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Cell polarity: models and mechanisms from yeast, worms and flies

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Summary

Determinants of cell polarity orient the behaviour of many cell types during development. Pioneering genetic screens in yeast, worms and flies have identified key polarity determinants that are evolutionarily conserved across the animal kingdom. Recent work in these three model organisms has combined computer modelling with experimental analysis to reveal the molecular mechanisms that drive the polarisation of determinants. Two key principles have emerged: the first is the requirement for a positive-feedback loop to drive self-recruitment of determinants to the plasma membrane; the second is the requirement for mutual antagonism between determinants that localise to opposite ends of the cell.

Key words: Cdc42, Crumbs, Lgl, PAR, Cell polarity, Epithelia

Introduction

Cell polarity is a fundamental feature of almost all cells. Different cell types employ polarity to orient their behaviour in a variety of different ways. For example, cells of an epithelial sheet display both apico-basal and planar polarity, while migrating mesenchymal cells have a clear front-to-back organisation. At one extreme are highly polarised neurons with clearly segregated dendritic and axonal domains; at the other are round cells, such as those in budding yeast, that display obvious polarity only during certain phases of their life cycle. However polarity is manifested, cells rely on molecular polarity determinants (see Glossary, Box 1) that localise to specific domains of the plasma membrane and then act to polarise the action of other cellular systems (Etienne-Manneville and Hall, 2002; Mellman and Nelson, 2008; Knoblich, 2010; St Johnston and Ahringer, 2010; Goodrich and Strutt, 2011; McCaffrey and Macara, 2011; Vichas and Zallen, 2011). These polarity determinants can orient a whole host of cellular functions, such as cell shape, cell adhesion, cell migration, cell division, cell fate determination, and the uptake and release of molecules. Yet, how polarity determinants manage to organise their own polarised locations within cells remains a major unsolved problem.

One key feature of polarity determinants is their ability to respond to extracellular cues from neighbouring cells or from the environment. Such cues can guide the localisation of these proteins to orient cell behaviour. However, polarity determinants can also become polarised in the absence of any external cues, indicating that their localisation can be determined simply by an intrinsic ability to polarise spontaneously.

The molecular mechanisms that confer these special abilities upon polarity determinants are now beginning to be revealed through a combination of computer modelling with experimental testing of hypotheses. This review summarises early breakthroughs with this approach from the yeast *Saccharomyces cerevisiae*, the worm *Caenorhabditis elegans* and the fly *Drosophila melanogaster*.

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Cell polarity in budding yeast

S. cerevisiae are symmetrical cells that become polarised in order to undergo asymmetric cell division, a process known as 'budding' (reviewed by Slaughter et al., 2009). The mother cell divides by producing a small bud that grows into a daughter cell and then detaches after cytokinesis by hydrolysis of the cell wall. Just prior to budding, the cytoskeleton and membrane trafficking machinery become polarised in order to deliver cargo selectively into the bud, promoting growth of the daughter cell. The master regulator of cell polarity in budding yeast is the small GTPase Cdc42 (cell division control protein 42), which was discovered in genetic screens for mutants with defects in the cell division cycle (Adams et al., 1990). Loss of Cdc42 activity causes cells to grow without budding, so that they arrest as large symmetric cells (Adams et al., 1990). In addition, loss of Cdc42 disrupts another polarised process, known as 'shmoo' formation, which occurs during yeast cell mating (Adams et al., 1990).

The Cdc42 protein contains a C-terminal CAAX-linked Geranylgeranyl membrane anchor and is uniformly distributed around the plasma membrane in symmetric interphase cells, as well as being present in the cytoplasm. When yeast cells initiate cell division, Cdc42 polarises to a single plasma membrane domain that defines the site of the future bud (reviewed by Slaughter et al., 2009; Johnson et al., 2011) (Fig. 1A). Although Cdc42 can be oriented by cues, such as the 'bud scar' from previous divisions (Chant and Herskowitz, 1991), it can spontaneously polarise in the

Box 1. Glossary

Cooperativity. The tendency of a molecule to increase its activity non-linearly according to its concentration.

Mutual antagonism. The ability of two sets of molecules to inhibit the activity or localisation of one another.

Non-linearity. A process whose output is not directly proportional to its input. For example, an equation that depends on the concentration of a factor raised to the power of 2 or 3.

Polarity cue. An extracellular signal that orients the direction of polarity but is not necessarily essential for polarization.

Polarity determinant. An intracellular or transmembrane molecule that is localised in a polarized manner and is essential for polarity.

Positive feedback. Self-reinforcing loop in which the mathematical sign of the net gain around the feedback loop is positive: input 'A' produces more of 'A'. This is a process in which a small disturbance of the system can induce an increase in the magnitude of the perturbation.

Self-recruitment. The tendency of a molecule to localise to the position at which it is already most concentrated. This is one type of positive-feedback loop.

