

## Similar requirements for CDC-42 and the PAR-3/PAR-6/PKC-3 complex in diverse cell types

David P. Welchman<sup>a</sup>, Laura D. Mathies<sup>b</sup>, Julie Ahringer<sup>a,\*</sup>

<sup>a</sup> *The Gurdon Institute and Department of Genetics, University of Cambridge, Cambridge, CB2 1QN, UK*

<sup>b</sup> *Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614, USA*

Received for publication 5 February 2007; accepted 14 February 2007

Available online 21 February 2007

### Abstract

During animal development, a complex of Par3, Par6 and atypical protein kinase C (aPKC) plays a central role in cell polarisation. The small G protein Cdc42 also functions in cell polarity and has been shown in some cases to act by regulating the Par3 complex. However, it is not yet known whether Cdc42 and the Par3 complex widely function together in development or whether they have independent functions. For example, many studies have implicated Cdc42 in cell migrations, but the Par3 complex has only been little studied, with conflicting results. Here we examine the requirements for CDC-42 and the PAR-3/PAR-6/PKC-3 complex in a range of different developmental events. We found similar requirements in all tissues examined, including polarised growth of vulval precursors and seam cells, migrations of neuroblasts and axons, and the development of the somatic gonad. We also propose a novel role for primordial germ cells in mediating coalescence of the *Caenorhabditis elegans* gonad. These results indicate that CDC-42 and the PAR-3/PAR-6/aPKC complex function together in diverse cell types.

© 2007 Elsevier Inc. All rights reserved.

**Keywords:** *Caenorhabditis elegans*; Cell migration; Polarity; Vulva; Anchor cell; Gonad; Cdc42; Par3; Par6; aPKC

### Introduction

A conserved complex of Par3 and Par6 (PDZ domain proteins) and an atypical protein kinase C (aPKC) plays a central role in the establishment and maintenance of cell polarity in animal cells (Macara, 2004). In some systems, this complex has been shown to be activated by the small GTPase Cdc42 (Etienne-Manneville, 2004). In mammalian epithelia Cdc42 and the Par3 complex are required for apical–basal polarity and junction formation (Joberty et al., 2000; Lin et al., 2000). In migratory mammalian cells, Cdc42 and a Par-6/aPKC complex mediate polarisation of the microtubule organising centre towards the leading edge (Etienne-Manneville and Hall, 2001; Solecki et al., 2004). In *Caenorhabditis elegans*, CDC-42 and the PAR-3/PAR-6/PKC-3 complex regulate polarity in the one celled embryo (Nance, 2005).

Although there are examples where Cdc42 and the Par3 complex are known to function together in cell polarisation, the

picture regarding other processes is not clear. For example, Cdc42 has been widely implicated in cell migration, but components of the Par3 complex have been little studied. Furthermore some studies have given conflicting results. Fibroblasts generated from Cdc42 null ES cells show no migration defects (Czuchra et al., 2005), whereas primary fibroblasts from conditional Cdc42 knockout mice show strong defects in wound recruitment and chemotaxis (Yang et al., 2006). Overexpression of mPar6 $\alpha$  prevents the migration of glial guided neurons in culture (Solecki et al., 2004). Axon outgrowth, which precedes cell body migration, is also inhibited and might be the cause of the migration defect. Axon outgrowth is also blocked by Par3 complex overexpression in hippocampal neuron cultures (Shi et al., 2003), but evidence from *Drosophila* mutants suggests that the complex is not required for axon outgrowth or dendrite morphology (Rolls and Doe, 2004). By contrast Par6 and Par3 (Bazooka) are required for the migration of *Drosophila* border cells (Pinheiro and Montell, 2004), although earlier experiments suggest that Cdc42 is not required for this process (Murphy and Montell, 1996). Thus the role of the Par3

\* Corresponding author. Fax: +44 1223 334089.

E-mail address: [j.ahringer@gurdon.cam.ac.uk](mailto:j.ahringer@gurdon.cam.ac.uk) (J. Ahringer).

complex in migration and polarised growth, and its relationship to Cdc42 are unclear.

To address this question, we investigated whether CDC-42 and the PAR-3 complex act in the same set of developmental processes in *C. elegans*. We found that inhibition of CDC-42 or components of the PAR-3/PAR-6/PKC-3 complex causes similar defects in multiple cell types, including somatic gonad precursors, vulval precursors, seam cells and neurons. We suggest that CDC-42 and the PAR-3 complex widely act together in cell migration and polarised cell growth.

## Materials and methods

### Strains and worm handling

Standard methods have been used for culturing *C. elegans* on NGM plates (Lewis and Fleming, 1995). Bristol strain N2 (Brenner, 1974) has been used as wild type throughout. The *ehn-3A::GFP* reporter (pRA230) contains 3003 bp upstream of the *ehn-3A* translational start and the first two exons of *ehn-3A*. Genomic sequences were cloned into pPD95.75 using XbaI and PstI sites. pRA230 was injected with pRF4 (Mello et al., 1991) and integrated to generate *rdIs2 [ehn-3A::GFP; rol-6(su1006)] V*.

Other strains used were *bnIs1[pie-1::GFP::pgl-1; unc-119(+)] I, let-23(sy1) II, syls77[zmp-1::YFP] II, mulS32[mec-7::GFP, lin-15(+)] II, cdc-42(gk388)/mIn1[mIs14 dpy-10(e128)] II, lin-12(n302) III, lin-12(n137) dpy-19(e1259)/lin-12(n137n720) unc-32(e189) III, lin-12(n941) III/hT2[qIs48] (I; III), jcls1[ajm-1::GFP; rol-6(su1006)] IV, lin-3(n378) IV, lin-45(n2018) IV, syls49[zmp-1::GFP; dpy-20(+)] IV, syls67[zmp-1::CFP; unc-119(+)] V, syls59[egl-17::CFP] X, zulS77[par-6::GFP; unc-119(+)], mIs[let-413::GFP; rol-6(su1006)], arIs51[cdh-3::GFP; dpy-20(+)], syEx[lin-3::GFP; pha-1(+)], qIs56[lag-2::GFP; unc-119(+)], arIs92[egl-17p::CFP::lacZ; unc-4(+); ttx-3p::GFP] and arIs82[lin-12::GFP; unc-4(+); egl-17p::lacZ]. For compound microscopy, live specimens were mounted by standard procedures (Sulston and Hodgkin, 1988) on 3% agar pads in 5 mM tetramisole in M9. Photomicrographs were generated as described under Immunofluorescence.*

### RNAi

RNAi was performed by feeding as described previously (Kamath et al., 2001), with minor modifications. Briefly, plates containing NGM agar, 1 mM IPTG and 25 µg/ml carbenicillin were inoculated with bacterial cultures grown overnight at 37 °C in LB medium supplemented with 50 µg/ml ampicillin. Synchronised L4 animals were placed on plates at 15 °C for 48 h for *cdc-42*, *par-3* and *pkc-3(RNAi)*, 24 h for *par-6(RNAi)*, and 40 h at 25 °C for *mes-1(RNAi)*. Longer incubations (72 h) result in 100% embryonic lethality for *cdc-42* or the *par-3* complex. After the first incubation adults were transferred to fresh plates and allowed to lay eggs for 24 h at 20 °C before removal. Progeny were incubated at 20 °C until they reached the required stage for analysis. Constructs used for RNAi are as previously described (Kamath et al., 2003). These clones are predicted to show no off-target effects (no primary or secondary off-targets in Wormbase, [www.wormbase.org](http://www.wormbase.org)).

### Vulval assays

The Muv phenotype was scored under a dissecting microscope. Vulval induction was scored at L4 as previously described (Poulin et al., 2005). 1° fate was scored on the basis of *egl-17::CFP* expression at the Pn.pxx stage.

### Immunofluorescence

Fixation for AJM-1/MH27 and LIN-12::GFP stainings was carried out using a modified version of the Finney–Ruvkun fixation procedure (Shaye and Greenwald, 2002), L1 larval staining and embryo staining was carried out as in Le Bot et al. (2003) and LIN-12 and *egl-17::CFP* stainings as in Hurd and Kemphues (2003). The following antibodies were used: MH27 (Francis and

Waterston, 1985), anti-GFP (Molecular Probes or Nacalai Tesque), anti-Pgl-1 (Kawasaki et al., 1998), NE8/4C6.3 (Goh and Bogaert, 1991), and anti-LIN-12 (gift of Stuart Kim). All conjugated secondary antibodies were from Jackson Immunoresearch. Stained worms were mounted in Mowiol (Merck) and viewed with a Zeiss Axioplan 2 microscope. Photomicrographs were obtained using either a Hamamatsu Orca C4742-95 camera and Improvision Openlab software or a Zeiss LSM 500 Meta confocal attachment.

