smg-7 Is Required for mRNA Surveillance in Caenorhabditis elegans

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ABSTRACT

Eukaryotic mRNAs that contain premature translation termination codons are usually less stable than their wild-type counterparts, a phenomenon termed “nonsense-mediated mRNA decay” (NMD) or “mRNA surveillance.” NMD may protect cells from the deleterious effects of truncated proteins by degrading the aberrant mRNAs that encode them (Pulak and Anderson 1993; Cali and Anderson 1998). NMD was first identified in yeast (Losson and Lacroute 1979) and has since been described in all eukaryotes tested (for recent reviews, see Maquat 1995; Jacobson and Peltz 1996; Ruiz-Echevarria et al. 1996). Because of NMD, the steady-state levels of nonsense mutant mRNAs are substantially reduced relative to normal mRNAs.

Genes whose functions are required for NMD have been described in both Caenorhabditis elegans (smg-1 through smg-6) and Saccharomyces cerevisiae (UPF1, UPF2, NMD2, UPF3; Leeds et al. 1991, 1992; Pulak and Anderson 1993; Cui et al. 1995; He and Jacobson 1995; Lee and Culbertson 1995). Loss-of-function alleles of smg or UPF genes eliminate NMD and prevent the normally rapid decay of mRNAs containing nonsense or frameshift mutations. Thus, the smg/UPF genes encode trans-acting factors needed for NMD. NMD is nonessential in both yeast and nematodes, as upf and smg mutants are viable yet defective for NMD. At least one of the proteins required for NMD is both structurally and functionally conserved among yeast and nematodes. SMG-2 of C. elegans is about 50% identical to Upf1p of yeast (M. F. Page, B. Carr, K. R. Anders and P. Anderson, unpublished results). Both SMG-2 and Upf1p are also ~50% identical to a human protein that is likely involved in mammalian NMD (Perlick et al. 1996; Applquist et al. 1997; Sun et al. 1998).

Most smg mutations were identified because of their suppression phenotype. smg mutations are allele-specific but gene-nonspecific suppressors of mutations affecting a variety of C. elegans genes (Hodgkin et al. 1989). A small fraction of nonsense mutations and certain gene rearrangements that express mRNAs with aberrant 3’ untranslated regions are phenotypically suppressed by smg mutations (Hodgkin et al. 1989; Kuwabara et al. 1992; Pulak and Anderson 1993; Zarower et al. 1994; Rougvie and Ambros 1995; Barnes and Hodgkin 1996). Genetic screens for smg mutants are likely not saturated. In a genome-wide screen for smg mutations, only one allele of smg-4 and two alleles of smg-6 were isolated (Hodgkin et al. 1989). Efforts to identify additional smg genes have been limited by the fact that almost 90% of smg mutations identified in genome-wide screens are alleles of smg-1, smg-2, or smg-5. We describe a modified screen for smg mutations that precludes isolating alleles of smg-1, smg-2, and smg-5. Using this screen, we have identified and cloned smg-7, a previously uncharacterized gene that we show is required for NMD. smg-7 is predicted to encode a novel protein that contains an acidic carboxyl terminus and two probable tetratricopeptide repeats. We provide evidence that smg-7 is cotranscribed with the previously characterized gene lin-45 and show that null alleles of smg-7 confer a temperature-sensitive defect in NMD.

MATERIALS AND METHODS

Strains and general methods: C. elegans strains were maintained as previously described (Brenner 1974). All strains were maintained at 20° except where noted. Mutations and chromo-
somal aberrations used in this study have been previously described (Hodgkin 1988, 1997) or are noted below:

LGI: dpy-5(e1), fog-1(q180) (Barton and Kimble 1990), let-11(x12), smg-1(r861) (Hodgkin et al. 1989), smg-2(r863), smg-5(r860), unc-13(e51), unc-15(e1402), unc-54(r293), r295, r315 (Eide and Anderson 1985a; Pulak and Anderson 1988, 1993), unc-73(e36).

LG II: dpy-10(e218), mut-5(st701), vab-9(e744), tra-2(e209), unc-4(e20).

LG III: dpy-1(e), smg-6(r886, r896) (Hodgkin et al. 1989).

LG IV: bli-6(zt16), deb-1(st555), dpy-9(e16), dpy-13(zt84), lag-1(q385) (Christensen et al. 1996), lin-45(yu6), smg-3(r867, ma17), unc-22(d6), unc-24(e338), unc-33(e204), unc-44(e862).

LG V: dpy-11(e224), him-5(e490), smg-4(ma116), unc-46(e177), unc-60(e723), unc-70(e524), unc-76(e111).


Mutations isolated and mutagens used in this study are as follows:

LGI: Ethylmethane sulfonate (EMS)-induced, unc-54(r1189, r1190, r1191, r1192); N-ethyl-N-nitrosourea (ENU)-induced, unc-54(r1195); mut-5 induced, unc-54(r1130, r1132, r1174).

LG III: EMS-induced, smg-6(r1165, r1188); ENU-induced, smg-6(r1178, r1187, r1198, r1203, r1204, r1205, r1206, r1214); diepoxycetane (DEO)-induced, smg-6(r1171); mut-5 induced, smg-6(r1172, r1177).

LG IV: EMS-induced, smg-3(r1081, r1163, r1164, r1175, r1176, r1177); ENU-induced, smg-3(r1179, r1180, r1183, r1184, r1186, r1199, r1201, r1202, r1203, r1213, r1215, smg-7(r1182, r1197); DEO-induced, smg-3(r1168, r1170); mut-5 induced, smg-3(r1149, r1173, r1218); unc-7(r1131).

LG V: ENU-induced, smg-4(r1181); DEO-induced, smg-4(r1169).

Chromosomal aberrations isolated in this study, rT1(IV;V) and rDf3.

