

## REVIEW

# The PAR proteins: from molecular circuits to dynamic self-stabilizing cell polarity

Charles F. Lang<sup>1,2</sup> and Edwin Munro<sup>1,2,\*</sup>

## ABSTRACT

PAR proteins constitute a highly conserved network of scaffolding proteins, adaptors and enzymes that form and stabilize cortical asymmetries in response to diverse inputs. They function throughout development and across the metazoa to regulate cell polarity. In recent years, traditional approaches to identifying and characterizing molecular players and interactions in the PAR network have begun to merge with biophysical, theoretical and computational efforts to understand the network as a pattern-forming biochemical circuit. Here, we summarize recent progress in the field, focusing on recent studies that have characterized the core molecular circuitry, circuit design and spatiotemporal dynamics. We also consider some of the ways in which the PAR network has evolved to polarize cells in different contexts and in response to different cues and functional constraints.

**KEY WORDS:** PAR, Polarity, *C. elegans*, *Drosophila*

## Introduction

PAR proteins constitute a highly conserved network of scaffolds, adaptors and enzymes that control cell polarity in a wide variety of developmental and physiological contexts across the metazoa (Goldstein and Macara, 2007; Suzuki and Ohno, 2006). A key feature of the PAR network is that many of its members are asymmetrically localized in polarized cells. PAR asymmetries are established in response to many different kinds of polarizing cues; they are reinforced by complex networks of mutually supportive and antagonistic interactions, and they feed forward to control the localization and activities of downstream targets to elaborate polarized phenotypes.

In the past few decades, we have made substantial progress in identifying and characterizing the core molecular players and interactions that govern the formation and stabilization of PAR polarity. Advances in live imaging and biophysical analyses have allowed us to probe PAR protein dynamics in living cells. This has set the stage for using mathematical models to frame and test hypotheses for how specific interactions lead to robust and dynamically stable PAR polarity. Although much of the key work to define core mechanisms has been performed in a few model organisms and in a few specific developmental contexts, comparisons across these different developmental and organismal contexts are beginning to reveal a broader view of how the same core circuitry/mechanisms can be tuned differently to perform different tasks.

Here, we review recent progress in the field, emphasizing an emerging view of the PAR network as a dynamic pattern-forming module, the circuit logic and molecular interactions of which endow it with the ability to form and stabilize asymmetric distributions of its components. We focus on studies in the *C. elegans* zygote, where the most comprehensive picture of mechanism has emerged. However, we also discuss how core elements of this circuit are modified and/or extended in other contexts to achieve functional variants of the same core mechanism.

## PAR proteins form a highly conserved, but versatile, polarity module

PAR proteins were first discovered in screens for mutations that affect asymmetric cell division in the *C. elegans* zygote (Kemphues et al., 1988; Tabuse et al., 1998; Watts et al., 1996). During polarization of the *C. elegans* zygote, a sperm-derived cue induces redistribution of cell fate determinants along the future anterior-posterior (AP) axis that are then inherited unequally during cell division (Rose and Gönczy, 2014). In seminal studies, Ken Kemphues and colleagues showed that PAR genes are required both for the initial segregation of cell fate determinants and to position the cleavage plane so these determinants are partitioned correctly into the anterior and posterior daughters of the zygote (Guo and Kemphues, 1996). They showed that, during polarization, a subset of PAR proteins becomes asymmetrically enriched at the cell cortex within complementary anterior and posterior domains, and that these asymmetries are controlled by mutual antagonism between anterior and posterior PAR proteins (Boyd et al., 1996; Etamad-Moghadam et al., 1995; Guo and Kemphues, 1995; Tabuse et al., 1998; Watts et al., 1996). Subsequent work showed that PAR protein homologues are asymmetrically localized in many other organisms and cell types (Denker et al., 2013; Nakaya et al., 2000; Tomancak et al., 2000; Wodarz et al., 2008). Complimentary PAR domains are associated with axis formation before and during fertilization in some oocytes, with contact-dependent polarities in early embryonic cells, with asymmetric cell divisions, and with apico-basal and planar polarities in embryonic epithelia. In other cells, such as neurons and neuroblast stem cells, subsets of PAR proteins can localize in a unipolar fashion, without an opposing domain. The polarizing inputs and functional outputs of PAR asymmetry are strikingly different in these different contexts. However, what appears to be most highly conserved is the core set of molecular interactions by which PAR proteins promote or inhibit the localization or activities of one another to convert transient polarizing inputs into stable cell polarity.

## An overview of polarization in the *C. elegans* zygote

The most complete understanding of how PAR proteins mediate the establishment and maintenance of cortical polarity comes from studies in the *C. elegans* zygote. The core players in this system include the original proteins discovered by Kemphues et al.

<sup>1</sup>Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637, USA. <sup>2</sup>Committee on Genetics, Genomics and Systems Biology, University of Chicago, Chicago, IL 60637, USA.

\*Author for correspondence (emunro@uchicago.edu)

 E.M., 0000-0001-5535-5675

(Kempthues et al., 1988) plus later additions (Beatty et al., 2010; Gotta et al., 2001; Hoegge et al., 2010; Kumfer et al., 2010; Tabuse et al., 1998); we refer to these collectively as PAR proteins (Table 1). Anterior PAR proteins (aPARs) include the oligomeric scaffold PAR-3, the adaptor PAR-6, the kinase PKC-3 and the small GTPase CDC-42. Posterior PAR proteins (pPARs) include the kinase PAR-1, the RING domain protein PAR-2, the tumor suppressor LGL-1 and a putative GTPase-activating protein (GAP) for CDC-42, called CHIN-1. Two additional proteins (the kinase PAR-4 and the 14-3-3 protein PAR-5) are not asymmetrically localized but control asymmetries of the other PARs. All of these proteins are highly conserved across the metazoa, with the exception of PAR-2, but even PAR-2 may have functional analogues in other organisms

The *C. elegans* zygote polarizes in two distinct phases, referred to as establishment and maintenance phases, which coincide with mitotic interphase and M phase, respectively (Fig. 1). Before polarity establishment, the aPARs are enriched throughout the cortex while pPARs are uniformly cytoplasmic. This symmetry is broken in response to multiple signals from a transient centrosome/microtubule-organizing center (the sperm MTOC) that forms near the site of sperm entry (Bienkowska and Cowan, 2012; Cowan and Hyman, 2004; Hamill et al., 2002; O'Connell et al., 2000). One of these signals, still poorly defined, acts locally to inhibit RhoA-dependent cortical actomyosin contractility (Motegi and Sugimoto, 2006; Munro et al., 2004). This creates a spatial gradient of contractility and anterior-directed cortical flows that segregate PAR-3, PAR-6 and PKC-3 towards the anterior pole, allowing PAR-1, PAR-2 and LGL-1 to accumulate on the posterior cortex (Cheeks et al., 2004; Goehring et al., 2011b; Mayer et al., 2010; Munro et al., 2004). A second signal from the sperm MTOC can induce symmetry-breaking in the absence of contractility (Motegi et al., 2011); during this event, microtubules associated with the sperm MTOC act locally to promote PAR-2 association with the posterior cortex, and PAR-2 in turn recruits PAR-1, which phosphorylates and promotes the dissociation of PAR-3.