## Results

### *cdc-42* and *par-3(RNAi)* result in hyperinduction of the vulva

Strong maternal reduction of *cdc-42*, *par-3*, *par-6* or *pkc-3* causes defects in the polarity of the first cell division and embryonic lethality (Gotta et al., 2001; Kemphues et al., 1988; Tabuse et al., 1998; Watts et al., 1996). Components of the PAR-3 complex are expressed widely during later development (Nance, 2005), suggesting additional roles, but such roles show maternal rescue: homozygous loss of function mutants of *par-3* and *par-6* grow into morphologically normal adults that give rise to 100% dead embryos (Kemphues et al., 1988; Watts et al., 1996) and a *cdc-42* null mutant is viable but homozygous sterile (data not shown). Consistent with this, a previous study using RNAi to inhibit zygotic but not maternal *par-3* function only identified defects in epithelia that develop in the last larval stage (Aono et al., 2004). In order to more broadly investigate somatic functions of these genes we reduced both their maternal and zygotic activities by carrying out RNAi of mothers for short periods and looked for phenotypes in their surviving progeny (see Materials and methods). We found that RNAi of *cdc-42*, *par-3*, *par-6* or *pkc-3* results in multiple ventral protrusions in adult progeny, characteristic of a Multiple vulva (Muv) phenotype (Table 1). For this paper, we focus on defects induced by RNAi of *cdc-42* and use *par-3* or *par-6* as representative of the PAR-3/PAR-6/PKC-3 complex, as the phenotypes induced by RNAi of *par-3*, *par-6* and *pkc-3* are similar.

The *C. elegans* vulva develops from three of six equipotent vulval precursor cells (VPCs), numbered P3.p–P8.p, induced and patterned by the Ras and Notch signalling pathways (Wang and Sternberg, 2001). A LIN-3/EGF signal produced by the Anchor Cell in the overlying gonad induces vulval fate in the three closest VPCs (P5.p–P7.p) by activating Ras signalling via the EGF receptor LET-23. Lateral signalling between the VPCs, mediated by LIN-12 Notch, results in the central cell (usually P6.p) adopting the primary (1°) vulval fate, while the flanking cells adopt the secondary (2°) fate. The uninduced P3.p, P4.p, and P8.p cells fuse with the surrounding hypodermis after one division, whereas the induced VPCs divide three times to produce 22 adult vulval cells. Counting the number of vulval cells confirmed that RNAi of *cdc-42*, *par-3*, *par-6* or *pkc-3* results in greater than three induced VPCs (Table 1).

Although CDC-42 and the PAR-3 complex play conserved roles in epithelial polarity, the vulval hyperinduction phenotypes observed following their knockdown do not appear to be caused by defects in vulval cell polarity. We found that apical, adherens junction and basal markers are localised to the correct

Table 1

VPCs are hyperinduced following RNAi of *cdc-42*, *par-3*, *par-6* or *pkc-3*

Treatment	Length of exposure (h)	% Animals hypo-induced	% Animals hyper-induced	Average induction (range)	n	% Muv <sup>a</sup>	n
<i>Vector(RNAi)</i>	48–72	0	0	3.0 <sup>b</sup> (3.0–3.0)	30	0	1793
<i>cdc-42(RNAi)</i>	48–72	10	47	3.4 <sup>b</sup> (2.0–5.0)	30	28	1971
<i>par-3(RNAi)</i>	48–72	0	10	3.1 <sup>b</sup> (3.0–5.0)	30	7	188
<i>par-6(RNAi)</i>	24–48	0	10	3.2 <sup>b</sup> (3.0–6.0)	30	4	233
<i>pkc-3(RNAi)</i>	48–72	0	13	3.1 <sup>b</sup> (3.0–4.5)	30	6	151
<i>mes-1(RNAi)</i>	40–64 <sup>c</sup>	n.d.	n.d.	n.d.	n.a.	7 <sup>d</sup>	206
<i>cdc-42(RNAi)</i> 1 AC	48–72	4	4	3.0 <sup>e</sup> (1.0–4.0)	55	n.d.	n.a.
<i>cdc-42(RNAi)</i> 2+ AC	48–72	4	80	4.1 <sup>e</sup> (2.0–6.0)	45	n.d.	n.a.

Greater than 3 induced VPCs is hyperinduced and fewer than 3 is hypoinduced. Hypoinduction may be a result of gaps between VPCs or abnormal AC positions. AC: Anchor Cell.

<sup>a</sup> Muv, multiple protrusions scored using a dissecting microscope.

<sup>b</sup> The number of VPCs induced (from 0–6) was scored by counting vulval cells at L4.

<sup>c</sup> *mes-1(RNAi)* incubations were at 25 °C.

<sup>d</sup> Previously reported by Capowski et al. (1991).

<sup>e</sup> The number of VPCs induced (from 0–6) was scored by counting vulval cells at L3 Pn.pxx.

domains in hyperinduced vulval cells of *cdc-42(RNAi)* and *par-3(RNAi)* animals (Supplementary Figure 1).

#### Polarised growth defects of the VPCs and seam cells

Although the apical junctions of individual VPCs are intact, *cdc-42* or *par-6(RNAi)* results in the occurrence of gaps between VPCs during L3 (Fig. 1B). VPCs are not born in contact with one another but elongate during L2 to form a continuous array (Liu et al., 2005). The finding of large gaps between VPCs during L3 suggests that CDC-42 and the PAR-3 complex are required for the normal polarised growth of these cells.

To ask whether a requirement in polarised growth is a general property of these genes, we examined seam cell development. The seam cells divide at the beginning of each larval stage, with the anterior daughters fusing with the hypodermis, while the posterior daughters retain a seam cell fate (Sulston and Horvitz, 1977). The continuous array of seam cells is restored by elongation of the posterior daughters in the anterior/posterior axis. Following RNAi of *cdc-42* or *par-6*, many seam cells do not extend to the neighbouring cell, leading to large gaps between them (Fig. 1D). In addition, seam cells have a rounded rather than an elongated morphology consistent with a defect in polarity of growth (Fig. 1D). Although apical junctions are continuous, they

appear abnormal, with bright spots of AJM-1::GFP at the apical membranes (Fig. 1D). Thus CDC-42 and the PAR-3 complex are required for the polarised growth of both VPCs and seam cells and may play a role in regulating the recruitment of junctional components.

#### Both AC/VU precursors adopt the Anchor Cell fate

We next explored the basis for the Muv phenotype induced by RNAi of *cdc-42*, *par-3*, *par-6*, and *pkc-3*. Because epistasis analyses showed that CDC-42 acts at or upstream of the LIN-3 signal produced by the Anchor Cell (AC) (Supplementary Table 1), we examined AC development.

The AC is derived from one of two equipotent cells, Z1.ppp and Z4.aaa, descendants of the somatic gonad precursors (SGPs), Z1 and Z4 (Kimble and Hirsh, 1979). Z1.ppp and Z4.aaa are brought together by the stereotyped division pattern of the SGPs. Through contact-dependent LIN-12 Notch signalling, either Z1.ppp or Z4.aaa adopts a ventral uterine (VU) fate, while the other forms the AC (Seydoux and Greenwald, 1989). Disruption of this signalling causes both cells to develop as ACs and can result in changes in vulval fate through excess LIN-3 signalling.

We found that instead of the single AC found in wildtype, two ACs were often present following RNAi of *cdc-42*, *par-3*, *par-6* or *pkc-3* (Fig. 2, Table 2). This suggests that

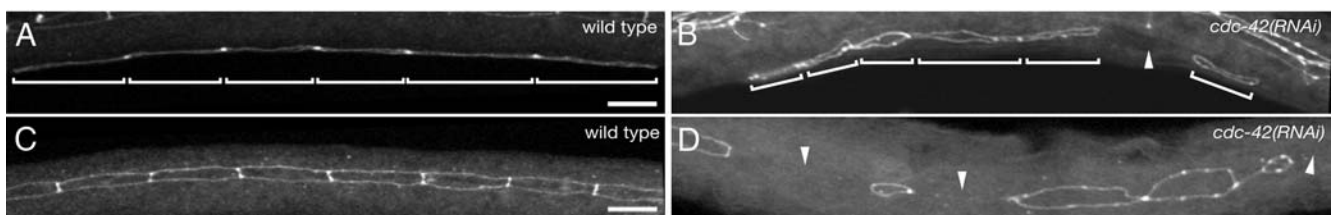


Fig. 1. Abnormal VPC and seam cell elongation. (A, B) AJM-1 at the apical junction of Pn.p stage VPCs (brackets). (A) Wild-type: VPCs form a continuous array ( $n=31$ ). (B) *cdc-42(RNAi)* animal with a large gap between VPCs (arrowhead); 39% of *cdc-42(RNAi)* animals ( $n=28$ ) and 17% of *par-6(RNAi)* animals ( $n=30$ ) have such gaps. (C, D) AJM-1 at the apical junction of L3 stage seam cells. (C) Wild-type: seam cells form a continuous array ( $n=31$ ). (D) *cdc-42(RNAi)* animal with large gaps (arrowheads) and rounded seam cells; seam cell gaps occur in 86% of *cdc-42(RNAi)* animals ( $n=28$ ) and 13% of *par-6(RNAi)* animals ( $n=30$ ). Anterior, left; ventral, down; scale bars, 10  $\mu\text{m}$ .

