Structure and Function of Suppressor tRNAs in Higher Eukaryotes

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I. INTRODUCTION

Suppression is readily defined and recognized in microorganisms as the reversal of mutations deleterious to life or cell proliferation, and it results in the production of functional gene products. Suppression occurs at the level of translation by different processes, and in several of these processes tRNA functions to read mRNA in a new way. In higher eukaryotes, with their greater complexity of genome organization and gene expression, it is not surprising that viability most likely does not result from the translational correction of mutational errors, and hence the role of naturally occurring suppressor tRNAs in multicellular organisms may be to operate in very specialized events and not in correcting deleterious mutations.

The classes of suppression that involve tRNA are

1. *Nonsense suppression* — In nonsense suppression, a termination or stop codon is translated with the incorporation of an amino acid consistent with a functional gene product. Alternatively, a detectable readthrough product is synthesized. The termination codons in higher eukaryotes are UAG (amber), UAA (ochre), and UGA (opal).
2. *Missense suppression* — In missense suppression, a missense codon is translated with the incorporation of an amino acid consistent with a functional gene product.
3. *Frameshift suppression* — In frameshift suppression, the reading frame is altered in the 5′ or 3′ direction, which may occur in a variety of ways, as examined further below.

This review is concerned with tRNAs that serve as suppressors in higher eukaryotes. For the purpose of this review, higher eukaryotes are defined as animals and plants above the level of yeasts and protists. There are several excellent reviews covering suppression primarily in bacteria and yeast to which the reader is referred.[1-8] Since our knowledge is most advanced with nonsense suppression in higher eukaryotes,[9-11a] we begin the review with this class of suppressors, then focus on ribosomal frameshifting, and then subsequently on missense suppression. Functional aspects of suppressor tRNAs in protein synthesis are also considered and are discussed in a separate section.

A. Nonsense Suppressor tRNAs

1. Naturally Occurring Suppressors

Seven tRNAs that occur naturally within the tRNA population of higher eukaryotes have been described that are capable of suppressing a stop codon in protein synthesis. Four of these are amber suppressors belonging to three amino acid families. They are glutamine,[12-15] tyrosine,[16-23] and two leucine tRNAs.[24] Each of the amber suppressors accomplishes suppression by misreading the stop codon, that is, the major function of each isoacceptor is to translate codons within the respective amino acid family (see Figures 1 to 3). The other suppressor tRNAs described to date are opal suppressors, which are a tryptophan,[25] and two minor tRNAs that are aminoacylated with serine.[26,27] The minor serine isoacceptors that read the termination codon, UGA, have recently been shown to be selenocysteine tRNAs,[28] while the tryptophan tRNA misreads the opal codon.[25] Recent evidence demonstrates that ochre suppression also occurs naturally in mammalian cells (see below). An interesting suppressor tRNA has been described recently in a dimorphic fungus, *Candida albicans*, which reads both amber and opal codons.[29] However, since this suppressor occurs in a lower eukaryote, it will not be considered further. Additional characteristics of each of the nonsense suppressors identified in higher eukaryotes are presented below.

a. AMBER SUPPRESSOR tRNAs

I. Glutamine tRNA

Glutamine tRNA serves as an amber suppressor *in vivo*, which has been demonstrated by sequencing the amino-terminal residues of the protease isolated from Moloney murine leukemia virus (MuLV)[12] and from feline leukemia virus.[13] The MuLV protease occurs at the end of the *gag* gene[12,13] and expression of the protease and the *pol* gene depend upon suppression of a UAG termination codon at the end of *gag*.[30] Comparison of the amino-terminal residues of the protease[12,13] with the viral DNA sequence[30] demonstrated that the first four amino acids are encoded at the 3′ end of the *gag* gene.[12,13] The fifth amino acid is glutamine, which is coded by the *gag* termination codon. Thus, a glutamine isoacceptor serves as a

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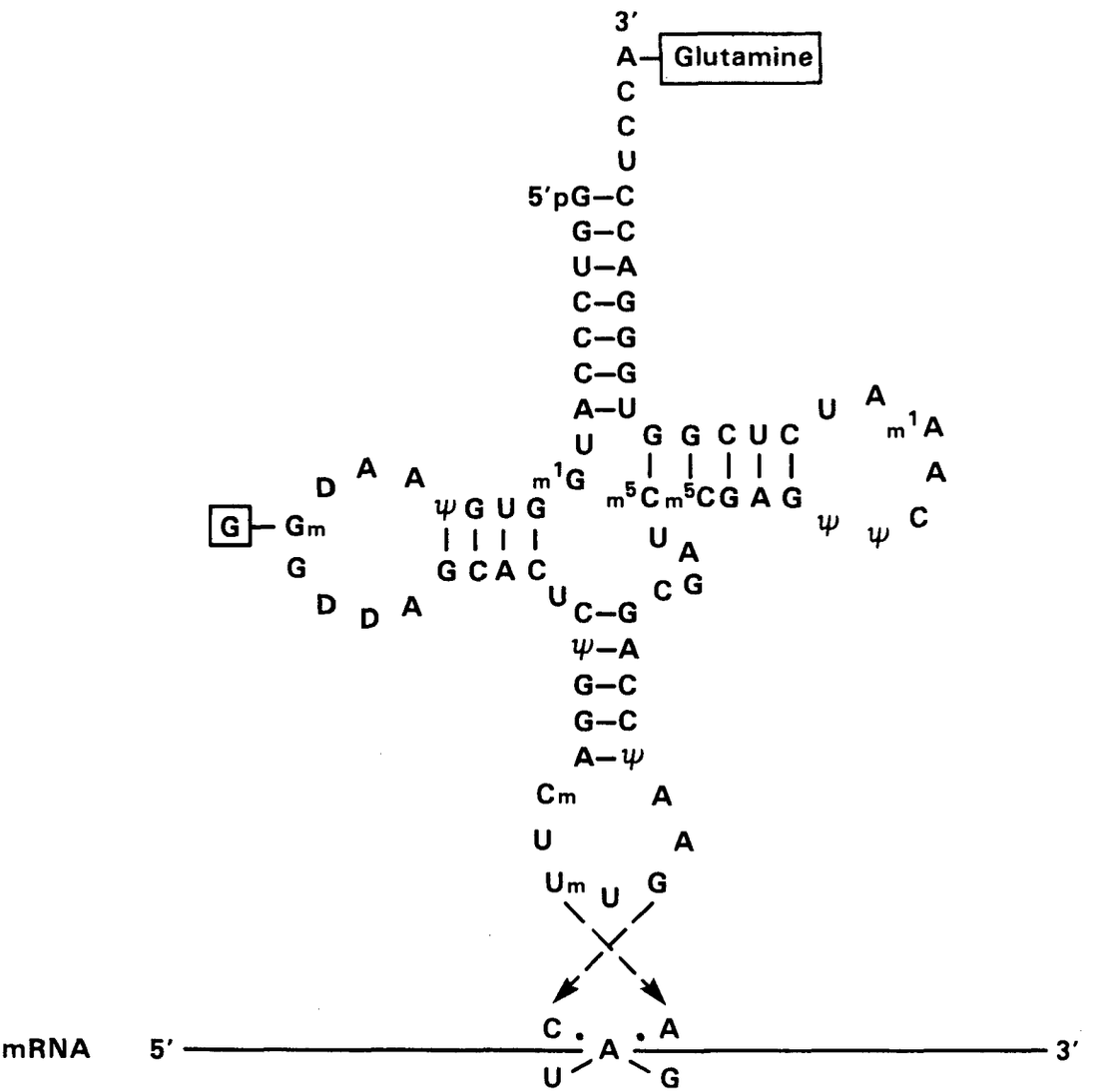

FIGURE 1. Structure of the glutamine tRNA from mouse liver that suppresses the UAG termination codon of TMV RNA *in vitro*.[14] The fully modified isoacceptor that represents most of the CAA reading tRNA in mouse liver has a methylG at position 18, while the minor, hypomodified isoacceptor has a G at this position. As shown in the figure, these isoacceptors read the glutamine codon, CAA, and the termination codon, UAG.

naturally occurring amber suppressor in mammalian cells.[12,13] It should also be noted that Philipson et al.[31] first demonstrated the occurrence of the UAG *gag* termination codon by addition of a yeast amber suppressor to reticulocyte lysates programed with MuLV mRNA, which resulted in an enhanced expression of the *gag-pol* fusion protein.

Kuchino et al.[14] and Feng et al.[15] have examined the glutamine tRNA involved in expression of the *gag-pol* fusion protein in MuLV. The former investigators observed that a minor CAA glutamine isoacceptor that represents 1 to 2% of the total glutamine tRNA population is induced manyfold in MuLV-infected cells.[14] Furthermore, this isoacceptor was identified as an amber suppressor by its ability to suppress the UAG codon in TMV RNA *in vitro*.[14] It was also reported that the level of the glutamine suppressor tRNA was substantially reduced in both MuLV-[32] and HIV-[33] infected cells when these cells were treated with avarol, which is a sesquiterpenoid hydroquinone. The reduction in glutamine suppressor paraleled inhibition of viral expression. In contrast, Feng et al.[15] observed that the level of total glutamine tRNA and the distribution of the glutamine CAG and CAA decoding isoacceptors were the same in MuLV-infected and -uninfected cells. At present, we cannot explain the reason for the different results obtained in these two laboratories, although it should be noted

