A Doctoral Thesis

SRC-1, a non-receptor type of protein tyrosine kinase, controls the direction of cell and growth cone migration in *C. elegans*

線虫 SRC-1 は細胞移動と軸索伸長の方向を制御する

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Summary

Src family tyrosine kinase (SFK) has been implicated in the regulation of cell adhesion and migration during animal development. Here I show that SRC-1, an orthologue of SFK, plays an essential role in directing cell migration in *Caenorhabditis elegans.* The mutation in the *src-1* gene results in defective distal tip cell (DTC)-directed gonad morphogenesis in an activity-dependent and DTC cell-autonomous manners. In the *src-1* mutants, DTCs fail to turn and continue their centrifugal migration along the ventral muscles. The effect of the *src-1* mutation is suppressed by mutations in genes that function in the CED/Rac pathway, suggesting that SRC-1 in DTCs is an upstream regulator of a Rac pathway that controls cytoskeletal remodeling. In the *src-1* mutant, the expression of *unc-5*/netrin receptor is normally regulated, and either the precocious expression of UNC-5 or the mutation in the *unc-5* gene does not significantly affect the DTC migration defect. These data suggest that SRC-1 acts in the netrin signaling in DTCs. The *src-1* mutant also exhibits cell-autonomous defects in the migration and growth cone path finding of Q neuroblast descendants AVM and PVM. However, these roles of SRC-1 do not appear to involve the CED/Rac pathway. These findings show that SRC-1 functions in responding to various extracellular guidance cues that direct the cell migration via disparate signaling pathways in different cell types.

General introduction

The tyrosine phosphorylation events have been implicated in the regulation of a variety of cellular functions including cell proliferation, migration, differentiation, survival and neuritegenesis. On the other hand mutation and changing of the expression levels of PTKs or its substrates are often observed in the tumor cells and some congenital anomalies in vertebrates. Therefore protein tyrosine kinases play pivotal roles for developing and maintaining the order of the cell society.

PTKs are classified into two groups, that is to say, receptor type PTKs and non-receptor type PTKs. Receptor type PTKs are further divided to subfamilies by their structural characteristics, they commonly function as follows; ligand binding to their extracellular domain triggers activation of intracellular kinase domain and causes phosphorylation of downstream substrates. On the other hand, non-receptor type PTKs are cytoplasmic or membrane associated proteins. Various ways of activation mechanism are known for this class of PTKs, but it has not been fully dissolved. Typically, they are activated by interacting with the upstream proteins like cell surface receptors or by the change of phosphorylation states by other PTKs and protein phosphatases. To date, ninety unique PTK genes were identified in a human genome as a result of the genome project. Those genes were classified into twenty subfamilies of receptor PTKs and ten subfamilies of non-receptor PTKs (Robinson et al., 2000). Of these, *src* was the first identified gene that encodes a protein tyrosine kinase, and at the same time it was the discovery of tyrosine phosphorylation event in a cell.

The *src* gene product, Src was originally identified as the transforming protein of a chicken tumor retrovirus, Rous sarcoma virus (RSV), discovered in 1910 by Peyton Rous (Brugge and Erikson, 1977; Purchio et al., 1978; Rous, 1979). Although RSV has only four genes, it has an ability to cause tumors in

chicken and transform cells *in vitro*. Of these genes, three of them are needed for viral life cycle and only one gene named *v-src* is required for triggering cell transformation (Martin et al., 1971; Vogt, 1971; Lai et al., 1973). A major breakthrough for understanding the *v-src* gene product (v-Src) came from the findings that v-Src possesses an intrinsic PTK activity (Collett and Erikson, 1978; Levinson et al., 1978; Collett et al., 1980; Hunter and Sefton, 1980) and Src PTK activity was found important for cellular transformation (Snyder et al., 1983). Moreover, a homologous sequence of the *src* gene was discovered in normal avian genomic DNA (Stehelin et al., 1976). The cellular form of Src, c-Src, in normal cells also possesses the PTK activity. However, the PTK activity of c-Src was lower than that of v-Src and c-Src did not cause cell transformation (Iba et al., 1984; Tanaka and Fujita, 1986). The most striking difference between c-Src and v-Src was found at the carboxy-terminal amino acids sequence containing a negative regulatory region of c-Src activity (Takeya and Hanafusa, 1983; Brown and Cooper, 1996). Further evidence showed that *v-src* gene was originated from *c-src* gene by DNA recombination during the retroviral life cycle.

In vertebrates eight proteins were found to share common structural features and significant sequence homology to Src: Fyn, c-Yes, Lyn, Lck, Hck, Blk, c-Fgr and c-Src itself (Brown and Cooper, 1996). The group of these non-recepter PTKs was named as Src family tyrosine kinases (SFKs). SFKs are 52-62 kDa proteins and have six distinctive functional regions in their structure (Fig. 1). In the N-terminus they have a 15-amino acid sequence (SH4 domain) that is myristoylated and/or palmitoylated serving as a membrane-targeting signal. Following the SH4 domain there is a unique domain where the sequence homology is not obviously seen among SFK members. This domain has been proposed to be important for recognition of specific interactant for each family member. The four regions follow the unique domain. They are SH3 domain, SH2 domain, catalytic (SH1) domain and the negative regulatory C-terminal tail. The

SH3 and SH2 domain binds to proline-rich motif (P-X-X-P motif) and phosphotyrosine containing motif (pY-X-X-y motif), respectively. Both of these serve as the protein binding domains and exist in the diverse signaling proteins (Cohen et al., 1995). In addition, the SH3 and SH2 domains play a central role in the negative regulation of SFKs' own activity, as described later. The catalytic (SH1) domain possesses the PTK activity. Biochemical and X-ray crystallographic analyses have revealed that the conserved tyrosine residue within the activation loop of the catalytic domain is important for positive regulation of SFKs' activity. This tyrosine residue is autophosphorylated when SFKs are activated and its autophosphorylation leads them to fully active state.

SFKs were studied in mammal prior to other animals. SFKs were conserved in all multicellular organisms. *C. elegans* is known for the most simplest multicellular model organisms. All cell linages was revealed, genome project was finished, experimental methods, such as RNAi, were established. *C. elegans* have 19.000 genes. Ortholog of SFK are only two genes, *src-1* and *src-2*. Although the function of SFKs is critical in many signal transduction, Src KO mice was viable. Knockout of multi SFKs brings lethality. These things suggest that there are redundancy and complementation of SFKs' function in mammal. However, loss of function of one SFK*, src-1*, resulted in embryonic lethal in *C. elegans*. Using nematode is solution for avoiding the problem of redundancy.

In this study, I investigated the postembryonic roles of *src-1* which is the major SFK in *C.elegans*. SRC-1 is necessary for the migration of cells, and regulation of polarity.

Introduction

Cell migration is crucial for the development of multicellular animals (Hedgecock et al., 1987; Antebi et al., 1997). Concerted cell movements are required to establish the tissue layers during gastrulation, individual cell migration contributes to tissue organization and the establishment of the body pattern, and growth cone migrations help construct the neural network. Such cell and growth cone migrations are guided by extracellular cues that provide polarity information to the cell in the form of substratum-bound or diffusible molecules (Tessier-Lavigne and Goodman, 1996; Varela-Echavarria and Guthrie, 1997). However, it remains unclear how these extracellular cues are transduced by the cell into the cytoskeletal and molecular motor activities that result in cell migration.

The gonad of a *Caenorhabditis elegans* hermaphrodite is located in the anterior-right and posterior-left areas of the body cavity (Fig. 1A). This bilobed gonad develops during larval development from a 4-cell primordium positioned in the ventral midbody. The shape of the two gonad arms is determined by the migratory path of a distal tip cell (DTC) at the leading edge of each arm (Kimble and Hirsh, 1979; Hedgecock et al., 1987). DTC migration proceeds through three sequential linear phases (Fig. 1B). Phase I is the centrifugal migration along the ventral bands of the body wall muscles away from the midbody. Phase II begins with a right-angled turn of DTC, after which it migrates along the inner surface of the epidermis from the ventral to the dorsal muscle bands. Finally, in phase III, DTC makes another right-angled turn and then migrates centripetally along the dorsal muscle bands back towards the midbody (Fig. 1B) (Kimble and Hirsh, 1979; Hedgecock et al., 1987).

DTC migration is regulated by several extracellular guidance cues (Hedgecock et al., 1987; Leung-Hagesteijn et al., 1992; Blelloch et. al., 1999; Montell, 1999; Lehmann, 2001). The ventral-to-dorsal migration of DTC during

phase II is mediated in part by the netrin family protein UNC-6 and its receptors UNC-5 and UNC-40 (Hedgecock et al., 1990; Culotti and Merz, 1998). UNC-5 and UNC-40 are expressed by DTC, whereas UNC-6 is expressed by the ventral body wall muscles. These observations suggest that UNC-5 and UNC-40 mediate the chemorepulsion of DTC away from the ventrally expressed UNC-6 (Wadsworth et al., 1996; Su et al., 2000). It has also been suggested that UNC-129/TGF-b is involved in DTC migration during phase II (Colavita et al., 1998; Nash et al., 2000). These guidance cues are transduced into cell signaling pathways to achieve cell migration. One of these pathways is the small G protein Rac signaling pathway, which is part of the cellular machinery involved in remodeling the actin cytoskeleton during DTC migration in response to these guidance cues. Upstream regulators of CED-10/Rac include CED-2/CrkII, CED-5/Dock180, and CED-12/Elmo, which control DTC migration by regulating the actin cytoskeleton via an evolutionally conserved mechanism (Wu and Horvitz, 1998; Reddien and Horvitz, 2000; Gumienny et al., 2001; Wu et al., 2001; Zhou et al., 2001; Reddien and Horvitz, 2004).

Guidance cues also regulate the neuronal cell and growth cone migration in *C. elegans*. The two Q neuroblasts, QL and QR, are born on opposite sides of the animal in bilaterally symmetric positions (Sulston and Horvitz, 1977; Chalfie and Sulston, 1981). The Q cells migrate and then each divides to give rise to three neuronal descendants that also migrate. QL and its descendants migrate toward the posterior body, whereas QR and its descendants migrate anteriorly. Guidance cues that function in these cell migrations and axon guidances include a novel protein MIG-13 (Sym et al., 1999), UNC-6/Netrin family (Chan et al., 1996; Wadsworth et al., 1996) and SAX-3/Robo receptor and its ligand SLT-1/Slit (Zallen et al., 1998; Hao et al., 2001).

The extracellular cues directing the migration of neuronal cells and

growth cones also modulate the cytoskeletal organization. It has been shown that the Rac family proteins CED-10, MIG-2 and RAC-2 and their regulator UNC-73/Trio influence the actin cytoskeleton and regulate the migration of neuronal cells and growth cones in *C. elegans* (Zipkin et al., 1997; Steven et al., 1998; Lundquist et al., 2001; Lundquist, 2003). Furthermore, the regulation of the actin cytoskeleton by UNC-34/Enabled and UNC-115/abLIM, which act downstream of UNC-40/DCC, is implicated in the axon guidance of the AVM and DA/DB motor neurons and the axon pathfinding of the CAN and PDE neurons (Yu et al., 2002; Gitai et al., 2003; Struckhoff and Lundquist, 2003; Chang et al., 2004). Although these molecules, together with small G proteins, are potential effectors that drive cell migration, the signaling pathways that directly relay the extracellular guidance cues to these effectors of cell migration are still unclear.