LIN-12 Notch signalling between AC/VU precursors might be disrupted. CDC-42 does not appear to be required for activity of the LIN-12 Notch pathway downstream of LIN-12, as RNAi of *cdc-42* cannot rescue the AC-less phenotype of weak *lin-12(gf)* mutants (*lin-12(n302gf)*  $n=186$ , *lin-12(n137gf)/lin-12(n137n720lf)*  $n=206$ ). This suggests that *cdc-*

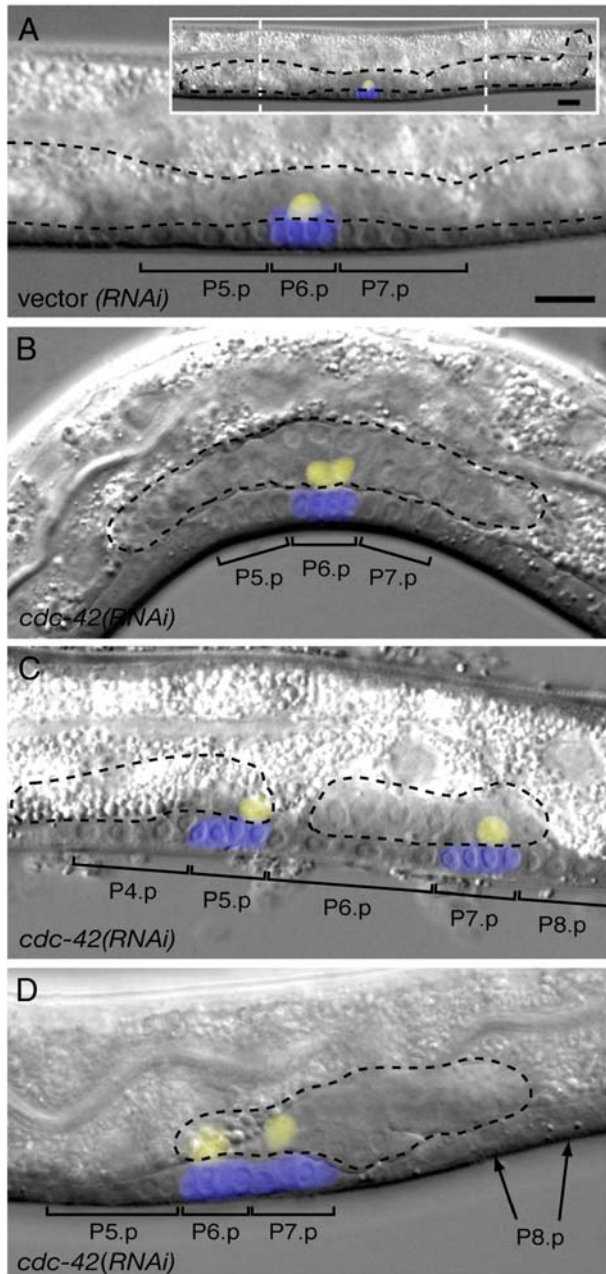
*42(RNAi)* does not cause multiple ACs by downregulating LIN-12 Notch signal transduction.

However, we observed that the two ACs in *cdc-42(RNAi)* animals were frequently separated from one another (Figs. 2C, D; Table 2). This contrasts with the direct contact of multiple ACs in *lin-12* mutants (Greenwald et al., 1983), suggesting that RNAi of *cdc-42* might cause ACs or their precursors to separate, preventing contact-dependent signalling. Indeed, we found that RNAi of *cdc-42* caused separation of the multiple ACs in *lin-12* mutants (Table 2).

The additional ACs in *cdc-42(RNAi)* animals appear to be derived from the normal AC/VU precursors because *cdc-42(RNAi)* does not increase the number of ACs in a background that already has multiple ACs derived from the normal precursors (*lin-12(n941)*; Table 2). The additional ACs formed in *cdc-42(RNAi)* animals also occur in the presence of normal distal tip cells (data not shown), whose precursors can instead, in certain mutant backgrounds, give rise to additional ACs (Miskowski et al., 2001).

#### *AC/VU precursors are separated and gonads are fragmented*

To further investigate the basis of AC duplication and separation, we examined the development of the somatic gonad in *cdc-42(RNAi)* and *par-3(RNAi)* animals. We found that, whereas wild-type animals have a single gonad, *cdc-42(RNAi)* animals often have a gonad split into two separate sections (23%;  $n=100$ ), each of which usually contains an AC (91%,  $n=23$ ; Fig. 2C). This suggests that the AC/VU precursors might have been physically separated, preventing them from signalling to one another and resulting in the formation of two ACs. Indeed, at the time of signalling between AC/VU precursors (L2), *cdc-42(RNAi)* and *par-3(RNAi)* animals showed frequent separation of these precursors (Figs. 3B, C; 34%,  $n=44$ , 44%,  $n=16$ , respectively) whereas separation was never seen in control animals ( $n=20$ ). AC duplication occurs both in animals with split gonads (47%;  $n=45$ ) and in those with single, non-split gonads (53%;  $n=45$ ) suggesting a requirement for CDC-42 and the PAR-3 complex in somatic cell positioning within the gonad. We conclude that RNAi inhibition of CDC-42 or components of the PAR-3 complex causes separation between the AC/VU precursors, resulting in



E	1° vulval fate cells			n
	Single	Adjacent	Separate	
vector ( <i>RNAi</i> ) <sup>1</sup>	100%	0%	0%	25
<i>cdc-42(RNAi)</i> <sup>1</sup>	77%	16%	7%	95
<i>par-3(RNAi)</i> <sup>1</sup>	85%	15%	0%	13
<i>cdc-42(RNAi)</i> 1 AC <sup>2</sup>	89%	11%	0%	55
<i>cdc-42(RNAi)</i> 2 AC <sup>2</sup>	20%	31%	51%	45

Fig. 2. *cdc-42(RNAi)* results in multiple ACs, adjacent 1° VPC fates and gonad splitting. Overlays of Nomarski images of L3 Pn.pxx stage animals with projections of *zmp-1::YFP* expression (yellow) marking the Anchor Cell (AC) and *egl-17::CFP* expression (blue) marking 1° fate VPCs. Brackets indicate the descendants of induced VPCs and black dotted outlines the morphology of the gonad. (A) Wild-type with extended gonad (inset), a single AC, and three induced VPCs, one of which (P6.p) has adopted the 1° fate (marked by *egl-17::CFP*). (B–D) *cdc-42(RNAi)* animals with (B) two touching ACs and a single 1° fate VPC, (C) two ACs in separate gonad fragments and two, non-adjacent, 1° fate VPCs, (D) two separated ACs in an intact, but short, gonad and two adjacent 1° fate VPCs. VPCs adjacent to 1° fate VPCs sometimes fail to adopt vulval fates (arrows in panel D), perhaps because of a gap between the VPCs. Anterior, left; ventral, down; scale bar, 10 μm. (E) Table of 1° vulval fate phenotypes. <sup>1</sup>1° fate scored using *arl592[egl-17::CFP::lacZ]* (Yoo et al., 2004). <sup>2</sup>1° fate scored using *syIs59[egl-17::CFP]* (Inoue et al., 2002). AC: Anchor Cell.

Table 2  
Multiple Anchor Cells induced by RNAi of *cdc-42*, *par-3*, *par-6* or *pkc-3*

Strain	% Animals with			n	% 2+ AC animals with		n	% 2+ separate AC animals with split gonad	n
	1 AC	2 AC	>2 AC		Touching ACs	Separate ACs			
Vector (RNAi) <sup>a</sup>	100	0	0	30	n.a.	n.a.	n.a.	n.a.	n.a.
<i>cdc-42</i> (RNAi) <sup>a, b</sup>	55	44	1 <sup>c</sup>	100	16	84	15	55	38
<i>par-3</i> (RNAi) <sup>d</sup>	85	15	0	26	50	50	4	n.d.	n.a.
<i>par-6</i> (RNAi) <sup>a</sup>	81	19	0	26	0	100	5	40	5
<i>pkc-3</i> (RNAi) <sup>a</sup>	79	21	0	29	33	67	6	n.d.	n.a.
<i>lin-12</i> (n941) <sup>c</sup>	0	78	22 <sup>c</sup>	80	100	0	80	n.d.	n.a.
<i>lin-12</i> (n941); <i>cdc-42</i> (RNAi) <sup>d</sup>	0	87	13 <sup>c</sup>	23	78	22	23	n.d.	n.a.
<i>mes-1</i> (RNAi) <sup>a</sup>	62	38	0	26	10	90	10	50	8
<i>hmd-1</i> (q740) <sup>a</sup>	92	8	0	85	57	43	7	44	9

Split gonads have two distinct fragments of gonadal tissue separate from one another.

<sup>a</sup> Anchor Cells (AC) scored using *syIs77* [*zmp-1::YFP*] (Inoue et al., 2002).

<sup>b</sup> AC duplication was confirmed with *arIs51*[*cdh-3::GFP*] (Karp and Greenwald, 2003) and *syEx*[*lin-3::GFP*] (Wang and Sternberg, 2000) (data not shown).

<sup>c</sup> Additional ACs are derived from the AC/VU precursors (Z1.ppp and Z4.aaa) and occasionally from their sibling cells (Z1.ppa and Z4.aap) (Seydoux et al., 1990).

<sup>d</sup> Anchor Cells scored using *syIs49* [*zmp-1::GFP*] (Wang and Sternberg, 2000).

the formation of multiple ACs, presumably by preventing LIN-12 Notch signalling.

#### Multiple Anchor Cells and vulval fate changes

To investigate whether the Muv phenotype of *cdc-42*(RNAi) is due to the presence of multiple ACs, we recovered *cdc-42*(RNAi) animals with either one or two ACs at L3 and then scored their vulval phenotype at adulthood: 78% ( $n=27$ ) of 2

AC animals were Muv, compared to only 2% ( $n=100$ ) of 1 AC animals. Similarly, scoring vulval induction directly showed that 80% ( $n=45$ ) of 2 AC animals have extra induced VPCs compared with only 4% ( $n=55$ ) of 1 AC animals (Table 1). These results strongly suggest that the additional ACs induce hyperinduction of the vulva.

