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MIG-13 controls anteroposterior cell migration by interacting with UNC-71/ADM-1 and SRC-1 in *Caenorhabditis elegans*

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**A B S T R A C T**

The transmembrane protein MIG-13 is a key regulator required for anterior migration of neural cells in *Caenorhabditis elegans*, but the signaling mechanisms involved remain unknown. Here, we isolated a suppressor mutation in the unc-71/adm-1 gene, which rescued the AVM neuron migration defect in mig-13 mutants. Genetic analyses revealed that UNC-71 at least partly acts downstream of MIG-13 and has an inhibitory effect on the anterior cell migration. The unc-71 mutation also rescued the anterior migration defect of AVM neuron in src-1 mutants. These findings suggest that MIG-13 controls anteroposterior cell migration by interacting with UNC-71 and SRC-1 in *C. elegans*.

**1. Introduction**

Cell migration is crucial for normal development of multicellular organisms and its underlying mechanism is functionally conserved during evolution [1,2]. Aberrant cell migration leads to severe embryonic malformations and defects in tissue organization and homeostasis that cause various disorders [3]. The nematode *Caenorhabditis elegans* is an excellent model for studying how cell migration is regulated during development [4,5]. A number of proteins related to cell migration in *C. elegans* have been identified. For example, the netrin/UNC-6, slit/SLT-1 and TGF-

FGF-like ligand EGL-17 [10] and a receptor belonging to the FGF receptor subfamily EGL-15 [11] are crucial for proper positioning of SM. Nonetheless, signaling mechanisms that control global guidance along the A/P axis still remain obscure.

*C. elegans* Q neuroblasts, QR and QL are born at similar anterior-posterior positions on the right and left side of the body, but they migrate in opposite directions; QL and its descendants migrate toward the posterior, whereas QR and its descendants migrate toward the anterior [12]. These migrations are regulated cell autonomously by two homeobox genes, *mab-5* and *lin-39*. Expression of *mab-5* is induced specifically in the QL lineage by Wnt/EGL-20 signaling and promotes the posterior migration of QL and its descendants [13–15]. *mab-5* is not expressed in the QR lineage; instead, *lin-39* promotes anterior migration of the QR lineage [16]. However, extracellular signalings acting in these migrations need to be addressed.

The *mig-13* gene is known to play a key role in promoting anterior cell migration along the A/P axis. *mig-13* encodes a novel transmembrane protein with an LDL receptor repeat and a CUB domain, and is expressed in the anterior body region. *mig-13* acts non-cell autonomously as a component of the global guidance system along the A/P axis. *mig-13* also acts in parallel to *lin-39*, and interacts generally with the Hox co-factor orthologs, *ceh-20* and *unc-62* [17,18]. However, the molecular mechanisms underlying *mig-13* function remain unknown.
To address the mechanisms of the MIG-13 pathway, we screened for mutations that suppress the defects in \textit{mig}-13 mutants. We isolated a suppressor mutant with a loss-of-function mutation in the \textit{unc-71/adm-1} gene, which encodes a disintegrin and metalloprotease (ADAM) protein [19]. We found that UNC-71 at least partly acts downstream of MIG-13 through being downregulated in its inhibitory effect on the anterior cell migration. Moreover, UNC-71 acts in the same pathway as SRC-1, a signaling tyrosine kinase required for controlling cell migration. These findings suggest that MIG-13 controls A/P cell migration via a novel signaling interaction with UNC-71/ADM-1 and SRC-1 in \textit{C. elegans}.

2. Materials and methods

2.1. Isolation of \textit{mig}-13 suppressor mutants

Animals homozygous for \textit{mig-13(mu225)} were mutagenized with 50 mM ethylmethane sulfonate (EMS) [5]. F2 progeny were screened for mutations that rescue the aberrant position of AVM neuron (QR.paa) in \textit{mig-13} mutants. The \textit{mecl-7-gfp} transcriptional fusion, \textit{muIs32}, is strongly expressed in the mechanosensory neurons including AVM neuron [20], and was used to visualize AVM neuron position by fluorescence stereomicroscopy.

2.2. Cloning and transformation rescue of \textit{unc-71}

Genomic DNA from N2 animals was isolated following standard procedures [21]. The \textit{unc-71} genomic region was amplified using a long range PCR kit (Roche). The DNA mixture for \textit{unc-71} rescue consisted of three overlapping PCR products covering the entire \textit{unc-71} coding region, as well as 5 kb 5'-flanking and 2 kb 3'-flanking sequences. To rescue the \textit{ov10} phenotype, the DNA mixture (50 ng\textmul) was injected with a \textit{Plin-44::gfp} injection marker (50 ng\textmul) into the \textit{sup-1(ov10); mig-13(mu225); mls32[Pmecl-7::gfp]} animals in the young adult stage. Transgenic lines were obtained using standard protocol [22].

