Mutations in genes involved in nonsense mediated decay ameliorate the phenotype of sel-12 mutants with amber stop mutations in <i>Caenorhabditis elegans</i>

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Abstract

**Background:** Presenilin proteins are part of a complex of proteins that can cleave many type I transmembrane proteins, including Notch Receptors and the Amyloid Precursor Protein, in the middle of the transmembrane domain. Dominant mutations in the human presenilin genes PS1 and PS2 lead to Familial Alzheimer's disease. Mutations in the <i>Caenorhabditis elegans</i> sel-12 presenilin gene cause a highly penetrant egg-laying defect due to reduction of signalling through the lin-12/Notch receptor. Mutations in six spr genes (for suppressor of presenilin) are known to strongly suppress sel-12. Mutations in most strong spr genes suppress sel-12 by de-repressing the transcription of the largely functionally equivalent hop-1 presenilin gene. However, how mutations in the spr-2 gene suppress sel-12 is unknown.

**Results:** We show that spr-2 mutations increase the levels of sel-12 transcripts with Premature translation Termination Codons (PTCs) in embryos and L1 larvae. mRNA transcripts from sel-12 alleles with PTCs undergo degradation by a process known as Nonsense Mediated Decay (NMD). However, spr-2 mutations do not appear to affect NMD. Mutations in the smg genes, which are required for NMD, can restore sel-12(PTC) transcript levels and ameliorate the phenotype of sel-12 mutants with amber PTCs. However, the phenotypic suppression of sel-12 by smg genes is nowhere near as strong as the effect of previously characterized spr mutations including spr-2. Consistent with this, we have identified only two mutations in smg genes among the more than 100 spr mutations recovered in genetic screens.

**Conclusion:** spr-2 mutations do not suppress sel-12 by affecting NMD of sel-12(PTC) transcripts and appear to have a novel mechanism of suppression. The fact that mutations in smg genes can ameliorate the phenotype of sel-12 alleles with amber PTCs suggests that some read-through of sel-12(amber) alleles occurs in smg backgrounds.
Background

Presenilin proteins are part of a complex of proteins that can cleave many type I transmembrane proteins with short extra-cellular domains [1]. Two of the best characterized substrates of the presenilin complex are the amyloid precursor protein (APP) and Notch-type receptors. Presenilin activity is necessary for the generation of b-amyloid, the major constituent of the senile plaques found post-mortem in the brains of patients who had suffered from Alzheimer’s disease [1]. Mutations in APP, and in the two human presenilin genes PS1 and PS2, dominantly cause early onset familial Alzheimer’s disease. Presenilins are also absolutely required for the signalling through Notch receptors and in all animals examined, the complete loss of presenilin activity is lethal due to developmental defects arising from the absence of Notch signalling.

In Caenorhabditis elegans there are three presenilin genes. Mutations in spe-4 lead to sterility and this gene appears to have a very specific role in spermatogenesis [2,3]. The two other presenilin genes, sel-12 and hop-1, each have broader, and partially redundant roles throughout the organism and are both required for proper Notch signalling [4-8]. In the absence of hop-1, animals have reduced fertility [5,6] while sel-12 mutants show a defect in egg-laying behaviour [4,7,8]. hop-1; sel-12 double mutants have a lethal phenotype and show defects in all known LIN-12 and GLP-1 signalling decisions [5,6]. The phenotype of sel-12 mutants is due to defects in two lin-12/ Notch-dependent developmental decisions that occur in the mid/late larval stages. In sel-12 animals there is often a defect in determination of the p cell fate and in the development of the connection between the vulva and the uterus [7]. In these animals the passage way between the uterus and the vulva remains blocked by thick tissue, which everts in the early adult stage giving rise in the adult stage to a protruding vulva phenotype (Pvl). However, this Pvl phenotype, which can easily be seen in a dissecting microscope, is only partially penetrant and even in the strongest alleles only 3/4 of all animals display it [8]. sel-12 animals also display a highly penetrant defect in the orientation of the vulval muscles that open the vulva to allow egg-laying. The muscle defects vary from animal to animal, but in strong sel-12 alleles no animals show a completely normal pattern of sex muscle orientation and consequently, strong sel-12 alleles display a completely penetrant egg-laying (Egl) defect [8].

In order to find out more about the biological roles of presenilins, we and others have done several screens for suppressors of the egg-laying defect of sel-12. Six genes (spr-1-spr-5 and sel-10) have been identified that strongly suppress the sel-12 defects [9-14]. In the strongest suppressor mutations, the penetrance of sel-12 defects is reduced to less than 10% and in the spr-3 gene to almost 0% [13]. However, many spr mutations, especially those that suppress the sel-12 phenotype more weakly, remain uncharacterized.

Mutations in the strong sel-12 suppressor genes spr-3, spr-4 and spr-5 have been shown to de-repress the transcription of the hop-1 presenilin gene and probably suppress sel-12 by replacing the activity of one presenilin with another [11,13]. SPR-1, SPR-3, SPR-4 and SPR-5 resemble components of the mammalian REST-CoREST transcriptional repressor complex [11-13]. Furthermore, SPR-1 physically interacts with SPR-5 in GST pull down and in yeast two hybrid experiments [11]. This suggests that SPR-1 probably functions together with SPR-3, SPR-4 and SPR-5 to regulate hop-1 transcription [14]. An additional suppression mechanism is provided by sel-10. SEL-10 is an F-box protein that is part of an E3 ubiquitin ligase complex [15] that targets proteins for degradation by the proteasome. Mutations in sel-10 result in the stabilization of its target proteins which include the presenilins and the intracellular domain of Notch receptors [9,16-20]. Thus sel-10 probably suppresses sel-12 by either increasing HOP-1 protein levels, by increasing the half-life of LIN-12 intracellular fragment, or by both.

Although spr-2 was the first spr gene to be cloned, how it suppresses sel-12 on a molecular level remains unknown [10]. Unlike sel-10 mutations, mutations in spr-2 have no obvious effects on lin-12 signaling [10]. However, genetic evidence indicates that, like spr-1, spr-3, spr-4 and spr-5, spr-2 does not bypass the requirement for presenilin activity, because a hop-1;spr-2;sel-12 triple mutant is as unviable as is a hop-1;sel-12 double mutant [10]. SPR-2 is a Nucleosome Assembly Protein (NAP) orthologous to the human SET/Taf-1beta/INHAT oncogene [10]. SET has been implicated in a wide range of processes including facilitating transcription, transcriptional repression, inhibition of Protein Phosphatase 2A, regulation of the cell cycle and the membrane recruitment of Rac1 during cell migration [21-26]. However, apart from the fact that spr-2 mutations can suppress sel-12, all the spr-2 mutants isolated so far are viable and have no strong phenotypes [10].

