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Switching States: dynamic remodelling of polarity complexes as a toolkit for cell polarization

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Abstract

Polarity is defined by the segregation of cellular components along a defined axis. To polarize robustly, cells must be able to break symmetry and subsequently amplify these nascent asymmetries. Finally, asymmetric localization of signaling molecules must be translated into functional regulation of downstream effector pathways. Central to these behaviors are a diverse set of cell polarity networks. Within these networks, molecules exhibit varied behaviors, dynamically switching among different complexes and states, active vs inactive, bound vs unbound, immobile vs diffusive. This ability to switch dynamically between states is intimately connected to the ability of molecules to generate asymmetric patterns within cells. Focusing primarily on polarity pathways governed by the conserved PAR proteins, we discuss strategies enabled by these dynamic behaviors that are used by cells to polarize. We highlight not only how switching between states is linked to the ability of polarity proteins to localize asymmetrically, but also how cells take advantage of 'state switching' to regulate polarity in time and space.

Introduction

Cell polarity describes the functional asymmetry of cells along a defined axis. Polarized cells typically possess complex molecular networks that orchestrate the asymmetric segregation of molecules, classically in response to local cues, and translate these molecular asymmetries to spatially control downstream effector pathways. Polarity networks are incredibly diverse, with distinct pathways operating not only in different species, but also within different cells of an organism or even at different times in the same cell. Although we will focus on the polarity of animal cells, and in particular, pathways related to the conserved PAR cell polarity network, we hope to illustrate principles common to the diversity of cell polarity networks and the cells in which they operate.

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Animal cell polarity is typically associated with conserved networks of polarity regulators. One of the most widespread is the *par*-titioning defective or PAR proteins pathway, which includes the scaffolds Par3 and Par6, the atypical protein kinase C (aPKC) and the small GTPase Cdc42. This PAR complex is typically opposed by lethal giant larva (Lgl) and Par1 (Mark) kinase, along with context-specific components such as PAR-2 in *C. elegans*, or Slmb (SCF E3 ubiquitin ligase) and Lulu/Yurt in higher animals [1–5]. These core PAR proteins are associated with a variety of other players. In epithelia, aPKC associates with the apical Crumbs complex - Crumbs, Stardust (Pals1) and PatJ, while basolateral membranes are defined by Scribble and Discs-Large (Dlg), in concert with Lgl and Par1 [6,7]. Together these molecules are involved in both the integration of geometric cues and the regulation of numerous downstream processes to functionally polarize cells. Work over the past decades has revealed that far from existing as stable complexes with a single function, these molecules are engaged in dynamic changes in interactions, localization patterns, and even molecular function [8].

This mini-review will focus on the dynamic nature of these polarity complexes and related molecules in the pathways they regulate. Specifically, we focus on their capacity to switch between states and how such switching enables polarity to be both robust and adaptable to the diversity of contexts in which these molecules act. Whether this switching is between active and inactive forms, bound and unbound, clustered and non-clustered, condensed and soluble, it is increasingly clear that such switching events control the ability of molecules to segregate in space and enable their function to be regulated in time and space. Ultimately, it is this adaptability of these networks that has enabled their use across a broad range of morphological events, including, but not limited to asymmetric cell division and fate specification, establishment of cell and tissue architecture and directional cell migration.

Pathways to Asymmetry

Entropy dictates that the random motion of molecules will tend to dissipate asymmetries in a system. Therefore, the generation of polarized distributions of molecules across the cell requires mechanisms to induce and amplify local concentration differences. Cells have consequently developed active processes for inducing asymmetry, including spatial varying production/degradation (source-sink), biased transport or local retention. Here we focus on the latter two mechanisms in which state switching allows for local accumulation.

