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The Role of eIF1 in Translation Initiation Codon Selection in *Caenorhabditis elegans*

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ABSTRACT

The selection of a proper AUG start codon requires the base-pairing interactions between the codon on the mRNA and the anticodon of the initiator tRNA. This selection process occurs in a pre-initiation complex that includes multiple translation initiation factors and the small ribosomal subunit. To study how these initiation factors are involved in start codon recognition in multicellular organisms, we isolated mutants that allow the expression of a GFP reporter containing a non-AUG start codon. Here we describe the characterization of mutations in *eif-1,* which encodes the *Caenorhabditis elegans* translation initiation factor 1 (eIF1). Two mutations were identified, both of which are substitutions of amino acid residues that are identical in all eukaryotic eIF1 proteins. These residues are located in a structural region where the amino acid residues affected by the *Saccharomyces cerevisiae* eIF1 mutations are also localized. Both *C. elegans* mutations are dominant in conferring a non-AUG translation initiation phenotype and lead to growth arrest defects in homozygous animals. By assaying reporter constructs that have base changes at the AUG start codon, these mutants are found to allow expression from most reporters that carry single base changes within the AUG codon. This trend of non-AUG mediated initiation was also observed previously for *C. elegans* eIF2β mutants, indicating that these two factors play a similar role. These results support that eIF1 functions in ensuring the fidelity of AUG start codon recognition in a multicellular organism.

TRANSLATION initiation is thought to be one of the most complex cellular processes in eukaryotes. It involves at least 12 translation initiation factors (eIFs) comprising over 30 polypeptides (Pestova *et al.* 2007). These factors bring together an initiator methionyl tRNA (Met-tRNAi), the small ribosomal subunit, and a mRNA to form a 48S initiation complex. An important role performed by this complex is to select an AUG codon to initiate translation of the mRNA. Since the first AUG at the 5′ end of most mRNAs is selected as the start site, it is believed that the initiation complex scans for an AUG start codon as it moves from the 5′-capped end of the mRNA toward the 3′ end, as proposed in the ribosomal scanning model (Kozak 1978; Kozak 1989). The recognition of the AUG start codon is mediated by the anticodon of the Met-tRNAi, and the matching base-pairing interactions between the codon of the mRNA and the anticodon determine the site of initiation (Cigan *et al.* 1988). These base-pairing interactions are essential, but are likely not the only components required for accurately selecting the correct AUG start codon. Numerous initiation factors along with base-pairing interactions have been shown to aid in the AUG recognition process (Pestova *et al.* 2007).

Translation initiation factors involved in start codon selection fidelity were first identified through genetic studies performed in the yeast *Saccharomyces cerevisiae.* Mutant strains with a modified *His4* gene that had an AUU instead of an AUG at the native start site were selected for the ability to survive on media lacking histidine (Donahue *et al.* 1988; Castilho-Valavicius *et al.* 1990). These mutants were found to be able to produce the *His4* protein by using a downstream inframe UUG codon (the third codon within the *His4* coding region) as the translation start site. Further analyses determined that non-AUG initiation occurred mostly from a UUG codon and not significantly from other codons (Huang *et al.* 1997). These mutants defined five genetic loci and were named *sui1-sui5* (*su*ppressor of *i*nitiation codon) on the basis of their ability to initiate translation at a non-AUG codon.

The *sui1* suppressors were found to have missense mutations in eIF1. These missense mutations showed semidominant or codominant properties in non-AUG translation initiation while deletion of the eIF1 gene led to lethality in yeast (Yoon and Donahue 1992). eIF1 is a highly conserved protein with a size of approximately 12 kDa that plays a vital role in multiple translation initiation steps. eIF1 is incorporated into a multifactor complex that includes eIF1A, eIF3, and eIF5 and stimulates the recruiting of the ternary complex (consisting of eIF2 · GTP and the charged Met-tRNAi) to the small ribosomal

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subunit to form the 43S pre-initiation complex (Singh *et al.* 2004). eIF1 acts synergistically with eIF1A to promote continuous ribosomal scanning for AUG codons by stabilizing an open conformation that allows mRNA to pass through the complex (Maag *et al.* 2005; Cheung *et al.* 2007; Passmore *et al.* 2007). It also mediates the assembly of the ribosomal initiation complex at the AUG start codon (Pestova *et al.* 1998). eIF1 dissociates from the complex upon recognition of the AUG codon and this dissociation is necessary to trigger a series of conformational changes leading to the translation elongation phase (Algire *et al.* 2005). Consistent with these roles, *sui1* mutations reduce the affinity of eIF1 for the ribosome and cause premature release of eIF1 at non-AUG codons (Cheung *et al.* 2007). Other *sui* mutations support the involvement of four additional genes in translation initiation fidelity in yeast. Mutations have been isolated in the heterotrimeric eIF2 as *SUI2* (α-subunit) (Cigan *et al.* 1989), *SUI3* (β-subunit) (Donahue *et al.* 1988), and *SUI4* (γ-subunit) (Huang *et al.* 1997), and a mutation in eIF5 corresponds to the *SUI5* mutant (Huang *et al.* 1997).

However, the genetic studies that identified these translation fidelity mutants were conducted only in yeast. It is not known if there are similar mechanisms regulating translation initiation fidelity in multicellular organisms. To address this question, we designed a genetic system to isolate *C. elegans* mutants that have reduced fidelity in AUG start codon selection (Zhang and Maduzia 2010). Mutants were selected on the basis of their ability to express a GFP reporter that contains a GUG codon in place of its native translation start site. Here we report the characterization of two mutants that have mutations in eIF1. Unlike yeast *sui1* mutants, which preferred the UUG codon, these mutants are capable of using a subset of non-AUG codons for translation initiation. Our results are consistent with eIF1 playing a role in the fidelity of AUG codon selection, perhaps by discriminating base-pairing interactions between the codon and anticodon during start-site selection.

