CDC-42 controls early cell polarity and spindle orientation in *C. elegans*

Monica Gotta, Mary C. Abraham and Julie Ahringer

Background: Generation of asymmetry in the one-cell embryo of *C. elegans* establishes the anterior–posterior axis (A-P), and is necessary for the proper identity of early blastomeres. Conserved PAR proteins are asymmetrically distributed and are required for the generation of this early asymmetry. The small G protein Cdc42 is a key regulator of polarity in other systems, and recently it has been shown to interact with the mammalian homolog of PAR-6. The function of Cdc42 in *C. elegans* had not yet been investigated, however.

Results: Here, we show that *C. elegans cdc-42* plays an essential role in the polarity of the one-cell embryo and the proper localization of PAR proteins. Inhibition of *cdc-42* using RNA interference results in embryos with a phenotype that is nearly identical to *par-3*, *par-6*, and *pkc-3* mutants, and asymmetric localization of these and other PAR proteins is lost. We further show that *C. elegans* CDC-42 physically interacts with PAR-6 in a yeast two-hybrid system, consistent with data on the interaction of human homologs.

Conclusions: Our results show that CDC-42 acts in concert with the PAR proteins to control the polarity of the *C. elegans* embryo, and provide evidence that the interaction of CDC-42 and the PAR-3/PAR-6/PKC-3 complex has been evolutionarily conserved as a functional unit.

Address: Wellcome/CRC Institute, Tennis Court Road, Cambridge CB2 1QR, UK.

Correspondence: Julie Ahringer
E-mail: jaa@mole.bio.cam.ac.uk

Received: **8 January 2001**
Revised: **2 February 2001**
Accepted: **21 February 2001**

Published: **3 April 2001**

Current Biology 2001, 11:482–488

0960-9822/01/$ – see front matter

Background

The polarity of the *C. elegans* embryo is established during the first cell cycle. Sperm entry, which determines the posterior end, drives cytoplasmic rearrangements that result in a highly polarized one-cell embryo. For example, the first mitotic spindle is asymmetrically placed and germline-associated P granules are localized to the posterior end. After the first cleavage, the two resulting blastomeres AB and P1 have different developmental potential, different cell cycle times, and different spindle orientations. The overall polarity of the one-cell embryo is governed by the *par* (partitioning-defective) genes. Mutations in these genes result in a symmetric first cleavage, failure in P granule segregation to P blastomeres, spindle orientation defects, and equal cell cycle times in the daughter cells. In addition, PAR proteins themselves exhibit an asymmetric distribution (reviewed in [1]). How the PAR proteins are asymmetrically localized is not well understood, but many localizations are interdependent.

Cdc42 is a highly conserved small G protein. In yeast, Cdc42 is important for cell polarity during mating and budding, and in mammalian cells it has been shown to play a role in establishing and maintaining epithelial polarity [2, 3]. Given this commonality of function, we decided to test whether *C. elegans cdc-42* has a function in controlling the polarity of the early embryo.

Results and discussion

The *C. elegans* homolog of Cdc42, encoded by the predicted gene R07G3.1, was previously identified by Chen *et al.* [4]. Recently, Jantsch-Plunger *et al.* noted variable early embryonic phenotypes after the reduction of *cdc-42* function [5]. Neither of these studies investigated a possible role for *cdc-42* in early embryonic polarity, however.

We used RNA-mediated interference (RNAi) to inhibit the function of *cdc-42* [6]. We first employed a standard RNAi injection protocol, where embryos were examined 24–48 hr after injection of the mothers. We found a temperature dependence of the phenotype. At 25°C, embryonic lethality was 86% (n = 353); embryos were frequently osmotically sensitive and displayed abnormal eggshell shapes, making the analysis of early polarity difficult (see Materials and methods; see Figure S1 in Supplementary material published with this article on the Internet). In embryos that could be analyzed, the spindle orientations of AB and P1 were variably defective (see Materials and methods). At 15°C, osmotic sensitivity and abnormal eggshells were not observed, but embryonic lethality was 81% (n = 1264). Because RNAi leads to mRNA degradation, we wondered whether the variable phenotypes could be due to residual CDC-42 protein in the embryos. Indeed, it has been reported that small G proteins are very

Table 1

Spindle orientation, position of first division, division time, and aster morphology in wild-type and *cdc-42(RNAi)* embryos.

	% egg length	Aster morphology			Difference in AB-P1 division time (sec)	Spindle orientation			
						Wild-type (AB, P1)	Longitudinal	Reversed	Transverse
Wild-type n=11	56.0+/-1.0	100%	0%	0%	129+/-20	11 (100%)	0 (0%)	0 (0%)	0 (0%)
cdc-42(RNAi) n=30	52.5+/-1.4	8%	88%	4%	57+/-20	3 (10%)	25 (84%)	1 (3%)	1 (3%)

