mRNA surveillance by the *Caenorhabditis elegans* smg genes

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mRNAs that contain premature stop codons are unstable in most eukaryotes, but the mechanism of their degradation is largely unknown. We demonstrate that functions of the six *C. elegans* smg genes are necessary for rapid turnover of nonsense mutant mRNAs of the *unc-54* myosin heavy chain gene. Nonsense alleles of *unc-54* express mRNAs that are unstable in *smg(+)* genetic backgrounds but have normal or near normal stability in *smg(-)* backgrounds. *smg* mutations also stabilize mRNA of *unc-54(r293)*, a small deletion that removes the *unc-54* polyadenylation site and expresses an aberrant mRNA. Most *unc-54* nonsense mutations are recessive in both *smg(+)* and *smg(-)* genetic backgrounds. However, four specific alleles are recessive when *smg(+)* and dominant when *smg(-)*. These *smg*-dependent dominant alleles express nonsense mutant polypeptides that disrupt thick filament and/or sarcomere assembly. All four alleles are predicted to express nonsense fragment polypeptides that contain most of the myosin globular head domain without an attached rod segment. By degrading messages that contain premature stop codons, the *smg* genes eliminate mRNAs that encode potentially toxic protein fragments. We propose that this system of mRNA turnover protects cells from their own errors of transcription, mRNA processing, or mRNA transport.

[Key Words: mRNA turnover; *Caenorhabditis elegans*; smg genes; mRNA surveillance]

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The steady-state level of a eukaryotic mRNA is established by its relative rates of synthesis and degradation. It is increasingly apparent that mRNA degradation is an important aspect of gene expression and its regulation (for reviews, see Atwater et al. 1990; Peltz et al. 1991). The half-lives of different mRNAs can vary from a few minutes to a few weeks. For example, the half-lives of *c-myc* and *c-fos* can be as short as 30 mins (Kruijer et al. 1984; Muller et al. 1984; Kindy and Sonnenshein 1986), the half-life of β -globin mRNA is >24 hr (Ross and Pizarro 1983), and the half-life of *Xenopus* vitellogenin mRNA in the presence of estrogen is ~3 weeks (Brock and Shapiro 1983). The stability of many mRNAs is regulated by cellular and environmental stimuli. For example, the half-lives of certain histone mRNAs change during the cell cycle (Hereford et al. 1981), tubulin mRNA turnover is regulated by the concentration of unpolymerized tubulin (Cleveland 1988), estrogen increases the half-life of vitellogenin mRNA (Brock and Shapiro 1983), and heat shock stabilizes HSP70 mRNA (DiDomenico et al. 1982). Regulated mRNA stability is widespread, but we know very little about the molecular mechanisms involved.

mRNA degradation presumably involves both *cis*-acting sequences that identify a mRNA for degradation and *trans*-acting factors that degrade (or regulate degradation of) the message. A number of *cis*-acting sequences that

are required for regulated or constitutive mRNA turnover have been defined. The iron-responsive element regulates stability of transferrin receptor mRNA (Owen and Kuhn 1987; Mullner and Kuhn 1988), and an AU-rich element mediates stability of GM-CSF (Shaw and Kamen 1986), *c-fos* (Wilson and Treisman 1988), and *c-myc* mRNAs (Jones and Cole 1987). While these *cis*-acting sequences are located within 3' translated regions, other stability determinants of *c-fos* (Shyu et al. 1989), *c-myc* (Wisdom and Lee 1991), β -tubulin (Gay et al. 1989b), *MAT α 1* (Parker and Jacobson 1990), and *STE3* (Heaton et al. 1992) are located within translated exons. Little is known about the *trans*-acting factors that interact with these stability determinants to accomplish selective mRNA turnover. Proteins that bind near stability determinants have been identified (Malter 1989; Bohjanen et al. 1991; Brewer 1991; Vakalopoulou et al. 1991), but their roles in degradation are unknown. Two central questions remain unanswered: What are the degradative enzymes and how is their activity controlled such that only specific mRNAs are degraded?

Several lines of evidence indicate that translation plays an important role in degrading many mRNAs: Drugs that inhibit protein synthesis cause many mRNAs to be superinduced (for review, see Peltz et al. 1991); mutations that impair translation have a similar effect (Peltz et al. 1992); autoregulated degradation of tubulin mRNA occurs when tubulin message is loaded onto polysomes, although the ribosomes need not be elongat-

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ing (Pachter et al. 1987); ribonucleases that are required for mRNA turnover in vitro are polysome associated (Ross and Kobs 1986); the abundance of rare codons causes a message to be unstable (Hoekema et al. 1987); and nonsense mutations cause mRNAs to be unstable in most organisms (discussed below). Collectively, these observations make a compelling case that most, if not all, mRNA degradation is intimately coupled to translation.

The importance of translation to mRNA turnover is particularly clear in the case of mRNAs that contain premature stop codons. The steady-state levels of most cellular or viral mRNAs that contain either a nonsense or frameshift mutation are dramatically reduced in most eukaryotes, including yeasts, plants, *Drosophila*, mice, hamsters, and humans (Losson and Lacroute 1979; Maquat et al. 1981; Baumann et al. 1985; Voelker et al. 1986; Scallan et al. 1987; Schneuwly et al. 1989; Urlaub et al. 1989; Washburn and O'Tousa 1989). Numerous studies demonstrate that these mutant mRNAs have reduced half-lives (Losson and Lacroute 1979; Maquat et al. 1981; Baumann et al. 1985; Barker and Beemon 1991; Gaspar et al. 1991; Leeds et al. 1991; Lim et al. 1992). It is not known where nonsense mutant mRNAs are degraded in a cell or how they are targeted for selective decay. Because premature translation termination triggers turnover, it seems likely that cytoplasmic ribosomes are involved. The cytoplasmic half-life of many nonsense mutant mRNAs is reduced. Observations concerning several mammalian genes, however, indicate that the mechanism might be more complex. Nonsense mutations affecting β -globin (Humphries et al. 1984; Takeshita et al. 1984), dihydrofolate reductase (Urlaub et al. 1989), triosephosphate isomerase (Cheng et al. 1993), and fibrillin (Dietz et al. 1993) mRNAs appear to influence metabolism of these mRNAs in the nucleus. Nuclear, rather than cytoplasmic, mRNAs seem to be unstable. It is puzzling how nonsense mutations can affect nuclear mRNA processing, but perhaps there are distinct cytoplasmic and nuclear mechanisms for degrading nonsense mutant mRNAs.