MATERIALS AND METHODS

Growth and handling of worms and *C. elegans* strains: Worms were grown on nematode growth medium (NGM) agar plates following standard procedures (Wood 1988). Relevant strains used are as follows:

MT464: *unc-5(e53) IV; dpy-11(e224) V; lon-2(e678) X.*
MT465: *dpy-5(e61) I; bli-2(e768) II; unc-32(e189) III.*
JW105: *dpy-10(e128) sup-39(je5) unc-4(e120)/unc-104(e1265) II.*
AG152: *unc-85(e1414) bli-2(e768) dpy-10(e128) II.*
MT5104: *lin-31(n301) clr-1(e1745) dpy-10(e128) II.*
CB4856: wild type, Hawaiian isolate.
IP415: *nbIs4; eif-1(nb132)/+.*
IP417: *nbIs4; eif-1(nb134)/+.*
IP443: *nbIs4; eif-1(nb132) bli-2(e768)/+ bli-2(e768).*
IP522: *nbIs4; lin-31(n301) eif-1(nb132) + unc-4(e120) +/+ clr-1(e1745) + dpy-10 (e128).*
IP597: *nbIs4; lin-31(n301) eif-1(nb132) + bli-2(e760) +/lin-31(n301) + clr-1(e1745) + dpy-10(e128).*

Mutant screen: Both *eif-1(nb132)* and *eif-1(nb134)* strains were isolated from a screen described previously (Zhang and Maduzia 2010). This screen mutagenized approximately 75,000 genomes with *N*-ethyl-*N*-nitrosourea (ENU) in a reporter strain carrying the integrated reporter transgene *nbIs4*.

Mapping of mutants and gene identification: To map *eif-1(nb132)* to a chromosome, double heterozygous males [*eif-1(nb132)/+*; *nbIs4/+*] were crossed with strains MT464 and MT465, both of which have chromosomes marked with specific mutant genes. On the basis of segregation of these chromosomal markers from the GFP expression phenotype of *eif-1(nb132)* in the F2 generation, *eif-1(nb132)* was found to be linked to chromosome II. Several three-factor or multifactor crosses further localized the mutation to the left of *bli-2*. From strains carrying the double markers *dpy-10 unc-4,* 12 non-Dpy-10 Unc-4 recombinants were isolated and all of them contained *eif-1(nb132)*. From strains carrying the markers *bli-2 dpy-10,* 10 Bli-2 non-Dpy-10 recombinants were isolated and none of them segregated *eif-1(nb132)*. Data from both of these mapping results suggested that *eif-1(nb132)* was located to the left of *bli-2*. From worms with the genotype *lin-31+ dpy-10/+ eif-1(nb132)+*, 19 of 20 Lin-31 non-Dpy-10 recombinants segregated the *eif-1(nb132)* mutation, indicating that *eif-1(nb132)* was between Lin-31 and Dpy-10 (Figure 1A). SNP mapping with CB4856 and *eif-1(nb132) bli-2* strains resulted in a total of 46 Bli-2 non-Eif-1 recombinants and each of these recombination events were found to occur to the right of SNP nbP102 (−2.50), consistent with the location of the *eif-1(nb132)* mutation being to the left of this marker. When SNP mapping with the triple mutant *lin-31 eif-1(nb132) unc-4*, 15 recombinants with phenotype Lin-31 non-Eif-1 non-Unc-4 were found to occur to the left of SNP marker HW25014 (−3.01). Altogether, these mapping results consistently located *eif-1(nb132)* between −3.01 and −2.50 map units.

Candidate gene T27F7.3 was sequenced using DNA lysate prepared from worms either heterozygous or homozygous for the *eif-1(nb132)* or *eif-1(nb134)* mutations. Worms were digested for 60 min at 65° with proteinase K (200 μg/ml) in 5 μl of lysis buffer (10 mm Tris–HCl, pH 8.3, 50 mm KCl, 2.5 mm MgCl2, 0.45% Nonidet P-40, 0.45% Tween 20, 0.01% gelatin). After heat inactivating the proteinase K at 95° for 15 min, 1 μl of the lysate was used as DNA template to amplify the candidate gene in a 25-μl PCR reaction using LongAmp Taq DNA polymerase (New England Biolabs, Ipswich, MA). The sequence obtained from the mutants was compared with the *C. elegans* wild-type reference sequence available at Wormbase (http://www.wormbase.org) using SeqMan from the LaserGene software package (DNASTAR, Inc.).

Construction of plasmids carrying wild-type and mutant eIF1 genes: The T27F7.3 gene was amplified using Phusion high-fidelity DNA polymerase (New England Biolabs, NEB) with wild-type genomic DNA as a template under the following conditions: 98° for 30 sec followed by 35 cycles of 98° for 5 sec, 58° for 30 sec, and 72° for 3 min with a final incubation at 72° for 10 min. The primer pair (TTTCTGCAGACATCTCTCG CATC and TAGTTATGACGATGATGACTGGG) amplified a 4967-bp fragment that included a 1123-bp sequence 5′ to the first predicted exon of eIF1, a 2719-bp region containing all exons and introns and a 1125-bp sequence 3′ to the last exon. USER cloning sequence adaptors were attached to the above PCR product using Taq DNA polymerase following a short PCR at 94° for 30 sec followed by 5 cycles of 94° for 5 sec, 53° for 30 sec, and 65° for 4 min, and a final step at 65° for 10 min. The same primer pair was used except they also contained USER cloning sequence adaptors [*GGAGACA*(dU) and *GGGAAAG* (dU)] at their 5′ ends. The PCR product was inserted into the pNEB206A vector following the instructions in the USER Friendly cloning kit (NEB).

Constructs containing eIF1 mutants were generated from the above plasmid carrying the wild-type gene with primers containing the mutant nucleotides using the Phusion site-directed mutagenesis kit (NEB). The primers are (mutant nucleotide bases are boldface):

nb132(C65G): GGAACTATTGTCGAGCATCCAG, ATTGC**C**ACTGTGCTTCTGAAATC; *nb134(G83R)*: GTTATCCAATTG ACA**A**GAGATCAGCGTGAC, CTCTCCATATTCTGGATGCTC GACAATAG; *sui1-1(D84G)*: CAATTGACAGGAG**G**TCAGCGT GACAAG, GATAACCTCTCCATATTCTGGATGCTC; *sui1-4 (D84Y)*: CAATTGACAGGA**T**ATCAGCGTGACAAG, GATAAC CTCTCCATATTCTGGATGCTC; *sui1-17(Q85P)*: GACAGGAG ATC**C**GCGTGACAAGGTC, AATTGGATAACCTCTCCATATT CTG; *mof2-1(G108R)*: CAGAGTGCAC**C**GTTTCTAAGC, CAGT TGGACTCATTTACGATAC.