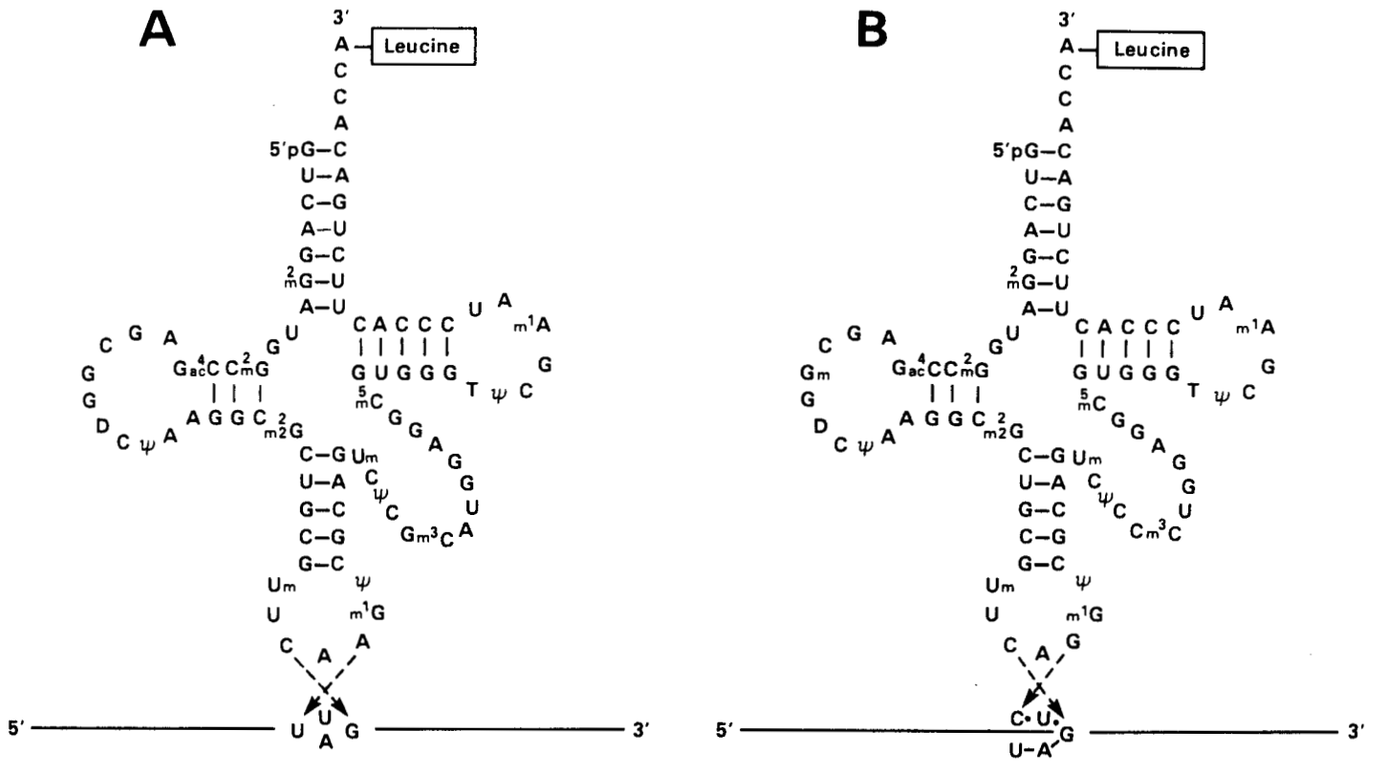

FIGURE 2. Structures of the novel leucine nonsense suppressor sequenced from calf liver.[24] These isoacceptors read the leucine codons, UUG (A) or CUG (B), as well as the termination codon, UAG. These isoacceptors have very similar structures and only differ by a methylG at nucleotide 17, a G at the 3′ position of the anticodon, and a C at nucleotide 49 that is within the variable loop in the CUG reading tRNA, compared with a G at nucleotide 17, an A at the 3′ position of the anticodon, a G at nucleotide 49, and an extra base (an A) at nucleotide 51 within the variable loop in the UUG reading tRNA.

that different chromatographic procedures were used in separating the glutamine isoacceptors.[14,15] Feng et al.[15] also used a rabbit reticulocyte lysate programed with a MuLV mRNA containing sequences from the *gag-pol* region to further study suppression of the *gag* termination codon. They observed that equivalent amounts of tRNA from MuLV-infected and -uninfected NIH-3T3 cells stimulated readthrough to virtually the same extent. Similar findings have been reported *in vivo* by Panganiban.[34] This investigator found that transfection of a construct containing a portion of the MuLV *gag-pol* region (which included the UAG codon) fused to lacZ into the cells of several vertebrates leads to an amount of β-galactosidase production that represents about 10% suppression. Use of MuLV-infected cells did not enhance enzyme synthesis. Thus, both the *in vitro* results of Feng et al.[15] and the *in vivo* results of Panganiban[34] demonstrate that suppression of the MuLV amber codon is not dependent on virus-induced qualitative or quantitative modification of suppressor tRNA.

The primary structures of two glutamine tRNAs from mouse liver were determined and the anticodon of the major isoacceptor was CUG, while that of a minor isoacceptor (which represented 1 to 2% of the glutamine tRNA population) was UmUG.[14] These tRNAs differed in structures in the wobble position of the anticodon and at position 4 and 68 of the acceptor stem. Two additional $tRNA^{Gln}$ species were also sequenced that corresponded identically to the ones described above, with the exception of a single hypomodification at position 18.[14] Only the $tRNA_{UmUG}$ isoacceptor and its hypomodified counterpart promoted suppression of the UAG codon in TMV RNA. The primary sequence of the proposed suppressor $tRNA_{UmUG}$ is presented in Figure 1.

A wobble in the first and third position of glutamine $tRNA_{UmUG}$ is required for its interaction with UAG. The occurrence of a wobble in G at the 3′ anticodon position with a U at the 5′ codon position has been reported in several other cases. For example, a yeast glutamine tRNA that reads CAA can weakly suppress UAA *in vivo* when the corresponding gene for $tRNA^{Gln}$ is present in high copy number.[35] Additionally, the yeast glutamine tRNA that reads CAG is capable of suppressing UAG codons *in vivo* under normal physiological conditions (i.e., when the tRNA is present in normal concentrations).[36-38] As described below, a calf liver $tRNA^{Leu}$ with anticodon CAG can suppress UAG *in vitro*. The unique feature in the mouse glutamine suppressor tRNA is the requirement for a wobble in the first and third positions to enable translation of the UAG codon.[14]

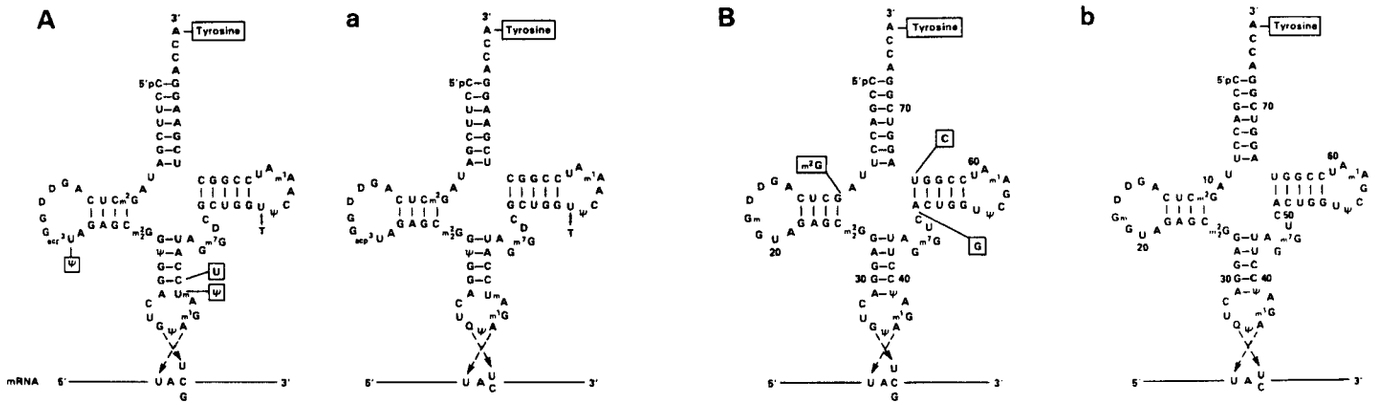

FIGURE 3. Structures of tyrosine tRNAs from *Drosophila*[16,17] (shown in A and a), from *A. pernyi*[23] (shown only in A, where the small boxes contain the bases in *A. pernyi*, which are different from those in *Drosophila*), from tobacco plants[18] (shown in B and b; tobacco plants contain two tyrosine tRNAs that differ from each other by the bases given in the boxes), from wheat germ,[19] from wheat leaves,[19] and from lupin seeds[22] (shown in B and b; bases given in the boxes only only occur in tobacco plant tyrosine tRNAs). The tyrosine tRNAs capable of suppressing UAG are shown in A and B, while the corresponding nonsuppressor species is shown in a and b. The only difference in the sequence of the suppressor (Q base deficient) and nonsuppressor (Q base containing) tRNA is the presence of Q base.

II. Leucine tRNAs

Calf liver contains two novel amber suppressor leucine tRNAs.[24] Valle, Morch, and Haenni fractionated total calf liver tRNA on a BD-cellulose column and assayed the eluted fractions for their ability to suppress the UAG termination codon in TMV RNA and in beet necrotic yellow vein virus (BNYVV) RNA in a cell-free protein synthesis system.[24] Column fractions that caused readthrough of TMV and BNYVV were further purified by polyacrylamide gel electrophoresis. The two tRNA species that suppressed UAG were isolated, sequenced, and found to be leucine isoacceptors. Since $tRNA^{Tyr}$, which lacks Q base, has been shown to be an amber suppressor in numerous other higher eukaryotic systems (see below), Valle et al.[24] purified and partially sequenced two calf liver $tRNA^{Tyr}$ species. Q base was present in the anticodon of both isoacceptors and neither promoted suppression of the UAG codon in TMV RNA. These investigators also showed that leucine $tRNA_{CAG}$ from bovine mammary tissue,[39] which differs in its primary structure from the corresponding calf liver suppressor, also suppressed the UAG termination codon in TMV and BNYVV RNAs.[24] However, leucine $tRNA_{IAG}$ from bovine mammary tissue did not function as an amber suppressor.[24] The structures characterized by Valle et al. are shown in Figure 2.

The calf liver amber suppressor tRNAs have the anticodons CAG and CAA,[24] both of which cannot, according to the wobble hypothesis,[41] base pair with the middle position of UAG. Therefore, base pairings between the anticodon in these novel suppressor tRNAs and the UAG termination codon must occur between the first and third positions. Wobble of G in the 3′ anticodon position of one of the suppressors must occur to allow reading of U. Wobble in the third position of the anticodon has been reported for other suppressor tRNAs, as discussed in the above section on glutamine tRNA. A more detailed presentation of the possible interactions between these novel leucine suppressor tRNAs and UAG is given in Valle et al.[24]

Each of the amber suppressor tRNAs that misread termination codons and are dependent on wobble in the third position of the anticodon for recognition of a nonsense codon lack a highly modified base in the 3′ position adjacent to the anticodon. Many tRNAs contain a highly modified base in this position[42] and the role of this highly modified base must be, at least in part, to restrict wobble. In support of this observation, Wilson and Roe[43] have recently shown that a phenylalanine tRNA from *Escherichia coli* that lacks the highly modified Wye base in the 3′ position adjacent to the anticodon is capable of misreading a leucine codon(s), while the corresponding isoacceptor that contains Wye base and is normally found in *E. coli* does not misread. Björk and collaborators[44] have recently published an excellent review on the occurrence and role of modified bases in tRNA, including those within the anticodon loop.

III. Tyrosine tRNA

The most extensively studied amber suppressor in higher eukaryotes is tyrosine tRNA. Tyrosine tRNA normally contains a hypermodified nucleotide in the wobble or 5′ position of its anticodon designated as Queuine or Q base. Q base is 7-[[[4,5-*cis*-dihydroxy-2-cyclopenten-1-yl]-amino]-methyl]-7-deazaguanosine (see Reference 45 for review on Q base). Without Q base in the anticodon of $tRNA^{Tyr}$, the undermodified tRNA misreads the termination codon, UAG. This very important observation was first demonstrated with a *Drosophila* $tRNA^{Tyr}$ that lacked Q base.[16] Adult *Drosophila* normally contain two $tRNA^{Tyr}$ species, one with and one without Q base.[16,17] Coinjection of purified $tRNA^{Tyr}$ Q^- from *Drosophila* and tobacco mosaic viral (TMV) RNA into *Xenopus* oocytes promoted syn-

thesis of the viral 180-kDa readthrough protein by suppressing a UAG termination codon.[16] *Drosophila* $tRNA^{Tyr}$ Q^+ did not cause suppression.