The six *Caenorhabditis elegans* *smg* genes identify a new kind of informational suppression (Hodgkin et al. 1989). *smg* mutations are allele-specific, but not gene-specific, suppressors of mutations affecting a variety of different genes. Genetic analysis of the *smg* genes indicates that (1) suppressor mutations are loss-of-function (or reduction-of-function) alleles; (2) the wild-type *smg* genes function in most, if not all, cells of the animal; (3) all six *smg* genes likely function in the same biochemical process or pathway; and (4) other than their suppression phenotype, *smg* mutants are nearly normal. *smg* mutants have reduced brood sizes and exhibit a mild morphogenetic defect (*smg* denotes suppressor with morphogenetic effects on genitalia). Otherwise, their growth and development is nearly normal.

We have investigated the molecular basis for suppression by *smg* mutations. We demonstrate that wild-type function of six *smg* genes is necessary for rapid turnover of a number of mutant mRNAs of the *unc-54* myosin

heavy chain gene. Most notably, mRNAs that contain *unc-54* nonsense or frameshift mutations are unstable in *smg(+)* genetic backgrounds but stable in *smg(-)* strains. We provide genetic evidence that when nonsense mutant mRNAs are translated, the resulting truncated polypeptides can have disruptive activities. We suggest that the *smg* genes constitute a mRNA surveillance system that protects cells from its own errors of mRNA synthesis or processing.

Results

unc-54(r293) mutants contain a small amount of an unusually large mRNA

We isolated *smg* mutations as extragenic suppressors of *unc-54(r293)* (Hodgkin et al. 1989). *unc-54* encodes myosin heavy chain B (MHC B), one of two myosin heavy chain isoforms expressed in body-wall muscles. *r293* is a spontaneous small deletion that was isolated in a general screen for *unc-54* loss-of-function mutations. The region deleted by *r293* (Pulak and Anderson 1988) is shown in Figure 1. *r293* deletes 256 bp of DNA entirely 3' of the *unc-54* open reading frame. The deleted material includes the 3' cleavage and polyadenylation site and most of the *unc-54* 3'-untranslated region (3'UTR) (Karn et al. 1983; Okkema et al. 1993).

We analyzed the quantity and size of *unc-54(r293)* mRNA using ribonuclease protection and Northern blot analyses. Figure 2 shows an RNase protection assay demonstrating that the steady-state level of *r293* mRNA is reduced relative to wild type. We hybridized samples of wild-type and *r293* total RNA with an excess of both *unc-54* and *act-1* radiolabeled probes. After RNase digestion and electrophoresis, we quantified the radioactivity contained in each protected fragment. Using the *act-1* hybridization signal to control for lane-to-lane variation in the amount of nematode RNA per assay, we estimate

Figure 1. The gene structure of *unc-54(r293)*. *r293* deletes the *unc-54* polyadenylation site but does not affect the *unc-54* translated region. Boxes represent *unc-54* exons. (■) Translated regions; (□) 5' and 3' UTRs. Connecting lines represent introns or flanking DNA. Wild-type mRNA is indicated by a wavy line. *unc-54* does not contain the sequence AAUAAA upstream of its polyadenylation site; rather, the related sequence GAUAAA is likely to be its polyadenylation signal (Okkema et al. 1993).

Figure 2. RNase protection assay of *unc-54(r293)* and *act-1(+)* steady-state mRNAs in *smg(+)* and *smg(-)* backgrounds. *r293* (lane 3) contains ~8% of the wild-type quantity of *unc-54* mRNA. *r293; smg(-)* strains contain approximately normal amounts. The negative control strain *r259* (lane 2) deletes *unc-54* and contains no mRNA. *smg* alleles used: *smg-1(r861)*, *smg-2(r863)*, *smg-3(r867)*, *smg-4(ma116)*, *smg-5(r860)*, and *smg-6(r896)*.

that *r293* contains 8% of the wild-type quantity of *unc-54* mRNA (Fig. 2, cf. lanes 1 and 3).

The motility of *unc-54(r293)* mutants confirms that they express a small but detectable quantity of functional MHC B protein. *r293* is a leaky mutation; homozygotes are slightly more motile than *unc-54* null alleles, which are nearly paralyzed. The motility of *r293* resembles that of *unc-54* amber mutants in the presence of *sup-5*, a tryptophan-inserting amber suppressor tRNA gene [Kondo et al. 1988] that restores ~5% of wild-type levels of MHC B [MacLeod et al. 1979].

The Northern blot shown in Figure 3 demonstrates that *unc-54(r293)* mRNA is unusually large. We estimate that *r293* mRNA is 1.8 ± 0.2 kb larger than wild-type mRNA (Fig. 3, cf. lanes 1 and 2). We have not analyzed the structure of *r293* mRNA in detail. Because the *unc-54* cleavage and polyadenylation site is deleted by *r293*, we presume that *r293* mRNA is larger than normal owing to inclusion of "downstream" sequences at the mRNA 3' end. However, we have not mapped the 3' end of *r293* mRNA nor have we established that it is polyadenylated. The effect of *r293* on the size and quantity of *unc-54* mRNA is similar to the effects of mutations disrupting cleavage and polyadenylation in both yeasts and humans [Zaret and Sherman 1982; Higgs et al. 1983; Rund et al. 1992].

smg mutations increase the steady-state level of *unc-54(r293)* mRNA

Figure 2 shows an RNase protection assay with which we quantified the steady-state levels of *unc-54(r293)*; *smg(-)* mRNAs. After normalizing lane-to-lane varia-

tion in the quantity of added RNA (using the *act-1* hybridization signal as a standard), we estimate that five strains, *r293; smg-1* through *r293; smg-5* contain 120–140% of the wild-type quantity of *unc-54* mRNA (Fig. 2, cf. lane 1 with lanes 4–8). Experiments described below demonstrate that *smg* mutations do not affect the relative abundance of *act-1* mRNA. *r293; smg-6(r896)* contains considerably less *unc-54* mRNA than the other *smg* mutants, ~85% of wild type (Fig. 2, cf. lanes 1 and 9). Throughout the experiments described here and below, the two extant alleles of *smg-6(r896)* and *r886*) consistently elevate *unc-54* mutant mRNA levels less than all alleles tested of *smg-1*, *smg-2*, *smg-3*, *smg-4*, or *smg-5*.

smg mutations affect the quantity, but not the size, of *unc-54(r293)* mutant mRNA. The Northern blot shown in Figure 3 demonstrates that in *smg(-)* backgrounds, *r293* mRNA is the same large size as it is in *smg(+)* backgrounds. But, the steady-state level of this mRNA is increased dramatically in *smg(-)* strains [Fig. 3; cf. lanes 1 and 2 with lanes 5, 7, and 9].

