toca-1 Is in a Novel Pathway That Functions in Parallel with a SUN-KASH Nuclear Envelope Bridge to Move Nuclei in *Caenorhabditis elegans*

Yu-Tai Chang, Daniel Dranow, Jonathan Kuhn, Marina Meyerzon,

Minh Ngo, Dmitry Ratner, Karin Warltier, and Daniel A. Starr¹

Department of Molecular and Cellular Biology, University of California, Davis, California 95616

ABSTRACT Moving the nucleus to an intracellular location is critical to many fundamental cell and developmental processes, including cell migration, differentiation, fertilization, and establishment of cellular polarity. Bridges of SUN and KASH proteins span the nuclear envelope and mediate many nuclear positioning events, but other pathways function independently through poorly characterized mechanisms. To identify and characterize novel mechanisms of nuclear migration, we conducted a nonbiased forward genetic screen for mutations that enhanced the nuclear migration defect of *unc-84*, which encodes a SUN protein. In *Caenorhabditis elegans* larvae, failure of hypodermal P-cell nuclear migration results in uncoordinated and egg-laying-defective animals. The process of P-cell nuclear migration in *unc-84* null animals is temperature sensitive; at 25° migration fails in *unc-84* mutants, but at 15° the migration occurs normally. We hypothesized that an additional pathway functions in parallel to the *unc-84* pathway to move P-cell nuclei at 15°. In support of our hypothesis, forward genetic screens isolated eight *emu* (*enhancer of the nuclear migration defect of unc-84*) mutations that disrupt nuclear migration only in a null *unc-84* background. The *yc20* mutant was determined to carry a mutation in the *toca-1* gene. *TOCA-1* functions to move P-cell nuclei in a cell-autonomous manner. *TOCA-1* is conserved in humans, where it functions to nucleate and organize actin during endocytosis. Therefore, we have uncovered a player in a previously unknown, likely actin-dependent, pathway that functions to move nuclei in parallel to SUN-KASH bridges. The other *emu* mutations potentially represent other components of this novel pathway.

NUCLEAR migration is a tightly controlled event where the nucleus moves through the cytoplasm to its specific location within a cell. Moving nuclei to specific intracellular locations is critical to many fundamental cell and developmental processes, including mitosis, meiosis, cell migration, differentiation, fertilization, and establishment of cellular polarity (Morris 2000a; Wilhelmsen *et al.* 2006; Starr and Fridolfsson 2010). While nuclear migration events are essential for proper development in the central nervous system and muscle (Morris *et al.* 1998; Tsujikawa *et al.* 2007; Valiente and Marin 2010; Metzger *et al.* 2012), abnormal nuclear migration can lead to developmental defects and human diseases, such as the smooth brain disease lissencephaly, which results in severe mental retardation and epilepsy (Morris 2000b; Wynshaw-Boris 2007).

The best-characterized mechanism for nuclear migration is how the **Linker of Nucleoskeleton and Cytoskeleton** (LINC) complex of SUN proteins in the inner nuclear membrane and KASH proteins in the outer nuclear membrane connects the nucleus to the cytoskeleton (Roux *et al.* 2009; Starr and Fridolfsson 2010). Mutations in either of the *Caenorhabditis elegans* *unc-83* and *unc-84* genes disrupt nuclear migration in a number of cells, including embryonic hyp7 precursors, intestinal primordial cells, the distal tip cell of the somatic gonad, the one-cell embryo, and larval hypodermal P cells (Sulston and Horvitz 1981; Malone *et al.* 1999; Starr *et al.* 2001; Xiong *et al.* 2011). In our nuclear-envelope bridging model, the SUN protein *UNC-84* is targeted to the inner nuclear membrane with its conserved SUN domain in the perinuclear space and its N terminus in the nucleoplasm, where it can interact with lamin (Malone *et al.* 1999; Lee *et al.* 2002; McGee *et al.* 2006; Tapley *et al.* 2011). *UNC-84* then recruits the KASH protein *UNC-83* to the outer nuclear membrane through a direct interaction between SUN and KASH domains in the

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¹Corresponding author: Department of Molecular and Cellular Biology, One Shields Ave., University of California, Davis, CA 95616. E-mail: dastarr@ucdavis.edu

perinuclear space (Starr *et al.* 2001; McGee *et al.* 2006; Mellad *et al.* 2011). In the cytoplasm, *UNC-83* acts as the nuclear-specific cargo adaptor to recruit the microtubule motors kinesin-1 and dynein to the cytoplasmic surface of the nucleus. Kinesin-1 then provides the mechanical forces to move nuclei while dynein mediates bidirectional movements of nuclei to avoid cellular roadblocks (Meyerzon *et al.* 2009; Fridolfsson and Starr 2010; Fridolfsson *et al.* 2010). Similar SUN-KASH nuclear-envelope bridges are conserved from yeast to humans (Starr and Fridolfsson 2010; Starr 2011). For example, SUN-KASH bridges recruit microtubule motors to move nuclei in *Drosophila* (Patterson *et al.* 2004; Kracklauer *et al.* 2007), in *Schizosaccharomyces pombe* (Chikashige *et al.* 2006), and in mammalian cells (Roux *et al.* 2009).

Many nuclear migration events rely on mechanisms other than the microtubule motor-based mechanism discussed above. For example, in polarizing fibroblasts, retrograde actin flow is coupled to nuclei by SUN-KASH bridges to move nuclei (Gomes *et al.* 2005; Luxton *et al.* 2010). In other cases, nuclear migration is propelled by SUN-KASH-independent mechanisms. Examples include unknown mechanisms used to move nuclei in *Arabidopsis* root hairs (Chytilova *et al.* 2000), actomyosin contraction at the rear of nuclei in migrating neurites (Bellion *et al.* 2005; Schaar and McConnell 2005; Tsai *et al.* 2007; Schenk *et al.* 2009; Martini and Valdeolmillos 2010), and kinesin-driven microtubule sliding in developing myotubes (Metzger *et al.* 2012). Many additional SUN-KASH-independent mechanisms of nuclear migration are likely unknown. Here, we employed a forward genetic screen in *C. elegans* to identify novel mechanisms of nuclear migration.

Mutations in *unc-83* and *unc-84* were originally found in cell-lineage mutant screens where P-cell lineages were missing (Horvitz and Sulston 1980; Sulston and Horvitz 1981). Normally, P-cell nuclei migrate from a lateral position to the ventral cord during the mid-L1 larval stage (Figure 1A) (Sulston 1976; Sulston and Horvitz 1977). After nuclear migration, P cells normally divide and their lineages develop into hypodermal cells, ventral neurons, and vulval cells (Sulston and Horvitz 1977). In *unc-83* and *unc-84* mutant animals, failure of P-cell nuclear migration leads to the death of P cells, which causes a loss of P-cell lineages and leads to **uncoordinated** (**Unc**) and **egg-laying-deficient** (**Egl**) phenotypes due to the loss of neurons and vulva, respectively (Sulston and Horvitz 1981; Malone *et al.* 1999; Starr *et al.* 2001). As originally described by Sulston and Horvitz, null mutations in *unc-83* or *unc-84* are temperature sensitive (Sulston and Horvitz 1981). At the restrictive temperature of 25°, <50% of P-cell nuclei migrate to the ventral cord, resulting in **Egl** and **Unc** animals (Figure 1B, top line). However, at the permissive temperature of 15°, ~90% of P-cell nuclei migrate to the ventral cord and the animals have no obvious phenotype (Figure 1B, middle line) (Sulston and Horvitz 1981; Malone *et al.* 1999; Starr *et al.* 2001). These observations lead to our central hypothesis—that an

uncharacterized pathway is sufficient to facilitate P-cell nuclear migration at 15° in the absence of *unc-83* or *unc-84* (Figure 1B, bottom line).

