A Genome-Wide RNAi Screen for Enhancers of *par* Mutants Reveals New Contributors to Early Embryonic Polarity in *Caenorhabditis elegans*

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ABSTRACT The *par* genes of *Caenorhabditis elegans* are essential for establishment and maintenance of early embryo polarity and their homologs in other organisms are crucial polarity regulators in diverse cell types. Forward genetic screens and simple RNAi depletion screens have identified additional conserved regulators of polarity in *C. elegans*; genes with redundant functions, however, will be missed by these approaches. To identify such genes, we have performed a genome-wide RNAi screen for enhancers of lethality in conditional *par-1* and *par-4* mutants. We have identified 18 genes for which depletion is synthetically lethal with *par-1* or *par-4*, or both, but produces little embryo lethality in wild type. Fifteen of the 18 genes identified in our screen are not previously known to function in *C. elegans* embryo polarity and 11 of them also increase lethality in a *par-2* mutant. Among the strongest synthetic lethal genes, polarity defects are more apparent in *par-2* early embryos than in *par-1* or *par-4*, except for *strd-1(RNAi)*, which enhances early polarity phenotypes in all three mutants. One strong enhancer of *par-1* and *par-2* lethality, *F25B5.2*, corresponds to *nop-1*, a regulator of actomyosin contractility for which the molecular identity was previously unknown. Other putative polarity enhancers identified in our screen encode cytoskeletal and membrane proteins, kinases, chaperones, and sumoylation and deubiquitylation proteins. Further studies of these genes should give mechanistic insight into pathways regulating establishment and maintenance of cell polarity.

THE regulation of cell polarity is essential for development and function of diverse cell types. The six *par* genes, first identified for their role in regulating polarity in the one-cell *Caenorhabditis elegans* embryo (Kemphues *et al.* 1988; Watts *et al.* 1996; Morton *et al.* 2002) are central to the coordinated organization of polarity in a variety of cell types, including epithelial tissues, migrating cells, neurons, and oocytes (reviewed in Johnston and Ahringer 2010; Nance and Zallen 2011). In the *C. elegans* zygote, the *par* genes are required for segregation of cytoplasmic components including developmental determinants and cell timing regulators. Because of the high degree of conservation of the PAR proteins and their partners, *C. elegans* remains an excellent system for identifying key regulators of polarity.

The genes *par-1* and *par-4* encode conserved serine-threonine kinases. PAR-1 is related to mammalian MARK kinases; PAR-4 is the *C. elegans* homolog of LKB1, the upstream regulatory kinase of the AMPK kinase family, including MARKs (reviewed by Alessi *et al.* 2006). LKB1 is a known tumor suppressor for which mutations lead to Peutz-Jegher syndrome and predisposition to cancer in epithelial tissues such as the intestine (Hemminki *et al.* 1998; Jenne *et al.* 1998). In the *C. elegans* embryo, PAR-1 and PAR-4 are essential for asymmetric localization of P granules and developmental regulators such as MEX-5 and PIE-1 and for asynchronous cell divisions of the early blastomeres (Kemphues *et al.* 1988; Morton *et al.* 1992; Guo and Kemphues 1995; Tenenhaus *et al.* 1998; Cuenca *et al.* 2003; Cheeks *et al.* 2004; Budirahardja and Gonczy 2008; Rivers *et al.* 2008; Tenlen *et al.* 2008). However, mutations in either *par-1* or *par-4* do not strongly disrupt localization of the anterior PAR proteins (PAR-3, PAR-6, and PKC-3), or of the posteriorly localized PAR-2 protein (Etemad-Moghadam *et al.* 1995; Boyd *et al.* 1996; Hung and Kemphues 1999; Cuenca *et al.* 2003). While PAR-1 is itself localized to the posterior cortex during the first cell cycle, PAR-4 is not asymmetrically

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doi: 10.1534/genetics.112.143727

Manuscript received July 11, 2012; accepted for publication August 8, 2012

Supporting information is available online at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.143727/-/DC1>.

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession no. JX293831.

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distributed in the early embryo (Guo and Kemphues 1995; Watts *et al.* 2000). That the anterior and posterior PAR proteins can become asymmetrically localized without PAR-1 or PAR-4 function suggests that PAR-1 and PAR-4 act downstream of the other PARs in the *C. elegans* embryo and are not essential for excluding the anterior PAR proteins, or that there are additional polarity components with redundant functions for these kinases.

Screens for maternal effect lethal mutations or RNAi knockdowns for phenotypes similar to the *par* mutants have enabled identification of many key polarity regulators and provided mechanistic insight into polarity establishment and maintenance (Cowan and Hyman 2007; St Johnston and Ahringer 2010). Yet there are likely to be additional gene products whose role in polarity is masked either by having redundant functions or by inefficient RNAi knockdown.

RNAi-based screens for enhancers or suppressors of existing mutations provide a powerful means to identify additional genes with previously undetected roles in specific developmental processes. RNAi screens have identified suppressors of polarity mutants (Labbe *et al.* 2006; Spilker *et al.* 2009) and RNAi screens for synthetic lethality with conditional lethal mutants involved in morphogenesis (Sawyer *et al.* 2011), embryo elongation (Zahreddine *et al.* 2010), and neddylation (Dorfman *et al.* 2009) have also proved fruitful. (For a general discussion of RNAi screens, see Boutros and Ahringer 2008.)

To uncover additional genes involved in early embryo polarity, we chose to perform an RNAi-based genome-wide screen for synthetic lethality with *par-1* or *par-4* conditional mutants. Such genes may give us insights into the functions of *par-1* and *par-4* as well as providing a more complete picture of the polarity system of the early embryo.

We have identified a set of 18 genes that when depleted by RNAi result in synthetic embryo lethality with *par-1* or *par-4* temperature-sensitive mutants under semipermissive conditions, but have little effect in wild type. Several genes enhance lethality in *par-1*, *par-4*, and *par-2* conditional mutants, suggesting that they may have polarity functions in *C. elegans*.

Materials and Methods

Strains and culture

C. elegans strains were maintained by standard procedures (Brenner 1974). Strains include N2 (Bristol) as wild type (WT), KK300 *par-4(it57ts)* V (Morton *et al.* 1992), KK418 *par-2(it5ts)* III (Kemphues *et al.* 1988), KK196 *lon-1(e185)* *par-3(e2074)III*; *sup-7(st5)* X (Cheng *et al.* 1995), KK725 *nop-1(it142)* III (Rose *et al.* 1995), KK822 *par-1(zu310ts)* V, and DH189 *emb-9(b189ts)* III (Wood *et al.* 1980).

RNAi screen

Individual RNAi clones from the library of Kamath *et al.* (2003) were grown in 96-well trays overnight in LB broth containing 100 µg/ml ampicillin and 50 µg/ml tetracycline

for RNAi by feeding (Timmons *et al.* 2001). The overnight cultures were diluted 1:100 in fresh media and grown for 3 hr, induced to express dsRNA by addition of isopropyl β-D-thiogalactoside (IPTG) to 100 µM for 2 more hours, and concentrated fivefold for feeding.

To prepare worms for screening, KK300 *par-4(it57ts)* and KK822 *par-1(zu310ts)* worms were propagated at permissive temperature (16°), the embryos isolated by bleach treatment, and hatched into M9 medium to synchronize populations. L1 larvae were transferred to NGM plates with OP50 bacteria to grow to L3–L4 stages. The larvae were washed and suspended in M9 containing 50 µg/ml ampicillin and 60 µl containing ~10 worms were pipetted into each well of 96-well dishes. A total of 30 µl of induced RNAi feeding bacteria from individual clones was added per well and plates were incubated at semipermissive temperature (19° for *zu310* and 17° for *it57*). Plates were scored ~4 days later for production of unhatched embryos and results were compared to those of Kamath *et al.* (2003) for N2, and to a parallel screen performed by J. Liu using *emr-1* and *lem-2* mutant strains, which do not affect embryo viability (J. Liu, unpublished results). Positive controls included RNAi clones for *par-2* and *par-4*. L4440 empty vector was the negative control (Timmons and Fire 1998). In our hands, ~1–2% of the clones in the RNAi library did not grow; thus the number of genes that we tested represents ~16,500 genes, or 85% of the *C. elegans* genome.

For those RNAi clones that resulted in embryo lethality in *par-1(zu310)* or *par-4(it57)*, RNAi tests were repeated in liquid as above, but viability of N2 embryos was assessed in parallel. RNAi clones causing lethality in *par* mutants but not in N2 were sequenced and compared to WormBase annotations (Harris *et al.* 2010) to verify identity and were retested by feeding L4 larvae on agar plates containing minimal medium with 12.5 µg/ml tetracycline and 50 µg/ml carbenicillin. Worms were transferred to fresh plates each day, and larvae and unhatched eggs were counted to determine percentage of embryo lethality. Percentage of embryo lethality was determined from two or more independent RNAi inductions. Differences in lethality from L4440 vector-fed worms were computed by one-tailed Student's *t* test, and a cut-off of *P* < 0.05 was used to determine whether to include the RNAi clone in our set of enhancers. For RNAi in *par-3(e2074)*; *sup-7*, two-tailed *t* tests were performed, since either suppression or enhancement of lethality might result. Some genes related to those in our screen were subsequently tested from the ORFeome-based RNAi library of Rual *et al.* (2004).

For RNAi of *par-1*, a clone containing nucleotides 1358–2952 (central linker domain) was used (Hurd and Kemphues 2003). For RNAi of *par-4*, we used a feeding construct of *par-4* cDNA beginning at nucleotide 15 and extending through the gene into the 3'-UTR (gift of D. Hurd, Cornell University).

A fragment of *F25B5.2* cDNA (nucleotides 1–425) was inserted into the L4440 vector and used for RNAi by feeding

to confirm the Nop (no pseudocleavage) phenotype observed upon depletion of *F25B5.2* from the RNAi feeding library (Kamath *et al.* 2003), which targets a different region of the gene.

Sequence analysis of *nop-1*

F25B5.2 cDNA was isolated from a *C. elegans* cDNA library (Gibco Proquest) by PCR amplification using iProof polymerase (Bio-Rad) with a forward primer containing ATGTCGGCGCC GAAGAAACGAAAAAG and reverse primer containing AGCTC AAATTTTAGCTCCAAACACAGTTG, cloned into a TOPO blunt vector (Invitrogen) and sequenced. This cDNA sequence was compared to the predicted transcript in WormBase (version WS231) by sequence alignment and has been deposited with GenBank (accession no. JX293831). For genomic DNA sequencing, individual wild type and *nop-1(it142)* worms were lysed by Proteinase K treatment and their DNA was amplified using primers to cover the *F25B5.2* gene.

Immunofluorescence and microscopy

Primary antibodies included rabbit anti-PAR-1 (Guo and Kemphues 1995), monoclonal mouse anti-PAR-3 (Nance *et al.* 2003), and OIC1D4 monoclonal mouse anti-P granule (Strome 1986). Monoclonal antibodies were obtained from the Developmental Studies Hybridoma Bank, University of Iowa. Secondary antibodies included Alexa Fluor 594-labeled goat antimouse or antirabbit (Invitrogen) and Cy3-labeled donkey antimouse or antirabbit (Jackson ImmunoResearch). Fixations were done in methanol at -20° for 15 min. For P granule staining, a postmethanol acetone step was included (Strome and Wood 1983). Slides were mounted with Vectashield containing DAPI (Vector Laboratories). Images and time-lapse movies were acquired on a Leica DM RA2 microscope fitted with a Hamamatsu Orca-ER digital Camera using OpenLab software (Improvision). OpenLab software was used to measure length of PAR-3 anterior-posterior domain and overall length of embryos. Measurements were taken twice for each embryo. For live imaging, embryos were mounted on 2% agarose pads on microscope slides and covered with a coverslip. The temperature of the microscope room was controlled to the semipermissive temperature of the mutant being analyzed.