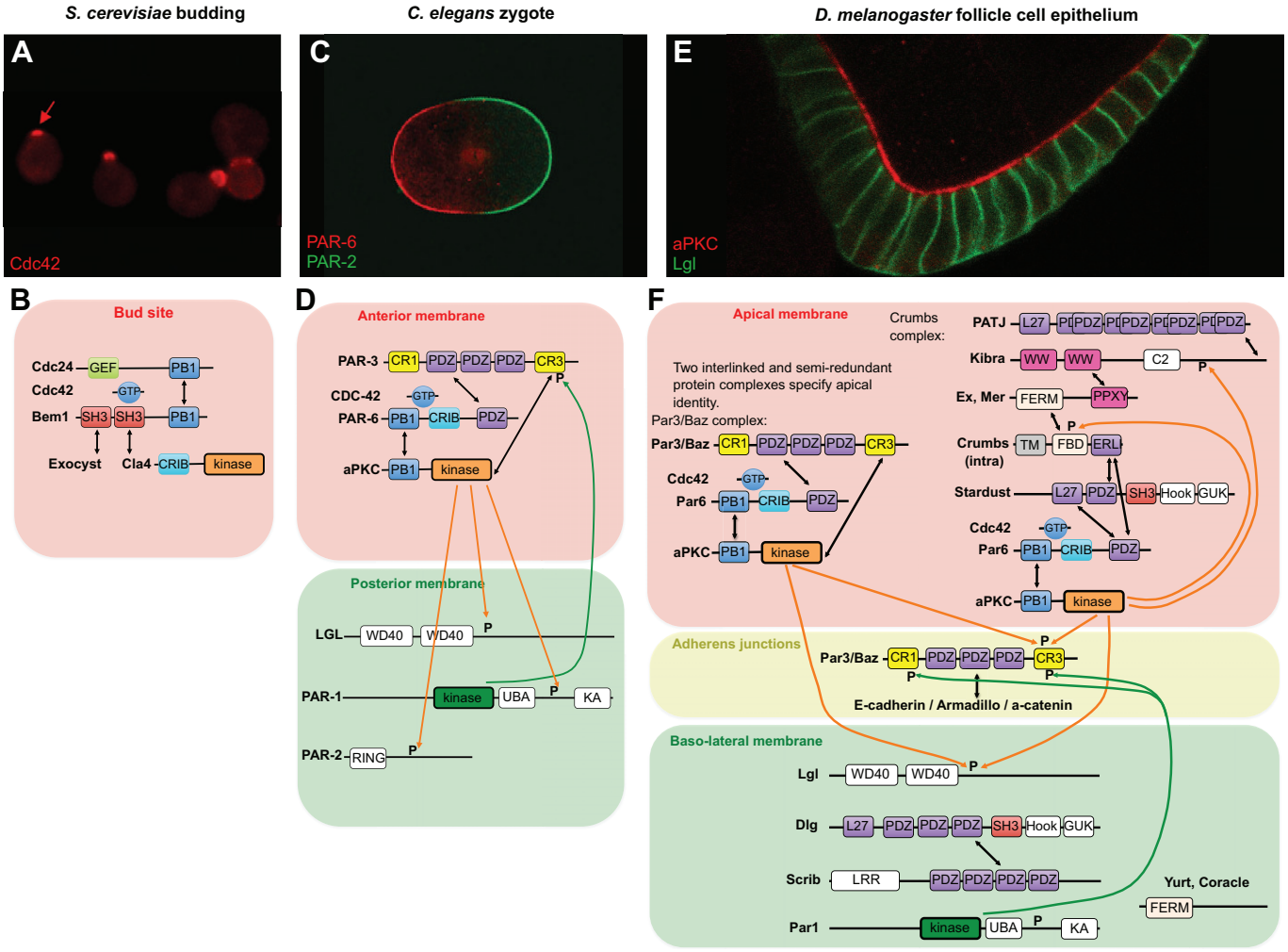

Fig. 1. Cell polarity in budding yeast, the worm zygote and the fly follicular epithelium. (**A-E**) The localisation of polarity determinants (A,C,E) and the domain structure and interactions between determinants (B,D,F) are shown for budding yeast (A,B), worm zygotes (C,D) and fly epithelia (E,F). aPKC, atypical protein kinase C; Baz, Bazooka; Bem1, bud emergence mediator 1; Cdc/CDC, cell division control protein; CR1/CR3, conserved region 1/3; CRIB, Cdc42/Rac interactive binding domain; Dlg, Discs large; Ex, Expanded; FERM, 4.1 protein, Ezrin, Radixin, Moesin; GUK, guanylate kinase domain; KA, kinase-associated domain; L27, Lin2 and Lin7 domain; Lgl/LGL, lethal giant larvae; Mer, Merlin; P, phosphorylation site; PAR/Par, partitioning defective; PATJ, PALS1-associated TJ protein; PB1, Phox and Bem1 domain; PDZ, post-synaptic density protein (PSD95), *Drosophila* discs large tumour suppressor (Dlg1) and zona occludens 1 protein (ZO1); Scrib, Scribbled; SH3, Src homology 3; UBA, ubiquitin-associated domain.

absence of these cues to a single site with a random orientation; budding proceeds normally from that site (Irazoqui et al., 2003). Insights into how Cdc42 can break symmetry to become polarised have come from a combination of mechanistic studies identifying Cdc42-interacting proteins that are essential for polarity and from computer modelling of Cdc42 polarisation.

