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Article

MIG-10/Lamellipodin and AGE-1/PI3K Promote Axon Guidance and Outgrowth in Response to Slit and Netrin

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Summary

Background: The cytoplasmic C. elegans protein MIG-10 affects cell migrations and is related to mammalian proteins that bind phospholipids and Ena/VASP actin regulators. In cultured cells, mammalian MIG-10 promotes lamellipodial growth and Ena/VASP proteins induce filopodia.

Results: We show here that during neuronal development, mig-10 and the C. elegans Ena/VASP homolog unc-34 cooperate to guide axons toward UNC-6 (netrin) and away from SLT-1 (Slit). The single mutants have relatively mild phenotypes, but mig-10; unc-34 double mutants arrest early in development with severe axon guidance defects. In axons that are guided toward ventral netrin, unc-34 is required for the formation of filopodia and mig-10 increases the number of filopodia. In unc-34 mutants, developing axons that lack filopodia are still guided to netrin through lamellipodial growth. In addition to its role in axon guidance, mig-10 stimulates netrin-dependent axon outgrowth in a process that requires the age-1 phosphoinositide-3 lipid kinase but not unc-34.

Conclusions: mig-10 and unc-34 organize intracellular responses to both attractive and repulsive axon guidance cues. mig-10 and age-1 lipid signaling promote axon outgrowth; unc-34 and to a lesser extent mig-10 promote filopodia formation. Surprisingly, filopodia are largely dispensable for accurate axon guidance.

Introduction

During neuronal development, the neuronal growth cone responds to conserved extracellular guidance cues with cytoskeletal rearrangements and directed cell movements [1–7]. In a typical growth cone, finger-like filopodia with bundled actin filaments extend from a lamellipodial region with loosely crosslinked actin webs [4]. In both neuronal and nonneuronal filopodia, the Ena/VASP family of actin regulators promote the formation of long unbranched actin filaments by protecting actin barbed ends from capping proteins [5, 8–13]. Genetic and biochemical results suggest that Ena/VASP proteins have important roles in guidance to the conserved axon guidance factor UNC-6/netrin via its receptor UNC-40/DCC [7, 13]. UNC-34/Ena also functions in repulsive axon guidance downstream of the Slit receptor Robo at the Drosophila midline and in C. elegans AVM axons, and in repulsive axon guidance downstream of the netrin receptor UNC-5 in motor neurons [5, 11, 14].

Members of the Ena/VASP family share a conserved domain structure with an Ena/VASP homology 1 (EVH1) domain, a proline-rich region, and an Ena/VASP homology 2 (EVH2) domain [15–18]. The EVH1 domain binds a consensus FPPPP proline-rich motif that is present in focal adhesion proteins and in the guidance receptor Robo [5, 15, 16] and in the cytoplasmic protein Lamellipodin (Lpd) [19]. Lpd colocalizes with Ena/VASP in fibroblast lamellipodia and filopodia and in neuronal growth cones, and Lpd stimulates F-actin formation during fibroblast lamellipodial protrusion. A Lpd-related molecule, RIAM, stimulates integrin-dependent actin polymerization, lamellipodia formation, and cell spreading in T cells [20]. Lpd and RIAM each have multiple FPPPP motifs, a Ras/Rap GTPase association domain, a lipid binding pleckstrin homology (PH) domain, and a profilin binding proline-rich domain (Figures 1A and 1B). The PH domain of Lpd binds PI(3,4)P2 (PIP2) phospholipids [19, 20].

The C. elegans genome encodes a single molecule homologous to mammalian Lpd and RIAM, MIG-10. mig-10 mutants have disrupted embryonic cell migrations [21] that resemble those in animals mutant for unc-34, the sole C. elegans Ena/VASP ortholog [22]. We show here that mig-10 has important functions in axon outgrowth and guidance that are masked by partial redundancy between mig-10 and unc-34 genes. Our results suggest that MIG-10 and UNC-34 have overlapping but distinct roles in organizing filopodial and lamellipodial growth in developing axons.
Figure 1. MIG-10/Lamellipodin Affects Axon Guidance

(A) Phylogram of the evolutionary relationships of *C. elegans* MIG-10 and its mammalian and *Drosophila* homologs. The horizontal distance represents the degree of sequence divergence, and the scale bar at the bottom corner corresponds to 10% substitution events.