To test our hypothesis and to identify and characterize novel nuclear migration pathways, we performed a forward genetic screen for *enhancer of the nuclear migration defect of unc-84* (*emu*) mutations that disrupt P-cell nuclear migration. We isolated eight *emu* mutations, representing multiple loci, and used whole-genome sequencing (WGS) to determine that one mutation, *yc20*, is in the *toca-1* gene. Transducer of Cdc42-dependent actin assembly (TOCA)-1 protein contains an F-BAR membrane-binding scaffold domain and domains that interact with the Rho family GTPase Cdc42 and the actin-nucleating WASP complex (Ho *et al.* 2004; Fricke *et al.* 2009; Giuliani *et al.* 2009). We conclude that *TOCA-1* is the founding member of a novel SUN-KASH-independent pathway for nuclear migration. Furthermore, this study demonstrates that the *C. elegans* P cell is a powerful model to identify novel and partially redundant mechanisms for nuclear migration.

Materials and Methods

C. elegans strains and genetics

Worms were cultured on OP50-seeded NGM plates at specific temperatures and unless otherwise noted, strains were derived from the N2 strain (Brenner 1974). Integrated *p_{unc-47}::gfp* transgenic animals (*oxIs12*; strain EG1285) (McIntire *et al.* 1997) were used as wild-type controls in this study. *oxIs12[p_{unc-47}::gfp]* was crossed into *unc-84(n369); ycEx60[odr-1::rfp; unc-84(+)]* to create the strain UD87. The *odr-1::rfp* plasmid was a gift from Noelle Etoile [University of California, San Francisco (UCSF)] (Etoile and Bargmann 2000). UD87 animals were used to perform the *emu* forward genetic screen and for backcrosses. Some nematode strains used in this work were provided by the *Caenorhabditis* Genetics Center (CGC), which is funded by the National Institutes of Health National Center for Research Resources. See Table 1 for genotypes of strains in this study.

For the *emu* forward genetic screen, standard EMS mutagenesis (Brenner 1974) was performed with UD87 (*unc-84(n369) oxIs12[p_{unc-47}::gfp]* X) animals at 15°. Since *ycEx60[odr-1::rfp; unc-84(+)]* rescues *unc-84*, F₂ progeny were blindly picked and screened in the F₃ generation for **Egl**, non-RFP animals at 15°. After a candidate mutant was isolated, *p_{unc-47}::GFP*-labeled GABA neurons were counted as a secondary screen.

To determine whether an *emu* mutation was on the X chromosome, *emu; unc-84(n369) oxIs12[p_{unc-47}::gfp]* X hermaphrodites were crossed to wild-type males with a GFP-marked pharynx (*p_{myo-2}::gfp* or *ajm-1::gfp*). Male progeny were hemizygous for *unc-84(n369)* and *emu* if the *emu* mutation was on chromosome X. *p_{unc-47}::GFP*-labeled GABA neurons were counted in hemizygous males. If the number of GABA neurons was significantly decreased, we concluded that the *emu* mutation was on chromosome X.

Figure 1 P-cell nuclear migration is temperature sensitive in an *unc-83* or *unc-84* background. (A) A conceptual diagram of the migration of nuclei (green) in P cells (blue) during the mid-L1 larval stage. A lateral view of the mid-body of an L1 animal (orange) is shown; ventral is down. In wild type, P-cell nuclei migrate from a lateral position to the ventral cord; the cytoplasm retracts following the nuclear migration. After migration, P-cell lineages normally develop into ventral neurons and vulva cells. In *unc-83* or *unc-84* null, mutant animals, failure of P-cell nuclear migration at 25°C causes the death of many P cells and leads to **egg-laying-deficient (Egl)** and **uncoordinated (Unc)** phenotypes. (B) Our central hypothesis for an enhancer pathway mediating nuclear migration is shown. (Top line) At 25°C, P-cell nuclear migration fails in *unc-83* or *unc-84* null animals. (Middle line) However, at 15°C, *unc-83* or *unc-84* null animals behave normally, suggesting that a second, uncharacterized pathway (the enhancer or *emu* pathway) is sufficient for nuclear migration at 15°C. (Bottom line) When both the *unc-83/84* and *emu* pathways are disrupted, we hypothesized that the resulting animals would be Egl and Unc. (C) *p_{unc-47}::GFP*-labeled GABA neurons were used as a marker of P-cell-derived lineage. On the left, in *yc20 unc-84(n369)* animals with the *unc-84*-rescuing extrachromosomal array *ycEx60[odr-1::rfp; unc-84(+)]*, 19 neurons were counted in the ventral cord between the pharynx and the anus (marked by dashed lines). However, failure of P-cell nuclear migration caused loss of GABA neurons as shown on the right where only 8 neurons were seen in a *yc20 unc-84(n369)* animal. Anterior is left. (D) An F₂ clonal screen for *emu* in *unc-84(n369)* animals was performed using an extrachromosomal array *ycEx60[odr-1::rfp; unc-84(+)]* containing an *unc-84(+)* rescuing construct (see *Materials and Methods* for details).

To assign *emu* mutations to chromosomes, two-point single-nucleotide polymorphism (SNP) mapping was performed (Fay and Bender 2008). First, UD87 was backcrossed with Hawaiian CB4856 males 10 times to create UD350 that carries the *unc-84(n369)* null mutation, but otherwise is mostly CB4856, Hawaiian, for nonlinked SNPs. For two-point SNP mapping, *unc-84(n369); emu* animals were mated with *unc-84(n369)*-Hawaiian males and Egl F₂ progeny were singled onto individual plates. PCR and restriction-enzyme digests for known SNPs were performed on F₃ Egl animals and individual *emu* mutations were assigned to chromosomes.

Assays for quantifying P-cell nuclear migration defects

The *oxIs12[p_{unc-47}::gfp]* marker was used as an assay to label P-cell-derived GABA neurons (McIntire *et al.* 1997) and to

approximate P-cell nuclear migration defects. L4 or young adult worms were mounted on a 2% noble agar pad in 0.5 mM levamisole M9 solution. The number of *p_{unc-47}::GFP*-labeled GABA neurons in the ventral cord was counted using a 10× objective on an epifluorescence compound microscope (DM6000; Leica). The most posterior neuron (DVB) and the seven neurons in the head (out of the plane of the ventral cord) were not counted, as none of them are derived from P-cell lineages (McIntire *et al.* 1997; Sulston and Horvitz 1977). Thus we counted 19 GABA neurons in wild type, 12 of which were derived from P cells (VD2–VD13) (McIntire *et al.* 1997; Sulston and Horvitz 1977). A complete loss of P-cell lineages resulted in only 7 GABA neurons in the ventral cord. Images were taken using a CCD camera (DC350 FX; Leica) and analyzed with LAS AF (Leica) software. Statistical analysis was performed by using the two-tailed