Nonsense mediated decay (NMD) is a normal cellular process in eukaryotes in which messenger RNAs containing Premature translation Termination Codons (PTCs) are targeted for degradation [27-29]. In C. elegans seven smg (suppressor mutations with morphological effects on genitalia) genes were identified and found to be essential for NMD [30-32]. More recently, two additional genes that play a role in NMD were identified but reduction of function of these genes is lethal so they were given the names smg-1 and smg-2 (for smg and lethal) [33].
Cycles of phosphorylation and de-phosphorylation of the protein SMG-2, the ortholog of yeast UPF1 [34,35], plays a central role in the process of NMD [36]. In the nematode, SMG-1, SMG-3 and SMG-4 act to phosphorylate SMG-2, whereas smg-5, smg-6 and smg-7 are required for SMG-2 dephosphorylation. smg-2 encodes an ATPase/helicase that can bind to mRNAs containing PTCs with greater affinity than those without. This process is enhanced by the presence of SMG-3 and SMG-4 [37], which are known as UPF2 and UPF3, respectively, in yeast [38,39]. SMG-1, a conserved Phosphatidylinositol Kinase-Related Protein Kinase [36], then phosphorylates SMG-2 in a SMG-3- and SMG-4-dependent manner [40]. smg-5, smg-6 and smg-7 encode related proteins all containing an N-terminal 14-3-3 like domain [41], and are thought to recognize phosphorylated SMG-2 and trigger its dephosphorylation by recruiting protein phosphatase 2A (PP2A) into a SMG-5-PP2A sub-complex [42-44]. SMG5 and SMG6 proteins further share a C-terminal PINc domain into a SMG-5-PP2A sub-complex [42-44]. SMG5 and SMG6 are known as UPF2 and UPF3, respectively, in yeast [37]. In order to determine if spr-2 functions through a similar mechanism to other spr genes to suppress sel-12, we looked at the effect of mutations in spr-2 on presenilin transcript levels. We find that spr-2 mutations do not increase the amounts of hop-1 or spr-4 transcripts (Gontijo and Lakowski unpublished data). However, spr-2 mutations increase the levels of sel-12(ar171) mutant transcripts in the L1 stage (Figure 1A). The sel-12 allele tested, ar171, contains a Premature Translation Termination Codon (PTC) that is predicted to truncate the SEL-12 protein before the large cytoplasmic loop region (See Figure 1B for scheme). Transcripts containing a PTC are known to be targeted for degradation in a translation dependent manner by the nonsense mediated decay (NMD) machinery [52].

Here, we present experiments in which we tried to determine better how the strong suppressor gene spr-2 suppresses sel-12. We show that, although spr-2 does not affect hop-1 transcript levels, spr-2 mutations do increase sel-12(PTC) transcript levels in the embryo and in the L1 larvae. This study led us to examine the effects of smg mutations on sel-12 transcript levels and in modulating the sel-12 phenotype. We find that the transcripts of three sel-12 alleles with PTC mutations are subject to NMD and that smg mutations restore nearly normal sel-12 transcript levels. Furthermore, we show that a new weak suppressor mutation we had identified, spr-8(pf52), is an allele of smg-6 and that another mutation identified in a putative weak spr strain, by146, is an allele of smg-1. smg mutations can weakly ameliorate the phenotype of sel-12 alleles with amber stop mutations but do not affect the phenotypes of missense, deletion or opal stop alleles. This suggests that some read through of sel-12(amber) alleles occurs in smg backgrounds. Furthermore, we show that spr-2 does not appear to be affecting NMD and that the suppression of sel-12 by spr-2 can not be explained by the effect of spr-2 mutations on sel-12(amber) transcript levels. We further suggest that spr-2 suppresses sel-12 by a novel mechanism.

**Results**

**Presenilin transcript levels in spr-2 mutants**

In order to determine if spr-2 functions through a similar mechanism to other spr genes to suppress sel-12, we looked at the effect of mutations in spr-2 on presenilin transcript levels. We find that spr-2 mutations do not increase the amounts of hop-1 or spr-4 transcripts (Gontijo and Lakowski unpublished data). However, spr-2 mutations increase the levels of sel-12(ar171) mutant transcripts in the L1 stage (Figure 1A). The sel-12 allele tested, ar171, contains a Premature Translation Termination Codon (PTC) that is predicted to truncate the SEL-12 protein before the large cytoplasmic loop region (See Figure 1B for scheme). Transcripts containing a PTC are known to be targeted for degradation in a translation dependent manner by the nonsense mediated decay (NMD) machinery [52].

**sel-12(PTC) transcripts are subject to NMD**

Sel-12 is expressed strongly in all stages but most strongly in eggs. sel-12(PTC) mutations in the wild type and in five sel-12 alleles: three, ar171, ty11, and by125 containing PTC mutations, one missense allele, ar131, and a deletion, lg1401 (Figure 1B, C). The lg1401 deletion eliminates the annealing sites of the primers used to detect the sel-12 transcript (Figure 1C and Table 1) so in this strain sel-12 is undetectable (Figure 1D). In all three of the PTC alleles, the transcript levels are greatly reduced (Figure 1A and 1D and Additional file 1). On the other hand, the missense ar131 mutation has transcript levels comparable to the wild type (Figure 1D). This clearly shows that PTC mutations in sel-12 are subject to NMD. sel-12 is the upstream gene in an operon with F35D12.5 but sel-12(PTC) mutations do not strongly affect the transcript levels of F35H12.5 (data not shown).

**The effect of spr-2 on sel-12(PTC) transcripts is stage specific**

The initial observation of mutant sel-12 mRNA stabilization by spr-2 mutations was made in synchronous L1 sel-12(ar171) animals (Figure 1A and data not shown). To examine the effect of spr-2 on sel-12 transcript levels more thoroughly, we determined the sel-12 transcript levels in the wild type (N2), LA242 sel-12(ty11), LA259 spr-2(ar199); sel-12(ty11) and smg-2(e2008); sel-12(ty11) strains at different times during development (Figure 2A). The sel-12 is expressed strongly in all stages but most strongly in eggs. These results are similar to what we have seen by Northern blot [13] when we correct for the fact that the
The effect of spr-2 and sel-12 mutations on sel-12 transcript levels. A) mRNA levels determined by qRT-PCR relative to the average of control transcripts (ama-1, nhx-4 and eft-2). sel-12 transcripts were detected from synchronized L1s. Three different amplicons, sel-12-3, sel-12-5 and sel-12-6, (see Table 1 for primer names and sequences) were tested. The error bars represent the standard deviation between the normalized control primers. sel-12 transcript levels are greatly reduced by the ar171 mutation, while both spr-2 mutations reproducibly increase ar171 transcript levels. B) A model of the SEL-12 presenilin protein where the position of the point mutations are depicted. Stop mutations are shown by open squares and amino acid substitutions by black squares (Modified from [6]). C) The gene structure of sel-12 (F35H12.3). Exons are denoted by blue rectangles joined by a jointed line. The 3' UTR of sel-12 is noted in lighter blue. The position of alleles PTC mutations are indicated by yellow vertical bars, the ar171 missense mutation is indicated with a green vertical bar and the extent of the ok2078 and lg1401 deletions is indicated with black horizontal bars. The position of the primer pairs used in qRT-PCR studies presented in this paper are indicated with small arrows above the gene structure. D) The relative mRNA levels of sel-12 detected by qRT-PCR with the primer pair for amplicon sel-12-6 (Table 1) are plotted on a logarithmic scale for N2, and strains with five different sel-12 mutations.
control in the Northern blot, ama-1, is expressed at higher levels in the eggs. The ty11 mutation greatly reduces sel-12 transcript levels at all developmental stages. smg-2 mutations greatly increases sel-12(ty11) levels close to those of the wild type, except at 44 hours. However, the effect of the spr-2 mutation on the ty11 transcript is much more modest and essentially limited to the eggs and L1 stage.

**spr-2 mutations do not affect NMD more generally**

To see if spr-2 mutations had a more general effect on RNA levels, we tested whether spr-2 could influence NMD in several ways. Firstly, we made an unc-54(r293); spr-2(ar199) double-mutant strain. unc-54 codes for a major muscle myosin. In strains containing the r293 allele, the unc-54 transcript is unstable and subject to NMD, yielding paralyzed adult animals. Mutations in any smg gene stabilize unc-54(r293) transcripts so that the animals can move more normally [30]. unc-54; spr-2 double mutants are still strongly paralyzed, suggesting that spr-2 is not a smg gene.

The ribosomal protein gene, rpl-12, is normally alternatively spliced to produce two isoforms [53]. One form is unproductively spliced and contains a piece of an intron (PTC) (Figure 2B). Although the spr-2 PTC transcript is constitutively produced, it is actively degraded by NMD, so that its levels are normally very low with respect to the other isoform. In the absence of a smg gene, however, the rpl-12 PTC transcript accumulates and can be readily detected by reverse transcriptase PCR (RT-PCR), yielding a robust test for Smg activity [53].