2.3. Phenotypic analysis

AVM (QR.paa), PVM (QL.paa) and BDU neurons were visualized using a \textit{mecl-7-gfp} transcriptional fusion, \textit{mls32}, which is expressed in mechanosensory neurons including AVM neuron and PVM, FLP, PVD, and BDU neurons. Their positions were scored relative to V-cell daughters, which were used as stationary landmarks at the end of the L1 stage [20]. QR lineage migration in living animals was directly observed on a 5% agar pad in M9 buffer using Nomarski optics.

2.4. RNA interference

RNAi was performed in the background of \textit{rrf-3(pk1426)}, which are more sensitive to RNAi than wild-type animals [23]. The \textit{unc-71} dsRNA sequence was designed as previously described [24], and was synthesized from pCZ418 (gift of Dr. Y. Jin) [19], as previously described [25]. The dsRNA (50 ng\textmul) was injected with a \textit{Plin-44::gfp} injection marker (50 ng\textmul) into \textit{mig-13(mu225); rf-3(pk1426); mls32[Pmecl-7-gfp]} and \textit{rf-3(pk1426); mls32[Pmecl-7-gfp]} animals in L4. Injected animals were put onto plates and their late L1 progeny were analyzed for AVM neuron position.

2.5. Ectopic expression of \textit{mig-13}

Ectopic expression of \textit{mig-13} was carried out as described previously [18]. The \textit{mig-13} cDNA was amplified by PCR, and the \textit{mig-13-gfp} translational fusion was constructed by inserting the GFP coding region of pPD95.75 into the XhoI site in the last exon of the \textit{mig-13} cDNA. The resulting construct was inserted into the H20 promoter vector. The \textit{PH20::mig-13::gfp} (50 ng\textmul) was injected with a \textit{Plin-44::gfp} injection marker (50 ng\textmul) into the \textit{muls32} young adults. We first made the extrachromosomal array in wild-type background, and then crossed these animals into \textit{mig-13(mu225), lin-39(n1760)}, and \textit{src-1(cj293)}. We analyzed a homozygous \textit{src-1(cj293)} mutant produced from the balanced heterozygote \textit{src-1(cj293)/+}, as the \textit{src-1(cj293)} mutant showed a maternal embryonic lethal phenotype [26].

3. Results

3.1. Isolation of a mutant that suppresses the migration defect in \textit{mig-13} mutants

In wild-type animals, QR and its descendants migrate anteriorly along the A/P axis, and the AVM neuron (QR.paa) ends up between the V1.p and V2.a cells (Fig. 1). In \textit{mig-13(mu225)} mutants, QR migrates anteriorly a short distance as in wild-type animals, but its descendants, particularly QR.a, QR.p, and QR.pa, end their migration prematurely. As a result, QR descendants, including AVM neurons, end up being located in a more posterior position that in wild-type animals (Fig. 1) [13,17]. To identify genes involved in the \textit{mig-13} pathway, we screened mutants that rescue these migration defects of QR descendants in \textit{mig-13(mu225)} mutants. We mutagenized \textit{mig-13(mu225); mls32[Pmecl-7-gfp]} animals and screened mutants with rescued AVM neuron position, using the \textit{mecl-7-gfp} transcriptional fusion \textit{mls32} as a marker of mechanosensory neurons, including the AVM neuron [20]. From more than 10000 mutagenized haploid genomes screened, we isolated a suppressor mutant, \textit{sup-1 (ov10)}, which partially rescues the AVM neuron position defect (Fig. 1A).

In \textit{sup-1(ov10); mig-13(mu225)} double-mutants, AVM neurons were located in a more anterior position than in \textit{mig-13(mu225)} mutants (Fig. 1C). Live observation revealed that the \textit{ov10} mutation rescued the \textit{mig-13(mu225)} AVM neuron position defect by extending the migration distance of QR descendants (Fig. 1B). In the double-mutants, however, the majority of QR descendants stopped migration at the position of the V2.a cell (Fig. 1C). Also, the migration defects of BDU neurons in the anterior body region of \textit{mig-13} mutants were not significantly rescued by the \textit{ov10} mutation (Fig. 1D). These observations indicate that the \textit{ov10} mutation can rescue the early phase of the anterior migration of these cells up to the mid-body region, and suggest that \textit{ov10} is at least partly linked to the \textit{MIG-13} pathway.

3.2. Mapping and cloning of \textit{ov10}

We next identified the gene responsible for the \textit{ov10} mutation. SNP mapping analysis revealed that the \textit{ov10} mutation is located to the right of the SNP allele pK3114, which is at +17.99 on LGIII [27,28]. We narrowed down the location of the \textit{ov10} mutation to between +17.99 and +19.53 by deficiency mapping of \textit{ctdF3} (Fig. S1A) [29]. The corresponding genomic region from 12497894 to 12951561 bp on LGIII was then subjected to next-generation sequencing. We found a single C-to-T substitution in the genomic region of \textit{ov10}.

