

# *smg* mutants affect the expression of alternatively spliced SR protein mRNAs in *Caenorhabditis elegans*

(alternative pre-mRNA splicing/alternative stop codons/RNA stability)

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**ABSTRACT** The expression of alternatively spliced mRNAs from genes is an ubiquitous phenomenon in metazoa. A screen for trans-acting factors that alter the expression of alternatively spliced mRNAs reveals that the *smg* genes of *Caenorhabditis elegans* participate in this process. *smg* genes have been proposed to function in degradation of nonsense mutant mRNAs. Here we show that *smg* genes affect normal gene expression by modulating the levels of alternatively spliced SRp20 and SRp30b mRNAs. These SR genes contain alternatively spliced exons that introduce upstream stop codons. The effect of *smg* genes on SR transcripts is specific, because the gene encoding the catalytic subunit of the cAMP-dependent protein kinase, which also contains an alternatively spliced exon that introduces upstream stop codon, is not effected in a *smg* background. These results suggest that the levels of alternatively spliced mRNAs may, in part, be regulated by alternative mRNA stability.

In multicellular organisms many genes express developmentally regulated or tissue-specific alternative messenger RNAs. Through the use of different splice sites, alternative pre-mRNA splicing can dramatically alter the coding capacity of a gene (1). Many proteins have been identified as alternative splicing factors, and cis-acting elements are important for splice site selection (2–4). Though modulation of splicing is required for the synthesis of alternatively spliced mRNAs, differential stability of the resulting mRNAs could determine the level of expression of any particular alternative message.

Regulation of mRNA stability is thought to play a critical role in a variety of processes, including development (5), growth and differentiation (6), and cytoskeleton dynamics (7). Untranslated sequences as well as sequences within coding regions are necessary and/or sufficient for proper mRNA turnover (8–12). Trans-acting factors have been identified that positively or negatively regulate mRNA degradation (8, 13). However, genetic description of trans-acting factors involved in metazoan mRNA turnover is limited to the *smg* genes of *Caenorhabditis elegans*. The *smg* mutants (suppressor with morphogenetic effects on genitalia) exhibit allele-specific suppression of certain nonsense mutations (14). Molecular analysis suggested that some *unc-54* alleles containing premature stop codons exhibit reduced mRNA expression. This reduced expression could be restored by mutations in any of the six *smg* genes (15). For this reason it has been proposed that the *smg* genes participate in the degradation of mutant mRNAs.

We present results of a genetic screen designed to identify factors involved in alternative pre-mRNA processing. Using a method analogous to yeast screens for temperature-sensitive pre-mRNA splicing mutants (16, 17), we screened mRNA

from temperature-sensitive embryo and early larval lethal worm mutants by Northern blotting with alternative exon probes. Through this analysis we found that *smg* mutants affect the expression of alternatively spliced mRNAs. These results indicate that, in addition to their role in degradation of mutant mRNAs, *smg* genes may play a role in the expression of wild-type genes.

## MATERIALS AND METHODS

***C. elegans* Culture.** Worm handling and husbandry were described previously (18). The strains used in these experiments were derived from N2 strain Bristol (18). The *g43* allele of *emb-14* was described previously (19). *smg* mutants were kindly provided by the *Caenorhabditis* Genetics Center (University of Minnesota, St. Paul) and the laboratory of Phil Anderson (University of Wisconsin, Madison).

**RNA Isolation and Analysis.** Mixed stage worms were washed in M9 buffer and floated on 30% sucrose (20). Embryos were collected by hypochlorite treatment of gravid adults (20). RNA isolation (21), poly(A)<sup>+</sup> mRNA enrichment (22), Northern blotting (22), and reverse transcription-PCR (RT-PCR) (23) were described previously. Signals were quantitated on a Molecular Dynamics PhosphorImager. Oligonucleotides used for SRp30b RT-PCR are 105-GTATGATC-GACCATC and 110-CACGACGTACCGGTG.

