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# The PAR Proteins: Fundamental Players in Animal Cell Polarization

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## Abstract

The par genes were discovered in genetic screens for regulators of cytoplasmic partitioning in the early embryo of *C. elegans*, and encode six different proteins required for asymmetric cell division by the worm zygote. Some of the PAR proteins are localized asymmetrically and form physical complexes with one another. Strikingly, the PAR proteins have been found to regulate cell polarization in many different contexts in diverse animals, suggesting they form part of an ancient and fundamental mechanism for cell polarization. Although the picture of how the PAR proteins function remains incomplete, cell biology and biochemistry are beginning to explain how PAR proteins polarize cells.

## 25 Years of par Genes

In February 1983, the first par mutant was spotted by Ken Kemphues and Jim Priess in a *C. elegans* mutant screen. Kemphues was a postdoc, and Priess was a graduate student at the University of Colorado at Boulder, in David Hirsh's lab. The few years before 1983 had seen two key advances in developmental biology that had prompted such screens: Nüsslein-Volhard and Wieschaus had shown that large numbers of key developmental genes could be identified by mutant screening in *Drosophila* (Nusslein-Volhard and Wieschaus, 1980), and evidence had accumulated that cell divisions in early animal embryos served to carefully partition developmental determinants (Whittaker, 1980 and Strome and Wood, 1982). Kemphues and Priess were keen to find the genes that controlled early development of the *C. elegans* embryo. They sought mutants that disrupted the earliest-acting gene products, those contributed by the mother during egg construction.

Kemphues and Priess considered a number of schemes to screen for maternal embryonic lethal mutants. After attending a lecture about ongoing mutant screens in yeast, Priess estimated based on the yeast numbers how many worms they would need to examine to find the relevant mutants. The number was disappointingly vast, and Priess spent a weekend regretting his decision to study worms for his Ph.D. He also thought about ways that the *C. elegans* screen might be streamlined, and came up with an inventive trick that still forms the basis for such screens in *C. elegans*. The trick took advantage of mutants that failed in egg-laying (the Egl phenotype), which had been found by others (Horvitz et al., 1983). In these mutants, embryos that were not released instead hatched inside their mothers, and devoured their mothers as they fed, resulting eventually in bags of young worms filling their mothers' cuticles. Priess reasoned that if Egl worms were mutagenized, and a penetrant maternal embryonic lethal mutation

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appeared, the mother would be spared—and that it would be easy to spot a plate that included such worms by the presence of crawling mothers on the plate. Any recessive lethal mutations that were found could be maintained by picking viable heterozygous siblings of these worms, and outcrossing these mutants could be facilitated by including in the mutagenized strain a mutation that results in a high incidence of males (the Him phenotype).

Priess tested his idea by crossing an existing conditional embryonic lethal mutation into an Egl Him strain, and saw that a plate with embryonic lethals could indeed be spotted easily in this background. He gave Kemphues the strain, and in the first screen, Kemphues and Nurit Wolf, a research technician, found six embryonic lethals. Among these was one strain with an exciting phenotype: the embryos had abnormally equal and synchronous cell divisions, suggesting that partitioning of important cytoplasmic components had failed during early divisions (Figure 1). This phenotype had been seen just once before, in a *C. elegans* mutant that Kemphues had been studying, zyg-11. The new gene was eventually named *par-1*, after its partitioning phenotype. The rest of the par genes fell out of similar screens, in some cases after streamlining the screening method even more, by searching for maternal embryonic lethal mutants that failed to form endoderm, an easily scored phenotype seen in some of the first par mutants (Kemphues et al., 1988 and Kemphues, 2000). In total, six par genes were identified in these screens, and multiple alleles of each par gene have since been isolated. Several other genes share the par genes' partitioning phenotypes (Tabuse et al., 1998,Schubert et al., 2000,Gotta et al., 2001 and Tagawa et al., 2001). For brevity, we will discuss these only where direct biochemical interactions with the proteins encoded by the six par genes have been found.

The initial mutant analyses revealed some important functions of par genes in *C. elegans*. Most of these genes were required for two related aspects of cell polarization—the asymmetric positioning of the mitotic spindle that results in unequal cell division, and the asymmetric positioning of a set of proteins and RNAs that are important for cell fate distinctions between specific cells. Because most of the par genes acted upstream of both of these aspects of cell polarization, it was likely that they encoded machinery at the heart of cell polarization in the one-cell stage *C. elegans* embryo. Indeed, a separate set of machinery has since been found to act downstream of the pars, functioning only in mitotic spindle positioning (Colombo et al., 2003, Gotta et al., 2003 and Srinivasan et al., 2003). Later work determined that the PAR proteins polarize additional cells in *C. elegans*, including cells involved in gastrulation, epithelial cells, and migrating cells (Nance, 2005 and Welchman et al., 2007).

## The Molecular Identities of the PAR Proteins

Were the par genes a *C. elegans*-specific phenomenon, or part of a more broadly used mechanism for animal cell polarization? Cloning the par genes was the first step toward answering this question. Kemphues and coworkers reported the cloning of all six par genes between 1994 and 2002 (Levitan et al., 1994, Etemad-Moghadam et al., 1995, Guo and Kemphues, 1995, Hung and Kemphues, 1999, Watts et al., 2000 and Morton et al., 2002). The sequences immediately suggested that these genes encode components of a novel intracellular signaling pathway. PAR-1 and PAR-4 encode serine threonine kinases. PAR-5 is a member of the 14-3-3 family of proteins, which are recruited to phosphorylated serines and threonines. PAR-3 and PAR-6 have PDZ domains, suggesting that they could act as part of a signaling scaffold, and PAR-2 has a RING finger domain that may act in the ubiquitination pathway (Moore and Boyd, 2004).

This set of protein identities did not immediately suggest links to cellular machinery, such as molecular motors or microfilaments or microtubules, that could drive asymmetry. The only possible exception to this was PAR-1, which has a quite distant relative in yeast that functions in cell polarization (Tassan and Le Goff, 2004). PAR-1 also has sequence identity with

mammalian microtubule affinity-regulating kinases (MARKs), which phosphorylate microtubule-associated proteins. However, there is as yet no clear evidence that PAR-1 functions as a MARK in *C. elegans* cell polarization (Labbe et al., 2003). The PAR proteins appeared, for the most part, to comprise a new signaling pathway. Further study of where the PAR proteins localize, along with their cell biological roles and biochemical interactors, would be important for understanding how they function.

## Asymmetric Localization of PAR Proteins

All of the PAR proteins are enriched to some degree at or near the cell cortex, and most of the proteins adopt asymmetric localization patterns as cell polarization develops (Kemphues, 2000). PAR-3 and PAR-6 become enriched in the anterior cortex during the one-cell stage, and PAR-1 and PAR-2 become enriched in the posterior cortex. PAR-4 and PAR-5 remain symmetrically localized through this period, and are both cortical and cytoplasmic (Figure 2).

Since par mutants are defective in cell polarization, and some PAR proteins become asymmetrically localized themselves, it appeared possible that PAR proteins drive their own asymmetric localization by regulating motors or by establishing the conditions necessary in the cell cortex for asymmetric movements of cellular components. Examining protein localization in par mutants showed that some PAR proteins do indeed function in localizing others, and these and later studies ordered a *C. elegans* PAR pathway (Figure 2). The anterior PAR proteins are required to prevent posterior PAR proteins from localizing anteriorly, and vice versa (Kemphues, 2000). PAR-5 is required for the mutual exclusion of anterior and posterior PAR domains (Morton et al., 2002 and Cuenca et al., 2003). In the posterior, PAR-1 membrane association is dependent in part on PAR-2 (Boyd et al., 1996). The next big questions were, “How are these PAR domains established, how is mutual exclusion of the PAR domains mediated, and do other organisms use PAR proteins similarly?”

