smg-7 Is Required for mRNA Surveillance in *Caenorhabditis elegans*

Brian M. Cali,*,[1] Sherry L. Kuchma,[†] Jonathan Latham[†] and Philip Anderson*,[†]

**Program in Cell and Molecular Biology and [†]Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706*

Manuscript received August 3, 1998
Accepted for publication October 26, 1998

ABSTRACT

Eukaryotic mRNAs that contain premature stop codons are degraded more rapidly than their wild-type counterparts, a phenomenon termed "nonsense-mediated mRNA decay" (NMD) or "mRNA surveillance." Functions of six previously described *Caenorhabditis elegans* genes, *smg-1* through *smg-6*, are required for NMD. Whereas nonsense mutant mRNAs are unstable in *smg*(+) genetic backgrounds, such mRNAs have normal stability in *smg*(−) backgrounds. Previous screens for *smg* mutations have likely not identified all genes involved in NMD, but efforts to identify additional *smg* genes are 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.

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; Applequist *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; Zarkower *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 such screens are alleles of *smg-1*, *smg-2*, or *smg-5*. We describe here a modified screen for *smg* mutants that precludes isolating alleles of *smg-1*, *smg-2*, and *smg-5*. Using this modified screen, we have identified, cloned, and molecularly characterized a previously uncharacterized gene, *smg-7*, which we show is required for 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-

Corresponding author: Philip Anderson, Department of Genetics, University of Wisconsin, 445 Henry Mall, Madison, WI 53706.
E-mail: andersn@facstaff.wisc.edu

[1]*Present address:* Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142.

somal aberrations used in this study have been previously described (Hodgkin 1988, 1997) or are noted below:

LGI: *dpy-5(e61), fog-1(q180)* (Barton and Kimble 1990), *lev-11(x12), smg-1(r861)* (Hodgkin *et al.* 1989), *smg-2(r863), smg-5(r860), unc-13(e51), unc-15(e1402), unc-54(r293, r259, r315)* (Eide and Anderson 1985a,b; Pulak and Anderson 1988, 1993), *unc-73(e936)*.
LG II: *dpy-10(e128), mut-5(st701), vab-9(e1744), tra-2(e1209), unc-4(e120)*.
LG III: *dpy-1(e1), smg-6(r886, r896)* (Hodgkin *et al.* 1989).
LG IV: *bli-6(sc16), deb-1(st555), dpy-4(e1166), dpy-9(e12), dpy-13(e184), lag-1(q385)* (Christensen *et al.* 1996), *lin-45(sy96), smg-3(r867, ma117), unc-22(e66), unc-24(e138), unc-33(e204), unc-44(e362)*.
LG V: *dpy-11(e224), him-5(e1490), smg-4(ma116), unc-46(e177), unc-60(e723), unc-70(e524), unc-76(e911)*.
Chromosome deficiencies: *nDf41*, *stDf7*, *stDf8* (Williams and Waterston 1994).

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)*; diepoxyoctane (DEO)-induced, *smg-6(r1171)*; *mut-5*-induced, *smg-6(r1172, r1217)*.
LG IV: EMS-induced, *smg-3(r1081, r1163, r1164, r1175, r1176, r1177)*; ENU-induced, *smg-3(r1179, r1180, r1183, r1184, r1186, r1199, r1200, r1201, r1202, r1213, r1215, r1216)*; *smg-7(r1182, r1197)*; DEO-induced, *smg-3(r1168, r1170)*; *mut-5*-induced, *smg-3(r1149, r1173, r1218)*; *smg-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)*; +/*szT1 (I;X)* [+ + + *unc-54(r293)*; *lon-2(e678)*]] is homozygous for *unc-54(r293)* and heterozygous for *szT1*. *szT1* 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, DEO, 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(e1744)*. 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 F_2 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 dominant. All extragenic 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°, 20°, and 25° as the scored phenotype.

Mapping of *smg-7(r1131)*: Hermaphrodites of genotype *unc-13(e51) dpy-5(e61) fog-1(q180) unc-54(r293); +/szT1 [+ + + unc-54(r293); lon-2(e678)]; mut-5(st701) vab-9(e1744); 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°, the nonpermissive temperature of *r1131*. *smg-7(r1131)* homozygotes 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(+)* homozygote was identified among the offspring based upon (i) its protruding vulva (pVul) phenotype and (ii) its wild-type motility at 20°. 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(e138); unc-54(r293)* hermaphrodites at 25°. 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* homozygotes), and the remainder were fully motile at 25°, confirming the failure of *r1131* to complement *nDf41*. To test *r1131* for complementation of *stDf7* and *stDf8*, *smg-7(r1131) unc-24(e138)*/++; *unc-54(r293)*/+ males were crossed with *unc-24(e138) unc-22(e66)*/*stDf7* or *stDf8* hermaphrodites. *smg-7(r1131) unc-24(e138)*/*stDf7* or *stDf8* cross-progeny were identified as Unc-24 animals whose offspring were approximately one-fourth inviable (*Df* homozygotes) 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(e120)*/+ *tra-2(e1209)* + hermaphrodites were mated with N2 males, and single F_1 males were mated with *smg-7(r1131)* hermaphrodites. Resultant *tra-2(e1209)*/+; *smg-7(r1131)*/+ hermaphrodites were selfed at 25°, and *smg-7(r1131)* homozygotes picked as pVul hermaphrodites. *smg-7(r1131); tra-2(e1209)* offspring were examined at 20° and 25° for suppression of the fertility and vulval and tail morphology defects characteristic of *tra-2(e1209)*. Animals raised at 20° were self-sterile [essentially identical to *tra-2(e1209)*], whereas animals raised at 25° 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°. Brood sizes of *smg-7(r1131); tra-2(e1209)* and *smg-1(r861); tra-2(e1209)* animals at 25° were less than those reported for *smg* suppression of *e1209* at 20° (*i.e.*, brood size of 1–5 animals at 25° *vs.* a mean of 11 animals at 20°; 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)*/+ cross-progeny were picked and selfed at 25°. Unc-54 offspring were picked and selfed at 25°. *smg-7(r1131)* homozygotes were identified in the next generation by their normal motility. *dpy-5(e61) unc-54(r293); smg-7(r1131)* homozygotes 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)* homozygotes and indistinguishable from *dpy-5(e61); smg-3(r867)* control animals.

