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(+)/ 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]

Received July 9, 1993, revised version accepted August 6, 1993.

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 [Krujier 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 HSF70 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], MATo1 [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 et al. 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-
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; Scallon et al. 1987; Schneuwly et al. 1989; Urlaub et al. 1989; Washburn and O’Toasa 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 genespecific, 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
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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-

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(+)/Df(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 RNA 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.].

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 diagramed 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, c1115, an ochre mutation located 220 codons upstream of the normal terminator, accumulates 4 ± 0.4% of the wild-type quantity of mRNA [mean ± s.d., n = 3]. The quantity of mRNA contained in c1115 is typical of all nonsense mutations located farther in the 5' direction. Two nonsense mutations, c1328 (ochre) and c1300 (amber), accumulate significantly more mRNA. Both c1328 and c1300 are located near the 3' end of unc-54. c1328 is 104 codons upstream of the normal translation terminator, it accumulates 13 ± 1%

Table 1. unc-54 nonsense mutations analyzed

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

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).
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mRNA degradation and stag gene
tion on mRNA abundance is reproducible using independ-
ent 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 accumu-
late little, if any, of the unstable MHC B protein (Bej-
sovec 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 autoregula-
tion, a phenomenon that might otherwise have ex-
plained the lowered abundance of unc-54 nonsense mu-
tant 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
stag(+) and stag(−) 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 diagramed
in Figure 6 (open circles). All unc-54(nonsense); stag(−)
strains contain increased amounts of mRNA. Steady
state quantities ranged from 189% [for unc-54(r316);
smg-4(mal16)] 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 stag-5 mutations contain sub-
stantially more mRNA than strains carrying smg-6 mu-
tations. In the following discussion and in Figure 6, we

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

| unc-54 Allele | smg(+) | smg-1 (r861) | smg-2 (r863) | smg-3 (r867) | smg-4 (ma116) | smg-5 (r860) | smg-6 (r896) | smg(−) Mean ± s.d. |
|---------------|--------|-------------|-------------|-------------|--------------|-------------|-------------|-------------------|
| unc-54(+)     | 1.00   | 0.94        | 0.92        | 1.08        | 0.89         | 0.94        | 0.83        | 0.95 ± 0.07       |
| r316          | 0.09   | 1.79        | 1.77        | 1.64        | 1.89         | 1.49        | 0.26        | 1.72 ± 0.15       |
| e1420         | 0.03   | N.D.        | N.D.        | 1.25        | 1.58         | 0.95        | 0.29        | 1.26 ± 0.32       |
| e1419         | 0.05   | 1.21        | 1.24        | 1.15        | 1.17         | 0.96        | 0.54        | 1.15 ± 0.11       |
| r274          | 0.03   | lethal      | lethal      | lethal      | lethal       | lethal      | lethal       | —                 |
| r308          | 0.04   | 0.89        | N.D.        | 1.01        | N.D.         | 0.92        | 0.36        | 0.94 ± 0.06       |
| e1092         | 0.06   | 1.16        | 1.31        | N.D.        | 0.92         | 0.81        | 0.43        | 1.05 ± 0.23       |
| r315          | 0.05   | 1.10        | 1.12        | 1.03        | 1.00         | 0.87        | 0.50        | 1.02 ± 0.10       |
| r318          | 0.04   | 0.87        | 0.59        | 0.93        | 0.76         | 0.80        | 0.61        | 0.79 ± 0.13       |
| r310          | 0.04   | 0.75        | 0.81        | 0.42        | 0.57         | 0.60        | 0.45        | 0.63 ± 0.15       |
| r309          | 0.08   | 0.82        | 0.77        | 0.92        | 0.85         | 0.90        | 0.29        | 0.85 ± 0.06       |
| e1213         | 0.05   | 0.55        | 0.77        | 0.76        | 0.41         | 0.72        | 0.48        | 0.64 ± 0.16       |
| e1115         | 0.04 ± 0.004 | 0.65 | 0.85 | 0.71 | 0.72 | 0.77 | 0.45 | 0.74 ± 0.07 |
| e1328         | 0.13 ± 0.01 | 0.59 | 0.57 | N.D. | 0.55 | 0.51 | 0.43 | 0.56 ± 0.03 |
| e1300         | 0.21 ± 0.03 | 0.65 | 1.05 | 0.87 | 0.73 | 0.41 | 0.74 | 0.74 ± 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.
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 ~5% of the wild-type quantity of unc-54 mRNA in smg(+) backgrounds; r306; smg-2(r863) and e190; smg-2(r863) contain ~74% and ~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
heterozygotes are muscle defective and uncoordinated. The strength of dominance varies among the four \textit{unc-54} alleles. \textit{r274} is the most strongly dominant. \textit{r274}/+; \textit{smg}(−) heterozygotes move very slowly, and polarized light microscopy indicates that they have severely disrupted body-wall muscle ultrastructure. \textit{r274}; \textit{smg-1}, \textit{smg-2}, \textit{smg-3}, or \textit{smg-5} homoyzogotes (\textit{smg} alleles \textit{r861}, \textit{r863}, \textit{r867}, \textit{ma116}, and \textit{r860}, respectively) are inviable; they arrest development as late embryos or early larvae. The phenotypes of \textit{r274} heterozygotes and homozygotes in a \textit{smg}(−) background resemble a previously described class of strongly dominant missense alleles of \textit{unc-54} (Bejsovec and Anderson 1988). \textit{r274}; \textit{smg-6(r896)} and \textit{r274}; \textit{smg-6(r886)} mutants are severely paralyzed and grow poorly, but they are viable (\textit{r886} double mutants grow more slowly than \textit{r896}). \textit{e1420}, \textit{e1419}, and \textit{r308} are more weakly dominant than \textit{r274}. In most \textit{smg}(−) backgrounds (see below), \textit{e1420}/+, \textit{e1419}/+, and \textit{r308}/+ move more slowly than \textit{smg}(+) heterozygotes, and polarized light microscopy indicates that they have moderately disrupted body-wall muscle ultrastructure. \textit{e1420}; \textit{smg}(−), \textit{e1419}, \textit{smg}(−), and \textit{r308}; \textit{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 \textit{smg}(+) genetic backgrounds. We believe that the dominance of \textit{r274}, \textit{e1420}, \textit{e1419}, and \textit{r308} in \textit{smg}(−) backgrounds is caused by increased expression of nonsense fragment polypeptides (see Discussion).

