

The *Caenorhabditis elegans* *ect-2* RhoGEF gene regulates cytokinesis and migration of epidermal P cells

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A reduction-of-function mutation in *ect-2* was isolated as a suppressor of the Multivulva phenotype of a *lin-31* mutation. Analysis using markers indicates that this mutation causes defects in both the cytokinesis and migration of epidermal P cells, phenotypes similar to those caused by expressing a *rho-1* dominant-negative construct. *ect-2* encodes the *Caenorhabditis elegans* orthologue of the mouse Ect2 and *Drosophila* Pebble that function as guanine nucleotide exchange factors (GEFs) for Rho GTPases. The *ect-2::GFP* reporter is expressed in embryonic cells and P cells. RNA interference of *ect-2* causes sterility and embryonic lethality, in addition to the P-cell defects. We have determined the lesions of two *ect-2* alleles, and described their differences in phenotypes in specific tissues. We propose a model in which ECT-2GEF not only activates RHO-1 for P-cell cytokinesis, but also collaborates with UNC-73GEF and at least two Rac proteins to regulate P-cell migration.

Keywords: Pebble; Etc2; Rho; Rac; *unc-73*

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INTRODUCTION

The small GTPases of the Rho family have roles in fundamental cellular functions such as cytokinesis, migration and polarization (Schmidt & Hall, 2002). Molecular and genetic studies have shown that the *Drosophila* RhoGEF Pebble (PBL) and its mammalian orthologue, the proto-oncogene product ECT2, are necessary for cytokinesis (Miki *et al*, 1993; Prokopenko *et al*, 1999; Tatsumoto *et al*, 1999). This function is mediated by PBL binding to Rho1, but not to Rac1 or Cdc42. More recently, PBL has been shown to genetically and physically interact with the Rho family GTPase-activating protein (GAP), RacGAP50C (Somers & Saint, 2003). In *Caenorhabditis elegans*, LET-502/Rho-binding kinase has been shown to regulate embryonic elongation (Wissmann *et al*, 1997). A weak loss-of-function (*lf*) allele of *ect-2* (also known as *let-21*), which encodes the likely PBL/ECT2 orthologue, has been shown to enhance the cytokinesis defect

during the first embryonic cell division caused by RNA interference (RNAi) of the *par-2* gene (Dechant & Glotzer, 2003), suggesting a potential role for *ect-2* in mitosis.

Rho and Rac GTPases have crucial roles during the development of epidermal P cells. At hatching, 12 P cells are present on the sublateral sides of the larvae. In the mid-first larval (L1) stage, these cells migrate to the ventral midline. After migration, each P cell divides along the anterior–posterior axis to produce two daughter cells, Pn.a and Pn.p. The Pn.a cells continue to divide for up to three rounds to generate motor neurons, whereas the Pn.p cells of hermaphrodites will later either be induced to adopt a vulval cell fate or fuse to the surrounding epidermal syncytium (Sulston, 1976). The RHO-1 GTPase appears to be required for both P-cell migration and cytokinesis, as these processes are disrupted in animals that carry a dominant-negative (dn) Rho mutant transgene (Spencer *et al*, 2001). Consequently, the Pn.a and Pn.p cells are missing in these animals and they are therefore defective in vulval formation and movement. Two Rac homologues, MIG-2 and CED-10, have been shown to act redundantly to regulate P-cell migration, but not cytokinesis (Spencer *et al*, 2001; Wu *et al*, 2002). These Rac proteins, along with a third Rac homologue, RAC-2, also act redundantly in the migration, axon guidance and morphogenesis of several other cells (Zipkin *et al*, 1997; Lundquist *et al*, 2001; Kishore & Sundaram, 2002; Wu *et al*, 2002). These studies indicate that Rho and Rac small GTPases collaborate to regulate P-cell migration, whereas Rho also functions in P-cell cytokinesis. Additionally, *unc-73*, which encodes a Trio-like guanine nucleotide exchange factor (GEF) protein, also has a role in axon guidance and cell migration (Zipkin *et al*, 1997; Steven *et al*, 1998; Lundquist *et al*, 2001; Spencer *et al*, 2001; Kishore & Sundaram, 2002; Wu *et al*, 2002). Although UNC-73 has two GEF domains that have been shown to catalyse the exchange reaction for both Rho and Rac *in vitro*, *unc-73(lf)* mutations do not disrupt P-cell cytokinesis (Spencer *et al*, 2001). Therefore, another GEF protein may interact with RHO-1 for the cytokinesis function. In this report, we describe our analysis of the *ect-2*/RHO GEF functions in P-cell development.

