/2p, Rho3p and Rho4p) coordinate the selection of the membrane growth site (the bud), and orientation of the cytoskeleton and targeting of vesicles to that site^{1,5} (Fig. 2b, c). Cdc42p is crucial in these events — in the absence Cdc42p the actin cytoskeleton is disorganized, and large, unbudded cells are formed as a result of isotropic growth of the mother cell⁶. A critical question is how is Cdc42p activity restricted to one site on the plasma membrane? One possibility is that Cdc42p is restricted to the landmark through the sequential recruitment of guanine-exchange factors (GEFs), which control GTPase activity locally⁷. Bud5p and Bud2p, the GEF and GTPase-activating protein for Bud1p/Rsr1p, respectively, co-localize with landmark proteins, and disruption of landmark proteins results in mislocalization of Bud5p^{8–10}. Recruitment of Bud5p to the landmark would locally activate the uniformly distributed Bud1p/Rsr1p. *In vitro* studies¹¹ show that the GTP-bound form of Bud1p/Rsr1p binds Cdc24p, the GEF for Cdc42p, which in the cell could restrict Cdc42p activity to the landmark. But cells lacking the Bud1p/Rsr1p machinery or any of the landmark genes form daughter cell buds, albeit randomly on the mother cell, in a process that requires activated Cdc42p^{2–4}. Polarization of Cdc42p and actin polymerization could be controlled through a positive feedback loop initiated by a stochastic increase in Cdc42p on the membrane¹², and landmarks normally adapt this feedback loop for bud formation at a prescribed, rather than random, site on the membrane.

Activation of Cdc42p at a site on the membrane unleashes global changes in cytoskeleton organization within the bud, involving assembly of a cap of actin filaments, and the organization of actin cables that extend into the mother cell^{1,13} (Fig. 2b, c). Ste20p and Cla4p are downstream effectors of Cdc42p that are members of the p21-activated kinase (PAK) family¹⁴. The type-I myosins Myo3p and Myo5p¹⁵ are PAK substrates, which together with homologues of Wiskott–Aldrich syndrome protein or WASP (Bee1p/Las17p) and WASP-interacting protein or WIP (Vrp1p) interact with the Arp2/3 complex to assemble and organize an actin cap at the bud site^{16–18} (for details of Arp2/3 function, see review in this issue by Gruenheid and Finlay, page 775).

A core scaffolding complex of Spa2p, Pea2p and Sph1p (the polarisome complex¹⁹) is assembled at the bud. The formin homologues Bni1p and Bnr1p bind this complex and, in turn, interact with proteins that assemble actin cables between the bud and mother cell^{20–22}: profilin (Pfy1p)²³, which promotes GDP to GTP exchange in monomeric actin and hence provides a local pool of GTP-actin for addition to the barbed end of growing actin filaments; Bud6p/Aip3p, an actin filament-binding protein²⁴; and activated GTPases including Cdc42p, Rho1p/2p, Rho3p and Rho4p^{20,25}. Loss of Bni1p and Bnr1p activity correlates with loss of actin cables throughout the bud and mother, and re-activation of Bni1p and Bnr1p results in re-growth of actin cables from the bud into the mother cell^{21,22}. *In vitro*, Bni1p can also nucleate actin polymerization and bind the barbed (growing) end of actin filaments^{26,27}. Taken together, Bni1p and Bnr1p and associated proteins seem to nucleate and anchor actin cables at the bud site (Fig. 2c).

Orientation of actin cables from the bud into the mother cell also drives microtubule capture in the bud and spindle orientation in the mother–bud axis for cytokinesis²⁸ (Fig. 2b). Astral microtubules contain at their distal, plus ends a protein complex of Kar9p and Bim1p that binds to the type-V myosin Myo2p, which, in turn, binds to actin cables and moves the microtubule-attached complex into the bud²⁹.