Identifying new smg mutations: Strain TR2034 [genotype unc-13(e51) dpy-5(e61) fog-1(q180) unc-54(r293) ; +/sz71 (I;X) (+ + + unc-54(r293); lon-2(e678))] is homozygous for unc-54(r293) and heterozygous for sz71. sz71 balances the right half of LG X and the left half of LG I, which includes the wild-type alleles of smg-1, smg-2, and smg-5. TR2034 is paralyzed due to unc-54(r293), a smg-suppressible allele of the unc-54 myosin heavy chain gene (Hodgkin et al. 1989). Intragenic and extragenic suppressors of r293 were identified as motile revertants of TR2034. Animals were mutagenized with EMS, DED, and 1 mm or 5 mm ENU as described previously (Brenner 1974; De Stasio et al. 1998). Spontaneous mutations were isolated in strain TR2038, which is identical to TR2034 but also contains mut-5(st701) vab-9(e744). Populations were established with 20–40 mutagenized young adults, and subsequent generations were screened for animals that exhibited increased motility and/or egg-laying proficiency. To ensure independence, no more than one mutant was retained from a single population. Each homozygous revertant was outcrossed to wild-type (N2) males, and F2 progeny were examined for the presence of Unc-54 animals. The absence of such animals indicated that a suppressor was tightly linked to unc-54, while their presence indicated that a suppressor was unlinked. All intragenic suppressors were found to be recessive and were tested for complementation of canonical alleles of smg-1 through smg-6 using suppression of unc-54(r293) at 15°C, 20°C, and 25°C as the scored phenotype.

Mapping of smg-7(r1131): Hermaphrodites of genotype unc-13(e51) dpy-5(e61) fog-1(q180) unc-54(r293); +/sz71 (+ + + unc-54(r293); lon-2(e678)); mut-5(st701) vab-9(e744); smg-7(r1131) were mated to N2 males. Wild-type cross-progeny hermaphrodites were picked singly and allowed to self-fertilize. Unc-54 offspring were then picked and allowed to self at 25°C, the nonpermissive temperature of r1131. smg-7(r1131) homoyzogotes were identified among progeny as fully motile animals. Non-Vab non-Dpy offspring were picked, and the outcross to N2 was repeated three additional times, yielding a strain of genotype smg-7(r1131); unc-54(r293). This strain was crossed with N2 males and a smg-7(r1131) unc-54(+ ) homoyzogote was identified among the offspring based upon (i) its protruding vulva (pVul) phenotype and (ii) its wild-type motility at 20°C. This smg-7 single mutant was used in mapping experiments described in Table 1.

To test nDf41 for complementation of smg-7(r1131), nDf41/+ males were mated to smg-7(r1131) unc-24(e338); unc-54(r293) hermaphrodites at 25°C. One-half of the cross-progeny were pVul, indicating failure of nDf41 to complement smg-7(r1131). Approximately one-fourth of the offspring of these heterozygotes were lethal (nDf41 homoyzogotes), and the remainder were fully motile at 25°C, confirming the failure of r1131 to complement nDf41. To test r1131 for complementation of stDf7 and stDf8, smg-7(r1131) unc-24(e338)/ +/+ ; smg-5(r293)/ +/+ males were crossed with unc-24(e338) unc-22(66b)/stDf7 or smg-28 hermaphrodites, smg-7(r1131) unc-24(e338)/stDf7 or smg-28 crossprogeny were identified as Unc-24 animals whose offspring were approximately one-fourth inviable (DF homoyzogotes) but never Unc-22. Approximately one-half of such heterozygotes yielded Unc-54 offspring, indicating that both stDf7 and stDf8 complement smg-7(r1131).

Testing suppression of tra-2(e1209) by smg-7(r1131): dpy-10(e128) + unc-4(e1201) + tra-2(e1209) + hermaphrodites were mated with N2 males, and single F1 males were mated with smg-7(r1131) hermaphrodites. Resultant tra-2(e1209) +/+ ; smg-7(r1131)/ +/+ hermaphrodites were seld at 25°C, and smg-7(r1131) homoyzogotes picked as pVul hermaphrodites. smg-7(r1131); tra-2(e1209) offspring were examined at 20°C and 25°C for suppression of the fertility and vulval and tail morphology defects characteristic of tra-2(e1209). Animals raised at 20°C were self-sterile (essentially identical to tra-2(e1209)), whereas animals raised at 25°C were self-fertile and had more normal vulval and tail morphology. smg-1(r861); tra-2(e1209) was included as a control and showed equivalent suppression at 25°C. Brood sizes of smg-7(r1131); tra-2(e1209) and smg-1(r861); tra-2(e1209) animals at 25°C were less than those reported for smg suppression of e209 at 20°C (i.e., brood size of 1–5 animals at 25°C vs. a mean of 11 animals at 20°C; Hodgkin et al. 1989).

Testing suppression of dpy-5(e61): dpy-5(e61) +/+ males were crossed with fog-1(q180) dpy-5(e61) unc-13(e51) unc-54(r293); smg-7(r1131) females, fog-1(q180) dpy-5(e61) unc-13(e51) unc-54(r293); +/+ dpy-5(e61) +/+ ; smg-7(r1131)/ +/+ crossprogeny were picked and seld at 25°C. Unc-54 offspring were picked and seld at 25°C. smg-7(r1131) homoyzogotes were identified in the next generation by their normal motility. dpy-5(e61) unc-54(r293); smg-7(r1131) homoyzogotes were identified among these suppressed animals as those that failed to yield Fog or Unc-13 offspring. Such animals were noticeably longer and less Dpy than dpy-5(e61) homoyzogotes and indistinguishable from dpy-5(e61); smg-3(r867) control animals.

