Drosophila Nonsense Suppressors: Functional Analysis in Saccharomyces cerevisiae, Drosophila Tissue Culture Cells and Drosophila melanogaster

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ABSTRACT

Amber (UAG) and opal (UGA) nonsense suppressors were constructed by oligonucleotide site-directed mutagenesis of two Drosophila melanogaster leucine-tRNA genes and tested in yeast, Drosophila tissue culture cells and transformed flies. Suppression of a variety of amber and opal alleles occurs in yeast. In Drosophila tissue culture cells, the mutant tRNAs suppress hsp70:Adh (alcohol dehydrogenase) amber and opal alleles as well as an hsp70: β-gal (β-galactosidase) amber allele. The mutant tRNAs were also introduced into the Drosophila genome by P element-mediated transformation. No measurable suppression was seen in histochemical assays for Adhneo (amber), Adhneo (opal), or an amber allele of β-galactosidase. Low levels of suppression (approximately 0.1–0.5% of wild type) were detected using an hsp70:cat (chloramphenicol acetyltransferase) amber mutation. Dominant male sterility was consistently associated with the presence of the amber suppressors.

SUPPRESSOR mutations, which reduce or eliminate abnormalities resulting from other mutations in the genome, are a powerful tool in genetic analysis. The class of nonsense suppressors results from mutational alterations in genes coding for tRNA molecules (usually in the anticodon), rendering them capable of inserting an amino acid at the site of a nonsense mutation that would otherwise result in premature polypeptide chain termination and an inactive product (Geis and Smith 1979). Since suppression can often restore the wild-type phenotype, nonsense mutations are conditional mutations.

Nonsense suppressors have a variety of applications in genetic analysis in bacteria, yeast and nematodes. They were instrumental in establishing the colinearity between genes and polypeptides (Smith 1979), and the use of nonsense suppressors derived from different tRNA genes in Escherichia coli has allowed the insertion of different amino acids into the sites of nonsense mutations for analyzing the relationship between protein structure and function (Miller et al. 1979; Cupples and Miller 1988). Nonsense suppressors have also been useful in studies of yeast tRNA gene expression (e.g., Kurjan et al. 1980; Shaw and Olson 1984; Strobel and Abelzon 1986a,b; Colby, Schedl and Guthrie 1976.) Other applications of nonsense suppressors in yeast genetics have been reviewed by Sherman (1982).

In the nematode worm Caenorhabditis elegans, suppressors are routinely used to identify nonsense mutations and have been used to study the tRNA<sup>Trp</sup> gene family in C. elegans (reviewed in Hodgkin, Kon do and Waterston 1987). By examining the allelic patterns of suppression obtained using different tRNA<sup>Trp</sup> amber suppressors, evidence has been obtained for tissue-specific tRNA expression (Kon do, Hodgkin and Waterston 1988). Nonsense suppressors also provide small, convenient, selectable markers for germline transformation of C. elegans (Fire 1986).

Nonsense suppressors are also of potential value in Drosophila genetics, for example, in identifying point mutations with null (loss of function) phenotypes and as a means of studying tRNA gene expression in Drosophila. In an analysis of existing suppressor mutations in Drosophila, none was found to be tRNA dependent (Bi enz 1981). Subsequently, a few suppressors were shown to act specifically on mutations caused by transposable elements [see Kubli (1986) for a review]. Although the absence of nonsense suppressors in Drosophila stocks may simply be due to chance, they may be absent because they act as dominant lethals or dominant steriles or are too inefficient to give detectable suppression (Kubli 1986).

Screening for unlinked suppressors of nonsense mutations would seem a straightforward strategy for isolating nonsense suppressors, but the frequency of the required base substitution is expected to be very low (quite apart from possible difficulties due to lethality or sterility). As an alternative, we have constructed suppressors from two Drosophila tRNA<sup>Leu</sup> genes (Robinson and Davidson 1981) using oligonucleotide site-directed mutagenesis (Zoller and Smith 1982). The suppressors were analyzed for function in yeast (Saccharomyces cerevisiae), in Drosophila tissue culture cells, and in transformed lines of D. melanogaster. We find that these and other heterologous suppressors function efficiently in yeast and in Drosophila tissue culture cells, but they are very in-
efficient when transformed into the genome. Furthermore, the presence of an amber suppressor causes variable levels of male sterility.

**MATERIALS AND METHODS**

**Mutagenesis and plasmid constructions:** Nonsense suppressors were constructed from two Drosophila tRNA<sup>Leu</sup> genes (ROBINSON and DAVIDSON 1981), here designated tRNA<sup>Leu<sub>46</sub></sup> and tRNA<sup>Leu<sub>49</sub></sup>. These particular tRNA genes were chosen because homologous genes in yeast give rise to strong amber suppressors by the same anticodon change that we intended to introduce (LIEBMAN et al. 1984). The two Drosophila tRNA genes differ by an insertion of seven base pairs in the intron of the tRNA<sup>Leu<sub>46</sub></sup> gene and a single base transition (ROBINSON and DAVIDSON 1981). Plasmids were constructed using standard molecular cloning techniques (MANIATIS, FRITSCH and SAMBROOK 1982). Fragments containing each of the tRNA genes were subcloned from plasmid p50AB, which contains the two leucine tRNA genes and five isoleucine tRNA genes within a 3.4-kb EcoRI-SalI fragment (ROBINSON and DAVIDSON 1981). The tRNA<sup>Leu<sub>46</sub></sup> gene was isolated in a 740-bp DpnI fragment, and the tRNA<sup>Leu<sub>49</sub></sup> gene in a 1005-bp Rsal fragment, and each was inserted into the small site of bacteriophage mp8 (MESSING 1985). Oligonucleotide mutagenesis was carried out as described (ZOLLER and SMITH 1982), and each tRNA gene was converted to an amber suppressor using an oligonucleotide with the sequence 5'-GCCAGACTCTAGATT-3'.