Active and Passive Directed Transport

An intuitively simple mechanism to drive asymmetry is through active and directed transport of polarity molecules. This is typically accomplished by molecular motors running on cytoskeletal networks, with directional transport arising from bias in the orientation of cytoskeletal tracks and association of cargo with motors that move preferentially in one direction along them. A classic example is transport of *oskar* mRNA by Kinesin 1 in the *Drosophila* oocyte. Here, the plus-end directed motion of kinesin, combined with a weak PAR-1-dependent bias in microtubule orientation, drives *oskar* accumulation at the posterior of oocyte[9,10] (Figure 1A). Directional motor-driven transport similarly underlies transport of Cdc42- and Rac-enriched vacuoles to the nascent apical surface of endothelial cells in

support of tubule formation[11,12], and polarized transport of PAR-3 to the apical and junctional membranes in endothelia and epithelia[13], and to axon growth cones in neural cells[14].

In addition to asymmetric transport networks, polarized transport requires regulation of cargo binding and switching between transport competent and incompetent states. For Par3, a switch in motor preferences allows for cell-type specific orientation of Par3 polarity relative to the underlying polarity of the microtubule network. A preference for the microtubule minus end-directed motor dynein allows Par3 to reach polarized junctions during epithelial cellularisation, junctions in collectively migrating fibroblasts, and the anterior of the *Drosophila* oocyte [13,15,16]. By contrast, a switch to the plus end-directed kinesin motor KIF3A allows Par3 to segregate to the tip of future axons in neurons [14]. PAR proteins in turn can regulate asymmetric transport. In migratory cells, leading edge enrichment of PAR proteins switches the relative forward (kinesin) and rearward (dynein) flux of intermediate filaments (IF) to promote transport towards the leading edge [17] (Figure 1B).

Directed transport can also arise indirectly through bulk flows, independently of direct motor-driven transport. For example, in lamellipodia of migrating cells, preferential nucleation and polymerization of actin at the cell front is coupled to contractile activity of myosin at the rear to drive a rearward bulk flow of actin filaments in a thin layer under the membrane known as the cortex [18–20]. Retrograde flow can induce gradients of a broad range of molecules associated with actin or even simply entangled or embedded within this viscous layer, to sustain long-lasting cell polarization of migrating cell [21]. Ultimately, the magnitude of polarity for a given species was shown to be directly related to the timescale of switching between actin-associated and free states, revealing how bias in switching rates can help shape distributions of molecules in such systems [13] (Figure 1C).

Such flow-dependent transport drives polarization of *C. elegans* zygote. Here, PAR-3/PAR-6/PKC-3(aPKC) are actively segregated into the anterior, not through direct association with motors, but by anterior-directed flows of the membrane-associated actomyosin cortex [22,23]. A recent trio of studies revealed PAR-6 and PKC-3 switch between alternate PAR-3- and CDC-42-associated states. Cortical flow-dependent transport or ‘advection’ of these molecules by flow depends specifically on the PAR-3 state, which is characterized by long-lived, slowly diffusing clusters, that are carried by the flow and actively promoted during polarization [24–26] (Figure 1C).

Local Retention

State switching also plays a key role in generating cell polarity through diffusion-based retention mechanisms, which require the interconversion of molecules between slow- and fast-moving states. Spatial regulation of the rates at which molecules shift between these two states biases the effective diffusion of molecules across the cell, and provides a mechanism for asymmetric accumulation (Figure 2).

One common paradigm that illustrates this concept is the case of switching between inactive, freely diffusing cytoplasmic states and an active, slowly diffusing membrane states. Because

of their reduced diffusion, molecules loaded onto the membrane in one location will not immediately diffuse away, allowing them to accumulate locally. This local accumulation creates an effective sink for cytoplasmic molecules as they are recruited to the membrane, causing net diffusion of cytoplasmic molecules from elsewhere in the cell to balance local binding events. This local accumulation, which may be driven by upregulation of membrane binding or reduction in dissociation, when coupled to diffusive transport from elsewhere in the cell, gives rise to a polarized distribution.