Table 1 Strains used in this study

Strain	Genotype
CB4856	A wild-type strain from a divergent population in Hawaii (Koch <i>et al.</i> 2000)
EG1285	<i>lin-15B(n765) oxIs12[p_{unc-47}::gfp; lin-15(+)] X</i> (McIntire <i>et al.</i> 1997)
MH1654	<i>unc-83(e1408) V; oxIs12 X</i> (a gift from Min Han, University of Colorado, Boulder)
PD4788	<i>mIs13[p_{myo-2}::gfp; pes-10::gfp; gut::gfp] I</i> (a gift from Kelly Liu, Cornell University)
UD4	<i>yc3; unc-83(e1408); oxIs12 X</i>
UD59	<i>unc-84(n369); ycls11[odr-1::gfp; p_{hlh-3}::vab-10-ABD::venus; p_{hlh-3}::nls::tdTOMATO]</i>
UD87	<i>unc-84(n369) oxIs12 X; yEx60[odr-1::rfp; unc-84(+)]</i>
UD230	<i>yc22; unc-84(n369) oxIs12 X</i>
UD277	<i>yc3; unc-84(n369) oxIs12 X</i> (10× backcross with UD87)
UD278	<i>yc16; unc-84(n369) oxIs12 X; yEx60</i> (10× backcross with UD87)
UD279	<i>yc21; unc-84(n369) oxIs12 X; yEx60</i> (10× backcross with UD87)
UD283	<i>yc18; unc-84(n369) oxIs12 X; yEx60</i> (10× backcross with UD87)
UD284	<i>yc15; unc-84(n369) oxIs12 X; yEx60</i> (10× backcross with UD87)
UD346	<i>toca-1(yc20) unc-84(n369) oxIs12 X; yEx60</i> (4× backcross with UD87)
UD347	<i>yc17; unc-84(n369) oxIs12 X; yEx60</i> (2× backcross with UD87)
UD350	<i>unc-84(n369)</i> in a mostly CB4856 background (10× backcross with CB4856)
UD352	<i>toca-1(tm2056) unc-84(n369) oxIs12 X; yEx60</i>
UD354	<i>toca-2(tm2088) III; unc-84(n369) oxIs12 X; yEx60</i>
UD355	<i>toca-1(tm2056) unc-84(n369) oxIs12 X; yEx60; yEx189[odr-1::gfp; p_{hlh-3}::toca-1::gfp]</i>
UD356	<i>toca-1(tm2056) unc-84(n369) oxIs12 X; yEx60; yEx190[odr-1::gfp; p_{col-10}::toca-1::gfp]</i>
UD357	<i>toca-1(yc20) unc-84(n369) oxIs12 X; yEx60; yEx189</i>
UD358	<i>toca-1(yc20) unc-84(n369) oxIs12 X; yEx60; yEx190</i>
UD373	<i>toca-1(tm2056) unc-84(n369); ycls11; yEx60</i>
UD381	<i>ycls11[odr-1::gfp; p_{hlh-3}::vab-10-ABD::venus; p_{hlh-3}::nls::tdTomato]</i>

distribution Student's *t*-test function in Excel (Microsoft, Redmond, WA).

To directly follow P-cell nuclei during migration, the plasmid pSL619 (*p_{hlh-3}::nls::tdTomato*) was created using the 3.4-kb *hlh-3* promoter (encoding the first 8 aa of *hlh-3*) (Doonan *et al.* 2008) and *nls::tdTomato* sequence. *nls::tdTomato* was PCR cloned from a mammalian pCAB vector (Spear and Erickson 2012) and the 5' primer contained an SV40 nuclear localization signal. pSL619 (*p_{hlh-3}::nls::tdTomato*) (2 ng/μl) was coinjected with pSL630 (*p_{hlh-3}::vab-10-ABD::venus*) (5 ng/μl) and *odr-1::gfp* (100 ng/μl) into N2 animals to create the extrachromosomal array *yEx202[odr-1::gfp; p_{hlh-3}::vab-10-ABD::venus; p_{hlh-3}::nls::tdTomato]*. The *odr-1::gfp* plasmid was a gift from Noelle LEtoile (UCSF) (LEtoile and Bargmann 2000). *yEx202* was integrated by a UV/TMP method (Gengyo-Ando and Mitani 2000) to create *ycls11*, which labeled P-cell nuclei throughout L1. The integrated strain was outcrossed four times to make the strain UD381 (*ycls11* in N₂). Subsequently *ycls11* was crossed into *unc-84(n369)* and *toca-1(tm2056) unc-84(n369)*, to create the strains UD59 and UD373, respectively.

To perform P-cell nuclei counting assays, transgenic animals carrying *ycls11* were synchronized at the beginning of the L1 stage by starvation in S-basal media for 36, 30, or 24 hr at 15°, 20°, or 25°, respectively (Stiernagle 2006). The early L1 larval arrest was released by the addition of food (*Escherichia coli* OP50) for 16, 13, or 10 hr at 15°, 20°, or 25°, respectively. Late L1 larvae, after the completion of nuclear migration, were collected by centrifugation, anesthetized with 0.5 mM levamisole, and mounted on a 2% agar pad. The positions of tdTOMATO-labeled nuclei were

examined using a 63× Plan Apo 1.40 NA objective on an epifluorescence compound microscope (DM6000; Leica). Nuclei that failed to migrate could be seen at a lateral position on either side of the animals.

Whole-genome sequencing and analysis

emu; unc-84(n369) oxIs12[p_{unc-47}::gfp] X; yEx60[unc-84(+); odr-1::rfp] homozygous animals were grown at 15° and washed with M9 from NGM plates. A worm pellet of ~200 μl was mixed with lysis buffer [100 mM NaCl, 100 mM Tris (pH 8.5), 50 mM EDTA, 1% SDS, 1% BME, 100 μg/ml Proteinase K] and incubated at 60° for 60 min. After phenol/chloroform extraction and RNase A (20 μg/ml) treatment at 30° for 30 min, genomic DNA was purified by ethanol precipitation. For WGS, DNA libraries were made using the next-generation DNA library kit (New England Biolabs, Beverly, MA). The DNA library from each mutant strain was subjected to WGS on an Illumina GAI sequencing platform at the University of California (UC) Davis Genome Center Core Facility, using paired-end 85-nt reads. Raw sequencing files were analyzed using MAQGene software (Bigelow *et al.* 2009). The MAQGene output data were imported into Excel (Microsoft) to filter out background mutations and identify candidates for the *emu* mutation.

RNA interference tests

dsRNA was made from PCR templates amplified from Ahringer library clones (Fraser *et al.* 2000; Kamath *et al.* 2003) or genomic DNA, using T7 RNAPol (Promega, Madison, WI) as described (Fire *et al.* 1998). For *toca-1(RNAi)*, Ahringer library clone X-1B09 was used. dsRNA was microinjected into

the gonads of L4 or young adult UD87 hermaphrodites as previously described (Fire *et al.* 1998). F₁ progeny grown at 15° and produced between 24 and 48 hr postinjection were collected and their GABA neurons were counted as above.

