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Communication

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ANK Repeat-Domain of SHN-1 Is Indispensable for *In Vivo* SHN-1 Function in *C. elegans*

Won Chan Oh[1,6,7], Hyun-Ok Song[2,7], Jeong Hoon Cho[3,7], and Byung-Jae Park[4,5,*]

Shank protein is one of the postsynaptic density (PSD) proteins which play a major role in proper localization of proteins at membranes. The *shn-1,* a homolog of Shank in *Caenorhabditis elegans*, is expressed in neurons, pharynx, intestine, vulva and sperm. We have previously reported a possible genetic interaction between Shank and IP_3 receptor by examining *shn-1* RNAi in IP_3 receptor (*itr-1*) mutant background. In order to show the direct interaction of Shank and IP_3 receptor as well as to show the direct *in vivo* function of Shank, we have characterized two different mutant alleles of *shn-1*, which have different deletions in the different domains. *shn-1* mutants were observed for Ca^{2+}-related behavioral defects with *itr-1* mutants. We found that only *shn-1* mutant defective in ANK repeat-domain showed significant defects in defecation, pharyngeal pumping and fertility. In addition, we found that *shn-1* regulates defecation, pharyngeal pumping and probably male fertility with *itr-1*. Thus, we suggest that Shank ANK repeat-domain along with PDZ may play a crucial role in regulating Ca^{2+}-signaling with IP_3 receptor.

INTRODUCTION

Scaffolding proteins contain multiple domains for protein-protein interactions such as PDZ domains and SH3 domains (Pawson and Scott, 1997). The function of these proteins is to bring various proteins togther in a signaling pathway and facilitate their interaction to enhance specificity of the signal. It has been found that scaffolding proteins bind to a variety of membrane and cytoplasmic proteins. The Shank/ProSAP/SSTRIP/Synamon/Spank/CortBP family of scaffolding proteins has been identified and extensively studied (Boeckers et al., 1999a; 1999b; Du et al., 1998; Lim et al., 1999; Naisbitt et al., 1999; Tu et al., 1999; Yao et al., 1999; Zitzer et al., 1999a; 1999b). Shank contains multiple ANK (ankyrin) repeats near the N-terminus, a SH3 (Src-homology) domain, a PDZ (PSD-95, Dlg and ZO-1) domain, a long proline rich motif and a SAM (sterile alpha motif) domain at the C-terminus (Naisbitt et al., 1999). Each domain is responsible for a specific protein interaction that is important for the formation and maintenance of postsynaptic densities (PSD) in nervous system.

The SH3 domain of Shank has been known to interact with GRIP, a scaffold protein that interacts with AMPA receptors (Bruckner et al., 1999). The highly conserved PDZ domain has been shown to interact with PSD-95 associated GKAP, a scaffold protein that binds directly to NMDA receptors (Naisbitt et al., 1999). The proline-rich motif has been known to interact with Homer, which interacts with phospholipase C (PLC)-coupled metabotropic glutamate receptors. This physical interaction is required for efficient signaling between metabotropic glutamate receptors and its downstream effector, inositol 1,4,5-triphosphate (IP_3) receptor (Du et al., 1998; Tu et al., 1999; Xiao et al., 1998). Finally, the SAM domain is responsible for multimerization of Shank (Sheng and Kim, 2000). The oligomerization of Shank may induce the cross-linking of multiple sets of protein complexes (Sheng and Kim, 2000). Thus, these specific protein-protein interactions including three major postsynaptic glutamate receptors via their associated scaffold proteins imply that Shank may be a key organizer of PSD at synapses.

