Review

Polarity and endocytosis: reciprocal regulation

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The establishment and maintenance of polarized plasma membrane domains is essential for cellular function and proper development of organisms. The molecules and pathways involved in determining cell polarity are remarkably well conserved between animal species. Historically, exocytic mechanisms have received primary emphasis among trafficking routes responsible for cell polarization. Accumulating evidence now reveals that endocytosis plays an equally important role in the proper localization of key polarity proteins. Intriguingly, some polarity proteins can also regulate the endocytic machinery. Here, we review emerging evidence for the reciprocal regulation between polarity proteins and endocytic pathways, and discuss possible models for how these distinct processes could interact to create separate cellular domains.

Introduction

In biology, the distinction between separate regions of individual cells is of utmost importance, as it impacts cell fate specification, cell movement, cell-based tissue function, and developmental processes. Some central examples include asymmetric cell division as in self-renewing stem cell populations and dividing zygotes, cell migration during wound healing and the immune response, and apical–basal polarity of cells providing absorptive and protective functions in epithelial tissues. In each case, the asymmetric distribution of biological molecules such as proteins, RNAs, organelles or lipids along a specific axis is crucial for proper polarized cellular function, whether it is in the apical–basal or anterior–posterior axis, in the plane of the tissue (planar cell polarity), or in the direction of migration. Although the global organization of intracellular components and compartments is also key to functional cell polarity, we will focus on the role of determinants that influence the polarity of the plasma membrane.

How does a cell achieve distinct polarized membrane domains? Models of targeted exocytosis, including transcytosis, have been most prominent in discussions of the mechanisms underlying plasma membrane polarity (reviewed in Ref. [1]). However, growing evidence has revealed an additional and essential role for endocytosis itself in maintaining polarized membrane domains. Moreover, proteins whose primary role seems to be in controlling cell polarity ('polarity regulators') have been found to participate in the positioning of endocytic machinery and also to regulate various steps in the endocytic pathway. We review here recent work in metazoan systems illuminating the connections between endocytosis and cell polarity, concentrating on advances in two areas: the role of canonical endocytic regulators in controlling polarity, and the role of recognized polarity regulators in controlling endocytosis.

Overview of endocytosis and polarity regulators

Endocytosis is the process of transporting extracellular or membrane-bound cargoes from the plasma membrane into the cell interior via vesicular transport. Several endocytic routes exist, but here we briefly outline a canonical endocytic pathway. Endocytic vesicles form as invaginations of the plasma membrane that subsequently pinch off, move internally, and fuse with other endocytic vesicles to form an early endosomal compartment (Box 1, Figure I). Cargo that enters the early endosome can take two different routes. Cargo destined for degradation remains in the early endosome as it matures into a multivesicular body (MVB), where cargo-containing vesicles invaginate into the interior of the organelle. The MVB ultimately fuses with the lysosome, where internalized cargoes are degraded. Alternatively, cargo destined for recycling rather than degradation is sorted into a specialized recycling endosome and then returned via vesicles to the plasma membrane. Notably, each of these steps is independently regulated by the interaction of multiple proteins (see Box 1 and Refs [2–4] for details).

Polarity regulators, as we define them here, are those proteins that show primary and widely conserved roles in polarizing various cell types. Three key polarity modules are the Crumbs (Crumbs/Stardust/PatJ), Scribble (Scribble/Discs Large/Lethal Giant Larva) and PAR (Par6/Par3/aPKC) modules [5]. These are referred to as modules because not all the constituent proteins are constantly found associated within a single complex, although they functionally act together. Each of these modules localizes to a distinct sub-domain of polarized cells. For instance, in the *Caenorhabditis elegans* (*C. elegans*) embryo, the PAR module localizes to the anterior cortex, whereas a distinct set of PAR proteins (PAR-1, PAR-2) localize to and specify the posterior cortex (reviewed in Ref. [6]). Similarly, epithelial cells are polarized along an apical–basolateral axis, with the apical surface facing a free surface or lumen, and the basolateral surfaces contacting neighboring cells and the underlying extracellular matrix [7]. In such apicobasally polarized cells, the PAR and Crumbs module proteins localize to the apical domain, whereas the Scribble module

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Box 1. Vesicle trafficking and key players in the endocytic pathway