These asymmetries persist during maintenance phase as the centrosomes, (i.e. the original cue) move towards the cell center. During this time, several new asymmetries appear. Active CDC-42 becomes enriched with other aPARs at the anterior pole (Kumfer et al., 2010). At the same time, CHIN-1 becomes highly enriched in a complementary posterior domain. In addition to interacting directly with other PAR proteins, CDC-42 acts through the conserved kinase MRCK-1 to activate myosin II, creating a gradient of contractility that drives anterior-directed cortical flows (Kumfer et al., 2010; Sailer et al., 2015). As discussed below, both flow-dependent and -independent mechanisms contribute to

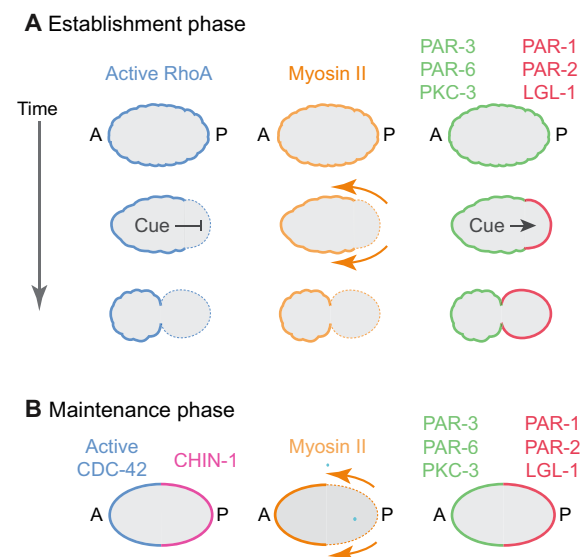
positioning and stabilizing the boundary between anterior and posterior PAR domains during maintenance phase.

### The molecular basis for complementary PAR domains

Biophysical studies, using fluorescence recovery after photobleaching (FRAP) (Cheeks et al., 2004; Goehring et al., 2011a; Nakayama et al., 2009) or single-molecule imaging (Arata et al., 2016; Robin et al., 2014; Sailer et al., 2015), have shown that PAR proteins exchange dynamically between the cytoplasm and the cell surface, where they move locally by diffusion or transport. At the same time, genetic and biochemical studies have revealed a complex web of local interactions through which PAR proteins modulate the recruitment or dissociation of one another (see below). Together, these data support a conceptual model for dynamic stabilization of cell polarity in which PAR proteins exchange locally with the cell surface, and either synergize to promote the recruitment of one another or compete to promote the dissociation of one another. Below, we review the mechanisms that mediate local recruitment of anterior and posterior PARs, and then discuss how mutual interactions between aPARs and pPARs reinforce their enrichment within complementary domains.

### The hierarchical recruitment of anterior PARs

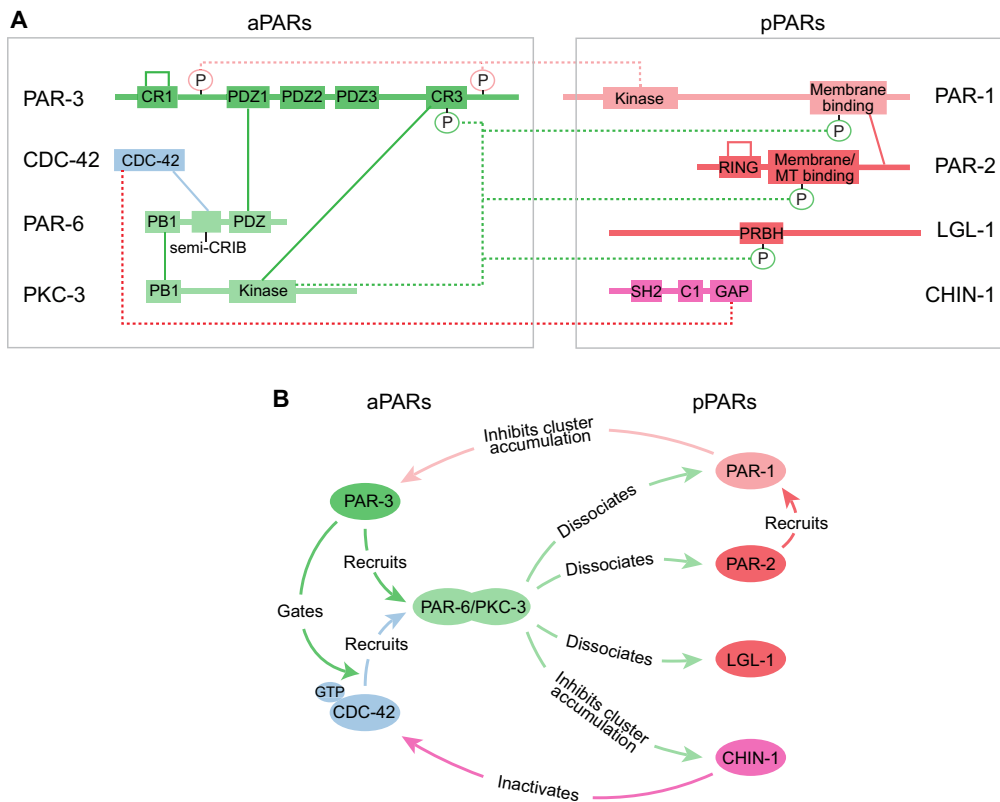
Cortical recruitment of aPARs is governed by a complex hierarchy of protein-protein interactions in which PAR-3 and CDC-42 bind independently to the cortex and synergize to recruit PAR-6 and PKC-3 (Fig. 2) (Kumfer et al., 2010; Sailer et al., 2015). Structure/function studies in *C. elegans*, *Drosophila* and vertebrate cells



**Fig. 1. Overview of polarization in the *C. elegans* zygote.** Polarization of the *C. elegans* zygote involves two distinct phases: establishment phase (A) and maintenance phase (B). (A) Before polarity establishment, anterior PAR proteins (green), active RhoA and Myosin II are uniformly enriched at the cell cortex. During polarity establishment, a transient sperm-derived cue acts locally to inhibit RhoA activity and induce actomyosin-based cortical flows that segregate anterior PAR proteins towards the anterior pole, and to promote local accumulation of posterior PAR proteins (red) on the posterior cortex where they act to inhibit local accumulation of anterior PAR proteins. (B) During maintenance phase, complementary distributions of anterior PAR proteins and posterior PAR proteins are maintained in the absence of a cue. RhoA activity is low, and CDC-42 activity becomes enriched at the anterior cortex, while the CDC-42 GAP CHIN-1 becomes enriched on the posterior cortex. CDC-42 acts through the kinase MRCK-1 to activate Myosin II on the anterior cortex, leading to persistent cortical flows.

**Table 1. The PAR proteins**

Name	Description	Localization in the polarized zygote	Conserved in Metazoa?
PAR-1	Kinase	Posterior cortex	Yes
PAR-2	RING protein	Posterior cortex	No
PAR-3	Scaffold protein	Anterior cortex	Yes
PAR-4	Kinase	Uniform	Yes
PAR-5	14-3-3 protein	Uniform	Yes
PAR-6	Adaptor protein	Anterior cortex	Yes
PKC-3	Kinase	Anterior cortex	Yes
CDC-42	Small GTPase	Anterior cortex	Yes
CHIN-1	Putative Rho GAP	Posterior cortex	Yes
LGL-1	Tumor suppressor	Posterior cortex	Yes



**Fig. 2. Core molecular interactions that underlie the dynamic stabilization of PAR asymmetries.** (A) A schematic view of the PAR network indicating key domains and phosphorylation sites involved in protein-protein interactions. Solid lines indicate direct binding interactions, whereas dotted lines terminating in circles represent enzymatic action, either phosphorylation or GAP activity. In the case of PAR-3 and PAR-2, self-connecting loops indicate oligomerization. (B) A functional view of the same circuit emphasizing the consequences of protein-protein interactions. For clarity, some interactions documented in other contexts (e.g. inhibition of aPKC by LGL or by PAR-3) have been omitted here.