So we prepared mixed stage RNA from a spr-2 homozygous strain and tested whether there would be any detectable enrichment of the rpl-12 PTC isoform, but found none (Figure 2C), confirming that spr-2 mutants are proficient in NMD. As the effect of spr-2 on sel-12 transcripts was found only in early stages, it could be that spr-2 affects NMD only in these particular stages of development. However, NMD of rpl-12 happens normally in a spr-2(ar199) strain at all the stages in which we have looked, even in embryos and L1s (data not shown), suggesting no general role for spr-2 in NMD at any developmental stage.

**smg mutations ameliorate the phenotype of sel-12(ty11)**

In the process of doing these experiments we discovered that a smg-2 mutation partially ameliorates the phenotype of sel-12(ty11). To see if other smg genes could also ameliorate the sel-12(ty11) phenotype, we made additional smg; sel-12(ty11) double mutants. Indeed, mutations in all smg genes that we tested greatly increased the levels of sel-12(ty11) transcripts (Figure 3A). Most smg; sel-12(ty11) double mutants also appeared to have some amelioration of the sel-12 phenotype. The ability of sel-12 animals to lay eggs can be scored in different ways which we quantified in both a smg-1 and a smg-2 background. More smg; sel-12(ty11) double mutants can lay some eggs than sel-12(ty11) animals (Figure 3B). Furthermore, fewer smg; sel-12(ty11) animals display a strong protruding vulva phenotype (Figure 3C), even though smg mutations often affect vulval morphology. Finally, most sel-12 animals die during the reproductive period due to internal hatching of progeny. These progeny develop further in the mother, feeding off her tissue, leading to a terminal 'bag-of-worms' phenotype where the developing larvae are encased by the empty cuticle of the mother. The internal hatching of progeny severely limits the lifespan of animals. However, in smg; sel-12(ty11) animals, many animals survive the reproductive period (Figure 3D). In smg; sel-12(ty11) ani-

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**Table 1: PCR primers used in this study**

| Product     | Sense primer | Antisense primer | enzyme |
|-------------|--------------|------------------|--------|
| Rpl-12      | GN1100 GCTGACGTCCAGGAAACAGTCG | GN1101 GATGATGTCGTGTTGGTGTTC | Drel |
| ama-1       | GN701 GTCACTGAAAAAGATGGCCATATAA | GN702 CATCATGATTTTGTGGTAAAGT | Drel |
| Efr-2       | GN739 ACTTGTTGGAGTCGATCAATCTT | GN740 GACCTGGAGATACGGAGACATTCC | Drel |
| nhx-4       | GN753 GGCTTGTTACCATAGTGGTTCAT | GN754 TCTTGTCTGATCTGGAGTAAAGC | Drel |
| sel-12-1    | GN854 GGACATCTCAGGTTATCTCACT | GN855 CCGGACAAAGAGTGGTATAGTA | Drel |
| sel-12-3    | GN876 TGGAGTCTGATGATGCGAGATTCT | GN877 GTAAGACCGAGACACTAGTGC | Drel |
| sel-12-5    | GN880 AGGAGAGGAGGAAACAGGGG | GN881 AGTGAACCGAGAGACATAGAAT | Drel |
| sel-12-6    | GN882 CCCTCCAGAAAAGACAGACAG | GN883 AATACGGTGAGATGCTAGCCATTT | Drel |
| Y22D7AL[4]  | GN37 CTATGCCCAAGGACGCTAG | GN38 GTATTTGTTCCCTAGGTCGCG | KpnI |
| pkp2006     | GN553 TTAGAAGAAGATCTGACAGGCGG | GN554 AAATGATGGCCTAGAAATCCGAGGTTCG | KpnI |
| Y71H2B[2]   | GN400 CTGAAAACTTGGAAATCTGGGC | GN401 GACGATGCTGAGATGCGAGTAC | KpnI |
| C48B6[1]    | GN124 GTCAAGAAGGACTGTTACAG | GN125 GGGACTTTAGTGGTGGTGG | KpnI |
| pyl4        | GN1008 GAATCTTGCAGGCGGTCAT | GN1009 CTAGGCGACAACTCATTAGTGC | KpnI |
| smg-1(by146) | GN1222 GCTTCTTTTATGAAAGGAGATGTCG | GN1233 AGAAGTGAGAAGGGAGCTATAGCAG | KpnI |
| smg-1(by146) | GN1222 GCTTCTTTTATGAAAGGAGATGTCG | GN1233 AGAAGTGAGAAGGGAGCTATAGCAG | KpnI |
The effect of smg-2 on sel-12 transcripts levels. A) The expression of sel-12 transcripts during development in: N2, LA242 sel-12(ty11), LA259 spr-2(ar199); sel-12(ty11) and smg-2(e2008); sel-12(ty11) strains. The relative mRNA levels were determined by qRT-PCR with primer pairs for amplicon sel-12-6 (Table 1). The X axis represents time in hours after feeding synchronous starved L1s as described in the Methods section. (--) is asynchronous eggs. B) A scheme of the alternative splicing of rpl-12. Coding exons are shown in dark blue, the alternative 3' extension of exon 2 is shown in light blue with a bar and a * to indicate a stop codon. The locations of the primers used to detect rpl-12 transcripts are noted. C) rpl-12 mRNA splicing assay for Smg activity. RNA was prepared from synchronous L1s. Pictures are from Ethidium bromide-stained 2% agarose gel. The sizes of DNA fragments from the loading ladder are shown at the left. The gN2 lane is a control with N2 genomic DNA.

Isolation and characterization of smg-6(pf52)

In our spr screens we have recovered many weaker mutations that allow some sel-12 animals to start laying eggs as young adults. However, most of these animals eventually become Egl and die from internal hatching of progeny. In the process of doing the studies on the effect of smg genes on sel-12 phenotype, we realized that one of the weak sel-12 suppressors that we had been trying to positionally clone mapped near smg-6. The pf52 allele complemented all of the known sel-12 suppressor genes, so we tried to map it genetically. pf52 is linked to the left arm of LGIII, where no other spr genes have been mapped. So we gave pf52 the gene name spr-8. The spr-8(pf52) mutation was mapped by a combination of classical and Single Nucleotide Polymorphism (SNP) mapping (see Methods) about mid way between dpy-1 and daf-2. The SNP mapping data was most consistent with a position between the SNPs pkP3086 and SNP_Y711H2B[2] (Figure 4A). The gene smg-6, which is involved in nonsense mediated decay (NMD), maps to the same genetic interval as spr-8 (Figure 4A). The pf52 mutation was recovered as a suppressor of the amber stop allele ty11. So we tested whether pf52 could be allelic to smg-6. We tested complementation of pf52 to smg-6(r896) for sel-12(ty11) suppression and found that some spr-8(pf52)/smg-6(r896); sel-12(ty11) animals were clearly suppressed in the first heterozygous generation and their progeny appeared to lay more eggs than sel-12(ty11). Moreover, pf52 strongly suppresses the movement defects of unc-54(r293) animals and displays a partial maternal effect for unc-54 suppression (See Methods). Consistent with this, smg-6 is known to have a maternal effect [30,54]. Finally, the PTC form of rpl-12 clearly accumulates in pf52 strains but that pf52 has a weaker defect in NMD than most other smg genes (Figure 4B and data not shown). Interestingly, smg-6(r896) is known not to completely abolish NMD [54], and also