RESULTS AND DISCUSSION

Isolation of *ku427* as a suppressor of a *lin-31* mutant

We isolated the mutation *ku427* as a suppressor of the Multivulva (Muv) phenotype of a *lin-31* allele (Fig 1; see the supplementary information online for details). The suppression of the Muv

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Table 1 | The migration and cytokinesis defect in the P-cell derivatives

Genotype ^a	Average number of Pn.p cells (n)	Percentage with ectopic P cells ^b (n)	Percentage with cytokinesis defects ^c (n)
<i>N2 wild type</i>	11.0 (20)	0 (50)	0 (50)
<i>col-10:rho-1(T17N, dn)</i>	3.6 (15)	68 (50)	100 (50)
<i>ect-2(RNAi)</i>	5.2 (25)	10 (50)	88 ^e (50)
<i>ect-2(ku427)</i>	7.1 (31)	17 (100)	70 ^e (100)
<i>ect-2(e1778)</i>	ND	6 (50)	34 ^e (50)
<i>unc-73(e936)</i>	7.6 (25) ^d	82 (50)	0.0 (50)
<i>unc-73(RNAi)</i>	ND	6 (50)	0.0 (50)
<i>unc-73(e936); ect-2(RNAi)</i>	ND	72 (50)	64 (50)
<i>let-502(RNAi)</i>	ND	12 (50)	0.0 (50)
<i>zen-4(RNAi)</i>	ND	0.0 (50)	63 (50)
<i>cyk-4(RNAi)</i>	ND	0.0 (50)	64 (50)
<i>ced-10(n3246); mig-2(RNAi)</i>	9.0 (10) ^d	64 (50)	0 (50)
<i>unc-73(RNAi); ect-2(ku427)</i>	ND	48 (50)	76 (50)

^aAll strains contain the integrated *unc-47::GFP* transgene (McIntire et al, 1997).

^bThe percentage of animals with ectopic P cells due to failed P-cell migration, indicated by the lateral position of the *unc-47::GFP* marker. *n* is the number of animals examined.

^cPercentage of animals with cytokinesis defects in P cells (Fig 3E). The same animals examined for P cell migration were examined.

^dData from Spencer et al (2001).

^eFor *ect-2(RNAi)*, *ect-2(ku427)* and *ect-2(e1778)*, the average P cells with visible cytokinesis defects per animal are 7.1, 2.1 and 1.4, respectively.

ND, not determined.

GEF proteins for Rho family GTPases (Miki et al, 1993; Prokopenko et al, 1999). Similarly to ECT2 and Pebble, ECT-2 has BRCT1 and BRCT2 domains in its amino-terminal half and a GEF domain in its carboxy-terminal half. The GEF domain contains a Dbl homology (DH) domain and a pleckstrin homology (PH) domain. BRCT domains are thought to be important for cell-cycle regulation, whereas the DH/PH domain interacts with Rho family proteins and catalyses the GDP to GTP exchange reaction (Prokopenko et al, 1999). In addition, ECT-2 has two putative nuclear localization signals (amino acids 9–16 and 361–367) in the N-terminal and middle regions.

Because *ku427* has a lesion in the promoter region, we compared the sequence of the promoter region of *ect-2* with that in another *Caenorhabditis* species, *C. briggsae*. This region is highly conserved between the two species and contains the FOXO-binding consensus site (Fig 1B) (Pierrou et al, 1994). We then used quantitative reverse transcription–PCR to determine whether the mutation results in a decrease in the transcription level of *ect-2*. We observed that the level of *ect-2* in the *ku427* mutant was sevenfold lower than that in wild type (Fig 1D). We also failed to detect green fluorescent protein (GFP) fluorescence from an *ect-2::GFP* reporter transgene that contained this lesion (data not shown).