Both amber suppressors were converted to UGA suppressors in a second step of mutagenesis, using the oligonucleotide 5'-GCCGACCTCAGATT-3'. Suppressor plasmids were isolated as EcoRI-SalI fragments from mp8 derivatives and subcloned into the yeast centromere-containing (CEN) vectors YCP50 (for the amber suppressors) or pTC3 (for the UGA suppressors) (HIETER et al. 1985) and the integrating vector YIP5 (STRAHL et al. 1979). To provide a yeast control, the yeast amber suppressor SUP53 (LIS et al. 1984) was subcloned in a 1.3-kb EcoRI-SalI fragment from the plasmid YEp13-am (FISCHHOFF, WATTERSON and OLSON 1984). Drosophila controls consisted of the wild-type Drosophila tRNA genes subcloned in a 3.4-kb EcoRI-SalI fragment from the cluster in p50AB (ROBINSON and DAVIDSON 1981) into both YCP50 and pTC3, yielding plasmids yCP50AB and pTC50AB.

The yeast CEN plasmids containing each of the four Drosophila suppressors, as well as pTC50AB containing the wild-type genes, were digested with EcoRI, the overhanging ends repaired with the Klenow fragment of DNA polymerase I, and the blunt ends ligated with nonphosphorylated SalI linkers using T4 DNA ligase. After transformation of E. coli strain HBl01, DNA from transformants was prepared by the boiling method (HOLMES and QUIGLEY 1981), and transformants were identified that had the original EcoRI site replaced with a SalI site, allowing further subcloning of each suppressor as a SalI fragment. The Drosophila suppressors and the wild-type tRNA cluster were subcloned into the SalI site of tissue culture vectors pCopNeo and pCopHyg (RIO and RUBIN 1983; RIO, LASKI and RUBIN 1985) and the transposable P element-translation vectors Carnegie 20 (RUBIN and SPRADLING 1983), here abbreviated Car20, and pUCHneo (STELLER and PIRROTTA 1985).

Plasmids with chimeric genes containing the hsp70 promoter fused to nonsense mutations of Adh were constructed using a SalI-BamHI fragment containing the hsp70 promoter fused to the 5' end of the wild-type Adh gene. This fragment was obtained from plasmid pCAH, which is a Carnegie 20 derivative containing an hsp70:Adh fusion that gives high levels of Adh expression when transformed into the germline of D. melanogaster (KLEEMENZ, HULTMARK and GEHRING 1985). The 3' ends of the nonsense constructs were obtained from the alleles Adh<sup>a</sup> (UGA) (MARTIN et al. 1985) and Adh<sup>a,436A</sup> (amber) (origin described below). The 3' end of the Adh<sup>a</sup> gene was purified as a BamHI-SalI fragment from the cloned Adh<sup>a</sup> allele in plasmid pBR-NB5.5, which contains a tryptophan-to-UGA mutation at codon position 235 (MARTIN et al. 1985, numbering of codon positions as in BENYAHTI et al. 1981).

For the Adh<sup>a,436A</sup> construct, a BamHI-SalI fragment containing the 3' end of the wild-type Adh gene was subcloned from plasmid pFA-1 (GOLDBERG, POSAKONY and MANIATIS 1983) into mp19 (YANISCH-PERRON, VIERA and MESSING 1985). The leucine at codon position 239 was changed into an amber codon by in vitro site-directed mutagenesis (BURKE and OLSON 1986) using the oligonucleotide 5'-CTGGCAGTAGGCACC-3', and the mutation was confirmed by direct nucleotide sequencing using the dyeoxy chain termination method (SANGER, NICKLEN and COULSON 1977). The 3' Adh fragments corresponding to either Adh<sup>a</sup>, Adh<sup>a,436A</sup> or the wild-type Adh sequence were ligated onto the SalI-BamHI fragment containing the hsp70:Adh fusion and inserted into the SalI site of pUC18 for transformation into E. coli strain DH5 (HANAHAN 1983). Clones containing the appropriate fusions were identified by colony hybridization (GRUNSTEIN and HOGNESS 1975). The Adh<sup>a</sup> mutation (GRELL, JACOBSON and MURPHY 1968), which contains a glutamineto-UGA mutation at codon position 82, was isolated as a Hpal-BamHI fragment from plasmid pDhn4 (W. CHIA, personal communication) and the fragment exchanged for the corresponding wild-type fragment in plasmid pAdhD (KLEEMENZ, HULTMARK and GEHRING 1985), in which hsp70 and Adh are fused. A SalI-BamHI fragment containing the 5' region of the fusion and the Adh<sup>a</sup> amber mutation was isolated and ligated with the wild-type BamHI-SalI fragment from pAdh1 and inserted into the SalI site of pUC18. The SalI fragments containing each of the four hsp70:Adh fusion genes (Adh<sup>a</sup>, Adh<sup>a,436A</sup>, Adh<sup>a,436A</sup> and wild type) were subcloned into the SalI site of pCopHyg and Carnegie 20.

Plasmids containing the hsp70 promoter fused onto a β-galactosidase coding region containing an amber mutation (hsp70:β-gal<sup>a,436A</sup>) or wild type (hsp70:β-gal) were constructed in the following way. A HindIII fragment containing the hsp70:β-gal wild-type fusion was isolated from ep19.1 (LIS, SIMON and SUTTON 1983) and subcloned into pGC1 (MYERS, LERMANN and MANIATIS 1985). Full-length plasmid molecules were gel purified following partial digestion with PvuII, and a nonphosphorylated NheI linker containing amber codons in all three reading frames was ligated onto the blunt ends and transformed into E. coli strains BD2012 and BD2018 (provided by T. KAZIC), both of which are deleted for the endogenous lacZ gene and fail to form blue colonies in the presence of the indicator X-Gal (MILLER 1972). BD2018 contains the amber suppressor supF, and therefore gives rise to blue colonies when transformed with β-gal constructs bearing amber mutations. DNA prepared from individual blue colonies resulting from transformation of BD2018 were retransformed into BD2012 and BD2018. Transformation of plasmid DNA having an amber mutation in β-gal should give white colonies in BD2012 and blue colonies in BD2018. Subsequent examination of putative amber mutations for the loss of a PvuII site and gain of an NheI site (present in the nonsense linker) identified an amber mutation (designated hsp70:β-gaL<sup>a</sup>) containing the non-
sense linker inserted into the PuchII site at position 4308 in lacZ (Casanadad, Chou and Cohen 1980). SalI-XhoI fragments containing the mutant and wild-type hisp70-β-gal genes were subcloned into the SalI cloning sites of Carnegie 20 and pCopHyg.