The various steps of vesicle trafficking through the canonical receptor-mediated endocytic pathway are each regulated by the activity of specific proteins. Endocytosis begins at the plasma membrane, where transmembrane proteins such as receptors and other membrane-associated proteins cluster into invaginating clathrin-coated pits (CCP). This clustering is dependent on recognition of appropriate cargo proteins by adaptor proteins such as the Adaptin complex (AP-2), which is also responsible for recruiting the clathrin coat subunits to the forming vesicle. Proteins such as the GTPase Dynamin (Dyn) are then recruited to the neck of the budded vesicle and promote membrane scission, freeing the vesicle from the plasma membrane. Once released, the clathrin coat disassembles from the vesicle, enabling interaction between the vesicle and the early endosome (EE) target membrane. The small GTPase Rab5 promotes cargo delivery into the early endosome through effector proteins that activate membrane fusion via a SNARE complex. From the early endosome, cargoes can be recycled back to the cell surface by a Rab4- or Rab11-mediated mechanism. Cargoes that are not recycled remain in the endosome, which acquires the marker Hrs. Proteins such as the ESCRT (endosomal sorting complex required for transport) complexes are recruited to the endosome and promote the budding and scission of intraluminal vesicles (ILVs), forming the late endosome/MVB compartment. Fusion between the MVB and the lysosome, which contains digestive enzymes such as proteases and lipases, ultimately leads to cargo degradation (Figure I).

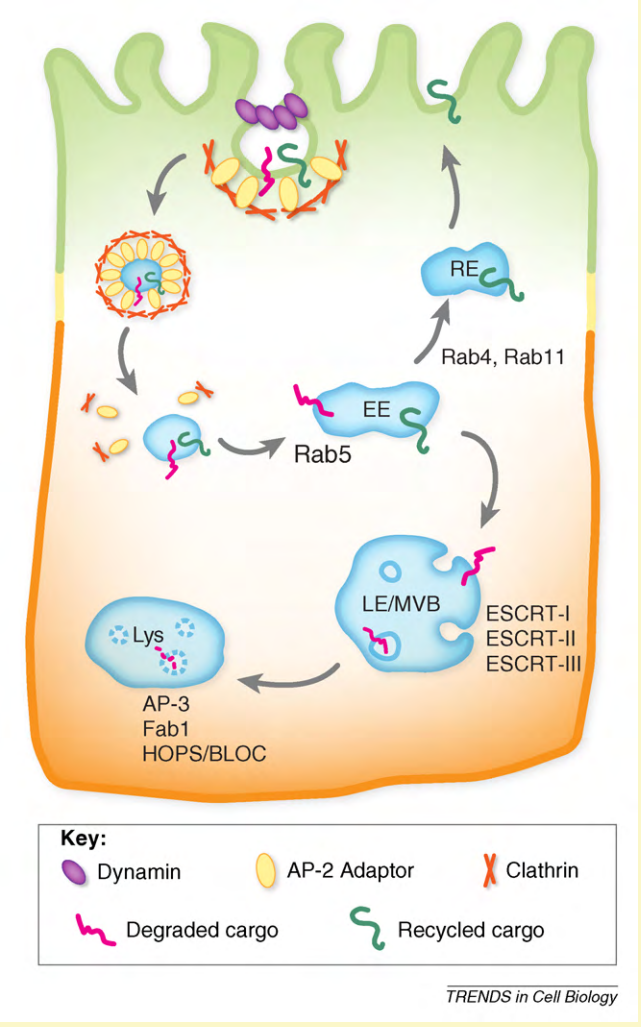

Figure I. A canonical endocytic pathway.

proteins localize to the basolateral domain. These segregation patterns are maintained both by complex regulatory interactions between the protein components [8,9], as well as interactions with additional factors – namely, small GTPases [10] and plasma membrane phosphoinositides [11].

These two seemingly independent systems – intracellular trafficking and polarity control – are now thought to work together, with recent data specifically emphasizing the importance of crosstalk between polarity proteins and endocytic regulators.

Endocytic regulators control polarity

Epithelial cells

Epithelial cells, with their distinct apical and basolateral membrane domains, have been a key system for the investigation of cell polarity. Groundbreaking work on epithelial polarity utilized cultured cell models, including Madin-Darby canine kidney (MDCK) cells [12]. Exocytic mechanisms of polarity generation in MDCK cells have received the most attention, but alternatives to a 'direct' biosynthetic delivery route have been found. Most notably, some polarized proteins are initially delivered from the *trans*-Golgi network to the basolateral surface, but are then endocytosed and re-delivered to the apical surface in a process called transcytosis (examples of apical-to-basolateral transcytosis also exist) [13]. For example, the polymeric immunoglobulin receptor (pIgR) transports its extracellular ligand dimeric immunoglobulin A (dIgA) across epithelial barriers by binding dIgA at the basal surface and then carrying it via a transcytotic pathway to the apical surface for release [14]. Transcytosis seems to require post-endocytic entry into a specialized recycling route, in which a sorting step specifies the internalized cargo for apical redelivery rather than basolateral recycling or transport to the lysosome (reviewed in Ref. [15]). The relative importance of the endocytosis-dependent transcytotic route versus the direct route varies in different cell types. For instance, epithelial liver cells rely heavily on basolateral-to-apical transcytosis to polarize the apical membrane domain [16]. One might expect that blocking regulators of endocytosis or the recycling pathway would result in retention of transcytotic cargo on an inappropriate cell surface, although there are few examples where this has been examined. In MDCK cells depleted of clathrin, cell junctions form properly but basolateral proteins become mislocalized to the apical surface [17]. However, this phenotype could be attributed to the role of clathrin in sorting in the biosynthetic pathway rather than its role in endocytosis, because cells depleted of AP-2 were not reported to have polarity defects [17]. Thus, evidence for the functional importance of endocytosis in overall epithelial polarity in mammalian cells is currently inconclusive.