Generation of transgenic worms coexpressing eIF1 mutations and non-AUG reporters: Extrachromosomal arrays were generated by injecting into the gonad with a mixture of 5 ng/μl of eIF1 plasmid, 12 ng/μl of non-AUG reporter, and 50 ng/μl of pRF4 following standard *C. elegans* transgenic procedures (Mello and Fire 1995). Non-AUG reporters were described previously (Zhang and Maduzia 2010).

Suppression of unc-62(t2012) by eif-1(nb132): A strain with the genotype *nbIs4;+/eif-1(nb132); +/eT1; unc-62(t2012) dpy-11(e224)/eT1* was constructed to obtain Dpy worms with the homozygous genotype *unc-62(t2012) dpy-11(e224)*. Two types of Dpy worms were found: Dpy that expressed GFP due to the presence of *eif-1(nb132)*/+ and Dpy worms that did not and thus lacked the *eif-1* mutation. These two types of Dpy worms were individually placed onto plates to examine if they produced progeny.

Determination of GFP expression: Visual scoring of GFP levels was performed using a Leica MZFLIII microscope with the objective set at 5× zoom. Fluorescent images were captured with a Zeiss Axiovert 200M microscope using a 10× objective. The intensities of the fluorescent signals of entire images containing between 5 and 10 worms were analyzed and displayed in bar graphs as an average GFP intensity, which represents a mean gray value per pixel. GFP protein expressed from an identical number of worms for each mutant strain using Western blot analysis. Detailed procedures for determining GFP expression were described previously (Zhang and Maduzia 2010).

RESULTS

Missense mutations in a conserved structural interface of eIF1 lead to expression of a non-AUG reporter: We have previously described a reporter system for isolating mutants that permits the use of non-AUG start codons in translation initiation (Zhang and Maduzia 2010). This reporter contains a GTG codon rather than an ATG codon at the start site of the GFP coding region. The GFP is not expressed from the reporter in wild-type worms due to a robust fidelity in translation initiation site selection, which allows translation to start only from the AUG codon. Mutants were isolated on the basis of their ability to allow GFP expression from this reporter in classic genetic screens. Here we describe the characterization of two mutants, *eif-1(nb132)* and *eif-1(nb134)*.

To determine the identity of genes mutated in strains carrying the *nb132* allele, we performed standard three-factor and SNP mappings to determine its chromosomal position. These mapping results consistently placed *eif-1(nb132)* between DNA polymorphisms HW25014 and nbP102 on chromosome II (Figure 1A). Close examination of the genes within this region focused our attention on T27F7.3, since it is predicted to encode the *C. elegans* initiation factor eIF1. The genomic DNA region of T27F7.3 was PCR amplified from mutant worms and sequenced. A single base mutation was found in *eif-1(nb132)* changing a T to a G. Since the *eif-1(nb134)* mutant strain had abnormalities similar to that of *eif-1(nb132)*, we sequenced T27F7.3 in *eif-1(nb134)* worms and found a single base change, a G to an A, at a different position. To confirm identification of this gene, both of the identified mutations were engineered into T27F7.3 by site-directed mutagenesis and introduced into wild-type worms together with the GUG–GFP reporter. Transgenes containing either the *eif-1(nb132)* or *eif-1(nb134)* mutations, but not the wild-type gene, allowed GFP expression from this non-AUG reporter (Figure 1B).

T27F7.3 has a unique gene structure and its largest intron within this gene is predicted to contain an unrelated open reading frame that consists of the coding sequence of T27F7.4 (Figure 1C). T27F7.3 is predicted to encode the only eIF1 in the *C. elegans* genome. The splicing pattern of T27F7.3 was confirmed by our RT–PCR analysis (data not shown) and numerous EST clones in Wormbase. The T27F7.4 transcript is predicted to have all of the exons of T27F7.3, but the eIF1 coding frame is interrupted by an out-of-frame coding region that encodes a mannosyltransferase III involved in glycosylphosphatidylinositol anchor biosynthesis. The *eif-1(nb132)* and *eif-1(nb134)* mutations affect only the eIF1 coding region although they are likely present in the 3′-UTR of the mannosyltransferase mRNA (Figure 1C). Interestingly, we surveyed the presence of this special gene structure and found that it is conserved in all examined nematode genomes including *C. briggsae*, *C. remanei*, *Pristionchus pacificus*, and *Brugia malayi*. We examined the expression of these two genes by fusing GFP in frame with each of the respective coding regions. GFP expression was observed in a number of cells in transgenic animals carrying the eIF1::GFP fusion with higher expression levels appearing in the pharynx and intestine (Figure 1C), but was undetectable in worms carrying the mannosyltransferase III::GFP fusion (Figure 1D).

C. elegans eIF1 is a 109-amino acid protein that shares high-sequence homology with other eIF1s found in eukaryotes ranging from yeast to humans (Figure 2A). The *eif-1(nb132)* mutation substitutes an amino acid of similar size changing a cystine to a glycine at position 65 (C65G), and *eif-1(nb134)* leads to a G83R substitution that increases both the size of the side chain and the overall net charge of the protein (Figure 2A). Both affected residues are identical among all eukaryotic eIF1s examined. These mutations occur at residues not affected by previously identified yeast *sui1* mutations. Strikingly, when the mutations are mapped to the human eIF1 crystal structure, both the *C. elegans* and

