

Note

Smg1* Nonsense Mutations Do Not Abolish Nonsense-Mediated mRNA Decay in *Drosophila melanogaster

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ABSTRACT

Smg1 is a key component of nonsense-mediated decay (NMD) in *Caenorhabditis elegans* and mammals. Here we report that two nonsense alleles of the ortholog of *Smg1* do not affect NMD in *Drosophila melanogaster*.

NONSENSE-MEDIATED decay (NMD) is an mRNA surveillance mechanism whereby transcripts with premature termination codons (PTC) are degraded (PULAK and ANDERSON 1993; WILUSZ *et al.* 2001; MAQUAT 2002). The key genes of NMD in higher eukaryotes were originally defined genetically in three unrelated suppressor screens of *Caenorhabditis elegans*, which uncovered overlapping subsets of seven genes (*Smg1–7*) (HODGKIN *et al.* 1989). *Smg* mutations can act as suppressors by allowing transcripts with PTCs to be translated into functional, albeit truncated, proteins. They can also make recessive mutants dominant by allowing the production of truncated dominant-negative proteins whose transcripts would normally be degraded (CALI and ANDERSON 1998). Orthologs to all *Smg* genes except *Smg7* have been identified in *Drosophila melanogaster* (GATFIELD *et al.* 2003), although mutations have not previously been documented.

Here we report that two nonsense mutations in the *Drosophila* ortholog of *Smg1* (CG32473) do not affect NMD. Thus *Smg1* does not have a role in NMD in *Drosophila* or its role is redundant in at least some tissues of the adult fly. The mutants were recovered from a screen for cyromazine resistance in flies exposed to ethyl methanesulfonate mutagenesis (ADCOCK *et al.* 1993; DABORN *et al.* 2000). The nonsense mutations were identified by positional cloning of two recessive alleles that failed to complement (Z. CHEN, C. ROBIN and P. BATTERHAM, unpublished results) and would yield proteins truncated at 70% and 22% of the length

of the predicted wild-type protein (Figure 1). CG32743 encodes a phosphatidylinositol kinase-like kinase (PIKK) and has been annotated as the ortholog of *Smg1* from worms and mammals (FlyBase, <http://www.flybase.org>; PULAK and ANDERSON 1993; YAMASHITA *et al.* 2001; GATFIELD *et al.* 2003). There is only a low level of amino acid sequence identity between CG32743 and human *Smg1* (23%) and *C. elegans* *Smg1* (17%), but it is greater than the level of identity between the human and *C. elegans* orthologs (16%) and the argument of orthology is supported by phylogenetic analyses of (i) complete sequences of all *D. melanogaster*, human, and *C. elegans* PIKKs; (ii) the individual kinase, FAT, and FATC domains; and (iii) regions of similarity outside these domains (not shown). Furthermore, there is no evidence for any other *Smg1*-like sequence in the *D. melanogaster* genome or for any of the other *Drosophila* species genome sequences currently available.

Microarray analysis: We set out to test whether the *dSmg1* mutations modified the steady-state levels of other transcripts. Microarrays containing 5849 non-redundant cDNA clones of DGC Release 1.0 were used to compare the transcriptomes of *dSmg1^{rst1}* flies and *w¹¹¹⁸* flies. We performed two replicates of a dye swap experiment (four arrays in total). The overall correlation coefficient between experiments was 0.8 with 72 genes upregulated and 40 genes downregulated in *dSmg1^{rst1}* when a B-statistic value of 1 was used as a cutoff (LONNSTEDT and SPEED 2002). To address whether the differentially regulated transcripts were a result of disruption in NMD, we examined transcripts that would be degraded by NMD if they were in mammals (NAGY and MAQUAT 1998). Specifically, a bioinformatics analysis of the *Drosophila* genome uncovered 365 genes with

