Developmental Biology 424 (2017) 18–27

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

The *Caenorhabditis elegans* matrix non-peptidase MNP-1 is required for neuronal cell migration and interacts with the Ror receptor tyrosine kinase CAM-1

Teresa R. Craft, Wayne C. Forrester*

Medical Sciences Program, Indiana University, Bloomington, IN 47405, United States

ARTICLE INFO

Keywords:
Caenorhabditis elegans
MNP-1
Cell migration
Secreted peptidase

ABSTRACT

Directed cell migration is critical for metazoan development. During *Caenorhabditis elegans* development many neuronal, muscle and other cell types migrate. Multiple classes of proteins have been implicated in cell migration including secreted guidance cues, receptors for guidance cues and intracellular proteins that respond to cues to polarize cells and produce the forces that move them. In addition, cell surface and secreted proteases have been identified that may clear the migratory route and process guidance cues. We report here that *mnp-1* is required for neuronal cell and growth cone migrations. MNP-1 is expressed by migrating cells and functions cell autonomously for cell migrations. We also find a genetic interaction between *mnp-1* and *cam-1*, which encodes a Ror receptor tyrosine kinase required for some of the same cell migrations.

1. Background

Cell migration is critical for metazoan development. Many cells migrate long distances during animal development. Cell migration is of particular importance in the developing nervous system, where many neuronal precursors move from their sites of birth to the positions they occupy in mature animals. In a related process the migrations of growth cones, specialized structures at the leading ends of axons and dendrites, establish the connections of the nervous system. Biochemical, genetic and molecular studies have identified many genes involved in cell migration but our understanding of the process remains incomplete.

We have utilized genetic screens to identify genes required for cell migration in the small nematode *Caenorhabditis elegans*. During *C. elegans* development, many cell types migrate extensively. For example, embryonic muscle cells migrate from lateral positions to flank the dorsal and ventral midlines (Hresko et al., 1994; Moerman et al., 1996; Sulston et al., 1983). Cell migration is also important during *C. elegans* nervous system development where several neuronal precursors migrate long distances during embryonic development (Fig. 1, Sulston et al., 1983) and migrating growth cones establish the connectivity of the nervous system (Durbin, 1987; White et al., 1986).

Different classes of proteins have been implicated in the process of cell migration. For example, putative guidance cues and their receptors direct migrating cells along their proper pathways. Intracellular proteins coordinate signals from guidance cues with the proteins that regulate the polymerization of actin at the leading edges to direct migrating cells. Also implicated in cell migration are secreted proteases. Originally these proteins were thought to function by clearing the pathway of obstacles for migrating cells (Brooks et al., 1996; Sato et al., 1994) but more recent results suggest that the roles of these proteins is more complex (reviewed in McCawley and Matrisian (2001), Seiki (2002)).

The *mnp-1* gene encodes a membrane-associated member of the M1 aminopeptidase family that is required for embryonic muscle cell migration (Tucker and Han, 2008). Aminopeptidases remove one or a few amino acids from the N-termini of target proteins or peptides (Hooper, 1994). They are implicated in tumorigenesis, regulation of blood pressure, angiogenesis, cell migration, and immune response. M1 aminopeptidases include a conserved GXMEN catalytic domain and an HENNH+E sequence motif that functions in the coordination of zinc, an essential cofactor for their proteolytic activity (Hooper, 1994; Iturrioz et al., 2001; Laustsen et al., 2001). Interestingly, *C. elegans* MNP-1 lacks a recognizable GXMEN motif and replaces three of the four conserved amino acids of HENNH+E motif and is therefore presumed to be catalytically inactive (Tucker and Han, 2008).