There is considerable evidence that $tRNA^{Tyr}$ Q^- serves as an endogenous suppressor in the expression of TMV in tobacco plants. Beier et al.[18] have shown that tobacco leaves contain two major tyrosine isoacceptors that suppress the UAG termination codon in TMV RNA in rabbit reticulocyte lysates and both tRNAs lack Q base. Addition of purified $tRNA^{Tyr}$ Q^- to the lysates resulted in a 3.5-fold increase in the 180-kDa readthrough protein. The viral major 126-kDa protein and minor 180-kDa readthrough protein synthesized *in vitro* in response to TMV RNA are identical to those synthesized *in vivo* in tobacco protoplasts.[19] Wheat germ extracts do not promote synthesis of the TMV readthrough protein in the absence of exogenous $tRNA^{Tyr}$ Q^-, and examination of $tRNA^{Tyr}$ in wheat germ revealed that 85% of this isoacceptor contained Q base.[19] Wheat leaves, on the other hand, contain a high level of Q^- $tRNA^{Tyr}$.[19,20] Yellow lupin seeds also contain $tRNA^{Tyr}$ that lacks Q base, and this species suppresses TMV RNA when both components are co-injected into *Xenopus* oocytes.[22] The sequence of the $tRNA^{Tyr}$ of lupin seeds[22] is identical to the corresponding isoacceptors observed in tobacco leaves and wheat germ.[18-20] Lupin seeds do not contain another amber suppressor tRNA or an opal suppressor.[22] Although the amino acid incorporated into the TMV readthrough protein at the UAG termination codon has not been identified, each of these studies[18-20,22] provides evidence that the endogenous suppressor in plants that promotes readthrough and viral maturity is tyrosine tRNA Q^-.

A tyrosine tRNA that lacks Q base has been isolated and sequenced from the posterior silk gland of *Antheraea pernyi*.[23] This tRNA promotes suppression of TMV RNA *in vitro*. It is important to note that the lesions caused by TMV infection are reduced by 20 to 30% in tobacco leaves pretreated with tRNA Q^-, compared with untreated leaves or leaves treated with yeast or *E. coli* tRNA.[46] It would appear that readthrough of TMV RNA is carefully regulated *in vivo* and that alteration of the level of this suppressor reduces viral expression.[46-48]

Tyrosine tRNA Q^- from mammalian cells also suppresses the UAG termination signal of TMV RNA. Shindo-Okada et al.[21] isolated $tRNA^{Tyr}$ Q^- from mouse tumor cells in culture and co-injected this tRNA with TMV RNA into *Xenopus* oocytes. The mouse $tRNA^{Tyr}$ supported synthesis of the viral 180-kDa readthrough protein. Interestingly, a tyrosine tRNA that contained 6-thioqueuine was found to be a more efficient suppressor of the TMV RNA UAG termination codon than the tyrosine tRNA that lacked Q base.[21]

The structures of *Drosophila*,[16,17] *A. pernyi*,[23] tobacco plant,[18] wheat germ,[19] wheat leaves,[19] and lupin seed[22] tyrosine tRNAs are shown in Figure 3. The single difference between suppressor and nonsuppressor is the presence or absence of Q base in the wobble position of the anticodon. The suppressor species lack Q base. Thus, the molecular basis for suppression of the TMV RNA termination codon is the substitution of G for Q in the wobble position of the $tRNA^{Tyr}$ anticodon. Furthermore, *these studies demonstrate unequivocally that the degree of base modification on tRNA regulates the expression of certain proteins at the level of translation.*

The interaction between the GψA anticodon in $tRNA^{Tyr}$ and the UAG termination codon is not well understood, particularly since G:G base pairings are not permitted in the wobble hypothesis.[41] However, the G in the anticodon must participate in the recognition process since the GΨA anticodon does not translate UAA codons.[20] It has been suggested that the G in the anticodon may interact with the G in UAG[20] in the syn conformation[49,50] (see review in Reference 11 for further discussion). The Ψ in the middle position of the anticodon, which is unique to $tRNA^{Tyr}$ of eukaryotes,[42] apparently also participates in the ability of the Q-deficient isoacceptor to read UAG codons. Changing Ψ to U in yeast $tRNA^{Tyr}$ has been shown to result in loss of suppressor activity.[51] It would appear that a more stable anticodon:codon complex is formed with the Ψ:A base pairing in the middle position than with the U:A base pairing[52] and thus Ψ:A may be required for suppression of the UAG termination codon.[16-23] The coding properties of Q-containing and Q-lacking Asn-, Asp-, and His-tRNAs that, unlike ^{Tyr}-tRNA, contain U in the middle position of their anticodon are discussed in the section on missense suppression.

Kubli and co-workers have contributed greatly to our understanding of the role of suppressor tRNAs[16,17,53-55] and suppression in *Drosophila*[54-56] and to the regulation of *Drosophila* $tRNA^{Tyr}$ gene expression during development.[55,57,58] In their studies, a total of eight *Drosophila* tyrosine tRNA genes have been sequenced.[55,57,58] Although the coding sequence of all eight genes is identical, each contains an intron that may vary in length. Six of the genes contain introns 20 to 21 nucleotides in length, while a seventh and eighth contain introns of 48 and 113 nucleotides, respectively. The introns are required for formation of Ψ in the middle position of the anticodon,[55,57,58] as has also been shown recently for human $tRNA^{Tyr}$.[59] Since the introns within the different *Drosophila* genes varied in size and sequence, a technique was devised by S1 mapping of the unprocessed transcripts to measure the transcriptional activity of different genes with identical mature products.[58] By this technique, expression of the *Drosophila* gene containing the 113-bp intron was determined to be switched on during embryogenesis and its expression continued through subsequent developmental stages. The gene containing the 48-bp intron was expressed during the larval stages only. Several of the tyrosine tRNA genes occur within or near the control regions of developmentally expressed RNA polymerase II genes. These observations suggest that the Pol III transcribed tRNA genes may be part of the regulatory system of the adjacent Pol II transcribed genes.[57,58]

The gene for the major $tRNA^{Tyr}$ from tobacco plants, *Ni-*

cotiana rustica, has been isolated and sequenced.[60] It contains an intron 13 nucleotides in length and the signals for transcription termination occur immediately after the coding sequence. The 5′ and 3′ flanking sequences of the primary transcript are processed first in HeLa cell extracts, which is then followed by excision of the intron.[61] This pathway of maturation in HeLa cell extracts is reversed from that observed with a human $tRNA^{Tyr}$, that is, the intron of the human gene is excised first from the primary transcript, followed by processing of the flanking sequences.[61] Recent evidence suggests that the intron of human $tRNA^{Tyr}$ may be removed autocatalytically and not enzymatically.[62]

b. OCHRE SUPPRESSOR tRNAs

The naturally occurring UAG termination codon at the end of the *gag* gene in MuLV[63] and at the end of the 130-kDa protein in TMV,[48] and the naturally occurring UGA termination codon in an open reading frame of Sindbis virus,[64] have been mutated to UAA. Introduction of the mutated genomes into the corresponding host cell resulted in formation of mature virus in all three cases. In the former two studies, suppression of the UAA termination codon also occurred *in vitro* in rabbit reticulocyte lysates. These results demonstrate that mammalian cells and tobacco plants contain tRNAs capable of serving as ochre suppressors and it is of considerable significance to identify the tRNA or tRNAs that suppress UAA. Furthermore, it will be of interest to determine if suppression of UAA codons occurs by misreading, as has been observed with each amber suppressor characterized thus far (see above), or if an authentic ochre suppressor is present in mammalian cells.

It should also be noted that yeast contains a naturally occurring ochre suppressor.[35] As noted above, the glutamine tRNA that normally reads CAA in yeast is capable, when present in high copy number, of misreading UAA codons.

c. OPAL SUPPRESSOR tRNAs

i. Serine tRNAs (Selenocysteine $tRNA^{Ser}$)

Two minor seryl-tRNAs in vertebrate cells that read UGA in protein synthesis[26,27] and have also been designated as phosphoseryl-tRNAs[10] have recently been shown to form selenocysteyl-tRNA; hence, they are now designated as selenocysteyl-$tRNA^{Ser}$[28] (and see below). The occurrence of these tRNAs in mammalian and avian cells was originally reported in 1970[65] when a late-eluting peak of seryl-tRNA from a reverse phase chromatographic column was found to recognize specifically the nonsense codon, UGA, in a ribosomal binding assay. At about this same time, a minor seryl-tRNA from avian cells was shown to be phosphorylated on its serine moiety to form phosphoseryl-tRNA.[66] Subsequently, the late-eluting peak of seryl-tRNA was reported to represent two species of seryl-tRNA that recognized UGA[67] and a minor seryl-tRNA from bovine mammary tissue was reported to form phosphoseryl-tRNA in the presence of ATP and a kinase preparation from the homologous tissue.[68] The seryl-tRNA that recognized UGA in a ribosomal binding assay was found to read UGA in protein synthesis[26,27] and its primary sequence showed that it was 90 nucleotides long, with relatively few modified bases.[26,27,69] It was not until 1982, however, that this tRNA and the minor seryl-tRNA that was phosphorylated on its serine moiety were shown to be the same molecule.[27] The kinase that phosphorylates this seryl-tRNA has been purified from bovine liver and it specifically phosphorylates the minor seryl-tRNA.[70]

There are two species of selenocysteine $tRNA^{Ser}$ in tissues of higher vertebrates that differ from each other by several pyrimidine transitions,[27] even though the two isoacceptors are encoded within the same gene that is present in single copy in the genomes of higher vertebrates.[71-73] The pyrimidine transitions that occur in the two gene products[27] must therefore arise posttranscriptionally. The primary sequences of the two isoacceptors are shown in Figure 4. Their anticodons are NCA and CmCA[26,27] and N is probably a modified U.[69] The corresponding tRNAs have also been sequenced from mouse and human cells[69] and their sequences are similar to those from bovine liver shown in the figure. The gene encoding these isoacceptors has been isolated and sequenced from human,[72] rabbit,[73] chicken,[71] *Xenopus*,[74] nematode (*Caenorhabditis elegans*),[74] and *Drosophila* genomes.[74] The vertebrate genes are identical in sequence, with the exception that the human and rabbit genes contain a T at position 11, while those of chicken and *Xenopus* contain a C at this position. The nematode and *Drosophila* genes have 79.3 and 74.7% homology, respectively, to the vertebrate genes and 77.0% to each other, demonstrating that the gene has undergone substantial evolutionary change. Each gene contains a TCA sequence corresponding to the anticodon of the gene product, demonstrating unequivocally that higher eukaryotes contain tRNAs that read a termination codon in their genomes. The human[72] and rabbit[73] genomes contain a pseudogene in addition to the opal suppressor tRNA gene. The gene and pseudogene are present on human chromosomes 19 and 22, respectively.[75] In addition, restriction analysis of DNAs obtained from ten different humans demonstrates that the suppressor tRNA gene contains a *SalI* restriction fragment length polymorphism.[75]

