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Review Article

PAR polarity: From complexity to design principles

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ABSTRACT

The *par*-titioning-defective or PAR proteins comprise the core of an essential cell polarity network that underlies polarization in a wide variety of cell types and developmental contexts. The output of this network in nearly every case is the establishment of opposing and complementary membrane domains that define a cell's polarity axis. Yet, behind this simple pattern is a complex system of interactions, regulation and dynamic behaviors. How these various parts combine to generate polarized patterns of protein localization in cells is only beginning to become clear. This review, part of the Special Issue on Cell Polarity, aims to highlight several emerging themes and design principles that underlie the process of cell polarization by components of the PAR network.

Contents

Introduction

The '*par*-titioning-defective' (PAR) proteins were discovered in pioneering genetic screens performed in *Caenorhabditis elegans* over 25 years ago. We now know that they comprise a conserved pathway that is essential for cell polarity throughout the animal kingdom. PAR proteins sit at major cellular crossroads, integrating upstream polarity cues and serving as spatial organizers for downstream pathways, including asymmetric transport, polarization of the cell cytoskeleton and trafficking pathways, and the

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segregation of cell fate determinants during asymmetric cell divisions [1–5].

Despite a large body of research documenting the molecules and molecular activities involved, a systems-level understanding of how a polarized pattern emerges from this molecular network remains beyond reach. This is in part due to complexity and variability of the PAR system, which complicates identification of core design principles. There are multiple sets of physical and regulatory interactions and several distinct 'PAR complexes.' Both the number and identity of molecular players can vary between cell type and organism, and in many cases there is functional redundancy. Given their central role in cellular organization, PAR proteins also interact with other cellular organizational systems including the cytoskeleton and endomembrane systems, which can potentially feed back onto the polarization process. Finally, while polarization can be viewed as a single cell phenomenon, in many contexts, the cell environment plays a key role, whether this be physical or chemical aspects of this environment, such as the basal lamina or contacts with neighboring cells [6,7].

Despite this complexity, it is important to note that in nearly all systems studied to date, it is the ability of PAR proteins to define discrete membrane domains that underlies the functional organization of the cell along the polarity axis. In addition, in most systems, this organization hinges on antagonistic interactions between various PAR components. Given these apparent universal features, a key goal going forward will be to distill core conserved features of the network that allow pattern formation, while at the same time trying to make sense of its inherent complexity. The past few years have seen significant progress on this front. Additional components continue to be identified. New details have emerged regarding interactions between PAR components. We have begun to define the mobility and dynamic behavior of PAR components in cells. And the regulatory mechanisms that modulate PAR activity are becoming more clear. Alongside these new results, there is increased interest in the application of theory and modeling to help identify emergent properties and design principles of these complex networks.

Given the limited scope of this review, I will not seek to comprehensively address detailed molecular mechanisms. Rather, given the context of this special issue on polarity, I will try to address a few emerging themes that simultaneously highlight core features of the PAR system as well as its complexity, chiefly focusing on feedback and cross-regulation, balancing PAR protein amounts and activity, PAR protein mobility and dynamics, as well as recent theoretical analysis that has attempted to bring these ideas together into a coherent, quantitative framework.

The PAR network

Core components of the PAR network include the kinases, PAR-1 and aPKC, the 14-3-3 protein PAR-5, which binds phosphorylated substrates, the PDZ-containing scaffold proteins PAR-3/Bazooka and PAR-6, the small Rho-family GTPase CDC-42, and Lethal Giant Larva (LGL). There are also a number of context-specific players. In the *C. elegans* embryo, the RING-finger containing protein, PAR-2 plays an essential role in anterior–posterior polarization and polarization of the germ lineage, where it acts together with PAR-1. In epithelia, one finds the additional involvement of the apical Crumbs-Pals1-PatJ complex and the basolateral proteins DLG-1 and Scribble, as well as a number of other PAR regulators that include Yurt and Slmb [8–10]. This list is by no means exhaustive and I encourage interested readers to consult a variety of excellent recent reviews [1–5].