suggest that PAR-3 recruitment depends on direct binding to membrane phospholipids, self-oligomerization and specific protein/protein interactions (Feng et al., 2007; Li et al., 2010a; McKinley et al., 2012; Mizuno et al., 2003; Wu et al., 2007; Yu and Harris, 2012). PAR-3 contains several conserved functional domains, including an N-terminal (CR1) oligomerization domain, three PDZ domains and a C-terminal (CR3) domain that interacts with PKC-3. Specific domains/residues that mediate membrane binding have been characterized in flies (Krahn et al., 2010b; McKinley et al., 2012), mammalian cells (Mizuno et al., 2003; Wu et al., 2007) and to a lesser extent in *C. elegans* (Li et al., 2010a), but do not appear to be tightly conserved. In *C. elegans* zygotes, both C-terminal sequences and PDZ2 are required for normal membrane association (Li et al., 2010a). Accumulation of PAR-3 also depends crucially on its ability to assemble oligomers via its CR1 domain. Live imaging studies show that PAR-3 accumulates dynamically at the membrane in small clusters of indeterminate sizes (Achilleos et al., 2010; Beers and Kemphues, 2006; Dickinson et al., 2017; Hung and Kemphues, 1999; McGill et al., 2009; Rodriguez et al., 2017; Sailer et al., 2015; Wang et al., 2017). The CR1 domain of PAR-3 assembles into helical filaments *in vitro* (Feng et al., 2007; Zhang et al., 2013). Deleting the CR1 domain or introducing point mutations that abolish oligomerization *in vitro* (Zhang et al., 2013) strongly attenuates membrane accumulation of PAR-3 in the *C. elegans* zygote (Dickinson et al., 2017; Li et al., 2010a; Rodriguez et al., 2017), as in *Drosophila* or mammalian epithelial cells (Benton and St Johnston, 2003a; Feng et al., 2007; McKinley et al., 2012; Mizuno et al., 2003). These data suggest that PAR-3 monomers can bind weakly to the membrane, but that increased binding avidity due to oligomerization is essential for strong accumulation.

In *C. elegans* zygotes, fluorescently tagged CDC-42 transgenes localize to the cortex throughout the cell cycle, independently of other aPARs (Aceto et al., 2006; Nakayama et al., 2009; Schonegg

and Hyman, 2006). As in other cells, only a subset of membrane-localized CDC-42 is likely to be in a GTP-bound active state. Analysis of a biosensor for CDC-42 activity suggests that active CDC-42 is enriched at the posterior pole during polarity establishment and at the anterior pole during polarity maintenance (Kumfer et al., 2010; Sailer et al., 2015). CDC-42 activation requires the GEF cGEF-1, which is uniformly enriched at the cortex of zygotes (Chan and Nance, 2013; Kumfer et al., 2010), while spatial restriction of CDC-42 activity during maintenance phase depends on its local inhibition by CHIN-1 (Beatty et al., 2013; Kumfer et al., 2010; Sailer et al., 2015) (see below). The mechanism(s) that localize CDC-42 activity during polarity establishment remain unclear.

PAR-6 and PKC-3 form heterodimers through conserved PB1 domain interactions (Hirano et al., 2005), and depend on one another for cortical accumulation in polarizing zygotes (Hung and Kemphues, 1999; Tabuse et al., 1998). Normal PAR-6/PKC-3 accumulation also depends on both PAR-3 and active CDC-42. Both PAR-6 and PKC-3 bind directly to PAR-3 (Joberty et al., 2000; Li et al., 2010b; Lin et al., 2000); however, direct binding of PAR-6 to PAR-3 is not required for PAR-6/PKC-3 accumulation in *C. elegans* (Li et al., 2010b). PAR-6/PKC-3 can also bind active CDC-42 through the conserved semi-CRIB domain of PAR-6 (Aceto et al., 2006). In the zygote, PAR-6/PKC-3 accumulate in two distinct pools: a punctate pool that requires PAR-3, but not CDC-42 (Aceto et al., 2006); and a diffuse pool that requires both PAR-3 and CDC-42 (Beers and Kemphues, 2006; Hung and Kemphues, 1999; Motegi and Sugimoto, 2006; Tabuse et al., 1998). PAR-6/PKC-3 punctae colocalize extensively with PAR-3 oligomers (Beers and Kemphues, 2006; Hung and Kemphues, 1999; Li et al., 2010b; Tabuse et al., 1998); they segregate to the anterior with cortical flow during polarity establishment and then decay during polarity maintenance (Motegi and Sugimoto, 2006; Munro et al., 2004). A



more diffuse pool of PAR-6/PKC-3 becomes progressively enriched on the anterior cortex during polarity establishment and persists through maintenance phase. Recent work shows that PAR-6/PKC-3 punctae represent PAR-6/PKC-3 bound directly to oligomeric PAR-3 (Dickinson et al., 2017), which can be blocked by genetic or pharmacological inhibition of PKC-3 activity (Rodriguez et al., 2017). In contrast, the diffuse accumulation of PAR-6/PKC-3 requires direct binding of PAR-6 to CDC-42 (Aceto et al., 2006). Interestingly, inhibition of PKC-3 or depletion of the cytoplasmic chaperone CDC-37, allows CDC-42 to recruit PAR-6/PKC-3 independently of PAR-3 (Beers and Kemphues, 2006). These and other observations suggest that PAR-3 may promote the association of PAR-6/PKC-3 with CDC-42 by competing with CDC-37 (and/or other cytoplasmic factors) for an active (or activatable) form of PKC-3 (Beers and Kemphues, 2006; Rodriguez et al., 2017).

### The hierarchical recruitment of posterior PARs

The mechanisms that govern pPAR recruitment have also been extensively characterized (Fig. 2). PAR-2 binding involves three distinct domains. A membrane localization domain binds to phosphoinositide lipids, while a microtubule-binding domain facilitates PAR-2 loading onto the posterior cortex by the sperm MTOC during polarity establishment. This is achieved through a mechanism in which microtubule binding protects PAR-2 from phosphorylation by PKC-3 (Motegi et al., 2011). Finally, a RING domain stabilizes PAR-2 binding to the cortex and mediates positive feedback in which membrane-localized PAR-2 facilitates recruitment of cytoplasmic PAR-2 (Hao et al., 2006; Motegi et al., 2011). Recent single molecule imaging studies indicate that PAR-2 forms oligomers at the membrane that appear to undergo size-dependent dissociation (Arata et al., 2016). Because oligomerization is common to many other RING domains (Deshaies and Joazeiro, 2009), RING domain-mediated oligomerization of PAR-2 may both stabilize PAR-2 binding and potentiate recruitment of cytoplasmic PAR-2.

PAR-1 can associate with the membrane via a conserved C-terminal domain that binds acidic phospholipids (Griffin et al., 2011; Moravcevic et al., 2010). The binding of PAR-2 to this domain is both necessary and sufficient for PAR-2 to promote PAR-1 recruitment during polarity establishment (Motegi et al., 2011), and contributes to recruiting PAR-1 during polarity maintenance (Boyd et al., 1996; Griffin et al., 2011).

The mechanism(s) that govern localization of LGL-1 in the *C. elegans* zygote remain unclear. Both vertebrate and invertebrate orthologues of LGL-1 bind non-muscle Myosin II (Betschinger et al., 2005; Dahan et al., 2014, 2012; Strand et al., 1994, 1995). Cortical localization of Lgl in *Drosophila* neuroblasts or S2 cells is myosin-dependent, but may not require direct binding to Myosin II (Barros et al., 2003; Betschinger et al., 2005). More recent studies of *Drosophila* Lgl identified a short conserved phospho-regulated polybasic and hydrophobic (PRBH) motif that is conserved in *C. elegans* LGL-1, binds negatively charged phospholipids, and is necessary and partially sufficient for cortical localization (Bailey and Prehoda, 2015; Dong et al., 2015). Thus, normal cortical recruitment of LGL-1 is likely to be mediated by direct binding to membranes and at least one other cortical target.

CHIN-1 is largely absent from the cortex during polarity establishment but accumulates in the form of larger clusters in the posterior cortex during polarity maintenance (Kumfer et al., 2010; Sailer et al., 2015). The mechanisms for cortical accumulation and cluster assembly remain unclear, although it is known that cortical accumulation does not require any of the other pPARs (Kumfer

et al., 2010; Sailer et al., 2015) and that the spatial distribution of clusters is determined through local inhibition by aPARs (discussed below).