Noncomplementation screen for new *smg-7* alleles: *smg-5(r860) unc-15(e1402) unc-54(r293); him-5(e1409)* males reared at 15° (*e1402* is temperature sensitive) were mutagenized with EMS and mated to *unc-54(r293); smg-7(r1131) unc-24(e138)* hermaphrodites at 25°. Self-progeny exhibit an Unc-24 phenotype, and, as both *smg-5(r860)* and *smg-7(r1131)* are recessive, most cross-progeny [genotype *smg-5(r860) unc-15(e1402) unc-54(r293)*/++ *unc-54(r293); smg-7(r1131) unc-24(e138)*/++; *him-*

5(e1490)/+] 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(e138)* 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(e362) smg-7(r1131) unc-24(e138); +/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 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:

LG I: *lev-11* (4/4), *unc-73* (3/6).
LG II: none tested.
LG III: *dpy-1* (4/5), *dpy-18* (7/9).
LG IV: *unc-24* (0/10).
LG V: *unc-70* (0/9), *dpy-11* (0/7).

Tests of recombination suppression: Heterozygous *m1 m2*/++ males, where *m1* and *m2* denote linked markers between which recombination was being tested (see Table 2), were mated to *unc-44(e362) lag-1(q385); +/rT1 (IV;V) [++;+]* hermaphrodites, and *m1 m2; +/rT1 (IV;V) [++;+]* heterozygous cross-progeny were isolated. Among the self-progeny of such heterozygotes, 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 quantified 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 contains 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 and 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 *deb-1* interval, yielding the following distribution of crossovers: *unc-44* (15/20) *smg-7(r1131::Tc1)* (4/20) *rP14::Tc1* (1/20) *deb-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 *Eco*RV 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° for 50 min, followed by a 30-min incubation at 22°. Roller larvae (those that retain the transforming array) and nonroller larvae (those that have lost it) were isolated singly, raised at 25° for 1–2 days, and scored for motility. All roller animals (10/10) grew to be Unc-54 Egl adults (non-Smg phenotype), while all nonroller animals (24/24) grew to be fully motile, egg-laying proficient adults (Smg phenotype).

Polyclonal anti-SMG-7 antibodies and Western blots: An 800-bp *Eco*RV fragment from *smg-7* cDNA clone TR#288, corresponding to SMG-7 amino acids 174–448, was cloned in-frame into the *Sma*I 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 semidry 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