In order to understand the genetic information of Shank, we have previously reported the *in vivo* function of Shank by using *Caenorhabditis elegans* as a model organism (Jee et al., 2004). In that study, we demonstrated that the *C. elegans* homolog of Shank, *shn-1* is expressed in neurons, pharynx, intestine, vulva and sperm and so on. We have also shown, by using *shn-1* RNAi, a genetic interaction between *shn-1* and *itr-1*, the IP_3 receptor gene, in the control of defecation rhythms. In this study, we further analyze the *in vivo* function of *shn-1* by analyzing *shn-1* mutants. We investigated the genetic interaction between *shn-1* and *itr-1* in various Ca^{2+}-related signaling pathways including defecation. Interestingly, we found that *shn-1* regulates defecation, pharyngeal pumping and probably male fertility along with *itr-1* in *C. elegans*. Furthermore, we also found that only the *shn-1* mutant having a complete deletion in ANK repeat-domain displayed defects in all examined phenotypes. Thus, our data suggest that the ANK repeat-domain of *shn-1* is crucial for the normal function of Shank in various tissues such

[1]Department of Life Science, Gwangju Institute of Science and Technology, Gwangju 500-712, Korea, [2]Department of Infection Biology, Zoonosis Research Center, Wonkwang University School of Medicine, Iksan 570-749, Korea, [3]Division of Biology Education, College of Education, Chosun University, Gwangju 501-759, Korea, [4]Department of Life Science, Hallym University, Chunchon 200-702, Korea, [5]Institute of Bioscience and Biotechnolgoy, Hallym University, Chunchon 200-702, Korea, [6]Present address: Center for Neuroscience, Cell and Developmental Biology Graduate Group, University of California, Davis, CA 95616, USA, [7]These authors contributed equally to this work.
*Correspondence: bjparkkk@hallym.ac.kr

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as intestine, pharynx and reproductive organs.

MATERIALS AND METHODS

C. elegans strains and culture conditions

Standard methods for maintaining and growing *C. elegans* strains were used as described by Brenner, 1974. Wild type Bristol N2, *shn-1(ok1241)* II, *shn-1(gk181)* II, *itr-1(sa73)* IV, *dpy-5(e907)* I, *unc-13(e1091)* I, *him-8(e1489)* IV strains were obtained from the *Caenorhabditis* Genetics Center (CGC) at the University of Minnesota, USA. The *shn-1 (tm488)* II mutant was obtained from the National BioResourse Project, Japan. All strains were out-crossed more than seven times.

Genotyping *shn-1* and *itr-1* deletion mutants by PCR

Deleted regions of *shn-1* mutants were confirmed by a nested PCR method. Primers were designed based on the predicted sequences spanning the full genomic DNA of *shn-1*. Primers for *shn-1(tm488)* were as follow: outer upstream primer (5′-ACT ACG GGC TCT TCC TTC-3′) and downstream primer (5′-TGT GAG TGA ATG GTG ACG-3′); inner upstream primer (5′-AAT TCC TTC CGA TCC AAC-3′) and downstream primer (5′-CAA CCC AAA TCT CAA CAA-3′); deletion upstream primer (5′-GCT GTG AAC AAG CAA GGA CA-3′) was paired with inner downstream primer to confirm homozygous deletion mutant. The primers used for *shn-1(ok1241)* were: outer upstream primer (5′-CCA ATT GGA CTT ACA CCG CT-3′) and downstream primer (5′-AGC AAA AAT CGG ACA CAA CC-3′); inner upstream primer (5′-TTC GAA TGA TCA AGT CGC TG-3′) and downstream primer (5′-TCC CGA ACA GTT TCT CGA AG-3′); deletion upstream primer (5′-CCG GTG ACA CGA TTA CAC TG-3′) was paired with inner downstream primer to confirm homozygous deletion mutant. The primers for *shn-1(gk181)* were: outer upstream primer (5′-AGG CAA ATA GGC AGG CAC AT-3′) and downstream primer (5′-CCC CCA TTC AAC GAC TCC TG -3′); inner upstream primer (5′-CGA CCA ACC CGA CCT TAT GT-3′) and downstream primer (5′-TGC GCA TCA TGC GTG TCT GA-3′); deletion upstream primer (5′-GCT GTG AAC AAG CAA GGA CA-3′) was paired with deletion downstream primer (5′-CAA CCC AAA TCT CAA CAA-3′) to confirm homozygous deletion mutant.

