unc-53 controls longitudinal migration in *C. elegans*

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**SUMMARY**

Cell migration and outgrowth are thought to be based on analogous mechanisms that require repeated cycles of process extension, reading and integration of multiple directional signals, followed by stabilisation in a preferred direction, and renewed extension. We have characterised a *C. elegans* gene, unc-53, that appears to act cell autonomously in the migration and outgrowth of muscles, axons and excretory canals. Abrogation of unc-53 function disrupts anteroposterior outgrowth in those cells that normally express the gene. Conversely, overexpression of unc-53 in bodywall muscles leads to exaggerated outgrowth. UNC-53 is a novel protein conserved in vertebrates that contains putative SH3- and actin-binding sites. unc-53 interacts genetically with *sem-5* and we demonstrated a direct interaction in vitro between UNC-53 and the SH2-SH3 adaptor protein SEM-5/GRB2. Thus, unc-53 is involved in longitudinal navigation and might act by linking extracellular guidance cues to the intracellular cytoskeleton.

Key words: Cell migration, Axonal guidance, Growth cone steering, Cytoskeleton, *C. elegans*

**INTRODUCTION**

The development of higher organisms involves the movement of cells and the extension of cellular processes, such as axons, along complex pathways to defined destinations. Cell migrations and the outgrowth of cellular processes appear to be driven by common mechanisms that have been conserved through evolution. For both, in addition to a general non-directional motility, there is an element of pathfinding, which is associated with the capacity to respond to repulsive and attractive cues from multiple directions (Chisholm and Tessier-Lavigne, 1999; Tessier-Lavigne and Goodman, 1996). The mechanisms by which guidance cues are interpreted, to produce organised rearrangements of the cytoskeleton and hence directional movement are not fully understood.

The nematode *C. elegans* has proved to be an extremely useful genetic model with which to dissect the molecular basis of long-range migrations (Branda and Stern, 2000), especially those associated with the growth of axons along the dorsoventral axis. Not only have guidance cues and their receptors been identified, such as the Netrin UNC-6 and its receptors UNC-5 and UNC-40 (Hedgecock and Norris, 1997), but insights have also been obtained into the complex interplay between signalling pathways. For example, it has been shown that expression of SAX-3 (the worm Robo homologue) and UNC-40 allows the axon of the laterally situated AVM neurone to grow towards UNC-6 expressed by ventral cord axons and away from the SLT-1 cue originating from dorsal muscle cells (Hao et al., 2001).

Less is known about the anteroposterior (AP) migrations of either axons or cells. One of the better characterised examples is that of the anterior migration of the hermaphrodite sex myoblasts (Branda and Stern, 2000). During their journey to the position of the future vulva, they are subject to a gonad-dependent attraction. The attractive cue is EGL-17/FGF, which mediates its effect via EGL-15/FGFR and a downstream signalling pathway that passes through the GRB2 adapter homologue SEM-5. Several other genes, which also function in axon guidance, are known to impinge on the sex myoblast EGL-17 pathway. Their corresponding proteins, UNC-51 (a serine/threonine kinase), its interactor UNC-14 (Ogura et al., 1997), UNC-44 (an ankyrin) (Otsuka et al., 1995), UNC-33 (a protein related to collapsin-response-mediator protein) (Li et al., 1992) and UNC-34, are believed to be localised to the cytoplasm in the sex myoblasts and to act together to transduce the EGL-17 signal (Branda and Stern, 2000). The sex myoblasts are also subject to a gonad-independent attraction that, in addition to *sem-5*, requires the activity of *unc-53*, *unc-71* and *unc-73* (Chen et al., 1997). All of these *unc* genes have been linked to multiple guidance phenotypes, including defects in axon outgrowth or fasciculation (Hedgecock et al., 1987; McIntire et al., 1992; Siddiqui, 1990). While the identity of
unc-71 has yet to be reported, UNC-73 and its fly homologue TRIO are guanine nucleotide exchange factors (GEFs) that have been shown to regulate Rho GTPases and thereby actin cytoskeleton dynamics (Lin and Greenberg, 2000; Steven et al., 1998). The receptor for this pathway is currently unknown, and until now the role of unc-53 was unclear. unc-53 mutants also display premature arrest of the excretory canals (Hedgecock et al., 1987) and of the axons of the mechanosensory neurones (Hekimi and Kershaw, 1993), suggesting that it has a specific role in AP guidance.

We describe the isolation of an additional allele of unc-53 and the detailed characterisation of the role of unc-53 in AP axonal and cell migration. We show that the gene encodes a novel protein with several conserved domains associated with signal transduction and actin binding, and that UNC-53 is capable of interacting directly with the adapter protein SEM-5. We show that unc-53 is expressed in those cells that require its function. Furthermore, we demonstrate that overexpression of unc-53 results in exaggerated cellular outgrowth. Our results clearly define a role for unc-53 in AP migration and raise the possibility that UNC-53 acts cell autonomously to integrate multiple directional cues.

MATERIALS AND METHODS

Strains

All strains, including the wild type N2 Bristol, those containing the unc-53 alleles e2432, e404 (Brenner, 1974), n152 (Trent et al., 1983), and the deficiencies mmD90 (SP70), mmD87 (SP753), mmD97/mmnl1[dpy-10(e128);mxIs14[myo-2::GFP]]) (CZ1989) and mmD77 (SP705), were grown and maintained at 20°C as described previously (Brenner, 1974). The new EMS-induced allele e2432 was isolated in a screen using Nomsarsi (DIC) and polarised microscopy for mutants with specific attachment or pattern defects in a subset of the male sex muscles but without defects in body wall muscle pattern or myofilament organisation. The e2432 allele was mapped to the left arm of chromosome II and was found not to complement unc-53(e404). Transgenic arrays were generated in a wild-type or mutant background using standard techniques (Mello et al., 1991). Transgenic animals were identified by the dominant Roller phenotype conferred by co-injected plasmid pRF4 (that contains rol-d(su1006) DNA).

