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Caenorhabditis elegans SUR-5, a Novel but Conserved Protein, Negatively Regulates LET-60 Ras Activity during Vulval Induction

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The *let-60 ras* gene acts in a signal transduction pathway to control vulval differentiation in *Caenorhabditis elegans*. By screening suppressors of a dominant negative *let-60 ras* allele, we isolated three loss-of-function mutations in the *sur-5* gene which appear to act as negative regulators of *let-60 ras* during vulval induction. *sur-5* mutations do not cause an obvious mutant phenotype of their own, and they appear to specifically suppress only one of the two groups of *let-60 ras* dominant negative mutations, suggesting that the gene may be involved in a specific aspect of Ras activation. Consistent with its negative function, overexpressing *sur-5* from an extragenic array partially suppresses the Multivulva phenotype of an activated *let-60 ras* mutation and causes synergistic phenotypes with a *lin-45 raf* mutation. We have cloned *sur-5* and shown that it encodes a novel protein. We have also identified a potential mammalian SUR-5 homolog that is about 35% identical to the worm protein. SUR-5 also has some sequence similarity to acetyl coenzyme A synthetases and is predicted to contain ATP/GTP and AMP binding sites. Our results suggest that *sur-5* gene function may be conserved through evolution.

The Ras-mediated signal transduction pathway plays important roles in specifying cell fates in a number of developmental events in the nematode *Caenorhabditis elegans* including vulval cell differentiation (17, 31), male spicule cell differentiation (4), germ nucleus exit from pachytene (5), sex-myoblast migration (32), and excretory duct cell differentiation (38). Vulval differentiation has been the main system used in a number of laboratories to identify new components of this pathway and study its regulation. Vulval differentiation in *C. elegans* hermaphrodites is controlled by the combination of several cell-cell signaling events (Fig. 1A) (17, 31). In particular, an inductive signal from the anchor cell induces three of the six vulval precursor cells (VPCs, P3.p to P.8p) to differentiate into vulval cells (Fig. 1A). The *let-60 ras* gene acts in a conserved signal transduction cascade to transduce the anchor cell signal encoded by the *lin-3* gene (Fig. 1C). Previous genetic screens in several laboratories have identified many components that are either key factors acting in the main backbone of the signaling cascade (Fig. 1C) or factors that regulate the activity of these key players in the pathway (17, 31). The functions of several genes as regulators of the signaling pathway (e.g., *unc-101*, *sli-1*, and *ksr-1*) have been identified only by the genetic suppressor phenotypes and the synergistic effects of their mutations in other mutant backgrounds, since mutants with loss-of-function mutations in these genes show few or no abnormalities in vulval development (14, 18, 19, 30).

To identify negative factors that down regulate the *let-60 ras* activity, we screened for mutations that suppress the Vulvaless phenotype caused by a *let-60 ras* dominant negative *(dn)* mutation, *let-60(K16N)*. In this paper, we describe the gene *sur-5*, defined by three such suppressor mutations. Our genetic study of *sur-5* indicates that it acts negatively on Ras, possibly by down regulating one of its activators. Our molecular analysis of the gene suggests that *sur-5* encodes a novel and conserved protein.

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MATERIALS AND METHODS

Strains and genetic methods. Methods for culturing, handling, and genetically manipulating *C. elegans* were previously described (3). All genetic experiments were done at 20°C except as otherwise noted. Methods for analyzing vulval defects under dissecting microscopes were performed as described previously (7, 9). Unless otherwise noted, the mutations used in this study are as described by Riddle et al. (23) and are as follows: *lon-2(e678)*, *unc-24(e138)*, *dpy-6(e14)*, *dpy-7 (e88)*, *unc-6(e78)*, *unc-18(e81)*, *let-60(n1046gf)*, *lin-15(n765)*, *lin-10(e1439)*, *lin-1 (e1275)*, *dpy-20(e1282)*, *dpy-20(e1362)*, *him-5(e1490)*, *mpk-1/sur-1(ku1)*, *let-23(sy1)*, *lin-45(sy96)*, *lin-45(ku112)* (30), *let-65(s254)*, *unc-22(s7)*, *unc-119(ed3)*, *let-60(sy94)*, *let-60(sy93)*, *let-60(sy101)*, *let-60(sy100)*, *let-60(n1531)*, *let-60(n2031)*, and *uDf1* (25).

Isolation and genetic characterization of suppressor mutants. Animals of the *unc-24(e138) let-60(sy94 dn)/let-65(s254) unc-22(s7)* genotype were mutagenized with 50 mM ethyl methanesulfonate (3), and the F_2 progeny were screened for egg-laying revertants. Candidates were picked and further characterized. The Vul percentage was determined by picking L4-stage animals and counting the number of Vul animals in the next 2 days. Two outcrosses were performed to eliminate false candidates and clean the genetic background. The first outcross was done by mating each candidate with *dpy-20(e1282)*; *him-5(e1490)* males. F_1 Vul animals from the first outcross were picked out to produce F_2 animals. At this point, candidates for intragenic suppressors were identified as dominant suppressors of Vul but not the lethal phenotype. Non-Dpy non-Egl$^-$ F_2 animals were allowed to propagate and were used for the second outcross. The second outcross was done by mating each surviving candidate from the first outcross to *dpy-20(e1282)/dpy-20(e1362) unc-31(e169)*; *him-5(e1490)*/+ males. Non-Dpy F_1 Vul animals were picked to produce F_2 animals. Non-Dpy non-Egl$^-$ F_2 animals were picked and allowed to propagate. These animals should have the genotype *unc-24(e138) let-60(sy94 dn)/dpy(e1362) unc-31(e169)*; *suppressor/suppressor*. We screened 24,000 haploid genomes and found 3 potential intragenic revertants and 10 extragenic suppressors including three *sur-5* alleles.

Genetic mapping and complementation tests. We constructed genetic mapping strains for each chromosome except chromosome IV. The genotypes of the two-point mapping strains are *dpy-20(e1282)IV*; *dpy-5(e61) unc-101(m1)*I, *dpy-20*

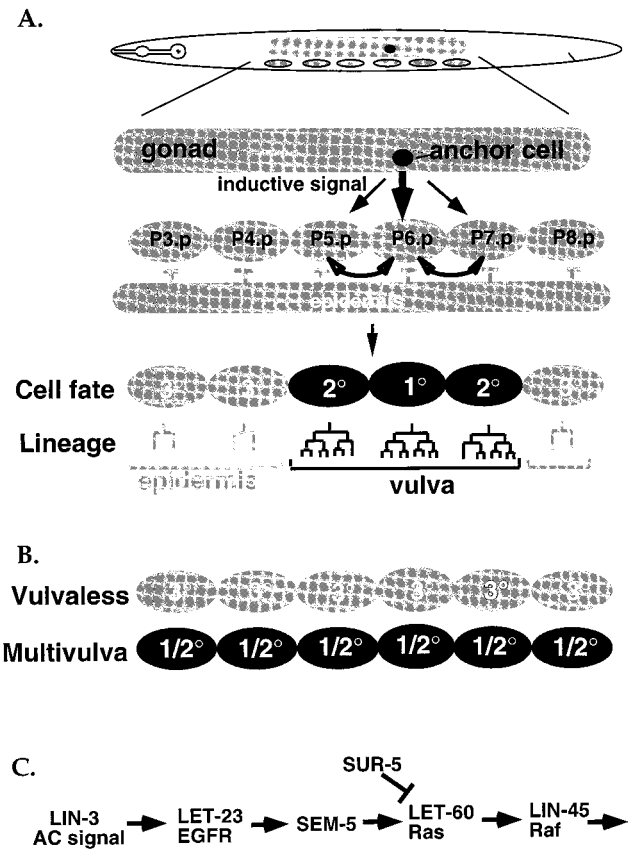

FIG. 1. Vulval differentiation induced by cell-cell signaling. (A) Three-signal model for vulval cell fate specification (17, 31). A negative signal has been proposed to act from the surrounding epidermis to inhibit vulval differentiation of the six VPCs. An inductive signal from the anchor cell in the gonad induces the nearest three of six VPCs (P3.p to P8.p) to adopt the vulval cell fate, and the stronger signal received by P6.p due to its proximity to the anchor cell induces this cell into 1° vulval cell fate. A lateral signal acting between neighboring VPCs induces 2° cell fate in P5.p and P7.p and thus also prevents them from adopting the 1° cell fate. (B) In Vulvaless (Vul) mutants, more than three VPCs (often all six) adopt the nonvulval epidermal cell fate (3°), while Multivulva (Muv) mutations causes more than three VPCs (often all six) to adopt vulval cell fates. A block in the pathway transducing the inductive signal from the anchor cell causes the Vul phenotype, while constitutive activity of the pathway leads to the Muv phenotype. (C) Proposed *sur-5* function (based on the work described in this paper) relative to some key factors in the main backbone of the pathway transducing the anchor cell (AC) signal.