Actin cables between the bud and mother cell provide tracks for exocytic vesicle transport from the mother cell Golgi complex into the bud^{1,13} (Fig. 2b, c). This is critical for polarized membrane growth as SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) located on the target membrane (t-SNAREs; Sec9p, Sso1p/2p) are not restricted to the bud, but are localized over the surface of the mother cell and bud³⁰. Vesicle delivery along actin cables requires Myo2p^{31,32}. Vesicle docking/fusion complex at the bud is further specified by a large complex termed the exocyst, which is localized initially to the bud tip³³; Sec4p, Rho1p/2p, Rho3p, Rho4p and Cdc42p regulate these late stages in vesicle delivery, and localization and function of the exocyst in the bud^{5,34}. Disruption of genes encoding the exocyst complex results in accumulation of transport vesicles in the bud, indicating that the exocyst complex may tether arriving vesicles or activate the SNARE complex in preparation for SNARE-dependent vesicle fusion with the plasma membrane³³. The yeast homologues of *Drosophila* lethal giant larva (a protein regulating epithelial polarity, see below), *sro7/sro77*, bind the t-SNARE Sec9p, and *sro7/sro77* mutants accumulate post-Golgi transport vesicles in the cytoplasm³⁵. Thus, both t-SNAREs and the exocyst are required for vesicle fusion at the bud, and localized vesicle docking/fusion in the bud is specified by vesicle delivery along actin cables to the exocyst.

Although these overlapping regulatory mechanisms involving different GTPases may seem overly complex, and in some cases redundant, they coordinate a series of processes that link, in time and space, the initial selection of the bud site to localized assembly and orientation of the actin and microtubule cytoskeletons in the bud–mother axis, and localized docking/fusion of vesicles at that site of membrane growth (Fig. 2c).

Compared to the highly polarized bud growth of *S. cerevisiae*, the rod-shaped fission yeast *S. pombe* divides in the middle by septation and, therefore, might be expected to grow in a more isotropic manner during the cell cycle. However, growth of fission yeast is also spatially polarized, first during G1 at the ‘old’ end opposite the site of septation, and then at the other end during G2 (Fig. 3a).

In fission yeast, a network of actin cables is oriented in the long axis of the cell, and in cortical patches at the cell ‘ends’³⁶. Little is known about the machinery for localizing actin polymerization to cortical patches, although there are tantalizing indications of mechanisms similar to those in budding yeast (Fig. 3b). Cortical actin patches contain Orb2p, a member of the PAK family that may be downstream of Cdc42p³⁷, the actin-binding protein Bud6p³⁸, and myosin-I and Arp2/3 complex³⁹ (Fig. 3b). For3p⁴⁰, a member of the formin family (like Bni1p in budding yeast; see above), localizes to cell ‘ends’ and cortical actin patches and is required for actin cable assembly and organization of actin patches. In *for3Δ* mutants, most daughter cells grow at both ‘old’ and ‘new’ ends simultaneously, indicating that, as in budding yeast, localization of a formin (For3p) at cell ‘ends’ could be part of a pathway that interprets and builds upon ‘landmarks’ for polarized membrane growth⁴¹.

Microtubules, like actin cables, are organized in the long axis of the cell and are uniformly polarized with their plus ends at the cell ends and minus ends around the nucleus. Disruption of microtubules causes cells to bend and branch abnormally as they grow³⁷, suggesting that they monitor the progress of cell growth. Microtubules have a 4–6 minute cycle of rapid polymerization towards a cell end, followed by catastrophic depolymerization ~100 seconds

after contacting the end^{41,42}. Several proteins seem to control this cycle (Fig. 3b). Tip1p⁴² and Mal3p⁴³ bind to the plus ends of growing, but not shrinking microtubules, and may control microtubule length and dynamics.

How are polarized membrane growth and actin/microtubule cytoskeletons linked structurally and functionally? A candidate is the kelch repeat protein Tea1p, which localizes to microtubule plus ends and is deposited at cell ends when microtubules start to shrink⁴⁴. Tea1p is in a complex with Bud6p³⁸, which localizes to cell ends coincident with first 'old', and then 'new', end growth. Tea1p, Bud6p and perhaps For3p may provide a functional link between actin and microtubule cytoskeletons, and actin assembly and membrane growth at cell 'ends' (Fig. 3b). At present, little is known about how transport vesicle delivery is polarized for membrane growth in fission yeast, although the exocyst complex functions in vesicle delivery to cell ends and at the site of septation⁴⁵ (Fig. 3b).