Results

Identification of enhancers of conditional *par-1* or *par-4* mutants

To identify new components of the PAR polarity system in the *C. elegans* embryo, we screened a library of RNAi clones (Kamath *et al.* 2003) for those that increased embryo lethality in the weak *par* mutants, *par-1(zu310ts)* or *par-4(it57ts)*. These *par* mutations each result in a single amino acid substitution in the mutant proteins (Watts *et al.* 2000; Spilker *et al.* 2009) and the mutant embryos show typical *par* phenotypes at the nonpermissive temperature of 25° . Under semipermissive conditions, 19° for *par-1(zu310)* and 17°

for *par-4(it57)*, ~ 10 – 20% of *par-1(zu310)* embryos and 20 – 35% of *par-4(it57)* embryos fail to hatch. Among the $\sim 16,500$ genes that we screened by RNAi (see *Materials and Methods*), we initially identified 709 candidate clones that appeared to increase embryo lethality of a *par* mutant, but had not been reported to cause embryo lethality in wild-type worms in the screen of Kamath *et al.* (2003). Most of these 709 candidates were identified as *par-4*-enhancing clones, perhaps because our screen was done closer to the temperature-sensitivity threshold for *par-4(it57)*, and this strain is more sensitive to temperature fluctuation than is *par-1(zu310)*. We retested the candidate clones, comparing them side by side with embryo lethality in the wild-type N2 strain, and narrowed our list to 105 that appeared to cause increased lethality in *par-1* or *par-4*, or both, but little embryo lethality in wild type (Supporting Information, Table S1). We then used RNAi feeding of L4 stage worms on plates, where we could assess more accurately the percentage of lethality by counting embryos and larvae, to further test these RNAi clones for their effects on lethality in *par-1(zu310)* and *par-4(it57)* compared to wild-type worms. In performing RNAi tests with worms at this late larval stage, we may have eliminated some RNAi clones for which longer periods are required for depletion, but our goal was to identify the strongest enhancers of the *par* mutants. Through this final step of screening, we identified 19 RNAi clones that significantly increased lethality in *par-1(zu310)* or *par-4(it57)* compared to RNAi in wild-type worms (Table 1). Seventeen of 19 RNAi clones significantly increase lethality in *par-1(zu310)* under semipermissive conditions, and among these, 7 also enhance lethality of *par-4(it57)*. RNAi of two genes increased lethality in *par-4(it57)* but not in *par-1(zu310)*.

The putative functions of the lethality-enhancing genes identified in our screen include phosphorylation (*strd-1*, *pig-1*, ZK856.8, and *kin-10*), membrane or cytoskeletal proteins (*mes-1*, *unc-59*, *T24F1.2*, *npp-22*, *str-43*, *ooc-3*, and *lgl-1*), sumoylation and ubiquitination (*gei-17*, *math-33*, and *uba-2*), chaperones (*hsp-2* and *sti-1*), and unknown (*ntl-9* and *F25B5.2*) (Table 1). The RNAi effect of *hsp-2*, a pseudogene with high sequence similarity to the *C. elegans* HSP-70A family gene *hsp-1* (Heschl and Baillie 1989), is probably not specific for *hsp-2*. We tested an *hsp-1* RNAi clone, and as with *hsp-2* RNAi, observed increased embryo lethality in *par-4(it57)* (Table S2). Thus, the increased lethality in *par-4(it57)* from *hsp-2* RNAi is most likely due to knockdown of *hsp-1*. We also see nonspecific RNAi effects from clone *sjj_F23F12.2*, which corresponds to an intronic region of *F23F12.3* and has multiple gene targets for RNAi. We were unable to distinguish among these possible targets the source of the *sjj_F23F12.2* RNAi effect. Eliminating that clone leaves us with 18 genes identified by RNAi as enhancers of embryo lethality in *par-1*, *par-4*, or both mutant backgrounds (Table 1, Figure 1, A and B).

Three of the genes we identified, *mes-1*, *lgl-1*, and *ooc-3*, have known polarity functions in the early embryo, and their

Table 1 Percentage of embryonic lethality in conditional *par* mutants treated with RNAi of screen positives

RNAi gene target	Function	Genotype		
		N2 (19°)	<i>par-1</i> (zu310) (19°)	<i>par-4</i> (it57) (17°)
L4440 (negative control)	RNAi feeding vector	0.8 ± 0.6	14.0 ± 1.6	26.7 ± 13.8
<i>sad-1</i> (negative control)	AMPK-related kinase, neuronal polarity	0.6 ± 0.2	19.0 ± 7.2	21.0 ± 9.5
<i>par-4</i> (positive control)	PAR-4 kinase, embryo cell polarity	26.2 ± 8.9**	84.8 ± 5.2**	87.1 ± 1.9**
Phosphorylation				
<i>strd-1</i>	PAR-4 kinase cofactor	0.6 ± 0.4	98.4 ± 1.6**	90.2 ± 6.2**
<i>pig-1</i>	AMPK-related kinase, neuronal polarity	1.8 ± 0.4*	95.7 ± 3.6**	40.8 ± 7.2
<i>kin-10</i>	Casein Kinase II regulatory subunit	26.3 ± 8.3**	36.8 ± 2.4 ^a	68.8 ± 4.5*
ZK856.8	Calcium-binding phosphatase-like	0.4 ± 0.2	38.1 ± 9.1*	11.3 ± 3.8
Cytoskeletal and membrane proteins				
<i>mes-1</i>	Membrane protein, embryo cell polarity	0.7 ± 0.1	88.0 ± 2.6**	64.0 ± 17.5*
<i>unc-59</i>	Septin GTPase	2.2 ± 0.3*	54.0 ± 6.2**	43.7 ± 9.7
<i>lgl-1</i>	Cell polarity	0.7 ± 0.4	51.1 ± 2.2*	23.5 ± 4.0
<i>T24F1.2</i>	Membrane protein	1.6 ± 1.3	34.5 ± 3.7**	52.0 ± 6.7*
<i>npp-22</i>	Nucleoporin	0.9 ± 0.8	33.6 ± 5.2*	49.8 ± 17.2
<i>str-43</i>	Membrane protein	0.8 ± 0.3	29.9 ± 6.9*	55.2 ± 15.9
<i>ooc-3</i>	ER membrane protein, cell polarity	0.8 ± 0.4	24.6 ± 4.4*	16.1 ± 6.1
Ubiquitination, sumoylation				
<i>math-33</i>	USP7/HAUSP ubiquitin hydrolase	1.9 ± 1.2	75.3 ± 6.3**	89.6 ± 6.1*
<i>uba-2</i>	SUMO-activating protein	24.4 ± 2.6**	74.9 ± 19.8*	84.1 ± 7.2*
<i>gei-17</i>	SUMO ligase	5.0 ± 1.2**	81.1 ± 0.8**	83.7 ± 4.5*
Chaperones				
<i>sti-1</i>	HSP90/HSP70 co-chaperone	0.9 ± 0.4	4.9 ± 0.5***	88.4 ± 11.4*
<i>hsp-2</i>	Pseudogene, also targets HSP-1	2.8 ± 1.7	17.5 ± 0.7	58.7 ± 16.6*
Unknown function				
<i>ntl-9</i>	Rcd1 superfamily	1.0 ± 0.4	87.4 ± 3.3**	49.8 ± 1.9
<i>F25B5.2 (nop-1)</i>	Unknown; nematode specific	0.8 ± 6.8	80.1 ± 11.0*	44.0 ± 7.2
<i>F23F12.3</i> intron	Unknown; secondary targets	0.6 ± 0.1	76.0 ± 6.3**	39.9 ± 0.9

Percentage of embryo lethality ± SD from mean following RNAi feeding of clones corresponding to the indicated genes. * $P < 0.05$, enhanced lethality, compared to L4440 control in the same genetic background. ** $P < 0.001$, enhanced lethality, compared to L4440 control. *** $P < 0.01$, suppression of lethality, compared to L4440 control.

^a Not significantly different from result in N2, though lethality is increased compared to L4440 in *par-1*(zu310).

detection supports the idea that genes encoding new polarity components could be identified by our screen. *mes-1* encodes a transmembrane protein required for asymmetric divisions of the germline precursor cells P2 and P3 (Berkowitz and Strome 2000; Bei *et al.* 2002) and for localizing PAR-2 at the four-cell stage (Arata *et al.* 2010). LGL-1 is a conserved polarity protein that has been shown to act redundantly with PAR-2 in the one-cell embryo (Beatty *et al.* 2010; Hoege *et al.* 2010) and *ooc-3* encodes an endoplasmic reticulum membrane protein required for proper localization of PAR proteins in the P1 cell of the two-cell embryo (Basham and Rose 1999; Pichler *et al.* 2000). While RNAi of *mes-1* strongly enhanced embryo lethality of both *par-1* and *par-4* temperature-sensitive mutants, RNAi of *ooc-3* only weakly increased lethality of *par-1*(zu310) and did not increase lethality of *par-4*(it57). RNAi depletion of *lgl-1*, which has been shown to enhance lethality in weak *par-2* mutants (Beatty *et al.* 2010; Hoege *et al.* 2010) caused increased lethality in *par-1*(zu310) but not in *par-4*(it57).

Two genes identified in our screen, *strd-1* and *pig-1*, although not known previously to have roles in the early embryo, have been reported to act in neuronal cell polarity (Cordes *et al.* 2006; Kim *et al.* 2010) and in a caspase-independent programmed cell death pathway in *C. elegans* (Denning *et al.* 2012). *strd-1*, which encodes the *C. elegans* ortholog of the mammalian STRADα cofactor for LKB1, is

a strong RNAi enhancer of embryo lethality in both *par-1* and *par-4*, consistent with a previous report of synthetic lethality between *strd-1* mutants and *par-1* or *par-4* temperature-sensitive mutants at permissive temperature (Narbonne *et al.* 2010). While in mammalian systems STRAD and LKB1 act together in polarization of neurons (Barnes *et al.* 2007; Shelly *et al.* 2007), in *C. elegans*, *strd-1* has been shown to act independently of *par-4* in regulating neuronal polarity (Kim *et al.* 2010). *pig-1*, a strong enhancer of *par-1* lethality in our screen, encodes a serine–threonine kinase with similar structure to PAR-1 and is important for polarized divisions of certain neuroblasts during *C. elegans* larval development (Cordes *et al.* 2006). *pig-1* is also required for cell shedding in caspase-independent apoptosis of specific cells in the embryo and both *par-4* and *strd-1* appear to act upstream of *pig-1* in this process (Denning *et al.* 2012). RNAi of another PAR-1-related kinase gene, *sad-1*, required for *C. elegans* neuronal cell polarity (Crump *et al.* 2001; Hung *et al.* 2007; Shelly and Poo 2011), which is not expressed in early embryos (Crump *et al.* 2001), did not enhance *par-1*(zu310) lethality. *sad-1* is as similar in sequence to the *par-1* gene as *pig-1* and was used as a negative control in our RNAi experiments (Table 1).