(e1282)IV; *dpy-10(e128) unc-4(e120)II*, *dpy-20(e1282)IV*; *dpy-17(e164) unc-32 (e189)III*, *dpy-20(e1282)IV*; *dpy-11(e224) unc-76(e911)V* and *dpy-20(e1282)IV*; *lon-2(e678)X*. Standard complementation tests were performed for suppressors that map to the X chromosome (3).

The *sur-5(ku105)* allele was further mapped by using three-point mapping. To map *sur-5* relative to *lon-2* and *dpy-6*, we constructed the *let-60(sy94dn)/unc-22 (s7)*; *sur-5(ku105)/lon-2(e678) dpy-6(e14)* strain. Among 13 Lon non-Dpy recombinants, 6 contain the *sur-5* mutation, indicating that *sur-5* lies between *lon-2* and *dpy-6*. A *lon-2 sur-5/unc-18(e81) dpy-6* strain was also constructed for mapping. While none of the five Unc non-Dpy recombinants contained the *sur-5* mutation, all five Dpy non-Unc recombinants contained the *sur-5* mutation, indicating that *sur-5* is located close to or to the left of *unc-18*. Finally, a *lon-2 sur-5/long-2 unc-6(e78) dpy-7(e88)* strain was constructed to map *sur-5* relative to *unc-6* and *dpy-7*. While 6 of the 10 Unc non-Dpy recombinants contained *sur-5(ku105)*, 3 of the 7 Dpy non-Unc recombinants contained the *sur-5* mutation, indicating that *sur-5* lies between *unc-6* and *dpy-7*. To determine the presence of the *sur-5* allele in the recombinants from the last two mapping strains, animals homozygous for the recombinant chromosomes were first obtained. They were then crossed with *let-60(sy94 dn)/let-60(sy130 gf)*; *lon-2(e678) sur-5(ku105)* males for a complementation test. The F_1 progeny were picked individually and scored for the Vul phenotype. The genotype of each F_1 progeny was determined by observing the segregation of genetic markers in their F_2 progeny.

Deficiency study. The deficiency *uDf1* uncovers *unc-6* and *dpy-7*. Thus, *uDf1* uncovers *sur-5*. To determine the phenotype of *sur-5(ku74)*/deficiency, we constructed a strain of *lon-2(e678) sur-5(ku74)/uDf1. lon-2(e678) sur-5(ku74)*; *him-5 (e1490)* males were crossed with a single *+/szT1*[*lon-2(e678)*]I; *uDf1/szT1X* hermaphrodite. Each non-Lon F_1 progeny was picked individually onto a plate, and its phenotype was recorded. A total of 10% of both *uDf1/+* and *uDf1/lon-2(e678) sur-5(ku74)* strains have a Egl^- phenotype that is independent of VPC induction, indicating that *sur-5(ku74)/uDf1* is wild-type for VPC induction. The genotype of each F_1 progeny was determined by examining genetic markers in the F_2 generation.

To determine the suppression of *let-60(sy94 dn)* Vul phenotype by *sur-5(ku74)/ uDf1*, we constructed animals whose genotype was *let-60(sy94 dn)/+*; *lon-2(e678) sur-5(ku74)/uDf1*. Males of the *let-60(sy94 dn)/let-60(sy130gf) dpy-20(e1282)*; *lon-2(e678) sur-5(ku74)*; *him-5(e1490)* genotype were crossed with a single *+/szT1*[*lon-2(e678)*]I; *uDf1/szT1X* hermaphrodite. Non-Lon F_1 progeny were picked onto microscope slides and scored for VPC induction percentage under a compound microscope. Each animal was then recovered from the microscope slide and placed onto an individual plate, where the animal produced F_2 progeny. The genotype of each F_1 animal was identified by examining genetic markers of its F_2 progeny. Only data from F_1 progeny with the desired genotype were considered in our experimental results.

Double-mutant constructions. We describe here how we constructed a *lin-10 (n1390)*; *sur-5(ku74)* double mutant as an example of our general procedure for constructing *sur-5(ku74)* double mutants. To construct the *lin-10(n1390)*; *sur-5 (ku74)* double-mutant animals, males of the genotype *lon-2(e678) sur-5(ku74)*; *him-5(e1490)* were crossed with the *lin-10(n1390)* hermaphrodites. F_1 progeny were then picked to individual plates. Double-mutant candidates were then selected by picking F_2 progeny that are Lon and Vul, which indicated homozygous *lin-10* and possible homozygous *sur-5(ku74)*. Each candidate's progeny was tested for the presence of the *sur-5(ku74)* molecular lesion, a 295-bp deletion, by PCR. Candidates that are Lon and Vul and whose progeny showed only the *ku74* molecular lesion on agarose gel were considered to be *lin-10(n1390)*; *sur-5(ku74)* double mutants.

Microinjection transformation. All cosmids used in this study were obtained from A. Coulson and J. Sulston (Sanger Center, Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom). DNA isolation, analysis, and subcloning were performed by standard molecular biology methods. Microinjection of cloned DNAs into the gonadal syncytia of *C. elegans* hermaphrodites was carried out as described previously (21). All the cosmids listed in Fig. 2 were initially injected as a pool of four overlapping cosmids at a concentration of ~12 ng/μl each. Positive cosmids were then injected individually at a concentration of ~60 ng/μl. The subclones were first injected as a pool of four nonoverlapping subclones at a concentration of ~15 ng/μl each. Subclones from positive pools were then injected individually at a concentration of ~15 ng/μl. A strain of *let-60(sy94 dn)/dpy-20(e1362) unc-31(e169)*; *lon-2(e678) sur-5(ku105)* was used as the host strain for microinjection. This strain is <15% Vul. Rescue of the *sur-5* mutant phenotype was indicated by a >40% Vul phenotype. Each injection was scored with at least four independent transgenic lines. The extent of rescue varied among different transgenic lines, ranging from 10 to 66% Vul phenotype. Plasmid pTG1 also rescues a strain of *let-60(sy94 dn)/dpy-20(e1362) unc-31(e169)*; *sur-5(ku74)*. Plasmid pTG1_1, which has an additional 1.8 kb 5′ upstream DNA sequence added to the *sur-5* 5′-flanking sequence in pTG1, also rescues the strain *let-60(sy94 dn)/dpy-20(e1362) unc-31(e169)*; *sur-5(ku74)*. When pTG1_1 was injected at a concentration of ~45 and ~60 ng/μl into wild-type animals, no mutant phenotypes were observed. For all the above microinjections, pRF4, which contains the dominant *rol-6* mutant gene, was used as the transformation marker injected at a concentration of ~100 ng/μl.

To overexpress *sur-5(+)* in the *let-60 (n1046gf)* background, we used the *let-60 (sy130gf) dpy-20(e1282)* host strain as well as the *let-60(n1046gf)*; *unc-119(ed3op)* strain. The molecular lesions of *sy130* and *n1046* alleles are identical (G13E) (1). When the *let-60(sy130gf) dpy-20(e1282)* mutant was used as a host strain, pMH86, which contained a *dpy-20(+)* gene (11), was injected as a transformation marker at a concentration of ~15 ng/μl, and pTG1_1 was injected at a concentration of ~15 ng/μl. When the mutant *let-60(n1046gf)*; *unc-119(ed3op)* was used as the host strain, pDP#MM016B, which had the *unc-119(+)* gene (20), was injected as the transformation marker at a concentration of ~30 ng/μl and pTG1_1 was injected at a concentration of 100 ng/μl. In both cases, we observed similar suppression of the Muv phenotype in independent transgenic lines.