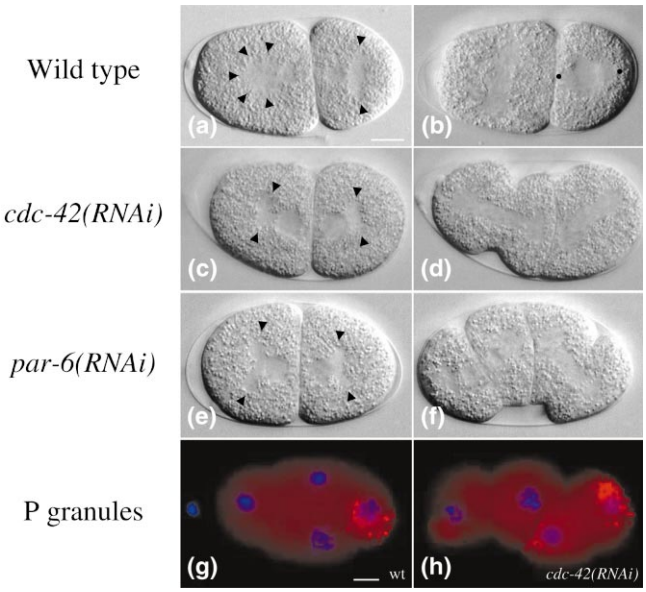
cdc-42(RNAi) data are from 30 embryos videorecorded from 25 different mothers. The position of cleavage is expressed as a percentage of egg length (mean ± standard deviation) with anterior equal to 0%. The position of the cleavage plane in *cdc-42(RNAi)* embryos is more central than in wild type, and is similar to *par* mutants (for example, % egg length in *par-3* is 52% ± 1.0) [27]. The difference between the AB and P1 division timing (s) was measured as the time taken from nuclear envelope breakdown in AB to that in P1. The position of cleavage and aster morphology were measured just after the completion of cytokinesis (this was not scorable in five embryos because the recordings began at the beginning of the two-cell stage). In the aster morphology column, the circle represents a round aster and the oval a flat aster; the anterior aster is on the left and the posterior one is on the right. Spindle orientations were determined by the positions of the centrosomes at nuclear envelope breakdown.

stable, with a half-life of about 15 hr [7]. We thus repeated RNAi of *cdc-42* at 15°C, but waited longer before analyzing the resulting embryos. We found that 48–72 hr postinjection, 100% of *cdc-42(RNAi)* embryos die (n = 560), and that these embryos have a consistent mutant phenotype; rare osmotic sensitivity and eggshell defects were seen after more than 72 hr postinjection at 15°C. Unfortunately, our attempts to raise an antibody to CDC-42 or to localize CDC-42 with published antibodies were not successful. Therefore, although *cdc-42(RNAi)* embryos will have a reduction of *cdc-42* function, we cannot assay whether a small amount of CDC-42 protein might remain. Because the 15°C 48–72 hr protocol gives a strong and consistent polarity phenotype, we used this method for our analysis of the role of CDC-42 in embryonic polarity. We have not further characterized the osmotic sensitivity and eggshell defects seen at 25°C.

In *cdc-42(RNAi)* embryos, the first mitotic spindle is symmetrically placed compared to wild type (Table 1) and both the anterior and posterior asters have the flat appearance of a wild-type posterior aster (Figure 1c; Table 1). In wild-type two-cell embryos, the AB spindle is oriented perpendicular to the first division, but the P1 division is along the same axis as the first division because of nuclear–centrosome rotation in that cell (Figure 1b). In most *cdc-42(RNAi)* embryos, both the AB and P1 daughter cells undergo rotation of the nuclear–centrosome complex (Figure 1d; Table 1). In addition, AB and P1 divide nearly synchronously in contrast to the 2 min difference in division time between wild-type AB and P1 blastomeres (Table 1). The defects in placement and morphology of the first mitotic spindle, and the fact that AB and P1 behave similarly with respect to spindle orientation and cell cycle timing indicate that *cdc-42* has a role in establishing embryonic polarity. The failure of a small percentage of analyzed embryos to display all of the identified defects may be due to incomplete silencing by RNAi.

The phenotypes of *cdc-42(RNAi)* embryos are reminiscent of those lacking PAR-3, PAR-6, or PKC-3 (see Figure 1e,f). In *par-3*, *par-6*, or *pkc-3* mutants the first cleavage is symmetric, both anterior and posterior asters are flat, cell cycle times are equal, and nuclear–centrosome rotation occurs in both AB and P1 cells [8–11]. In mammalian cells, homologs of these three proteins form a functional complex [12–14]. This complex probably exists in *C. elegans* as well, because PKC-3 has been shown to physically associate with PAR-3 [11], and a complex of the three has been detected (T. Hung and K. Kemphues, personal communication). To see whether CDC-42 might have a role in regulating this complex, we examined the distribution of PAR-3, PAR-6, and PKC-3 in *cdc-42(RNAi)* embryos. In wild-type embryos, these proteins are all localized to the anterior cortex of one-cell embryos (Figure 2a) [8, 10, 11]. In two-cell embryos, they are found all around the cortex of the anterior AB cell and are restricted to the anterior cortex of P1 (Figure 2e) [8, 10, 11]. Localization of these proteins is interdependent, as the loss of one component results in the mislocalization of the other components [8–11]. In one-cell *cdc-42(RNAi)* embryos, PAR-6 is initially localized correctly to the anterior (Figure 2b). From metaphase of the first division onward, however, anterior localization is lost and PAR-6 is found