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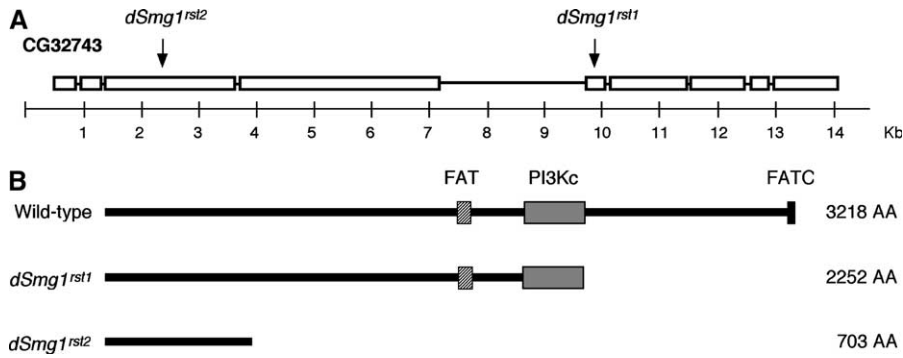


FIGURE 1.—(A) The genomic structure of the *dSmg1* gene, showing the position of the nonsense mutations in *dSmg1^{rst1}* (codon 2253 AAA → TAA) and *dSmg1^{rst12}* (codon 704 AAA → TAA) alleles. (B) The predicted ORFs of wild-type and mutant *dSmg1*. The three characteristic domains of PIKKs—FAT, PI3Kc, and FATC—are shown (BOSOTTI *et al.* 2000).

transcripts that had termination codons located >50 bp upstream of the last exon/exon junction (S. PROCHNICK and N. HARRIS, personal communication). Although the definition of a PTC is not known in *Drosophila*, we may still expect to have an overrepresentation of PTC transcripts in this subset. Of these 365 genes, 213 were represented on the microarray, but only 8 of these were considered differentially regulated in *Smg1^{rst1}* vs. wild-type flies (*i.e.*, B-statistic >1). Thus the distribution of these 213 PTC-containing genes was not significantly different from that of all other genes ($\chi^2 = 3.4$, $P > 0.5$).

PTC-bearing *Adhⁿ⁴* transcripts not affected by *dSmg1^{rst1}* or *dSmg1^{rst12}*: To test more directly the role CG32743 plays in NMD in *Drosophila*, we measured the effect that the *dSmg1^{rst1}* and *dSmg1^{rst12}* mutations had on the abundance of specific transcripts thought to be

degraded by NMD. The *Adhⁿ⁴* allele contains a premature stop codon located 258 bp upstream from the boundary between exon 3 and exon 4 and Northern data show that it has 7% of the steady-state mRNA of the wild-type allele (BROGNA 1999), presumably due to the action of NMD. *Adhⁿ⁴* males were crossed with *dSmg1^{rst1}* females and the *Adh* mRNA levels of F₁ offspring were measured. If CG32743 had a role in NMD, then we might expect that *dSmg1^{rst1}* flies would have impaired NMD and a greater steady-state abundance of PTC transcripts. We compared the ratio of *Adhⁿ⁴* transcripts to *Adh⁺* transcripts in flies that were heterozygous for these *Adh* alleles and either hemizygous for *dSmg1^{rst1}* (males) or heterozygous for *dSmg1^{rst1}* (females). The relative abundance of the *Adh* transcripts was determined by sequencing cDNA preparations and assessed

