Cell polarity: **Scaffold proteins *par excellence***

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Par proteins are involved in determining cellular asymmetry. Recent studies have identified one of these proteins, Par6, as a key regulator of cell polarity and transformation via its interactions with small GTPases and atypical forms of protein kinase C.

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The establishment and maintenance of cellular asymmetry, or polarity, is a critical event in the development of multicellular organisms. Neurons and epithelial cells represent the clearest examples of polarised cell types, where extracellular signals are normally presented to one end of neurons and to one surface of epithelial cells [1]. Polarity in epithelial cells is generated by the asymmetric distribution of specific proteins, creating apical and basolateral domains. The suggestion that the basolateral and apical domains of epithelial cells correspond to the somatodendritic and axonal surfaces of neurons, respectively, leads to the proposal that similar cellular processes are involved in generating polarity in both cell types [2]. The exact mechanisms involved in polarity establishment have not yet been fully deciphered, but proteins such as *Caenorhabditis elegans* PAR-3 (for 'partitioning defective') and its homologues in other species (Bazooka in *Drosophila melanogaster* and ASIP in rats) have been shown to be involved. A new member of the Par family, Par6, has now been identified in mammals and several recent reports [3–6] have described the role of this protein in the establishment of cell polarity and other cellular processes, such as tight junction formation and transformation. A model has emerged in which Par6 forms a multimeric protein complex with a small GTPase, another member of the Par family, Par3, and a member of the atypical protein kinase C (aPKC) family.

Before summarising the new reports on Par6 signalling, it is worthwhile to review briefly the data on cellular polarity and asymmetric cell division to date. Many of the key early observations emerged from the study of asymmetric cell divisions in the *C. elegans* zygote, and of *Drosophila* embryonic development. The *par* family of genes (*par1–6*) has been implicated in asymmetric cell division in early *C. elegans* development [1]. A summary of the properties of the Par proteins is presented in Table 1. A multimeric complex of PAR-3, PAR-6 and an aPKC has been shown to localise to the anterior pole of the polarised *C. elegans* one-cell embryo, whereas other members of this family (PAR-1 and PAR-2) localise the opposite pole of the embryo [5]. Disruption of these proteins can lead to asymmetric cell division and defective cell-fate determination [7].

An analogous group of proteins has been identified in *Drosophila*, where the PAR-3 homologue, Bazooka, forms a complex with two other proteins, Inscuteable and Partner of Inscuteable (Pins), at the apical pole of the dividing neuroblast [5]. As with *C. elegans*, this complex is crucial for correct cell-fate determination. The small GTPase Cdc42 regulates the establishment and maintenance of the bud site in *Saccharomyces cerevisiae*, whereas in more complex organisms, Cdc42 regulates epithelial cell polarity and axon guidance [3]. The specific role for many of the proteins mentioned above in cell polarity has remained unclear. Now, several reports provide evidence that a Par3–Par6–GTPase–aPKC multimeric complex is essential for the establishment of cell polarity and tight junctions in epithelial cells, and furthermore, may be involved in cellular transformation ([3–6], S. Ohno, personal communication).

Joberty *et al.* [3] identified mammalian Par6 as a binding partner for TC10, a small GTPase related to Cdc42. Par6 proteins bound to activated, GTP-bound forms of TC10 and Cdc42 *in vivo* and *in vitro*, and the Cdc42/Rac interactive binding (CRIB) motif and PDZ domain of Par6 seem to be important for this interaction. The previous observation that PAR-6 colocalised with PAR-3 in *C. elegans* led

Table 1

Summary of the characteristics of Par family members.

Par1	Contains Ser/Thr kinase-like motifs Localises to the posterior pole of the *C. elegans* zygote during early cell division [10]
Par2	Contains a myosin-like ATP-binding site and a cysteine-rich domain Co-localises with Par1 in early *C. elegans* zygote development [10]
Par3	Contains three PDZ domains Interacts with Par6 Localises to the anterior peripheral cytoplasm of the zygote [10] Binds members of the aPKC family [10]
Par4, Par5	Little is known about these proteins
Par6	Co-localises with Par3 at the anterior pole of the *C. elegans* one-cell embryo Mammalian Par6 associates with Par3 and small GTPases via its CRIB and PDZ domains Directly regulates members of the aPKC family [3–6]

