Mutations in Eukaryotic Release Factors 1 and 3 Act as General Nonsense Suppressors in Drosophila

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ABSTRACT

In a screen for suppressors of the Drosophila *wingless*^{PE4} nonsense allele, we isolated mutations in the two components that form eukaryotic release factor. eRF1 and eRF3 comprise the translation termination complex that recognizes stop codons and catalyzes the release of nascent polypeptide chains from ribosomes. Mutations disrupting the Drosophila eRF1 and eRF3 show a strong maternal-effect nonsense suppression due to readthrough of stop codons and are zygotically lethal during larval stages. We tested nonsense mutations in *wg* and in other embryonically acting genes and found that different stop codons can be suppressed but only a subset of nonsense alleles are subject to suppression. We suspect that the context of the stop codon is significant: nonsense alleles sensitive to suppression by *eRF1* and *eRF3* encode stop codons that are immediately followed by a cytidine. Such suppressible alleles appear to be intrinsically weak, with a low level of readthrough that is enhanced when translation termination is disrupted. Thus the *eRF1* and *eRF3* mutations provide a tool for identifying nonsense alleles that are leaky. Our findings have important implications for assigning null mutant phenotypes and for selecting appropriate alleles to use in suppressor screens.

TRANSLATION termination is controlled by two different classes of release factors (reviewed in Kisselev and Buckingham 2000; Bertram *et al.* 2001). Class I release factors recognize the nonsense codons and catalyze the peptidyl-tRNA hydrolysis necessary for chain termination. Despite their functional similarity, no clear sequence homology exists between the different class I release factors. Two prokaryotic class I release factors, RF1 and RF2, have different stop codon specificity. RF1 catalyzes release at UAA and UAG stop codons and RF2 at UAA and UGA codons. The single eukaryotic class I release factor, eRF1, recognizes all three stop codons. Class II release factors, represented by RF3 in prokaryotes and eRF3 in eukaryotes, enhance the efficiency of protein termination. Although the prokaryotic and eukaryotic RF3 are limited in sequence similarity, they are functionally analogous. Both are thought to remove and recycle class I release factors from the ribosome after peptide release in a GTP-dependent fashion (Freistoffer *et al.* 1997; Zavialov *et al.* 2001).

The structure of human eRF1, deduced from crystallographic data (Song *et al.* 2000), resembles the structure of tRNA: the polypeptide chain of eRF1 is made up of three domains with an αβ sandwich architecture organized in a Y shape. Domain 1 corresponds to the tRNA anticodon stem loop and contains the highly conserved TASNIKS motif, which is the proposed stop codon recognition site (Knight and Landweber 2000; Ito *et al.* 2002; Kisselev 2002). Domain 2 of eRF1 forms the bottom of the Y shape and has a highly conserved tripeptide stretch at its most distal tip. This GGQ motif is invariant in all known class I release factors and is thought to represent the catalytic center for peptidyl-tRNA hydrolysis (Frolova *et al.* 1999; Song *et al.* 2000; Seit-Nebi *et al.* 2001). This domain is the structural equivalent of the aminoacyl acceptor stem loop of tRNA (Song *et al.* 2000). Domain 3 corresponds to the T stem of tRNA, which interacts with elongation factor eEF1a, a protein with GTPase activity. Similarly, domain 3 of eRF1 is necessary for binding to eRF3 (Song *et al.* 2000).

The C-terminal region of eRF3 is highly conserved and is important for translation termination and for interaction with eRF1 (Ter-Avanesyan *et al.* 1993). This region contains the GTP-binding site and is very similar to the GTP-binding region found in eEF1a. The switch from a GDP- to a GTP-bound form may affect the ability of eRF3 to bind the ribosome during translation (Zavialov *et al.* 2001). The N-terminal region is not conserved and is not essential for viability in yeast.

In the yeast *Saccharomyces cerevisiae*, translation termination factors were first identified genetically in screens for omnipotent suppressors that recognize all three stop codons, as well as for allosuppressors that enhance the effect of weak tRNA nonsense suppressor mutants (re-

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viewed in Stansfield and Tuite 1994). The *eRF1* and *eRF3* genes in yeast are known as *SUP45* and *SUP35*, respectively. Mutations in both of these genes show pleiotropic effects. In addition to suppressing premature stop codons, mutants are deficient in respiration, sensitive to temperature and osmotic pressure, and susceptible to the microtubule depolymerizing drug, benomyl (Tikhomirova and Inge-Vechtomov 1996). These effects could be secondary consequences of termination suppression at normal stop codons or may implicate SUP35 and SUP45 in cellular processes independent of translation.

To date, nonsense suppressor screens have not identified translation release factors in any multicellular eukaryote. Genetic screens for specific Drosophila phenotypes have uncovered a mutation in *eRF3*, also known as *Elf*, that produces meiotic spindle defects (Basu *et al.* 1998) and an overexpression phenotype in the embryonic nervous system for a locus subsequently found to be *eRF1* (Maixner *et al.* 1998; Abdelilah-Seyfried *et al.* 2000), but a role for these mutations in nonsense suppression was not assessed. Nonsense suppression at the Drosophila *rosy* locus was accomplished by introducing an amber tRNA suppressor transgene (Doerig *et al.* 1988). Naturally occurring readthrough of premature stop codons has also been observed in the absence of any nonsense suppressors at the *ninaE* rhodopsin locus (Washburn and O'Tousa 1992) and at the *elav* locus in Drosophila (Samson *et al.* 1995).

In this study we describe two suppressors of a hypomorphic nonsense allele of the fly segment polarity gene, *wingless*. Using standard genetic mapping and complementation and cloning strategies, we demonstrate that these two suppressors represent mutations in the Drosophila orthologs of *eRF1* and *eRF3*. In addition, we have characterized a series of *eRF1* alleles isolated in a saturation screen of the region (Lukinova *et al.* 1999). The molecular defects in these alleles confirm previous speculations about functional domains within the eRF1 protein. Finally, we have tested an assortment of nonsense mutations in *wg* and in other embryonically acting genes and find that 8 of 20 alleles tested are suppressed by the *eRF1* and *eRF3* mutations. We show that different stop codons can be suppressed and that suppression appears to depend on the context of the premature stop codon. We propose that the sequence following the stop codon is of critical importance in determining whether the ribosome will release the peptide chain or read through the stop.

MATERIALS AND METHODS

Drosophila stocks and culture: The *wg* allele used in the suppressor screen, wg^{PE4}, carries a nonsense mutation that changes a nonconserved arginine at position 250 into a UGA termination codon (Bejsovec and Wieschaus 1995). The resulting truncated Wg molecule retains some signaling activity at lower temperatures and shows a transport defective phenotype (Hays *et al.* 1997). The deficiency stock [*Df(3L)rdgC-co2, th*^{1} *st*^{1} *in*^{1} *kni*$^{ri\text{-}1}$ *p*^{p}*/TM6C, cu*^{1} *Sb*^{1} *Tb*^{1} *ca*^{1}] and two *P*-element stocks, *l(3)00103* and *l(3)neo28* (recently redesignated as *eRF1* alleles; *P{ry+t7.2 = PZ}eRF*^{100103} *ry*^{506}*/TM3, ry*^{RK} *Sb*^{1} *Ser*^{1} and *mwh*^{1} *P{hsneo}eRF1*^{neo28} *red*^{1} *e*^{1}*/TM3, ry*^{RK} *Sb*^{1} *Ser*^{1}), were obtained from the Bloomington Stock Center. Eight of the *eRF1* alleles described in this article were generated in a saturation screen for cytological region 77A–D (Lukinova *et al.* 1999) and were kindly provided by Mark Fortini. Stocks carrying nonsense alleles of other genes used in our nonsense suppression analysis were obtained from the Bloomington and Umea stock centers (see Table 2). Transgenic stocks used were *UAS-wg*$^{+}$ (Hays *et al.* 1997), *UAS-wg*^{PE4}, and *UAS-wg*^{PE13}, described below. *UAS* transgene-bearing females were crossed with *E22C-Gal4* males to drive high ubiquitous transgene expression in the resulting embryos.

Flies were reared on standard cornmeal-agar-molasses medium and eggs were collected on apple juice-agar plates. To examine cuticle patterns, embryos were allowed to develop fully (24 hr at 25°), dechorionated in bleach, and then transferred to a microscope slide bearing a drop of Hoyer's medium mixed 1:1 with lactic acid (Wieschaus and Nüsslein-Volhard 1986). Cuticle preparations were heated at 65° overnight before viewing with a Zeiss Axioplan microscope.