To test whether SUR-5:GFP fusion protein retains the *sur-5* function, we injected pTG96_1 into a *let-60(sy94 dn)/dpy-20(e1362) unc-31(e169)*; *sur-5(ku74)* strain at a concentration of ~100 ng/μl. The resulting transgenic line is 74% Vul, indicating the rescue of the *sur-5* mutant phenotype.

To overexpress *sur-5* in the *lin-45(ku112)* background, we injected a *lin-45 (ku112) dpy-20(e1282)* strain with pTG96_1 at a concentration of ~100 ng/μl. All transgenic lines had similar phenotypes. *kuEx76* was then crossed into various genetic backgrounds for other studies.

Northern analysis, cDNA isolation, and DNA sequencing. pTG1 was used as a template for making radioactive probes for Northern blots. Low levels of *sur-5* transcript were detected in poly$(A)^+$ early embryonic RNAs and total RNAs. pTG1 was used to screen approximately 10^6 plaques from a λgt11 early embryonic cDNA library (a gift from P. Okkemma and A. Fire). Four clones were identified. All four clones lack one or two nucleotides that caused deletion of the

first amino acid, methionine, based on the sequence data and on a prediction by the *C. elegans* genome project.

cDNA and genomic DNA were sequenced by directly sequencing the PCR products purified from agarose gels. To determine the molecular lesions of the *sur-5* mutation alleles, we sequenced all *sur-5* exons and exon/intron boundaries by using gel-purified DNAs which were PCR amplified from *sur-5* mutants and wild-type animals.

Plasmid construction. The rescuing plasmid, pTG1, has a 8-kb *Bam*HI fragment with the overlapping region of cosmids K03A1 and R12E12 cloned into pBluescript. Plasmid pTG1_1 was constructed by first inserting an 1.8-kb *Nsi*I-*Bam*HI fragment upstream of *sur-5* into the *Pst*I-*Bam*HI site of the pUC19 vector. The 8-kb *Bam*HI fragment of pTG1 was then inserted into the *Bam*HI site of the resulting plasmid.

To make a translational SUR-5–GFP fusion construct, we fused the *sur-5* genomic DNA sequence just before the Stop codon in-frame to the sequence of green fluorescent protein (GFP) fusion vectors (8a). We first amplified a 339-bp DNA fragment with PCR primers 5′AACTGCAGGAGGGCATGGACGAGG AA3′ and 5′TCCCCCCGGGAAGTCTGTATTGAACGAAAT3′ from pTG1. This fragment includes the last 327 nucleotides just before the Stop codon. This fragment was cloned into the *Pst*I-*Sma*I sites of pBluescript. After we sequenced this fragment, the 231-bp *Bsp*E1-*Sma*I fragment was cut out and used to replace the *Sma*I-*Bsp*EI fragment of pTG1_1 to create plasmid pTG1_3. The *Sph*I-*Sma*I fragment of pTG1_3 was then cloned into the *Sph*I-*Sma*I sites of GFP containing the vector pPD95.70 to create pTG96 and into the *Sph*I-*Sma*I sites of pPD95.79 to create pTG96_1. To create a SUR-5–GFP transcriptional fusion protein, we used PCR to amplify from pTG1_1 the 3.68-kb 5′ upstream sequence of *sur-5* with PCR primers 5′GCCAAGCTTGCATGCCTGCA3′ and 5′GCTCTAGA CATTCTGAAAACAAAATCTAAA3′. The 3′ end of this PCR fragment included the first amino acid, methionine, of SUR-5 in frame with linker amino acids whose DNA sequence represents a *Xba*I site. This PCR product was cut with *Xba*I and *Sph*I and then cloned into the *Sph*I-*Xba*I sites of pPD95.69 to create pTG96_2.

Mammalian *sur-5* homology search. We use the BLAST computer program to search for homologs. Only in the EST database did we find mammalian sequences that are similar to SUR-5. They consist of mouse and human cDNA sequences. Based on these sequences, it is likely that all the EST sequences are incomplete cDNAs made from the same transcript from each organism. We then cloned additional human and mouse sequences by PCR by screening about 3×10^6 plaques of a mouse thymus and a human brain cDNA library (Stratagene), using lambda reverse or forward primers as the 5′-end primers and specific EST sequences as the downstream 3′ primer. We finally cloned an additional 1.4-kb 5′ human cDNA fragment by PCR from a psport I human brain cDNA library, using psport forward primer as the 5′-end primer and specific sequences as the downstream 3′ primer.

RESULTS

Isolation of suppressors of dominant negative *let-60 ras*. To identify negative regulators in the *let-60 ras* pathway, we screened for suppressors of a *let-60* dominant negative allele, *let-60(K16N dn)* (genetic allele name, *sy94* [12]). Homozygous *let-60(K16N dn)* mutations are completely lethal, while heterozygous *let-60(K16N dn)*/+ worms display a moderately reduced LET-60 protein activity as indicated by the partial defect in vulval development (about 30% VPC induction and 97% egg-laying defective [Egl$^-$]) (Table 1) (11). This allele was chosen for the screen because (i) it does not poison the *let-60* (+) Ras activity completely in the heterozygous mutant, which may allow us to isolate a wide variety of mutants with mutations in different *let-60* negative regulators; and (ii) it has a relatively tight Egl$^-$ phenotype (97%), which would facilitate the screening for egg-laying competent (non-Egl$^-$) revertants.

We mutagenized *let-60(K16N)*/+ heterozygous worms with EMS and then screened for mutations that suppress the Vul phenotype by selecting non-Egl$^-$ progeny (see Materials and Methods). We screened 24,000 mutagenized haploid genomes and isolated 13 independent mutations. Ten suppressors are extragenic, recessive, suppressor mutations, and the remaining three suppressors are probably intragenic revertants. The *sur-5* gene is defined by three allelic suppressor mutations on X: *ku74*, *ku105*, and *ku131* (Table 1). We mapped *sur-5* to a region between the *unc-6* and *dpy-7* genes on the X chromosome. It is approximately 0.18 map units to the left of the *dpy-7* marker (see Materials and Methods).

All three *sur-5* alleles are similar in their genetic properties.

TABLE 1. Suppression of *ras(dn)* by *sur-5* alleles

sur-5 genotype[a]	*let-60* genotype[a]	% Egl$^-$ (*n*)[b]	% Vulval differentiation (*n*)[c]
+/+	+/+	0	100
ku74/*ku74*	+/+	0 (100)	100 (20)
ku105/*ku105*	+/+	0 (200)	100 (21)
ku131/*ku131*	+/+	0 (100)	ND[d]
+/+	*K16N dn*/+	97 (300)	32 (20)
ku74/*ku74*	*K16N dn*/+	2 (314)	99 (25)
ku105/*ku105*	*K16N dn*/+	8 (309)	83 (20)
ku131/*ku131*	*K16N dn*/+	3 (398)	ND
ku74/*uDf1*	*K16N dn*/+	ND	88 (10)

[a] The complete genotypes for the four groups shown are (from top to bottom) wild-type N2, *sur-5(kuX)*/*sur-5(kuX)*, *let-60(sy94)*/*dpy-20(e1326) unc-31(e169)*; *sur-5(kuX)*/*sur-5(kuX)*, and *let-60(sy94)*/*dpy-20(e1326) unc-31(e169)*; *lon-2(e678) sur-5(ku74)*/*uDf-1*. + indicates wild type.

[b] Percentage of hermaphrodites that are Egl$^-$. *n* indicates the number of animals scored.

[c] Percentage of VPCs (P3.p to P8.p) that differentiate into vulval cells relative to the wild type (100%) (9).

[d] ND, not determined.

They all cause the *let-60(K16N)* dominant Egl$^-$ phenotype to revert to mostly wild type. None of them have obvious mutant phenotypes on their own (Table 1), and they all fail to suppress the homozygous lethal phenotype and the mating defect of the *let-60(K16N)*/+ males (data not shown). The suppression of the Egl$^-$ phenotype is due to increased vulval cell induction, as indicated by data from examining two of the *sur-5* mutations under Nomarski optics (Table 1). All *sur-5* alleles are recessive (data not shown). *sur-5(ku74)* appears to be the strongest mutation since it suppresses the *let-60(K16N dn)* Vul phenotype to nearly wild-type levels. *ku74* could be a null allele or a severe loss-of-function allele since a *ku74*/deficiency strain shows a similar mutant phenotype. However, since the deficiency (*uDf1*/+) heterozygote itself is not as healthy and has a Egl$^-$ phenotype, this test is not conclusive.