mals, the restoration of egg-laying behavior is variable and animals can display severe side-effects seldom seen in spr; sel-12(ty11) or sel-12(ty11) mutants alone, such as exploding during the L4 to adult molt and increased sterility (data not shown). Although animals with these problems were excluded from our analysis, we found that these smg mutations do not obviously increase brood size (Figure 3E). The brood size remained very similar to that of sel-12 with only very modest increases in a few of the smg; sel-12(ty11) double mutants that were not statistically significant (Additional file 2, Expt.1). The phenotypes of smg; sel-12(ty11) strains clearly contrasts with that of the LA259 spr-2(ar199) IV; sel-12(ty11) strain. For all phenotypes examined, smg mutations suppress sel-12(ty11) much more weakly than spr-2(ar199) mutation (Figures 3B, C and 3E).
Figure 3

smg mutations ameliorate the phenotype of sel-12(ty11). A) qRT-PCR analysis of sel-12 mRNA (amplicon sel-12-6) from asynchronous eggs from different genotypes. Results are shown for three independent Egg preparations, except for smg-5; sel-12, where only two biological repeats were performed. By a paired sample one-tailed Student’s T-test, all four smg mutations significantly increase sel-12 transcript levels. B) Phenotypic quantification of the fraction of animals laying eggs (see Additional file 2, expt. 1 for the data) and C) the fraction of animals with a pronounced (sel-12-like) protruding vulva (Pvl); 55/137, 0/20, 21/115 and 15/99 for LA242 sel-12(ty11), LA259 spr-2(ar199); sel-12(ty11), LA613 smg-1(r861); sel-12(ty11) and smg-2(e2008); sel-12(ty11) animals respectively ± 95% confidence limits. Values statistically significantly higher than sel-12(ty11) are indicated with an * and those values significantly lower than sel-12(ty11) are indicated with **. Many N2 animals were observed but none showed a strong Pvl phenotype and all laid eggs. D) Survival curve of adult worms. At least 20 worms of each genotype were singed as L3s and checked daily for death. E) Total brood size quantification of a panel of mutants ± the 95% confidence limits of the mean. Values statistically significantly higher than sel-12(ty11) are indicated with an *. Results are also tabulated in Additional file 2 experiment 1.
shows this same weak effect on rpl-12 PTC transcript accumulation (data not shown).

smg-6 corresponds to the predicted gene Y54F10AL.2 (Philip Anderson personal communication). There are three predicted isoforms of smg-6 so we sequenced all coding exons in the longest form of the gene (Y54F10AL.2A) and found that the pf52 has a G → A mutation at position 3660 of the transcript [GenBank:NM_065165] that leads to an G → D amino acid substitution at position 1216 in the protein [GenBank:NP_497566.3]. smg-6 has also been named est-1 as it is similar to the Saccharomyces cerevisiae gene Ever Shorter Telomeres-1. An alignment of the PINc domain of SMG-6 proteins from the Human, and the nematode species Brugia malayi and C. elegans are shown in Figure 4C. The smg-6(pf52) mutation affects an amino acid very close to the final aspartate of the putative catalytic domain.

Isolation and characterization of smg-1(by146)
To test whether any other mutations we recovered in the different spr screens were additional mutations in smg genes, we prepared mixed stage RNA from the original isolate strain of all previously uncharacterized spr mutations and tested the effects of these mutations on the stability of the rpl-12 PTC isofrm. All of these strains, except one, show no effect on the stability of the long form of rpl-12 (Figure 4B and data not shown). However, the BR2097 by146; sel-12(ar171) unc-1(e538) X strain has high levels of the rpl-12 PTC isofrm that are comparable to that seen with the strongest smg mutations we have tested and much stronger than smg-6(pf52) (Figure 4B and data not shown).

To confirm that BR2097 contains a smg mutation, we outcrossed BR2097 and could recover weakly Pell non-Unc strain that did not contain either sel-12(ar171) or unc-1(e538). All other smg mutations have been shown to have mild vulva defects. by146 can completely suppress the paralyzed phenotype of unc-54(r293), confirming that it is a smg mutation. by146 is tightly linked to SNP_C48B8[1] and by4 (see Table 1), two SNPs in the cluster of LG I (data not shown) close to smg-1, smg-5 and smgl-1. No mutations have been isolated in smgl-1 so we tested complementation of smg-1(r861) and smg-5(r860) to by146. We found that by146 complements smg-5(r860), but fails to complement smg-1(r861) for the Pel phenotype and for suppression of unc-54(r293).

As the by146 mutation was recovered in a UV/TMP mutagenesis, we thought that there would be a good possibility that this allele would have a chromosomal rearrangement, such as a deletion, that could be detected by PCR. So we amplified the smg-1 genomic region in fragments of about 2–3 kb in both an N2 and by146 background and subjected these fragments to restriction digest with different enzymes. We determined that the by146 mutation had a small deletion in the 787 bp AvaII fragment (Figure 5A). So we sequenced this fragment and determined that by146 has a simple 190 bp deletion. The by146 deletion has the following breakpoints which are denoted by a slash:

GTGCTACACC/aatgttccac ... ccagcaaca/TGTATCACAA,
where retained sequences are shown in capitals and deleted sequences in lower case. The smg-1(by146) deletion changes the reading frame and truncates the predicted SMG-1 protein so that it lacks the C-terminal 1/4 of the protein including most of the PI3 kinase domain (Figure 5B). The PI3 kinase domain is essential for SMG-2 phosphorylation and thus for NMD. Consequently, by146 is likely to be a strong loss of function or null allele of smg-1, consistent with its strong effect on the rpl-12(PTC) transcript (Figure 4B).

smg mutations only suppress sel-12(amber) alleles
To test whether the effect of smg genes on the sel-12 phenotype was only through the stabilization of sel-12(PTC) transcripts, we tested whether smg-2 affected hop-1 or spe-4 mRNA stability, but we found that smg-2 had little or no effect on hop-1 or spe-4 mRNA levels at any developmental stage (data not shown). If smg mutations suppress sel-12 by stabilizing sel-12(PTC) transcripts then they should only suppress sel-12(PTC) alleles. So we compared the effects of placing smg mutations into the background of six different sel-12 alleles. ty11 and by125 are amber PTC alleles, ar171 is an opal PTC allele, oh2078 and lg1401 deletions that delete a large part of the sel-12 gene and should be null alleles and ar131 is an C605 amino acid substitution with a partial loss of function phenotype (see Figure 1C). In one experiment we directly compared the effects of smg-6(pf52) on sel-12(by125) and sel-12(ty11) alleles. Consistent with our hypothesis, pf52 equally ameliorated the proportion of animals that lay eggs as well as increasing average brood size of sel-12(by125) and sel-12(ty11) alleles (Figure 6 and Additional file 2 experiment 3).

In another experiment we found that smg-2(e2008), smg-5(r860) and smg-6(pf52) mutations do not ameliorate the sel-12(ar131) phenotype. smg-12(ar131) animals have very similar Egl phenotypes to ar131 animals (Figures 6A and Additional file 2 experiment 2). Smg mutations do not increase the brood size of sel-12(ar131) animals and indeed actually might slightly reduce the number of progeny of sel-12(ar131) animals. The strains LA726 smg-2(e2008); sel-12(ar131) and LA722 smg-5(r860); sel-12(ar131) had statistically lower brood sizes than the LA728 sel-12(ar131) control strain (Additional file 2 experiment 2). However, the LA725 smg-2(e2008); sel-12(ar131) and LA723 smg-5(r860); sel-12(ar131) strains
had brood sizes that were not statistically different from the LA728 sel-12(ar131) control strain (Additional file 2 experiment 2), indicating that the reduced brood sizes are not simply caused by the presence of the smg mutation. Additionally, Student's T-tests indicate that the brood sizes of the LA726 and LA725, as well as the LA723 and LA722 strains are not statistically different. Furthermore, when the results of LA722 and LA723 strains are pooled and when the results of the LA726 and LA725 strains are pooled, the brood size of smg-2(e2008); sel-12(ar131) and smg-5(r860); sel-12(ar131) animals does not statistically differ from that of LA728 sel-12(ar131) (P = 0.100 and P = 0.103 respectively, Student's T-test).