Isolation and characterization of release factor mutations: We isolated two suppressors of wg^{PE4} in the course of an EMS mutagenesis designed to recover suppressors of *wg* mutant phenotypes. This was a standard F_3 lethal screen performed in a *wg* mutant background and involved examining cuticle preparations from individual isogenized lines. Thus recessive mutations either linked to *wg* on the second chromosome or segregating independently could be assessed for their ability to alter the *wg* mutant phenotype. The original stock designations for the mutagenized lines bearing these suppressors were KY7 and LR16, which are subsequently used to denote the alleles recovered from the lines. KY7 was mapped to the third chromosome, and LR16 was linked to *wg* on the second chromosome. Both mutations are recessive lethal, but show a dominant maternal-effect suppression. To test for suppression of nonsense mutations in *wg* and in other genes, balanced stocks were crossed to $eRF1^{KY7}$, $eRF1^{F2}$, or $eRF3^{LR16}$. Nonbalancer virgin F_1 females were then crossed back to the nonsense allele-bearing stock and F_2 embryos resulting from the cross were collected. Nonbalancer F_1 males were independently crossed back to the nonsense allele-bearing stock to produce unsuppressed embryos in a similar genetic background for comparison. In the case of *wg* and *en* alleles, nonbalancer F_1 females and males were also independently mated to flies bearing RNA-null alleles of the genes (wg^{CX4} and en^{SFX31}).

Plasmid rescue and cDNA cloning: We obtained genomic sequence flanking the *eRF1*-associated *P* elements using a standard plasmid rescue protocol (Bellen *et al.* 1989). Briefly, we extracted genomic DNA from ~50 flies of the *l(3)00103* and *l(3)neo28* stocks. We digested the DNA with *Xba*I and *Eco*RI, respectively, followed by religation, phenol/chloroform extraction, and precipitation. The religated DNA containing the rescued plasmids was resuspended and used to transform DH5α cells. Plasmid DNA was extracted from at least five colonies and subjected to sequence analysis using a modified primer to the terminal repeat sequence (5′-GACGGGACCAC CTTATG-3′). We identified the *eRF1* cDNA by screening a 0- to 24-hr embryonic λZap II library (kindly provided by Andrew Andres), using a rescued flanking genomic fragment from *l(3)00103*. Briefly, we screened ~50,000 plaques and identified 10 positive plaque-forming units. These were converted to plasmids using the λZap II excision strategy (Stratagene, La Jolla, CA). We isolated DNA from the individual

clones and sequenced several of them, including the largest clone with an insert of ~2.3 kb. BLAST searches identified homology to several known eRF1 sequences. Comparison of the flanking sequence from the noncomplementing *P*-element insertions, *l(3)00103* and *l(3)neo28*, shows that they are inserted at −416 and −599, respectively, relative to the unique starting ATG for *eRF1* (Table 1) in the same of two alternative noncoding first exons. The two alternative transcripts predicted by GadFly correspond well with the two bands of ~1.8 and 2.3 kb that we observed on Northern blots probed with an *eRF1* cDNA clone (data not shown).

RT-PCR, PCR, and sequence analysis: RNA was extracted from 0- to 24-hr embryos in TriZol (GIBCO BRL, Gaithersburg, MD). One to five micrograms of total RNA was denatured for 10 min at 70°, quick chilled on ice, and reverse transcribed for 1 hr at 37° or 42° in a 20-μl reaction containing 1 mM of each dNTP, 2.5 μM of oligo(dT)(15) primer, 1× RT buffer, and 5–10 units of avian myeloblastosis RT (Promega, Madison, WI). Five microliters of the reaction was used in a 50-μl PCR reaction containing 200 μM of each dNTP, 1× PCR buffer (with 1.5 mM $MgCl_2$), 2.5 units AmpliTaq (Perkin-Elmer, Norwalk, CT), and 0.2 μM of both forward and reverse primers (5′-CCCAAATTCTTAATCCCCATC-3′ and 5′-GGAA GAAGGTTTGTGTTTTCTC-3′) to amplify an ~1.5-kb cDNA fragment covering the complete open reading frame (ORF) of eRF1. We used the following amplification conditions: initial denaturation of 5 min at 94°, followed by 30 cycles of 94° for 1 min, 58° for 1 min, and 72° for 2 min, followed by a final extension step at 72° for 7 min. For genomic DNA analysis, three fragments were amplified covering the entire coding region of the *eRF1* gene using DNA extracted from adult flies. Fragment 1 covers exons 2–5 and was amplified using primers (5′-GTCCAATAACCGAATGTCAAG-3′ and 5′-AAAGCAGCA TGAGGGAAGAGG-3′) to yield a product of ~1.7 kb. Cycling conditions were as above. Fragments 2 and 3 cover the coding part of the first and last exon, respectively. PCR reactions were as above: for fragment 2, the same forward primer as the one used to amplify the cDNA and the reverse primer 5′-TGCC ATGCATTGTGTATACC-3′ amplify a 272-bp fragment; for fragment 3, 5′-GAGGATGGGGTATCCGTTTAT-3′ and 5′-CAA ATTTGTTCACGTAAAACGG-3′ yield a product of 576 bp. Both *LR16/CyO* adults and *LR16/LR16* embryos (identified by the absence of *CyO-actin-GFP* balancer fluorescence) were used to isolate genomic DNA to sequence the *eRF3* region. Primers for *LR16/CyO* PCR synthesis were 5′-CTGAAGCAGT TTGTAGAAGGAG-3′ and 5′-TTTGTTGACCAGCAGGAC-3′ to amplify a 3.5-kb fragment covering exons 1, 2, and part of 3 and 5′-ATCTGGTCGTGCTGGTCAAC-3′ and 5′-GTTCC GTTCCGTTTTGAATC-3′ to amplify a 1.3-kb fragment covering part of exon 3 to the end of the coding region in exon 7. Primers for *LR16/LR16* PCR synthesis were 5′-TCACCA CCAAGCACAGTAG-3′ and 5′-TTGGTCTTTCAGCCCGTAT CCG-3′ to amplify a 0.9-kb fragment used to verify the sequence mutation. All PCR products were purified using Wizard Prep PCR purification columns (Promega) or the QIAquick purification kit (QIAGEN, Chatsworth, CA) according to the manufacturer's protocol. The purified product was resuspended in 50 μl TE and 2 μl was used for sequencing. All sequencing reactions were performed using the ABI Prism cycle sequencing protocol (ABI). Sequences were viewed using EditView software (ABI).

Transgenic strain construction: Transgenic flies were constructed and analyzed as described in Dierick and Bejsovec (1998), with the following exception. Complementary oligonucleotides containing the single base change corresponding to the wg^{PE4} (5′-GTGAAGACCTGCTGAATGCGACTGGC-3′) or wg^{PE13} (5′-GTGAAGACCTGCTGGATGTGACTGGC-3′) were used to prime PCR synthesis with a hemagglutinin (HA)-tagged *wg* cDNA clone (p*SP65-wg*$^+$; Hays *et al.* 1997) as the template. PCR cycling conditions were as follows: initial denaturation at 95° for 30 sec followed by 12 cycles of 95° for 30 sec, 55° for 1 min, and 68° for 12 min. PCR products were distinguished from the template by digestion with the methylation-sensitive *Dpn*I restriction enzyme. After transformation in XL-1 blue cells (Stratagene), positive clones were sequenced to verify the base change corresponding to the mutant allele and to ensure that no other mutations were introduced during PCR. Insert DNA corresponding to wg^{PE4} or wg^{PE13} was subcloned into a modified form of the pUAST vector, QIAGEN column purified, and used to transform Drosophila *w; Δ2-3/TM3 e* embryos. Several lines for each mutant allele were recovered and tested for expression with the *E22C-Gal4* driver. Lines that showed strong levels of expression as assayed by Western blots were used for further analysis.

Immunoblot analysis: A group of 5- to 9-hr-old embryos were collected and frozen at −80° until use. Proteins were isolated using SDS buffer (50 mM Tris-HCl, pH 6.8/100 mM dithiothreitol/2% SDS/0.1% bromophenol blue/10% glycerol) supplemented with protease cocktail (Roche complete mini-tablets). Protein samples were electrophoresed on 10% polyacrylamide gels and electroblotted to nitrocellulose. Filters were stained with Ponceau S to monitor the transfer and preincubated in Odyssey blocking buffer (LI-COR Biosciences) diluted 1:1 with TBS (10 mM Tris/150 mM NaCl). Filters were incubated with Odyssey blocking buffer diluted 1:1 with TBS-tween (TBS/0.1% Tween-20) and probed with antihemagglutinin antibody (Sigma, St. Louis) at a dilution of 1:1000 or with antitubulin E7 antibody (Developmental Studies Hybridoma Bank) at a dilution of 1:5000. Filters were washed in TBS-tween and reprobed with peroxidase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) at a dilution of 1:10,000. Signals were detected and quantified using the Odyssey infrared imaging system (LI-COR Biosciences). Similar results were obtained using standard Western blotting procedures with the Pierce (Rockford, IL) SuperSignal chemiluminescent substrate detection system.