***ect-2::GFP* is strongly expressed in P cells**

To investigate the expression patterns of *ect-2* in *C. elegans*, we made transgenic worms containing a transcriptional *ect-2::GFP* fusion construct (pKM37 in Fig 1A). In these worms, GFP expression was detected from embryogenesis to adulthood. In embryogenesis, almost all cells, other than those in the gut lineage, showed GFP expression (Fig 2A). Before P-cell migration in the L1 stage, expression was detected only in Q cells (Fig 2B).

After P-cell migration and division, expression was seen in the P-cell derivatives (Fig 2C) and distal tip cells (data not shown). In L3, GFP expression was present only in the vulval precursor cells and their derivatives (Fig 2D). In L4 and adulthood, GFP fluorescence was detected only in some neuronal cells (data not shown).

***ect-2* is required for cytokinesis in P cells**

ect-2(ku427) and *ect-2(RNAi)* caused the absence of Pn.p cells (Table 1). To determine the cause of this phenotype, we used *unc-47::GFP* to mark GABAergic neurons (Fig 3C), as 13 of the 26 GABAergic neurons are derived from P cells (Pn.a cells) (McIntire et al, 1997), and followed the P-cell lineage in *ku427* mutants. We observed that in *ect-2(ku427)* and *ect-2(RNAi)* animals, although nuclear divisions of P cells seemed to be completed at the late larval stage, cytokinesis seemed to be disrupted (Fig 3; Table 1). Owing to the likely failed cytokinesis of some P cells (and also Pn.a derivatives) in *ku427* mutants, multinuclear cells were formed, as visualized by *UNC-47::GFP* fluorescence (Fig 3E). The Pn.p nuclei were observed in these cells, but the stereotypical Pn.p nuclear morphology was no longer seen. We previously showed that, when expressed under an epidermis-specific promoter, a *rho-1(T19N dn)* transgene caused the same cytokinesis defect in P cells (Spencer et al, 2001) (Table 1). To confirm the role of *ect-2* in cytokinesis, we also examined the effect of *ect-2(RNAi)* on divisions of early embryos. Examination of tubulin::GFP-marked live embryos showed a strong cytokinesis defect in these embryos, whereas mitosis seemed to be normal (supplementary Fig 1 online). Our data are consistent with the evidence that Pebble in *Drosophila* and ECT2 in the mouse activate Rho GTPases to regulate cytokinesis (Prokopenko et al, 1999; Tatsumoto et al, 1999). Interestingly, as