Mechanistically, the spontaneous polarisation of Cdc42 does not require microtubules or F-actin, indicating that Cdc42 can act upstream of the polarisation of the cytoskeleton (Irazoqui et al., 2003). However, Cdc42 polarisation does require the PB1 (Phox and Bem1 domain) domain-containing GTP exchange factor (GEF) Cdc24, which induces GTP loading of Cdc42, and several effector proteins for Cdc42, including the Pak-family kinases Cla4 and Ste20 (sterile 20), which act redundantly (Gulli et al., 2000; Bose et al., 2001; Kozubowski et al., 2008), and the SH3 (Src homology 3) domain- and PB1 domain-containing scaffold protein Bem1 (bud emergence mediator 1) (Chenevert et al., 1992; Irazoqui et al., 2003), which binds to Cdc42, Cla4, Cdc24 and other proteins (Peterson et al., 1994; Zheng et al., 1995; Bose et al., 2001; Kozubowski et al., 2008; Slaughter et al., 2009) (Fig. 1B). These results show that a GEF-Cdc42-scaffold-kinase complex has an intrinsic ability to polarise spontaneously in budding yeast, but do not provide an answer as to how this occurs. Understanding how such a complex can polarise has required the use of computer models of cell polarity.

Mathematical and computational models have been crucial for establishing the notion that positive-feedback loops (see Glossary, Box 1) can promote polarisation of polarity determinants in various contexts. Early mathematical models made use of positive-feedback loops and a variety of other abstract concepts from mathematics and physics to generate patterns of different kinds (Turing, 1952; Gierer and Meinhardt, 1972; Meinhardt and Gierer, 2000). These early models have inspired more recent efforts to combine equation-based computer modelling of polarity determinants with experimental approaches to understand cell polarity (reviewed by Mogilner et al., 2012).

Altschuler et al. modelled spontaneous polarisation of Cdc42 molecules that localise either to the plasma membrane or in a homogeneous cytoplasmic pool (Altschuler et al., 2008). The homogeneity of the cytoplasm allows the plasma membrane to be simulated as a one-dimensional line, along which Cdc42 molecules can diffuse and appear on/disappear from as they bind/unbind the plasma membrane (Altschuler et al., 2008) (Fig. 2). Polarisation is achieved via a simple positive-feedback loop in which the on rate of Cdc42 from the cytoplasm to a particular region of the plasma membrane depends linearly on the concentration of Cdc42 already present at that point on the membrane (Altschuler et al., 2008) (Fig. 2). Polarity in this model is unstable and the model only polarises with relatively few (~200) molecules in the cell. Interestingly, a more-detailed model, in which Cdc42 self-recruits (see Glossary, Box 1) in a cooperative nonlinear fashion, can polarise in the presence of larger numbers of molecules (Goryachev and Pokhilko, 2008); non-linearity (see Glossary, Box 1) is a common feature of many models of polarity in different cell types (Jilkine and Edelstein-Keshet, 2011; Mogilner et al., 2012).

Together, the experimental data and computer modelling suggest that the GEF-Cdc42-scaffold-kinase complex self-recruits to the plasma membrane to polarise spontaneously in budding yeast (Altschuler et al., 2008; Goryachev and Pokhilko, 2008; Kozubowski et al., 2008). How, at the molecular level, one of these complexes promotes recruitment of the next remains to be explored (Johnson et al., 2011). These explorations should keep in mind one complication, which is that this rapid Bem1-mediated polarisation of Cdc42 acts redundantly with a second, slower, mechanism of Cdc42 polarisation that involves polarisation of the actin cytoskeleton itself, but whose mechanistic details are still unclear (Wedlich-Soldner et al., 2004; Johnson et al., 2011; Layton et al., 2011). Interestingly, computer modelling by Brandman et al. (Brandman et al., 2005) suggests that redundant, but interlinked, fast and slow positive-feedback loops may help ensure the robustness of polarisation. Thus, actin-dependent polarisation of Cdc42 may be a second level of positive feedback that is superimposed upon the first level to stabilise polarity in yeast (Slaughter et al., 2009). Finally, recent work has combined computer modelling with experiments to suggest that a negative-feedback loop exists that mediates competition between initial clusters of Cdc42 so that a single polarised domain emerges in a robust fashion (Howell et al., 2009; Howell et al., 2012).

Many of the discoveries made in yeast that have been highlighted above are also important for cell polarity in other organisms. In particular, analyses in worms and flies have confirmed that principles identified in yeast also apply in multicellular organisms, and have identified new molecules and mechanisms that are necessary to mediate cell polarity in animals. These are reviewed below.

Cell polarity in the *C. elegans* zygote

The *C. elegans* egg is initially symmetrical along the anterior-posterior axis, but becomes polarised after fertilisation, an event that also triggers the first cell division, which is therefore an asymmetric one (reviewed by St Johnston and Ahringer, 2010) (Fig. 1C). The entry of the sperm provides the second of each chromosome pair and a pair of centrioles to initiate the cell cycle. The position of the sperm centrosome provides a cue that orients the cell by defining the posterior pole in the one-cell zygote. How the sperm centrosome acts as a polarity cue (see Glossary, Box 1) is still not fully understood, as there appear to be multiple redundant mechanisms at work (Cowan and Hyman, 2007; Zonies et al., 2010; Motegi et al., 2011). Nevertheless, the key polarity determinants that respond to these early signals and maintain polarity were discovered in pioneering genetic screens for mutants that affect the asymmetric partitioning of granules during the first cell division (Kemphues et al., 1988). The genes identified were named ‘partitioning defective’ (PAR) genes.