RESULTS

Isolation of two maternal-effect suppressors of a *wg* nonsense allele: We have performed a series of genetic screens to isolate modifiers of *wg* mutant phenotypes. These screens have mutationally identified new components of the Wg signaling pathway, such as the transcriptional effector *dTCF* (van de Wetering *et al.* 1997; Cavallo *et al.* 1998) and the human tumor-suppressor homolog *dAPC2* (McCartney *et al.* 1999). In addition, mutations that disrupt other aspects of epidermal patterning and cuticle deposition have been isolated and characterized (Ostrowski *et al.* 2002). Several different alleles of *wg* have been used in these screens, all of which make a protein product and some of which retain partial signaling activity. The wg^{PE4} mutation results from a nonsense change that truncates the molecule roughly halfway through the coding region, altering arginine 250 to a UGA stop codon. The homozygous mutant embryos show pattern defects similar to those of a *wg* null mutant at 25° with a continuous lawn of denticles on the ventral surface of the embryonic cuticle (Bejsovec and Wieschaus 1995). Embryos raised at 18° ex-

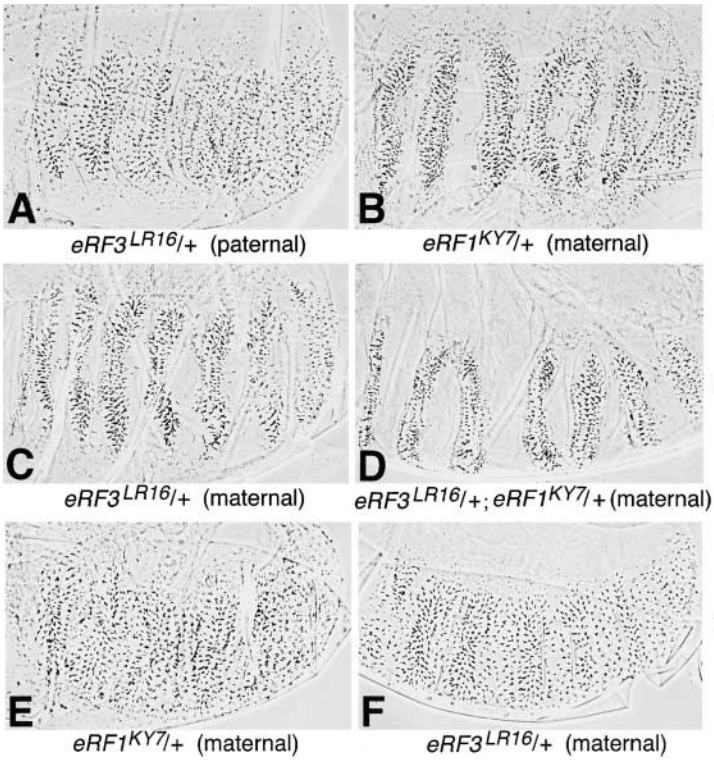

FIGURE 1.—KY7 and LR16 mutations suppress the wg^{PE4} but not the wg^{PE13} mutant phenotype. (A) Embryos derived from wg^{PE4} $eRF3^{LR16}$ heterozygous fathers crossed to wg^{PE4} heterozygous mothers show the typical wg^{PE4} cuticle pattern, which at 25° resembles a *wg* null mutant lawn of denticles. wg^{PE4} homozygous embryos derived from mothers heterozygous for wg^{PE4} and either $eRF1^{KY7}$ (B) or $eRF3^{LR16}$ (C) mutations show a suppressed phenotype with regions of naked cuticle separating the denticle belts. wg^{PE4} homozygous embryos derived from mothers heterozygous for wg^{PE4} and both $eRF1^{KY7}$ and $eRF3^{LR16}$ show even greater suppression (D). wg^{PE13} homozygous embryos derived from mothers heterozygous for wg^{PE13} and either $eRF1^{KY7}$ (E) or $eRF3^{LR16}$ (F) show no change from the original wg^{PE13} mutant phenotype. In this and subsequent figures, embryos are oriented with anterior to the left.

hibit less severe defects and secrete cuticles with small expanses of naked cuticle separating the denticles. This cuticle pattern is similar to that observed for *wg* mutations, which retain signaling activity but disrupt movement of the secreted protein through the ventral epidermal cells (DIERICK and BEJSOVEC 1998). Thus the premature stop codon encoded by the wg^{PE4} allele does not abolish gene activity, but instead results in a product that is compromised for intercellular distribution (HAYS *et al.* 1997).

Flies heterozygous for the wg^{PE4} allele were mutagenized with EMS, and isogenized lines were derived from their progeny as described in OSTROWSKI *et al.* (2002). Two lines, originally designated KY7 and LR16, showed suppression of the wg^{PE4} mutant phenotype at 25° (Figure 1A), with a restoration of naked cuticle separating the denticle belts (Figure 1, B and C). Embryos bearing both mutations show a more complete suppression of the wg^{PE4} mutant phenotype (Figure 1D). For both suppressor lines, the suppression is manifested as a dominant, maternal effect: that is, all of the wg^{PE4} homozygous embryos laid by a female heterozygous for wg^{PE4} and either suppressor mutation show suppression of the *wg* phenotype, whether or not the male parent carries the suppressor mutation. The locus affected by the LR16 suppressor mutation is linked to *wg* on the second chromosome, whereas the KY7 mutation segregated independently and maps to the third chromosome.

Because wg^{PE4} is a nonsense allele, we immediately tested for suppression of other *wg* nonsense mutations. Four other *wg* nonsense alleles, all of which change an amino acid codon to the UGA stop codon, were examined and showed no suppression. This includes wg^{PE13} (Figure 1, E and F), which changes a conserved tryptophan to a premature stop codon at position 248, truncating the gene product two residues prior to the position of the wg^{PE4} lesion (BEJSOVEC and WIESCHAUS 1995). Therefore, we initially concluded that the KY7 and LR16 suppressor mutations were not involved in general nonsense suppression. However, the molecular identities of these suppressor loci, described below, proved otherwise and suggest an interesting explanation for our observations.

Genetic and molecular characterization of the KY7 suppressor mutation: Using standard meiotic mapping and deficiency analysis, we mapped KY7 to cytological region 77A1–77D1 on the third chromosome. *Df(3L) rdgC-co2*, a deficiency for the region, shows the same dominant, maternal-effect suppression of wg^{PE4} as does our KY7 mutation (data not shown), indicating that the dominance is due to haplo-insufficiency for the gene product. Since KY7 segregates independently of *wg*, it was straightforward to assess the mutant phenotype on its own. The KY7 mutation causes recessive larval lethality and the phenotype does not increase in severity when placed *in trans* with *Df(3L)rdgC-co2*. Embryos have no morphological defects and hatch normally. The first instar larvae also look completely normal but fail to grow and eventually die after 7–8 days.

We evaluated the maternal contribution of KY7 by generating germ line mutant clones with the FLP-FRT system (XU and RUBIN 1993). An FRT-bearing chromo-

TABLE 1

***eRF1* mutations**

Allele[a]	Nucleotide change	Amino acid change	Suppression of wg^{PE4}	Comment
K3	G to A	W15@	+	
C1	G to A	A59T	+	
KY7	C to T	Q162@	+	RNA not detected
V2	G to A	R192H	++	
C2	T to A	L345@	+	
U3	G to A	3′ splice site intron 5	++	Premature stop[b]
F2	C to T	Q398@	++	
K7	ORF intact		−	
A7	ORF intact		−	
l(3)00103	*P* element		+	Insert at −416[c]
l(3)neo28	*P* element		+/−	Insert at −599[c]

@, stop codon.

[a] All EMS alleles except KY7 are from Lukinova *et al.* (1999).

[b] Splice site mutation is predicted to cause readthrough into intron 5, adding 28 nonspecific amino acids after E362 before encountering a stop codon in exon 6.

[c] *P*-element insertion sites are relative to the ATG start codon on the basis of expressed sequence tag sequence comparison with the genomic sequence. There are two alternative splice forms in the 5′ untranslated region of *eRF1*, and both insertions are located within the same large first exon.

some carrying the KY7 mutation was constructed and mitotic recombination with an ovo^{D} FRT chromosome was induced by expressing a heat-shock-driven FLP transgene. The resulting female adults were mated with males carrying either the KY7 mutation or a deficiency removing the locus. In both cases none of the flies produced eggs, suggesting that the wild-type gene product is essential for normal egg formation.

Complementation tests with known *P*-element insertions in the region revealed two independently generated *P* elements, *l(3)00103* and *l(3)neo28*, that fail to complement the KY7 lethality (Table 1). We used flanking sequences to probe a cDNA library and characterized clones that define a single transcript with high sequence similarity to human eukaryotic release factor 1. The amino acid identity between Drosophila and other eRF1 proteins ranges from 67% identity with yeast and 72% with Arabidopsis to >84% identity with humans and frogs (Figure 2).