(B) Schematic representation of domain organization of Lamellipodin, RIAM, and MIG-10. N, amino-terminal region; RA, Ras-association domain; PH, pleckstrin homology domain; Proline, proline-rich domain; FP4, motifs fitting the FPPPP consensus for EVH1 binding.

(C) Axon guidance defects in *mig-10* mutants. Left, schematic diagrams of wild-type and mutant axons. Right, percentage of defective neurons in *mig-10(ct41)* and controls. For HSN, most lateral axons grew either anteriorly (b) or posteriorly (c) before growing ventrally; only 10% failed to reach the ventral midline entirely (a). For AVM, only axons that failed to reach the ventral midline were scored. For CAN posterior axons, 66% extended between 50%–75% of their normal length, and 20% extended less than 50% of their normal length. For ADL, 8% of the cells lacked ventral branches and 7% lacked dorsal branches. Transgenes are described in Experimental Procedures. In parentheses are the number of animals or neurons scored.

(D) Cross-section showing cells and molecules that affect ventral guidance of AVM axon (green). Dorsal muscles express the repellent SLT-1/Slit (red). Ventral axons express the attractant UNC-6/netrin (purple).

(E) Quantitation of AVM axon guidance defects. All mutations represent the strongest available loss-of-function alleles. In all figures, error bars represent standard error of the proportion. Asterisks and brackets represent p < 0.05 by t test or Bonferroni t test (in the case of multiple comparisons).
Results

MIG-10 Affects Netrin-Dependent and Slit-Dependent Axon Guidance

The mig-10(ct41) mutation leads to an early stop codon in all three known splice forms of mig-10 and is a candidate null mutation [21]. We examined axon structure in these mutants by using a panel of transgenic lines expressing the green fluorescent protein (GFP) in different neurons. Many axons had mild guidance defects in mig-10 mutants (Figure 1C). In HSN motor neurons, which are guided ventrally in response to netrin, about half of the axons of mig-10 mutants had aberrant lateral or ventral-lateral trajectories. This result suggests that mig-10 could play a role in netrin signaling in HSN (see below). A fraction of AVM mechanosensory neurons, which are guided ventrally by attraction to netrin and repulsion from Slit, were also defective in mig-10 mutants. The branches of the ADL sensory neuron grow dorsally and ventrally in response to netrin and Slit [23]; both branches were occasionally lost in mig-10 mutants. The posterior process of the bipolar CAN neuron, whose guidance cues are unknown, often terminated prematurely in mig-10 mutants. HSN and CAN also have cell migration defects in mig-10 mutants [21], but their axon defects cannot be explained purely as indirect effects of mismigration; both HSN axon defects and CAN axon defects are much more severe in mig-10 mutants than they are in several other mutants in which cell migration is comparably abnormal [22]. Among other defects observed at low frequency were excessive midline crossing by the PVQ and PVP interneurons and de-fasciculation of the ventral nerve cord (data not shown). These defects suggest that mig-10 contributes to many guidance decisions.

The AVM pioneer axon is easily visualized and convenient for detailed analysis of C. elegans axon guidance. To determine whether mig-10 acts directly in migrating axons or indirectly in other cells, we expressed a mig-10 cDNA under the control of a mec-7 promoter, which is expressed strongly in six neurons including AVM [24]. A mec-7::mig-10 transgene rescued the mild ventral guidance defect in AVM, suggesting that mig-10 can act cell autonomously in developing neurons (Figure 1E).

The AVM axon is guided ventrally by attraction to UNC-6 (netrin) and repulsion from dorsal SLT-1 (Slit) (Figure 1D) [25–27]. Mutations in unc-6, its receptor unc-40/DCC, slt-1, or its receptor sax-3/Robo cause ventral guidance defects in 30%–40% of AVM axons; in double mutants in which both unc-6 and slt-1 pathways are inactivated, AVM ventral guidance fails completely. Thus, enhancement of an unc-6(null) mutant can identify genes that affect slt-1-dependent guidance in AVM, while enhancement of a slt-1(null) mutant can identify genes involved in unc-6 signaling [11, 28]. mig-10; unc-6 double mutants displayed strongly enhanced AVM guidance defects similar to those of slt-1 unc-6 double mutants (Figure 1E). In contrast, mig-10; slt-1 double mutants resembled slt-1 single mutants. These results suggest that mig-10 functions in the slt-1 axon guidance pathway in AVM.