In the *C. elegans* zygote, PAR proteins ultimately segregate into two opposing membrane domains. Fluorescence recovery after photobleaching (FRAP) provided the first measures of membrane diffusion and dissociation in the embryo, supporting a model in which spatial variation in membrane binding allowed accumulation of slower diffusing species within domains drawn from a rapidly diffusing cytoplasmic pool [27]. Recent single molecule methods have provided further insight into the spatially biased turnover of PAR proteins, suggesting that while posterior proteins primarily bias membrane association of anterior proteins, most likely through limiting availability of membrane-binding adapter molecules PAR-3 and Cdc42 [28], posterior protein distributions are driven primarily by an anterior bias in dissociation downstream of the kinase PKC-3 [29,30].

In such models, understanding the mechanisms of membrane association and how it restricts diffusion becomes paramount. Regulated binding of plasma membrane lipids has emerged as a common theme. The polarity-related proteins Par1, Lgl, Miranda, Numb, PAR-2 and the anterior protein Par3 share a common ability to bind anionic phospholipids, most likely the plasma membrane-enriched PIP2, via dedicated domains [31–36]. A shared characteristic of numerous polarity-related proteins, these domains are often the direct targets of regulatory kinases, allowing membrane association to be regulated in time and space. Miranda, Numb, Lgl and PAR-2 membrane binding domains are direct targets of the polarity-related kinase aPKC, providing a mechanism for exclusion from aPKC-enriched domains [32,34,36,37].

The affinity of single lipid binding domains is often insufficient to allow stable membrane association and restriction of diffusion. Instead, molecules typically engage in multivalent association with the membrane through the formation of homo- or hetero-dimeric complexes (Figure 2A). PAR-2 and Par3 form homo-oligomers [30,38], while PAR-1 and the related MARK kinases are thought to require coincident binding to plasma membrane lipids along with additional accessory factors, such as the *C. elegans* PAR-2 protein, to achieve maximal membrane enrichment [31]. In the case of Par3, it is precisely these higher-order, multivalent interactions that allow formation of stable, slow-diffusing, membrane-associated clusters that are segregated by cortical flows as discussed above and which likely play a role in numerous systems [38,39]. Assembly of complexes then becomes an additional point for regulating switching between fast cytoplasmic and slow membrane-associated states, with aPKC proposed to disfavor PAR-2 oligomerization [30] and PAR-1/PAR-2 heterodimer formation [31], and PAR-1 impeding PAR-3 clusters [28].

While membrane association is a common paradigm for regulating mobility in polarity networks in bacteria, yeast, plants, and animals, mechanisms for inducing spatial variation in diffusivity are much broader. One such case is the formation of asymmetries of cytoplasmic

proteins, the most well-studied examples occurring the *C. elegans* zygote. Here, during the first cell division, asymmetries of various fate determinants are set up along the anterior-posterior axis to induce cell fate differences in the two daughters. Despite not ostensively requiring membrane binding, the general paradigm of regulated switching between fast and slow diffusing states is similar, with slow diffusing species accumulating preferentially on one size of the cell. The key difference is in how the slow diffusing state is achieved.

One way to restrict mobility is through binding of cytoplasmic RNA (Figure 2B). mRNAs diffuse very slowly in eukaryotic cytoplasm (<0.4μm^2/s [40,41] vs >20μm^2/s for soluble GFP [42]) and thus reversible RNA binding would allow for local switching of mobility between slow, RNA-associated states and fast, unbound states. In the zygote, biases in mobility were first observed for two cytoplasmic fate determinants downstream of PAR polarity, MEX-5 and PIE-1 [43,44]. Polarity of MEX-5 was subsequently shown to require RNA-binding, with the switch between bound and unbound states regulated by the opposed activity of the polarized kinase PAR-1, which promotes the unbound, fast state, and a uniform phosphatase PP2A that catalysed the reverse [45]. Quantitative imaging and single particle tracking have now extended this paradigm of RNA-binding and spatial variation in switching rates to PIE-1, POS-1, and MEG-3 [46–49].

