spr-2, a suppressor of the egg-laying defect caused by loss of *sel-12* presenilin in *Caenorhabditis elegans*, is a member of the SET protein subfamily

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Edited by Michael S. Levine, University of California, Berkeley, CA, and approved October 23, 2000 (received for review September 18, 2000)

Presenilin plays critical roles in the genesis of Alzheimer's disease and in LIN-12/Notch signaling during development. Here, we describe a screen for genes that influence presenilin level or activity in *Caenorhabditis elegans*. We identified four *spr* (suppressor of presenilin) genes by reverting the egg-laying defective phenotype caused by a null allele of the *sel-12* presenilin gene. We analyzed the *spr-2* gene in some detail. We show that loss of *spr-2* activity suppresses the egg-laying defective phenotype of different *sel-12* alleles and requires activity of the *hop-1* presenilin gene, suggesting that suppression is accomplished by elevating presenilin activity rather than by bypassing the need for presenilin activity. We also show that SPR-2 is a nuclear protein and is a member of a protein subfamily that includes human SET, which has been identified in numerous different biochemical assays and at translocation breakpoints associated with a subtype of acute myeloid leukemia.

A key factor in the development of Alzheimer's disease is the deposition of plaques formed from Aβ peptides. These peptides are released when the single-pass transmembrane protein β-amyloid precursor protein (β-APP) is cleaved at the β site in the extracellular domain and at a γ site in the transmembrane domain. Studies of familial Alzheimer's disease led to the identification of the presenilin 1 and presenilin 2 genes (reviewed in ref. 1). Presenilin is required for γ-secretase cleavage of β-APP (2, 3), and the two presenilins seem to be functionally interchangeable (4–6). Studies with putative aspartyl protease-active site inhibitors indicate that presenilin may itself be the long elusive γ-secretase (7, 8).

Presenilin is being intensively pursued as a key therapeutic target for the amelioration or prevention of Alzheimer's disease. The identification of factors that influence presenilin activity, synthesis, and stability will be important for maximizing the efficacy of drugs that are targeted against γ-secretase and perhaps for identifying new therapeutic targets. Genetic analysis of presenilin in *Caenorhabditis elegans* offers one approach to identifying such factors.

In *C. elegans*, there are two presenilins, *sel-12* and *hop-1*, that can be studied by virtue of their involvement in LIN-12/Notch signal transduction (9, 10). LIN-12/Notch proteins are transmembrane proteins that act as receptors for intercellular signals that specify cell fates. Ligand binding to LIN-12/Notch receptors leads to proteolytic cleavage within the transmembrane domain, which releases the intracellular domain so that it may translocate to the nucleus and activate transcription of target genes (see 11, 12). The transmembrane-cleavage event is analogous to the γ-secretase-processing event that generates Aβ from β-amyloid precursor protein, and presenilin is essential also for the proteolytic cleavage that releases the intracellular domain of LIN-12/Notch proteins (11, 12). Indeed, in *C. elegans*, concomitant depletion of both *sel-12* and *hop-1* activity causes the phenotypes associated with the loss of all LIN-12/Notch signaling (10), and this observation, along with similar findings from genetic studies in *Drosophila* (12, 13) and mice (3, 6, 14, 15), has suggested that the key essential function of presenilin during animal development may be in LIN-12/Notch signaling.

We describe here one genetic approach to identifying factors that influence presenilin activity, synthesis, and stability: the identification of suppressors of the egg-laying defective (Egl) phenotype caused by loss-of-function mutations in the *C. elegans* presenilin *sel-12*. The suppressor approach mitigates potential difficulties arising from functional redundancy of members of gene families, functional redundancy of different regulatory mechanisms, or pleiotropy. We analyze one such suppressor of presenilin, *spr-2*, in some detail. We show that *spr-2* seems to suppress the Egl phenotype resulting from the absence of *sel-12* activity by elevating the level or activity of another *C. elegans* presenilin, *hop-1*. We also show that SPR-2 is a nuclear protein that is related to mammalian SET, a protein that has been identified in numerous different contexts.

Materials and Methods

Genetic Materials and Methods. Standard methods were used for handling, maintenance, ethyl-methanesulfonate mutagenesis, and genetic analysis. The wild-type parent for most strains used in this study is *C. elegans* var. Bristol strain N2 (16). Experiments were conducted at 20°C unless otherwise indicated. The following single-nucleotide polymorphism (SNP) from LG *IV* were used for mapping as described below; these SNPs were identified in *C. elegans* var. Hawaii strain CB4856 by the Genome Sequencing Consortium (http://genome.wustl.edu/gsc/CEpolymorph/snp.shtml): vm23 g02.s1, vc86f02.s1, vd48b08.s1, v125f08.s1, and vr89 g03.s1. *sel-12* alleles are described in ref. 9. The markers used for mapping or for facilitating genetic analysis mentioned in the text are described at http://biosci.umn.edu/CGC/CGChomepage.htm.

Mapping of *spr* Mutations. The *spr* mutations were initially mapped to autosomal linkage groups with visible *dpy* markers (data not shown) and then to LG *V*, LG *IV*, LG *X*, and LG *I* intervals.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: Egl, egg-laying defective; Egl$^+$, normal egg-laying; GFP, green fluorescent protein; RNAi, RNA-mediated interference.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF321546).