By contrast, striking evidence for the importance of endocytosis in regulating cell polarity has come from several forward genetic screens in *Drosophila*. These studies demonstrate that regulators acting at multiple stages of the endocytic pathway are required for global apicobasal polarity in the imaginal disc and follicle cell epithelia (Figure 1). The first to be reported, Avalanche (Avl), is a

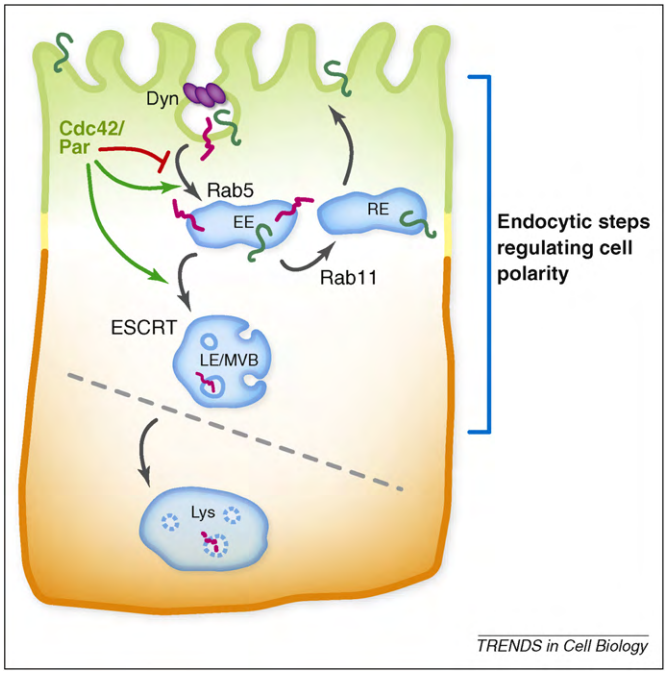

Figure 1. Reciprocal regulation between polarity proteins and endocytic regulators in epithelial cells. The plasma membrane in polarized epithelial cells is divided into apical (green) and basolateral (orange) domains, separated by cell–cell junctions (yellow). This segregation requires endocytosis, as does the apical localization of Cdc42 and the Par module. Proteins are removed from the plasma membrane by endocytosis, trafficked through the endocytic pathway (blue compartments) and then either degraded in the lysosome (pink protein) or recycled back to the plasma membrane (green). Transmembrane proteins are shown for simplicity, but the same steps could apply to peripheral membrane proteins. Key polarity regulators such as Cdc42 and Par module proteins positively or negatively (depending on the tissue context) regulate traffic from the plasma membrane to early endosomes, regulated by Dynamin (purple) and Rab5. Cdc42 and the Pars also promote the ESCRT-regulated endosome-to-MVB maturation. Green arrows represent positive regulation; red bars represent negative regulation. The gray dashed line indicates the division between endocytic steps known to regulate polarity (above) and those that do not (below); for models of the role of endocytosis in controlling epithelial polarity, see Figure 2. Note that only representative regulatory proteins are shown for the endocytic pathway. EE, early endosome; RE, recycling endosome; LE/MVB, late endosome/multivesicular body; Lys, lysosome.