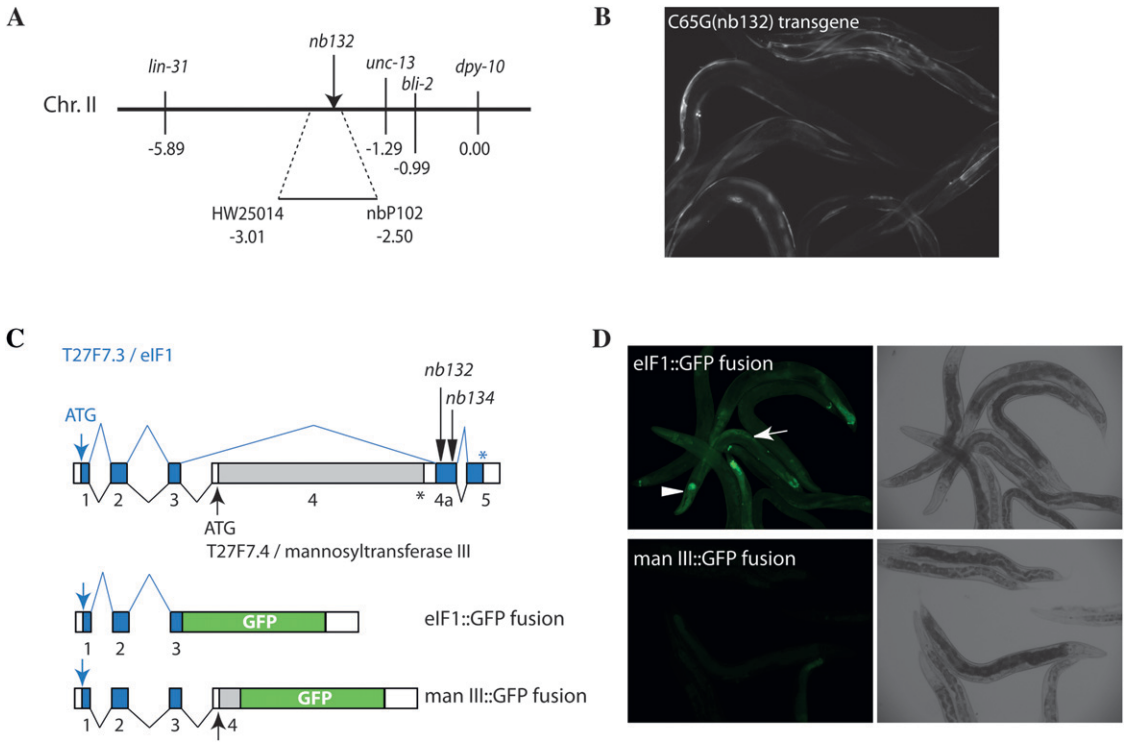

Figure 1.—Identification of mutations in eIF1. (A) Chromosomal location of *eif-1(nb132)*. (B) GFP expression from extrachromosomal arrays coexpressing GUG–GFP reporter and the *eif-1(nb132)* (C65G) mutant eIF1. (C) eIF1 is an alternatively spliced open reading frame. T27F7.3 (exons 1, 2, 3, 4a, and 5 with blue lines indicating splicing) contains the continuous coding region of eIF1. T27F7.4 (exons 1, 2, 3, 4, and 5 with black lines indicating splicing) contains the coding region of mannosyltransferase III (shown as shaded region in exon 4), which interrupts the coding region of eIF1. GFP fusion constructs for examining the expression of the two open reading frames are shown at the bottom. (D) Fluorescent images (left) and corresponding DIC images (right) of worms expressing GFP-fusion transgenes. Arrowhead, GFP expression in the pharynx; arrow, expression in the intestine.

yeast mutations localize to the same structural interface (Figure 2B). Thus, the *C. elegans* eIF1 mutations and *sui1* mutations may affect a similar molecular process.

Mutations in eIF1 are dominant for GFP expression of non-AUG reporters and recessive for growth defects: Both *eif-1(nb132)* and *eif-1(nb134)* strains showed a high level of GFP signal expression from the GTG-containing GFP reporter in heterozygous (Figure 3, B and C) or homozygous (Figure 3, E and F) animals. Worms carrying the *eif-1(nb132)* allele had a higher level of GFP expression than that of *eif-1(nb134)* mutant animals, both as heterozygotes or homozygotes (Figure 3G). Western blot analyses with heterozygous animals also indicated that the GFP protein expressed is more abundant in mutant animals than in wild-type animals. Additionally, the GFP produced is the same size as the GFP synthesized from a reporter carrying a native ATG start codon (Figure 3H).

Homozygotes of either mutant also show growth arrest defects. *eif-1(nb132)* worms were able to grow through the four larval stages. However, they arrested as late L4 worms (9/9 L1 larvae examined) and could not develop into adults. The developing vulva and gonad, both characteristic of L4 development, were visible in these arrested worms but no oocytes were found in the gonad. In contrast, *eif-1(nb134)* homozygous worms arrested at the L1 larval stage (10/10 L1 larvae examined). A similar L1 larval arrest phenotype was observed when eIF1 activity was knocked down by RNA interference upon injecting eIF1 dsRNA (data not shown). This suggests that the growth arrest phenotype associated with these two mutations resembles the loss-of-function phenotype resulting from the RNAi knockdown of eIF1, and the *eif-1(nb134)* mutation appears to cause more severe detrimental defects.

Genetic suppression of an initiator AUG codon mutation in *unc-62(t2012)* by *eif-1(nb132):* Since *eif-1* mutants allowed GFP expression from transgenes containing a non-AUG reporter, we wondered whether they might also allow expression of endogenous mRNAs that contain a non-AUG codon at the translation start site. Previously, we used genetic suppression experiments to demonstrate that the eIF2β mutant *iftb-1(nb101)* was able to initiate translation from endogenous mutant *unc-62(t2012)* mRNAs containing an AUA codon in place of its AUG start codon (Zhang and Maduzia 2010). We performed similar genetic suppression experiments with *eif-1(nb132)*. Mutation of the AUG start codon to AUA in *unc-62(t2012)* animals causes a maternal-effect lethal phenotype such that homozygous worms grow up normally but their progeny arrest as either embryos or early larvae (Van Auken *et al.* 2002).