Noncomplementation screen for new smg-7 alleles: smg-5(r860) unc-15(e402) unc-54(r293); him-5(e409) males reared at 15°C (e402 is temperature sensitive) were mutagenized with EMS and mated to unc-54(r293); smg-7(r1131) unc-24(e338) her-
5(s490/v +) exhibit an Unc-54 phenotype. Rare cross-progeny carrying a new mutation in smg-7 are fully motile, because the paralysis of unc-54(r293) is suppressed in smg-7(r1131)/smg-7(new) "homozygotes." smg-7(new) can be subsequently distinguished from smg-7(r1131), as smg-7(r1131) is coupled to unc-24(e183) in the screen.

**Analysis of rT1**: Tests of linkage. Heterozygous m/+ males (where m denotes one of several recessive visible markers tested) were mated with unc-44(e626) smg-7(r1131) unc-24(e183) / + ; / rT1 (IV; V) [ + / + ; + ] hermaphrodites. Cross-progeny of genotype m/ + ; / rT1 [ + / + ] animals were isolated and allowed to self, and m/ m offspring were picked. Such animals were examined for whether a large number of dead eggs were present among their progeny, as this is characteristic for rT1/+ animals. If m is unlinked to rT1, two-thirds of m/ m homozygotes are expected to be rT1/+ heterozygotes. If m is linked to rT1, less than two-thirds of m/ m homozygotes are expected to be rT1/+ heterozygotes, with the actual proportion determined by the frequency of crossing over between m and the breakpoint of rT1. Markers used in mapping rT1, and the fraction of m/ m that were heterozygous for rT1 were as follows:

<table>
<thead>
<tr>
<th>LG</th>
<th>Step</th>
<th>Marker</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG I: le-11 (4/4), unc-73 (3/6)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LG II: none tested.</td>
<td></td>
<td></td>
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<tr>
<td>LG III: dpy-1 (4/5), dpy-18 (7/9)</td>
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<tr>
<td>LG IV: unc-24 (0/10)</td>
<td></td>
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<tr>
<td>LG V: unc-70 (0/9), dpy-11 (0/7)</td>
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**Tests of recombination suppression**: Heterozygous m2/++ males, where m1 and m2 denote linked markers between which recombination was being tested (see Table 2), were mated to unc-44(e626) lag-1(q385); / rT1 (IV; V) [ + / + ] hermaphrodites, and m1 m2; / rT1 [ IV; V ] [ + / + ] heterozygous cross-progeny were isolated. Among the self-progeny of such heterozygous animals, the numbers of m1 non-m2 and m2 non-m1 offspring were scored.

**RNase protection assays**: RNA isolation and RNase protection assays were performed as previously described (Pulak and Anderson 1993). Twenty micrograms of total RNA was tested in each assay. RNase-protected products were quantitated with a PhosphorImager, and the unc-54 signal was normalized to that of act-1.

**Identification, cloning, and mapping of dimorphic Tc1 elements near smg-7**: smg-7(r1131), which was isolated in a strain that retains a high copy number of Tc1, was outcrossed five times to N2 prior to analysis. Dimorphic Tc1 elements linked to smg-7(r1131) were identified on Southern blots and mapped relative to two crossovers between bli-6 and unc-24 (see Figure 2). Two dimorphic Tc1 elements, designated rP14::Tc1 and r1131::Tc1, were inseparable from smg-7 in these tests of linkage. We isolated genomic DNAs flanking rP14::Tc1 and r1131::Tc1 by inverse PCR (Ochman et al. 1988), cloned them into plasmid vectors, and sequenced the Tc1 insertional junction fragments. When compared to C. elegans genomic cDNA sequences (Waterston et al. 1997), rP14::Tc1 proved to derive from cosmid C25A8, while r1131::Tc1 (contained on plasmid clone TR#235) had not yet been sequenced. We refined the genetic positions of rP14::Tc1 and r1131::Tc1 using PCR tests of their inheritance among 20 crossovers in the ~0.4 map unit unc-44 to db-1 interval, yielding the following distribution of crossovers: unc-44 (15/20) smg-7(r1131::Tc1) (4/20) rP14::Tc1 (1/20) db-1. Analysis of 23 additional crossovers in the same interval failed to separate r1131::Tc1 from smg-7.

**Transformation rescue of smg-7**: The insert of full-length cDNA clone TR#288 was amplified by PCR and cloned into the EcoRV site of expression vector pPD49.78 (Mello and Fire 1995), yielding plasmid clone TR#302. Plasmids TR#302 and pRF4 were microinjected into unc-54(r293); smg-7(r1131) and heritable transformants established. In the absence of heat shock, such transformants have normal motility, indicating that they are non-Smg. A small fraction of animals was slightly slow, suggesting that the transgene partially rescues smg-7(r1131) under non-heat-shock conditions. To test for heat-shock-dependent rescue, a mixed-stage population was heat-shocked at 33°C for 50 min, followed by a 30-min incubation at 22°C. Roller larvae (those that retain the transforming array) and nonroller larvae (those that have lost it) were isolated singly, raised at 25°C for 1–2 days, and scored for motility. All roller animals (10/10) grew to be Unc-54 EG1 adults (non-Smg phenotype), while all nonroller animals (24/24) grew to be fully mobile, egg-laying proficient adults (Smg phenotype).

**Polynuclear anti-SMG-7 antibodies and Western blots**: An 809-bp EcoRV fragment from smg-7 cDNA clone TR#288, corresponding to SMG-7 amino acids 174–448, was cloned in-frame into the Smal site of pGEX-2T (Pharmacia, Piscataway, NJ) and transformed into the Escherichia coli host strain BL21. The resulting SMG-7 GST fusion protein was induced, found to be insoluble, and purified as inclusion bodies as previously described (Williams et al. 1995). Two New Zealand White rabbits were immunized with 1.0 mg of fusion protein and boosted 2, 6, 10, and 14 wk thereafter. Sera was collected after the fourth boost and affinity purified as previously described (Williams et al. 1995) using both SMG-7/GST- and GST-affinity columns to purify anti-SMG-7 antibodies. Protein samples for Western blots were prepared by boiling 30 adult worms in SDS loading buffer, after which they were run on denaturing SDS-polyacrylamide gels and transferred to immobilon-P membranes (Millipore Corp., Bedford, MA) using a semidy blotter. Membranes were incubated for 1 hr in PBS containing 0.1% Tween-20, 5% powdered milk, and primary and secondary antibodies at appropriate dilutions. Signals were detected by chemiluminescence (ECL system, Amersham Life Sciences, Arlington Heights, IL) according to the manufacturer’s instructions.