## Fundamental Players in Animal Cell Polarization

Until 1998, roles for PAR proteins in cell polarization were known almost exclusively in *C. elegans*. In 1998, the fly cell polarization gene bazooka was cloned and was found to encode a protein closely resembling PAR-3 (Kuchinke et al., 1998). A mammalian PAR-3 homolog that can bind an atypical protein kinase C (aPKC) was found to adopt an apico-basal asymmetrical localization in mammalian epithelial cells (Izumi et al., 1998), and this led to the identification of *C. elegans* aPKC as a new protein with a Par loss-of-function phenotype and an asymmetric localization in the anterior cortex, like PAR-3 and PAR-6 (Tabuse et al., 1998). Soon after, mammalian PAR-3, PAR-6, and aPKC were found to exist in a complex with the small GTPase CDC42 (Joberty et al., 2000 and Lin et al., 2000), an ancient polarity protein that was first identified in budding yeast (Johnson, 1999). In *C. elegans*, CDC-42 binds PAR-6 and plays a role in associating the complex with the cell cortex (Cowan and Hyman, 2007). *Xenopus* oocytes and ascidian embryonic cells also have members of this complex localized asymmetrically (Nakaya et al., 2000 and Patalano et al., 2006), suggesting that this complex has a fundamental and ancient role in animal cell polarization. The other PAR proteins also turned out to be highly conserved, and to function in cell polarization in diverse contexts (Figure 3 and below).

## How Do PAR Proteins Polarize Cells, and How Are the PAR Proteins Polarized?

“Perhaps the most significant gap in our understanding has been information linking the PAR proteins to downstream localization events. That is, how do the PAR proteins mediate asymmetric distribution of other molecules?”

—Kemphues, 2000

This gap has not yet been closed, although recent cell biological and biochemical work has begun to narrow it. Studies of mechanisms in diverse systems have revealed a core signaling pathway that intersects with numerous other pathways in diverse ways, indicating that the PAR proteins polarize cells by a number of different mechanisms. The converse question, of how the PAR proteins themselves are segregated to different locations within the cell, has to date remained unanswered in most of the systems where it has been studied. Here, we review some of the cases in which mechanisms of PAR-dependent cell polarization have been well studied.

## *C. elegans* One-Cell Stage

The *C. elegans* one-cell stage (Figure 2) becomes polarized by a contraction of the actomyosin cortex to one side of the cell (Munro, 2006). The fertilizing sperm delivers two putative cues for this asymmetric contraction: the sperm-derived centrosomes, and a RhoGAP called CYK-4. Rho affects myosin organization and activates myosin-based contractility in this system as in other systems (Motegi and Sugimoto, 2006 and Schonegg and Hyman, 2006). The sperm-supplied CYK-4 RhoGAP appears to locally inactivate Rho, which results in an inhibition of myosin, breaking the contractile actomyosin meshwork on one side of the cell, and allowing the cortex to contract away from the site of sperm entry (Jenkins et al., 2006 and Motegi and Sugimoto, 2006). How the sperm-derived centrosomes contribute to this is not yet clear. Centrosome-nucleated microtubules can induce movements of a microfilament-rich cortex in a number of systems (Rodriguez et al., 2003), but treatments that eliminate all but a small density of centrosome-associated microtubules fail to prevent cell polarization in this system, raising the possibility that centrosomal components signal more directly to the cortex (Cowan and Hyman, 2004).

The contraction of the cortex to one side appears to move the cortically associated PAR-3/PAR-6/aPKC complex toward the anterior, although the finding that PAR-6 and PAR-2 associate dynamically with the moving cortex suggests that PAR proteins may exchange rapidly on and off of moving cortical scaffolds, rather than be moved themselves (Cheeks et al., 2004 and Munro et al., 2004). Little is known about how the anterior complex associates with the cell cortex, except that this association depends in part on interactions between members of the complex (Cowan and Hyman, 2007). Once anterior cortical PAR proteins become localized on one side of the cell, posterior PAR proteins can then associate with the posterior cortex, from which the anterior PAR proteins have cleared (Cuenca et al., 2003, Cheeks et al., 2004 and Munro et al., 2004). All of the PAR proteins except PAR-1 play roles in this movement of the cortex, but how they affect cortical movement is not known. Understanding how PAR proteins function in cortical motility will be key to understanding how PAR proteins drive cell polarization in this system.

Once two PAR domains are established, the PAR proteins have functions on each end of the embryo that serve to maintain polarity. A positive feedback loop involving two kinases, aPKC at the anterior and PAR-1 at the posterior, appears to maintain two distinct domains; each kinase inhibits specific proteins from associating locally. In the anterior, aPKC phosphorylation of PAR-2 prevents its cortical association (Hao et al., 2006). In the posterior, PAR-2 prevents reverse-directed cortical flow, and blocks anterior PARs from associating in part by allowing PAR-1 to accumulate at the posterior cortex, where this kinase may cause local disassembly of the anterior complex (Cuenca et al., 2003).

PAR proteins also have local functions that are important for cellular behaviors that differ between the anterior and posterior ends of the early embryo. PAR-1 locally affects the concentration of several critical regulators of development, although whether it does so by direct phosphorylation of these regulators is not yet known (Guo and Kemphues, 1995,

Schubert et al., 2000, Huang et al., 2002, DeRenzo et al., 2003, Cheeks et al., 2004 and Wu and Rose, 2007). PAR-2 and PAR-3 modulate cortical pulling forces on microtubules on either side of the embryo, during both spindle movement to the posterior and an ensuing phase of spindle rocking (Grill et al., 2003 and Labbe et al., 2004). How PAR-2 and PAR-3 mediate forces that position the spindle is not yet completely clear, although several important downstream players have been identified (Tsou et al., 2002, Colombo et al., 2003, Gotta et al., 2003, Srinivasan et al., 2003 and Du and Macara, 2004). These PAR proteins appear to work at least in part by determining the asymmetric localization of regulators of G protein signaling (GPR-1/2) in the posterior cortex and a DEP domain protein (LET-99) in a circumferential cortical band just posterior of the center of the embryo (Figure 2). GPR-1/2 may function as a critical, asymmetrically localized link between the cortex and microtubule motors, as it binds both a cortical G protein alpha subunit and LIN-5, a protein that associates with microtubules and dynein motors. This link might promote association of microtubules with cortical dynein motors in the posterior (Colombo et al., 2003, Gotta et al., 2003, Srinivasan et al., 2003, Du and Macara, 2004, Nguyen-Ngoc et al., 2007 and Couwenbergs et al., 2007). The PAR proteins may also work by locally affecting dynamics of microtubules: *C. elegans* PAR-3 acts, presumably indirectly, to stabilize microtubules that reach the anterior cortex (Labbe et al., 2003). Stable microtubules might play a role in movement of the spindle by locally occluding pulling forces, although this has yet to be tested.