The point mutation of *itr-1(sa73)* was confirmed by PCR and sequencing. The primers used for *itr-1(sa73)* were: outer upstream primer (5′-AAG TTG ACG AGA AGG AGA GAA CG-3′) and downstream primer (5′-GTG ATA TTG TGC TCT TCC GCT AG-3′); inner upstream primer (5′-TCA AAG GGA CGT AGT TCT GAC AGC-3′) and downstream primer (5′-TGA AGA GCT GAA TAT GGC GCT TC-3′).

Bacteria-mediated feeding RNAi of *shn-1*

The *shn-1* RNAi clone was purchased from Geneservice. For the bacteria-mediated feeding RNAi, L2 to L3 worms (P_0) were transferred onto plates seeded with *E. coli* HT115 (DE3) strain harboring L4440 control plasmids or *shn-1* RNAi plasmids. Phenotypes were analyzed by observing F_2 progenies.

Phenotype analysis

The defecation cycles of one day old adult animals were observed at 20°C. The interval time was measured from the pBoc to the next pBoc as described (Park et al., 2001). The pharyngeal pumping was measured by counting terminal bulb contractions at 20°C as previously described (Modified from Abada et al., 2009; Walker et al., 2004). Hermaphrodite fertility was analyzed by measuring brood size. The brood size was measured by counting progenies (F_1) after placing and daily transferring individual worms (P_0) on seeded plates at 16°C, 20°C and 25°C as described (Kim et al., 2008; Lee et al., 2004). Male fertility was analyzed by mating efficiency test, sperm competition and male mating behavior. Mating efficiency tests were performed as described earlier (Liu and Sternberg, 1995) with minor modifications. Three hermaphrodites of *dpy-5(e907)* were mated with one male of each strain at 20°C. After 24 h crossed progenies were counted as described by Park et al. (2001). Sperm competition experiments were performed as previously described (Singson et al., 1999). Briefly, one hermaphrodite of *dpy-5(e907)* was mated with three males of each strain. Crossed progenies were scored after two days to avoid scoring an ambiguous Dpy phenotype. The percentage of self-progeny versus crossed progeny produced from each individual was determined. Male mating behavior (Gower et al., 2005) was assayed by placing a single young adult male on a mating plate containing 20 young adults of *unc-13(e1091)* hermaphrodites. To score turning ability, turns were scored in the 3 min observation period for each male. Each turn was examined and categorized as described by Loer and Kenyon (1993). Briefly, when the tail remained in contact with the hermaphrodite throughout the turn, it was scored as “good”. When the tail lost contact for a while but quickly got contact to the opposite side, it was scored as “sloppy”. A “missed” was scored when the male tail completely lost contact.

RESULTS AND DISCUSSION

The identification of three alleles of *shn-1* mutants in *C. elegans*

Previously we have shown the genetic interaction of *shn-1* and *itr-1* by treating *shn-1* RNAi on *itr-1* mutants. In this study, in order to show more direct interaction between *shn-1* and *itr-1*, we characterized three loss-of-function mutant alleles of *shn-1*: *shn-1(tm488)*, *shn-1(gk181)* and *shn-1(ok1241)*. The *shn-1 (tm488)* mutant has a 1,537 bp deletion from the second intron to the end of the sixth exon, which covers the ANK repeat and PDZ domain. The *shn-1(gk181)* mutant has a 1,458 bp deletion from the most of the fourth exon to the sixth intron, which includes most of ANK repeat and the complete PDZ domain. The *shn-1(ok1241)* mutant has a deletion of 2,245 bp from the end of the fifth exon to the most of the eighth exon covering the entire PDZ domain and the Proline rich region (Figs. 1A and 1B). The genotypes of all three different mutant alleles were confirmed by nested and deletion PCR of single worm using specific primer sets designed upon the deleted regions (Fig. 1C). Interestingly, we found that the *shn-1(gk181)* mutant still showed a product from deletion PCR. It has been previously reported that Shank contains multiple sites for alternative splicing (Boeckers et al., 1999a; Lim et al., 1999; Yao et al., 1999; Zitzer et al., 1999a) and thus the transcript encoding proteins are alternatively spliced. Therefore, this may explain the positive PCR band in *shn-1(gk181)* mutant. However, we cannot rule out the possibility that the deleted fragment is inserted somewhere else in other chromosomes. Thus, we characterized for further study only two deletion mutants of *shn-1*, *shn-1(tm488)* and *shn-1(ok1241)*, and excluded the *shn-1(gk181)* mutant allele.