3.3. \textit{ov10} is \textit{unc-71/adm-1} mutant

To confirm that the \textit{unc-71} mutation underlies the suppressor phenotype in \textit{ov10} animals, we examined whether the \textit{unc-71} gene
is able to rescue the ov10 phenotype by germline transformation. The ov10 phenotype was rescued in the transgenic animals, with the AVM neuron position similar to that in mig-13(mu225) mutants (Fig. 2A). Furthermore, we crossed mig-13(mu225) mutants to other unc-71 alleles, ju156 and e541 [19]. ju156 has a premature stop before the metalloprotease domain due to a 170 bp deletion in Exon2 and is thought to a null mutation. e541 is a missense mutation in the cysteine-rich domain. Both alleles could rescue
the AVM neuron position defect in mig-13 mutants, although single mutants of these alleles exhibited no defects (Fig. 2B). These results indicate that the unc-71 mutation can serve as a suppressor of the mig-13 mutation.

We also found that the ov10 mutation more strongly rescued the mig-13 phenotype than did the e541 mutation (Fig. 2B), suggesting that both of the disintegrin domain and the cysteine-rich domain are required for the activity that disrupts the migration of QR descendants. However, the ov10 phenotype was also stronger than that of ju156. In some of the unc-71(ov10) animals, the AVM neuron position was shifted more anteriorly than its normal position (Fig. 2B). These observations suggest that the unc-71(ov10) mutation, but not the unc-71(e541) or unc-71(ju156) mutation, may have another epistatic effect by expressing the truncated form of UNC-71.

To determine whether these phenotypes indeed resulted from the reduced unc-71 activity, we carried out unc-71 RNAi using the rrf-3(pk1426) strain, in which neuronal RNAi is enhanced [23]. In the rrf-3(pk1426) background, wild-type and mig-13(mu225) exhibited the same phenotype as in the N2 background (Fig. 2C). unc-71 RNAi suppressed the migration defect of AVM neurons in mig-13 mutants, as seen in unc-71(ov10); mig-13(mu225) animals (Fig. 2C); however it had no effect on the rrf-3(pk1426) strain (data not shown). These results indicate that the suppressor effect of ov10 on the defect in mig-13 mutants is due to a loss of unc-71 function.

To further confirm the functional link between unc-71 and mig-13, we examined the effect of unc-71 mutation on lin-39 mutants, which exhibit anterior migration defects of QR descendants by acting in parallel to mig-13 [17]. If unc-71 acts in the same pathway as mig-13, unc-71 should also act in parallel to lin-39. Consistent with this hypothesis, the migration defect of AVM neuron in lin-39(n1760) mutants was not rescued by the unc-71(ov10) mutation (Fig. S2). Taking together, these findings suggest that UNC-71 at

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**Fig. 2.** ov10 is unc-71 mutant. (A) Final AVM neuron positions in the indicated strains are scored. unc-71 DNA was injected into sup-1(ov10); mig-13(mu225); muIs32[Pmec-7::gfp] animals. The AVM neuron position in unc-71 transgenic animals reverted to that of mig-13 mutants (bottom panel). (B) Final AVM neuron positions for three different unc-71 alleles (ov10, ju156 and e541) are scored. The results for unc-71 single mutants (left panels) and unc-71; mig-13 double mutants (right panels) are shown. The AVM neuron position defect in mig-13 mutants is suppressed by these unc-71 alleles. (C) Effects of unc-71 RNAi on the mig-13 phenotype are scored.

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least partly acts downstream of MIG-13 during anterior cell migration up to the mid-body region, and that its activity has an inhibitory effect on the anterior migration of the QR and its descendants.

3.4. UNC-71 is expressed in a different pattern than MIG-13 and may act non-cell autonomously

To learn whether UNC-71 is expressed in QR and its descendants, we used an unc-71-gfp transcriptional fusion, jul866. Previous work showed that unc-71 is expressed in several head neurons, excretory cell, excretory gland cells and sphincter muscle cells in L1 to adult animals [19]. Though we were able to confirm unc-71 expression in these cells, no significant expression was detected in the QR lineage at any stage (Fig. S3A). This suggests that UNC-71 is likely to act non-cell autonomously. We also confirmed the mig-13 expression pattern using a mig-13-gfp translational fusion and the jul866 transgenic animal which expresses a mig-13-mKO transcriptional fusion. However, we could not detect co-localization of UNC-71 and MIG-13 in any cell lineages (Fig. S3B–F). These observations suggest that UNC-71 acts non-cell autonomously in cells different from MIG-13-expressing cells.