Transgenic strains made for this study are the following:

UG1 unc-53(n152);bgEx1 [T28D2;pRF4]
UG191 unc-53(n152);bgEx9 [T28D2;pNP3;pRF4]
UG21 unc-53(e2432);bgEx9 [S4;pRF4]
UG54 unc-53(e2432);bgEx12 [S4;pRF4]
UG83 unc-53(e2432);bgEx16 [S4;pRF4]
UG13 wt;bgEx3 [pNP3;pRF4]
UG14 wt;bgEx4 [pNP3;pRF4]
UG17 unc-53(n152);bgEx3 [pNP3;pRF4]
UG18 unc-53(e2432);bgEx3 [pNP3;pRF4]
UG38 wt;bgEx17 [pNP3;pNP8;pRF4]
UG39 wt;bgEx18 [pNP3;pNP8;pRF4]
UG41 unc-53(n152);bgEx18 [pNP3;pNP8;pRF4]
UG174 unc-53(n152);bgEx24 [pNP3;pNP8;pRF4]
UG175 unc-53(n152);bgEx25 [pNP3;pNP8;pRF4]
UG176 unc-53(n152);bgEx26 [pNP3;pNP8;pRF4]
UG177 unc-53(n152);bgEx27 [pNP3;pNP8;pRF4]
UG51 wt;bgEx10 [pNP10;pRF4]
UG52 wt;bgEx11 [pNP10;pRF4]
UG53 unc-53(n152);bgEx10 [pNP10;pRF4]
UG62 unc-53(n152);bgEx20 [pNP9;pNP10;pRF4]
UG63 unc-53(n152);bgEx22 [pNP9;pNP10;pRF4]
UG42 wt;bgEx21 [pNP21;pRF4]
UG85 unc-53(n152);bgEx21 [pNP21;pRF4]
UG186 unc-53(n152);bgEx34 [pNP24;pNP21;pRF4]
UG187 unc-53(n152);bgEx35 [pNP24;pNP21;pRF4]
UG188 unc-53(n152);bgEx36 [pNP24;pNP21;pRF4]
UG189 unc-53(n152);bgEx37 [pNP24;pNP21;pRF4]
UG190 unc-53(n152);bgEx38 [pNP24;pNP21;pRF4]
UG100 wt;bgEx48 [pTB113;pRF4]
UG101 wt;bgEx49 [pTB113;pRF4]
UG102 wt;bgEx50 [pTB113;pRF4]
UG103 wt;bgEx51 [pPD93.48;pRF4]
UG104 wt;bgEx52 [pPD93.48;pRF4]

Analysis of unc-53 phenotypes

To visualise the sex muscle phenotype of unc-53 mutants, young adults were mounted on 2% agarose pads containing 0.2% phenoxoypropanol as described ( Sulston and Horvitz, 1981) and observed using polarised light (with a Brace Kohler compensator) or DIC microscopy. In addition, adults were fixed, incubated with FITC-coupled phalloidin and mounted for fluorescence microscopy, as described (Goh and Bogaert, 1991). The stop points of the excretory canals were recorded in young adult hermaphrodites by DIC microscopy. Egg-laying was assayed by directly counting the number of progeny. As unc-53 hermaphrodites are not sterile, sick or starved, this accurately reflects defects in egg-laying (Trent et al., 1983). The effect of the different reporter GFP constructs (pAB::GFP, pA::GFP and pB::GFP) was assayed in the wild-type and n152 background and found not to influence the number of progeny (results not shown). The ALN and PLN axons were visualised in young adult hermaphrodites in transgenic strains carrying the pA::GFP reporter construct. The stop points of these axons were scored with reference to the position of the vulva and the anus.

Mapping and cloning of unc-53

unc-53(e2432) was mapped by three-factor crosses between unc-4 and sqt-1, 0.23 map units to the left of sqt-1, and relative to three deficiencies in the region. mmD87 and mmD90 do not complement unc-53(e2432), whereas mmD77 does. Southern blots of genomic DNA from the wild-type and deficiency strains were probed with cosmids throughout the region. Cosmid K02F7 is deleted in mmD90 but not deleted in mmD87 and mmD77, thus identifying a leftmost location for unc-53. The genomic region covered by cosmids W10G4, T08D11 and F33G3 are not deleted in mmD77 but are deleted in mmD87 and mmD90. Cosmid K04H9 is deleted in mmD77 and identifies a rightmost location for the gene. Further mapping of unc-53 relative to RFLPs placed unc-53 in an interval of 80 kb. Among cosmids from this region tested for their capacity to rescue unc-53(n152), T28D2 was found to rescue the Unc and Egl phenotypes. A genomic library of N2 in lambda2001 (the kind gift of A. Coulson) was screened with T28D2 and flanking overlapping cosmids. Lambda clone S4 carrying a 16 kb insert was shown to partially rescue unc-53(n152). Deletions in alleles n152 and e2432 were identified by Southern analysis and confirmed by sequencing. The allele n152 corresponds to a deletion of 319 bp from the alternative exon 18 to intron 19, while e2432 carries a deletion of 375 bp from exon 12 to intron 13, giving the breakpoint sequencesCCCCACGAGATCAATTTAC and TACGATTGTCTCTAACATCC, respectively.

Characterisation of unc-53 cDNAs

The 9 kb Xhol genomic fragment from the S4 phage was cloned into pBKS to give X16. Using this Xhol restriction fragment as a probe, cDNA clones CE5, CE6 and CE7 were isolated from a Lambda MGU1 cDNA library (a gift from R. Barstead). The insert of the largest clone, CE5, was entirely sequenced. It contains part of the SL1 trans-spliced leader sequence (Krause and Hirsh, 1987), a 5’ UTR of 42 bp and the entire coding sequence of unc-53, corresponding to 23 exons (GBAFA504312). To confirm the 5’ end of the unc-53 transcript,
was counted. Lethality was never observed in two p
allowed to lay eggs for 48 hours at 20°C. The number of mutant
UG102) were generated in a wild-type background. The presence of
unc-54::unc-53
vector pPD30.38 (a gift from A. Fire), which contains the myosin
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Nhe
I sites of the expression
in bodywall muscle, we cloned an
to express
Unc-53
53(n152 or e2432)

unc-53 ::GFP constructs
To monitor unc-53 expression, several translational unc-53::GFP fusions were constructed. pAB::GFP (pNP3) contains 6.2 kb of genome from the Xhol site in exon 5 to the Sp/I site in exon 13. To construct it, a PCR amplifier, with flanking SpII sites, corresponding to the GFP-coding region and poly A tail of unc-53 from pPD95.75 (a gift from A. Fire) was inserted into the unique SpI site of KX16 (see above). pA::GFP (pNP10) contains 2.8 kb of genome from the Xhol site in exon 5 to the Nhel site in exon 8 fused to the GFP coding region and poly A tail of unc-53 from pPD95.75. pB::GFP (pNP21) contains 3.4 kb of genome sequence from the Nhel site in exon 13 to the Sp/I site in exon 13 fused to the GFP-coding sequence; it was generated by deleting the Apol-Nhel fragment from pNP3. Further details of the constructs are available upon request. Several independent strains were generated in a wild-type or unc-53(n152 or e2432) background with the constructs pAB::GFP (UG13, UG14, UG17, UG18), pA::GFP (UG51, UG52, UG53) and pB::GFP (UG42, UG85).