TOCA-1::GFP rescue and localization

toca-1 was expressed specifically in P cells with the promoter of *hlh-3* to assay the ability for wild-type *toca-1* to rescue *toca-1(yc20)*. *toca-1* genomic DNA containing all exons and introns except for the first intron was amplified by PCR from fosmid WRM0623aA03 from the Moerman fosmid library (Source BioScience LifeSciences), using primers ods1546 (GGTACCCCCATGAACGACAGTTGCAGTTGGGACCAGCTATGGGACCAACAAGGTACTCTCA) and ods1547 (GGTACCGA CTTTTCCGAACCAGGTGA). The *toca-1* PCR product was cloned into the *KpnI* site of pPD95.79 (Addgene) to make pSL614, a promoterless construct to express GFP fused to the C terminus of TOCA-1. Next, 3.4 kb of the promoter for *hlh-3*, which is expressed specifically in embryonic and larval P cells, was obtained from pRD1 (Doonan *et al.* 2008) and cloned into the *SmaI* site of pSL614 to make *p_{hlh-3}::toca-1::gfp* (pSL626). Alternatively, 1.2 kb of the promoter for *col-10*, which is expressed in embryonic and larval hypodermal cells, was obtained from pOS12 (Spencer *et al.* 2001) and cloned into the *SphI* and *SmaI* sites of pSL614 to make *p_{col-10}::toca-1::gfp* (pSL627). *p_{hlh-3}::toca-1::gfp* (2 ng/μl) or *p_{col-10}::toca-1::gfp* (5 ng/μl) was microinjected with *odr-1::gfp* (100 ng/μl) as a marker into the gonads of young adult hermaphrodites (Mello and Fire 1995). Multiple, independent stable transgenic lines were isolated and GABA neurons were counted as above. TOCA-1::GFP images were acquired using a 63× objective on a Leica DM6000 microscope as above.

RT-PCR and Western analysis

Total RNA from N2, UD87, *toca-1(tm2056) unc-84(n369)*, and *toca-1(yc20) unc-84(n369)* animals was extracted using the RNeasy kit (Qiagen, Valencia, CA) and cDNA was made using an oligo(dT)_{12–18} primer (Invitrogen, Carlsbad, CA). RT-PCR was performed as previously described (Fridolfsson *et al.* 2010). For Western analysis, total worm lysate was made, separated by SDS-PAGE, and transferred to a membrane as described (Tapley *et al.* 2011). Anti-TOCA-1 rabbit serum (1:1000) (Giuliani *et al.* 2009) and monoclonal anti-α-tubulin mouse antibody (1:1000) (Clone DM 1A; Sigma, St. Louis) were used as primary antibodies. IRDye 680LT donkey anti-rabbit IgG (1:5000) and IRDye 800CW donkey anti-mouse IgG (1:5000) (LI-COR Biosciences) were used as secondary antibodies. Images of Western blotting were scanned by the Odyssey Infrared Imaging System (LI-COR Biosciences).

Results

Forward genetic screens for enhancers of the nuclear migration defect of *unc-83* or *unc-84*

Mutations in *unc-83* or *unc-84* disrupt P-cell nuclear migration in a temperature-sensitive manner and are not additive

(Sulston and Horvitz 1981). In *unc-83* or *unc-84* mutant animals raised at 25°, P-cell nuclear migration fails, leading to the death of P cells and a loss of neurons and the vulva, which leads to *Unc*, *Egl* animals (Figure 1, A and B) (Sulston and Horvitz 1981). However, if *unc-83* or *unc-84* null mutant animals are raised at 15°, ~90% of P-cell nuclei migrate normally and adults have no obvious phenotypes (Figure 1B) (Sulston and Horvitz 1981; Malone *et al.* 1999; Starr *et al.* 2001). Since most alleles of *unc-83* and *unc-84* are molecular nulls (Malone *et al.* 1999; Starr *et al.* 2001), the process of P-cell nuclear migration itself is temperature sensitive in an *unc-83* or *unc-84* mutant background. We therefore hypothesized that there is an unknown pathway(s) that functions in parallel to the *unc-83/84* pathway to move P-cell nuclei (Figure 1B, middle line).

To test our hypothesis, we carried out genetic screens for enhancer of the nuclear migration defect of *unc-84* (*emu*) mutations. We reasoned that simultaneous mutations in both the *unc-84* and the parallel pathway would synthetically lead to a strong P-cell nuclear migration defect at 15°, resulting in *Egl*, *Unc* animals missing P-cell lineages (Figure 1B, bottom line). We screened for *emu* mutations, using a powerful synthetic approach (Fay *et al.* 2002). In this approach, we mutagenized an *unc-84(n369)* starting strain containing an extrachromosomal array carrying an *unc-84* rescuing construct (McGee *et al.* 2006) and marked with *odr-1::rfp* (LEtoile and Bargmann 2000). After mutagenesis, F₂ animals potentially homozygous for *unc-84* and an *emu* mutation, but also carrying the *unc-84* rescuing transgene and therefore phenotypically *unc-84*(+), were individually plated. In the F₃ generation, non-RFP animals (mutant for *unc-84*) were compared to RFP-positive siblings carrying the extrachromosomal *unc-84* rescuing array. A candidate *emu* mutation that acted synthetically with *unc-84* was isolated when the non-RFP animals were *Egl*, but the RFP-positive siblings were not *Egl* (Figure 1D). About 4500 mutagenized genomes were screened in this manner and seven *emu* mutations (*yc15–18* and *-20–22*) were isolated. An eighth mutation, *yc3*, was isolated in an independent screen in *unc-83(e1408)* and crossed into *unc-84(n369)*. This rate of about one *emu* mutant animal for every ~650 genomes screened was similar to rates observed in other enhancer and synthetic screens in *C. elegans* (Ferguson and Horvitz 1989; Rocheleau *et al.* 2002).

Since *Egl* can result from defects other than a failure of P-cell nuclear migration, a secondary screen was performed to directly count cells derived from P-cell lineages. *p_{unc-47}::GFP* is expressed in 19 GABA neurons in the ventral cord, 12 of which are derived from P cells (Figure 1C) (Sulston and Horvitz 1977; McIntire *et al.* 1997). Mutants with significantly fewer GABA neurons at 15° (Figure 1C, right) than the *unc-84(n369)* starting strain were saved as *emu* mutant strains. The strongest mutants had only 10–14 GABA neurons per animal at 15°, similar to *unc-84* mutants at 25°, and significantly fewer than the 18.5 neurons in the starting background at 15° (Figure 2 and Table 2).

Figure 2 *emu* mutations enhance the nuclear migration defect of *unc-84*. GABA neuron numbers were used to quantify P-cell nuclear migration defects. All strains are in the *unc-84(n369)* background. The animals counted for the data on the left half contained an *unc-84(+)* rescuing array, so they were effectively wild type for *unc-84* and mutant for the *emu* indicated (*yc3*, -15–18, and -20–22). The data on the right are from *emu; unc-84(n369)* double mutants. All *emu; unc-84* double mutants had significantly fewer GABA neurons compared to *unc-84* alone at 15°, 20°, and 25° (t-test; $P < 0.0001$ for all comparisons to *unc-84* mutants with no *emu* mutation). Sample sizes are all >20 L4 or young adult animals, and error bars show the standard error of the mean (SEM).