Liquid-liquid phase separation (LLPS) has emerged as another common theme for structuring the cytoplasm, allowing the local enrichment or sequestration of molecules within membraneless compartments. Liquid droplets diffuse at extremely slow rates. Thus, when a soluble molecule partitions into the liquid droplet phase, they are effectively immobilized, allowing them to accumulate locally. LLPS governs asymmetric segregation of germ granules in a number of systems (Figure 2C). In the *C. elegans* zygote, LLPS is critical for asymmetry of the germline associated P granules, which are large phase separated droplets composed of diverse proteins and mRNAs [50]. Here LLPS is controlled by MEX-5. Anterior-enriched MEX-5 locally suppresses phase separation through competition with P granule components for RNA binding sites, ultimately restricting P granules to the posterior [51,52]. LLPS has been proposed to play a role in numerous polarity pathways, including in the segregation of basal fate determinants Pon/Numb in Drosophila neuroblasts [53]. Theoretical work suggests a unique feature of LLPS in cell polarity may be its ability to lock in a polar distribution of phase separated material following a transient stimulus without additional energy input [54].

When taken to the extreme, this paradigm of local switching between rapid and slow diffusing states morphs into a simple diffusion and capture model in which an immobile scaffold binds to and thereby locally traps an otherwise randomly moving species (Figure 2D). Such a mechanism guides anterior and posterior trapping of the anterior marker Bicoid and the posterior marker Oskar, downstream of PAR polarization of the Drosophila oocyte, allowing randomly or weakly oriented transport to be converted to stable asymmetry [55,56]. Thus, consideration of the mobilities of molecules and their spatial regulation supports a generic mechanism for the polarization of molecules in cells.

Regulation of polarity in space and time

The ability of polarity complexes to switch between states provides cells with flexible and highly controllable systems for regulating the polarization and downstream outputs of polarity networks in space and time. This is particularly critical as conserved polarity molecules are often reused and reconfigured as development proceeds. Even within the *C. elegans* embryo, the PAR polarity machinery is deployed across multiple contexts, first to drive cell-autonomous symmetry-breaking and anterior-posterior axis specification in the zygote, then to specify contact dependent radial polarity in early blastomeres, and subsequently to specify apical-basal polarity in a variety of epithelial tissues [57]. In each case, the configuration of polarity proteins and the symmetry-breaking signals to which the PAR network respond vary. Thus, spatiotemporal control over polarity networks themselves is central to their proper function.

Developmental switches

Developmentally regulated changes in PAR polarity are often associated with cell type specific switches in the composition of PAR complexes and changing demands on their functions in cells. We have already seen how directionality of Par3 transport on microtubules is subject to cell-type specific regulation. In *Drosophila*, sensory organ precursors (SOP) derive from PAR-polarized epithelia. Before entry in mitosis, Par3 (Baz in fly) which is normally positioned uniformly at the apical junctions in the epithelia, becomes planar polarized thanks to its interaction with Meru, a SOP-specific planar cell polarity (PCP) adaptor [58]. Baz therefore shifts from responding to apical basal polarity (ABP) components to PCP modules to polarize the SOP cell along the PCP axis prior to cell division (Figure 3A). Interestingly such a functional switch between ABP and PCP also occurs in vertebrates, although the molecular details differ [59](Figure 3A). In developing epithelia, Baz again is subject to regulation of its localization and function, here through its phosphorylation by aPKC, which alters the balance of Par-containing functional complexes. Baz initially localizes apically to direct recruitment of Par6, aPKC and other apical components [6,60]. Subsequently, phosphorylation by aPKC triggers the release of Baz from these apical complexes and its relocation to the apical junctions; while Par6/aPKC associates with Crumbs to help specify apical membrane identity in a Cdc42-dependent fashion [61,62] (Figure 3B). In yet another example, the polarity component Yurt (Lulu in mammals) undergoes a developmental switch in localization. Early on, Yurt plays a role in preventing invasion of apical components such as Crumbs and aPKC into basolateral regions. Yurt activity relies on its oligomerized state and is restricted basolaterally by negative aPKC phosphorylation. Epithelium maturation leads to Yurt oligomers resisting aPKC phosphorylation, thus extending apically to suppress local function of Crumbs, and thereby limiting apical domain size [5,63](Figure 3B).