Figure 1



Early defects in *cdc-42(RNAi)* embryos. **(a)** In wild-type two-cell embryos, the anterior aster is round and the posterior one is flat (marked by arrowheads). **(b)** In wild-type two-cell embryos, the anterior AB spindle is oriented along the short axis of the egg (perpendicular to the previous division), whereas the posterior P1 nuclear–centrosome complex rotates to be oriented along the anterior–posterior axis (dots mark the centrosomes in P1). **(c)** In *cdc-42(RNAi)* embryos, both asters are flat, as in **(e)** *par-6(RNAi)* embryos. **(d)** In most *cdc-42(RNAi)* embryos, both AB and P1 undergo nuclear–centrosome rotation so that the cells divide along the anterior–posterior axis, as also occurs in **(f)** *par-6(RNAi)* embryos. **(g)** Wild-type four-cell embryo with P granules localized to P1. **(h)** *cdc-42(RNAi)* four-cell embryo with P granules in both EMS and P2. In 96% of *cdc-42(RNAi)* two-cell embryos, P granules are found only in P1 (n = 46). In most four-cell embryos, they are found in both P2 and EMS (84%, n = 45). In some cases they are found in all four cells (11%) or only in P2 (5%). Posterior is to the right. The scale bar in (a) represents 10 μm.

weakly around the entire cortex (Figure 2d). Similarly, in two-cell embryos, PAR-6 is weakly found at the cortex of both AB and P1 rather than being anteriorly restricted (compare Figure 2f with 2e). PAR-3 and PKC-3 follow an identical localization pattern in *cdc-42(RNAi)* embryos (see legend to Figures 2 and S2). Therefore, CDC-42 is required for the asymmetric distribution of PAR-3, PAR-6, and PKC-3. The normal anterior localization of these proteins in early one-cell embryos suggests that CDC-42 may not be required for the initial asymmetric localization of the complex, but appears to be necessary to maintain this localization.

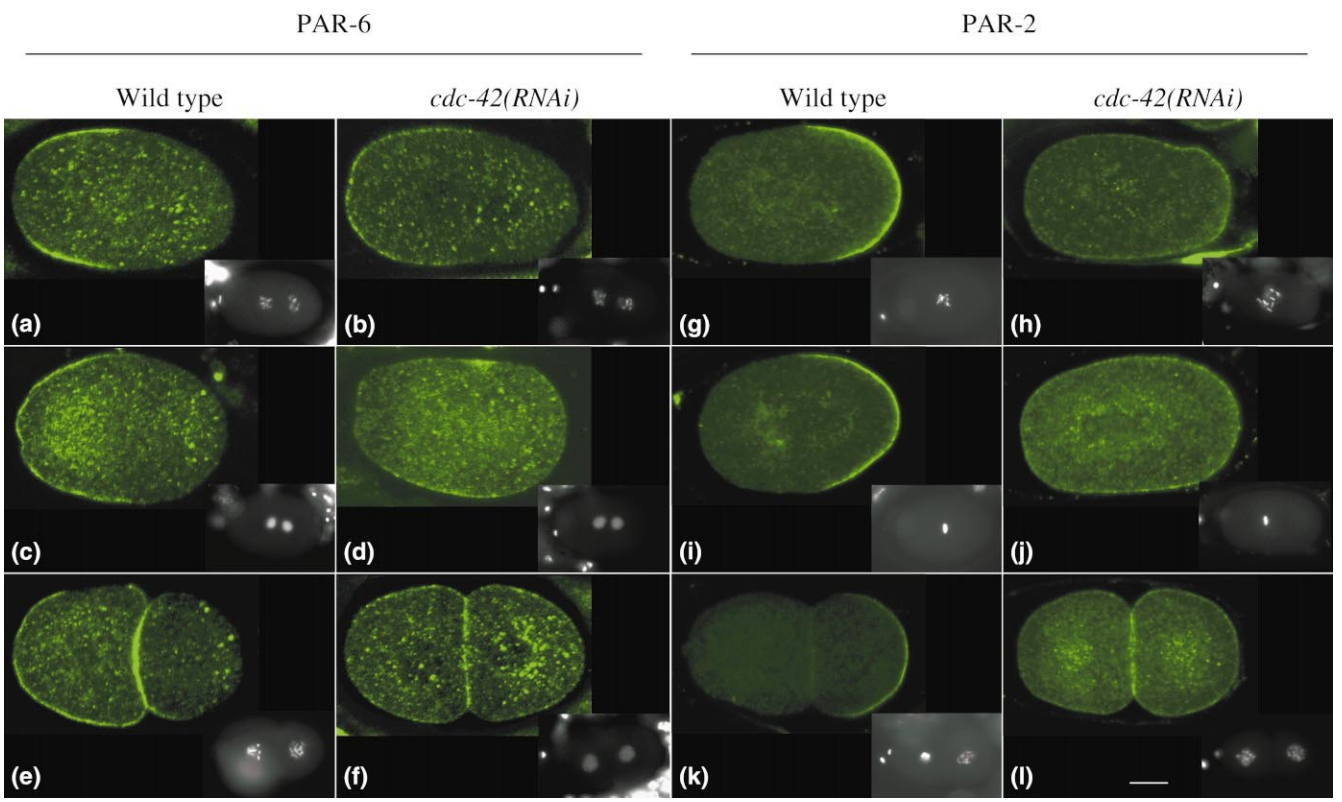
As the localization of different PAR proteins is interdependent, we wondered whether the localization of the posteriorly localized PAR proteins PAR-1 and PAR-2 [15, 16] was also affected in *cdc-42(RNAi)* embryos. Up to metaphase of one-cell *cdc-42(RNAi)* embryos, PAR-2 is found weakly at both the anterior and posterior cortices but is often richer at the posterior cortex (Figure 2h). In later one-cell embryos and in two-cell embryos, PAR-2 is found at both anterior and posterior cortices (Figure 2j,l) rather than being restricted to the posterior. In addition, PAR-1, which is normally localized to the posterior, is uniformly distributed in one-cell and two-cell *cdc-42(RNAi)* embryos (Figure 3).