In analyzing vulval hyperinduction in *cdc-42*(RNAi) and *par-3*(RNAi) animals, we observed unusual patterns of vulval cell fates. In the wild-type and in animals where VPCs are hyperinduced due to excessive Ras signalling, 1° fate VPCs never have 1° fate neighbours but are instead flanked by 2° fate neighbours due to LIN-12 Notch lateral signalling (Wang and Sternberg, 2001). In contrast, 1° fate cells are often adjacent to one another in *cdc-42*(RNAi) or *par-3*(RNAi) animals (Figs. 2D,E). These adjacent primary cells both downregulate LIN-12 (Supplementary Figure 2), as do normal 1° cells in wildtype animals (Shaye and Greenwald, 2002).

Adjacent 1° fates usually result from a defect in LIN-12 Notch mediated lateral signalling between VPCs. In a *lin-12* null mutant all three induced VPCs adopt the 1° fate (Greenwald et al., 1983). We found that the incidence of adjacent primary fates in *cdc-42*(RNAi) animals is related to the distance between the ACs. Most *cdc-42*(RNAi) animals with two touching ACs have only a single 1° fate (71%,  $n=7$ , Fig. 2B), indicating that the presence of two ACs is not alone sufficient to induce multiple 1° fates. The percentage of these *cdc-42*(RNAi) animals, with touching ACs, showing adjacent 1° fates (29%,  $n=7$ ) is similar to the percentage having gaps between VPCs (39%;  $n=28$ ). Because LIN-12/Notch signalling is largely contact dependent (Greenwald, 2005), the presence of a gap between VPCs would be expected to prevent lateral signalling and lead to adjacent 1° fates. Gaps between VPCs could also explain the observation of adjacent 1° fates in 11% ( $n=55$ ) of 1 AC *cdc-42*(RNAi) animals. Where two ACs are separated by more than one VPC width (21  $\mu$ m,  $n=55$ ), then most animals display multiple, non-adjacent 1° fates (84%,  $n=24$ , Fig. 2C).

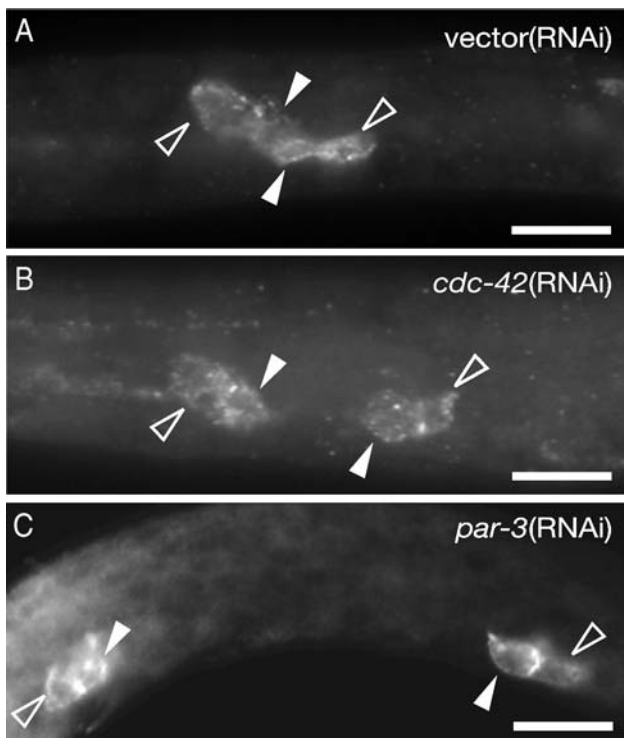


Fig. 3. AC/VU precursors are separated. AC/VU precursor cells (filled arrowheads) and their siblings (unfilled arrowheads) at the L2 stage, visualised by LIN-12::GFP expression. (A) Wild-type: AC/VU precursors are in contact. (B) *cdc-42*(RNAi) and (C) *par-3*(RNAi) animals with separated AC/VU precursors. Anterior, left; ventral, down; scale bars, 10  $\mu$ m.

However, we observed a different phenotype in animals where the two ACs are not touching but are separated by less than the width of a VPC. Most of these animals have adjacent 1° fates (89%,  $n=9$ , Fig. 2D), a rate more than double that of VPC gaps. This result suggests that when neighbouring VPCs each underlie an AC, the AC signal might override lateral signalling between the VPCs.

#### Multiple ACs result from disruption of the L1 gonad primordium

To understand the cause of the somatic gonad cell position defects, we examined earlier stages of gonad development. At hatching the gonad primordium is composed of a linear array of four cells, with two SGPs at the poles and two central primordial germ cells (PGCs) (Kimble and Hirsh, 1979). During embryogenesis the SGPs migrate from their anterior birth positions to the PGCs in the posterior region (Sulston et al., 1983).

We found that the L1 gonad primordium was disrupted in 55% ( $n=49$ ) of *cdc-42(RNAi)* and 38% ( $n=60$ ) of *par-6(RNAi)* animals (Figs. 4A–E and H). Three different defects were seen: 1) disorganised primordia, with one of the SGPs in a central rather than polar position (Figs. 4C, D); 5/5 *cdc-42(RNAi)* animals of this type developed a single gonad and one had two ACs. 2) separated primordia, with each SGP independently associated with one or more PGCs (Fig. 4B); 2/2 *cdc-42(RNAi)* animals of this type developed split gonads and 2 ACs. 3) SGPs with no associated PGCs (Fig. 4E); 8/8 *cdc-42(RNAi)* animals of this type had two ACs. This PGC-less phenotype was the most frequent (Fig. 4H) and associated with SGP separation in 77% ( $n=13$ ) of such animals. *cdc-42(RNAi)* animals with a normally organised L1

primordium developed intact gonads (18/18) and only a single AC (17/18), suggesting that CDC-42 is no longer required to maintain gonad integrity subsequent to hatching. These results show that *cdc-42* and *par-6* are required for proper cell positions within the four cell gonad primordium. In addition, reduction of their function leads to the absence of PGCs.

#### Loss of PGCs is due to mis-segregation of P-granules during embryogenesis

What is the cause of the striking absence of PGCs at L1 following RNAi of *cdc-42* or *par-6*? During normal development the germ-line P-granules are segregated into the germline precursor P4, which divides to give rise to the PGCs, Z2 and Z3 (Strome and Wood, 1982). In contrast to control animals, where P-granules are found in only the two normal PGCs ( $n=67$ , Fig. 5A), 55% ( $n=44$ ) of *cdc-42(RNAi)* animals contain P-granules in multiple cells that were separated from the SGPs (Fig. 5B). These abnormal PGL-1 positive cells coincided with body wall muscles in all cases ( $n=13$ , Fig. 5B).

The presence of P-granules in body wall muscles is reminiscent of mutations in *mes-1*, wherein P-granules are mis-segregated into the germline precursor P4 and its muscle precursor sibling D during the division of P3, usually resulting in both cells adopting a D-like muscle fate and the corresponding loss of PGCs (Strome et al., 1995). Studies of the early embryonic roles of CDC-42 and the PAR-3 complex have shown earlier mis-segregation of P-granules between the germline and somatic lineages (Gotta et al., 2001; Kempthues et al., 1988; Tabuse et al., 1998; Watts et al., 1996). Under our RNAi conditions, P-granules also mis-segregate between D and

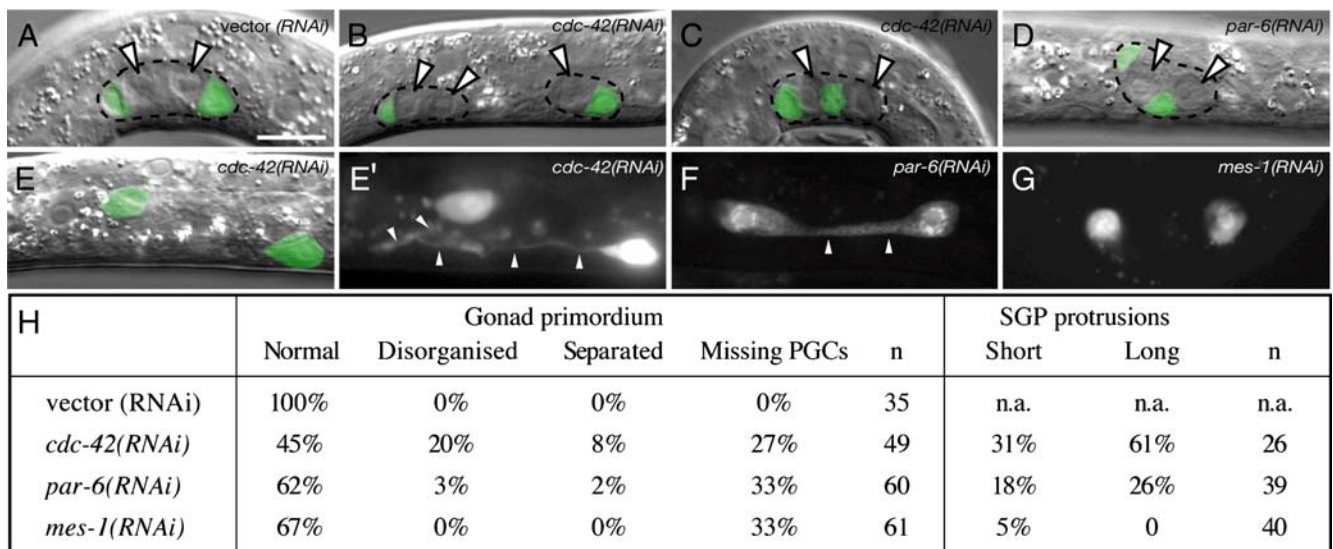


Fig. 4. Abnormal SGP positions, gonad splitting and loss of PGCs. (A–E) SGPs (green) marked by *ehm-3A::GFP* over Nomarski images of L1 gonad primordia; arrowheads mark PGCs, dotted outlines mark the primordium. (A) Wild-type primordium has polar SGPs and central PGCs. (B) *cdc-42(RNAi)* animal with two separate primordia (C) *cdc-42(RNAi)* or (D) *par-6(RNAi)* animals with disorganised single primordia. (E) *cdc-42(RNAi)* animal with SGPs lacking associated PGCs. (E'–G) Projections of *ehm-3A::GFP* expression in SGPs. In (E') *cdc-42(RNAi)* or (F) *par-6(RNAi)* animals lacking PGCs, SGPs extend long processes. (G) In *mes-1(RNAi)* animals lacking PGCs, processes are absent. Anterior, left; ventral, down; scale bar, 10  $\mu$ m. (H) Table of gonad primordium phenotypes.