**Yeast strains and methods:** Standard yeast media and growth conditions were used (Sherman, FUNX and HICKS 1986). Yeast colonies were grown at 30°C, and nutritional markers were scored on omission (drop-out) plates consisting of synthetic media deficient in particular amino acids or nitrogen bases. CEN plasmids were transformed into appropriate yeast strains by the lithium acetate method (ITO et al. 1983). Primary transformants were plated on synthetic media lacking either uracil or tryptophan to select for the plasmid marker genes URA3 (c5p50 plasmid derivatives) or TRP1 (pTC3 plasmid derivatives). Primary transformants were subcloned to single colonies on selective media. Three independent transformants for each construct were patched to selective media and replica plated to tester plates to assay suppression of nonsense alleles. Plater plates were grown for 2–3 days at 30°C and assayed for growth. Cells from replica plates were diluted in sterile water and plated on nonselective media (YPD) to compare the sectoring phenotypes of the resulting cells.

Designations for yeast nonsense mutations are: ochre (o), amber (a) and UGA (u). Yeast strain AB1124 (a ade3-26a ilv1-1a met8-1a trpl-1a tyr7-1a lys1-1o leu2-1o his5-1o ura3-1o) was obtained from M. V. OLSON. Yeast strain AB429 (a ade2-14a lys2-1o ura4-1o leu2-1u can1-1u arv6-1a trpl-1-1) was obtained from E. S. SCHMIDT. Yeast strain AB1840 (a ade2-101o lys2-801a his3D200 ura3-52 leu2 tyr1-1 trpl-1-1) was obtained from M. JOHNSTON. AB1841 was constructed from AB1840 by conversion of the ade2-101o ochre mutation into an amber mutation. This was accomplished in two steps. First, a deletion derivative of the yeast tyrosine-inserting ochre suppressor SUP4-o, carried on the pTC5 vector (pDB-SUP4-o of D. BURKE and M. V. OLSON, personal communication), was converted into an amber suppressor. This was accomplished by transforming pDB-SUP4-o into yeast strain AB-6C (a ade2-101o lys2-1o can1-1o leu2-1o his5-1o trpl-1-1 ilv1-1a tyr7-1a) and selecting revertants on medium lacking tryptophane. A pDB-SUP4-a derivative was identified by simultaneous loss of ochre suppression of the ade2-10 and lys2-10 mutations and the gain of amber suppression of the ilv1-1a and tyr7-1a mutations. The mutation was further confirmed by cosegregation of the plasmid marker (TRP1) with the amber suppressor. DNA was prepared from AB-6C pDB-SUP4-a and the plasmid transformed into E. coli strain K1. The pDB-SUP4-a plasmid was also transformed into AB1840 and revertants were selected on medium lacking adenine. ADE° revertants that were also TRP° (containing the plasmid) and LYS° (containing the amber suppressor) were then tested for the red/white sectoring phenotype on nonselective media (YPD). The pDB-SUP4-a plasmid was eliminated from one such revertant by segregation on nonselective media and subcloning a red sector, and the resulting strain was designated AB1841. This strain was transformed with amber and ochre suppressors to confirm the specificity of the nonsense mutation.

Inhibition of the amber suppressors into AB1841 was carried out with linear fragments made by digestion of YIp15 suppressor plasmids with SmaI. SmaI cuts the plasmids in yeast sequences flanking the URA3 gene, directing the integration events to the homologous sequences in the chromosomal copy of the URA3 gene in the recipient strain (ORR-WEAVER, Szostak and Rothstein 1983). The integration events include multiply integrated, tandem copies of the plasmid sequences. A preliminary set of independent transformants was selected on the basis of colony color, assuming that increased copy number of the suppressor would give higher levels of phenotypic suppression. Yeast genomic DNA was prepared from these transformants, cut with HindIII, and fractionated on 1% agarose gels using field inversion gel electrophoresis (FIGE) (CARE, FRANK and OLSON 1986) with constant switching time (0.3 s) and alternating voltage (350 V forward, 250 V back). The gels were blotted (SOUTHERN 1975) to nylon membranes (Amersham) and hybridized with pBR322 labeled to high specific activity with [32P]ATP using the random oligomer method (FLEISCHER and VOGELSTEIN 1984). High molecular weight DNA markers were used as size standards. Preliminary blots carried out on AB1841 using the URA3 gene as a probe indicated the size of the genomic HindIII fragment into which the plasmid sequences were expected to integrate. This fragment was 1.9 kb, and so multicopy integrants of the DIL° Su°(amber) in YIP were expected to be 8.4 kb (one copy integrated), 14.9 kb (two copies), 21.4 kb (three copies) and 27.9 kb (four copies).

**Drosophila strains and methods**: Flies were cultured on standard medium containing cornmeal, sucrose, dextrose, brewer's yeast and agar (LEWIS 1960) at 25°C unless otherwise noted. Genetic markers, balancers, and chromosome rearrangements are as described (LINDSLEY and GRELLE 1968). The ry° transformation host strain was obtained from G. RUBIN. The cn, ry transformation host strain was obtained from P. M. BINGHAM. A Cy0°/Df(2L)64j strain was obtained from the Mid-America Sock Center. A MKRS/ Ubx strain was obtained from R. DUNCAN. The P[ry°, his70- cat] wild-type strain and the P[ry°, hisp70-cat(am33)]i amber mutant strain (LASKI et al. 1989) were generously provided by F. LASKI. Transformation with P-element vectors was carried out essentially as in RUBIN and SPRADLING (1982). Transposition notation is as described in HAZZLETT, LEVIS and RUBIN (1984). Each integrated transposon was mapped by following the segregation of ry° (Carnegie 20 derivatives) or G418 resistance (pUCHMNEO derivatives), using a Cy0°/ Df(2L)64j b Adh-2° strain. Transposons not mapping to the X or second chromosome were subsequently mapped to chromosome 3 using a MKRS/ry°° strain. Once mapped, each transposon was maintained over balancers (Cy0° or MKRS).