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Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.011446498. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.011446498

LG V. spr-1(ar200) maps between *unc-68(e540)* and *rol-3(e754)*: *unc-68* (6/10) *spr(ar200)* (4/10) *rol-3*. Because *ar201* [*unc-68* (5/11) *spr(ar201)* (6/11) *rol-3*] and *ar205* [*unc-68* (14/18) *spr(ar205)* (4/18) *rol-3*] map to the same genetic interval, they may also be *spr-1* alleles. Preliminary results suggest that *spr(ar212)* maps much closer to *rol-3*, and, hence, may not be an allele of *spr-1* (data not shown). Because *spr(ar212)* has not been mapped to a different interval, however, we have not given it an independent *spr* gene designation.

LG IV. spr-2(ar199), *spr-2(ar211)*, and *spr-2(ar214) IV* were all mapped between *unc-8(e49)* and *dpy-20(e1282)*. *ar199*: *unc-8* (7/17) *ar214* (10/17) *dpy-20*. *ar214*: *unc-8* (7/12) *ar214* (5/12) *dpy-20. ar211*: *unc-8* (2/12) *ar211* (10/12) *dpy-20*. The *ar216* mutation was not mapped further but was shown to be an allele of *spr-2* by DNA sequence analysis. *spr-2(ar211)* was mapped between the single-nucleotide polymorphisms (SNPs) markers vd48b08.s1 and vl25f08.s1, and *spr-2(ar199)* was mapped between the SNPs markers vr89 g03.s1 and vm23 g02.s1, essentially as described (17).

LG X. spr-3(ar209) was mapped between *dpy-3(e27)* and *unc-2(e55)*: *dpy-3* (9/13) *spr-4(ar209)* (4/13) *unc-2*. *ar198* also maps in this interval: *dpy-3* (20/23) *spr(ar198)* (3/23) *unc-2*. *ar217* was found to be X-linked but was not further mapped.

LG I. Three *spr* mutations, *ar197*, *ar204*, and *ar208*, mapped to LG *I*, and we have tentatively assigned *ar208* as the canonical allele of *spr-4*. In our initial experiments, we were unable to map any of these mutations unambiguously to a single interval; further work will be necessary to determine why.

Identification of *hop-1(0)* Alleles. We used the protocol of R. Barstead (http://pcmc41.ouhsc.edu/Knockout/) to screen for an internal deletion within the *hop-1* gene. We used the primers TMP-F_2 (5′-CACAGTAACCTTCAAAACCACAC) and TMP-R2 (5′-GTTAGACGATCTCCACCATC), which give a wild-type PCR product of 2,509 bp and recovered two deletion alleles, *hop-1(ar179)* and *hop-1(ar180)* (data not shown). *hop-1(ar179)* is predicted to be a protein null and was used for the genetic studies described in this paper. *hop-1(ar179)* is a 716-bp deletion with breakpoints in exon 2 (at codon Ser-57) and exon 4 (at codon Tyr-218). This deletion shifts the reading frame after the first transmembrane domain, resulting in a stop codon immediately after residue Asp-56 and a newly introduced residue Thr.

We note that *ar179* affects only sequences internal to the *hop-1* locus. In contrast, the coordinates reported for other *hop-1*-deletion alleles suggest that neighboring genes are affected. *hop-1(nr2003)*, a deletion from 23,744 (or 23,743) to 22,549 (or 22,548) of cosmid C18E3 (18), should remove part of C18E3.2; *hop-1(lg1501)*, a deletion of 20,359–22,238 of cosmid C18E3 (19), should remove part of C18E3.3.

Transgenic Lines. DNA was injected into the germline of *C. elegans* hermaphrodites (20). PMH86[*dpy*-20(+)] (21) was used as a cotransformation marker so recipient strains would contain the chromosomal marker *dpy-20(e1282)*.

Rescue experiments. Cosmid DNA spanning the *spr-2* region was injected at 5 μg/ml each in pools together with pMH86 (10 μg/ml) and pBluescript (80 μg/ml) into recipient strain *spr-2(ar211) dpy-20(e1282)*; *sel-12(ar171) unc-1(e538)*. Transgenic lines were established and their egg-laying ability was checked for antisuppression, which is indicative of *spr-2(+)* activity. When rescue was seen with a pool at the F_2 stage, individual cosmid DNAs from that pool were injected into the same strain at a higher concentration (50 μg/ml) together with pMH86 (10 μg/ml) and pBluescript (40 μg/ml). Only one cosmid clone, F18C7, showed rescuing activity in 6 of 12 transgenic lines at the F_2 stage. Five predicted genes are completely contained in F18C7, and two others are partially contained. Five PCR fragments, each of which contains one or two of these predicted genes, were injected into the recipient strain at 50 μg/ml. Four of these PCR fragments generated three to eight transgenic lines each, and none showed antisuppression. The fifth PCR fragment contained C27B1.1 and C27B2.2; lines could not be obtained at 50 μg/ml, but a single line was obtained at 5 μg/ml, and antisuppression was seen in the F_2 generation. In all cases where antisuppression was seen at the F_2 generation, the antisuppression activity subsequently disappeared, although the *dpy-20(+)* marker was still functional. We did not investigate this behavior further.