At a coarse-grained level, we can consider these components to fall broadly into antagonistic groups that demarcate the cell membrane into polarity domains (Fig. 1A). In the *C. elegans* embryo, as well as the *Drosophila* oocyte and neuroblasts, PAR-3/PAR-6/aPKC co-segregate to one cell half and exclude PAR-1 and LGL (and PAR-2 in *C. elegans*). PAR-1, LGL, and PAR-2 co-segregate to the other cell half and reciprocally exclude PAR-3/PAR-6/aPKC. PAR-5 is localized throughout the cytoplasm and is thought to act throughout the cell to modulate the activity of diverse phosphorylated substrates, including PAR proteins.

Current data support a complex web of interactions between these various proteins (Fig. 1D). The core players PAR-3/PAR-6/aPKC co-purify. However, in most contexts they only partially co-localize, suggesting that the three proteins do not form a constitutive complex [11–13]. CDC-42 can bind PAR-6 and is required to maintain PAR-6/aPKC at the cell membrane [14,15]. PAR-1 and PAR-2 appear to interact [16], however, there is no evidence that either interacts with LGL. Instead, LGL co-purifies with its antagonists PAR-6 and aPKC [17,18].

In epithelia, the picture is complicated by additional players, including the apical Crumbs(Crb)–Stardust(Sdt)–Pals1 complex, which associates with PAR-6/aPKC, and Scribbled (Scr) and Discs Large (Dlg), which colocalize with LGL and PAR-1 at basolateral membranes (Fig. 1B). In these cells, PAR-3 localizes primarily to the junctions, defining an additional junctional region. Nonetheless, the overall theme is similar to non-epithelia: aPKC, as part of the Crumbs complex, excludes basolateral proteins from the apical domain, while LGL/Scr/Dlg exclude PAR-6/aPKC containing complexes, perhaps through preventing PAR-6/aPKC from associating with other apical components such as Crb and PAR-3 [2]. There appears to be an additional level of cross-talk between PAR-3- and Crb-containing complexes to ensure that PAR-3 and Crb localize to distinct regions in the apico-junctional domain [13,19].

Feedback

We have already seen that a central feature of the PAR polarity network is mutual exclusion between PAR-6/aPKC containing complexes on one hand, and variously PAR-1, PAR-2 and LGL on the other. Intuitively, this mutual exclusion helps explain their anti-symmetric localization in the cell. For example, in the *C. elegans* zygote, phosphorylation of PAR-1, PAR-2 and LGL by asymmetrically localized aPKC is thought to exclude them from the anterior membrane. In support of this model, loss of PAR-3, PAR-6, aPKC or CDC-42 results in enrichment of PAR-1, PAR-2, and LGL throughout the cell membrane. Conversely, loss of PAR-1 or PAR-2 leads to aberrant spreading of PAR-3, PAR-6, and aPKC, which are thought to be excluded through the PAR-1-dependent phosphorylation of PAR-3 (recently reviewed in [1,20]). PAR-5/14-3-3 has been directly implicated in moderating this antagonism. By binding these phosphorylated substrates, PAR-5 can displace or sequester them from membranes [21–23]. The activity of LGL remains poorly understood, but likely involves its ability to displace PAR-3 from the PAR-3/PAR-6/aPKC complexes and inhibit aPKC activity [17,24].