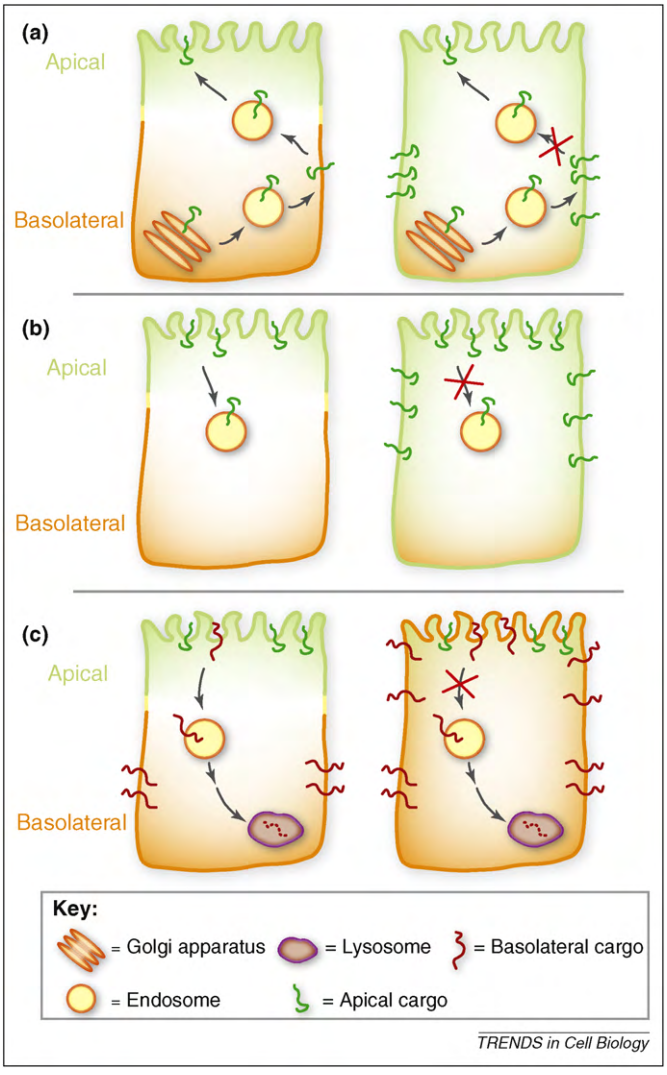

Figure 2. Possible models for endocytosis in controlling polarity in epithelia. **(a)** Newly synthesized apical proteins (dark green) could first be delivered from the Golgi network to the basolateral membrane (orange) before being endocytosed, sorted, and transported to the apical membrane (light green) in a process called transcytosis. The apical membrane is separated from the basolateral membrane by cell–cell junctions (yellow). When endocytosis is blocked, these apically-destined proteins may aberrantly accumulate on basolateral surfaces, disrupting apical–basal polarity. **(b)** Surface levels of hypothetical 'master polarity regulators' (dark green) might normally be kept in check by endocytosis. When endocytosis is blocked, these master regulators could accumulate on the cell surface, leading to mispolarization of the cell. **(c)** Intrinsic exocytosis error rates or lateral diffusion between membrane domains could occasionally lead to mislocalized basolateral polarity proteins (dark red) on the apical surface, which are removed from the incorrect domain via endocytosis and degraded in the lysosome (purple compartment). Disruptions in endocytosis would lead to unmitigated delivery errors and subsequent polarity perturbation.

syntaxin associated with and required for entry into the early endosome [18]. Avl functions with Rab5, Rabenosyn-5 (Rbsn) and Vps45 to regulate this process, and epithelia lacking any of the four gene products show identical phenotypes, which include disruption of adherens junctions (AJs), mislocalization of apical proteins at basolateral surfaces and failure to maintain a regular cellular monolayer [19]. Components of the ESCRT machinery, which control the subsequent sorting of endocytic cargoes into the MVB en route to the lysosome, are also required for epithelial polarity and show a similar null phenotype [20–23]. Finally, recent results indicate that polarity-regulating endocytic steps include the earliest step in the endocytic pathway, namely AP-2 dependent sorting into clathrin-coated vesicles and their scission from the plasma membrane [24], (S. Windler and J. Schoenfeld, pers. commun., G. Fletcher and B. Thompson, pers. commun.). Conversely, proteins that act downstream of ESCRT sorting, including Fab1 and the AP-3/HOPS/BLOC complex which regulate entry into the lysosome, are not required for epithelial polarity [25–27].

Why would canonical proteins that control the endocytic pathway be required for epithelial polarity in the fly? One possibility is that fly epithelia, like mammalian liver cells, rely on transcytosis as an apical transport route. Blocking endocytosis would prevent the post-surface-delivery internalization, sorting, and recycling required to segregate apical- from basolateral-destined cargo (Figure 2a). A second possibility is that polarity requires carefully controlled levels of certain transmembrane proteins that act as 'master regulators' of polarized domains or downstream signaling events. Endocytosis could function to restrict surface levels of these proteins by mediating their transport to and degradation in the lysosome (Figure 2b). A third possibility is that polarized exocytosis has a certain 'error rate', and endocytosis is constantly required to remove the

misplaced proteins (either for lysosomal degradation or transcytosis) to prevent the intermixing of domains (Figure 2c). Current data do not allow distinction between these and other speculative models. Studies of proteins required in the recycling pathway such as Rab11 would help to distinguish between the first two models, but analyses of Rab11-depleted conditions are hampered by the general requirement of Rab11 for cell viability in *Drosophila*, and also complicated by its additional role in polarized biosynthetic delivery pathways [28,29]. The polarity phenotypes associated with loss of ESCRT proteins, which would be expected to have their major impact on sorting of cargo destined for degradation, lend some weight to the requirement of lysosomal transport rather than recycling. Other manipulations that would distinguish these routes have yet to be reported.