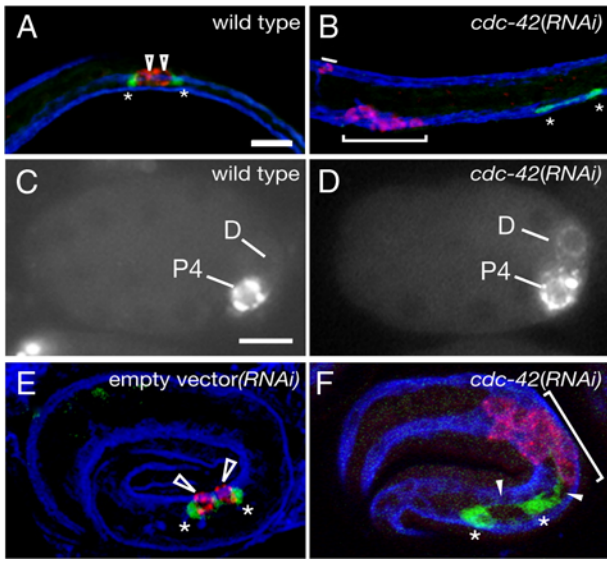


Fig. 5. Germ cells adopt muscle fates and are not associated with SGPs in *cdc-42(RNAi)* animals. SGPs (*ehm-3A::GFP*, green), P-granules (anti-PGL-1 (Kawasaki et al., 1998), red in panels A, B, E, F, white in panels C, D), and body wall muscles (NE8/4C6.3 marker (Goh and Bogaert, 1991), blue). (A) Wild-type L1 gonad primordium with polar SGPs (asterisks) and central PGCs (unfilled arrowheads) (B) *cdc-42(RNAi)* L1 animal with P-granules (red, brackets) in muscle cells (blue), and SGPs (green) lacking associated PGCs (no P-granule containing cells). (C) Wild-type embryo after the division of P3, the P granule marker *pgl-1::GFP* (Cheeks et al., 2004) is only in the germline precursor P4. (D) *cdc-42(RNAi)* embryo with P-granules in both P4 and its sibling D. (E) Wild-type 3-fold embryo; SGPs are associated with PGCs (F) *cdc-42(RNAi)* embryo; SGPs extend processes that appear to be directed towards the PGL-1 staining cells (filled arrowheads). Anterior, left; scale bar, 10  $\mu$ m.

P4 following *cdc-42(RNAi)* (Fig. 5C, D) and are found in body wall muscles later in embryogenesis (Fig. 5F).

In *cdc-42(RNAi)* embryos lacking PGCs, the SGPs are found in the posterior of the embryo but are usually separated from one another (Fig. 5F). This separation and lack of association with PGCs presumably leads to the formation of independent gonad primordia, each containing one of the SGPs (but no PGCs).

#### Gonad development in the absence of PGCs

To ask whether CDC-42 and the PAR complex have roles in gonad development independent of their roles in PGC formation, we compared their RNAi phenotypes to those of *mes-1(RNAi)* animals, which also lack PGCs but should not disrupt CDC-42 or the PAR-3 complex. Similar to *cdc-42(RNAi)* and *par-6(RNAi)* animals, we found that *mes-1(RNAi)* animals often have two ACs and split gonads, and become Muv adults (Tables 1 and 2). However, unlike *cdc-42(RNAi)* or *par-6(RNAi)* animals, neither abnormal cell positions in single primordia nor separated primordia containing both SGPs and PGCs were seen in *mes-1(RNAi)* animals. These account for 51% of affected *cdc-42(RNAi)* and 13% of affected *par-6(RNAi)* animals. Therefore *cdc-42* and *par-6* are required for normal SGP cell positions and/or coalescence of the gonad.

We also observed that SGPs in *cdc-42(RNAi)* and *par-6(RNAi)* L1 animals extend long protrusions when they are not associated with PGCs (Figs. 4E', F, H). In contrast, protrusions were not seen in *mes-1(RNAi)* animals lacking PGCs (Figs. 4G, H). Abnormal SGP protrusions were also observed in *cdc-42(RNAi)* embryos lacking PGCs (4/5 embryos after the one-fold stage) (Fig. 5F). Interestingly, these protrusions appear to be directed towards the abnormal P-granule containing muscle cells (3/4). These observations suggest that the PGCs might normally provide a cue for positioning the SGPs and that the P-granule containing muscle cells still provide this cue. Since the SGPs in *mes-1(RNAi)* animals that lack PGCs do not extend long processes, it seems likely that their formation in *cdc-42(RNAi)* and *par-6(RNAi)* animals is not the result of loss of the PGCs, but of a separate effect upon cell architecture.

The separation between SGPs observed in the absence of PGCs suggests that PGCs may play an attractive role during normal coalescence of the gonad. However, previous studies that eliminated PGCs by ablating the PGC precursor P4 did not cause SGP separation: the SGPs generated a single small, but normal, somatic gonad (Junkersdorf and Schierenberg, 1992; Sulston et al., 1983). One explanation for the normal coalescence of SGPs following P4 ablation is that the corpse of P4 might provide an attractive cue. Consistent with this possibility, following ablation of P4 we observed that its corpse was clearly associated with the L1 gonad primordium in 5/8 individuals (Supplementary Figure 3). We hypothesise that the PGCs provide an attractive cue that aids in the final steps of gonad coalescence.

#### Q cell and axon migration defects

Numerous studies have implicated Cdc42 in regulating cell migrations. Whether or not this cell migration role involves the PAR-3 complex has been little studied. In order to investigate whether CDC-42 and the PAR-3 complex act together during cell migrations in *C. elegans*, we asked whether RNAi of *cdc-42*, *par-3* or *par-6* affected migration of neurons and axons. We used the marker *mec-7::GFP* (Ch'ng et al., 2003) to visualise the descendants of the migratory Q neuroblasts during L4. In the wild type, QR migrates anteriorly and QL posteriorly during L1. At L4 the descendants of these cells QR.paa (AVM) and QL.paa (PVM) have distinctive anterior and posterior positions respectively and both express *mec-7::GFP*. In 21% of *cdc-42(RNAi)* and 5% of *par-3(RNAi)* animals, AVM failed to complete migration and was posterior to its normal location (Figs. 6B, E, F). In addition, 12% of *cdc-42(RNAi)* and 21% of *par-3(RNAi)* animals displayed axon migration and/or branching defects in ALM, PLM or A/PVM neurons (Figs. 6D, E). We also observed axon migration and/or branching defects of ALM and PLM neurons in 12% of *par-6(RNAi)* animals (Fig. 6F). These phenotypes are unlikely to be a consequence of gonad abnormalities as they are not displayed by *mes-1(RNAi)* animals (Fig. 6F). These results show that CDC-42 and the PAR-3 complex are required for neuroblast and axon migration.

## Discussion

CDC-42 and the conserved PAR-3/PAR-6/aPKC complex were previously shown to function together in establishing cell polarity in a variety of cell types (Etienne-Manneville, 2004; Etienne-Manneville and Hall, 2003b). Here, we show that a range of different polarised growth and cell migration processes show similar requirements for CDC-42 and the PAR-3 complex. We also demonstrate multiple roles for these proteins in

formation of the *C. elegans* gonad. Our results suggest that these proteins are likely to act together widely in cell migration and polarised cell growth in animals.

*CDC-42 and the PAR-3/PAR-6/PKC-3 complex act together broadly to regulate cell migration and polarised growth in C. elegans*

When *cdc-42*, *par-3* or *par-6* are knocked down the migration, polarised growth or positioning of several cell types is perturbed. Abnormal elongation of the VPCs and seam cells results in gaps between cells, and seam cells have a rounded rather than an elongated morphology. In ALM, PLM, and A/PVM neurons, axon migration is abnormal and PVM neuroblast migration is incomplete. Somatic gonad precursors (SGPs) adopt abnormal positions within the gonad primordium or form separated primordia. Thus, for each of these cell types, CDC-42 and the PAR-3 complex function in the same processes.