**Cloning of SRp20 and 30b Genes and Generation of CeCat and Actin Hybridization Probe.** cDNAs encoding *C. elegans* SR proteins (SRp20 and SRp30b) were identified in expression sequence tag libraries by homology with mammalian SR cDNAs (24). These cDNAs were used to isolate genomic fragments containing these genes from a genomic library (Stratagene). DNA sequences of the relevant DNAs appear in GenBank under accession nos. U23484 and U41007. Chromosome mapping of SR genes was done by Southern hybridization to filters of cloned genomic DNAs provided by Alan Coulson (Sanger Center, Cambridge, U.K.). A hybridization probe for both isoforms of CeCat was made by random-priming a fragment of a CeCat cDNA that contains the alternative exon and the two flanking common exons. The following oligonucleotides were used for this work: CR-63-GACTTGCTGAA-GAATTTGCTC and CR64-GTGCCCGAGATACGAA-GCGG (25).

## RESULTS

To better understand the mechanisms that affect alternative mRNA expression we screened a collection of temperature-sensitive *C. elegans* strains for mutants with altered expression of alternatively spliced mRNAs. As test genes we chose two

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This paper was submitted directly (Track II) to the *Proceedings* office. Abbreviations: RT-PCR, reverse transcription-PCR; RRM, RNA recognition motifs.

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genes that encode SR proteins (SRp20 and SRp30b) and the gene that encodes the catalytic subunit of the cAMP-dependent protein kinase (CeCat) (25). Each is subject to alternative pre-mRNA splicing of a single exon bearing an in-frame stop codon (Fig. 1). We were able to obtain probes to *C. elegans* SR protein genes because the primary sequence of SR proteins is highly conserved in the animal kingdom (24). For example, human and *C. elegans* SRp20 are more related to each other than *C. elegans* SRp20 is to *C. elegans* SRp30b. cDNAs encoding *C. elegans* SRp20 and SRp30b were identified in expression sequence tag databases (26, 27). The cDNAs were used to isolate genomic clones and to determine that SRp20 is located on chromosome IV and SRp30b is located on chromosome II.

SR proteins are essential pre-mRNA splicing factors that contain amino terminal RNA recognition motifs (RRM) and a carboxy terminal domain composed of alternating serine and arginine (SR). In mammals, SRp30a pre-mRNA is alternatively spliced, and alternative stop codons are used to encode proteins containing either the RRM domain alone or the RRM and an SR domain (28). These proteins are expressed *in vivo* and have distinct functions (J. L. Manley, personal communication; ref. 29). The organization of exons and isolation of cDNAs suggest that pre-mRNAs from *C. elegans* SRp20 and SRp30b genes are alternatively spliced to encode similar protein isoforms (Fig. 1).

In wild-type animals the exon-skipped mRNAs for SRp20 and SRp30b are more abundant than the alternative exon-containing mRNAs. However, mutant analysis revealed that the levels of these alternatively spliced mRNAs may be subject to regulation. Northern blots probed with cDNAs containing the alternative exon shows that one of the temperature-sensitive mutants we screened, *emb-14* (*g43*) (19), contains much higher levels of the exon-included SR mRNAs than wild-type animals (Fig. 2*A* and *B*, lanes 1 and 2). This result also is supported by RT-PCR of mRNA from *g43* and wild-type animals using oligonucleotides that allow for detection of both

SRp30b messages (Fig. 3, lanes 1 and 2). The sizes of the resulting RT-PCR products are consistent with the DNA sequence of SRp30b alternative cDNAs. The results indicate that the exon-included SRp30b mRNA represents approximately 0.6% of the total SRp30b mRNA in wild-type and 15% in *g43* animals.