Molecular analysis of *eRF1* alleles: To determine whether the KY7 suppressor line carries a mutation within the *eRF1* coding region, we isolated genomic DNA from heterozygous mutant flies and amplified the *eRF1* gene in three overlapping fragments. We sequenced these fragments and identified a heterozygous C-to-T transition that changes a glutamine at position 162 into a stop codon (Table 1, Figure 2). In addition, we detected several heterozygous nucleotide changes that do not alter the amino acid sequence. To test for nonsense-mediated decay of the KY7 mutant transcript, we extracted RNA from embryos derived from the KY7 mutant stock and performed RT-PCR, amplifying the entire *eRF1* cDNA. Sequence analysis of this fragment showed only the wild-type sequence for Q162 and the silent polymorphic positions, suggesting that the RNA derived from the mutant allele is unstable and that only RNA derived from the wild-type *eRF1* allele on the balancer chromosome is detected. On the basis of this result and the observation that there is no phenotypic difference between KY7 homozygous and KY7/Df embryos, we conclude that the KY7 mutant represents a null allele of *eRF1*.

Subsequently, we characterized eight more alleles of *eRF1* (Table 1) that had been generated in a saturation screen for lethal mutations within the 77A–D cytological region (Lukinova *et al.* 1999). All eight EMS-induced mutations failed to complement the lethality of our KY7 suppressor mutation, and all showed similar homozygous mutant lethal phenotypes. Two alleles, K7 and A7, do not suppress wg^{PE4}. Three alleles, C1, C2, and K3, suppress wg^{PE4} as well as does our original KY7 mutation and the deficiency for the locus. Thus these three alleles are presumed to be loss-of-function alleles. Three alleles, F2, U3, and V2, suppress wg^{PE4} more strongly than do the loss-of-function alleles or the deficiency, and thus their protein products may possess novel or antimorphic properties.

We detected molecular lesions within the coding region (Figure 2) in all alleles except K7 and A7, which do not suppress wg^{PE4} (Table 1). C2 and K3 encode nonsense mutations terminating the polypeptide prematurely. In contrast, C1, which also behaves as a loss-of-function mutation, encodes a missense change in the highly conserved TASNIKS motif that is thought to be essential for stop codon recognition. Two of the antimorphic alleles, F2 and U3, are predicted to cause trun-

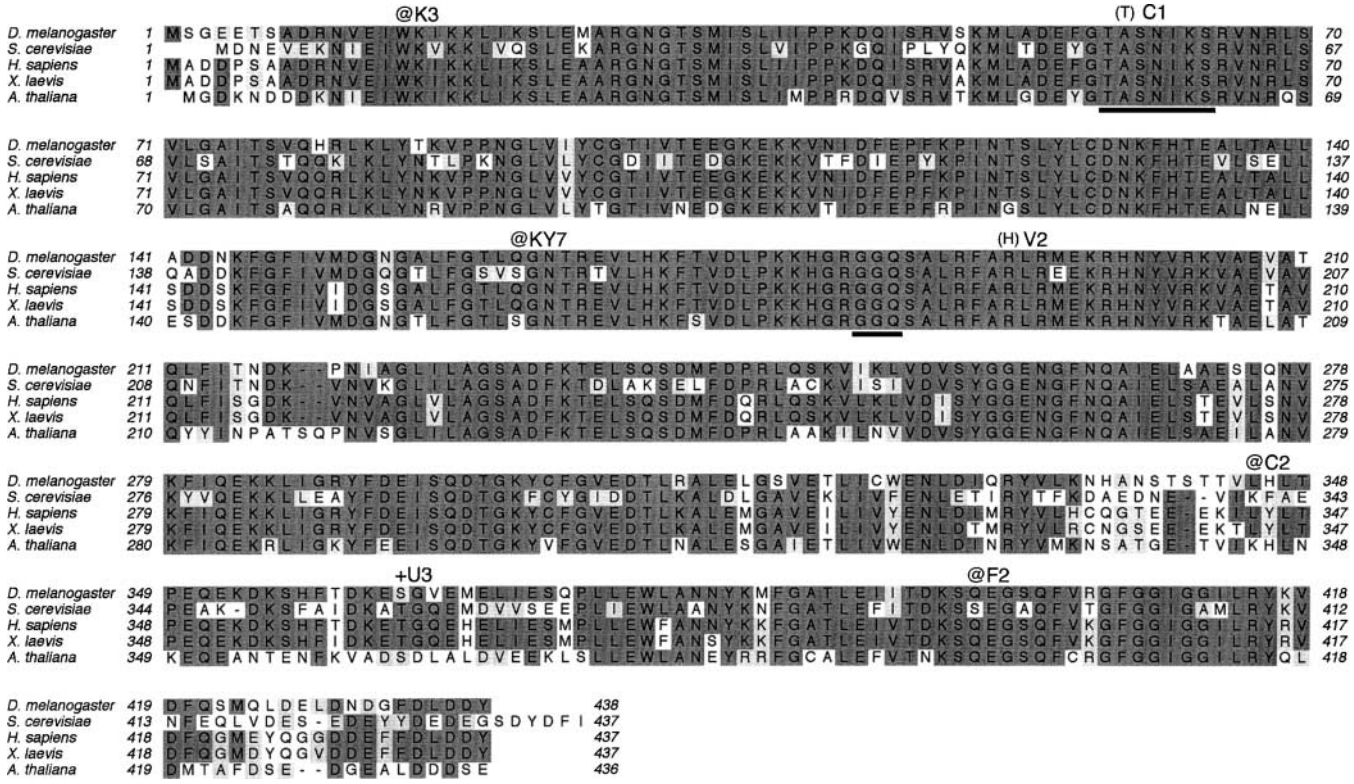

FIGURE 2.—ClustalW alignments for eukaryotic release factor 1. Identical residues are darkly shaded, and similar residues are lightly shaded. The highly conserved TASNIKS and GGQ motifs are underlined. Molecular lesions associated with the *eRF1* alleles are indicated above the Drosophila sequence. @ denotes a stop codon and + a splice site alteration. Missense changes are indicated in parentheses above the altered residue. Protein accession numbers are *Drosophila melanogaster* (Q9VPH7), *S. cerevisiae* (CAA85101), *Homo sapiens* (AF095901-1), *Xenopus laevis* (P35615), and *Arabidopsis thaliana* (AAA91169).

cation at the very end of the protein: F2 replaces Q398 with a stop codon, and U3 alters a splice acceptor site for the last intron. Failure to splice out intron 5 would cause insertion of 28 nonspecific amino acids after position 362 and then termination at a now in-frame stop codon in exon 6. The third antimorphic allele, V2, changes a highly conserved arginine residue at position 192 into a histidine. This region is very close to the presumed catalytic domain of the release factor represented by the GGQ motif. It is curious that this missense change has a dominant negative effect whereas the C1 missense change does not.

Genetic and molecular characterization of the LR16 suppressor mutation: The LR16 mutation was linked to *wg* on the second chromosome and had to be recombined away from the *wg* mutation to determine whether the suppressor mutation causes a detectable phenotype on its own. A recessive lethal mutation was isolated from the LR16 line and found to be associated with suppression by recombining it back onto a different chromosome carrying the wg^{PE4} allele. At the same time, we recombined the lethal mutation onto a chromosome carrying the wg^{PE13} chromosome and found that LR16, like KY7, does not suppress the wg^{PE13} nonsense mutation at position 248 (Figure 1F). As with KY7 homozygous mutants, LR16 homozygous mutants appear morphologically normal but die after several days during the first larval instar.

The LR16 lethal mutation was mapped by meiotic recombination to a position very close to *black* on the second chromosome. Deficiency analysis revealed that the recessive lethal phenotype fails to complement both *Df(2L)Prl* (32F01-3;33F01-2) and *Df(2L)prd1.7* (33B02-03;34A01-2). The region of overlap between these two deficiencies, 33B02–33F02, was scanned for candidate genes. This interval contains the gene encoding eukaryotic release factor 3 (*eRF3*), called *Elf* in the fly (ADAMS *et al.* 2000). Because we had recently characterized *eRF1* mutations from the same wg^{PE4} suppression screen, as described above, we considered *eRF3* the likeliest candidate. A *P*-element insertion at the locus, *P{lacW}Elf[k069091]*, was obtained from the Bloomington Stock Center and was found to be allelic with the LR16 lethal mutation. Sequence analysis reveals that the LR16 line carries a mutation in the *eRF3/Elf* coding region, altering a highly conserved glycine at position 282 to an aspartic acid. This lesion is predicted to disrupt the GTPase activity of the eRF3 protein as it lies within the GTP-binding region that is conserved with EF-1a (Figure 3).