As indicated in Table 1, none of the *sur-5* alleles can cause complete reversion of the *let-60(sy94 dn)* Vul phenotype; a small percentage of the suppressed animals are still Egl$^-$. If these alleles are null or severe-loss-of-function mutations, this result may indicate that rather than being a major negative regulator in the Ras pathway such as the *lin-1* gene (2), *sur-5* may play a role in fine-tuning the level of *let-60 ras* activity in the pathway. On the other hand, the *sur-5* function could be redundant or partially redundant in *C. elegans*, so that eliminating its function may cause only a small increase in signaling activity. We also do not exclude the possibility that the *sur-5* alleles we have isolated are not null alleles. It is conceivable that a *sur-5* null mutation is lethal and that we were unable to isolate such an allele in our screen because of this.

Genetic interactions between *sur-5* and mutations in other genes in the signaling pathway. To determine where *sur-5* acts in the *let-60* Ras pathway, we constructed double mutants between mutants containing *sur-5(ku74)* and other loss-of-function mutations of the *let-60 ras* pathway and then examined the vulval phenotype of these double mutants (Table 2).

sur-5(ku74) fails to suppress *lin-45(sy96)* and *mpk-1*/*sur-1 (ku1)*. Since *lin-45* and *mpk-1* are known positive factors downstream of *let-60*, this result could suggest that *sur-5* does not act downstream of *lin-45* and *mpk-1*. However, *sur-5(ku74)* also fails to suppress mutations in two genes upstream of *let-60 ras*, *let-23(sy1)* and *sem-5(n2019)*. These results suggest that the *sur-5(ku74)* mutation does not cause a significant increase of *let-60 ras* activity in the *let-60(+)* background which would

TABLE 2. Genetic interactions between *sur-5(ku74)* and mutations in other genes

sur-5 genotype[a]	Other genotype[a]	% Egl$^-$ (*n*)[b]	% VPC induction (*n*)[c]
+	*let-23(sy1)*	71 (187)	15 (12)
ku74	*let-23(sy1)*	75 (175)	13 (10)
+	*sem-5(n2019)*	92 (234)	ND[d]
ku74	*sem-5(n2019)*	91 (295)	ND
+	*let-60(n2021)*	21 (141)	88 (20)
ku74	*let-60(n2021)*	27 (124)	90 (19)
+	*lin-45(sy96)*	96 (124)	30 (12)
ku74	*lin-45(sy96)*	97 (140)	28 (10)
+	*mpk-1(ku1)*	ND[e]	85 (15)
ku74	*mpk-1(ku1)*	ND[e]	81 (11)
+	*lin-15(n765)*	ND[e]	110 (12)[c]
ku74	*lin-15(n765)*	ND[e]	119 (17)[c]
+	*lin-10(e1439)*	84 (124)	ND
ku74	*lin-10(e1439)*	88 (212)	ND
+	*lin-8(n111)*	0 (180)	100 (20)
ku74	*lin-8(n111)*	0 (200)	100 (22)
+	*lin-9(n112)*	0 (200)	100 (22)
ku74	*lin-9(n112)*	0 (200)	100 (48)

[a] The complete genotypes for the 18 strains shown are (from top to bottom) *let-23(sy1)*; *lon-2(e678)*, *let-23(sy1)*; *lon-2(e678) sur-5(ku74)*, *lon-2(e678) sem-5(n2019)*, *lon-2(e678) sur-5(ku74) sem-5(n2019)*, *let-60(n2021)*; *lon-2(e678)*, *let-60(n2021)*; *lon-2(e678) sur-5(ku74)*, *unc-24(e138) lin-45(sy96)*; *lon-2(e678)*, *unc-24 (e138) lin-45(sy96)*; *lon-2(e678) sur-5(ku74)*, *dpy-17(e164) mpk-1(ku1)*, *dpy-17 (e164) mpk-1(ku1)*; *lon-2(e678) sur-5(ku74)*, *lon-2(e678) lin-15(n765)*, *lon-2(e678) sur-5(ku74) lin-15(n765)*, *lin-10(e1439)*; *lon-2(e678)*, *lin-10(e1439)*; *lon-2(e678) sur-5(ku74)*, *dpy-10(e128) lin-8(n111)*, *dpy-10(e128) lin-8(n111)*; *lon-2(e678) sur-5 (ku74)*, *dpy-17(e164) lin-9(n112)*, and *dpy-17(e164) lin-9(n112)*; *lon-2(e678) sur-5 (ku74)*.

[b] Percentage that are egg-laying defective (Egl$^-$). *n* indicates the number of animals scored.

[c] All *lin-15* experiments were done at 17°C.

[d] ND, not determined.

[e] The percentages of Egl$^-$ animals are not recorded because *mpk-1(ku1)* has an Egl$^-$ phenotype that is not due to lineage defects (36) and *lin-15(n765)* homozygous worms often have an exploding gonad phenotype that is difficult to distinguish from Egl$^-$ phenotypes.

have suppressed the *let-23* alleles (12). However, since it is possible that *sur-5(ku74)* is not a null allele, a null allele might have a stronger effect on mutations in some of the genes tested.

A synthetic Multivulva (syn-Muv) pathway in vulval induction has been described previously (8). There are two classes of mutations in this pathway, class A and class B. Mutations in either class cause no phenotype by themselves, but double mutants containing a mutation in both classes shows a Muv phenotype. Class A and class B genes thus define two functionally redundant pathways that negatively regulate vulval induction (8). To determine if *sur-5* belongs to the syn-Muv gene groups, we made double mutants between *sur-5(ku74)* and the class A mutation *lin-8(n11)* or the class B mutation *lin-9(n112)*. We did not detect any mutant vulval phenotypes in these strains (Table 2). We conclude that *sur-5* is not one of the syn-Muv genes.

It is also possible that the *sur-5* function is partially redundant with respect to these syn-Muv genes. To test this possibility, we made a double mutant between *sur-5(ku74)* and *lin-15(n765)* to see if *sur-5* can enhance the Muv phenotype caused by *lin-15*. The *lin-15* locus contains both class A and class B genes of the syn-Muv gene family (8). The *n765* allele genetically mutates both class A and class B genes, and it is a temperature-sensitive allele: the mutant is 100% Muv at 20°C and about 78% Muv at 15°C (7, 13). We examined the vulval induction of a *sur-5(ku74) lin-15(n765)* double mutant at 17°C by using Nomarski optics, and we found that *sur-5(ku74)* fails to enhance the Muv phenotype of *lin-15(n765)* at 17°C (Table 2).

To test if the *sur-5* mutations can suppress a loss-of-function *ras* allele, we constructed and examined the *sur-5(ku74)*; *let-60 (n2021 G75S)* double mutant. *let-60(G75S)* is a partial-loss-of-function mutation that causes 98% death. Of the 2% of animals escaping death, some are Vulvaless as adults (1). We found that *sur-5(ku74)* failed to suppress the Vulvaless phenotype of *let-60(G75S)* escapees (Table 2). It is possible that *let-60(G75S)* is also mutant in responding to negative regulation by *sur-5*. This result may suggest that *sur-5* is involved in only a specific aspect of regulation of *ras* activity.

