OPINION

Principles of PAR polarity in *Caenorhabditis elegans* embryos

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Abstract | A hallmark of cell polarity in metazoans is the distribution of partitioning defective (PAR) proteins into two domains on the membrane. Domain boundaries are set by the collective integration of mechanical, biochemical and biophysical signals, and the resulting PAR domains define areas of cytosol specialization. However, the complexity of the signals acting on PAR proteins has been a barrier to uncovering the general principles of PAR polarity. We propose that physical studies, when combined with genetic data, provide new understanding of the mechanisms of polarity establishment in the *Caenorhabditis elegans* embryo and other organisms.

Partitioning defective (PAR) proteins are conserved and common to all metazoans. They function to control asymmetric cell division of several cell types[1,2] and the partitioning of components in the cytosol, and they are crucial to the establishment of cell polarity and the control of cell fate[1]. The mechanisms behind the formation of PAR domains and their maintenance remain key questions that lie at the heart of polarity control.

The *Caenorhabditis elegans* embryo has provided a valuable model for addressing these questions. In a *C. elegans* embryo at the one-cell stage of development, PAR proteins form two distinct domains on the cell membrane. These domains result from the partitioning of PAR proteins between anterior and posterior portions of the embryo before the first cell division. Such distribution of PAR proteins depends on the activation of several genetic pathways and biochemical interactions acting together, but how these act collectively is unclear. PAR genes were discovered in *C. elegans* nearly 25 years ago in genetic screens[3] (for reviews, see REFS 1,4–6). The PAR-3, PAR-6 and atypical protein kinase C-like 3 (PKC-3) proteins form the anterior domain, and the PAR-1, PAR-2 and lethal giant larvae-like 1 (LGL-1) proteins form a posterior domain[7–13]. The subsequent identification of PAR domains in many other metazoans showed that division of the cellular cortex into two PAR domains is a common way to control cell polarity during early differentiation[1,2].

PAR domains on the membrane drive the asymmetric distribution of cytosolic components to control cellular differentiation. However, understanding how PAR domains control cellular differentiation is complicated by the fact that the formation of PAR domains and the polarization events downstream of PAR proteins involve the collective behaviour of large numbers, and different species, of molecules that often interact with each other only transiently and/or weakly.

How then can we understand the mechanisms by which PAR domains form and then polarize the embryo? Several studies in recent years have suggested that the analysis of physical principles that govern PAR polarization can be used to address both of these questions. This approach allows the construction of models that predict the collective behaviour of large numbers of individual components and bridge the length scale from local molecular mechanisms to the patterning of an embryo during morphogenesis. Importantly, the power of live imaging and molecular genetics in different systems allows these models to be tested. In many cases physical studies provide simple answers to what at first sight seem intractable problems, such as the question of how a protein gradient that depends on PAR membrane domains is formed in the cytosol. Moreover, they provide testable ideas for future experiments.

In this Opinion article, we examine the properties of PAR domain proteins, how they form stable domains and how they exert their effect on embryo polarity. In particular, we focus on the insights that physical studies have provided into these processes, mainly concentrating on *C. elegans*. We start by discussing the mechanisms that maintain the boundaries between the PAR domains. We outline the upstream signals that trigger polarization and the mechanical forces that lead to the establishment of two PAR domains. Finally, we discuss how PAR domains on the cell membrane are translated into protein gradients and downstream differentiation events.

PAR domains on the membrane

Polarization events are rapid. By roughly 40 minutes after fertilization, and 10 minutes after polarization has been initiated, two distinct PAR domains have formed on the cell cortex; the domain in the anterior part of the embryo consists of PAR-3, PAR-6 and PKC-3 (anterior PARs (aPARs)) and the domain in the posterior part consists of PAR-1, PAR-2 and LGL-1 (posterior PARs (pPARs))[7–13] (FIG. 1). How are these domains kept separate on the membrane? The physical properties of PAR proteins, their distinct biochemical interactions and their potential mechanisms of action all contribute to maintain PAR domains in a steady state.

Diffusion of PAR proteins on the membrane. Fluorescence recovery after photobleaching (FRAP) experiments have shown that PAR-2 and PAR-6 move freely on the membrane by lateral diffusion[14]. In the cytosol, they diffuse about an order of magnitude faster than they do on the membrane and they exchange with the membrane pool of PAR proteins by different rates of binding and unbinding[14,15] (FIG. 1a). The boundary between PAR domains on the membrane is not discrete: instead there is an overlapping gradient of PAR-2 and PAR-6 in the boundary region. On the membrane, the typical travel distances of PAR-2 and PAR-6 are 6 μm and 10 μm, respectively. This indicates that both PAR-2 and PAR-6 can travel over the boundary region down a concentration gradient (FIG. 1a,b). However, if both proteins can travel over the domain boundaries down their concentration gradient, how are the concentration gradients and the boundary maintained?

One simple explanation could be asymmetric binding and unbinding of PAR molecules to the membrane. Molecules that diffuse down the concentration gradient might detach from the membrane after they reach the other domain and then be replaced by new molecules, which would be recruited from the cytosol to the membrane in their own domain. Importantly, this process becomes asymmetric because binding and unbinding rates of both PAR-2 and PAR-6 are different between anterior and posterior membrane domains[14,16].

Other potential mechanisms could involve diffusion barriers, which would impose lateral sorting of proteins between domains.