Genetic characterization of *emu* mutations

None of the eight *emu* mutant strains had a nuclear migration defect in *unc-84* rescued animals at 15° (Figure 2, left). These data suggest that the *emu* mutants have mutations in genes that are synthetic to the *unc-84* pathway at 15° and likely represent genes that previous screens for nuclear migration mutants would have missed. The *yc3* mutation also enhanced the nuclear migration defect of *unc-83(e1408)*. *yc3; unc-83(e1408)* double-mutant animals had only 11.8 ± 0.4 , 9.7 ± 0.3 , and 8.6 ± 0.2 GABA neurons ($\pm$ SEM) in the ventral cord at 15°, 20°, or 25°, respectively, compared to 18.2 ± 0.2 , 17.1 ± 0.3 , and 13.9 ± 0.3 GABA neurons in *unc-83(e1408)* single-mutant animals (Table 2). Furthermore, *toca-1(RNAi)* (*yc20* is a mutation in *toca-1*, see below) also enhanced the nuclear migration defect of *unc-83* at 15° (17.7 ± 0.1 GABA neurons; $n = 94$; t-test; $P = 0.012$), suggesting that the *emu* mutations function in parallel to the SUN-KASH bridge pathway, rather than in an *UNC-83*-independent function of *UNC-84*. To test whether any *emu* mutations had caused defects in P-cell lineages prior to nuclear migration, we counted P cells in comma-stage embryos or young L1 larvae. No P-cell lineage defects were observed prior to the middle of the L1 larval stage when nuclear migration would normally occur (Figure 3). In all *emu* single or *emu; unc-84* double mutants, 6 P cells (if only one side could be observed) or 12 P cells (both sides) were present (Figure 3). We therefore concluded that the *emu* phenotypes occur after the proper numbers of P cells were born and that they function normally through embryogenesis.

Next, we aimed to determine whether the *emu* mutations were dominant or recessive and to clean up their mutagenized backgrounds. All eight *emu* mutant lines acted as recessive mutations since heterozygous *emu/+* animals in an *unc-84* homozygous background were neither *Egl* nor *Unc* at 15°. Also, no decrease in the number of GABA neurons was observed in heterozygous animals compared to *unc-84*

(*n369*) animals alone raised at 15° (data not shown). To remove unlinked mutations that were a result of mutagenesis, the eight *emu* mutant lines in the *unc-84* rescuing array background were outcrossed to *unc-84(n369)* animals.

To compare the severity of the P-cell nuclear migration defects of *emu* mutant lines, the number of GABA neurons in the ventral cord was quantified at 15°, 20°, and 25° (Figure 2). At 15°, the permissive temperature for *unc-84(n369)*, *emu; unc-84(n369)* double mutants had significantly fewer GABA neurons than did *unc-84* mutants alone, which had 17.4 ± 0.2 ($\pm$ SEM) on average. *yc15* was the strongest enhancer of *unc-84* at 15°; occasionally (5 of 62) only 7

Table 2 Number of GABA neurons ($\pm$ SEM) of *emu* mutations

	15°	20°	25°
<i>unc-84</i> screen			
Wild type	18.5 ± 0.1	18.8 ± 0.1	18.3 ± 0.4
<i>yc3</i>	18.4 ± 0.2	18.2 ± 0.3	16.9 ± 0.2
<i>yc15</i>	17.8 ± 0.2	18.0 ± 0.1	17.3 ± 0.3
<i>yc16</i>	18.7 ± 0.1	18.1 ± 0.2	17.4 ± 0.2
<i>yc17</i>	18.3 ± 0.2	18.3 ± 0.3	17.8 ± 0.2
<i>yc18</i>	18.2 ± 0.2	17.9 ± 0.3	16.3 ± 0.3
<i>yc20</i>	18.4 ± 0.1	18.4 ± 0.3	17.9 ± 0.2
<i>yc21</i>	18.1 ± 0.1	18.9 ± 0.1	17.9 ± 0.2
<i>yc22</i>	18.6 ± 0.2	17.3 ± 0.2	16.4 ± 0.3
<i>unc-84(n369)</i>			
<i>yc3, unc-84</i>	11.7 ± 0.3	11.4 ± 0.3	9.8 ± 0.4
<i>yc15, unc-84</i>	10.3 ± 0.2	9.6 ± 0.4	9.5 ± 0.3
<i>yc16, unc-84</i>	12.1 ± 0.6	11.6 ± 0.3	8.3 ± 0.3
<i>yc17, unc-84</i>	15.4 ± 0.2	12.4 ± 0.8	10.2 ± 0.5
<i>yc18, unc-84</i>	12.7 ± 0.3	12.5 ± 0.2	9.6 ± 0.2
<i>yc20, unc-84</i>	13.9 ± 0.5	10.5 ± 0.3	10.0 ± 0.5
<i>yc21, unc-84</i>	12.4 ± 0.4	10.8 ± 0.5	9.1 ± 0.4
<i>yc22, unc-84</i>	15.8 ± 0.2	11.3 ± 0.4	10.1 ± 0.4
<i>unc-83</i> screen			
<i>unc-83(e1408)</i>	18.2 ± 0.2	17.1 ± 0.3	13.9 ± 0.3
<i>yc3, unc-83</i>	11.8 ± 0.4	9.7 ± 0.3	8.6 ± 0.2

Figure 3 *emu* mutations do not disrupt P-cell specification. The morphology of P cells is shown in (A) a wild-type embryo, (B) a *yc18; unc-84(n369)* embryo, (C) a *yc3; unc-84(n369)* embryo, (D) a *yc3; unc-84(n369)* early-L1 larva, (E) a *yc21; unc-84(n369)* mid-L1 larva, and (F) a *yc3; unc-84(n369)* mid-L1 larva prior to the onset of nuclear migration. P-cell boundaries were marked using MH27 immunostaining to mark adherens junctions. Asterisks or arrowheads mark six P cells on one ventral-lateral side of the embryo or L1 larvae. Sample size of each mutant strain is between 18 and 50. A variety of stages were observed for each of the eight *emu* mutants; all worms had normal P-cell shapes.

GABA neurons were found in *yc15; unc-84* double mutants (10.3 ± 0.2 on average). Since 7 of the 19 GABA neurons originate from lineages other than P cells, 7 GABA neurons in the ventral cord represent a complete loss of P-cell lineages and likely a complete failure of P-cell nuclear migration. *emu; unc-84* double mutants were also cultured at 20° and 25° to see how *emu* mutations enhance *unc-84* P-cell nuclear migration defects at restrictive temperatures for *unc-84(n369)*. At both 20° and 25°, *emu; unc-84* double mutants had significantly fewer GABA neurons compared to *unc-84(n369)* single mutants (Figure 2, right). At 20°, there was a range of averages for the double mutants from 9.6 to 12.5 [compared to *unc-84(n369)* single mutants, which had 16.6 ± 0.2 on average], but *emu; unc-84(+)* mutants had near wild-type numbers of GABA neurons. In many cases (*yc3*, *yc17*, *yc20*, and *yc22*) the phenotypes of the *emu; unc-84* doubles were significantly more severe at 20° than at 15° (*t*-test; $P < 0.01$) (Figure 2, right). At 25°, all eight *emu; unc-84* double mutants had significantly fewer GABA neurons than *unc-84* single mutants, which had 13.41 ± 0.37 on average. However, at 25°, some *emu* single mutants [in the presence of the *unc-84(+)* rescuing array] had a mild phenotype of their own when compared to wild type (Figure 2, left). In summary, *unc-84* P-cell nuclear migration defects were significantly enhanced by all eight *emu* mutations at 15°, 20°, and 25°.