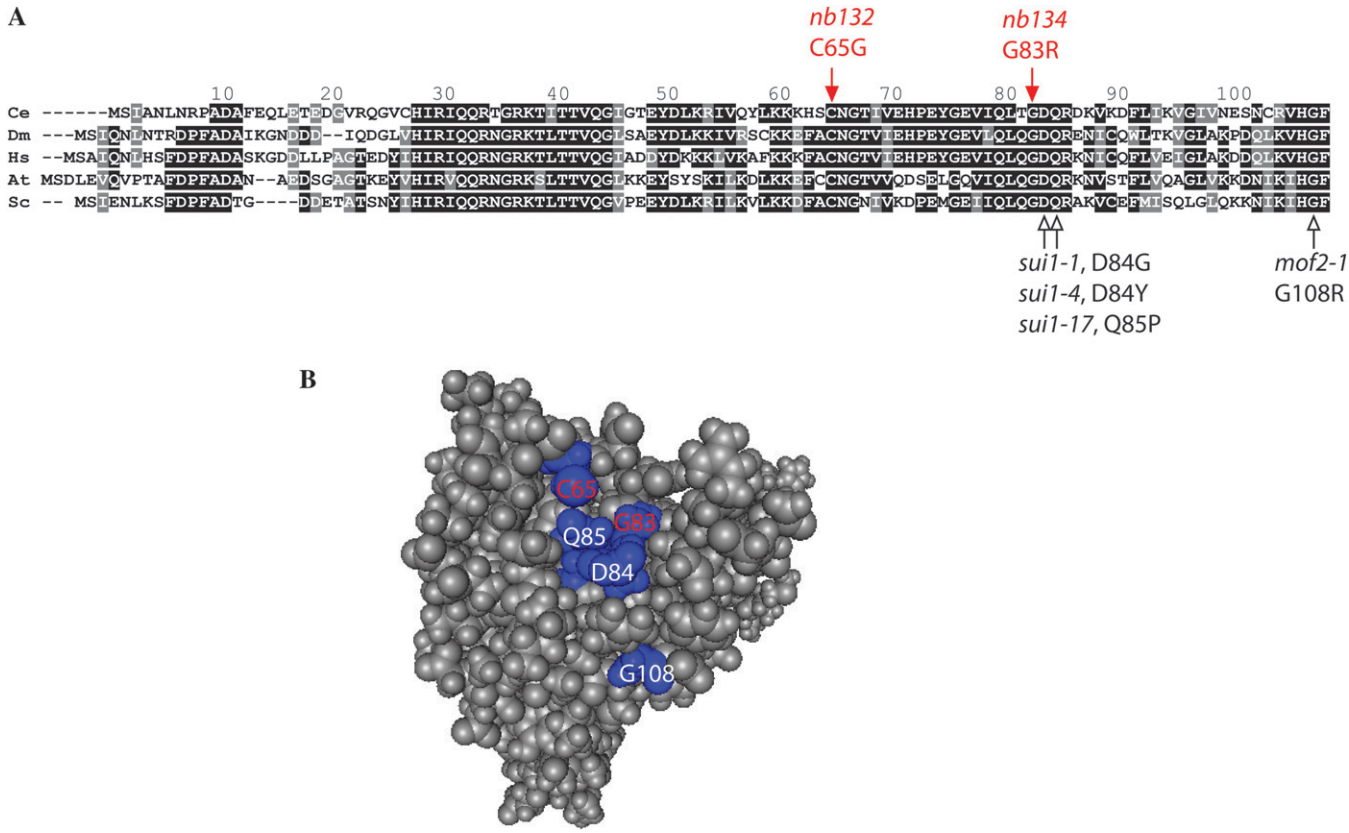

FIGURE 2.—Amino acid substitutions in eIF1 mutants. (A) Multiple sequence alignment of selected eukaryotic eIF1s. The amino acid changes in *eif-1(nb132), eif-1(nb134),* and yeast eIF1 mutations (*sui1-1*, *sui1-4*, *sui1-17*, and *mof2-1*) are shown and labeled according to the *C. elegans* sequence. Short names for species are: Ce, *C. elegans*; Dm, *Drosophila melanogaster*; Hs, *Homo sapiens*; At, *Arabidopsis thaliana*; Sc, *Saccharomyces cerevisiae*. Identical residues are on a black background and similar residues are on a gray background. (B) Positions of amino acid residues of eIF1 mutations on the human eIF1 structure (2IF1) (FLETCHER *et al.* 1999). Side chains affected by mutations are highlighted blue and the remaining structure gray. The residues affected by *C. elegans eif-1(nb132)* (C65) and *eif-1(nb134)* (G83) mutations are numbered in red and yeast mutations (D84, Q85, and G108) in white.

We found that the presence of the *eif-1(nb132)* mutation improves the viability of *unc-62(t2012)* worms. Out of nine *unc-62(t2012)* worms that are also heterozygous for the *eif-1(nb132)* mutation, two of them produced a total of four larvae that grew to either L4 larvae or sterile adults. In contrast, all 11 *unc-62(t2012)* worms with wild-type eIF1 in the background produced only dead embryos. Suppression of the maternal effect lethal phenotype of *unc-62(t2012)* by *eif-1(nb132)* indicates that, similar to *iftb-1(nb101)*, the *eif-1(nb132)* mutation also allows translation of endogenous mRNAs containing non-AUG codons at the translation start site.

eIF1 containing the *eif-1(nb132)* or *eif-1(nb134)* mutations allows translation to start at a subset of non-AUG codons: Since the eIF1 mutants were isolated using a non-AUG reporter that contains a GUG codon at the site of the native AUG start codon, we set out to determine if these mutants might also permit expression of GFP from reporters carrying other non-AUG codons using a transgenic assay we previously used to study dominant eIF2β mutants (ZHANG and MADUZIA 2010). In this assay, transgenic worms that coexpress a mutant eIF1 and a GFP reporter containing an altered start codon were examined for GFP expression. The GFP expression level was visually scored under a fluorescent microscope (Table 1). The scoring results generally correlated well with a separate analysis using fluorescent images to estimate GFP brightness on the basis of average intensity of fluorescent signals (Figure 4).

We assayed reporters containing all possible single base changes in the AUG start codon as well as a reporter with two base changes. When the wild-type eIF1 transgene is present, little to no GFP expression is detected in transgenic worms containing these reporters (Table 1, column *wt*; Figure 4A). With either the *eif-1(nb132)* or *eif-1(nb134)* mutant transgene, some of the reporters show increased GFP expression. Reporters that have one base change at the first or the third base position within the AUG codon express GFP (Table 1, lines 1–3 and 7–9; Figure 4, A, C, and D). An exception was observed in multiple transgenic lines where *eif-1(nb134)* did not result in an increase in GFP expressed from a reporter containing AUC at the translation start site (Table 1, line 8; Figure 4D). Changes at the second base position to a C (and thus the codon ACG) allow GFP expression; however, changes to either an A or G (codons AAG or