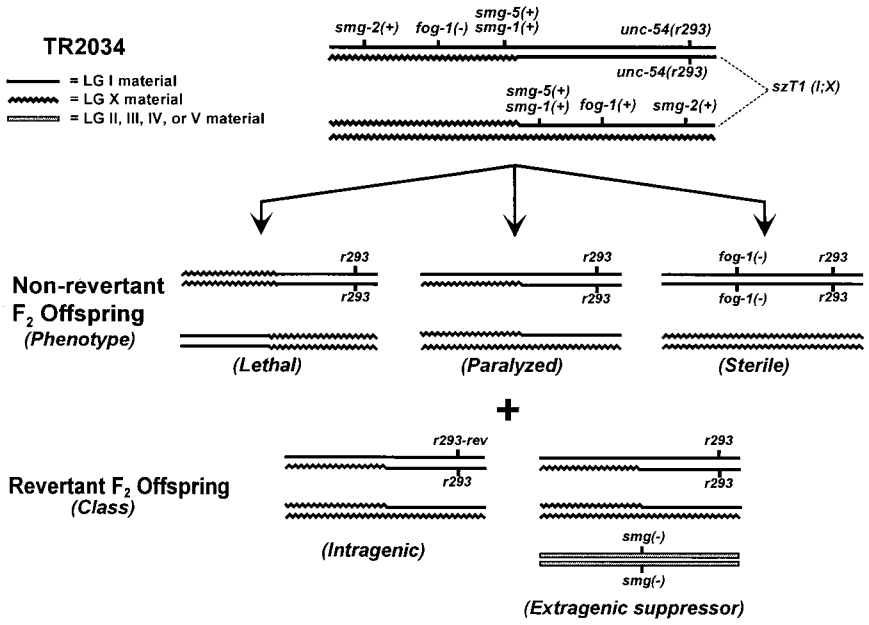

Figure 1.—A modified screen for *smg* mutations that precludes isolating alleles of *smg-1*, *smg-2*, and *smg-5*. TR2034 is heterozygous for the reciprocal translocation *szT1* and homozygous for *unc-54(r293)*. *smg-1*, *smg-2*, *smg-5*, and *fog-1* are located on LG I (left), within the region balanced 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 F_2 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 1). *szT1* is a reciprocal translocation that balances the left half of LG I and the right half of LG X (Fodor and Deak 1985). *smg-1*, *smg-2*, and *smg-5* are all located on the left half of LG I, and *szT1* contains the wild-type alleles of those genes. The unrearranged chromosome I of TR2034 contains *fog-1(q180)*, which causes sterility when homozygous (Barton and Kimble 1990). TR2034 contains additional mutations coupled to *fog-1(q180)* and *szT1* to mark their presence (see materials and methods). Isolating recessive alleles of *smg* genes located on the left half of LG I or the right half of LG X is precluded from this screen, because animals homozygous for *szT1* are inviable and those homozygous for *fog-1* are sterile. To facilitate isolating *smg* mutations not previously described, we mutagenized TR2034 with EMS, DEO, or ENU, each of which yields a different spectrum of mutations (Anderson 1995; De Stasio *et al.* 1998). In screens for spontaneous *smg* mutations, the mutator mutation *mut-5(st701)* was included in the background, because it activates the transposable element Tc1 (Mori *et al.* 1988).

We isolated a total of 49 suppressors of *unc-54(r293)* using this screen. Eight are dominant and inseparable by recombination from *unc-54(r293)* (RF $<$ 0.1 map unit; see materials and methods). The tight linkage and dominance of these mutations suggest that they are intragenic suppressors affecting *unc-54* itself. 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.

Genetic identification of *smg-7*: Three suppressors (*r1131*, *r1182*, and *r1197*) complement canonical alleles of *smg-1* through *smg-6* yet fail to complement each other. Three lines of genetic evidence demonstrate that these alleles define *smg-7*, a previously unidentified *smg* gene. First, *smg-7* mutations exhibit the same pattern of allele-specific suppression as other *smg* mutations. *smg-7(r1131)* suppresses *tra-2(e1209)* and *dpy-5(e61)*, but does not suppress *unc-54(r308)*, *unc-54(r315)*, or *unc-54(r259)*. Second, *smg-7(r1131*, *r1182*, and *r1197)* hermaphrodites have protruding vulvae and males have abnormal bursae, morphogenetic defects characteristic of *smg* mutants. Third, *smg-7* maps to a genetic position that is distinct from previously described *smg* genes (see Figure 2). *smg-7* maps between *bli-6* and *deb-1*, ~0.1 map unit to the right of *smg-3*. Although *smg-7* is within the same three-factor interval as *smg-3* (see Table 1), the two genes are clearly separable. From a heterozygote of genotype *unc-44* + *smg-7 unc-24*/+ *smg-3* + +, one of three Unc-24 non-Unc-44 recombinant chromosomes was a *smg-3 smg-7* double mutant. The *unc-24 smg-3 smg-7* chromo-

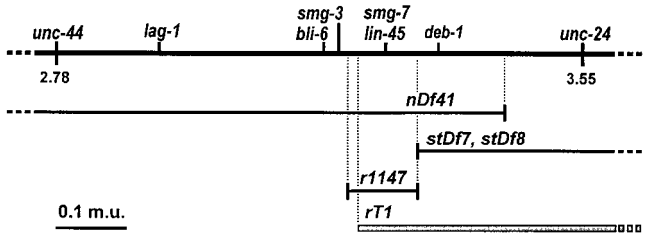

Figure 2.—Linkage map near *smg-7*. Except for *smg-7*, genes shown above the chromosome have been previously ordered by a combination of three-factor crosses and deficiency mapping. We mapped *smg-7* relative to *bli-6*, *smg-3*, *lin-45*, and *deb-1* by three-factor crosses, and relative to *stDf7*, *stDf8*, and *nDf41* by complementation tests.

TABLE 1

Three-factor mapping of *smg-7*

Heterozygote genotype	Recombinant class	Distribution of crossovers[a]
unc-22 + dpy-13/+smg-7 +	Unc non-Dpy	*unc-22* (1/5) *smg-7* (4/5) *dpy-13*
	Dpy non-Unc	*unc-22* (1/5) *smg-7* (4/5) *dpy-13*
bli-6 + unc-24/+ smg-7 +	Unc non-Bli	*bli-6* (1/6) *smg-7* (5/6) *unc-24*
+ lin-45 unc-24/smg-7 ++	Unc-24 non-Lin	*smg-7* (0/6) *lin-45* (6/6) unc-24
unc-44 + deb-1/+ smg-7 +	Unc-44 non-Deb	*unc-44* (32/43) *smg-7* (11/43) *deb-1*

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

some fails 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(r867* 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