As an additional assay of polarity in *cdc-42(RNAi)* embryos, we examined the distribution of the P granules, which in wild-type embryos are segregated to the P lineage (for example, to P1 and then to P2) [17]. In *cdc-42(RNAi)* embryos, P granules are partitioned normally to the P1 cell. In the majority of embryos, however, they are incorrectly partitioned to both P2 and EMS at the second cell cycle rather than being segregated exclusively to P2 (Figure 1h); in most of the remainder, P granules are found in all four cells (see legend to Figure 1). This phenotype is shared with *par-6* mutants [9].

One proposed role of the PAR-3/PAR-6/PKC-3 complex is to inhibit nuclear–centrosome rotation in AB [8]. In *par-2* mutants, these proteins are found at the cortex of P1 as well as of AB, and no nuclear–centrosome rotation occurs in P1. Conversely, where the complex is disrupted (for example, in *par-3* mutants), nuclear–centrosome rotation occurs in both AB and P1. Thus, it was surprising to find nuclear–centrosome rotation in AB and P1 in *cdc-42(RNAi)* embryos, given that PAR-3, PAR-6, and PKC-3 were all localized to the cortex of both AB and P1, as in *par-2* mutants, where no rotation occurs. We suggest that CDC-42 is required both for the proper localization of these proteins and for their activity. Consistent with this idea, PAR-3 is found around AB and P1 cortices in *par-6* mutants where spindles do rotate, but where the complex is thought to be inactive due to lack of PAR-6 [9].

To further investigate the connection between CDC-42 and the PAR-3/PAR-6/PKC-3 complex, we tested whether a physical interaction between these might occur. Recently, it was shown that mammalian Cdc42 is able to interact with PAR-6 in a two-hybrid system, and that in mammalian cells, Cdc42 coprecipitates with the mammalian PAR-3/PAR-6/PKC-3 complex [12–14]. A fragment of PAR-6 containing the PDZ domain and the so-called semi-CRIB domain, a domain with homology to the CRIB domain but that lacks some of the most conserved residues, is necessary and sufficient for this interaction, but the semi-CRIB domain is not sufficient. We tested whether *C. elegans* CDC-42 could interact with PAR-6. CDC-42 is active and interacts with downstream effectors when bound to GTP but is inactive when bound to GDP. We constructed an activated form of CDC-42 that should mimic the GTP-bound form, CDC-42(Q61L), as well as a dominant-negative form that should be constitutively