Thus our wg^{PE4} suppression screen has yielded mutations in the two components of the eukaryotic release factor required for translation termination. Both muta-

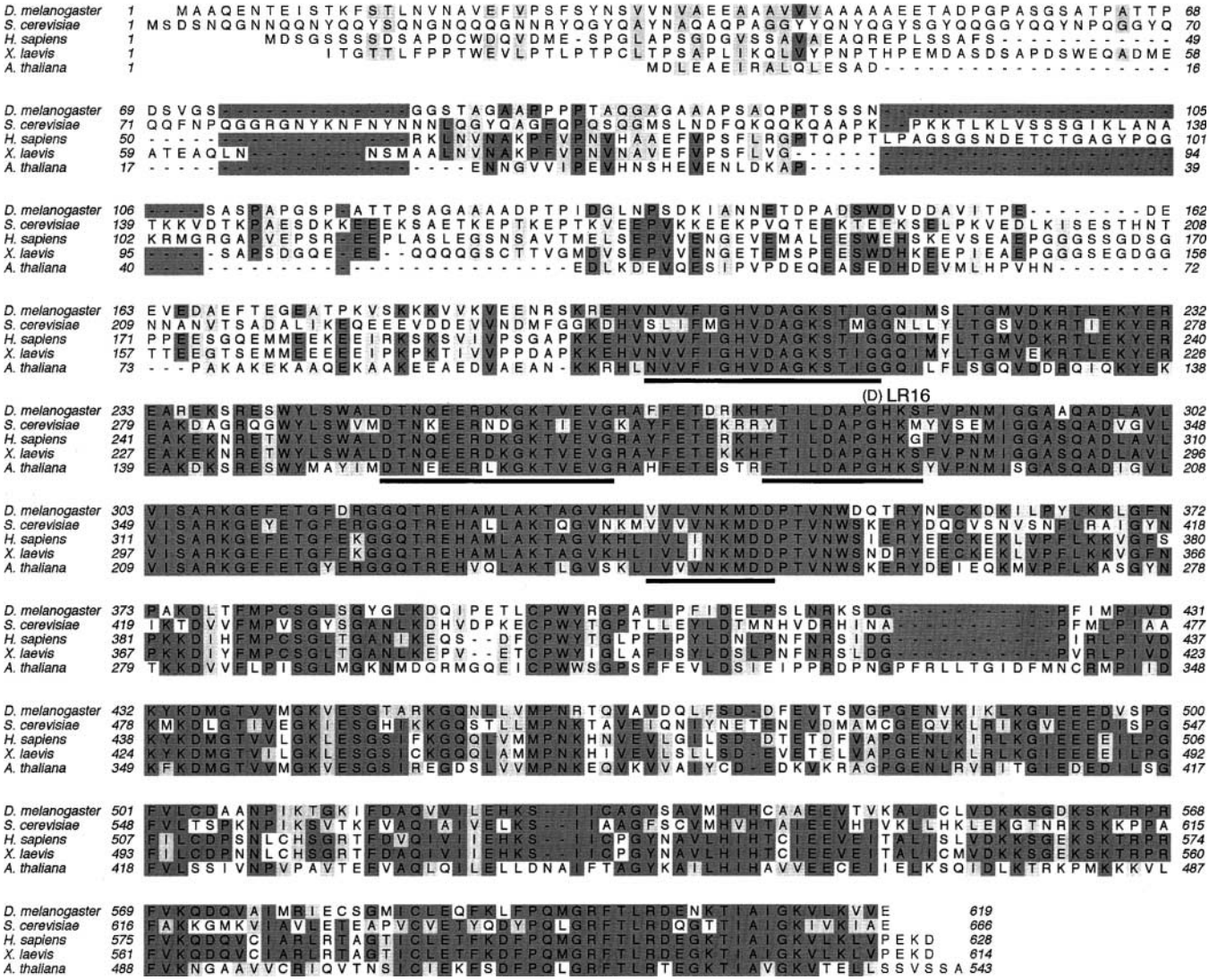

FIGURE 3.—ClustalW alignments for eukaryotic release factor 3. The four domains associated with GTP binding are underlined, and the position of the glycine-to-aspartic acid change found in *eRF3*^{LR16} is indicated above the Drosophila sequence. The Arabidopsis sequence, At1g18070, was identified through a BLAST search of the National Center for Biotechnology Information database and most likely represents the *eRF3* ortholog. The Xenopus sequence is a partial fragment identified by its homology with yeast SUP35. Protein accession numbers are *D. melanogaster* (AAC24943), *S. cerevisiae* (AAK26178), *H. sapiens* (BAA91612), *X. laevis* (S58444), and *A. thaliana* (AAF97824).

tions cause partial suppression of the *wg*^{PE4} mutant phenotype in a haplo-insufficient, maternal-effect fashion. Thus reduction of the maternal contribution of either *eRF1* or *eRF3* may allow some readthrough of termination codons. To test this idea and to reexamine our earlier observation that *wg*^{PE4} is suppressed but *wg*^{PE13} is not suppressed even though these two nonsense mutations lie in close proximity, we analyzed translation products from suppressed and unsuppressed fly strains.

***eRF1* and *eRF3* mutants show increased levels of readthrough *wg* gene product:** Both *wg*^{PE4} and *wg*^{PE13} truncate the Wg protein prior to the 85-amino-acid nonconserved region, which is the primary epitope detected by anti-Wg antibodies (HAYS *et al.* 1997). Therefore, to detect the truncated products derived from these alleles, we engineered the *wg*^{PE4} and *wg*^{PE13} mutations into an HA-epitope-tagged wild-type *wg* transgene. Transgenic lines that express the constructs at high levels were chosen for further analysis. A wild-type HA-tagged *wg* transgene causes naked cuticle to replace the normal denticle belts when it is ectopically overexpressed in a wild-type embryo (HAYS *et al.* 1997 and Figure 4A). Under the same conditions, neither the *wg*^{PE4} nor the *wg*^{PE13} transgene alters the wild-type embryonic cuticle pattern (Figure 4, D and G). However, when the transgenes are crossed into the backgrounds of either the *eRF1* or the *eRF3* mutations, the *wg*^{PE4} transgene now shows detectable signaling activity (Figure 4, E and F). Ectopic expression of the transgene replaces the wild-type cuticle pattern with excess naked cuticle, although not to the

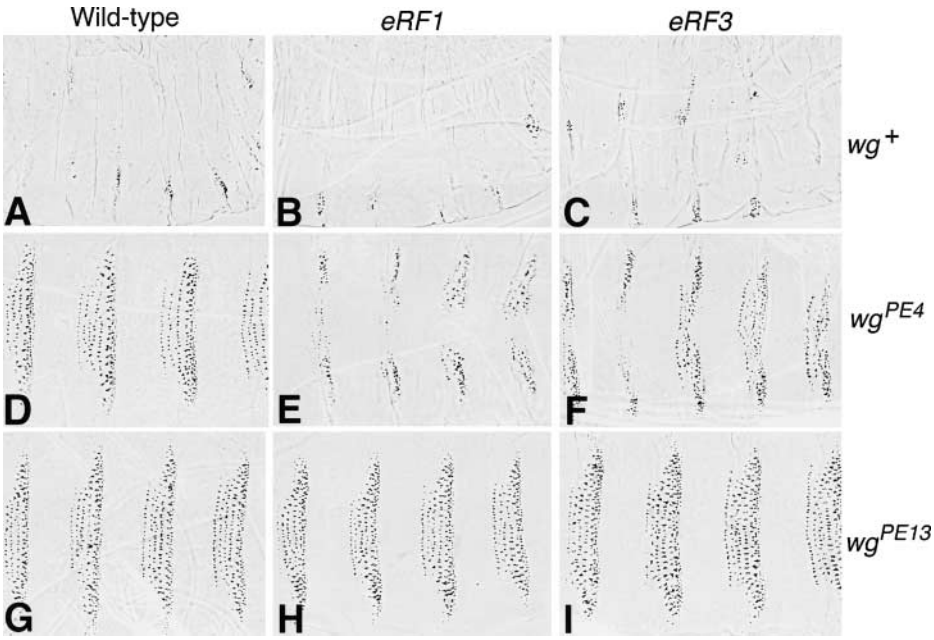

FIGURE 4.—Overexpression of the wg^{PE4} but not the wg^{PE13} transgene hyperactivates the Wg pathway. Embryos derived from mothers homozygous for the *UAS* HA-tagged transgenes crossed to fathers homozygous for the *E22C-Gal4* driver express high levels of transgenic gene product throughout the epidermis. In a wild-type embryo or in embryos mutant for *eRF1* or *eRF3*, ectopic overexpression of the *UAS-wg*$^{+}$ transgene has the same effect: transforming all epidermal cells to the naked cuticle cell fate (A–C). Ectopic overexpression of the *UAS-*wg^{PE4} transgene in a wild-type background does not alter the cuticle pattern (D). However, ectopic expression of the *UAS-*wg^{PE4} transgene produces excess naked cuticle in embryos derived from mothers heterozygous for $eRF1^{KY7}$ (E) or $eRF3^{LR16}$ (F), indicating the presence of functional product. The *UAS-*wg^{PE13} transgene does not alter the cuticle pattern in any genetic background (G–I).

same extent as the wild-type *wg* transgene does (Figure 4, B and C). In contrast, the wg^{PE13} transgene produces no phenotypic effect in either the *eRF1* or the *eRF3* mutant background (Figure 4, H and I). Thus suppression remains allele specific even when the mutant *wg* mRNAs are highly overexpressed.