Synthetic Lethality and Synergistic Axon Guidance Defects in mig-10; unc-34 Double Mutants

The mammalian Lpd and RIAM proteins bind directly to Ena/VASP proteins through multiple FPPPP motifs [19, 20]. unc-34 and mig-10 share similar patterns of axon and cell migration defects, and FPPPP-containing N-terminal and C-terminal fragments of MIG-10 can bind UNC-34 in far Western assays (see Figure S1 in the Supplemental Data available with this article online). To understand the relationship between these two genes, we generated animals with null mutations in both genes. Strikingly, mig-10; unc-34 double mutants were lethal, unlike either single null mutant (Figure 2). Double mutants arrested with a spectrum of phenotypes between midembryogenesis and the third larval stage. Some embryos failed to undergo morphogenesis, arresting with a mixture of differentiated cells and nonadherent cells (Figure 2A). Others arrested later in development with deformed heads and lumpy cuticles (Figure 2C). These phenotypes are characteristic of defects in epidermal enclosure, a process in midembryogenesis in which epidermal cells surround other embryonic tissues through actin-based motility [29]. The synthetic lethality of unc-34 and mig-10 suggests that they have parallel functions in embryonic epithelial morphogenesis.

The AVM axon grows ventrally late in the first larval stage. Although most mig-10; unc-34 double mutants arrested early in development, ~5% arrested at or after the second larval stage, after AVM guidance was complete. These animals showed severe defects in AVM axon ventral guidance, with more than half of the axons...
affected (Figure 1E). The defect in mig-10; unc-34 double mutants represents a minimal estimate of their importance in AVM guidance, since some maternal gene product may persist in these animals derived from heterozygous unc-34/+ mother [30]. It is possible that the AVM defect in these animals has indirect contributions from mig-10 effects in other cells. However, the mig-10 interaction appears specific to unc-34 and not all actin regulators; for example, ced-10/Rac; mig-10 double mutants had the mild AVM guidance defects of mig-10 single mutants (Figure 1E), although ced-10 has strong genetic interactions with other axon guidance genes [7].

The strongly enhanced axon defect in mig-10; unc-34 double mutants relative to single mutants suggests that these genes have overlapping functions in AVM axon guidance that are masked by redundancy between them. It is inconsistent with a linear pathway in which either unc-34 or mig-10 only regulates the other gene.

**mig-10 Promotes UNC-40-Stimulated Axon Outgrowth**

Because of Lpd’s role in lamellipodial growth, we asked whether mig-10 has outgrowth-promoting activity in developing neurons. Netrin signaling through DCC receptors stimulates axon outgrowth as well as guidance [31]. AVM neurons expressing MYR::UNC-40, a dominant active form of UNC-40/DCC in which the cytoplasmic domain of UNC-40 is fused to a myristoylation signal, have excessive outgrowth with misguided axons, extra axons, and deformed cell bodies [7] (Figures 3A and 3B and Figure S2). A mig-10(ct41) mutation partially suppressed the excessive AVM axon outgrowth caused by MYR::UNC-40 (Figure 3C), suggesting that mig-10 has outgrowth-promoting activity. The extent of suppression was similar to that caused by null mutations in actin regulators in the netrin pathway such as unc-34 or ced-10 [7] (Figure 3C). Expression of a mig-10
cDNA under the specific mec-7 promoter restored the exaggerated outgrowth of the MYR::UNC-40 strain (Figure 3C), but the same mig-10 transgene did not cause outgrowth defects on its own (Figure 1E, Figure S2). These results suggest that MIG-10 can promote axon outgrowth cell autonomously in the AVM neurons.

Two conserved motifs in the UNC-40 cytoplasmic domain called P1 and P2 contribute additively to the outgrowth-promoting activity of MYR::UNC-40 [7] (Figure 3A). Deleting either the P1 motif or the P2 motif from MYR::UNC-40 reduces the severity of the outgrowth defect in AVM neurons; genetic results suggest that P1 is required for activation of UNC-34, and P2 is required for activation of CED-10 [7]. mig-10 did not suppress all defects caused by P1-deficient or by P2-deficient MYR::UNC-40 transgenes (Figure 3D), but it did change the spectrum of defects in the P1-deficient strain (Figure S2). A possible interpretation of this result is that both the P1 element and the P2 element of UNC-40 contribute to mig-10 activation.