Figure 2

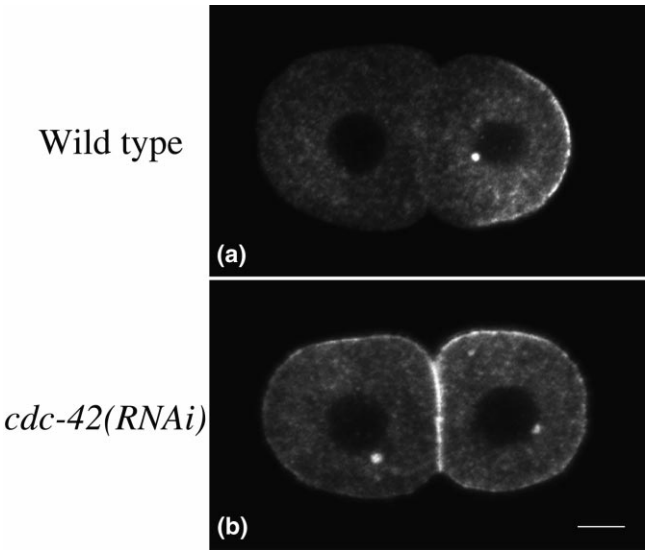


Asymmetric localization of PAR-6 and PAR-2 is lost in *cdc-42(RNAi)* embryos. (a–f) PAR-6 (green) and DNA (visualized with DAPI; inset at lower right of each panel). In wild-type one-cell embryos during pronuclear migration **(a)** or at telophase **(c)**, PAR-6 is enriched at the anterior periphery. **(b)** *cdc-42(RNAi)* embryo during pronuclear migration. PAR-6 is localized at the anterior periphery as in wild type (100%, n = 18). PAR-3 (100%, n = 10) and PKC-3 (95%, n = 19) are also localized as in wild-type embryos at this stage of *cdc-42(RNAi)* embryos. **(d)** *cdc-42(RNAi)* one-cell embryo at telophase. PAR-6 is found around both anterior and posterior cortices of 85% of one-cell *cdc-42(RNAi)* embryos from metaphase onward (n = 13); in the remainder there is no detectable PAR-6 at the cortex. PAR-3 and PKC-3 are also found around both anterior and posterior cortices (100%, n = 6 and 60%, n = 10, respectively) from metaphase of one-cell *cdc-42(RNAi)* embryos. **(e)** In two-cell embryos, PAR-6 is found at the periphery of the anterior AB cell and is restricted at the anterior cortex of P1. PAR-3 and PKC-3 show the same localization pattern as PAR-6 in wild-type one- and two-cell embryos [8, 11]. **(f)** *cdc-42(RNAi)* two-cell embryo. In 74% of embryos, PAR-6 is found around the cortices of AB and P1; in the remainder, no detectable PAR-6 is found at the cortex (n = 27). Again, PAR-3 and PKC-3 follow the same pattern of localization in two-cell *cdc-42(RNAi)* embryos, being found around AB and P1 cortices in 92% (n = 26) and 78% (n = 23) of two-cell embryos, respectively. In the remainder, no detectable PAR-3 or PKC-3 are found at the cortex. See Figure S2 for PAR-3 and PKC-3 staining. (g–l) PAR-2 (green) and DNA (visualized with DAPI; inset at lower right of each panel). In wild-type one-cell embryos at pronuclear meeting **(g)** or at metaphase **(i)**, PAR-2 is enriched at the posterior periphery. **(h)** *cdc-42(RNAi)* one-cell embryo at pronuclear meeting. Before metaphase, PAR-2 is weakly enriched at the posterior in 38% of embryos and uniformly distributed in the remainder (n = 13). **(j)** In *cdc-42(RNAi)* one-cell embryos at metaphase or later, PAR-2 is found around both anterior and posterior cortices (100%, n = 12). **(k)** Wild-type two-cell embryo with PAR-2 localized to the posterior cortex of P1. **(l)** In 77% of *cdc-42(RNAi)* two-cell embryos, PAR-2 is found around the cortices of both AB and P1. In 15%, PAR-2 is found around both AB and P1 cortices but is enriched at the posterior of P1 cortex, and in 8%, PAR-2 is not detectable at the cortex (n = 26). Posterior is to the right. The scale bar in (l) represents 10 μm.

in the GDP-bound state [CDC-42(T17N)] [18–20]. Using a two-hybrid system, we found that the activated CDC-42(Q61L) interacts with both full-length PAR-6 and with a fragment containing the semi-CRIB domain and the PDZ domain. No interaction was detected between these clones and the inactive CDC-42(T17N) form of CDC-42 (Figure 4). The semi-CRIB domain alone was not sufficient for the interaction (Figure 4). These results are similar to those seen between the mammalian counterparts of these proteins. The interaction of *C. elegans* CDC-42 with PAR-6 could explain how CDC-42 regulates the complex.

To help in understanding CDC-42's role in regulating the PAR proteins, we tested whether *cdc-42* was epistatic to *par-2*, as these genes have opposite effects on spindle orientation. Surprisingly, we found that the inhibition of *cdc-42* in *par-2(it46)* mutants caused the partial rescue of both mutant phenotypes. The progeny of *par-2(it46)* mutants showed 2% (n = 196) embryonic hatching. After

Figure 3



PAR-1 localization is symmetric in *cdc-42(RNAi)* embryos. **(a)** Wild-type two-cell embryo. PAR-1 is enriched at the posterior of P1. **(b)** *cdc-42(RNAi)* two-cell embryo. PAR-1 is found around AB and P1 cortices (100%, n = 18). In early and late one-cell embryos, PAR-1 is found around anterior and posterior cortices (100%, n = 15). Posterior is to the right. The scale bar in (b) represents 10 μm.

the injection of *cdc-42* dsRNA, however, 30% (n = 702) of the progeny of *par-2(it46)* mothers hatched. For comparison, the injection of *cdc-42* dsRNA into wild-type mothers resulted in 0% hatching (n = 255). Interestingly, it was previously shown that the reduction of *par-6* function also partially suppresses the lethality and sterility of *par-2* mutants [9], suggesting that the inhibition of CDC-42 might cause suppression through the reduction of PAR-6 activity or that of a PAR-3/PAR-6/PKC-3 complex.