Genetic interactions between *sur-5(ku74)* and *let-60 dn* mutations. To determine whether suppression by *sur-5(ku74)* of the dominant Vul phenotype caused by *let-60(K16N dn)* is allele specific, we tested whether *sur-5(ku74)* can suppress other *let-60 dn* mutations (1, 11, 12). We found that *sur-5(ku74)* was able to suppress four *let-60 dn* alleles, *sy94 (K16N)*, *sy101(G10R)*, *n1531(G15D)*, and *n2301(G15S)*, but failed to suppress two other *let-60 dn* alleles, *sy93(E119N)* and *sy100(S89F)* (Table 3). We have also constructed and tested double mutants between *let-60(S89F)* and the other two *sur-5* alleles and found that these *sur-5* alleles also fails to suppress the dominant Vul phenotype caused by *let-60(S89F)* (data not shown). All of the *let-60(dn)* alleles that can be suppressed by *sur-5(lf)* (called group I alleles) altered the residues within the first conserved loop of the Ras protein that is involved in GTP/GDP binding (35). One of the *sur-5(lf)* nonsuppressible alleles (group II

TABLE 3. Interaction between *sur-5(ku74)* and *let-60(dn)* mutations

Genotype[a]			Phenotype	
sur-5	*let-60 dn* allele	Lesion	% Egl (*n*)	% Vulval differentiation (*n*)
+/+	*sy94*/+	*K16N*	97 (300)	32 (20)
ku74/*ku74*	*sy94*/+	*K16N*	2 (314)	99 (25)
+/+	*sy101*/+	*G10R*	80 (170)	35 (18)
ku74/*ku74*	*sy101*/+	*G10R*	9 (247)	90 (20)
+/+	*n1531*/+	*G15D*	88 (228)	44 (17)
ku74/*ku74*	*n1531*/+	*G15D*	4 (418)	89 (14)
+/+	*n2301*/+	*G15S*	33 (220)	81 (16)
ku74/*ku74*	*n2301*/+	*G15S*	1 (310)	99 (21)
+/+	*sy93*/+	*D119N*	95 (200)	5 (12)
ku74/*ku74*	*sy93*/+	*D119N*	95 (180)	6 (10)
+/+	*sy100*/+	*S89F*	25 (418)	70 (22)
ku74/*ku74*	*sy100*/+	*S89F*	25 (257)	65 (14)

[a] The complete genotypes for the 12 strains shown are (from top to bottom) *let-60(sy94)/dpy-20(e1282) unc-31(e169)*, *let-60(sy94)/dpy-20(e1362) unc-31(e169)*; *sur-5(ku74)*, *let-60(sy101) dpy-20(e1282)/unc-22(s9)*; *lon-2(e678)*, *let-60(sy101) dpy-20(e1282)/unc-22(s9)*; *lon-2(e678) sur-5(ku74)*, *let-60(n1531)/dpy-20(e1282)*; *lon-2(e678)*, *let-60(n1531)/dpy-20(e1282)*; *lon-2(e678) sur-5(ku74)*, *let-60(n2301)/ dpy-20(e1282)*; *lon-2(e678)*, *let-60(n2301)/dpy-20(e1282)*; *lon-2(e678) sur-5(ku74)*, *let-60(sy93)/dpy-20(e1282)*; *lon-2(e678)*, *let-60(sy93)/dpy-20(e1282)*; *lon-2(e678) sur-5(ku74)*, *let-60(sy100) dpy-20(e1282)/unc-22(s9)*; *lon-2(e678)*, and *let-60(sy100) dpy-20(e1282)/unc-22(s9)*; *lon-2(e678) sur-5(ku74)*. + indicates wild type.

TABLE 4. Phenotype of strains with extrachromosomal arrays containing *sur-5(+)*

Genotype[a]	Phenotype[b]					
	% Muv	*n*	% Egl	% Unc	% Lethality	*n*
let-60(gf);unc-119(lf); Ex unc-119(+)	89	301				
let-60(gf);unc-119(lf); Ex unc-119(+) sur-5(+)	34	345				
lin-45(ku112) dpy-20(lf)			0	0	0	100
lin-45(ku112) dpy-20(lf); Ex sur-5(5′)			0	0	0	124
lin-45(ku112) dpy-20(lf); Ex sur-5(+)			15	64	6	80

[a] The complete genotypes of the five strains shown are (from top to bottom) *let-60(n1046G13E)*; *unc-119(ed3)*; *Ex pUNC-119(+)*; *let-60(n1046G13E)*; *unc-119(ed3)*; *Ex pUNC119(+)* and *pTG1_1*, *lin-45(ku112) dpy-20(e1282)*, *lin-45 (ku112) dpy-20(e1282)*; *ExpMH86*, *pTG96_2 [sur-5(5′)-GFP]*, *lin-45(ku112) dpy-20(e1282)*; *ExpMH86 pTG96_1[sur-5(+)]. pTG96_2 [surr-(5′)]* contains GFP fused to the *sur-5* promoter and the first codon. Ex indicates extrachromosomal array.

[b] Muv, animals with a multivulva phenotype observed as multiple ventral protrusions under dissecting microscopes. *n* is the number of animals scored. Egl, egg-laying defective; Unc, uncoordinated movement.

alleles), *E119N*, mutates residue 119 on loop 8, which is also involved in nucleotide binding, while the other group II allele S89F mutates residue 89, which might be involved in interacting with other factors (35). It is interesting that the group II allele *let-60(S89F)* is the weakest dominant negative allele, based on the percentage of vulval differentiation, while the other group II allele *let-60(E93N)* is the strongest dominant negative allele (Table 3) (11). Therefore, it is unlikely that the difference in *sur-5(lf)* suppression between the two groups is due to the strength of the dominant negative effect of the *let-60 ras(dn)* alleles. The results in Table 3 may suggest that the dominant negative effects of these two groups of *ras(dn)* mutations are due to two different mechanisms (see Discussion).

Phenotypes due to overexpression of *sur-5*. If *sur-5* is a negative regulator of *let-60 ras*, it is possible that elevating *sur-5* activity will suppress the Muv phenotype caused by an activated *let-60 ras* mutation. Since an extrachromosomal array can contain hundreds of copies of injected plasmids (21), we tested if we could observe an effect of overexpressing *sur-5* transgenes (see below for cloning the *sur-5* gene). The data in Table 4 indicates that an extrachromosomal array carrying a *sur-5(+)* plasmid partially suppresses the Muv phenotype of *let-60 (G13E gf)* from 89 to 34%. This result is consistent with the notion that *sur-5* plays a negative role in the *let-60 ras* pathway.

We also tested if overexpression of *sur-5* could suppress the Muv phenotype due to mutations in two other negative regulators, *lin-15* and *lin-1*, by introducing an extrachromosomal array carrying the *sur-5* transgene (*kuEx76*) into *lin-15(n765)* and *lin-1(e1275)* mutant animals. We observed no reduction in the percentage of Muv in these transgenic animals (data not shown). We also observed no mutant phenotype when *sur-5* was overexpressed from various extrachromosomal arrays in the wild-type background. These results are consistent with the notion that *sur-5* is specifically involved in modifying certain aspects of *let-60 ras* activity and that its effect on the signaling pathway is limited.

Although *sur-5* overexpression generates no obvious phenotype in a *let-60(+)* genetic background, it may still reduce the signaling activity when *let-60 ras* is wild type. To observe such a possible effect, we introduced the extrachromosomal array *kuEx76* into a strain with a partial-loss-of-function mutation of *lin-45raf, ku112. lin-45(ku112)* also causes no mutant phenotypes in a *let-60(+)* genetic background but suppresses the Muv phenotype caused by *let-60(G13E gf)* (30), indicating that the Ras-mediated signaling is reduced but not eliminated in the *lin-45(ku112)* mutant. The *sur-5* transgene in the *lin-45 (ku112)* mutant causes some synergistic mutant phenotypes (Table 4). A total of 64% of the transgenic animals move very slowly and often show no movement for hours. Such an uncoordinated (Unc) phenotype was unexpected since it is not known that a decrease in *let-60 ras* pathway activity can lead to an Unc phenotype. This result may suggest that both *sur-5* and *lin-45raf* play a role in cellular functions (e.g., neuronal cell differentiation) that are important for mobility.

Of the transgenic *lin-45(ku112)*; *kuEx76* animals, 6% die between the L1 and L3 developmental stages. Since severe-loss-of-function mutations in *lin-45 raf* cause a larval lethal phenotype similar to that of many *let-60 ras* mutations (reference 10 and unpublished data), the synthetic lethal phenotype caused by overexpression *sur-5* in the *lin-45(ku112)* mutant suggests that *sur-5* may also function with *let-60 ras* during early development.