The phenotypes of *unc-54(r293); smg* mutants confirm that they express large amounts of functional MHC B mRNA and protein. Under appropriate conditions, the motility of an animal can reflect the quantity of MHC B that it expresses. For example, *unc-54* null heterozygotes [genotype *unc-54(0)/+*] express 50% of the normal amount of MHC B and are very nearly wild type [Bejsovec 1988; Bejsovec and Anderson 1988]. Normal motility is achieved only when MHC B levels approach 50% of

Figure 3. Northern blot of wild-type and mutant mRNAs. Wild-type and *r293* mRNAs are shown at left. To visualize the signal of *r293* (lane 2), approximately eight-fold more RNA was loaded than for wild type (lane 1). By comparing these mRNAs to RNA size markers (not shown), we estimate that *r293* mRNA is 1.8 ± 2 kb larger than wild-type mRNA. *unc-54(+); smg(-)* and *r293; smg(-)* mRNAs are shown at right. *smg* mutations affect the quantity but not the size of *r293* mRNA.

that found in the wild type (see Hodgkin et al. 1989). *r293; smg(-)* homozygotes exhibit normal motility. Suppressed hemizygotes [genotype *unc-54(r293)/Df(unc-54); smg(-)*], which contain only a single suppressed allele of *r293*, also have normal motility. These motility phenotypes confirm that *r293; smg(-)* strains express substantial quantities (>50% wild type) of MHC B.

smg mutations do not affect the quantity of unc-54(+) mRNA

The increased level of *unc-54(r293)* mRNA in *smg* mutant backgrounds could be either a transcriptional or post-transcriptional effect. If *smg* mutations increased the rate of *unc-54* transcription, they should also increase the quantity of wild-type *unc-54* mRNA. To test this, we crossed a representative allele of each of the six *smg* genes into an *unc-54(+)* genetic background and quantified the steady-state level of *unc-54(+)* mRNA. Figure 4 shows one such RNase protection assay. After normalizing *unc-54* hybridizing signals to those of *act-1*, we estimate that the six *smg* mutants contain 83–108% of the wild-type quantity of *unc-54* mRNA (Fig. 4, cf. lane 1 with lanes 2–6). Northern blots demonstrate that the size of *unc-54(+)* mRNA is normal in *smg(-)* strains (Fig. 3, lanes 4, 6, and 8).

Figure 4 also demonstrates that *smg* mutations do not affect the abundance of *act-1* mRNA relative to total RNA. We assayed approximately equal amounts of RNA in all six strains in Figure 4, as judged both by spectrophotometry and the intensity of rRNA bands on acridine orange-stained agarose gels. All *smg* mutants contained about wild-type quantities of *act-1* mRNA. Similar experiments demonstrate that the relative abundance of *mlc-1(+)* and *mlc-2(+)* mRNAs are normal in *smg(-)* strains (A. Rushforth and P. Anderson, unpubl.).

Figure 4. RNase protection assay of *unc-54(+)* and *act-1(+)* mRNAs in *smg(+)* and *smg(-)* genetic backgrounds. The steady-state level of these mRNAs is unaffected by *smg* mutations. Similar results were obtained with *smg-6(r896)*.

Table 1. *unc-54 nonsense mutations analyzed*

<i>unc-54</i> Allele	Affected nucleotide	Wild-type codon	Mutant codon	Reference
<i>r316</i>	3325	Gln-420	UAA	Bejsovec and Anderson (1990)
<i>e1420</i>	3960	Gln-614	UAA	Bejsovec and Anderson (1990)
<i>e1419</i>	4011	Gln-631	UAG	Bejsovec and Anderson (1990)
<i>r274</i>	4401	Gly-761	UGA	this paper
<i>r308</i>	4619	Trp-833	UGA	Bejsovec and Anderson (1990)
<i>e1092</i>	5343	Gln-1075	UAA	Dibb et al. (1985)
<i>r315</i>	5907	Gln-1263	UAG	Bejsovec and Anderson (1990)
<i>r318</i>	6264	Gln-1382	UAA	Bejsovec and Anderson (1990)
<i>r310</i>	6777	Gln-1553	UAA	Bejsovec and Anderson (1990)
<i>r309</i>	6840	Gln-1574	UAG	Bejsovec and Anderson (1990)
<i>e1213</i>	7116	Gln-1666	UAA	Dibb et al. (1985)
<i>e1115</i>	7359	Gln-1747	UAA	Dibb et al. (1985)
<i>e1328</i>	7812	Gln-1863	UAA	Dibb et al. (1985)
<i>e1300</i>	7993	Gln-1906	UAG	Dibb et al. (1985)

unc-54 nonsense mutants contain reduced quantities of mRNA

mRNAs that contain nonsense mutations are unstable in many organisms (see introductory section). We tested whether the steady-state levels of *unc-54* nonsense mutant mRNAs are low and whether the levels are elevated in *smg(-)* backgrounds. We prepared total RNA from 14 different *unc-54* nonsense mutants and measured their *unc-54* mRNA levels using an RNase protection assay. The position and sequence of each tested nonsense allele are shown in Table 1. A typical RNase protection experiment, involving the amber allele *unc-54(r315)* is shown in Figure 5. The steady-state levels of the 14 nonsense mutant mRNAs are listed in Table 2 (column 2) and diagrammed in Figure 6 (closed circles).