Rescuing minigenes constructs
Fragments derived from the three constructs used to monitor GFP expression were used to construct three equivalent minigenes to test the rescue of the different phenotypes. pAB::unc-53 (pN8), pA::unc-53 (pNP9) and pB::unc-53 (pNP24) contain the same upstream genomic region as pAB::GFP, pA::GFP and pB::GFP, respectively, but fused to a unc-53 cDNA contained in pTB72 (derived from CE5, see below) at the Sp/I, BstXI or SpII sites, respectively (Fig. 3). Further details of the constructs are available upon request. Several independent strains were generated in an unc-53(n152) background with the constructs pAB::unc-53 (UG41, UG174, UG175, UG176 and UG177), pA::unc-53 (UG62 and UG63) and pB::unc-53 (UG186, UG187, UG188, UG189 and UG190).

Overexpression constructs
To express unc-53 in bodywall muscle, we cloned an Xhol-KpnI fragment derived from CE5 into the Nhel-KpnI sites of the expression vector pPD30.38 (a gift from A. Fire), which contains the myosin heavy chain unc-54 promoter, to obtain punc-54::unc-53 (pTB113). This construction contains the entire unc-53 CE5 cDNA, starting from the first initiation codon and including the unc-53 stop codon and 60 bp of the 3’ UTR, but lacks the 5’ UTR and the SL1 leader. Three independent punc-54::unc-53-containing strains (UG100, UG101 and UG102) were generated in a wild-type background. The presence of the transgene was associated with a marked degree of lethality. To evaluate this, ten healthy adult hermaphrodite transgenic worms were allowed to lay eggs for 48 hours at 20°C. The number of mutant larvae, unhatched embryos and the total number of transgenic progeny was counted. Lethality was never observed in two punc-54::GFP
(pPD93.48) control strains (UG103 and UG104), also generated in a wild-type background.

Expression constructs
To optimise in vitro translational initiation at the first methionine, a mammalian KOZAK consensus sequence was engineered upstream of the unc-53 initiation codon by PCR amplification with BG03 (ataagatgggcccgggcatctgcatcagataattgatta) that contains the KOZAK consensus sequence and a NotI restriction site and BG02 (gaggctataacaggggggctgatgattgata) that contains a BamHI site. The digested amplicon, which contains the first 139 codons of unc-53, was then fused to the complementary fragment of the unc-53 cDNA, derived from CE5, to reconstitute the entire coding sequence. This was then cloned as a 5.1 kb NotI-Apal cassette in the mammalian expression vector pCDNA3 (Invitrogen) to generate plasmid pBT72. The construct pTB50 contains a C-terminal deletion of the last 71 codons (UNC-53Δ71C) cloned into the NotI and Apel sites of pBluescript II-KS.

A convenient NdeI site was generated immediately upstream of the unc-53 initiation codon, by PCR amplification with BG01 (gaaaaccttcatagctgcctgtaattgataattgata) and BG02. The NdeI-BamHI-digested amplicon, which contains the first 139 codons of unc-53, was then cloned into the prokaryotic expression vector pRK172 (McLeod et al., 1987) to generate construct pTB57. pTB61 contains the 5’ PCR amplicon-derived end of the unc-53 cDNA from pTB57 joined at the level of the common SacII site to the truncated 3’ end of the cDNA present in pTB50 (UNC-53AΔ1C). Further details of the constructs are available upon request.

Generation of an anti-UNC-53 monoclonal antibody, mAb 16-48-2
A Ndel-EcoRI fragment of the unc-53 cDNA CE5 (corresponding to amino acids 1043 to 1465) was subcloned in pRK172 to generate pTB66 and expressed in E. coli strain BL21DE3. The corresponding recombinant fusion protein was purified over a DEAE column equilibrated in 8 M urea, emulsified in complete Freund’s adjuvant and injected in Lou rats (Ausubel et al., 1993). All derived antisera were shown to be active at titres of 1:30,000 on western blots of extracts from bacteria expressing recombinant UNC-53. Rat-mouse hybridomas were prepared as described (Ausubel et al., 1993) and a hybridoma cell line producing a monoclonal antibody to UNC-53, designated Mab 16-48-2 was identified using the western blot assay. The specificity of the antibody was confirmed by immunostaining of UNC-53 in wild-type embryos, the absence of reactivity in the n152 deletion mutant and restoration of the signal in transgenic overexpression experiments. Mab 16-48-2 failed to detect antigen of the correct size on western blots of total nematode proteins or proteins fractionated by progressive extraction with detergents, urea and SDS.

Immunostaining and microscopy
To detect UNC-53, embryos were washed, freeze-cracked, fixed and permeabilised as previously described (Goh and Bogaert, 1991). Fixed specimens were incubated overnight in primary antibody solution (1:200 dilution of Mab 16-48-2), washed three times in TBS-Tween (0.1%), and incubated for between 1 and 16 hours in a secondary antibody solution (1:200 dilution of Cy3-conjugated donkey anti-mouse; Jackson Laboratories). The nematodes were washed three times in TBS-Tween and mounted in 2% propyglylate, 80% glycerol (pH 8.0) or in a commercially available mounting medium (Vectorshiel, Vector Laboratories) and observed using a Zeiss axiophot.

Immunoprecipitations and SEM-5/GRB2 binding experiments
UNC-53Δ71C protein, labelled radioactively with 35S, was produced by in vitro translation in rabbit reticulocyte lysates from the construct pTB50. It was immunoprecipitated with the MAB 16-48-2 under
denaturing conditions (0.4% SDS, 2.0% Triton X-100) for 1 hour at room temperature, then incubated with protein G sepharose for 2 hours at room temperature. The beads were washed three times with PBS and the bound products analysed by SDS-PAGE and fluorography. As a control, a reaction without MAb 16-48-2 was treated identically. For a GST pull-down assay, the same protein was incubated with glutathione resin bound to GST (glutathione-S-transferase) protein or to a GST-SEM-5 fusion protein for 1 hour at 20°C. After incubation, the beads were washed four times with phosphate-buffered saline (PBS)/Triton X-100 (0.2%) and the bound proteins analysed by SDS-PAGE and fluorography. For the western overlays, UNC-53Δ71C protein was expressed in E. coli from the pTB61 construct and incubated with either GST or GST-GRB2 proteins that had been biotinylated. The blots were revealed using an alkaline phosphatase-linked anti-streptavidin antibody and chromogenic substrate following standard procedures. The SEM-5 and GRB2 GST fusion constructs (Lowenstein et al., 1992; Stern et al., 1993) were a kind gift from M. Stern.