The selenocysteine $tRNA^{Ser}$ isoacceptors are synthesized by a unique metabolic pathway unlike that of other tRNAs, as demonstrated by the observation that the primary transcript does not contain a 5′ leader sequence and that the 5′ terminal triphosphate is transported from the nucleus to the cytoplasm.[76] The 3′ trailer sequence is cleaved by the 3′ processing enzyme. The kinetics of transport were also determined for these isoacceptors and found to be similar to those of other tRNAs.[76] The *Xenopus* gene is more actively expressed than the human, rabbit, and chicken genes both *in vitro* (in HeLa cell extracts) and *in vivo* (after microinjection into *Xenopus* oocytes).[77] Expression of the primary transcript of the *Xenopus* gene is governed by several upstream regulatory elements, including

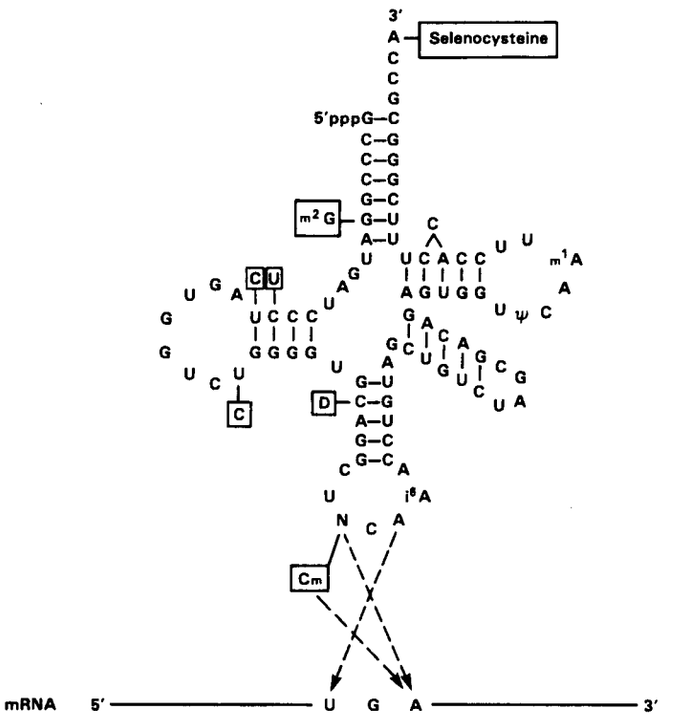

FIGURE 4. Structure of selenocysteine tRNA^{Ser} isoacceptors from bovine liver.[26,27] The isoacceptors differ from each other by several pyrimidine transitions, as well as a methylG at nucleotide 6, as shown by the bases in the small boxes. Both isoacceptors read UGA in protein synthesis,[26,27] and both form phosphoseryl-tRNA^{Ser}[27,70], while the CmCA isoacceptor preferentially forms selenocysteyl-tRNA^{Ser}[28].

an AT-rich region between −62 and −75, a GC-rich region, and a TATA box near −30.[77] An internal control region designated as the A box determines the initiation start site of the gene.[77]

Several functions have been proposed for the mammalian and avian phosphoseryl-tRNAs.[10,27,66,78,79] Even though the phosphoseryl-tRNA appears to be capable of donating its phosphoserine moiety to protein,[80] recent studies suggest that its major role is to serve as an intermediate in the biosynthesis of selenocysteyl-tRNA^{Ser} (see Reference 28 and below).

The minor seryl-tRNAs that read UGA in protein synthesis and are phosphorylated on their serine moiety to form phosphoseryl-tRNA have been shown recently to exist *in vivo* as selenocysteyl-tRNA.[28] They are now designated as selenocysteyl-tRNA^{Ser}. This observation, along with the finding that both seryl-tRNA^{Ser} and phosphoseryl-tRNA^{Ser} were also isolated *in vivo*, provide strong evidence that the cellular role of the minor opal suppressor tRNAs are twofold: (1) to serve as carrier molecules upon which selenocysteine is biosynthesized and (2) to serve as direct donors of selenocysteine to protein in response to specialized UGA codons.[28] Interestingly, a TGA codon occurs in mouse,[81,82] human,[82-84] bovine,[82] and rat[85] glutathione peroxidase genes and the TGA codon codes for a selenocysteine moiety that is present in the active site of the corresponding protein product. The selenocysteine in glutathione peroxidase is known to arise from serine and selenium.[88] In this connection, it is important to note that selenocysteine occurs at the active site of formate dehydrogenase in *E. coli*, which is coded by UGA.[87-89] The selenocysteine moiety is inserted co-translationally in response to the UGA codon,[86] which is correlated with the presence of a specific tRNA.[89] The tRNA is aminoacylated with serine[89] and also forms selenocysteyl-tRNA^{Ser90} (see review in Reference 91). The human glutathione peroxidase gene has been mapped to human chromosomes 3, 21, and X, and these studies confirm that the active gene occurs on chromosome 3 and suggest that the other loci are pseudogenes.[92]

The possible means by which UGA can code for selenocysteine in some circumstances and as a stop codon in others has been discussed recently by several investigators.[11,95-97] Recent studies in our laboratory, however, suggest that a specific factor (e.g., an antirelease factor) may also be required for the incorporation of selenocysteine into protein from selenocysteyl-tRNA.[262]

Mizutani and Hitaka[98] have determined the association constants for UGA and phosphoseryl-tRNA^{Ser} and for UGA and the release factor. The K_a value for UGA and tRNA^{Ser} was

quite high ($8 \times 10\ M^{-1}$), while that for UGA and the release factor was much lower ($1.26 \times 10^5\ M^{-1}$). Although these data were interpretated that $tRNA^{Ser}$ functions to read only UGA codons that favor readthrough, they may also be interpreted that a specific factor is required for translation of UGA codons that occur in an open reading frame in proteins such as glutathione peroxidase and for insertion of selenocysteine into the growing polypeptide chain. Mizutani and co-workers have also reported that the UGA-recognizing isoacceptors are aminoacylated with serine by seryl-tRNA synthetase.[93]

II. Tryptophan tRNA

Transfer $tRNA^{Trp}$ was isolated from rabbit reticulocytes and shown to suppress the UGA termination codon in rabbit β-globin mRNA *in vitro*.[25] This study also indicated that the readthrough protein of β-globin occurs naturally in rabbit reticulocytes and that the $tRNA^{Trp}$ may be the mediator of this suppression *in vivo*. Rabbit reticulocyte $tRNA^{Trp}$ has not yet been sequenced.

It is important to note that the minor tRNA that is aminoacylated with serine forms selenocysteyl-tRNA and reads UGA in protein synthesis (see above section) also occurs in rabbit reticulocytes,[99-101] making this isoacceptor a potential candidate to participate in the production of the β-globin readthrough protein. To determine the amino acid at the site of readthrough, antibodies were generated against the readthrough protein and used to isolate this protein from rabbit reticulocytes.[102] The readthrough β-globin molecule and a second protein, which appears to be a degradation product of the readthrough molecule, were isolated from rabbit reticulocytes for characterization.

2. Codon Context Effects in Nonsense Suppression

The level of suppression at a termination codon is presumably determined by the competition between the release factor, which promotes the cessation of protein synthesis, and a suppressor tRNA, which promotes readthrough, for a stop codon. In addition, the nucleotides surrounding stop codons (i.e., the nucleotide context) play a role in the efficiency of suppression in microorganisms.[103,104] In higher eukaryotes, however, the available evidence suggests that the nucleotide context may not be a determining factor in whether a termination codon will be suppressed, nor how effciently it will be suppressed.[82,105,106] Some stop codons occur in an open reading frame[81-85,107,108] and must be translated efficiently for expression of the resulting protein product. Glutathione peroxidase (GPx) mRNA, for example, contains a UGA codon that corresponds to a selenocysteine moiety in the protein product[81-85] and this codon must be translated efficiently for expression of the enzyme. The nucleotide sequences surrounding the opal codon in human, bovine, and mouse GPx mRNA are identical to those surrounding the natural opal termination codons in a number of other eukaryotic mRNAs, suggesting that nucleotide context does not play a role in translation of the GPx UGA codon.[82] However, comparison of the secondary structure of GPx mRNA and 12 other eukaryotic mRNAs that utilize UGA as a termination codon revealed that GPx mRNA may have a unique conformation in which UGA occurs in a stem that may favor readthrough.[82]

Among the first studies to show that nucleotide context may not be involved in the efficiency of readthrough in higher eukaryotes was that in which a UGA mutation was identified in alcohol dehydrogenase mRNA isolated from *Drosophila*.[106] The mutation was efficiently suppressed *in vitro* by an opal suppressor tRNA. However, the UAA termination codon for this protein was resistant to suppression even though the nucleotide context of the opal mutation and the ochre termination codon were virtually identical, i.e., AUCUGAAAA and AUCUAAGAA. The observation that the UAA termination signal was resistant to suppression substantiated an earlier report that most termination codons *in vivo* are also resistant to readthrough.[109] Bienz et al.[109] injected a mixture of purified amber, ochre, and opal suppressor tRNAs into *Xenopus* oocytes and then compared the protein populations in injected and uninjected cells by two-dimensional gel electrophoresis. In over 600 proteins resolved by electrophoresis, there were very few "new" proteins that arose from suppression, demonstrating that most termination codons favor release of the growing polypeptide chain. To provide further insight into the reason that most termination codons are resistant to suppression *in vivo*, the nucleotide contexts of naturally occurring termination signals in numerous eukaryotic mRNAs were examined.[105] The latter study did not reveal any consensus sequences surrounding stop codons[105] that might in part explain the observation made in *Xenopus* oocytes.[109]

Several naturally occurring termination codons in higher eukaryotes are leaky or are weakly suppressed. These include the UAG termination codons in the plant viral RNAs, TMV[110] and BNYVV,[111] and in the *gag-pol* RNAs of mammalian type C viruses, MuLV[12,14,15,31] and FeLV.[13] In addition, the UGA termination codon of rabbit β-globin mRNA is weakly suppressed *in vivo*.[25,102] The nucleotide contexts of the leaky UAG termination codons in these mRNAs[30,112-114] are the same or are very similar to those of natural termination codons,[105] which presumably are resistant to readthrough. Hence, it would seem from these observations and those reported above that the nucleotide contexts of stop codons do not play a major role in the level of suppression in higher eukaryotes. However, more information must be obtained about the nature of the suppression process of nonsense codons in higher eukaryotes before we can definitively assess the effect of the codon context. Since the nucleotide context may be important in missense suppression and there are other parameters in which the context of termination codons should be considered in translation, this topic is further discussed below in Section I.D, *Function of Suppressor tRNAs in Translation*. In addition, the reader is

referred to an excellent review by Valle and Morch[11] on the termination process and to additional considerations of UGA as a termination codon and as a codon for selenocysteine.[95-97]

3. Identification of Nonsense Suppressor tRNAs and Nonsense Mutations

a. *IN VITRO* ASSAYS

Nonsense suppressor tRNAs and nonsense mutations have been readily identified *in vitro* using nuclease-treated rabbit reticulocyte lysates[115] and wheat germ extracts[116] programed with mRNA and to which is added a suppressor tRNA. Opal and ochre suppressor tRNAs have been characterized by adding rabbit globin mRNA and a suppressor tRNA to nuclease-treated reticulocyte lysates and separating the globin products by polyacrylamide gel electrophoresis.[117] Rabbit α-globin mRNA terminates in UAA and the next termination signal is 21 amino acids downstream,[118] while rabbit β-globin mRNA terminates in UGA and the next termination signal, which consists of two tandem UAA codons, is 22 amino acids downstream.[119] The α- and β-globins are 141 and 146 amino acids in length, respectively, and thus the β-globin readthrough protein would be expected to migrate more rapidly on a polyacrylamide gel than the corresponding α-globin protein. The reverse occurs, however, which is attributed to the large number of proline residues located in the α-globin readthrough protein.[117] Amber suppressor tRNAs have also been identified in reticulocyte lysates. The nuclease-treated lysates are programed with TMV RNA[110] or BNYVV RNA[111] and the protein synthesis products then examined on a polyacrylamide gel. Wheat germ extracts programed with the same set of mRNAs used in reticulocyte lysates have also been employed to assay for nonsense suppressor tRNAs.[120] The lysate system, however, has been used more widely.