Cell cycle control

The cell cycle places constraints on the function of PAR polarity-related protein complexes. Reviewed extensively elsewhere in this issue, mitosis poses unique challenges for polarized cells due to the need to coordinate cell polarity with the dramatic spatial re-organization cell division entails. We already saw a glimpse of this in the regulation of asymmetric division of

epithelial SOP cells. In fact, even in symmetrically-dividing epithelia, polarity regulators play important roles, often associated with control of the mitotic spindle alignment. In this case they ensure division occurs parallel to the plane of the epithelial tissue to maintain tissue architecture. In Drosophila follicular epithelia, spindle alignment requires remodelling of the Dlg/Scrib/Lgl complex. In non-mitotic epithelia, the Dlg/Scrib/Lgl complex is implicated in apical basal polarity [64]. However, during mitosis of various epithelial cells, Dlg/Scrib become important for proper spindle alignment in the plane of the epithelia [65,66]. New work suggests that at least in Drosophila follicular epithelia, this mitotic shift in function requires phosphorylation of Lgl by the cell cycle kinase Aurora A, which releases Lgl from the Dlg/Scrib complex [67,68]. As Lgl and the spindle orientation factor Pins are bound by the same phosphoserine binding region within the guanylate cyclase domain of Dlg [69,70], phosphorylation of Lgl by Aurora A could allow Dlg to associate with Pins to orient the spindle in these cells. Following division, activity of the PP1 phosphatase allows Lgl back onto the membrane to act in concert with Scrib in maintaining basolateral membrane identity [71]. Finally, recent data suggest that Aurora and Polo kinases can tune activity of PAR complex proteins. In Drosophila neural precursors, Aurora A modulates PAR complex composition by phosphorylation of PAR-6 to activate aPKC [72], while in the *C. elegans* zygote, Aurora A and the Polo kinase PLK-1 control the levels and oligomeric state of PAR-3 to ensure proper symmetry-breaking in response to spatial cues [24,73].

Outlook

Far from being stable complexes, recent work has revealed the dynamic behaviour of polarity proteins such as PARs, to be integral in their function. This capacity for dynamic remodelling also favours their ability to segregate in space, and to adapt to changing contexts, such as during tissue development, within the cell cycle, or in different cell types. We are only beginning to understand the complex regulation of these dynamic behaviours.

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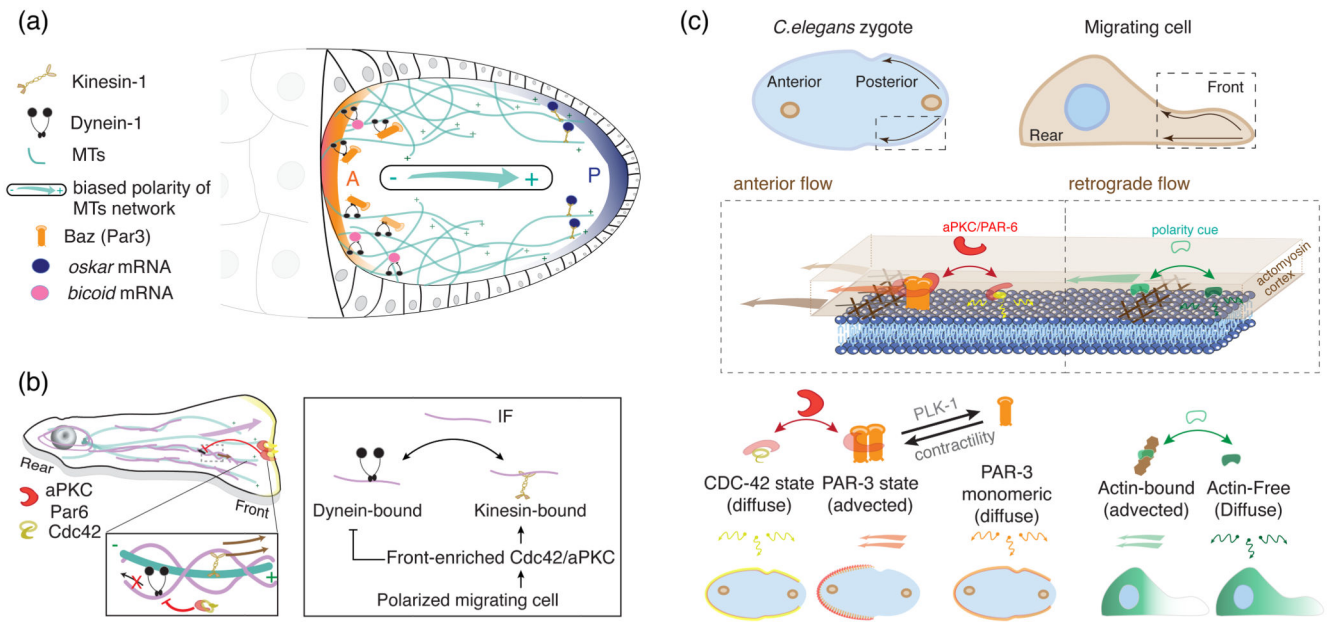