The phenotypes observed in this study are in the context of partial gene knockdown because complete RNAi inhibition of CDC-42 or members of the PAR-3 complex results in embryonic lethality. Furthermore, the cells assayed retain some polarity. For example, VPCs and seam cells have apical junctions and neurons have axons. Our results do not distinguish whether the observed defects are due to impaired cell polarisation, or are a direct effect on cell migration or polarised growth, or both. We also do not yet know the site of action of these genes. Since RNAi after hatching does not induce the effects we observed here (data not shown), maternal protein is sufficient for these processes. In the future, the use of cell specific constitutively active or dominant negative proteins could address this question. Nevertheless, our results indicate that CDC-42 and the PAR-3 complex function together broadly in a range of different developmental processes.

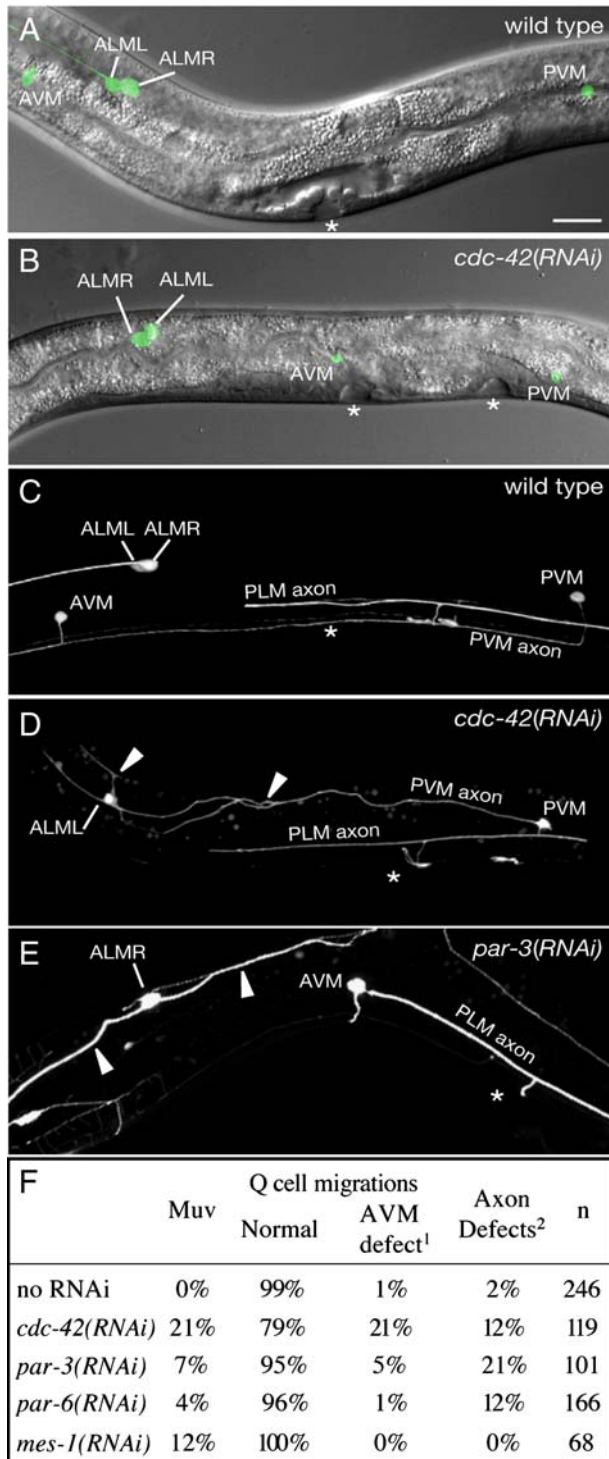


Fig. 6. Q cell migrations and axon guidance are disrupted by *cdc-42(RNAi)*. (A, B) Overlays of Nomarski images of L4 animals with projections of *mec-7::GFP* expression (green). (A) Wild-type; AVM neuron is anterior of the ALM neurons. (B) *cdc-42(RNAi)* animal with AVM neuron in the mid-body, posterior to ALM. (C–E) Projections of *mec-7::GFP* expression in L4 animals marking neurons and axons. In wild type animals the PVM axon migrates ventrally and enters the ventral nerve cord and both PVM and ALM axons are unbranched (C). Some *cdc-42(RNAi)* (D) and *par-3(RNAi)* (E) animals show defects of mechanosensory neurons, including dorso-ventral guidance defects of the PVM axon (D), branching defects of both PVM and ALML axons (arrowheads in panel D) and neuron polarity defects with extended anterior and posterior processes of ALMR (arrowheads in panel E). Only the left side of the body is shown in panel D and the right side in panel E. Anterior, left; ventral, down; vulvae are marked by asterisks; scale bar, 20  $\mu$ m. (F) Table of Q cell migration and axon guidance defects. <sup>1</sup>All affected control and *par-6(RNAi)* animals had only mild defects, while 7/25 affected *cdc-42(RNAi)* and 2/5 affected *par-3(RNAi)* animals had strong defects. <sup>2</sup>Defects include dorso-ventral and anterior–posterior guidance defects, abnormal branching and defasciculation of ALM axons (*cdc-42*, *par-3* or *par-6(RNAi)*), PLM axons (all treatments) and PVM axons (*cdc-42* or *par-3(RNAi)*). AVM: great granddaughter (QR.paa) of the anterior migrating QR neuroblast. PVM: great granddaughter (QL.paa) of the posterior migrating QL neuroblast.



### *CDC-42 and the PAR-3/PAR-6/PKC-3 complex together regulate multiple aspects of gonad formation*

In many *cdc-42(RNAi)* or *par-6(RNAi)* animals, PGCs are missing due to conversion of the germ cell lineage to a muscle fate at the 25-cell stage, a phenotype originally described for *mes-1* mutants (Strome et al., 1995). The majority of these animals also show separation of SGPs suggesting that the PGCs are required for coalescence of the SGPs into a single primordium. This partial reliance upon PGCs for coalescence is supported by the fact that the same phenotype is observed in *mes-1(RNAi)* animals which have a specific defect in PGC specification (Strome et al., 1995). A role for PGCs in attracting SGPs is also suggested by the observation that the abnormal processes generated by *cdc-42(RNAi)* or *par-6(RNAi)* SGPs in the absence of PGCs, are usually directed towards the P-granule containing descendants of D and P4.

Our suggestion that PGCs are important for gonad coalescence conflicts with previous studies suggesting that PGCs are dispensable for somatic gonad development: ablation of P4, the precursor of the PGCs, caused no apparent somatic gonad defects (Junkersdorf and Schierenberg, 1992; Sulston et al., 1983). However, in repeating these ablation experiments, we found that the P4 corpse is often incorporated into the gonad primordium, and therefore could be the source of an SGP cue. We propose that the PGCs provide an attractive cue for gonad coalescence.

In addition to those individuals lacking PGCs, *cdc-42(RNAi)* or *par-6(RNAi)* also result in animals which retain PGCs but have abnormal SGP positions within the gonad primordium or separate primordia. One possible explanation for these phenotypes is that the SGPs fail to migrate to their normal positions. However we have not detected significant differences in SGP positions during embryogenesis following *cdc-42(RNAi)* (data not shown). Alternately the primordium might initially form normally but become disrupted by subsequent movement of the SGPs. Whether this defect is a result of abnormal SGP behaviour or abnormal cohesion of the PGCs is an intriguing question for future studies.

### *CDC-42 and the PAR-3 complex in neuronal development and migration*

Cdc42 was first identified in *Saccharomyces cerevisiae* by a mutant that failed in polarised growth, arresting as large unbudded cells (Adams et al., 1990; Johnson and Pringle, 1990). Subsequently, it was shown that Cdc42 is also important in polarised cell shape changes in *Drosophila* epithelia, indicating a conserved function in animals (Eaton et al., 1995). Members of the Par3 complex were identified by their requirement for the polarity of the *C. elegans* zygote (Gotta et al., 2001; Kempfues et al., 1988; Tabuse et al., 1998; Watts et al., 1996). Although it is now well-established that Cdc42 and the Par3 complex act together in establishing animal cell polarity, there has been little previous work investigating their shared functions in other processes. We discuss here studies in neuronal and other systems showing

roles for members of the Par3 complex or Cdc42 and their possible links and effectors.

Although a role for the Par3 complex in polarised epithelial cell growth has not yet been reported, its requirement in axon formation supports such a function. Overexpression of Par3 or Par6 in mammalian hippocampal neurons in culture inhibits axon formation (Shi et al., 2003). Similarly, overexpression of Par6 in glial-guided neurons inhibits axon formation, causes shorter neurites and impairs migration (Solecki et al., 2004). In contrast, *Drosophila* interneurons mutant for Par-3 (Baz), Par-6 or aPKC have normal axon development *in vivo* (Rolls and Doe, 2004). None of these studies investigated involvement of Cdc42, but work in other neuron types in culture has shown a requirement for Cdc42 in neurite outgrowth (Li et al., 2002; Yuan et al., 2003). Cdc42 has also been shown to be involved in axon guidance in *Xenopus* and *Drosophila* (Kim et al., 2002; Yuan et al., 2003). In the present study we show that CDC-42 and the PAR-3 complex are both needed for axon guidance and neuronal migration in *C. elegans*. We did not uncover a role in axon formation, but our RNAi conditions may not have been sufficient to inhibit this process. Our findings, along with data from mammalian systems discussed above, suggest that CDC-42 and the PAR-3 complex act together in regulating neuronal polarity and axon guidance. It may be the case that these functions are also conserved in *Drosophila* but that low levels of residual maternal protein are sufficient to allow normal development of zygotic mutant neurons.