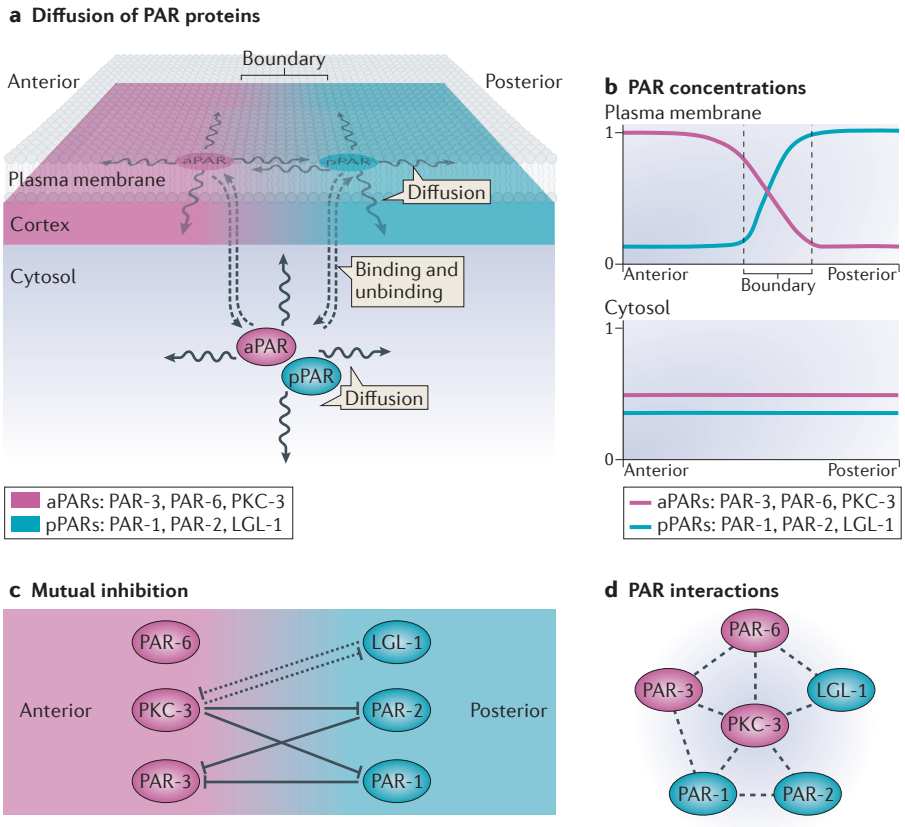

Figure 1 | **Properties of PAR proteins in *Caenorhabditis elegans* embryos.** **a** | Schematics of partitioning defective (PAR) protein diffusion in polarized embryos. PARs move on the membrane by lateral diffusion and exchange with the cytosolic pool by binding and unbinding. PARs in the cytosol move freely by diffusion. The membrane diffusion velocities for PAR-2 and PAR-6 are 0.15 $\mu m^2\ s^{-1}$ and 0.28 $\mu m^2\ s^{-1}$, respectively, and the average distances that they can travel on the membrane are 6 µm and 10 µm, respectively. Diffusion in the cytosol is rapid and about a magnitude higher. Arrows indicate diffusion directions, and binding and unbinding between membrane and cytosol. **b** | PAR concentrations in polarized embryos. Anterior PAR (aPAR) and posterior PAR (pPAR) concentrations are high in anterior and posterior membrane regions, respectively. At the domain boundary, aPARs and pPARs show overlapping localization. PAR concentrations in the cytosol are lower than on the membrane and do not show a gradient, with the exception of PAR-1, which has been suggested to form a cytosolic concentration gradient[53]. **c** | Mutual inhibition of PAR proteins at the domain boundary. Inhibition by phosphorylation (solid arrows) or mutual elimination (dashed arrows, in which LGL-1 removes PAR-6, atypical protein kinase C-like 3 (PKC-3) and itself from the membrane) lead to altered affinities and relocalization from the boundary region to the cytosol pool. The inhibition of PAR-3 by PAR-2 and PKC-3 by LGL-1 has been proposed[11,30] but has not yet been demonstrated directly. **d** | Physical interactions between *C. elegans* PAR proteins. aPARs interact with each other to form the PAR complex (PAR-3–PAR-6–PKC-3) or interact with the pPAR LGL-1 to form the LGL complex (PAR-6–PKC-3–LGL-1) that does not contain PAR-3. The pPARs PAR-1 and PAR-2 interact with each other, whereas interactions between PAR-1 or PAR-2 with aPARs have not been not reported. Dashed lines indicate physical interactions based on biochemical experiments. Other transient interactions might also exist.

However, the overlap between PAR domains and the travel distances of PARs on the membrane do not support such a model. Moreover, inverse fluorescence recovery after photobleaching (iFRAP) experiments in which molecules around the domain boundary are photobleached have shown that PAR-2 and PAR-6 do not move out of the boundary region[17], indicating that lateral sorting or transport is not a major contributor to domain maintenance. Diffusive behaviour of PAR proteins is independent of a functional contractile actomyosin cortex (which lies under the plasma membrane), excluding actomyosin as a scaffold for PAR organization[14]. However, a functional actomyosin cortex is required for the establishment of polarity, as discussed below.