**RESULTS**

unc-54(r293) is one of several previously identified alleles suppressed by mutations of smg-1 through smg-6 (Hodgkin et al. 1989). unc-54 encodes myosin heavy chain B, which is required in body-wall muscle for normal locomotion (Epstein et al. 1974). unc-54(r293) is a small deletion that deletes the unc-54 polyadenylation signal but does not affect the unc-54 open reading frame (Pulak and Anderson 1988). unc-54(r293) expresses an unusually large mRNA that is unstable due to the action of NMD. smg mutations phenotypically suppress unc-54(r293) by eliminating NMD. Increased accumulation of unc-54(r293) mRNA in smg mutants causes increased synthesis of a myosin heavy chain B whose amino acid sequence is normal (Pulak and Anderson 1993).

**A modified screen for smg mutations**: We designed a modified screen for smg mutations for two purposes: (i) to preclude isolating additional alleles of smg-1, smg-2, and smg-5, which collectively comprise almost 90% of smg mutations identified in genome-wide screens (Hodgkin et al. 1989); and (ii) to facilitate the cloning by transposon tagging of any new smg genes that we might identify. We constructed strain TR2034, which is homozygous for unc-54(r293) and heterozygous for szT1 (see
H. Shang

...will be described elsewhere (characterization of these mutations confirms this assignment). The tight linkage and dominance of these mutations suggest that they are intragenic suppressors affecting unc-54. Molecular characterization of these mutations confirms this assignment and will be described elsewhere (S. O'Connor, H. Shang and P. Anderson, unpublished results). The remaining 41 suppressors are recessive and unlinked to unc-54. Complementation tests with canonical alleles of smg-1 through smg-6 demonstrated that 23 are alleles of smg-3, 13 are alleles of smg-6, and 2 are alleles of smg-4. The remaining 3 suppressors define a new gene, smg-7, which is described below.

Figure 1.—A modified screen for smg mutations that precludes isolating alleles of smg-1, smg-2, and smg-5. TR2034 is heterozygous for the reciprocal translocation szT1 and homozygous for unc-54(r293). smg-1, smg-2, smg-5, and fog-1 are located on LG I (left), within the region balanced by szT1. Among the euploid offspring of TR2034, szT1 and fog-1 homozygotes are lethal and sterile, respectively, whereas szT1 heterozygotes are viable and fertile. Aneuploid offspring are inviable. Mobile revertants of TR2034 identified among the F2 offspring are either alleles of unc-54 itself or extragenic suppressors of unc-54(r293). In the absence of crossing over (which is suppressed by szT1), recessive smg mutations must be located on LGs II, III, IV, or V. smg mutations on LGs I (right) and X (left) can in principle be identified, but they must occur concomitantly with an appropriate crossover. See materials and methods for a complete description of the screen.

Figure 2.—Linkage map near smg-7. Except for smg-7, genes shown above the chromosome have been previously ordered by a combination of three-factor crosses and deficiency mapping. We mapped smg-7 relative to bli-6, smg-3, lin-45, and deb-1 by three-factor crosses, and relative to stD7, stD8, and nD41 by complementation tests.

<table>
<thead>
<tr>
<th>smg-4</th>
<th>smg-3</th>
<th>smg-7</th>
<th>smg-2</th>
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<tbody>
<tr>
<td>unc-24</td>
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<tr>
<td>bli-6</td>
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<td>lin-45</td>
</tr>
<tr>
<td>deb-1</td>
<td>deb-1</td>
<td>deb-1</td>
<td>deb-1</td>
</tr>
<tr>
<td>stD7, stD8</td>
<td>stD7, stD8</td>
<td>stD7, stD8</td>
<td>stD7, stD8</td>
</tr>
<tr>
<td>nD41</td>
<td>nD41</td>
<td>nD41</td>
<td>nD41</td>
</tr>
</tbody>
</table>

Legend:
- LG I material
- LG X material
- LG II, III, IV, or V material

Non-revertant F1 Offspring (Phenotype)
- (Lethal)
- (Paralyzed)
- (Sterile)

Revertant F1 Offspring (Class)
- (Intragenic)
- (Extragenic suppressor)
some fail to complement alleles of both smg-3 and smg-7, whereas the smg-3 and smg-7 alleles involved in the cross fully complement each other. Furthermore, all known alleles of smg-7, including two deletions (see below), fully complement smg-3 (r1867 and ma117) for both suppression and morphogenetic phenotypes.

smg-7(r1131, r1182, and r1197) are temperature sensitive, in contrast to all previously described smg mutations. At 15° and 20°, unc-54(r293); smg-7 homozygotes are slightly more motile and egg-laying proficient than unc-54(r293); smg(+) controls, but the suppression is very weak. At 25°, however, unc-54(r293); smg-7 animals have normal motility and egg laying. Suppression of unc-54(r293) by smg-7(r1131) is affected by maternal genotype. unc-54(r293); smg-7(r1131) homozygotes recovered from unc-54(r293); r1131/+ heterozygous mothers are less motile (more weakly suppressed) than offspring of unc-54(r293); smg-7(r1131) homozygous mothers. A similar maternal-presence effect has been described for alleles of smg-3, smg-4, and smg-6 (Hodgkin et al. 1989).

smg-7 is required for nonsense-mediated mRNA decay: To test whether smg-7 is required for NMD, we measured the steady-state levels of unc-54 wild-type and nonsense mutant mRNAs in a smg-7(r1131) background. mRNA of unc-54(r315), an amber mutation at unc-54 codon 1263 (out of 1966), is known from previous work to be unstable in smg(+) backgrounds but stable in smg(−) backgrounds (Pulak and Anderson 1993). The RNase protection experiment shown in Figure 3 demonstrates that smg-7(r1131) eliminates the rapid degradation of unc-54(r315) mRNA. unc-54(r315) mRNA is reduced in this experiment to 11% of wild type in an smg(+) background (Figure 3, lane 4), whereas it is of normal abundance in smg-7(r1131) animals raised at 25° (Figure 3, lane 5). smg-7(r1131) does not significantly affect the quantity of unc-54(+) mRNA (Figure 3, lane 3). These results demonstrate that smg-7(r1131), like mutations affecting smg-1 through smg-6, prevents the rapid decay of nonsense mutant mRNAs.