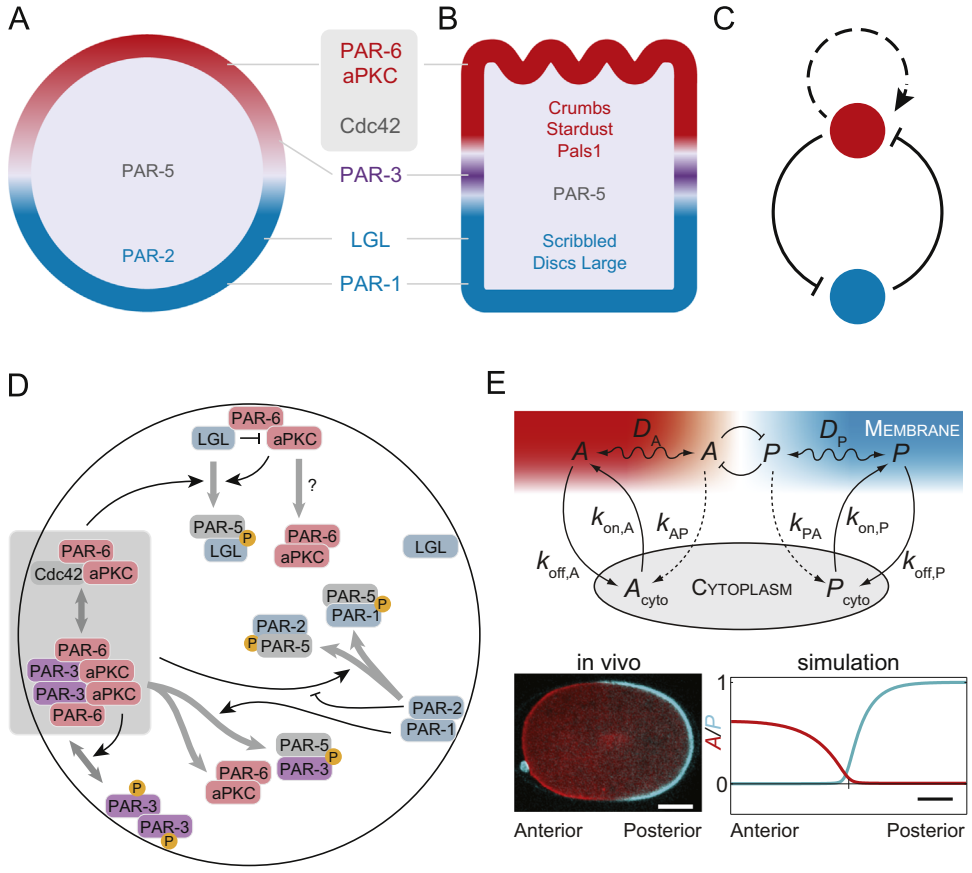

Fig. 1 – (A) PAR polarity in non-epithelial cells such as the *C. elegans* embryo. PAR-3, PAR-6 and aPKC localize coincidently to one hemisphere and are opposed by PAR-1, LGL and PAR-2 on the other. PAR-5 does not exhibit asymmetry but facilitates PAR cross-inhibition. (B) PAR polarity in epithelial cells comes with the addition of the Crumbs and Scribbled/Discs Large complexes, which define the apical and basolateral domains, respectively. Here, PAR-3 shows an apico-junctional localization distinct from the Crumbs complex. (C) A simplified coarse-grained PAR network that has formed the basis of several PAR models. It consists of a mutually antagonistic negative feedback loop between two opposing PAR species. In several models, this negative feedback loop is coupled to a positive feedback loop centered on one of the two species. (D) A detailed molecular PAR network based on empirical data showing a complex web of interactions. See text for details. Grey arrows indicate species conversion events, typically associated with changes in phosphorylation state. (E) A reaction–diffusion model for PAR polarity based on measured kinetic properties of PAR dynamics in *C. elegans* embryos and assumed cross-inhibitory interactions between anterior and posterior PAR components qualitatively captures the key behavior of the PAR network in vivo (modified from [44]).

In epithelia, PAR-6/aPKC, as part of the Crumbs complex, is similarly thought to phosphorylate PAR-1 and LGL to exclude them from the apical domain. PAR-1, in turn, phosphorylates PAR-3 and prevents its spread beyond its apical/junctional localization, while LGL is thought to inhibit formation and spread of Crumbs complex, again, presumably through its sequestration and inhibition of PAR-6/aPKC.

