The C2H2 zinc-finger protein SYD-9 is a putative posttranscriptional regulator for synaptic transmission

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Chemical synapses mediate neuronal communication through the regulated release of neurotransmitters from synaptic vesicles. Extensive studies have focused on the molecular machinery that mediates and regulates exocytosis, the process in which membrane fusion between synaptic vesicles and the plasma membrane leads to neurotransmitter release and endocytosis, the process that recovers synaptic vesicles from the plasma membrane (1, 2). The release of neurotransmitters is stimulated by the influx of Ca2+ at the presynaptic active zones. Protein components of the vesicle membranes (synaptobrevin, SNB) and plasma membranes [syntaxin and SNAP-25 (synaptosome-associated protein of 25 kDa)] form a SNARE complex that functions as a minimal fusion machine to drive vesicle fusion (3–5). The formation and conformational change of the SNARE complex requires modulation by other proteins (6–9). Synaptotagmin (SNT) is a vesicle membrane protein that binds to Ca2+- and forms complexes with phospholipids and SNARE complexes. It is a proposed Ca2+- sensor for Ca2+- dependent exocytosis (10–12). SNT-1 knockout mice display selective loss of Ca2+-triggered fast exocytosis, and loss of SNT function in Caenorhabditis elegans and Drosophila leads to a large reduction of exocytosis (13, 14). Disrupting the function of UNC-13/mUNC13 and UNC-18/mUNC18 (UNC, uncoordinated) also leads to a reduction in exocytosis and an accumulation of unreleased synaptic vesicles (15, 16). After exocytosis, the recycling of synaptic vesicles serves as a major mechanism to replenish synaptic vesicle pools (2, 17, 18). The best-characterized mechanism is clathrin-mediated endocytosis, in which the adaptor proteins AP2 and AP180 recruit a clathrin matrix at the sites of endocytosis, generally considered to be at plasma membranes flanking the active zones, to initiate the membrane invagination and budding of vesicle precursors. The budded vesicles are cleaved from the plasma membrane and stripped of clathrin, and they mature into synaptic vesicles. Synaptojanin, a phosphoinositid phosphate that forms a protein complex with AP2, is required for the budding and uncoating of vesicle precursors and the maturation of synaptic vesicles from the resulting endosomes (19, 20). Endophilin binds both synaptojanin and dynamin and is required for multiple steps of endocytosis through localizing synaptojanin at the synapse (21, 22). In Drosophila and C. elegans, synaptojanin and endophilin mutants show severe depletion of synaptic vesicles, and synaptic vesicle precursors are arrested at various endocytotic stages (21–23). SNT also regulates endocytosis through its interaction with AP2 (24). Drosophila and C. elegans SNT mutants display depletions of vesicles at synapses (13, 25). Furthermore, SNT is required during endocytosis for the replenishment of vesicles at Drosophila neumuscular junctions (NMJs) (26).

Recent studies suggest that specific regulation or fine-tuning of synaptic activity can also be achieved at posttranscriptional levels (27). Rapid protein synthesis is associated with changes in synaptic activity (28–31). Alternative splicing of ion channels and other synaptic proteins plays a key role in modulating the activity of synapses (27, 32). Identification of a vertebrate splicing factor, Nova, which is not only nervous system-specific but also exhibits a profound preference for RNAs encoding synaptic proteins (33), suggests that transcriptional regulatory machinery dedicated to synaptic genes is present. Two neuronal nuclei-restricted RNA-binding proteins, UNC-75 and EXC-7, are required for synaptic transmission without affecting neuronal development or synapticogenesis in C. elegans (34). UNC-75 is the C. elegans ortholog of the C. elegans/Brnunol family of proteins that control pre-mRNA splicing in mammals (35, 36) and mediate translation repression in Drosophila oocytes (37, 38). EXC-7 is the C. elegans ELAV (embryonic lethal abnormal vision)/Hu family protein, a neuron-specific alternative splicing factor in Drosophila (39–42) and neuromuscular RNA-processing regulator in mammals (43–45). We identified a previously uncharacterized nuclear protein, syd-9 (syd, synaptic defective), in C. elegans that specifically regulates synaptic transmission: in particular, endocytosis. syd-9 mutants are synthetically lethal with unc-75 mutations. We propose that syd-9 may regulate RNA splicing events that are specific for synaptic transmission.