Isolating alleles of smg-7 in a noncomplementation screen: All smg-7 alleles described above are temperature sensitive for suppression. At 25°, r1131, r1182, and r1197 are strong suppressors of unc-54(r293), while at 15° and 20° they are very weak suppressors. Our failure to isolate nonconditional alleles of smg-7 might indicate that, in the absence of SMG-7, NMD is an inherently temperature-sensitive process. Alternatively, the smg-7 alleles isolated above might be weak or altered-function alleles of a gene whose null phenotype is more severe, possibly even lethal. In order to isolate smg-7 alleles in a manner that does not require their viability when homozygous, we isolated additional smg-7 alleles in a

\[ \text{TABLE 1} \]

<table>
<thead>
<tr>
<th>Heterozygote genotype</th>
<th>Recombinant class</th>
<th>Distribution of crossovers*</th>
</tr>
</thead>
<tbody>
<tr>
<td>unc22 + dpy-13/+smg-7+</td>
<td>Unc non-Dpy</td>
<td>unc22 (1/5) smg-7 (4/5) dpy-13</td>
</tr>
<tr>
<td>bl-6 + unc24/+smg-7+</td>
<td>Dpy non-Unc</td>
<td>unc22 (1/5) smg-7 (4/5) dpy-13</td>
</tr>
<tr>
<td>+ lin-45 unc24/sm-7++</td>
<td>Unc non-Lin</td>
<td>bl-6 (1/6) smg-7 (5/6) unc24</td>
</tr>
<tr>
<td>unc44 + dbe1/+smg-7+</td>
<td>Unc-44 non-Dbe</td>
<td>smg-7 (0/6) lin-45 (6/6) unc24</td>
</tr>
</tbody>
</table>

* Fractions shown in parentheses indicate the proportion of crossovers that occurred between each pair of adjacent genes in a three-factor cross.

Figure 3.—Effect of smg-7(r1131) on nonsense-mediated mRNA decay. RNase protection experiments using total RNA of the indicated strains were performed as previously described (Pulak and Anderson 1993). Lane 1, wild-type strain N2; lane 2, unc-54(r259), a deletion that removes most of unc-54; lane 3, smg-7(r1131); unc-54(+); lane 4, unc-54(r514), an amber mutation at unc-54 codon 1263; lane 5, unc-54(r315); smg-7(r1131); unc-54 hybridization signals were quantified by phosphorimaging. To adjust for lane-to-lane variation in the amount of assayed RNA, unc-54 hybridization signals were measured relative to those of act-1 and normalized to the quantity of act-1 mRNA found in wild type (lane 1).
Third, we crossed a transgenic array of clone \textit{M}olecular analysis of \textit{7(r1131)}. \noncomplementation" screen. We knew that we could isolate null alleles of \textit{smg-7} heterozygous to \textit{smg-7(r1131)}, because nDf41 deletes \textit{smg-7} (see Figure 2) and \textit{smg-7(r1131)/nDf41} heterozygotes are viable, fertile, and Smg. We mutagenized \textit{smg-7(+) males with EMS and mated them with \textit{smg-7(r1131)} hermaphrodites at 25°. Additional mutations were present in the parent strains to distinguish self-progeny from cross-progeny, to allow \textit{unc-54(r293)} homozygous males to mate, to mark the \textit{r1131}-containing chromosome, and to phenotypically distinguish Smg from non-Smg offspring (see materials and methods). We isolated two \textit{smg-7} alleles in this screen among \~4400 mutagenized genomes. Both of these alleles, \textit{r1147} and \textit{r1148}, are lethal when homozygous.

\textit{smg-7(r1147)} fails to complement both \textit{smg-7(r1131)} and the nearby mutation \textit{lin-45(sy96)}. \textit{smg-7(r1147)} complements \textit{smg-3(r867), bli-6(sc16), and deb-1(st555)}. \textit{lin-45} encodes a member of the \textit{raf} family of serine/threonine kinases, and \textit{lin-45} mutants exhibit a zygotic vulvalless and maternal-effect lethal phenotype due to defects of ras-mediated signal transduction (H\textit{an et al.} 1993). Three lines of evidence indicate that \textit{lin-45} and \textit{smg-7} are distinct genes. First, \textit{smg-7} viable mutations do not exhibit a \textit{Lin-45} phenotype and fully complement \textit{lin-45(sy96)} for all phenotypes. Second, \textit{lin-45(sy96)}, a strong loss-of-function allele (H\textit{an et al.} 1993), does not suppress \textit{unc-54(r293)} or exhibit the morphogenetic phenotypes of \textit{smg-7} mutants. Third, we crossed a transgenic array of clone \textit{\lambda}raf-E, which contains \textit{lin-45(+) and rescues \textit{lin-45(sy96)} (H\textit{an et al.} 1993), into \textit{smg-7(r1131); unc-54(r293)} and determined that the \textit{lin-45(+) array does not rescue \textit{smg-7(r1131)}. Molecular analysis of \textit{smg-7} (see below) demonstrates that \textit{smg-7(r1147)} is a deletion that removes all transcribed sequences of both \textit{smg-7} and \textit{lin-45}.