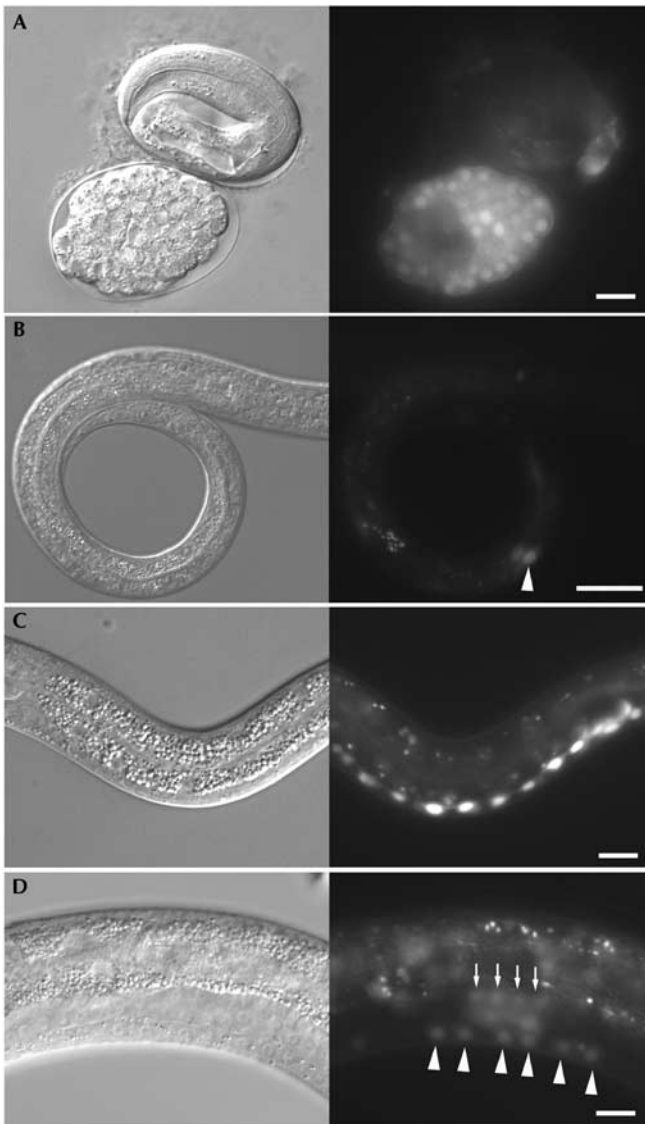


Fig 2 | *ect-2::GFP* expression pattern. Differential interference contrast (left column) and fluorescence (right column) images of a twofold embryo (A), L1 larvae (B,C) and an L3 larva (D). The arrowhead in (B) indicates Q cell, arrowheads in (D) indicate daughters of three Pn.ps and the arrows in (D) indicates gonadal cells. Scale bars, 10 μ m.

reported for the *Drosophila rho* gene (Schumacher et al, 2004), we failed to rescue either the cytokinesis or the migration defects in P cells in the *ku427* mutant by expressing a hyperactive *rho-1* mutant gene (*G14V gf*) (data not shown). This may indicate that cycling between the GDP-bound and the GTP-bound forms is important for the proper functioning of Rho.

As mentioned above, *ect-2::GFP* is expressed in a limited number of cell lineages during postembryonic development. This raises the possibility that ECT-2-mediated cytokinesis is not required for all cell divisions. Such an idea is consistent with a recent report that states that activation of Rho A for cytokinesis is tissue specific in mammals (Yoshizaki, 2004).

The P-cell cytokinesis defect was not observed in the mutants of two *rac* genes (*ced-10* and *mig-2*) or in *unc-73* (Table 1).

Similarly, in *Drosophila*, mutations in *Rac* genes do not result in any obvious cytokinesis defects (Hakeda-Suzuki et al, 2002).

ect-2 is also involved in P-cell migration

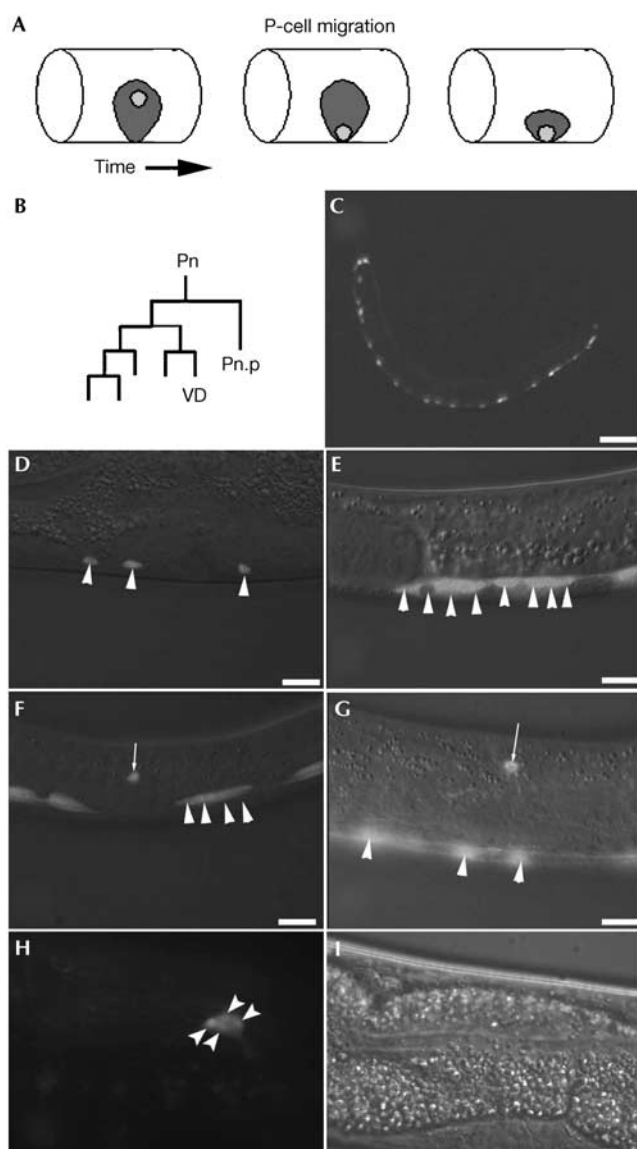
We have previously shown that *rho-1(dn)* causes a strong defect in P-cell migration from the sublateral position to the ventral midline, shortly after hatching (Spencer et al, 2001). The worms show P-cell-derived neurons on the lateral surface instead of the normal ventral midline positions (Table 1). We also observed misplaced neurons in the *ect-2(ku427)* and *ect-2(RNAi)* animals, although this phenotype is significantly weaker than that of *rho-1(dn)* (Fig 3F,G; Table 1). In addition, we have also observed multinucleated P cells in the lateral positions in late-stage larvae (Fig 3H,I), which suggests that the cytokinesis and migration defects can be associated with the same P cells. An *unc-73* mutation also displaced neurons in the lateral position in 58% of animals (Table 1) (Spencer et al, 2001). These data indicate that *ect-2* is also involved in regulating P-cell migration, but the contribution by this gene to P-cell migration is minor compared with that of *rho-1* or *unc-73*. Consistent with a proposal that both ECT-2 and UNC-73 contribute to the exchange reaction of RHO-1 during P-cell migration (Fig 4), *ect-2(ku427); unc-73(RNAi)* showed a stronger cell migration defect than either *ku427* or *unc-73(RNAi)* alone (Table 1).