Baseline principles for cell polarity

Core mechanisms for actin polymerization and vesicle transport are adapted for polarized membrane growth around a landmark at the plasma membrane. The landmark is recognized and reinforced by localized assembly of a signalling complex of small GTPases (for example, Bud1p/Rsr1p, Cdc42p and Rho), which relays signals to modular protein complexes that concentrate machinery for actin cytoskeleton assembly (Arp2/3, WASP/WIP, type-I myosins and profilin), anchoring/nucleation of actin cables (formin proteins, including Bni1p and Bnr1p/For3p, and Bud6p), microtubule assembly/capture (Kar9p/Bim1p and Tea1p/Tip1p/Mal3p) and vesicle delivery (type-V myosins, Sec4p, SNAREs and exocyst). Assembly of this hierarchy of complexes links landmarks to local assembly and orientation of the cytoskeleton, vesicle delivery for membrane growth, spindle orientation and inheritance of landmarks by daughter cells.

Generation of cell polarity in a multi-cell epithelium

So far, I have considered cell polarity in the context of single cells preparing to divide. Do the same principles of cell polarity that emerged from studies of single cells apply to polarized epithelial cells in multi-cell organisms? Clearly, there is increased complexity in multicellular tissues, as more than one membrane domain is formed (Fig. 4a), often concurrently, and the cell must use additional mechanisms to initially distinguish these domains, target and organize different proteins in each domain and, ultimately, keep the identities of the domains separate. I focus here on two control points. First, the apical junctional complex, a structure conserved between *Caenorhabditis elegans*, *Drosophila* and vertebrates (Fig. 4a), which initiates cell–cell adhesion and regulates the identities of different membrane domains. Second, mechanisms involved in sorting and targeting proteins to different membrane domains. As examples, I will examine formation of polarized epithelia during *de novo* assembly of plasma membrane domains in the *Drosophila* blastoderm and cultured mammalian epithelial cells.

Extracellular contacts between epithelial cells define the bounded (basolateral) and free (apical) cell surface⁴⁶. Although full development of apicobasal polarity requires organization cues from both cell–cell and cell–extracellular matrix adhesions^{47,48}, assembly of the apical junctional complex, at the boundary between apical and (basal-)lateral membranes (Fig. 4a), recognizes and reinforces the principal landmark formed by cadherin-mediated cell–cell contacts. The apical junctional complex is a multifunctional, modular structure containing protein sub-complexes located at the boundary between the apical and lateral membrane domains⁴⁹ (Fig. 4a). In general, each protein sub-complex comprises an integral membrane protein bound to scaffold protein modules, each of which has multiple protein–protein binding motifs that potentially could inter-link different membrane protein

sub-complexes. Scaffold proteins generally bind to the actin cytoskeleton^{50–52}, which is regulated by Rho-family small GTPases⁵³, although links to microtubules may also be present⁵⁴; some proteins at the apical junctional complex also act as transcriptional (co-)activators of gene expression⁵⁵.

Genetic studies in *Drosophila* and *C. elegans* have identified classes of proteins in the apical junctional complex that regulate development of epithelial cell polarity⁵⁶: cadherin/catenins^{57,58}, Crumbs (Crb)/Stardust⁵⁹, Bazooka (Baz/Par3)/Par6/atypical protein kinase C (aPKC)^{57,60,61} and Lethal giant larva (Lgl)⁶²/Scribble (Scrib)⁶³/Discs large (Dlg)^{64,65}/LET-413⁶⁶ (Fig. 4b). Recent studies show that the proteins in each class function in a common pathway, and the complexes seem to be integrated into a regulatory pathway that instructs apical and lateral membrane formation^{67,68}. Cell–cell adhesion mediated by the cadherin/catenin complex^{57,58,67,68} is required to initiate these events, as maternal and zygotic deletion of one of the catenins, *armadillo*, in *Drosophila* inhibits formation of all cellular structures including the first epithelium⁵⁸; note, however, the other complexes are required subsequently to maintain function and integrity of cadherin junctions^{67,68}.

The Baz complex is recruited to cadherin junctions^{57,67,68} and initiates formation of the apical membrane. Spread of apical membrane identity down the lateral membrane is impeded by activity of the Lgl/Scrib complex^{60,67,68}, which is recruited to a position below the Baz complex/cadherin junction^{60,67,68}. The Lgl/Scrib complex seems to antagonize functions of the Baz and, later, Crb complexes, thereby maintaining lateral membrane identity^{67,68} below the adherens junction. The Crb complex is recruited apically to the Baz complex/cadherin junction^{57,59} and seems to further antagonize activity of the Lgl/Scrib complex by blocking the spread of lateral membrane, thereby maintaining apical membrane identity^{67,68} above the adherens junction (Fig. 4b).