Tyrosine phosphorylation is required for crucial functions in multicellular animals such as cell differentiation, cell adhesion and migration, axon guidance and cell-cell communication (Hunter, 2000). The Src family of non-receptor protein tyrosine kinases (SFKs) serves as a critical molecular switch that transmits extracellular cues into the intracellular tyrosine phosphorylation events that lead to the cellular responses (Brown and Cooper, 1996; Sicheri and Kuriyan, 1997; Thomas and Brugge, 1997). In *C. elegans*, there are two SFK orthologues, *src-1* and *src-2/kin-22* (Bei et al., 2002; Hirose et al., 2003). A deletion allele of *src-1*, *cj293*, was originally isolated by imprecise transposon excision (Bei et al., 2002). The *src-1(cj293)* allele lacks the SH2 and kinase domains and is potentially a null allele. Homozygous *src-1(cj293)* hermaphrodites are themselves viable but produce inviable embryos, suggesting SRC-1 plays essential roles in early development. It has been shown that SRC-1 is required for the accumulation of tyrosine-phosphorylated proteins at the membrane boundary between the P2

and EMS cells, and functions in parallel with Wnt/Wg signaling to specify the endoderm and to orient the division axis of EMS in the early embryo (Bei et al., 2002). However, the functions of SRC-1 in organogenesis and the development of nervous system, where SRC-1 is abundantly expressed, remain unknown (Hirose et al., 2003).

To address the roles SRC-1 plays in the later stages of *C. elegans* development, I have characterized homozygous *src-1(cj293)* hermaphrodites. I found that SRC-1 is essential for directing the migration of DTCs and a subset of neuronal cell bodies, and the growth cone path findings, which are regulated by different guidance cues including UNC-6/netrin. Furthermore, analyses of the genetic interactions between SRC-1 and potential downstream factors revealed that SRC-1 transduces the various extracellular guidance cue that direct cell migration *via* different pathways that depend on the cell type.

Materials and methods

Strains and genetics

C. elegans strains were cultured at 20°C as described by Brenner (1974). N2 Bristol served as the wild-type strain. The alleles used are listed by linkage groups. The strains used include LGI: *src-1(cj293), ced-12(k149), src-2(ok819), hT2[qIs48]* (I;III); LGII: *muIs32[mec-7::gfp, lin-15(+)];* LGIV: *ced-2(n1994), ced-10(n1993), unc-5(e53)*, *ced-5(n1812), rac-2(ok326), kyIs179[unc-86::gfp, lin-15(+)];* LGV: *qIs19[lag-2::gfp, rol-6(su1006)], evIs54[unc-5B::lacZ; rol-6(su1006dm)]* ; and LGX: *mig-2(mu28), kyIs4[che-23::gfp, lin-15(+)].* The *hT2* translocation chromosome balances *src-1*; the *qIs48* insertion onto *hT2* allows *src-1(cj293)*/+ heterozygotes to be distinguished from *src-1(cj293)* homozygotes by the presence or absence of the GFP marker inserted onto *hT2* (Wang and Kimble, 2001). Double mutant strains were constructed by standard methods. The mutations in the double mutants were confirmed by DNA sequencing or PCR for the *src-1(cj293)* mutation.

Phenotypic analysis

Gonad morphology was observed on a 5% agar pad in M9 buffer by Nomarski differential interference contrast microscopy. The DTC migration pattern was inferred from the gonadal morphology of young adults at 20°C except as indicated. At the L1 stage, the positions of the AVM, ALM and PVM neurons were determined by using *mec-7::gfp*, *muIs32* (Ch'ng et al., 2003), the position of CAN was determined by using *che-23::gfp*, *kyIs4* (Forrester and Garriga, 1997), and the position of HSN was determined by using *unc-86::gfp, kyIs179* (Shen and Bargmann, 2003). The images of the neuronal cells were captured by a LSM-510 confocal laser-scanning microscope (Zeiss). The positions of AVM, ALM, CAN, HSN and PVM were scored on the basis of their positions relative to the V cell daughters, since these are stationary landmarks.

A defect in the AVM axon morphology was scored when it failed to extend in the anterior direction after the nerve ring branch. A defect in the PVM axon morphology was scored when it failed to turn in the anterior direction after reaching the ventral nerve cord. Defects in ALM, CAN, HSN and PLM migration were determined by comparisons with their wild-type morphology. This morphology was defined by electron microscopic reconstitution of the *C. elegans* nervous system (White et al., 1986).

Feeding RNAi

RNAi was performed essentially as described by Timmons and Fire (1998). After preparing the *src-1* RNAi feeding plate, some parental worms were allowed to lay eggs on the plates for 3-4 hours and were then removed. The remaining eggs were cultured into young adults and then assessed for gonad morphology.

Plasmid construction

To express *src-1*, the *src-1* cDNA was amplified by PCR from the plasmid yk117f2 and cloned into pPD49.26 carrying the *src-1* promoter (4.9 kbp) to generate *Psrc-1::src-1*. pPD49.26 carrying the *lag-2* promoter (3.0 kbp) was kindly provided by J. Kimble and was used to generate *Plag-2::src-1*. pPD95.86 and pPD96.41 were used to generate *Pmyo-3::src-1* and *Pmec-7::src-1*, respectively. To generate the kinase-negative form of *src-1*, the lysine 290 residue was substituted with methionine to produce *src-1K290M*. *src-1K290M* was then inserted into the vectors described above instead of *src-1*.

Transgenic strains

Transgenic lines were generated using standard techniques (Mello et.

al., 1991). For rescue experiments, a *lin-44::gfp* construct was co-injected as a marker (50 mg/ml). To rescue the defect in DTC migration, the expression construct was injected at 1 mg/ml into the *src-1/hT2[qIs48]* heterozygote with an injection marker. The defect in DTC migration was then scored in the *src-1(cj293)* homozygote carrying the extrachromosomal array. To rescue the defects in the positioning of AVM cell body, *src-1* or *src-1K290M* in pPD96.41 was injected at 50 mg/ml into the *src-1/hT2[qIs48]* heterozygote with an injection marker. To rescue the aberrant growth cone migration of PVM, *src-1* or *src-1K290M* in pPD96.41 was injected at 50 mg/ml into the *src-1/hT2[qIs48]* heterozygote with an injection marker. The axon trajectories of PVM were observed by fluorescence microscopy and were compared with those in the *src-1(cj293)* homozygote. For the precocious expression of *unc-5,* the plasmid pSU16 (*emb-9::unc-5*) construct (a gift from J. G. Culotti and L. Brown) was injected at 1 mg/ml into the *src-1/hT2[qIs48]* worms with an injection marker. The gonad morphology of the resulting heterozygotes *(src-1*/+) and homozygotes (*src-1*/*src-1*) was then analyzed.

Tissue staining

Adult worms were dissected and fixed essentially as described by Francis et al. (1995). Briefly, the dissected gonads were fixed with 3.7% formaldehyde for 1 hr and postfixed with 100% methanol for 5 min. The specimens were then blocked with 3% BSA in 0.1% Tween TBS, and stained with anti-phosphotyrosine monoclonal antibody, 4G10 (Upstate), followed by detection with secondary antibodies conjugated with Texas Red. The images were captured by confocal laser-scanning microscopy on a Fluoview FV1000 (Olympus). For the *unc-5* reporter assay, staining for b-galactosidase activity was carried out as described previously (Fire et al., 1990).

RESULTS

Defects in DTC migration caused by the *src-1* mutation.

To examine the roles of SRC-1 in the development of postembryonic stages, I analyzed a homozygous *src-1(cj293)* mutant produced from the balanced heterozygote *src-1(cj293)*/+, since the *src-1(cj293)* mutant showed a maternal embryonic lethal phenotype (Bei et al., 2002). The *src*-1(*cj293*) mutants grew into adults but showed an apparent defect in gonad morphogenesis (Fig. 2). DTCs in the *src-1(cj293)* mutant migrated normally during the first phase but frequently failed to turn at subsequent phases of gonad development. Consequently, DTCs continued their centrifugal migration along the ventral muscles and the tips of the anterior and posterior lobes eventually reached the pharynx and anus, respectively (Fig. 2F). I here term this defect as “no-turn phenotype”. Perhaps because of space limitations, these extended gonads in the adult worms became randomly bent and accordionated (Fig. 2H).

I also analyzed worms that had developed on *src-1* RNAi feeding plates. The *src-1(RNAi)* worms had a similar phenotype to the *src-1(cj293)* mutants. Observed DTC migrations were classified into three categories (Table I). The first is the normal migration path (100% in N2, 19% in *src-1(cj293),* 28% in *src-1(RNAi)*). The second type represents the migration path that turns in the opposite direction during the third phase (0% in N2, 16% in *src-1(cj293),* 8% in *src-1(RNAi)*). The third type is the no-turn phenotype, which was the most frequently exhibited phenotype for the *src-1(cj293)* mutant and the *src-1(RNAi)* worms (0% in N2, 65% in *src-1(cj293),* 64% in *src-1(RNAi)*). These results raise the possibility that DTCs in the *src-1(cj293)* mutants are not being controlled by the guidance cues that direct their turning at the appropriate time and position.

As indicated above, the defect in DTC migration in the *src-1(cj293)* mutant was phenocopied in the *src-1(RNAi)* worms (Table I; Fig. 3), but there were some differences in the frequencies of the defects. The frequency of DTC

migration defects in the *src-1(RNAi)* worms was 72% for both the anterior and posterior lobes: those in the *src-1(cj293)* mutant were 50% and 80%, respectively (Fig. 3I). The lower frequency of anterior lobe defects in the *src-1(cj293)* mutant may be due to some maternal effects in this mutant. The mutation or RNAi of another SFK, SRC-2*,* did not result in any defects in DTC migration (Fig. 3E, F, I), suggesting that SRC-1 plays a specific role in regulating DTC migration. Moreover, in contrast to the hermaphrodites, the gonads in male *src-1(cj293)* worms were apparently normal (Fig. 3G-I). This suggests that the mechanism involved in the migration of the male linker cells is different from that guiding DTCs**.**

To determine whether the defects in the *src-1(cj293)* mutant are due to the loss of SRC-1 function in DTCs, SRC-1 was expressed in the mutant under the *src-1* promoter or the DTC-specific *lag-2* promoter (Fig. 4). The expression of SRC-1 under either promoter rescued the defects, with the defect frequency being decreased to 12%/33% in the anterior/posterior lobes by the *src-1* promoter and to 10%/41% by the *lag-2* promoter (Fig. 4G). However, the defect was not rescued by expression of a kinase-negative form of SRC-1 (SRC-1K290M) or by the expression of SRC-1 under the body wall muscle-specific *myo-3* promoter. These results demonstrate that SRC-1 acts in an activity-dependent and DTC cell-autonomous manner.

To further examine the contribution of SRC-1 to DTC migration, the expression of SRC-1 was determined by detecting the expression of a reporter gene (GFP) expressed under the *src-1* promoter. The expression of *src-1* gene was clearly detected in DTCs (Fig. 5A, B) as described previously (Hirose et al., 2003). Furthermore, immunostaining with an anti-phosphotyrosine antibody (4G10), which specifically recognizes phosphorylated tyrosine residues of various proteins, revealed high levels of tyrosine phosphorylated proteins in DTCs (Fig. 5C). In contrast, DTCs in the *src-1(cj293)* mutant gave only faint

signals (Fig. 5D), showing that SRC-1 activity is a major source of tyrosine phosphorylation in DTCs.