SPR-2::GFP expression. p21XXGFP encodes a SPR-2::GFP protein and contains all genomic sequences from C27B7.1 described above, with green fluorescent protein (GFP) inserted in frame after the last codon of SPR-2. p21XXGFP is a derivative of p21XX, which contains a 2.1-kb *Xba*I/*Xho*I genomic fragment containing 0.7 kb of 5′ flanking region and 0.35 kb of 3′ flanking region. The coding region of p21XX was sequenced and confirmed. Additional details are available on request.

We generated transgenic lines in a *dpy-20(e1282)* background. p21XXGFP was injected at 50 μg/ml into *dpy-20(e1282)* hermaphrodites with pMH86 [*dpy-20(+)*] as a cotransformation marker. Six independent extrachromosomal arrays were generated, and (GFP) fluorescence was observed in all six lines; however, the expression pattern from the extrachromosomal arrays was highly mosaic. We used a standard method (20) to generate the integrated transgene *arIs57*, used for analyzing the SPR-2::GFP expression pattern. *dpy-20(e1282)*; *arIs57* showed a reproducible expression pattern from animal to animal. In addition, the SPR-2::GFP fusion protein expressed from this transgene is functional, because it displays antisuppression activity: 40% of hermaphrodites of genotype *spr-2(ar199) dpy-20*; *sel-12(ar131) unc-1*; *arIs57* [*spr-2::gfp*] are Egl.

RNA-Mediated Interference (RNAi). RNAi was performed as described (22). cDNA clone yk81B12 was used for *spr-2* double-stranded (ds)RNA; genomic DNA was used to generate *D2096.8* dsRNA. dsRNA was microinjected into the pseudocoelomic space of L4 hermaphrodites. Injected hermaphrodites were cultured individually overnight and then transferred to fresh plates. Progeny of injected hermaphrodites were scored for their ability to lay eggs.

Results

Identification of Extragenic Suppressors of *sel-12(ar171)*. Hermaphrodites that are homozygous for the putative null allele *sel-12(ar171)* are Egl. The Egl phenotype of *sel-12(−)* resembles that of a *lin-12* partial loss-of-function mutant (9). The cellular basis for the Egl phenotype in either case is not completely understood, although it is likely to reflect cell fate abnormalities involving the π cells of the ventral uterus (ref. 23 and A. Newman, personal communication).

We screened for suppressors of *sel-12(ar171)* by mutagenizing *sel-12(ar171) unc-1(e538)* hermaphrodites with ethyl methanesulfonate (16) and screening for normal egg-laying (Egl^+) revertants in the F_1, F_2, and F_3 generations. Approximately 13,800 F_1 hermaphrodites (representing 27,600 mutagenized haploid genomes) and their progeny were examined for suppression, and Egl^+ hermaphrodites or eggs were picked to establish potentially suppressed strains. We kept only strains displaying relatively high penetrance, a minimum of approximately 80% Egl^+. Then 14 independent, highly penetrant Egl^+ revertants were obtained, for a frequency of approximately 5×10^{-4} suppressor mutations/mutagenized haploid genome. Because these suppressor mutations seem to define four distinct loci, this frequency is comparable to the average ethyl methanesulfonate mutagenesis-induced forward mutation rate of 1.3×10^{-4} per gene (16, 24), suggesting that at least some *spr* mutations recovered in this screen are likely to be hypomorphic or null alleles.

Our initial assessment suggested that most of the *spr* mutations are somewhat semidominant (data not shown); thus, we relied principally on genetic-map position rather than complementation

Table 1. Allele-nonspecific suppression

Relevant genotype	Egl$^+$/total, %
sel-12(ar171)	0/30 (0%)
sel-12(ar131)	0/33 (0%)
spr-2(ar199); sel-12(ar171)	28/30 (93%)
spr-2(ar199); sel-12(ar131)	31/31 (100%)
spr-2(ar211); sel-12(ar171)	30/30 (100%)
spr-2(ar211); sel-12(ar131)	32/33 (97%)

All strains also contained *unc-1(e538).*

tests to assess the number of *spr* genes. Initial linkage experiments identified potential loci on LG *I* (three alleles), *IV* (four alleles), *V* (four alleles), and *X* (three alleles); two alleles were lost. We have tentatively designated four *spr* genes: *spr-1 V*, *spr-2 1V*, *spr-3 X*, and *spr-4 I*. The map data for most of the *spr* mutations are consistent with their being alleles of one of these four loci, with possible exceptions described in *Materials and Methods*.

We note that the LG *V spr* mutations map to a distinct position from *sel-10*, a partial suppressor of the Egl phenotype of *sel-12(ar171)* that had originally been identified based on genetic interactions with *lin-12* (see ref. 25). We did not expect to recover *sel-10* here, because loss of *sel-10* activity results in only 20% suppression of *sel-12(ar171)* (25), less than the 80% threshold of this screen.

The genomic location of *spr-2* helped make it especially tractable to molecular analysis, and the remainder of this report is concerned with this gene.