It is of some surprise that, with the exception of Rab11, fly epithelial cells lacking central regulators of endocytosis can survive, and not only carry out the physiological functions required for cell growth and division but in many cases even cause continuous, tumorous overgrowth. This indicates that the process of endocytosis *per se* is not completely blocked, and that mutant cells have alternative pathways through which membrane can be internalized. While these alternative pathways are capable of allowing sufficient function for viable cell physiology and division, what is lacking is a mechanism capable of maintaining the separation of apical and basolateral domains, which relies heavily on the canonical endocytic pathway.

Neuroblasts and embryos

Endocytic regulators control polarity in multiple *Drosophila* epithelia, suggesting that this requirement is not tissue-specific and may apply to epithelial cells in general. Interestingly, there is no evidence thus far for endocytic pathways in controlling the polarity of non-epithelial *Drosophila* neuroblasts, despite the fact that other elements of the polarizing machinery (e.g. the PAR and Scribble modules) are shared between the two cell types. For instance, the Dynamin-associated protein Dap160 controls localization of the key polarity regulator aPKC in neuroblasts, but this function appears to be independent of endocytic functions [30]. Moreover, temperature-sensitive *dynamin* mutants do not alter the polarized localization of aPKC, suggesting that endocytosis may not be important for polarity in dividing neuroblasts [30]. One rationale for the different requirements for endocytosis in neuroblast and epithelial apicobasal polarity could be the use of divergent polarizing mechanisms. Extrinsic cues mediated by transmembrane proteins play a major role in establishing epithelial cell polarity. Although extrinsic cues can orient the axis of asymmetric neuroblast division, intrinsic cues mediated by cortical proteins are sufficient to orient neuroblast apicobasal polarity in the absence of extrinsic cues [31].

Nevertheless, endocytosis can control polarity in a non-epithelial cell. In the *C. elegans* embryo, membrane-associated polarity determinants specify the cortical anterior–posterior axis. The activity of Rho family small GTPases generates opposing PAR protein domains – PAR-6 in the anterior cortex and PAR-2 in the posterior – which in turn reinforce embryonic asymmetry. The cortical domains form in two distinct phases: establishment and maintenance. The establishment phase seems to rely primarily on RHO-1 and actomyosin contractility to localize the anterior determinants [32,33]. Meanwhile, CDC-42 is required specifically during the maintenance phase [34]. Endocytosis from the anterior membrane is significantly enriched specifically during the polarity maintenance phase, and this is measurably reduced in embryos partially depleted of dynamin/DYN-1 [35]. This reduction is associated with a decrease in anterior PAR-6 signal and expansion of PAR-2 into the anterior region. Also, the small GTPase CDC-42 expands beyond the anterior domain during the maintenance phase while the RHO-1 signal decreases. Finally, the presence of PAR-6-labeled endocytic puncta near DYN-1-positive foci suggests that PAR-6 is largely removed from the cortex in a DYN-1-dependent manner [35] and might indicate a mechanism in which cortical polarity cues are endocytosed and recycled to maintain PAR asymmetry. In budding yeast cells, efficient endocytosis is necessary to correct diffusion of Cdc42 from the bud site (Box 2) [36]. Remarkably, a similar phenotype is observed in *dyn-1* RNAi-treated *C. elegans* embryos [35]. One explanation for this could be that endocytosis mitigates diffusion of CDC-42 in *C. elegans* embryos, as it does in yeast. In embryos where endocytosis is compromised, spreading of CDC-42 could also lead to dispersal of PAR-6 cortical signal. Alternatively, it is possible that a decrease in

Box 2. Reciprocal regulation of endocytic recycling and polarity in budding yeast

Saccharomyces cerevisiae has provided a great deal of insight into the reciprocal regulatory mechanisms existing between polarity cues and endocytic recycling (reviewed in Refs [60,61]). The budding yeast cell polarizes during bud growth by targeting a specific set of proteins, including the conserved small GTPase Cdc42, to the bud site. Once these polarity cues are established, two distinct mechanisms maintain their localization [57]. The Rho guanine-nucleotide dissociation inhibitor Rdi1 maintains Cdc42 polarity through a non-endocytic, fast recycling mechanism. Meanwhile, Cdc42 polarity is also maintained by a slower recycling pathway, mediated by endocytosis at actin patches near the membrane which corrects diffusion of the polarity cues [36,56,57]. Indeed, yeast cells in which the endocytic actin patches were specifically depolymerized failed to prevent Cdc42 from diffusing beyond the bud tip [36,62]. Cdc42 polarization is required for the formation of both the bud and the 'shmoo' mating projection, but the decision to adopt either of these morphological fates appears to be determined by the rate of Cdc42 recycling [57]. These data demonstrate how a cell can finely modulate its morphology by transporting cell polarity cues.

