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

David P. Welchman [a], Laura D. Mathies [b], Julie Ahringer [a,*]

[a] *The Gurdon Institute and Department of Genetics, University of Cambridge, Cambridge, CB2 1QN, UK*
[b] *Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614, USA*

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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.

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 mPar6α 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 (J. Ahringer).

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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, *syIs77[zmp-1::YFP]* II, *muIs32[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), *jcIs1[ajm-1::GFP; rol-6(su1006)]* IV, *lin-3(n378)* IV, *lin-45(n2018)* IV, *syIs49[zmp-1::GFP; dpy-20(+)]* IV, *syIs67[zmp-1::CFP; unc-119(+)]* V, *syIs59[egl-17::CFP]* X, *zuIs77[par-6::GFP; unc-119(+)], mcIs[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::lac-Z]*. 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).

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