## *Drosophila* Oocyte Development

Anteroposterior axis determination in *Drosophila* occurs during oogenesis, rather than after fertilization as in *C. elegans*, but, with the sole exception of the nematode-specific protein PAR-2, the same set of PAR proteins is involved (Tomancak et al., 2000 and Cox et al., 2001). Some of the PARs also play earlier roles in the maintenance of oocyte identify (Huynh et al., 2001). All of these functions appear to involve microtubules. For example, the anteroposterior axis is governed by a bias in the polarized orientation of microtubules, in which most minus ends at midoogenesis are directed toward the anterior, and the plus ends, toward the posterior. Cell fate determinants are carried to either end along the microtubules. *par-1* mutants disrupt this organization, which leads to the mislocalization of germline determinants such as oskar mRNA away from the posterior end of the oocyte (Shulman et al., 2000). Although PAR-1 affects microtubule stability in both the *Drosophila* oocyte and mammalian cells, the fly protein is unlikely to function biochemically in the same way as the mammalian MARKs. PAR-1 affects microtubule organization by stabilizing rather than destabilizing microtubules, for instance, and microtubule-associated proteins of the class that MARKs phosphorylate do not appear to play a role in oocyte polarity (Doerflinger et al., 2003).

Interestingly, PAR-1 is initially recruited to the posterior by microfilaments, prior to microtubule polarization, and is retained there at later times by the Oskar protein (Doerflinger et al., 2006). At the same time, PAR-1 phosphorylates and stabilizes Oskar, and is necessary for its posterior accumulation (Riechmann et al., 2002); Oskar can then retain PAR-1 and amplify the microtubule polarization in a classic positive feedback loop (Zimyanin et al., 2007). Unexpectedly, PAR-1 also regulates the anterior localization of Bicoid mRNA, through phosphorylation of a mediator called Exuperantia (Riechmann and Ephrussi, 2004). *C. elegans* PAR-1 might also be involved in controlling mRNA localization, because it locally stabilizes germline ribonucleoprotein complexes that appear to move to the posterior in a countercurrent flow driven by the movement of the cortex (Cheeks et al., 2004). PAR-1 in the *Drosophila* oocyte also phosphorylates PAR-3, excluding it from a posterior cortical domain (Benton and Johnston, 2003). These studies clearly demonstrate that PAR-1 function extends beyond the regulation of microtubule dynamics, and it will be of interest to identify additional substrates in worms and flies and in mammalian epithelial cells and neurons.

## Epithelial Polarization in *C. elegans*, *Drosophila*, and Mammals

The formation of epithelial sheets with apico-basal polarity is a fundamental process in development. Such sheets form the contact surface between the organism and its environment, and internal sheets can fold into villi, ducts, and cysts during organogenesis. Junctions between the epithelial cells provide adhesion and control the permeability of the sheets to ions and molecules. An important unifying principle in cell biology has emerged through the discovery that a conserved set of gene products, which includes the PAR proteins, controls epithelial polarization throughout the metazoa. In *C. elegans*, the anteroposterior segregation of PAR proteins changes by the end of the four-cell stage of embryogenesis, so that PAR-3 and PAR-6 become localized to the apical surface and are essential for the apico-basal asymmetries associated with gastrulation (Nance et al., 2003). As discussed above, the fly ortholog of PAR-3 functions in *Drosophila* epithelial cells also to drive apico-basal polarization (Kuchinke et al., 1998); in mammalian epithelia, PAR-3 is localized to tight junctions at the apical/lateral boundary (Izumi et al., 1998), and functions in their assembly (Chen and Macara, 2005). *Drosophila* epithelial cells possess septate junctions rather than tight junctions, and their position relative to the adherens junction is quite different; yet, PAR-3 is situated at the apical/lateral boundary in both cell types.

This molecular conservation extends to other polarity proteins that were identified from fly screens, and which play more contextually restricted roles in polarization. For example, the Crumbs protein (CRB) controls the extension of the apical surface or apical junction assembly (or both) in worms, flies, and vertebrates (Macara, 2004, Segbert et al., 2004 and Chalmers et al., 2005). CRB associates with a complex consisting of Stardust and Patj (PALS1 and PATJ in mammals), which is located at the apical/lateral boundary in both fly and mammalian epithelia and is essential for normal polarization (for review, see Macara, 2004). A second set of proteins, which includes Scribble (SCRB), Discs-large (DLG), and Lethal-giant-larvae (LGL), is associated with the lateral cortex of epithelial cells across the metazoa (Macara, 2004). Elegant experiments by the Tepass and Perrimon groups showed that these different sets interact genetically to define the apical and basolateral surfaces of epithelial cells in *Drosophila* (Bilder et al., 2003 and Tanentzapf and Tepass, 2003). The SCRB group suppresses apical membrane identity on the basolateral surface by inhibiting the function of the PAR-3 complex, while the PAR-3 complex recruits CRB, which antagonizes SCRB activity at the apical surface.

These genetic interrelationships reflect physical interactions between the three groups of polarity proteins that were first identified through their mammalian orthologs, and they relate to the signaling function of the PAR proteins. PAR-6 acts as a targeting subunit for aPKC, and it recruits both CRB (Hurd et al., 2003, Lemmers et al., 2004 and Sotillos et al., 2004) and LGL as substrates (Betschinger et al., 2003, Plant et al., 2003 and Yamanaka et al., 2003). The purpose of CRB phosphorylation is not yet known, although it is important for apico-basal polarization in *Drosophila* (Sotillos et al., 2004). However, an important consequence of LGL phosphorylation is that it triggers dissociation of LGL from the cell cortex. Because the PAR-6/aPKC complex is apical and LGL is normally basolateral, only LGL molecules that have strayed into the wrong territory become phosphorylated. Thus, PAR-6/aPKC maintains the integrity of the apical domain. A similar mechanism operates to keep the PAR-1 polarity protein off the apical surface. In this case, however, the mechanism is known in more detail. The phosphorylation of PAR-1 by aPKC permits binding of PAR-5, which both inhibits the PAR-1 kinase activity and blocks membrane binding (Hurov et al., 2004 and Suzuki et al., 2004). Conversely, PAR-1 present at the lateral cortex can phosphorylate any PAR-3 that diffuses down into its territory. The phosphorylated PAR-3 binds PAR-5 and is released from the cell cortex, thereby preventing the spread of PAR-3 into the lateral domain occupied by PAR-1 (Figure 4). Similarly, LGL can—in fly epithelial cells—exclude PAR-6 from the basolateral

cortex (Hutterer et al., 2004), possibly by binding to PAR-6 and displacing PAR-3 (Yamanaka et al., 2006). Thus, the initial segregation of the PAR proteins into separate domains is maintained (and possibly established) by a system of mutual exclusion, driven by phosphorylation, PAR-5 association, and competitive binding. This system nicely explains the anteroposterior segregation of the PAR proteins in the *C. elegans* zygote, and accounts for why PAR-5, which is not distributed in an asymmetric manner, is nonetheless necessary for polarization.

The model is incomplete, however, because it does not explain how the PAR-6/aPKC complex is regulated. What maintains it at the apical surface, and what prevents it from phosphorylating substrates throughout the cell? A key factor is the small GTPase, CDC42. Strikingly, aPKC activity is almost entirely suppressed by its association with PAR-6, but this suppression is partially relieved by CDC42, which binds to the Crib domain of PAR-6 (Yamanaka et al., 2001, Garrard et al., 2003 and Atwood et al., 2007). CDC42 also increases the association of PAR-6 with PALS1, which might increase CRB phosphorylation (Hurd et al., 2003). In addition, CDC42 plays a crucial role in localizing PAR-6/aPKC at the cell cortex. CDC42-GTP is essential to recruit PAR-6 to the apical cortex in both neuroblasts and epithelial cells of *Drosophila* (Hutterer et al., 2004 and Atwood et al., 2007). Keith Mostov's laboratory, which is working on MDCK mammalian epithelial cells, has also found that CDC42 is necessary for correct PAR-6/aPKC localization, and for normal apico-basal polarization of cells grown in 3D cultures (Martin-Belmonte et al., 2007).