All 14 *unc-54* nonsense mutants, including amber (UAG), ochre (UAA), and opal (UGA) alleles, have low steady-state levels of mRNA. The quantity of mRNA contained by any given mutant depends on its position within *unc-54*. Nonsense alleles located throughout most of *unc-54* (12 of 14 tested alleles) accumulate very low levels of mRNA (between 3% and 8% of wild type). For example, *e1115*, an ochre mutation located 220 codons upstream of the normal terminator, accumulates $4 \pm 0.4\%$ of the wild-type quantity of mRNA (mean $\pm$ S.D.; $n = 3$). The quantity of mRNA contained in *e1115* is typical of all nonsense mutations located farther in the 5' direction. Two nonsense mutations, *e1328* (ochre) and *e1300* (amber), accumulate significantly more mRNA. Both *e1328* and *e1300* are located near the 3' end of *unc-54*. *e1328* is 104 codons upstream of the normal translation terminator; it accumulates $13 \pm 1\%$

Figure 5. RNase protection assay of *unc-54(r315)* mRNA in *smg(+)* and *smg(-)* genetic backgrounds. *r315* is an amber (UAG) mutation at codon 1263 of *unc-54*. *r315* (lane 3) contains ~5% of the wild-type quantity of mRNA. *r315; smg(-)* strains (lanes 4–9) contain 50–112% of wild-type amounts. The negative control strain *r259* deletes *unc-54* and contains no mRNA.

of the wild-type quantity of mRNA (mean $\pm$ S.D.; $n = 3$). *e1300* is 61 codons upstream of the normal terminator; it accumulates $21 \pm 3\%$ of the wild-type quantity of mRNA (mean $\pm$ S.D.; $n = 3$). The effect of posi-

tion on mRNA abundance is reproducible using independent RNA preparations and in multiple repetitions of the same RNA sample.

unc-54 missense mutants express normal quantities of *unc-54* mRNA, despite the fact that they often accumulate little, if any, of the unstable MHC B protein (Bejsovec 1988; Bejsovec and Anderson 1988, 1990). Thus, the abundance of *unc-54* mRNA is not autoregulated by MHC B. The expression of MHC B, furthermore, is strictly gene dose dependent (Bejsovec and Anderson 1988). This confirms the absence of *unc-54* autoregulation, a phenomenon that might otherwise have explained the lowered abundance of *unc-54* nonsense mutant mRNA.

smg mutations increase the quantity of *unc-54* nonsense mutant mRNAs

We crossed each of the 14 *unc-54* nonsense mutations into all six *smg(-)* backgrounds and constructed most of the *unc-54; smg* double mutant combinations. We then quantified the amount of *unc-54* mRNA in each strain as described above. A typical RNase protection experiment, involving the amber mutation *unc-54(r315)* in both *smg(+)* and *smg(-)* backgrounds, is shown in Figure 5. The quantities of *unc-54* mRNA contained in all strains tested are listed in Table 2 (columns 3–8) and diagrammed in Figure 6 (open circles). All *unc-54*(nonsense); *smg(-)* strains contain increased amounts of mRNA. Steady state quantities ranged from 189% [for *unc-54(r316); smg-4(mal116)*] to 25% [for *unc-54(r274); smg-6(r896)*] of that found in wild type. As with *unc-54(r293)*, strains carrying *smg-1* through *smg-5* mutations contain substantially more mRNA than strains carrying *smg-6* mutations. In the following discussion and in Figure 6, we

Table 2. Steady-state abundance of *unc-54* nonsense mutant mRNAs

<i>unc-54</i> Allele	<i>smg</i> Genotype							<i>smg(-)</i> Mean $\pm$ S.D.
	<i>smg(+)</i>	<i>smg-1</i> (<i>r861</i>)	<i>smg-2</i> (<i>r863</i>)	<i>smg-3</i> (<i>r867</i>)	<i>smg-4</i> (<i>mal116</i>)	<i>smg-5</i> (<i>r860</i>)	<i>smg-6</i> (<i>r896</i>)	
<i>unc-54(+)</i>	1.00	0.94	0.92	1.08	0.89	0.94	0.83	0.95 $\pm$ 0.07
<i>r316</i>	0.09	1.79	1.77	1.64	1.89	1.49	0.26	1.72 $\pm$ 0.15
<i>e1420</i>	0.03	N.D.	N.D.	1.25	1.58	0.95	0.29	1.26 $\pm$ 0.32
<i>e1419</i>	0.05	1.21	1.24	1.15	1.17	0.96	0.54	1.15 $\pm$ 0.11
<i>r274</i>	0.03	lethal	lethal	lethal	lethal	lethal	0.25	—
<i>r308</i>	0.04	0.89	N.D.	1.01	N.D.	0.92	0.36	0.94 $\pm$ 0.06
<i>e1092</i>	0.06	1.16	1.31	N.D.	0.92	0.81	0.43	1.05 $\pm$ 0.23
<i>r315</i>	0.05	1.10	1.12	1.03	1.00	0.87	0.50	1.02 $\pm$ 0.10
<i>r318</i>	0.04	0.87	0.59	0.93	0.76	0.80	0.61	0.79 $\pm$ 0.13
<i>r310</i>	0.04	0.75	0.81	0.42	0.57	0.60	0.45	0.63 $\pm$ 0.15
<i>r309</i>	0.08	0.82	0.77	0.92	0.85	0.90	0.29	0.85 $\pm$ 0.06
<i>e1213</i>	0.05	0.55	0.77	0.76	0.41	0.72	0.48	0.64 $\pm$ 0.16
<i>e1115</i>	0.04 $\pm$ 0.004	0.65	0.85	0.71	0.72	0.77	0.45	0.74 $\pm$ 0.07
<i>e1328</i>	0.13 $\pm$ 0.01	0.59	0.57	N.D.	0.55	0.51	0.43	0.56 $\pm$ 0.03
<i>e1300</i>	0.21 $\pm$ 0.03	0.65	1.05	0.87	0.73	0.41	0.74	0.74 $\pm$ 0.24

The steady-state quantities of *unc-54* mRNA are expressed relative to wild-type strain N2. The means and standard deviations of *smg(-)* strains (last column) were calculated using only *smg-1*, *smg-2*, *smg-3*, *smg-4*, and *smg-5* measurements (see text). The *smg(+)* strains *e1115*, *e1328*, and *e1300* were determined in triplicate using two independent RNA preparations. The mean and standard deviation for these measurements are shown. (N.D.) Not determined; (lethal) the indicated double mutant is lethal and cannot be measured.