The PAR proteins were found to localise to one or the other pole of the zygote (reviewed by Suzuki and Ohno, 2006) (Fig. 1C). PAR-1 is a kinase (Guo and Kemphues, 1995) and PAR-2 is a RING domain protein (Boyd et al., 1996); both localise to the posterior of the zygote along with the lethal giant larvae (LGL) protein (Hoege et al., 2010), named after its *Drosophila* homologue (see below) (Fig. 1D). PAR-3 (Etemad-Moghadam et al., 1995), called Bazooka (Baz) in *Drosophila*, is a multiple PDZ-domain protein that forms a complex with another PDZ-domain protein, PAR-6, atypical protein kinase C (aPKC) and Cdc42 at the anterior pole of the zygote (Watts et al., 1996; Izumi et al., 1998; Joberty et al., 2000; Lin et al., 2000; Gotta et al., 2001; Welchman et al., 2007; Li et al., 2010) (Fig. 1D).

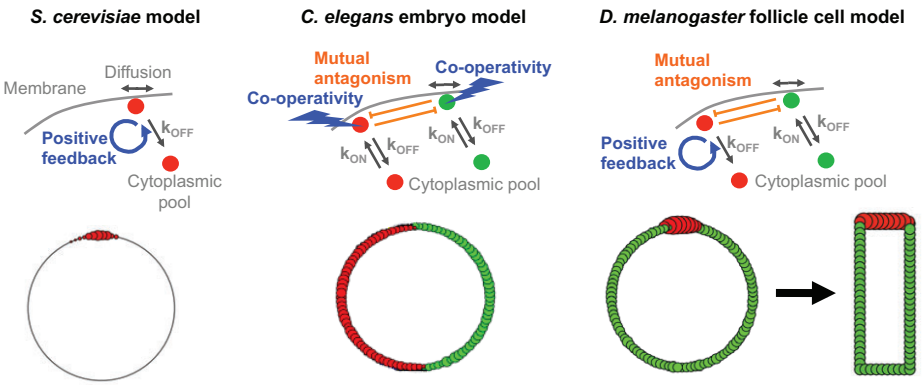

Fig. 2. Comparison of computational models of cell polarity in budding yeast, the worm zygote and the fly follicular epithelium. For each of the three different computational models (yeast, Altschuler et al., 2008; *C. elegans*, Goehring et al., 2011; *D. melanogaster*, Fletcher et al., 2012), a schematic summary diagram of the basic components of the model is shown. Simulated polarity determinants are shown as red or green dots that can diffuse along a simulated one-dimensional membrane. Binding and unbinding the plasma membrane from a cytoplasmic pool is determined by k_{ON} and k_{OFF} rates, by positive feedback loops of self-recruitment to the point of highest concentration, and/or by mutual antagonism between red and green determinants. The outcome of each polarity model is shown in the simulated cells below (circular for yeast or worm zygote, rectangular for epithelial cells). Either positive feedback or non-linearity is required for polarisation in each of the models.

Loss of the anterior PAR-3 complex causes the posterior polarity determinants to spread abnormally around the entire plasma membrane (see Gotta et al., 2001). Conversely, loss of the posterior LGL/PAR-2/PAR-1 complex causes aberrant spreading of anterior determinants around the entire plasma membrane (see Motegi et al., 2011). These findings indicate that anterior and posterior polarity determinants act in a mutually antagonistic manner to exclude one another from the plasma membrane (reviewed by Suzuki and Ohno, 2006; St Johnston and Ahringer, 2010). Following on from work in *Drosophila* (see below), mutual antagonism (see Glossary, Box 1) between anterior and posterior polarity determinants appears to involve aPKC-mediated phosphorylation of LGL, PAR-1 and PAR-2 (Hurov et al., 2004; Hoege et al., 2010; Motegi et al., 2011), and PAR-1-mediated phosphorylation of PAR-3 (Cuenca et al., 2003; Motegi et al., 2011) – phosphorylation events that are thought to inhibit plasma membrane association directly (Betschinger et al., 2005; Krahn et al., 2010b; Motegi et al., 2011) (Fig. 3). Curiously, the aPKC phosphorylation sites in LGL are required not only to remove it from the anterior plasma membrane, but also for the function of LGL in removing anterior PARs from the posterior membrane (Hoege et al., 2010). This led to the proposal that an interaction between anterior and posterior polarity complexes at the border between these two domains leads to mutual elimination of the two complexes from the plasma membrane (Fig. 3) (Hoege et al., 2010).

Whether the principle of mutual antagonism between two groups of polarity determinants is sufficient to explain how they polarise to opposite ends of the cell requires testing with computer models. Goehring et al. devised a model of mutual antagonism between anterior and posterior determinants in the zygote (Goehring et al., 2011). In this model, determinants can localise either to the plasma membrane, simulated as a one-dimensional line upon which they can diffuse, or in a homogeneous cytoplasmic pool, similar to the yeast models (Altschuler et al., 2008; Goryachev and Pokhilko, 2008; Goehring et al., 2011; Mogilner et al., 2012) (Fig. 2). Surprisingly, providing two different determinants with the ability to antagonise the membrane association of the other was not sufficient for generating polarity (Goehring et al., 2011). Polarisation was achieved only by the addition of cooperativity (see Glossary, Box 1), a mathematical function in which the strength of antagonism increased in a non-linear fashion with the concentration of each determinant at the plasma membrane (Goehring et al., 2011). The requirement for non-linearity in this model appears to correspond to the requirement for positive-feedback loops in both yeast (see above) and *Drosophila* (see below), suggesting that self-recruitment of polarity determinants may also underpin polarity in *C. elegans* embryos (Fig. 2).