A role for *ect-2* in cell migration is consistent with the recent finding in *Drosophila* that Pebble is involved in the cell migration of the mesoderm in embryogenesis (Schumacher et al, 2004; Smallhorn et al, 2004). However, it was suggested that Rho is not involved in this Pebble-mediated cell migration, as a cell migration defect was not observed when a dominant-negative (*dn*) form of Rho was expressed in the cells (Schumacher et al, 2004).

Phenotypic differences among the two *ect-2* alleles and RNAi

The *ect-2(ku427)* mutant showed a strong Egl phenotype and a relatively weak Unc phenotype owing to the cytokinesis and migration defects of P cells. The average brood size was 68 ($n=14$) and no obvious germline defects were observed. In addition, this strain showed no obvious embryonic lethal phenotype (1%, $n=222$). In contrast, 47% ($n=299$) of the *ect-2* (RNAi) animals were embryonic lethal. Escapers showed strong Unc and Ste (sterile) phenotypes (100%, $n=50$) as well as stronger cytokinesis defects in P cells (Table 1). These animals sometimes showed a ball-like gonad (data not shown). The differences in phenotypes may be due to the difference in the extent of loss of *ect-2* gene activity under the two conditions. As described earlier, the *ku427* mutant has a lesion in the promoter region that causes a decrease in transcription of the gene. The residual low expression level of *ect-2* in *ku427* animals appears to be sufficient to cover the gene functions in the embryo as well as in the germ line, and P cells may be more sensitive to the reduction in the gene dosage. Alternatively, it is also possible that the reduction of the transcription level by the *ku427* lesion is more drastic in the P-cell lineage, as we failed to detect any expression from the GFP reporter driven by the mutant promoter in P cells.

Interestingly, the *ect-2(e1778)* allele caused only a weak Unc phenotype, but a complete Ste phenotype. This allele also resulted in P-cell defects that were weaker than those of *ku427* (Table 1). The difference between the two alleles may indicate that there is some tissue specificity in the effects of these mutations.