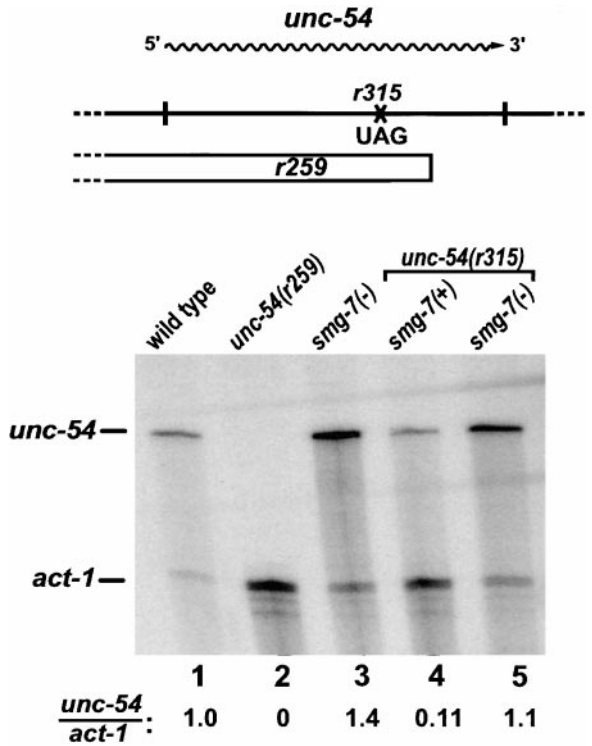

Figure 3.—Effect of *smg-7(r1131)* on nonsense-mediated mRNA decay. RNase protection experiments using total RNA of the indicated strains were performed as previously described (Pulak and Anderson 1993). Lane 1, wild-type strain N2; lane 2, *unc-54(r259)*, a deletion that removes most of *unc-54*; lane 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).

TABLE 2

Effect of *rT1(IV;V)* on LG IV and LG V crossing over

Heterozygote genotype	Number of self-progeny				Calculated map distance (cM)[a]
	WT	DpyUnc	Unc	Dpy	
unc-60 dpy-11/++	391	89	37	38	14.6 ± 2.9
unc-60 dpy-11/rT1(V);++/rT1(IV)	276	68	0	0	0
unc-46 dpy-11/++	685	210	4	9	1.4 ± 1.2
unc-46 dpy-11/rT1(V);++/rT1(IV)	318	102	0	0	0
dpy-11 unc-76/++	692	192	25	15	4.4 ± 1.3
dpy-11 unc-76/rT1(V);++/rT1(IV)	69	15	6	2	9.1 ± 5.9
unc-24 dpy-4/++	504	151	32	44	11.0 ± 2.3
unc-24 dpy-4/rT1(IV);++/rT1(V)	274	63	0	0	0
dpy-9 unc-33/++	330	54	46	53	23.2 ± 3.8
dpy-9 unc-33/rT1(IV);++/rT1(V)	112	47	18	17	20.0 ± 5.7

[a] Map distances were calculated as described in Hodgkin (1988, 1997). Confidence intervals of 95% were calculated using the normal approximation of the binominal distribution.

"noncomplementation" screen. We knew that we could isolate null alleles of *smg-7* heterozygous to *smg-7(r1131)*, because *nDf41* deletes *smg-7* (see Figure 2) and *smg-7(r1131)/nDf41* heterozygotes are viable, fertile, and Smg. We mutagenized *smg-7(+)* males with EMS and mated them with *smg-7(r1131)* hermaphrodites at 25°. Additional mutations were present in the parent strains to distinguish self-progeny from cross-progeny, to allow *unc-54(r293)* homozygous males to mate, to mark the *r1131*-containing chromosome, and to phenotypically distinguish Smg from non-Smg offspring (see materials and methods). We isolated two *smg-7* alleles in this screen among ~4400 mutagenized genomes. Both of these alleles, *r1147* and *r1148*, are lethal when homozygous.

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

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

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

TABLE 3

Transformation rescue of *smg-7(r1131)*

Host genotype	Injected DNA	Motility phenotype following: No heat shock	Motility phenotype following: Heat shock	*smg-7* Activity following heat shock
unc-54(r293)	None	Paralyzed	Paralyzed	+
unc-54(r293); smg-7(r1131)	None	Motile	Motile	−
unc-54(r293); smg-7(r1131)	*hsp16-2::smg-7(+)*	Motile	Paralyzed	+
wild type	*hsp16-2::smg-7(+)*	Motile	Motile	+

Heritable transformants were heat shocked at 33° for 50 min, after which individual larvae were isolated, grown at 25° for 1 to 2 days, and scored for motility.

as a hybridization probe, we identified a phage lambda genomic clone of the wild-type region and subsequently subcloned a 1.6-kb *Eco*RI 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. Sternberg, 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 (Mello 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 (Mello *et al.* 1991), into *unc-54(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(+)* expression. 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 pI 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-