Goehring et al. also use their model (Goehring et al., 2011) to investigate how the sperm centrosome acts as a cue to orient polarity by triggering cortical flows of acto-myosin that generate a bulk fluid motion in the cytoplasm, called ‘advection’, that pulls the PAR-3 complex to the anterior (Jenkins et al., 2006; Cowan and Hyman, 2007). However, this mechanism appears to be redundant with another microtubule-based mechanism that stabilises PAR-2 at the posterior (Zonies et al., 2010; Motegi et al., 2011). In addition, Goehring et al. have investigated the issue of domain size, using their model to show that the relative levels of anterior and posterior determinants can define the relative size of each domain (Goehring et al., 2011).

Thus, the application of genetics, biochemistry and cell biology, as well as computational modelling, has been crucial for establishing the key principle of mutual antagonism in polarisation of the *C. elegans* zygote. Further work is needed to test whether the principle of positive feedback acts in these cells to drive self-recruitment of polarity determinants. Excitingly, results from *Drosophila* support the notion that PAR proteins and other polarity determinants do indeed act in this way, as described below (Benton and St Johnston, 2003a; Fletcher et al., 2012).

Cell polarity in *Drosophila* epithelia

Epithelial tissues are composed of polarised cells with distinct apical and basolateral plasma membrane domains, and a ring of adherens junctions located at the interface of these two domains. A distinct basal domain can also appear where epithelial cells contact a basement membrane. In the fruit fly *Drosophila*, apico-basal polarity is first established during cellularisation of the early embryo and is thereafter maintained in epithelia that derive from the embryo, such as imaginal disc epithelia. In this Review, I focus mainly on the follicular epithelium, a well-established model system for epithelial polarity that is derived from apparently symmetrical stem cells that then develop epithelial polarity in response to cues from the germline (apical) and basement membrane (basal) (Tanentzapf et al., 2000; Franz and Riechmann, 2010) (Fig. 1E). Given the conservation of polarity mechanisms across evolution, it is likely that lessons learned from recent analyses in the follicular epithelium will apply in other *Drosophila* epithelia, as well as in equivalent systems in other species.

Many of the key polarity determinants discovered in yeast (Cdc42) and worms (the PAR proteins) also control polarity in fly epithelia, and genetic screens in *Drosophila* have uncovered many other important polarity determinants (reviewed by St Johnston and

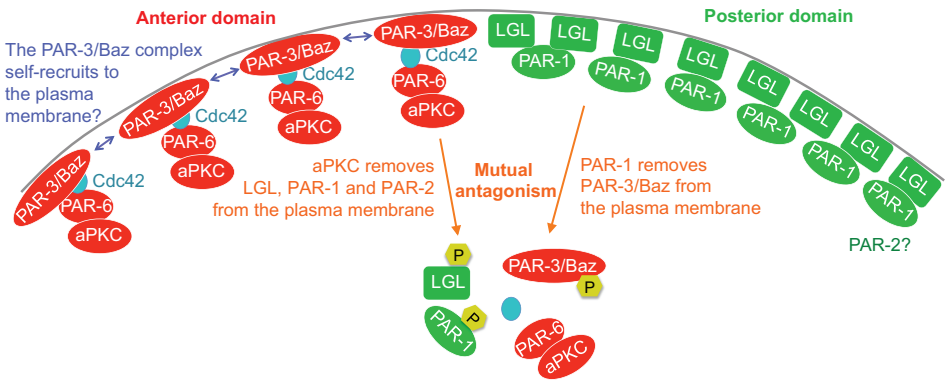

Fig. 3. Potential mechanisms for positive feedback and mutual antagonism in the worm zygote. In *C. elegans* zygotes, the anterior and posterior determinants polarise via mutual antagonism – each complex phosphorylates components of the other to promote its removal from the plasma membrane. PAR-3 may also engage in a positive-feedback loop of self-recruitment via oligomeric interactions, based on its similarity to *Drosophila* Baz. aPKC, atypical protein kinase C; Baz, Bazooka; Cdc42, cell division control protein 42; LGL, lethal giant larvae; P, phosphorylation site; PAR, partitioning defective.

Ahringer, 2010; Tepass, 2012) (Fig. 1F). For example, mutation of the basolateral determinants Lgl [L(2)gl – FlyBase], Scribble (Scrib) or Discs-large (Dlg) causes abnormal spreading of apical determinants around the plasma membrane and consequently a failure to localise the belt of adherens junctions or maintain cell shape in fly epithelia (Bilder et al., 2000; Bilder and Perrimon, 2000; Bilder et al., 2003). However, mutation of the core apical determinants Cdc42, aPKC or Par6 causes loss of the apical domain and consequent localisation of the basolateral determinants all around the plasma membrane (Wodarz et al., 2000; Rolls et al., 2003; Hutterer et al., 2004; Harris and Tepass, 2008; Franz and Riechmann, 2010; Fletcher et al., 2012). These core determinants form an apical complex with either the Bazooka (Baz) protein (Müller and Wieschaus, 1996; Joberty et al., 2000; Lin et al., 2000; Wodarz et al., 2000; Petronczki and Knoblich, 2001; Abdelilah-Seyfried et al., 2003; Franz and Riechmann, 2010) or the transmembrane protein Crumbs and its PDZ domain-containing binding partner Stardust (the Crb-Sdt complex) (Müller and Wieschaus, 1996; Tepass, 1996; Tanentzapf et al., 2000; Tanentzapf and Tepass, 2003; Fletcher et al., 2012) (Fig. 1F). Thus, apical and basolateral determinants appear to act in a mutually antagonistic manner in *Drosophila* epithelia, a striking parallel with polarity in the *C. elegans* zygote.