### The molecular basis for mutual antagonism

In the *C. elegans* zygote, as in many other contexts, PKC-3 plays a dominant role in enforcing anterior cortex identity by phosphorylating and excluding pPARs (Fig. 2). Phosphorylation of PAR-1, PAR-2 and LGL-1 by PKC-3 is both necessary and sufficient to prevent them from binding to the anterior cortex (Hao et al., 2006; Hoege et al., 2010; Motegi et al., 2011). In all three cases, PKC-3 phosphorylates residues within the motifs that are known to mediate membrane binding (Bailey and Prehoda, 2015; Hao et al., 2006; Hoege et al., 2010; Motegi et al., 2011). PAR-6/PKC-3 also restrict local accumulation of CHIN-1 clusters to the posterior pole (Kumfer et al., 2010; Sailer et al., 2015), although the molecular basis for this restriction remains to be determined (discussed below).

The pPARs act in multiple ways to prevent posterior accumulation of PAR-6/PKC-3. Early studies assigned a key role for PAR-2 in promoting local dissociation of PAR-6/PKC-3, because loss of PAR-2 leads to rapid posterior accumulation of PAR-6/PKC-3 during maintenance phase, whereas loss of other pPARs does not (Beatty et al., 2010; Hoege et al., 2010). However, recent single molecule imaging studies have shown that during maintenance phase, PAR-6/PKC-3 asymmetries are determined by faster anterior association, rather than by faster posterior dissociation (Robin et al., 2014; Sailer et al., 2015). Thus, PAR-2 does not act by promoting posterior dissociation of PAR-6/PKC-3. Instead, live imaging studies show that the loss of asymmetry in *par-2* mutants is due to the redistribution of PAR-6/PKC-3 by ectopic posterior-directed cortical flows, which can be reversed by inactivating Myosin II during maintenance phase (Beatty et al., 2010; Munro et al., 2004; Sailer et al., 2015). Interestingly, overexpressing LGL-1 rescues loss of polarity and embryonic lethality in *par-2* mutants, suggesting that LGL-1 can substitute functionally for PAR-2 (Beatty et al., 2010; Hoege et al., 2010).

In contrast, PAR-1 and CHIN-1 act redundantly to exclude aPARs during maintenance phase through mechanisms that do not involve local inhibition of actomyosin contractility. Depletion of CHIN-1 causes uniform activation of CDC-42 during maintenance phase (Beatty et al., 2013; Kumfer et al., 2010; Sailer et al., 2015), but PAR-3, PAR-6 and PKC-3 are still excluded from the posterior cortex (Sailer et al., 2015). Depletion of PAR-1 causes weak posterior accumulation of PAR-3 clusters, and a slight increase in posterior association of PAR-6/PKC-3, but has no effect on active CDC-42 (Kumfer et al., 2010). However, depletion of both PAR-1 and CHIN-1 leads to uniform CDC-42 activation and weak posterior accumulation of PAR-3 clusters, and this is accompanied by rapid posterior association of PAR-6/PKC-3 and complete loss of PAR-6/PKC-3 asymmetry.

These observations imply that active CDC-42 is not sufficient to recruit PAR-6/PKC-3 to the cortex, but in the presence of active CDC-42, a small amount of PAR-3 is both necessary and sufficient to achieve maximum rates of PAR-6/PKC-3 recruitment. Importantly, most of the diffuse pool of PAR-6/PKC-3 that accumulates on the posterior pole in embryos depleted of CHIN-1 and PAR-1 cannot be directly associated with the few PAR-3 clusters that accumulate there. Thus, cortical PAR-3 somehow gates the local association of PAR-6/PKC-3 with active CDC-42. How this works remains an interesting puzzle. An attractive hypothesis is that cortical PAR-3 competes with cytoplasmic factors such as

CDC-37 to bind PAR-6/PKC-3 dimers, then transfers those dimers to CDC-42. In other systems, PAR-3, CDC-42 and PAR-6/aPKC can form a quaternary complex (Joberty et al., 2000; Lin et al., 2000), PAR-3 can bind and inhibit the active form of aPKC, and this inhibitory bond can be released by inducing phosphorylation of PAR-3 by aPKC (Morais-de-Sá et al., 2010; Soriano et al., 2016; Walther and Pichaud, 2010). Thus, one possible scenario is that PAR-6/aPKC dock initially on PAR-3, followed by formation of a quaternary complex, and then phosphorylation of PAR-3 by aPKC releases CDC-42/PAR6/aPKC.

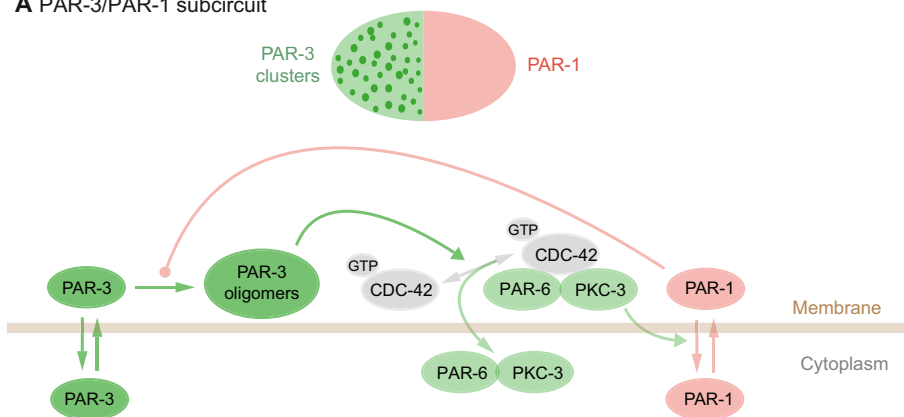
Regardless of the exact mechanism, the requirement for PAR-3 to gate the local association of PAR-6/PKC-3 with CDC-42 plays a key role in shaping the spatial gradient of active PKC-3 in the polarized zygote. Older work (Aceto et al., 2006; Gotta et al., 2001; Kay and Hunter, 2001; Schonegg and Hyman, 2006) and an elegant recent study (Rodriguez et al., 2017) suggest that the pool of PAR-6/PKC-3 that associates with PAR-3 is inactive, whereas the diffuse CDC-42-bound pool of PAR-6/PKC-3 is the active form that phosphorylates and excludes posterior PARs. Single-molecule imaging and particle-tracking analyses suggest that PAR-3/PAR-6/PKC-3-containing clusters couple tightly to the actin cortex and are efficiently segregated by cortical flow (Dickinson et al., 2017; Motegi and Sugimoto, 2006; Munro et al., 2004; Rodriguez et al., 2017; Sailer et al., 2015; Wang et al., 2017), whereas the pool of PAR-6/PKC-3 that associates with CDC-42 diffuses freely and rapidly (Robin et al., 2014). Thus, the gradient of active PKC-3 is likely shaped by two mechanisms: the distribution of relatively immobile PAR-3 defines the local source for PKC-3 recruitment, while diffusion and dissociation shape a broader gradient of active PKC-3 that acts upon its posterior targets.

**A circuit-level view of PAR polarity**

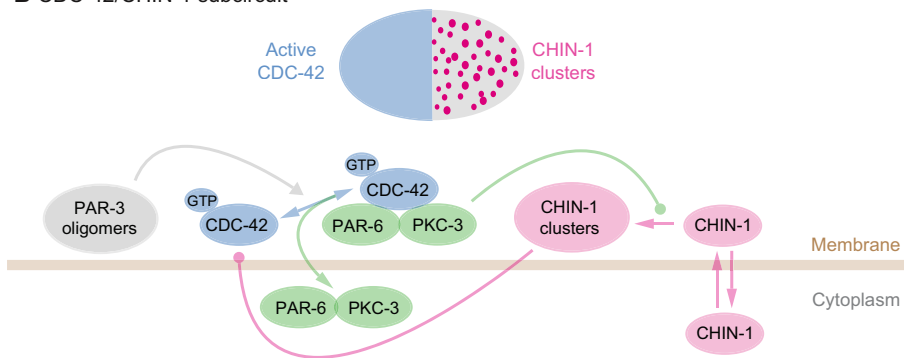
Although many important molecular and biochemical questions remain to be answered, the above studies support a model in which two distinct subcircuits act redundantly to stabilize cortical polarity (Fig. 3). In the PAR-1/PAR-3 subcircuit, PAR-3 promotes PAR-6/PKC-3 association with CDC-42, PKC-3 phosphorylates and promotes dissociation of PAR-1 (Sailer et al., 2015), while PAR-1 phosphorylates and promotes the dissociation of PAR-3. In a second CDC-42/CHIN-1 subcircuit, active CDC-42 recruits PAR-6/PKC-3, which locally inhibits the accumulation of CHIN-1 clusters (Beatty et al., 2013; Sailer et al., 2015), while CHIN-1 inactivates CDC-42. The PAR-1/PAR-3 and CDC-42/CHIN-1 subcircuits are coupled by their shared use of PKC-3 to phosphorylate and exclude pPARs, and by the dual requirement for PAR-3 and CDC-42 to recruit PAR-6/PKC-3, which allows either PAR-1 or CHIN-1 to prevent posterior accumulation of PAR-6/PKC-3.