To identify genes required for cell migration, we performed a genetic screen for mutations that disrupted canal-associated neuron (CAN) cell migration (Forrester and Garriga, 1997; Forrester et al., 1998). One such mutation we called *fam-1(gm85)*, for fasciculation

* Corresponding author.
E-mail address: wforrest@indiana.edu (W.C. Forrester).

http://dx.doi.org/10.1016/j.ydbio.2017.02.016
Received 23 November 2016; Received in revised form 21 February 2017; Accepted 22 February 2017
Available online 24 February 2017

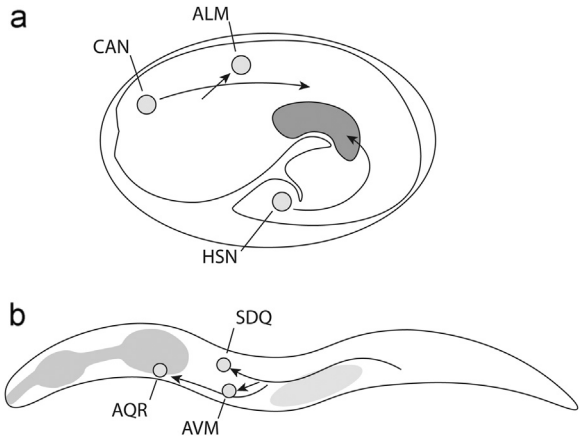

Fig. 1. Cell migrations. A. Schematic lateral view of a 450-min old embryo showing the final positions of CAN, HSN and ALM (circles) and their migratory routes (arrows). B. Schematic lateral view of the right side of a late first stage larva. QR divides to produce AQR, AVM and SDQ and two cells that die (not shown). Their final positions and approximate migratory routes are shown (arrows).

Table 1
mnp-1 is required for multiple cell migrations[a].

Strain	CAN	HSN		ALM	QR	QL
		posterior	anterior			
wild type	0 (38)	2.7 (38)	0	0 (38)	2.6 (38)	0 (42)
mnp-1(gm85)	46.7 (60)	18.2 (56)	9.1	20.7 (59)	59.3 (27)	7.1 (28)
mnp-1(ok2434)	36.3 (81)	27.0 (75)	2.7	15.8 (77)	39.3 (28)	0 (34)

[a] Data presents percentage of cells misplaced. The number in parentheses is the number of cells scored. CAN was scored as misplaced if it was anterior to V3. HSN was scored as posterior if it was posterior to V4 and anterior if it was anterior to P5/6. ALM was scored as misplaced if it was anterior to V2. QR was scored as misplaced if it was posterior to V2.a. QL was scored as misplaced if it was anterior to V4.p.

and cell migration defective. *fam-1(gm85)* mutants are defective in the migrations of multiple cell types and axon fasciculation, where axons fail to remain tightly bundled together (Forrester and Garriga, 1997). We cloned *fam-1* and found that *fam-1(gm85)* is a mutation in the *mnp-1* gene. We find that MNP-1 functions cell autonomously for CAN cell migration. Interestingly we find that *mnp-1* interacts genetically with another gene, *cam-1*, that also is required for the migrations of multiple cell types including CAN (Forrester et al., 1999; Forrester and Garriga, 1997).

2. Materials and methods

2.1. Strains

Nematodes were grown at 20 °C as described (Brenner, 1974). Strains used in this study include: the N2 wild-type strain and *cam-1(cw82), cam-1(gm105), cam-1(gm122)* (Forrester et al., 1999, 1998; Forrester and Garriga, 1997), *mIn1*[mIs14 *dpy-10(e128)*] (Edgley and Riddle, 2001), *mnp-1(gm85)* (Forrester and Garriga, 1997; Forrester et al., 1998), *mnp-1(ok2434), otIs33[kal-1::gfp]* (Bulow et al., 2002), *gmEx129[ceh-10::gfp], gmIs18[ceh-23::gfp, rol-6(su1006)]* (Lai and Garriga, 2004), *juIs76[unc-25::gfp, lin-15]* (Jin et al., 1999), *jcIs1[ajm-1::gfp, unc-29, rol-6(su1006)]* (Koppen et al., 2001), *cwEx488[Pmnp-1::mnp-1::gfp], cwEx486[Pmnp-1::mnp-1::gfp], cwEx487[Pmnp-1::mnp-1::gfp], cwEx526[Punc-119::mnp-1::gfp], cwEx526[Punc-119::mnp-1::gfp], cwEx527[Punc-119::mnp-1::gfp],* cwEx495*[Pceh-10::mnp-1::gfp], cwEx498[Pceh-10::mnp-1::gfp], cwEx502[Pceh-23::mnp-1::gfp], cwEx510[Pceh-23::mnp-1::gfp], cwEx490[Phlh-1::mnp-1::gfp], cwEx489[Phlh-1::mnp-1::gfp], cwEx491[Pajm-1::mnp-1::gfp], cwEx491[Pajm-1::mnp-1::gfp], cwEx518[Pajm-1::mnp-1::gfp], cwEx519[Pajm-1::mnp-1::gfp + Phlh-1::mnp-1::gfp],* and *cwEx520[Pajm-1::mnp-1::gfp + Phlh-1::mnp-1::gfp].* Germline transformants were produced by microinjection as described (Mello et al., 1991).