A total of 15% of the transgenic animals are Egl$^-$. However, this synthetic Egl$^-$ phenotype appears not to be caused by a reduction in the VPC induction, since we observed only wild-type VPC induction in the transgenic animals under Nomarski optics (data not shown). Although we cannot determine the cause of this synthetic Egl$^-$ phenotype, the phenotype may be consistent with the reduced activity of the signaling pathway. Egl$^-$ phenotypes that are not caused by defects in vulval induction are also caused by some other genes functioning in vulval fate specification. For example, certain *lin-12* and *mpk-1* mutants have a wild-type vulval induction but are Egl$^-$ for reasons that are yet to be determined (29, 36).

The *sur-5* gene encodes a novel gene product. We genetically mapped *sur-5* to a small chromosome region between two cloned genes, *unc-6* and *dpy-7* (0.37 map unit) (Fig. 2). *sur-5* is estimated to reside between 0.14 and 0.16 map unit away from *dpy-7* (see Materials and Methods). We used DNA-mediated microinjection methods to identify cosmids that span the region containing the *sur-5* gene, with the dominant *rol-6* mutant DNA as a marker (21). The host strain has the genotype *let-60 (K16N dn)/dpy-20(e1362) unc-31(e169)*; *lon-2(e678) sur-5(ku105)*. We assayed DNA-mediated rescue by scoring transformants for reversion of the suppressor phenotype of *sur-5(ku74)* on the *let-60* dominant Vul phenotype. We determined that each of two overlapping cosmids, K03A1 and R12E12, is capable of rescuing the *sur-5(ku74)* suppression phenotype (data not shown). After further subcloning and injection, we determined that an 8-kb *Bam*HI restriction fragment (pTG1) within the overlapping region of cosmids K03A1 and R12E12 rescues the *sur-5* mutant phenotype. Using this DNA fragment as a probe to screen a cDNA library (a gift from P. Okkema and A. Fire), we isolated four positive clones from about 10^6 plaques. All four cDNA clones had the same *sur-5* gene sequence. The *sur-5* cDNA sequence was predicted to encode a protein that has 700 amino acids (Fig. 3). Northern analysis with the *sur-5* genomic DNA as a probe detected a single transcript of approximately 2.2 kb which is present in the embryos and larvae (data not shown).

The computer program PROSITE (distributed by EMBL) predicts that SUR-5 has one potential ATP or GTP binding motif and two potential AMP binding motifs. Amino acids 3 to 10, AVSANGKT, fit a consensus sequence (A/G)-X_4-G-K-(S/T) for an ATP/GTP binding motif (P-loop [24, 34]). Amino acids 315 to 327, VMFSSGTTGIPK, are predicted to be an AMP binding motif and fit the consensus (L/I/V/M/F/Y)-X_2-

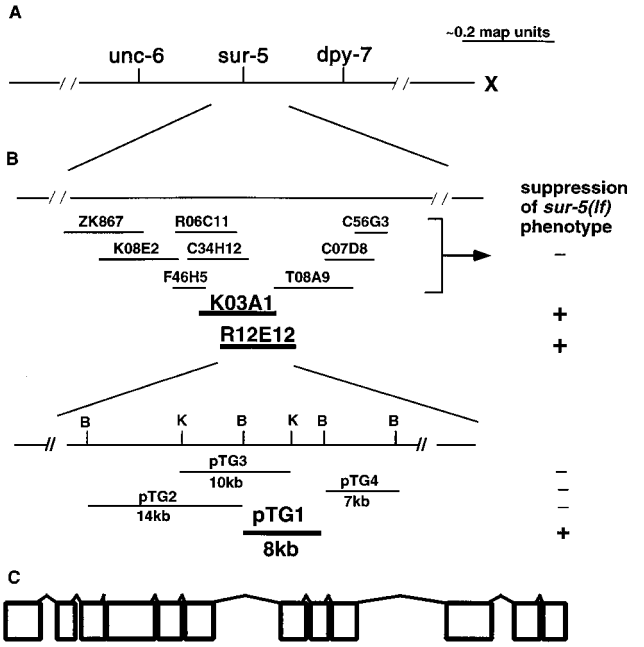

FIG. 2. Positional cloning of the *sur-5* locus. (A) Genetic map position of *sur-5* on the X chromosome. (B) Identification of DNA clones containing the *sur-5* gene. A collection of cosmid clones near the *sur-5* gene were used in microinjection transformation tests. A partial restriction map within the overlapping region of the two positive cosmids is depicted. B, *Bam*HI; K, *Kpn*I. (C) Exon and intron structure of the *sur-5* gene. A total of 12 exons (open boxes) and 11 introns (lines) are shown proportional to their actual size. The genomic sequence of *sur-5* was determined by the *C. elegans* genome project. The intron/exon boundaries were predicted by Genefinder software and confirmed by sequencing a cDNA clone.

(S/T/G)-(S/T/A/G)-G-(S/T)-(S/T/E/I)-(S/G)-X-(P/A/S/L/I/U/M)-(K/R) (26, 28, 33). Another possible AMP binding motif is at the C terminus of the SUR-5 amino acid sequence. SUR-5 protein has a relatively weak but probably significant sequence similarity to acetyl coenzyme A (acetyl-CoA) synthetases (~21 to 24% amino acid identity). At the C terminus of acetyl-CoA synthetases, there is an AMP binding-site consensus (16). Amino acids 658 to 669 of SUR-5, PYTSSGKKVEV, have similarity to the AMP binding site consensus P-K-T-(R/V/L)-S-G-K-(I/V/T)-(T/M/V/K)-R-(R/N). These sequence features may suggest that SUR-5 performs a biochemical function that requires ATP or AMP binding. The similarity to acetyl-CoA synthetases may suggest a function that involves the molecule acetyl-CoA.

We have identified the molecular lesions of two *sur-5* alleles, *ku74*, and *k105*, by sequencing the coding region and intron/exon boundaries of the mutant genomic DNA (Fig. 2). We found that the *ku74* allele has a 295-bp in-frame deletion mutation that deletes 70 amino acids (residues 76 to 145) and creates an extra threonine. Also, the *ku105* allele has a point mutation, a T-to-A change, causing methionine 370 to be replaced by lysine. Methionine 370 is conserved between *C. elegans sur-5* and its potential mammalian homologs (Fig. 3).

Isolation of a potential human SUR-5 homolog. Using BLAST software, we identified partial DNA sequences of potential mouse and human *sur-5* homologs in the EST database. We then used PCR screening of mouse thymus and human brain cDNA libraries to clone additional cDNA sequence 5′ to the EST fragments. The total cloned cDNA length (>3.2 kb including >1.2 kb of the 3′ untranslated region of the EST clones) is similar to the size of a single transcript displayed in a Northern blot, suggesting that the cDNA clone is either full-length or close to full-length. The predicted amino acid sequence of the human clone is close to the size of the *C. elegans* protein. The predicted human protein is about 38% identical to the worm protein and contains all the key features mentioned above (Fig. 3). There is about 90% amino acid identity between the potential human SUR-5 homologs and the partial mouse sequence. This result suggests that the *sur-5* gene may be conserved from *C. elegans* to humans. Northern analysis also shows that the human gene was expressed ubiquitously with higher abundance in brain and testis (data not shown). We did not identify a yeast gene that has a high degree of similarity in overall structure to the *sur-5* gene.

***sur-5* is strongly expressed in most of the cells in *C. elegans*.** To visualize *sur-5* expression in vivo, we constructed several SUR-5–GFP fusion protein constructs. One construct, pTG96, includes a 3.68-kb fragment of the 5′-flanking sequence and the full-length *sur-5* genomic sequence fused at its C terminus to GFP containing a potent, artificial nuclear localization signal (NLS) sequence. When transgenic animals carrying pTG96 on an extrachromosomal array (*kuEx75*) were examined, the fusion protein, as judged by fluorescence of GFP, was observed tightly localized to the nuclei of most cells. SUR-5 appeared to be expressed in the VPCs, consistent with a function in regulating Ras activity during vulval induction (Fig. 4). However, we have no data demonstrating that SUR-5 functions in the VPCs.