Different rates of PAR protein binding and unbinding to the membrane seem to account for PAR concentration differences in different parts of the embryo, and this implies that binding and unbinding should be regulated processes. Indeed, several PAR proteins associate with the membrane directly by electrostatic interactions or indirectly by binding to membrane-bound proteins, such as the GTPase CDC-42; in the case of indirect binding to CDC-42, CDC-42 recruits PAR-6, which in turn recruits PKC-3 (REFS 18,19). In addition, MAP/microtubule affinity-regulating kinase 1 (MARK1; the human homologue of PAR-1), *C. elegans* PAR-2 and *Drosophila melanogaster* PAR-3 bind phosphatidylinositol phosphates (PIPs), a family of negatively charged membrane lipids, *in vitro*[20–22]. However, we do not yet know whether they bind membrane or specific PIPs in *C. elegans in vivo* or whether this could be sufficient for their localization to the cell periphery. Interestingly, the PIP_2-producing enzyme PPK-1 (PIP kinase 1) is enriched on the posterior membrane, and this enrichment depends on the presence of functional PARs[23]. PAR-2 on the membrane has a heterogeneous distribution that consists of at least two populations with different diffusive properties[15]. Mammalian PAR-3 can form oligomers[24] that could recruit PAR-6 and PKC-3 and/or other binding partners to form large assemblies on the membrane, and this might be required to establish the anterior PAR domain. Similar mechanisms might control PAR-3 distribution in *C. elegans*.

In summary, several PAR proteins probably bind to the plasma membrane *in vivo*, where they could associate into large and dynamic protein complexes. However, there is currently no evidence showing that differences in the composition of anterior and posterior membranes affect the asymmetric distribution of PAR proteins. Therefore, future work should concentrate on how PAR proteins associate with the membrane, and whether membrane composition changes during polarization.

Biochemical activity underlies mutual inhibition. 'Mutual inhibition' is used to describe the process by which proteins from one domain antagonize the colocalization of proteins from the other domain in a reciprocal way. For example, the anterior proteins PAR-3, PAR-6 and PKC-3 are required for the localization of PAR-1, PAR-2 and LGL-1 on the posterior membrane of the embryo. At the same time, PAR-1, PAR-2 and LGL-1 are also required together to restrict the localization of PAR-3, PAR-6 and PKC-3 to the anterior region of the membrane. Thus, they mutually inhibit each other[8–10,12,13] (FIG. 1c,d).

Several genetic pathways seem to provide negative or positive feedback signals that together contribute to the fine-tuning

Table 1 | **PAR proteins and other polarity proteins are both regulators and substrates in the *C. elegans* one-cell embryo**

Regulator	Substrate	Regulatory mechanism*	Effect	Species other than *Caenorhabditis elegans* in which this regulation has been shown	Refs
Membrane					
PKC-3‡	PAR-1§	Phosphorylation	Inhibition‖	*Drosophila melanogaster* and mammals	53,63,64
PKC-3‡	PAR-2§	Phosphorylation	Inhibition	NA	30
PKC-3‡	LGL-1§	Phosphorylation	Inhibition	*D. melanogaster* and mammals	11,33
PKC-3‡	PAR-3‡	Phosphorylation	Inhibition	*D. melanogaster*	65,66
PAR-1§	PAR-3‡	Phosphorylation	Inhibition	*D. melanogaster*	22,67
LGL-1§	PKC-3‡	Genetic and biochemical interaction	Inhibition	*D. melanogaster*	11,68
PAR-2	PAR-3‡	Unknown	Inhibition	NA	30
PAR-5	PAR-3‡	14-3-3 binding	Inhibition	*D. melanogaster* and mammals	65,67,69
Cytosol					
PAR-1§	MEX-5	Phosphorylation	Activation	NA	53
LET-92	MEX-5	Dephosphorylation	Inhibition	NA	53
PLK-1	MEX-5	Phosphorylation	Activation	NA	70
Cell cortex					
LGL-1§	NMY-2	Genetic and biochemical interaction	Inhibition	*D. melanogaster* and mammals	7,71,72
PAR-1§	NMY-2	Genetic and biochemical interaction	Inhibition	NA	73
PAR-2§	NMY-2	Genetic interaction	Inhibition	NA	35
PAR-3‡	NMY-2	Genetic interaction	Activation	NA	35
PAR-4	ANI-2 (acts indirectly on NMY-2)	Genetic interaction	Activation	NA	74
PAR-6‡	CDC-42	Biochemical interaction	Activation	*D. melanogaster* and mammals	18,19,75

ANI-2, anillin 2; CDC-42, cell division control protein 42 homologue; NA, not available; NMY-2, non-muscle myosin heavy chain 2; PAR, partitioning defective; PKC-3, atypical protein kinase C-like 3. *Genetic interactions indicate that the type of regulation can be indirect. Locations of regulation are inferred. ‡Anterior PARs are indicated. §Posterior PARs are indicated. ‖Phosphorylation at Thr983 has been proposed[53].

of PAR domains during polarization[22,25–29]. We focus on recent work that has provided biochemical data to support the proposed genetic framework of mutual inhibition. PAR-1, PAR-2 and probably LGL-1 are all phosphorylated by PKC-3 (REFS 7,11,22,30) (TABLE 1). Phosphorylation changes the net charges of the target proteins, and this could reduce their association with the membrane or the cell cortex. Such reduced association could be either due to changes in protein conformation or due to decreased binding affinities for the membrane or the cell cortex. So, one possibility is that PAR-1, PAR-2 or LGL-1 diffuse on the posterior membrane (where PKC-3 concentrations are low) but become phosphorylated after they cross the domain boundary into the anterior domain (where PKC-3 concentrations are high), and this triggers their asymmetric binding and unbinding to membrane.

How are aPARs restricted to the anterior part of the embryo? PAR-1 phosphorylates PAR-3 *in vitro*[22], and LGL-1 could directly regulate PKC-3 (REF. 11). PAR-2, which requires a functional RING finger[30] that is characteristic of ubiquitin E3 ligases for its function, could ubiquitylate aPARs, although ubiquitylation targets have not yet been identified (FIG. 1c,d; TABLE 1).