These observations raise the inevitable question as to what recruits or activates CDC42. PAR-3 is upstream of CDC42 in the *Drosophila* neuroblast (Atwood et al., 2007) and acts upstream of PAR-6/aPKC in embryonic epithelial cells (Harris and Peifer, 2005), but the molecular linkage between these proteins remains unclear. For historical reasons, reviews often refer to a PAR-3/PAR-6/aPKC complex, with the implication that these three proteins are constitutively linked to one another. This complex can in fact be isolated from mammalian cells through its association with CDC42. Yet it is clear that they also can act independently, and can be both functionally and spatially separate. For example, in embryonic *Drosophila* epithelial cells, PAR-3 localizes with the adherens junctions, below PAR-6 and aPKC (Harris and Peifer, 2005), and acts upstream of these proteins during polarization. Moreover, PAR-3 positioning is dynein dependent, while positioning of PAR-6 and aPKC is independent of dynein. The dynein-dependent localization of PAR-3 suggests that PAR polarity is initially determined by cytoskeletal polarity (Figure 5). An analogous situation occurs in mammalian MDCK epithelial cells undergoing apico-basal polarization in 3D cultures. PAR-3 in these cells localizes predominantly to the tight junctions between adjacent cells, whereas aPKC and PAR-6 are also found on the apical surface and in the cytoplasm (Yamanaka et al., 2003 and Martin-Belmonte et al., 2007). We expect, therefore, that additional factors are engaged in signaling between PAR-3 and CDC42.

Clues from studies in both flies and mammalian cells suggest that these factors control phosphoinositide metabolism. *Drosophila* PAR-3 binds directly to the phosphoinositide phosphatase PTEN (von Stein et al., 2005), and in photoreceptor epithelial cells of *Drosophila*, the recruitment of PTEN to the lateral adherens junctions by PAR-3 leads to an enrichment of PIP3 on the apical surface, which is necessary for organization of the microvilli (Pinal et al., 2006). Independently, work on MDCK cells suggests that an enrichment of PIP2 at the apical membrane is responsible for the recruitment of CDC42 (Martin-Belmonte et al., 2007). In these cells PI-3 kinase, which generates PIP3, is associated with the adherens junctions on the lateral surface, while PTEN is situated on the apical surface where it can destroy PIP3 (Figure 5). Remarkably, the phosphoinositides can define cortical identity; the addition of exogenous PIP2 to the basolateral surface triggers the recruitment of apical proteins to that surface (Martin-Belmonte et al., 2007). Conversely, PIP3 can specify basolateral identity

(Gassama-Diagne et al., 2006 and Martin-Belmonte et al., 2007), because addition of exogenous PIP3 to the apical surface is sufficient to drive the transient localization of basolateral proteins to this surface and exclude apical proteins. Endogenous PIP3 is exclusively present on the basolateral membrane in MDCK cells. Perhaps a feedback system enables the PAR proteins to determine the balance of phosphoinositides in different cortical domains, which in turn regulates PAR protein localization. However, the molecular details that define these relationships in different organisms are probably distinct. For example, mammalian PAR-3 does not colocalize with PTEN, and has not been found to associate with this phosphatase; also, apical identify in *Drosophila* retinal epithelium is not defined by PIP2. Moreover, nothing is yet known about the GEFs and GAPs that control the level of CDC42-GTP (Figure 5). These factors might also be spatially segregated, and recruited or activated by phosphoinositides. It will be interesting to determine whether similar mechanisms control PAR localization and polarization in the *C. elegans* embryo.

Two other PAR proteins, PAR-1 and PAR-4, also participate in epithelial polarization. In mammalian cells PAR-1 determines the organization of microtubules, which in turn establish the position of the luminal surface (Cohen et al., 2004). High PAR-1 activity converts columnar epithelial cells with vertical microtubules and an apical luminal surface into a hepatic type of epithelial cell with horizontal microtubules and lumens that form between adjacent cells. PAR-1 is phosphorylated and activated by PAR-4. Mammalian PAR-4 is also known as LKB1, and has received intense scrutiny because of its linkage to Peutz-Jeghers cancer syndrome (PJS). This disease involves primarily the epithelial cells of the gastrointestinal tract, pancreas, lungs, and reproductive organs (Baas et al., 2004), although PAR-4 is expressed ubiquitously (Alessi et al., 2006). PJS patients frequently possess mutations that truncate the kinase domain or result in loss of expression. In mammals, PAR-4 functions as a master kinase that can phosphorylate and activate at least 14 downstream kinases, including PAR-1, and it has been implicated in metabolic control, cell growth, and mitosis, in addition to cell polarization and asymmetric cell division (Alessi et al., 2006). It seems likely that distinct downstream kinases regulate these different processes. High PAR-4/LKB1 activity can drive the polarization of isolated epithelial cells even in the absence of cell-cell contacts, but one of the key downstream kinases necessary for this process appears to be not PAR-1, but AMPK, a kinase previously thought of primarily as a metabolic regulator that responds to energy deprivation (Lee et al., 2007 and Mirouse et al., 2007). Remarkably, a constitutively active AMPK can rescue many of the polarity defects that occur in *Drosophila* lkb1 null mutants, apparently through phosphorylation of the myosin light chain, and loss of either AMPK or PAR-4/LKB1 results in a stress-dependent loss of epithelial cell polarity. Conversely, activation of AMPK by energy deprivation can drive epithelial cell polarization. These results reveal an unexpected link between energy metabolism and the polarity/proliferation pathways, and an important goal is now to identify the links between AMPK and the PAR polarity machinery.

## Neuronal Development in Flies and Mammals

Neurons derive from progenitor neuroepithelial cells that undergo asymmetric cell divisions. Daughter cells that are destined to become neurons produce extensions that appear initially isotropic, but begin to polarize such that one extension continues to grow and becomes an axon, while the others branch and become dendrites. A growth cone at the tip of the axon detects attractive and repulsive signals in the environment that guide the direction of growth. Axons and dendrites from neighboring neurons make synaptic contacts, enabling information to be transmitted from the axon to the dendrites. In mammals, the excitatory synapses usually form on dendritic spines, which are actin-rich structures like microscopic mushrooms, and which are essential for cognition. Neuronal development is, therefore, an attractive model in which to study a range of polarity problems, since it combines cell fate specification (as occurs in the *C. elegans* zygote) with polarization into axon and dendrites, reminiscent of apico-basal

polarization, and with synaptogenesis, which has parallels to intercellular junction assembly in epithelia.