**RESULTS**

### Isolation of a new unc-53 allele

In a search for C. elegans genes involved in specific aspects of cell adhesion or migration, we screened for mutants with defects in the sex muscles but without defects in the body-wall muscles, myofilament organisation or overall anatomy. The sex muscles bridge the hypodermis and gonads, develop post-embryonically and are not required for viability. From this screen, the allele e2432 was isolated. Upon further characterisation, it was found to be an allele of unc-53. Several unc-53 alleles have been previously identified. The unc-53(e404) allele was isolated by Brenner based on its uncoordinated (Unc) phenotype, being incapable of correct reverse locomotion (Brenner, 1974). Homozygous mutants are healthy if slightly ‘dumpyish’ in size, and males present a defect in mating (Hodgkin, 1983). In a screen for egg-laying defective (Egl) mutants, the unc-53 allele n152 was identified (Trent et al., 1983). A further screen, for genetic enhancers of sex myoblast migration defects in a sensitised sem-5 background, yielded three additional unc-53 alleles, ay10, ay11 and ay62 (Chen et al., 1997). Consistent with the results of the previous screens, homozygous unc-53(e2432) mutants are Unc, Egl and exhibit poor male mating.

### Only two types of sex muscles are defective in unc-53 males

In the male, there are 41 sex-specific muscles that develop post-embryonically and function during mating. In the wild type, the 15 diagonal muscles are distributed along the posterior body parallel to each other, seven on the left and eight on the right, and contribute to the characteristic ventral arching of the male tail (Sulston et al., 1980) (Fig. 1A). In unc-53 mutants, the diagonal muscles are frequently not parallel to one another, or have a dorsal attachment point that is more ventrally positioned than in wild type (Fig. 1B). All unc-53(n152) homozygous males display at least one defective muscle.

During copulation, two spicules are inserted through the hermaphrodite vulva. In the wild type, each spicule is attached at its proximal end to two associated protractor and retractor muscles. The retractor muscles are attached anteriorly to the body wall (Sulston et al., 1980). In unc-53 mutants, the
retractor muscles are shorter than in wild-type worms. The attachment point to the body wall is shifted posteriorly and frequently more ventrally to the edge between the seam and body wall muscles (Fig. 1B), while their attachment to the spicules is normal. In addition, in unc-53 mutants, the protractor muscles sometimes extend processes onto the attachment point of the retractor muscles on the hypodermis, suggesting that the defect is not in these attachment points, but rather in the extension of the muscles towards their normal point of attachment. Moreover, in unc-53 males, the fan and pattern of the rays are normal, suggesting that the sex muscle defect is not likely to be due to generalised defects in the hypodermis. However, the observed defects in muscles required for copulation explain the observed mating phenotype of unc-53 males.

Longitudinal migration of the developing sex myoblasts is affected in the unc-53 hermaphrodite

In the hermaphrodite, the vulval muscles are a set of four pairs of cells arranged symmetrically in a cross-pattern around the vulval slit (Fig. 1E). Their simultaneous contraction enables the vulva to open and the eggs to be laid. Each pair consists of one vm1 and one vm2 muscle cell (Sulston and Horvitz, 1977). The vm muscles attach proximally to the vulva and distally to the hypodermis (White et al., 1986), at the dorsal margin of the ventral body wall muscle cells for vm1, or in between the two ventral body wall muscle cells for vm2 (Fig. 1C,E). In all unc-53(n152) mutants, the vm1 and vm2 muscles are shorter than in wild type. Their proximal attachment is normal, but their distal attachment is abnormal, being in an apparently random position closer to the vulva (Fig. 1D).

To understand the origin of this phenotype, sex muscle development was examined throughout the L4 stage using a GFP reporter gene pAB::GFP (see below), which is expressed in the sex myoblasts and their descendants, and permits the visualisation of cell shape and growth cone spikes not visible by DIC microscopy. In wild-type hermaphrodites, after their migration to the position of the future vulva, the two sex myoblasts divide three times, giving rise to two groups of cells on each side of the vulva: the future vm1 and vm2, and the future uterine muscles um1 and um2 (Sulston and Horvitz, 1977). During division, these groups of myoblasts migrate along the longitudinal axis away from the vulva, leading to four clearly separate groups of cells (Fig. 1G). This migration fails to occur in unc-53(n152) mutants, resulting in a cluster of myoblasts that flanks the vulva too closely on either side of the animal (Fig. 1H). The vulval myoblasts then send cellular extensions ventrally to attach to the vulva, while remaining attached at the other end to the hypodermis. This ventral extension is wild type in unc-53 mutants. Concomitantly, the vulval myoblasts extend thin distal growth cone extensions longitudinally along the seam. These feel-like structures may serve to further anchor the muscles to the hypodermis (Fig. 1E and see Fig. 4J). In unc-53 mutants, these feel-like structures are not made, and the muscles only attach over the width of the myofilaments, yielding rounded muscle attachments (Fig. 1F). Occasionally, the vm1 muscles do not remain connected to the seam and attach more ventrally in between the body wall muscles, at the site left vacant by the vm2 muscles. These observations thus reveal a specific problem in AP migration and extension of the sex muscles.

Fig. 2. Longitudinal migration defects in unc-53 mutants. (A) The wild-type excretory canal. Two posterior canals extend from the excretory cell on each side along the lateral cord from the head to the tail. The boxes show the regions represented in B,C. These are DIC photomicrographs of adult worms showing the trajectory of the excretory canal (arrowheads). (B) In the wild type, the canal stops at the level of the anus (arrow). (C) An unc-53(n152) mutant, with a canal that stops at the level of the vulva (arrow). (D) The wild-type ALN and PLN projections. ALN and PLN send anteriorly directed axons up to the head along the sublateral dorsal and ventral cords, respectively. The box shows the region represented in E-H. These are fluorescence photomicrographs of adult wild-type (E,G) and unc-53(n152) (F,H) worms expressing a pA::GFP reporter construct, showing the trajectory of an ALN (E,F) or PLN (G,H) axon (arrows). (F) The ALN axon has stopped its anterior outgrowth before the mid-body and sends several dorsal branches directly to the dorsal cord (arrowhead). (H) The PLN axon has stopped its anterior outgrowth before the mid-body and sends several dorsal branches to the ventral cord (arrowhead). c, cell body; pag, pre-anal ganglion. Scale bars: 10 μm.