Upon concentration at the bud site, Cdc42 induces the reorientation of cytoskeletal elements, including actin cables and microtubules, and recruits vesicular transport machinery to the bud site (reviewed in Ref. [61]). In addition to the well-established links between Cdc42 and the exocytic machinery, Cdc42 may also actively regulate endocytosis at the bud site by stimulating the formation of actin patches [63]. Current data suggest that the Cdc42 cap is maintained, in part, by an endocytic positive feedback loop in which Cdc42 recruits cytoskeletal elements, thereby promoting efficient endocytosis and transport of polarity cues back to the bud site [56,57]. Although the specific proteins and modules that determine the bud site differ somewhat from the polarity determinants used in higher eukaryotes, budding yeast has provided important clues as to how a cytosolic protein can exist in a dynamically polarized state at the plasma membrane.

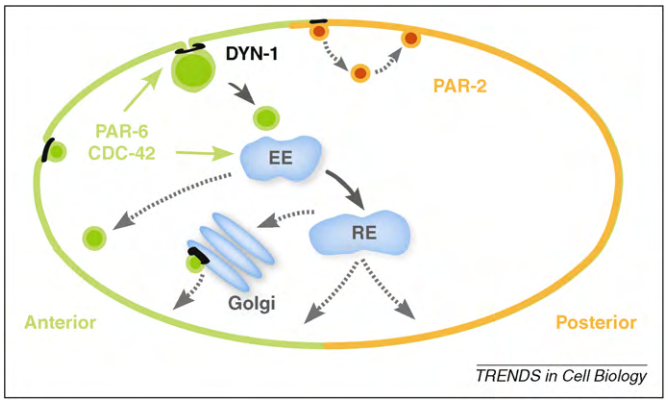

Figure 3. Reciprocal regulation between polarity proteins and endocytic regulators in non-epithelial cells. In the *C. elegans* embryo, separate domains are marked by distinct, opposing PAR proteins, including PAR-6 in the anterior (green) and PAR-2 in the posterior (orange). Dynamin promotes the anterior enrichment of endocytosis. PAR-6 maintains the anterior enrichment of dynamin (black membrane patches) and early endosomes (green, solid arrows). PAR-6/CDC-42 may be internalized and trafficked via association with the early endosomes (gray arrows), which are enriched in the anterior. The polarizing cargo could be recycled rapidly from the early endosome, or associate with the recycling endosome and be recycled back to the anterior or posterior cortex. Speculatively, PAR-6/CDC-42 could associate with the Golgi apparatus, and dynamin could participate in vesicle scission here. Another putative function of dynamin-dependent endocytosis is preventing the expansion of PAR-2 into the anterior by promoting PAR-2 removal and recycling back to the posterior cortex, as indicated by the dotted gray arrows. EE, early endosome; RE, recycling endosome. Solid gray arrows indicate areas of the model supported by data. Dashed gray arrows indicate areas of speculation that are supported by observations in other polarized cells.

DYN-1 levels could affect vesicular traffic at another endosomal compartment, such as the Golgi (Figure 3), however, current data do not allow distinction between these two speculations.

In addition to dynamin, the correct polarization of PAR complex proteins in *C. elegans* also requires the endocytic recycling regulator, RAB-11 [37]. In RAB-11-depleted embryos, the posterior PAR-2 domain is measurably reduced and PAR-3 is no longer restricted to the anterior cortex, often even colocalizing with PAR-2 at the posterior pole. Interestingly, this contrasts with the dynamin depletion phenotype, in which the posterior PAR-2 domain is ectopically extended into the anterior of the embryo, and suggests that different endocytic mechanisms may be controlling cortical PAR localization at opposite poles of the embryo. The exact details of this distinction are currently unresolved.

From the above examples, it is clear that while transmembrane proteins are natural targets for endocytosis-dependent localization, endocytosis can also play a key role in regulating the localized concentration of cortical proteins. Although it is not clear exactly how the PAR complex associates with the plasma membrane, binding to lipid-modified Cdc42 is one probable mechanism. Phosphoinositides could also play a role in linking polarity cues to the membrane [38], raising the intriguing possibility that endocytosis might influence polarity through preferential internalization of plasma membrane domains with specific phosphoinositide content. Identifying the factors involved in tethering the PAR complex to the membrane and determining how endocytic regulators might recognize PAR-enriched domains may greatly advance our understanding of how these mechanisms cooperate and interact, whether directly or indirectly.