The asymmetric cell division of the neuroblast has been studied most deeply in *Drosophila*. Indeed, this powerful model system has provided much of our current knowledge about the machinery of polarization and cell fate determination (for a recent review see Yu et al., 2006). Creation of distinct daughter cells requires that cell fate determinants be segregated to opposite ends of the mother cell and that the mitotic spindle be oriented such that the plane of cell division is orthogonal to the axis of polarization. This ensures that only one daughter cell will receive the fate determinants that drive differentiation. PAR-3 determines both of these processes: the basal localization of determinants Numb and Miranda, and the orientation of the mitotic spindle (Yu et al., 2006), while PAR-6/aPKC seem to be required only for the localization of determinants (Rolls et al., 2003). Spindle orientation involves a distinct signaling pathway in which PAR-3 localizes to a crescent at the apical surface of neuroblasts, during the delamination of the cell from the overlying epithelium, and recruits a protein called Inscuteable (Insc) (Schober et al., 1999, Schaefer et al., 2000, Yu et al., 2000 and Cai et al., 2003). Insc in turn recruits a protein called Pins, which associates both with heterotrimeric G protein αi subunits (Gαi) and with Mud, a microtubule-associated protein (Bowman et al., 2006, Izumi et al., 2006 and Siller et al., 2006) (Figure 6). A similar pathway is conserved in mammals and in *C. elegans.* In mammalian cells, PAR-3 binds a protein with weak homology to Insc, which in turn binds to LGN, the mammalian Pins ortholog. Pins can associate both with NuMA, a microtubule-binding protein, and with Gαi, which is constitutively present at the cell cortex. This association triggers Pins to switch from a closed to an open conformation, permitting more stable association with NuMA (Du et al., 2001, Du and Macara, 2004 and Lechler and Fuchs, 2005). As discussed above, GPR-1/2 performs similar functions to LGN/Pins in *C. elegans*. The resulting protein complex then alters the tension on, or cortical attachment of, astral microtubules through an unknown mechanism, such that the metaphase chromosomes are moved into the correct orientation for asymmetric cell division. In this case, therefore, PAR-3 appears to act as a spatially localized signaling platform.

A key decision in neuronal differentiation occurs when one neurite extension becomes an axon while all other extensions become dendrites. The molecular basis for this decision has been the subject of intensive study in mammalian cells, and several PAR proteins have been implicated in the decision. PAR-3 is translocated along microtubules to the growing end of axons by a plus end-directed kinesin, KIF3A, and its localization to the nascent axon tips requires both a plus end binding protein called APC, and a protein kinase, GSK3β (Nishimura et al., 2004 and Shi et al., 2004) (Figure 6). The molecular details of PAR-3 localization remain unclear, however. The phosphoinositides appear to be involved, though exactly how they operate remains unclear as well. Also, dominant-interfering fragments of PAR-3, or inhibition of aPKC, can block axon specification (Shi et al., 2003). However, the aPKC inhibitor used in this study is not specific, and the ectopic expression of PAR-3 mutants might act indirectly by mislocalizing other proteins, so the case for PAR-3 playing a critical, direct role in axon specification is not watertight. No knockdown or knockout studies have been reported that implicate PAR-3 in this process. Moreover, axon specification is independent of PAR-3 in *Drosophila* (Rolls and Doe, 2004).

Neuronal polarization also depends on the PAR-4 polarity protein, and is stimulated by the phosphorylation of PAR-4 via protein kinase A, which transduces neurite-outgrowth-promoting signals (Shelly et al., 2007). Interestingly, the same phosphorylation site on PAR-4 is required for cell cycle regulation and for polarity establishment in *Drosophila*, but its function remains mysterious (Martin and St Johnston, 2003), since it does not stimulate PAR-4 kinase activity. One possibility is that it targets the kinase to specific cellular locations or substrates. Two kinases downstream of PAR-4, called SAD-A and SAD-B, are expressed at

high levels in the brain, and mediate the effects of PAR-4 on neuronal polarization (Barnes et al., 2007).

As mentioned above, another kinase downstream of PAR-4 is PAR-1, which is important for the regulation of microtubule organization in mammals and flies. Indeed, the first mammalian PAR-1 orthologs, MARKs, were purified as kinases that phosphorylate microtubule-associated proteins such as tau, MAP2, and MAP4 (Drewes et al., 1997). These microtubule-associated proteins stabilize microtubules when bound, and the phosphorylations cause their dissociation from microtubules. The consequent change in microtubule dynamics has important ramifications, which differ depending on the cell type. Tau is the key microtubule-associated protein in axons, and PAR-1 can affect neurite outgrowth, axon specification, and axonal transport (Biernat et al., 2002). Hyperphosphorylation of tau is believed to play a role in Alzheimer's disease, and there is evidence that PAR-1 can initiate the hyperphosphorylation cascade. However, SAD-A and SAD-B also mediate the phosphorylation of microtubule-associated proteins in neurons, and the relative contributions of all these kinases to neuronal polarization and function remain to be delineated. It is clear from studies in *Drosophila* oocytes that PAR-1 has many other functions distinct from regulating microtubule stability, and it will be of interest to determine, for example, if it controls RNA localization or stability in neurons.

One known target for PAR-1 of particular interest is Dishevelled (DVL). This scaffold protein is a central component of the Wnt signaling pathways. Wnts act through one signaling pathway (the so-called canonical pathway) to regulate gene expression and cell fate determination, while multiple noncanonical forms of Wnt signaling can affect cell polarity. Remarkably, PAR-1 can phosphorylate DVL and modulate noncanonical WNT signaling, while a distinct isoform of PAR-1 regulates canonical WNT signaling through a separate but so far unknown mechanism (Sun et al., 2001 and Ossipova et al., 2005). In an important new twist to this story, Zhang and coworkers recently discovered that in hippocampal neurons, DVL binds to and activates aPKC (Zhang et al., 2007). Strikingly, this interaction appears to be direct, and is not mediated through PAR-6. The association with DVL also stabilizes aPKC, and is promoted by WNT5a, which increases the phosphorylation of PAR-1 and promotes axon differentiation. As discussed below, this interaction also appears to be important in cell migration. Thus, a number of PAR proteins impact WNT signaling, and it will be important to unravel how these inputs are integrated and/or insulated from one another.

Another function for PAR-3 in mammalian neurons is in dendritic spine morphogenesis (Figure 6). Spines grow out from the dendritic shafts as filopodia, which upon contact with an axon form synapses and swell into mature, mushroom-shaped spines. Silencing of PAR-3 expression in rat hippocampal neurons phenocopies the expression of an activated mutant of Rac, which prevents maturation, resulting in filopodial-like extensions from the dendrites that do not form functional synapses (Zhang and Macara, 2006). PAR-3 acts through TIAM1, a RacGEF, and the effects of silencing PAR-3 expression can be reversed either by reducing TIAM1 levels or by the expression of a dominant-negative Rac mutant. TIAM1 binds to the C-terminal region of PAR-3, and both proteins normally localize to the tips of the spines. Thus, in this case, PAR-3 functions to spatially restrict a signaling protein, which otherwise could activate Rac in the wrong region of the neuron. Overexpression of PAR-3 can produce a similar phenotype to loss of PAR-3, because the overexpressed protein does not localize correctly, which compromises the spatial restriction of TIAM1.

Interestingly, PAR-6 is also essential for dendritic spine morphogenesis, but it seems to operate independently of PAR-3 and perform a distinct function, because depletion of PAR-6 results in the loss of spines rather than in the formation of immature spines. Conversely, overexpression of PAR-6 increases spine density along the dendrites. WhilePAR-3 regulates Rac activity via TIAM1, PAR-6 appears to control spine formation via the p190 RhoGAP,

which regulates RhoA activity (H. Zhang and I.G. Macara, unpublished data). Whether this regulation involves phosphorylation of p190 by aPKC remains to be determined.

PAR-3 also performs other additional functions in brain development. For example, it has been implicated in the differentiation of the Schwann cells that form an insulating sheath around axons. PAR-3 localizes in Schwann cells to the axonal/glial boundary, where it binds to the p75 neurotrophin receptor (Chan et al., 2006). This interaction is essential for myelination, which is triggered by secretion of brain-derived neurotrophic factor (BDNF), an agonist for p75. PAR-3 in this case may function to either spatially restrict p75 activity or restrict other effectors of the receptor that are essential for turning on the myelination program.