This cellular defect explains the observed egg-laying defective phenotype of unc-53 mutants. All adult homozygous n152 and e2432 hermaphrodites become bloated with late stage embryos that hatch internally and eventually kill the mother, the so-called ‘bag of worms’ phenotype. The Egl phenotype was equally strong for unc-53(n152) mutants as for heterozygous n152/mnDf87 worms (see Fig. 5A), suggesting that n152 is a strong loss-of-function or null allele for this phenotype.

Further abnormal AP migrations in unc-53 mutants

The excretory cell in C. elegans is a large H-shaped cell whose
ectopic branches joined the ventral cord (Fig. 2H). For another phenotype was observed for the PLN axon, but in this case, the branches that joined the dorsal cord (Fig. 2F). A similar neurones with the appearance of dorsally directed ectopic Such premature arrest was associated in the case of the ALN and PLN axons frequently stopped prematurely (Fig. 2F,H), with 75% and 48% of axons, respectively, failing to reach the mid-body region in 2F,H), with 75% and 48% of axons, respectively, failing to reach the mid-body region in unc-53(n152) mutants (Fig. 5C). In worms homozygous for the alleles unc-53(n152) (Nelson et al., 1983). Although the dorsal migration was normal in unc-53(n152), in 83% of mutants, the posterior canals of the excretory cell terminated their AP migration prematurely at the level of the vulva (n=100; Fig. 2A and see Fig. 5C). In n152/mdf87 heterozygotes, 96% of the canals stopped at the same level (n=52), suggesting that n152 is a strong loss-of-function allele for this phenotype as well (Fig. 5C). In worms homozygous for the alleles e2432 and e404, the majority of canals progressed further past the vulva but terminated before the tail (T. B. and I. Maillet, unpublished) (Hedgecock et al., 1987). Frequently, the canal processes in unc-53 mutants meander and even reverse their path before stopping prematurely. These data suggest that unc-53 is required for the excretory canal to grow in the correct direction, particularly during its trajectory from the level of the presumptive vulva to the tail.

ALN and PLN are two pairs of neurones located in the tail ganglion. Normally, they send axons anteriorly as far as the head along the sublateral cord (Fig. 2D,E,G). In the mutants, the ALN and PLN axons frequently stopped prematurely (Fig. 2F,H), with 75% and 48% of axons, respectively, failing to reach the mid-body region in unc-53(n152) mutants (Fig. 5B). Such premature arrest was associated in the case of the ALN neurones with the appearance of dorsally directed ectopic branches that joined the dorsal cord (Fig. 2F). A similar phenotype was observed for the PLN axon, but in this case, the ectopic branches joined the ventral cord (Fig. 2H). For another anteriorly directed neurone, the PLM, ventral ectopic branching of prematurely arrested axons has previously been reported in unc-53 mutants (Hekimi and Kershaw, 1993).

unc-53 might play a minor role in DV migration, as the dorsal outgrowth of the DA and AS motoneurone axons was sometimes abnormal in unc-53(n152) mutants (see Table 1). However, ventral migrations of the sex myoblasts and DV outgrowth of excretory canals (see above), mechanosensory neurones (Siddiqui, 1990; Hekimi and Kershaw, 1993), HSN (Wightman et al., 1997) and PDE (N. P., unpublished) are not affected in unc-53 mutants. Taken together, these results suggest that unc-53 is required for the navigation of cells and cellular processes along the AP axis.

unc-53 encodes a conserved protein of 1583 amino acids

Genetic mapping had previously placed unc-53 between bli-1 and rol-1, 3 cM to the right of the centre of LG II (Brenner, 1974). The position of the gene was further refined by mapping deletion end points and RFLPs (see Materials and Methods). Transformation rescue of unc-53(n152) by microinjection of cosmids from the candidate region of the physical map showed that the gene is contained within T28D2 (Fig. 3A and see Fig. 5A,C). The overlapping cosmids C09H10 partially rescued the Unc and Egl phenotypes, as did the phage S4. The cosmids T28D2 contains a single predicted gene that spans over 16 kb. The generation and sequencing of the phage S4. The overlapping cosmids C09H10 partially rescued the Unc and Egl phenotypes, as did the phage S4. The cosmids T28D2 contains a single predicted gene that spans over 16 kb. The generation and sequencing of

Table 1. Cells that express unc-53 reporter constructs in adults are associated with a phenotype in unc-53 mutants

<table>
<thead>
<tr>
<th>Neurones</th>
<th>Muscles and other cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells</td>
<td>Express unc-53::GFP Require unc-53</td>
</tr>
<tr>
<td>Two ALN</td>
<td>pA +</td>
</tr>
<tr>
<td>Two PLN</td>
<td>pA +</td>
</tr>
<tr>
<td>Two PVP</td>
<td>pA nd</td>
</tr>
<tr>
<td>Two PVQ</td>
<td>pA nd</td>
</tr>
<tr>
<td>Two BDU</td>
<td>pA nd</td>
</tr>
<tr>
<td>Two PVM</td>
<td>pA nd</td>
</tr>
<tr>
<td>Nine DA</td>
<td>pB +*</td>
</tr>
<tr>
<td>11 AS</td>
<td>pB +*</td>
</tr>
<tr>
<td>ALA</td>
<td>pB +</td>
</tr>
<tr>
<td>RID</td>
<td>pB nd</td>
</tr>
<tr>
<td>Two PDE</td>
<td>pB +†</td>
</tr>
<tr>
<td>Two HSN</td>
<td>pB +†</td>
</tr>
<tr>
<td>Two ALM</td>
<td>nd +†</td>
</tr>
<tr>
<td>Two PLM</td>
<td>nd +†</td>
</tr>
<tr>
<td>Two PVM</td>
<td>nd +</td>
</tr>
<tr>
<td>Two PVQ</td>
<td>nd +</td>
</tr>
</tbody>
</table>

Two promoters, pA and pB, give rise to an expression in different cell types. The promoter pAB which is a fusion of both pA and pB presents an additive pattern of the expression seen with pA and pB.