Identification of the molecular lesion in the *emu* mutation *yc20*

To identify molecular lesions underlying *emu* mutations, we undertook a WGS approach. The *C. elegans* genome is ~100 Mb, making next-generation WGS well suited to rapidly and cost-effectively identify molecular lesions (Sarin *et al.* 2008; Doitsidou *et al.* 2010; Zuryn *et al.* 2010). To narrow our focus for the WGS data analysis, we mapped *yc15*, -16, -20, and -21 to chromosome X and *yc3* and -18 to chromosome I (see *Materials and Methods*). We sequenced the *yc20* genome to 54.6× coverage (Figure 4A). In addition, we have sequenced other genomes derived from mutagenesis of the UD87 starting strain, which allowed us to filter out changes in the *yc20* genome that were also found in other *emu* strains that originated from UD87 when compared to the reference *N2* genome. Using the MAQGene software (Bigelow *et al.* 2009) to analyze the sequence data and filter out background mutations, we identified 122 unique mutations on chromosome X in the *yc20* genome. This list included only 5 predicted missense mutations and no predicted nonsense mutations (Figure 4A). We then analyzed the WGS data to further map *yc20* on chromosome X. The backcrossed *yc20* strain was expected to have a cluster of G/C to A/T transitions from the EMS mutagenesis linked to the *emu* locus (Zuryn *et al.* 2010). There was a cluster of such transitions toward the left end of chromosome X in the *yc20* genome (Figure 4B). This clustering approach is based on a small number of mutations and is therefore subject to false positives. Nonetheless, it is supportive of the hypothesis that *yc20* maps to the left end of chromosome X. One predicted missense mutation in the gene *toca-1* (transducer of Cdc42-dependent actin assembly) was found in the region of chromosome X implicated by the mapping data for *yc20*. Thus, *toca-1* was an excellent candidate for the *yc20* mutation.

TOCA-1 is conserved across animals and contains an F-BAR domain that binds to membranes, an HR domain that recruits the Rho-family GTPase Cdc42, and an SH3 domain that binds the WASP/WAVE complex (Ho *et al.* 2004; Giuliani *et al.* 2009). Both Cdc42 and WASP/WAVE regulate actin filament polymerization. The point mutation in *toca-1* (*yc20*) is a C-to-T mutation that is predicted to cause an Ala-to-Val amino acid change in the putative F-BAR domain at position 100 of the *TOCA-1* protein. Thus, the *yc20* mutation may disrupt *TOCA-1* function and actin dynamics at membranes.

Disruption of *toca-1* enhances the *unc-84* nuclear migration defect

To further confirm that *TOCA-1* functions to move P-cell nuclei, additional alleles of *toca-1* were examined in an *unc-84* mutant background. Like *yc20*, both *toca-1(RNAi)* and the deletion allele *toca-1(tm2056)* had significant P-cell nuclear migration defects in an *unc-84(n369)* background at 15° (Figure 5, center), but no phenotype in the presence of the *unc-84(+)* transgene (Figure 5, left). These *yc20* data are slightly different from those in Figure 2 because they

Figure 4 Whole-genome sequencing (WGS) and mapping of *yc20*. (A) Summary of *yc20* WGS. (B) The *yc20* mutation was mapped to the *toca-1* region. Each peak represents a cluster of G-to-A or C-to-T mutations identified by WGS characteristic of EMS mutagenesis. Since *yc20* was backcrossed after mutagenesis, it is expected that the remaining EMS-induced mutations would be linked to *yc20* on chromosome X.

were obtained at different times. These data show that *toca-1* is an *emu* gene. *toca-1* is partially redundant to *toca-2* (Giuliani *et al.* 2009) and no phenotype has previously been reported for a single mutation in either gene, although the double mutant blocks endocytosis of yolk granules in oocytes, resulting in sterility (Giuliani *et al.* 2009). *toca-2(tm2088); unc-84(n369)* animals did not have a significant nuclear migration defect (Figure 5, center), suggesting that the role of *toca-1* in P-cell nuclear migration is not redundant with that of *toca-2*.

The above data are consistent with the hypothesis that *yc20* is an allele of *toca-1*. Two experiments were performed to further test this hypothesis. First, we performed a complementation test. *yc20/tm2056* heterozygous animals in an *unc-84(n369)* background raised at 15° had only 16.8 ± 0.2 GABA

neurons on average (Figure 5, right). This was significantly fewer than that in either *yc20/+ unc-84(n369)* or *tm2056/+ unc-84(n369)* animals, which were similar to those in the *unc-84(n369)* background alone (Figure 5, right). Second, we tested whether wild-type *toca-1* could rescue the nuclear migration defect of the *yc20* mutation. Wild-type *toca-1* was expressed in P cells prior to nuclear migration from two independent promoters: the pan-hypodermal promoter of *col-10* (Spencer *et al.* 2001) and the P-cell-specific promoter of *hlh-3* (Doonan *et al.* 2008). The *yc20 unc-84(n369)* nuclear migration defect was rescued in transgenic animals expressing either *p_{col-10}::toca-1::gfp* or *p_{hlh-3}::toca-1::gfp*. The average number of GABA neurons significantly increased from 13.2 ± 0.4 to 15.1 ± 0.3 in the *p_{col-10}::toca-1::gfp* rescued lines and from 13.59 ± 0.28 to 15.0 ± 0.2 in

Figure 5 *toca-1* RNAi or a deletion allele enhance *unc-84*. (Left) *yc20*, *toca-1* (RNAi), *toca-1(tm2056)*, and *toca-2(tm2088)* have similar average GABA neuron numbers in comparison with *unc-84(n369)* *ycEx60[odr-1::rfp; unc-84(+)]* animals at all temperatures. (Center) At 15°, *yc20*, *toca-1(RNAi)*, and *toca-1(tm2056)* showed significantly lower GABA neuron numbers (15.6 ± 0.4 , 16.4 ± 0.2 , and 15.9 ± 0.2 , respectively) than *unc-84* (17.5 ± 0.2) (*t*-test; $P < 0.0001$). However, at 15° *toca-2(tm2088)* and *unc-84* showed similar GABA neuron numbers (17.5 ± 0.2 vs. 17.5 ± 0.2). At 20°, *yc20* and *toca-1(tm2056)* showed significantly fewer GABA neuron numbers (10.5 ± 0.3 and 12.9 ± 0.4 , respectively) than *unc-84* (15.8 ± 0.2) (*t*-test; $P < 0.0001$). At

25°, *yc20* showed significantly lower average GABA neuron numbers (10.0 ± 0.5) than *unc-84* (13.0 ± 0.3) (*t*-test; $P < 0.0001$) but *toca-1(tm2056)* showed similar numbers to *unc-84* (12.4 ± 0.6 vs. 13.0 ± 0.3). (Right) Both *yc20/tm2056* and *tm2056/yc20* (male X chromosome/hermaphrodite X chromosome) *trans*-heterozygous animals showed significantly lower average GABA neuron numbers (16.8 ± 0.2 and 17.4 ± 0.2 , respectively) than *yc20/+* and *tm2056/+* (18.7 ± 0.1 and 18.7 ± 0.2 , respectively) (*t*-test; $P < 0.0001$). Sample sizes are all >20 L4 or young adult animals, and error bars show the standard error of the mean (SEM).