Figure 6. Steady-state abundance of *unc-54*(nonsense) mRNAs in *smg*(+) and *smg*(-) genetic backgrounds. mRNA levels are plotted relative to position of the nonsense mutation within *unc-54* mRNA. The normal *unc-54* translational terminator occurs at codon 1967. The steady-state levels of *smg*(+) and *smg*(-) mRNAs are indicated by ● and ○, respectively. The point and error bars for *smg*(-) measurements indicate the mean and standard deviation of *unc-54*; *smg-1*, *smg-2*, *smg-3*, *smg-4*, and *smg-5* double mutants considered as a group (see text). The arrows indicate the positions of *unc-54* introns (Karn et al. 1983). (A) Amber; (O) ochre; (U) UGA.

consider only the effect of *smg-1* through *smg-5* on mRNA abundance.

The quantity of mRNA contained in any specific *unc-54*(nonsense); *smg*(-) double mutant depends on the position of the nonsense mutation within the gene (see Fig. 6). *smg*(-) strains containing *unc-54*(*r316*), located nearest the mRNA 5' end, accumulate $172 \pm 15\%$ of the wild-type quantity of mRNA [mean $\pm$ s.d. for the five *smg*(-) double mutants]. *smg*(-) strains containing *unc-54*(*e1300*), located nearest the mRNA 3' end, accumulate $74 \pm 24\%$ of the wild-type quantity. Between these two extremes, mutations tend to contain intermediate amounts of mRNA. Because the error bars for many of these measurements overlap, we cannot be certain whether nonsense mutations constitute discrete groups of alleles having similar mRNA quantities or whether they have a graded effect throughout the entire length of the mRNA. It is reproducible and statistically significant, however, that *unc-54*(*r316*); *smg*(-) strains contain substantially more mRNA than wild type. We discuss this observation below.

Frameshift mutants contain reduced amounts of unc-54 mRNA; the reduction is smg- dependent

Frameshift mutations almost always cause premature translation termination, and they affect mRNA stability similar to nonsense mutations. We analyzed the effect of *smg* mutations on two *unc-54* out-of-frame deletions. *unc-54*(*r306*) is a 4-bp deletion (nucleotides 2936–2939; Bejsovec and Anderson 1990) that shifts the reading

frame and terminates at a UGA 28 codons downstream of the deletion junction. *unc-54*(*e190*) is a 401-bp deletion (nucleotides 6746–7146; Dibb et al. 1985) that shifts reading frame and terminates at a UGA stop 5 codons downstream of the deletion junction. We analyzed *r306* and *e190* mRNA in both *smg*(+) and *smg*(-) backgrounds using RNase protection assays. After normalizing the *unc-54* signals to those of *act-1*, we estimate that both *r306* and *e190* contain $\sim 5\%$ of the wild-type quantity of *unc-54* mRNA in *smg*(+) backgrounds; *r306*; *smg-2*(*r863*) and *e190*; *smg-2*(*r863*) contain $\sim 74\%$ and $\sim 94\%$, respectively, of the wild-type amount of mRNA (data not shown).

Certain unc-54 nonsense mutations are dominant in smg(-) genetic backgrounds

Although *smg* mutations increase the quantity of *unc-54* nonsense mutant mRNAs, they do not suppress any of them phenotypically. Most *unc-54*(nonsense); *smg*(-) double mutants are paralyzed; their phenotypes are identical to *unc-54*(nonsense) single mutants. These strains contain increased levels of mRNA, but that mRNA is still mutant and, when translated, fails to produce functional myosin. The phenotypes of four *unc-54* nonsense mutants, however, are affected by *smg* mutations. *e1420*, *e1419*, *r274*, and *r308* are all recessive in *smg*(+) genetic backgrounds but dominant in *smg*(-) backgrounds. All four of these nonsense mutations are located within *unc-54* at a position that predicts translation of nonsense fragment polypeptides containing most of the myosin globular head domain without an attached rod segment (see Fig. 7). A polarized light micrograph demonstrating the *smg*-dependent dominance of *unc-54*(*e1420*), a typical allele exhibiting *smg*-dependent dominance, is shown in Figure 8.

In *smg*(+) genetic backgrounds, *e1420*, *e1419*, *r274*, and *r308* are indistinguishable from each other and from all other *unc-54* null alleles. Homozygotes are paralyzed and heterozygotes are wild type. In most *smg*(-) genetic backgrounds (see below), *e1420*, *e1419*, *r274*, and *r308*

Figure 7. The location of *unc-54* *smg*-dependent dominant mutations relative to the domain structure of myosin. Of 14 tested nonsense mutations, 10 are recessive in both *smg*(+) and *smg*(-) genetic backgrounds. Four mutations, all located near the myosin head/rod junction are recessive (R) when *smg*(+) and dominant (D) when *smg*(-).

Figure 8. Polarized light micrographs of *unc-54(e1420)/+* heterozygotes in *Smg*⁺ and *Smg*[−] genetic backgrounds. (Top left) The oblique striations of wild-type body-wall muscle. (Top right) *e1420* homozygotes. *e1420* exhibits moderate-to-severe disruption of body-wall muscle ultrastructure. Its phenotype is typical of *unc-54* null alleles. (Bottom) *e1420/+* muscle in *Smg*⁺ and *Smg*[−] backgrounds. *smg-3(r867)* is recessive. The strain shown at the lower right is phenotypically *Smg*⁺, whereas the strain shown at the lower left is phenotypically *Smg*[−]. *e1419*, *r274*, and *r308* exhibit similar muscle-defective phenotypes when heterozygous in a *smg*([−]) background.