Genetic interaction of the *src-1* gene with other genes involved in DTC migration.

To position SRC-1 in a cell signaling pathway involved in DTC migration, I first analyzed the genetic interaction of *src-1* with components of the Rac pathway, including *ced-2*/CrkII, *ced-5*/Dock180, *ced-12*/Elmo, and *ced-10*/Rac. Mutations in these genes induce unregulated turns of DTC migration (Wu and Horvitz, 1998; Reddien and Horvitz, 2000; Gumienny et al., 2001; Wu et al., 2001; Zhou et al., 2001; Reddien and Horvitz, 2004). I analyzed DTC migration in the following double mutants: *src-1;ced-2*, *src-1;ced-5*, *src-1;ced-10* and *src-1;ced-12*. In these experiments, the phenotypes of these mutants were evaluated by assessing the occurrence of typical no-turn phenotype of the *src-1(cj293)* mutant (Table II). The *ced* mutations alone did not induce no-turn phenotype. However, the no-turn phenotype of *src-1(cj293)* mutant was drastically suppressed in the *src-1;ced-2*, *src-1;ced-5*, and *src-1;ced-12* double mutants. The effects were evident in both gonad lobes with the frequency being decreased from 13%/70% (anterior/posterior lobes) to 2%/13%, 2%/5% and 2%/7% in the *src-1;ced-2*, *src-1;ced-5*, and *src-1;ced-12* double mutants, respectively. These results suggest that SRC-1 potentially acts as a suppressor of the Rac signaling pathway. Furthermore, in these double mutants, DTCs turned twice as in wild-type worm, raising the possibility that there exists an alternative pathway that functions in parallel to the *src-1/ced* pathway. In *src-1;ced-10* double mutant, the no-turn phenotype was only partially suppressed, with the frequency in the anterior/posterior lobes being 20%/45%, suggesting that other genes function redundantly with *ced-10* in this signaling pathway.

There are two additional *ced-10*–related genes in *C. elegans*, namely, *mig-2* and *rac-2.* It has been reported that the *mig-2* mutant has the same kind of DTC migration defect observed in the *ced* mutants, suggesting the functional redundancy between MIG-2 and CED-10 (Lundquist et al., 2001; Lundquist, 2003). However, since clear role for RAC-2 in DTC migration has not been reported, I examined the relationship between *src-1* and these Rac relatives. In *src-1;mig-2* mutants, the no-turn phenotype was suppressed in the posterior lobes to the same extent as in *src-1;ced-10* mutants, while it was significantly enhanced in *src-1;rac-2* mutants (98%) (Table II). These results suggest that RAC-2 functions in parallel with SRC-1, but do not rule out the possibility that it also functions in the same signaling pathway as SRC-1.

It is well established that the chemorepulsive mechanism mediated by UNC-6/netrin and its receptors UNC-5 and UNC-40 guide DTC migration, particularly during phase II (Hedgecock et al, 1990). The programmed expression of *unc-5* in DTCs is shown to time the turning of DTC migration (Su et al., 2000). Mutations in *unc-5(e53)* and *unc-6(ev400)*, which are putative null alleles, cause specific defects in the ventral-to-dorsal migration of DTCs. In either *src-1(cj293);unc-5(e53)* mutants or *src-1(cj293);unc-6(ev400)* mutants, the no-turn defect characteristic for *src-1(cj293)* mutant was not significantly affected (Table II). These observations demonstrate that *src-1(cj293)* mutation is epistatic to *unc-5(e53)* or *unc-6(ev400)* mutations.

Since the timing of DTC turning is affected by regulation of *unc-5* expression (Su et al., 2000), I examined the effect of the *src-1(cj293)* mutation on the expression of *unc-5* by detecting the *unc-5* promoter activity (*unc-5B::lacZ*). As shown in Fig. 6B, the *src-1(cj293)* mutant at the L3 stage expressed *unc-5* at the appropriate time, while still showing the no-turn phenotype. Furthermore, the precocious turning of DTCs that is induced by the precocious expression of *unc-5* under the *emb-9* promoter (*emb-9::unc-5*) was

substantially decreased from 30%/70% (anterior/posterior lobes) in the *src-1(cj293)*/+ heterozygotes to 7%/14% in the *src-1(cj293)* homozygotes (Fig. 6C, D; Table III). These results suggest that the *src-1* mutant phenotype is epistatic to not only precocious but also normal DTC turning induced by UNC-5, and that SRC-1 is required for UNC-5 mediated signaling pathway that directs DTC migration.

Defects in neuronal cell migration caused by the *src-1(cj293)* mutation.

To examine the roles SRC-1 plays in the migration of other cell types, I analyzed the migration of the QR and QL neuroblasts in the *src-1(cj293)* mutant. In the wild-type worm, the QR neuroblast and its descendants migrate over long distances. As a result, the AVM neuron, a QR descendant, eventually becomes positioned anteriorly to the ALM neuron (Fig. 7A, B, C). In the *src-1(cj293)* mutant, however, the QR neuroblast and its descendants can migrate only a short distance and AVM is located posteriorly to ALM (Fig. 7D, E). When SRC-1 was expressed in the *src-1(cj293)* mutant under the control of the *mec-7* promoter, which specifically acts in the mechanosensory neurons, including AVM, this cell migration defect was significantly rescued (42%) (Table IV). The expression of a kinase-negative SRC-1K290M could not rescue the defect at all, indicating that SRC-1 acts cell-autonomously and in an activity-dependent manner in AVM.

To examine the contribution of the Rac pathway to the migration of the QR neuroblast and its descendants, the *ced-5(n1812)* and *ced-12(k149)* mutants, which are molecular null alleles, were scored for cell migration defect (Fig. 7F). However, no apparent defect was detected in either mutant. Furthermore, unlike the DTC defects, the AVM defect in the *src-1(cj293)* mutant was not significantly modified by either the *ced-5(n1812)* or *ced-12(k149)* mutations. These findings suggest that SRC-1 differentially employs

downstream pathways depending on the cell types. The migration of the PVM cell body, a QL neuroblast descendant, was normally positioned in either *src-1* or *ced-5/-12* mutants (Fig. 7F). Moreover, the migration of CAN and HSN, was not significantly affected by the *src-1(cj293)* mutation (data not shown). These observations reveal that SRC-1 functions in the migration of a particular subset of neuronal cells.

In this experiment, I found the misplacement of both ALMR and ALML in *ced-5(n1812)* and *ced-12(k149)* mutants (Fig. 7G). In the wild-type worm, ALMR and ALML , which are sister cells, migrate posteriorly. *In ced-5/-12* mutants, half of ALMs can migrate only short distance. However, no apparent defects was detected in *src-1(cj293)* mutant. It is known that mig-2/Rac influence the embryonic migration of the ALM cells (Hedgecock et al., 1987). Unlike AVM and PVM placement, ALM placement may be regulated by rac signaling pathway. Interestingly, the ALM defects were suppressed in *src-1;ced-5* and *src-1;ced-12* double mutants. The wandering migration of DTC in ced mutant was also suppressed by *src-1* mutation. These observations suggest that less signal of SRC-1 affect returning to normal in case of ced mutant background.

Defects in growth cone migration due to the *src-1(cj293)* mutation.

The migration and direction of growth cones are also regulated by specific extracellular guidance cues. To examine whether SRC-1 is involved in these processes, we observed the effect of the *src-1(cj293)* mutation on the axon trajectories of the AVM, ALM, CAN, HSN, PVM and PLM neurons. In the *src-1(cj293)* mutant, apparent defects in the axon trajectory directions were observed for AVM, ALM, CAN and PVM with a penetrance of 60%, 21%, 38%, and 46%, respectively (Fig. 8). AVM axons failed to extend anteriorly after the nerve ring branch, while ALM axons were slightly bent probably because of

mislocation of the ALM cell body to ventral side. In CAN neurons, axons terminated prematurely and often branched inappropriately. Normally, the growth cones of PVM neurons make a right-angled turn anteriorly after reaching the ventral nerve cord (Fig. 8H). In the *src-1(cj293)* mutant, however, about half of the PVM growth cones (46%) turn in the opposite direction (Fig. 8I-K). During this inappropriate migration, a substantial number of the PVM axons (26%) make a reverse turn in the anterior direction, thus allowing the growth cones to reach the anterior body (Fig. 8J). These observations suggest that the PVM growth cones in the *src-1(cj293)* mutant fail to respond to the guidance cues that direct their migration along the anterior-posterior axis. This defect was completely rescued by the expression of SRC-1 but not the kinase-defective form of SRC-1 under the *mec-7* promoter (Table V), indicating again the cell-autonomous and kinase-dependent role of SRC-1 in cell migration. However, as in the case of AVM cell body migration, the *ced-5/-12* mutations did not significantly alter the normal PVM growth cone migration, nor did they modify the *src-1(cj293)* phenotype (Table V). These observations suggest that SRC-1 utilizes different downstream pathway in PVM to the one it uses in DTCs.

Discussion

DTC migration in the *src-1(cj293)* mutants

In this study, I first showed that the kinase activity of SRC-1 is required for the patterned DTC migration that occurs during gonad morphogenesis. In the *src-1(cj293)* mutant, the motility of DTCs seems to be normal because these cells migrate normally during phase I. In subsequent phases, however, DTCs fail to change direction, eventually traveling to the pharynx and anus (Fig. 2). These observations suggest that SRC-1 is required for DTC response to the guidance cues that direct its migration, including UNC-6/netrin and UNC-129/TGF-b. A probable candidate upstream regulator of SRC-1 is UNC-5/netrin receptor, which is known to regulate the ventral-to-dorsal phase II migration of DTCs (Hedgecock et al, 1990; Culotti and Merz, 1998). The genetic interactions of *src-1* with *unc-6* or *unc-5* suggest that SRC-1 acts in the UNC-6/netrin signaling pathway through UNC-5, which is supported by the observations that the *src-1(cj293)* mutation does not affect the timing of the *unc-5* expression and suppresses DTC turning induced by not only precocious but also normal expression of *unc-5*. Previously, it was shown that UNC-5 functions require phosphorylation of a cytoplasmic tyrosine residue, suggesting a critical role of a tyrosine kinase(s) in the UNC-6/netrin signaling pathway (Killeen et al., 2002). It was also reported that the activation of a vertebrate Src family kinase is required for the phosphorylation of the netrin receptor and its subsequent cell signaling (Li et al., 2004; Liu et al., 2004; Meriane et al., 2004), and that SRC-1 could mediate UNC-5 signaling in *C. elegans* by directly interacting with UNC-5 (Lee et al., 2005). These findings together with our observations support a model in which SRC-1 acts as a component of a netrin signaling pathway, at least during a particular time period in gonad development. However, it is also notable that the precocious expression of UNC-5 is still able to induce the precocious turning of DTC in the *src-1(cj293)* mutants, albeit with low frequency

(7%/14% in anterior/posterior lobes). Thus, it remains possible that alternative SRC-1 independent pathways can be involved in the guidance of DTC migration. Furthermore, the fact that the *src-1(cj293)* mutants fail to make the second turn that usually occurs during phase II reveals that SRC-1 may also be required for the DTC to respond to some other signaling mechanism that directs the secondary turning of DTCs.