Western blot analysis with an antihemagglutinin antibody reveals that both transgenes produce a truncated product of the expected size (Figure 5). Even in the wild-type background, a small amount of transgenic full-length Wg is detected in the wg^{PE4} line. Spontaneous readthrough of the nonsense mutation may explain the partial signaling activity observed in the original wg^{PE4} mutant embryos. The temperature sensitivity of the phenotype suggests that either readthrough occurs more frequently or the readthrough product is more active at lower temperature. Due to reduced activity of the Gal4-UAS system at 18°, we cannot test this directly with our transgenes and we are currently exploring other avenues to determine the basis for the temperature-sensitive phenotype.

No readthrough product is detected in the wg^{PE13} transgenic line, even when the transgene is placed in the *eRF1* or *eRF3* maternal mutant backgrounds. In contrast, the low level of full-length readthrough product from the wg^{PE4} transgene increases 2.5-fold when it is placed in the *eRF1* maternal mutant background and increases 2.0-fold when placed in the *eRF3* maternal mutant background. This correlates well with the activity of the transgenes in the cuticle assay. Thus we conclude that the failure of either *eRF* mutant to suppress wg^{PE13} is not due to production of an inactive readthrough product, a formal possibility since wg^{PE13} alters a conserved tryptophan residue whereas wg^{PE4} alters an arginine residue that is not well conserved among the Wnt proteins. Rather, we propose that the failure to suppress wg^{PE13} results from a difference in the context of the UGA stop codon, such that the ribosome responds to the UGA stop codon in wg^{PE4} less stringently. That is, the context of the UGA stop codons in the two mutant alleles must be different even though they are separated by only three nucleotides. To test this idea, we searched for other nonsense mutations with which we might detect suppression. Since the *eRF* mutants show maternal-effect suppression and homozygotes die as larvae, it seemed likely that only embryonic phenotypes would be susceptible to suppression. Therefore, we concentrated our efforts on embryonic lethal mutations that had been characterized at the molecular level and curated in FlyBase.

***eRF* mutations suppress other stop codons in a context-dependent fashion:** We searched FlyBase for zygotic lethal mutations that had been molecularly characterized and found to result from nonsense changes. Since the antimorphic $eRF1^{F2}$ allele gave the strongest suppression of the wg^{PE4} mutant phenotype, we used this allele to test for suppression of nonsense mutations in other genes. We crossed each nonsense mutation into the $eRF1^{F2}$ mutant background to construct female flies heterozygous for both mutations and then crossed these females to males carrying the single nonsense mutation. Simultaneously, we crossed the doubly heterozygous males to females carrying the single nonsense mutation to provide an unsuppressed control for the zygotic lethal phenotype in the same genetic background. Of the 20 nonsense mutations tested, 12 were not suppressed by *eRF1*, confirming that suppression is a rare event (Table 2). Strong suppression was observed for two out of three

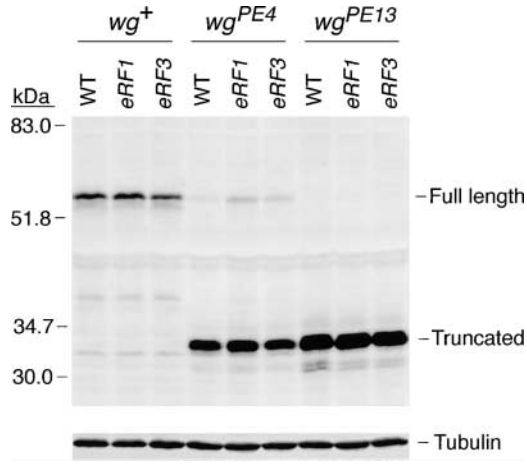

FIGURE 5.—Immunoblot analysis reveals full-length readthrough product from the wg^{PE4} HA-tagged transgene but not from the wg^{PE13} HA-tagged transgene. Embryo lysates derived from the same crosses described in Figure 4 were subjected to Western blot analysis, using antihemagglutinin to detect the transgenic Wg protein. Both the wg^{PE4} and the wg^{PE13} transgene produce abundant truncated product of the predicted size. In addition, full-length product migrating at the same position as wild-type Wg is detected in embryos expressing the wg^{PE4} transgene. This full-length product is detected even in a wild-type background and is more abundant in embryos derived from mothers heterozygous for $eRF1^{KY7}$ or $eRF3^{LR16}$.

engrailed (*en*) nonsense alleles tested (Figure 6) and for two alleles of *schnurri* (*shn*). Two of three *armadillo* (*arm*) alleles and one of three *Epidermal growth factor receptor* (*Egfr*) alleles show slight suppression. Among the alleles suppressed were amber (UAG) nonsense mutations as well as opal (UGA) mutations. Thus mutations in the Drosophila *eRF1* act as general nonsense suppressors, like the yeast *sup45* mutations, in that they can suppress termination at different stop codons and can act on many unrelated genes.

In studying the difference between nonsense alleles that are suppressed and those that are not, we noted that all of the stop codons that are strongly suppressed are immediately followed by a cytidine (Table 2). This result is consistent with the tetranucleotide stop codon hypothesis, which proposes that the identity of the base immediately following natural stop codons may influence the efficiency of translation termination (BROWN *et al.* 1990). Experiments with mammalian genes have shown that the efficiency of termination *in vitro* is lower when a C or a U follows the stop codon than when an A or a G is present in that position (MCCAUGHAN *et al.* 1995). These observations underscore the importance of stop codon context and lead us to propose that our *eRF* mutations allow readthrough preferentially at stop codons that are inherently weak.

DISCUSSION

We have characterized mutations disrupting the Drosophila translation termination machinery and have demonstrated that deficient embryos show increased translational readthrough of a premature stop codon in the *wg* gene. This readthrough correlates with a low level of active gene product, detectable as suppression at the phenotypic level. Even in the absence of the release factor mutations, the wg^{PE4} stop shows a small quantity of readthrough product, suggesting that this stop codon does not terminate translation as efficiently as do other nonsense alleles of *wg*. Curiously, the wg^{PE4} nonsense mutation is temperature sensitive, showing a much weaker phenotype at lower temperatures. This property is shared by nonsense mutations in other genes that are suppressible by *eRF1* and *eRF3* mutations, such as arm^{XM19} and en^{7}. Since these genes encode very different protein products, it seems likely that the temperature sensitivity observed may result from altered termination efficiency at lower temperatures, rather than from increased functionality of the gene product.

Reducing the dose of maternal eRF1 or eRF3 product is sufficient to produce phenotypic suppression of nonsense alleles, but heterozygosity in an otherwise wild-type background does not affect viability. Zygotic loss of function, however, results in a failure to progress beyond the first larval instar. The animals remain alive for roughly a week but do not grow in size or proceed through larval molts. We suspect that this developmental arrest results from accumulation of defective proteins. Natural stop codons may suffer readthrough

TABLE 2

Stop codon context of alleles tested for *eRF1* suppression

Alleles	Mutation	5′	Stop	3′
Suppressed				
en^{4}	W422@	AUG	UGA	CCC
en^{7}	Q52@	CAA	UAG	CAA
shn^{1}	Q1898@	GAC	UAG	CAG
shn^{2}	Q779@	GCA	UAG	CAG
wg^{PE4}	R250@	AUG	UGA	CUG
Weakly suppressed				
arm^{XK22}	K402@	ACU	UAG	GUG
arm^{XM19}	Q681@	CCG	UAG	GAU
$Egfr^{f3}$	W1075@	GUC	UGA	GCC
Unsuppressed				
arm^{YD35}	Q387@	GUG	UAA	AAC
$Egfr^{f2}$	Q267@	ACG	UAA	AAG
$Egfr^{f5}$	W186@	CAG	UAG	UCG
en^{58}	Q497@	GCG	UAG	AUC
exd^{1}	Q62@	GCC	UAG	GCC
os^{upd-4}	Q60@	AAU	UAG	GGC
smo^{3}	W366@	CAU	UGA	AGG
sna^{1}	C285@	GAG	UGA	GGA
wg^{PE3}	R356@	GGA	UGA	AAU
wg^{PE7}	C428@	UGC	UGA	GGG
wg^{PE13}	W248@	UGC	UGA	AUG
wg^{PE16}	W449@	CAC	UGA	UGC

Allele information was derived from FlyBase (http://flybase.bio.indiana.edu/) and the references cited therein. @, stop codon.