heterozygotes are muscle defective and uncoordinated. The strength of dominance varies among the four *unc-54* alleles. *r274* is the most strongly dominant. *r274/+*; *smg*([−]) heterozygotes move very slowly, and polarized light microscopy indicates that they have severely disrupted body-wall muscle ultrastructure. *r274*; *smg-1*, *smg-2*, *smg-3*, *smg-4*, or *smg-5* homozygotes (*smg* alleles *r861*, *r863*, *r867*, *ma116*, and *r860*, respectively) are inviable; they arrest development as late embryos or early larvae. The phenotypes of *r274* heterozygotes and homozygotes in a *smg*([−]) background resemble a previously described class of strongly dominant missense alleles of *unc-54* (Bejsovec and Anderson 1988). *r274*; *smg-6(r896)* and *r274*; *smg-6(r886)* mutants are severely paralyzed and grow poorly, but they are viable (*r886* double mutants grow more slowly than *r896*). *e1420*, *e1419*, and *r308* are more weakly dominant than *r274*. In most *smg*([−]) backgrounds (see below), *e1420/+*, *e1419/+*, and *r308/+* move more slowly than *smg*(⁺) heterozygotes, and polarized light microscopy indicates that they have moderately disrupted body-wall muscle ultrastructure. *e1420*; *smg*([−]), *e1419*; *smg*([−]), and *r308*; *smg*([−]) homozygotes are viable but severely paralyzed. They are

more severely paralyzed, grow more slowly, and have smaller brood sizes than the same mutations in *smg*(⁺) genetic backgrounds. We believe that the dominance of *r274*, *e1420*, *e1419*, and *r308* in *smg*([−]) backgrounds is caused by increased expression of nonsense fragment polypeptides (see Discussion).

The severity of *smg*-dependent dominance of *r274*, *e1420*, *e1419*, and *r308* also depends on the *smg* mutation involved. The tested *smg* mutations can be separated into three groups based on their effect on *unc-54(r274)* dominance. *smg-1(r861)*, *smg-2(r863)*, *smg-3(r867)*, and *smg-4(ma116)* cause *r274* to be strongly dominant. *smg-5(r860)* and *smg-6(r886)* causes *r274* to be more weakly dominant. *smg-6(r896)* does not elicit synthetic dominance at all. Although genetic analysis of the *smg* mutations indicates that they are loss-of-function alleles, we do not know whether the apparently weaker mutations *smg-5(r860)*, *smg-6(r886)*, and *smg-6(r896)* are null alleles.

Discussion

Genetic analysis of the six *C. elegans smg* genes identi-

fied them as informational suppressors (Hodgkin et al. 1989). *smg* mutations are allele-specific, but not gene-specific, suppressors of mutations affecting a variety of different genes. Informational suppressors are classically described as mutations affecting the cellular machinery that transmits information from DNA to protein (Gorini 1970). Informational suppressors affecting the translational apparatus are well known, but in theory suppressors might act at any stage in decoding genetic information. We demonstrate in this paper that *smg* mutations influence the stability of many different *unc-54* mutant mRNAs.

We isolated *smg* mutations as extragenic suppressors of *unc-54(r293)* (Hodgkin et al. 1989). *r293* deletes the *unc-54* polyadenylation signal and site, without affecting the *unc-54* translated region (Fig. 1). *r293* mRNA is unusually large (Fig. 3), presumably because of inclusion of downstream sequences at its 3' end. In *smg(+)* strains, the steady-state level of *r293* mRNA is only ~8% of that found in wild type (Fig. 2). In *smg(-)* strains, the levels are approximately normal. The effect of *smg* mutations on *r293* mRNA appears to be posttranscriptional (Fig. 4). Because *smg* mutations are reduction- or loss-of-function alleles, and because the six *smg* genes appear to act in the same biochemical pathway or process (Hodgkin et al. 1989), the most likely explanation for these observations is that a system for degrading *r293* mRNA is eliminated in *smg* mutants.

smg mutations dramatically affect the abundance of *unc-54* mRNAs that contain nonsense or frameshift mutations (Figs. 5 and 6). This effect is the strongest evidence that *smg* mutations eliminate a system of mRNA turnover. The steady-state level of mRNAs that contain premature stop codons is low in most eukaryotes; such mRNAs are rapidly degraded (see introductory section). Although we have not directly measured half-lives of any *C. elegans* mRNAs, we assume by analogy to these other organisms that the low steady-state level of *unc-54* nonsense and frameshift mutation-containing mRNAs is the result of their instability. The abundance of these mRNAs is normal or near normal in *smg(-)* backgrounds. Thus, wild-type function of the *smg* genes is necessary to degrade the messages. The effect of *C. elegans smg* genes on mRNA stability appears to be similar to that of *UPF1* and *UPF3* in the yeast *Saccharomyces cerevisiae*. *upf1⁻* and *upf3⁻* mutations selectively stabilize mRNAs that contain nonsense or frameshift mutations in many different genes without affecting the decay rates of most cellular messages (Leeds et al. 1991, 1992).

The DNA sequences of additional *smg*-suppressible mutations confirm the importance of nonsense mutations and gene rearrangements involving the 3'UTR in *smg* suppression. *tra-3(e1107)* and *tra-3(e1903)* are *smg*-suppressible amber (UAG) mutations (T. Barnes and J. Hodgkin, pers. comm.). *glp-1(q35)*, *tra-2(el209)*, and *lin-29(n546)* are *smg*-suppressible opal (UGA) mutations (Mango et al. 1991; Kuwabara et al. 1992; A. Rougvie and V. Ambros, pers. comm.). Although it is unknown precisely why suppression occurs in each case, translation

of the increased quantities of nonsense mutant mRNAs found in *smg(-)* backgrounds likely increases the amount of a functional (or partially functional) gene product. In different cases of suppression, the functional proteins might be either amino-terminal polypeptide fragments resulting from translation termination at the premature stop codon, carboxy-terminal polypeptide fragments resulting from translational reinitiation downstream of a nonsense codon, or full-length proteins resulting from mistranslation (readthrough) of a nonsense codon (Mango et al. 1991; J. Hodgkin; A. Rougvie and V. Ambros, both pers. comm.). *unc-17(p1156)*, and *unc-17(md1447)* are *smg*-suppressible deletions that remove the *unc-17* polyadenylation site (J. Rand, pers. comm.). It is likely that such mutations are suppressed in a manner similar to that of *unc-54(r293)*.