In *C. elegans*, defects in DTC migration have been observed in the *ced-2*/CrkII, *ced-5*/Dock180, *ced-10*/Rac and *ced-12*/Elmo mutants (Wu and Horvitz, 1998; Reddien and Horvitz, 2000; Gumienny et al., 2001; Wu et al., 2001; Zhou et al., 2001; Reddien and Horvitz, 2004). CED-2, CED-5 and CED-12 form a ternary complex that can trigger the localized remodeling of the actin cytoskeleton through CED-10. It has also been shown that the Dock180-Elmo complex functions as a guanine nucleotide-exchanging factor for Rac, and that CrkII binding enhances the functions of this complex (Brugnera et al., 2002). Src may act upstream of this pathway to regulate cell migration through the actin cytoskeleton since the binding of CrkII to Dock180 is enhanced in v-Src transformed 3Y1 cells (Kiyokawa et al., 1998). Furthermore, Hck, a Src family member, binds to Elmo1 through its SH3 domain in vitro and phosphorylates Elmo1 in cultured cells (Scott et al., 2002). These lines of evidence strongly support our proposal that SRC-1 functionally acts in the Rac pathway in DTCs. Of the three *rac*–related genes in *C. elegans* (*ced-10*, *mig-2* and *rac-2*), CED-10 and MIG-2 have been shown to redundantly control the migration of some cells, including DTCs (Lundquist et al., 2001; Wu et al., 2002). Consistent with this is our observation, that a mutation in either *mig-2* or *ced-10* only partially suppressed the no-turn phenotype of DTC migration in the *src-1(cj293)* mutant, whereas mutations in *ced-2, ced-5* or *ced-12* robustly suppressed this phenotype. These results suggest that CED-10 and MIG-2 function redundantly downstream of the CED-2 complex under the control of

SRC-1. In contrast, a mutation in the *rac-2* gene enhanced the DTC migration defect in the *src-1(cj293)* phenotype, suggesting that RAC-2 is involved in DTC migration but acts in an alternative pathway independently of SRC-1.

Our analysis of the genetic interaction between *src-1* and the genes in the Rac pathway revealed that the DTC migration defect of *src-1(cj293)* mutants was suppressed in all the double mutants. This raised the possibility that SRC-1 may act as an upstream suppressor of the Rac signaling pathway. If this is the case, it may be that the Rac pathway is constitutively active in migrating DTCs, allowing a continuous remodeling of the cytoskeleton. Upon exposure to some guidance cues that activate SRC-1 (potentially netrin), the Rac pathway is suppressed, thereby inhibiting actin remodeling; this may be required for the DTC to change cell polarity and turn. This is consistent with the previous observation that loss-of–function of the Rac pathway induces an extra-turn phenotype (Wu and Horvitz, 1998). To verify this hypothesis, the mechanism by which SRC-1 suppresses Rac activity should be elucidated. Notably, it is generally accepted that Src positively regulates the Rac signaling pathway in mammals. This inconsistency may be resolved by identifying downstream effectors of SRC-1 that inactivate the Rac pathway in *C. elegans*.

Neuronal cell and growth cone migration in the *src-1(cj293)* mutants.

In the *src-1(cj293)* mutant, I also observed apparent defects in the migration of neuronal cells. In particular, the positioning of AVM, a descendant of the QR neuroblast, is strongly affected by the *src-1(cj293)* mutation. As with DTC migration, the kinase activity of SRC-1 is required for its role in the directional migration of QR and its descendants. While the cues that guide Q cell migration along the anterior-posterior axis are still unknown, it has been reported that the transmembrane protein MIG-13 is a key determinant in the final positioning of AVM (Sym et al., 1999). MIG-13 expression is restricted to

the anterior and central body regions and functions in a non-cell-autonomous manner to promote migration in the anterior direction. Like the *src-1(cj293)* mutant, a *mig-13* mutant shows defects in the migration of QR and its descendants, but not in the migration of QL and its descendants (ALM, CAN and HSN). Furthermore, the final position of AVM in the *mig-13* mutant is quite similar to that in the *src-1(cj293)* mutant. These observations raise the interesting possibility that SRC-1 mediates the cell signaling induced by extracellular MIG-13 cues. Mutations in *ced-5/-12* genes that affected DTC turning did not alter the migration defects of QR and its descendants in the *src-1(cj293)* mutants. This shows that the signaling pathways that are dependent on SRC-1 vary depending on cell type.

The *src-1(cj293)* mutant also showed defects in the migration of the growth cones of some neuronal cells, namely, AVM, ALM, CAN and PVM. For example, while the growth cone of PVM normally makes a right-angled turn in the anterior direction after reaching the ventral nerve cord, in the *src-1(cj293)* mutant it frequently makes a turn in the opposite direction (Fig. 8). It appears that the axon of PVM in the *src-1(cj293)* mutant may randomly determine the direction in which it turns. SRC-1 may be involved in the regulation of cell signaling evoked by the guidance cues that direct the migration of a growth cone, suggesting that the migration of a neural growth cone uses the same mechanisms employed by migrating cell bodies like DTCs. However, identification of the guidance cues involved in the attraction or repulsion of the PVM growth cone within the ventral nerve cord will be required to confirm the role of SRC-1 in this process.

In this study, I showed initially that SRC-1 plays a potential role in transducing the netrin signal to the Rac pathway at a particular time of DTC migration. However, our subsequent observations of neuronal cells and growth cones suggest that SRC-1 plays a more general role in directing cell migration,

and that it does so *via* different pathways depending on the cell types. Thus, in response to various extracellular guidance cues, activated SRC-1 may transduce the signals into an appropriate intracellular pathway that probably regulates the cytoskeletal remodeling required for providing polarity information to the cells. In vertebrates, Src family kinases have been shown to respond to a wide variety of extracellular cues, including guidance cues, extracellular matrices and growth factors, and to play roles in regulating a wide variety of cellular functions, including cell adhesion, migration, secretion, endocytosis, proliferation, and differentiation. These multifunctional aspects of Src family kinases have hampered the unraveling of their most critical function(s). Further analysis of the functions of SRC-1, particularly by focusing on the process of cell migration, may help elucidate its basal role, which may be conserved during animal evolution.

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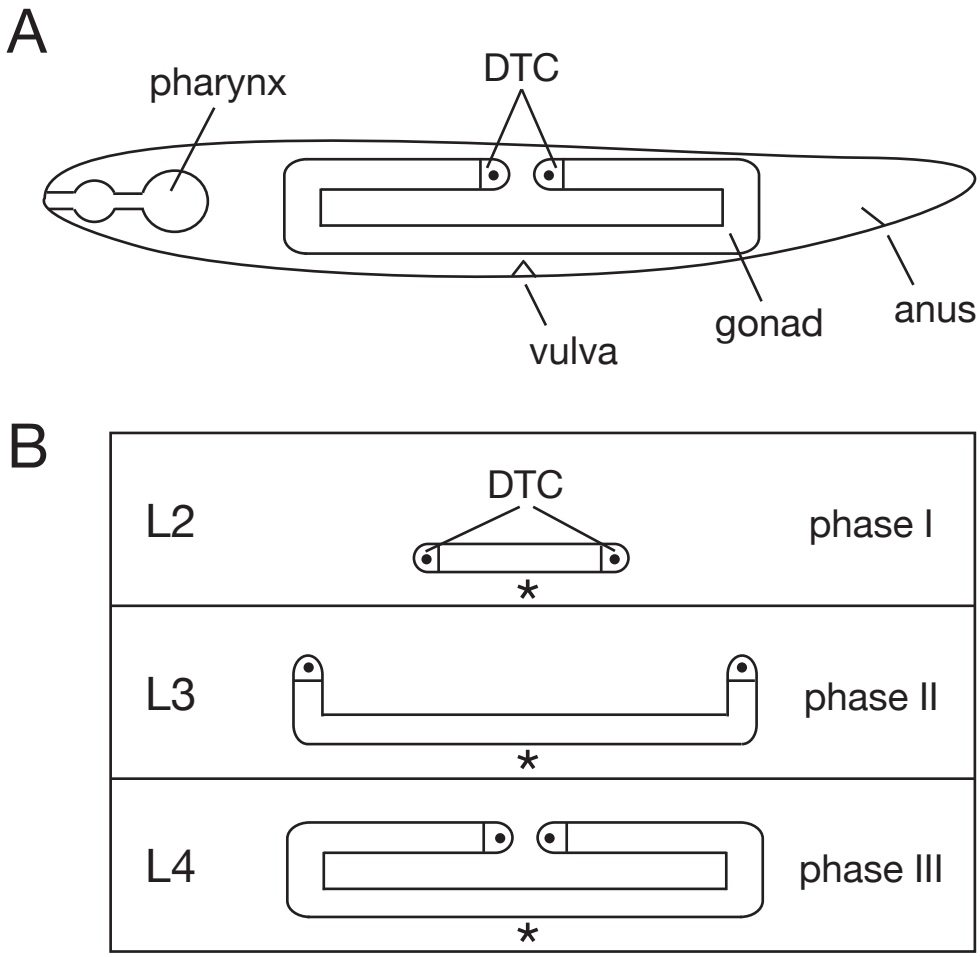


Fig. 1. (A) Schematic representation of the gonad structure of a wild-type hermaphrodite. The U-shaped gonad lobes are rotationally symmetrical around the dorsal-ventral axis at the center of the body. DTC, distal tip cell. The dorsal side is up. (B) Schematic illustration of the time course and pattern of gonad lobe extensions in wild-type hermaphrodites (lateral view). The DTCs lead the extension of the gonad lobes in three migration phases. First, the DTCs are generated at the ventral mid-body during the L2 stage and migrate in opposite directions along the ventral body wall muscle (phase I). They then turn orthogonally and migrate over the lateral hypodermis towards the dorsal muscles during the L3 stage (phase II). After reaching the dorsal side, the DTCs turn again and migrate towards the midbody along the dorsal body wall muscles during the L4 stage (phase III). The ventral midbody is marked with an asterisk.