MIG-10 Acts with AGE-1/PI3K in AVM Outgrowth and Guidance

A potential regulator of MIG-10 suggested by work in other systems is phosphoinositide-3-kinase, or PI3K. During chemotaxis of neutrophils and Dictyostelium amoebae, Rac GTPases cooperate with PI3K to define the leading edge of the migrating cell [32, 33]. PI3K stimulates the production of PI(3,4)P2, the lipid ligand of the Lamellipodin PH domain [19]. A null mutation in age-1, the C. elegans PI3K homolog, significantly suppressed the effects of a MYR::UNC-40 transgene in AVM axons (Figure 4A). This result suggests that age-1 acts with MYR::UNC-40 to promote AVM outgrowth. Expression of an age-1 cDNA under the mec-7 promoter restored the defects to MYR::UNC-40 animals, consistent with an autonomous function of age-1 in AVM (Figure 4A). mec-7::age-1 did not cause AVM defects in a wild-type background (Figure 4B). MYR::UNC-40 suppression in age-1; mig-10 double mutants was comparable to suppression in either age-1 or mig-10 alone, consistent with function in a common process.
To understand the activity of MIG-10 in more detail, we overexpressed mig-10 in developing neurons and examined the effects on axon morphology. When mig-10 was overexpressed in AVM neurons by high-copy injection of a mec-7::mig-10 transgene, it caused defects suggestive of uncontrolled outgrowth and disrupted guidance. Neurons overexpressing MIG-10 had extra axons and deformed cell bodies, an excess outgrowth phenotype similar to the phenotype of neurons expressing MYR::UNC-40 (Figures 4C and 4D, Figure S2). In addition, 41% of MIG-10-overexpressing neurons lacked the characteristic ventral axon in AVM, suggesting a primary axon guidance defect. These defects were not suppressed by null mutations in unc-34, but were almost entirely suppressed by an age-1 null mutation (Figure 4E). These results support the proposal that age-1 and mig-10 act in a common process in developing axons.

Loss-of-function mutations in age-1 had no effect on AVM guidance on their own but had significant effects on ventral guidance in combination with other guidance mutations. In general, age-1 mutants acted like mild mig-10 mutants (Figure 4B). age-1; mig-10 double mutants were similar to mig-10 single mutants, consistent with action in a common pathway. age-1; unc-34 double mutants had substantially enhanced defects compared to single mutants, but less severe defects than mig-10; unc-34 double mutants. age-1; unc-6 double mutants were slightly more severe than unc-6 alone and less severe than mig-10; unc-6 double mutants (Figure 4B). These results suggest that age-1 is one of several regulators of mig-10 in AVM guidance.

MIG-10 and UNC-34 Cooperate in Ventral Filopodia Formation Downstream of Netrin

Most studies of axon guidance examine final axon morphology, but the detailed effects of cytoskeletal regulators like MIG-10 and UNC-34 could be more apparent if the actual process of axon guidance is observed. It has not been possible to visualize AVM guidance in real time, but for another neuron, HSN, it is possible to visualize the progress by which the axon navigates to the ventral nerve cord [34]. The HSN neuron grows ventrally toward UNC-6 during the L2 and L3 stages of development. mig-10 is important for normal HSN ventral guidance (Figure 1C), while unc-34 has a minor role [7]; both genes also have an earlier function in neuronal polarization prior to axon formation [34].

Filopodia are a prominent feature of neuronal growth cones. Throughout the L2 and L3 stages, the leading edge of HSN has an array of filopodia that extend and retract from the ventral side of the cell body (Figure 5A). mig-10 mutants had a reduced number of filopodia during HSN ventral growth, with an average of 0.8 filopodia per cell in the late L2 stage, instead of the 1.8 filopodia seen in wild-type animals (Figures 5B and 5D). Moreover, the occasional filopodia in mig-10 mutants were often present in abnormal dorsal locations, whereas normal HSN filopodia were always localized to the ventral side of the cell (Figure 5B, arrowhead). This result suggests that MIG-10 promotes filopodia formation and acts with netrin to localize filopodia to the ventral HSN.

unc-34 mutants had a severe defect in filopodia formation during HSN ventral guidance. Virtually all...
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PI3K. Previous results with pharmacological inhibitors overexpression were strongly suppressed by *unc-34* and *mig-10* mutations in (Figures 5E and 5F). The excessive filopodia caused by lopodia per cell (Figure 5D).