◀ **Fig 3** | Cytokinesis and migration defects in the P cells of *ect-2* mutants. (A) Diagram illustrating P-cell migration during L1. P cells are present on the sublateral surface of the larvae on hatching. During mid-L1, the leading edges cytoplasm moves to a ventral contralateral position. P-cell nuclei and the rest of the cytoplasm sequentially move ventrally and come to rest at the ventral midline (Sulston, 1976). (B) Typical P-cell division pattern. Once migration is complete, each P cell divides into Pn.a and Pn.p cells. Pn.a cells undergo further rounds of division and generate five neurons including GABAergic neurons. (C) *unc-47::GFP* expression in the P-cell-derived GABAergic neurons on ventral cord. A total of 13 of the 26 GABA neurons are P-cell derived. (D) L4 wild-type hermaphrodite. Individual GABA neurons are seen (arrowhead). (E) An L4 larva treated with *ect-2(RNAi)*. *UNC-47::GFP* is seen in cells containing many nuclei (arrowheads), indicating failed P-cell cytokinesis. (F) An L4 larva treated with *ect-2(RNAi)*. A *unc-47::GFP* expression cell is seen in the lateral position, indicating failed P-cell migration (arrow) and failed P-cell cytokinesis (arrowheads). (G) Same as (F), except that an *ect-2(ku427)* mutant worm is shown. The arrow indicates failed P-cell migration. Scale bars in (C–G): 10 μ m. (H,I) Fluorescent (H) and differential interference contrast (I) images of an *ect-2(ku427)* L4 larva that contains the *unc-47::gfp* integrated transgene, showing a multinucleated P cell mislocalized in a lateral position. The arrowheads indicate the nuclei.

RhoGAP, both of which are part of a central spindle complex that is involved in the formation of the central spindles and cleavage furrow during cytokinesis of early embryos (Raich *et al*, 1998; Jantsch-Plunger *et al*, 2000; Severson *et al*, 2000; Mishima *et al*, 2002; Portereiko *et al*, 2004). As indicated in Table 1, worms treated with *zen-4* RNAi or *cyk-4* RNAi showed the cytokinesis defect in P cells, but not the migration defect, suggesting that *ect-2/RhoGEF* and *rho-1* interact with different factors for the migration function.

In *Drosophila*, Pebble, Racgap50C (homologue of *cyk-4*) and Pavarotti (homologue of *zen-4*) form a complex that is involved in the positioning of the contractile ring and coordinates F-actin and microtubule remodelling during cytokinesis (Somers & Saint, 2003). Our results indicate that the functions of Rho, its regulators and associated proteins in cytokinesis are conserved.

Speculations

A model for *ect-2* function, in relationship with other genes, is shown in Fig 4. The mechanism by which the Rho GEF proteins are regulated for the cell migration function is at present not clear in *C. elegans* or other systems (Raftopoulou & Hall, 2004). The mechanism acting downstream of Rho for the P-cell migration function also remains to be understood. We have shown that an *lf* mutation in *let-502*, which encodes a Rho-activated kinase (Wissmann *et al*, 1997), shows a partially penetrant defect in P-cell migration but not a defect in cytokinesis (Spencer *et al*, 2001) (Table 1), suggesting that *let-502* is involved in the migration process. However, its weak phenotype suggests that there are other Rho effectors acting in the process.

METHODS

Methods for scoring phenotypes. For scoring P-cell numbers, we counted the Pn.p cells of L2 larvae under Nomarski optics. For P-cell migration and cytokinesis defects, we examined L4 larvae

ect-2(e1778) has an in-frame deletion that removes the BRCT1 domain (111–207 aa; Fig 1E). Thus, it is possible that the BRCT1 domain has a more crucial role in the germ line. Alternatively, the *e1778* allele could have eliminated the gene activity. However, a strong maternal effect masks the requirements of the gene during embryogenesis and in other lineages, but not during the germline development that occurs in the later stages. This leads to the strong Ste phenotype associated with the homozygous *e1778* animals from the heterozygous mother. A similar phenomenon has been observed in several essential *C. elegans* genes (Fay & Han, 2000; Antoshechkin & Han, 2002).

zen-4 and cyk-4 function in cytokinesis of P cells

ect-2 and *rho-1* mutants have defects in both P-cell migration and cytokinesis. To determine whether the migration defect is related to the role of *ect-2* and *rho-1* in the cytokinesis mechanism, we examined the effects of RNAi of two other genes, *zen-4* and *cyk-4*. *zen-4* encodes a kinesin-like protein and *cyk-4* encodes a