In addition to preventing active redistribution of aPARs by cortical flow, PAR-2 plays an integral role in the PAR-3/PAR-1 subcircuit through its ability to recruit PAR-1, and positive feedback on PAR-2 recruitment can further reinforce posterior identity (Arata et al., 2016; Motegi and Seydoux, 2013) (and see below). Likewise, LGL-1 may contribute to both subcircuits by limiting the availability of PAR-6/PKC-3, either by reducing their total abundancies (Beatty et al., 2013) or through sequestration of cytoplasmic PAR-6/PKC-3 (Hoegel et al., 2010; Wirtz-Peitz et al., 2008). Both PAR-2 and LGL-1 could also contribute by inhibiting the actions of PKC-3 towards other posterior targets (Hao et al., 2006; Soriano et al., 2016; Wirtz-Peitz et al., 2008). Finally, other uncharacterized feedback mechanisms must contribute to promoting local accumulation of PAR-3, because PAR-3 remains

**A PAR-3/PAR-1 subcircuit**



**B CDC-42/CHIN-1 subcircuit**



**Fig. 3. Decomposition of the PAR network into two smaller, mutually inhibitory subcircuits.**

(A) The PAR-3/PAR-1 subcircuit involves mutual antagonism between PAR-3 oligomers and PAR-1. PAR-3 promotes local association of PAR-6/PKC-3 with active CDC-42; PKC-3 phosphorylates and inhibits membrane association of PAR-1; PAR-1 phosphorylates PAR-3 and inhibits membrane association and/or oligomerization. (B) The CDC-42/CHIN-1 subcircuit involves mutual antagonism between active CDC-42 and clustered CHIN-1. Active CDC-42 binds PAR-6/PKC-3 in the presence of PAR-3; PAR-6/PKC-3 inhibits local growth and accumulation of CHIN-1 clusters; CHIN-1 inactivates CDC-42.

stably asymmetric during maintenance phase in embryos co-depleted of CHIN-1 and PAR-1, when all the other PARs are absent or uniformly distributed (Sailer et al., 2015).

Why all this redundancy? Theoretical studies and experimental analyses of synthetic circuits suggest that positive feedback and mutual inhibition are both in principle sufficient to stabilize cortical polarity, but the combination of both circuit motifs makes the process more robust with respect to variations in protein abundancies, diffusivities or interaction strengths (Chau et al., 2012). Redundancy may also have evolved to allow the same network to respond to a larger range of symmetry-breaking cues. For example, as discussed above, PAR asymmetries can be established in the *C. elegans* zygote by active transport of aPARs, or through local exclusion of PAR-3 by PAR-2/PAR-1, both directed by the sperm MTOC (Motegi et al., 2011). However, similar PAR asymmetries also form later in development but they do so from different starting points and in response to different cues. For example, in *C. elegans* P2 cells, extracellular signals from an adjacent cell (the EMS cell), mediated by SRC-1 and MES-1, bias PAR-2 recruitment to the EMS/P2 boundary to induce symmetry breaking (Arata et al., 2010; Seirin Lee, 2016). In contrast, somatic *C. elegans* blastomeres exhibit contact-dependent radial polarity in which pPARs are localized to cell-cell contacts and aPARs are localized to contact-free zones. In this case, the cue is mediated by junctional proteins that accumulate at cell-cell contacts, and recruit the GAP PAC-1 to inactivate CDC-42 (Anderson et al., 2008; Klompstra et al., 2015). Finally, redundancy may provide a way to partition specific polarity functions across different phases of the cell cycle, while maintaining overall continuity of cell polarity. For example, the PAR-3/PAR-1 circuit plays a key role in responding to polarity cues during interphase, while the CDC-42/CHIN-1 circuit may have distinct M-phase-specific roles.

### From biochemical circuits to cell polarity

Genetic and biochemical approaches have revealed much of the core biochemical circuitry that mediates the formation and stabilization of PAR domains. However, deeper dynamic and mechanistic insights into how stable polarity emerges from circuit logic and pairwise molecular interactions has come from mathematical models, backed by quantitative imaging and biophysical analysis. Thus far, most models for Par polarity have focused on simplified versions of the polarity circuit in which anterior and/or posterior PARs are lumped together and assumed to exchange, diffuse and act locally to promote the dissociation of one another (Blanchoud et al., 2015; Dawes and Munro, 2011; Goehring et al., 2011b; Seirin Lee and Shibata, 2015). Several key insights have emerged from these studies. First, to stabilize complementary domains through mutual inhibition, the PAR circuit must exhibit locally bistable dynamics. That is, in the absence of diffusion, it must be able to stabilize either of two states in which aPARs win or pPARs win. Second, to achieve bistable dynamics, the strengths of opposing interactions must be sufficiently balanced and the effective exchange rates of one or more factors must have sigmoidal (S-shaped) or ‘ultrasensitive’ dependence on the concentrations of reactants (Blanchoud et al., 2015; Dawes and Munro, 2011; Goehring et al., 2011b; Seirin Lee and Shibata, 2015). Finally, given locally bistable dynamics, additional mechanisms are required to ensure a stably positioned AP boundary in the face of diffusion or transport by cortical flow (Dawes and Munro, 2011; Goehring et al., 2011b; Mori et al., 2008). Below, we discuss sources of ultrasensitivity and bistability in the PAR circuit and then discuss mechanisms that help to stabilize boundary position.

### Sources of ultra-sensitivity and bistability in the PAR circuit

Ultrasensitivity underlies bistable dynamics in many biochemical control circuits and can arise in many different ways, e.g. through multi-site phosphorylation, stoichiometric inhibition or in phosphorylation/dephosphorylation reactions, when kinase and phosphatase operate near saturation (Bryant and Mostov, 2008; Ferrell and Ha, 2014). All of these mechanisms are potentially relevant to generating bistable dynamics in the PAR circuit. For example, multi-site phosphorylation is common to many targets of aPKC, including LGL (Graybill and Prehoda, 2014) and PAR-2 (Hao et al., 2006). PAR-3, LGL and likely PAR-2 can act as stoichiometric inhibitors of PKC-3 (Hao et al., 2006; Soriano et al., 2016; Wirtz-Peitz et al., 2008), but whether these interactions are tuned to yield ultrasensitive responses has not been explored.

One source of ultrasensitivity that is particularly relevant to stabilizing PAR asymmetries emerges from the observation that multiple PARs, including PAR-2 (Arata et al., 2016), PAR-3 (Li et al., 2010a; Sailer et al., 2015), PAR-6/PKC-3 (Hung and Kemphues, 1999) and CHIN-1 (Kumfer et al., 2010; Sailer et al., 2015), form oligomers at the membrane. Oligomers will bind more avidly to the membrane than single monomers (Lemmon, 2008), and this can lead to ultrasensitive dependence of effective dissociation rates on the local concentration of the oligomerizing species. Indeed, a simple model of the PAR circuit suggests that oligomerization of PAR-3 could endow this circuit with bistable dynamics (Dawes and Munro, 2011).