Similarly we found that smg-5(r860); him-8(e1489); sel-12(lg1401) hermaphrodites were as Egl and Pvl as those in a control him-8(e1489); sel-12(lg1401) strain. Finally we directly compared the effects of the smg-1(by146) mutation on the sel-12 alleles: ar171, by125, ok2078 and ty11. In two experiments, we see that by146 ameliorates both the egg laying phenotype and the brood size of by125 and ty11 (Figures 6A, B and Additional file 2 experiments 5 and 6). However, by146 has no consistent effect on the phenotype of ok2078 and ar171 (Figures 6A, B and Additional file 2 experiments 5 and 6). Thus our results show that smg mutations do not ameliorate the phenotype of sel-12 missense and deletion alleles as expected. Surprisingly, smg mutations ameliorate the phenotypes of amber PTC sel-12 alleles but not that of the ar171 opal PTC mutation.

Discussion

All smg genes affect sel-12 similarly

In the course of this study we have shown that sel-12 transcripts with premature termination codons are subject to NMD. The loss of nonsense mediated decay can restore sel-12(PTC) transcripts to near wild type levels. The different smg genes have different roles in mediating NMD. In particular, smg-1, smg-3 and smg-4 help to promote SMG-2 phosphorylation, while smg-5, smg-6 and smg-7 promote SMG-2 de-phosphorylation. Furthermore, smg-2, smg-5 and smg-6 have been implicated in the maintenance of RNAi while smg-1 is not [55]. However, in our initial study (Figure 3), we found no strong differences in the effects of the different smg genes on sel-12(PTC) transcript levels and sel-12 suppression. This suggests that smg mutations ameliorate the sel-12(ty11) phenotype simply by stabilizing sel-12(ty11) transcripts.

smg mutations poorly suppress sel-12

Genes involved in NMD weakly ameliorate the phenotype of sel-12(amber) mutations. However, this effect is much weaker than the effect of mutations in strong spr genes such as spr-2. In the spr screens, we have identified only two smg mutations as weak suppressors of sel-12 in over 100 spr alleles indicating that screening for sel-12 suppression is an inefficient way to recover smg alleles. Although smg mutations weakly suppress sel-12, most smg; sel-12(PTC) animals still become Egl and die of internal hatching of progeny. Thus at any time, only a proportion of smg; sel-12 animals lay eggs and can be isolated for this phenotype. This may explain the relative paucity of smg alleles recovered in screens for suppressors of sel-12(ty11). Furthermore, the absence of clear effects of smg-1(by146)
on the phenotype of sel-12(ar171) suggests that smg should only be very poorly selected for in ar171 suppressor screens.

The by146 mutation was recovered serendipitously
Curiously, the by146 allele was isolated in the BR2097 by146; sel-12(ar171) unc-1(e538) strain. However, our results show that by146 does not obviously suppress sel-12(ar171), so it is unclear why it was recovered in a sel-12(ar171) background. The BR2097 strain was frozen soon after it was isolated and was not examined further until this study. Not all of our putative weak suppressor alleles have been characterised carefully, so we re-examined the phenotype of BR2097. BR2097 animals have a similar proportion of egg laying animals to all of the sel-12 null alleles including ar171 (Figures 6A and Additional file 2 experiment 6). Furthermore, the brood size of BR2097 is not elevated and is in fact significantly smaller than ar171 itself (Figures 6B and Additional file 2 experiment 6), an effect that might be due to the unc-1(e538) background mutation. These results indicate that the BR2097 strain does not appear to have a suppressor mutation that can suppress sel-12(ar171) and that the presence of the smg-1(by146) mutation could not have been selected for. This suggests that the isolation of by146 could have been due to the fortuitous presence of a background smg-1 mutation in the BR2097 strain that did not strongly affect the Egg-laying defect of sel-12(ar171). However, our subsequent studies indicate that smg-1(by146) can ameliorate the phenotype of sel-12(ty11) and sel-12(by125). Thus all of our results are consistent with smg-1(by146) being a weak amber allele specific suppressor of sel-12. We note that our results conflict with those of Levitan et al. who saw that sel-12(ar171) is somewhat suppressed by

**Figure 5**
The nature of the smg-1(by146) mutation. A) The structure of the smg-1 gene with the position of the primers, GN1212, GN1213 and GN1264 used to detect and genotype the by146 mutation. The gene structure was captured from Wormbase [http://www.wormbase.org/](http://www.wormbase.org/) Wormbase release WS187). Below the gene structure the position of AvaI and Ddel restriction sites are shown by vertical bars along with the approximate position of the by146 mutation by a horizontal bar. B) The domains within the SMG-1 protein according to [40] along with the expected affect of the by146 mutation on the protein. NLS = Nuclear Localization Signal, FAT = FRAP, ATM, and TRRAP domain, PIK domain = phosphatidylinositol kinase (PIK) domain and FATC = FAT carboxyl terminus domain.
strain and it was subsequently lost. Our alleles have been high that we were not able to continue propagating the
1), may suppress
The sterility in the
from the CGC collection we saw a high degree of sterility. Furthermore, this activity is dependent on the PINc domain and the three catalytic aspartate residues in this domain [57,58]. There are also several basic residues in the PINc domain that may bind to RNAs [45]. The addition of an acidic amino acid in this region may destabilize RNA binding. So the pf52 mutation could affect the putative RNAse activity of SMG-6 by either disrupting the catalytic core, by affecting RNA binding, or both. The fact that pf52 does not eliminate NMD as well as alleles of other genes, including smg-1(by146) (see Figure 4B) suggests that either smg-6 is not completely required for NMD in C. elegans, or that alternately, the pf52 allele may be a partial loss of function mutation. However, in a non-complementation screen for smg-6 alleles several alleles were recovered including three homozygous lethal mutations [54]. This suggests that the null phenotype of smg-6 may be lethal and that pf52 does not completely eliminate smg-6 function.

Smg mutations appear to affect fertility
Although they were not directly compared, the suppression of sel-12(amber) mutations by our alleles, smg-1(by146) and smg-6(pf52) was clearer, especially for brood size (see Figure 6B and Additional file 2 experiments 5 and 6), than with reference alleles ordered from the Caenorhabditis Genetics Center (CGC) (Figure 3E). Indeed, in many smg; sel-12(ty11) strains with smg alleles from the CGC collection we saw a high degree of sterility. The sterility in the smg-2(e2008); sel-12(ty11) strain was so high that we were not able to continue propagating the strain and it was subsequently lost. Our alleles have been propagated for a limited number of generations while the reference alleles of the smg genes were isolated over 20 years ago [30]. Our data also suggest that pf52, the allele with a weak effect on NMD (Figure 4B and Additional file 1), may suppress sel-12 better than the by146 allele which has completely lost NMD (Figures 4B, 6B and Additional file 2). All of this suggests that there might be negative effects on fertility of eliminating NMD and maintaining strains without NMD for many generations. Consistent with this, our results show that LA920 smg-1(by146) has a clearly reduced brood size as compared to N2 (Figure 6B and Additional file 2). Additionally, when the LA920 smg-1(by146) strain was cultured at the stressful temperature of 25°C for several generations, we saw that some animals had greatly reduced number of progeny (Lakowski data not shown), suggesting that some epigenetic effect may underlie the severely reduced fertility seen in some smg; sel-12 animals. As genes involved in NMD have been implicated in telomere maintenance in other systems, it would be interesting to investigate whether telomere length or sub-telomeric silencing is affected by maintaining strains without NMD for many generations in C. elegans.