***spr-2* Suppression of the Egl Phenotype of *sel-12(−)* Is Not Allele-Specific and Requires the Activity of *hop-1*.** There are two *C. elegans* presenilin genes, *sel-12* and *hop-1* (9, 10). *sel-12* is expressed in many different cell types (4); *hop-1* expression seems to be too low to detect in cells by using conventional GFP and lacZ reporter-gene approaches (X.L. and I.G., unpublished observations). Expression of a *hop-1* cDNA under the control of *sel-12* regulatory sequences rescues the *sel-12(ar171)* Egl phenotype (10).

In principle, mutations that suppress the Egl phenotype of *sel-12(ar171)* might have one of the following effects: (*i*) corrective interaction: the *spr* mutations might function as informational suppressors or somehow enable the truncated SEL-12(ar171) product to function; (*ii*) bypass mechanism: the *spr* mutations might bypass the need for presenilin activity altogether; or (*iii*) augment *hop-1* activity: the *spr* mutations might augment the activity, level, or stability of HOP-1 protein, either directly or indirectly.

These three possibilities may be distinguished by two genetic tests: allele-specificity and *hop-1* dependence. A corrective interaction or informational suppression would be allele specific and would not depend on *hop-1* activity. A bypass mechanism would be allele nonspecific and would not depend on *hop-1* activity. A mechanism that augments *hop-1* activity would be allele-nonspecific and would depend on *hop-1* activity.

When we performed these two genetic tests, we found a lack of allele specificity and dependence on *hop-1. First, spr-2* mutations efficiently suppress *sel-12(ar131)*, a C60S missense change in the first transmembrane domain, as well as *sel-12(ar171)*, a W225STOP change in the fifth transmembrane domain (ref. 9; Table 1). Second, the presence of the *hop-1(ar179)* null allele (see *Materials and Methods*) prevented *spr-2* mutations from suppressing the Egl phenotype of *sel-12(ar171)* (Table 2*A*). These results taken together indicate that *spr-2* mutations augment *hop-1* activity.

These observations raise the question of whether the effect of *spr-2* is specific to *hop-1*, or whether *spr-2* might be able also to augment *sel-12* presenilin activity. In principle, this question could be addressed genetically by examining whether *spr-2* can suppress the effects of removing *hop-1* activity, and whether such suppression depends on *sel-12*. Because there is no phenotype caused by removing *hop-1* activity in a *sel-12(+)* or *lin-12* mutant background (refs. 10 and 18; X.L. and I.G., unpublished observations), this question cannot be answered at this time.

Table 2. Genetic interactions with *hop-1* and with *lin-12

hop-1 dependence

Relevant genotype	No. of Egl$^+$/total, %
hop-1(ar179); sel-12(ar171)	0/28
hop-1(ar179); spr-2(ar199); sel-12(ar171)	0/30
hop-1(ar179); spr-2(ar211); sel-12(ar171)	0/40
No effect on the 0 AC defect caused by elevating *lin-12* activity	
Complete genotype	No. of 0 AC/total, %†
lin-12(n302)/unc-32; dpy-20/+	27/50 (54%)
lin-12(n302)/unc-32; spr-2(ar211) dpy-20	33/60 (55%)
lin-12(n302)/unc-32; spr-2(ar211) dpy-20/spr-2(+) dpy-20	88/168 (52%)
No effect on the 2 AC defect caused by reducing *lin-12* activity	
Complete genotype	No. of 2 AC/total, %§
lin-12(ar170)‡ (25°C)	39/50 (78%)
lin-12(ar170); spr-2(ar211) (25°C)	25/39 (64%)
lin-12(ar170)‡ (20°C)	10/52 (19%)
lin-12(ar170); sel-12(ar171) unc-1 (20°C)	39/43 (91%)
lin-12(ar170); spr-2(ar211); sel-12(ar171) unc-1 (20°C)	40/49 (82%)

*Hermaphrodites of the relevant genotype shown segregated from parents that also carried the free duplication *mnDp68 [sel-12(+)]*. Maternal *sel-12(+)* activity provided by the duplication enables *hop-1(−); sel-12(−)* progeny to survive to adulthood and to produce progeny, which arrest as embryos (see ref. 18). *spr-2* mutations do not suppress the maternal-effect lethality of embryos produced by *hop-1(−); sel-12(−)* mothers (data not shown). All strains also contained *unc-1(e538).*

†The 0 AC defect was scored by determining egg-laying ability; for *lin-12(n302),* the ability to lay eggs correlates absolutely with the presence of an AC (38). AC, anchor cell.

‡*lin-12(ar170)* behaves as a partial loss-of-function allele at all temperatures but is most hypomorphic at 25°C (39).

§The number of ACs was scored directly using Nomarski microscopy.

Genetic Interactions Between *spr-2* and *lin-12* Have Not Been Detected. We considered whether *spr-2* might seem to augment *hop-1* activity because *lin-12* activity, rather than *hop-1* presenilin activity *per se*, has been elevated. If *spr-2* affected *lin-12* directly, then we might expect to see genetic interactions between *spr-2* and *lin-12*. However, we have not detected any such interactions. First, the Egl phenotype of the partial loss-of-function allele *lin-12(n676n930)* is not suppressed by *spr-2(ar211)* and *spr-2(ar199)* (data not shown). Second, we did not see any evidence for *spr-2* involvement in a well characterized *lin-12*-mediated process, the decision of two gonadal cells between the anchor cell (AC) and ventral uterine precursor cell (VU) fates (reviewed in ref. 26): *spr-2(ar211)* and *spr-2(ar199)* do not influence the penetrance of AC/VU defects caused by elevating *lin-12* activity or by partially reducing *lin-12* activity (Table 2). However, *spr-2* may not function in the AC/VU pair, because *spr-2* mutations do not suppress the increased penetrance of the two-AC defect caused by combining a partial loss-of-function allele of *lin-12* with *sel-12(ar171)* (Table 2).