The conclusion to be drawn from this overview of neuronal development is that the PAR proteins function in diverse contexts and intersect multiple signaling pathways to control cellular morphogenesis. Similar pathways seem also to be involved in the control of directional cell movements, as discussed below.

## Polarized Cell Migration

Cell migration is of critical importance during development and morphogenesis, in wound healing, and in cancer. Migration can be directed or random (at least in vitro), but in each case the cells must become polarized, generating a front and a back end. Both actin and microtubule cytoskeletons are key players in migration, but their roles vary from cell type to cell type. Nonetheless, given the multiple ways in which the PAR proteins impact cytoskeletal dynamics, one might expect that they would be intimately involved in polarizing cells during migration. Surprisingly, however, this has turned out so far to be true only for certain cell types. To date there is no evidence that specialized migrators—the slime mold, Dictyostelium, and mammalian neutrophils—use PAR signaling at all, even though they harness phosphoinositides to polarize cell movment (Affolter and Weijer, 2005). However, cells such as fibroblasts and astrocytes, which migrate much more slowly, do need the PAR proteins to orient themselves in a directional fashion.

Although CDC42 had been shown by Catherine Nobes and Alan Hall to be important in establishing polarized migration in fibroblasts during wound healing (Nobes and Hall, 1999), the mechanism by which it did so remained unknown until the discovery that CDC42 could bind the PAR-6 polarity protein (Joberty et al., 2000 and Lin et al., 2000). Hall's group then demonstrated that in astrocytes, PAR-6/aPKC mediates directionality of migration and orientation of the centrosome with respect to the nucleus and axis of movement (Etienne-Manneville and Hall, 2001). A similar requirement has been found in fibroblasts (Schlessinger et al., 2007). Microtubule polarization and dynein-mediated tension are important for this process. In astrocytes, the protein kinase GSK3β acts downstream of PAR-6/aPKC, and other polarity proteins including DLG and SCRB have also been implicated, although it remains uncertain how these various proteins are linked to one another (Etienne-Manneville et al., 2005 and Osmani et al., 2006). Initially, centrosome orientation was thought to require the phosphorylation of GSK3β by aPKC, but it is now clear that GSK3β phosphorylation is not directly involved, and that GSK3β is instead inhibited by DVL, acting through aPKC downstream of WNT5a (Schlessinger et al., 2007). Again, therefore, the PAR pathway intersects with WNT signaling.

Of note, WNT signaling is also necessary for cell movements during vertebrate gastrulation, and it is tempting to speculate that it might function in this process through PAR-6/aPKC. Indeed, studies of cell movement during gastrulation in *Xenopus* embryos have revealed a new function for aPKC in phosphorylating an ArfGAP (Hyodo-Miura et al., 2006). This protein, XGAP (ArfGAP1), is required for gastrulation, and shows codependent localization with aPKC/PAR-6 to regions that undergo protrusive activity. The phosphorylated XGAP binds

PAR-5, but whether this association alters its membrane association remains to be tested. Protrusive activity requires the coordinated activation of the Rho and Rac GTPases, and DVL has been known for some years to activate both of these GTPases through independent and parallel pathways, but the underlying molecular mechanisms remain obscure (Wallingford and Habas, 2005). However, given that PAR-6/aPKC can regulate Rho activity through p190 and that PAR-3 can regulate Rac activity via TIAM1 (Chen and Macara, 2005), an interesting speculation is that the PAR proteins couple DVL to these GTPases. The PAR-DVL axis might, therefore, enable the integration of microtubule dynamics (through GSK3β) and actin dynamics (through the Rho/Rac GTPases) during polarization in many contexts.

PAR proteins might also impact cell migration through two entirely separate pathways—Numb-mediated endocytosis of integrins, and Smurf1-mediated degradation of Rho. The latter pathway has been implicated in a loss of polarity by mammary epithelial cells that is specifically induced by TGFβ. Smurf1 is an E3 ligase that ubiquitinates a number of proteins in the TGFβ pathway. Interestingly, it can also ubiquitinate RhoA, a small GTPase that controls actin dynamics (Wang et al., 2003). Smurf1 preferentially recognizes inactive, GDP-bound RhoA. aPKC recruits Smurf1 to the cell cortex, where destruction of RhoA permits protrusive activity and cell motility (Wang et al., 2003 and Sahai et al., 2007). This pathway appears to be linked to morphological changes, including a loss of tight junctions. The TGFβ receptor, which is present at the junctions, can bind and phosphorylate PAR-6 on Ser 345, near its C terminus, which enhances the binding of Smurf1 (Ozdamar et al., 2005); Smurf1 then triggers destruction of RhoA at the tight junctions, resulting in their disassembly. How the phosphorylation of PAR-6 results in recruitment of Smurf1 remains unclear, since Smurf1 also binds directly to aPKC. An important question is how widespread this pathway is in controlling Rho function, because RhoA is present (and activated) at the leading edge of at least some migrating cells (Kurokawa et al., 2005 and Pertz et al., 2006). Moreover, as noted above, PAR-6/aPKC can also regulate RhoA-GTP levels via p190, and it will be of interest to see how these two distinct mechanisms are balanced in different contexts.

Numb is an adaptor protein required for the endocytosis of specific cargoes, including Notch, a conserved, transmembrane receptor that controls cell fate specification in many contexts throughout metazoan development. In *Drosophila*, PAR proteins maintain the asymmetric location of Numb during divisions of neuroblasts and sensory organ precursor cells, which ensures that Notch signaling is suppressed in one of the two daughter cells. Just as for Lgl and PAR-1, aPKC phosphorylates Numb, thereby excluding it from the region of the cell cortex occupied by the PAR-6/aPCK complex (Smith et al., 2007). Although not yet demonstrated, it seems likely that—as for the other aPKC substrates described above—release will require the association of phosphorylated Numb with PAR-5. Recently, however, Numb has also been identified as a key regulator of polarized integrin internalization in migrating cells (Nishimura and Kaibuchi, 2007). In this case, phosphorylation by aPKC triggers its release from integrin and clathrin-coated structures, thereby blocking endocytosis. Nishimura and Kaibuchi also showed that Numb associates weakly with PAR-3 and PAR-6, and that the knockdown of either aPKC or PAR-3 impairs Numb localization during migration (Nishimura and Kaibuchi, 2007). They suggest that PAR-3 recruits Numb for phosphorylation by aPKC, but it is unclear how this might occur, since PAR-3 binds aPKC through the kinase domain, and is a substrate for aPKC phosphorylation. Thus, it would be predicted to act as a competitive substrate rather than as a recruitment factor. Nonetheless, the weak interaction of Numb with PAR-6 suggests that PAR-6 is not needed as a targeting subunit in this situation.

This overview shows that PAR proteins constitute a signaling pathway that intersects with numerous other pathways to organize the cytoskeleton, membrane traffic, and other cellular components so as to polarize cells during oriented migration. It seems quite likely, however,

that we have only begun to grasp the complexity of PAR signaling, and that many other proteins interact with these polarity proteins in specific cellular contexts.