Nonsense mutations and naturally occurring termination codons have been identified in viruses where the viral mutant or normal mRNA is assayed *in vitro* in the presence of the purified suppressor tRNAs from microorganisms. For example, two amber and one ochre mutations within the human adenoviral 30-kDa ND-1 protein were identified among a series of suspected nonsense mutations by assaying for restoration of the ND-1 protein in the presence of an amber and an ochre suppressor tRNA from yeast.[121] An amber and an opal mutation were similarly identified in the thymidine kinase gene of herpes simplex virus by assaying a series of mutant mRNAs for restoration of viral kinase activity.[122] Furthermore, the termination codon in Moloney murine leukemia[31,123] and sarcoma viruses,[123] which is at the end of the *gag* gene, that in TMV, which is at the end of the 126-kDa protein,[110] and that in BNYVV, which is at the end of the coat protein,[111] was identified as UAG by utilizing an amber suppressor from yeast.

The specific response of selenocysteyl-tRNA^{Ser} (formerly designated as opal suppressor seryl-tRNA or opal suppressor phosphoseryl-RNA) to UGA[65] in a ribosomal binding assay[124] has provided a simple and rapid means of identifying the occurrence of this tRNA in tissues and cells of higher eukaryotes.[10,26-28,65,99-101] Potential opal suppressor tRNAs within the tRNA population of bovine liver were detected by fractionating the tRNA and then identifying those isoacceptors that stimulated the binding of ^{3}H-UGA to ribosomes.[67] The assay is based on a previous finding that the attachment of a labeled trinucleotide diphosphate to ribosomes is significantly enhanced in the presence of the corresponding isoacceptor that recognizes the labeled codon.[125] Interestingly, the only tRNAs that were found to recognize UGA specifically were the selenocysteyl-tRNA^{Ser} isoacceptors.

b. *IN VIVO* ASSAYS

Mammalian cells in culture and *Xenopus* oocytes have been injected with purified suppressor tRNAs from microorganisms to identify nonsense mutations and to examine their effects on readthrough. For example, yeast and *E. coli* amber, ochre, and opal suppressors were injected independently into mouse L cells that were suspected of carrying a nonsense mutation in the HGPRT gene.[126] Only the ochre tRNAs restored HGPRT activity and thus the mutation was identified as UAA. Beinz and co-workers[109,127] have utilized *Xenopus* oocytes as an *in vivo* assay system for studying nonsense suppression and these investigators noted several advantages of *Xenopus* oocytes over *in vitro* and other protein synthesis systems involving intact cells.[127] In their initial studies, *Xenopus* oocytes were co-injected with either a yeast amber, ochre, or opal suppressor and with rabbit globin mRNA or TMV RNA.[127] The opal suppressor resulted in readthrough of rabbit β-globin mRNA and the amber and ochre suppressors resulted in readthrough of TMV RNA. The level of suppression in each case was dependent upon the amount of suppressor tRNA injected. Other studies utilizing *Xenopus* oocytes in nucleotide context studies[109] and in identifying tyrosine tRNA Q^{-} as a suppressor[16] have been described above. A thorough description of *Xenopus* oocytes as an *in vivo* system for assaying and screening nonsense mutations and suppressor tRNAs has been published.[128]

An assay based on suppression of a natural UAG termination codon in the NS1 protein of influenza virus that extends the protein by 20 amino acids has been developed for quantifying suppressor activity in intact cells.[129] An amber suppressor tRNA gene (see Reference 130 and below) and the influenza virus were introduced into mammalian cells that were then pulse labeled with ^{35}S-methionine. The labeled NS1 protein and readthrough protein were isolated from a cell extract by immunoprecipitation, electrophoresed on a polyacrylamide gel, and the percent readthrough determined. A high level of suppression (about 25%) occurred in cells where the suppressor gene was transiently introduced and a low level of suppression (about 2.5%) in cells where the suppressor was stably integrated into the cell genome. The reason for the low level of suppression observed in those cells in which the suppressor gene was permanently integrated is not known. The possible deleterious effects of high levels of suppressor tRNAs in cells

of higher eukaryotes is discussed further below in Section I.A.4, *Introduction of Nonsense Suppressor tRNA Genes into Intact Cells*.

An extremely clever and important assay for detecting nonsense mutations in *Drosophila* has been developed recently.[54] The assay is based on the partial reversal of mutant eye color to wild type following the introduction of a vector encoding an amber or ochre suppressor tRNA into the germ line of *Drosophila* stocks in which the xanthine dehydrogenase was characterized by not only being enzymatically negative, but also negative for cross-reacting material. The amber and ochre nonsense suppressors were generated by site-specific mutagenesis of a wild-type $tRNA^{Tyr}$ gene and the resulting suppressor genes subcloned into a transformation vector. Introduction of the vector into a series of *rosy* stocks resulted in an eye color change toward wild type in one of the stocks. The suspected amber codon in the xanthine dehydrogenase gene in this stock was confirmed by sequencing.[54]

The nematode, *C. elegans*, provides an excellent tool for studying nonsense suppression in higher eukaryotes. Suppressor tRNAs and nonsense mutations have been generated in *C. elegans*, and this subject has been thoroughly reviewed elsewhere.[131,132]

An *in vivo* assay for measuring the intracellular levels of aminoacylation of suppressor tRNAs following their amplification has been reported.[133] A suppressor tRNA gene is introduced into a cell and the resulting amplified, suppressor tRNA (which is aminoacylated within the intact cell) is extracted at low pH and the acylated and unacylated tRNA separated on a polyacrylamide gel. Amber $tRNA^{Gln}$,$tRNA^{Lys}$, and $tRNA^{Tyr}$ suppressors, which were expressed from the corresponding amplified genes, were aminoacylated 80, 40 to 50, and 100%, respectively. The lower levels of aminoacylation of $tRNA^{Gln}$ and $tRNA^{Lys}$ may be due to the effect of an altered base in the anticodon on the aminoacylation process.[133,134]

4. Introduction of Nonsense Suppressor tRNA Genes into Intact Cells

New approaches for studying nonsense suppressor tRNAs have been developed by introducing the corresponding suppressor genes, which have been constructed by site-specific mutagenesis, into cells of higher eukaryotes.[129,130,133-141] These studies have provided a means of (1) identifying and characterizing nonsense mutations, (2) quantifying levels of suppression of nonsense codons, (3) determining the long-range effects of suppressor tRNAs on cells following the permanent insertion of the corresponding genes into the host cell genome, (4) analyzing and characterizing viral genomes by classic genetic techniques, and (5) determining if suppressor tRNAs may provide an avenue for alleviating human diseases that result from nonsense mutations. The question of whether nonsense suppressors can be used in gene therapy experiments has been addressed by Kan and co-workers.[133,134,136] In a model system, an amber suppressor $tRNA^{Lys}$ gene[136] and, subsequently, an amber suppressor $tRNA^{Gln}$ gene,[134] and mRNA from a patient with β-thalassemia carrying a UAG mutation (AAG → UAG), were co-injected into *Xenopus* oocytes, which resulted in production of functional suppressors that translated the amber mutation. Further examination of this model system has shown that much greater insight into the ability of suppressors to function in cells of higher eukaryotes is required before actual gene therapy experiments to correct nonsense mutations in human globin can be undertaken.[134] For example, further characterization of the $tRNA^{Lys}$ suppressor has shown that it functioned inefficiently as a suppressor in mammalian cells when the corresponding gene was stably integrated in the host's genome, even though the gene and gene product were amplified manyfold.[133,134] The low suppressor activity was due to poor aminoacylation of the gene product. The reason for the poor aminocylation is not known, although the presence of a different base in the anticodon may affect the level of lysine attachment to the suppressor tRNA.[133] In addition, the levels of globin mRNA carrying a nonsense mutation vary in thalassemic patients and these levels are often quite low.[142] It is encouraging that introduction of a suppressor tRNA gene into cells containing a nonsense mutation in globin mRNA resulted in an enrichment in the level of mRNA (see Reference 142 and references therein). However, several questions must be resolved before gene therapy experiments using nonsense suppressor tRNAs can be undertaken. For example, it must be established if (1) the levels of production and subsequent aminoacylation of suppressor tRNAs are adequate to suppress nonsense codons providing sufficient amounts of product, but yet the suppressor activity is not at level that is deleterious to the cell, and (2) mRNAs harboring nonsense mutations can be maintained in sufficient levels so that suppression of the mutation would result in enough product to benefit the cell.

RajBhandary, Sharpe, and co-workers have contributed significantly to our understanding of the role and effects of suppressor tRNAs in mammalian cells.[129,130,135,137-141] These investigators prepared a number of suppressor tRNA genes by site-specific mutagenesis in the anticodon of the corresponding normal tRNA gene and cloned the resulting suppressor into an appropriate vector for introduction into different mammalian cell lines. The biological activity of the suppressor tRNAs were monitored by co-introducing a complementary set of nonsense codons into the same cell line. The suppressor tRNAs included an amber[130] and an ochre[135] suppressor that were derived from a *Xenopus laevis* tyrosine tRNA gene and an amber, ochre, and opal suppressor that were derived from a human serine tRNA gene.[139] The nonsense codons included naturally occurring amber and ochre termination codons encoded in different viral strains,[130,138,139] and amber, ochre, and/or opal mutations generated in viral[135,138,141] and *E. coli* genes,[135,140] and in the kanamycin resistance gene.[135,136] An extremely useful set of nonsense mutations was prepared in an *E. coli* CAT gene in

which the serine codon at position 27 was changed to either an amber, ochre, or opal codon.[140] Co-introduction of the mutant CAT genes, other mutant *E. coli* genes, mutant viral genes, or a virus containing a naturally occurring termination codon and the corresponding suppressor tRNA has shown that the level of suppression was efficient (ranging from about 20 to 50%) in transient systems,[129,140] but inefficient (about 3%) in cell types in which the suppressor tRNA gene was stably integrated into the host's genome.[129,134,135] The fact that different suppressor tRNA genes that are stably introduced into different mammalian cell lines result thus far in suppression levels of only about 3% suggests that cells cannot tolerate higher suppressor levels on a permanent basis. Such observations are also supported in studies with *Drosophila*. Nonsense suppressor tRNA genes that have been introduced into *Drosophila* cell lines function inefficiently[54,143] and the resulting flies in some cases are sterile.[54] Thus, it appears that higher eukaryotes may have evolved in such a way that they cannot utilize suppressor tRNAs to reverse deleterious effects from nonsense mutations. In this way, populations of organisms cannot arise that depend on the presence of suppressor tRNAs for survival.