Although these studies identify overarching roles for these different complexes in initiating polarity of the apical and lateral membrane domains, the underlying biochemical mechanisms remain unclear. It is noteworthy that many of the proteins involved contain protein-binding motifs, PDZ domains (for example, Baz (Par3), Par6, Scrib, LET-413 and Discs large)⁵⁶ and leucine-rich domains (Scrib and LET-413)⁶⁸ that act as scaffolds to bind and spatially order proteins in the apical junctional complex^{69–71}. The Baz complex contains an aPKC^{60,72}. In mammalian epithelia, dominant negative mutants of aPKC cause mislocalization of Par3 (Baz) and ZO-1 (a tight-junction protein), resulting in disruption of tight and adherens junctions, and defects in apical/basolateral polarity⁷³. The Baz complex is also regulated by Rho-family GTPases that activate aPKC^{74,75}. In addition, Lgl binds to the t-SNARE syntaxin-4 and the complex localizes to the lateral membrane⁷⁶, but a role of Lgl in syntaxin-4 function has not been tested in detail. An attractive possibility is that complexes determining apical (Baz and Crb) and lateral (Lgl/Scrib) membrane domain identity may regulate delivery of transport vesicles containing apical and (basal-)lateral proteins, respectively, thereby contributing to the generation and maintenance of membrane domain identity.

Although specific protein complexes associated with the apical junctional complex control the identity of apical and basolateral membrane domains, how do cells determine the distribution of different proteins and functions in those domains? The generation of epithelial cell polarity during cellularization in the *Drosophila* embryo and in cultured mammalian cells provide mechanistic insights.

The first 13 cell divisions of the *Drosophila* embryo generate a syncytial blastoderm comprising ~6,000 nuclei that are localized beneath the plasma membrane. During cellularization, the surface area of the plasma membrane increases >25-fold as it forms

between each nucleus to yield a sheet of columnar, polarized epithelial cells that are ~30 μm tall⁷⁷ (Fig. 5a). Cellularization occurs in two phases, 'slow' and 'fast'⁷⁸. In the slow phase, lasting 35–40 minutes and accounting for ~10 μm of lateral membrane, growth occurs by rapid invagination of the apical surface and redistribution of internalized protein to the lateral membrane (furrow canal)⁷⁸, perhaps by transcytosis. This phase of membrane growth is controlled by the product of the *slam* gene⁷⁹, which coordinates localized membrane insertion and membrane growth. The growing end of the furrow canal is marked by a small cluster of cadherin/catenin complexes that form a 'basal' junction that is organized by the product of the *nullo* gene⁸⁰; this junction also contains myosin-II and Discs lost⁸⁰. Loss of microtubules also disrupts cellularization⁸⁰. Thus, the force of membrane growth may be generated by insertion of membrane⁷⁸, with actin–myosin contraction⁸¹ and microtubules guiding and stabilizing the lateral membrane as it grows downwards⁷⁸.

Towards the end of the slow phase of growth, the depleted apical membrane is replenished directly from intracellular pools in the secretory pathway⁷⁸, and cadherin/catenin complexes begin to cluster towards a more apical aspect of the lateral membrane to form spot adherens junctions. During the subsequent fast phase of membrane growth, lasting 15–20 minutes and accounting for the remaining ~20 μm of membrane growth, new membrane is inserted into the more apical portion of the lateral membrane by direct transport of vesicles from the secretory pathway^{56,78} (Fig. 5a).

How are vesicles targeted to designated sites on different membrane domains, and how are proteins pre-sorted in the Golgi complex prior to their delivery in vesicles to the plasma membrane? Studies of cell polarity in cultured mammalian epithelial cells have provided some answers to these questions, as they can be used for detailed biochemical and cell biological analyses of protein sorting pathways⁴⁶.

Most cells use endocytic pathways to route proteins between different domains of the plasma membrane and intracellular organelles⁸². In polarized epithelial cells, these pathways are coupled to sorting of proteins between different plasma membrane domains (termed transcytosis) (Fig. 5b). Transcytosis can be specific for transporting subsets of proteins between the apical and basolateral membranes (for example, ligand–receptor complexes⁸³) or general for many classes of proteins⁷⁸. In hepatocytes, for example, all membrane proteins are delivered from the Golgi to the basolateral membrane, and then apical proteins are specifically internalized and resorted by transcytosis to the apical membrane domain⁸⁴.