Molecular Cloning of *spr-2*, Mutations, and RNAi. *spr-2* was mapped between single-nucleotide polymorphisms on cosmids D2096 and F49C12, and cosmids and derivative PCR fragments were assayed for antisuppression, the ability to cause an Egl phenotype in a recipient strain of genotype *spr-2(ar211) dpy-20(e1282)*; *sel-12(ar171) unc-1(e538)* (see *Materials and Methods*). Ultimately, a

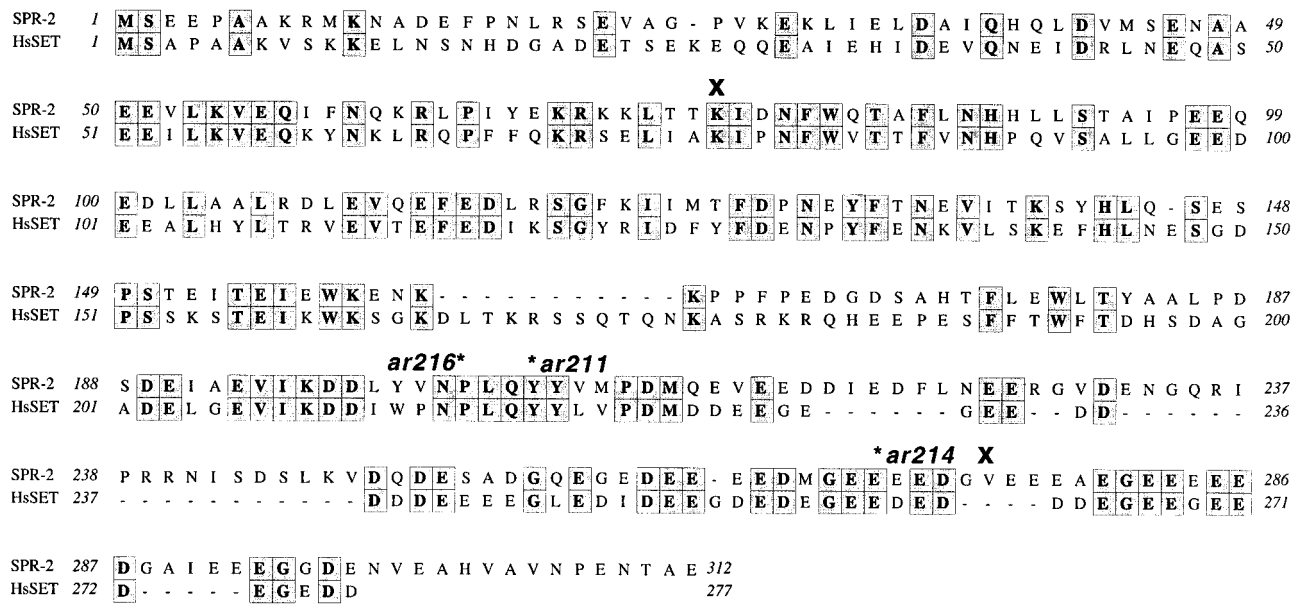

Fig. 1. Alignment of SPR-2 and human SET. Identical amino acids are shaded. The SPR-2 sequence is based on our cDNA sequence analysis (see text). The positions of *spr-2(ar211)* Y206N, *spr-2(ar214)* E270stop, and *spr-2(ar216)* P203S are indicated by asterisks (*). The region used for construction of the tree shown in Fig. 2 ranges from K76 to V275 of SPR-2 (each marked with X).

PCR fragment containing C27B7.1 and C27B7.2 showed antisuppression, suggesting that *spr-2* might correspond to one of these two genes. We therefore sequenced the coding regions of C27B7.1 and C27B7.2 from all four *spr* alleles that mapped to LG *IV*. In three alleles, we found single nucleotide changes in the coding region of C27B7.1 (see Fig. 1) but no change in the coding region of C27B7.2. The fourth allele, *ar199*, was found to contain a 134-bp deletion in the predicted 5′ flanking region of C27B7.1, 64 bp upstream of the predicted start codon.

We sequenced three cDNA clones corresponding to C27B7.1: yk81b12, yk312a7, and yk274d1, kindly provided by Yuji Kohara (http://www.ddbj.nig.ac.jp/htmls/c-elegans/html/CE INDEX.html). All intron/exon junctions predicted by GENEFINDER were confirmed with one exception: the 3′ acceptor site of the first intron appears to be three nucleotides downstream of the predicted acceptor. Our analysis suggests that the predicted SPR-2 protein has 312 amino acids, shown in Fig. 1.