Oocytes

Remarkably, in addition to cortical polarity cues, endocytic machinery is also involved in the polarization of cytoplasmic molecules in non-epithelial cells. In the *Drosophila* oocyte, polarization of certain mRNAs is necessary for the specification of the embryonic anterior–posterior axis. Localization of *oskar* mRNA to the posterior of the developing oocyte is required for its translation [39,40], and Oskar protein then polarizes the oocyte in part via an endocytic mechanism [41]. A long isoform of Oskar is associated with endocytic membranes at the posterior pole and is required for the observed high levels of endocytosis there [41,42]. When endocytosis is disrupted downstream of Oskar by loss of the Rab5 effector Rabenosyn-5 (Rbsn), the pole plasm proteins Vasa and Tudor are no longer anchored at the cortex but instead become diffusely localized in the cytosol [42].

Regulators of additional endocytic steps also participate in the polarization of the *Drosophila* oocyte. For instance, *Oskar* mRNA requires Rbsn, Rab11 and an intact microtubule array to maintain its posterior localization, and Rab11 as well as Rbsn are required for microtubule polarity [42,43]. Although this suggests an intriguing mechanism for oocyte polarization via cytoskeleton regulation, the molecular link between microtubules and Rab11 or Rbsn, as well as whether or not the roles of Rab11 and Rbsn in endocytic trafficking are distinct from their role in PAR module and microtubule polarity, is not known. The anterior localization of *bicoid* mRNA in the oocyte requires the ESCRT-II complex [44], but interestingly, this requirement seems independent of endosomal sorting since it is unaffected in ESCRT-I, ESCRT-III or *rabenosyn-5* mutants. Why endocytic regulators are required for the proper localization of certain mRNAs remains to be determined.

Why do such a variety of metazoan cells rely on endocytosis to polarize their cytoplasm and plasma membrane? It is interesting to speculate that evolution has taken advantage of the existing cell biological 'infrastructure' of endocytic machinery present even in single-cell organisms, such as budding yeast. Even in unpolarized cells, endosomes and other vesicles are motile and linked to the cytoskeleton [45]; so spatial regulation of this movement could be easily adapted to generate polarity via differential trafficking of transmembrane proteins. Moreover, even without their trafficking functions, endosomes, like other organelles, are 'singularities' within the cell cytoplasm. These endocytic organelles could have been adopted as anchors for cytoplasmic macromolecules lacking alternative polarity-maintaining membrane attachments. Finally, endosomes have also been adapted as signaling centers during metazoan evolution; an intriguing possibility is that endosomes are sites where polarity pathways and intercellular signaling pathways can interact with each other.

Polarity regulators affect endocytosis and recycling

While evidence for endocytic regulation of cellular polarity has been rapidly accumulating, concurrent work has also revealed that polarity proteins regulate the endocytic machinery. Interesting observations from *C. elegans* first

implicated polarity cues as endocytic regulators. For example, a marker for early endosomes, EEA-1, localizes to anteriorly enriched puncta in a manner that depends on PAR-3 [46]. Similarly, the *C. elegans* ortholog of dynamin, DYN-1, is enriched and maintained in the anterior cortex in a PAR-6- and PKC-3/aPKC-dependent manner [35]. The clearest evidence for a functional link emerged when the anterior PAR module and the Rho GTPase CDC-42 were shown to be required for efficient endocytosis in *C. elegans* [47]. Reduced function of any of these proteins also impairs endocytic traffic in non-polarized Chinese hamster ovary (CHO) cells. Cdc42 and Par-6, in particular, are required for the uptake of clathrin-dependent cargo as well as the recycling of clathrin-independent cargo, demonstrating specific roles for polarity cues in both endocytosis and trafficking [47]. These findings point toward a specific, unexpected and conserved role for PARs in regulating endocytic traffic.

Studies in *Drosophila* epithelial cells have emphasized the importance of PAR-regulated endocytosis, but have also revealed that the details of the mechanisms could vary between cell types. Disruption of Cdc42 function in the embryonic ectoderm leads to a loss of apical proteins from the plasma membrane and their accumulation in enlarged endocytic structures within affected cells [48]. Similar accumulations of apical cargo are observed in *par6* and *baz*/*par3* mutant embryos, suggesting that Cdc42 and the Par proteins function as negative regulators of apical endocytosis. Consistent with this, reducing endocytosis by disrupting Rab5 function rescued the phenotypes of Cdc42-compromised cells. Surprisingly, the compartment in which apical cargo accumulated contained markers of early endosomes, suggesting that further advancement through the endocytic pathway was blocked. Thus, in the *Drosophila* embryo Cdc42 and the PAR module appear to function in two distinct roles: negatively regulating early apical endocytosis, and positively regulating early endosome to late endosome maturation (Figure 1), although an additional role in regulating recycling cannot be ruled out.