The recent measurements of PAR protein diffusive properties[14,31] have allowed the description of PAR polarity as a reaction–diffusion system[16,32]. Importantly, simulations that incorporate PAR diffusion rates, their mutual inhibition at the domain boundary and feedback regulation by limiting PAR protein pools give rise to PAR domains with characteristics very similar to those measured *in vivo*[16].

The theoretical description of PAR polarity has shown that PAR polarity depends on mutual inhibition[16], but mutual inhibition does not require specific biochemical mechanisms in these theoretical models. This suggests that *in vivo* cells could apply different biochemical mechanisms for mutual inhibition. The flexibility of the biochemical mechanisms that maintain a domain boundary is shown by studies on LGL-1 (REFS 7,11). LGL-1 is part of the posterior PAR domain along with PAR-1 and PAR-2, but if moderately overexpressed it can alone maintain a posterior domain in the absence of PAR-2 and without PAR-1 localized to the membrane[11]. This has brought up the question of how LGL-1 can inhibit aPARs from entering the posterior domain. Posterior LGL-1 can form a stable protein complex with the anterior proteins PAR-6 and PKC-3 (FIG. 1d) and requires PKC-3-dependent phosphorylation for its activity. The most plausible explanation for these observations is that LGL-1 binds to PAR-6 and PKC-3 at the domain boundary where it is phosphorylated by PKC-3, and that this induces the relocation of the LGL-1–PAR-6–PKC-3 complex to the cytosol. In this way, LGL-1, PAR-6 and PKC-3 could mutually eliminate each other from the membrane and stabilize the PAR domain boundary. Importantly, this mechanism would require a LGL-1 phosphorylation cycle to remove the LGL-1–PAR-6–PKC-3 complex from the membrane. A mutated version of LGL-1 that is resistant to phosphorylation is able to associate with the membrane and bind to PAR-6 and PKC-3, but it cannot remove

them from the membrane[11] and, therefore, it cannot maintain the PAR domain boundary. Mutual elimination might be a conserved mechanism, as LGL-1, PAR-6 and PKC-3 are present in other polarity systems studied[33,34].

Genetic data indicate that LGL-1 and PAR-2 could act as actomyosin inhibitors and suggest that the actomyosin cortex might also have a role in maintaining PAR domains, although actomyosin contractility, which is regulated by non-muscle myosin 2 (NMY-2), is downregulated after PAR domains have formed. However, NMY-2–GFP concentrations are still increased in an anterior cap-like structure when PAR domains are maintained during mitosis. In double mutant embryos of *par-2* and *lgl-1* this anterior cap-like structure seems to span the whole embryo, and aPARs become localized to the posterior[7,35]. Thus, although a functional actomyosin cortex seems dispensable for localization of PAR-2 and PAR-6 to the cell membrane[14], actomyosin interactions could contribute to the maintenance of PAR domain boundaries.

It is possible that the biochemical mechanisms that maintain PAR domains could be quite diverse in different metazoan clades and are constrained only by the functional requirement to form two opposing domains. Different mechanisms have probably evolved to account for the physical and scalar constraints of different systems, such as cell size or the amount of time for which a PAR domain must be maintained. For example, the length scale of any particular PAR gradient on the membrane will only be appropriate for a certain cell size and could be adjusted by a change in PAR diffusive properties.

Upstream signals as polarity triggers

Before fertilization, pPARs (PAR-1, PAR-2 and possibly LGL-1) localize uniformly to the membrane of oocytes[8,10] and aPARs (PAR-3, PAR-6 and PKC-3) are cytoplasmic. After fertilization by sperm the embryo is still unpolarized and undergoes meiosis, and pPARs remain on the membrane (FIG. 2a). When meiosis is completed and the embryo enters the mitotic cell cycle, actomyosin contractions of the cell cortex underlying the plasma membrane are triggered by an unknown signal[4,36]. At this point, aPARs become localized on the cell membrane; however, the signals that induce rapid aPAR membrane recruitment are unknown. While aPARs are being recruited, pPARs leave the membrane, probably as a result of PKC-3 activation (REFS 25,29,30) (FIG. 2a). A few minutes later, polarity triggers lead to the local inhibition of actomyosin contractions, the flow of actomyosin and aPARs away from the initial polarization site and the accumulation of pPARs in a growing posterior domain in the region of actomyosin inhibition[35].

What are the polarity triggers that lead to PAR domain establishment in the first place? The establishment of polarity probably requires several signals that rely on a functional centrosome, which is brought in by the sperm in the form of centrioles. These centrioles mature in the embryo to a functional centrosome by recruiting pericentriolar material (PCM) from the cytoplasm[37–40]. PCM proteins are required for polarity[40,41], but it remains unclear how they modify actomyosin contractions on the cell cortex. Before polarity triggering, centrosomes perform random walks on cytoplasmic microtubules, which are enriched around the cell cortex[42]. The first indication of polarization so far described is an expanding small bleb of cell membrane, in which foci of NMY-2 are absent[35,42]. At this stage, centrosomes are on average 4 μm away from the cell cortex, and microtubule asters at the centrosome are still small. This suggests that the centrosome might signal polarity by a diffusible signal[42]. Shortly after, centrosomes move closer to the cell cortex, possibly driven by cytosolic flow on microtubule tracks. Close to the cortex, astral microtubules seem to support pPAR domain growth for robust polarization[22,43]. Although centrosomes seem to be able to trigger polarization from any position within the embryo[42], polarization is faster and more robust if centrosomes are closer to the cell cortex. This might be due to the access of short astral microtubules to the cell cortex (FIG. 2b).