## The Evolutionary Origin of PAR Polarity Systems

The functions of PAR proteins in diverse animals suggest that there existed, in ancient animals, mechanisms for cell polarization that depended on every PAR protein except PAR-2 (which is to date a nematode-specific protein). How early did the PAR polarity systems evolve, and what were their original functions? Outside of the animals, there is little evidence for PAR polarity systems. Yeast has a kinase that distantly resembles PAR-1 and 14-3-3 proteins that resemble PAR-5, and these proteins have roles in cytoskeletal polarization, among other roles (Lottersberger et al., 2007), but yeast PAR-1-like and PAR-5-like proteins are not known to work together as they do in animal systems. Choanoflagellates, which are among the closest relatives of early multicellular animals (Lang et al., 1999), encode a protein that is strikingly PAR-1-like, and one that is PAR-5-like, but there is no evidence as yet suggesting that these proteins function in cell polarization in choanoflagellates. There is also no evidence as yet that PAR proteins function in cell polarization in any of the prebilateral animal phyla, such as the cnidarians or ctenophores.

Given what is known about PAR protein functions in animal systems, a picture emerges in which all of the PAR proteins except PAR-2 were likely fundamental players in cell polarization mechanisms more than 500 million years ago, in the ancestors of *C. elegans*, *Drosophila*, mammals, and all other bilateral animals. It is possible that some of these proteins, such as PAR-1 and PAR-5, might have had even earlier roles in cell polarization. Whether the early roles for these proteins were in cell migration, embryo polarization, neuronal polarization, epithelial polarization, or polarization of entire one-celled organisms is impossible to resolve based on current data.

The remarkable diversity of biochemical roles that the PAR proteins play in cell polarization is impressive, and one can imagine that an ancient system of precisely polar-localized kinase activities could have been exploited through evolutionary tinkering to produce this remarkable diversity. While study of PAR proteins in model organisms can further resolve the mechanisms by which PAR proteins function in cell polarization, study of PAR protein roles outside of the traditional model organisms may resolve the ancient roles of PAR proteins, as well as the paths by which evolution has elaborated new roles for PAR proteins in cell polarization.

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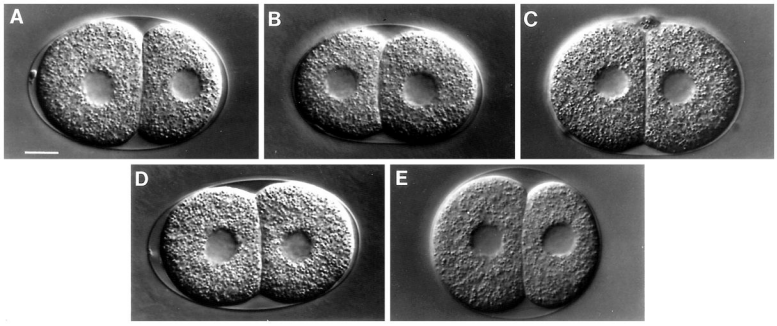


**Figure 1. Nomarski Micrographs of Two-Cell Embryos**

Nomarski micrographs of two-cell embryos from wild-type mothers (A) and mothers of genotypes *par-1* (B), *par-2* (C), *par-3* (D) and *par-4* (E). Taken from Kemphues et al. (1988).

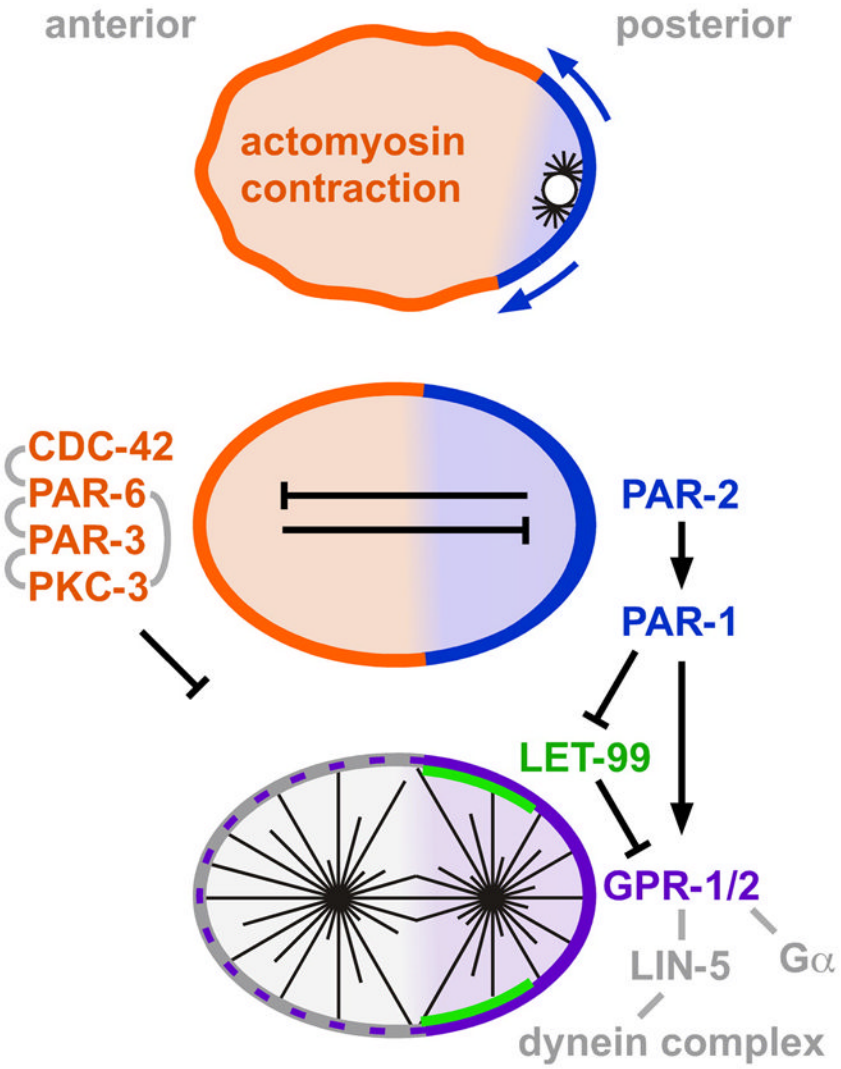


**Figure 2. Some Mechanisms That Localize PAR Proteins and Specific Downstream Proteins at the One-Cell Stage in *C. elegans***

(Top) Sperm-contributed nucleus and centrosome-nucleated microtubules are on the right side. Arrows indicate spread of cortex to which PAR-2 associates as actomyosin contraction proceeds. (Bottom) The mitotic spindle adopts an asymmetric position under control of the proteins shown. Black lines indicate genetic interactions; short gray lines indicate biochemical interactions. Proteins indicated in gray are not localized to just one side of the embryo.

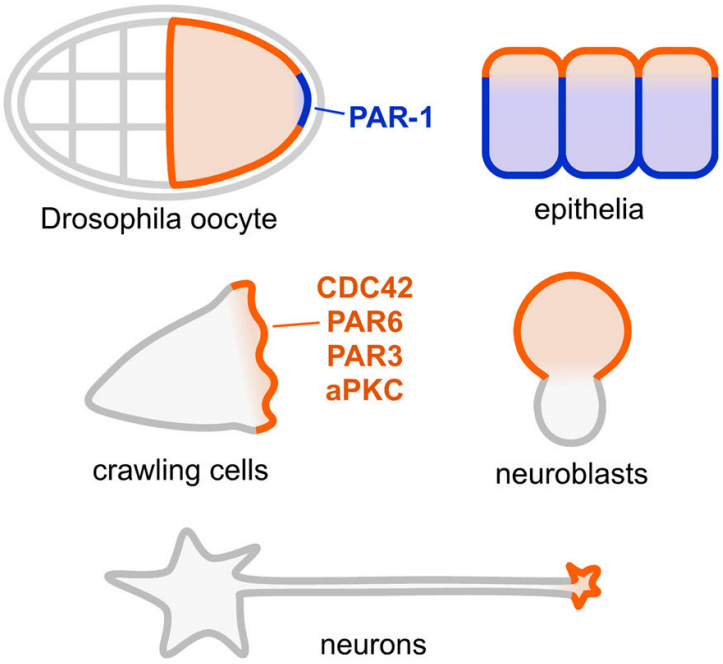


**Figure 3. PAR Localization in Multiple Systems**

PAR-3, PAR-6, aPKC, CDC42, or some or all of these in combination are enriched at the places indicated with orange lines, and PAR-1 is enriched at the places indicated with blue lines.