Phenotypic suppression of Adh nonsense alleles in adult flies was assayed by testing resistance to 6% ethanol as described by VIGUE and SOFER 1976. For the P[ry°, DIL° Su°(amber)]D7 line, this was done by crossing P[ry°, DIL° Su°(amber)]D7 females to b Adh°; ry°° males and comparing the survival of phenotypically ry° vs. ry°° individuals. The 'P[Su°(opal)]' lines with the suppressor on the X or third chromosome and containing the second chromosome balanced lethal Adh° chromosomes (Cy0°/Df(2L)64j) or Adh°-L° were tested similarly for the suppression of the Adh°° allele. (In this case the nonsense allele could not be made homozygous, since it resides on the balancer chromosome.)

Male sterility was tested with males reared at various temperatures and crossing them individually with 3–4 Oregon R or b Adh°; ry°° females. Putative sterile males were transferred after 1 week to fresh medium and provided new females. Each vial was kept for either 3 weeks (25°C) or 5 weeks (17°C) to test for the presence of progeny. Most transformed lines were tested in heterozygous condition with a balancer chromosome (either Cy0° or MKRS). The P[ry°, DIL° Su°(amber)]R5-bearing chromosome was also tested in homozygous condition. A strain containing both the P[ry°, DIL° Su°(amber)]R3 transposon and the P[G418°, DIL° Su°(amber)]1 transposon in cis configuration on the third chromosome was obtained in two steps by recombi-
nation. The P[G418, plasmid] I line (derived from Oregon-R) was first made by crossing with b Adh1 and selecting the F2 of P[G418, plasmid] recombinants on G418. The resulting b Adh1, P[G418, plasmid] strain was then crossed with the P[+], plasmid strain, and F2 of P[G418, plasmid] recombinants were selected and used to establish a balanced stock. Heat shock of larvae and adults was carried out for β-galactosidase (LIS, SIMON and SUTTON 1983) and chloramphenicol acetyl transferase (CAT) (LASKI et al. 1989) assays. Tissue dissections and histochemical assays were performed as described for ADH (URSPRUNG, SOFER and BURROUGHS 1970) and β-galactosidase (LIS, SIMON and SUTTON 1983).

Preparation of fly extracts and determination of CAT activity was carried out essentially as described in MSMBER and RUBIN (1988) and LASKI et al. (1989), except that flies were heat shocked for 8 hr at 37°C and the chloramphenicol acetyl transferase reactions were allowed to proceed for 8–12 hr. Extracts from flies containing the wild-type hsp70:cat were diluted 1000-fold with extract from the P[+] strain, and the resulting protein was assayed on a nitrocellulose membrane using the Bradford method (BRADFORD 1976). This method was used to establish a balanced stock. Heat shock of larvae and adults was carried out for β-galactosidase (LIS, SIMON and SUTTON 1983) and chloramphenicol acetyl transferase (CAT) (LASKI et al. 1989) assays. Tissue dissections and histochemical assays were performed as described for ADH (URSPRUNG, SOFER and BURROUGHS 1970) and β-galactosidase (LIS, SIMON and SUTTON 1983).

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We found that the appropriate combinations of ade2 nonsense allele and Drosophila suppressor [ade2(amber) with amber suppressor, ade2(UGA) with UGA suppressors] gave rise to sectoring colonies (Figure 1); nonsuppressed mutants [ade2(amber) with UGA suppressors and ade2(UGA) with amber suppressors], as well as all combinations of ade2 nonsense alleles with the wild-type Drosophila tRNA cluster), yielded nonsectoring, red colonies.

Yeast cells bearing the yeast SUP53 amber suppressor gave rise to white colonies with red sectors, while colonies derived from cells transformed with the Drosophila amber or opal suppressors produced pink colonies with sectors that were red or white (Figure 1). This phenotype is characteristic of low level suppression, in which cells containing one suppressor plasmid form pink colonies, and the white and red sectors derive, respectively, from segregants containing two or more suppressor plasmids and segregants that have lost the plasmid (SHAW and OLSON 1984; HIETER et al. 1985). Similar pink colonies with red and white sectors were observed (data not shown) when using CEN plasmids containing the sup7(amber) suppressor from C. elegans (WATERSTON 1981; BOLTON et al. 1984) and a Drosophila tRNA_{Tyr} amber suppressor [DtT Su+(amber)] (LASKI et al. 1989).

Drosophila amber suppressors were also tested for suppression of other nonsense mutations (Table 1). Each strain was transformed with Dtl Su\(^+\) plasmids and individual transformants were tested for their ability to grow on selective media. Only the expected combinations of Dtl Su\(^+\) plasmid and nonsense allele permitted growth on each of the selective media.

Qualitative differences in suppression could also be seen, with SUP53 > Dtl\(^b\) Su\(^+\)(amber) > Dtl\(^b\) Su\(^+\)(amber). Suppressor efficiencies per copy cannot be directly compared in this way because of variation in plasmid copy number. Most colonies derived from cells grown under selective conditions (requiring suppression) have white and pink sectors whereas colonies derived from cells grown under conditions selecting only for the plasmid (URA\(^+\) or TRP\(^+\)) generally have pink and red sectors. This suggests that selection for suppression leads to increased plasmid copy number, making comparisons of suppressor strengths difficult. Relative suppressor efficiency was therefore determined using ade2(amber) strains with multiple copies of the Dtl Su\(^+\)(amber) suppressors integrated into the yeast genome. In Figure 2, the number of integrated copies is indicated by the size of the hybridizing fragment: lanes 1–4 are from strains with 4, 2, 3 and 1 integrated copies, respectively.

Suppressor efficiency was estimated in two ways. First, relative levels of suppression were estimated by direct comparison of colony color for the different strains. Strains with four copies of Dtl\(^b\) Su\(^+\)(amber) give the same level of suppression as one copy of SUP53 (off-white), while seven copies of Dtl\(^b\) Su\(^+\)(amber) give even higher levels of phenotypic suppression (white). Strains with five copies of Dtl\(^b\) Su\(^+\)(amber) give less phenotypic suppression than a single copy of SUP53, giving rise to colonies that are light pink. When the growth of the Dtl\(^b\) strains was compared on selective media, levels of suppression again correlated with the number of copies of the Dtl\(^b\) genes integrated into the URA3 locus (Table 1).