Cell types that express this fusion protein include neurons, hypodermis, Pn.p cells, body muscles, many cells of the pharynx, and a few cells of the somatic gonads. Cells that do not display the fluorescence include B, F, K′, K.a, K.p, hyp3, the germ line, and the excretory duct cells (reference 37 and data not shown). In nonmosaic animals, the intensity varies among the cells. The intestinal cells and excretory cells are almost always very bright, whereas neurons are almost always fainter. Uterine cells and many of the cells derived from the M cell are very faint and often difficult to see. The SUR-5–GFP fusion proteins are expressed in all stages of *C. elegans* development. The earliest expression is at the 100- to 150-cell embryonic stage (5a), and the fusion proteins are expressed throughout development from that stage on. The same expression pattern is seen when this array is integrated into one of the chromosomes (data not shown). The intense and broad expression of the *sur-5–GFP* fusion construct makes it a useful marker for mosaic analysis and for microinjection transformation (37).

When we expressed another SUR-5–GFP construct, pTG96_1 which differs from pTG96 in that it lacks the artificial NLS, we found that the extrachromosomal array (*kuEx76*) carrying this fusion gene appeared to have SUR-5 function since it could efficiently cause reversion of the suppression of the *let-60 (K16N dn)* dominant Vul phenotype by *sur-5* mutations (data not shown). This fusion protein is still localized in the nuclei of most cells. The expression pattern is the same as that seen from the array containing pTG96 (with NLS), but the nuclear localization is not as tight, and there appears to be some diffusion of SUR-5–GFP proteins from the nucleus to the cytoplasm. Although *sur-5* appears to be expressed strongly in the nucleus, a function of SUR-5 in the cytoplasm is still possible since a relatively small amount of SUR-5–GFP fusion protein is detected in cytoplasm.

DISCUSSION

Multiple negative factors influence the *let-60 ras* signaling pathway. We have screened for suppressors of a dominant negative *ras* mutation to identify negative regulators of the

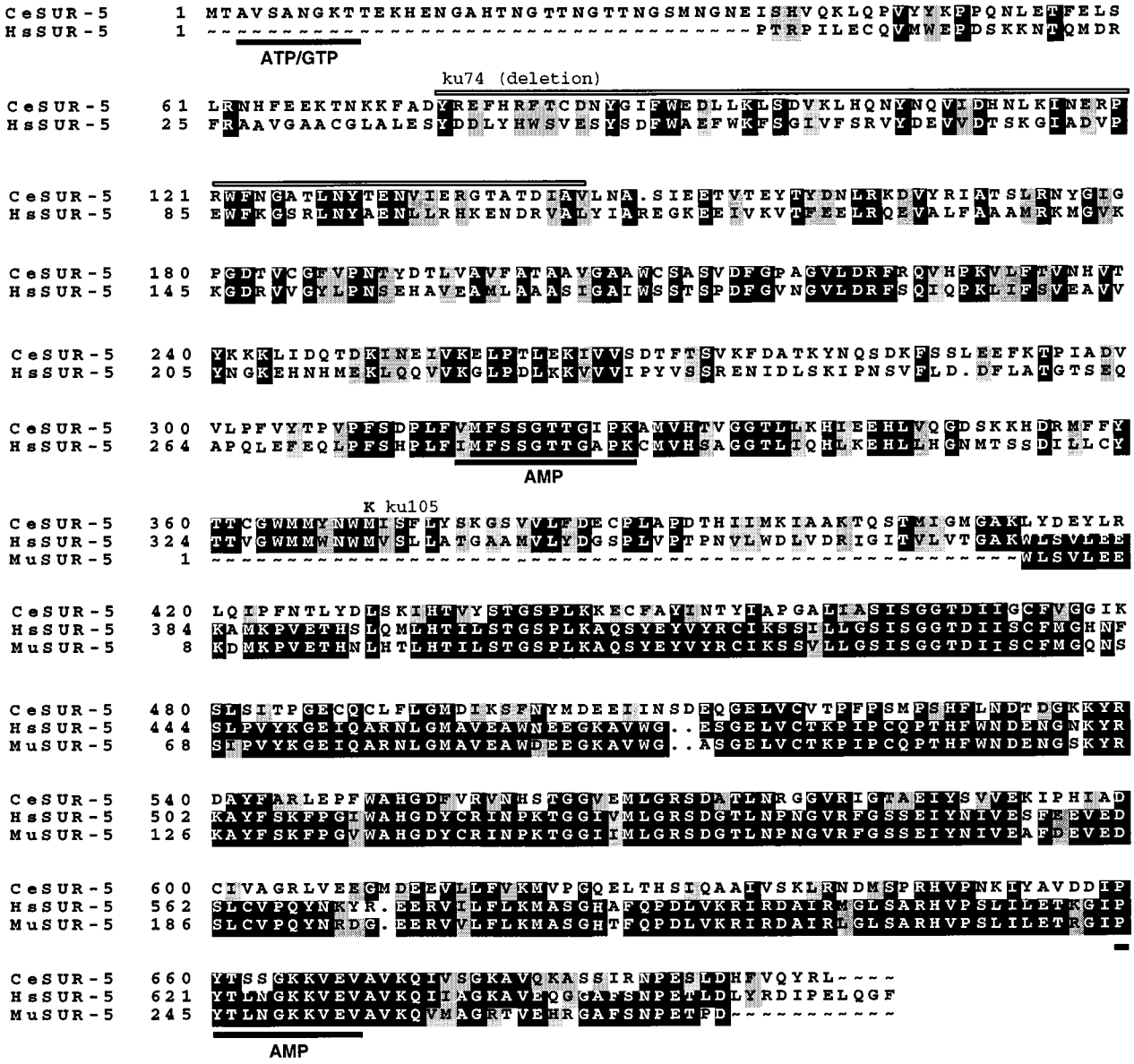

FIG. 3. Amino acid sequence alignment of SUR-5 and its potential mammalian homologs. SUR-5 is shown aligned with a potential human homolog (HsSUR-5) and a C-terminal partial mouse sequence (MuSUR-5). Boxed letters indicate amino acid identity (black) or similarity (gray) between at least two organisms. Gaps are represented by dots. Potential ATP or GTP binding motifs and AMP binding motifs are indicated by bars below the sequences. The deleted region (amino acids 76 to 145) in the *ku74* allele is indicated by the open bar above the letters. The point mutation in the *ku105* allele is also indicated.

Ras-mediated signal transduction pathway. We have isolated 10 extragenic suppressors that may define six genes involved in the Ras-mediated signaling process. Since the number of worms screened was relatively small (~24,000 haploid genome) and since it is possible that only rare mutations in some essential or multifunctional genes were recovered in the suppressor screen, the screen is probably not close to saturation. None of the 10 mutations we isolated displayed any mutant phenotypes except suppression of the Vulvaless phenotype caused by the *let-60(K16N dn)* mutation. If these mutations are null or severe-loss-of-function alleles, this result suggests that negative regulators defined by these mutations play a limited regulatory role or that there is functional redundancy among two or more negative regulators. In the case of the *sur-5* gene, our genetic and molecular data do not exclude the possibility that the mutations isolated are only partial-loss-of-function mutations.

Role of *sur-5* in regulating *ras* activity. Although *sur-5(ku74)* is an effective suppressor of several dominant negative *let-60 ras* mutations, it does not suppress mutations in *sem-5* or *let-23* receptor tyrosine kinase, both of which act upstream of *let-60 ras*. Since *sem-5(n2019)* and *let-23(sy1)* homozygous mutants do not display a more severe Vul phenotype than that of *let-60 (K16N dn)*/+ animals, loss of *sur-5* appears to elevate vulval induction more in the *let-60 ras (dn)*/+ background than in the lowered *let-60(+)* background.