Several lines of evidence suggest that *g43* contains an allele of a *smg* gene. First, *g43* worms have protruding vulvae and abnormal male copulatory structures, phenotypes exhibited by all *smg* mutants (14). Second, *g43* suppresses the paralyzed phenotype of *unc-54* (*r293*) (A. K. Hopper, personal communication), another phenotype common to all *smg* mutants (14). Third, *g43* maps to chromosome I near the *smg-1* and *smg-5* genes (19, 14). The fact that alleles of *smg* genes all have very similar phenotypes led us to test whether the molecular SR phenotype was a general *smg* phenomenon. Northern blot analysis indicates that representative alleles of six *smg* mutants express higher levels of the alternatively spliced messages to the same extent as *g43* (Fig. 2*A* and *B*). The *smg-6* allele shows a much weaker effect, which is consistent with its weaker effect in other *smg* assays (15). RT-PCR of mRNA isolated from *smg-1* mutant animals using oligonucleotides that detect both SRp30b mRNAs also supports the conclusion that *g43* contains an allele of a *smg* gene (Fig. 3, lane 3).

To determine whether the presence of an alternative stop codon containing exon in an mRNA always reveals a *smg*-dependent effect we assayed the expression of the catalytic subunit of the cAMP-dependent protein kinase (CeCat). Like SR genes the CeCat gene expresses two messages, one of which contains an alternative exon that introduces an alternative stop codon (25). Northern blot analysis shows that the relative ratio of the two alternative CeCat mRNAs does not vary between wild-type and a *smg-1* mutant (Fig. 4). Indistinguishable results were obtained for *g43* (data not shown). The failure of *smg* to affect the level of these alternate mRNAs indicates that *smg* acts on a specific subset of mRNAs.