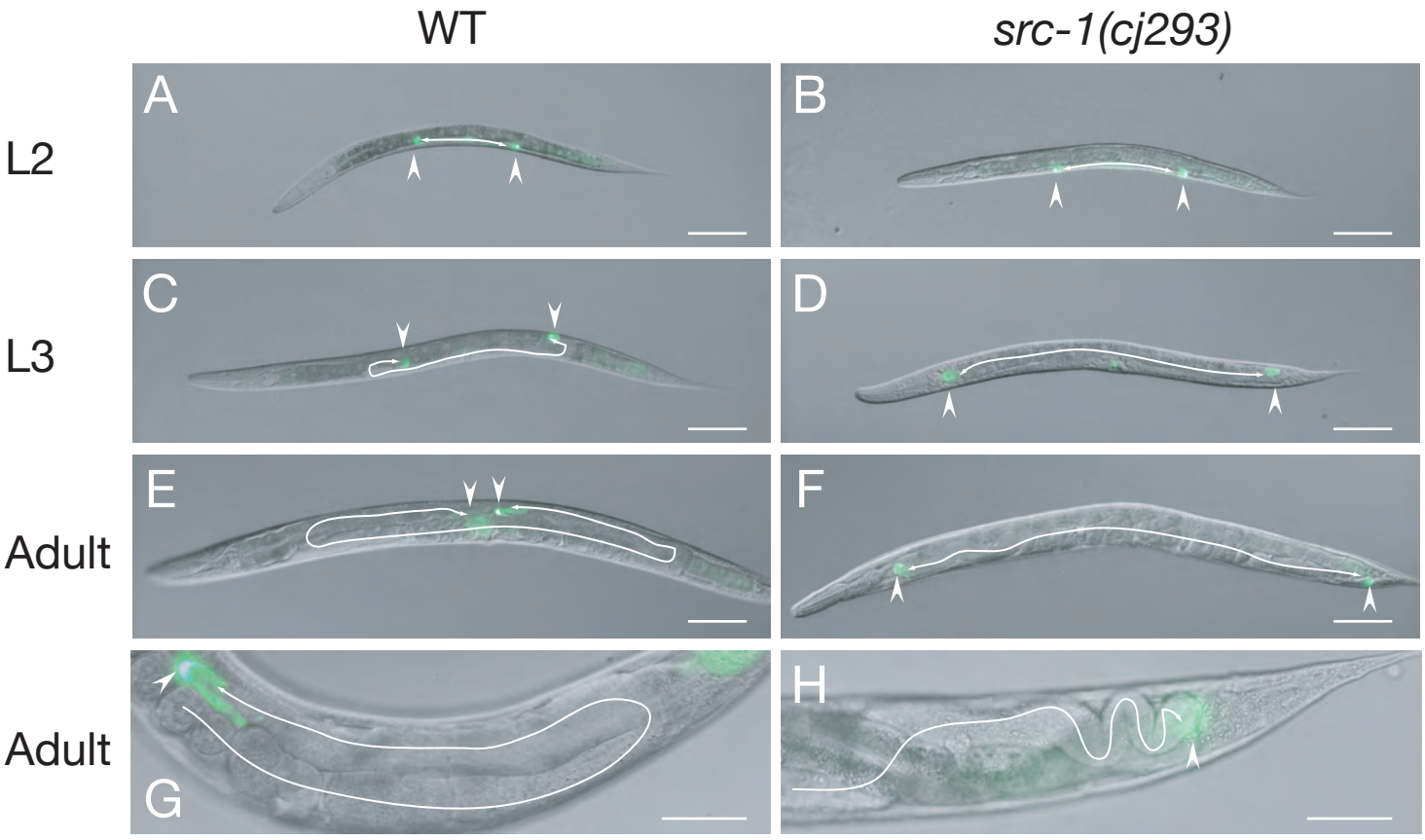
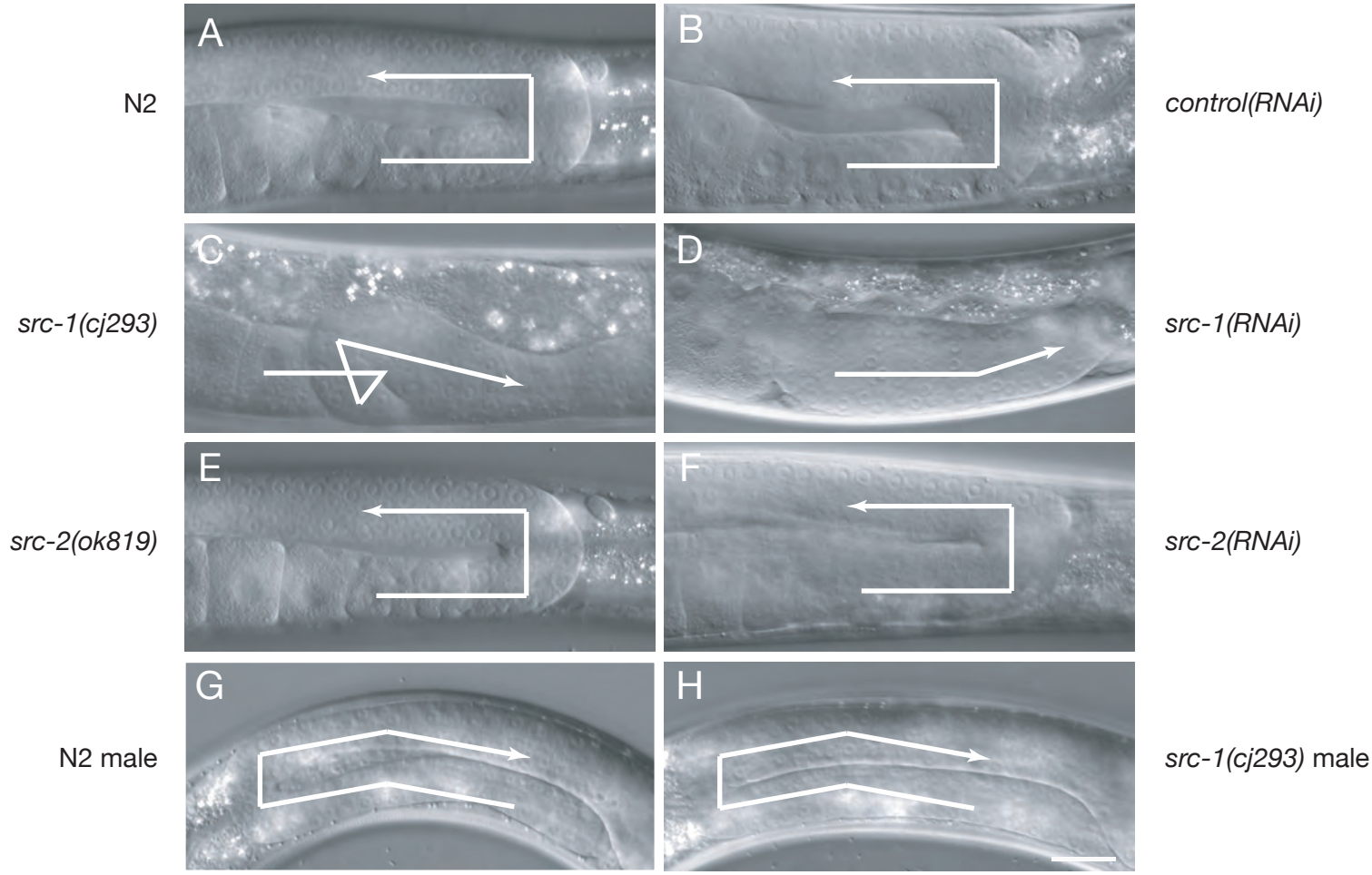


Fig. 2. Defective DTC migration in the *src-1(cj293)* mutant. The position of DTC was monitored by analyzing the expression of *lag-2::gfp* in wild-type (A, C, E, G) and *src-1(cj293)* mutant worms (B, D, F, H). The DTCs migrate in opposite directions along the ventral body wall muscle during the L2 stage in both the wild-type and mutant (A, B). However, the DTCs fail to turn dorsally in the *src-1(cj293)* mutant (D) during the L3 stage and continue their centrifugal migration in the anterior and posterior directions (F). In contrast, in the wild-type worm at the adult stage, the DTCs migrate centripetally along the dorsal muscle bands back toward the midbody (E). In the adult *src-1(cj293)* mutant, the extended gonads are randomly bent and accordionated (H). The arrowheads indicate the DTCs. Arrows show the migration pattern of the DTCs. Scale bar: 100 μm.



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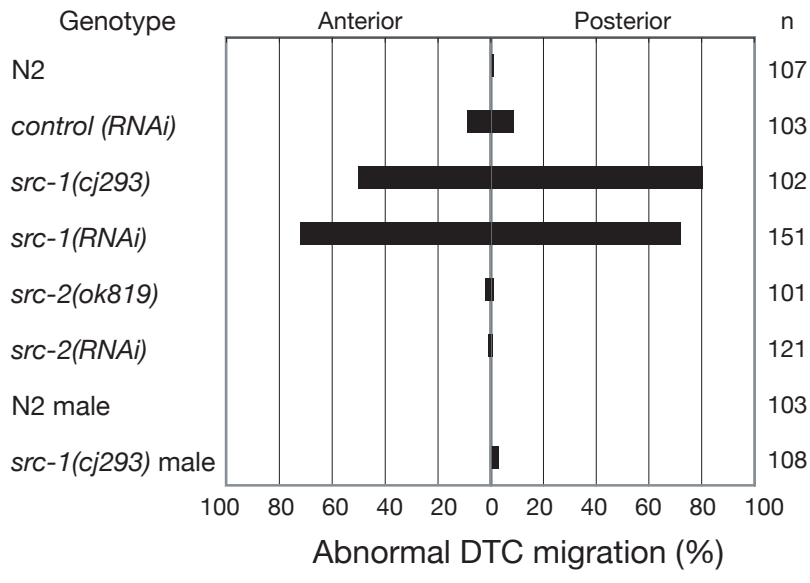
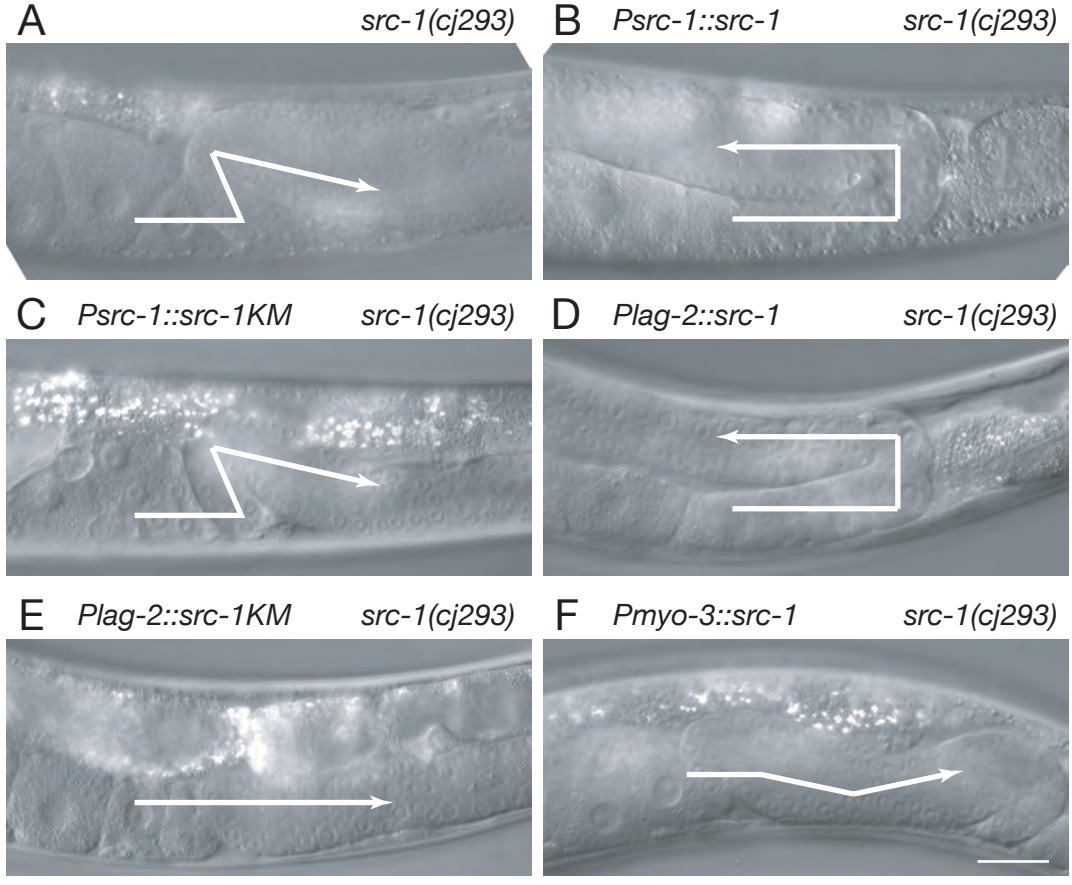


Fig. 3. The DTC migration defect in the *src-1(cj293)* mutant is phenocopied in *src-1(RNAi),* but neither the *src-2* mutation or the *src-2 RNAi* impairs DTC migration. (A-H) Shown are Nomarski images of the posterior gonad lobe at the young adult stage in the wild-type N2 (A), the control *gfp (RNAi)* worm (B), the *src-1 (cj293)* mutant (C), the *src-1(RNAi)* worm (D), the *src-2(ok819)* mutant (E), and the *src-2(RNAi)* worm (F). Also shown are the male gonads of the wild-type N2 (G) and the *src-1(cj293)* mutant (H). Arrows show the migration pattern of the DTCs. Scale bar: 20 μm. (I) Percentages of the various worm types that have a DTC migration defect in their anterior or posterior lobe. The total numbers of worms observed (n) are also indicated.