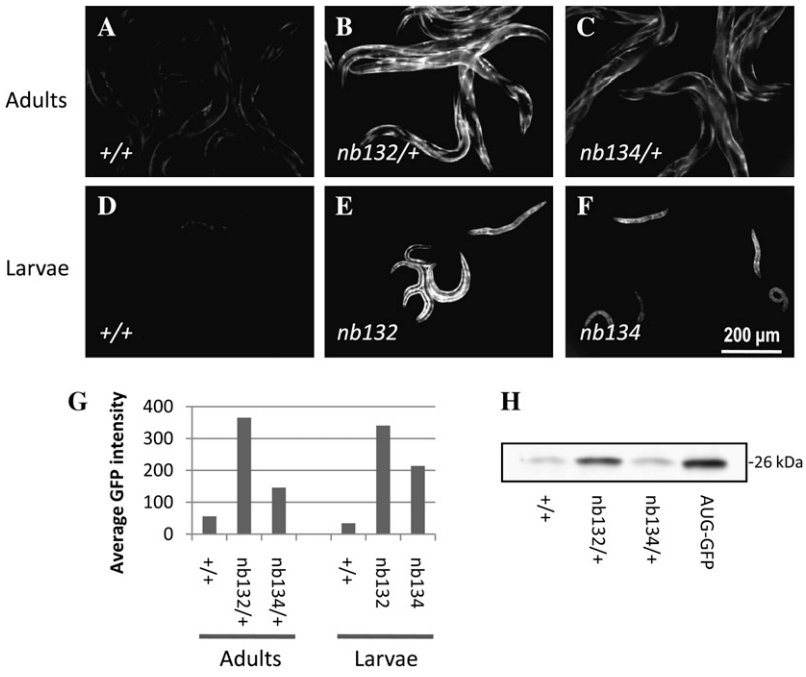

FIGURE 3.—GFP expression from a GFP reporter containing a GUG codon at the start site in eIF1 mutants. GFP images are shown for adult worms (A–C) and L1 larvae (D–F) with respective genotypes. (A) +/+, wild type. (B) *eif-1(nb132)/+*. (C) *eif-1(nb134)/+*. (D) +/+, wild type. (E) *eif-1(nb132)*. Image also contains a few L2 larvae. (F) *eif-1(nb134)*. (G) GFP signal intensity from images A–F determined by digital image analysis. (H) Western Blot analyses showing GFP expression. Each lane contains protein lysate from 100 adult worms with the exception of the AUG–GFP reporter, which contains lysate from 40 worms. A much higher level of GFP expression is observed from the AUG–GFP reporter, which contains a proper translation start codon. All worms carry the *nbIs4* integrated chromosomal array consisting of the GUG–GFP reporter with the exception of the AUG–GFP sample, which contains extrachromosomal arrays.

AGG) do not (Table 1, lines 4–6; Figure 4, B and D). Also, no expression was observed from the reporter that contained two simultaneous base changes (Table 1, line 10; Figure 4D). The relative GFP levels from most reporters co-injected with the *eif-1(nb132)* transgene appeared to be higher than the levels of those same reporters co-injected with the *eif-1(nb134)* transgene (Figure 4D).

***C. elegans* eIF1 carrying an equivalent yeast *sui1* mutation allows translation initiation at a similar subset of non-AUG codons:** In contrast to what we observed with the *eif-1* mutants described above, non-AUG initiation by yeast eIF1 *sui1* mutants occurs at UUG but not with other non-AUG codons (HUANG *et al.* 1997). To address whether eIF1 carrying these *sui1* mutations are able to confer a similar phenotype in our reporter system, we constructed mutations in the *C. elegans* eIF1 gene (Figure 2A) corresponding to all known *sui1* alleles (*sui1-1*, *sui1-4*, and *sui1-17*) isolated in the *His4* suppressor screens (YOON and DONAHUE 1992) and *mof2*-1, which was first isolated as a mutant with an altered frameshift efficiency but later found to have a reduction in start codon recognition fidelity as well (CUI *et al.* 1998). Since *sui1* mutations are dominant or codominant in yeast (YOON and DONAHUE 1992), it is possible that these mutations also behave dominantly in *C. elegans*, allowing the detection of their defects in our transgenic assay.

These mutants were initially assayed with non-AUG GFP reporters carrying GUG or UUG codons. The *C. elegans* eIF1 transgene carrying the *sui1-4* mutation promoted GFP expression from both GUG and UUG reporters unlike transgenes carrying the wild-type eIF1 and the mutant eIF1 genes containing the *sui1-1*, *sui1-17*, or *mof2-1* mutations (data not shown). These results indicate that only the *sui1-4* mutant was able to confer non-AUG translation in the *C. elegans* reporter system, which is in contrast to that observed in yeast where all these mutations allowed translation to start at the UUG codon.

We further assayed the ability of the *sui1-4* mutant to allow GFP expression from reporters containing other non-AUG start codons. GFP expression was observed from additional reporters that changed a single base either at the first or the third base position of the AUG codon (Table 1, lines 1–3 and 7–9; Figure 5, A, C, and D). An exception to this trend was seen in worms carrying a reporter containing the codon AUC. No GFP was expressed in these worms (Figure 5, C and D), similar to what was observed with this reporter in worms containing

TABLE 1

GFP expression from non-AUG reporters coexpressing eIF1 mutants

Line	Reporter	*wt*	*nb132 C65G*	*nb134 G83R*	*sui1-4 D84Y*
1	CUG	+/−	++	+++	++
2	GUG	+/−	+++	+++	++
3	UUG	—	+++	+++	++
4	AAG	—	—	—	—
5	ACG	—	++	++	++
6	AGG	—	—	—	—
7	AUA	—	+++	++	++
8	AUC	—	+++	—	—
9	AUU	+/−	+++	++	++
10	UUC	—	—	—	—

The GFP expression intensity was scored under a dissecting microscope as follows: —, no expression; +/−, weak expression in rare animals; +, weak; ++, moderate; +++, high.