Two groups independently determined that Cdc42, Par6, and aPKC control *Drosophila* epithelial organization through regulation of endocytosis, although the results of their experiments in imaginal discs differ somewhat from the observations in neuroectodermal cells. In these studies, cells lacking functional Cdc42, Par6 or aPKC contained enlarged Rab5- and Rab11-positive endosomes, as well as discontinuous AJs and E-cadherin-rich junctional extensions and puncta [49,50]. Similar AJ discontinuities and E-cadherin-rich structures were observed in cells with disrupted shibire/Dynamin function, suggesting that the structures observed in the Cdc42-, Par6- or aPKC-compromised cells are arrested endocytic structures and that E-cadherin is endocytosed in a dynamin- and Cdc42-regulated manner. It thus seems that in the imaginal disc, in contrast to the embryonic ectoderm, the PAR proteins positively regulate scission of endocytic vesicles containing junctional cargo from the membrane, and this contributes to the maintenance of junctions and proper localization of polarity cues.

How could polarity cues affect endocytic trafficking? One possibility is that the cortically localized Cdc42/PAR module regulates regions of endocytic activity, perhaps through specific polarized GTPase-activating proteins such as Rich1 [51]. Par6 and aPKC might also act in conjunction with other Cdc42 effectors after recruitment to the apical or anterior membrane. The kinase activity of aPKC could phosphorylate targets, such as endocytic regulators or adaptor proteins, which specify the rate and identity of endocytosed cargo; one example is the AP-2-interacting endocytic adaptor Numb [52,53]. Another possibility is that proper polarity protein function is required for cytoskeletal organization. In the imaginal disc, the phenotypic similarity between Cdc42, the Cdc42 effector Cip4, dynamin, WASp and Arp2/3 suggests that Cdc42 regulates E-Cad endocytosis in part through regulated actin polymerization [49,50]. Although this might suggest that a general misregulation of cortical actin contributes to the endocytic defects, the lack of endocytic phenotypes in tissues with disrupted cortical actin, such as those mutant for Rac or Scar, suggests that WASP and Arp2/3 regulate actin in a more specific context, perhaps in promoting Dynamin-dependent vesicle scission. The PAR module might also promote the efficient endocytic trafficking of cargo by affecting microtubule organization and/or dynamics [54] (reviewed in Ref. [55]). Whereas the evidence for polarity cues organizing endocytic traffic is convincing, the details of the molecular mechanisms remain unclear. Identifying interactions, both direct and indirect, between the polarity machinery and endocytic regulators will lead to a clearer view of how certain polarity proteins act as positive or negative regulators of endocytosis in different contexts.

Concluding remarks

The emerging data suggest a synergistic model in which polarity and endocytosis regulators mutually contribute to the proper functioning of polarized cells. Owing perhaps to the apparently context-specific behavior of some molecules, no simple, central model has yet emerged. However, it seems that dialogue between the two regulator classes reinforces the proper positioning and activity of both polarity cues and endocytic machinery (Figures 1 and 3). In the clearest example, the PAR module may promote dynamin-dependent endocytosis, while dynamin-dependent endocytosis may be required to maintain correct localization of the PAR module. As work in these fields continues, similar examples are likely to arise and yield a more cohesive and complete view of the interplay between polarity and endocytosis.

The data from various experimental systems suggest that the cooperation between cell polarity and general endocytic traffic is conserved across different cell types and organisms. There is, however, variability in whether a particular polarity regulator acts positively or negatively on trafficking steps, and on what step of endocytic traffic the polarity regulators exert their influence and vice versa. Moreover, the interactions between the endocytic and polarizing proteins could be direct or indirect, and no studies have yet explicitly addressed this uncertainty. Further work will also distinguish between the possibilities of polarity regulators impacting the endocytic and trafficking pathways through actin and/or microtubule

organization, or by more directly affecting endocytic regulators, perhaps through aPKC-dependent phosphorylation. Finally, the importance of the relation between endocytosis and polarity regulators in establishing versus maintaining polarity based on other cellular cues remains ambiguous. While models derived from *in silico* and *in vivo* data in budding yeast imply that endocytosis and recycling can be an integral component of polarity maintenance mechanisms (Box 2) [56,57], more extensive analyses are necessary to determine the importance of endocytosis in establishing polarized axes during metazoan development.

Overall, the work reviewed here indicates that the standard cellular process of endocytosis exists in a flexible, reciprocal relation with polarity regulators and is utilized by organisms in differing ways to organize asymmetric domains. Although we have focused our discussion on animal models, it is important to note that growing research emphasizes the importance of cell polarity in plants as well (reviewed in Ref. [58]). Future advances toward elucidating polarization mechanisms will benefit from the introduction of quantitative, real-time fluorescent trafficking assays to invertebrate systems, and potent *in vivo* disruption of the endocytic pathway to vertebrate systems. Ultimately, a more complete understanding of the interplay between endocytosis and polarity and the reasons why particular cell types employ specific regulatory strategies will clarify the mechanisms necessary for proper organismal development and potentially provide new treatments for the numerous diseases associated with disruptions in cell polarity (reviewed in Ref. [59]).

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