The mutant phenotype (e.g., Vul in *C. elegans*) caused by a dominant negative *ras* mutation in a *ras(dn)*/+ heterozygote is due to the toxic effect of the mutant protein on the wild-type protein. Previous studies on *dn* mutations in mammalian, yeast,

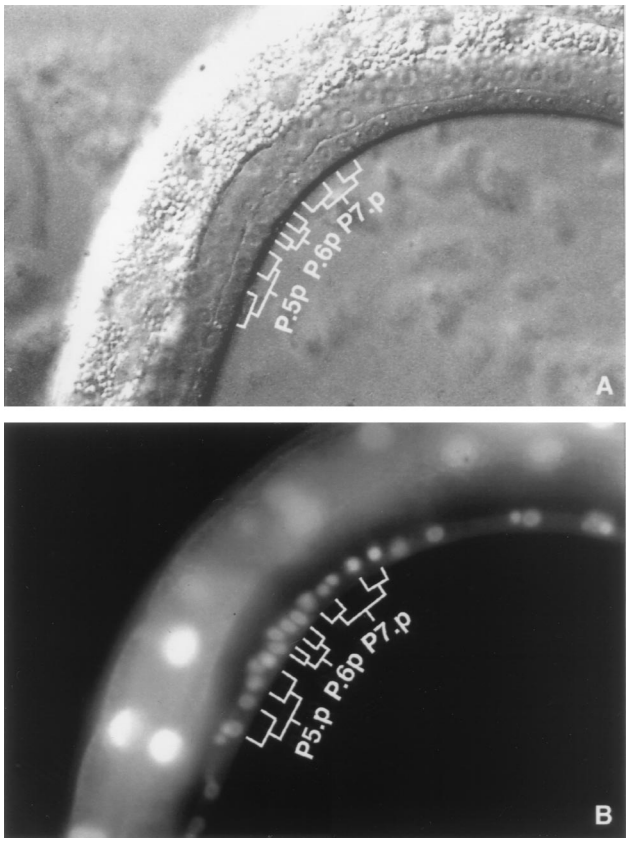

FIG. 4. SUR-5–GFP expression in vulval cells in a transgenic animal carrying a SUR-5–GFP construct (N2; kuEx75) examined under a compound microscope with Nomarski optics. (A) Nomarski image of the mid-body. The three VPCs (P5.p to P7.p) have just completed the second round of cell division. (B) The same animal examined under a fluorescent light source. The SUR-5 and GFP proteins are expressed mostly in the nuclei, and all cells derived from VPCs express the fusion protein.

and *C. elegans ras* genes indicated that the toxic effect of at least some *dn* alleles (e.g., the G15A mutation in the mammalian proteins) is probably due to the titration of a Ras activator such as a guanine nucleotide exchange factor (GNEF) by the mutant protein (6, 11, 15, 22, 27). Therefore, there are perhaps two possible mechanisms by which *sur-5* mutations suppress the Vul phenotype in *let-60 (dn)*/+ mutants. By one mechanism, SUR-5(+) protein may suppress LET-60 Ras(+) activity (e.g., it may directly suppress its ability to bind to an effector or may suppress the expression of the effector); loss of *sur-5* activity may result in an increase of LET-60 Ras(+) activity that either competes better with the LET-60 Ras(dn) for the activator or becomes less dependent on the activator. By the other mechanism, SUR-5(+) may inhibit the activity of an activator of Ras (e.g., it may inhibit the expression level of the activator or its interaction with Ras); loss of *sur-5* gene activity may increase its expression or alter its interaction with *ras* and thus reduce the toxicity of the LET-60(dn) protein. LET-60(+) is less likely to be independent of an activator such as a GNEF in *sur-5* mutants because a *sur-5* mutation does not suppress the Vul phenotype of mutations in *sem-5* or *let-23 RTK*.

The second mechanism in which *sur-5* is involved in activation of Ras by a specific activator is supported by the selective suppression of one of the two groups of *ras(dn)* mutations by *sur-5* mutations. If loss of *sur-5* activity simply elevates wild-type Ras activity or activity downstream of Ras, the suppression is not likely to be discriminative, since all Ras(dn)/+ strains are likely to have similar amounts of Ras(+) proteins. The selective suppression is probably not due to the strength of dominant negative effects between the two groups of *let-60 ras (dn)* alleles, since the nonsuppressible group (group II) has the weakest (S89F) as well as the strongest (E119N) dn alleles (Table 3) (11).

Indication of multiple Ras activators. The observation that *sur-5* mutations fail to suppress the Vul phenotype caused by one of the two groups of *let-60 dn* mutations suggests there are at least two mechanisms which account for the dominant effect of *ras(dn)* mutations and that *sur-5* mutations affect only one of these mechanisms. Since all *let-60 ras(dn)* mutations are completely suppressed by the *let-60(G13E gf)* mutation in *trans*-heterozygotes (1, 11, 12), both mechanisms are likely to involve toxic effects on upstream activators rather than downstream effectors. One such activator would probably be a GNEF, since previous studies showed that increased expression of the *Saccharomyces cerevisiae CDC25* (a GNEF) gene can suppress the toxic effect caused by either mammalian H-Ras (G15E dn) or yeast RAS2(G22A dn) (22). Residue 22 of *S. cerevisiae* RAS2 corresponds to residue 15 of H-Ras. We could thus imagine that the *sur-5(lf)*-suppressible *let-60 ras(dn)* mutations (group I), which are all located on loop 1 of Ras, behave similarly to the G15A mutations of H-Ras. The *sur-5(lf)* nonsuppressible LET-60 Ras(dn) proteins (S89F and E119N) may be toxic to a different activator. The possibility of having multiple positive regulators was raised previously. Overexpression of GNEF can suppress only one of the two types of Ras dominant negative mutants in *Schizosaccharomyces pombe* (15). Although alternative explanations are possible, this work suggests that there could be more than one upstream Ras activator in *S. pombe*. There is as yet no direct evidence that *C. elegans* has multiple Ras activators.

***sur-5* overexpression phenotypes.** Consistent with the role of *sur-5* as a negative regulator of Ras-mediated vulval signaling, multiple copies of *sur-5* in an extrachromosomal array partially suppress the Muv phenotype caused by the *let-60(G13E gf)* mutation. This result is still consistent with the potential role of *sur-5* in interacting with a Ras activator, since vulval induction in the *let-60(G13E gf)* mutant is known to be influenced to a certain degree by gene activities upstream of Ras (30, 36). The level of suppression of the Muv phenotype of the *let-60(gf)* allele by the *sur-5* transgenes (89 to 38% Muv) is significant, considering that the *sur-5(+)* transgenes do not cause any phenotype in wild-type worms. It is possible that vulval induction is more sensitive to elevated *sur-5* activity in the *let-60 ras (G13E gf)* mutant than in the *let-60 ras(+)* strains due to the *sur-5* function on a specific regulatory aspect of Ras. Mutations in the *ksr-1* gene were also shown to have a stronger effect on the *let-60 ras(gf)* gene than on the *let-60 ras(+)* gene (30), suggesting that the function of *ksr-1* is closely related to Ras activity.

We have also shown that multiple copies of the SUR-5 transgene cause some interesting synergistic phenotypes in a weak-loss-of-function *lin-45 raf* mutant including larval death, uncoordinated movement, and egg-laying defects. This result may suggest that *sur-5* is involved in controlling other aspects of development that probably also involve functions of Ras and Raf. Since *sur-5* mutations alone do not cause these phenotypes, the contribution of *sur-5* to in these developmental aspects appears to be limited or redundant and can be observed only when activities in other genes are compromised.

The *sur-5* gene product may be conserved during evolution. We show in this paper that *sur-5* encodes a novel protein, and we have identified candidate mammalian homologs of *sur-5*.

The human protein sequence is about 38% identical to the SUR-5 sequence (Fig. 3). There is about 90% identity between the human sequence and the partial mouse sequence. Like *C. elegans sur-5*, which is expressed in most of the cell lineages, the potential human *sur-5* homolog is also expressed ubiquitously in many tissues. Further studies of these mammalian genes may determine whether they are also involved in negative regulation of Ras functions. We have not identified an obvious *sur-5* homolog in *S. cerevisiae*.

The sequences of SUR-5 and its potential mammalian homologs do not give us clear clues about its biochemical functions. However, SUR-5 has several interesting properties. It contains one potential ATP binding motif and two potential AMP binding motifs, suggesting that it may function as an enzyme involved in binding to ATP and AMP and catalyzing ATP- and AMP-dependent reactions. *C. elegans* SUR-5 has a low but possibly significant similarity to the acetyl-CoA synthetase protein families (21 to 23% overall identities). Therefore, SUR-5 function may be in some way related to that of the acetyl-CoA synthetase.

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