Figure 6 *yc20* is a mutation in *toca-1*. The number of GABA neurons counted in each indicated genotype is shown. Solid bars indicate the presence of *ycEx189[p_{col-10}::toca-1::gfp; odr-1::gfp]* that is broadly expressed in all hypodermal cells (left) or *ycEx190[p_{hlh-3}::toca-1::gfp; odr-1::gfp]* that is specifically expressed in P cells (right). ***P* < 0.001, *t*-test; **P* < 0.003, *t*-test. Sample sizes are all >30 L4 or young adult animals, and error bars show the standard error of the mean (SEM).

the *p_{hlh-3}::toca-1::gfp* rescued animals (Figure 6). Moreover, the P-cell-specific rescue of the *p_{hlh-3}::toca-1::gfp* transgene suggested that *TOCA-1* functions to move P-cell nuclei in a cell-autonomous manner. Based on these data, we concluded that *yc20* mutation is in *toca-1*.

To further explore the nature of the *toca-1(yc20)* allele, we examined *toca-1* mRNA and *TOCA-1* protein levels in *toca-1(yc20)* animals. Using RT-PCR, full-length mRNA with a missense mutation from Ala to Val at residue 100 was detected in *toca-1(yc20)* animals (Figure 7A). Moreover, a slightly smaller mRNA was detected by RT-PCR in the deletion allele *toca-1(tm2056)* (Figure 7A). *TOCA-1* protein was visualized using a polyclonal antibody against *TOCA-1* residues 362–592 (Giuliani *et al.* 2009). In both wild-type and *toca-1(yc20)* animals grown at either 15° or 25°, *TOCA-1* was detected as a doublet of ~70 kDa on Western blots. However, no *TOCA-1* bands were detected in *toca-1(tm2056)* animals (Figure 7B). Based on these data, we concluded that *toca-1(yc20) unc-84(n369)* animals made full-length *toca-1* mRNA and protein with an Ala-to-Val mutation at residue 100 that is responsible for the defect in nuclear migration.

To examine the subcellular localization of *TOCA-1* in larval P cells, *TOCA-1::GFP* was expressed specifically in P cells of L1 wild-type animals, using the promoter *p_{hlh-3}*. In early L1 larval stage, we found that *TOCA-1::GFP* localization was clearly decorating the cell–cell contacts with few noticeable punctate spots (Figure 7D) and was also concentrated at the front end (close to the ventral cord) and the rear end (lateral side) of cytoplasm (Figure 7D'). We also found *TOCA-1::GFP* was surrounding the P-cell nuclei (seen as dark holes) at the rear end of P cells (Figure 7D'). Later in the mid-L1 larval stage just prior to P-cell nuclear migration, *TOCA-1::GFP* similarly localized to the cell–cell contact boundaries and to the region of both the front and the rear ends (Figure 7E). The localization of *TOCA-1::GFP* suggests

its function could occur at the sites of cell–cell contacts, at the ventral-cord front end, or at the lateral rear end of P cells in the vicinity of the nucleus.

Finally, to more directly analyze the defects of P-cell nuclear migration in *toca-1 unc-84* double mutants, we developed a P-cell nuclear counting assay, using an integrated array, *ycIs11*, expressing tdTOMATO in nuclei of P cells and their descendants throughout the L1 stage (see *Materials and Methods*) (Figure 8). Nuclear migration of 12 P cells starts at the mid-L1 stage and ends at the late L1 stage (Sulston 1976; Sulston and Horvitz 1977). We therefore reasoned that in late L1 animals, nuclei that failed to migrate would be seen at the lateral sides of late L1 larvae and <12 P-cell nuclei or their divided nuclei would be seen in the ventral cord. In wild-type *N2* animals, almost all P-cell nuclei migrated normally at 15°, 20°, and 25° (only 2 of 2232 nuclei failed to migrate; Table 3). Furthermore, *toca-1(tm2056)* single-mutant animals had no P-cell nuclear migration defects (Table 3). In contrast, *unc-84(n369)* animals had only 11.7 ± 0.1 (average $\pm$ SEM), 10.5 ± 0.2 , or 9.5 ± 0.3 nuclei that migrated normally at 15°, 20° or 25°, respectively (Figure 8A and Table 3). As expected, *toca-1(tm2056) unc-84(n369)* animals had only 11.7 ± 0.1 , 9.7 ± 0.2 , or 6.9 ± 0.3 nuclei that migrated at 15°, 20°, or 25°, respectively (Table 3). Therefore, the *toca-1(tm2056)* mutation significantly enhanced the defects of P-cell nuclear migration in *unc-84(n369)* animals, at least at 20° (*t*-test; *P* < 0.01) and 25° (*t*-test; *P* < 0.0001).

Discussion

Overcoming genetic redundancy is a major hurdle to the study of many cell and developmental processes, including nuclear migration. For example, <20% of predicted genes in *C. elegans* are thought to play essential roles (Kamath *et al.* 2003; Sonnichsen *et al.* 2005). However, one recent study

Figure 7 *toca-1* expression and localization. (A) RT-PCR from mRNA amplified a 1.7-kb band of the expected size (arrowhead) in N2, *unc-84(n369)* and *toca-1(yc20) unc-84(n369)* animals. A 1.6-kb band of the expected size (arrow) was amplified from *toca-1(tm2056) unc-84(n369)* RNA. (B) On Western blots, anti-TOCA-1 antibodies recognized a doublet (red, marked with arrowheads) of the expected size of ~70 kDa in both *unc-84(n369)* and *toca-1(yc20) unc-84(n369)* animals. As expected, both bands of the TOCA-1 doublet were absent from *toca-1(tm2056) unc-84(n369)* and *toca-1(tm2056) toca-2(ng11)* animals. Nonspecific bands were marked with asterisks. α -Tubulin (55-kDa band in green, marked with an arrow) was used as loading control. (C) Schematized section of an early L1 animal. Underneath a sheath of cuticle, P cells are the epidermis that covers the lateral-to-ventral side of the animal. P cells are very thin where they are over muscles. P-cell nuclei localize to the lateral side before the nuclear migration occurs. (D and E) TOCA-1::GFP expression in P cells of early (D and D') and mid-L1 (E) animals. Two planes of focus as diagramed in the cartoon, one at the lateral surface (D) and one farther inside (D'), are shown for the same animal. Insets are enlargements of the indicated boxes. Bars: 10 μ m.

found that 38% of the predicted genes in the *C. elegans* genome have clear human orthologs (Shaye and Greenwald 2011). Thus, there are many genes in the *C. elegans* genome that are clearly conserved to humans, but have no known function on their own. It is likely that many or most of these conserved, nonessential genes function redundantly to other pathways. Identification of such genes requires relatively difficult synthetic or enhancer screens, such as the one employed here.