Two RNAi clones in our set, corresponding to *uba-2* and *kin-10*, cause >20% embryo lethality in wild-type *C. elegans*, but the lethality is significantly increased in combination

Figure 1 Embryonic lethality produced by RNAi of screen positives in weak *par* mutants. Percentage of embryonic lethality upon RNAi of the indicated genes in (A) *par-1(zu310)*, (B) *par-4(it57)*, (C) *par-2(it5)*, (D) *par-3(e2074); sup-7*, at the temperature shown for each genotype. Red bars indicate enhanced lethality compared to L4440 control RNAi ($P < 0.05$), while those in light gray are not significantly different from the control. Blue bars indicate suppression of embryo lethality compared to L4440 control RNAi ($P < 0.05$). Genes depleted by RNAi are ordered along the x axis for each *par* mutant by level of embryo lethality produced. Error bars indicate standard deviation from the mean. *sad-1* RNAi is an additional negative control, while *par-4* RNAi is a positive control.

with *par* mutations. For *kin-10*, which encodes a regulatory subunit of casein kinase II, this effect is stronger for *par-4(it57)*. RNAi of the *uba-2* gene, encoding a SUMO-activating protein, results in similar embryonic lethality levels in either *par-1(zu310)* or *par-4(it57)* mutants. To assess whether this was due to its role in the sumoylation pathway, we also tested RNAi of *aos-1*, which was not present in the RNAi library used for screening. *AOS-1* forms a heterodimer with *UBA-2* and both subunits act at the first step of protein sumoylation by activating SUMO (Johnson and Blobel 1997; Muller *et al.* 2001). We found that *aos-1* RNAi also increases embryo lethality of *par-1(zu310)* and *par-4(it57)* (Table S2). *gei-17*, which encodes a sumo ligase (Holway *et al.* 2005), was also identified in our screen, suggesting that sumoylation may be important for polarity or PAR protein function in *C. elegans*.

ntl-9 encodes a protein with high similarity to Rcd1-like proteins, part of the CCR4–NOT complex, which has diverse functions including transcription, deadenylation, and ubiquitination (reviewed by Parker and Sheth 2007; Collart and Panasenko 2012). Some *C. elegans* CCR4–NOT proteins are involved in RNA processing (Schmid *et al.* 2009), but the function of *ntl-9* has yet to be elucidated.

One gene of unknown function identified in our screen, *F25B5.2*, has no obvious homologs outside of nematodes and corresponds to *nop-1*, a gene previously identified by mutation (Rose *et al.* 1995) that functions in cortical contractility of the one-cell embryo (see below).

Seven of the genes that we identified in our screen enhanced lethality of both *par-1(zu310)* and *par-4(it57)*. However, one strong *par-4*-specific enhancer, corresponding to the HSP90/HSP70 co-chaperone *sti-1*, not only failed to enhance *par-1(zu310)* lethality but resulted in slightly higher viability of *par-1(zu310)* embryos relative to controls (Table 1, Figure 1A).

Most of the enhancers of *par-1* and *par-4* embryo lethality also increase lethality in a weak *par-2* mutant, but not in a *par-3* mutant

We tested whether the genes that we identified could also enhance another conditional *par* mutant, *par-2(it5ts)*. *par-2*

encodes a RING-domain-containing ubiquitin ligase that associates with the posterior cell cortex of the zygote, is required to keep the anterior PAR proteins from reentering that domain, and for *PAR-1* to localize to the posterior cortex (Levitani *et al.* 1994; Boyd *et al.* 1996; Cuenca *et al.* 2003; Hao *et al.* 2006). We found that RNAi of 11 of the 18 genes identified in our screen also caused increased embryo lethality in a *par-2* mutant background (Table 2, Figure 1C); five of them (*strd-1*, *math-33*, *uba-2*, *gei-17*, and *T24F1.2*) increase embryo lethality in all three *par* mutants (Figure 2).

Because several of the genes identified in our screen appear to act synergistically with two posterior domain protein-encoding genes, *par-1* and *par-2*, we wondered what their effect might be if an anterior *par* gene were compromised instead. We do not have temperature-sensitive mutants of *par-3*, *par-6*, or *pkc-3* that would enable us to test this, but we were able to make use of a nonsense allele of *par-3* that can be partially suppressed by the *sup-7* tRNA amber suppressor (Cheng *et al.* 1995). In *par-3(e2074); sup-7(st5)*, ~45% of embryos are viable at 22°. We determined the percentage of embryo viability when worms of this genotype were treated with RNAi for each of the genes that enhanced lethality in *par-1* or *par-4*. We found that RNAi of *uba-2*, *ntl-9*, *gei-17*, *nop-1*, *sti-1*, and *kin-10* caused a significant increase in embryo lethality in the *par-3* mutant compared to control RNAi, whereas others did not (Table 2, Figure 1D). The embryonic lethality observed for *kin-10* RNAi, however, is not significantly higher than that expected by adding the lethality produced in wild type (26%) to the lethality levels of *par-3(e2074); sup-7* controls (56.5%).

We also found that two RNAi clones, those corresponding to *lgl-1* and *math-33*, suppressed embryo lethality in the *par-3(e2074) sup-7* strain, suggesting that these two genes act antagonistically to *par-3*.

Several of the genes identified in our screen enhance lethality of multiple *par* mutants. Such genes may be acting in polarity processes or could be nonspecific in their effect on conditional mutations. To address this, we tested whether the enhancers could also increase lethality in *emb-9(b189ts)*,

Table 2 Percentage of embryo lethality in *par-2(it5ts)* and *par-3(e2074); sup-7* treated with RNAi of enhancers of *par-1* and *par-4* embryo lethality

RNAi gene target	<i>par-2(it5)^a</i>	<i>Par-3(e2074); sup-7^b</i>
L4440 (negative control)	9.0 ± 1.9	56.5 ± 11.1
<i>sad-1</i> (negative control)	16.0 ± 5.8	58.5 ± 15.1
<i>par-4</i> (positive control)	61.8 ± 11.3**	97.5 ± 1.4**
<i>strd-1</i>	79.6 ± 1.3**	65.8 ± 9.3
<i>pig-1</i>	96.5 ± 0.1**	69.5 ± 15.8
<i>mes-1</i>	28.7 ± 19.3	70.5 ± 13.0
<i>ntl-9</i>	81.0 ± 12.5**	89.4 ± 4.6**
<i>gei-17</i>	42.1 ± 3.7*	81.0 ± 7.5*
<i>F25B5.2 (nop-1)</i>	99.4 ± 0.4**	79.8 ± 3.5*
<i>F23F12.3</i> intron	45.9 ± 15.1*	69.0 ± 5.3
<i>math-33</i>	99.1 ± 1.3**	33.2 ± 7.6***
<i>uba-2</i>	71.9 ± 6.9*	94.2 ± 5.3**
<i>unc-59</i>	15.2 ± 7.1	62.5 ± 16.5
<i>lgl-1</i>	98.9 ± 0.1**	25.9 ± 13.2***
<i>ZK856.8</i>	23.4 ± 6.4	66.2 ± 13.0
<i>kin-10</i>	23.9 ± 12.0	84.4 ± 39.0 ^d
<i>T24F1.2</i>	50.6 ± 5.6*	62.0 ± 5.0
<i>npp-22</i>	57.0 ± 8.6*	54.3 ± 14.1
<i>str-43</i>	11.1 ± 5.6	58.1 ± 17.2
<i>ooc-3</i>	28.7 ± 15.6	64.9 ± 14.2
<i>hsp-2</i>	21.1 ± 9.3	66.8 ± 7.5
<i>sti-1</i>	29.3 ± 12.7*	77.5 ± 9.4*

Percentage of embryo lethality ± SD from mean following RNAi feeding of clones corresponding to the indicated genes, ordered by degree of lethality produced in *par-1(zu310)*. **P* < 0.05, enhanced lethality, compared to L4440 control in the same genetic background. ***P* < 0.001, enhanced lethality, compared to L4440 control. ***Suppression of lethality, *P* < 0.05, compared to L4440 control.

^a 16°.

^b 22°.

^c Not significantly different from expected lethality produced by RNAi in wild type added to control levels of lethality.

which has a temperature-sensitive period late in embryogenesis (Wood *et al.* 1980). *emb-9* encodes the α-chain of type IV collagen (Guo *et al.* 1991) and is required for epidermal elongation during late embryogenesis as well as for gonadogenesis and larval development, but mutations do not compromise the early embryo (Schierenberg *et al.* 1980; Gupta *et al.* 1997). From *emb-9(b189)* worms shifted to the semipermissive temperature of 21° at the L4 stage, we found that 3% of *emb-9(b189)* embryos fail to hatch (*n* = 2476), while young adults shifted to 25° produce 100% embryonic lethality (*n* = 1244), and L4 worms shifted to 25° are sterile. Testing the effects of depleting our enhancer genes on *emb-9(b189)* at 21°, we found that only *gei-17* and *hsp-2* significantly increased embryo lethality (to 15 and 7% lethality, respectively), though to a much lower extent than their effects on the *par* mutants (Table S3). Thus these two genes may be nonspecific in their effect, or may act at multiple times in embryogenesis. Because RNAi of the remaining genes more specifically enhances lethality of weak *par* mutants, they are good candidates for early functions in embryogenesis that influence processes mediated by the PAR proteins.

F25B5.2* is *nop-1

One of the strongest enhancers of *par-1* and *par-2* conditional mutants to come out of our screen is *F25B5.2*. Most of

Figure 2 RNAi enhancers of embryonic lethality affect multiple *par* genes. Venn diagram showing the genes identified by RNAi that significantly increase lethality (*P* < 0.05) in *par-1*, *par-2*, and *par-4* conditional mutants.

the genes identified in our screen have homologs that suggest how they may function; *F25B5.2* does not. We observed that in *par*; *F25B5.2*(RNAi) one-cell embryos lack pseudocleavage, which is an actomyosin-driven constriction of the cell cortex early in the first cell cycle, at approximately the time of polarity establishment (Hill and Strome 1988; Hird and White 1993; Munro *et al.* 2004). Because *F25B5.2* is located in the same genetic region as a mutant lacking pseudocleavage, *nop-1(it142)* (Rose *et al.* 1995), for which the molecular identity was unknown, we examined one-cell embryos produced by wild-type worms treated with *F25B5.2* RNAi, and found that even in the absence of a *par* mutation, depletion resulted in a Nop (no pseudocleavage) phenotype remarkably similar to the *nop-1* mutant (Figure 3, File S1).

A wild-type cDNA clone corresponding to *F25B5.2* shows a subtle splice variant resulting in two additional codons at the beginning of exon 6 compared to the predicted transcript for *F25B5.2* in WormBase (version WS231). The coding region is the same in other respects and encodes a predicted 759-amino-acid protein of unknown function, conserved among nematodes, but without obvious homologs in other animals. Sequence analysis of the *F25B5.2* gene in the *nop-1(it142)* mutant revealed a G-to-A mutation predicted to change the codon for W698 (or W696 in the shorter form) to a UGA stop, to produce a truncated protein. Searches for conserved domains in the predicted NOP-1 protein by BLAST (Altschul *et al.* 1990), Pfam (Finn *et al.* 2010), or SMART (Letunic *et al.* 2009) database searches failed to identify any protein motifs that could suggest function.