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

**Discussion**

Genetic analysis of the six \textit{C. elegans smg} genes identi-
fied them as informational suppressors (Hodgkin et al. 1989). smg mutations are allcdc-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 (Corini 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(tr293) [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(q35j), tra-2(e1209), and lin-29(n546) are smg-suppressible opal [UGA] mutations [Mango et al. 1991; Kuwahara et al. 1992, A. Rougivie 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. Rougivie 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(tr293).

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 [Mulliner 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(fr316), an ochre mutation located 420 codons from the initiator methionine [21% of the translated length], accumulate 172 ± 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 ± 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 [Kaback 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 [Raymond 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; Coquerelle 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 polysomes. 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 mutants 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 Smal–KpnI frag-

ment [nucleotides 696–3203] inserted into vector pBluescript II KS(–) [Stratagene]. We linearized TR#128 with EcoRV and transcribed an antisense RNA probe from the T3 promoter. The probe extends from the unc-54 KpnI site at nucleotide 3203 to the EcoRV 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 HinCII–Hinfl fragment [nucleotides 1448–1665 of GenBank accession number X16796] from plasmid pCeA103 [Krause et al. 1989] inserted into vector pT7/T3-18 [BLR, 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 EcoRI 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 × 10^5 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.

Acknowledgments

We thank Kirk Anders, Brian Cali, Sioux Christensen, Mike Krause, Andy Papp, and Kevin Hill for their generous gifts of strains and clones, Marv Wickens and Andrea Bulger for technical advice and reagents for RNase protection, Alice Rushforth for polarized light micrographs, and Rolf Samuels, Alice Rushforth, Kirk Anders, and Brian Cali for help with the manuscript. This work was supported by National Institutes of Health research grant GM41807 and by an institutional training award from the Lucille B. Markey Charitable Trust.

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*Genes Dev.* 1993, 7:
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