3.5. src-1 acts in the same pathway as mig-13 and unc-71

To further examine the potential contribution of other components to the MIG-13 pathway, we focused on the src-1 gene. We have previously shown that SRC-1 controls the direction of cell and growth cone migration of cells including the Q neuroblasts [26]. Notably, we showed that src-1 mutants exhibited almost the same AVM neuron distribution as mig-13 mutants. Thus, we examined the genetic interaction of src-1 with mig-13 and unc-71. We found that the migration defect in src-1 mutants was partially rescued in the unc-71(ov10) background (Fig. 3). Furthermore, src-1(cj293); mig-13(mu225) double mutants showed a distribution of the AVM neuron similar to that in src-1(cj293) mutants.

We then tested whether src-1 is required for cells to respond to MIG-13 by expressing a mig-13-gfp translational fusion in src-1(cj293) mutants using the pan-neural H20 promoter. The mig-13 expression promoted anterior migration of AVM neurons in wild-type, mig-13 and lin-39 animals; however it had no effect on src-1(cj293) animals (Fig. 4). These results suggest that UNC-71 acts in the same pathway as SRC-1, and that SRC-1 is tightly associated with the MIG-13 pathway.

4. Discussion

To address the mechanisms underlying anterior cell migration along the A/P axis, we screened for suppressors of mig-13 mutants and identified an unc-71/adm-1 mutation. The unc-71 mutation suppressed the migration defect in mig-13 mutants, allowing QR descendants to migrate anteriorly without MIG-13 activity. Expression of an unc-71 transgene in unc-71; mig-13 double mutants inhibited this anterior migration. These observations suggest that an unknown factor promotes anterior migration independently of MIG-13, and the activity of this factor may be suppressed by UNC-71. The fact that unc-71 single mutants (ju156 and e541) exhibit no QR anterior migration defects further indicates that UNC-71 function is suppressed in the presence of MIG-13. Taken together, these findings suggest that MIG-13 promotes the anterior migration of the QR descendants by downregulating UNC-71 function (Fig. S4).

We also found that unc-71 mutation rescued the migration defect in mig-13 mutants only up to the region around the V2.a cell. Also, the migration defects of the BDUR neuron in the anterior body region of mig-13 mutants were not significantly rescued by the unc-71 mutation. These results suggest that unc-71 is involved in anterior migration only up to the mid-body region, and that the MIG-13 pathway may require additional factor(s) to accomplish migration to the anterior body region (Fig. S4). Thus, a more comprehensive analysis of the components of this pathway will be necessary in order to fully unravel the mechanisms of MIG-13-mediated cell migration.

UNC-71 is a member of the ADAM family of proteins, and is probably a homolog of mammalian ADAM14. Previous work has revealed that UNC-71 functions in axon guidance, axonal morphogenesis, and sex myoblast migration [19]. Since UNC-71 appears
not to have proteolytic activity, it may regulate target molecules via protein–protein interactions. Since mutations in the unc-71 disintegrin and cysteine-rich domains suppress the mig-13 mutant phenotype, it is likely that UNC-71 interacts with other components via these domains. It should also be noted that ov10, which likely produces an extracellular domain with an inactive metalloprotease domain, showed a stronger phenotype than that of the null ju156. Previous work has shown that expression of an UNC-71 mutant lacking the transmembrane domain and cytoplasmic tail induces an unc-71 mutant-like phenotype in wild-type animals [19]. Thus, it is possible that UNC-71 must be anchored to the membrane to control the anterior migration of QR descendants, and that the inactive metalloprotease domain of the truncated UNC-71 may have another effect to enhance the unc-71 phenotype. In this study, we showed that UNC-71 is likely to act non-cell autonomously, as reported previously [19]. These findings indicate that UNC-71 affects QR and its descendants by interacting with other targets on the surface of these cells or through some mediators. Identification of such UNC-71-related factors will be necessary to elucidate the function of UNC-71.

Recently, a novel membrane glycoprotein, LRP12/MIG13a, was identified as a mammalian homolog of MIG-13 [30]. LRP12/MIG13a is a member of the low-density lipoprotein receptor related protein (LRP) family, and regulates cell migration during neural development similarly to MIG-13. Interestingly, Reelin signaling mediated by Src family tyrosine kinases (SFKs) is required to regulate LRP12/MIG13a-positive cells. We also found that SRC-1, a C. elegans homolog of SFKs, is related to the MIG-13 pathway. SRC-1 controls the direction of cell and growth cone migration, including the anterior migration of the QR descendants [26,31]. However, the target molecules of SRC-1 have yet to be identified. Thus, it would be interesting to determine which components of the MIG-13 pathway can serve as substrates of SRC-1. Further mechanistic analysis of this pathway will help elucidate the molecular basis of normal cell migration in multicellular animals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2012.01.031.

References