Two lines of evidence suggest that \textit{smg-7(r1148)}, the second allele isolated in the noncomplementation screen, is a reciprocal translocation involving chromosomes IV and V. In the following discussion, we refer to \textit{smg-7(r1148)} as \textit{rT1}. First, \textit{rT1} exhibits linkage to markers on two different chromosomes (\textit{unc-24 IV, unc-70 V, and dpy-11 V}; see materials and methods). \textit{rT1} does not exhibit linkage to \textit{lev-11 I, unc-73 I, dpy-1 III, or dpy-18 III. Second, \textit{rT1} inhibits recombination on the right arm of LG IV and on the left arm of LG V. We placed \textit{rT1} heterozygous to various chromosome IV and V double-mutant chromosomes and measured the apparent frequency of crossing over. As shown in Table 2, \textit{rT1} reduces the frequency of crossing over on LG IV (right) and LG V (left), but not LG IV (left) or LG V (right). Such effects are consistent with \textit{rT1} being a reciprocal translocation having breakpoints on LG IV near \textit{smg-7} and on LG V between \textit{dpy-11} and \textit{unc-76}. \textit{rT1} fails to complement both \textit{smg-7(r1131)} and \textit{lin-45(sy96)}, suggesting that the LG IV breakpoint of \textit{rT1} disrupts both of these genes. Molecular analysis of \textit{smg-7} demonstrates that the breakpoint of \textit{rT1} is located within \textit{lin-45} and that all \textit{smg-7} sequences are deleted in \textit{rT1} (see below).

\textbf{Transposon tagging of \textit{smg-7} and identifying \textit{smg-7} genomic and cDNA clones:} \textit{smg-7(r1131)} was identified as a spontaneous mutation arising in a \textit{mut-5(st701)} genetic background. As many \textit{mut-5} induced mutations are due to insertion of the transposon \textit{Tc1} (M\textit{ori et al.} 1988), we investigated whether a \textit{Tc1} element is associated with \textit{smg-7(r1131)}. We mapped dimorphic \textit{Tc1} elements near \textit{r1131} using a collection of 45 crossovers that we isolated in the \~0.4 map unit intervals between \textit{bli-6} and \textit{unc-24} or between \textit{unc-44} and \textit{deb-1} (see Figure 2). Two \textit{Tc1} elements mapped near \textit{smg-7}. \textit{rP14::Tc1} was separated from \textit{smg-7(r1131)} by 4 of the 45 crossovers. \textit{rP14::Tc1} maps rightward of \textit{smg-7}, between \textit{smg-7} and \textit{deb-1}. A second \textit{Tc1} element was not separated from \textit{smg-7(r1131)} and, as shown below, is located within \textit{smg-7} itself. We designated this element \textit{r1131::Tc1 and cloned one of its insertion junctions by inverse PCR using \textit{Tc1}-specific primers (O\textit{chman et al.} 1988). Using this clone

\begin{table}
\centering
\caption{Effect of \textit{rT1(IV;V)} on LG IV and LG V crossing over}
\begin{tabular}{|l|c|c|c|c|}
\hline
Heterozygote genotype & Number of self-progeny & & & \\
\hline
 & WT & DpyUnc & Unc & Dpy & Calculated map distance (cm) \\
\hline
unc60 dpy-11/++ & 391 & 89 & 37 & 38 & 14.6 ± 2.9 \\
unc60 dpy-11/rT1(IV);+++/rT1(IV) & 276 & 68 & 0 & 0 & 0 \\
unc46 dpy-11/++ & 685 & 210 & 4 & 9 & 1.4 ± 1.2 \\
unc46 dpy-11/rT1(IV);+++/rT1(IV) & 318 & 102 & 0 & 0 & 0 \\
dpy11 unc-76/++ & 692 & 192 & 25 & 15 & 4.4 ± 1.3 \\
dpy11 unc-76/rT1(IV);+++/rT1(IV) & 69 & 15 & 6 & 2 & 9.1 ± 5.9 \\
unc24 dpy-4/++ & 504 & 151 & 32 & 44 & 11.0 ± 2.3 \\
unc24 dpy-4/rT1(IV);+++/rT1(IV) & 274 & 63 & 0 & 0 & 0 \\
dpy9 unc-33/++ & 330 & 54 & 46 & 53 & 23.2 ± 3.8 \\
dpy9 unc-33/rT1(IV);+++/rT1(IV) & 112 & 47 & 18 & 17 & 20.0 ± 5.7 \\
\hline
\end{tabular}
\end{table}

\footnote{Map distances were calculated as described in Hodgkin (1988, 1997). Confidence intervals of 95\% were calculated using the normal approximation of the binominal distribution.}
as a hybridization probe, we identified a phage lambda genomic clone of the wild-type region and subsequently cloned a 1.6-kb EcoRI wild-type fragment into which Tc1 had inserted in smg-7[r1131].

To determine whether r1131::Tc1 was inserted into an expressed sequence, we identified eight cDNA clones that hybridized to the r1131::Tc1 junction fragment from a mixed-stage wild-type cDNA library (Barstead and Waterston 1989). DNA sequencing revealed that seven of these cDNAs correspond to transcripts of lin-45 (Han et al. 1993), while one (clone TR#274) defined a previously uncharacterized expressed sequence. As described below, further analysis of TR#274 demonstrated that it corresponds to smg-7. Comparing the sequence of TR#274 to genomic sequence of lin-45 (A. Golden and P. Stemberg, personal communication) indicated that smg-7 is located immediately downstream of and transcribed in the same relative orientation as lin-45 raf. To test whether lin-45 and smg-7 constitute an operon, and to potentially isolate a smg-7 cDNA clone complete to the 5' end, we amplified by RT-PCR the 5' end of smg-7 cDNA using an SL2-specific and a smg-7-specific primer pair, yielding cDNA clone TR#285. Comparing the sequence of TR#285 to lin-45 genomic sequence demonstrated that smg-7 is trans-spliced to SL2 at a 3' splice site 95 nt downstream of the lin-45 poly(A) addition site. We conclude that lin-45 and smg-7 constitute an operon, with smg-7 being a downstream gene and trans-spliced to SL2.