Our goal here was to identify novel mechanisms employed to move nuclei. Previous studies in multiple systems demonstrated that SUN and KASH proteins function at the nuclear envelope to connect and move nuclei along the cytoskeleton (Roux *et al.* 2009; Starr and Fridolfsson 2010). The screen presented here was designed to identify novel mechanisms that function in parallel to SUN-KASH bridges. Our model system, *C. elegans* larval P-cell nuclear migration, is better suited for identifying such pathways than embryonic dorsal hyp7 precursors where the mechanisms of

UNC-84 and UNC-83 were elucidated (McGee *et al.* 2006; Meyerzon *et al.* 2009; Fridolfsson *et al.* 2010; Fridolfsson and Starr 2010; Tapley *et al.* 2011). SUN-KASH bridges are absolutely required for nuclear migration in the embryonic hypodermis at all temperatures. However, the process of P-cell nuclear migration is temperature sensitive in the background of SUN (*unc-84*) or KASH (*unc-83*) mutants (Sulston and Horvitz 1981; Malone *et al.* 1999; Starr *et al.* 2001), allowing us to identify *emu* mutations that enhance P-cell nuclear migration defects.

The temperature-sensitive nature of nuclear migration defects in *unc-84* or *unc-83* mutants led to our hypothesis that an unknown pathway or pathways work, in part, redundantly to the SUN-KASH pathway. In support of our hypothesis and fulfilling our goal, we identified eight, independently isolated, mutant lines that carry mutations in genes that appear to function parallel to the SUN-KASH pathway. We determined that one of these mutations is in the *toca-1* gene. Thus, our screen was successful in

Figure 8 P-cell nuclear migration defects in *toca-1(tm2056) unc-84(n369)* are shown. (A) tdTOMATO labels nuclei in *unc-84(n369)* animals at 15° at the late L1 stage. Non-P-cell nuclei are marked by asterisks. The ventral cord is marked by the dotted line. (B) Two nuclei that failed to migrate to the ventral side in a *toca-1(tm2056) unc-84(n369)* L1 larva at 25° are shown. Migrated nuclei and their descendants can be seen in the ventral cord in B'.

identifying conserved, nonessential genes that function to move nuclei. The remaining *emu* mutations are likely to be a continuing source of interesting genes that also function in parallel to *unc-84* to move nuclei.

We quantified nuclear migration in the *emu* mutants by two different assays. For most of our experiments, we counted L4/young adult GABA neurons that were derived from P-cell lineages and concluded that fewer GABA neurons represented failed nuclear migrations. To more directly observe P cells in L1 larvae, we employed a P-cell-specific nuclear marker. The nuclear migration defect of *toca-1(tm2056) unc-84(n369)* appeared less severe using the L1 larval P-cell nuclear marker (Figure 8 and Table 3) than when the GABA neuron assay was used (Figure 2 and Table

2). There are two possible reasons to explain this discrepancy. First, *toca-1* could play an important role in the GABA lineage after nuclear migration, such as in a polarized cell division in the ventral cord. We favor an alternative, where our assay missed defects in nuclear migration. Because we waited until all P-cell nuclear migration was completed before scoring, we would have missed a significant delay in P-cell nuclear migration, which could lead to a later loss of the P-cell lineage. Therefore, further live imaging will be required to determine the specific effects of the loss of *toca-1* on P-cell lineages.

The P-cell defect quantified by both of our assays is likely to represent a nuclear migration defect, not a more general cell migration defect. *rho-1(dn)* mutants, which disrupt cell

Table 3 *toca-1* enhances the P-cell nuclear migration phenotype of *unc-84*

	15°	20°	25°
Wild type	12.00 ± 0.0 (n = 45) ^a	12.0 ± 0.0 (n = 50)	12.00 ± 0.0 (n = 91)
<i>unc-84(n369)</i>	11.7 ± 0.1 (n = 39)	10.5 ± 0.2 (n = 73)	9.5 ± 0.3 (n = 66)
<i>toca-1(tm2056)</i>	12.0 ± 0.0 (n = 21)	12.00 ± 0.0 (n = 17)	12.0 ± 0.0 (n = 30)
<i>toca-1(tm2056) unc-84(n369)</i>	11.7 ± 0.1 (n = 100)	9.7 ± 0.2 (n = 56)	6.9 ± 0.3 (n = 35)

^a P-cell nuclei that migrated normally were counted using an *nls::tdTomato* marker expressed in P-cell nuclei. The means ± SEM are shown.

migration, were previously shown to lead to ~60% of L2 larvae with lateral P-cell derivatives (Spencer *et al.* 2001). In our observations of GABA neurons in L2 larvae, we very rarely (eight neurons in 149 L2 *toca-1 unc-84* double-mutant animals) observed misplaced neurons at the lateral sides, suggesting we are not studying a general defect in cell migration and can genetically separate nuclear migration from cell migration.

toca-1 is conserved from nematodes to humans, but does not have a phenotype on its own (Giuliani *et al.* 2009). It was previously shown that *toca-1* functions redundantly to *toca-2* to mediate the essential process of endocytosis of yolk proteins in oocytes (Giuliani *et al.* 2009). Here, we showed that *toca-1*, but not *toca-2*, functions redundantly to *unc-84* at 15° to move P-cell nuclei. Four observations are consistent with the conclusion that the nuclear migration defect in the *yc20* mutant isolated in the *emu* screen is caused by a missense mutation in *TOCA-1*: (1) The *yc20* mutation mapped to a region of chromosome X using EMS-linked mutations; (2) WGS identified a missense mutation in *toca-1*; (3) *toca-1(RNAi)* and the *toca-1(tm2056)* deletion allele both enhanced the nuclear migration defect of *unc-84(n369)* to a similar extent to that of the *yc20* allele; and (4) most importantly, the *yc20* nuclear migration defect was rescued with a wild-type copy of *toca-1* from a transgene expressed specifically in P cells. We therefore conclude that *yc20* is an allele of *toca-1*.

The genetics show that *TOCA-1* functions in parallel to the SUN-KASH nuclear-envelope bridge. To our knowledge, this is the first report of a role for *TOCA-1* in nuclear migration. *TOCA-1* is conserved across animals and contains an F-BAR domain that is thought to bind curved membranes and two domains that recruit cdc42 and N-WASP (Ho *et al.* 2004; Giuliani *et al.* 2009). How *TOCA-1* functions in nuclear migration remains unclear. One possibility is that because the *yc20* lesion is predicted to change an alanine in the F-BAR domain to a valine, the mutation could affect *TOCA-1* localization to membranes. However, *TOCA-1::GFP* did not obviously associate with the nuclear envelope. A second possibility is that the *toca-1(yc20)* mutation disrupts actin dynamics by disrupting the function of the cdc42 and/or N-WASP/WAVE interaction domains. In support of this hypothesis, depletion of the actin nucleator Arp2/3 or WAVE leads to the loss of one to two GABA neurons at 25°, suggesting a weak P-cell nuclear migration defect (Xiong *et al.* 2011). A third possibility is that *TOCA-1* is functioning through its known roles in membrane tubulation and clathrin-mediated endocytosis (Ho *et al.* 2004; Kakimoto *et al.* 2006; Bu *et al.* 2009, 2010; Fricke *et al.* 2009; Giuliani *et al.* 2009). In *Drosophila*, Rab-mediated endocytosis and vesicular transport contribute to nuclear positioning in the developing eye (Houalla *et al.* 2010). Future experiments will help clarify the mechanism of *TOCA-1* in P-cell nuclear migration. Molecular characterization of the additional *emu* mutations from this screen should significantly increase our general understanding of the mechanisms of nuclear migration.

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