shn-1 regulates defecation with *itr-1*

We have previously shown that *shn-1* RNAi treatment enhanced defecation defect only in the *itr-1* loss-of-function mutant but not in wild-type *C. elegans*. In this study, we examined whether or not the *shn-1* mutant alone exhibits defecation defect. As shown in Table 1, the *shn-1(tm488)* mutant showed a

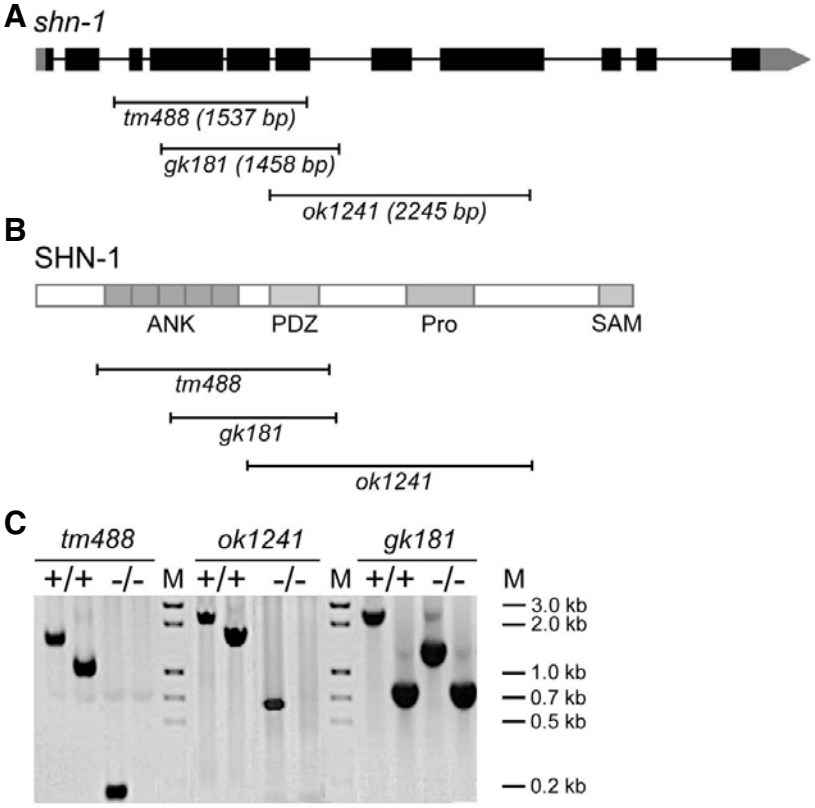

Fig. 1. Identification of *shn-1* mutants. (A) Genomic organization and gene structure of *shn-1*. Exons are indicated by black boxes. Each of the deleted regions of the three *shn-1* mutant alleles are indicated with black line flanked by vertical lines. (B) Functional domain of SHN-1 and *shn-1* mutants. *shn-1(tm488)* mutant has a deletion in the ANK repeats and PDZ domain. *shn-1(gk181)* mutant has a deletion covering half of the ANK repeats and the entire PDZ domain. *shn-1(ok1241)* mutant has a deletion covering the entire PDZ domain and the Proline rich motif. (C) *shn-1* deletion mutants (-/-) were confirmed by nested and deletion PCR (each first and second lane in the gel image). Both *shn-1(tm488)* and *shn-1(ok1241)* mutants showed a smaller band in nested PCR and no band in deletion PCR compared to wild-type animals (N2, +/+) suggesting that both *shn-1* deletion mutants are homozygous mutant lines. However, the *shn-1(gk181)* mutant still had a band similar in length to that of the wild-type animal in deletion PCR. M, DNA size marker.