**Transformation rescue of smg-7**: Transformation rescue experiments establish that cDNA clones TR#274 and TR#285 correspond to smg-7. We derived a full-length smg-7 cDNA clone by PCR-mediated ligation of TR#274 and TR#285, yielding plasmid clone TR#288. We cloned the insert of TR#288 into the C. elegans expression vector pPD49.78 (Melilo and Fire 1995) downstream of the hsp16-2 promoter, which is inducible by heat shock (Stringham et al. 1992). The resulting plasmid (TR#302) was microinjected together with plasmid pRF4, which carried a dominant allele of rol-6 as a marker for successful transformation (Melilo et al. 1991), into unc54[r293] smg-7[r1131] hermaphrodites. Heritable transformants were identified by their roller phenotype and subsequently assessed for their Smg phenotype before and after heat shock. Non-Smg animals are expected to be Unc-54 (indicating absence of r293 suppression), whereas Smg animals are expected to have normal motility (Hodgkin et al. 1989). The results are shown in Table 3. Following heat shock and growth at 25°, hsp16-2::smg-7(+), transformants are indistinguishable from unc-54[r293], indicating efficient rescue of smg-7[r1131] (Table 3, line 3). In the absence of heat shock, transformants have normal motility, indicating absence of smg-7(+). Control transformants demonstrate that heat shock of hsp16-2::smg-7(+) does not confer a dominant Unc-54 phenotype (Table 3, line 4). We conclude that cDNA clone TR#288 contains a complete smg-7 coding region.

**SMG-7 is a novel acidic protein containing two probable tetratricopeptide repeats**: The DNA sequence and deduced amino acid sequence of cDNA clone TR#288 (GenBank accession no. AF089729) is shown in Figure 4. TR#288 is 1521 nt long, contains SL2 at its 5' end, and an oligo(A) tract at its 3' end. TR#288 hybridizes on Northern blots to a wild-type smg-7 mRNA that we estimate to be 1.6 kb long. Thus, we believe that TR#288 is full length, or very nearly so. TR#288 contains a single long open reading frame predicted to encode a 53,080-D protein. Two features of SMG-7 are noteworthy. First, the carboxyl terminus of SMG-7 is predicted to have a high net negative charge. One-third of the 75 carboxyl terminal amino acids are aspartic acid or glutamic acid residues, and the pl of SMG-7 is predicted to be 4.98. Second, SMG-7 contains two probable tetratricopeptide repeats (TPRs). TPRs form pairs of amphipathic α-helices and have been shown to mediate numerous protein-protein interactions in both prokaryotes and eukaryotes (Sikorski et al. 1990; Lamb et al. 1995; Das et al. 1998). While the primary sequence of TPRs is not highly conserved, several features characterize TPR motifs. They are 34 amino acids long and often arranged as tandem clusters. Positions 8, 20, and 27 are preferentially small or small hydrophobic residues, while certain other positions (especially 4, 11, and 24) tend to be bulky hydrophobic residues. Proline residues are often found near the end of helical regions, espe-
Figure 4.—Sequence of smg-7 cDNA clone TR#288. Sequences of the transspliced leader SL2 are underlined with dashes. The two TPR domains (amino acids 130–163 and 164–197) are boxed. The acidic carboxyl terminus is underlined. Arrowheads indicate the positions of smg-7 introns, which are noted in Figure 4. smg-7(r1131::Tc1) is identical, 62% similar) to TPR domains of a diverse set of proteins, the most similar of which are shown in a Tc1 insertion into a TA dinucleotide at position 368. smg-7(r1197) is a T→A transversion at position 644.

Figure 5.—Alignment of SMG-7 TPR domains. TPR1 and TPR2 of SMG-7 are similar to TPR domains of a variety of prokaryotic and eukaryotic proteins, the most similar of which are shown here. A TPR consensus was derived from the 42 TPR domains contained in the Pfam protein domain families database release 2.1 (Sonnhammer et al. 1998) and represents a slight refinement of the previous consensus (Lamb et al. 1995). Positions of conservation within TPR domains are numbered above and below the alignments. Positions of identity between SMG-7 TPRs and the aligned or consensus TPRs are shaded. The two TPR domains of SMG-7 (amino acids 130–163 and 164–197) are arranged in tandem and related (up to 38% identical, 62% similar) to TPR domains of a diverse set of proteins, the most similar of which are shown in Figure 5.

The smg-7 null phenotype is a temperature-sensitive defect in NMD: To investigate the nature of viable smg-7 mutations, we amplified genomic fragments of r1131, r1182, and r1197 by PCR and determined their sequences. These data define the positions of smg-7 introns, which are noted in Figure 4. smg-7(r1131::Tc1) is a Tc1 insertion into a TA dinucleotide at position 368. smg-7(r1197) is a T→A transversion at position 644, which changes a TTA (Leucine) codon to a TAA (Stop) codon. We did not detect a sequence change in smg-7...
temperature-sensitive defect in NMD. prove useful for manipulating expression. mutations constituted 87% of previously described alleles of *smg-7*. The *smg-7* cDNA clone TR#288 represents the product of the *smg-7* gene. These results confirm that cDNA clone TR#288 represents smg-7 and establish that *smg-7* (r1131, r1182, and r1197) eliminate expression of SMG-7. The smg-7 null phenotype, therefore, is a temperature-sensitive defect in NMD.