It was previously reported that *nop-1(it142)* mutants have variable embryonic lethality (Rose *et al.* 1995) up to 23%; we observe only 5.3% embryo lethality (*N* = 2548) in

Figure 3 *F25B5.2(RNAi)* embryos exhibit a Nop (No Pseudocleavage) phenotype like that of *nop-1(it142)* mutant embryos. The top row shows a wild-type one-cell embryo at (A) the time of ruffling, ~6.5 min prior to nuclear envelope breakdown (NEBD); (B) pseudocleavage (arrow-heads), ~2.5 min prior to NEBD; (C) NEBD; and (D) the two-cell stage. (E–H) *nop-1(it142)* embryo at the same times relative to NEBD (G) as those in A–D. (I–L) *F25B5.2(RNAi)* embryo at the same intervals. Cortical ruffling and pseudocleavage are not observed in either *nop-1(it142)* or *F25B5.2(RNAi)* embryos. Anterior is to the left for all embryos. Time-lapse movies of *F25B5.2(RNAi)* and wild-type embryos from which these images are taken can be viewed in File S1 and File S2.

the KK725 *nop-1(it142)* strain, and this is increased to 15.3% embryonic lethality when *nop-1(it142)* worms are treated with *nop-1(RNAi)* ($N = 1680$). A second RNAi construct representing a different portion of *F25B5.2* than that used for our screen (see *Materials and Methods*) produces similar embryonic lethality: 1% in N2 ($N = 827$) and 8% in *nop-1(it142)* ($N = 752$) and also results in embryos lacking a pseudocleavage furrow when knocked down in wild type (40/40). It seems likely that *nop-1* is not absolutely essential for viability, but is important for actomyosin contractility in the zygote.

Most enhancers do not increase polarity phenotypes in early embryos of *par-1* and *par-4*

RNAi depletions that enhance lethality of weak *par-1* and *par-4* mutants may or may not do so by compromising *PAR-1* and *PAR-4* function in early embryonic polarity. Therefore, we investigated whether the enhancers affected the diagnostic early polarity phenotype of mislocalization of P granules. P granules, nonmembranous ribonucleoprotein organelles, are segregated to the posterior during the first cell cycle and subsequently to the germline lineage in *C. elegans* embryos (Strome and Wood 1982), but are destabilized in embryos of strong *par-1* or *par-4* mutants and fail to localize during the first cell cycle (Kemphues *et al.* 1988; Cheeks *et al.* 2004; Gallo *et al.* 2008). We examined P granules in 52 *par-1(zu310)* two-cell embryos at semipermissive temperature by immunolocalization, and found that P granules were segregated properly to the P1 cell in all of them (Table 3, Figure 4). Choosing clones for RNAi treatment that resulted in >70% lethality in *par-1(zu310)* at the semipermissive temperature, we assessed whether the embryos exhibited P granule distribution defects similar to loss-of-function *par-1* mutations at the two-cell stage. Contrary to our expectations, we found that RNAi of most of the *par-1* enhancers had no effect on P granule distribution at this stage in the *par-1* sensitized background in spite of producing high rates of lethality (Table 3, left column). Only *strd-1* RNAi caused a high frequency of P granule mislocalization and loss of P granule aggregation in *par-1(zu310)* two-cell embryos at 19° (39/43 embryos; Figure 4, Table 3).

In *par-4(it57)* at semipermissive temperature, none of the 53 two-cell embryos examined showed P granule mis-

localization (Table 3). Consistent with our results with *par-1(zu310)*, we found that most of the enhancers of *par-4(it57)* lethality had little or no effect on P granule distribution. *strd-1* RNAi caused *par-4(it57)* embryos to have P granules that appeared much fainter than those of control embryos and that failed to localize to the posterior cell (Figure 4), similar to phenotypes produced by reduction of *par-1* or *par-4* by RNAi. The co-chaperone gene *sti-1* also produced an abnormal P granule phenotype in *par-4(it57)* two-cell embryos when knocked down by RNAi, with very faint P granules by immunofluorescence. These unaggregated granules were mostly localized, but some ectopic granules remained in the anterior (Figure 4G; Table 3, middle column). The relatively normal P granule localization observed in *par-1(zu310)* and *par-4(it57)* mutants treated with RNAi for the remaining lethality-enhancing genes suggests that the lethality is not due to loss of polarity in the first cell cycle.

The enhancement of P granule mislocalization defects in *par-1* and *par-4* embryos by *strd-1* RNAi is higher than that previously reported by Narbonne *et al.* (2010) for the effects of *strd-1* mutations on these *par* mutants. One possible explanation for this difference is that our tests were carried out at the semipermissive temperatures of 19° for *par-1(zu310)* and 17° for *par-4(it57)* rather than at 15°. We repeated *strd-1(RNAi)* on *par-1(zu310)* and *par-4(it57)* at 15° and observed lethality and P granule phenotypes very similar to those reported by Narbonne *et al.* (2010) for *strd-1*; *par* double mutants: 82% embryonic lethality for *strd-1(RNAi)par-1(zu310)*, ($N = 745$), and 84% lethality for *strd-1(RNAi)par-4(it57)*, ($N = 640$), and only 23/43 in *strd-1(RNAi); par-4(it57)* and 0/62 in *strd-1(RNAi); par-1(zu310)* early embryos showed P granule mislocalization. Thus there appears to be a threshold temperature for this polarity phenotype in the *par-1* and *par-4* mutants.

We also asked whether two other aspects of early asymmetry, cell division asynchrony, and P1 spindle orientation at the two-cell stage, were affected by the enhancers. In *par-1* mutant embryos at semipermissive temperature, we observed that cells of two-cell embryos exhibited closer cell division times than wild-type embryos (45 sec on average, instead of 2 min). This loss of asynchrony was further enhanced by RNAi knockdown of *strd-1*, and to a lesser extent by *pig-1* and *mes-1* (Table 4). In *par-4(it57)*, *strd-1*, or *sti-1* RNAi

Table 3 P Granule mislocalization in early *par* embryos treated with RNAi of enhancers of lethality

RNAi	Genotype		
	% <i>par-1(zu310)</i> , 19°, two cell (N)	% <i>par-4(it57)</i> , 17°, two cell (N)	% <i>par-2(it5)</i> , 16°, four cell (N)
L4440 control	0 (52)	0 (53)	0 (59)
<i>par-1</i>	100 (18)	100 (27)	100 (22)
<i>par-4</i>	100 (20)	91 (12)	100 (16)
<i>strd-1</i>	91 (43)	88 (24)	54 (24)
<i>pig-1</i>	3 (36)	—	70 (37)
<i>mes-1</i>	0 (50)	—	—
<i>ntl-9</i>	0 (22)	—	62 (13)
<i>gei-17</i>	0 (16)	0 (22)	—
<i>nop-1</i>	6 (33)	—	90 (30)
<i>math-33</i>	0 (43)	0 (17)	100 (18)
<i>uba-2</i>	4 (27)	0 (18)	0 (15)
<i>lgl-1</i>	—	—	100 (23)
<i>sti-1</i>	—	48 (44)	—

Percentage of embryos with mislocalized P granules is indicated. RNAi clones producing >70% embryonic lethality in the *par* mutant at semipermissive temperature were used for this analysis. —, lethality was <70%.

increased cell division timing defects, consistent with their enhancement of the P granule mislocalization phenotype. These early alterations in cell division timing could result in defects in developmental pattern, leading to the embryonic lethality caused by RNAi of these genes. We also examined P1 spindle orientation in *par-1* and *par-4* two-cell embryos treated with RNAi for the enhancing genes. In wild-type embryos, the spindle of the anterior AB cell orients transversely to the long axis of the embryo while the posterior P1 cell centrosome–nuclear complex rotates 90° to orient along the long axis, and P1 always divides later than AB. A transverse P1 spindle orientation is sometimes observed in stronger *par-1* or *par-4* mutants (Kemphues *et al.* 1988) or with *par-1* RNAi, but we did not see this phenotype in either *par-1(zu310)* or *par-4(it57)* embryos at semipermissive temperatures. Although we noted a very low frequency of transverse P1 spindles in *par-1(zu310)* embryos following RNAi with some of the enhancers, we did not observe this in *par-4(it57)* for any of them (Table 4).

Strong enhancers of *par-2* lethality contribute to *par-2* polarity phenotypes

In contrast to our findings for *par-1* and *par-4*, the enhancers of *par-2(it5ts)* embryo lethality strongly enhance polarity defects in *par-2(it5)* early embryos. In *par-2(it5)* at 25°, P granules are generally segregated into the posterior cell at the first division, but are mislocalized at the four-cell stage (Kemphues *et al.* 1988). At 16°, 100% of *par-2(it5)* four-cell embryos contain P granules only in the P2 cell (N = 59), like wild-type embryos. When treated with RNAi for the seven genes that caused >70% lethality in *par-2(it5ts)* at 16°, we observed a high percentage of four-cell embryos with mislocalized P granules present in two or more cells for all but *uba-2* (Figure 4; Table 3, right column). The penetrance of the P granule mislocalization phenotype correlates with the degree of lethality produced by RNAi in *par-2(it5)*, with *lgl-1*, *math-33*, and *F25B5.2* producing the highest frequency of

abnormal P granule mislocalization (Table 3). Thus the embryonic lethality of the *par-2*-enhancing genes, except for *uba-2*, is most likely due to their effects on early embryo polarity.

To assess whether the enhancers of *par-2* lethality also enhanced other *par-2* phenotypes, we examined spindle orientation and cell division timing at the two- to four-cell stage. In strong *par-2* mutants, the P1 rotation fails and both cells divide synchronously with spindles oriented transverse to the long axis of the embryo. In *par-2(it5)* embryos treated with control RNAi, 96% of embryos (N = 45) showed a normal, longitudinal P1 spindle orientation at the permissive temperature of 16°. However, when treated with RNAi for *lgl-1*, *math-33*, or *F25B5.2*, 100% of *par-2(it5)* embryos had transverse spindle orientations in P1 (Figure 4, Table 4). RNAi of *par-1* or *par-4* in *par-2(it5)* at 16° also causes both cells of two-cell embryos to divide transversely. *pig-1* or *ntl-9* RNAi also caused a high incidence of transverse P1 spindle orientations, and about half of the *strd-1* RNAi embryos showed this phenotype, but *uba-2* showed only a slight increase in the penetrance of this phenotype (4/27 embryos). *par-2(it5)* embryos treated with RNAi for the genes that result in transverse P1 spindle orientations also exhibited a greater degree of synchrony between AB and P1, as determined by DAPI staining of two-cell embryos and time-lapse videomicroscopy (Figure 4, Table 4).

par-2 is required for restricting *PAR-3* the anterior domain in the zygote (Etemad-Moghadam *et al.* 1995; Cuenca *et al.* 2003) and for *PAR-1* localization to the posterior cortex (Boyd *et al.* 1996). We investigated whether the enhancers that increase lethality in *par-2(it5)* increase mislocalization of *PAR-3* and *PAR-1* in the *par-2* early embryos at permissive temperature. Consistent with the other polarity defects seen with these enhancers, all but *uba-2*(RNAi) embryos exhibited abnormal extension of cortical *PAR-3* and associated loss of *PAR-1* in the posterior one-cell and two-cell *par-2(it5)* embryos (Figure S1, Table 5).