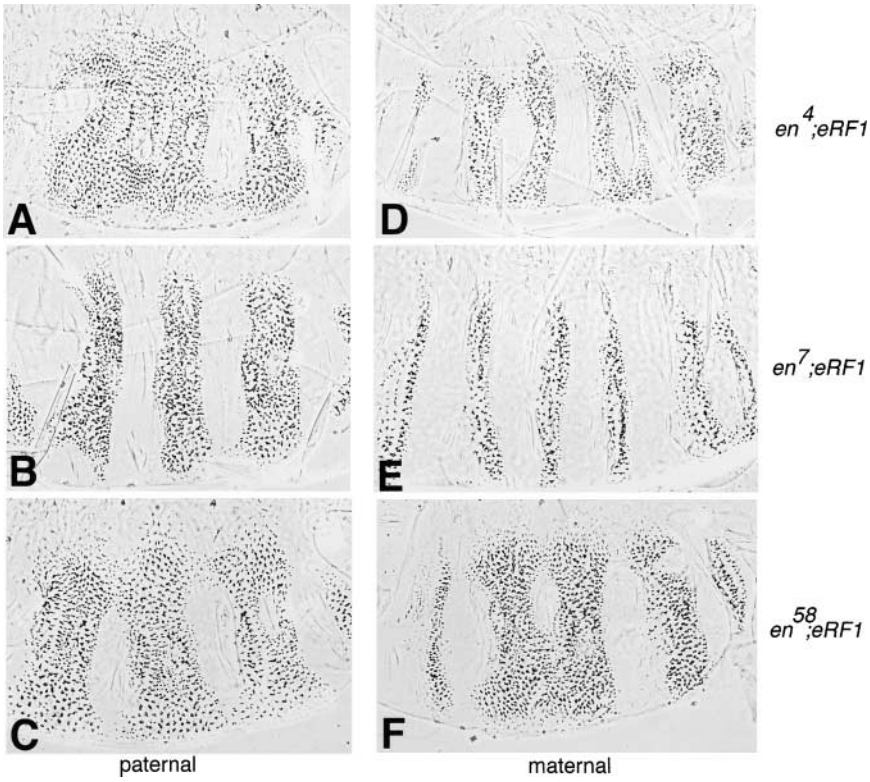

FIGURE 6.—Two of three *engrailed* nonsense mutations are suppressed by $eRF1^{KY7}$. Homozygous *en* mutant embryos derived from fathers heterozygous for $eRF1^{KY7}$ show typical *en* loss-of-function cuticle patterns. Note that the en^{7} mutant phenotype (B) is weaker than that of en^{4} (A) and en^{58} (C). Homozygous en^{4} (D) and en^{7} (E) mutant embryos derived from mothers heterozygous for $eRF1^{KY7}$ show less severe cuticle pattern defects, whereas homozygous en^{58} mutant embryos are not rescued by maternal reduction of *eRF1* (F).

when the maternal contribution of release factor in the zygotic mutants is depleted. In addition, accumulation of defective mRNAs may contribute to the demise of the mutants. eRF1 and eRF3 have been implicated in nonsense-mediated decay as both have been shown to interact with Upf1p, a central component in this process (CZAPLINSKI *et al.* 1998).

Nonsense-mediated decay may also play a role in the allele specificity of suppression. In higher eukaryotes, mRNA-binding proteins added in the nucleus during the splicing process remain associated with the transcript and mark it for destruction unless they are displaced by ribosomal movement in the first round of translation (reviewed in WILUSZ *et al.* 2001). Premature stop codons that terminate translation before these marks are removed result in degradation of the mRNA. Thus premature stop codons that permit readthrough would rescue some transcripts and allow continued translation of the truncated as well as readthrough protein product. While this may account for some of the phenotypic suppression that we observe *in vivo*, we know that it cannot explain the phenotypic difference between wg^{PE4} and wg^{PE13}. Both mutations were engineered into a wild-type *wg* cDNA that lacks introns. Thus the transgenic gene products would not be subject to nonsense-mediated decay. Moreover, substantial amounts of both transgenic truncated proteins are detected on Western blots, but no activity of the wg^{PE13} molecule can be detected in embryos. Therefore, whether or not the endogenous wg^{PE13} transcript is susceptible to decay, blocking that decay would not rescue activity of the resulting mutant protein.

The activity observed for the wg^{PE4} gene product either must result from the small amount of full-length readthrough product or must reside in the truncated molecule itself. In either case, the resulting protein product is compromised for movement (HAYS *et al.* 1997). Thus either the truncation removes signals for proper trafficking of the ligand or the insertion of an inappropriate amino acid at this position disrupts trafficking, as has been demonstrated for missense changes at other positions in the Wg molecule (DIERICK and BEJSOVEC 1998). We do not know if a specific amino acid is inserted during readthrough. In bacteria, plants, and animals, selenocysteine can be incorporated at UGA stop codons in specific contexts (BOCK 2000; TUJEBAJEVA *et al.* 2000; FU *et al.* 2002). Many viral genes contain in-frame stop codons that are read through to produce functional viral proteins, with tryptophan frequently incorporated at UGA codons (ZERFASS and BEIER 1992; HARRELL *et al.* 2002). Tryptophan has also been found to be incorporated at UGA termination codons in bacterial expression systems (MACBEATH and KAST 1998).

These instances of "recoded" stop codons point out the importance of sequence context for translation termination. Our work indicates that nonsense mutations in Drosophila that are followed by a C may be inherently weak and therefore more likely to be suppressed by *eRF1* and *eRF3* mutations. This is consistent with work in yeast showing that a consensus sequence of CA (A/G) N (U/

C/G) A following a stop codon increases the readthrough frequency from a normal background rate of 0.3 to >5% (Namy *et al.* 2001). Viral genomes also harbor a consensus sequence similar to that of yeast associated with recoded stops, and a survey of 91 such viral stop codons reveals that the majority are followed by a C (Harrell *et al.* 2002). Our data suggest that translation termination context in Drosophila is similar to that observed in human cells, where the context preference for the 3′ nucleotide is C < G < U = A (Phillips-Jones *et al.* 1995); this order of preference differs from that of either *Escherichia coli* or *S. cerevisiae* (McCaughan *et al.* 1995).

In our Drosophila experiments, we suspect that the level of gene product is critical in determining suppressibility of a mutant phenotype. Small amounts of readthrough product might not alter the mutant phenotype of certain loci. Some genes for which we tested suppression of nonsense alleles yielded no suppressed phenotypes. In all cases, these alleles contained stop codons that were not followed by a C and so would be predicted to terminate translation more efficiently. However, we cannot definitively state that the context of the stop codon was important for these nonsense alleles because we do not know if a low level of readthrough product would suffice to give a detectable change in phenotype. Therefore, only those genes for which we found suppressible alleles can be considered significant in this analysis: *wingless, engrailed, armadillo, EGF receptor,* and *schnurri.*

Our results indicate that premature stop signals do not necessarily truncate the protein and render the gene product defective. The presence of a nonsense mutation in a gene of interest, even one that occurs very early in the coding region, cannot be taken as proof that the mutant allele is null for function. This point should also be taken into consideration when planning genetic screens for modifiers. In retrospect, wg^{PE4} was not the best allele to use in a screen for modifiers of Wingless protein transport, but was an excellent choice for identifying general nonsense suppressor mutations.

We thank M. Fortini for sending the alleles from his saturation screen of the 77A–D region, some of which we found to be allelic with our $eRF1^{KY7}$ mutation. We also thank Trudi Schüpbach for providing the $Egfr^{f3}$ and $Egfr^{f5}$ alleles. As always, we are deeply grateful to the Bloomington Stock Center for cheerfully sending us legions of flies and to the curators of FlyBase for organizing information so efficiently. We thank the Fehon lab for technical assistance and use of their LI-COR imaging system. We also thank R. Wharton and members of the Bejsovec laboratory for discussions and comments on the manuscript. This work was supported by National Institutes of Health grant GM-59068 and National Science Foundation grant IBN 97-34072 to A.B.

LITERATURE CITED

Abdelilah-Seyfried, S., Y.-M. Chan, C. Zeng, N. J. Justice, S. Younger-Shepherd *et al.*, 2000 A gain-of-function screen for genes that affect the development of the Drosophila adult external sensory organ. Genetics **155:** 733–752.

Adams, M. D., S. E. Celniker, R. E. Holt, C. A. Evans, J. D. Gocayne *et al.*, 2000 The genome sequence of *Drosophila melanogaster.* Science **287:** 2185–2195.

Basu, J., B. C. Williams, Z. X. Li, E. V. Williams and M. L. Goldberg, 1998 Depletion of a *Drosophila* homolog of yeast sup35p disrupts spindle assembly, chromosome segregation and cytokinesis during male meiosis. Cell Motil. Cytoskeleton **39:** 286–302.

Bejsovec, A., and E. Wieschaus, 1995 Signaling activities of the Drosophila *wingless* gene are separately mutable and appear to be transduced at the cell surface. Genetics **139:** 309–320.