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Abbreviations: AP, adaptor protein; NMJ, neuromuscular junction; SNB, synaptobrevin; SNT, synaptotagmin; SNG, synaptojanin; RNAI, RNA interference.

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Results

Identification and Behavioral Phenotypes of syd-9 Mutants. jul(s1) is a synaptic vesicle marker that expresses the GFP-labeled vesicle protein SNB (SNB-1::GFP) in GABAergic motoneurons of C. elegans (46). We identified a mutant named syd-9(ju49) that displays moderate diffusion and reduction in the brightness of the individual SNB-1::GFP punctum at presynaptic termini (Fig. 1 A–C). A second syd-9 allele, ok1110, was later generated by the C. elegans Gene Knockout Consortium (Oklahoma City, OK). Both alleles of syd-9 mutants display a drastic decrease in locomotion and a significant delay in development. Wild-type (WT) C. elegans displays a continuous and smooth sinusoidal wave that does not vary during development. By contrast, syd-9 mutant animals are sluggish, with very slow locomotory activity and no obvious sinusoidal wave (Fig. 1 M–O). Although the locomotion defects are obvious upon hatching for ok1110 animals, ju49 animals start to display obvious defects from the late second-larval stage. Both alleles of syd-9 mutants are also slow to develop, exhibiting a thinner and smaller body size and producing very few progeny, with ok1110 being slower than ju49 animals (Fig. 1 M–O). All syd-9 animals display a starved appearance characterized by enlarged gut lumen as well as weak and sporadic pharyngeal pumping and enteric activities. syd-9 mutants also retain eggs. Severity of the phenotypes and complementation tests by deficiencies suggest that ju49 represents a severe loss-of-function allele, whereas ok1110 represents a close to null allele of the syd-9 gene. Because ok1110 was obtained toward the end of the study, some studies were carried out only in the ju49 allele.

syd-9 Regulates Synapse Morphology and Presynaptic Protein Distribution. C. elegans locomotion, pharyngeal pumping, and egg laying are controlled by defined neural circuits that innervate specific muscle cells. These phenotypes suggest a decrease in general neuronal or muscle activities in syd-9 animals. The number, position, and morphology of neurons and their axonal projections, as visualized by a panneural GFP marker, edl20, and a GABAergic-specific GFP marker, jul(s76), were normal in both alleles of syd-9 mutants (Fig. 7, which is published as supporting information on the PNAS web site). The ventral and dorsal nerve cords appeared slightly defasciculated, with some extra branching in ok1110 animals, which is likely a secondary phenotype caused by synapse defects (see below). We did not detect gross abnormality in body wall, uterine, and vulva muscles by phalloidin staining.

The generally normal morphology of the nervous system and musculature led us to examine whether the behavioral defects were caused by defects at synapses. In addition to the SNB-1::GFP marker, abnormalities were observed in the distribution of other synaptic proteins, including the vesicle protein synaptogyrin SNG-1 (47), the presynaptic Ser/Thr kinase SAD-1 (48, 49), and the postsynaptic GABA receptor UNC-49. In ju49 mutants, SNB-1::GFP, SNG-1::GFP, SAD-1::GFP, and UNC-49::GFP puncta were moderately dimmer and diffuse along the axons (Fig. 1 B, E, H, and K). These markers were severely diffuse and reduced in brightness in ok1110 animals (Fig. 1 C, F, I, and L). There was also abnormal accumulation of SNG-1::GFP in some neuronal cell bodies (Fig. 1 D and E).