Figure 1. Generating asymmetry through directed transport

(a, b) Examples of directional transport driven by polarized cytoskeletal tracks and preferential cargo-motor association. **(a)** In the oocyte, an asymmetric MT network drives polarized transport of *oskar* and *bicoid* mRNA as well as PAR proteins. Their differential accumulation arises from preferential association of cargoes with distinct motors: dynein drives accumulation of *bicoid* and Bazooka to the anterior, while kinesin drives accumulation of *oskar* to the posterior. **(b)** A PAR-dependent switch in motor affinity allows polarized accumulation of intermediate filaments (IF) during cell migration. **(c)** Polarized transport by bulk cortical flow (advection). At left, cortical actomyosin flow in the *C. elegans* zygote preferentially transports a pool of PAR-6/aPKC that is associated with oligomers of PAR-3 to the anterior. The oligomeric state of PAR-3 is tightly regulated by cell-cycle kinase PLK-1 and cortex contractility itself and is essential for its transport. At right – direct association with the actin cortex allows polarity cues to be advected by retrograde actin flow during cell migration. The binding-affinity of molecules for F-actin shapes their concentration gradients, reinforcing the polarity of the moving cell.

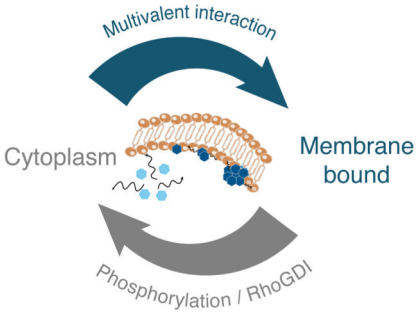
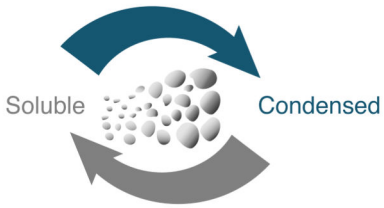
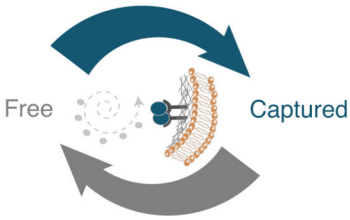
State 1 (Fast) ⇄ State 2 (Slow)	Molecular Mechanisms	Examples
(a) Membrane Interaction 	Homo-oligomerization	Par3 [38,74-76] PAR-2 [30] Lgl [77] Yurt [63]
	Hetero-oligomerization	PAR-1/PAR-2 [31] aPKC/Par6/Par3 [24] Cdc42/Bem1/Cdc24 [78]
	aPKC-dependent phosphorylation	PAR-1 [31] PAR-2 [32] Lgl [34,35] Miranda, Numb [34,37]
	Par1-dependent phosphorylation PLK-1-dependent phosphorylation	Par3 [32,79] Par3 [24]
	RhoGDI-dependent membrane extraction	Cdc42 [80] ROPs (root hair [81], pollen tube [82])
(b) Cytoplasmic Trapping 	RNP trapping PP2A-dependent binding	MEX-5 [45] PIE-1 [43], POS-1, MEX-1 [46-48] MEG-3[49]
	PAR-1-dependent unbinding	MEX-5 [45]
	MEX-5 / PLK-1-dependent unbinding	PIE-1, MEX-1, POS-1 [48]
(c) Liquid-Liquid Phase transition 	RNA-protein interactions MEX-5 dependent	*P-granules:* PGL-1/GLH-1 [50], MEG-3 [51]
	Oskar dependent	*Nuclear granules:* Oskar/Vasa [83]
	Protein-protein interactions	Numb/Pon [53]
(d) Diffusion and Capture 	Myosin-dependent anchor	*oskar* mRNA/ Staufen [56]
	Oskar-dependent anchor	Germ Granules [84] *nanos* mRNA [85]
	Unknown anchor	*bicoid* mRNA [55]