The suppression of sel-12 by smg mutations may involve translational read through
It is surprising that smg mutations can sometimes ameliorate sel-12(PTC) phenotypes as these transcripts still retain a stop codon and cannot encode full length sel-12 proteins. Presenilin proteins have eight predicted transmembrane (TM) domains and are normally cleaved in the large cytoplasmic loop between TMs 6 and 7 (for a scheme of the protein see Figure 1B) to generate N and C terminal fragments that remain associated in a protein complex known as the γ-secretase [1]. The γ-secretase also contains three other proteins known as APH-2/Nicastrin, APH-1 and PEN-1. The γ-secretase is an unusual protease complex and all four proteins are required for activity and trafficking to the cell surface of the complex [1]. Partial protein fragments of presenilin proteins are not known to retain protease activity, or to be efficiently assembled in to γ-secretase complexes. This suggests that the suppression of sel-12 by smg mutations could involve translational read through. We did not directly determine SEL-12 protein levels as we had no antibodies that could detect SEL-12 either in Immunoflorescence or on Western blots. However, our results strongly implicate translational read-through in the mechanism of sel-12 suppression by smg mutations. The efficiency of read through may be dependent on the type of sel-12 allele. The fact that by146 ameliorates the phenotype of both amber stop alleles but not that the ar171 opal stop allele is consistent with a read through mechanism specific to sel-12(amber) alleles. Proteins involved in NMD have been implicated in modulating read-through of transcripts containing PTCs in yeast [53]. Although all three types of stop codons could be read through in these experiments, the levels of read through of ochre stop mutations was consistently lower than those of amber or opal stop codons. This indicates that the efficiency of read through can be dependent on the type of stop mutation in yeast. In C. elegans, amber suppressor tRNA mutations are readily recovered, while opal and ochre specific suppressors are unknown [59]. Thus, in C. elegans, informational suppression of amber mutations is more common than that of opal or ochre
mutations, consistent with what we see in the smg; sel-12 strains.

The effects of spr-2 on sel-12 transcript levels do not explain the spr-2 phenotype
Curiously, we also found that mutations in spr-2 increase the transcript levels of sel-12(ar171) and sel-12(ty11) but only in the embryo and the L1 larva. The increase in sel-12(ar171) or sel-12(ty11) transcript levels in the embryo and the L1 stage in a spr-2 background is robust, but much weaker than the near restoration of sel-12 levels seen in the smg-2 background at almost all developmental stages. However, the effects of spr-2 on sel-12 transcript levels can not explain the suppression of sel-12 by spr-2 mutations. Firstly, mutations in strong spr genes suppress all sel-12 alleles that have been tested. For spr-2, it was initially shown that spr-2 mutations strongly suppress the phenotypes of the sel-12(ar131) missense and the sel-12(ar171)opal stop mutations [10]. We show that spr-2 mutations also strongly suppress the sel-12(ty11) amber allele. Secondly, smg mutations, which greatly increase sel-12(PTC) transcript levels, do not suppress sel-12 anywhere near as well as spr-2 mutations. This indicates that stabilizing sel-12(PTC) transcripts can only weakly ameliorate the phenotype of sel-12 mutants. Thus, although the effect of spr-2 mutations on sel-12 transcript levels might in some cases contribute to the suppression of sel-12 phenotypes, it is wholly insufficient to explain the strong spr-2 suppressor phenotype. As spr-2 mutations do not affect lin-12 signaling [10] or hop-1 transcript levels (Gontijo and Lakowski unpublished) spr-2 mutations suppress sel-12 through another, as yet uncharacterized, mechanism that is distinct from both that of NMD and that of the other known spr genes.

How spr-2 mutations might affect sel-12(PTC) transcript levels
The effect of spr-2 mutations on sel-12(PTC) transcript levels could be explained if spr-2 mutations directly, or indirectly, affect transport out of the nucleus of the mutant sel-12 transcripts. Proteins with Nucleosome Assembly Protein (NAP) domains are known to shuttle between the nucleus and the cytoplasm and can function as histone chaperones. The Aspergillus nidulans protein most similar to SPR-2, binds to the A. nidulans importin α ortholog [60]. Most, if not all, NMD takes place in the cytoplasm and if the export of sel-12(PTC) transcripts is affected by spr-2 mutations, more of the transcript could be retained in the nucleus and be protected from degradation. In this mechanism, the excess sel-12 transcript should have no effect on SEL-12 protein levels as translation also takes place in the nucleus and the cytoplasm and can function as histone chaperones. Alternatively, since NMD requires a primary round of translation, spr-2 could be necessary for optimal sel-12 translation after nuclear export. Reducing translation could thus reduce NMD.

Another possible mechanism could be if spr-2 mutations increase sel-12 transcription in the embryo and early larva. The human homolog of SPR-2 is known as SET/TAF1beta and has been shown to facilitate transcription [61]. So the effect of spr-2 mutations on sel-12 transcript levels could be at the level of decreasing transcription of a repressor of sel-12 transcription. Paradoxically, SET is also a component of the INHAT (Inhibitor of Histone Acetyltrans-
ferase) transcriptional repressor complex, which represses transcription of some loci by blocking the acetylation of histones and thus the activation of transcription [23]. In the absence of this activity, the transcription of a gene could be de-repressed. So spr-2 could also be a stage specific repressor of sel-12 transcription. However, spr-2(ar199) does not obviously affect sel-12(+) transcript levels in the embryonic L1 stages (data not shown), suggesting that it is unlikely that spr-2 affects sel-12 transcription.

Why spr-2 mutations have a stage specific effect on sel-12(PTC) transcript levels is also unclear. All evidence [10] (and Gontijo and Lakowski unpublished) indicates that spr-2 is broadly expressed at all developmental stages. Thus our results suggest that the stage specific effect of spr-2 mutations on sel-12(PTC) transcript levels might be due to an effect of spr-2 on the interaction of sel-12(PTC) transcripts and some unknown factor with a stage specific expression pattern. Alternatively, the effect could be due to developmental, or tissue-specific, differences in the effects of spr-2 mutations. Determining the role of spr-2 in the stage specific regulation of sel-12(PTC) transcript levels will require further study.

Conclusion
Mutations in spr-2 increase sel-12(PTC) transcript levels in embryos and L1 larvae. However, this effect does not explain the spr-2 suppressor phenotype and spr-2 does not affect nonsense mediated decay (NMD). The mechanism by which spr-2 suppresses sel-12 is still unclear, but appears to be novel. Mutations in smg genes weakly suppress the phenotype of sel-12 animals with amber PTC alleles by stabilizing the sel-12 transcripts which are otherwise subject to NMD. Some read through of sel-12(amber) PTCs appears to occur in smg backgrounds. Over 100 spr mutations have been recovered in screens for suppressors of either the sel-12(ar171) opal mutation or the sel-12(ty11) amber mutation. However, mutations in smg genes are only very infrequently recovered in direct screens for sel-12 suppressors. This is probably due to the weakness of their effects on the sel-12(ty11) phenotype and the absence of clear effects on the sel-12(ar171) phenotype.

Methods
Culture conditions, mutations used and strain construction
All worm strains were cultured as previously described [62] and were maintained at 20 ºC during all experiments unless otherwise stated. The full genotypes of strain used in this study are listed in Additional file 3. The following mutations were used for genetic analysis:

LGII: daf-7(e1372), dpy-1(e1), smg-6(r896), daf-2(e1370) unc-32(e189)

LGIV: smg-3(ma117), smg-7(r1197), let-92(ok1537)

LGV: smg-4(ma116), him-5(e1490)

LGV: sel-12(ar131[4], ar171[4], by125[8], ok2078, lg1401[8], ty11[7]).

unc-1(e538, e719)

pf126 is a late Egl and weak smg-like Pvl mutation that arose spontaneously in a VC13 dog-1(gk10) 1 mutator strain (Lakowski unpublished).