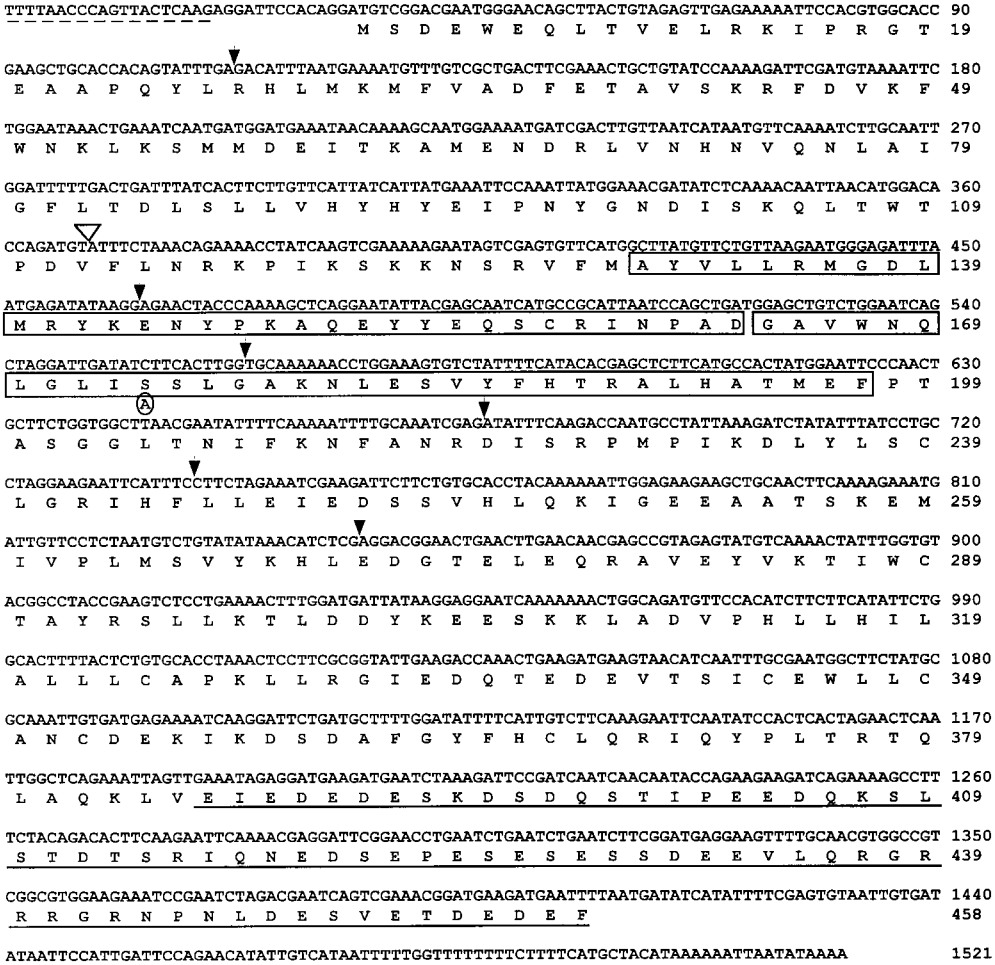

Figure 4.—Sequence of *smg-7* cDNA clone TR#288. Sequences of the *trans*-spliced leader SL2 are underlined with dashes. The two TPR domains (amino acids 130–163 and 164–197) are boxed. The acidic carboxyl terminus is underlined. Arrowheads indicate the positions of *smg-7* introns, and the site of Tc1 insertion in *r1131* (nt 368) is indicated by an open triangle. Nucleotide 644 is changed from T to A (circled) in *smg-7(r1197)*.

cially at position 32 near the end of helix B. 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 defined 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-*

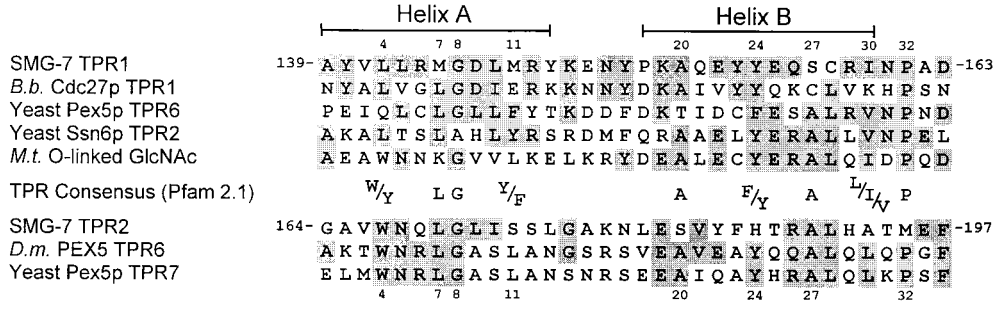

Figure 5.—Alignment of SMG-7 TPR domains. TPR1 and TPR2 of SMG-7 are similar to TPR domains of a variety of prokaryotic and eukaryotic proteins, the most similar of which are shown here. A TPR consensus was derived from the 42 TPR domains contained in the Pfam protein domain families database release 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. Helices A and B were defined by the crystal structure of TPR domains of protein phosphatase 5 (Das *et al.* 1998). Aligned sequences are as follows: *Borrelia burgdorferi CDC27* (Fraser *et al.* 1997), yeast *PEX5* (Van der Leij *et al.* 1993), yeast *SSN6* (Schultz and Carlson 1987), *Methanobacterium thermoautotrophicum* O-linked GlcNAc (Dubois *et al.* 1997), and *Drosophila melanogaster* PEX5 (GenBank accession no. 2832771).

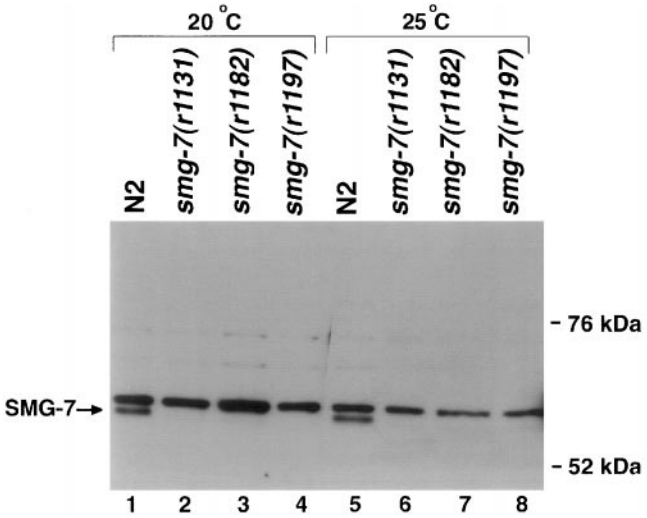

Figure 6.—Western blot of SMG-7. Total proteins of wild types (lanes 1 and 5) were probed with anti-SMG-7 antibody AS74. SMG-7 (indicated by arrow) is absent in *smg-7(r1131*, *R1182*, and *r1197)* grown at both 20° and 25° (lanes 2–4 and 6–8). AS74 cross-reacts with a protein slightly larger than SMG-7 that is not affected by *smg-7* mutations.