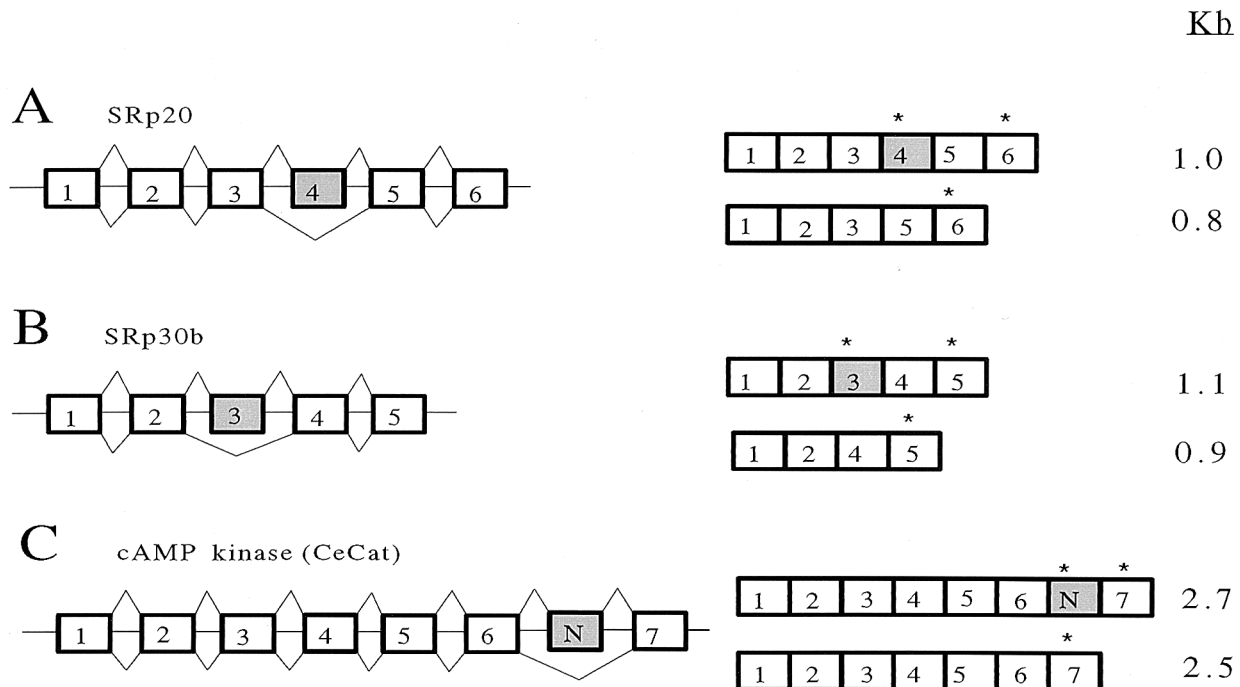


FIG. 1. SR pre-mRNAs are alternatively spliced. Schematic representation of the genomic organization, alternative splicing, and message expression from (A) SRp20, (B) SRp30b, and (C) catalytic subunit of the cAMP-dependent protein kinase (CeCat) genes. (Left) Gene structures. (Right) mRNAs. Exons are boxes and introns are lines. The sizes of the exons and introns are not drawn to scale. \* indicate positions of alternative stop codons. The RRM and SR domain of SRp20 are encoded by exons 1–3 and 5–6, respectively. The RRM and SR domain of SRp30b are encoded by exons 1–2 and 3–4, respectively.

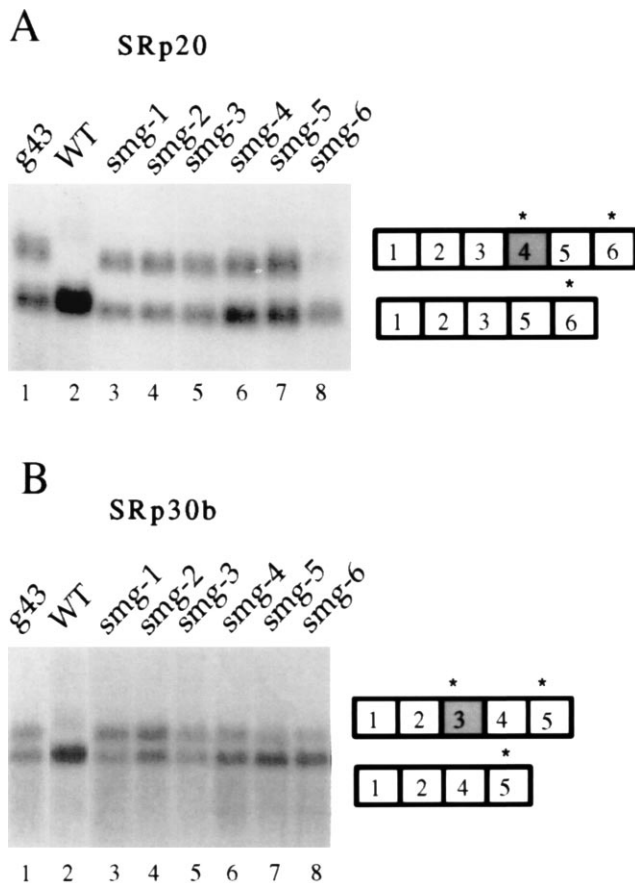


FIG. 2. Expression of SRp20 and SRp30b mRNAs in g43, wild-type, and *smg* mutant embryos. Total RNA was probed with cDNAs from the (A) *SRp20* and (B) *SRp30b* genes. Lane 1: *emb-14* (g43). Lane 2: Wild type (N2). Lane 3: *smg-1* (r861). Lane 4: *smg-2* (e2008). Lane 5: *smg-3* (ma117). Lane 6: *smg-4* (ma116). Lane 7: *smg-5* (r860). Lane 8: *smg-6* (r896). (Left) Northern blots. (Right) mRNAs.

DISCUSSION

We report that mutations in *smg* genes have dramatic and specific effects on the level of naturally occurring alternatively spliced mRNAs from the SRp20 and SRp30b genes. Most prominent is the increase in abundance of the SR mRNAs that contain alternative exons in *smg* mutants. Though we find a slight decrease in the level of the exon-skipped SRp30b and