Figure 4 P granule phenotypes and AB/P1 cell cycle synchrony in *par* mutants treated with RNAi for screen positives. Embryos are immunostained with anti-P granule antibodies (green) and the DNA costained with DAPI (blue). (A–D) *par-1* (*zu310*) two-cell embryos from worms at semipermissive temperature (19°) after the indicated RNAi treatment. L4440 is empty vector control RNAi. (E–H) *par-4* (*it57*) two-cell embryos from worms at semipermissive temperature (17°) after the indicated RNAi treatment. (I–K) *par-2* (*it5*) two-cell embryos and (M–O) four-cell embryos from worms at semipermissive temperature (16°) after the indicated RNAi treatment. (L and P) Two-cell and four-cell *par-2* (*it5*) embryos grown at the nonpermissive temperature, 25°. (Q and R) Wild-type two-cell and four-cell embryos are shown for comparison.

Thus six enhancers of lethality in *par-2(it5)* strongly enhance polarity defects in *par-2* in spite of the fact that they were initially isolated as enhancers of lethality in *par-1* or *par-4*. Only one of these six, *strd-1*, also causes early polarity phenotypes in *par-1* and *par-4*. We infer that the other five enhancers of *par-2* phenotypes, *nop-1*, *lgl-1*, *math-33*, *pig-1*, and *ntl-9* have roles in early polarity that are redundant with *par-2*, but not with *par-1* or *par-4*, and that their depletion must enhance *par-1* or *par-4* lethality due to defects at or after the two-cell stage, perhaps by alterations of relative cell cycle rates in these mutants.

Discussion

We have identified 18 RNAi clones that strongly enhance embryo lethality of conditional mutations of *par-1* or *par-4* or both. Three clones correspond to genes with previously known roles in embryonic polarity (*lgl-1*, *mes-1*, and *ooc-3*) and 15 correspond to genes with previously unknown roles.

Surprisingly, the only enhancer identified in our screen that clearly functions with *par-1* and *par-4* in polarity during the first cell cycle is *strd-1*. *strd-1* encodes an ortholog of STRAD α , a pseudokinase cofactor of the mammalian ortholog of PAR-4, LKB1. Binding of STRAD has been shown to increase activity of LKB1 in mammalian systems (Baas *et al.* 2003; Boudeau *et al.* 2003a; Hawley *et al.* 2003; reviewed by Alessi *et al.* 2006). In *C. elegans*, STRD-1 can be immunoprecipitated with PAR-4 and colocalizes with PAR-4 at the cortex of early embryos (Narbonne *et al.* 2010). However, it is unclear whether STRD-1 acts in the early embryo to increase activity of PAR-4. *strd-1* mutations do not on their own result in embryonic lethality, but do result in increased

lethality when combined with conditional *par-1* and *par-4* mutants (Narbonne *et al.* 2010). By examining phenotypes at semipermissive temperatures, we have found that *strd-1* does contribute to polarity processes regulated by *par-1* and *par-4* in the zygote such as asymmetric P granule localization and cell cycle timing. The high lethality but normal early polarity observed in *par-1(zu310)*; *strd-1(RNAi)* embryos at the lower temperature of 15° suggests that *strd-1* synergizes with *par-1* in other processes of embryogenesis that are more sensitive to reductions in PAR-1 activity, which may or may not be occurring at the one-cell stage. Recently Kim *et al.* (2010) have reported that *strd-1* functions independently of *par-4* in establishing *C. elegans* neuronal polarity, by acting in a complex with SAD-1, a kinase with high structural similarity to PAR-1, but which does not appear to act in the early embryo. Whether the enhancing effects of *strd-1* depletion in the early embryo are due to effects on activities of PAR-4, PAR-1, or another kinase, will be of future interest.

Besides *strd-1*, the only other RNAi enhancer for which lethality correlated with early polarity defects in *par-4(it57)* was *sti-1*. STI-1 is a highly conserved stress-induced phosphoprotein that can interact directly with HSP-70 or HSP-90 in *C. elegans* (Gaiser *et al.* 2009; Song *et al.* 2009) to act as a co-chaperone for target proteins. HSP-90 has been shown to act with the co-chaperone CDC-37 to regulate stability and activity of LKB1 (Boudeau *et al.* 2003b; Nony *et al.* 2003; Gaude *et al.* 2012). In *C. elegans*, CDC-37 has been shown to affect steady-state levels of PKC-3 and phosphorylated PAR-4, and is essential for regulating the mutual restriction of the anterior and posterior PAR proteins during polarity establishment (Beers and Kemphues 2006). The fact

Table 4 Spindle orientation and cell cycle phenotypes of two-cell *par* embryos treated with RNAi for the indicated genes at semipermissive temperatures

RNAi	Genotype					
	<i>par-1(zu310)</i> , 19°		<i>par-4(it57)</i> , 17°		<i>par-2(it5)</i> , 16°	
	% with P1 transverse spindle (N)	% with AB/P1 synchrony (N)	% with P1 transverse spindle (N)	% with AB/P1 synchrony (N)	% with P1 transverse spindle (N)	% with AB/P1 synchrony (N)
L4440	0 (30)	40 (25)	0 (27)	18 (27)	4 (45)	18 (45)
<i>par-1</i>	65 (23)	100** (22)	6 (16)	100** (16)	100** (25)	100** (25)
<i>par-4</i>	0 (30)	100** (30)	0 (12)	83** (12)	100** (8)	100** (8)
<i>strd-1</i>	0 (31)	97** (31)	0 (25)	89** (19)	56** (30)	63** (30)
<i>pig-1</i>	0 (19)	74* (19)	—	—	88** (27)	100** (26)
<i>mes-1</i>	4 (26)	61* (23)	0 (18)	13 (18)	—	—
<i>ntl-9</i>	0 (25)	32 (25)	—	—	83** (24)	78** (23)
<i>gei-17</i>	4 (26)	48 (25)	0 (17)	18 (17)	—	—
<i>nop-1</i>	2 (41)	54 (41)	—	—	100** (19)	100** (19)
<i>math-33</i>	4 (24)	41 (22)	0 (17)	20 (15)	100** (29)	97** (29)
<i>uba-2</i>	0 (16)	43 (16)	0 (25)	5 (22)	15* (27)	22 (27)
<i>sti-1</i>	—	—	0 (38)	55** (38)	—	—
<i>kin-10</i>	—	—	0 (27)	11 (27)	—	—
<i>lgl-1</i>	—	—	—	—	100** (23)	96** (25)

RNAi clones producing >60% embryonic lethality in the *par* mutant at semipermissive temperature were used for this analysis. —, lethality was <60%. Percentages are rounded to the nearest whole number. * $P < 0.05$, χ^2 test; ** $P < 0.0001$, χ^2 test.

that RNAi of either *hsp-1*, which encodes an HSP-70A type protein, or *sti-1* reduces viability of weak *par-4* mutants could mean that HSP-70 and STI-1 chaperones are also important for PAR-4 stability, phosphorylation status, or function. *sti-1* RNAi did not increase polarity defects in *par-1* (*zu310*); indeed, *sti-1* RNAi even suppressed lethality. Thus, while *sti-1* may be acting through another protein to affect PAR-4 function, it seems unlikely that it is acting through PAR-1.

Most of the *par-1* and *par-4* enhancing genes did not exhibit strong early polarity defects when depleted in *par-1* and *par-4* backgrounds in spite of high levels of embryonic lethality. There are two possible explanations for this. First, their effects on polarity might be too subtle to detect in our assays, but sufficient to lead to cascading defects in later stages. For example, the slight loss of asynchrony at the two-cell stage could result in a development defect only detectable in later stage embryos. Alternatively, the lethality may reflect a role for these genes with PAR-1 and PAR-4 in a later essential function of the two kinases. For example, the absence of intestine in *par-4* and *par-1* mutants could reflect a role in specification of the EMS or E cell fates. MES-1, for example, is known to have an important function in polarity and intercellular signaling between EMS and P2 at the four-cell stage (Berkowitz and Strome 2000; Bei *et al.* 2002; Arata *et al.* 2010); other enhancers in our set may also be acting at this stage or later.

It is noteworthy that genes we initially identified as enhancers of *par-1* or *par-4* or both, but that do not show increased polarity phenotypes in those mutants when depleted by RNAi, nonetheless very effectively enhance *par-2* early polarity phenotypes. One interpretation of this result is that modest reductions in efficiency of processes that *par-2*

mediates can enhance lethality in sensitized *par-1* and *par-4* backgrounds. This interpretation is supported by the example of *lgl-1*, which has been shown to act redundantly with PAR-2 in early polarity (Beatty *et al.* 2010; Hoege *et al.* 2010).

Another possible explanation for the differences we see in polarity phenotypes among the *par* mutants depleted of the enhancers could be due to differences in the *par* mutations used for our studies. While each of the mutants shows strong early polarity phenotypes at nonpermissive temperature, they may have different sensitivities at lower temperatures for their early polarity functions. We note that *strd-1* RNAi in *par-1(zu310)* and *par-4(it57)* produced strikingly different frequency of P granule localization defects depending on temperature, despite producing similar levels of lethality. The enhancers may affect localization of some components of the cell that we did not assay, independent of P granule localization, in these mutants.

Nonmuscle myosin is critical for driving cytoplasmic and cortical flows that transport the PAR proteins and other components to their polarized domains shortly after fertilization (Munro *et al.* 2004). After this establishment phase, PAR-2 is essential for maintaining the polarized domains (Cuenca *et al.* 2003). It has recently been shown, however, that when myosin-directed cortical flows are compromised, PAR-2 is able to direct polarity establishment via a microtubule-directed process (Zonies *et al.* 2010; Motegi *et al.* 2011); the redundant genes that we have identified that increase polarity phenotypes in the *par-2* mutant may be particularly important for polarity maintenance or for robustness of actomyosin flows.

One of the enhancers with the highest levels of lethality and polarity phenotypes in the *par-2* background is *nop-1*. In

Table 5 PAR-3 posterior extension in *par-2(it5); enhancer(RNAi)* one-cell and two-cell embryos (16°)

RNAi	One-cell stage		Two-cell stage
	% prophase–metaphase (N) ^a	% anaphase (N) ^a	% frequency of mislocalization in P (N) ^b
Control	50.5 ± 5.3 (28)	57.6 ± 5.0 (10)	0 (41)
<i>strd-1</i>	62.8 ± 6.4* (18)	58.0 ± 7.2 (8)	71 (25)
<i>pig-1</i>	70.0 ± 5.8* (14)	71.2 ± 6.7* (7)	96 (26)
<i>ntl-9</i>	61.7 ± 8.3* (17)	71.3 ± 4.9* (9)	93 (46)
<i>nop-1</i>	65.4 ± 7.6* (24)	68.1 ± 7.6* (8)	94 (50)
<i>math-33</i>	62.4 ± 6.0* (18)	74.7 ± 6.6* (7)	83 (40)
<i>lgl-1</i>	55.9 ± 2.7* (10)	63.7 ± 6.9* (7)	89 (35)
<i>uba-2</i>	50.2 ± 5.4 (18)	57.0 ± 8.5 (3)	0 (53)
Control, 25°	62.8 ± 6.4* (11)	89.8.0 ± 11* (2)	100 (22)

* Value significantly different from control RNAi at 16°, $P < 0.01$, Student's t test.