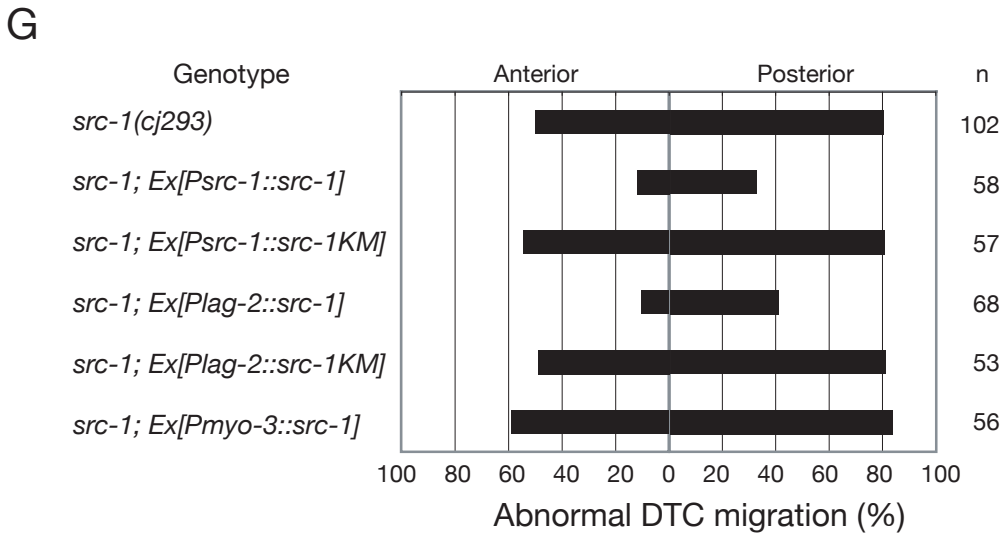


Fig. 4. Rescue of the DTC migration defect by the DTC-specific expression of active SRC-1. (A-F) Shown are Nomarski images of the posterior gonad lobe at the young adult stage in the *src-1(cj293)* mutant (A), the *src-1(cj293)* mutant expressing src-1 (B) or *src-1K290M* (C) under the *src-1* promoter, the *src-1(cj293)* mutant expressing *src-1 (*D) or *src-1K290M* (E) under the *lag-2* promoter, and the *src-1(cj293)* mutant expressing *src-1* under the *myo-3* promoter (F). Arrows show the migration pattern of the DTCs. Scale bar: 20μm. (G) Percentages of worms with DTC migration defects in their anterior or posterior lobe are indicated. For each transgene, two independent lines were scored; similar results were obtained. The total numbers of worm observed (n) are also indicated.

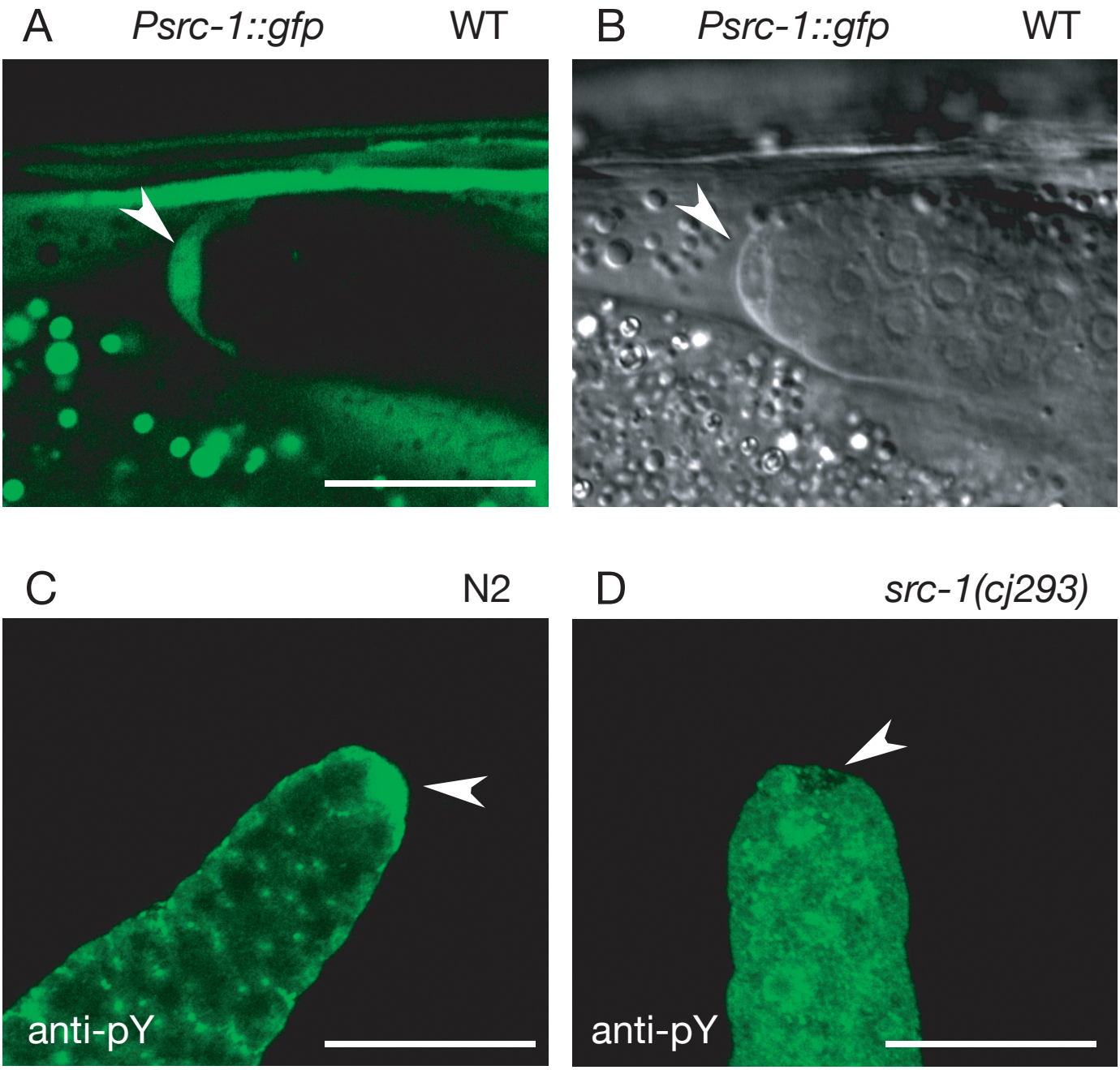


Fig. 5. DTC expression of SRC-1 in the wild-type DTCs, and limited tyrosine phosphorylation in the DTCs of the *src-1(cj293)* mutant. (A. B) The expression of SRC-1 in wild-type DTCs was determined by detecting the expression of a reporter gene expressed under the *src-1* promoter (*Psrc-1::gfp*). The DIC image is shown in B. (C, D) The dissected gonads of a wild-type worm (C) and the *src-1(cj293)* mutant (D) were stained with the anti-phosphotyrosine antibody 4G10. The arrowheads indicate the DTCs. Scale bar: 40 μm.