Ultrasensitive dependence of CHIN-1 cluster accumulation on PAR-6/PKC-3 may account for bistable dynamics in the CHIN-1/CDC-42 subcircuit (Kumfer et al., 2010; Sailer et al., 2015). CHIN-1 clusters nucleate and grow on the posterior cortex during polarity maintenance (Kumfer et al., 2010; Sailer et al., 2015). Strikingly, cluster growth has threshold dependence on local concentration of PAR-6/PKC-3: below a certain concentration of PAR-6/PKC-3, clusters grow, whereas above that concentration, they shrink. This threshold dependence converts a shallow gradient of PAR-6/PKC-3 into a sharp step change in the local density of CHIN-1 clusters. Incorporating these findings into a detailed mathematical model of the CDC-42/CHIN-1 subcircuit, in which exchange rates and interactions strengths are constrained by experiments, shows that threshold-dependence of CHIN-1 cluster growth of PAR-6/PKC-3 endows this circuit with locally bistable dynamics (Sailer et al., 2015). While the mechanisms that govern CHIN-1 cluster growth and its inhibition by PAR-6/PKC-3 remain unknown, threshold dependence of cluster growth on PKC-3 emerges naturally from a model in which CHIN-1 clusters (and/or factors that recruit CHIN-1) are dynamic polymers that assemble through reversible monomer addition. A characteristic feature of polymer assembly is the existence of a critical monomer concentration, above which polymers grow and below which they shrink (Edelstein-Keshet and Ermentrout, 1998). Threshold dependence of polymer growth on kinase activity can emerge if the kinase modulates either the availability of monomers relative to a fixed critical concentration or the critical concentration relative to a fixed-size pool of available monomers.

A similar mechanism may also lead to ultrasensitive dependence of PAR-3 accumulation on PAR-1. The PAR-3 boundary is exceedingly sharp relative to the opposing gradient of PAR-1 (Beers and Kemphues, 2006; Motegi et al., 2011; Sailer et al., 2015). In flies and vertebrate cells, PAR-1 phosphorylates conserved N-terminal residues adjacent to the CR1 domain of PAR-3 to inhibit oligomerization (Benton and St Johnston, 2003a; Mizuno et al., 2003). This is consistent with the possibility that



PAR-1 could modulate the concentration of monomers that are competent to oligomerize, and thus the effective critical concentration for PAR-3 oligomer assembly. Whether it does so during polarization of the *C. elegans* zygote remains to be seen. As discussed above, additional mechanisms must shape PAR-3 asymmetries because PAR-3 remains asymmetric in embryos lacking PAR-1.

### Stabilizing the position of the polarity boundary

Once polarity is established, the zygote can maintain a normally positioned boundary in the absence of contractility and flow (Kumfer et al., 2010; Sailer et al., 2015) or when actin filaments are depolymerized by acute drug treatments (Goehring et al., 2011a). However, wild-type embryos maintain the same boundary position in the face of persistent contractile asymmetries and redistribution of polarity proteins by cortical flow. Moreover, in embryos that fail to establish normal asymmetries during interphase, cortical flows can restore normal polarity and a normally positioned boundary during maintenance phase (Zonies et al., 2010). Thus, there appear to be both flow-dependent and -independent mechanisms for stabilizing the same boundary position.

Mathematical models suggest one possible mechanism for stabilizing boundaries in the absence of flow (Mori et al., 2008). In a bistable reaction-diffusion system based on mutual inhibition, if the reactants are abundant, then one domain will tend to spread at the expense of the other, unless the competition between domains is finely tuned (Dawes and Munro, 2011; Goehring et al., 2011b; Mori et al., 2008). However, if the total abundance of one or more factors that 'feed' the expanding domain is limiting, then depletion of those factors as the domain expands can slow and stall domain expansion, 'pinning' the boundary at a characteristic position (Dawes and Munro, 2011; Goehring et al., 2011b; Mori et al., 2008). A number of observations are consistent with this mechanism operating in the *C. elegans* zygote: the AP boundary position shifts when aPARs or pPARs are overexpressed (Goehring et al., 2011b; Kumfer et al., 2010; Sailer et al., 2015; Zonies et al., 2010); when the PAR boundary is mis-positioned during polarity establishment by increasing or decreasing RhoA activity, it corrects rapidly during maintenance phase, as predicted by a wave-pinning model (Spiga et al., 2013; Zonies et al., 2010); and the progressive depletion of cytoplasmic PAR-2 as the cortical PAR-2 domain expands during polarity establishment is consistent with the idea that PAR-2 could act as a limiting factor (Goehring et al., 2011b). However, in embryos that lack maintenance phase contractility, boundary position is insensitive to posterior PAR depletion (Sailer et al., 2015), and boundary correction during maintenance depends on actomyosin contractility (Zonies et al., 2010). Thus, whether and how individual PAR proteins act as limiting components to stabilize boundary position in the absence of actomyosin contractility remains to be determined.

Indeed, a growing body of data suggests that boundary position is strongly shaped by feedback loops in which cortical flow redistributes PAR proteins, while PAR proteins modulate contractility and cortical flow. Particle tracking analyses reveal strong dependencies of PAR-3 and CHIN-1 cluster mobilities on actin assembly and cluster size, and strong correlations between cluster movement and cortical flow (Dickinson et al., 2017; Rodriguez et al., 2017; Sailer et al., 2015; Wang et al., 2017). Local gating of PAR-6/PKC-3 by PAR-3 and local inhibition of CDC-42 by CHIN-1 ensure tight coupling between movements of PAR-3 and CHIN-1 clusters and movements of the polarity boundary. Thus, local clustering of polarity proteins plays a central role in coupling PAR asymmetries to cortical flow.

Localizing cluster assembly to the cell surface may also provide a way to avoid counter-transport of polarity proteins by cytoplasmic flows.

During polarity establishment, PAR proteins modulate a gradient of RhoA-dependent actomyosin contractility induced by the sperm cue (Motegi and Sugimoto, 2006; Schmutz et al., 2007; Schonegg and Hyman, 2006; Tse et al., 2012). PAR-3, PAR-6 and PKC-3 promote anterior contraction and thus their own segregation, while PAR-2 inhibits posterior contractility to promote segregation of anterior PARs. The molecular mechanisms remain unclear, although recent work suggests that PAR-3 may promote contractility by inhibiting the TAO kinase KIN-18, a putative negative regulator of RhoA activity (Spiga et al., 2013).

During polarity maintenance, actomyosin contractility is primarily controlled by CDC-42 through MRCK-1 (Kumfer et al., 2010). Anterior restriction of CDC-42 activity by CHIN-1 biases Myosin II activation at the anterior cortex. In addition, PAR-2 (and possibly LGL-1) act downstream of CDC-42 to inhibit posterior accumulation and/or activation of Myosin II (Beatty et al., 2010; Munro et al., 2004; Sailer et al., 2015). PAR-3/PAR-6/PKC-3 indirectly promote anterior contractility by locally inhibiting PAR-2 and CHIN-1 accumulation as discussed above (Beatty et al., 2010; Munro et al., 2004; Sailer et al., 2015). Whether they also act more directly to promote contractility during polarity maintenance remains unclear (Beatty et al., 2013; Zonies et al., 2010).

Regardless of the molecular mechanism(s), local activation of Myosin II by anterior PARs and local inhibition by posterior PARs, will synergize to promote anterior-directed cortical flows and anterior movement of the PAR boundary. This positive-feedback loop could help to explain the ability of the zygote to amplify an initially weak PAR asymmetry, and hence rescue polarity during maintenance phase, in embryos that lack RhoA activity (Zonies et al., 2010). However, it does not explain why the boundary stabilizes at a unique position in the face of persistent contractile asymmetry and cortical flow. Additional mechanisms must exist to counteract the anterior movement of the PAR boundary. One theoretical possibility (Bois et al., 2011) is that anterior transport of some factors, such as PAR-3 and CHIN-1 clusters, could be balanced by diffusive spread of other factors, such as a CDC-42/PAR-6/PKC-3 complex. Indeed quantitative measurements and mathematical modeling suggest that the counterbalance of directed and diffusive transport could mediate boundary stabilization by the CDC-42/CHIN-1 subcircuit when PAR-1 is missing (Sailer et al., 2015). However, it remains to be explained how this balance is achieved, in the face of persistent contractile asymmetries, at a unique AP position. Understanding how a stable boundary position is determined by the dynamic interplay of PAR proteins and contractility remains a challenging problem for future studies.