This list is incomplete as there are several unidentified cells in the head that express pAB::GFP.

*DA and AS motoneurones are involved in backwards movement, which is impaired in unc-53. In 46% of unc-53(n152) mutants (n=65), between one and three of the visible motoneurone commissures failed to join the dorsal cord. Overall, 13% of the visible commissures (n=372) were abnormal, and exhibited highly variable phenotypes: some axons stopped prematurely, others turned anteriorly along the lateral cords.

† Hedgecock et al., 1987.
‡ Wightman et al., 1997.

A variable gonad morphology defect was observed rarely in unc-53(e2432) homozygotes (T. B., unpublished).
Fig. 3. Molecular organisation of the \textit{unc-53} gene. (A) The exon-intron structure of the \textit{unc-53} gene. The different SL1 transspliced sites are indicated, as well as the regions deleted in alleles \textit{n152} and \textit{e2432}. Alternatively spliced exons are in pink. The extent of the phage and several cosmids clones are shown. The structure of the different GFP and minigenes constructs is also presented. (B) Structure of UNC-53. UNC-53 contains a calponin homology domain (CH, light green; amino acids 11-109), two proline-rich SH3-binding motifs, (SH3 b, dark green; 487-495 and 537-545), two coiled-coil regions (CC, red; 890-923 and 1078-1113; predicted by COILS at http://www.ch.embnet.org) and an ATPase associated with diverse cellular activities domain (AAA, yellow; 1292-1425) that contains a NTP-binding motif (1300-1307). The positions of the two potential LKK motifs (green, 114-133; 1097-1116) are indicated. Arrowheads mark the positions corresponding to the start of the \textit{e2432} and \textit{n152} deletions. (C) Alignment of the CH domain (PF00307), SH3-, LKK and NTP-binding motifs (PS00017). The Genbank Accession numbers for all the sequences used are: CeUNC-53, AF504312; HsNAV2, AX009326; Hst-actin, P12814; GgDystrophin, P11533; MnSOS, Q62245; DmSOS, P26675; MmDynamin, P39053; RnPI3K-p85, Q63787; HsVillin, XP_010866; HsDematin, Q08495; CcThymosin, T32473.

representing both alternative SL1 trans-splicing events and internal splicing of alternative exons (Fig. 3A). Southern analysis revealed the presence of polymorphisms in the T28D2 region of two mutant alleles \textit{n152} and \textit{e2432}, and subsequent sequencing revealed the corresponding molecular alterations. In allele \textit{e2432} a deletion of 374 bp removes part of exon 12, and all of exon 13. Allele \textit{n152} carries a 319 bp deletion, which would be predicted to remove part of alternatively spliced exon 18, and exon 19, thereby introducing a premature stop codon in exon 20.

The largest \textit{unc-53} transcript encodes a putative protein product of 1583 amino acids for which three human
homologues have recently been identified (Maes et al., 2002). In terms of sequence, UNC-53 is overall most closely related to human NAV2/RAINB1 (Maes et al., 2002; Merrill et al., 2002). The highly conserved N- and C-terminal regions of UNC-53 (residues 11-143 and 164-1533), which together represent 95% of the protein, are (respectively) 35% and 24% identical (56% and 41% similar) to the corresponding regions of NAV2/RAINB1 (residues 90-213 and 941-2340). The human protein contains an additional ~720-residue sequence between these regions, not present in UNC-53. The two proteins share several identifiable domains and motifs (Fig. 3B,C). There is a calponin homology (CH) domain in the N terminus, at amino acids 11 to 109. CH domains are present in various actin-binding proteins, including α-actinin and dystrophin, proteins known to crosslink actin filaments into bundles and networks. They share an additional putative actin-binding site of the LKK consensus (Van Troys et al., 1999). Both UNC-53 and NAV2/RAINB1 have a second LKK consensus actin-binding site, although these are not in equivalent positions in the two proteins. The same is true for two proline rich sequences, potential SH3-binding domains (Yu et al., 1994), that in UNC-53 are found at residues 487-495 and 537-545. UNC-53 also shares with its three human homologues two regions (890-923 and 1078-1113) predicted to adopt a coiled-coil configuration that could mediate homomeric or heteromeric protein-protein interactions (Maes et al., 2002), together with a nucleotide (NTP)-binding site contained within an ATPases-associated with diverse cellular activities (AAA) domain. Despite extensive sequence conservation, the members of the AAA family are implicated in diverse cellular functions, including cell cycle regulation and vesicle-mediated transport (Confalonieri and Duguet, 1995).

**unc-53 expression pattern**

The expression pattern of *unc-53* was characterised using several GFP reporter fusions (Fig. 3A). A region 2.3 kb upstream of the most 5′ SL1 splice site produced weak GFP expression in half a dozen unidentified neurones in the head. As this expression appeared not to correlate with the different *unc-53* phenotypes, other regions of the gene were assayed for their ability to drive GFP expression. We focused on the proximal part of phage S4 (Fig. 3A), as this clone was able to rescue partially the Unc and Egl phenotypes of *unc-53(n152)*, indicating that it contains regulatory elements necessary for the correct expression of UNC-53. When the first 6.2 kb of the S4 clone was used, in the pAB::GFP fusion, expression was seen in some pioneering neurones of the nerve ring, beginning at the early comma stage (Fig. 4A,B). At the two-fold stage, expression was detected in