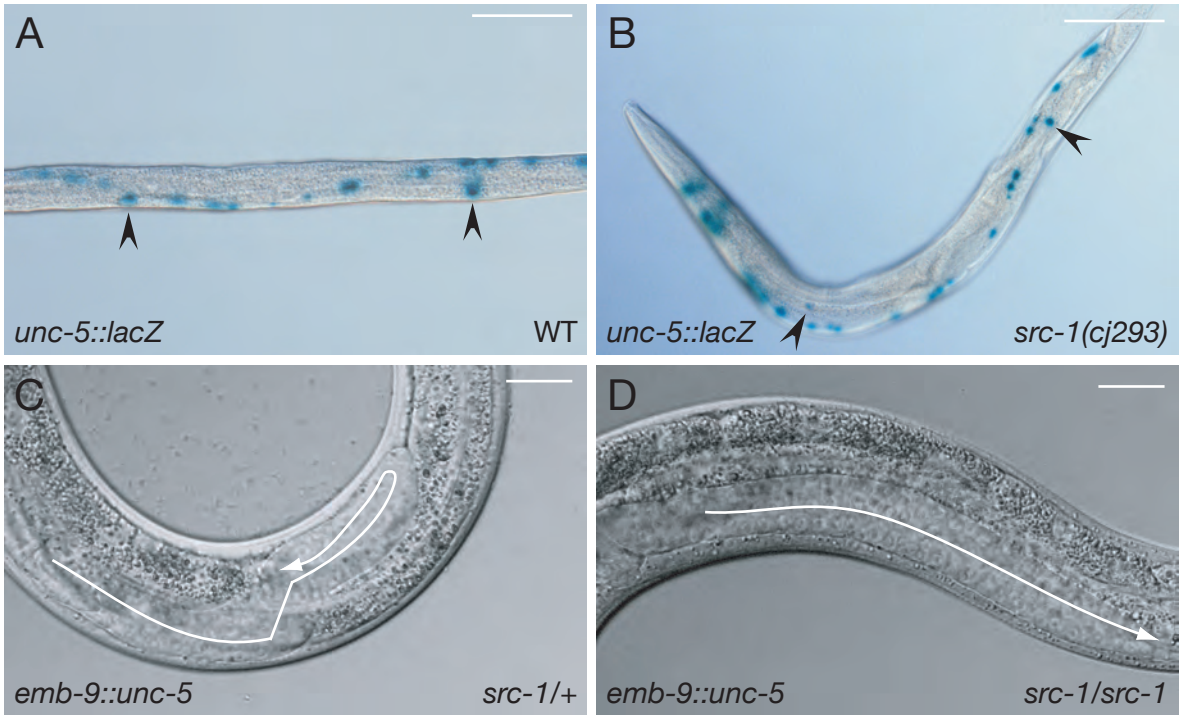
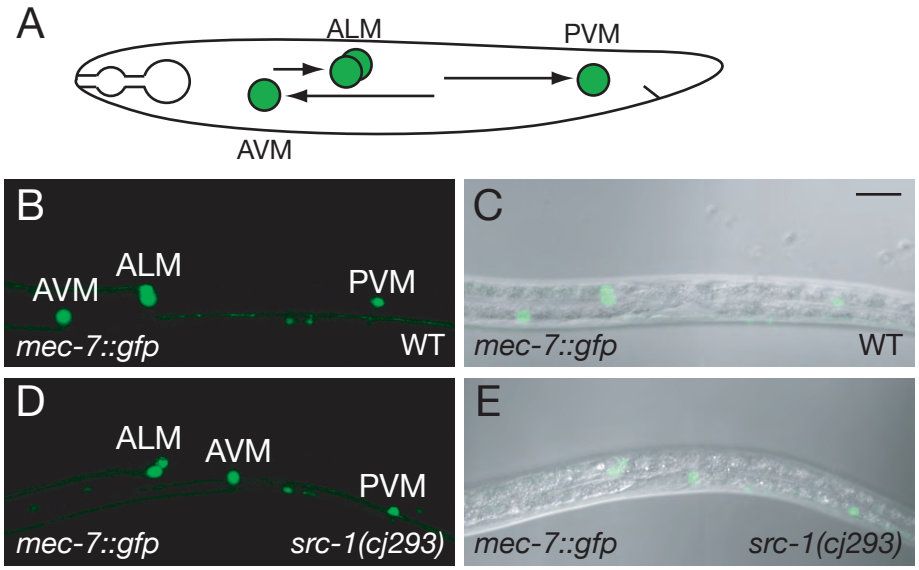
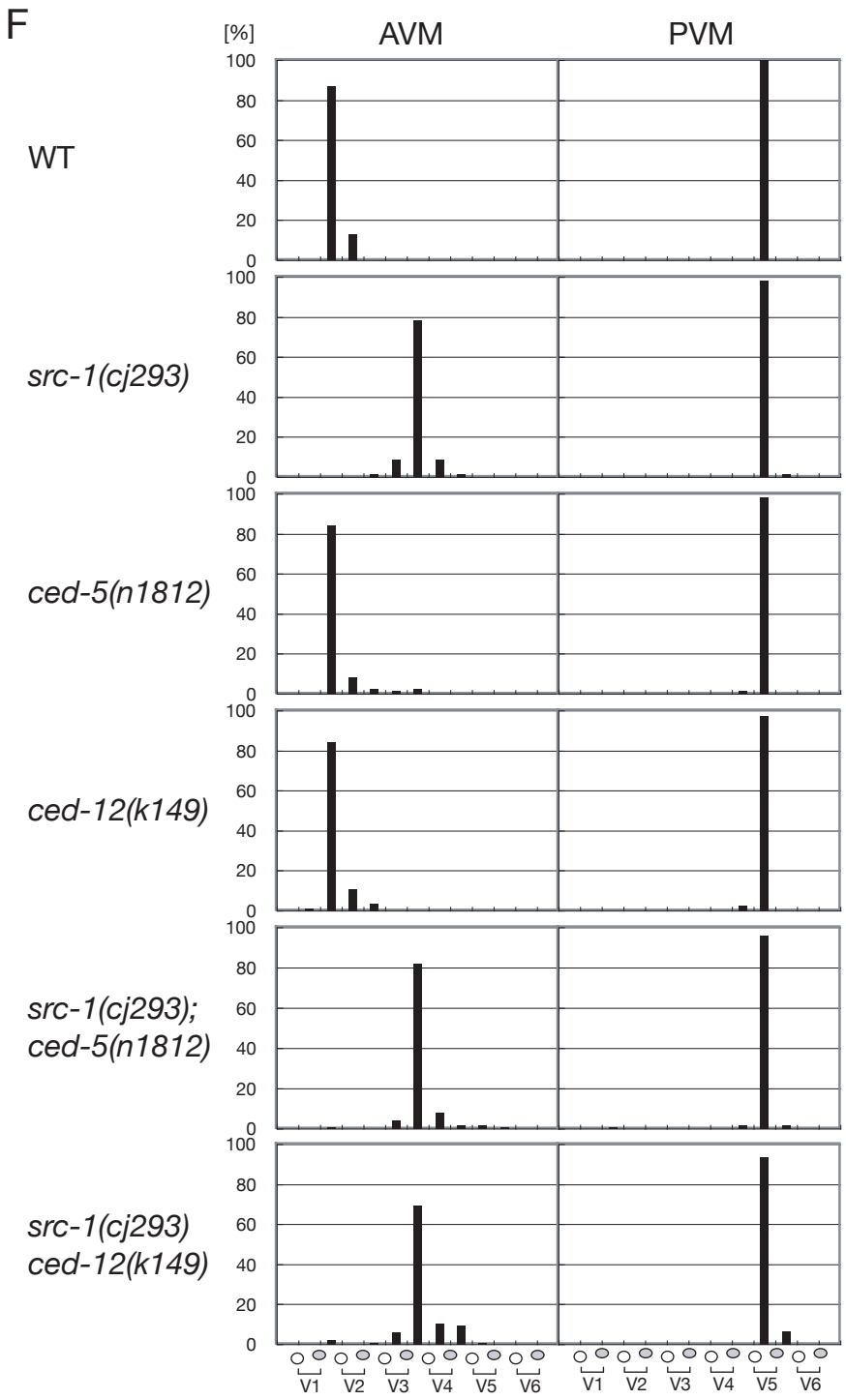


Fig. 6. (A, B) The expression of unc-5 is not affected by the *src-1(cj293)* mutation. The expression of *unc-5::lacZ* in wild-type worms (A) and the *src-1(cj293)* mutant (B) at around L3 stage was detected by staining for β-galactosidase activity. The arrowheads indicate the DTCs. Scale bar: 100 μm. (C, D) The precocious turning of DTCs induced by the precocious expression of UNC-5 is not observed in the *src-1(cj293)* homozygote background. The expression vector for UNC-5 under the *emb-9* promoter (*emb-9::unc-5*) was injected into the *src-1/hT2* worm, and the gonad morphology of the resulting heterozygote (*src-1*/+; C) and homozygote (*src-1/src-1*; D) was analyzed. The arrows show the migration pattern of the DTCs. Scale bar: 40 μm.


A
ALM
PVM
AVM
B
AVM
ALM
PVM
mec-7::gfp
WT
C
mec-7::gfp
WT
D
ALM
AVM
PVM
mec-7::gfp
src-1(cj293)
E
mec-7::gfp
src-1(cj293)


F
[%]
AVM
PVM
WT
src-1(cj293)
ced-5(n1812)
ced-12(k149)
src-1(cj293);
ced-5(n1812)
src-1(cj293)
ced-12(k149)
100
80
60
40
20
0
V1
V2
V3
V4
V5
V6

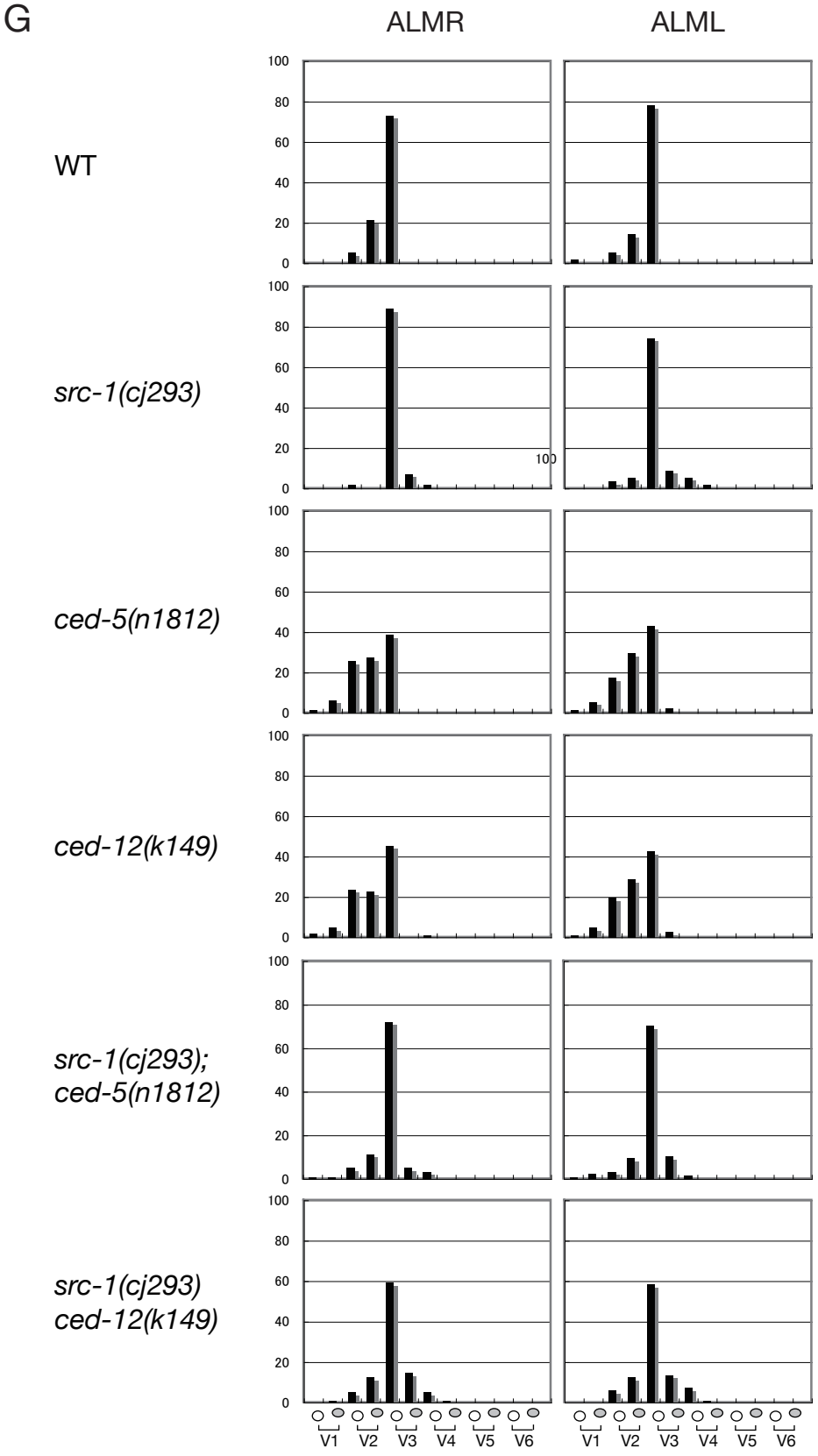


Fig. 7. Defects in the migration of the QR neuroblast and its descendants in the *src-1(cj293)* mutant. (A) Schematic representation of the AVM and ALM neuronal cells that originate from the QR neuroblast and the PVM descendant of the QL neuroblast. (B-E) The GFP signals produced from *mec-7::gfp* in wild-type worms (B) and the *src-1(cj293)* mutant (D) were visualized by epifluorescence. The merged GFP and DIC images are shown in C and E. Scale bar: 20 μm. (F, G) The genetic interaction of the aberrant AVM phenotype in the *src-1(cj293)* mutant with the *ced-5/-12* mutations. The final positions of AVM ,PVM (F), ALMR, and ALML (G) in the wild-type and indicated mutant worms were scored according to their relative distance from the stationary Vn.a and Vn.p cells shown on the x-axis.