These cross-inhibitory interactions support a general notion of mutual antagonism between asymmetrically localized PAR proteins, which provides a useful conceptual framework for understanding the PAR network. This framework forms the basis of several mathematical models that qualitatively reproduce core features of the polarization process (Fig. 1C, E, see below). However, this simple reciprocal feedback model obscures complexity in both proposed molecular mechanisms and genetic interactions.

One complication, as noted above in the case of LGL, is that the molecular mechanisms underlying mutual exclusion remain unclear. Even in the case where phosphorylation is known to regulate membrane association, precisely how cross-phosphorylation leads to exclusion is only partially understood. In the simplest case, phosphorylation of a target, e.g. PAR-3 by PAR-1, induces its dissociation from the membrane. Phosphorylation site mutants in PAR-1, PAR-2, PAR-3 and LGL generally support this view—phosphomemetic mutants lose membrane association, while non-phosphorylatable mutants remain membrane enriched and extend beyond their normal domain boundaries [18,23,25–29]. However, recent single molecule analysis of PAR-6 in *C. elegans* embryos provides evidence that at least for this molecule it is not membrane dissociation but membrane association that is affected by the presence of PAR-1/PAR-2, a finding which must be reconciled with the phosphorylation-induced detachment models discussed above [3]. Clearly, additional quantitative analysis of the behaviors of PAR proteins in vivo will be necessary to clarify the mechanism of mutual exclusion.

There are also additional feedback pathways that operate in conjunction with mutual antagonism. As we shall discuss below,

aPKC can phosphorylate itself, PAR-3, and Crb, indicating a role for aPKC activity beyond simply antagonizing PAR-1, PAR-2 and LGL. In epithelia, there is additional cross-regulation between PAR-3 and Crb containing complexes, which is required to maintain distinct apical and junctional membrane domains, indicating that multiple levels of cross-inhibition operate in epithelial cells. Oligomerization is important for the function of both PAR-3 and Crb, and it has therefore been suggested that each could actively recruit more of itself to the cortex as part of a positive feedback loop (Fig. 1C) [31,32]. In the case of Crumbs complex, this model envisages a Crb–Cdc42–PAR-6–aPKC complex that recruits (via Crb) and stabilizes (via PKC) new Crb molecules [32]. Such positive feedback is presumably overlaid upon classical cross-inhibition between this Crumbs–Cdc42–PAR-6–aPKC complex at the apical domain and PAR-1/LGL in the basolateral domain [33].

Not only are known regulatory pathways complex, but there are also distinct differences in the polarity phenotypes of *par* mutants in different species or even tissues within the same species. Some of this is due to redundancy. The role of LGL in *C. elegans* was obscured by the fact that it is dispensable for polarization in otherwise wild-type embryos due to the presence of PAR-2. LGL has only recently has been shown to have maintained its ability to antagonize PAR-3/PAR-6/PKC in this system [18,26]. Redundancy is also found in many epithelial tissues, where loss of either PAR-3 or Crbs yields relatively normal polarity with only double mutants showing strong polarity phenotypes [34].

The requirements for a given PAR species can also be tissue-specific. For example, Crumbs complex function is specifically involved in polarizing epithelial cells. Here, Crb and PAR-3 appear to be mutually exclusive members of PAR-6/aPKC-containing complexes, with PAR-3 localizing primarily to the junctions and Crumbs defining the apical domain [2,3]. By contrast, no role for Crumbs complex has been seen in non-epithelial cells such as the one cell *C. elegans* embryo divisions or *Drosophila* neuroblasts [35,36]. In these cases, PAR-3 segregates co-incidentally with the PAR-6/PKC domain. This potential difference in PAR-3 function appears to be regulated, at least in part, by PKC-dependent phosphorylation of PAR-3. PAR-3 phosphomutants rescue PAR-3 function in two non-epithelial contexts, the *C. elegans* embryo and the *Drosophila* oocyte, but fail to rescue later developmental stages in the same organism [13,37]. Thus, developmentally regulated shifts in PAR complex composition appear to be critical for polarization in diverse contexts, further highlighting the complexity of regulatory cross-talk within this network.