Conclusions

We have shown that *cdc-42* plays an essential role in early embryonic polarity in *C. elegans* (see also Kay and Hunter, 2001 [this issue of *Current Biology*]) [28]. Our phenotypic and two-hybrid data suggest that CDC-42 might activate the PAR-3/PAR-6/PKC-3 complex through interaction with PAR-6. CDC-42 appears to be necessary for the activity of the complex as well as for its correct localization. It is also possible that the PAR-3/PAR-6/PKC-3 complex has a role in activating CDC-42, as its initial anterior localization seems CDC-42 independent. One way that CDC-42 and the PAR-3/PAR-6/PKC-3 complex might direct polarity is through the regulation of the actin cytoskeleton. In *par-3* mutants and in *cdc-42(RNAi)* embryos, enrichment of actin at the anterior of early embryos is lost (M.G. and J.A., unpublished data) [21]. Further, co-suppression of *par-2* and *cdc-42(RNAi)* mutant phenotypes suggests that CDC-42 and PAR-2 have counterbalancing, antagonistic activities. Because PAR-2 has a RING finger [22], a motif that has been proposed to be involved in ubiquitin-mediated protein degradation [23], CDC-42 might normally activate a protein that is a target of PAR-2. Future biochemical and in vivo studies should help to reveal the nature of these interactions and identify downstream targets.

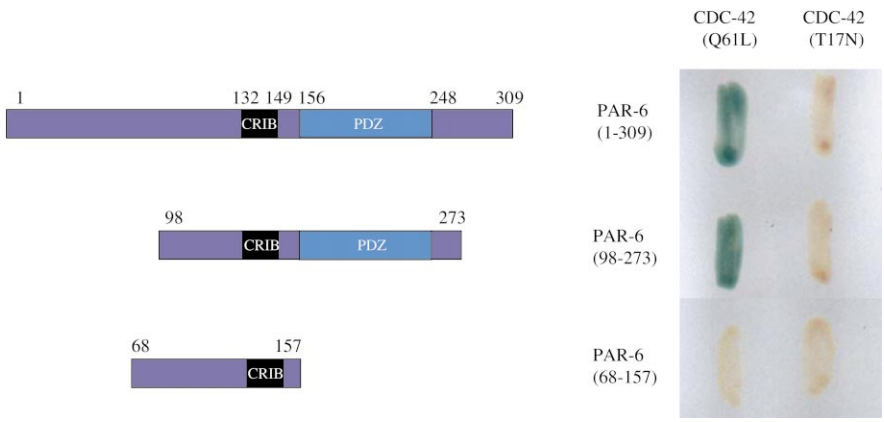
Materials and methods

Strains and constructs

The Bristol strain N2 was used as the standard wild-type strain. The *par-2* mutant strain used is LV19 [*unc-45(wc2) dpy-1(e1)/daf-7(e1372) par-2(it46)* III]. *cdc-42* cDNA was amplified by PCR from yk109f2 (kindly provided by Yuji Kohara) using the following primers: 5′-CGGGATCCG TATGCAGACGATCAAGTGCGT-3′ and 5′-CGGAATTCTAGAGAAT ATTG**G**ACTTCTTC-3′. The 3′ oligonucleotide contains a C to G substitution (in bold) to create a Cys to Ser substitution (C188S) that would prevent prenylation as it was used in two-hybrid experiments (see Two-hybrid assays section below). The cDNAs were cloned into the BamH1 and EcoRI sites of pBSKS(−) (Stratagene) to give pBS-CDC-42. The two-hybrid plasmids were constructed by subcloning a BamHI-SalI fragment containing the cdc42 coding region from pBS-CDC-42 into the BamHI and SalI sites of pAS-2 to create pAS-2-CDC-42. The T17N and Q61L mutations were made by PCR. Full-length PAR-6 and the fragment containing the semi-CRIB and PDZ domain (PAR-6 [98–273]) were amplified from total cDNA using the following primers: 5′-CGG GATCCGTATGTCCTACAACGGCTCCTACC-3′ and 5′-AAACCCGG TCGACTCAGTCCTCTCCACTGTCCG-3′ for full-length PAR-6 and

Figure 4

PAR-6 interacts with CDC-42. Shown is the interaction of full-length PAR-6 (1–310) and a fragment containing the semi-CRIB and PDZ domains (98–273) with activated CDC-42 [CDC-42(Q61L)] (blue). The same two fusion proteins do not interact with the dominant-negative CDC-42 [CDC-42(T17N)] (white). A fragment containing the semi-CRIB domain (68–157) is not sufficient to interact with activated CDC-42. β-Galactosidase activity indicating interaction was measured on a colony lift filter assay (see Materials and methods).



5′-CGGGATCGAATCATGGGAAGAGAAATATGG-3′ and 5′-AAAC CCGGTCGACCTGAATTCCACCTGTAGCG-3′ for PAR-6 (98–273). The fragments were cloned into the BamHI and XhoI sites of pACT2 (Clontech).

Immunofluorescence

Antibody staining was carried out as in [15] with the following modifications: the slides were blocked in PBS, 0.2% Tween, and 1% milk and all primary and secondary antibodies were diluted in PBS Tween before use. The following primary antibodies were used: rabbit anti-PAR-6 [10]; rabbit anti-PAR-2 [16]; chicken anti-PAR-3 [10]; rabbit anti-PKC-3 [11]; rabbit anti-PAR-1 (gift of P. Gönczy); mouse anti–P granule monoclonal cell supernatant (OIC1D4) [17]. For antibody staining, embryos were fixed at least 70 hr after mothers were injected with *cdc-42* dsRNA.