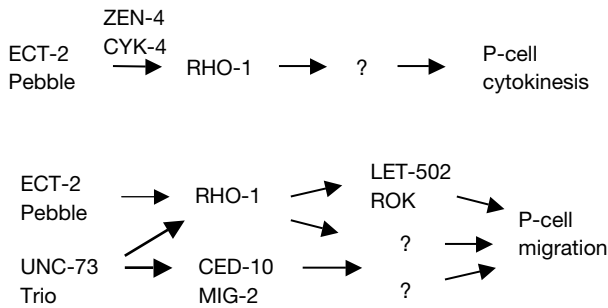


Fig 4 | Model for the small GTPase functions in P-cell migration and cytokinesis. ECT-2 is proposed to act as an activator of RHO-1 for roles in both P-cell cytokinesis and migration on the basis of their similar phenotypes and probable biochemical functions known to be associated with their homologues in other organisms. ZEN-4 and CYK-4 are proposed to act as regulators of RHO-1 on the basis of their genetic phenotype and biochemical functions. Rac family small GTPase CED-10 and MIG-2 are proposed to act downstream of UNC-73 to regulate P-cell migration. UNC-73, with the two DH/PH domains, may act on both Rac and Rho proteins for the migration function. See text for more information.

containing the *unc-47::GFP* transgene for abnormal lateral positions of GFP-positive cells and for the appearance of multinuclear GFP-positive cells, respectively.

Mutant isolation and position cloning. *ect-2(ku427)* was isolated as a suppressor of the Muv phenotype of the *lin-31(n301); eff-1(hy21)* double mutants. Details regarding the strains and the methods for the screen, mapping as well as cloning, are given in the supplementary information online.

Quantitative RT-PCR, RNAi and construction of plasmids for transgenic animals. See the supplementary information online for details.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

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REFERENCES

Antoshechkin I, Han M (2002) The *C. elegans evl-20* gene is a homolog of the small GTPase ARL2 and regulates cytoskeleton dynamics during cytokinesis and morphogenesis. *Dev Cell* **2**: 579–591

Dechant R, Glotzer M (2003) Centrosome separation and central spindle assembly act in redundant pathways that regulate microtubule density and trigger cleavage furrow formation. *Dev Cell* **4**: 333–344

Fay DS, Han M (2000) Mutations in *cye-1*, a *Caenorhabditis elegans* cyclin E homolog, reveal coordination between cell-cycle control and vulval development. *Development* **127**: 4049–4060

Hakeda-Suzuki S, Ng J, Tzu J, Dietzl G, Sun Y, Harms M, Nardine T, Luo L, Dickson BJ (2002) Rac function and regulation during *Drosophila* development. *Nature* **416**: 438–442

Jantsch-Plunger V, Gonczy P, Romano A, Schnabel H, Hamill D, Schnabel R, Hyman AA, Glotzer M (2000) CYK-4: a Rho family gtpase activating

protein (GAP) required for central spindle formation and cytokinesis. *J Cell Biol* **149**: 1391–1404

Kishore RS, Sundaram MV (2002) ced-10 Rac and mig-2 function redundantly and act with unc-73 trio to control the orientation of vulval cell divisions and migrations in *Caenorhabditis elegans*. *Dev Biol* **241**: 339–348

Lundquist EA, Reddien PW, Hartweg E, Horvitz HR, Bargmann CI (2001) Three *C. elegans* Rac proteins and several alternative Rac regulators control axon guidance, cell migration and apoptotic cell phagocytosis. *Development* **128**: 4475–4488

McIntire SL, Reimer RJ, Schuske K, Edwards RH, Jorgensen EM (1997) Identification and characterization of the vesicular GABA transporter. *Nature* **389**: 870–876

Miki T, Smith CL, Long JE, Eva A, Fleming TP (1993) Oncogene *ect2* is related to regulators of small GTP-binding proteins. *Nature* **362**: 462–465

Mishima M, Kaitna S, Glotzer M (2002) Central spindle assembly and cytokinesis require a kinesin-like protein/RhoGAP complex with microtubule bundling activity. *Dev Cell* **2**: 41–54