How might CDC-42 and the PAR-3 complex regulate cell migrations and polarised growth? In mammalian astrocytes, activation of Cdc42 at the leading edge leads to recruitment of a Par-3/Par-6/aPKC complex and reorientation of the MTOC in the direction of migration (Etienne-Manneville and Hall, 2001, 2003a). Orientation of the centrosome also plays a role in axon outgrowth and neuronal migrations. In glial guided neurons centrosomal re-orientation precedes axon outgrowth and overexpression of Par6 or aPKC impairs both this centrosome movement and the subsequent axon outgrowth and cell migration (Solecki et al., 2004). In the case of astrocytes, reorientation of the MTOC involves GSK-3 $\beta$  regulation of APC (Etienne-Manneville and Hall, 2003a). As the *C. elegans* APC homologue APR-1 plays a role in the migration and elongation of hypodermal cells during embryogenesis (Hoier et al., 2000), it is a potential effector of CDC-42 and the PAR-3 complex in regulating cell migration and polarised growth.

Other possible effectors are Rac and Rho GTPases. Rac GTPases regulate cell migration through inducing actin polymerisation at the leading edge (Wittmann and Waterman-Storer, 2001). In mammalian neural cells Cdc42 and Par-3 activate Rac through interaction with Rac GEFs (Chen and Macara, 2005; Nishimura et al., 2005). In *C. elegans* the three Rac homologues, CED-10, MIG-2 and RAC-2 have partially redundant roles in the migration of a wide-range of cell types (Lundquist et al., 2001). MIG-2, for example, is required for axon guidance and Q cell migrations, with mutants displaying abbreviated migrations of QR and QL (Zipkin et al., 1997). Recently, Norman et al. (2005) showed that CDC-42 acts with MIG-2 and CED-10 in the migration of gonadal distal tip cells,

supporting a possible role for MIG-2 and CED-10 as CDC-42 effectors. In addition, Cram et al. (2006) recently identified *cdc-42* as a gene required for distal tip cell migration in an RNAi screen. In contrast to Rac, Rho regulates actin behaviour at the rear of migrating cells and is excluded from the leading edge (Wittmann and Waterman-Storer, 2001). In migrating fibroblasts the Par6/aPKC complex promotes protrusive activity by recruiting the E3 ubiquitin ligase Smurf-1, which locally degrades RhoA (Wang et al., 2003). In *C. elegans*, the Rho homologue, RHO-1, plays a role in P cell migration (Spencer et al., 2001). Thus CDC-42 and the PAR-3 complex might regulate cell migrations in *C. elegans* via modulation of Rac and/or Rho activity.

Finally, in addition to regulation of the cytoskeleton, Cdc42 has also been shown to be involved in polarised secretion via the exocyst complex (Zhang et al., 2001). Members of this complex are also potential candidates for mediating elongation of the VPCs and seam cells.

In summary, we have provided evidence that CDC-42 and the PAR-3 complex act together in a range of cell types for polarised cell growth, cell migrations and cell positioning. The challenge for the future will be to link these proteins to their activators and effectors to understand how they together carry out these diverse processes.

## Acknowledgments

Thanks to Dan Shaye, Neil Hopper, Renaud Legouis, Paul Sternberg, Iva Greenwald, Henrik Korswagen, Jim Priess, Judith Kimble, Susan Strome and Stuart Kim for strains, reagents and advice. We are grateful to Gino Poulin, Monica Gotta, David Rivers, Nathalie Le Bot and Sarah Bray for help and useful discussions and especially to DMR and Pouly for comments on the manuscript. Thanks also to Ravi Kamath and Andy Fraser who made the initial observation of a Muv phenotype in *cdc-42(RNAi)*. Some strains used in this work were provided by the *Caenorhabditis* Genetics Center, which is funded by the NIH National Center for Research Resources (NCR), and the International *C. elegans* Gene Knockout Consortium. DW was supported by a Wellcome Trust PhD studentship. LM is supported by a grant from the National Science Foundation (IOB-0617897). JA is a Wellcome Trust Senior Research Fellow (054523).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2007.02.022.

## References

- Adams, A.E., Johnson, D.I., Longnecker, R.M., Sloat, B.F., Pringle, J.R., 1990. CDC42 and CDC43, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* 111, 131–142.
- Aono, S., Legouis, R., Hoose, W.A., Kempfues, K.J., 2004. PAR-3 is required for epithelial cell polarity in the distal spermatheca of *C. elegans*. *Development* 131, 2865–2874.
- Brenner, S., 1974. The genetics of *Caenorhabditis elegans*. *Genetics* 77, 71–94.
- Capowski, E.E., Martin, P., Garvin, C., Strome, S., 1991. Identification of grandchildless loci whose products are required for normal germ-line development in the nematode *Caenorhabditis elegans*. *Genetics* 129, 1061–1072.
- Ch'ng, Q., Williams, L., Lie, Y.S., Sym, M., Whangbo, J., Kenyon, C., 2003. Identification of genes that regulate a left–right asymmetric neuronal migration in *Caenorhabditis elegans*. *Genetics* 164, 1355–1367.
- Cheeks, R.J., Canman, J.C., Gabriel, W.N., Meyer, N., Strome, S., Goldstein, B., 2004. *C. elegans* PAR proteins function by mobilizing and stabilizing asymmetrically localized protein complexes. *Curr. Biol.* 14, 851–862.
- Chen, X., Macara, I.G., 2005. Par-3 controls tight junction assembly through the Rac exchange factor Tiam1. *Nat. Cell Biol.* 7, 262–269.
- Cram, E.J., Shang, H., Schwarzbauer, J.E., 2006. A systematic RNA interference screen reveals a cell migration gene network in *C. elegans*. *J. Cell Sci.* 119, 4811–4818.
- Czuchra, A., Wu, X., Meyer, H., van Hengel, J., Schroeder, T., Geffers, R., Rottner, K., Brakebusch, C., 2005. Cdc42 is not essential for filopodium formation, directed migration, cell polarization, and mitosis in fibroblastoid cells. *Mol. Biol. Cell* 16, 4473–4484.
- Eaton, S., Auvinen, P., Luo, L., Jan, Y.N., Simons, K., 1995. CDC42 and Rac1 control different actin-dependent processes in the *Drosophila* wing disc epithelium. *J. Cell Biol.* 131, 151–164.
- Etienne-Manneville, S., 2004. Cdc42—the centre of polarity. *J. Cell Sci.* 117, 1291–1300.
- Etienne-Manneville, S., Hall, A., 2001. Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. *Cell* 106, 489–498.
- Etienne-Manneville, S., Hall, A., 2003a. Cdc42 regulates GSK-3beta and adenomatous polyposis coli to control cell polarity. *Nature* 421, 753–756.
- Etienne-Manneville, S., Hall, A., 2003b. Cell polarity: Par6, aPKC and cytoskeletal crosstalk. *Curr. Opin. Cell Biol.* 15, 67–72.
- Francis, G.R., Waterston, R.H., 1985. Muscle organization in *Caenorhabditis elegans*: localization of proteins implicated in thin filament attachment and I-band organization. *J. Cell Biol.* 101, 1532–1549.
- Goh, P.Y., Bogaert, T., 1991. Positioning and maintenance of embryonic body wall muscle attachments in *C. elegans* requires the *mup-1* gene. *Development* 111, 667–681.
- Gotta, M., Abraham, M.C., Ahringer, J., 2001. CDC-42 controls early cell polarity and spindle orientation in *C. elegans*. *Curr. Biol.* 11, 482–488.
- Greenwald, I., 2005. LIN-12/Notch signaling in *C. elegans*. In: T.C.e.R. Community (Ed.), “WormBook”. doi/10.1895/wormbook.1.7.1, <http://www.wormbook.org>.
- Greenwald, I.S., Sternberg, P.W., Horvitz, H.R., 1983. The *lin-12* locus specifies cell fates in *Caenorhabditis elegans*. *Cell* 34, 435–444.
- Hoier, E.F., Mohler, W.A., Kim, S.K., Hajnal, A., 2000. The *Caenorhabditis elegans* APC-related gene *apr-1* is required for epithelial cell migration and Hox gene expression. *Genes Dev.* 14, 874–886.
- Hurd, D.D., Kempfues, K.J., 2003. PAR-1 is required for morphogenesis of the *Caenorhabditis elegans* vulva. *Dev. Biol.* 253, 54–65.
- Inoue, T., Sherwood, D.R., Aspöck, G., Butler, J.A., Gupta, B.P., Kirouac, M., Wang, M., Lee, P.Y., Kramer, J.M., Hope, I., Burglin, T.R., Sternberg, P.W., 2002. Gene expression markers for *Caenorhabditis elegans* vulval cells. *Mech. Dev.* 119 (Suppl. 1), S203–S209.
- Joberty, G., Petersen, C., Gao, L., Macara, I.G., 2000. The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. *Nat. Cell Biol.* 2, 531–539.
- Johnson, D.I., Pringle, J.R., 1990. Molecular characterization of CDC42, a *Saccharomyces cerevisiae* gene involved in the development of cell polarity. *J. Cell Biol.* 111, 143–152.
- Junkersdorf, B., Schierenberg, E., 1992. Embryogenesis in *C. elegans* after elimination of individual blastomeres or induced alteration of the cell-division order. *Roux's Arch. Dev. Biol.* 202, 17–22.
- Kamath, R.S., Martinez-Campos, M., Zipperlen, P., Fraser, A.G., Ahringer, J., 2001. Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. *Genome Biol.* 2, research0002.1–research0002.10.
- Kamath, R.S., Fraser, A.G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., Kanapin, A., Le Bot, N., Moreno, S., Sohrmann, M., Welchman, D.P.,