The apical Baz and Crb-Sdt complexes act in a semi-redundant fashion in fly epithelia, such that removal of both is necessary to eliminate completely the apical domain in a fully penetrant manner (Tanentzapf and Tepass, 2003; Fletcher et al., 2012). In tissues undergoing morphogenetic movements that involve relocalisation of Baz to the adherens junctions, such as the gastrulating embryo or developing photoreceptors, Crb-Sdt becomes essential for maintaining epithelial polarity (Müller and Wieschaus, 1996; Tepass, 1996; Pellikka et al., 2002; Campbell et al., 2009). In the case of the embryo, Baz then functions in regulating the localisation of adherens junctions during morphogenetic movements rather than apical identity (Harris and Peifer, 2005; Simões et al., 2010; Wang et al., 2012). In tissues where Crb is not expressed, such as the cellularising embryo, neuroblasts or very early stage follicle cells, Baz is necessary for polarity establishment (Müller and Wieschaus, 1996; Schober et al., 1999; Wodarz et al., 1999; Wodarz et al., 2000; Harris and Peifer, 2004; Atwood et al., 2007; Franz and Riechmann, 2010; Morais-de-Sá et al., 2010). Thus, the Baz complex and Crb-Sdt complexes can act independently to specify the apical domain. Nevertheless, as both complexes contain the same core components (Cdc42-Par6-aPKC) and can colocalise at the apical membrane, one complex can assist the polarisation of the other (Benton and St Johnston, 2003b; Harris