Table 1. Analysis of the defects of *shn-1* mutants in defecation and pharyngeal pumping

	Defecation interval (s)	Pharyngeal pumping (pumps/min)
N2	53 ± 01 (10)	248 ± 05 (50)
shn-1(tm488)	71 ± 06 (10)	203 ± 18 (50)
shn-1(ok1241)	52 ± 01 (10)	241 ± 12 (40)
N2_L4440	53 ± 01 (10)	256 ± 09 (10)
N2_*shn-1* RNAi	71 ± 02 (10)	213 ± 14 (10)
itr-1(sa73)(lf)	109 ± 07 (10)	176 ± 18 (50)
itr-1(sa73)(lf); shn-1(tm488)	113 ± 14 (10)	187 ± 18 (50)*

Mean ± STDEV
n number is shown in parenthesis.
**shn-1(tm488)* vs *shn-1;itr-1(lf)*, $p < 10^{-6}$; *itr-1(lf)* vs *shn-1;itr-1(lf)*, $p = 0.002$

significantly prolonged defecation interval compared to wild-type animal. However, *shn-1(ok1241)* mutant did not show any defect in defecation suggesting a phenotypic variation between the two different mutant alleles. We further confirmed whether the *shn-1* mutation is indeed responsible for the defecation defect by using bacteria-mediated feeding RNAi, despite previously reporting no effect of *shn-1* RNAi in wild-type animals by microinjection method. We used a genomic fragment of *shn-1* in bacteria-mediated feeding RNAi experiment instead of a cDNA fragment of *shn-1* that we have used previously. The bacteria-mediated feeding RNAi method may reduce possible damage to the *C. elegans* which could occur during microinjection thus thereby keeping animals relatively healthy. Thus, we speculate that the feeding RNAi method could produce different results from the former microinjection-mediated RNAi. As shown in Table 1, *shn-1* RNAi fed wild-type animals phenocopied the defecation defect suggesting that *shn-1* is indeed responsible for normal defecation in *C. elegans*.

Next, we further explored the genetic interaction of *shn-1* and *itr-1* in regulating defecation. Since only the *shn-1(tm488)* mutant allele displayed defecation defect, we constructed a *shn-1(tm488);itr-1(sa73)(lf)* double mutant and observed its defecation behavior. As described previously (Dal Santo et al., 1999; Lee et al., 2004), *itr-1(sa73)(lf)* mutant showed much prolonged defecation intervals as compared to wild-type animal (Table 1). Interestingly, we found that *shn-1(tm488);itr-1(sa73) (lf)* double mutant showed a similar defecation interval to that of *itr-1(sa73)(lf)* single mutant (Table 1). This suggests that *shn-1* acts at upstream of *itr-1* in regulating defecation.

shn-1 regulates pharyngeal pumping with *itr-1*

itr-1 has been also involved in pharyngeal pumping in *C. elegans* (Walker et al., 2002) and *shn-1* is known to be expressed in pharynx (Jee et al., 2004). Thus, we analyzed whether *shn-1* is also involved in regulation of pharyngeal pumping with *itr-1*. In order to elucidate the function of *shn-1* in pharyngeal pumping, we measured the pharyngeal pumping rate in *shn-1* and *shn-1;itr-1* double mutants. As shown in Table 1, *shn-1(ok1241)* mutants showed similar pharyngeal pumping to that of wild-type animals. However, *shn-1(tm488)* mutants displayed significantly reduced pharyngeal pumping rate as compared to wild-type animals. This reduced pharyngeal pumping of *shn-1 (tm488)* mutants was confirmed by *shn-1* feeding RNAi therefore suggesting that *shn-1* is required for normal pharyngeal pumping. We further examined the pharyngeal pumping of the