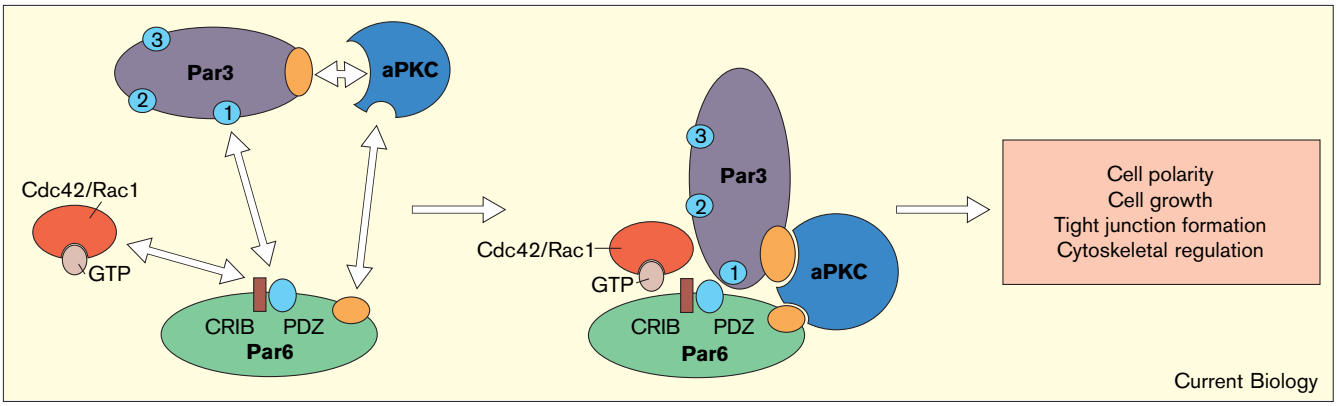
Joberty *et al.* [3] to examine whether the mammalian versions of these proteins could interact. Mammalian Par6 and Par3 did indeed associate, and deletion of the CRIB or PDZ domains of Par6 abolished this binding. The first PDZ domain of Par3 was necessary and sufficient for this interaction. Association of Cdc42, Par6 and Par3 *in vivo* was demonstrated by co-immunoprecipitation, and PKCζ was also detected in the complex. Surprisingly, the interaction of Par6 and PKCζ was shown to be direct, and not via Par3, which had previously been shown to bind members of the aPKC family. The Par6–PKCζ interaction seems to occur between the amino termini of both proteins. Furthermore, the physiological significance of these interactions was demonstrated by the finding that epithelial cell tight junctions were disrupted when one or more of these proteins was overexpressed.

Human Par6 was also identified as a binding partner for Cdc42 by Johansson *et al.* [4]. At least three isoforms of Par6 were detected, and Par6 was shown to interact with activated Cdc42 and Rac1. The authors went on to suggest that Par6 is a physiological target for Cdc42, but not Rac1. Subcellular localisation studies of epithelial cells revealed that Par6 was detected in tight junctions, and also in the nucleus. Stimulation of these cells with hepatocyte growth factor, previously shown to induce disruption of cell–cell contacts, led to a displacement of tight junctions, and a concomitant loss of Par6 from this region of the cell. Binding of Par6 to Par3, another member of the *par* family found in tight junctions, was also detected.

Comparable findings have been presented by Lin *et al.* [5]. Three different splice variants of Par3 were identified in a screen of an embryonic mouse cDNA library, using a sequence corresponding to a known PDZ-domain-binding site as a probe. The authors demonstrated a similar expression pattern of Par3 and Par6 in the rat central nervous system, and co-localisation of these proteins in the same neuronal cell compartments. Furthermore, these two proteins were localised within a similar neuronal population — in the fibres of the cortex and the hippocampus. Similar to Joberty *et al.* [3], the role of the PDZ domains of both Par3 and Par6 in their interaction was highlighted, as was the formation of a protein complex containing Par3, Par6 and Cdc42. The importance of the Par6 CRIB domain for Cdc42 binding was also demonstrated. Interestingly, Lin *et al.* [5] not only showed that aPKC family members bind to Par3 and Par6, but also that Par3 is a substrate for PKCζ. Phosphorylation of Par6 by PKCζ was not observed, however, although evidence was also presented to suggest that Par proteins can influence the activity of aPKC *in vitro*.