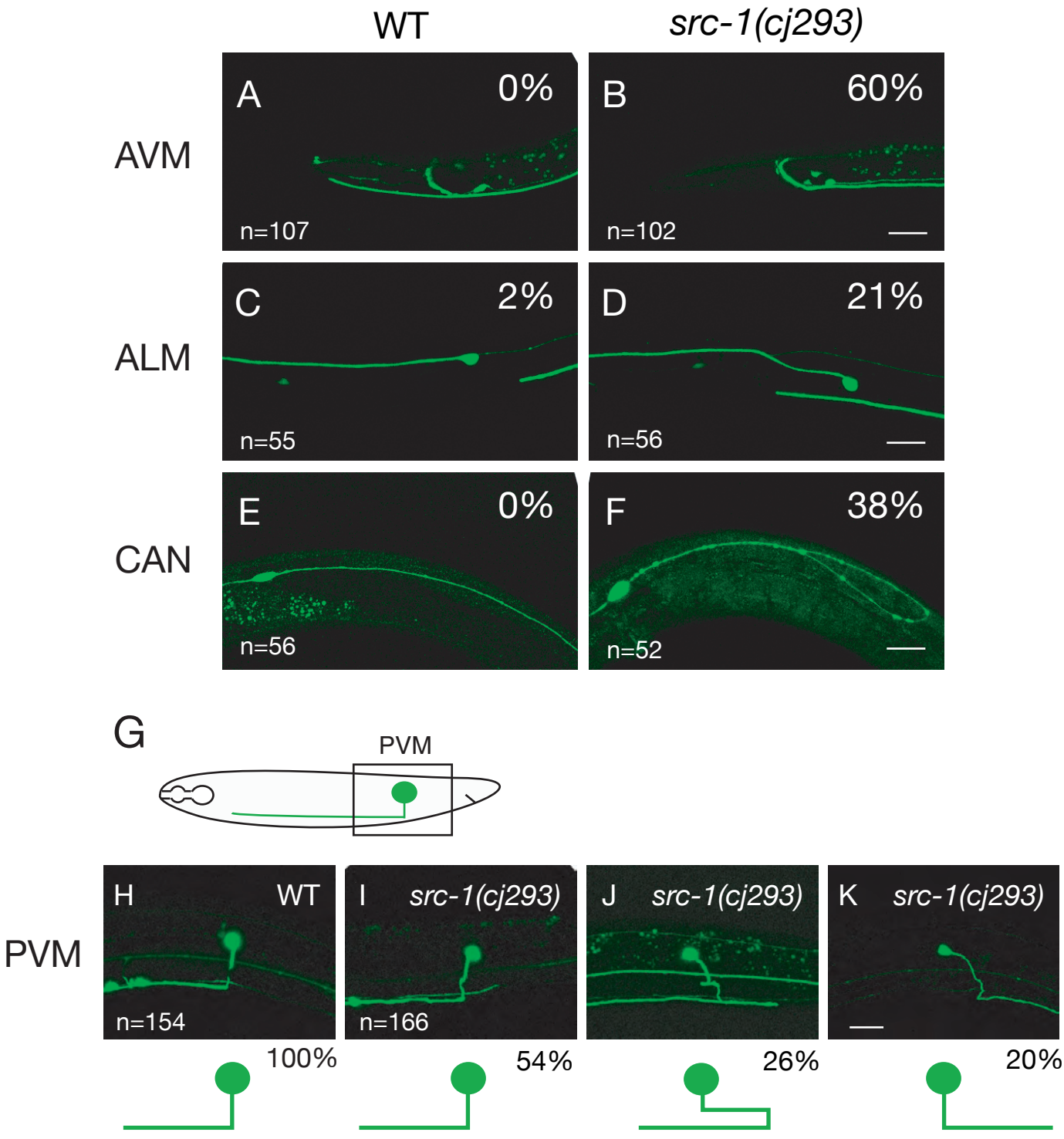
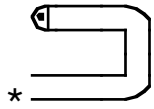
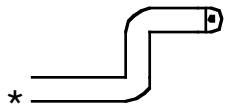
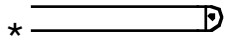


Fig. 8. Defective axon guidance in the *src-1(cj293)* mutant. (A-F) The typical axon trajectories of the AVM (A, B), ALM (C, D) and CAN (E, F) neurons in the wild-type (A, C, E) and *src-1(cj293)* mutant were visualized as described in the MATERIALS AND METHODS. Defects in the nerve ring branch of the AVM trajectory (B), the ALM cell body and its trajectory (D) and the CAN cell body and posterior axon trajectory (D) were observed. The percentages of worms with defective axon guidance and the total numbers of worm observed (n) are indicated in each panel. Scale bar: 20 μm. (G) Schematic representation of the PVM axon trajectory in a wild-type worm. (H-K) The defective PVM axon trajectory in the *src-1(cj293)* mutant. The defects were classified into two patterns as indicated schematically at the bottom of the panels. Some of the PVM axons in the *src-1(cj293)* mutant turn in the posterior direction on the ventral cord and then makes a reverse turn in the anterior direction (J), while others turn in the posterior direction on the ventral cord and continues in that direction (K). The percentages of worms with the various PVM axon trajectory patterns are shown. Scale bar: 20 μm.

Table 1. Classification of abnormal DTC migration in *src-1(cj293)* mutant.

	N2	*src-1(cj293)*	*src-1(RNAi)*
	100%	19%	28%
	0%	16%	8%
	0%	65%	64%
n	112	102	285

The schematic representations of DTC migration routes of posterior lobe of gonad are shown. The ventral midbody is marked with an asterisk. Percentages of each type of DTC migration at L4 stage of wild-type worm (N2), *src-1(cj293)* and *src-1(RNAi)* are shown. The total numbers of worms observed (n) are also shown.

Table 2. Genetic interaction of *src-1(cj293)* with *rac*-related and *unc-5/-6* genes.

Genotype	Anterior	Posterior	n
WT	0%	0%	112
src-1(cj293)	13%	70%	120
ced-2(n1994)	0%	0%	60
ced-5(n1812)	2%	2%	60
ced-10(n1993)	0%	2%	60
ced-12(k149)	0%	0%	60
src-1(cj293); ced-2(n1994)	2%	13%	60
src-1(cj293); ced-5(n1812)	2%	5%	60
src-1(cj293); ced-10(n1993)	20%	45%	60
src-1(cj293) ced-12(k149)	2%	7%	60
mig-2(mu28)	0%	3%	60
rac-2(ok326)	0%	0%	60
src-1(cj293); mig-2(mu28)	23%	42%	60
src-1(cj293); rac-2(ok326)	60%	98%	60
unc-5(e53)	0%	0%	111
unc-6(ev400)	7%	4%	106
src-1(cj293); unc-5(e53)	10%	61%	164
src-1(cj293); unc-6(ev400)	13%	51%	100

Percentages of no-turn DTC migration defects at the L4 stage in the anterior and posterior lobes of the gonad are shown. The total numbers of worms observed (n) are also shown.

Table 3. Effect of precocious *unc-5* expression on DTC migration in the *src-1(cj293)* mutant.

	Precocious turning		
Genotype	Anterior	Posterior	n
WT	0%	0%	112
src-1(cj293)	0%	0%	120
src-1/+; Ex[emb-9::unc-5]	30%	70%	56
src-1/src-1; Ex[emb-9::unc-5]	7%	14%	71

Percentages of precocious turning phenotypes in the anterior and posterior lobes of the gonad are shown. The total numbers of worms observed (n) are also shown.

Table 4. SRC-1 rescues the AVM position defect in a cell-autonomous manner.

Genotype	Abnormal AVM position	n
WT	1%	159
src-1(cj293)	99%	156
src-1; Ex[Pmec-7::src-1]	42%	101
src-1; Ex[Pmec-7::src-1KM]	98%	152

Defects in the positioning of AVM was scored at the larval stage in *muIs32* animals in the *src-1(cj293)* or wild-type background. For each transgene, two independent lines were scored; similar results were obtained. The total numbers of worms observed (n) are also shown.

Table 5. Effect of the *src-1(cj293)* and *ced-5/-12* mutaions on the axon trajectory of PVM.

Genotype				n
WT	100%	0%	0%	154
src-1(cj293)	54%	26%	20%	166
src-1(cj293); Ex[Pmec-7::src-1]	92%	5%	3%	103
src-1(cj293); Ex[Pmec-7::src-1KM]	55%	24%	22%	139
ced-5(n1812)	99%	1%	0%	106
ced-12(k149)	98%	2%	0%	103
src-1(cj293); ced-5(n1812)	62%	18%	21%	107
src-1(cj293) ced-12(k149)	63%	14%	23%	104

Percentages of the worms with the indicated axon trajectory are shown. The total numbers of worms observed (n) are also shown.

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I thank J. G. Culotti and L. Brown for the generous gift of the *C. elegans* stain *evIs54 [unc-5B::lacZ; rol-6(su1006dm)]* and the pSU16[*emb-9::unc-5*] vector, C. I. Bargmann for the *kyIs179[unc-86::gfp, lin-15(+)]* strain for the HSN marker, J. Kimble for the *lag-2* promoter, N Ringstad for discussion and comments on the manuscript, N. Hisamoto and K Matsumoto for technical advice about genetic balancers, T. Mitamura and T. Horii for a microscope, Y. Kohara for EST clones, A. Coulson and J Sulston for physical mapping, cosmid and YAC clones, and A. Fire for the vectors. Some of the strains used in this work were provided by the *Caenorhabditis* Genetic Center, Center for Research Resources and the *C. elegans* Gene Knockout Consortium.

Achievement

（１）学術雑誌等に発表した論文及び著書

［査読あり］

Bunsho Itoh, Takashi Hirose, Nozomu Takata, Kiyoji Nishiwaki, Makoto Koga, Yasumi Oshima and Masato Okada.

SRC-1, a non-receptor type of protein tyrosine kinase, controls the direction of cell and growth cone migration in *C. elegans*

Development **132**, 5161-5172 (2005)

（２）学術雑誌等又は商業誌における解説、総説

小谷武徳，　伊東文祥，　岡田雅人

チロシンキナーゼ

『タンパク質科学イラストレイテッド』，pp105-118，羊土社，2005年

（3）国内学会・シンポジウム等における発表

［査読あり］

伊東文祥，高田望，岡田雅人

線虫 SRC-1 は wnt シグナルに関与する

第３０回日本分子生物学会年会，No.4W8-3，パシフィコ横浜，2007 年 12 月，ワークショップ口頭発表

［査読なし］

伊東文祥，高田望，岡田雅人

線虫 SRC-1 は wnt シグナルに関与する

第３０回日本分子生物学会年会，No.1P-0342，パシフィコ横浜，2007 年 12 月，ポスター発表

伊東文祥，　広瀬隆，　岡田雅人

線虫 *C.elegans* SRC-1 は distal tip cells の移動を制御する

第２７回日本分子生物学会年会，No. 1PB-334，神戸ポートアイランド，2004 年 12 月，ポスター発表

伊東文祥，　広瀬隆，　高田望，岡田雅人

SRC-1, a non-receptor type of protein tyrosine kinase, controls the direction of cell and growth cone migration in *C.elegans*.

第２８回日本分子生物学会年会, No. 3P-0854，福岡ヤフードーム, 2005 年 12 月，ポスター発表

（4）国際会議における発表

［査読なし］

Bunsho Itoh, Takashi Hirose, Masato Okada

SRC-1, a non-receptor type of protein tyrosine kinase, controls the direction of cell and growth cone migration in *C.elegans*.

15th international *C.elegans* Meeting, No. 947A, University of California, Los Angeles, USA, June, 2005，ポスター発表

Bunsho Itoh, Takashi Hirose, Masato Okada

SRC-1 controls migration of distal tip cells in *C.elegans*.

East Asia *C.elegans* Meeting, No. 091, Awaji Island, Japan, June, 2004，ポスター発表