Why is *r293* mRNA unstable? *r293* deletes most of the *unc-54* 3' UTR, including the site of cleavage and polyadenylation (Fig. 1). The resulting *r293* mRNA is unusually large; its 3' UTR contains ~1.8 kb of downstream sequences that are not normally found in mature *unc-54* mRNA. Perhaps *r293* mRNA is unstable because a *cis*-acting stabilizing element normally present in the 3' UTR of *unc-54* mRNA is deleted by *r293*. In wild type, such a stabilizing element might antagonize the action of the *smg* genes. Interestingly, sequences within the *unc-54* 3' UTR are necessary for efficient expression of the gene in transgenic animals (Okkema et al. 1993). Although numerous *cis*-acting mRNA destabilizing elements have been identified (for review, see Peltz et al. 1991), *cis*-acting stabilizing elements are uncommon. One possible exception is the iron-responsive element, which is located within the 3' UTR of transferrin receptor mRNA and acts in *cis* to regulate mRNA stability in response to iron regulation (Mullner and Kuhn 1988). Alternatively, perhaps the large 3' UTR of *r293* mRNA destabilizes the message in a manner more strictly analogous to nonsense-mediated mRNA decay. Discrete sequence elements or general structural features of the *r293* 3' UTR might cause the normal *unc-54* stop codon to appear as a premature stop codon to the *smg*-encoded degradative system. Sequences downstream of a stop codon can be necessary for nonsense-mediated mRNA decay. In the human triosephosphate isomerase gene, intron sequences must be downstream of a stop codon for rapid decay (Urlaub et al. 1989; Cheng et al. 1990). In yeast, required downstream sequences may be translation reinitiation sites (Peltz et al. 1993). Perhaps the *r293* 3' UTR contains similar *cis*-acting elements that destabilize the message.

Two aspects of *unc-54* nonsense-mediated mRNA decay depend on the position of the nonsense codon within the transcript. First, nonsense mutations located near the normal *unc-54* terminator destabilize the message less than nonsense codons located farther upstream (Fig. 6). This effect is similar to that seen in other genes and organisms (Losson and Lacroute 1979; Baumann et al. 1985; Leeds et al. 1991), except that in other systems nonsense alleles near the natural terminator can have nearly normal levels of mRNA. The graded effect of *unc-*

54 nonsense mutations is unrelated to the position of *unc-54* introns, as has been observed for certain mammalian genes (Urlaub et al. 1989; Cheng et al. 1990). Either of the two models discussed above (elements in the 3' UTR that antagonize nonsense-mediated decay or downstream elements that destabilize a message) satisfactorily explains this aspect of position dependence. Second, a very different position effect is evident in *smg(-)* backgrounds. Nonsense mutations nearer the *unc-54* 5' end cause more mRNA to accumulate than mutations nearer the 3' end. *smg(-)* strains containing *unc-54(r316)*, an ochre mutation located 420 codons from the initiator methionine (21% of the translated length), accumulate $172 \pm 15\%$ of the wild-type quantity of mRNA [*smg-6* data excluded; see above]. *smg(-)* strains containing six nonsense mutations located within a similar distance (420 codons) of the normal terminator accumulate $70 \pm 16\%$ of the wild-type quantity of mRNA. Nonsense mutants between these extremes tend to have intermediate levels of mRNA (see Fig. 6). Because many of the error bars in Figure 6 overlap, we are unsure whether nonsense mutations constitute discrete groups of alleles having similar mRNA quantities or whether they have a graded effect throughout the entire length of the message.

We interpret this latter position effect to indicate that translation of *unc-54* mRNA contributes to its instability. Translation for short distances (nonsense mutations nearer the 5' end) yields a more stable message than translation for long distances (wild-type or nonsense mutations nearer the 3' end). The effect of translation on *unc-54* mRNA stability could be a generalized effect, such that elongating ribosomes have a low but significant probability of causing the mRNA to be degraded. Alternatively, *cis*-acting destabilizing elements might be present within the mRNA. Discrete exon sequences that cause a message to be unstable have been defined in a number of eukaryotic genes (Kabnick and Housman 1988; Gay et al. 1989a; Shyu et al. 1989; Herrick et al. 1990; Parker and Jacobson 1990; Shyu et al. 1991; Heaton et al. 1992; Herrick and Jacobson 1992). Such instability determinants must be translated to have their effect. Perhaps one or more such elements is present in *unc-54* mRNA downstream of *r316*. Early nonsense mutations would prevent translation of the elements and stabilize the message. Activity of such elements would not require *smg* gene function, because this phenomenon is evident in *smg(-)* strains.

Four *unc-54* nonsense mutations (*r274*, *r308*, *e1420*, and *e1419*) are recessive when *smg(+)* but dominant when *smg(-)*. Ten other tested alleles are recessive either when *smg(+)* or *smg(-)*. As heterozygotes in *smg(-)* backgrounds, the dominant alleles are muscle defective and uncoordinated. They exhibit moderate to severe disruption of body-wall muscle ultrastructure (Fig. 8). The most strongly dominant allele, *r274*, is lethal as a *smg(-)* homozygote, similar to *unc-54* missense mutations affecting the ATPase or actin-binding functions of the myosin globular head (Bejsovec and Anderson 1988,1990). The dominance of *r274*, *r308*,

e1420, and *e1419* indicates that they express mutant polypeptides that disrupt thick filament and/or sarcomere assembly. We believe that the disruptive proteins of these alleles are the amino-terminal nonsense fragments, not carboxy terminal reinitiation fragments or mistranslation (readthrough) of the nonsense codons. Reinitiation by eukaryotic ribosomes is usually inefficient (Liu et al. 1984; Peabody and Berg 1986; Kozak 1987), except in special circumstances and usually near the 5' end of a message (for review, see Hershey 1991; Kozak 1992). If mistranslation of *unc-54* nonsense codons occurred at appreciable frequency, *smg* mutations would likely suppress nonsense alleles, not render them dominant. This assumes that the full-length proteins resulting from mistranslation of nonsense codons would be functional. Most single amino acid substitutions of the myosin rod segment are functional (within limits of the genetic selection), because such mutations are not isolated among a large collection of *unc-54* loss-of-function alleles (Dibb et al. 1985; Bejsovec and Anderson 1990). None of the 14 tested *unc-54* nonsense mutations is even slightly suppressed by *smg* mutations. Thus, the most likely disruptive polypeptides are the amino-terminal nonsense fragments.