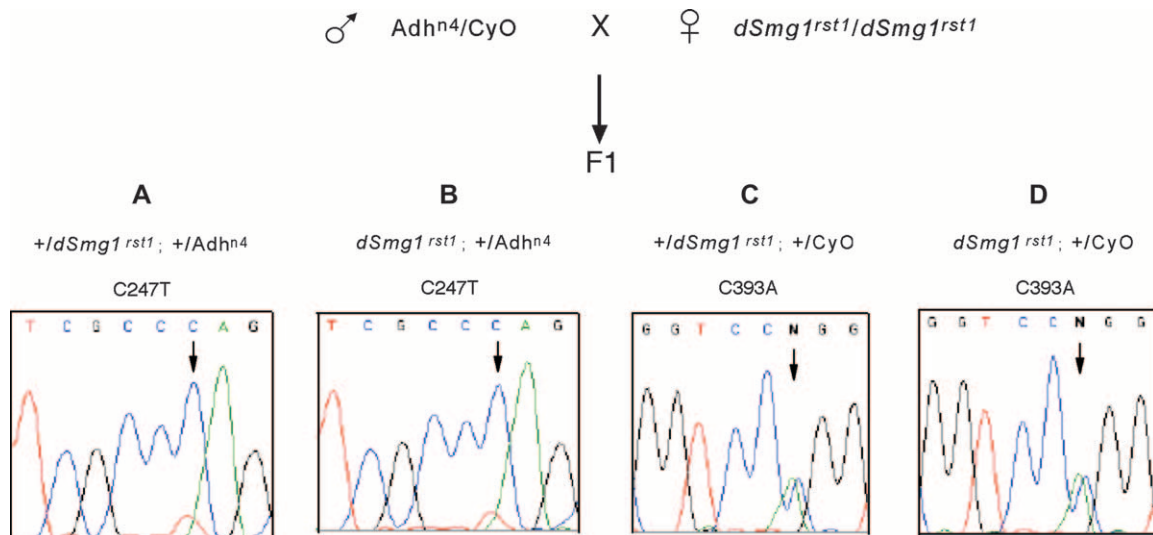


FIGURE 2.—Genetic crosses between flies harboring the *Adhⁿ⁴* allele (BL-2240 and BL-3410), which has a PTC and is degraded by NMD, and flies carrying *Smg1^{rst1}*. F₁ males do not carry a wild-type *Smg1* allele (because *Smg1* is on the X chromosome) whereas F₁ females do. (A) Sequence trace of *Adh* cDNA extracted from females heterozygous for *Smg1⁺* and *Adhⁿ⁴* shows that the *Adhⁿ⁴* transcript (T247) is barely detectable, consistent with NMD activity. (B) Sequence trace of *Adh* cDNA extracted from males hemizygous for *Smg1^{rst1}* and heterozygous for *Adhⁿ⁴* also shows that the *Adhⁿ⁴* transcript appears to be degraded by NMD. (C and D) Sequence traces of *Adh* cDNA extracted from progeny classes that do not contain *Adh*-PTC but do have a SNP between *Adh* alleles (C393A), demonstrating that sequencing is quantitative. Total RNA was isolated with the RNeasy mini kit (QIAGEN, Chatsworth, CA). RT-PCR for *Adhⁿ⁴* was performed using the Access RT-PCR System (Promega, Madison, WI). The products of the RT-PCR were directly sequenced with BigDye Terminator V3.1 (Applied Biosystems, Foster City, CA).

by examining chromatograms, as each of the *Adh* alleles under investigation carried specific SNPs. No difference could be seen in the ratio of *Adhⁿ⁴*/*Adh⁺* transcripts between males and females. In both cases, the *Adhⁿ⁴* peaks were much smaller than the wild-type *Adh⁺* (Figure 2), even though they are equally abundant in sequence of genomic DNA (not shown). These results were also observed in crosses using *dSmg^{rst2}* and using stocks containing *Adhⁿ⁴* in different genetic backgrounds. In 16 experiments on four independent crosses, each with two independent cDNA preps, the chromatogram peak height of the *Adhⁿ⁴* allele ranged from undetectable to ~10% of the wild-type allele regardless of the *dSmg1* allele.

Quantitative PCR of *Adh^{fn6}* in *dSMG1^{rst2}* vs. wild-type flies: To confirm these results, we investigated the effect of the *dSmg^{rst2}* mutation on a different *Adh*-PTC allele using real-time quantitative PCR. The *Adh^{fn6}* allele has a mutation with a sequence repeat and deletion that prevents the splicing of the first intron and leads to a premature stop codon (BENYAJATI *et al.* 1983). As in *Adhⁿ⁴* flies, the level of *Adh^{fn6}* mRNA is drastically reduced (BROGNA 1999). We created a strain (*Adh^{fn6}*; *dSmg1^{rst2}*) in which homozygous *dSmg1^{rst2}* was placed into a homozygous *Adh^{fn6}* background. Quantitative real-time RT-PCR showed that the level of *Adh^{fn6}* mRNA is only 6.2% that of wild-type *w¹¹¹⁸*, consistent with previous reports. However, the message level of the *Adh^{fn6}* allele was not elevated as a consequence of *dSmg1^{rst2}*. The transcript expressed in *Adh^{fn6}*; *dSmg1^{rst2}* flies is 6.9% of *w¹¹¹⁸* and not significantly different from *Adh^{fn6}* ($P > 0.05$) (Figure 3). We conclude from the above results that *dSmg1^{rst1}* and *dSmg1^{rst2}* do not abolish NMD even though they almost certainly knock out the ortholog of one of the key players in *C. elegans* and human NMD.