syd-9 Specifically Regulates Synaptic Transmission. The localized defects in synapse morphology in syd-9 mutants suggest that SYD-9 regulates synaptic activity specifically. We examined the spontaneous and evoked neurotransmitter release at NMJs in syd-9(ju49) animals (50, 51). The evoked response was measured by recording the postsynaptic current in voltage-clamped muscles resulting from stimulation of the ventral nerve cord (Fig. 2 A). syd-9 mutants showed a 60% decrease in the amplitude of the evoked response,
indicating a dramatic decrease in regulated release. Compared with WT animals, the frequency of endogenous synaptic events at syd-9 mutant NMJs was reduced by 70%, whereas the amplitudes of the remaining release events remained similar (Fig. 2B). Thus, the reduced synaptic transmission in syd-9 mutants likely results mainly from a decrease in presynaptic vesicle fusion rather than from alterations in postsynaptic response to neurotransmitters. Exposure to aldicarb, an inhibitor for acetylcholine esterase, causes hyper-contraction and lethality in C. elegans adults displayed sensitivity to aldicarb, an inhibitor for acetylcholine esterase, causes hyper-contraction and lethality in C. elegans adults (Fig. 3A). Syd-9 protein expression is out of the focusing plane. (C–E) Double staining with GFP and DAPI in hps6 animals carrying only the SYD-9::GFP isoform. SYD-9::GFP signal was predominantly localized at the interchromatin region (arrows). (F–H) Staining of WT animals with SYD-9 antibody N (F and G) and SYD-9 antibody C (H). SYD-9 is expressed in the nuclei of body wall muscles (arrow) and ventral cord motorneurons (arrowhead). At higher magnification, SYD-9 expression shows a distinct subnuclear pattern similar to that of SYD-9::GFP; in particular, antibody C shows a speckle-like pattern (G and H). Antibody N staining in the retrovesicular ganglion neurons (G) and antibody C staining in the nerve ring ganglion neurons (H) are shown. (I and J) Western blot analysis of mixed-stage C. elegans lysates by using antibody N (I) and antibody C (J). Tubulin is the loading control for lysate. (Scale bars: A, 20 μm; B and F, 3 μm; C–E, G, and H, 1 μm.)

Two antibodies were generated against SYD-9 proteins to analyze their expression (Fig. 4 F–H). One antibody, which was developed against the N-terminal region of the large isoforms, recognized SYD-9C and -9D (Fig. 4F). The other antibody, generated against the C-terminal portion, recognized only the smallest isoform, SYD-9B (Fig. 4G). We were unable to detect SYD-9A. In ok1110 animals, a deletion from exon 2 results in a frameshift for SYD-9A, -9C, and -9D transcripts that abolishes their expression. Whole-mount staining using the antibody against SYD-9B also showed a drastic reduction in ok1110 animals (not shown). In the ju49 allele, a point mutation converts Arg-339 (as in SYD-9D) into a premature stop codon immediately before the last C2H2 domain that is shared by all SYD-9 isoforms (Fig. 3B and C). This mutation led to a truncation of SYD-9C and SYD-9D and a severe reduction in the level of SYD-9B (Fig. 4J and J).

To examine whether all SYD-9 isoforms contribute to the syd-9(ju49) phenotype, we generated minigenes that express each isoform with their predicted endogenous promoter regions (Fig. 3B; see also Table 1, which is published as supporting information on the PNAS web site).
SYD-9 Protein Is Predominantly Expressed in the Nuclei of Neurons and Muscles. Whole-mount staining with SYD-9 antibodies showed specific staining in the nuclei of neurons and muscles in WT animals (Fig. 4 F–H), whereas in ju49 and ok1110 animals, the staining either disappeared completely or was drastically reduced. Both antibodies revealed staining patterns enriched at specific subdomains within the nuclei (Fig. 4 G and H). The C-terminal antibody detected a speckle-like pattern exclusively in the nuclei (Fig. 4H). The N-terminal antibody stained a broader region but also included the speckle-like pattern in the nuclei (Fig. 4G), suggesting that SYD-9 isoforms may have slightly different subcellular distribution.