Native myosin contains two very distinct domains. An amino-terminal globular domain contains the myosin ATPase enzyme and actin and myosin light chain-binding functions. A carboxy-terminal rod segment promotes myosin-myosin alignment within thick filaments (for review, see Titus 1993). The locations of the four synthetic dominant *unc-54* nonsense mutations predict translation of nonsense fragment polypeptides containing most (but not all) of the myosin globular domain without an attached rod segment (see Fig. 7). The most strongly dominant protein (from allele *r274*) would express a nonsense fragment that contains most of the myosin globular domain but would not contain the regulatory and alkali myosin light chain-binding sites (Rayment et al. 1993) or the entire rod segment. Perhaps the *r274* toxic fragment disrupts muscle assembly by binding to filamentous actin in an unregulated fashion.

smg mutants exhibit mild morphogenetic defects and reduced brood sizes, but their growth and development is otherwise normal. What, then, is the *in vivo* role of the *smg* system of mRNA degradation? We suggest that *smg* genes function to identify and degrade aberrant mRNAs resulting from errors of transcription, mRNA processing, or mRNA transport. For example, unspliced or incompletely spliced mRNAs cannot be translated for their full length, because most introns contain stop codons in all reading frames. mRNAs that have been spliced incorrectly will often contain translational frameshifts at the incorrect spliced junctions. Frameshift mutations or stop codons introduced by transcriptional errors will cause premature translation termination. The frequencies of such transcriptional or mRNA processing errors is largely unknown, but they might be substantial (Nigro et al. 1991; Cocquerelle et al. 1992). We suggest that the *smg* system degrades these (and other) aberrant mRNAs to prevent their translation. The cell is thereby protected

from the potentially disruptive effects of nonsense fragment polypeptides. As in the case of myosin, we envision that many protein fragments are toxic. Work in progress demonstrates that among a collection of dominant visible mutations isolated in a *smg*(-) background, about half of them depend on the *smg* mutation for their dominance (B. Cali and P. Anderson, unpubl.). By degrading aberrant mRNAs, *smg* genes may "fine tune" gene expression and render certain mistakes less costly. Similar *in vivo* roles have been proposed for the yeast *UPF1* gene (He et al. 1993). Unspliced pre-mRNAs are more stable in *upf*⁻ mutants and are associated with polyosomes. This suggests that one role of *UPF1* is to degrade unspliced pre-mRNAs. An additional, or perhaps alternative, *in vivo* role for the *smg* gene system might be to mitigate the potentially deleterious effects of somatic nonsense and frameshift mutations. Such mutations might express protein fragments that would otherwise be toxic.

We propose the term "mRNA surveillance" to describe this system of nonsense-mediated mRNA decay. mRNA surveillance increases the fidelity of gene expression by eliminating incompletely translated mRNAs. Not all truncated polypeptides are disruptive to a cell, but by eliminating all nonsense mutant mRNAs the cell is protected from those that are. Although the frequency of errors for any single gene may be low, the accumulated effect of errors introduced during expression of tens of thousands of genes could be significant. Because mRNAs that contain nonsense mutations are unstable in all eukaryotes, the components of mRNA surveillance should be found in all of them.

Materials and methods

General procedures

The conditions for growth, maintenance, and genetic manipulation of *C. elegans* are described by Brenner (1974). DNA sequences of most *unc-54* mutations have been reported previously (Dibb et al. 1985; Pulak and Anderson 1988; Bejsovec and Anderson 1990). We sequenced *unc-54(r274)* as part of this study. *r274* is a G → T transversion at nucleotide 4401, resulting in a UGA stop codon (Gly-761 → UGA). The C → T transition of *unc-54(r316)* (nucleotide 3325) produces a UAA stop codon (Gln-420 → ochre, not a UAG stop codon as described previously [Bejsovec and Anderson 1990]).

Northern blots

We isolated total RNA using methods described by Ross (1976) and modified by Cummins and Anderson (1988). Our Northern blot procedure is described in Maniatis et al. (1982). RNA was glyoxylated in 6 M glyoxal, separated in a 1% agarose gel in 0.01 M NaH₂PO₄ (pH 7.0), and transferred to nitrocellulose filters. RNA was fixed to the filters by UV illumination for 2 min using a Fotodyne transilluminator. Filters were hybridized with radiolabeled probes TR#128 and pT7/T3-18-103, which are described below.

Hybridization probes

Plasmid TR#128 contains an *unc-54* genomic *Sma*I-*Kpn*I frag-

ment (nucleotides 696-3203) inserted into vector pBluescript II KS(-) (Stratagene). We linearized TR#128 with *Eco*RV and transcribed an antisense RNA probe from the T3 promoter. The probe extends from the *unc-54 Kpn*I site at nucleotide 3203 to the *Eco*RV site at nucleotide 2777. In RNase protection assays, 348 nucleotides of this 438-nucleotide probe is protected by hybridization to *unc-54* mRNA. Plasmid pT7/T3-18-103 was kindly provided by M. Krause. This plasmid contains an *act-1* genomic *Hin*CII-*Hin*FI fragment (nucleotides 1448-1665 of GenBank accession number X16796) from plasmid pCeA103 (Krause et al. 1989) inserted into vector pT7/T3-18 (BRL, Inc.). *act-1* is one of four *C. elegans* actin genes and was used as a normalization standard in our experiments. We linearized pT7/T3-18-103 with *Eco*RI and transcribed antisense RNA from the T3 promoter. In RNase protection assays, ~90 nucleotides of this 250-nucleotide probe is protected by hybridization to *act-1* mRNA.

RNase protection assays

Ribonuclease protection analysis is described by Sambrook et al. (1989). Ten micrograms of total RNA was tested in each assay. The RNA was dissolved in 30 µl of hybridization buffer containing an excess of both TR#128 and pT7/T3-18-103 hybridization probes (5 × 10⁵ cpm of each probe). Hybridization mixtures were incubated at 50°C for 12 hr and then digested with a mixture of both RNase T1 and RNase A. The resultant samples were treated with proteinase K, extracted with phenol/chloroform, and precipitated with ethanol, using 20 µg of yeast carrier tRNA. The precipitated RNase-protected products were resuspended in 10 µl of 80% formamide loading buffer, heated for 5 min at 95°C, transferred to ice, and analyzed by electrophoresis through a 6% polyacrylamide/7 M urea gel. This gel was transferred to 3MM paper and dried under vacuum. The quantities of protected probe were measured on a Betascope model 603 Blot Analyzer (Betagen Corp.). To control for lane-to-lane variation in the quantity of *C. elegans* RNA, the amounts of *unc-54* protected fragments were normalized to those of *act-1*. Control experiments demonstrated that the *unc-54* and *act-1* signals were linear with the amount of added RNA, up to 40 µg of total RNA.

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