^a Average size of PAR-3 domain along the anterior–posterior axis of the embryo as percentage of embryo length followed by (N), determined by immunostaining. Stage was determined by DAPI staining. Genes producing >70% embryonic lethality by RNAi in *par-2(it5)* at 16° were used for this analysis.

^b Percentage of embryos exhibiting abnormal PAR-3 extension in the posterior P1 cell.

addition to lacking pseudocleavage, *nop-1(RNAi)* or *nop-1(it142)* embryos have reduced ruffling and cortical flows (File S1). The effects of *nop-1(RNAi)* on actomyosin contractility may explain its synthetic lethality with the *par* mutants, particularly *par-1* and *par-2*. Since PAR-1 and PAR-2 act together in PAR-2's redundant role with myosin contractility in polarity establishment (Motegi *et al.* 2011), *nop-1* would be expected to enhance polarity defects and embryo lethality in both *par-1* and *par-2* mutants, as we observe.

The strong *par-1* enhancer *pig-1*, (*par-1*-like gene), encodes a kinase in the AMP kinase family with very similar structure to PAR-1 (Cordes *et al.* 2006). The closest mammalian homolog to PIG-1 is MELK/KP38, a kinase for which the RNA is highly enriched in mouse oocytes and very early embryos (Heyer *et al.* 1997, 1999) and is also related to the egg-enriched *Xenopus* pEg3 (Blot *et al.* 2002). PIG-1 in *C. elegans* is important for asymmetric division of certain neuroblasts in the Q lineage (Cordes *et al.* 2006), and its enhancement of embryo lethality in *par* mutants suggests that it is also playing a role in early embryo polarity. Like *nop-1*, *pig-1* RNAi increases embryonic lethality in both *par-1(zu310)* and *par-2(it5)* at semipermissive temperatures but has little effect on viability in *par-4(it57)*, and early polarity defects are seen only when *par-2* is compromised. Although we did not see effects on P granule localization in early *par-1* embryos treated with *pig-1* RNAi, one possibility, currently under investigation, is that PIG-1 acts redundantly with PAR-1 in the early *C. elegans* embryo in other aspects of embryo polarity. It will also be of interest to learn whether the enriched MELK in vertebrate embryos regulates cellular asymmetries.

Among the other enhancers identified in our screen is the deubiquitylase gene *math-33*. MATH-33 is closely related to mammalian USP7 (HAUSP), which has multiple targets for its deubiquitylating activity that include the tumor-suppressor p53 and its ubiquitin E3 ligase, MDM2, as well as FOXO4, PTEN, and other proteins important for oncogenesis and stem cell maintenance (reviewed by Nicholson and Suresh Kumar 2011). Changes in ubiquitylation status may regulate signaling pathways either by directing proteins for degrada-

tion by the proteasome or by altering protein conformation and activity (Haglund and Dikic 2005; Mukhopadhyay and Riezman 2007). RNAi of several proteasome components has been shown to suppress embryonic lethality in *par-2(it5)* (Labbe *et al.* 2006), suggesting that proteasomal function is indeed important for polarity in *C. elegans* embryos. *par-2* itself encodes a ubiquitin E3 ligase (Hao *et al.* 2006), but reducing activity of *math-33* by RNAi enhances, rather than suppresses, the lethality of the *par-2* mutant, suggesting that MATH-33 does not act in opposition to PAR-2 activity. Determining the targets of the MATH-33 deubiquitylase in *C. elegans* will be important for understanding its function in the early embryo.

We identified two sumoylation genes in our screen, encoding the SUMO-activating protein subunit UBA-2 and the SUMO ligase GEI-17. RNAi of these genes causes low levels of embryonic lethality in wild type, and each synergistically enhances embryonic lethality in each of the four *par* mutant strains tested. However, we did not observe striking polarity phenotypes in early embryos, suggesting that the essential role for sumoylation in *par*-mediated processes may be occurring after the two-cell stage. Because cytoskeletal proteins made up 7% of sumoylated *C. elegans* proteins identified in a proteomic study (Kaminsky *et al.* 2009), we speculate that one or more of these cytoskeletal proteins could be the relevant target responsible for the enhancing phenotype. We also note that *gei-17* increases lethality in *emb-9(b189)* embryos, which have a temperature-sensitive period late in embryogenesis, suggesting that the function of *gei-17* is not specific to the early embryo.

Three of the genes identified in our study, *math-33*, *ntl-9*, and *pig-1*, are also among 14 genes found by RNAi to increase lethality in a weak *vab-10A*/Plakin mutant (Zahreddine *et al.* 2010). VAB-10A is required for attachment of intermediate filaments to membranous organelles to form hemidesmosomes during *C. elegans* embryo elongation (Bosher *et al.* 2003). We have shown that RNAi of *math-33*, *ntl-9*, or *pig-1* strongly increases early embryo phenotypes in a weak *par-2* mutant; their enhancement of lethality during morphogenesis suggests that they might also function in epithelial

polarity. However, none of our 18 *par*-enhancing genes overlap with a set of recently identified *C. elegans* genes acting redundantly in gastrulation (Sawyer *et al.* 2011).

Our set of 18 genes that enhance lethality of *par-1*, *par-4*, or both is certainly not an exhaustive set of genes with roles in embryonic cell polarity. Our screen was biased toward genes with no or very weak RNAi phenotypes on their own. Many genes with important roles in early embryonic polarity will have strong lethal phenotypes including pleiotropic effects that could obscure a role in polarity. Furthermore, because our goal was to identify genes with strong effects on the *par* system, we performed our screens in a way that would eliminate genes with subtle effects or for which RNAi depletion must begin earlier in larval development to see an effect. There are also likely to be genes that we missed due to incomplete coverage of the genome in the set of RNAi genes that we used. Indeed, *aos-1* and *unc-61*, the heterodimeric partners of enhancers *uba-2* and *unc-59*, respectively (Johnson and Blobel 1997; Jones *et al.* 2002; John *et al.* 2007), were missing from the library, but are effective enhancers of *par-1* and *par-4* (Table S2). Nevertheless, our screen has identified a number of candidates for polarity function that have not previously been known. Most of these genes are conserved, and may function redundantly with key polarity regulators, ensuring the robustness of polarity establishment and maintenance. Investigating their roles in *C. elegans* embryogenesis and in polarity in other animals will enable us to develop a more complete understanding of this highly complex process.

Acknowledgments

We thank Jun Kelly Liu for sharing unpublished results from a parallel RNAi enhancer screen; Rich McCloskey, Alex Beatty, Jun Kelly Liu, and the Cornell Worm Group for helpful discussions; Daryl Hurd for *par-1* and *par-4* RNAi feeding constructs; and Mona Hassab for media preparation. This research was supported by National Institutes of Health grants HD27689 and GM079112 to K.J.K.

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Communicating editor: D. Greenstein

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Supporting Information

<http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.143727/-/DC1>

A Genome-Wide RNAi Screen for Enhancers of *par* Mutants Reveals New Contributors to Early Embryonic Polarity in *Caenorhabditis elegans*

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Figure S1 PAR-3 extension and reduction of PAR-1 in the posterior of *par-2(it5)* early embryos treated with enhancer(RNAi). One-cell anaphase stage (left column) and two-cell (right column) embryos from worms grown at 16°, treated with control (L4440), *nop-1* or *pig-1* RNAi, co-immunostained with PAR-3 (green) and PAR-1 (red) antibodies. DNA is stained with DAPI (blue). In *par-2(it5)* embryos treated with *nop-1(RNAi)* or *pig-1(RNAi)*, PAR-3 extends further into the posterior in anaphase one-cell embryos and is around the posterior of the P1 cell at the two-cell stage. PAR-1 is reduced at the posterior cortex in control *par-2(it5)* embryos compared to wild type and is not detectable at the cortex of the RNAi treated embryos.

File S1

***nop-1(RNAi)* embryo lacking pseudocleavage and anterior cortical ruffling.**

Time-lapse DIC movie with images taken every 10 sec from early one-cell to four-cell stage of embryogenesis.

File S1 is available for download as a Quicktime movie (MOV) at

<http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.143727/-/DC1>.

File S2

Wild-type (N2) control embryo showing pseudocleavage and anterior cortical ruffling.

Time-lapse DIC movie with images taken every 10 sec from early one-cell to four-cell stage of embryogenesis.

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Table S1 RNAi screen candidates for increasing embryonic lethality in *par-1(zu310)* or *par-4(it57)*

RNAi target	Encodes ^a	<i>par-1</i>	<i>par-4</i>
B0240.4	NPP-22 nucleoporin	EMB/+	EMB/+
B0334.11	OOC-3 embryo endoplasmic reticulum protein	EMB	EMB/+
<i>B0393.7</i>	LDL receptor-related	EMB	WT
<i>B0511.10</i>	EIF-3.E translation initiation factor	EMB/+	EMB/+
<i>C06C6.7</i>	Uncharacterized, nematode-specific	Ste/+	WT
<i>C07E3.10</i>	Uncharacterized, nematode-specific	WT	EMB
<i>C10C6.1</i>	KIN-4 PDZ-containing kinase	WT	EMB/+
<i>C12D8.10</i>	AKT-1 kinase	WT	EMB/+
<i>C12D8.9</i>	Uncharacterized	WT	EMB/+
<i>C17C3.6</i>	F-box domain protein	WT	EMB
<i>C24H12.5</i>	Uncharacterized	EMB	EMB/+
<i>C25H3.4</i>	Hits RGS-3 regulator of G protein signaling	WT	Ste/+
C26E6.3	Rcd1-related	WT	EMB
<i>C27C12.1</i>	Uncharacterized	EMB/+	WT
<i>C27F2.5</i>	VPS-22 sorting protein	WT	EMB/+
<i>C27H6.1</i>	UNC-41 adaptin-related protein	WT	EMB/+
<i>C27H6.2</i>	RUVB-1 ATPase	EMB/+	EMB
<i>C31B8.8</i>	Metalloprotease	WT	EMB/+
<i>C34F11.1</i>	Uncharacterized, nematode-specific	Ste/EMB	WT
<i>C38H2.2</i>	Galactosyltransferase	WT	EMB/+
<i>C44C3.3</i>	SRW-138 7-transmembrane receptor	WT	EMB/+
<i>C56E6.1</i>	ABCX-1 ABC transporter	Ste	Ste
<i>D1065.3</i>	Uncharacterized ankyrin repeat protein	WT	EMB
<i>D2045.5</i>	Tau tubulin kinase-related	EMB/+	WT
<i>F08E10.6</i>	SRH-111 serpentine receptor	Ste/+	WT
<i>F10D2.7</i>	UGT-38, UDP-GlucuronosylTransferase	WT	EMB/+
<i>F10E7.10</i>	CUTL-5 cuticulin	WT	Ste/+
<i>F11D11.1</i>	CLEC-255 C-type lectin	WT	EMB/+
<i>F11G11.9</i>	Sulfurtransferase	WT	EMB/+
<i>F13E6.4</i>	Ubiquitin protein ligase	Ste/+	WT
<i>F14H8.6</i>	CNG-1 cyclic nucleotide-gated channel protein	EMB/+	WT
<i>F16B12.5</i>	Casein Kinase I-like	WT	EMB/+
<i>F16D3.4</i>	Putative tubulin-specific chaperone	EMB/+	WT
<i>F16G10.4</i>	Uncharacterized, nematode-specific	WT	EMB/+
<i>F18A1.7</i>	Uncharacterized, nematode-specific	gro	EMB/+
<i>F20B4.6</i>	CGT-2 ceramide glucosyltransferase	WT	EMB