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**Fig. 4.** *unc-53::GFP expression pattern.** DIC (A) and fluorescence images of pAB::GFP expression in transgenic animals (B-H). (A-F) Embryos of successive stages. (A,B) 1.5-fold stage embryo showing a pioneering neurone growing in the head (B, arrow). (C,D) A view between the end of the pharynx and the mid-body of a three-fold stage embryo, showing expression in DA motoneurones pioneering the dorsal cord; C and D are two different focal planes. (E,F) Late three-fold stage embryo: (E) shows the ventral side, (F) the dorsal side, revealing expression in all DA motoneurones and some neurones in head and tail ganglia. (G-L) Adult stage; anterior is towards the left. (G,H) Adult tail, ventral (G) and mid-plane (H) views showing expression in a phasmid socket (PHso), sphincter muscle (sph) and neurones in the preanal ganglion (pag). (I) Adult head showing expression in the amphid socket (AMso), ALA and several neurones in the dorsal, ventral and retrovesicular ganglion (rvg), as well as pharyngeal neurones, including M5. nr, nerve ring. (J) Ventral view of the mid-body showing vulval sex muscles (vm1 and vm2) of an adult hermaphrodite. One of the four foot-like attachments is highlighted. Ventral (K) and dorsal (L) views of the male tail, showing specific expression in diagonal (diag) and spicule retractor (sp) muscles. Scale bars: 10 μm.
Although the mechanosensory neurones are affected in the presenting a phenotype in
Additional cells expressing the pAB::GFP construct and n152; unc-53 and n152; pA::unc-53, respectively. For each of these, the progeny of 12, 23, 23 and 19 individuals from two, five, two and five independent strains, respectively, were counted. Each independent strain of a given genotype gave similar results. Both unc-53 alleles show a strong Egl phenotype, indistinguishable from that of n152/mmDf87 heterozygotes. The unc-53(n152) phenotype was largely rescued by the cosmid T28D2, by pAB::unc-53 and by pB::unc-53, but not by pA::unc-53. (B) Quantification of ALN and PLN axonal outgrowth defects. The worm was divided into three regions (1-3) with reference to the vulva and the anus. The stop point of ALN axons was determined using a pA::GFP reporter carried by wild-type (n=100), n152 homoygous (n=50) and two independent strains of n152::pA::unc-53 (n=100) worms. The percentage of axons stopping at each of the three positions was then calculated. The PLN defect was scored similarly for 70, 44 and 80 worms, respectively. The two n152::pA::unc-53 strains gave comparable results. The unc-53(n152) phenotype was largely rescued by pA::unc-53.

(C) Quantification of the excretory canal outgrowth defect. The worm was divided into four regions (1-4) between the vulva and the anus. The stop point of canals was determined by DIC microscopy for wild type (n=100), n152 (n=100), n152/mmDf87 (n=52), n152;T28D2 (n=48), n152::pAB::unc-53 (n=50), n152;::pA::unc-53 (n=62) and n152::pB::unc-53 (n=57). For the transgenic strains, two independent lines for each were examined. The percentage of canals stopping at each of the four positions was then calculated. unc-53(n152) was associated with a stereotyped arrest of the canal at the level of the vulva, that was almost as penetrant as n152/mmDf87 heterozygotes. The phenotype was largely rescued by the cosmid T28D2. The pAB::unc-53 and pA::unc-53 constructs gave a degree of rescue, in contrast to pB::unc-53.

some 10 neurones in the head that extend axons into the nerve ring, and in two neurones in the tail that extend processes anteriorly. This expression pattern was confirmed by immunohistochemistry with MAb Ab16-48-2 (results not shown). At the three-fold stage, expression was seen in all DA motoneurones and persisted while they pioneered the dorsal nerve cord (Fig. 4C-F). It was also seen in four to six neurones in each of the four head ganglia, including ALA and RID in the dorsal ganglion, and four of the six neurones of the terminal bulb, including M5. In the tail, two neurones in the pre-anal ganglion and six in the lumbar ganglion, including PVQL and PVQR, showed pAB::GFP expression. Additionally, a transient expression was seen in the four rows of bodywall muscle cells in the embryo. After hatching, in L1 larvae, the expression domain extended to amphid and phasmid socket cells (Fig. 4G-I), and subsequently in L2 larvae to all the newly born AS motoneurones. In hermaphrodite L3 larvae, expression was seen in the sex myoblasts subsequent to their anterior migration towards the position of the presumptive vulva, and in adult worms at a high level in the vulval muscles vm1 and vm2 (Fig. 4J). In males, expression was seen in the diagonal (Fig. 4K) and spicule retractor muscles (Fig. 4L). Significantly, these are the only two muscle types in the male tail to be affected in the unc-53 mutant. Additional cells expressing the pAB::GFP construct and presenting a phenotype in unc-53 mutants are shown in Table 1. Although the mechanosensory neurones are affected in the unc-53 mutant, they did not show expression of the pAB::GFP construct. It is therefore possible that there is an additional uncharacterised promoter upstream of the most 5' SL1 trans-splice site. Putative expression of unc-53 in the mechanosensory neuorones could not be confirmed with the MAb 16-48-2 antibody, as we were unable to obtain staining in larvae or adults.

The existence of two transcripts with different SL1 spliced 5' ends in the region covered by pAB::GFP suggested a bipartite nature for the unc-53 promoter (Fig. 3A). Consequently, two non-overlapping reporter constructs, pA::GFP and pB::GFP were produced. These were associated with non-overlapping expression domains, that together recapitulated the expression pattern of pAB::GFP (Table 1).

**unc-53 act cell autonomously**

As unc-53 is expressed in cells that present a mutant phenotype in unc-53 animals, the gene might be supposed to act in a cell-autonomous fashion. To test this hypothesis, we constructed mini genes that contained the cell-specific promoters pA or pB, or the combined pAB fused to an unc-53 cDNA. Although the combined pAB promoter construct rescued the Egl and the excretory canal extension phenotype of n152 at levels comparable with the rescue obtained with cosmid T28D2, when the promoters were tested individually, differential rescue of phenotypes was observed (Fig. 5). The pB::unc-53 fusion, that is expressed in the sex muscles, showed the same level of rescue

of the Egl phenotype as pAB::unc-53. At a cellular level, this rescue was associated with a restoration of wild-type morphology and attachment of the sex muscles (results not shown). By sharp contrast, the pA::unc-53 fusion, which is not expressed in the sex muscles, failed to rescue the Egl phenotype (Fig. 5A). However, the pA promoter does drive expression in the ALN and PLN neurones, and the pA::unc-53 fusion gave a high degree of rescue of their extension phenotypes in n152 (Fig. 5B). Furthermore, while pB was not associated with expression in the excretory cell, pA was occasionally, and only pA::unc-53 produced some rescue of excretory canal extension (Fig. 5C).