The nature of the three point mutations, *spr-2(ar211)* Y206N, *spr-2(ar214)* E270stop, and *spr-2(ar216)* P203S, does not allow us to conclude that any are likely molecular-null alleles. However, loss of *spr-2* function can result in suppression, because *spr-2(RNAi)* has suppressor activity: many Egl$^+$ progeny were produced after injection of *sel-12(ar131)* hermaphrodites or *sel-12(ar171) unc-1(e538)* hermaphrodites (summarized in Table 3).

Table 3. Summary of RNAi experiments

dsRNA injected	Wild type	*sel-12(ar171)**	*sel-12(ar131)*
none†	+ (many)	Egl (4/4)	Egl (5/5)
spr-2	ND	Egl$^+$ (6/15)	Egl$^+$ (10/10)
D2096.8	ND	Egl (13/13)	Egl (10/10)
spr-2 + *D2096.8*‡	+ (10/10)	Egl$^+$ (9/15)	Egl$^+$ (10/10)

See text for details. +, no novel phenotypes, such as overt lethality, were seen. Egl, egg-laying defective. Egl$^+$, egg-laying ability was restored, and no novel phenotypes were seen. The numbers in parentheses indicate the number of broods displaying the phenotype indicated/total injected hermaphrodites.

*Also carried *unc-1(e538).*

†Distilled water lacking dsRNA was injected.

‡The extent of suppression of the Egl phenotype *sel-12(ar171)* and *sel-12(ar131)* was comparable to the extent of suppression seen with *spr-2(RNAi)* alone (data not shown).

SPR-2 Is a Member of the SET/NAP Protein Family. BLAST searches of the GenBank database and CLUSTALW analysis have revealed that SPR-2 is a member of a large family of proteins that includes human SET and yeast Nap1 (Fig. 1). SPR-2 falls within a distinct subfamily of SET/Nap proteins, referred to here as the SET subfamily. The SET subfamily includes mammalian SET and TSPY proteins, as well as proteins from eukaryotes as diverse as *Arabidopsis* and *Plasmodium*. (SET does not contain a "SET domain," a motif found in certain DNA binding proteins.)

SET has been identified in many different assays and experimental systems, but its function *in vivo* is not clear. SET was identified first as the product of a gene located at a translocation breakpoint associated with a subtype of acute myeloid leukemia (27). SET was identified also in HeLa cell extracts as template-activating factor in a biochemical assay for proteins that enable replication of the adenovirus genome complexed with viral core proteins (28), as an inhibitor of PP2A (29), in a yeast two-hybrid screen for proteins that bind to the human chromatin protein HRX (30), and in *Xenopus* extracts as a protein that binds to cyclin B (31).

Another group of proteins related in sequence to SET and identified in BLAST searches with SPR-2 are the Nap proteins. SET and Nap1 share many biochemical activities, but the implication of the biochemical activities of Nap1 for its *in vivo* function is not clear. Nap1 was first identified as a nucleosome assembly protein (32), and this activity has been the focus of biochemical studies of Nap1 and closely related Naps. In yeast, Nap1p has nucleosome-assembly activity (33), but, in addition, Nap1p was found to bind cyclin B and to be involved in the specific functions of cyclin B/p34cdc2 kinase complexes (31, 34). BLAST searches and CLUSTALW analyses reveal that there are multiple genes in mammals that are more related to Nap1 than to SET, i.e., these Nap-related genes seem to be distinct from SPR-2 and the SET subfamily (Fig. 2). (We note that another protein, Nck Associated Protein, has been called "Nap1" also, but is unrelated to SET/Nap proteins.) BLAST searches per-