The induction of high levels of suppressor activity in mammalian cells has been reported recently.[141] An amber suppressor $tRNA^{Ser}$ gene was cloned into SV40 near its origin of replication and the resulting plasmid co-transfected into monkey kidney cells with a SV40 plasmid carrying a temperature-sensitive mutation in the large T-antigen gene. Cells that stably integrated both DNAs were selected. Suppression levels as high as 70% of an amber codon were observed when the suppressor gene was amplified by changing the cells from a nonpermissive to a permissive temperature. The technique was used to suppress an amber mutation in the replicase gene of poliovirus, which resulted in an efficient production of virus.[141]

5. Natural Termination Codons That Are Suppressed, Occurrence of Nonsense Mutations, Effects of Polyamines and Antibiotics on Suppression, and Posttranscriptional Generation of Termination Codons in mRNA

Several termination codons have been shown to be read through in higher eukaryotes. These include the termination codons characterized in plant viruses,[110,111] mammalian type C viruses,[12-15,31] Middleburg and Sindbis viruses,[107,108] rabbit β-globin mRNA,[25,102] and glutathione peroxidase mRNA.[81-85] Recently, UAG has been shown to occur in an open reading frame of a C-hordein gene from barley[141a] and of a 21-kDa Zein gene from maize.[141b]

Nonsense mutations have also been characterized in a number of organisms. In *Drosophila*, mutations have been generated by mutagenesis and a UGA[106,144] and a UAG mutation[145] were characterized in the alcohol dehydrogenase gene, a UGA mutation in an actin gene,[146] and a UAG mutation in the xanthine dehydrogenase gene.[54] In nematodes, several nonsense mutations have been generated and characterized, and this subject has been reviewed elsewhere.[131,132] In humans, the occurrence of nonsense mutations in α- and β-globin mRNA genes have been known for some time and have been summarized elsewhere (see References 142 and 147 and references therein). Recent examples of nonsense mutations in human globin genes include variants in the α-globin gene, a TAC → TAA change at codon 140[145] and at codon 35,[94] and in the β-globin gene, a GAA → TAA change at codon 121.[149] Other examples of nonsense mutations identified in humans are (1) a Trp TGG → TGA mutation in the gene for the receptors for plasma low-density lipoprotein,[150] (2) an Arg CGA → TGA mutation at codon 306 in the gene for protein C that is an anticoagulant serine protease,[151] (3) a Lys AAG → TAG mutation at codon 217 in the I-antitrypsin gene,[152] (4) an Arg CGA → TGA mutation at codon 150 in the Clq B chain,[153] and (5) a TGG → TGA mutation in the N-terminal region of 17 α-hydroxylase cytochrome *P*-450.[154] Recently, a series of nonsense mutations were prepared in human β-globin gene to study the effects of nonsense mutations on the cellular accumulation of mRNA.[147] Each nonsense mutation resulted in a decreased accumulation of β-globin mRNA, whereas missense mutations had no effect on the level of accumulation.

Polyamines, as well as certain antibiotics, can stimulate suppression of nonsense codons. For example, spermine and spermidine have been shown to stimulate readthrough of the UGA termination codon in rabbit β-globin mRNA[155] and the UAG termination codon in TMV and TYMV *in vitro*.[156] In addition, the aminoglycoside antibiotics, G-418 and paromomycin, enhance suppression of an amber mutation transformed into monkey cells and the level of readthrough was about 20% of that observed with wild type.[157]

Generation of a stop codon in mRNA that occurs posttranscriptionally and results in the presence of a molecularly distinct protein has been observed in mammalian cells.[158-162] A single protein gene encodes human apolipoprotein (apo) B-100 and apoB-48 proteins, where apoB-48 is approximately 48% of the molecular weight of apoB-100.[158] Human apoB-100 contains a glutamine codon (CAA) at position 2153 that is converted to the termination codon UAA by a single pyrimidine transition.[158] This reaction is tissue specific[159-162] and is hormonally modulated in rat liver.[162]

a. CONCLUDING REMARKS ABOUT NONSENSE SUPPRESSOR tRNAs

Our knowledge about nonsense suppressor tRNAs and nonsense suppression in higher eukaryotes is summarized in Table 1. Table 1A shows the aminoacyl-tRNAs that suppress termination codons and Table 1 shows the mRNAs that are read through in higher eukaryotes. The major function of the Gln-, Leu-, Trp-, and Tyr-tRNAs shown in Table 1A is to read assigned codons within the amino acid family and these isoacceptors serve as nonsense suppressors by misreading termination codons. The function of the selenocysteyl-tRNAs is apparently twofold, as discussed above. The latter two iso-

Table 1
Natural Suppression of Termination Codons in Mammalian Cells[a]

Aminoacyl-tRNAs that Suppress Termination Codons

tRNA	Source	Anticodon	Codons read	Comments
Tyrosine	*Drosophila*[16,17] *A. pernyi*,[23] tobacco plant,[18] wheat germ and leaves,[19] lupin seeds,[22] and mouse tumor cells[21]	GΨA	UAU, UAC, UAG	Q^- isoacceptor suppresses UAG stop codon in TMV RNA either *in vivo* (in *Xenopus* oocytes) or *in vitro*[16,18,19,21,22]
Glutamine	Mammalian cells	UmUG	CAA, UAG	Kuchino et al.[14] reported this tRNA suppresses UAG stop codon in TMV RNA *in vitro;* their report that this tRNA is enriched manyfold in MuLv-infected cells was not substantiated by *in vitro*[15] or *in vivo* studies[34]
Leucine	Calf liver, bovine mammary tissue[24]	CAG	CUG, UAG	Suppress UAG stop codon in TMV and BNYVV RNAs *in vitro*[24]
Leucine	Calf liver[24]	CAA	UUG, UAG	
Tryptophan	Rabbit reticulocytes	?	UGG, UGA	Suppresses UGA stop codon in rabbit β-globin *in vitro*[25]
Selenocysteine	Mammalian tissues[26-28]	CmCA	UGA	Suppress UGA codon in rabbit β-globin mRNA *in vitro*;[26,27] form phosphoseryl-tRNA[27,70] and selenocysteyl-tRNA[28]
Selenocysteine	Mammalian tissues[26-28]	NCA	UGA	

mRNAs that are suppressed

mRNA	Codon	Aminoacyl-tRNA	Comments
Glutathione peroxidase	UGA	Selenocysteine (see text)	cDNA from human,[82-84] mouse,[81,82] bovine,[82] and rat[85] sources has been sequenced and a TGA codon occurs at the active site of the gene product that corresponds to a selenocysteine moiety
Rabbit β-globin	UGA	?	Readthrough protein occurs in rabbit reticulocytes[25,102]
Sindbis and Middleburg viruses	UGA	?	These viruses contain a UGA codon in an open reading frame[107,108]
Moloney, feline and AKR leukemia viruses	UAG	Glutamine	The amino acid at the stop codon in *gag-pol* fusion protein in MuLV and feline LV is Gln,[12,13] but it has not been determined in AKR[163]
Moloney murine leukemia virus	UAA, UGA	?	The UAG stop codon at the end of the *gag* gene has been changed to UAA and UGA and both codons are suppressed intracellularly as well as *in vitro*[63]
Sindbis virus	UAA	?	The UGA codon that occurs in an open reading frame of this virus has been changed to UAA and it is suppressed intracellularly[64]
TMV	UAA		The UAG codon at the end of the 130-kDA protein has been changed to UAA and it is suppressed intracellularly as well as *in vitro*[48]

[a] References to original work are given in the table.

acceptors should be considered as suppressors until we better understand how they utilize a termination codon as the code word for selenocysteine, but yet do not read through all UGA termination signals. Our knowledge about nonsense suppressors in higher eukaryotes will be enhanced by identification of the amino acids that are present at the site corresponding to the stop codons in rabbit β-globin readthrough protein,[25,102] in Sindbis and Middleburg viral proteins[107,108] that arise by suppression of a UGA codon, and in the altered stop codons of Moloney leukemia[63] and Sindbis[64] viruses. Another important area for further study is the reason why some termination codons are read through efficiently, compared with others that are poorly suppressed or are not suppressed at all.

It is unfortunate that high levels of nonsense suppressor tRNAs apparently cannot be tolerated on a permanent basis in the cells of higher eukaryotes. Thus, the optimism for use of suppressor tRNAs in gene therapy experiments to correct nonsense mutations must await further experimentation.

B. Ribosomal Frameshifting

Ribosomal frameshifting or frameshift suppression may operate in one of two directions by a variety of mechanisms

such that the reading frame is altered either in the 5′ or 3′ direction.[1,164-168] Bacteria and yeast are capable of frameshifting in both directions (see above references and, in addition, Reference 11 and references therein). The *gag* and *pol* genes of many vertebrate retroviruses occur in different reading frames and the frames are aligned or are suspected of being aligned by ribosomal frameshifting in the 5′ (or −1) direction.[166,169-174] Since frameshifting is best understood in retroviruses, we will examine this means of altering reading frames and then consider the possible involvement of tRNA in this process.

1. Ribosomal Frameshifting in Retroviruses

As noted above, the *gag* and *pol* genes of many vertebrate retroviruses occur in different reading frames (see references in legend to Table 2 and Reference 11). The alignment of the different reading frames for expression of the *gag-pro-pol* fusion protein requires a single frameshift event in the −1 direction in some retroviruses, while others require two frameshift events, one between *gag-pro* and the other between *pro-pol*, both of which are in the −1 direction. Table 2 documents the ribosomal frameshift sites and signals or suspected sites and signals in a number of retroviruses, in the mouse intracisternal A-particle,[175] in the nonretroviruses avian coronavirus (designated IBV),[176] and in the transposable elements in *Drosophila* designated *gypsy*[177] and 17.6.[178] The number of bases in each overlap window and the number of bases from the 3′ end of each window are also included in the table. The boundaries of each frameshift window are determined, for example, in a retrovirus requiring only one frameshift event, by the termination codon read in the zero frame at the end of the *gag* gene and the first upstream termination codon in the −1 frame. The occurrence of hypermodified bases in the anticodon loop of tRNAs at the ribosomal A- and P-sites within the frameshift signals are also shown in the table, which will be discussed further below. One of three common consensus sequences is found in each overlap window: either A AAC, U UUA, or U UUU, where asparagine (AAC), leucine (UUA), or phenylalanine (UUU) are read in the zero frame.[173]