To try to detect CDC-42, the following antibodies were tested: rabbit anti-CDC-42 raised against the *C. elegans* CDC-42 protein [24], goat anti-Cdc42 and rabbit anti-Cdc42 raised against *S. cerevisiae* Cdc42p (Santa Cruz), and mouse anti-Cdc42 raised against human Cdc42 (Transduction Laboratories).

RNA interference

The *cdc-42* template for RNA synthesis was produced by PCR with T3 and T7 oligonucleotides using the pBS-CDC-42 plasmid. Sense and antisense RNAs were produced using an RNA synthesis kit from Promega (Ribomax) or Ambion (MEGAscript) and were annealed before use. *cdc-42* dsRNA was injected at a concentration of 0.5–1 mg/ml. Embryos from injected mothers were analyzed at least 48 hr after injection. Worms were grown at 15°C and mated with males in order to maintain egg laying. This injection and mating protocol was also used for the *par-2* epistasis experiment.

When worms were grown at 25°C and embryos were analyzed 24–30 hr after injection, the defect in spindle orientation was variable (40% [6/15], no rotation in AB or P1; 27% [4/15], rotation in AB and no rotation in P1; 20% [3/15], wild-type orientation; and 13% [2/15], rotation in both AB and P1). Embryos that were analyzed at least 30 hr postinjection at 25°C often showed osmotic sensitivity, as evidenced by embryo swelling (32%, n = 34; see Figure S1); abnormal eggshell shapes and cytokinesis defects were also seen. None of these defects could be rescued by mating with wild-type males (6/17 and 5/17 embryos showed osmotic sensitivity with and without mating, respectively). Osmotic sensitivity and cytokinesis defects were also observed when worms were grown at 15°C with males, but only after more than a 72 hr incubation and in less than 10% of the embryos. Osmotic sensitivity was not observed in embryos (n = 30) from unmated hermaphrodites at 48 hr postinjection at 15°C.

Analysis of embryos by DIC 4-D videomicroscopy

Animals were dissected in a drop of M9 on poly-L-lysine-coated 18 × 18 mm coverslips, mounted over an agar pad, and sealed with Vaseline. Embryos were recorded from the one- to four-cell stage (12 focal planes every 30 s) with DIC optics on a Leica DMRBE microscope using Openlab software. Nuclear–centrosome rotation in AB and P1 were scored by following the positions of the centrosomes in 4D videorecordings up to the time that the nuclear envelope broke down. We scored a rotation event as happening if the nuclear–centrosome complex was clearly and obviously seen to rotate. In some cases, although a rotation event occurred, the spindles were not both longitudinal after they elongated. This may be caused because embryos were mounted on agar pads for analysis, which has been shown to decrease the penetrance of longitudinal spindles [25].

Two-hybrid assays

Two-hybrid assays were performed in yeast strain Y190 transformed with pAS2-1-based plasmids expressing DNA binding domain fusions, and pACT2-based plasmids expressing transcriptional activation domain fusions (Clontech). The pAS2-CDC-42 plasmids contained a Cys to Ser substitution (C188S) that prevented prenylation. β-Galactosidase activity was measured by a colony lift filter assay according to published protocols [26]. Positives were blue after 30 min incubations at 37°C. At least five independent transformants were analyzed in three independent experiments.

Supplementary material

Additional figures showing anti-PAR-3 and anti-PKC-3 staining in wild-type and *cdc-42(RNAi)* embryos and examples of the osmotic sensitivity and eggshell defects of *cdc-42(RNAi)* embryos are available at http://images.cellpress.com/supmat/supmatin.htm.

Acknowledgements

We would like to thank K. Kemphues, S. Strome, Y. Tabuse, L. Lim, and P. Gönczy for antibodies and Y. Kohara for cDNAs. Some strains used in this study were obtained from the *Caenorhabditis* Genetics Center, which is supported by the NIH National Center for Research Resources (NCRR). We also thank M. Sohrmann for critical comments on the manuscript. This work was supported by a Wellcome Senior Research Fellowship to J.A., an HFSP long-term fellowship to M.G., and an EC TMR network grant.