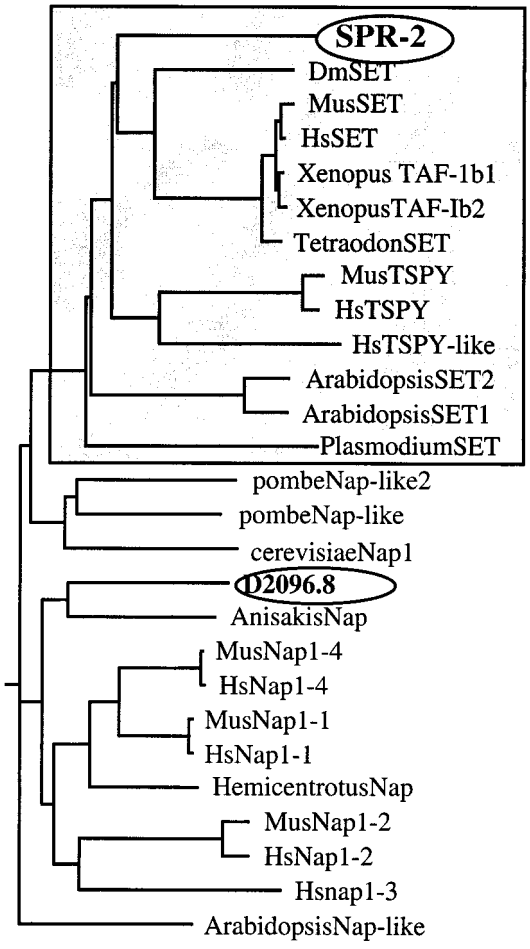

Fig. 2. The SET/Nap family. BLAST searches were done by using SPR-2, SET, and Nap1 as query sequences. To generate this tree, a region that seemed well conserved among all members of this family (as marked in Fig. 1) was used for CLUSTALW analysis. The two *C. elegans* SET/Nap proteins, SPR-2 and D2096.8, are encased within ovals, and the SET subfamily is boxed in gray. For clarity, we have in some cases renamed proteins, as various names are seen on the BLAST report because of the different synonyms for SET, and we have omitted sequences from other mammals, other species of *Plasmodium*, other plants, and relatively short expressed sequence tags. Accession numbers: SPR-2, AF321546; DmSET (*Drosophila melanogaster*), AE003708; MusSET (*Mus musculus*), AB015613; HsSET (*Homo sapiens*), Q01105; XenopusTAF-Ia (*Xenopus laevis*), AB022691; XenopusTFA-Ib, AB022692; TetraodonSET (*Tetraodon fluviatilis*), AF007219; MusTSPY, AF042180.1; Hs TSPY, U58096; HsTSPY-like, AAF03521.1; ArabidopsisSET2 (*Arabidopsis thaliana*), AC011765; ArabidopsisSET1, AC011809; PlasmodiumSET-like (*Plasmodium falciparum*), AJ238237; pombeNap-like2 (*Schizosaccharomyces pombe*), T40114; pombeNap-like, T41330; cerevisiaeNap1, NP 012974.1; CeD0296.8, T15896; AnisakisNap-like (*Anisakis simplex*), AJ237977; MusNap1–4, NP 032698.1; HsNap1–4 and MusNap1–1, NP 056596.1; HsNap1–1, NP 004528.1; HemicentrotusNap-like (*Hemicentrotus pulcherrimus*), D21877; MusNap1–2, NP 032697.1; HsNap1–2, BAA84706; HsNap1–3, NP 004529; and ArabidopsisNap-like, AAA50234.

formed using SPR-2 and mammalian Nap proteins also reveal another *C. elegans*-predicted protein, D2096.8, which is related in sequence to SET and Nap1-like proteins. D2096.8 is not clearly orthologous to any available Nap sequences and is quite divergent also from SPR-2 and its SET homologs (Fig. 2).

The relationship between SET and Nap proteins led us to investigate whether *D2096.8* is functionally related to *spr-2*, despite the considerable sequence divergence. We performed RNAi with double-stranded *D2096.8* RNA (Table 3). We saw no evidence for suppression of *sel-12(-)*: no Egl^+ progeny were produced after injection of *sel-12(ar131)* hermaphrodites or *sel-12(ar171) unc-1(e538)* hermaphrodites, suggesting that *D2096.8* activity is not a major influence on *hop-1* or *sel-12* activity (Table 3). Furthermore, we saw no synthetic lethality or other novel phenotypes when *D2096.8* activity was concomitantly depleted along with *spr-2* activity by RNAi in a wild-type (N2) background, suggesting that *D2096.8* and *spr-2* may not be functionally redundant.

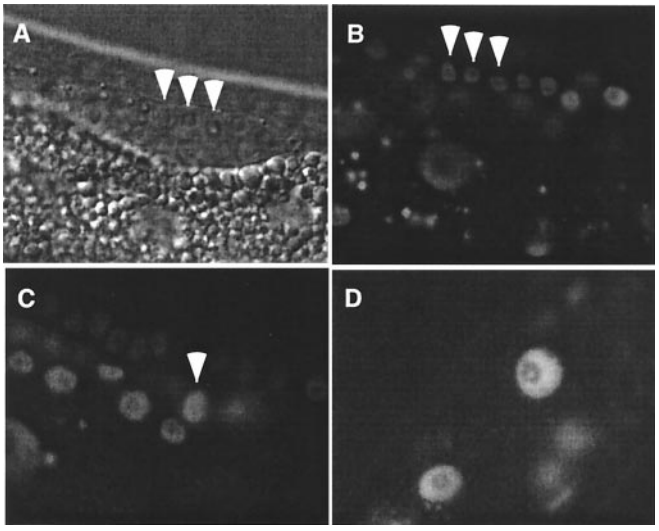

Fig. 3. Expression of SPR-2::GFP. All L3 stage. (*A*) Nomarski photomicrograph. The π cells (23) are indicated with arrowheads. (*B*) GFP fluorescence in the nuclei of π cells of the same hermaphrodite shown in *A*. (*C*) GFP fluorescence in the nucleus of the anchor cell (marked with arrowhead) and other gonadal cells. (*D*) GFP fluorescence in intestinal nuclei.

A Functional SPR-2::GFP Protein Is Localized to the Nucleus. To determine the subcellular localization of SPR-2, we constructed the integrated array *arIs57*, which expresses a SPR-2::GFP reporter protein. The SPR-2::GFP protein seems to retain *spr-2* antisuppression activity (see *Materials and Methods*). SPR-2::GFP was localized to the nucleus in all cells and at all stages in which it could be visualized (Fig. 3; data not shown). In particular, we note that SPR-2::GFP was visualized in the nuclei of π cells (Fig. 3), a likely cellular focus for the Egl phenotype of *sel-12* and *lin-12* mutants (ref. 23; A. Newman, personal communication). In mammals, SET is found predominantly in the cell nuclei (35), whereas yeast Nap1 has been found to localize to the cytoplasm (31), and *Drosophila* Nap1 has been found to change its subcellular localization from nucleus to cytoplasm in a cell cycle-dependent manner (33, 36). The finding of SPR-2::GFP in the nucleus is consistent with our assignment of SPR-2 to the SET subfamily based on sequence analysis.