SYD-9::GFP expression is consistent with the expression pattern of endogenous SYD-9 proteins. A functional SYD-9::GFP construct was generated by inserting GFP after the C-terminal zinc-finger domain so that it tags all SYD-9 isoforms (Fig. 3B). GFP expression was observed throughout the nervous system and muscle cells (Fig. 4 A and B). SYD-9::GFP also accumulates in speckle-like patterns. To further define the subnuclear pattern, we counterstained for DNA in hps6, a strain that expresses the smallest isoform, SYD-9B::GFP. SYD-9B::GFP preferentially accumulates at interchromatin regions (Fig. 4 C–E). Speckle-like patterns at the interchromatin regions are hallmarks for many RNA-binding proteins and proteins regulating pre-mRNA processing (56), suggesting that SYD-9 is likely involved in pre-mRNA processing.

SYD-9 Is Predominantly Required in Neurons to Rescue Synaptic and Behavioral Defects. The expression of SYD-9 in both muscles and neurons raises the question of where SYD-9 function is required. We performed mosaic analysis using syd-9(ju49);hpEx[sur-5::GFP;pH32] in which syd-9 mutants were rescued by an extrachromosomal array that carried the rescuing genomic fragment and a marker that expressed GFP in cell nuclei that inherit the array (57). We generated five syd-9 mosaic animals that had lost the array in the lineage that generates all neurons but retained it in most muscle cells. All these animals were reminiscent of syd-9 mutants, with severe locomotion defects, slow development, thin bodies, and low brood sizes. By contrast, 10 of 20 mosaic animals that maintained the rescuing array in the neuronal lineage, but not the muscle lineage, displayed normal sinusoid locomotion and healthy body morphology in larvae. As they approached adulthood, the rescue was less apparent and brood size was still low, suggesting that rescue was partial. The mosaic analysis suggests that neuronal SYD-9 plays a more dominant role in locomotion.

We next used RNA interference (RNAi) to determine the functional requirement for SYD-9. A tissue-specific down-regulation of SYD-9 expression is possible because the C. elegans nervous system is generally resistant, whereas muscles are susceptible, to RNAi effects (58). We used the hps6 line (Fig. 5A) to monitor the effectiveness of the RNAi on endogenous SYD-9 proteins. After treating hps6 animals with dsRNA against all syd-9 isoforms, GFP completely disappeared from the nuclei of muscles, whereas the level of GFP in neurons was unchanged (Fig. 5C). These animals displayed sinusoidal and active locomotion and healthy body morphology, suggesting that the muscle SYD-9 is largely dispensable (Fig. 5D; see also Movies 1 and 2, which are published as supporting information on the PNAS web site). Mutation in the rrf-3 gene leads to an increase in RNAi sensitivity in all tissues, including neurons (59). Consistently, >90% of rrf-3;hps6 animals treated with the same RNAi construct displayed a significant decrease of neuronal GFP intensity and absence of GFP in muscles (Fig. 5E). Sluggish locomotion was observed in these animals, with some displaying starved-looking body morphology and low brood size (Fig. 6F; see also Movie 3, which is published as supporting information on the PNAS web site), similar to syd-9

EM analysis of syd-9(ju49) mutants suggests that vesicle recycling is impaired. (A) Sections from WT and ju49 ventral nerve cord showing a NMJ. n, neuron; m, muscle; PD, presynaptic density; c, cisternae. (B) The average number of vesicles per synaptic profile is reduced in ju49 animals. (C) The number of large membrane-bound organelles (cisternae) is increased in ju49 profiles. (D) The distribution of plasma membrane-contacting vesicles in ju49 mutants is redistributed away from the presynaptic specialization toward a region involved in vesicle endocytosis. Error bars show SEM.

