High copy arrays containing a sequence upstream of mec-3 alter cell migration and axonal morphology in C. elegans

ABSTRACT

The mec-3 upstream sequence appeared to be sequestering (titrating out) a specific DNA-binding factor that is required for the ALMs to migrate correctly. Because titration of this factor could reverse the direction of ALM migrations, it may be part of a program that specifies both the direction and extent of ALM migrations. mec-3 is a master regulator of touch receptor neuron genes, so the factor or factors that bind this sequence may also be involved in specifying the fate of touch receptor neurons.

INTRODUCTION

Background Cell migration is one of the most important and complex cellular behaviors. It is essential for animal development, immune system function, and wound repair. Defects in cell migration can lead to human diseases such as birth defects, and failure to control cell migration is an important step in tumor metastasis. We currently believe that migrating cells extend and retract actin rich protrusions, lamellipodia and filopodia, into their environment. Protrusions that adhere strongly enough are stabilized and fail to retract. In this way, cells (or cell processes) can follow adhesive guidance cues. While this model has been around for some time, we are only now beginning to understand the molecular signals that cause cells to initiate movement, how cells move, the signals that guide the cell migrations, and the signals that stop cells at their appropriate positions. Recently, progress in understanding cell migration has come from studies of Caenorhabditis elegans and Drosophila. Most cell migration genes identified in these simple invertebrates are conserved in vertebrates, which confirms the efficacy of these genetically tractable systems for studying cell migration. C. elegans is a particularly attractive system for the study of cell migration. These animals are transparent and anatomically simple, so cell migrations can be followed in the living animal at all stages of development by fluorescence microscopy of GFP fusion proteins or by Nomarski microscopy. Both the cell lineage and the cell migrations are invariant from animal to animal, so migration defects can be easily identified. Several genetic screens performed with C. elegans have identified mutations that interfere with cell migrations (for reviews see). Some of these mutations affect all cell migrations, whereas others only affect the migrations of a limited subset of cells. Not surprisingly, many of these mutants also show defects in axon extension, bundling and pathfinding. The genes identified by these mutations encode extracellular proteins, cell surface receptors, fibroblast growth factor-like proteins and their receptors, adhesion molecules, small GTPases, non-muscle myosins and transcription factors. In C. elegans, three genes, unc-6, unc-5 and unc-40, guide cells and processes along the dorsal-ventral axis. All of these genes are conserved across broad groups of animals from C. elegans to man. UNC-6 protein, a laminin-like protein that is located in the ventral region of the animal, is a homolog of the vertebrate protein netrin. UNC-5 and UNC-40 are cell surface receptors that interact with UNC-6. UNC-5 promotes dorsal migrations, whereas UNC-40 promotes ventral migrations, both in response to UNC-6 signals. Based on studies in other species, the difference in how UNC-5 and UNC-40 guide cells and axons lies in their intracellular domains. Mutations in unc-129, a member of the transforming growth factor β (TGFβ) superfamily, were identified as genetic suppressors of ectopic UNC-6 signaling. unc-129 is expressed dorsally and loss of UNC-129 function disrupts dorsal axon migrations. In Drosophila, TGFβ

family members are also involved in controlling dorsal-ventral migrations. It appears, therefore, that the UNC-6/netrin and TGFβ guidance systems act redundantly and are conserved across species. The guidance of cells and axons along the anterior-posterior axis of C. elegans is not as well understood. Two genes involved in anterior-posterior cell migration are vab-8 and mig-13. VAB-8 is a kinesin-related protein that acts cell autonomously and is involved in posterior cell migrations. Therefore, VAB-8 is probably involved in the cellular response to guidance cues. MIG-13 is a novel transmembrane protein that acts non-cell autonomously and is involved in anterior cell migrations. The dose of MIG-13 appears to affect the extent of anterior cell migrations. MIG-13 may, therefore, signal to cells their direction and extent of migration. Chalfie and colleagues have identified a transcriptional cascade that leads to the activation of touch neuron-specific genes. UNC-86 is a POU homeodomain transcription factor needed to activate the mec-3 gene. mec-3 in turn encodes a LIM homeodomain protein that is expressed in the six touch receptor neurons, two FLP neurons and two PVD neurons. MEC-3 and UNC-86 proteins form a heterodimer that binds to and activates the mec-3 promoter and the promoters of touch receptor-specific genes such as mec-7 and mec-4. In this way, MEC-3 activates its own transcription, which probably prevents the dedifferentiation of the touch neurons. Later in development, mec-17 also contributes to the maintenance of mec-3 expression. We show here that a sequence upstream of mec-3, when transformed into C. elegans in high copy arrays, altered the extent and direction of ALM touch receptor neuron migrations. This sequence also disrupted extension of the PLM touch receptor axon. These defects did not result from RNA interference (RNAi), the heavy genetic load of carrying a transgenic array, the expression of GFP, or the rol-6 marker gene used to make the transgenic arrays. The ALM migration defects were due to a specific DNA sequence and only occurred when there were many copies of that sequence in the array. This sequence did not affect all cell migrations, the ALM/BDU cell division or the positions of the BDU cells. We conclude, therefore, that the sequence is sequestering a factor that helps control ALM migrations and PLM axon outgrowth. We also suggest that this factor may be differentially segregated into touch receptor neurons and that it may help specify the touch receptor neuron cell fate.

CONCLUSION

Conclusions The experiments described here show that transformation of C. elegans with many copies of a specific sequence located upstream of mec-3 induced cell migration and axonal guidance defects. This mec-3 upstream sequence appeared to be sequestering a factor involved in controlling ALM migration and PLM axonal outgrowth. This factor may also regulate mec-3 and thereby control touch receptor neuron fate. Titration of transcription factors with high copy arrays may become widely applicable in C. elegans once conditions that optimize this effect are found. The ALM defect described here may be useful for finding these conditions.