- Zipperlen, P., Ahringer, J., 2003. Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* 421, 231–237.
- Karp, X., Greenwald, I., 2003. Post-transcriptional regulation of the E/Daughterless ortholog HLH-2, negative feedback, and birth order bias during the AC/VU decision in *C. elegans*. *Genes Dev.* 17, 3100–3111.
- Kawasaki, I., Shim, Y.H., Kirchner, J., Kaminker, J., Wood, W.B., Strome, S., 1998. PGL-1, a predicted RNA-binding component of germ granules, is essential for fertility in *C. elegans*. *Cell* 94, 635–645.
- Kemphues, K.J., Priess, J.R., Morton, D.G., Cheng, N.S., 1988. Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. *Cell* 52, 311–320.
- Kim, M.D., Kolodziej, P., Chiba, A., 2002. Growth cone pathfinding and filopodial dynamics are mediated separately by Cdc42 activation. *J. Neurosci.* 22, 1794–1806.
- Kimble, J., Hirsh, D., 1979. The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. *Dev. Biol.* 70, 396–417.
- Le Bot, N., Tsai, M.C., Andrews, R.K., Ahringer, J., 2003. TAC-1, a regulator of microtubule length in the *C. elegans* embryo. *Curr. Biol.* 13, 1499–1505.
- Lewis, J.A., Fleming, J.T., 1995. Basic culture methods. In: Epstein, H.F., Shakes, D.C. (Eds.), *Caenorhabditis elegans: Modern Biological Analysis of an Organism*. Academic Press.
- Li, X., Saint-Cyr-Proulx, E., Aktories, K., Lamarche-Vane, N., 2002. Rac1 and Cdc42 but not RhoA or Rho kinase activities are required for neurite outgrowth induced by the Netrin-1 receptor DCC (deleted in colorectal cancer) in N1E-115 neuroblastoma cells. *J. Biol. Chem.* 277, 15207–15214.
- Lin, D., Edwards, A.S., Fawcett, J.P., Mbamalu, G., Scott, J.D., Pawson, T., 2000. A mammalian PAR-3–PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. *Nat. Cell Biol.* 2, 540–547.
- Liu, Z., Fujii, T., Nukazuka, A., Kurokawa, R., Suzuki, M., Fujisawa, H., Takagi, S., 2005. *C. elegans* PlexinA PLX-1 mediates a cell contact-dependent stop signal in vulval precursor cells. *Dev. Biol.* 282, 138–151.
- Lundquist, E.A., Reddien, P.W., Hartwig, E., Horvitz, H.R., Bargmann, C.I., 2001. Three *C. elegans* Rac proteins and several alternative Rac regulators control axon guidance, cell migration and apoptotic cell phagocytosis. *Development* 128, 4475–4488.
- Macara, I.G., 2004. Par proteins: partners in polarization. *Curr. Biol.* 14, R160–R162.
- Mello, C.C., Kramer, J.M., Stinchcomb, D., Ambros, V., 1991. Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* 10, 3959–3970.
- Miskowski, J., Li, Y., Kimble, J., 2001. The sys-1 gene and sexual dimorphism during gonadogenesis in *Caenorhabditis elegans*. *Dev. Biol.* 230, 61–73.
- Murphy, A.M., Montell, D.J., 1996. Cell type-specific roles for Cdc42, Rac, and RhoL in *Drosophila* oogenesis. *J. Cell Biol.* 133, 617–630.
- Nance, J., 2005. PAR proteins and the establishment of cell polarity during *C. elegans* development. *BioEssays* 27, 126–135.
- Nishimura, T., Yamaguchi, T., Kato, K., Yoshizawa, M., Nabeshima, Y., Ohno, S., Hoshino, M., Kaibuchi, K., 2005. PAR-6–PAR-3 mediates Cdc42-induced Rac activation through the Rac GEFs STEF/Tiam1. *Nat. Cell Biol.* 7, 270–277.
- Norman, K.R., Fazio, R.T., Mellem, J.E., Espelt, M.V., Strange, K., Beckerle, M.C., Maricq, A.V., 2005. The Rho/Rac-family guanine nucleotide exchange factor VAV-1 regulates rhythmic behaviors in *C. elegans*. *Cell* 123, 119–132.
- Pinheiro, E.M., Montell, D.J., 2004. Requirement for Par-6 and Bazooka in *Drosophila* border cell migration. *Development* 131, 5243–5251.
- Poulin, G., Dong, Y., Fraser, A.G., Hopper, N.A., Ahringer, J., 2005. Chromatin regulation and sumoylation in the inhibition of Ras-induced vulval development in *Caenorhabditis elegans*. *EMBO J.* 24, 2613–2623.
- Rolls, M.M., Doe, C.Q., 2004. Baz, Par-6 and aPKC are not required for axon or dendrite specification in *Drosophila*. *Nat. Neurosci.* 7, 1293–1295.
- Seydoux, G., Greenwald, I., 1989. Cell autonomy of lin-12 function in a cell fate decision in *C. elegans*. *Cell* 57, 1237–1245.
- Seydoux, G., Schedl, T., Greenwald, I., 1990. Cell–cell interactions prevent a potential inductive interaction between soma and germline in *C. elegans*. *Cell* 61, 939–951.
- Shaye, D.D., Greenwald, I., 2002. Endocytosis-mediated downregulation of LIN-12/Notch upon Ras activation in *Caenorhabditis elegans*. *Nature* 420, 686–690.
- Shi, S.H., Jan, L.Y., Jan, Y.N., 2003. Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. *Cell* 112, 63–75.
- Solecki, D.J., Model, L., Gaetz, J., Kapoor, T.M., Hatten, M.E., 2004. Par6alpha signaling controls glial-guided neuronal migration. *Nat. Neurosci.* 7, 1195–1203.
- Spencer, A.G., Orita, S., Malone, C.J., Han, M., 2001. A RHO GTPase-mediated pathway is required during P cell migration in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 98, 13132–13137.
- Strome, S., Wood, W.B., 1982. Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 79, 1558–1562.
- Strome, S., Martin, P., Schierenberg, E., Paulsen, J., 1995. Transformation of the germ line into muscle in mes-1 mutant embryos of *C. elegans*. *Development* 121, 2961–2972.
- Sulston, J.E., Hodgkin, J., 1988. Methods. In: Wood, W.B. (Ed.), *The Nematode Caenorhabditis elegans*. Cold Spring Harbor Laboratory Press.
- Sulston, J.E., Horvitz, H.R., 1977. Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Dev. Biol.* 56, 110–156.
- Sulston, J.E., Schierenberg, E., White, J.G., Thomson, J.N., 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 100, 64–119.
- Tabuse, Y., Izumi, Y., Piano, F., Kemphues, K.J., Miwa, J., Ohno, S., 1998. Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in *Caenorhabditis elegans*. *Development* 125, 3607–3614.
- Wang, M., Sternberg, P.W., 2000. Patterning of the *C. elegans* 1° vulval lineage by RAS and Wnt pathways. *Development* 127, 5047–5058.
- Wang, M., Sternberg, P.W., 2001. Pattern formation during *C. elegans* vulval induction. *Curr. Top. Dev. Biol.* 51, 189–220.
- Wang, H.R., Zhang, Y., Ozdamar, B., Ogunjimi, A.A., Alexandrova, E., Thomsen, G.H., Wrana, J.L., 2003. Regulation of cell polarity and protrusion formation by targeting RhoA for degradation. *Science* 302, 1775–1779.
- Watts, J.L., Etemad-Moghadam, B., Guo, S., Boyd, L., Draper, B.W., Mello, C.C., Priess, J.R., Kemphues, K.J., 1996. par-6, a gene involved in the establishment of asymmetry in early *C. elegans* embryos, mediates the asymmetric localization of PAR-3. *Development* 122, 3133–3140.
- Wittmann, T., Waterman-Storer, C.M., 2001. Cell motility: can Rho GTPases and microtubules point the way? *J. Cell Sci.* 114, 3795–3803.
- Yang, L., Wang, L., Zheng, Y., 2006. Gene targeting of Cdc42 and Cdc42GAP affirms the critical involvement of Cdc42 in filopodia induction, directed migration, and proliferation in primary mouse embryonic fibroblasts. *Mol. Biol. Cell* 17, 4675–4685.
- Yoo, A.S., Bais, C., Greenwald, I., 2004. Crosstalk between the EGFR and LIN-12/Notch pathways in *C. elegans* vulval development. *Science* 303, 663–666.
- Yuan, X.B., Jin, M., Xu, X., Song, Y.Q., Wu, C.P., Poo, M.M., Duan, S., 2003. Signalling and crosstalk of Rho GTPases in mediating axon guidance. *Nat. Cell Biol.* 5, 38–45.
- Zhang, X., Bi, E., Novick, P., Du, L., Kozminski, K.G., Lipschutz, J.H., Guo, W., 2001. Cdc42 interacts with the exocyst and regulates polarized secretion. *J. Biol. Chem.* 276, 46745–46750.
- Zipkin, I.D., Kindt, R.M., Kenyon, C.J., 1997. Role of a new Rho family member in cell migration and axon guidance in *C. elegans*. *Cell* 90, 883–894.