Pierrou S, Hellqvist M, Samuelsson L, Enerback S, Carlsson P (1994) Cloning and characterization of seven human forkhead proteins: binding site specificity and DNA bending. *EMBO J* **13**: 5002–5012

Portereiko MF, Saam J, Mango SE (2004) ZEN-4/MKLP1 is required to polarize the foregut epithelium. *Curr Biol* **14**: 932–941

Prokopenko SN, Brumby A, O'Keefe L, Prior L, He Y, Saint R, Bellen HJ (1999) A putative exchange factor for Rho1 GTPase is required for initiation of cytokinesis in *Drosophila*. *Genes Dev* **13**: 2301–2314

Raftopoulos M, Hall A (2004) Cell migration: Rho GTPases lead the way. *Dev Biol* **265**: 23–32

Raich WB, Moran AN, Rothman JH, Hardin J (1998) Cytokinesis and midzone microtubule organization in *Caenorhabditis elegans* require the kinesin-like protein ZEN-4. *Mol Biol Cell* **9**: 2037–2049

Riddle DL, Blumenthal T, Meyer BJ, Priess JR (eds) (1997) *C. elegans II*. Plainview, NY, USA: Cold Spring Harbor Laboratory Press

Schmidt A, Hall A (2002) Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. *Genes Dev* **16**: 1587–1609

Schumacher S, Gryzik T, Tannebaum S, Muller HA (2004) The RhoGEF Pebble is required for cell shape changes during cell migration triggered by the *Drosophila* FGF receptor Heartless. *Development* **131**: 2631–2640

Severson AF, Hamill DR, Carter JC, Schumacher J, Bowerman B (2000) The aurora-related kinase AIR-2 recruits ZEN-4/CeMKLP1 to the mitotic spindle at metaphase and is required for cytokinesis. *Curr Biol* **10**: 1162–1171

Smallhorn M, Murray MJ, Saint R (2004) The epithelial–mesenchymal transition of the *Drosophila* mesoderm requires the Rho GTP exchange factor Pebble. *Development* **131**: 2641–2651

Somers WG, Saint R (2003) A RhoGEF and Rho family GTPase-activating protein complex links the contractile ring to cortical microtubules at the onset of cytokinesis. *Dev Cell* **4**: 29–39

Spencer AG, Orita S, Malone CJ, Han M (2001) A RHO GTPase-mediated pathway is required during P cell migration in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* **98**: 13132–13137

Steven R, Kubiseski TJ, Zheng H, Kulkarni S, Mancillas J, Ruiz Morales A, Hogue CW, Pawson T, Culotti J (1998) UNC-73 activates the Rac GTPase and is required for cell and growth cone migrations in *C. elegans*. *Cell* **92**: 785–795

Sulston JE (1976) Post-embryonic development in the ventral cord of *Caenorhabditis elegans*. *Philos Trans R Soc London B* **275**: 287–297

Tatsumoto T, Xie X, Blumenthal R, Okamoto I, Miki T (1999) Human ECT2 is an exchange factor for Rho GTPases, phosphorylated in G2/M phases, and involved in cytokinesis. *J Cell Biol* **147**: 921–928

Wissmann A, Ingles J, McGhee JD, Mains PE (1997) *Caenorhabditis elegans* LET-502 is related to Rho-binding kinases and human myotonic dystrophy kinase and interacts genetically with a homolog of the regulatory subunit of smooth muscle myosin phosphatase to affect cell shape. *Genes Dev* **11**: 409–422

Wu YC, Cheng TW, Lee MC, Weng NY (2002) Distinct *rac* activation pathways control *Caenorhabditis elegans* cell migration and axon outgrowth. *Dev Biol* **250**: 145–155

Yoshizaki H, Ohba Y, Parrini MC, Dulyaninova NG, Bresnick AR, Mochizuki N, Matsuda M (2004) Cell type-specific regulation of RhoA activity during cytokinesis. *J Biol Chem* **279**: 44756–44762

Zipkin ID, Kindt RM, Kenyon CJ (1997) Role of a new Rho family member in cell migration and axon guidance in *C. elegans*. *Cell* **90**: 883–894