Discrepancy with dsRNAi in S2 cells: These results are in contrast with the work of GATFIELD *et al.* (2003) in which reporter genes with PTCs were transfected into S2 cell lines that were then treated with double-stranded RNA interference (dsRNAi) directed against the *Drosophila* orthologs of *Smg* genes. Two types of reporter genes were used in these experiments: *Adh* harboring exactly the same mutation that we used and a GFP transgene containing a PTC. Northern blots were used to determine whether RNAi against the *Smg* genes increases the abundance of PTC-containing transcripts. While the RNAi against *Smg2*, -3, -4, -5, and -6 appears to increase the abundance of PTC-containing reporter transcripts 4.6- to 6.9-fold, the effect on RNAi against *Smg1* is smaller (2.8-fold) and perhaps less convincing (MAQUAT 2004). However, it raises the possibility that S2 cells rely more heavily on *Smg1*'s role in NMD than adult male flies do. For instance, there may be a level of redundancy in adult flies caused by some other PIKK fulfilling *Smg1*'s role. Interestingly, *nanos* PTC transcripts have been shown to be targets for NMD in early embryos but not in later development (MEYER and

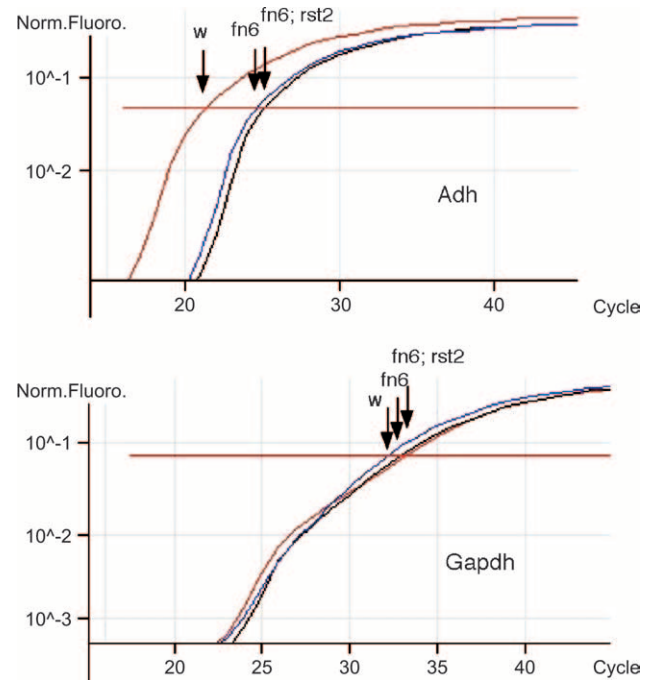


FIGURE 3.—Flies containing the *dSmg1^{rst2}* mutant do not have impaired nonsense-mediated decay of *Adh^{fn6}* null allele transcripts. Real-time quantification of *Adh* and *Gapdh* transcripts using dual-labeled fluorogenic gene probes (*Adh* probe 6-FAM-CTGGCGAAACTGGCCCCATTATAMRA, *Gapdh* probe TET-CCGCTTGGTTCTCCGCGCC-BHQ) in *w¹¹¹⁸*, *Adh^{fn6}*, and *dSmg1^{rst2}*; *Adh^{fn6}* flies. *Adh* transcript levels in *Adh^{fn6}* and *dSmg1^{rst2}*; *Adh^{fn6}* flies are similar and much lower than those in *w¹¹¹⁸*. The housekeeper control (*Gapdh*) transcript levels are the same in the three strains. Each combination of primer set and DNA was done in triplicate.

GAVIS 2005). Thus there is independent evidence that the molecular components regulating NMD may change throughout fly development. In conclusion, our data suggest that either *Smg1* is not involved in NMD in *Drosophila* or, if it has a role, it is developmentally limited.

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