7(r1182), but we did not sequence the *lin-45* to *smg-7* intergenic region, mutation of which might affect *smg-7* expression.

To confirm that *smg-7(r1197)* is a null allele and to identify SMG-7 on Western blots, we prepared affinity-purified polyclonal antibodies against an SMG-7 fusion protein expressed in bacteria (see materials and methods). As shown in Figure 6, this antibody (designated AS74) detects a protein migrating at ~57 kD that is absent in *smg-7(r1131*, *r1182*, and *r1197)* at both permissive (20°) and restrictive (25°) temperatures. AS74 also cross-reacts with an ~59-kD protein that is unaffected by *smg-7* mutations and is, therefore, not a 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

TABLE 4

Summary of *smg* mutations

Gene	Hodgkin *et al.* (1989)	This study
smg-1	23	0
smg-2	20	0
smg-3	4	23
smg-4	1	2
smg-5	4	0
smg-6	2	13
smg-7	0	3
unc-54 (intragenic)	1	8

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°, 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; Goebl and Yanagida 1991;

Lamb *et al.* 1995). About 25 different proteins with TPR domains have been identified to date. Individual TPR domains are 34 amino acids long and have a loose consensus sequence. TPRs form paired amphipathic helices (Das *et al.* 1998), and mutational studies suggest that TPRs mediate homo- and heteromeric protein-protein interactions. TPR domains may coordinate the assembly of multiprotein complexes, examples of which include mitochondrial and peroxisomal import receptor complexes (Fransen *et al.* 1995; Neupert 1997), anaphase promoting complex (Lamb *et al.* 1994; King *et al.* 1995), and transcription repression complexes (Smith *et al.* 1995; Tzamarias 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 (He 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; He and Jacobson 1995). The acidic carboxyl terminus of Upf2p has been shown to mediate direct interactions with Upf1p (He 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. $P58^{IPK}$ is a TPR-containing inhibitor of the double-stranded, RNA-activated, protein kinase PKR (Lee *et al.* 1994). $P58^{IPK}$ binds to PKR via one of its TPR domains and, in doing so, inhibits PKR activity (Gale *et al.* 1996). If SMG-7 were a negative regulator of the kinase that phosphorylates SMG-2 (analogous to the $P58^{IPK}$/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.

We thank A. Golden and P. Sternberg for providing *lin-45* strains and clones, S. O'Connor for help in constructing TR2034, and Michelle Page for editing the manuscript. Strains used for mapping *smg-7* were obtained from the Caenorhabditis Genetics Center, a resource supported by the National Institutes of Health (NIH) National Center for Research Resources. This work was supported by an NIH grant (GM-50933) to P.A., by a Howard Hughes Medical Institute Predoctoral Fellowship to B.M.C., and by the University of Wisconsin Training Grant in Genetics.

LITERATURE CITED

Anderson, P., 1995 Mutagenesis. Methods Cell Biol. **48:** 31–58.

Andjelkovic, N., S. Zolnierowicz, C. Van Hoof, J. Goris and B. A. Hemmings, 1996 The catalytic subunit of protein phosphatase 2A associates with the translation termination factor eRF1. EMBO J. **15:** 7156–7167.

Applequist, S. E., M. Selg, C. Raman and H. M. Jack, 1997 Cloning and characterization of HUPF1, a human homolog of the Saccharomyces cerevisiae nonsense mRNA-reducing UPF1 protein. Nucleic Acids Res. **25:** 814–821.

Barnes, T. M., and J. Hodgkin, 1996 The *tra-3* sex determination gene of *Caenorhabditis elegans* encodes a member of the calpain regulatory protease family. EMBO J. **15:** 4477–4484.

Barstead, R. J., and R. Waterston, 1989 The basal component of the nematode dense body is vinculin. J. Biol. Chem. **264:** 10177–10185.

Barton, M. K., and J. Kimble, 1990 *fog-1*, a regulatory gene required for specification of spermatogenesis in the germ line of *Caenorhabditis elegans*. Genetics **125:** 29–39.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

Cali, B. M., and P. Anderson, 1998 mRNA surveillance mitigates dominance in *C. elegans*. Mol. Gen. Genet. **260:** 176–184.

Christensen, S., V. Kodoyianni, M. Bosenberg, L. Friedman and J. Kimble, 1996 *lag-1*, a gene required for *lin-12* and *glp-1* signaling in *Caenorhabditis elegans*, is homologous to human *CBF1* and Drosophila *Su(H)*. Development **122:** 1373–1383.

Cui, Y., K. W. Hagan, S. Zhang and S. W. Peltz, 1995 Identification and characterization of genes that are required for the accelerated degradation of mRNAs containing a premature translational termination codon. Genes Dev. **9:** 423–436.

Czaplinski, K., M. J. Ruiz-Echevarria, S. V. Paushkin, X. Han, Y. Weng et al. 1998 The surveillance complex interacts with the translation release factors to enhance termination and degrade aberrant mRNAs. Genes Dev. **12:** 1665–1677.