Balance

A core principle of the PAR polarity pathway identified soon after the identification of the *par* genes is that of balance. In the *C. elegans* embryo, a temperature sensitive mutation in the posterior *par* gene, *par-2,* can be suppressed by reduction in the dosage of anterior associated PAR components, including PAR-3, PAR-6, aPKC, and CDC-42 [38–41]. In *Drosophila* epithelia, *crb* loss of function alleles can be suppressed by mutations in *lgl*/*dlg*/*scr* in a dose dependent fashion [35]. Conversely, *lgl* mutants can be suppressed by *apkc* mutants in neuroblasts [42]. The importance of balance makes sense in a system that relies on competition between the two opposing species—if one species is reduced, the opposing species gains advantage. Intriguingly, the response of polarizing systems to changes in dosage can be variable.

One impact of changes in PAR dosage is alteration of the threshold for polarization, a clear example of which can be seen in the one cell *C. elegans* embryo. In wild-type embryos, actomyosin flows trigger polarization [43,44]. However, when flows are compromised, a microtubule-dependent rescue pathway is revealed which can induce polarization by stabilizing a population of PAR-2 at the posterior [16,25]. While the actomyosin flow-dependent cue readily tolerates introduction of a GFP::PAR-6 transgene, the same GFP::PAR-6 transgene inhibits polarization in the absence of flows [45,46]. Importantly, further introduction of an extra copy of PAR-2 can restore polarization in embryos lacking flows, suggesting that the balance of PAR activity in the cell is tuning the threshold required for polarization and that the two cues are differentially sensitive to changes in this threshold.

Another effect of changing PAR dosage is seen in PAR domain size. In several systems, alteration of PAR protein dosage leads to corresponding changes in the relative size of PAR domains. In the *C. elegans* embryo, PAR-2 domain size correlates with PAR-2 dosage and inversely with PAR-6 dosage [44]. Similarly, in some *Drosophila* epithelial tissues, the ratio of PAR-1 to PAR-3 correlates with the relative size of the apical and basolateral domains [47]. Overexpression of Crb or PAR-6/PKC also can induce expansion of apical domains [48]. In other contexts, this relationship is less clear. Moderate overexpression of PAR-1 or PAR-3 in neuroblasts leads to partial inversion or rotation of the polarity domains, rather than domain loss or size changes [49]. Why some systems and not others exhibit this dependence of domain size on relative PAR dosage is unclear. Nonetheless, this does suggest that PAR systems have inherent abilities to regulate and control domain size and that one way to do this is through dosage.

Given the sensitivity of PAR polarity to changes in PAR dosage, an outstanding question is how the levels and activities of PAR proteins themselves are tuned. Indeed there is a variety of evidence supporting the existence of dosage regulation mechanisms. Screens in *C. elegans* have uncovered a number of pathways that may play a role in regulating total amounts of PAR-6 and aPKC [50,51]. In *Drosophila* epithelia, there is evidence that aPKC activity is titrated by LGL, which binds to and sequesters PAR-6/aPKC away from PAR-3 [52]. This 'buffering' of aPKC activity has been suggested to play a role in keeping aPKC levels low until mitotic entry. The aPKC substrate Yurt may be playing a similar role in regulating dosage of aPKC activity by binding to and inhibiting aPKC [8]. In both cases, one can imagine that rather than specifically acting to displace PAR-6/aPKC from the basolateral membranes, Yurt and LGL may tune levels of active aPKC complexes relative to values required for polarization. Finally, there are numerous connections between membrane trafficking pathways and PAR polarity [53,54], though whether trafficking plays an essential role in promoting cell polarity remains controversial and may be cell type specific [55]. One role for trafficking may be to regulate the availability of active PAR species at the plasma membrane [56], which would potentially allow for tuning of PAR activity. Whether cells directly sense PAR levels and, if so, how, remain unanswered questions.