**Transient assays in Drosophila tissue culture:** To
assess the ability of the tRNA\textsuperscript{Leu\textasciitilde} suppressors to function in Drosophila, we performed transient expression assays both without (Table 2) and with (Table 3) reporter genes. The results imply that each of the amber mutations is efficiently suppressed by the two Drosophila amber suppressors. This result is confirmed by the level of ADH protein observed in Western blots [Figure 3, compare ADH band A in suppressor-bearing strains (lanes 4 and 5) with wild-type control (lane 7) and negative control (lane 6)]. On the other hand, suppression of the Adh\textsuperscript{hy} (UGA) mutation is weak with either DlL\textsuperscript{a} Su\textsuperscript{+}(opal) or DlL\textsuperscript{b} Su\textsuperscript{+}(opal), as judged by enzyme activity (Tables 2 and 3). However, substantial amounts of full-length protein are detected in Western blots [Figure 3, compare ADH band A in suppressor-bearing strain (lane 1) with unsuppressed control (lane 2); band B results from premature chain termination in Adh\textsuperscript{hy} (MARTIN et al. 1985)].

Transformation of Drosophila germline: P element-meditated germline transformation (RUBIN and SPRADLING 1982) was carried out with each of the Drosophila tRNA\textsuperscript{Leu\textasciitilde} suppressors. Transformation efficiency with DlL\textsuperscript{a} Su\textsuperscript{+}(amber) was extremely low: from over 3000 injections, only three G\textsubscript{0} adults gave ry\textsuperscript{+} G\textsubscript{1} flies. One of these three G\textsubscript{0} adults produced only a single transformed male, which was sterile, so that only two independent lines were established from the ry\textsuperscript{+} transformants. A third independent line was obtained using a pUCHsneo DlL\textsuperscript{a} Su\textsuperscript{+}(amber) plasmid and Oregon R as host strain. With DlL\textsuperscript{b} Su\textsuperscript{+}(amber), none of 3000 injections yielded a transformed line. In marked contrast to the results with the amber suppressors, equivalent P vectors bearing UGA suppressors or the wild-type tRNA cluster (Su\textsuperscript{+}) transformed at normal frequencies.

The structure of the integrated transposons was examined by DNA blot analysis (Figure 4). Digestion of DNA from each transformed line examined with SalI liberates a diagnostic fragment of 1 kb containing the suppressor (panel A, lanes 1–6 and 9); digestion with enzymes that cut internally in the transposon (SstI, PvuII, and/or BgZII) was used to confirm the structural integrity of the integrated transposon (panel B). Suppressor transformed lines were cloned and retested in yeast to ensure that mutational lesions had not been introduced during the transformation. A suppressor cloned from the P[G418\textsuperscript{a}, DlL\textsuperscript{a} Su\textsuperscript{+}(amber)]1-transformed line gave a sectoring phenotype that was indistinguishable from that seen with the original Su\textsuperscript{+}(amber) CEN plasmid. Equivalent results were obtained for three UGA suppressor transformed lines.

Male sterility: We observed variable levels of male sterility in DlL\textsuperscript{b} Su\textsuperscript{+}(amber) transformed lines (Table 4). This was most pronounced for P[ry\textsuperscript{+}, DlL\textsuperscript{b} Su\textsuperscript{+}(amber)]1 transformed lines. InTABLE 1

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Nutrient tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ura</td>
</tr>
<tr>
<td>AB1124</td>
<td></td>
</tr>
<tr>
<td>YCP DlL\textsuperscript{a} Su\textsuperscript{+}(amber)</td>
<td>++</td>
</tr>
<tr>
<td>YCP DlL\textsuperscript{a} Su\textsuperscript{+}(amber)</td>
<td>++</td>
</tr>
<tr>
<td>AB1841</td>
<td></td>
</tr>
<tr>
<td>YCP DlL\textsuperscript{b} Su\textsuperscript{+}(amber)</td>
<td>++</td>
</tr>
<tr>
<td>YCP DlL\textsuperscript{b} Su\textsuperscript{+}(amber)</td>
<td>++</td>
</tr>
<tr>
<td>YCP SUP53am</td>
<td>++</td>
</tr>
<tr>
<td>AB1841 multicopy</td>
<td></td>
</tr>
<tr>
<td>YIP DlL\textsuperscript{a} Su\textsuperscript{+}(amber)-1</td>
<td>++</td>
</tr>
<tr>
<td>(1 copy)</td>
<td></td>
</tr>
<tr>
<td>YIP DlL\textsuperscript{a} Su\textsuperscript{+}(amber)-2</td>
<td>++</td>
</tr>
<tr>
<td>(2 copies)</td>
<td></td>
</tr>
<tr>
<td>YIP DlL\textsuperscript{a} Su\textsuperscript{+}(amber)-3</td>
<td>++</td>
</tr>
<tr>
<td>(3 copies)</td>
<td></td>
</tr>
<tr>
<td>YIP DlL\textsuperscript{a} Su\textsuperscript{+}(amber)-4</td>
<td>++</td>
</tr>
<tr>
<td>(4 copies)</td>
<td></td>
</tr>
<tr>
<td>YIP DlL\textsuperscript{a} Su\textsuperscript{+}(amber)-7</td>
<td>++</td>
</tr>
<tr>
<td>(7 copies)</td>
<td></td>
</tr>
<tr>
<td>YIP SUP53</td>
<td>++</td>
</tr>
<tr>
<td>(1 copy)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2.—DNA blot analysis of DNA from AB1841 YIP DlL\textsuperscript{a} Su\textsuperscript{+}(amber) integration strains. DNA fragments were separated by field inversion gel electrophoresis (FIGE), blotted, and hybridized with a pBR322 probe. The DNA was digested with HindIII, which releases a 1.9-kb genomic fragment in AB1841 and does not cut within the integrated plasmid sequences (data not shown). Each integration event introduces one or more copies of the 6.5-kb plasmid. Lane 1, DlL\textsuperscript{a} Su\textsuperscript{+}(amber)-4; lane 2, DlL\textsuperscript{a} Su\textsuperscript{+}(amber)-2; lane 3, DlL\textsuperscript{a} Su\textsuperscript{+}(amber)-3; lane 4, DlL\textsuperscript{a} Su\textsuperscript{+}(amber)-1.
also found that the dominant sterility phenotype of $P^{[py^+, \ DtL^b \ Su^+(umber)]R5}$-bearing third chromosome dominant male sterile at 17°C (Table 4, row 3). Dissection of testes from sterile males revealed the production of only nonmotile sperm. In contrast to the $P^{[G418^r, \ DtL^b \ Su^+(umber)]l}$ transposons give high levels of sterility as well (Table 4, rows 6 and 7). Since suppression appears to be cold sensitive in *C. elegans* (WATERSTON and BRENNER 1978; WATERSTON 1981), we also tested the $P^{[G418^r, \ DtL^b \ Su^+(umber)]l}$ line at lower temperatures and found that it was a partial dominant male sterile at 17°C (Table 4, row 4). We also found that the dominant sterility phenotype of $P^{[ry^+, \ DtL^b \ Su^+(umber)]D7}$ became less severe over time; after a period of approximately 1 year this led to recessive, partial male sterility, enabling the establishment of a homozygous stock. Lines transformed with the entire wild-type tRNA cluster or with the UGA suppressors were completely fertile.