Das, A. K., P. T. W. Cohen and D. Barford, 1998 The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. EMBO J. **17:** 1192–1199.

De Stasio, E., C. Lephoto, L. Azuma, C. Holst, D. Stanislaus *et al.*, 1998 Characterization of revertants of *unc-93(e1500)* in *C. elegans* induced by N-ethyl-N-nitrosourea. Genetics **147:** 597–608.

Dubois, J., T. Aldredge, R. Bashirzadeh, D. Blakely, R. Cook *et al.*, 1997 Complete genome sequence of *Methanobacterium*

thermoautotrophicum delta H: functional analysis and comparative genomics. J. Bacteriol. **179:** 7135–7155.

Eide, D., and P. Anderson, 1985a The gene structures of spontaneous mutations affecting a *Caenorhabditis elegans* myosin heavy chain gene. Genetics **109:** 67–79.

Eide, D., and P. Anderson, 1985b Transposition of Tc1 in the nematode *C. elegans*. Proc. Natl. Acad. Sci. USA **82:** 1756–1760.

Epstein, H., R. Waterston and S. Brenner, 1974 A mutant affecting the heavy chain of myosin in *Caenorhabditis elegans*. J. Mol. Biol. **90:** 291–300.

Fodor, A., and P. Deak, 1985 The isolation and genetic analysis of a *C. elegans* translocation (*szT1*) strain bearing an X-chromosome balancer. J. Genet. **64:** 143–157.

Fransen, M., C. Brees, E. Baumgart, J. C. Vanhooren, M. Baes *et al.*, 1995 Identification and characterization of the putative human peroxisomal C-terminal targeting signal import receptor. J. Biol. Chem. **270:** 7731–7736.

Fraser, C. M., S. H. W. M. Casjens, G. G. Sutton, R. A. Clayton, R. Lathigra *et al.*, 1997 Genomic sequence of a Lyme disease spirochete, *Borrelia burgdorferi*. Nature **390:** 580–586.

Gale, M., S.-L. Tan, M. Wambach and M. G. Katze, 1996 Interaction of the interferon-induced PKR protein kinase with inhibitory proteins $P58^{IPK}$ and vaccinia virus K3L is mediated by unique domains: implications for kinase regulation. Mol. Cell. Biol. **16:** 4172–4181.

Goebl, M., and M. Yanagida, 1991 The TPR snap helix: a novel protein repeat motif from mitosis to transcription. Trends Biochem. Sci. **16:** 173–177.

Han, M., A. Golden, Y. Han and P. W. Sternberg, 1993 *C. elegans lin-45 raf* gene participates in *let-60 ras*-stimulated vulval differentiation. Nature **363:** 133–140.

He, F., and A. Jacobson, 1995 Identification of a novel component of the nonsense-mediated mRNA decay pathway by use of an interacting protein screen. Genes Dev. **9:** 437–454.

He, F., A. H. Brown and A. Jacobson, 1997 Upf1p, Nmd2p, and Upf3p are interacting components of the yeast nonsense-mediated mRNA decay pathway. Mol. Cell. Biol. **17:** 1580–1594.

Hodgkin, J., 1988 Genetics, pp. 491–584 in *The Nematode Caenorhabditis elegans*, edited by W. B. Wood. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Hodgkin, J., 1997 Genetics, pp. 881–1047 in *C. elegans II*, edited by D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Hodgkin, J., A. Papp, R. Pulak, V. Ambros and P. Anderson, 1989 A new kind of informational suppression in the nematode *Caenorhabditis elegans*. Genetics **123:** 301–313.

Jacobson, A., and S. W. Peltz, 1996 Interrelationships of the pathways of mRNA decay and translation in eukaryotic cells. Annu. Rev. Biochem. **65:** 693–739.

King, R. W., J. M. Peters, S. Tugendreich, M. Rolfe, P. Hieter *et al.*, 1995 A 20S complex containing cdc27 and cdc16 catalyses the mitosis-specific conjugation of ubiquitin to cyclin B. Cell **81:** 279–288.

King, R. W, R. J. Deshaies, J. M. Peters and M. W. Kirschner, 1996 How proteolysis drives the cell cycle. Science **274:** 1652–1659.

Kuwabara, P. E., P. G. Okkema and J. Kimble, 1992 *tra-2* encodes a membrane-protein and may mediate cell communication in the *Caenorhabditis elegans* sex determination pathway. Mol. Biol. Cell **3:** 461–473.

Lamb, J. R., W. A. Michaud, R. S. Sikorski and P. S. Hieter, 1994 Cdc16p, Cdc23p and Cdc27p form a complex essential for mitosis. EMBO J. **13:** 4321–4328.

Lamb, J. R., S. Tugendreich and P. Hieter, 1995 Tetratricopeptide repeat interactions: to TPR or not to TPR? Trends Biochem. Sci. **20:** 257–259.

Lee, B. S., and M. R. Culbertson, 1995 Identification of an additional gene required for eukaryotic nonsense mRNA turnover. Proc. Natl. Acad. Sci. USA **92:** 10354–10358.

Lee, T. G., N. Tang, S. Thompson, J. Miller and M. G. Katze, 1994 The 58,000-dalton cellular inhibitor of the interferon-induced double-stranded RNA-activated protein kinase (PKR) is a member of the TPR repeat family of proteins. Mol. Cell. Biol. **14:** 2331–2342.