**DISCUSSION**

*C. elegans* smg genes are required for nonsense-mediated mRNA decay (Pulak and Anderson 1993). Despite having isolated over 50 smg mutations in previous screens (Hodgkin et al. 1989), the distribution of alleles among genes suggested that not all smg genes had been identified. For example, only one allele of smg-4 and two alleles of smg-6 were previously isolated. Discovery of additional smg genes by continuing such screens is inefficient, because almost 90% of recovered mutations are alleles of smg-1, smg-2, or smg-5. We designed a modified screen for smg mutants that precludes isolating alleles of smg-1, smg-2, and smg-5. The screen is based on the fortuitous circumstance that smg-1, smg-2, and smg-5 reside on the left arm of chromosome I and on properties of szT1, a reciprocal translocation involving LG I and LG X. In animals heterozygous for both szT1 and fog-1(q180), recessive mutations on the left arm of LG I and the right arm of LG X are not recovered, because szT1 homozygotes are lethal and fog-1 homozygotes are sterile. smg mutations located outside of the balanced region can be isolated, but not those within the balanced region. As shown in Table 4, the modified screen effectively eliminates smg-1, smg-2, and smg-5 mutations from the recovered sample. While smg-1, smg-2, and smg-5 mutations constituted 87% of previously described alleles, none were isolated among 41 smg mutations described here.

We have used this screen to define smg-7, a previously unidentified smg gene. Like smg-1 through smg-6, smg-7 is required for rapid decay of nonsense mutant mRNAs (Figure 3). *smg-7* (r1131, r1182, and r1197) are all temperature-sensitive, even though r1197 is an ochre nonsense mutation in the middle of the smg-7 open reading frame, and Western blots demonstrate that all three alleles express no detectable SMG-7. We conclude that, in the absence of SMG-7, NMD is an inherently temperature-sensitive process and that SMG-7 is required only for NMD at elevated temperature. The temperature-sensitive character of NMD in smg-7 mutants should prove useful for manipulating *C. elegans* gene expression. Expression of smg-sensitive mRNAs can be rendered conditional by incorporating smg-7 (or other) temperature-sensitive mutations into appropriate strains. Why were smg-7 mutations not recovered in previous screens? At 20°C, the temperature at which this and previous suppressor screens were done, smg-7 (r1131, r1182, and r1197) are weak suppressors. Their weak phenotype likely caused smg-7 alleles to be overlooked in previous screens and underrepresented in our modified screen.

SMG-7 is a member of the family of proteins that contain TPRs. TPRs are found in a variety of prokaryotic and eukaryotic proteins, including cell division cycle proteins, hsp90-binding immunophilins, transcription factors, and peroxisomal and mitochondrial import proteins (Sikorski et al. 1990; Goebel and Yanagida 1991;
in such complexes can occur between two TPR domains. We thank A. Golden and P. Sternberg for providing Smith 1995; Tzamarias promoting complex (mitochondrial and peroxisomal import receptor complex (Lamb et al. 1994; King et al. 1995), and transcription repression complexes (Smith et al. 1995; Tzamaras and Struhl 1995). TPR interactions in such complexes can occur between two TPR domains, or between TPR and non-TPR regions.

The presence of TPRs within SMG-7 suggests that they are part of a protein complex. Indeed, recent work demonstrates that SMG-7 and SMG-5 interact with each other. Both SMG-5 and SMG-7 are coimmunoprecipitated with anti-SMG-5 or anti-SMG-7 antibodies, although it is unknown if this interaction is direct or indirect (K. Anders and P. Anderson, unpublished results). A complex of proteins involving Upf1p, Upf2p, Upf3p, and translation release factors eRF1 and eRF3 has been implicated in yeast NMD (Eh and Jacobson 1995; He et al. 1997; Czaplinski et al. 1998). Although the primary sequences of SMG-7 and Upf2p are not similar, both proteins have strongly acidic carboxyl termini (Cui et al. 1995; Eh and Jacobson 1995). The acidic carboxyl terminus of Upf2p has been shown to mediate direct interactions with Upf1p (Eh and Jacobson 1995; He et al. 1997). Perhaps SMG-7 will prove to interact with SMG-2, which is homologous to Upf1p, via its acidic carboxyl terminus.

We have shown previously that activity of SMG-7 influences the phosphorylation status of SMG-2 (M. F. Page, B. Carr, K. R. Anders and P. Anderson, unpublished results). In smg-7 mutants, a phosphorylated isoform of SMG-2 accumulates to abnormally high levels. We note several TPR-containing proteins that, in other systems, influence the metabolism of certain phosphoproteins. For example, protein phosphatase 5 (PP5) contains three TPR domains. Protein phosphatase 2A (PP2A, which does not contain TPR domains) has been shown to interact with eRF1, which has in turn been shown to interact with Upf1p of yeast (Andjelkovic et al. 1996; Czaplinski et al. 1998). Perhaps phosphorylated SMG-2 is a substrate of PP5 or PP2A, and the TPR domain of SMG-7 targets the appropriate phosphatase to its SMG-2 substrate. According to this model, smg-7 mutants would be expected to accumulate phosphorylated SMG-2. P58PK is a TPR-containing inhibitor of the double-stranded, RNA-activated, protein kinase PKR (Lee et al. 1994). P58PK binds to PKR via one of its TPR domains and, in doing so, inhibits PKR activity (Gal et al. 1996). If SMG-7 were a negative regulator of the kinase that phosphorylates SMG-2 (analogous to the P58PK/PKR relationship), smg-7 mutants might express an overly active SMG-2 kinase, resulting in elevated levels of SMG-2 phosphorylation. Several subunits of anaphase-promoting complex are TPR-containing proteins (Lamb et al. 1994). Anaphase-promoting complex functions at specific times during the cell cycle to target certain proteins for ubiquitin-dependent proteolysis (King et al. 1996). If SMG-7 were involved in targeting phosphorylated SMG-2 for proteolysis, a phosphorylated isoform of SMG-2 might accumulate in smg-7 mutants. Refinement of these or other explicit models of smg-7 function must await its further characterization, especially defining the protein(s) with which it interacts.

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