Figure 2. Asymmetry by diffusion-based local retention mechanisms

Asymmetric switching between slow- and fast-moving states allow for polarized accumulation. **(a)** Interaction with the plasma membrane lipid bilayer restricts diffusion. Membrane association is often associated with oligomerization or complex formation providing multivalent interactions that enable stable membrane interaction and activity. Displacement from the membrane shifts molecules to a fast diffusing cytoplasmic state. This switch may be triggered by phosphorylation on basic hydrophobic residues or oligomerization domains important for membrane binding, or, in the case of RhoGTPases,

by their extraction via RhoGDI proteins. See [24, 30–32, 34, 35, 37, 38, 63, 74–82]. **(b)** Cytoplasmic fate determinants can be slowed by switching into an RNA-bound state. By spatially regulating interconversion between an RNA-trapped state and an unbound, fast diffusing state, molecules can be segregated asymmetrically in the cytoplasm. See [43, 45–49]. **(c)** Liquid-Liquid phase separation (LLPS) also allows for local retention as phase separated droplets diffuse very slowly. Spatial regulation of a molecule's ability to partition into droplets allows them to be retained asymmetrically. See [50, 51, 53, 83]. **(d)** Capture of randomly diffusing molecules by immobile anchors represents an extreme case of diffusion-based retention mechanism. See [55, 56, 84, 85].