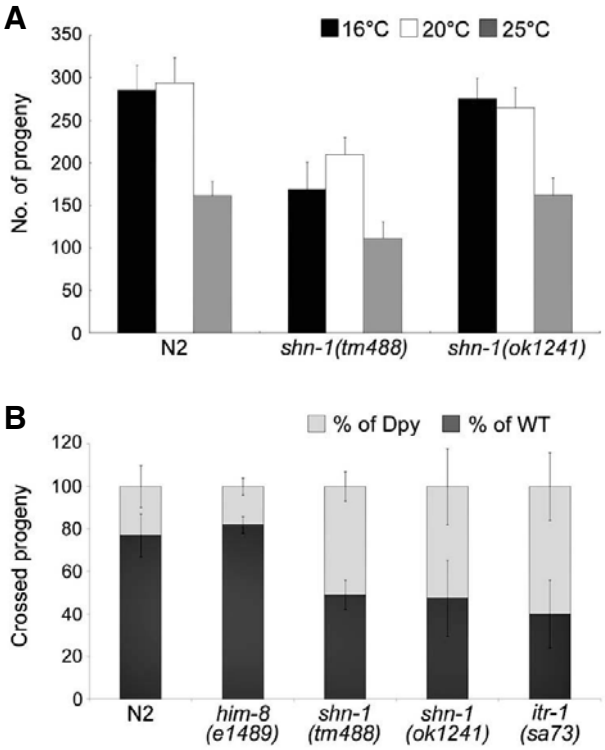

Fig. 2. *shn-1* is required for normal fertility in *C. elegans* hermaphrodites and males. (A) Hermaphrodite fertility of *shn-1* mutant was examined by measuring brood size at 16°C, 20°C and 25°C. *shn-1(tm488)* mutant was defective in hermaphrodite fertility at all three temperatures. (B) Male fertility of the *shn-1* mutant was tested by male mating efficiency. *dpy-5(e907)* hermaphrodites were crossed with *shn-1* mutant male and crossed progenies (WT-like animals) were scored. The percentage of crossed progeny was significantly reduced in both *shn-1(tm488)* and *shn-1(ok1241)* mutants.

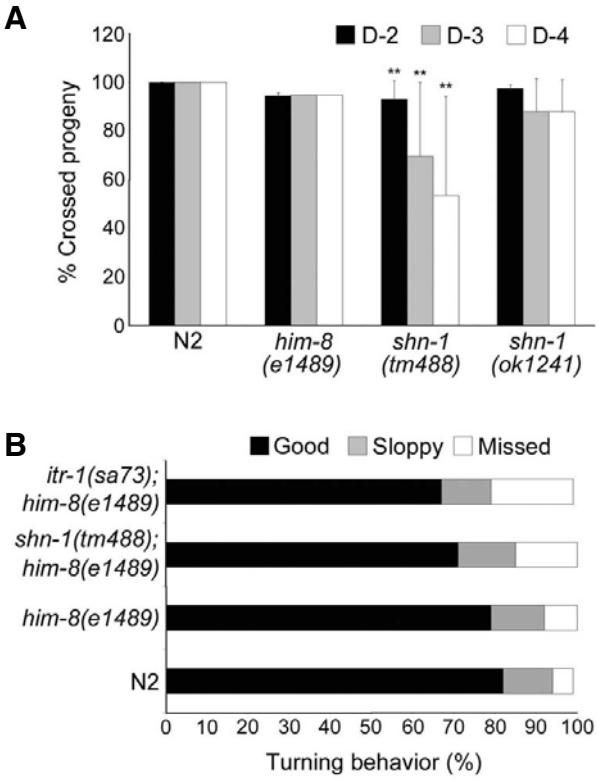

Fig. 3. *shn-1* is required for sperm function and male turning behavior. (A) Sperm function of *shn-1* mutants was investigated by observing sperm competition. *dpy-5(e907)* hermaphrodites were crossed with *shn-1* mutant male and crossed progenies (WT-like animals) were scored after 2 days. The *shn-1(tm488)* mutant displayed less competent sperm function compared to wild-type animals. N2 vs. *shn-1(tm488)* mutant; **, $p < 0.001$. (B) Male turning behavior of *shn-1* mutants was observed during mating. Each turn was categorized as good (black), sloppy (gray) or missed (white). Similar to the *itr-1(sa73)(lf)* mutant, the *shn-1(tm488)* mutant showed defective male turning behavior compared to wild-type animals.