To examine more insertion sites, we induced $P^{[ry^+, \ DtL^b \ Su^+(umber)]D7}$ to transpose to new genomic sites in a dysgenic cross. Four independent transpositions were obtained, one of which produced a single sterile male. Three independent lines, designated $H3, H4$ and $H5$ were established and the presence of the suppressor confirmed on Southern blots (Figure 4). Of these three new lines, two had high levels of sterility at 25°C and one at 17°C (Table 4, rows 8, 9 and 10). Including those cases where lines could not be established as a result of male sterility, 6/8 insertion sites gave male sterility.

### TABLE 2

**Suppression in *Drosophila* tissue culture cells (no reporter gene)**

<table>
<thead>
<tr>
<th>Constructs</th>
<th>Activity (units/mg)</th>
<th>Suppression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$hsp70:\beta$-gal construct</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Car20 \ hsp70:\beta$-gal*</td>
<td>$5.5 \pm 1.1$</td>
<td>0.0</td>
</tr>
<tr>
<td>$Car20 \ hsp70: \beta$-gal(amber)</td>
<td>$0.12 \pm 0.02$</td>
<td>0.0</td>
</tr>
<tr>
<td>$Car20 \ hsp70: \beta$-gal(amber) + pCopHyg $DtL^b \ Su^+(umber)$</td>
<td>$0.87 \pm 0.33$</td>
<td>0.0</td>
</tr>
<tr>
<td>$Car20 \ hsp70: \beta$-gal(amber) + pCopHyg $Dl\beta \ Su^+(umber)$</td>
<td>$3.37 \pm 0.59$</td>
<td>0.0</td>
</tr>
<tr>
<td>$Car20 \ hsp70: \beta$-gal(amber) + pCopHyg $Dl\beta \ Su^+(opal)$</td>
<td>$0.11 \pm 0.04$</td>
<td>0.0</td>
</tr>
<tr>
<td>pCopHyg $hsp70: \beta$-gal*</td>
<td>$35.2 \pm 3.5$</td>
<td>0.0</td>
</tr>
<tr>
<td>pCopHyg $hsp70: \beta$-gal(amber)</td>
<td>$1.1 \pm 0.30$</td>
<td>0.0</td>
</tr>
<tr>
<td>pCopHyg $hsp70: \beta$-gal(amber) + pCopHyg $Dl\beta \ Su^+(amber)$</td>
<td>$10.7 \pm 1.6$</td>
<td>0.0</td>
</tr>
<tr>
<td>pCopHyg $hsp70: \beta$-gal(amber) + pCopHyg $Dl\beta \ Su^+(amber)$</td>
<td>$17.8 \pm 10.4$</td>
<td>0.0</td>
</tr>
<tr>
<td>No DNA</td>
<td>$0.15 \pm 0.08$</td>
<td>0.0</td>
</tr>
</tbody>
</table>

### TABLE 3

**Suppression in *Drosophila* tissue culture cells (with reporter gene)**

<table>
<thead>
<tr>
<th>Constructs</th>
<th>Activity (units/mg)</th>
<th>Suppression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$hsp70: \beta$-gal construct (Adh reporter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCopHyg $hsp70: \beta$-gal*</td>
<td>$81.1 \pm 2.3$</td>
<td>0.0</td>
</tr>
<tr>
<td>pCopHyg $hsp70: \beta$-gal(amber)</td>
<td>$0.02$</td>
<td>0.0</td>
</tr>
<tr>
<td>pCopHyg $hsp70: \beta$-gal(amber) + pCopHyg $Dl\beta \ Su^+(amber)$</td>
<td>$21.3 \pm 1.8$</td>
<td>0.0</td>
</tr>
<tr>
<td>pCopHyg $hsp70: \beta$-gal(amber) + pCopHyg $Dl\beta \ Su^+(amber)$</td>
<td>$27.8 \pm 2.8$</td>
<td>0.0</td>
</tr>
</tbody>
</table>

$Su^+(amber)]D7$ males, where greater than 90% of the heterozygous males were sterile at 25°C (Table 4, row 3).
FIGURE 3.—Autoradiogram of a Western blot of total protein from transfected Drosophila SL2 tissue culture cells incubated with anti-ADH antibody and visualized with \(^{125}I\)-protein A. Transformations were carried out with 5–10 pg of each plasmid. Each lane contains approximately 200 pg of total protein. Bands A and B represent full-length and truncated ADH, respectively. Lane 1, Car20 hsp70:Adh\(^{ab}\) (UGA) and pCopHyg \(\text{DIL}\)\(^{Su}(\text{opal})\); lane 2, Car20 hsp70:Adh\(^{ab}\) (UGA); lane 3, no DNA; lane 4, Car20 hsp70:Adh\(^{ab}\) and pCopHyg \(\text{DIL}\)\(^{Su}(\text{amber})\); lane 5, Car20 hsp70:Adh\(^{ab}\); lane 6, Car20 hsp70:Adh\(^{ab}\); lane 7, Car20 hsp70:Adh\(^{ab}\).