In many epithelial cells, protein distributions in different membrane domains are also determined by direct sorting of apical and basolateral proteins in the *trans*-Golgi network (TGN) and subsequent targeting to the correct membrane domain⁸⁵ (Fig. 5b). Sorting of proteins into the apical pathway seems to involve clustering membrane proteins into lipid rafts, and a glycosylphosphoinositide (GPI) domain in one class of proteins is sufficient to direct sorting into the apical pathway in most cells⁸⁶. Additional studies in budding yeast also suggest that lipid rafts are important in protein sorting⁸⁷. Sorting of proteins into the basolateral pathway requires sorting signals in the cytoplasmic domain and may be mediated by clustering through adaptor proteins and clathrin coats in the TGN or endosomes^{88,89}. Note that protein sorting in the TGN seems to be a constitutive process that occurs in cells such as fibroblasts that have neither a defined apical or basolateral membrane domain, nor cadherin-mediated cell–cell adhesion⁹⁰. Therefore, after cell adhesion, re-organization of the cytoskeleton and the generation of targeting patches for directed delivery of vesicles from the Golgi complex are critical from post-Golgi sorting of proteins to the correct membrane domain⁴⁶.

Organization of the cytoskeleton is important in regulating epithelial polarity (Fig. 5b). The actin cytoskeleton localizes to the cell cortex of each membrane domain, and actin reorganization occurs in a bundle circumscribing the cell at the apical junctional complex. This reorganization depends, in part, on linkage of actin to components of the apical junctional complex, and regulation of actin polymerization by Rho-family small GTPases⁵³, Arp2/3 and additional proteins⁹¹. Lateral membrane scaffolds containing actin-binding proteins regulate the distribution of some membrane proteins. For example, Na⁺,K⁺-ATPase is localized to the lateral membrane in mammalian and *Drosophila* epithelial cells through linkage to ankyrin and the spectrin scaffold^{92,93}. The spectrin scaffold is recruited to cell–cell contacts by binding to the actin cytoskeleton that links to (cadherin) sub-complexes of the apical junctional complex⁴⁶.

Microtubules are organized into bundles aligned in the apicobasal axis of the cell, with plus ends at the basal pole and intertwined mats of short filaments underneath the apical membrane and on the basal membrane⁴⁶ (Fig. 5b). How microtubules adopt these distributions is not understood. Microtubules may also be attached to the apical junctional complex⁵⁴, which may be important in orienting the mitotic spindle (this must be perpendicular to the apicobasal axis of polarity to maintain the monolayer organization of the epithelium). Actin may be important locally in vesicle delivery at the membrane, while microtubules seem to provide long-range pathways for vesicle delivery to plasma membrane domains, especially in transcytosis, and roles for both microtubule minus-end (dynein) and plus-end (kinesin family) motors has been implicated in vesicle targeting^{94,95}.

Specification of vesicle docking/fusion with different plasma membrane domains involves the SNARE complex, in which binding between cognate vesicle v-SNAREs and target membrane t-SNAREs specifies vesicle delivery. In polarized epithelial cells, different t-SNAREs are localized to apical (syntaxin-3) and basolateral (syntaxin-4) membranes⁹⁶ and inhibiting their function blocks vesicle delivery. Little is known about how t-SNAREs become localized to different membrane domains. Direct analysis of vesicle delivery to the basal and lateral membranes during polarization of Madin–Darby canine kidney (MDCK) cells revealed that, before cell–cell adhesion, vesicles containing either apical or basolateral membrane proteins fused with the basal surface⁹⁷. But after induction of cadherin-mediated cell–cell adhesion, neither apical nor basolateral vesicles fused with the basal surface; observations of vesicle fusion in the apical membrane was technically impossible, but basolateral vesicles were found to fuse at the top half of the lateral membrane⁹⁷. Thus, cell–cell adhesion may act as a signal for polarizing either vesicle docking/fusion machinery or vesicle/cytoskeleton transport pathways to the apical and lateral membranes (Fig. 5c). Interestingly, maintenance of syntaxin-3 depends on microtubules, and disruption of microtubules causes syntaxin-3 to mislocalize to lateral membrane, with the consequence that apical vesicles fuse with the lateral membrane; no effect on syntaxin-4 distribution was observed⁹⁷.