A microtubule-dependent polarity signal becomes essential when actomyosin activity at the cell cortex is compromised[22,44] and enables a pPAR domain to form in the absence of actomyosin contractions. In this case, pPAR domains take longer to form and become established later in the cell cycle, indicating that actomyosin contractility is required for timely polarization[22,44]. Polarization in the absence of actomyosin contractions also depends on the presence of the RING finger protein PAR-2 (REFS 22,44). *In vitro*, PAR-2 binds microtubules, and it has been suggested that microtubules support the accumulation of PAR-2 on the membrane *in vivo*[22].

Taken together, studies on centrosome-dependent polarization suggest that there are probably redundant mechanisms that are required to polarize the embryo: a microtubule-independent signal and a microtubule-dependent signal that depends on PAR-2 interaction with microtubules. Further work is needed to identify the nature of the centrosome signal, how it leads to centrosomes becoming proximal to the cell cortex and whether, or how, it communicates with microtubule-dependent signals. Protein degradation could also play a part, although we do not yet know the direct implications of this degradation[43,45].

The physical basis of PAR segregation

It has long been known that polarity triggers signal a distinct set of events that are driven by actomyosin contractility and that lead to the formation of the two opposed PAR domains[36]. Models based on conventional protein–protein interactions have been unable to elucidate the method by which the actomyosin cell cortex drives PAR segregation; however, recent physical studies, which define the roles of tension and advection in the cortex, have established that PAR proteins move with a flow of fluid actomyosin cortex[35,46].

Tension and viscous forces at the cell cortex. The notion that the embryo cell cortex is under tension was proposed two decades ago[36,47]. It was suggested that a polarity trigger induces the formation of a gradient in mechanical tension along the anterior–posterior axis, which in turn creates a cortical flow of actomyosin towards the anterior side[36]. Recent work in which cortical actomyosin was ablated using laser radiation has shown that there is no such gradient in tension[48]. This is because the local balance of forces in the cortex prevents the formation of a tension gradient along the anterior–posterior axis, although tension is anisotropic. Rather, the cell cortex is characterized by viscous forces, and this underlies the contribution of hydrodynamic force balances and fluid dynamics to polarization events[48] (FIG. 2c).

Advection forces of the cell cortex. Previous work on cell cortex tension suggested that flows of actomyosin drive movement of the aPARs, but the mechanism has remained unclear[35,48,49]. Recent work has shown that flow velocities of the cell cortex driven by the actomyosin cortex are high enough to support passive transport of PAR protein complexes by advection[16,46]. The movement of protein complexes along a flow by advection can be likened to the movement of objects in a river. Sufficiently large objects, such as a tree trunk, will move with the flow, whereas small objects, such as small particles, might diffuse too quickly and not be subjected to flow forces. These studies have indicated