Many mutations used in this study were provided by the Caenorhabditis Genetics Centre (CGC). The original spr-2(ar199) strain was linked to dpy-20(e1282). To study better the phenotypic effects of the spr-2(ar199) mutation, we separated ar199 from e1282 by selecting for Spr non Dpy progeny from +/spr-2(ar199) dpy-20(e1282); sel-12(ty11) hermaphrodites. smg; sel-12 X strains were constructed by crossing the single mutant strains together and scoring the descendents for the presence of the sel-12 Egl phenotype and its amelioration by the smg gene. We also scored the mild Pvl phenotype often induced by smg mutations to score for their presence in a strain. For most strains, the presence of smg mutations was confirmed by the rpl-12 test (described below). For smg-1(by146); sel-12 strains, the by146 deletion was detected by PCR using GN1222 and GN1264 (see Table 1 for primer sequences) on crude worm extracts.

Isolation of weak spr mutations
All spr alleles starting with by were isolated in screens the lab of Ralf Baumeister as suppressors of the opal stop mutation sel-12(ar171). These screens are described in [11,13] however, only the isolation of strong spr mutations was reported in these papers. Additional weaker suppressors were also recovered in these screens but were not characterised at that time. In particular, the by146 mutation was recovered in an Ultra Violet light/Tetramethylpsoralen (UV/TMP) screen at 20 ºC in a sel-12(ar171) unc-1(e538) background. unc-1, which is closely linked to sel-12, was used to facilitate the isolation of suppressor mutations, as Unc-1 animals tend to remain near to the eggs that they have laid. unc-1 mutations do not strongly affect the proportion of sel-12 animals with an Egl or Pvl phenotype. All alleles starting with pf, with the exceptions of pf167, pf168 and pf169, were isolated in Nematode Genetics Group at the Institut Pasteur as suppressors of the amber stop allele sel-12(ty11). The suppressor screens in the ty11 background will be described in greater detail elsewhere. Briefly, the pf52 mutation was isolated after
Ethylmethanesulfonate (EMS) mutagenesis of a sel-12(yt11) strain in essentially the same manner as the screens reported in [13], except that animals were maintained at 25°C instead of the standard 20°C and we deliberately looked for both strong and weaker suppressors in this screen. The pf167, pf168 and pf169 mutations were isolated in a sel-12(ar171) unc-1(e538) background in the same screen as by146. These mutations were frozen at that time but were only recently given allele names.

Staging of worms
To stage worms, plates full of gravid adult hermaphrodites were treated with alkaline hypochlorite solution to kill and dissolve worms [62]. The more resistant eggs were washed three times with M9 buffer and then allowed to hatch overnight in M9 buffer with aeration. Synchronized L1s were then placed onto 9 cm Petri plates to feed and allowed to develop until the desired stage. Staged worms were then washed off the plates, and washed 3 times with M9 before freezing the worm pellet at -80°C until RNA preparation. Where not otherwise specified, L1 corresponds to 5 hours, L2 to 22 hours, L3 to 28 hours, L4 to 44 hours, L4/YA to 50 hours and YA (young adult) to 58 hours of growth. Eggs were not synchronized.

Isolation of RNA, PCR and Quantitative-PCR
To examine the effect of mutations on transcripts, we extracted total RNA from either mixed stage, or staged worms, depending on the experiment, using TRIZOL reagent following their protocol. Reverse transcriptase reactions and quantitative PCR were done using the two-step RT-qPCR Sybr Green low ROX kit by ABgene (reference: AB-1381/a, which is called AB-4116/b since 14/09/2007) according to the manufacturer's recommendations. To synthesize cDNAs we used a 3:1 ratio of random primers and oligo dT primers. Real time PCR reactions were done in 20 μl volumes and run on an Applied Biosystems AB7500 machine in 96 well plates.

For most Q-PCR experiments, the Ct levels of the control genes ama-1, nhx-4 and eft-2 were averaged and this average served as the internal control for the DeltaDeltaCt method to estimate relative fold differences in the target gene RNA levels. The RNA polymerase II large subunit, encoded by the ama-1 gene, has often been used as a control in Northern blots including by us [11,13]. ama-1 is expressed strongly in all developmental stages, however, we found that it does seem to be even more strongly expressed in embryos than the larval stages (data not shown) consistent with the high level of transcription in embryos and with the fact that ama-1 has a strong maternal component. In order to normalize better our studies, we sought additional controls that were broadly expressed housekeeping genes with expression levels both higher and lower than ama-1. As a control gene with expression levels lower than ama-1 we chose the gene encoding the presumptive housekeeping Na+H+ exchanger, nhx-4, which has been shown to be expressed in all cells at all times examined [64]. As a very highly expressed gene, we chose eft-2 [65], a gene that encodes a homolog of translation elongation factor 2 (EF-2), a GTP-binding protein essential for the elongation phase of protein synthesis that is broadly expressed in all stages of development [65].

rpl-12 NMD assay
To test the stability of the unproductive splice variant of rpl-12 in different strains, we used the protocol of [53]. Briefly, 2 μl of first strand cDNAs were used for PCR with the primers GN1100 and GN1101 to test for rpl-12, while amplification with GN701 and GN702 was run in parallel to determine ama-1 levels as a control. The PCR program was 5 min at 94°C and then 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 45 seconds followed by a final step of 7 minutes at 72°C. The products were then migrated on a 2% agarose gel. For ama-1, a product of 80 bp is expected, while for rpl-12, the reaction can amplify two products of approximately 450 and 550 bp. The 550-bp product retains part of an intron with a PTC codon and is subject to NMD in strains that are proficient in NMD. Strains that are deficient in NMD can be easily identified by the detection of increased amounts of the larger rpl-12 product. The original isolates of following alleles were tested: by106, by111, by115, by118, by120, by121, by122, by123, spr-4(by130), spr-3(by137), by140, by141, by142, by143, by144, by145, by146, pf9, pf14, pf21, pf33, pf48, pf50, pf52, pf53, pf54, pf56, pf57, pf58, pf59, pf60, pf61, pf62, pf63, pf66, pf69, pf70, pf71, pf72, pf73, pf74, pf77, pf119, pf120, pf139, pf145, spr-3(pf154), pf161, pf167, pf168, pf169, spr-3(pf193) (Figure 4B and data not shown). For all other spr alleles the gene affected, and the specific mutation, are known. Two additional mutants were also tested: pf126 is a spontaneous mutation that arose in a VC13 dog-1(gk10) strain [63] with a mild, smg-like protruding vulva (Lakowski unpublished) and
ok1537 is a mutation in let-92 the catalytic subunit of protein phosphatase 2A which has been shown to interact with SMG-5 and SMG-7 to dephosphorylate SMG-2.

**Genetic mapping of spr-8(pf52)**

To determine linkage for spr-8(pf52), we crossed CB4856 males to pf52; sel-12(ty11) unc-1(e719) hermaphrodites, isolated cross progeny and then placed F2 Uncs that were not Egl singly on plates. The Spr phenotype of the strains was confirmed in the F3 generation and lysates from 15-20 of the confirmed broods were pooled for analysis. These lysates, along with N2 and CB4856 control DNA were used to test for the presence of SNPs using a variation of the protocol of [66]. We found strong linkage of pf52 to SNP_Y22D7A[e4] on the left arm of chromosome III (see Figure 4A for a genetic map).