Like yeast, polarized epithelial cells express the exocyst complex (also referred to as the Sec6/8 complex)⁹⁸. In polarized MDCK cells, the exocyst is localized to the apical aspect of the lateral membrane⁹⁸, in the general region of basolateral vesicle docking/fusion⁹⁷. Inhibition of exocyst function inhibits delivery of vesicles containing a basolateral protein to the plasma membrane, but delivery of apical vesicles is unaffected⁹⁸. Cdc42 also has a role in delivery of basolateral, but not apical, transport vesicles⁹⁹. The polarized distribution of the exocyst complex depends on the maintenance of cell–cell contacts, and cadherin-mediated cell–cell adhesion recruits the exocyst specifically to sites of cell–cell contact⁹⁸. Similar to membrane growth during cellularization in the *Drosophila* embryo, polarization of MDCK cells results in a sixfold increase in the surface area of the lateral membrane, whereas those of apical and basal membrane domains do not increase¹⁰⁰ (Fig. 5c).

Conclusions

Cell polarity is portrayed in a wide variety of shapes and functions in single-cell organisms and cells in multi-cell tissues. Each cell type has not evolved a different mechanism to generate polarity, but has instead adapted a basic set of evolutionarily conserved core mechanisms, including: localized assembly of signalling complexes, cytoskeleton assembly and recruitment, mobilization of proteins from intracellular pools, and targeted vesicle delivery to sites of membrane growth. Polarized cells generate asymmetry around a cell-surface landmark by localized assembly of modular protein scaffolds that direct assembly and orientation of the cytoskeleton and specify vesicle delivery for membrane growth at that site.

Several principles of cell polarity can be deduced from these diverse examples. The pathway is hierarchical. It is initiated by a cell-surface landmark or spatial cue (the point of cytokinesis in budding and fission yeast, and cell(-cell) adhesion in epithelial cells from worms, flies and mammals), and defines a point on the cell surface to which the cell orients (mother-daughter axis in yeast, and the apicobasal axis in epithelial cells). This axis of polarity is propagated from the landmark throughout the cell by adaptation of core mechanisms that assemble and orient actin and microtubule cytoskeletons around the landmark.

Cytoskeleton reorganization around the landmark is determined by the local assembly at the landmark of signalling complexes of Rho-family small GTPases (Rho, Cdc42 and Rac1), which concentrate machinery for localized assembly of actin (profilin, Arp2/3 complex and type-I myosins), and modular protein scaffolds (the polarisome and Bni1p/Bud6p, and For3p/Bud6p in budding and fission yeast, respectively, and the apical junctional complex comprising Crb, Baz and Lgl/Scrib complexes in epithelia). Actin polymerization and assembly of protein scaffolds also reinforce the organization, retention and inheritance of the landmark. Actin cytoskeleton orientation around the landmark may regulate capture/reorientation of microtubules (involving complexes of Kar9p, Tip1p/CLIP170, Bim1p/Mal1p/EB1 and APC) for spindle orientation in the axis of cell polarity (in budding yeast, *Drosophila* neuroblasts and epithelia) and for vesicle delivery to different membrane domains (in transcytotic and exocytic vesicle transport in epithelia).

The efficiency and fidelity of vesicle delivery to the landmark depends on cytoskeleton assembly and orientation, and recruitment to the landmark of vesicle docking/fusion machinery (small GTPases such as Sec4p and RalA, SNARE protein complexes and the exocyst); in epithelia, the machinery is separated to the apical and lateral membrane domains. Localized vesicle delivery results in rapid plasma membrane growth around the landmark. The identity of the new membrane domain is maintained by the subset of (pre-sorted) proteins that had been delivered there in vesicles either directly from the Golgi complex or by transcytosis, and by retention through membrane scaffolds. Assembly of this hierarchy of complexes links landmarks to local assembly and orientation of the cytoskeleton, vesicle delivery for membrane growth, spindle orientation, and the inheritance of landmarks by daughter cells.

Acknowledgments

This review is dedicated to I. Herskowitz (University of California, San Francisco) who first inspired me to think broadly about cell polarity.

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Figure 1.