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on the PNAS web site). The robust rescue of syd-9(ju49) phenotypes could only be achieved by cotransforming plasmids expressing the small isoforms with either one of the large isoforms, suggesting that both large and small isoforms are required for syd-9 function.
mutants. These results also indicate that neuronal SYD-9 plays a
more important role in regulating locomotion.

Last, we generated constructs in which a panneuronal promoter
drives the expression of individual isoforms. Coexpression of these
constructs significantly improved the locomotion and body mor-
phology defects of *ju49* animals in larvae. It also restored the
accumulation of GABA receptors (axls22) at the NMJs in *ok1110*
animals (Fig. 9, which is published as supporting information on the
PNAS web site), suggesting that the postsynaptic receptor clustering
depends on neuronal SYD-9 proteins. These constructs did not
rescue morphology in adults and the low brood size of *syd-9*
mutants. By contrast, expressing SYD-9 in muscles did not rescue
locomotion or restore GABA receptors at NMJs in *ok1110* animals
(Fig. 9). In these animals, GABA receptors were increased in
abundance but failed to concentrate at synapses (Fig. 9). This result
supports the conclusion that SYD-9 is required in neurons and also
suggests a participating role of muscle SYD-9 in regulating GABA
receptors. Taken together, we demonstrated that *syd-9* functions
predominantly in neurons to regulate synaptic function.

### syd-9 Mutants Show Genetic Interactions with Endocytotic Mutants.

The predominantly neuronal requirement of SYD-9 and specific
defects for synaptic function in *syd-9* mutants suggest that *syd-9* may
regulate synaptic transmission through known synaptic vesicle
recycling pathways. We examined the genetic interactions between
*ju49* and known regulators of synaptic function, including SNT-1,
synaptotagmin (UNC-26), endophilin (UNC-57), AP180 (UNC-11),
syntaxin (UNC-64), SNAP-25 (RIC-4), UNC-13, RAB-3, SYD-2,
RPM-1, and SAD-1. *syd-9(ju49)* mutants showed no specific genetic
interactions with mutations in UNC-64, RIC-4, UNC-13, RAB-3,
SYD-2, RPM-1, and SAD-1 because the double mutants displayed the
locomotion and developmental characteristics of both single
mutants. The double mutants were “sicker” than the single mutant
alone, as expected from an additive effect; they proceeded in
development and propagated.

*syd-9(ju49)* mutants displayed specific genetic interactions with
*snt-1*, *unc-26*, *unc-57*, and *unc-11*, all genes that participate in
endocytosis (13, 14, 19, 21, 60). In fact, the morphological defects of *syd-9*
(specifically, the thin body and lack of sinusoidal wave) are
reminiscent of these mutants. SNT-1 is required for both exocytosis
and endocytosis in *C. elegans* (13, 14). *snt-1*/*syd-9* double mutants
were 100% lethal upon hatching. Although *snt-1(tm2290)*/*syd-
9(ju49)*/+ heterozygous animals appeared WT, they did not segre-
gate viable *snt-1*/*syd-9* progeny, and *snt-1*+/syd-9* and *snt-1*/*syd-9*
animals passed on so few progeny that the strain could not be
maintained. Because neither the *snt-1* nor *syd-9* gene is haploid-
insufficient in the WT background, this genetic interaction suggests
that SYD-9 functions either in the same pathway or in parallel to
SNT-1 to regulate synaptic transmission. A slightly different genetic
interaction between *syd-9* and *unc-26*, *unc-57*, and *unc-11* was
observed. *syd-9(ju49);unc-26(m2), *syd-9(ju49);unc-57(ok310),* and
*syd-9(ju49);unc-11(e47) animals could be recovered from *syd-
9;unc-26*+, *syd-9;unc-57*+, and *syd-9;unc-11*+ parents. They were
initially viable and behaviorally identical to *unc-26*, *unc-57*, and
*unc-11* null animals but became arrested and passed on few
progeny. The arrest displayed by these double mutants is different
from the additive “sickness” of the single mutants. These genetic
interactions support the hypothesis that *syd-9* regulates synaptic
transmission, particularly endocytosis.