To refine the position of pf52, we crossed +/pf52; sel-12(ty11) males to daf-7(e1372) dpy-1(e1); sel-12(ty11) hermaphrodites and isolated cross progeny. We singled all non-Egl F2 progeny from 8 plates and scored their progeny for the various phenotypes. The results of this cross was consistent with pf52 being to the right of dpy-1 (data not shown). So we crossed +/pf52 III; sel-12(ty11) X males to dpy-1(e1) daf-2(e1370) unc-32(e189)III hermaphrodites, isolated cross progeny and then isolated Egl F2 progeny. In the F3 we singled Unc non Dpy and Dpy non Unc recombinant progeny from those plates that had some eggs. The final scoring of these recombinants was dpy-1 31 spr-8 20 daf-2 41 unc-32, placing spr-8 about midway between dpy-1 and daf-2. We constructed a dpy-1(e1) spr-8(pf52) unc-32(e189) III; sel-12(ty11) X strain for further SNP mapping with polymorphisms from the CB4856 strain. However, the results of these experiments were very difficult to interpret due to the weak phenotype of pf52 and the presence of polymorphisms that modify the sel-12 phenotype in the CB4856 background. However, the results of these mapping experiments were most consistent with pf52 mapping between the SNPs pkP3086 and SNP_Y71H2B[e2] (data not shown).

**Testing the ability of pf52 to suppress unc-54(r293)**

To see if pf52 affected NMD more generally, we tested whether pf52 could suppress the smg suppressible unc-54(r293) allele. We crossed unc-54(r293+)/males to spr-8(pf52) III; sel-12(ty11) unc-1(e719) hermaphrodites and isolated wild type moving cross progeny. In the F2 generation we randomly picked 19 Unc-54 animals individually to plates and then examined the F3 and F4 progeny. All of the progeny in three of 19 F3 broods were nearly wild type, although slightly slow moving and displayed a mild protruding vulva phenotype similar to that seen in all previously identified smg mutations. Eight of the 16 remaining broods had some eggs and some animals moving better than unc-54 animals usually move. In the F4 generation these eight plates had many wild type moving progeny and 6/8 had some Unc-1 progeny. In the F4 two of the remaining eight broods had no wild type progeny but many animals suppressed for Unc-54 but with Unc-1 movement defects. The remaining six broods were phenotypically Unc-54 in the F3 and F4 generations. These results are consistent with pf52 strongly suppressing unc-54(r293) but displaying a partial maternal effect.

**Complementation tests for by146**

We crossed +/smg-1 and +/smg-5 males to by146 unc-54(r293) I; sel-12(ar171) unc-1(e538) X hermaphrodites. We placed 10 cross progeny hermaphrodites for each cross on individual plates as L3/L4 animals. 4/10 cross progeny from the +/smg-1 cross had a mild Pvl while none of the cross progeny from the +/smg-5 cross did. Three out of the four Pvl animals from the +/smg-1 cross segregated no phenotypically Unc-54 progeny in the next generation. The seven remaining + or smg-1(r861)/by146 unc-54(r293) plates segregated some phenotypically Unc-54 animals, while all 10 of the + or smg-5(r860)/by146 unc-54(r293) plates segregated Unc-54 animals.

**Linkage analysis of smg-1(by146)**

As we do not know the reason why the CB4856 background modifies the sel-12 phenotype, we examined if there were polymorphisms in this strain that could modulate NMD. We crossed CB4856 males to unc-54(r293) hermaphrodites, isolated cross progeny and then placed 30 Unc-54 F2 progeny singly on plates. All 30 animals gave rise to strains in which all animals were strongly paralyzed indicating that the CB4856 strain does not contain any polymorphisms that strongly affect NMD. We then crossed CB4856 males to by146 hermaphrodites. Using the protruding vulva (Pvl) phenotype of by146 we isolated cross progeny and then placed 60 F2 animals with Pvls individually on plates. We examined the F3 generation for a high penetrance of Pvl defect and found 45 strains that we were fairly confident were smg based on their highly penetrant mild Pvl phenotype. We made lysates from each of these strains and then pooled 2 μl of each lysate. We then tested these pooled lysates, along with N2 and CB4856 genomic DNA for the presence of SNPs near each of the known smg and smgl genes (data not shown).

**Reproducibility and statistical analysis**

Standard errors of the mean, 95% confidence limits and Student’s T-tests were performed using Excel 2003. ANOVA tests were calculated using http://www.physics.csbsju.edu/stats/anova.html. 95% confidence limits for proportions were calculated in Excel 2003 using formulas 22.26 and 22.27 for the confidence limits for proportions from [67].
For Q-RT-PCR experiments, unless otherwise specified, the normalized average of three biological repeats ± standard error of the mean is shown in the figures. In Figure 1A, we show sel-12 transcript levels as determined with three different amplicons (sel-12-3, sel-12-5 and sel-12-6). In this figure, both spr-2 mutations reproducibly increase sel-12(ar171) transcript levels. If we average the results for the two cDNAs and consider each amplicon as an independent assessment of sel-12 transcript levels, the probabilities that the means are similar are p = 0.047 and p = 0.062 (Student’s T-test) for comparison of the means of ar171 vs. ar199; ar171 and ar171 vs. ar211; ar171 respectively and at the point of being statistically significant. Additionally, results for the sel-12-1 amplicon were very similar (data not shown). For Figure 1D, error bars represent the standard deviation of the mean between at least two independent biological repeats, except for sel-12(ar171) where it represents the standard deviation of the mean between two independent qRT-PCR assays from one biological sample (same biological sample as in Figure 1A). In Additional File 2 the results of individual experiments for the determination of egg-laying and brood size are presented. In Figure 6 the pooled results for the individual strains are shown. For the brood size analysis, presenting the results in this manner is justified as no significant differences (p < 0.05) in results for individual strains were detected by T-tests for two repeats, or an ANOVA analysis of variance test for more than two repeats (see Additional file 2, totals).

List of abbreviations
APP: amyloid precursor protein; Egl: egg-laying defect; NMD: nonsense mediated decay; PTC: premature translation termination codons; Pvl: protruding vulva phenotype; smg: suppressor with morphological effects on genitalia; SNP: single nucleotide polymorphism; spr: suppressor of presenilin.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
AMG constructed the first set of smg sel-12(ty11) strains, designed and did the Q-PCR studies, analyzed the phenotypes of smg; sel-12(ty11) strains, analyzed data and helped write the paper. SA prepared RNA for all uncharacterized spr alleles, did the rpl-12 test, linkage analysis of smg-1(by146) and detection and sequencing of the by146 mutation. IR isolated pf52, did the complementation tests for spr-8(pf52) and the linkage analysis for pf52. BL isolated by146, did the genetic mapping of pf52 and by146, constructed and analyzed the unc-54 smg, smg-1(by146); sel-12 and smg-6(pf52); sel-12 double mutant strains and wrote the paper. All authors read and approved the final manuscript.

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Additional material

Additional file 1
Supplemental figure. sel-12(by125)transcripts are subject to NMD and smg-6(pf52)partially restores sel-12(by125)mRNA levels. The relative mRNA levels of controls (average between ama-1, nhx-4 and eft-2 transcript levels) and sel-12 transcripts detected with two different primer pairs (for the sel-12-5 and sel-12-6 amplicons) in mixed stage N2, LA54 sel-12(by125) and LA279 smg-6(pf52); sel-12(by125) strains. The error bars represent the standard deviation of the mean of two independent qRT-PCR assays from two biological samples per strain. By a one tailed Student’s T-test LA279 smg-6(pf52); sel-12(by125) transcript levels are significantly higher than LA54 sel-12(by125) (P = 0.02).
Click here for file
[http://www.biomedcentral.com/content-supplementary/1471-2156-10-14-S1.pdf]

Additional file 2
Supplemental table. Table of results of individual phenotype analysis experiments.
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[http://www.biomedcentral.com/content-supplementary/1471-2156-10-14-S2.doc]

Additional file 3
Supplemental table two. Strains used in this study. The strain names and genotypes of strains used in this study are noted. We also note the original parental strains and the sel-12 strains used to construct these strains along with the total number of times the mutation of interest and the sel-12 allele have been outcrossed.
Click here for file
[http://www.biomedcentral.com/content-supplementary/1471-2156-10-14-S3.xls]
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