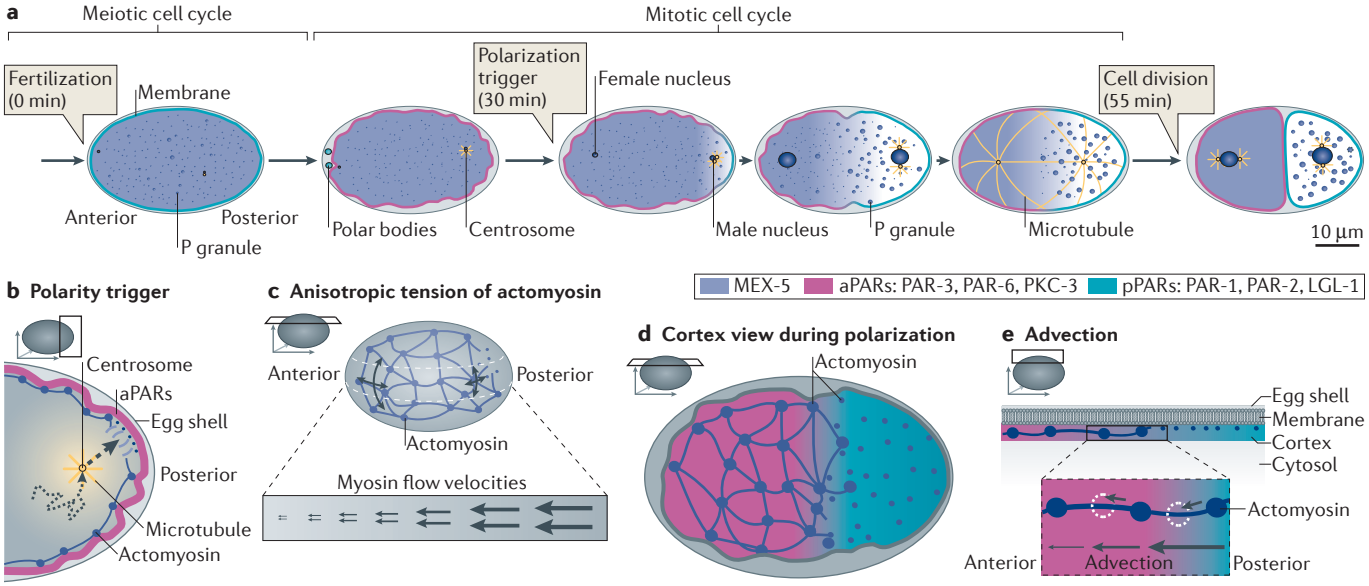

Figure 2 | **Polarization of the *Caenorhabditis elegans* embryo at the one-cell stage.** **a** | Schematic of meiotic and mitotic cell cycles. After fertilization (not shown) the zygote undergoes two rounds of meiotic divisions, which result in the extrusion of two polar bodies, and then enters the mitotic cell cycle. Contractions of the cell cortex begin and posterior PARs (pPARs: PAR-1, PAR-2 and LGL-1) leave the membrane, whereas anterior PARs (aPARs: PAR-3, PAR-6 and PKC-3) are relocated to the membrane. **b** | Polarization is triggered by signals of the centrosome and its microtubule asters. During polarization two PAR domains form on the cell cortex. In the cytosol an MEX-5 gradient forms during polarization and P granules segregate to the posterior part of the embryo (see part **a**). Centrioles are brought in by the sperm (not shown) and mature to centrosomes, which then duplicate and nucleate microtubules that form the mitotic spindle. Polarity is triggered by a signal (of not yet well defined nature) from the centrosome. The centrosomes move by random walk (grey track) and send a diffusible signal (yellow gradient) that reaches the cell cortex (dashed arrow), which induces the local cessation of actomyosin contractions. **c** | After triggering polarization, the actomyosin cortex displays anisotropic tension (arrows on the embryo diagram). Vertical tension is higher in the anterior part of the embryo. Actomyosin-dependent flow is directed towards the anterior part of the embryo. Flow velocities are high in the posterior embryo and low in the anterior embryo. **d** | View onto the cell cortex during polarization. Actomyosin is less crosslinked and less dense in the posterior embryo than in the anterior embryo during polarization, when PAR proteins on the membrane have adopted a polarized localization with a boundary region. **e** | A cell cortex cross-section. Flow of actomyosin towards the anterior part of the embryo during polarization leads to passive transport of aPAR proteins by advection. Close up of boundary region (lower part of figure): advective forces caused by actomyosin lead to a net flux of aPARs to the anterior. This depletes aPARs on posterior membrane and allows the binding of pPARs to the membrane.

that, because advective flow is sufficient to passively transport membrane aPARs to the anterior domain *in vivo*, this creates a net loss of aPARs in posterior membrane regions. Consequently, PARs with high concentrations in the cytosol (the pPARs PAR-1, PAR-2 and LGL-1) undergo a net flux from the cytosol to the posterior membrane, creating a growing posterior domain (FIG. 2d,e).

These results suggest more generally that the size of a protein complex can affect protein localization. If a protein complex is large enough it will move with flow, but if it is disassembled by cellular signals its mode of transport may change to simple diffusion. It will be interesting to identify PAR diffusion rates in other systems and see whether diffusion rates can change during development. Indeed, regulation of diffusion by the size and concentration of the protein complex seems to regulate cytoplasmic gradients, as discussed below.

If actomyosin flow and advective forces are absent at the cortex, PAR domains can still form *in vivo* via a redundant microtubule-dependent trigger, although they form more slowly and later[22,29,44]. However, simulations in absence of actomyosin flow have shown that local perturbations of PAR kinetics need to be strong to allow polarization in this case[16]. One possible explanation is that astral microtubules could locally change membrane-unbinding rates of PAR-2 (REF. 22) by protecting PAR-2 from PKC-3 activity by microtubules. The ability of PARs to respond to different polarity triggers might be one reason for their conservation among metazoans, and this may imply the existence of a diverse array of polarization triggers.

Downstream signalling events

PAR domains on the membrane guide asymmetric cell divisions by G protein signalling that positions the mitotic spindle slightly to the posterior (see REF. 50 for a review). PAR domain proteins on the membrane also induce polarization of the cytosol to determine cell fate via the formation of effector protein diffusion gradients.

Cytosolic protein gradients. PAR domains on the membrane lead to a cytosolic gradient of the RNA binding proteins muscle excess 5 (MEX-5)[51] (and possibly its paralogue MEX-6). This protein gradient seems to be driven by an underlying gradient of MEX-5 diffusion rates along the anterior–posterior axis; MEX-5 diffusion rates are higher in the posterior and lower in the anterior region of the cytosol[52]. PAR-1-dependent phosphorylation releases MEX-5 from some protein complexes, and diffusion of MEX-5 becomes faster in the posterior cytosol[53] (FIG. 3a).

It is important to understand how different diffusion rates result in a protein concentration gradient along the anterior–posterior axis. It has been suggested that PAR-1 itself forms a gradient in the cytosol by being enriched in the posterior region of the embryo[53]. Thus, MEX-5 would be preferentially phosphorylated in the posterior cytosol. Moreover, this faster-diffusing MEX-5 was proposed to explore a larger area and therefore enter the anterior cytosol

Glossary

Actomyosin
Crosslinked network of actin and non-muscle myosin filaments, which can contract by sliding along each other and which underlie the plasma membrane of the cell.

Advection
Passive transport of molecules by drag forces in liquids.

Anisotropic
Directionally dependent difference in a property of the material.

Cellular cortex
Layer on the inner side of the plasma membrane that mechanically supports the membrane. It consists of actin, non-muscle myosin (see actomyosin) and several actin-binding proteins.

Centrioles
Cylindrical structure in most cells that is typically composed of nine triplets of microtubules. Involved in the organization of the centrosome and the mitotic spindle.

Centrosome
Organelle that organizes microtubules in the cell and contains centrioles.

Effector
A product or process that operates after instructions issued by, for example, a control protein such as a kinase.