shn-1(tm488);itr-1(sa73)(lf) double mutant to determine their genetic epistasis and found that the double mutant exhibited similar pharyngeal pumping to that of *itr-1(sa73)(lf)* single mutant (Table 1). Thus, we suggest that *shn-1* acts at upstream of *itr-1* to regulate pharyngeal pumping.

shn-1 is essential for normal fertility in both hermaphrodite and male

Previously, we have used microinjection and observed no obvious abnormalities in *shn-1* RNAi treated animals. However, in this study we have demonstrated that the *shn-1* mutant displayed obvious abnormalities in defecation and pharyngeal pumping suggesting that using *shn-1* microinjection was not enough to induce specific defects. We further examined other phenotypes in *shn-1* mutant to determine any other specific functions of *shn-1*. We first observed fertility of *shn-1* mutant because we have previously shown that *shn-1* is expressed in reproductive organs in *C. elegans* (Jee et al., 2004). The two alleles of *shn-1* mutant, *shn-1(tm488)* and *shn-1(ok1241)*, were examined for fertility in hermaphrodites and males. As shown in Fig. 2A, the *shn-1(tm488)* mutants showed significantly reduced brood size of hermaphrodites (169 ± 32, n = 23 at 16°C; 210 ± 20, n = 47 at 20°C; 111 ± 20, n = 18 at 25°C) as compared to wild-type animals (285 ± 30, n = 44 at 16°C; 294 ± 30, n = 43 at 20°C; 161 ± 17, n = 18 at 25°C) at all three temperatures. However, the brood size of *shn-1(ok1241)* mutant hermaphrodites (276 ± 24, n = 28 at 16°C; 265 ± 24, n = 32 at 20°C; 162 ± 21, n = 18 at 25°C) was comparable to that of wild-type animals at all three temperatures (Fig. 2A). Unfortunately, we were not able to see the same defects in brood size by feeding RNAi of *shn-1* (N2 fed with L4440 control, 339 ± 15, n = 32; N2 fed with *shn-1* RNAi, 328 ± 26, n = 10). We assume that both *shn-1* RNAi by microinjection and feeding was not enough to knock *shn-1* down to show its function in hermaphrodite fertility.

We further investigated the function of *shn-1* in male fertility. *shn-1* mutant males were crossed with *dpy-5(e907)* hermaphrodites and resulting crossed progenies were scored. As shown in Fig. 2B, both alleles of *shn-1* mutant displayed significantly reduced crossed progenies (wild-type like animals) suggesting a much decreased male fertility. Particularly, the *shn-1(tm488)* mutant showed much more significantly reduced male fertility as compared to wild-type and *him-8(e1489)* male background control animals (% WT, $p < 10^{-5}$, % Dpy, $p < 10^{-5}$). Although the *shn-1(ok1241)* mutant also showed reduced male fertility, it exhibited a large variation among tested animals thereby the significance when compared to control animals (% WT, $p < 0.05$, % Dpy, $p < 0.05$) is relatively lower than the *shn-1(tm488)*

mutant allele. The defect in male fertility of *shn-1(tm488)* mutant was comparable to that of *itr-1(sa73)(lf)* mutant. As previously described, the *itr-1(sa73)(lf)* mutant showed a severe male fertility defect (Gower et al., 2005). Taken together, these results suggest that *shn-1* is essential for both hermaphrodite and male fertility in *C. elegans*.