References

1. Rose LS, Kemphues, KJ: **Early patterning of the *C. elegans* embryo**. *Annu Rev Genet* 1998, **32:**521-545.
2. Johnson DI: **Cdc42: an essential Rho-type GTPase controlling eukaryotic cell polarity.** *Microbiol Mol Biol Rev* 1999, **63:**54-105.
3. Kroschewski R, Hall A, Mellman, I: **Cdc42 controls secretory and endocytic transport to the basolateral plasma membrane of MDCK cells.** *Nat Cell Biol* 1999, **1:**8-13.
4. Chen W, Lim HH, Lim L: **The CDC42 homologue from *Caenorhabditis elegans*. Complementation of yeast mutation.** *J Biol Chem* 1993, **268:**13280-13285.
5. Jantsch-Plunger V, Gonczy P, Romano A, Schnabel H, Hamill D, Schnabel R, *et al.*: **CYK-4: a Rho family gtpase activating protein (GAP) required for central spindle formation and cytokinesis**. *J Cell Biol* 2000, **149:**1391-1404.
6. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC: **Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*.** *Nature* 1998, **391:**806-811.
7. Backlund PS Jr: **Post-translational processing of RhoA. Carboxyl methylation of the carboxyl-terminal prenylcysteine increases the half-life of Rhoa.** *J Biol Chem* 1997, **272:**33175-33180.
8. Etemad-Moghadam B, Guo S, Kemphues KJ: **Asymmetrically distributed PAR-3 protein contributes to cell polarity and spindle alignment in early *C. elegans* embryos.** *Cell* 1995, **83:**743-752.
9. Watts JL, Etemad-Moghadam B, Guo S, Boyd L, Draper BW, Mello CC, *et al.*: **par-6, a gene involved in the establishment of asymmetry in early *C. elegans* embryos, mediates the asymmetric localization of PAR-3.** *Development* 1996, **122:**3133-3140.
10. Hung TJ, Kemphues KJ: **PAR-6 is a conserved PDZ domain-containing protein that colocalizes with PAR-3 in *Caenorhabditis elegans* embryos.** *Development* 1999, **126:**127-135.
11. Tabuse Y, Izumi Y, Piano F, Kemphues KJ, Miwa J, Ohno S: **Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in *Caenorhabditis elegans*.** *Development* 1998, **125:**3607-3614.
12. Qiu RG, Abo A, Steven Martin, G: **A human homolog of the *C. elegans* polarity determinant Par-6 links Rac and Cdc42 to PKCζ signaling and cell transformation.** *Curr Biol* 2000, **10:**697-707.
13. Joberty G, Petersen C, Gao L, Macara IG: **The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42.** *Nat Cell Biol* 2000, **2:**531-539.
14. Lin D, Edwards AS, Fawcett JP, Mbamalu G, Scott JD, Pawson, T: **A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and αPKC signalling and cell polarity.** *Nat Cell Biol* 2000, **2:**540-547.
15. Guo S, Kemphues KJ: **par-1, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed.** *Cell* 1995, **81:**611-620.

16. Boyd L, Guo S, Levitan D, Stinchcomb DT, Kemphues KJ: **PAR-2 is asymmetrically distributed and promotes association of P granules and PAR-1 with the cortex in *C. elegans* embryos.** *Development* 1996, **122:**3075-3084.
17. Strome S, Wood WB: **Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*.** *Proc Natl Acad Sci USA* 1982, **79:**1558-1562.
18. Luo L, Liao YJ, Jan LY, Jan YN: **Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion.** *Genes Dev* 1994, **8:**1787-1802.
19. Ottilie S, Miller PJ, Johnson DI, Creasy CL, Sells MA, Bagrodia S, *et al.*: **Fission yeast pak1+ encodes a protein kinase that interacts with Cdc42p and is involved in the control of cell polarity and mating.** *EMBO J* 1995, **14:**5908-5919.
20. Ziman M, O'Brien JM, Ouellette LA, Church WR, Johnson DI: **Mutational analysis of CDC42Sc, a *Saccharomyces cerevisiae* gene that encodes a putative GTP-binding protein involved in the control of cell polarity.** *Mol Cell Biol* 1991, **11:**3537-3544.
21. Kirby C, Kusch M, Kemphues K: **Mutations in the par genes of *Caenorhabditis elegans* affect cytoplasmic reorganization during the first cell cycle.** *Dev Biol* 1990, **142:**203-215.
22. Levitan DJ, Boyd L, Mello CC, Kemphues KJ, Stinchcomb DT: **par-2, a gene required for blastomere asymmetry in *Caenorhabditis elegans*, encodes zinc-finger and ATP-binding motifs.** *Proc Natl Acad Sci USA* 1994, **91:**6108-6112.
23. Joazeiro CA, Weissman AM: **RING finger proteins: mediators of ubiquitin ligase activity.** *Cell* 2000, **102:**549-552.
24. Chen W, Chen S, Yap SF, Lim L: **The *Caenorhabditis elegans* p21-activated kinase (CePAK) colocalizes with CeRac1 and CDC42Ce at hypodermal cell boundaries during embryo elongation.** *J Biol Chem* 1996, **271:**26362-26368.
25. Cheng NN, Kirby CM, Kemphues KJ: **Control of cleavage spindle orientation in *Caenorhabditis elegans*: the role of the genes par-2 and par-3.** *Genetics* 1995, **139:**549-559.
26. Golemis EA, Gyuris J, Brent R: **Interaction trap/two hybrid system to identify two hybrid proteins.** In *Current Protocols in Molecular Biology.* Edited by Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. New York: John Wiley and Sons; 1996:20.21.21-20.21.23.
27. Kemphues KJ, Priess JR, Morton DG, Cheng NS: **Identification of genes required for cytoplasmic localization in early *C. elegans* embryos.** *Cell* 1988, **52:**311-320.
28. Kay AJ, Hunter CP: **CDC-42 regulates PAR protein localization and function to control cellular and embryonic polarity in *C. elegans*.** *Curr Biol* 2001, **11:**this issue, 474–481.