Fluorescence recovery after photobleaching
(FRAP). Microscope technique in which fluorescent molecules are studied in a defined area before and after fluorescent molecules are photobleached. The kinetics of fluorescence recovery is a readout of the mobility of the fluorescent molecules in space.

Inverse fluorescence recovery after photobleaching
(iFRAP). A special type of FRAP, in which fluorescent molecules are photobleached outside the region that is analysed.

Lateral diffusion
Two-dimensional diffusion (for example, on a surface); that is, the motion of molecules that causes flux and mixing of molecules from high to low concentrations.

P granules
Complex assembly of heterogeneous RNA and protein molecules that form spheres in the cytoplasm of the germ line, oocytes and embryos of *C. elegans*. Although their function is not yet solved, they seem to contribute to future germ cell fate (known as germ granules in other organisms).

Phase separation
A type of partitioning between thermodynamically similar regions of space (for example, P granules partition between two different kinds of liquids in the cytosol).

Random walks
The stochastic moving path of a molecule travelling through a medium.

Reaction–diffusion system
In biology a patterning process in which diffusive molecules (morphogens) undergo biochemical reactions (for example, phosphorylation, degradation and synthesis) and form well-defined spatial distributions.

Steady state
Dynamic equilibrium in which reactions do not change the composition of mixtures.

Tension
Pulling force that acts between solid objects.

Triggers
A cue or signal that initiates a process transforming an unpolarized cell into a polarized cell.

Viscous forces
A measure of resistance to flow. This describes the internal friction of a moving fluid (for example, fluids are more viscous than liquids).

more easily, where it would become incorporated into slower-diffusing complexes. The incorporation into these complexes depends on the activity of phosphatases, which do not show a concentration gradient along the anterior–posterior axis and which antagonize PAR-1 phosphorylation. A phosphorylation cycle of MEX-5 driven by PAR-1 and phosphatases could therefore define a reaction diffusion system that establishes a protein gradient in the cytosol by differential diffusion[53] (FIG. 3a). Thus, differential control of protein complex size by phosphorylation could provide a powerful mechanism to control protein localization.

Other proteins that are involved in cell fate decisions show asymmetric localization in the early embryo, and this depends on the MEX-5 gradient. The transcriptional repressors pharynx and intestine in excess protein 1 (PIE-1) and posterior segregation 1 (POS-1) show posterior enrichment and segregate to the P cell lineage that will become the germ line in the adult worm[54,55]. It will be interesting to see whether, in a similar way to MEX-5, their segregation also underlies differential diffusion.

Phase separation of P granules. The formation of a cytosolic gradient of MEX-5 is thought to lead to the segregation in the cytosol of transcriptional and translational repressors, which in turn contribute to cell differentiation in *C. elegans* embryos. Other factors that are segregated include P granules, which are non-membranous germ plasm assemblies composed of diverse maternal RNA and proteins, similar to processing bodies (P bodies), Cajal bodies or promyelocytic leukaemia (PML) bodies in mammalian cells[56,57]. In *C. elegans,* segregation of germ plasm components contributes to the formation of the future germ line, although we lack detailed knowledge about how germline cell fate is determined. The study of P granule localization has served as a model for PAR domain-mediated control of differentiation. Although P granules contain germ plasm components, their functions still remain unclear[57,58]. Previously, it was thought that P granules move and segregate by cytosolic flow — the flow being a result of actomyosin contractions of the cell cortex and feedback regulation by PARs[35,49,59]. P granules can indeed move with the cytosolic flow, but they grow or shrink depending on their position along the anterior–posterior axis of the embryo and, surprisingly, do not require cytosolic flow for posterior segregation. Compared with other protein and RNA complexes, such as ribosomes, they appear to have disordered structures with possibly low and multivalent affinities between individual components[60]. Importantly, they have liquid-like behaviour on minute timescales[61]. Granules can condense from freely diffusing protein and RNA molecules in a supersaturated cytosol to form a new liquid-like phase that defines the granules. Granules will also shrink by dissolution of their content back into the cytosolic phase if the cytosol is undersaturated[56,61] (FIG. 3b). The MEX-5 gradient set up by PAR domains on the membrane leads to net condensation or dissolution of the granules, depending on the granule position along the anterior–posterior axis in the cytosol. They condense at the posterior and dissolve at the anterior of the embryo.

The segregation of P granules can be considered as being driven by differential diffusion. P granules can only form at the posterior where the concentration of MEX-5 is low. After they form P granules, they condense into large assemblies that diffuse slowly. As the components condense into P granules, the concentration of individual P granule components becomes lower in the posterior embryo. Individual protein–RNA complexes in the embryo cytosol diffuse quickly (in the range of a few μm^2 per s). Therefore, diffusive flux will ensure that the concentration of individual components will evenly distribute throughout the embryo. In the posterior embryo, P granule components continue to condense into granules by diffusive flux until all P granule components are incorporated into the granules[61].

The movement of proteins between specific liquid phases in P granules is a simple and fast-acting way to sort complex mixtures over micrometre-length scales without the need for a complex sorting machinery. Sorting complex mixtures by phase separation might be a conserved mechanism to organize the cytosol in other organisms.