Discussion

We have found that loss of *spr-2* activity can suppress the Egl phenotype caused by loss of *sel-12* presenilin activity in *C. elegans.* Suppression is not *sel-12* allele specific and depends on the activity of *hop-1*, another *C. elegans* presenilin: when *hop-1* activity is removed also, *spr-2(−)* cannot suppress the Egl phenotype caused by *sel-12(−)*. This latter result implies that *spr-2(−)* does not bypass the need for presenilin *per se* and instead has the effect of augmenting *hop-1* presenilin activity. *spr-2(−)* might have a direct effect on the level of *hop-1* gene expression or on HOP-1 protein stability or activity. Alternatively, the elevation of *hop-1* activity might be indirect, reflecting an increase in the level of expression, stability, or activity of another component required for presenilin function in the development of the egg-laying system.

One of these other components might be, in principle, *lin-12* or limiting components of *lin-12* signaling. However, we have not been able to detect genetic interactions between alleles of *spr-2* and alleles of *lin-12*. In this context, we note that there is no evidence that presenilin activity is normally rate limiting for LIN-12/Notch signaling in *C. elegans*, such that elevating presenilin activity beyond the wild-type level may not increase *lin-12* activity, and might not be detectable via a genetic interaction with *lin-12* alleles. If presenilin activity is not rate limiting, then the lack of genetic interaction between *spr-2* and *lin-12* would favor the possibility that *spr-2* affects presenilin activity *per se* rather than *lin-12* activity.

We have found that SPR-2 is a member of the SET/Nap family of proteins. Our analysis suggests that there is a clear subfamily of SET-related proteins in multicellular organisms, which includes SPR-2 and human SET. This subfamily seems to be distinct from the nucleosome assembly protein Nap1 and Nap1-related proteins. Furthermore, we used RNAi to investigate the role of the *C. elegans* protein D2096.8, which is more Nap1-like, and found no evidence that *D2096.8* is a potential suppressor of *sel-12(−)* or functionally redundant with *spr-2*.

Biochemically, SET has been identified in different systems based on different properties. One group of properties pertains to chromatin structure: there is evidence that the template-activating factor activity of SET involves remodeling the chromatin structure of the adenovirus core (37), and SET is found in a protein complex with HRX, a chromatin-remodeling protein (30). Biochemical studies of Nap1 and Nap1-related proteins have also pointed to a role in chromatin structure (32, 33). However, SET has been identified also as an inhibitor of PP2A enzymatic activity (29), and both SET and Nap1 have been identified as factors that bind to cyclin B in *Xenopus* extracts (31). Thus, whether there is a single biochemical mechanism of SET function *in vivo* remains unclear.

We have found that the major site of accumulation of a functional SPR-2::GFP protein is in the nucleus. This observation suggests that SPR-2 functions in the nucleus to facilitate *hop-1* activity. In the context of biochemical data suggesting a role in chromatin structure, a simple hypothesis is that loss of *spr-2* activity alters chromatin structure, which derepresses expression of *hop-1*. We have not detected an alteration in the level of *hop-1* mRNA by Northern analysis (S. Jarriault, C.W., and I.G., unpublished observations); however, this method would not detect an alteration restricted to specific cells, a possibility we have not been able to explore further because we have been unable to detect expression of a *hop-1*::*gfp* transgene (X.L. and I.G., unpublished observations). It is possible also that loss of *spr-2* activity depresses expression of a gene that facilitates presenilin activity. Alternatively, another of the diverse biochemical properties of SET, or other properties that remain to be discovered, may underlie the mechanism of *sel-12(−)* suppression by *spr-2(−)*.

Our finding that loss of *spr-2* activity suppresses the Egl phenotype of *sel-12(−)* offers a system for the investigation of SET structure and function *in vivo*. *spr-2* is one of an apparently small group of genes that can be identified as strong suppressors of the Egl phenotype of *sel-12(−)*. Other *spr* genes identified in this screen may prove, therefore, to be conserved factors that cooperate with SPR-2/SET in regulating *hop-1* activity. If so, they may provide insight as to the mechanism of SET function or additional tools for the biochemical analysis of SET function in other systems.

We are grateful to Ilya Temkin for valuable technical assistance with experiments throughout the course of this project. We thank Denise Brousseau and Lesley Emtage for help with suppressor screens, Oliver Hobert and Ning Chen for advice on sequence analysis, Sophie Jarriault for help with Northern analysis, and Xantha Karp for help identifying π cells. We also thank Mark Gurney for interesting discussions when we began the suppressor screens, members of the Columbia Biochemistry Department worm community for advice and discussion throughout the project, and Barth Grant, Oliver Hobert, and Sophie Jarriault for comments on the manuscript. This work was supported by National Institutes of Health Grant NS35556 and by a gift from the Pharmacia & Upjohn Corporation. I.G. is an investigator and C.W. was a research specialist with the Howard Hughes Medical Institute.

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