### A comparison with other systems

To what extent are the mechanisms that operate in *C. elegans* embryos more widely conserved? Studies in *Drosophila* oocytes, in epithelia, and in neural precursors and stem cells (Prehoda, 2009; St Johnston and Ahringer, 2010; Tepass, 2012) reveal conservation of many core molecular interactions. At the same time, these studies highlight how the core circuitry has been modified in different contexts to serve distinct functional requirements of different cells or tissues.

### PAR asymmetries in *Drosophila* oocytes

In *Drosophila* oocytes, complementary domains are formed and stabilized through mutual antagonism between anterior (Par-3, Cdc-

42, Par-6/aPKC) and posterior (Par-1 and Lgl) PAR proteins. As in *C. elegans*, both Par-3 and Cdc-42 are required to recruit and activate Par-6/aPKC (Doerflinger et al., 2010; Leibfried et al., 2013); aPKC phosphorylates and excludes both Par-1 and Lgl (Doerflinger et al., 2010; Tian and Deng, 2008), and both Par-1 and Lgl contribute to excluding Par-3 and Par-6/aPKC from the posterior pole. Par-1 plays the dominant role, whereas Lgl plays a smaller role that can be enhanced by overexpression (Morais-de-Sá et al., 2014; Tian and Deng, 2008). While *Drosophila* lack a homologue of PAR-2, an E3 ubiquitin ligase called Slmb may play an analogous role in the sense that Slmb is required for posterior accumulation of Par-1 and the establishment of oocyte polarity, and loss of Slmb can be rescued by overexpressing Lgl (Morais-de-Sá et al., 2014; Skwarek et al., 2014). Whether posterior inhibition of Cdc-42 activity is also involved remains unclear. However, unlike the situation in *C. elegans*, actomyosin contractility and cortical flow do not appear to be involved in either establishing or maintaining *Drosophila* oocyte polarity (Doerflinger et al., 2010). Instead, local signals from posterior follicle cells are thought to break symmetry by displacing Par-6/aPKC from the posterior cortex, allowing local recruitment of Par-1 and local displacement of Par-3 (Doerflinger et al., 2010; Morais-de-Sá et al., 2014). Thus, while *Drosophila* oocytes appear to rely heavily on a variant of the Par-3/Par-1 subcircuit, some of the mechanisms that operate redundantly to establish and maintain PAR asymmetries in *C. elegans* zygotes may be missing, and additional factors (e.g. Slmb) may fulfill analogous roles.

#### PAR asymmetries in epithelia

Epithelial cells partition themselves into three domains: apical, junctional and basolateral. The apical domain is defined by co-enrichment of Cdc-42, Par-6/aPKC and members of the highly conserved Crumbs (Crb) complex, which contains Crumbs (Crb), Stardust (Std) and PatJ. The junctional domain is enriched in Par-3 and overlaps with adherens and tight or septate junctions. The basolateral domain is enriched in Par-1 and members of the Scribble polarity module, comprising Scribble (Scrib), Discs Large (Dlg) and Lgl. Not surprisingly, therefore, the molecular circuitry in epithelial cells is more complex than that in *C. elegans*, although the circuit design principles are strikingly analogous (Laprise and Tepass, 2011; Rodriguez-Boulán and Macara, 2014; Tepass, 2012).

During epithelial polarization *in vivo*, Par-3 serves as a crucial landmark that localizes early to the apical margin of cell-cell contacts, where it recruits components of adherens junctions and acts as a transient docking station for Par-6/aPKC and other members of the apical Crb/Cdc-42/Par-6/aPKC complex to facilitate their association with the apical membrane (Achilleos et al., 2010; Franz and Riechmann, 2010; Harris and Peifer, 2005; Horikoshi et al., 2009; Krahn et al., 2010a; Mccaffrey and Macara, 2009; McKinley et al., 2012). As in other contexts, Par-1 phosphorylates and excludes Par-3 from lateral membranes (Bayraktar et al., 2006; Benton and St Johnston, 2003b; McKinley et al., 2012). Par-3 is also excluded from the apical surface through competition with the apical Crumbs complex for binding to Par-6/aPKC (Morais-de-Sá et al., 2010; Soriano et al., 2016; Tepass, 2012; Walther and Pichaud, 2010). Binding to the conserved CR3 domain of Par-3 can transiently trap Par-6/aPKC in a high-affinity inhibited state (Soriano et al., 2016). However, unknown input(s) stimulate aPKC to phosphorylate Par-3, reducing their mutual affinity, and allowing Crumbs to out-compete Par-3 for binding to Par-6/aPKC (Morais-de-Sá et al., 2010; Walther and Pichaud, 2010). Cdc-42 may promote this segregation by binding

Par-6 and increasing its affinity for Crumbs (Whitney et al., 2016). Together, these interactions promote the accumulation of Par-6/aPKC with Crb and Cdc-42 on the apical surface, while restricting Par-3 to adherens junctions.

As in *C. elegans*, multiple forms of feedback contribute to reinforcing apical versus basolateral identities (Rodriguez-Boulán and Macara, 2014; Tepass, 2012). For example, aPKC enforces apical identity by phosphorylating and displacing Par-1, Lgl and other basolateral polarity factors (Gamblin et al., 2014; Suzuki and Ohno, 2006). In addition, Cdc-42/Par-6/aPKC can inhibit the endocytosis of Crb and other apical factors to reinforce their apical accumulation (Fletcher et al., 2012; Harris and Tepass, 2008). If left unchecked, this positive feedback can lead to apical expansion (Fletcher et al., 2012). However, multiple basolateral factors, including members of the Scribble module (Bilder and Perrimon, 2000; Bilder et al., 2003; Laprise et al., 2009; Tanentzapf and Tepass, 2003) and a later acting Yurt/Coracle module (Laprise et al., 2009), oppose expansion. The molecular basis for this opposition remains unclear, but several basolateral factors, including Lgl and Yurt, appear to play dual roles as substrates and inhibitors of aPKC. (Wirtz-Peitz et al., 2008; Yamanaka et al., 2006). Local inhibition of aPKC activity could indirectly prevent local accumulation of aPKC by inhibiting aPKC-dependent positive feedback (Fletcher et al., 2012) or by allowing the accumulation of aPKC targets that cross-inhibit aPKC accumulation.

Finally, a growing body of work has begun to reveal additional feedback interactions involving PAR proteins, the Crumbs and Scribble polarity modules, phosphoinositide lipids and polarized membrane traffic that reinforce apical versus basolateral identities in epithelia (Eaton and Martin-Belmonte, 2014; Rodriguez-Boulán and Macara, 2014; Shewan et al., 2011). In many epithelia, local enrichment of PIP2 [PtdIns(4,5)P<sub>2</sub>] and PIP3 [PtdIns(3,4,5)P<sub>3</sub>] play key roles in defining, respectively, apical and basolateral identities (Claret et al., 2014; Gassama-Diagne et al., 2006; Martin-Belmonte and Mostov, 2008; Martin-Belmonte et al., 2007). Polarity proteins can modulate the relative levels of PIP2 and PIP3 by promoting or inhibiting the local recruitment of lipid kinases (e.g. PI3 kinase or PPK-5) or phosphatases (e.g. PTEN) (Chartier et al., 2011; Claret et al., 2014; Martin-Belmonte et al., 2007; von Stein et al., 2005), and PIP2 and PIP3 can in turn bias the recruitment of polarity proteins, either by direct binding or by targeting secretion to apical versus basolateral domains (reviewed by Eaton and Martin-Belmonte, 2014). These additional slower forms of feedback may be essential to maintain compartmental identities on longer timescales against the continuous insertion and removal of membrane proteins that elaborates compartment identities, and during the continuous and active remodeling of epithelia during tissue morphogenesis. Indeed, membrane recycling appears to play a much smaller role in the *C. elegans* zygote where cortical polarities are formed and maintained on much faster timescales (Nakayama et al., 2009; Shivas and Skop, 2012).