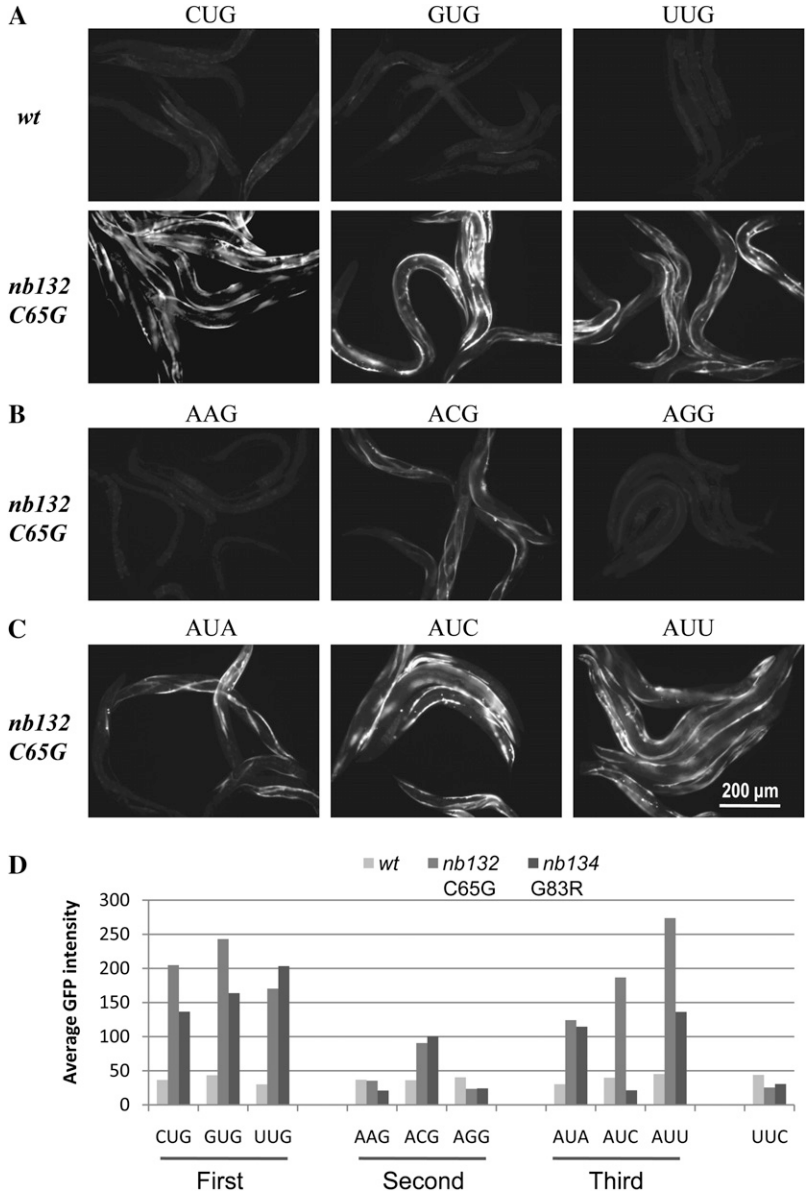

FIGURE 4.—Expression of GFP from non-AUG reporters coexpressing *eif-1(nb132)* or *eif-1(nb134)* mutant eIF1. (A–C) Fluorescent images for non-AUG reporters expressed with the *eif-1(nb132)* (C65G) transgene. (D) Expression for *eif-1(nb134)* (G83R) is summarized in the image analyses without showing GFP images. Fluorescent images for non-AUG reporters containing changes at the first base position within the AUG codon coexpressed with the wild-type eIF1 transgene (A, top row) are representative of reporters with changes at the second and third base positions (not shown) since low expression levels of GFP are comparable among these strains. (A) Changes at the first base position of the AUG codon (CUG, GUG, and UUG). (B) Changes at the second position (AAG, ACG, and AGG). (C) Changes at the third base position (AUA, AUC, and AUU). (D) Average intensity of GFP signals determined by digital image analysis.

an *eif-1(nb134)* mutant transgene (Figure 4D). When the second base was changed, increased GFP expression was observed only when it was changed to a C but not when it was changed to a G or an A. Thus, eIF1 containing the *sui1-4* mutation allows translation initiation to occur at a subset of non-AUG codons similar to what was observed for the *eif-1* alleles (*nb132* and *nb134*) in this *C. elegans* assay, as opposed to observations in yeast where non-AUG translation initiates only from the UUG codon.

DISCUSSION

In this study, we have isolated and characterized eIF1 mutants in the multicellular organism *C. elegans*. Several lines of evidence support that these eIF1 mutants have a defect in selecting the AUG start codon. First, the GUG reporter was constructed in such a way that the GFP mRNA has no in-frame AUG codon available for the synthesis of a functional GFP protein (ZHANG and MADUZIA 2010). Western blot analysis reveals that the size of GFP expressed from the GUG reporter is the same as that of GFP synthesized from the wild-type GFP reporter carrying a normal AUG start codon, consistent with translation initiating from similar positions on these mRNAs. We also showed that the *eif-1(nb132)* mutant is able to produce functional products from an endogenous mutant mRNA that contains an AUA codon at the translation start site in a genetic suppression experiment. Finally, in comprehensive assays of GFP reporters that have base changes only at the AUG start codon, these mutants allow GFP expression from some but not all reporters. These results are consistent with a notion that translation of these modified mRNAs likely initiates at the non-AUG codons due to a reduced fidelity in start codon recognition in eIF1 mutants, similar to yeast eIF1 *sui1* mutants that allow translation