Diversity of shapes of polarized cells (not to scale). *Fucus* zygote exposed to a light gradient showing polarized distributions of ion channels/dihydropyridine receptors (red circles) and F-actin (blue line) in the rhizoid cell (bottom) compared to the thallus cell (top). Fission yeast (*Schizosaccharomyces pombe*) showing polarized distributions of actin (purple) and microtubules (blue dotted line) in the long axis of the cell, and the nucleus positioned in the centre of the cell. *Drosophila* neuroblast delaminated from the ventral neuroectoderm with an asymmetric plane of division that will yield a large 'apical' neuroblast stem cell and a small 'basal' ganglion mother cell. *Caulobacter crescentus* predivisional cell showing polarized distributions of the flagellum (swarmer cell, top) and stalk (stalked cell, bottom). Invertebrate/vertebrate transporting epithelium showing organization of polarized epithelial cells (apical membrane, green; basolateral membrane, blue) in a tube that separates two biological compartments and regulates vectorial transport of ions/solutes (red arrow) between those compartments. Budding yeast (*Saccharomyces cerevisiae*) forming a daughter cell 'bud' from the mother cell next to the previous site of cytokinesis (bud scar, red disc), and orienting actin cables (purple) for transport of vesicles (black circles) from the mother to daughter cell. Mammalian basket cell interneuron showing the distribution of the soma/dendrite (black) and axon (red; image courtesy of D. Madison, Stanford University School of Medicine).

Figure 2.

Protein pathways for generating cell polarity in budding yeast. **a**, Axial and bipolar budding patterns. **b**, A complex of proteins is assembled at the bud tip that orients the actin cytoskeleton, astral microtubules and vesicle delivery to the bud. **c**, Hierarchical organization of regulators, processes and cellular machinery linking bud-site selection to assembly of an actin cap, anchoring/nucleation of actin cables, and vesicle targeting that results in polarized membrane growth at the bud.

Figure 3.

Protein pathways for generating cell polarity in fission yeast. **a**, Distribution of actin (cables and cortical patches) and microtubule cytoskeletons relative to the 'old' and 'new' ends of the cell, which grow during the G1 and G2 phases of the cell cycle, respectively. **b**, Hypothetical organization of proteins in cortical actin patches, and interactions of microtubule plus ends with the cell end/cortical actin patch (for details, see text).

Figure 4.

Organization of polarized epithelial cells and the apical junctional complex. **a**, Polarized epithelial cells form a monolayer in which the apical (unbounded surface) is separated at the boundary with the basal and lateral membranes (bounded surfaces) by the apical junctional complex (top). The main part of the panel shows molecular organization of the apical junctional complex. In vertebrates, the apical junctional complex is separated into structurally and functionally different sub-domains comprising membrane proteins (Crumbs, JAM (junctional adhesion molecule), nectin, occludin/claudin and cadherin) linked to modular protein scaffolds, which in turn bind mostly to the actin cytoskeleton, although

links to microtubules are possible. In invertebrates (*C. elegans* and *Drosophila*), the apical junctional complex is similarly organized, except that the ‘tight junction’ function is provided by the septate junction localized below the cadherin (adherens) junction. **b**, Simplified scheme for how different protein complexes in the apical junctional complex regulate cell–cell adhesion (cadherin complex), and apical membrane (Bazooka and Crumbs complexes) and lateral membrane (Lethal giant larvae, Scribble and Disc large complex) identity. For details see text.

Figure 5.

Generation of cell polarity in epithelia. **a**, Formation of the cellular blastoderm in early *Drosophila* embryogenesis (for details, see text). **b**, Schematic representation of polarized epithelial cells. Left, organization of the actin and microtubule cytoskeletons; right, organization of vesicle transport pathways to different plasma membrane domains either directly from the Golgi complex, or indirectly via apical or basal endosomes through endocytic or transcytotic pathways. **c**, Generation of the lateral membrane domain in cultured epithelial cells. Prior to cadherin-mediated cell–cell adhesion, the exocyst is cytosolic and vesicles fuse with the basal membrane. Upon cell–cell adhesion (step 1), the

exocyst is recruited to cell–cell contacts and vesicles fuse with the forming lateral membrane. As more vesicles fuse, the lateral membrane increases in area around sixfold. Later (step 2), the exocyst and vesicle delivery are located in the apical region of the lateral membrane.