Mobility and transport

The output of PAR feedback pathways is the formation of two, complementary membrane domains. PAR proteins must therefore

be enriched within their specified region of the membrane, while simultaneously being depleted from areas beyond this region. Understanding such spatial segregation requires addressing the intrinsic dynamic mobility of PAR proteins and how this mobility can be constrained and regulated in space to yield stable membrane domains with distinct protein concentrations.

Several PAR components, including PAR-2, PAR-6, and CDC-42 are very mobile, exchanging between membrane-associated and cytoplasmic pools and diffusing readily in both the cytoplasm (3D) and in the plane of the membrane (2D) [30,57–60]. Thus, PAR proteins do not appear to be anchored to specific sites in the cell. In fact, in the *C. elegans* zygote, both PAR-2 and PAR-6 appear free to diffuse across the boundary between domains [58], suggesting there are no barriers to diffusion as, for example, are seen at the yeast bud neck and which support stable polarization in that system [61]. This high mobility of PAR proteins and the lack of physical membrane compartmentalization suggest that transient induction of asymmetry is not sufficient for polarity. Rather, asymmetry must be actively maintained and enforced to counter diffusive mobility of PAR proteins in the cell.

The dynamic behavior of PAR proteins, coupled to extensive cross-regulatory feedback, and the lack of clear physical organizing structures have led to models in which polarity emerges from self-organizing properties of the PAR network [16,31,44,58,62]. In these models, PAR proteins, through feedback mechanisms discussed above, locally modulate the mobility of opposing PAR species by regulating transitions between slowly diffusing, membrane-associated 'active' states and more rapidly diffusing 'inactive' states, ultimately causing active species to be segregated within particular domains of the plasma membrane. These models will be discussed in further detail below.

The dynamic mobility of polarity proteins also has implications in the context of cellular transport processes. In yeast, transport of Cdc42 associated with secretory vesicles along actin cables plays a critical role in polarity during budding [63]. Symmetry-breaking in the *C. elegans* embryo also relies on transport using actin structures; however, rather than being transported along actin cables, PAR proteins are carried passively by flows induced by translocation of a cortical actomyosin network along the anterior–posterior axis [43,44,64]. There is also microtubule-dependent transport of PAR proteins—specifically PAR-3—in both developing epithelia and neurons [65,66]. Finally, PAR proteins can feedback onto their own transport or the transport of other PAR network components to modulate polarity establishment, for example by regulating the actomyosin network or the localization of microtubule nucleation [43,67,68]. Such active transport will tend to be opposed by diffusive processes. Therefore, it is increasingly essential to undertake a quantitative consideration of mobility of PAR proteins on the membrane and in the cytoplasm, their membrane dissociation rates, and potential transport speeds to determine whether mechanical transport mechanisms are sufficient to account for the distributions observed [69].

Reaction–diffusion models for cell polarity

Recent years have seen several groups construct quantitative models to describe PAR polarization in an effort to account for polarization by this dynamic and interconnected PAR network. To date, these models have taken the form of reaction–diffusion systems that integrate the mobility of PAR proteins with known or postulated feedback circuits (e.g. Fig. 1E).

Perhaps the most well known generic reaction–diffusion systems are so-called Turing models, which are well recognized for their ability to drive pattern formation in a variety of contexts. These systems are capable of reproducing a array of patterns, including spots, stripes and traveling waves, depending on the precise implementation [70]. Early on, it was speculated that such systems could also account for cell polarity, provided certain conditions were satisfied [71]. A key feature of Turing and similar reaction–diffusion models is a spatial separation of short and long range feedback [72].

Local or short-range self-amplifying feedback drives the formation of a domain, typically characterized by local auto-catalytic enrichment of one species, the so-called activator (Fig. 2A). Such local feedback may involve positive feedback, reciprocal negative feedback between two species (i.e. mutual exclusion), or combinations of the two [73]. Importantly, the mobility of this locally enriched species must be limited so that it does not simply disperse throughout the system (Fig. 2A) In many polarity models, this mobility shift is associated with membrane binding, resulting in membrane associated domains enriched in reduced mobility active species.