The site of the frameshift has been determined within the *gag-pro* overlapping reading frame in MMTV that involves the A AAC consensus sequence[170] and within the *gag-pol* frameshift sites of HIV[172,174] and RSV[173] that involve the U UUA consensus sequence. These determinations were made by sequencing the transframe protein expressed *in vivo* in MMTV[170] and *in vitro* in RSV[173] and HIV.[172] In MMTV, leucine occurs at the frameshift site that is coded by either UUG in the zero frame or by CUU in the −1 frame (where C is the 3′ base of the AAC codon in the zero frame and UU are the two 5′ bases of the UUG codon; see Table 2).[170] In HIV, the shift occurs at a leucine residue corresponding to the UUA codon (Table 2), but both leucine and phenylalanine occur at the frameshift site in a ratio of 7:3, making assignment of the precise site uncertain.[172] Arginine, which is coded by the 3′ base in UUA and the first two GG bases in the downstream codon, is translated in the −1 frame in HIV. The frameshift in RSV also occurs at a leucine codon in response to the U UUA consensus sequence.[173] The next amino acid residue in the RSV peptide generated from the frameshift site is isoleucine, which is read in the −1 frame. Isoleucine is coded by the 3′ A of UUA and the next two downstream bases, which are UA.[173]

Jacks, Varmus, and co-workers have proposed that the mechanism for ribosomal frameshifting involves a "slippage" of the translational machinery to the −1 reading frame[169,171-173] occurring within the heptanucleotide signal shown in Table 2. A series of mutations constructed within this signal in RSV and determination of the sequence of a peptide generated from the frameshift signal harboring a mutation at its 3′ end support this model.[173] That is, mutations constructed at each base within the heptanucleotide signal shown in Table 2 in RSV[173] (and at most of the corresponding bases in HIV[172,174]) show that frameshifting is inhibited by alterations within this region, with the exception of the 3′ terminal base. Changes at the 3′ terminal base (e.g., alteration of the A in this position in RSV to U, C, or G) do not inhibit frameshifting.[173] Sequence analysis of the RSV peptide generated from AAU UUU UA (where U represents the altered base) showed that asparagine (AAU) and phenylalanine (UUU) were decoded in the zero frame and leucine (UUA) in the −1 frame.[173] In the normal RSV frameshift event, asparagine (AAU) and leucine (UUA) are decoded in the zero frame and isoleucine (AUA) in the −1 frame. In light of the amino acids generated from the A to U mutated sequence, a logical means by which frameshifting occurs in RSV (and other retroviruses) is by slippage of the translational machinery to the −1 reading frame at the A AAU UUA sequence (and presumably at the heptanucleotide sequences within the overlapping reading frames of other retroviruses shown in Table 2).[173]

The observation that a single base change at the 3′ end of the RSV frameshift signal results in the presence of two new amino acids in the peptide generated from this sequence (see Reference 173 and the above paragraph) demonstrates unequivocally that the frameshift has to occur at this site. It also rules out the possibility that the different reading frames in retroviruses are aligned by two out of three base readings or by overlapping reading of a single base upstream of this site.[181] In fact, the alignment must occur by overlapping reading such that the base at the 3′ end of the heptanucleotide signal is decoded twice, once in the zero frame and once in the −1 frame, as was originally noted by Hizi et al. from their studies on sequencing the MMTV transframe protein.[170]

The frameshift event in HTLV-1 is inhibited by mutation of the A AAA AAC sequence in the *gag-pro* overlapping reading frame to A ATA TTC.[179] Thus, the homopolymeric A region that was predicted to be part of the frameshift site in BLV[191] and shown to be part of the frameshift site in MMTV[170,171] is also involved in frameshifting in HTLV-1.

Jacks et al.[173] demonstrated that each of the consensus

Table 2
Ribosomal Frameshift Sites And Signals in Vertebrate Viruses And in Transposable Elements of Higher Eukaryotes[a] And The Occurrence of Hypermodified Bases in The Anticodon Loop of tRNAs at The Ribosomal A- And P-sites

Source	Overlap window	Bases in overlap	Bases from 3′ end of overlap	Bases at and around the frameshift site	Class and subclass[b]	Hypermodified base at ribosomal A-site[c]	Hypermodified base at ribosomal P-site
MMTV	*gag-pro*	16	3	U C A A A A A C UUG	I A	Q	MCM5S2U and MS2T6A
BLV	*gag-pro*	49	0	U C A A A A A C UAA	I A	Q	MCM5S2U and MS2T6A
HTLV-1, STLV-1	*gag-pro*	37	18	C C A A A A A C UCC	I A	Q	MCM5S2U and MS2T6A
HTLV-2	*gag-pro*	28	18	G A A A A A A C UCC	I A	Q	MCM5S2U and MS2T6A
EIAV	*gag-pol*	241	195	C C A A A A A C GGG	I A	Q	MCM5S2U and MS2T6A
HTLV-1	*pro-pol*	178	156	C C U U U A A C CAG	I B	Q	None
STLV-1	*pro-pol*	121	99	C C U U U A A C CGG	I B	Q	None
HTLV-2	*pro-pol*	373	18	C C U U U A A C CUG	I B	Q	None
BLV	*pro-pol*	22	0	C C U U U A A C UAG	I B	Q	None
IBV		40	30	U A U U U A A C GGG	I B	Q	None
SRV-1	*gag-pro*	181	147	C A G G G A A A C GGA	I C	Q	?
SRV-2, MPMV	*gag-pro*	181	147	C A A A A A A A C GGG	I C	Q	?
VISNA	*gag-pol*	124	45	C A G G G A A A C AAC	I C	Q	?
Mouse IAP	*gag-pol*	34	3	C U G G G U U U U CCG	II A	Wye	None
SRV-1, MPMV	*pro-pol*	22	0	G G A A A U U U U UAA	II B	Wye	Q
SRV-2	*pro-pol*	22	0	G G A A A U U U U UAG	II B	Wye	Q
17.6	*gag-pol*	46	30	G A A A A U U U U CAG	II B	Wye	Q
RSV	*gag-pol*	58	0	A C A A A U U U A UAG	III A	None	Q
MMTV	*pro-pol*	13	0	C A G G A U U U A UGA	III A	None	Q
HIV-1	*gag-pol*	241(205)	234(198)	A A U U U U U U A GGG	III B	None	Wye
HIV-2	*gag-pol*	283	267	G G U U U U U U A GGA	III B	None	Wye
SIV	*gag-pol*	343	213	G G U U U U U U A UUG	III B	None	Wye
gypsy	*gag-pol*	70	51	A A U U U U U U A GGG	III B	None	Wye

[a] Underlined bases designate conserved heptanucleotide sequences within the overlaps that are associated with or are suspected of being associated with frameshifting (see Reference 173 and the text). Abbreviations and references to published work are MMTV, mouse mammary tumor virus;[170,171,184] HTLV-1 and -2, human T-cell leukemia virus-1[185-187] and -2;[188] STLV-1, simian T-cell leukemia virus;[187] EIAV, equine infectious anemia virus;[189,190] BLV, bovine leukemia virus;[191,192] SRV-1, simian acquired immunodeficiency syndrome ([SAIDS] designated as SRV);[193] and SRV-2;[194] MPMV, Mason-Pfizer monkey virus;[195] VISNA, VISNA VIRUS;[196] RSV, Rous sarcoma virus (an avian virus);[169,173,197] IBV, (coronavirus) infectious bronchitis virus (an avian nonretrovirus);[176] HIV-1 and -2, human immunodeficiency virus-1[172,198-200] and -2;[201] SIV, simian immunodeficiency virus;[202,203] *gypsy*, transposable element in *Drosophila* designated *gypsy*;[177] mouse IAP, mouse intracisternal A-particle;[175] 17.6, transposable element in *Drosophila* designated 17.6.[178]

[b] Class is based on whether a hypermodified base (Q base in Asn-tRNA or Wye base in Phe-tRNA) or lack of a hypermodified base (Leu-tRNA) is normally found at the ribosomal A-site of the frameshift signal. As noted in the text, Phe-tRNA minus Wye base is found in HIV-infected cells and Asn-tRNA minus Q base is found in HTLV-1- and BLV-infected cells.

[c] Subclass is based on whether a hypermodified base [Q base in Asn-, Asp-, and Tyr-tRNAs,[42] Wye base in Phe-tRNA,[42] 5-methoxycarbonyl methyl-2-thiouridine and *N*-((9-beta-D-ribofuranosyl-2-methylthiopurine-2-yl)carbamoyl)-threonine in Lys-tRNA$_3$,[42] unknown base in Gly-tRNA$_{UCC}$,[42] or lack of a highly modified base (Gly-tRNA$_{GCC}$,[42])] is normally found at the ribosomal P-site of the frameshift signal.

sequences found in the overlapping reading frames of retroviruses and other genetic elements (i.e., U UUA, U UUU, and A AAC [see above and Table 2]), when placed at the end of the RSV frameshift site, support efficient frameshifting, while A AAA and G GGG sequences are not as efficient. This observation led these investigators to propose that "only certain, specialized 'shifty' tRNAs" participate in frameshifting.[173] This proposal is further supported by the fact that only three codons, UUA, UUU, and AAC, are found at the ribosomal A-site within the frameshift sites of each overlapping reading frame examined (see Table 2). The possibility that the "shifty" tRNA may lack a hypermodified base in its anticodon loop is considered below.

Efficient frameshifting in RSV apparently requires the participation of a stem-loop region that is immediately downstream of the frameshift site.[173] Disruption of base pairings within the

stem by generating specific stem-destabilizing mutations in RSV resulted in a decrease in frameshifting, while restoring these base pairings by generating specific stem-restabilizing mutations rescued frameshifting. In HIV, on the other hand, there is no requirement for a downstream stem-loop effect on the frameshift event and, in fact, the only requirement for frameshifting is a short segment that includes the heptanucleotide signal.[174]

2. tRNAs Involved in Frameshifting

At least one, if not both, of the codons that are read in the zero frame within each frameshift signal shown in Table 2 corresponds to tRNAs that normally contain a hypermodified base in their anticodon loop. For example, Q base occurs in Asn-tRNA (which is coded by AAU or AAC in a number of the frameshift signals shown in Table 1) and in Asp-tRNA (which is coded by GAU in the *pro-pol* signal of MMTV), and Wye base occurs in Phe-tRNA (which is coded by UUU in a number of frameshift signals). The frameshift signals shown in Table 2 can be grouped into various classes, based on the occurrence of Q base (Asn-tRNA, class I), Wye base (Phe-tRNA, class II), or lack of a highly modified base (Leu-tRNA, class III) in the respective tRNA utilized (under normal cellular conditions) at the ribosomal A-site of each frameshift signal.[180] Whenever Leu-tRNA occurs at the A-site, either a tRNA with Q base (Asn-tRNA or Asp-tRNA) or a tRNA with Wye base (Phe-tRNA) occurs at the P-site. The frameshift signals can also be grouped into subclasses (i.e., IA, IB, etc.) that include those hypermodified bases that are found in the anticodon of the tRNA utilized (under normal cellular conditions) at the ribosomal P-site of each frameshift signal (see Table 2 and legend to Table 2).

The coding properties of tRNAs lacking Q[16,18,182] or Wye base[183] in their anticodon loop are altered. Furthermore, the leucine isoacceptors (a leucine tRNA that reads UUA is required for translation in many of the signals shown in Table 2) do not contain a hypermodified base in their anticodon loop (see Reference 24 and references therein). It seems reasonable to propose that the frameshift event may be facilitated if the tRNA at the "slippage site" does not have a highly modified base in the anticodon loop, i.e., such a tRNA may be more "shifty".