The data of Qiu *et al.* [6] extend the above observations. Similar to Joberty *et al.* [3], human Par6 was identified in a two-hybrid yeast screen using activated Cdc42 as bait. Par6 interacted with activated Cdc42 and activated Rac1 *in vitro* and *in vivo*, and this interaction was shown to be dependent on GTP. After confirming that Par6 associated with PKCζ, and that a ternary complex was formed between Par6, Rac1/Cdc42 and PKCζ, the authors demonstrated that overexpression of Par6 stimulated PKCζ activity. To examine the effect of Par6 on Rac1-mediated transformation, focus assays using NIH 3T3 fibroblasts were performed. Co-transfection of Par6 with activated Rac1 potentiated focus formation approximately 10-fold, and this effect was dependent on the direct interaction between Par6 and Rac1. The potentiation of focus formation by Par6 was then shown to require PKCζ activity, because a kinase-dead version of PKCζ blocked this

Figure 1



Model of Par6 signalling. A multimeric complex is formed using Par6 as a scaffold protein. The PDZ domains of Par3 and Par6 are represented by pale blue circles. The interaction of Par3 and Par6 occurs via the first PDZ domain of Par3 and the PDZ domain of Par6. Both the CRIB and PDZ domains of Par6 are important for Cdc42/Rac1 binding, and aPKC binding appears to involve the amino terminus of Par6 and the regulatory region of aPKC. Direct binding of Par3 to Cdc42/Rac1 has not been demonstrated. Formation of the Par3–Par6–GTPase–aPKC quaternary complex regulates cell polarity, tight junction formation, cytoskeletal rearrangements and cell growth.

effect. Similar results were seen when a second parameter of transformation — anchorage-independent growth — was examined. Finally, Qiu *et al.* [6] revealed that Par6 and PKCζ mediate a signalling pathway downstream of Rac1 that is distinct from that mediated by activated Raf in terms of focus formation and cell morphology.

From the data discussed above, a model can be proposed in which Par6 acts as a scaffold protein, nucleating a multiprotein complex consisting of Par3, Cdc42/Rac1 and PKCζ (Figure 1). Previous reports demonstrated that Cdc42 could associate with aPKC, without identifying the exact components of the multimeric complex [8]. The association of Par3 and Par6 appears to be a key interaction in the complex, because binding of these proteins seems to serve a scaffolding role, recruiting proteins such as Cdc42/Rac1 and aPKC into the complex. The apparent involvement of Cdc42 in cell polarity determination in *C. elegans* and human cells suggests that essential elements of the molecular machinery for the establishment of cell polarity have been conserved throughout evolution [4].

The binding of Par6 to Cdc42/Rac1 suggests that the Par3–Par6 complex may be involved in the regulation of the actin cytoskeleton. Indeed, cytoskeletal rearrangements have been suggested to play a role in the establishment of cell polarity in epithelial cells. The observation that Par6 also mediates cellular transformation suggests that a link between cell polarity and growth control may be present in cells. Of note, a recent paper from Wu *et al.* [9] has identified the γ-subunit of the coatomer complex as a downstream target of Cdc42 in mediating transformation. The exact mechanism of the regulation of aPKC by the Par3–Par6 complex, and the role of other upstream regulators of aPKC, such as phosphatidylinositol 3-kinase or 3-phosphoinositide-dependent protein kinase-1, in Par3–Par6 signalling remains to be determined. Other proteins involved in the Par3–Par6 complex, for example proteins that interact with the second and third PDZ domains of Par3, also need to be identified, as do membrane receptors that recruit these complexes to the tight junctions of epithelial cells.

In summary, we now have strong evidence for the formation of a Par3–Par6–Cdc42/Rac1–aPKC a multimeric protein complex in cells. This complex appears to be involved in the control of cell polarity determination, and perturbation of this signalling pathway may lead to cellular transformation. The challenge that lies ahead will be to determine exactly how this complex is regulating these cellular processes, and which ligands are involved in mediating signalling via this pathway. Answers to these questions will provide a fascinating insight into the biology of cell polarity and transformation and may ultimately lead to therapeutic agents that can interfere with the growth of transformed cells.

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