Bellen, H. J., C. J. O'Kane, C. Wilson, U. Grossniklaus, R. K. Pearson *et al.*, 1989 P-element-mediated enhancer detection: a versatile method to study development in Drosophila. Genes Dev. **3:** 1288–1300.

Bertram, G., S. Innes, O. Minnella, J. Richardson and I. Stansfield, 2001 Endless possibilities: translation termination and stop codon recognition. Microbiology **147:** 255–269.

Bock, A., 2000 Biosynthesis of selenoproteins—an overview. Biofactors **11:** 77–78.

Brown, C. M., P. A. Stockwell, C. N. Trotman and W. P. Tate, 1990 The signal for the termination of protein synthesis in procaryotes. Nucleic Acids Res. **18:** 2079–2086.

Cavallo, R. A., R. T. Cox, M. M. Moline, J. Roose, G. A. Polevoy *et al.*, 1998 *Drosophila* TCF and Groucho interact to repress Wingless signaling activity. Nature **395:** 604–608.

Czaplinski, K., M. J. Ruiz-Echevarria, S. V. Paushkin, X. Han, Y. Weng *et al.*, 1998 The surveillance complex interacts with the translation release factors to enhance termination and degrade aberrant mRNAs. Genes Dev. **12:** 1665–1677.

Dierick, H. A., and A. Bejsovec, 1998 Functional analysis of Wingless reveals a link between intercellular ligand transport and dorsal-cell-specific signaling. Development **125:** 4729–4738.

Doerig, R. E., B. Suter, M. Gray and E. Kubli, 1988 Identification of an amber nonsense mutation in the *rosy516* gene by germline transformation of an amber suppressor tRNA gene. EMBO J. **7:** 2579–2584.

Freistoffer, D. B., M. Y. Pavlov, J. MacDougall, R. H. Buckingham and M. Ehrenberg, 1997 Release factor RF3 in E. coli accelerates the dissociation of release factors RF1 and RF2 from the ribosome in a GTP-dependent manner. EMBO J. **16:** 4126–4133.

Frolova, L. Y., R. Y. Tsivkovskii, G. F. Sivolobova, N. Y. Oparina, O. I. Serpinsky *et al.*, 1999 Mutations in the highly conserved GGQ motif of class 1 polypeptide release factors abolish ability of human eRF1 to trigger peptidyl-tRNA hydrolysis. RNA **5:** 1014–1020.

Fu, L. H., X. F. Wang, Y. Eyal, Y. M. She, L. J. Donald *et al.*, 2002 A selenoprotein in the plant kingdom. Mass spectrometry confirms that an opal codon (UGA) encodes selenocysteine in *Chlamydomonas reinhardtii* glutathione peroxidase. J. Biol. Chem. **277:** 25983–25991.

Harrell, L., U. Melcher and J. F. Atkins, 2002 Predominance of six different hexanucleotide recoding signals 3′ of read-through stop codons. Nucleic Acids Res. **30:** 2011–2017.

Hays, R., G. B. Gibori and A. Bejsovec, 1997 Wingless signaling generates epidermal pattern through two distinct mechanisms. Development **124:** 3727–3736.

Ito, K., L. Frolova, A. Seit-Nebi, A. Karamyshev, L. Kisselev *et al.*, 2002 Omnipotent decoding potential resides in eukaryotic translation termination factor eRF1 of variant-code organisms and is modulated by the interactions of amino acid sequences within domain 1. Proc. Natl. Acad. Sci. USA **99:** 8494–8499.

Kisselev, L., 2002 Polypeptide release factors in prokaryotes and eukaryotes: same function, different structure. Structure **10:** 8–9.

Kisselev, L. L., and R. H. Buckingham, 2000 Translational termination comes of age. TIBS **25:** 561–566.

Knight, R. D., and L. F. Landweber, 2000 The early evolution of the genetic code. Cell **101:** 569–572.

Lukinova, N. I., V. V. Roussakova and M. E. Fortini, 1999 Genetic characterization of cytological region 77A-D harboring the presenilin gene of *Drosophila melanogaster.* Genetics **153:** 1789–1797.

MacBeath, G., and P. Kast, 1998 UGA read-through artifacts–when popular gene expression systems need a pATCH. Biotechniques **24:** 789–794.

Maixner, A., T. P. Hecker, Q. N. Phan and D. Wassarman, 1998

A screen for mutations that prevent lethality caused by expression of activated Sevenless and Ras1 in the *Drosophila* embryo. Dev. Genet. **23:** 347–361.

McCartney, B. M., H. A. Dierick, C. Kirkpatrick, M. M. Moline, A. Baas *et al.*, 1999 *Drosophila* APC2 is a cytoskeletally-associated protein that regulates Wingless signaling in the embryonic epidermis. J. Cell Biol. **146:** 1303–1318.

McCaughan, K. K., C. M. Brown, M. E. Dalphin, M. J. Berry and W. P. Tate, 1995 Translational termination efficiency in mammals is influenced by the base following the stop codon. Proc. Natl. Acad. Sci. USA **92:** 5431–5435.

Namy, O., I. Hatin and J.-P. Rousset, 2001 Impact of the six nucleotides downstream of the stop codon on translation termination. EMBO Rep. **2:** 787–793.

Ostrowski, S., H. A. Dierick and A. Bejsovec, 2002 Genetic control of cuticle formation during embryonic development of *Drosophila melanogaster*. Genetics **161:** 171–182.

Phillips-Jones, M. K., L. S. J. Hill, J. Atkinson and R. Martin, 1995 Context effects of misreading and suppression at UAG codons in human cells. Mol. Cell. Biol. **15:** 6593–6600.

Samson, M. L., M. J. Lisbin and K. White, 1995 Two distinct temperature-sensitive alleles at the *elav* locus of Drosophila are suppressed nonsense mutations of the same tryptophan codon. Genetics **141:** 1101–1111.

Seit-Nebi, A., L. Frolova, J. Justesen and L. Kisselev, 2001 Class-1 translation termination factors: invariant GGQ minidomain is essential for release activity and ribosome binding but not for stop codon recognition. Nucleic Acids Res. **29:** 3982–3987.

Song, H., P. Mugnier, A. K. Das, H. M. Webb, D. R. Evans *et al.*, 2000 The crystal structure of human eukaryotic release factor eRF1: mechanism of stop codon recognition and peptidyl-tRNA hydrolysis. Cell **100:** 311–321.

Stansfield, I., and M. F. Tuite, 1994 Polypeptide chain termination in *Saccharomyces cerevisiae*. Curr. Genet. **25:** 385–395.

Ter-Avanesyan, M. D., V. V. Kushnirov, A. R. Dagkesamanskaya, S. A. Didichenko, Y. O. Chernoff *et al.*, 1993 Deletion analysis of the SUP35 gene of the yeast *Saccharomyces cerevisiae* reveals two non-overlapping functional regions in the encoded protein. Mol. Microbiol. **7:** 683–692.

Tikhomirova, V. L., and S. G. Inge-Vechtomov, 1996 Sensitivity of *sup35* and *sup45* suppressor mutants in *Saccharomyces cerevisiae* to the anti-microtubule drug benomyl. Curr. Genet. **30:** 44–49.

Tujebajeva, R. M., P. R. Copeland, X.-M. Xu, B. A. Carlson, J. W. Harney *et al.*, 2000 Decoding apparatus for eukaryotic selenocysteine insertion. EMBO Rep. **1:** 158–163.

van de Wetering, M., R. Cavallo, D. Dooijes, M. van Beest, J. van Es *et al.*, 1997 Armadillo co-activates transcription driven by the product of the *Drosophila* segment polarity gene *dTCF*. Cell **88:** 789–799.

Washburn, T., and J. E. O'Tousa, 1992 Nonsense suppression of the major rhodopsin gene of Drosophila. Genetics **130:** 585–595.

Wieschaus, E., and C. Nüsslein-Volhard, 1986 Looking at embryos, pp. 199–227 in *Drosophila*: *A Practical Approach*, edited by D. B. Roberts. IRL Press, Oxford.

Wilusz, C. J., W. Wang and S. W. Peltz, 2001 Curbing the nonsense: the activation and regulation of mRNA surveillance. Genes Dev. **15:** 2781–2785.

Xu, T., and G. M. Rubin, 1993 Analysis of genetic mosaics in developing and adult *Drosophila* tissue. Development **117:** 1223–1237.

Zavialov, A. V., R. H. Buckingham and M. Ehrenberg, 2001 A posttermination ribosomal complex is the guanine nucleotide exchange factor for peptide release factor RF3. Cell **107:** 115–124.

Zerfass, K., and H. Beier, 1992 The leaky UGA termination codon of tobacco rattle virus RNA is suppressed by tobacco chloroplast and cytoplasmic tRNAs(Trp) with CmCA anticodon. EMBO J. **11:** 4167–4173.

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