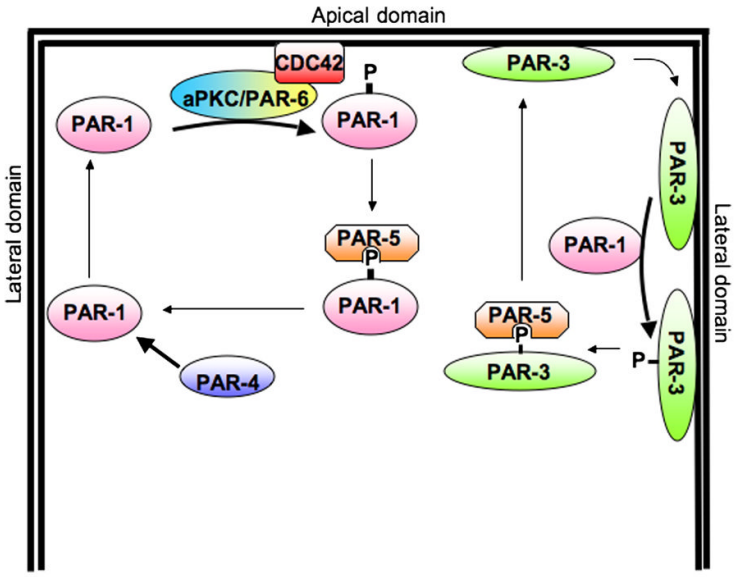


**Figure 4. The PAR Signaling Pathway: Phosphorylation and Binding**

The PAR signaling pathway; phosphorylation and binding of PAR-5 provides a mechanism for mutual exclusion from different PAR domains. On the left, PAR-1 that has diffused onto the apical domain is phosphorylated by aPKC, which inhibits the PAR-1 kinase activity and induces binding of PAR-5, which in turn triggers release into the cytoplasm. A similar mechanism probably controls the cortical attachment of LGL, Numb, and perhaps XGAP. In a reciprocal fashion, PAR-3 that diffuses into the basolateral domain is phosphorylated by PAR-1, which induces binding of PAR-5 and release into the cytoplasm.

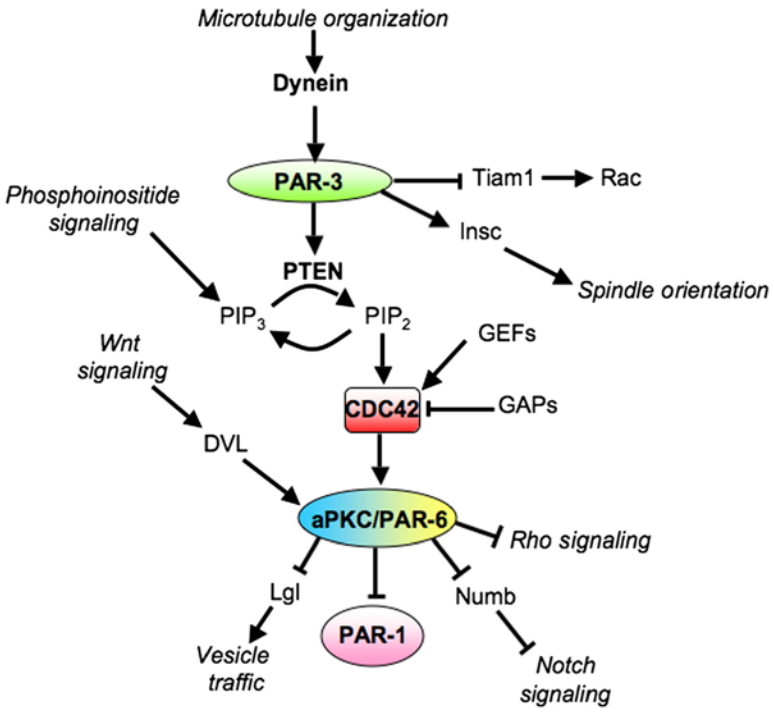


**Figure 5. The PAR Signaling Pathway: Inputs and Outputs**

This schematic shows links between the PAR-3/PAR-6/aPKC polarity proteins and a variety of other signaling networks. The components of this scheme are drawn from different model systems, and it is unlikely that the details are common to all organisms or polarization processes. It is therefore somewhat speculative, but does indicate a possible mechanism by which cytoskeletal organization could trigger localized changes in phosphoinositide metabolism, which in turn localizes PAR-6/aPKC through CDC43-GTP.

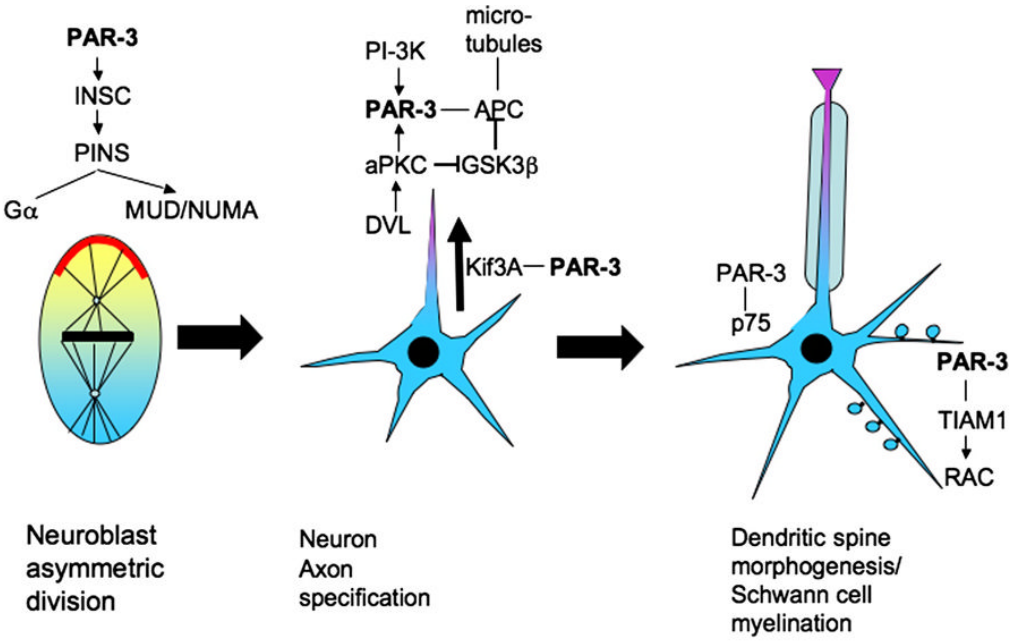


**Figure 6. The Multiple Functions of PAR-3 in Neuronal Differentiation**

PAR-3 plays critical roles in asymmetric division of the neuroblast, in axon specification, in dendritic spine maturation, and in Schwann cell function. Interactions with distinct partners appear to drive these separate functions.