<i>F21D5.2</i>	Similar to ovarian tumor cysteine protease	WT	EMB/+
<i>F23F12.2</i>	Targets intron of <i>F23F12.3</i> and 8 other genes	EMB/+	EMB/+
<i>F25B5.2</i>	Uncharacterized, nematode-specific	EMB/+	WT
<i>F26G1.7</i>	MSP-3 major sperm protein, also hits other MSPs	WT	EMB/+
<i>F37E3.2</i>	Uncharacterized	EMB/+	EMB
<i>F40B1.2</i>	<i>bath-18</i> pseudogene	WT	EMB/+
<i>F42C5.10</i>	Uncharacterized	WT	Ste
<i>F42G8.3</i>	PMK-2 MAP kinase	WT	EMB/+
<i>F49E8.6</i>	Uncharacterized	WT	Ste
<i>F54F7.5</i>	MES-1 receptor-tyrosine kinase-like protein	EMB/+	WT
<i>F55G11.9</i>	ABC transporter	Ste/+	WT
<i>F56F10.4</i>	LGL-1 posterior polarity protein	Stp	WT
<i>F59B1.3</i>	SRX-93 chemoreceptor	WT	EMB/+
<i>H19N07.2</i>	MATH-33 ubiquitin carboxyl-terminal hydrolase	EMB/+	EMB
<i>H43I07.2</i>	RNA polymerase subunit RPAC	EMB/+	EMB
<i>K06B4.3</i>	Uncharacterized, nematode-specific	WT	Ste
<i>K06B4.4</i>	Uncharacterized, nematode-specific	EMB/+	WT
<i>K08D12.4</i>	Uncharacterized, nematode-specific	WT	EMB/+
<i>K09C4.3</i>	<i>hsp-2</i> pseudogene, also targets HSP-1	Ste/EMB	Ste/EMB
<i>K09D9.1</i>	Uncharacterized	WT	EMB/+
<i>K10B3.2</i>	Transposon	EMB/+	WT
<i>M88.5</i>	Putative mRNA binding protein	WT	EMB/+
<i>R09E12.3</i>	STI-1 HSP70/HSP90 co-chaperone	WT	EMB
<i>R11G11.12</i>	NHR-210 nuclear hormone receptor family	WT	EMB/+
<i>R11G11.9</i>	SRH-37 serpentine receptor	WT	EMB/+
<i>T01G9.6</i>	KIN-10, casein kinase II regulatory subunit	EMB/+	EMB
<i>T02G5.8</i>	KAT-1 3-ketoacyl-coA thiolase	WT	Ste
<i>T04C10.4</i>	ATF-5 transcription factor	WT	Ste/+
<i>T06C12.4</i>	FBXA-197 F-Box protein	WT	EMB/+
<i>T06E6.11</i>	SRBC-63 serpentine 7-transmembrane receptor	WT	EMB/+
<i>T08D10.2</i>	LSD-1 histone demethylase	Ste/+	WT
<i>T08G11.5</i>	UNC-29 acetylcholine receptor subunit	Ste/EMB	Gro
<i>T09D3.2</i>	SRG-28 serpentine receptor	WT	EMB/+
<i>T14G8.4</i>	Uncharacterized, nematode-specific	Ste/+	WT
<i>T18D3.6</i>	Uncharacterized, nematode-specific	WT	EMB/+
<i>T19D12.1</i>	Mucin-like	WT	EMB/+
<i>T23D5.9</i>	STR-43 7-transmembrane receptor	WT	Ste/+
<i>T23F11.2</i>	Uncharacterized, nematode-specific	EMB/+	WT
<i>T23F11.3</i>	CDKA-1 Cyclin-dependent kinase-5 activator	WT	EMB/+

T24F1.2	Transmembrane protein	EMB	WT
T26E4.13	srbc-74 pseudogene; also hits srbc-75, srbc-76	Ste	WT
T28B11.1	F-box protein	WT	EMB
T28F3.4	Major facilitator superfamily protein	WT	EMB/+
W02A11.4	UBA-2 SUMO-activating enzyme	EMB	WT
W03G1.6	PIG-1 kinase, MELK-like	EMB/+	WT
W09C5.2	UNC-59 septin GTPase	EMB/+	EMB/+
W10D5.3	GEI-17 SUMO ligase	EMB	EMB
Y102A5C.17	CLEC-238 C-type lectin	WT	EMB/+
Y102A5C.31	SRH-131 serpentine receptor	EMB/+	WT
Y106G6H.3	RPL-30 large ribosomal subunit protein	Ste/+	Ste
Y116A8C.33	Uncharacterized	EMB/+	EMB/+
Y22F5A.1	NHR-232 nuclear hormone receptor	WT	Ste/+
Y40B10B.1	Vitellin membrane protein-like	WT	EMB/+
Y43F11A.6	Pseudogene	WT	EMB/+
Y52D3.1	STRD-1 ortholog of STRAD	EMB/+	EMB
Y57E12AL.5 & .6	MDT-6 transcriptional mediator & Uncharacterized protein	EMB	EMB
Y5H2B.1	Predicted glycosyltransferase	WT	EMB/+
Y67D8C.4	Uncharacterized	WT	EMB/Gro
Y70C5C.3	Predicted receptor	EMB/+	WT
Y87G2A.8	GPI-1 glucose-6-phosphate isomerase	EMB/+	WT
ZC239.6	Uncharacterized	EMB/+	WT
ZK1127.10	CTH-2 cystathionine gamma-lyase	WT	EMB
ZK377.2	SAX-3 sensory axon guidance receptor	EMB/+	Ste
ZK856.8	Putative calcium-binding protein phosphatase	Ste/+	WT

RNAi clone candidates for enhancing lethality of *par-1(zu310)* or *par-4(it57)* at semipermissive temperatures by liquid feeding assay (see Materials and Methods). RNAi phenotypes are shown for each genotype: EMB: Embryo lethal; EMB/+ : both unhatched embryos and larvae produced; Ste (Sterile): adult worms are gonad-defective with few progeny; Ste/+ : some worms are sterile; Stp: progeny are sterile; Gro: slow growth; WT: larvae produced from RNAi-treated worms, similar to empty vector control RNAi. Genes that resulted in significantly increased lethality in *par-1(zu310)* or *par-4(it57)* when retested by RNAi feeding on plates are indicated in bold (see Table 1).

^a encoded gene products determined by BLAST and Wormbase annotation (www.wormbase.org).

Table S2 Embryonic lethality in *par* mutants upon RNAi of genes related to screen positives

RNAi	Genotype			
	N2, 19°	<i>par-1(zu310)</i> , 19°	<i>par-4(it57)</i> , 17°	<i>par-2(it5)</i> , 16°
L4440 control	0.1% (840)	10.0% (829)	25.2% (848)	8.2% (809)
<i>hsp-1</i>	27.9% (43)	35.8% (53)	59.6% (109)	31.6% (95)
<i>aos-1</i>	46.6% (606)	96.0% (1097)	81.9% (756)	69.2% (386)
<i>unc-61</i>	2.2% (787)	71.6% (1012)	48.2% (676)	7.9% (1170)

Table S3 Percent embryonic lethality in *emb-9(b189)* treated with RNAi of screen positives

RNAi	N2 (21°)	<i>emb-9(b189)</i> (21°)
L4440	0.4 ± 0.3	3.0 ± 0.3
<i>sad-1</i>	0.3 ± 0.2	1.7 ± 1.1
<i>par-4</i>	36.2 ± 1.5	34.9 ± 7.3
<i>strd-1</i>	0.5 ± 0.2	3.1 ± 1.2
<i>pig-1</i>	0.4 ± 0.3	2.2 ± 1.8
<i>mes-1</i>	0.9 ± 0.5	1.5 ± 0.7
<i>ntl-9</i>	3.8 ± 1.1	11.0 ± 6.0
<i>gei-17</i>	5.4 ± 0.1	14.6 ± 0.6*
<i>F25B5.2</i>	1.4 ± 0.2	1.9 ± 0.8
<i>math-33</i>	0.7 ± 1.6	3.9 ± 2.9
<i>uba-2</i>	29.9 ± 12.3	30.3 ± 7.8
<i>unc-59</i>	4.1 ± 2.2	4.2 ± 0.5
<i>lgl-1</i>	0.7 ± 0.2	2.4 ± 0.4
<i>ZK856.8</i>	0.7 ± 0.1	1.5 ± 0.4
<i>kin-10</i>	29.0 ± 8.0	20.6 ± 3.6
<i>T24F1.2</i>	1.6 ± 0.5	1.0 ± 0.5
<i>npp-22</i>	0.8 ± 0.4	1.8 ± 0.3
<i>str-43</i>	1.2 ± 0.3	1.0 ± 0.1
<i>ooc-3</i>	0.3 ± 0.2	1.7 ± 1.0
<i>hsp-2</i>	2.0 ± 0.7	7.0 ± 1.1*
<i>sti-1</i>	0.8 ± 0.2	2.1 ± 1.9

* enhanced lethality, p<0.05 compared to L4440 control and to N2

Figure S1 PAR-3 extension and reduction of PAR-1 in the posterior of *par-2(it5)* early embryos treated with enhancer(RNAi). One-cell anaphase stage (left column) and two-cell (right column) embryos from worms grown at 16°, treated with control (L4440), *nop-1* or *pig-1* RNAi, co-immunostained with PAR-3 (green) and PAR-1 (red) antibodies. DNA is stained with DAPI (blue). In *par-2(it5)* embryos treated with *nop-1(RNAi)* or *pig-1(RNAi)*, PAR-3 extends further into the posterior in anaphase one-cell embryos and is around the posterior of the P1 cell at the two-cell stage. PAR-1 is reduced at the posterior cortex in control *par-2(it5)* embryos compared to wild type and is not detectable at the cortex of the RNAi treated embryos.

File S1

***nop-1(RNAi)* embryo lacking pseudocleavage and anterior cortical ruffling.**

Time-lapse DIC movie with images taken every 10 sec from early one-cell to four-cell stage of embryogenesis.

File S1 is available for download as a Quicktime movie (MOV) at

<http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.143727/-/DC1>.

File S2

Wild-type (N2) control embryo showing pseudocleavage and anterior cortical ruffling.

Time-lapse DIC movie with images taken every 10 sec from early one-cell to four-cell stage of embryogenesis.