Table 2
Tissue specific rescue of cell migration defects.

Strain	CAN*	HSN	Lumpy	N
wild type	0	1.0	0	104
mnp-1(gm85)	38.7	7.5	37.0	93
mnp-1(ok2434)	35.6	14.1	27.7	101
mnp-1; cwEx522[rol-6]	28.2	13.8	34.5	110
MNP-1 promoter[a]				
mnp-1; cwEx488[Pmnp-1::mnp-1]	0.9	13.9	0	109
mnp-1; cwEx486[Pmnp-1::mnp-1]	1.8	13.2	14.0	114
mnp-1; cwEx487[Pmnp-1::mnp-1]	3.0	17.2	13.5	101
Pan-neuronal				
mnp-1; cwEx526[Punc-119::mnp-1]	7.5	3.8	20.8	106
mnp-1; cwEx527[Punc-119::mnp-1]	9.3	7.5	29.6	108
Expression in CAN				
mnp-1; cwEx495[Pceh-10::mnp-1]	6.5	8.9	13.1	123
mnp-1; cwEx498[Pceh-10::mnp-1]	4.7	24.6	35.2	106
CAN + amphid neurons				
mnp-1; cwEx502[Pceh-23::mnp-1]	15.1	13.2	41.5	108
mnp-1; cwEx510[Pceh-23::mnp-1]	24.5	16.7	25.9	110
Muscle				
mnp-1; cwEx490[Phlh-1::mnp-1]	15.0	19.3	30.2	113
mnp-1; cwEx489[Phlh-1::mnp-1]	10.3	10.5	23.5	107
Hypodermis				
mnp-1; cwEx491[Pajm-1::mnp-1]	24.1	13.4	17.9	112
mnp-1; cwEx518[Pajm-1::mnp-1]	35.3	19.8	22.4	116
Muscle + Hypodermis				
mnp-1; cwEx519[Pajm-1 & hlh-1::mnp-1]	24.0	13.9	10.0	100
mnp-1; cwEx520[Pajm-1 & hlh-1::mnp-1]	20.8	9.4	12.5	96

[a] Subheadings generalize major tissue types that express the relevant promoters.
* CAN cell migration defects were rescued by *Pmnp-1::mnp-1*, *Punc-119::mnp-1* and *Pceh-10::mnp-1* ($p < 0.0001$) and partially rescued by *Phlh-1::mnp-1* ($p < 0.01$). It was not rescued by *Pajm-1* & *hlh-1::mnp-1*, *Pceh-23::mnp-1*, and *Pajm-1::mnp-1*. p values were calculated by *t*-test-test comparing each transgenic line to *mnp-1(ok2434)[rol-6].*

2.2. mnp-1 cloning

To determine the chromosomal location of *fam-1* we generated *unc-93(e1500sd) fam-1(gm85) dpy-17(e164)* animals and crossed them with CB4856, a polymorphic strain (Koch et al., 2000; Wicks et al., 2001). We identified Unc non Dpy and Dpy non Unc recombinant animals, determined whether they were also *fam-1(gm85)* homozygotes and asked whether they had the N2 or CB4856 polymorphism for SNPs in the interval to which *fam-1* mapped. We found seven recombinants that showed that *gm85* was to the right of a polymorphism within the C30D11 cosmid and four that showed it was to the left of F25F2. Of these, a single recombinant showed that *gm85* lay to the right of a polymorphism within R10E4 and a second recombinant showed that it was to the left of C28A5.

We next looked for polymorphisms within the interval to which *gm85* mapped by whole genome Illumina DNA sequencing (performed by the Indiana University Center for Genomics and Bioinformatics). This analysis identified four polymorphisms of which a single one altered the DNA sequence within a predicted gene. The mutation