**Suppression in transformed lines:** Transformed suppressors were first tested for suppression of the Adh amber and opal mutations Adh\(^{nt}\) and Adh\(^{ab}\) (W. CHIA, personal communication; MARTIN et al. 1986). No significant increase in resistance to 6% ethanol (VIGUE and SOFER 1976) was seen, and no increase in ADH activity above background levels was detected for any of the lines tested by either spectrophotometric or histochemical assays on both larval and adult tissues. Western blots carried out on Su\(^{+(opal)}\) + Adh\(^{nt}\) (UGA) or Su\(^{+(amber)}\) + Adh\(^{nt}\) (amber) combinations failed to yield full-length ADH polypeptide in quantities above background. [The unsuppressed Adh nonsense alleles exhibit 0.01–0.1% the wild-type level of full-length ADH polypeptide. We interpret this as due to readthrough translation, since the frameshift mutation Adh\(^{nt}\) gives no detectable full-length protein (data not shown).] To investigate the possibility of tissue-specific suppression, we transformed P vectors carrying the wild type and an amber mutation of an hsp70:β-gal fusion gene, which is expressed in virtually all tissues following heat shock (LIS, SIMON and SUTTON 1983). Histochemical and Western blot assays on both larval and adult tissues did not produce any evidence for suppression.

Finally, we obtained lines of *D. melanogaster* that had been transformed with wild-type and amber mutations of an hsp70:cat gene fusion (LASKI et al. 1989) and tested them for CAT activity in the presence of \(\text{DIL}\)\(^{Su}(\text{amber})\) (Figure 5). We consistently observed 0.1–0.4% of the wild-type levels of CAT activity with either the \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)D7 line (lane 2 vs. lane 1) or the line containing both the \(P[\text{(G418}^{*}, \text{DIL}\)\(^{Su}(\text{amber})]\)l and \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)R5 integrants.

FIGURE 4.—DNA blot autoradiogram from \(\text{DIL}\)\(^{Su}(\text{amber})\) transformed lines of *D. melanogaster*. Each lane contains genomic DNA from five flies digested with SalI (A) or PvuII (B) and probed with the 1.0-kb SalI fragment containing the rosy gene (B). The band at 1.0 kb in panel A is diagnostic of \(\text{DIL}\)\(^{Su}(\text{amber})\), that at 2.3 kb in panel B is diagnostic of \(y^{+}\). Panel A: lane 1, \(P[\text{G418}^{*}, \text{DIL}\)\(^{Su}(\text{amber})]\)l; lane 2, \(cn\); lane 3, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)R5/\(y^{+}\); lane 4, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)H3/\(y^{+}\); lane 5, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)H4/\(y^{+}\); lane 6, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)H5/\(y^{+}\); lane 7, \(cn\); lane 8, \(\text{Oregon-R}\); lane 9, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)D7/\(y^{+}\); lane 10, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)H3/\(y^{+}\); lane 11, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)H4/\(y^{+}\); lane 12, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)H5/\(y^{+}\); lane 13, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)R5/\(y^{+}\); lane 14, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)R3/\(y^{+}\); lane 15, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)H2/\(y^{+}\); lane 16, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)R1/\(y^{+}\).

Panel B: lane 1, \(cn\); lane 2, \(\text{Oregon-R}\); lane 3, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)D7/\(y^{+}\); lane 4, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)H3/\(y^{+}\); lane 5, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)H4/\(y^{+}\); lane 6, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)H5/\(y^{+}\); lane 7, \(b \text{Adh}^{nt}\); \(P[y^{+}, \text{DIL}\)\(^{Su}(\text{amber})]\)R5/\(y^{+}\).
Background levels of CAT-amber activity were consistently 5–10-fold lower than the suppressor-dependent CAT activity. [Some readthrough translation is indicated in Figure 5 in the trace of CAT activity observed in lane 3 as compared with the blank in the negative control (lane 4).]

**DISCUSSION**

**Heterologous suppressors function in yeast:** A variety of heterologous nonsense suppressors from Drosophila are capable of acting as allele-specific nonsense suppressors in yeast (Table 1, Figure 1). This was expected because of the high degree of DNA sequence conservation between the tRNA^{	ext{Lys}}^+ genes of yeast and Drosophila (ROBINSON and DAVIDSON 1981). Using suppression as a functional assay to confirm tRNA gene expression and function in vivo, we have extended the results of others showing that extracts of *S. cerevisiae* can transcribe and process heterologous tRNA genes in vitro (KLEKAMP and WEIL 1982; WILLIS et al. 1986).

Comparisons of suppressor efficiencies using Su^+ CEN plasmids are ambiguous since selection for suppression appears to lead to increased plasmid copy number. CEN plasmids are normally present at 1–2 copies per cell (CLARKE and CARBON 1983) and are routinely used to introduce and assay genes at low copy number per cell. Our results suggest that selection for increased expression of genes on the CEN plasmid may cause an increase in average copy number. A qualitative analysis of suppression was carried out by varying the number of copies of the heterologous suppressors integrated into the yeast genome. A potential problem with interpreting these results is that we have not measured absolute levels of suppression, and SUP53-mediated suppression of the ade2 (amber) mutation may be well above that required for the formation of off-white colonies. However, strains bearing seven copies of *Dl^+ Su^+(amber)* give higher phenotypic levels of suppression than the single copy SUP53 integrant (Table 2), suggesting that saturation levels of suppression are not reached in SUP53, and that SUP53 is roughly 5–6-fold stronger than *Dl^+ Su^+(amber)*.

**Suppression in Drosophila:** Transformed lines of *D. melanogaster* show extremely low levels of suppression—approximately two orders of magnitude lower than that seen in Drosophila tissue culture cells, yeast or *C. elegans*. This difference is presumably due to differences in the actual levels of suppression per copy. Three other explanations that could potentially account for our results are: (1) tissue or stage specificity of expression, (2) mutational lesions introduced at the time of transformation or (3) chromosomal position effects.