Despite the near-complete absence of filopodia, HSN growth cones reached the ventral nerve cord in 92% (n = 105) of *unc-34* animals. This observation indicates that filopodia are largely dispensable for the ventral guidance of HSN axons. It also separates the relative contributions of PI3K and UNC-34 to stimulate the formation of filopodia in HSN, while the analysis of AVM indicates that MIG-10 also has separate functions that do not require UNC-34.

Discussion

*C. elegans* MIG-10 plays roles in both Slit-dependent and netrin-dependent axon guidance pathways. In AVM neurons, MIG-10 promotes repulsion from Slit through the SAX-3/Robo receptor. In HSN neurons, MIG-10 promotes filopodia formation during attractive netrin signaling through the UNC-40 receptor. In addition to its effect on axon guidance, MIG-10 can stimulate cell and axon outgrowth in C. elegans neurons, as it can in cultured fibroblasts and T cells [19, 20].

*mig-10* activity in developing axons is stimulated by *age-1*, which encodes the lipid kinase PI3K. Lpd has an unusual lipid binding specificity with a strong preference for *P*[(3,4)P]2 phospholipids, which are products of PI3K. Previous results with pharmacological inhibitors have implicated PI3K in netrin signaling in Xenopus neurons [35, 36]; our genetic results provide evidence that PI3K affects axon guidance and outgrowth in vivo and also identify MIG-10 as a potential target of the lipids generated by PI3K. The positive feedback loop between the Rac GTPase and PI3K defined in migrating neutrophils provides one possible pathway from UNC-40/DCC to MIG-10 [33]. In addition, DCC can bind to the focal adhesion kinase (FAK) when phosphorylated by src family kinases [37–39], and FAK kinases can recruit and activate PI3K in other cell types [40]. Mammalian Lpd and RIAM interact with H+, K+, N-, and R-Ras [41], so a Ras-PI3K pathway could provide another mechanism for activation of MIG-10.

Biochemical and genetic results suggest that MIG-10 and UNC-34 (Ena/VASP) have related functions. However, *mig-10* and *unc-34* do not act in a simple linear pathway (Figure S3); each gene has separate functions, the double mutant is lethal, and double mutant escapers have strongly enhanced axon guidance defects. Studies of Lpd and Ena/VASP proteins in mammalian cells also suggest that they have distinct functions, although the experiments have not been conducted in the same cell types. Lpd is required for lamellipodia formation in melanoma cells [19]. Ena/VASP function is required for filopodia formation in fibroblast and neurons, but cells that lack Ena/VASP function can still form lamellipodia, though the dynamics are different [42, 13]. In addition to its synthetic lethality with *mig-10*, *C. elegans* unc-34 also has synthetic lethal interactions with the actin-regulatory proteins of the WASP and WAVE complexes [30]. These results suggest that these proteins belong to a broad network of cytoskeletal regulators with partly overlapping functions. Interestingly, WASP, WAVE, Ena, Lpd, and other actin-regulatory proteins can be copurified in a common complex from the mammalian brain [43].

Ena/VASP proteins are strongly implicated in filopodia formation [13, 17]. Our results support the importance of this interaction for UNC-34 and also show that MIG-10 enhances filopodia formation in vivo. Because filopodia are a general feature of growth cones, they have been thought to be critical for axon guidance. The sole *C. elegans* Ena/VASP protein UNC-34 is essential for generating filopodia downstream of netrin but is largely dispensable for HSN guidance toward the ventral nerve cord. Thus growth cone guidance in vivo can be accurate without many filopodia, perhaps through the alternative motility mechanisms stimulated by MIG-10 and by Rac pathways [7]. Although filopodia represent an elegant extended structure for exploring broad regions and evaluating shallow gradients with minimal cytoskeletal commitment, they may not be essential when guidance cues are relatively unambiguous or when the migration distances are relatively short.

We suggest that MIG-10 has two signaling modes, one in which it acts mainly with UNC-34 to organize filopodia, and one in which it acts mainly with AGE-1-dependent phospholipids to stimulate lamellipodial outgrowth (Figure S3). At this point, we do not know the exact rules for recruitment and activation of these molecules, but the genetic results show clear differences between the ways that MIG-10 functions even within a single neuron. The cooperative action of lipid-modulating enzymes, actin regulators, and multifunctional linkers such as MIG-10 provides multiple points of contact between specific guidance receptors and the shared signaling components in a developing neuron.