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Table S1 RNAi screen candidates for increasing embryonic lethality in *par-1(zu310)* or *par-4(it57)*

RNAi target	Encodes ^a	<i>par-1</i>	<i>par-4</i>
B0240.4	NPP-22 nucleoporin	EMB/+	EMB/+
B0334.11	OOC-3 embryo endoplasmic reticulum protein	EMB	EMB/+
<i>B0393.7</i>	LDL receptor-related	EMB	WT
<i>B0511.10</i>	EIF-3.E translation initiation factor	EMB/+	EMB/+
<i>C06C6.7</i>	Uncharacterized, nematode-specific	Ste/+	WT
<i>C07E3.10</i>	Uncharacterized, nematode-specific	WT	EMB
<i>C10C6.1</i>	KIN-4 PDZ-containing kinase	WT	EMB/+
<i>C12D8.10</i>	AKT-1 kinase	WT	EMB/+
<i>C12D8.9</i>	Uncharacterized	WT	EMB/+
<i>C17C3.6</i>	F-box domain protein	WT	EMB
<i>C24H12.5</i>	Uncharacterized	EMB	EMB/+
<i>C25H3.4</i>	Hits RGS-3 regulator of G protein signaling	WT	Ste/+
C26E6.3	Rcd1-related	WT	EMB
<i>C27C12.1</i>	Uncharacterized	EMB/+	WT
<i>C27F2.5</i>	VPS-22 sorting protein	WT	EMB/+
<i>C27H6.1</i>	UNC-41 adaptin-related protein	WT	EMB/+
<i>C27H6.2</i>	RUVB-1 ATPase	EMB/+	EMB
<i>C31B8.8</i>	Metalloprotease	WT	EMB/+
<i>C34F11.1</i>	Uncharacterized, nematode-specific	Ste/EMB	WT
<i>C38H2.2</i>	Galactosyltransferase	WT	EMB/+
<i>C44C3.3</i>	SRW-138 7-transmembrane receptor	WT	EMB/+
<i>C56E6.1</i>	ABCX-1 ABC transporter	Ste	Ste
<i>D1065.3</i>	Uncharacterized ankyrin repeat protein	WT	EMB
<i>D2045.5</i>	Tau tubulin kinase-related	EMB/+	WT
<i>F08E10.6</i>	SRH-111 serpentine receptor	Ste/+	WT
<i>F10D2.7</i>	UGT-38, UDP-GlucuronosylTransferase	WT	EMB/+
<i>F10E7.10</i>	CUTL-5 cuticulin	WT	Ste/+
<i>F11D11.1</i>	CLEC-255 C-type lectin	WT	EMB/+
<i>F11G11.9</i>	Sulfurtransferase	WT	EMB/+
<i>F13E6.4</i>	Ubiquitin protein ligase	Ste/+	WT
<i>F14H8.6</i>	CNG-1 cyclic nucleotide-gated channel protein	EMB/+	WT
<i>F16B12.5</i>	Casein Kinase I-like	WT	EMB/+
<i>F16D3.4</i>	Putative tubulin-specific chaperone	EMB/+	WT
<i>F16G10.4</i>	Uncharacterized, nematode-specific	WT	EMB/+
<i>F18A1.7</i>	Uncharacterized, nematode-specific	gro	EMB/+
<i>F20B4.6</i>	CGT-2 ceramide glucosyltransferase	WT	EMB

<i>F21D5.2</i>	Similar to ovarian tumor cysteine protease	WT	EMB/+
<i>F23F12.2</i>	Targets intron of <i>F23F12.3</i> and 8 other genes	EMB/+	EMB/+
<i>F25B5.2</i>	Uncharacterized, nematode-specific	EMB/+	WT
<i>F26G1.7</i>	MSP-3 major sperm protein, also hits other MSPs	WT	EMB/+
<i>F37E3.2</i>	Uncharacterized	EMB/+	EMB
<i>F40B1.2</i>	<i>bath-18</i> pseudogene	WT	EMB/+
<i>F42C5.10</i>	Uncharacterized	WT	Ste
<i>F42G8.3</i>	PMK-2 MAP kinase	WT	EMB/+
<i>F49E8.6</i>	Uncharacterized	WT	Ste
<i>F54F7.5</i>	MES-1 receptor-tyrosine kinase-like protein	EMB/+	WT
<i>F55G11.9</i>	ABC transporter	Ste/+	WT
<i>F56F10.4</i>	LGL-1 posterior polarity protein	Stp	WT
<i>F59B1.3</i>	SRX-93 chemoreceptor	WT	EMB/+
<i>H19N07.2</i>	MATH-33 ubiquitin carboxyl-terminal hydrolase	EMB/+	EMB
<i>H43I07.2</i>	RNA polymerase subunit RPAC	EMB/+	EMB
<i>K06B4.3</i>	Uncharacterized, nematode-specific	WT	Ste
<i>K06B4.4</i>	Uncharacterized, nematode-specific	EMB/+	WT
<i>K08D12.4</i>	Uncharacterized, nematode-specific	WT	EMB/+
<i>K09C4.3</i>	<i>hsp-2</i> pseudogene, also targets HSP-1	Ste/EMB	Ste/EMB
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<i>M88.5</i>	Putative mRNA binding protein	WT	EMB/+
<i>R09E12.3</i>	STI-1 HSP70/HSP90 co-chaperone	WT	EMB
<i>R11G11.12</i>	NHR-210 nuclear hormone receptor family	WT	EMB/+
<i>R11G11.9</i>	SRH-37 serpentine receptor	WT	EMB/+
<i>T01G9.6</i>	KIN-10, casein kinase II regulatory subunit	EMB/+	EMB
<i>T02G5.8</i>	KAT-1 3-ketoacyl-coA thiolase	WT	Ste
<i>T04C10.4</i>	ATF-5 transcription factor	WT	Ste/+
<i>T06C12.4</i>	FBXA-197 F-Box protein	WT	EMB/+
<i>T06E6.11</i>	SRBC-63 serpentine 7-transmembrane receptor	WT	EMB/+
<i>T08D10.2</i>	LSD-1 histone demethylase	Ste/+	WT
<i>T08G11.5</i>	UNC-29 acetylcholine receptor subunit	Ste/EMB	Gro
<i>T09D3.2</i>	SRG-28 serpentine receptor	WT	EMB/+
<i>T14G8.4</i>	Uncharacterized, nematode-specific	Ste/+	WT
<i>T18D3.6</i>	Uncharacterized, nematode-specific	WT	EMB/+
<i>T19D12.1</i>	Mucin-like	WT	EMB/+
<i>T23D5.9</i>	STR-43 7-transmembrane receptor	WT	Ste/+
<i>T23F11.2</i>	Uncharacterized, nematode-specific	EMB/+	WT
<i>T23F11.3</i>	CDKA-1 Cyclin-dependent kinase-5 activator	WT	EMB/+

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W02A11.4	UBA-2 SUMO-activating enzyme	EMB	WT
W03G1.6	PIG-1 kinase, MELK-like	EMB/+	WT
W09C5.2	UNC-59 septin GTPase	EMB/+	EMB/+
W10D5.3	GEI-17 SUMO ligase	EMB	EMB
Y102A5C.17	CLEC-238 C-type lectin	WT	EMB/+
Y102A5C.31	SRH-131 serpentine receptor	EMB/+	WT
Y106G6H.3	RPL-30 large ribosomal subunit protein	Ste/+	Ste
Y116A8C.33	Uncharacterized	EMB/+	EMB/+
Y22F5A.1	NHR-232 nuclear hormone receptor	WT	Ste/+
Y40B10B.1	Vitellin membrane protein-like	WT	EMB/+
Y43F11A.6	Pseudogene	WT	EMB/+
Y52D3.1	STRD-1 ortholog of STRAD	EMB/+	EMB
Y57E12AL.5 & .6	MDT-6 transcriptional mediator & Uncharacterized protein	EMB	EMB
Y5H2B.1	Predicted glycosyltransferase	WT	EMB/+
Y67D8C.4	Uncharacterized	WT	EMB/Gro
Y70C5C.3	Predicted receptor	EMB/+	WT
Y87G2A.8	GPI-1 glucose-6-phosphate isomerase	EMB/+	WT
ZC239.6	Uncharacterized	EMB/+	WT
ZK1127.10	CTH-2 cystathionine gamma-lyase	WT	EMB
ZK377.2	SAX-3 sensory axon guidance receptor	EMB/+	Ste
ZK856.8	Putative calcium-binding protein phosphatase	Ste/+	WT

RNAi clone candidates for enhancing lethality of *par-1(zu310)* or *par-4(it57)* at semipermissive temperatures by liquid feeding assay (see Materials and Methods). RNAi phenotypes are shown for each genotype: EMB: Embryo lethal; EMB/+ : both unhatched embryos and larvae produced; Ste (Sterile): adult worms are gonad-defective with few progeny; Ste/+ : some worms are sterile; Stp: progeny are sterile; Gro: slow growth; WT: larvae produced from RNAi-treated worms, similar to empty vector control RNAi. Genes that resulted in significantly increased lethality in *par-1(zu310)* or *par-4(it57)* when retested by RNAi feeding on plates are indicated in bold (see Table 1).

^a encoded gene products determined by BLAST and Wormbase annotation (www.wormbase.org).

Table S2 Embryonic lethality in *par* mutants upon RNAi of genes related to screen positives

RNAi	Genotype			
	N2, 19°	<i>par-1(zu310)</i> , 19°	<i>par-4(it57)</i> , 17°	<i>par-2(it5)</i> , 16°
L4440 control	0.1% (840)	10.0% (829)	25.2% (848)	8.2% (809)
<i>hsp-1</i>	27.9% (43)	35.8% (53)	59.6% (109)	31.6% (95)
<i>aos-1</i>	46.6% (606)	96.0% (1097)	81.9% (756)	69.2% (386)
<i>unc-61</i>	2.2% (787)	71.6% (1012)	48.2% (676)	7.9% (1170)

Table S3 Percent embryonic lethality in *emb-9(b189)* treated with RNAi of screen positives

RNAi	N2 (21°)	<i>emb-9(b189)</i> (21°)
L4440	0.4 ± 0.3	3.0 ± 0.3
<i>sad-1</i>	0.3 ± 0.2	1.7 ± 1.1
<i>par-4</i>	36.2 ± 1.5	34.9 ± 7.3
<i>strd-1</i>	0.5 ± 0.2	3.1 ± 1.2
<i>pig-1</i>	0.4 ± 0.3	2.2 ± 1.8
<i>mes-1</i>	0.9 ± 0.5	1.5 ± 0.7
<i>ntl-9</i>	3.8 ± 1.1	11.0 ± 6.0
<i>gei-17</i>	5.4 ± 0.1	14.6 ± 0.6*
<i>F25B5.2</i>	1.4 ± 0.2	1.9 ± 0.8
<i>math-33</i>	0.7 ± 1.6	3.9 ± 2.9
<i>uba-2</i>	29.9 ± 12.3	30.3 ± 7.8
<i>unc-59</i>	4.1 ± 2.2	4.2 ± 0.5
<i>lgl-1</i>	0.7 ± 0.2	2.4 ± 0.4
<i>ZK856.8</i>	0.7 ± 0.1	1.5 ± 0.4
<i>kin-10</i>	29.0 ± 8.0	20.6 ± 3.6
<i>T24F1.2</i>	1.6 ± 0.5	1.0 ± 0.5
<i>npp-22</i>	0.8 ± 0.4	1.8 ± 0.3
<i>str-43</i>	1.2 ± 0.3	1.0 ± 0.1
<i>ooc-3</i>	0.3 ± 0.2	1.7 ± 1.0
<i>hsp-2</i>	2.0 ± 0.7	7.0 ± 1.1*
<i>sti-1</i>	0.8 ± 0.2	2.1 ± 1.9

* enhanced lethality, p<0.05 compared to L4440 control and to N2