shn-1 is required for normal sperm function and male mating behavior

Male fertility is basically dependent on sperm function and male mating behavior which is composed of a set of steps. In order to elucidate the specific function of *shn-1* in male fertility, we examined sperm function and the mating behavior of *shn-1* mutants. We first investigated the sperm function of *shn-1* mutants by observing sperm competition between male-derived sperm and hermaphrodite-derived sperm (Singson et al., 1999). *shn-1* mutant males were crossed with *dpy-5(e907)* mutant hermaphrodites and resulting crossed progenies showing wild-type like phenotype were scored after two days for each of the next three days. As shown in Fig. 3A, the *shn-1(tm488)* mutant but not the *shn-1(ok1241)* mutant displayed significantly reduced crossed progenies compared to wild-type and *him-8(e1489)* male background control animals. This indicates that the sperm derived from *shn-1(tm488)* mutant males is less competent for fertilization than the sperm derived from *dpy-5 (e907)* hermaphrodites. The sperm competition is decided by sperm motility and the number of spermatozoa transferred from the male to hermaphrodite (Singson et al., 1999). Since we have previously shown that *shn-1* is expressed in sperm and pseudopodia, structures for motility of mature sperm, we speculate that *shn-1* male sperm may be less motile and therefore the amount of sperm transferred during mating is reduced. Next, we observed the mating behaviors of *shn-1* mutant males. Male mating behaviors are composed of a series of steps: hermaphrodite recognition, backing, turning, vulval location and copulation. We specifically examined the male turning behavior of *shn-1* males since it has been described that *itr-1* plays important roles in mating especially in male turning behavior (Gower et al., 2005). Similarly most of our studies also show the genetic relation of *itr-1* and *shn-1*. Furthermore, we have previously shown that *shn-1* is expressed in the mail tail especially in the presumptive rays 7-9, which are essential for turning behavior (Barr and Garcia, 2006). As shown in Fig. 3B, the *shn-1(tm488)* mutant male failed to turn properly compared to wild-type and *him-8(e1489)* male background control animals. Similar to the *itr-1(sa73)(lf)* mutant, *shn-1(tm488)* mutant showed increased number of sloppy and missed turns (See "Materials and Methods"). This demonstrates that *shn-1* has role(s) in regulating male turning behavior. Taken together, these data therefore suggest that defective sperm function and mating behavior may be responsible for the reduced male fertility of *shn-1(tm488)* mutant.

ANK repeat-domain is important for SHN-1 functions in various tissues

Here, we reported our continued study of *C. elegans shn-1* using two different *shn-1* mutant alleles. One [*shn-1(tm488)*] has a complete deletion of ANK repeat and PDZ domain and the other [*shn-1(ok1241)*] has a complete deletion of PDZ and proline-rich motif. This allele specificity made it possible to access the domain-specific function of *shn-1* in *C. elegans*. In this study, we investigated a variety of phenotypes in *shn-1* mutants based on our previous study showing the tissue expression pattern of *shn-1* and the genetic interaction of *shn-1* and *itr-1*. In this study we examined defecation, pharyngeal pumping, fertility, sperm competition and male mating behavior. Our results showed that the *shn-1(tm488)* mutant displayed severe defects in all phenotypes whereas the *shn-1(ok1241)* mutant exhibited a mild defect only in male fertility. Thus, we speculate that the ANK repeat-domain may be crucial for *shn-1* function in *C. elegans*.

Shank has multiple domains for protein-protein interaction. Most identified binding proteins of Shank interact with the SH3, PDZ and proline-rich domain of Shank. However, little is known about potential protein candidates interacting with the ANK repeat-domain of Shank. Interestingly, we have shown in this study that the *shn-1* mutant deleting entire ANK repeat-domain induced severe defects in various physiological phenotypes related with *itr-1* function. Although Homer is known to link IP_3 receptor with Shank via proline-rich motif of Shank to regulate dendritic spine maturation in brain (Sala et al., 2001), our results provide a plausible possibility that, at least in *C. elegans*, IP_3 receptor could directly interact with Shank. This IP_3 receptor interaction with Shank via its ANK repeat-domain in tissues including intestine, pharynx and reproductive organs may regulate the tissue-specific physiological function controlled by Ca^{2+}. Our novel findings that demonstrate the important role of ANK repeat-domain may help to elucidate new functions of Shank in the future.

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