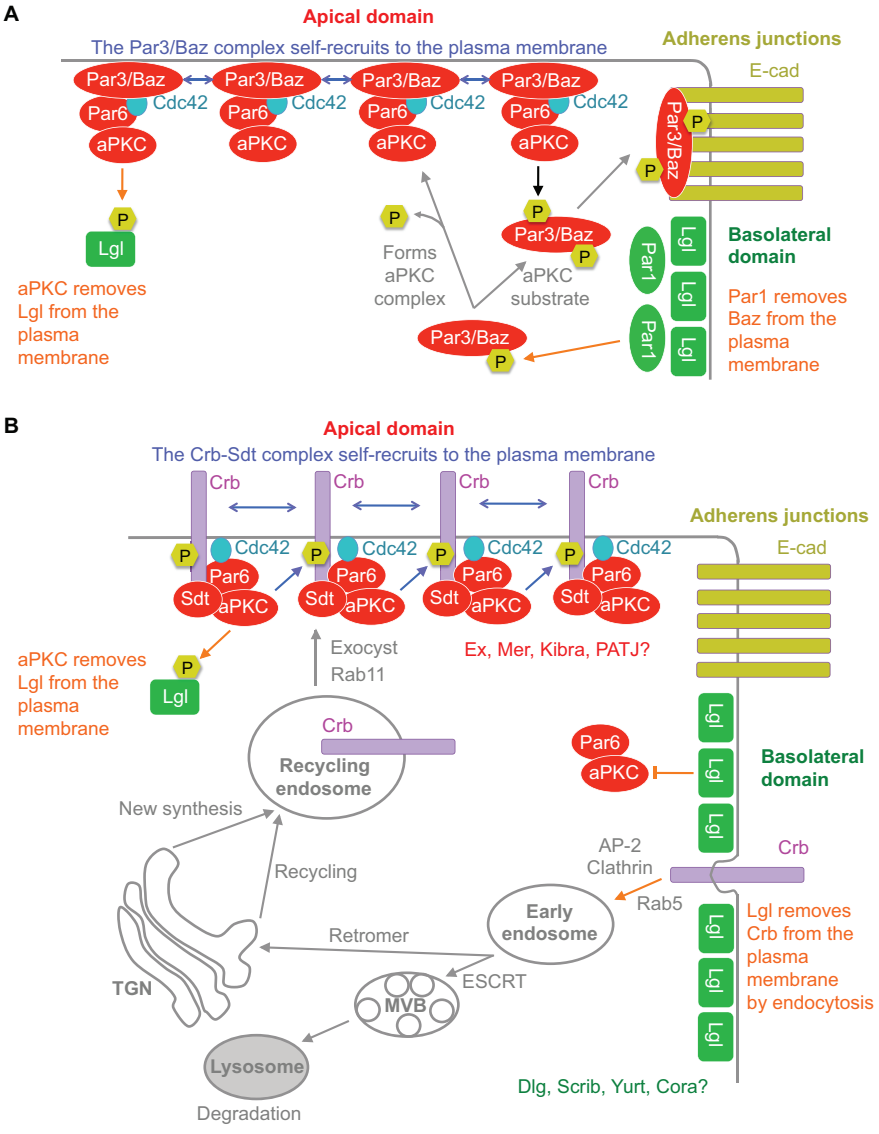

Fig. 4. Mechanisms for positive feedback and mutual antagonism in fly follicle cell epithelium. (**A**,**B**) In *Drosophila* follicle cells, the apical and basolateral domains are separated by a belt of adherens junctions. (**A**) The Par3/Baz complex is envisaged to self-recruit to the apical domain via oligomerisation of Par3/Baz in a positive-feedback loop. There is also mutual antagonism between the apical Par3/Baz complex and basolateral Par1, similar to *C. elegans*. One unique feature of *Drosophila* epithelia is that Par3/Baz can also localise to adherens junctions when phosphorylated by both aPKC and Par1 (Morais-de-Sá et al., 2010; Walther and Pichaud, 2010). In some tissues, such as the gastrulating embryo, Par3/Baz localises exclusively to adherens junctions. (**B**) The Crb complex is also able to specify the apical domain. Crb self-recruits through oligomeric interaction of its extracellular domain and phosphorylation of Crb by aPKC in a positive-feedback loop. The Crb complex also engages in mutual antagonism with asolateral determinants. aPKC, atypical protein kinase C; Baz, Bazooka; Cdc42, cell division control protein 42; Cora, Coracle; Crb, Crumbs; Dlg, Discs large; E-cad, E-cadherin; ESCRT, endosomal sorting complex required for transport; Ex, Expanded; Lgl, lethal giant larvae; Mer, Merlin; MVB, multi-vesicular body; P, phosphorylation site; Par, partitioning defective; PATJ, PALS1-associated TJ protein; Scrib, Scribbled; Sdt, Stardust; TGN, trans-Golgi network.

and Peifer, 2004; Franz and Riechmann, 2010) and the two can even directly interlink via Baz-Sdt interactions (Krahn et al., 2010a).

The involvement of Cdc42 as a polarity determinant that localises through two redundant but interlinked mechanisms is a common theme in yeast and *Drosophila* polarity. In yeast, Cdc42 polarises via positive-feedback loops (see above), implying that same mechanism may operate in *Drosophila* epithelia. A computer model of *Drosophila* epithelial polarity from Fletcher et al. (Fletcher et al., 2012) suggests that the combination of positive feedback among apical determinants plus mutual antagonism between apical and basal determinants is sufficient to spontaneously generate and maintain polarity (Fig. 2). The model of Fletcher et al. (Fletcher et al., 2012) is therefore similar to that of Goehring et al. (Goehring et al., 2011) in that it combines mutual antagonism with another essential principle (positive feedback in the case of *Drosophila* as opposed to non-linearity in the *C. elegans* model) to achieve polarisation (Fig. 2). Both models also raise the issue of how the Baz complex or Crb-Sdt complex might self-recruit to the plasma membrane to mediate positive feedback and how mutual antagonism between apical and basolateral determinants might occur.

In the case of Baz, there is potential for self-recruitment via a conserved N-terminal oligomerisation domain (CR1) that is essential for Baz to localise to the plasma membrane (Benton and St Johnston, 2003a; Mizuno et al., 2003) (Fig. 4A). The basolateral determinant Par1 phosphorylates S151 in the Baz CR1 oligomerisation domain, as well as S1085 in the Baz CR3 domain – which contains both lipid-binding and aPKC-binding regions (Krahn et al., 2010b) – to prevent Baz associating with the plasma membrane (Benton and St Johnston, 2003b). Phosphorylation is thought to recruit 14-3-3 proteins and thereby inhibit oligomerisation of Baz and prevent binding to either lipids or aPKC, thus disrupting self-recruitment of the Baz complex to the plasma membrane (Benton et al., 2002; Benton and St Johnston, 2003b; Krahn et al., 2010b). However, it remains unclear how apical determinants restrict Par1 to the basolateral domain. One possibility is that Par1 might bind to Lgl, which is excluded from the apical domain upon phosphorylation by aPKC (Betschinger et al., 2003; Betschinger et al., 2005). However, results from *C. elgans* and mammalian cells suggest that Par1 is directly excluded from the apical domain by aPKC phosphorylation (Hurov et al., 2004; Suzuki et al., 2004). Whatever the precise mechanism, these insights are consistent with a model of polarity that is driven by the combination of positive feedback and mutual antagonism.

In the case of the Crb-Sdt complex, there is evidence for oligomeric interactions between neighbouring Crb molecules via the Crb extracellular domain (Fletcher et al., 2012), as well as for potential trans-phosphorylation of the Crb intracellular domain by aPKC from a neighbouring Crb-Sdt complex, both of which appear to stabilise Crb at the plasma membrane (Fig. 4B) (Sotillos et al., 2004; Fletcher et al., 2012). Other undiscovered mechanisms may also exist to promote self-recruitment of Crb, and the multiple PDZ-domain protein PATJ (PALS1-associated TJ protein) is an interesting candidate that could conceivably promote a network of interactions between Crb-Sdt complexes (Roh et al., 2003; Shin et al., 2005; Richard et al., 2006; Zhou and Hong, 2012). The model of Fletcher et al. (Fletcher et al., 2012) suggests that basolateral determinants must in some way antagonise self-recruitment of apical determinants to the plasma membrane, and Lgl has been shown to bind to aPKC-Par6 and to inhibit the kinase activity of aPKC – an action that could directly disrupt the Crb-mediated positive-feedback loop (Betschinger et al., 2003; Plant et al., 2003; Yamanaka et al., 2006). The roles of Dlg and Scrib remain unclear, but one possibility is that these proteins antagonise the action of Sdt and PATJ – which have, respectively, similar domain structures to Dlg and Scrib (Fig. 1F).