Equally important is long-range feedback, classically conceived as a fast-diffusing inhibitor that is induced coordinately with the local self-amplifying process. The increased mobility of the inhibitor relative to the activator species is critical as it allows the inhibitor to spread beyond the zone of activation and both inhibit expansion of the activation domain and suppress the formation of additional activation domains. If this inhibitory process is sufficiently long-range (e.g. if diffusion of the inhibitor is sufficiently fast), it will suppress the formation of additional domains throughout the system, ensuring that one, and only one, domain is formed (Fig. 2A).

In the case of PAR polarity, various mechanisms could provide for locally self-amplifying feedback that would be consistent with this general class of models, and a number of specific models have been proposed. All rely on mutual antagonism between opposing PAR species, but typically also incorporate cooperativity, oligomer-/dimerization, and/or positive feedback [31,32,44,62]. This feedback permits so-called mutual exclusion: if one PAR species has a local advantage over the other, it will tend drive membrane dissociation of the second, opposing species. Now relatively depleted, the second species will be less able to inhibit the first species, allowing the latter to accumulate to even higher concentrations. Consequently, the membrane will locally tend to be in either a PAR-6/aPKC enriched, PAR-1/PAR-2/LGL depleted state or the reverse PAR-1/PAR-2/LGL enriched, PAR-6/aPKC depleted state (Fig. 2C).

No long-range molecular inhibitors have been identified for PAR polarity. Instead, recent evidence suggests that the role of a long-range inhibitor may be provided by limiting pools of PAR protein (Fig. 1E and Fig. 2C) [44,74]. Rather than of coupling activator and inhibitor production, if membrane activation is associated with depletion of a pool of rapidly diffusing substrate, this depletion effect can globally suppress activator production throughout a system, limiting both the size of the activated region and the formation of secondary domains (Fig. 2B) [71]. In the *C. elegans* embryo, three features of PAR polarity support this model. First, the pool of PAR protein is provided maternally. Thus, there is a fixed pool of PAR protein that can be converted into an active, membrane-associated state. Second, formation of the PAR-1/PAR-2 membrane domain is accompanied by cytoplasmic depletion of