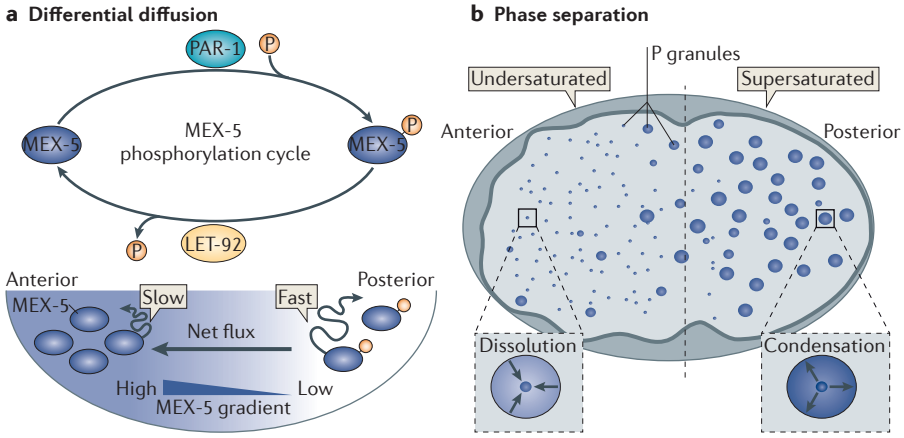

Figure 3 | **Polarizing events downstream of PAR proteins.** **a** | The MEX-5 protein gradient in the cytosol results from differential diffusion rates, which depend on a phosphorylation cycle. Partitioning defective 1 (PAR-1)-dependent phosphorylation and LET-92-dependent dephosphorylation compete on MEX-5 in the cytosol. MEX-5 is phosphorylated in the posterior cytosol and dephosphorylated in the anterior cytosol. Phosphorylated MEX-5 diffuses faster, which leads to a net flux of MEX-5 towards the anterior part of the embryo, where it becomes incorporated into slower diffusing complexes. MEX-6 (not shown), which is a MEX-5 paralogue, could behave in a similar manner. **b** | P granules partition by phase separation along the anterior–posterior axis. P granules are dense and homogeneous associations of RNA and protein molecules that diffuse in the cytoplasm. During polarization P granules dissolute in the anterior cytosol or condense P granule components into larger granules in the posterior cytosol, respectively. This behaviour depends on the cytosol being undersaturated or supersaturated for P granule components, which separate as a result of an existing MEX-5 protein gradient.

Conclusions

We propose that the events that follow the triggering of embryonic polarity and that lead to the segregation of components in the cytosol represent a cascade in which the steps are governed by different physical phenomena (FIGS 2,3). Moreover, simple physical principles help to explain the apparent complexity of *C. elegans* embryonic polarity establishment, and these principles might extend to other metazoans.

A model has emerged in which several signals that require the centrosome lead to anisotropic tension and cortical flow of the actomyosin cortex[35,37,48]. This flow then passively transports aPARs by advection to anterior membrane regions. After flow ceases, aPARs and pPARs work through a reaction–diffusion system that maintains the two domains, which are then stabilized by mutual inhibition and concentration, as well as by activity-dependent feedback loops[7,11,16,22,25,30,35,49,62]. PAR domains on the membrane use their phosphorylation activities to differentially modify the diffusion of target proteins in the cytosol[53]. This is sufficient to create protein concentration gradients in the cytosol that lead to the partitioning of downstream effector proteins[54,55] and the partitioning of P granules along the anterior–posterior axis by phase separation[61].

One of the principles that has emerged over the past few years from cell polarity studies using *C. elegans* is the importance of considering diffusion in understanding the mechanisms of PAR polarity, as shown by the establishment of a MEX-5 gradient and the formation of P granules. Cells seem to carefully control and adjust diffusion rates of different cellular components by fairly simple biochemical reactions, leading to large-scale changes in cellular organization.

The challenge for the next decade is to connect these large-scale physical properties to the underlying molecular mechanisms. We do not yet understand how cortical tension and advective forces are linked to biochemical and biophysical activities of PAR proteins on the membrane. Little is known about the behaviour of PAR proteins other than their ability to freely diffuse, self-organize, interact with other proteins and form protein complexes. How do they associate with the membrane or the cell cortex, and how are their diffusive properties and protein–protein interactions regulated? How do centrosome signals modulate actomyosin activity and PAR kinetics on the membrane? What is the role of the microtubules, and how could they interact with the membrane to change PAR kinetics?

There are also interesting questions about signalling downstream of the PARs. How do PARs regulate G protein signalling, and how does that control asymmetric cell division? Do other cytoplasmic properties change upon PAR signalling in addition to the establishment of a gradient of MEX-5 (and possibly of other effectors such as PIE-1 and POS-1)? How do the effectors determine cell fate? Furthermore, what is the function of the P granules, and how does an MEX-5 gradient lead to phase separation of P granule material in the posterior cytoplasm?

A key question for the future is how the different parts of the polarity cascade are integrated and can feed back information to each other. For example, aster microtubules seem to feed back onto pPARs to stabilize the posterior PAR domain on the membrane[22], both anterior and posterior membrane PARs and cytosolic MEX-5 feed back onto actomyosin activity and cytosolic flow[25,35,49], and the cytokinesis furrow feeds back by a LIN-5-dependent pathway to correct PAR domains during cell division[28]. Better understanding of how these mechanisms are coordinated should provide new insights into polarity control in diverse systems.

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doi:10.1038/nrm3558
Published online 18 April 2013

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Acknowledgements

The authors thank the anonymous reviewers, their colleagues in the laboratory and at the Max Planck Institute of Molecular Cell Biology for critical comments, suggestions and discussion on the manuscript. They apologize to their colleagues in the field for omitting their work due to space limitations. Work in the authors laboratory was supported by the Max-Planck-Gesellschaft (MPG) and the Deutsche Forschungsgemeinschaft (DFG).

Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

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