Unlike Baz, Crb is a transmembrane protein that polarises through regulated membrane trafficking. Endocytosis of Crb via the AP2/Clathrin machinery is essential to remove it from the basolateral domain (Lu and Bilder, 2005; Fletcher et al., 2012) and recycling via the retromer (Pocha et al., 2011; Zhou et al., 2011) and Rab11 endosomes (Fletcher et al., 2012), as well as polarised exocytosis via the exocyst machinery, help deliver Crb to the apical domain (Fig. 4B) (Blankenship et al., 2007). Recent studies have implicated roles for FERM (4.1 protein, Ezrin, Radixin, Moesin) domain proteins in regulating the localisation of Crb, which contains a FERM-binding motif in its intracellular domain (this motif is also the site at which aPKC phosphorylates Crb). The apically localised FERM domains Expanded and Merlin – which act redundantly (Hamaratoglu et al., 2006) – were found to bind to Crb (Sotillos et al., 2004; Ling et al., 2010; Robinson et al., 2010) and to promote localisation of Crb to the plasma membrane (Fletcher et al., 2012). Expanded and Merlin also bind to and function together with Kibra (Baumgartner et al., 2010; Genevet et al., 2010; Yu et al., 2010), a protein that can also be phosphorylated by aPKC (Büther et al., 2004), suggesting a possible mechanism by which Expanded and Merlin functions might be regulated. By contrast, the basolateral FERM-domain proteins Yurt and Coracle were found to inhibit Crb localisation at the basolateral membrane, presumably by inducing endocytosis of Crb (Laprise et al., 2006; Laprise et al., 2009). Precisely how these FERM domain proteins regulate Crb trafficking to promote polarisation remains to be discovered.

Conclusion

Pioneering genetic screens in yeast, worms and flies have uncovered key determinants of cell polarity that are responsible for orienting cell behaviour. More recent work has employed computational models to make sense of how molecular interactions between these determinants can organise their polarised localisations within cells, and hence how polarity is generated and maintained. The results of these studies point to central roles for positive feedback and mutual antagonism mechanisms in organising polarity. Nevertheless, several unresolved issues remain and these are summarised below.

Issues for future research

Understanding self-recruitment

Further work is needed to understand how polarity determinants can self-recruit to the plasma membrane in yeast, worms and flies. Computational models currently use very simple approximations for self-recruitment and these can be improved by making the computer models more closely resemble known mechanisms of interaction among apical determinants. How the Crb-Sdt system self-recruits is still not fully understood and the roles of proteins such as PATJ and the Ex/Mer/Kibra complex are particularly unclear. Moreover, the degree to which the principles uncovered in model organisms apply in other animal tissues has yet to be ascertained.

Understanding mutual antagonism

Although mutual antagonism is quite well understood in the case of the Par-3/Baz system in worms and flies, it is less clear for the

fly Crb-Sdt system. In particular, how the *Drosophila* proteins Dlg, Scrib, Yurt and Coracle are removed from the apical membrane by apical determinants and act to antagonise the Crb-Sdt complex in epithelia remains a mystery. The role of polarised lipids such as phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol (3,4,5)-triphosphate also requires further exploration.

How is domain size determined?

In the *C. elegans* zygote, computer modelling suggests that domain size is simply determined by the relative amounts of anterior and posterior determinants. In fly epithelia, there is evidence to support this notion, but the situation is complicated by the presence of adherens junctions between the apical and basolateral domains. Adherens junctions can be neatly re-localised by altering the levels of apical aPKC, or basolateral Par-1 – both of which appear to act via phosphorylating Baz, which then determines the position of adherens junctions at this stage of embryogenesis (Wang et al., 2012). Incorporating adherens junctions into computer models of epithelial polarity is an important priority, as they could conceivably play an important role in determining domain size.

What orients up?

The cues that provide the initial orientation of cell polarity are not always fully understood. For example, in the early *Drosophila* embryo, it is clear that the outside face of the forming epithelium becomes apical, whereas the inside face becomes basal, but it is not known how cells sense outside and inside, and transduce this information such that Baz localises to the apical region of the forming epithelium. Similarly, in the follicular epithelium, how cell polarity initially responds to cues from the overlying germline and underlying basement membrane remains unclear.

How do polarity determinants regulate downstream effectors?

Cell polarity is responsible for orienting many cellular functions, such as cell shape, cell adhesion, cell migration, cell division, cell fate determination, and the uptake and release of molecules. Many of these functions depend on effector proteins that localise in response to polarity determinants, yet – with a few exceptions (Schober et al., 1999; Wodarz et al., 1999; Smith et al., 2007; Atwood and Prehoda, 2009) – how they do so remains poorly understood. For example, it is still unclear how adherens junctions are positioned at the interface of apical and basolateral domains in epithelia.

Modelling polarity in other systems

Combining computational models with experiments has led to great progress in understanding cell polarity in yeast budding, the worm zygote and fly follicular epithelium. Other cell types polarise in different ways, but the same combination of modelling and experiments is a highly promising approach to understanding each of them. Progress is being made here (see Neilson et al., 2011) but much more work needs to be carried out in order to understand the commonalities and differences in the mode of cell polarisation in cells from different tissues and organisms.

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