Experimental Procedures

Strains

Nematodes were cultivated according to standard protocols and maintained at 20°C [44]. The following mutations and transgenes were used: LGI, zdIs5[mec-4::gfp, lin-15(+)], klys170[mec-220::gfp, lin-15(+)], LGII, kyIs262[unc-86::myr::unc-40(m15)], LGV, zdIs4[mec-4::gfp, lin-15(+)], LGIV, kyIs170[unc-86::myr::unc-40(m15)], klys170[mec-220::gfp, lin-15(+)], LGX, zdIs1[ceh-23::GFP, lin-15(+)], zdIs4[mec-4::gfp, lin-15(+)], klys170[unc-86::myr::unc-40(m15)], klys170[mec-220::gfp, lin-15(+)], LGX, unc-34::GFP, odr-1::dsred, LGX, unc-6(ov400), sli-1(eh15), sax-3(ky123). Transgenes maintained as extrachromosomal arrays included: cyEx21[mec-7::mig-10a, odr-1::dsred], kyEx458[unc-86::myr::unc-40, str-1::gfp], kyEx637[unc-86::myr::unc-40::P2], odr-1::dsred, kyEx639[unc-86::myr::unc-40::P1], odr-1::dsred, kyEx710[unc-86::GFP;unc-34, odr-1::dsred], and kyEx1694[mec-7::age-1, odr-1::dsred].

Transgenes used to characterize axon guidance defects in Figure 1 were klys262 (HSN), zdIs5 (AVM), klys170 (ADL), and zdIs1 (CAN).

Germline transformation of *C. elegans* was performed by standard techniques [44]. The *mec-7::mig-10a* and *mec-7::age-1* overexpressing lines were injected at 100 ng/ul along with the coinjection marker *odr-1::dsred* at 70 ng/ul. Transgenic lines were maintained by following *odr-1::dsred* fluorescence with a Zeiss M²BIO imaging system.
system. For the mig-10 and age-1 cell-autonomy experiments, two independent transgenic lines with similar properties were analyzed; the data shown are from one line, kyE456, kyE457, and kyE639 transgenes have been described previously [7]. Some mutant strains were kindly provided by Gian Garriga at Berkeley or Theresa Stiernagle of the Caenorhabditis Genetics Center.

Microscopy

Axonal processes of AVM neurons were visualized with the integrated mec-4::gfp transgene zdsfl in young adult animals, except for mig-10; unc-34 doubies, which were analyzed on the L2-L3 stage. The observer was not blind to the genotype. Animals were placed on 5% Noble Agar pads in M9 buffer containing 10 mM sodium azide and examined with a Plan-NEOFLUAR 40x objective on a Zeiss Axiosplan 2. Arrested mig-10; unc-34 double mutants were picked from agar plates where embryos laid from mig-10; unc-34/+ parents had developed for 48 hr. Arrested animals were analyzed with a Plan-APOCHROMAT 100x objective with Nomarski optics. Images for axons and body morphology were captured with a SPOT camera (RT Slider Diagnostic Instruments, Inc.). All pictures of HSN are projections of Z-stacks captured on a BioRad MRC1024 confocal with a 63x oil objective.

Developmental Analysis of HSN Neurons

Embryos were released from gravid adults by standard techniques [44], then synchronized by overnight starvation in M9 buffer. Animals were then fed and grown at 25°C until the late L2 stage [34]. In wild-type animals, this occurred ~20 hr after feeding; in mig-10 and unc-34 animals, this occurred ~25 hr after feeding. Populations were examined by standard epifluorescence on a Zeiss Axiosplan2 with a 63x oil objective.

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mec-7::mig-10a was generated by cloning the mig-10a cDNA into KpnI and XhoI sites of the pPD96.41 vector containing the mec-7 promoter [45] between SpnI and SalI sites. The mec-7::mig-10a cDNA was amplified by PCR and inserted at the 3’ end of GFP in a pDPD96.41 vector. pPD96.41 was a gift of Andrew Fire (Stanford University). To generate unc-86::GFP::unc-34, an unc-34 cDNA was amplified by PCR and inserted at the 3’ end of GFP in a pDPD96.41 vector containing a 5 kb fragment of the unc-86 promoter [45] between SpnI and SalI sites. The unc-34 cDNA was provided by Tim Yu and the mig-10a cDNA was provided by Yui Kohara (National Institute of Genetics, Japan).

Supplemental Data

Supplemental Data include three figures and Supplemental Experimental Procedures and can be found with this article online at http://www.current-biology.com/cgi/content/full/16/9/854/DC1/.

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