Tissue-specific expression of suppressors could give high levels of suppression in a particular cell or tissue type while giving only low levels of suppression when averaged over the entire organism. Tissue-specific tRNA expression has been shown for tRNA^{Ala} genes of *Bombyx mori* (MEZA et al. 1977; SPRAGUE, HAGENBUCHE and ZUNIGA 1977), and evidence for tissue-specific expression of suppressors has been obtained in *C. elegans*, where there is differential suppression.
of genes affecting different tissues (Kondo, Hodgkin and Waterston 1988). While we did not obtain evidence for tissue-specific suppression of a \( \beta \)-galactosidase amber mutation, expression in a small subset of cells or during a particular developmental stage cannot be ruled out. In particular, we have observed some embryonic lethality associated with the amber suppressor, which could result from higher levels of suppression during embryonic development (or perhaps from greater sensitivity to amber suppression during this period). Precedents for stage-specific expression do exist. For example, a Drosophila tRNA\( ^{\text{Tyr}} \) gene containing an unusually large intron (48 bp) has been shown to be expressed in a stage-specific fashion (Kubli et al. 1988), which is of interest since the \( \text{Dil}^+ \text{Su}^+ \) (amber) gene contains seven additional base pairs in the intron, relative to the \( \text{Dil}^+ \text{Su}^+ \) (amber) gene, from which we were unable to obtain transformants. In Xenopus laevis there is also stage specificity, with different oocyte and somatic tyrosine tRNA genes (Stutz, Gouilloud and Clarkson 1989). It remains to be seen whether these are special cases or reflect more general control mechanisms.

An apparent reduction in suppressor expression could also occur if the only viable transformants obtained were those in which suppressor expression was reduced. Since suppressor-bearing fragments cloned from the Drosophila genome still function when reintroduced into yeast, it is unlikely that inefficient expression of the suppressors occurred via the introduction of mutational lesions during the transformation process. Furthermore, the ability of the suppressors in P-element plasmids to function in transient assays argues against any major effects of the flanking vector sequences. A requirement for genomic integration sites to inefficiently express the suppressor genes would be consistent with the low transformation efficiency observed with the amber suppressors. The sensitivity of the suppressor to position effects is indicated by the variable expressivity of the male sterility phenotype associated with the \( \text{Dil}^+ \text{Su}^+ \) (amber) transformed lines. T. Washburn and J. O’Tousa (personal communication) have obtained evidence for low level suppression of an opal mutation in the ninaE gene by \( \text{Dil}^+ \text{Su}^+ \) (opal). Since normal transformation frequencies are seen for the UGA suppressors, inhibition of suppressor expression via position effects cannot account for the inefficient suppression seen in this case. From these results we conclude that, while position effects are probably responsible for some reduction in suppressor gene expression, such effects alone are not sufficient to explain the relatively weak suppression observed in Drosophila.

Results obtained with amber suppressors derived from a Drosophila tRNA\( ^{\text{Tyr}} \) gene provide additional evidence that amber suppressor efficiency is low in \( D. \) melanogaster and that amber suppressors cause sterility (Doeg et al. 1989; Laski et al. 1989). Doeg et al. (1989) constructed an amber suppressor from a tRNA\( ^{\text{Tyr}} \) gene and transformed recipient strains with a series of point mutations in the rosy gene, enabling the identification of an amber allele by partial suppression of the rosy eye color phenotype of \( ry^+ \). Even low levels of \( ry^+ \) activity can restore the phenotype to wild type (Chovnick, Gelbart and McCarron 1977), and the intermediate phenotype observed indicates extremely low levels of suppression. Both male and female sterility was seen in the GI transformants, and no stable transformed lines were obtained. Laski et al. (1989) also transformed \( D. \) melanogaster with an amber suppressor derived from the same tRNA\( ^{\text{Tyr}} \) gene and established stable transformed lines giving variable levels of male and female sterility. Levels of suppression determined with \( hsp70: \text{cat} \) amber mutations were approximately 0.4%. Moreover, transformation frequencies in this case were normal. Overall, these results support the idea that suppressor efficiency is low in \( D. \) melanogaster and that amber suppression results in sterility. Additionally, the differences in the ability to establish permanent transformed lines from identical suppressors points out the potential importance of flanking sequences and genetic background in modulating the sterility phenotype.

Our results with the two suppressors in Drosophila are similar to those seen with suppressors in mammalian tissue culture, where levels of suppression were high in transfection experiments (Temple et al. 1982; Laski et al. 1982, 1984; Hudziak et al. 1982; Capone, Sharp and Rajbhandary, 1985; Capone et al. 1986) but were low for integrated suppressors (Ho et al. 1986; Sedivy et al. 1987). Furthermore, reductions in growth rate were seen when levels of suppression were only a few percent of wild type. These results cannot easily be explained by selection for chromosomal integration at sites that repress tRNA gene transcription, since transformation frequencies were as high as wild-type tRNA controls (Sedivy et al. 1987).

Two models can account for the inefficient suppression observed in Drosophila relative to the efficient suppression observed in yeast and nematodes: (1) tRNAs may not compete as effectively for stop codons, and (2) the steady state concentration of mature, charged, suppressor tRNAs may be reduced. If tRNAs do not compete effectively for stop codons in Drosophila, we might expect to see less efficient suppression than in yeast or \( C. \) elegans. However, we observed significant amounts of translational readthrough occurring with both the Adh and cat nonsense mutations in \( D. \) melanogaster, suggesting that increased termination efficiency cannot account for the observed 50–
100-fold difference in suppressor efficiency between these organisms. It seems more likely that the inefficient suppression observed in Drosophila is due largely to relatively lower suppressor tRNA concentrations within the cell.

In Drosophila, the low efficiency of the nonsense suppressors limits their utility to those situations where only low levels of suppression are required. The sterility associated with the amber suppressors makes increasing the level of suppression difficult, requiring regulated expression. The prospects for increasing the levels of UGA suppression are somewhat better than for UAG suppression, because UGA suppression gives no evidence for associated sterility or toxicity, and increasing suppressor dosage could increase suppression without requiring regulation. On the other hand, although the efficiency of these Drosophila suppressors is not presently high enough to make suppressors generally useful genetic tools, they may aid in the study of tRNA gene expression and in determining whether the clustering of tRNA genes in the Drosophila genome has any functional significance.

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LITERATURE CITED


Drosophila Nonsense Suppressors


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