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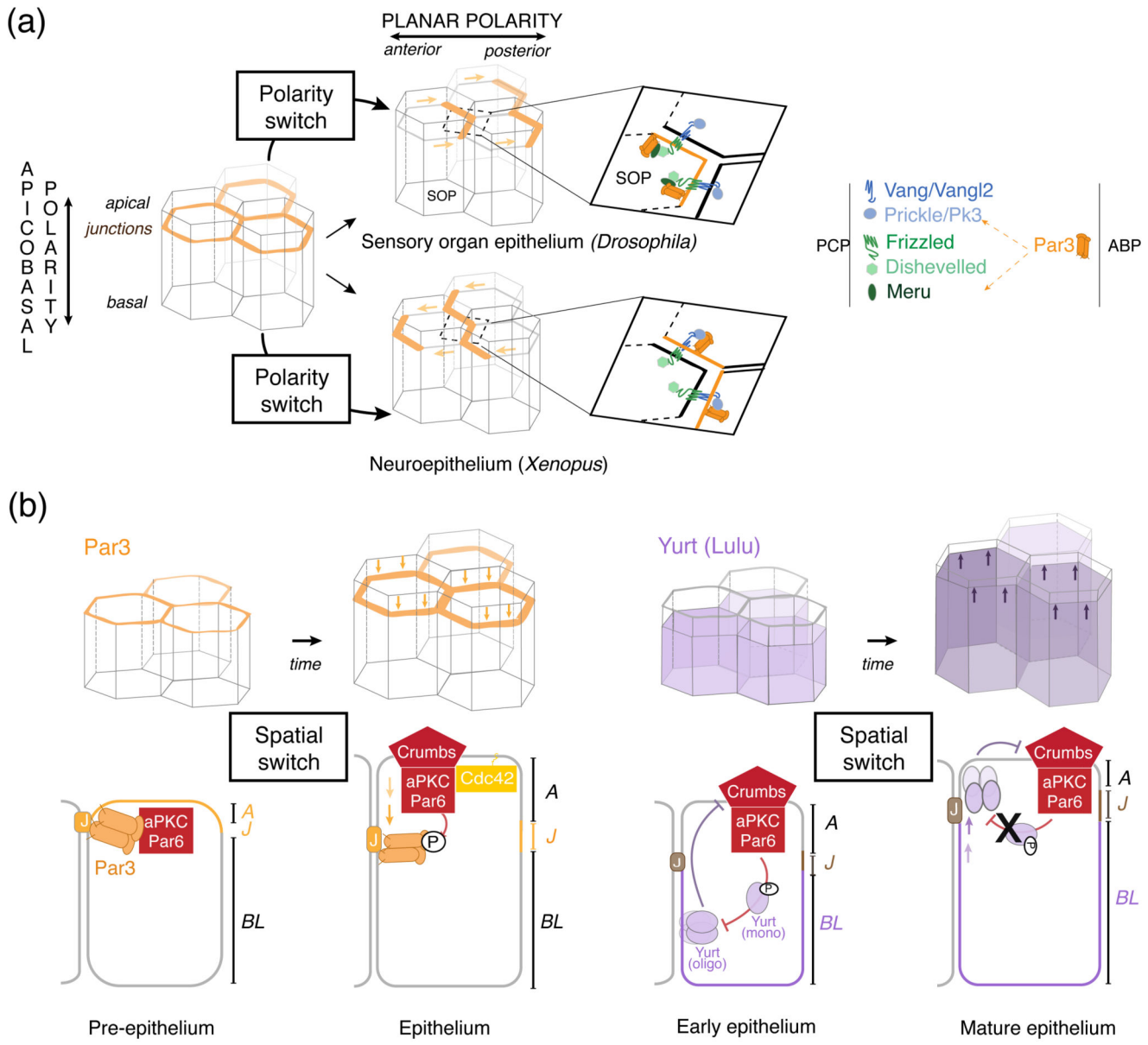

Figure 3. Functional switching of polarity modules during tissue development

(a) Context-specific switching of Par3 polarization from apical-basal to planar polarized during development. Switching is achieved by developmentally regulated expression of the polarity adaptors Meru in sensory organ precursor cells (*Drosophila*) and Prickle3 in vertebrate neuroepithelium, which link Par3 to components of the planar cell polarity pathways. **(b)** Temporal switches in polarity protein behaviour during development of apical-basal polarized epithelia. At left, Par3 (orange) is shown initially enriched at nascent cell-cell contacts (J) and recruits aPKC/Par6 apically (A). Par3 is then phosphorylated by aPKC, presumably upon activation by apical enrichment of Cdc42, which triggers its exclusion from apical membrane and relocation to the junctions. In parallel aPKC/Par6 is released from Par3 and retained apically via interaction with the Crumbs complex. At right,

Yurt (Lulu in mammals, purple) extends apically during epithelium maturation. Yurt inhibition of Crumbs depends on its oligomerized state (oligo). This state is suppressed by aPKC, which promotes the monomeric state (mono), restricting Yurt activity to the basolateral domain (BL). As the epithelium develops, phosphorylation of Yurt is reduced, possibly due to increased phosphatase activity or insensitivity to aPKC, promoting its oligomerization, which helps restrict the size of the Crumbs-enriched apical domain.

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