Overexpression of unc-53 in bodywall muscles leads to overgrowth

The body wall muscles of C. elegans are arranged longitudinally in four quadrants along the anteroposterior axis. In wild-type animals, body muscle cells are normally spindle shaped, while in unc-53(e2432) animals, these cells sometimes have reduced processes and fork-shaped tips (T. B., unpublished). Transient expression of unc-53 in embryonic body wall muscles was observed with the pAB::GFP reporter. No significant signal was detected using the anti-UNC-53 monoclonal antibody, however, suggesting that the endogenous level of UNC-53 is very low in these cells. To investigate further the role of unc-53, we tested the effect of its overexpression in the body wall muscles by expressing the unc-53 cDNA under the control of the unc-54 promoter (punc-54::unc-53). The unc-54 gene, which encodes the myosin heavy chain, is transcribed in body muscle from the comma stage onwards (Okkema et al., 1993). In comparison with wild-type growth cones, revealed by a punc-54::GFP reporter (Fig. 6A,C), the processes of punc-54::unc-53 embryos were overextended along the anteroposterior axis of the animal (Fig. 6B,D). The growth cones of punc-54::unc-53-expressing cells were on average 54% longer than the length of the growth cones of wild-type muscle cells in late embryos (Table 2). In addition, a variably penetrant lethality was observed, associated with severe morphological defects and detachment of muscle cells from the hypodermis (Fig. 6F; Table 2).

Interaction of UNC-53 with SEM-5/GRB2

The observation that certain alleles of unc-53 enhance the sex myoblast migration defect seen in sem-5 mutants suggests that UNC-53 and SEM-5 co-operate to regulate sex myoblast migration (Chen et al., 1997). SEM-5 contains SH2 and SH3 domains, while UNC-53 contains putative SH3-binding domains, migration (Chen et al., 1997). SEM-5 contains SH2 and SH3 binding domains. The interaction of UNC-53 with SEM-5/GRB2

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It has been shown that human GRB2 can rescue the sex myoblast migration defects of sem-5 mutants and as such can be considered a true orthologue (Stern et al., 1993). We therefore tested whether UNC-53A71C and GRB2 interact directly. UNC-53A71C produced in bacteria was used in western blot overlays, revealed with biotinylated GST or GST-GRB2 (Fig. 7C). A specific interaction with GRB2 was observed. Interestingly, no signal was detectable with an UNC-53 fusion protein containing the last 541 amino acids, thus lacking the polyproline repeats (data not shown). This provides supportive evidence that these could directly bind to the SH3 domains of a SEM-5/GRB2 adapter protein.

**DISCUSSION**

**UNC-53 affects many cell and axon migrations in C. elegans**

The gene unc-53 is required for cell migrations and the
outgrowth of cellular processes, predominantly along the AP axis. unc-53 is capable of acting in a fasciculation-independent manner in pioneering neurones, including PLM (Hekimi and Kershaw, 1993) and in ALN during its extension from the tail to mid-body region. In the absence of unc-53, not only does ALN arrest its growth prematurely, but it also sends projections to the dorsal cord, in stark contrast to wild type ALN axons, which never stray from their AP path. In some cases, mutant axons arriving at the dorsal cord, then turn back and extend towards the tail. Furthermore, unc-53 appears to be required only in last half of the trajectory of the excretory canal and ALA send posteriorly directed axons. The expression of unc-53 is involved in the response to as yet unidentified directional cues that originate from the mid-body. This provides evidence in support of a cell-autonomous interaction of gonad-dependent and -independent signals. Significantly, unc-53 is required for the gonad-independent attraction of the sex myoblasts to the mid-body (Chen et al., 1997). We have demonstrated a further role for unc-53 in the final longitudinal migrations of the sex myoblasts away from the vulva that accompany their division. This clearly suggests that unc-53 is involved in the response to as yet unidentified directional cues that originate from the mid-body.

**UNC-53 homology domains could link SEM-5 and the actin cytoskeleton**

Molecular analyses revealed multiple unc-53 transcripts. The longest encodes a predicted protein of 1583 amino acids containing a CH domain, two LKK motifs, two SH3-binding domain, two coiled-coil regions and a nucleotide-binding domain. Sequence analysis of two unc-53 alleles, n152 and e2432 showed that they probably correspond to severely truncated proteins, lacking the conserved nucleotide binding domain, the C-terminal LKK motif and the two coiled-coil regions. These modules are therefore likely to be functionally important. As the regions associated with a putative function make up only about 25% of the protein, molecular analysis of additional mutations may pinpoint further domains involved in specific molecular interactions.

Genetic evidence has suggested that sem-5 and unc-53 cooperate to control sex myoblast migration (Chen et al., 1997). SEM-5 is the C. elegans GRB2 homologue and consists exclusively of SH2 and SH3 domains (Clark et al., 1992). The biochemical experiments of the present study suggest that the
interaction between unc-53 and sem-5 is direct. UNC-53 protein physically associates with SEM-5 and its mammalian homologue GRB2 in vitro, presumably via its SH3-binding domain.

Although functional actin-binding modules cannot always be accurately predicted, UNC-53 does contain conserved a CH domain and LKK motifs (Van Troys et al., 1999). The first is found in a variety of cytoskeletal and signal transduction molecules implicated in the regulation of cell shape dynamics (Stradal et al., 1998). The actin-cross-linking proteins a-actinin, b-spectrin and dystrophin each contain two relatively dissimilar CH domains (Carugo et al., 1997), while UNC-53, in common with Vav and calponin, contains only one. Preliminary experiments suggest that UNC-53 is able to co-sediment actin in vitro (E. S., unpublished). It is therefore possible that UNC-53 could link SEM-5 to the actin cytoskeleton.

Models for UNC-53 activity

Studies in invertebrates and vertebrates have suggested that the leading edge of a migrating cell or the growth cone at the tip of an axon are steered in a similar manner by attractive and repulsive extracellular cues, which may be emitted along multiple axes. In the growth cone, cell-surface receptors receive specific and competing signals and transduce them to components within the cell. This leads to changes in the cytoskeletal architecture resulting in the localised accumulation of polymerised actin in the growth cone. Subsequent translocation of microtubules to this point drives the growth cone forward stepwise in that chosen direction (Bentley and O’Connor, 1994).

In this context, two models for the action of UNC-53 can be proposed. Within the cell, activation of UNC-53 by SEM-5 could lead to the recruitment of UNC-53 to the actin cytoskeleton and then regulate, perhaps via nucleotide binding, the crosslinking of actin molecules to stabilise a growth cone spike and promote extension in a specific direction. Alternatively, UNC-53 may act as a signal relay, associated with the cytoskeleton, but not directly responsible for the modifications of the actin cytoskeleton associated with growth cone extension. In both models, the unc-53 pathway integrates signals received at the cell surface and determines the direction and rate of growth cone extension. Preliminary experiments have suggested that UNC-53 does indeed localise to the cytoskeleton. Further characterisation of the interactions between UNC-53 and its molecular partners may shed more light on the mechanisms of cell migration and growth cone extension.

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unc-53 and growth cone migration


