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

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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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**ABSTRACT**

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.

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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-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 as a component of the global guidance system along the A/P axis.

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 [17]. However, the molecular mechanisms underlying MIG-13 function remain unknown.

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To address the mechanisms of the MIG-13 pathway, we screened for mutations that suppress the defects in mig-13 mutants. We isolated a suppressor mutant with a loss-of-function mutation in the 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 C. elegans.

2. Materials and methods

2.1. Isolation of mig-13 suppressor mutants

Animals homozygous for 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 mig-13 mutants. The mec-7::gfp transcriptional fusion, 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 unc-71

Genomic DNA from N2 animals was isolated following standard procedures [21]. The unc-71 genomic region was amplified using a long range PCR kit (Roche). The DNA mixture for unc-71 rescue consisted of three overlapping PCR products covering the entire unc-71 coding region, as well as 5 kb 5'-flanking and 2 kb 3'-flanking sequences. To rescue the ov10 phenotype, the DNA mixture (50 ng/μl) was injected with a Plin-44::gfp injection marker (50 ng/μl) into the sup-1(ov10); mig-13(mu225); muIs32[Pmec-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 mec-7::gfp transcriptional fusion, muIs32, which is expressed in mechanosensory neurons including AVM 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. RNAi interference

RNAi was performed in the background of rrf-3(pk1426), which are more sensitive to RNAi than wild-type animals [23]. The 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/μl) was injected with a Plin-44::gfp injection marker (50 ng/μl) into mig-13(mu225); rrf-3(pk1426); muIs32[Pmec-7::gfp] and rrf-3(pk1426); muIs32 [Pmec-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 mig-13

Ectopic expression of mig-13 was carried out as described previously [18]. The mig-13 cDNA was amplified by PCR, and the mig-13::gfp translational fusion was constructed by inserting the GFP coding region of pPD95.75 into the Xhol site in the last exon of the mig-13 cDNA. The resulting construct was inserted into the H20 promoter vector. The PH20::mig-13::gfp (50 ng/μl) was injected with a Plin-44::gfp injection marker (50 ng/μl) into the muIs32 young adults. We first made the extrachromosomal array in wild-type background, and then crossed these animals into mig-13(mu225), lin-39(n1760), and src-1(cj293). We analyzed a homozygous src-1(cj293) mutant produced from the balanced heterozygote src-1(cj293)/+, as the 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 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 mig-13(mu225) mutants, QR migrates anteriorly a short distance as in wild-type animals, but its descendants, particularly QRa, QRp, and QR.p, 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 MIG-13 pathway, we screened mutants that rescue these migration defects of QR descendants in mig-13(mu225) mutants. We mutagenized mig-13(mu225); muIs32[Pmec-7::gfp] animals and screened mutants with rescued AVM neuron position, using the mec-7::gfp transcriptional fusion muIs32 as a marker of mechanosensory neurons, including the AVM neuron [20]. From more than 10000 mutagenized haploid genomes screened, we isolated a suppressor mutant, sup-1 (ov10), which partially rescues the AVM neuron position defect (Fig. 1A).

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

3.2. Mapping and cloning of ov10

We next identified the gene responsible for the ov10 mutation. SNP mapping analysis revealed that the ov10 mutation is located to the right of the SNP allele pkP3114, which is at +17.99 on LGIII [27,28]. We narrowed down the location of the ov10 mutation to between +17.99 and +19.53 by deficiency mapping of 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 unc-71/adm-1 coding region (Fig. S1A), which changes a glutamine codon to a stop codon in the middle of disintegrin domain, suggesting that ov10 may express a truncated form of UNC-71 (Fig. S1B).

3.3. ov10 is unc-71/adm-1 mutant

To confirm that the unc-71 mutation underlies the suppressor phenotype in ov10 animals, we examined whether the 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 be 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

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.
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, juls66. 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 juls66 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.

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