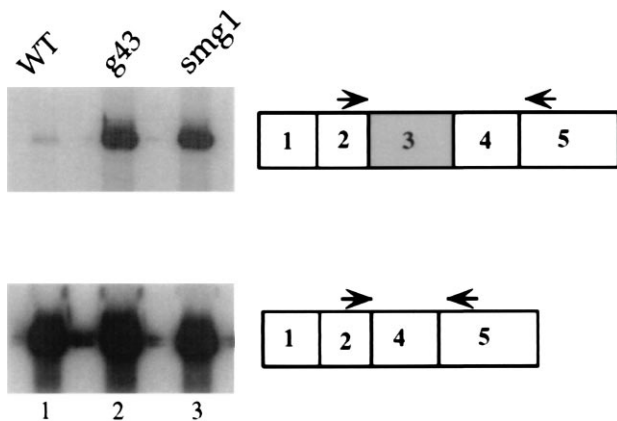


FIG. 3. RT-PCR detection of alternatively spliced SRp30b mRNAs in wild-type and mutant animals. Regions of SRp30b were amplified by PCR after reverse transcription of total mRNA from N2 (lane 1), g43 (lane 2), and *smg-1* (r861) (lane 3), using the primers designated by arrows.

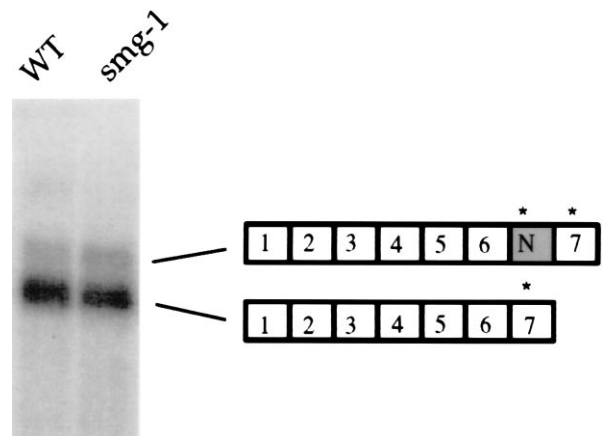


FIG. 4. cAMP-dependent protein kinase catalytic subunit (CeCat) expression is the same in wild-type and *smg-1* animals. Poly(A)<sup>+</sup> RNA was probed with a fragment of the *CeCat* gene. (Right) The alternatively spliced mRNAs. \* indicate alternative stop codons.

SR20 alternative mRNA in *smg* mutants, the absolute amount of mRNA remains approximately constant (less than a 2-fold change). These findings indicate that in wild-type nematodes *smg* activity may be involved in regulating the levels of the exon-included SRp20 and SRp30b mRNAs.

Previously *smg* genes were shown to have effects only on mutant genes. The finding that *smg* can effect the regulation of expression of wild-type genes raises the possibility that *smg* gene products play a role in processes in which RNA turnover is likely to be important, such as cell cycle progression and degradation of maternal mRNAs during early embryonic development. Because *smg* mutants affect the levels of SR protein mRNAs and because SR proteins participate widely in regulating alternative pre-mRNA splicing (30, 31), *smg* activity could have additional indirect effects on gene expression.

The alternative exons in SRp20, SRp30b, and CeCat all introduce upstream stop codons. Because this is a naturally occurring event we refer to it as an alternative stop codon and not a premature stop codon, a term used to describe nonsense mutations. The levels of alternative stop codon-containing mRNAs are known to be independently regulated and important for such processes as cell adhesion (32) and erythropoiesis (33). That *smg* genes affect the levels of exon-included alternative stop codon-containing mRNAs from SR genes, but not from CeCat, suggests either that some mRNAs are subject to regulation at the level of stability and others are not or that additional *smg*-independent degradation mechanisms exist to regulate the levels of alternative stop codon-containing mRNAs.

Though *smg* gene products may effect splicing, previous work on *unc-54* (15) favors the possibility that they effect turnover. We propose that significant amounts of both alternatively spliced SRp20 and SRp30b mRNAs are produced and *smg* activity destabilizes the exon-included mRNA. This would account for the low level of the exon-included message in wild-type animals and its increase in *smg* mutants. This work raises the possibility that independent regulation of the stability of alternatively spliced mRNAs may contribute to alternative pre-mRNA processing.

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