### syd-9(ju49) Mutants Display Endocytosis Defects.

We performed electron microscopy analysis to examine whether *syd-9* mutants
display ultrastructural features consistent with a defect in endocy-
tosis. Several characteristic ultrastructural defects have been ob-
served in *C. elegans* endocytosis mutants (13, 14, 19, 21, 60). These
defects include a reduction in the total synapse vesicle number,
reflecting the defective replenishment of synaptic vesicles, and an
altered distribution pattern of synaptic vesicles, with more mem-
brane-contacting vesicles present away from active zones. Vesicle
accumulation in these membrane regions is thought to reflect a
disruption or delay in the maturation and mobilization of endocy-
tosed vesicles. Endocytosis mutants also display an increased num-
ber of vesicles with large sizes and abnormal shape or clusters, which
reflects an arrest in various vesicle maturation and sorting stages.
These ultrastructural phenotypes are not observed in mutants for
genes with predominant roles in exocytosis.

Compared with WT synapses, the total vesicle number in *syd-
9(ju49)* mutants was reduced by 25% (Fig. 6C). The distribution of
the vesicles was further disrupted, with membrane-contacting ves-
icles shifting away from the presynaptic specialization and accu-
mulating 300–350 nm distally (Fig. 6). Furthermore, we detected the
presence of large and abnormal-sized vesicles, evidenced by
increased numbers of cisternae (Fig. 6). The combination of
ultrastructural changes in *syd-9* mutants is thus characteristic of
perturbations of the classical endocytotic pathway.

### syd-9 Functions in Parallel with unc-75/BrunoL.

The speckle-like expression pattern of *syd-9* isoforms suggests that at least some
SYD-9 isoforms may function through RNA-binding and mRNA
processing. Only two RNA-binding proteins, UNC-75 and EXC-7,
have been shown to function in synaptic transmission in *C. elegans*,
and both display speckle-like expression patterns (34). UNC-75 is
the *C. elegans* homologue of the CELF/Bruno family protein that
regulates tissue-specific alternative splicing in mammals and spe-
cific mRNA translation repression in *Drosophila* oocytes. *unc-
75(e950) null mutants display uncoordinated locomotion (34) but
are nonetheless healthy, with close to normal brood size. All
*syd-9;unc-75(e950)* double mutants were paralyzed and arrested
upon hatching. This synergistic genetic interaction suggests that the
*syd-9* gene functions in parallel with *unc-75* to regulate synaptic
transmission. ELAV is a neuronal-specific alternative splicing factor in
*Drosophila*, and most of its mammalian homologues, the Hu
family proteins, are RNA processing regulators in the nervous
system. In contrast to *unc-75* mutants, *exc-7* mutants did not
enhance or affect *syd-9* behavioral or developmental defects, which
supports the specificity of the genetic interactions between *unc-75*
and *syd-9*.

### Discussion

We report the identification of the SYD-9 nuclear proteins that
regulate synaptic transmission: specifically, endocytosis in *C. el-
egans*. In *syd-9* mutants, we were unable to detect obvious defects
in the differentiation of neurons and muscles. Terminal differen-
tiation at the synapse also takes place; however, the distribution
and level of several synaptic proteins are abnormal, which suggests
that the function of SYD-9 is highly specific for developmental main-
tenance of synaptic structures and functions.