Leeds, P., S. W. Peltz, A. Jacobson and M. R. Culbertson, 1991 The product of the yeast *UPF1* gene is required for rapid turnover of mRNAs containing a premature translational termination codon. Genes Dev **5:** 2303–2314.

Leeds, P., J. M. Wood, B. S. Lee and M. R. Culbertson, 1992 Gene products that promote mRNA turnover in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:** 2165–2177.

Losson, R., and F. Lacroute, 1979 Interference of nonsense mutations with eukaryotic messenger RNA stability. Proc. Natl. Acad. Sci. USA **76:** 5134–5137.

Maquat, L. E., 1995 When cells stop making sense: effects of nonsense codons on RNA metabolism in vertebrate cells. RNA **1:** 453–465.

Mello, C., and A. Fire, 1995 DNA transformation, pp. 451–482 in *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, edited by H. F. Epstein and D. C. Shakes. Academic Press, San Diego.

Mello, C. C., J. M. Kramer, D. Stinchcomb and V. Ambros, 1991 Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J. **10:** 3959–3970.

Mori, I., D. G. Moerman and R. H. Waterston, 1988 Analysis of a mutator activity necessary for germline transposition and excision of Tc1 transposable elements in *Caenorhabditis elegans*. Genetics **120:** 397–407.

Neupert, W., 1997 Protein import into mitochondria. Annu. Rev. Biochem. **66:** 863–917.

Ochman, H., A. S. Gerber and D. L. Hartl, 1988 Genetic applications of an inverse polymerase chain reaction. Genetics **120:** 621–623.

Perlick, H. A., S. M. Medghalchi, F. A. Spencer, R. J. Kendzior, Jr. and H. C. Dietz, 1996 Mammalian orthologues of a yeast regulator of nonsense transcript stability. Proc. Natl. Acad. Sci. USA **93:** 10928–10932.

Pulak, R., and P. Anderson, 1988 Structures of spontaneous deletions in *Caenorhabditis elegans*. Mol. Cell. Biol. **8:** 3748–3754.

Pulak, R., and P. Anderson, 1993 mRNA surveillance by the *C. elegans smg* genes. Genes Dev. **7:** 1885–1897.

Rougvie, A. E., and V. Ambros, 1995 The heterochronic gene *lin-29* encodes a zinc finger protein that controls a terminal differentiation event in *Caenorhabditis elegans*. Development **121:** 2491–2500.

Ruiz-Echevarria, M. J., K. Czaplinski and S. W. Peltz, 1996 Making sense of nonsense in yeast. Trends Biochem. Sci. **21:** 433–438.

Schultz, J., and M. Carlson, 1987 Molecular analysis of *SSN6*, a gene functionally related to the *SNF1* protein kinase of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:** 3637–3645.

Sikorski, R. S., M. S. Boguski, M. Goebl and P. Hieter, 1990 A repeating amino acid motif in *CDC23* defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. Cell **60:** 307–317.

Smith, R. L., M. J. Reed and A. D. Johnson, 1995 The tetratricopeptide repeats of Ssn6 interact with the homeo domain of alpha 2. Genes Dev. **9:** 2903–2910.

Sonnhammer, E. L., S. R. Eddy, E. Birney, A. Bateman and R. Durbin, 1998 Pfam: multiple sequence alignments and HMM-profiles of protein domains. Nucleic Acids Res. **26:** 320–322.

Stringham, E. G., D. K. Dixon, D. Jones and E. P. M. Candido, 1992 Temporal and spatial expression patterns of the small heat-shock (HSP16) genes in transgenic *Caenorhabditis elegans*. Mol. Biol. Cell **3:** 221–233.

Sun, X. L., H. A. Perlick, H. C. Dietz and L. E. Maquat, 1998 A mutated human homologue to yeast *UPF1* protein has a dominant-negative effect on the decay of nonsense-containing mRNAs in mammalian cells. Proc. Natl. Acad. Sci. USA **95:** 10009–10014.

Tzamarias, D., and K. Struhl, 1995 Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 corepressor complex to differentially regulated promoters. Genes Dev. **9:** 821–831.

Van der Leij, I., M. M. Franse, Y. Elgersma, B. Distel and H. F. Tabak, 1993 PAS10 is a tetratricopeptide-repeat protein that is essential for the import of most matrix proteins into peroxisomes of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **90:** 11782–11786.

Waterston, R. H., J. E. Sulston and A. R. Coulson, 1997 The genome, pp. 23–45 in *C. elegans II*, edited by D. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Williams, B. D., and R. H. Waterston, 1994 Genes critical for muscle development and function in *Caenorhabditis elegans* identified through lethal mutations. J. Cell Biol. **124:** 475–490.

Williams, J. A., J. A. Langeland, B. S. Thalley, J. B. Skeath and S. B. Carroll, 1995 Expression of foreign proteins in *E. coli* using plasmid vectors and purification of specific polyclonal antibodies, pp. 15–58 in *DNA Cloning 2: Expression Systems: A Practical Approach*, edited by D. M. Glover and B. D. Hames. Oxford University Press, Oxford.

Zarkower, D., M. De Bono, R. Aronoff and J. Hodgkin, 1994 Regulatory rearrangements and *smg*-sensitive alleles of the *C. elegans* sex-determining gene *tra-1*. Dev. Genet. **15:** 240–250.

Communicating editor: R. K. Herman