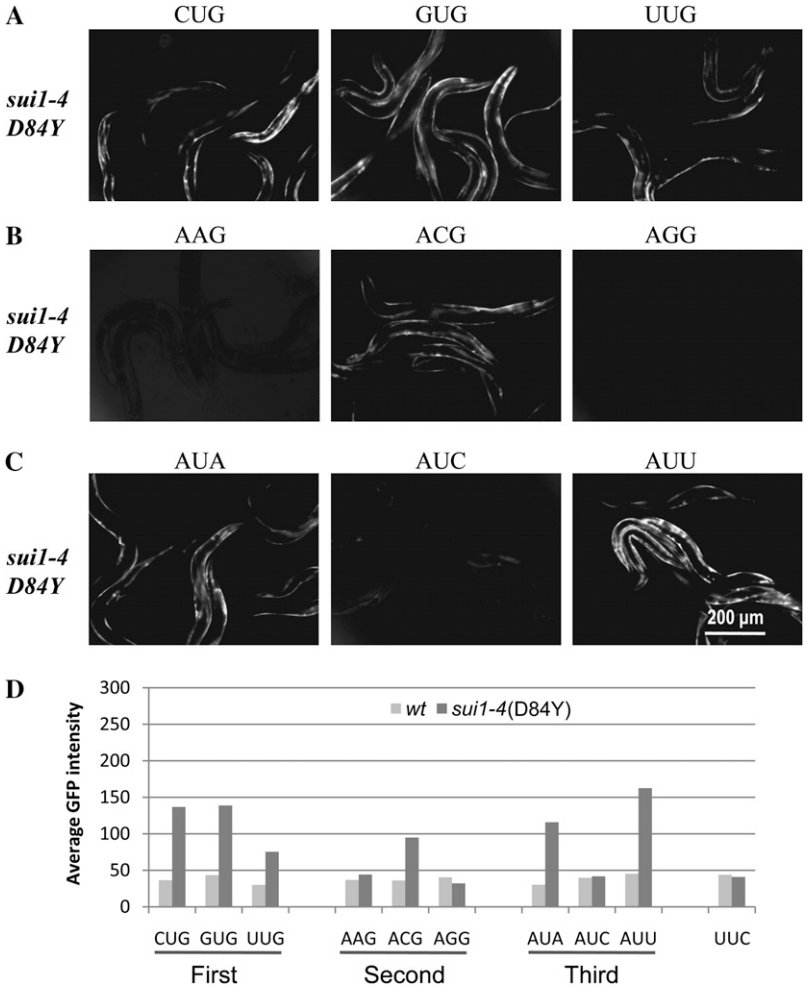

FIGURE 5.—Expression of GFP from non-AUG reporters coexpressing *sui1-4* (D84Y) mutant eIF1. Arrangement is similar to that in Figure 4 except that images from wild-type eIF1 transgene are not shown. (A) Changes at the first base position. (B) Changes at the second position. (C) Changes at the third base position. (D) Average intensity of GFP signals determined by digital image analysis.

initiation of a modified *His4* selection marker gene from a non-AUG start codon (YOON and DONAHUE 1992).

The ability of different non-AUG codons to initiate translation in these *C. elegans* eIF1 mutants is not random. GFP expression was observed only from a subset of reporters containing single base changes within the AUG either at the first or the third position of the start codon. In addition, changing the middle base position to a C (and thus resulting in the codon ACG) also gives considerable levels of expression. There is no detectable expression when two bases are changed. This phenomenon is not limited to mutants isolated from our *C. elegans* screen. The *sui1-4* mutant, the only *sui1* allele that permits non-AUG reporter expression when engineered into the *C. elegans* eIF1, follows a similar trend. These patterns of non-AUG codon usage are comparable with what we observed in *C. elegans* eIF2β mutants (ZHANG and MADUZIA 2010), but are different from that observed in yeast with the *sui1* and other *sui* mutants, which show a much higher rate of translation initiation only at the UUG codon (HUANG *et al.* 1997). As we proposed before (ZHANG and MADUZIA 2010), it is possible that the sequence context surrounding the non-AUG codons in the worm and yeast reporter constructs may contribute to this difference. Interestingly, analogous non-AUG usage is also observed in wild-type yeast (KOLITZ *et al.* 2009) and mammalian cells (PEABODY 1989) where sensitive assays allow the detection of low levels of protein expression from mRNAs with altered AUG start codons. This naturally occurring misrecognition indicates that discriminating two base-paired near-cognate codons from the perfect three-base-paired AUG codon is subject to mistakes. Mutations in translation initiation factors, such as eIF1 and eIF2β, further increase the levels of these mistakes.

The trend we observed in *C. elegans* mutants is best explained by a model where two base-pairing interactions between non-AUG codons and the anticodon of the Met-tRNAi are sufficient to trigger translation initiation, suggesting that wild-type eIF1 plays a role in monitoring proper base-pairing interactions when scanning for the AUG start site. It would be predicted that the Met-tRNAi, not a cognate tRNA matching an individual non-AUG codon, is used in translation initiation at these non-AUG start codons. This prediction is

consistent with evidence in the literature. The translation initiation complex will bind only the Met-tRNAi as opposed to other tRNAs because Met-tRNAi has unique sequence and structural features that allow it to be loaded onto eIF2 of the ternary complex and enable it to fit into the P site of the ribosome (Pestova *et al.* 2007). In addition, it has been demonstrated that Met-tRNAi is indeed used to initiate translation of mRNAs at UUG codons in the yeast *sui1* (Yoon and Donahue 1992) and *SUI3* mutants (Donahue *et al.* 1988).

Localization of mutations on the three-dimensional protein structure suggests that a particular structural interface on eIF1 plays an important role in AUG start codon selection. Both *C. elegans eif-1* and yeast *sui1* mutations are missense mutations affecting amino acid residues that are highly conserved among all eukaryotic eIF1 proteins. Importantly, all affected residues cluster together on a narrow interface (Figure 3B) of the eIF1 crystal structure (Fletcher *et al.* 1999). Since both *C. elegans* and yeast mutations lead to initiation at non-AUG start codons, the colocalization of these affected residues indicates that this structural surface is critically involved in translation start codon selection. It is unclear how this interface interacts with other components in the initiation complex. This interface is known to be important in ribosome binding and the affinity of eIF1 for the small ribosomal subunit is severely reduced when either the *sui1-1* or *sui1-7* mutations are present (Cheung *et al.* 2007). However, these affected residues appear to mediate ribosomal binding indirectly as they are located slightly to the side of the ribosomal binding site of eIF1 (Lomakin *et al.* 2003). Additionally, there is evidence that eIF1 interacts with eIF5 (Reibarkh *et al.* 2008). It is quite possible that this eIF1 interface is involved directly or indirectly in the binding of these two factors.

Interestingly, we find that not all yeast *sui1* mutations behave the same in the *C. elegans* assay as they do in yeast (Yoon and Donahue 1992). When engineered into the *C. elegans* eIF1 gene, only the *sui1-4*(*D84Y*) mutation, but not those of *sui1-1(D84G)*, *sui1-17(Q85P)*, and *mof2-1(G108R)*, allowed detectable expression levels from the non-AUG-codon GFP reporter. Peculiarly, *sui1-1(D84G)* and *sui1-4(D84Y)* mutations affect the same amino acid residue, both resulting in a reduction of the net negative charge of eIF1. D84Y also increases the surface hydrophobicity, which may be more detrimental to specific molecular interactions. It is unclear why the other *sui1* mutations did not show a similar phenotype in our assay. One possibility is that *C. elegans* eIF1 containing the *sui1-1*, *sui1-17*, or *mof2-1* mutations cannot form a proper pre-initiation complex, thus making it impossible to assay for non-AUG translation initiation activity. It is also possible that these *sui1* mutations simply do not allow translation initiation at non-AUG codons in the *C. elegans* reporter system. Either way, these results suggest that there are functional differences between yeast and *C. elegans* eIF1.

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