#### Unipolar PAR asymmetries in neuroblast stem cells

Studies of *Drosophila* neural stem cells (neuroblasts) highlight another interesting variation on PAR-mediated polarity. Embryonic and larval neuroblasts delaminate from a surface epithelium and then execute a sequence of asymmetric divisions, each producing a small ganglion mother cell and a self-renewed neuroblast (Hartenstein and Wodarz, 2013). As in epithelia, Par-3 forms an essential landmark that is enriched in an apical cap from late interphase through to the end of mitosis. During mitosis, Par-3 cooperates with Cdc-42 to recruit and activate Par-6/aPKC; aPKC in



turn phosphorylates and displaces basal determinants from the apical cortex (Atwood and Prehoda, 2009; Atwood et al., 2007; Petronczki and Knoblich, 2001; Wodarz et al., 2000).

Neuroblasts inherit an apical Par-3 cap when they first delaminate. This cap disappears as neuroblasts divide then reform in daughter neuroblast cells during late interphase in response to spatial cues from the overlying epithelium (in embryos) (Siegrist and Doe, 2006) or from an apical centrosome that ‘remembers’ the position of the previous apical cap (in larvae) (Januschke and Gonzalez, 2010; Januschke et al., 2013; Rusan and Peifer, 2007). In cells that lack these spatial cues, Par-3 caps form later in mitosis, with random orientation, but can still direct a normally asymmetric division (Januschke and Gonzalez, 2010; Siegrist and Doe, 2006).

Interestingly, apical polarity in neuroblasts does not appear to require basolateral inhibition. Phosphorylation by Par-1 is not required to restrict Par-3 to a polar cap, although Par-1 may help to align Par-3 caps with polarity cues (Krahn et al., 2009). Members of the Scribble polarity module are co-enriched with Par-3 at the apical cortex of neuroblasts, and mutating Dlg, Scrib or Lgl does not cause basal accumulation of apical factors or loss of Par-3 asymmetry (Lee et al., 2006; Ohshiro et al., 2000; Peng et al., 2000; Siegrist and Doe, 2005). So how do neuroblasts form and stabilize an apical PAR domain? Par-3 binds the adaptor Inscuteable (Insc) (Schober et al., 1999; Wodarz et al., 1999) to localize a conserved Pins/Ga/Dlg complex to the apical cap (Betschinger and Knoblich, 2004). This complex plays a key role in aligning the mitotic spindle with the polarity axis to ensure correct partitioning of cell fate determinants between daughter cells (reviewed by Lu and Johnston, 2013). In Insc mutants, Par-3 caps are lost, but a normal Pins cap forms late in mitosis at a random position relative to apical cues, possibly via positive feedback involving microtubules and the kinesin Khc-73 (Siegrist and Doe, 2005). On the other hand, in Pins or Ga mutants, Par-3 caps are positioned normally with respect to apical cues but are attenuated or lost later in the cell cycle (Cai et al., 2003; Schaefer et al., 2001, 2000; Yu et al., 2000). Thus, Pins feedback may reinforce the Par-3 cap that forms during interphase at a position dictated by apical cues; but in the absence of a polarizing cue, a Pins cap can form *de novo* at a random position and recruit Par-3 through the adaptor Insc. Whether the weaker Par-3 caps that forms in neuroblasts lacking Pins depends upon a polarizing cue, or reflects additional mechanism(s) for spontaneous formation of PAR-3 caps, remains unclear. Interestingly, Par-3 can spontaneously form polar caps during mitosis in *Drosophila* S2 cells, even in the absence of Insc (Wodarz et al., 1999).

#### Examples that do not involve mutual antagonism

While much of this Review has emphasized an essential role for mutual antagonism in reinforcing complementary PAR protein distributions, there are many examples in which aPARs localize to discrete subcellular domains in the apparent absence of mutual antagonism. A classic example comes from studies of hippocampal neurons *in vitro*, in which Par-3/Par-6/aPKC localize to the growing tips of multiple individual neurites, before becoming restricted to the tip of a single fast-growing axon (Shi et al., 2003). This localization is thought to be sustained in part by positive feedback in which Par-3 is delivered to neurite tips by microtubule-based transport (Nishimura et al., 2004; Shi et al., 2004), while oligomeric Par-3 feeds back by binding, bundling and stabilizing microtubules (Chen et al., 2013).

Discrete patches of Par-3 also associate with centrosomes in a variety of different cells, including *Drosophila* neuroblasts (Januschke and Gonzalez, 2010; Rebollo et al., 2007; Rusan and

Peifer, 2007), *C. elegans* intestinal epithelia (Feldman and Priess, 2012) and *Drosophila* male germline stem cells (Inaba et al., 2015; Yuan et al., 2012). While the underlying mechanisms remain unknown, studies of Par-3 localization in *Drosophila* aPKC mutant epithelia have provided some insights (Harris and Peifer, 2007; Jiang et al., 2015). In wild-type epithelia, Par-3 is uniformly distributed along apical cell-cell junctions. In aPKC mutant epithelia, Par-1 is no longer excluded from the apical surface and Par-3 is restricted to discrete patches that colocalize with apical centrosomes in aPKC mutants. This restriction appears to be governed by a positive-feedback loop between centrosomes and Par-3 (Jiang et al., 2015). Strikingly, Par-1 promotes this positive feedback by phosphorylating Par-3 (and also through its actions on centrosomal microtubule dynamics) (Jiang et al., 2015). This suggests that the Par-1/Par-3 interaction can undergo a context-dependent switch between mutual antagonism and synergism that supports positive feedback and the formation of discrete unipolar patches of Par-3. In the embryonic epithelium, aPKC enforces mutual antagonism by phosphorylating and excluding Par-1 from the apical surface. However, the synergistic mode is likely to play a functional role in other contexts, where Par-3 patches colocalize with centrosomes (Feldman and Priess, 2012; Inaba et al., 2015).

#### Conclusions

In the past decade, we have made rapid progress in understanding how cells form and stabilize PAR asymmetries. Molecular, genetic and biochemical approaches continue to supply new information about individual polarity proteins and their pairwise interactions. Mechanistic insights into the dynamics of polarization have come from biophysical and imaging approaches that allow one to quantify PAR protein dynamics in living cells, and from mathematical models that attempt to explain these dynamics in terms of known interactions. Looking forward, the system is now ripe for approaches from optogenetics, synthetic biology and biochemical reconstitution (Chau et al., 2012; Loose et al., 2017; Witte et al., 2017).

Despite (and because of) this progress, there is still much to learn from the study of traditional model systems. Recent work highlights key processes involved in the clustering of polarity proteins and raises many new questions. How does clustering shape the local recruitment of polarity proteins, their physical coupling to the cytoskeleton and their biochemical activities? How is this modulated through cell cycle control and developmental progression? Another pressing challenge is to uncover the molecular mechanisms by which PAR proteins modulate cytoskeletal dynamics and motor-based transport, how these in turn feed back to govern local recruitment and redistribution of PAR proteins, and how these feedback loops are integrated to ensure robust self-stabilizing polarity. The overarching question that remains to be addressed is how cells stabilize uniquely positioned boundaries between complementary domains (or a unique size and position for unipolar domains) through a dynamic balance of local exchange, assembly/disassembly, diffusion/transport and mechanical force.

Finding answers to these questions in simple and tractable model systems will provide a blueprint for understanding how the PAR network carries out its functions in many other contexts. Indeed, rapid advances in microscopy and genome editing make it increasingly possible to characterize the dynamic behaviors of PAR proteins in many other developmental and organismal contexts. Using our increasingly deep knowledge of individual systems to ground comparative analyses, we are now poised to gain deep insights into how this highly conserved and yet versatile circuit has evolved to do so many different variants of its core job in so many different contexts.

**Competing interests**

The authors declare no competing or financial interests.

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