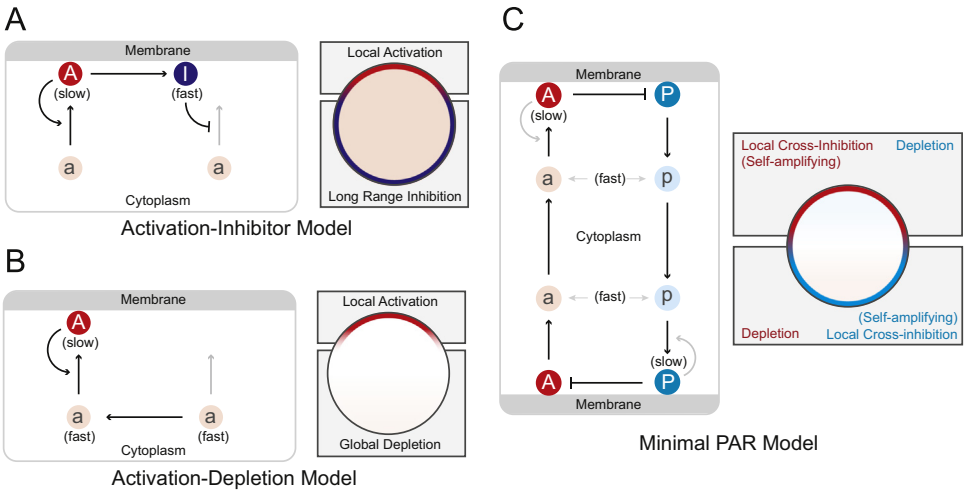

Fig. 2 – (A) An activator–inhibitor model relies on autocatalytic production of a slow-diffusing activator, *A*, and a fast-diffusing inhibitor, *I*. Here *A* is produced from a cytoplasmic substrate, *a*, and accumulates locally at the cell membrane in one region of the cell, while *I* dominates everywhere else. (B) An activator-depletion model resembles the system in Fig. 2A only no inhibitor is produced. Instead, production of *A* results in depletion of substrate, *a*, as *a* diffuses towards the activation domain from elsewhere in the cell. This global depletion of *a* limits the amount of *A* that can accumulate, thereby limiting the size of the activation domain and preventing secondary sites of activation. (C) A minimal PAR model based on self-amplifying cross-inhibition and limiting pools of available PAR proteins. On one side of the cell, *A* dominates, inhibiting *P* and allowing its own accumulation. The situation is inverted on the other side of the cell. The accumulation of membrane associated *A* and *P* results in a depletion of the corresponding pools of free cytoplasmic PAR species, *a* and *p*, respectively. This depletion limits the expansion of the two PAR domains such that neither can dominate the other. Positive feedback in which *A* and *P* stimulate their own production from cytoplasmic species *a* and *p*, respectively (grey arrows), can result in more robust polarization.

PAR-2. And third, changes in dosage of PAR-2 or PAR-6 cause a shift in the relative fraction of the membrane occupied by PAR-1/PAR-2 vs. PAR-6/PKC [44,74]. Whether this limiting pool/depletion model underlies regulation of PAR domain size in other systems remains to be determined, but the general sensitivity of PAR polarity and PAR domain size to changes in the balance between PAR components described above points in this direction.

As mentioned earlier, the mobilities of PAR species and the dynamic changes in these mobilities brought about by interactions between PAR species is integral to this class of models. Notably, all of the above models rely on the fact that cross-phosphorylation drives switching between alternate states of PAR species: a rapidly diffusing inactive state and a slowly diffusing active state. The slow diffusion of the active species allows them to be relatively concentrated or depleted from different regions of the membrane, while the rapid diffusion of the cytoplasmic species means that the effect of local conversion into the membrane state results in global depletion of the cytoplasmic pool. To date, such analysis of protein mobility has only been performed on PAR-2 and PAR-6 [57,58] as well as CDC-42, though CDC-42 data comes primarily from yeast where the PAR network is absent [59,60]. A key goal must be to analyze the behavior of additional components to examine whether their localization is consistent with this general framework of whether alternative frameworks will be necessary.

Outlook

The past 25 years since the discovery of the *par* genes have seen incredible progress in our understanding of PAR polarization. Out of this work, a few key concepts have been identified, which, in combination with new insights into the dynamic behaviors of PAR proteins, has enabled formulation of the first generation of quantitative models for PAR polarity. These models begin to explain how cell polarity can emerge from this collection of molecules, activities and interactions, some of which I have attempted to describe here. However, it would be naïve to ignore the chasm that persists between these coarse-grained models and our molecular understanding of PAR-related protein function. Bridging this gap will require us both to critically assess models in light of empirical data, challenging the predictions and assumptions of these models, and, importantly, obtaining quantitative data with which to parameterize them. This includes detailed study of the mobility and interactions of PAR pathway components. At the same time, models allow us to quantitatively challenge the feasibility of proposed molecular and genetic models and to understand the effects of a given activity in the context of a highly complex network.

One key outstanding question is why the PAR network is so complex, given that relatively simple models can capture the essential features of the polarization process. One reason may be our current inability to distinguish between core elements of the PAR network from regulatory or modulatory elements that control when and how the core PAR network functions. Another is likely that in different cell types, the cues, timescales, downstream pathways and even the organization of PAR domains themselves vary substantially, suggesting adaptability of this network has been key to its ability to operate in diverse contexts. Finally, given the extreme importance of PAR polarity to animal development and cell and tissue function, the PAR network must be robust. Theoretical studies indicate that combining multiple feedback loops, each of which can act as a minimal polarizing

system, results in more robust polarization responses than what is achieved by the minimal systems alone [73]. Understanding how these various feedback pathways are combined to achieve highly reproducible and reliable polarization of cells will be critical going forward.

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