Using both mosaic analysis and RNAi, we specifically eliminated
the expression of *SYD-9* proteins in muscle cells. Neither manip-
ulation resulted in obvious developmental and behavioral defects,
suggesting that the muscle expression of *SYD-9* is largely indis-
pensable in larvae. It remains possible that there are subtle changes that we did not analyze for (such as the level of GABA receptors)
or that the remaining SYD-9 proteins in muscles, although at a
much reduced level, are sufficient for development. However, the
potential defects in muscles did not contribute greatly to defects in
*syd-9* larvae, because neuronal-specific expression of *SYD-9* can
rescue *syd-9(ju49)* mutants to a similar degree as the *syd-9* genomic
fragment. Treatment of *hpy6* animals with RNAi against *syd-9* led to
“Egl” adults that displayed a delay in egg-laying, most likely
caused by a down-regulation of hermaphrodite-specific neuron
activity (Movies 1 and 2 and Fig. 10, which are published as
supporting information on the PNAS web site). *syd-9*, therefore, is
predominantly required in neurons to regulate synaptic function.

The phenotype of *syd-9* mutants and the genetic interactions
between *syd-9* and several endocytotic mutants strongly suggest that

syd-9 functions through, and in parallel to, cholinergic-mediated endocytosis during synaptic transmission. C2H2 domains have been implicated in binding DNA, RNA, and protein motifs. Because SYD-9 protein localizes to nuclear speckles, it is possible that SYD-9 regulates the posttranscriptional processing of transcripts that are specifically required for endocytosis. We determined that at a gross level, SNT-1 and other synaptic proteins in syd-9(ju49) animals (Fig. 11, which is published as supporting information on the PNAS web site) were not significantly altered. Therefore, it is unlikely that SYD-9 directly regulates the transcription of the most obvious synaptic regulators. SYD-9 proteins likely function together or in parallel with UNC-75, a BrunoL family pre-mRNA processor, to regulate the transcription of proteins that are specifically required for endocytosis. Many RNA-binding proteins and proteins that regulate pre-mRNA processing display speckle-like patterns located at the interchromatin regions (56). Biochemical studies in mammalian systems have identified several C2H2 zinc-finger motif-containing proteins that localize to nuclear speckles and play a role in transcription or posttranscriptional mRNA processing events, (54, 55). Interestingly, both ZNF74 and Glis2, the human proteins containing zinc-finger domains highly homologous to those in SYD-9 proteins, also localize to discrete speckles in nuclei. ZNF74 directly interacts with the hyperphosphorylated form of RNA polymerase II and colocalizes with splicing factors in the nuclear matrix, implicating its potential role in mRNA processing (54, 55). We speculate that at least certain isoforms of SYD-9 function as splicing factors or premRNA processors that specifically regulate the splicing and translation of RNA transcripts that are essential to mediate endocytosis.

Recent studies have just begun to reveal the specific roles for transcriptional and translational regulation in synaptic function. NOVA, Bruno/LUNC-75, and ELAV/EXC-7 were the only reported mRNA processing proteins that specifically regulate synaptic functions. Our studies revealed SYD-9 as a previously uncharacterized factor that functions in parallel to UNC-75 and regulates endocytosis through mRNA processing. syd-9, unc-75, and exc-7 mutants provide powerful genetic tools for uncovering other components and targets of the regulatory machinery specific for synapse function.

Methods

C. elegans Genetics and Molecular Biology. See Supporting Methods, which is published as supporting information on the PNAS web site.

RNAi Against syd-9. ph1477 was transformed in HT115 induced by 1 mM isopropyl β-D-thiogalactoside (IPTG) for 2 h and seeded on nematode growth medium plates with carbenicillin (25 µg/ml) and IPTG (1 mM). L4 animals were propagated on these plates at 15°C.

Generation of Transgenic Lines. nlp66 was generated by integrating pH35 and Lin-15 plasmid into the lin-15(e765) animals.

Aldicarb Tests. Twenty adults were transferred to plates containing 0.1–1.5 mM aldicarb and scored for lethality every hour for 4 h. An animal was scored as dead when it appeared hypercontracted, felt flaccid, and did not respond when probed.

Electrophysiology and Ultrastructural Analysis. See Supporting Methods.

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