The chromatographic properties of aminoacyl-tRNAs utilized at and around the frameshift site isolated from HIV-1-, BLV-, and HTLV-1-infected cells and from a corresponding set of uninfected (control) cells have been examined.[180,181] HIV-1 utilizes Phe- and Leu-tRNAs within the *gag-pol* frameshift signal,[172,198-200] while BLV[191,192] and HTLV-1[185-187] utilize Asn- and Lys-tRNAs within the *gag-pro* and Asn- and Leu-tRNAs within the *pro-pol* frameshift signals (see Table 2). These studies showed that virtually all of the Asn-tRNA from each set of infected cells was Q deficient, while a greater proportion from uninfected cells was Q containing.[180,181] Additionally, virtually all of the Phe-tRNA from HIV-1-infected cells was Wye deficient, while most of the Phe-tRNA from uninfected cells was Wye containing. The chromatographic properties of other aminoacyl-tRNAs at and around each frameshift site were not altered. It is tempting to speculate from these observations that the presence of 1-methylG in place of the hypermodified Wye base in the 3′ position next to the anticodon of Phe-tRNA[204] and of G in place of the hypermodified Q base in the 5′ position of the anticodon of Asn-tRNA[17,18,182] would facilitate the frameshift event.[180,181] Most certainly, in the absence of Q or Wye base, more space exists in and around the frameshift site. Furthermore, greater flexibility of movement of the respective tRNA anticodon might be expected in the absence of a highly modified base in the anticodon loop, such as is found in Leu-tRNA and in hypomodified Asn- and Phe-tRNAs.[180,181]

3. Concluding Remarks About Frameshift Suppression

A summary of the possible frameshift signals and sites in numerous retroviruses that have been sequenced and in other genetic elements from higher eukaryotes are shown in Table 2. A question was raised whether one or more of the tRNAs utilized in translation within the frameshift signal may lack a hypermodified base in its anticodon loop to facilitate the frameshift event. If some viruses require an alteration in the host tRNA population for ribosomal frameshifting, then interrupting this alteration may provide an avenue for inhibiting viral expression.[10] Important therapeutic value could then be gained by suppressing the expression of viral replicative enzymes in cells infected with HIVs or HTLVs by this or other means.

C. Missense Suppression and Misreading Genetic Code Words

Missense mutations are the most commonly occurring kind of point mutation in nature. Suppression of missense mutations, however, has not been detected thus far in higher eukaryotes. It is unfortunate that the large number of inborn errors in human metabolism that result from missense mutations do not appear, at least with the present knowledge of the utilization of aminoacyl-tRNAs in protein synthesis, to be approachable through gene therapy experiments involving tRNA. Although it seems reasonable that gene therapy experiments might be designed for correcting the hemoglobinopathies where a suppressor tRNA gene may be introduced selectively into red blood cell precursors rather than into the genome of intact organisms, it seems unlikely that the tRNA could be engineered to read more efficiently at a specific site to selectively correct a missense mutation. In designing gene therapy experiments involving missense suppressors in cells in culture, it would seem that several criteria must be met before attempting such experiments. Initially, the mutation to be corrected would ideally correspond to an infrequently used code word in order that the missense suppressor would hopefully not affect other proteins and, thus, only a small amount of isoacceptor would be ex-

pected to be present[101] for the suppressor to compete with in translating the missense code word. In addition, it would be advantageous if the protein that is to be restored to an active state is present in low levels and, thus, a missense suppressor in elevated levels could presumably completely suppress the mutation. Perhaps candidates to consider for "gene therapy" experiments are the $p21^{ras}$ proteins that occur in minor levels in mammalian cells and in which amino acid changes at specific positions cause the protein to become oncogenic (see Reference 205 for a review). An infrequently used codon could be generated at one of the "sensitive" sites, which would make the protein oncogenic; then, a tRNA could be generated with an appropriate anticodon to insert a "wild-type" amino acid in response to the infrequently used codon.

Since tyrosine tRNA, which lacks Q base in its anticodon, is capable of misreading (and suppressing) UAG codons, a question could arise as to whether asparagine, aspartic acid, and histidine tRNAs, which are Q^-, can also misread the corresponding XAG code words (where X may be either C, A, or G) and therefore serve as missense suppressors. This possibility seems highly unlikely because (1) even if these isoacceptors are capable of misreading XAG codins, they must compete with the corresponding isoacceptors that normally read XAG codons and (2) asparagine, aspartic acid, and histidine tRNAs contain U in the middle position of their anticodon, whereas tyrosine tRNA contains Ψ at this position, which apparently is essential for suppression to occur (see prior Section I.A.1.a, *Amber Suppressor tRNAs*).

Starvation of mammalian cells in culture for an essential amino acid results in the insertion of the wrong amino acid into protein.[206-208] The different types of amino acid changes that occurred in protein in the cells deprived of an essential amino acid were consistent with misreading of pyrimidines for purines at the 3′ codon position.[206-208] This technique has provided a means of measuring the fidelity of translation in mammalian cells[207] and of examining the levels of mistranslation in mammalian cells before and after transformation.[208]

Misrecognition of genetic code words has been shown to occur within the same amino acid family. This subtle type of misrecognition has been observed in cases where the preference of a tRNA for a codon within the same amino acid family may be altered by virtue of a hypomodification in the anticodon loop.[182,209] Examples of this form of "misreading" are a hypomodified mammalian lysine tRNA that reads AAA codons more readily than the corresponding fully modified isoacceptor[209] and a hypomodified *Drosophila* histidine tRNA that reads CAU and CAC codons with different preferences than the corresponding fully modified isoacceptor.[182] Misrecognition of code words within the same amino acid family, which is considered further in Section I.D below, *Function of Suppressor tRNAs in Translation,* may favor the expression of mRNAs rich in codons that are read more readily by the hypomodified isoacceptor. Thus, this form of "misreading" must be considered among the factors that are important in orchestrating the complex pattern of protein synthesis in gene expression.[183]

1. Concluding Remarks About Missense Suppression

Very little is known about the occurrence and role of missense suppressors in higher eukaryotes. A possible "gene therapy" experiment was proposed in which the effect of an oncogenic protein on a cell might be reversed by genetically engineering the correct missense suppressor tRNA into the cell. A subtle type of misrecognition of code words that occurs within the same amino acid family due to the hypomodification of tRNA bears further study as a possible factor in upsetting the balance of cellular protein synthesis.

D. Function of Suppressor tRNAs in Translation

Suppression should also be considered in the context of the tRNA function in translation. In fact, suppression represents special cases of tRNA function. Therefore, certain basic principles of tRNA function are discussed.

The incorporation of amino acids in response to the genetic code is a process of considerable fidelity. It is commonly stated that the rate of erroneous translation is 10^{-4}, which means, of course, only a single error in about 10,000 amino acids incorporated.[210] However, if we consider the mature erythrocyte with its 300 million molecules of hemoglobin, each containing 640 amino acids, it can be calculated that hemoglobin in an erythrocyte contains about 20 million erroneous amino acids. In fact, errors do not occur randomly and some errors are more likely to occur than others,[211-213] depending on details of tRNA structure and on the site being translated, as is discussed further below.

It must be recognized that the function of tRNA in translation is more complex than the reading of a linear sequence of information by a uniformly performing family of decoding molecules. The following factors affect translation and suppression. Examples are taken from both eukaryotes and prokaryotes to illustrate some of the principles of tRNA function.

1. Characteristics of tRNA

a. tRNA STRUCTURE

I. The Anticodon

Codon:anticodon interaction is a major factor in tRNA function and the major determinant of the fidelity of decoding.[41] Many, but not all, suppressor mutations involve base changes in the anticodon and result in a new meaning for the tRNA. The rules of the "wobble hypothesis"[41] in general apply, although, as discussed below, these have undergone some modification, with some of this modification being necessitated by studies on suppression.

The concept of the extended anticodon[214] is based on variations in the efficiency of nonsense suppression in bacteria caused by base changes not in the anticodon itself, but else-

where in the anticodon loop, in the anticodon stem, and even in more remote parts of the tRNA molecule. Changes in the extended anticodon affect efficiency, but not the fidelity of coding. Destailization of the distal base pair in the anticodon stem, which results in a nine-membered anticodon loop, causes frameshifting of variable efficiency as four bases rather than a triplet are read.[215]

ii. tRNA Base Modifications

Many modifications of the bases of tRNA are known, consisting of changes that are made posttranscriptionally. The changes range from an isomeric form of uracil, through methylated bases, to bases with large added moieties that are sometimes called hypermodifications.[216] Our laboratories have a longstanding interest in the effects of modifications involving hypermodified bases in and adjacent to the anticodons of tRNAs for several amino acids. In animals, fully modified tRNAs are generally found in postmitotic and slowly dividing cells, while hypomodified tRNAs are found in tumor and other rapidly dividing cells. Over the years, there has been much interest in whether these tRNA differences have some role in gene expression in cells or whether they are a consequence of the rate of cell division, with perhaps tRNA transcription outpacing tRNA modification in rapidly dividing cells.[217,218] Considering the overall utilization of fully modified and hypomodified tRNAs in translation, a pair of tRNA isoacceptors in which the fully modified species is preferred ($tRNA^{Lys}$) in hemoglobin translation,[209,219] a pair in which the hypomodified species is preferred ($tRNA^{Phe}$),[220,221] and a pair in which the level of modification seems to make no difference ($tRNA^{His}$)[222] have been observed. As described below, the utilization of these tRNAs is more usefully considered at the individual sites of incorporation than in overall incorporation, which involves many sites.

tRNA modifications affect codon reading. Perhaps most significantly, it has been shown that four codon families (i.e., NNA/C/G/U) can be translated by a single tRNA species with an unmodified U in the first anticodon position, while the more restricted reading of two codon families requires U modification.[223] A consequence of this is that the genetic code can be read by as few as 24 tRNA species. It has been reported that fully modified and hypomodified $tRNA^{His222}$ and $tRNA^{Phe221}$ do not distinguish C and U in the third codon position, while the hypomodified $tRNA^{Lys209}$ is more active than the fully modified species in the A/G wobble that occurs in reading lysine codons.

In nonsense suppression in higher eukaryotes, there is a well-investigated example described in the section on nonsense suppressors where a hypomodified tyrosine tRNA can suppress UAG codon, while the fully modified isoacceptor cannot.

iii. Base Sequence Microheterogeneity

Although tRNA isoacceptor diversity often results from posttranscriptional base modifications and from extensive base sequence differences, some of it results from differences in single bases or base pairs.[42] This microheterogeneity presumably occurs because of point mutations in tRNA genes, and tRNA genes in eukaryotes are often present in multiple copies in the genome.[224] Little is known about the effects of these base sequence differences in domains of tRNA remote from the anticodon. A case of much speculative interest is provided by the $tRNA^{Ala}$ isoaccepting species in the silkworm in which one $tRNA^{Ala}$ occurs in most of the tissues of the larva, while a unique $tRNA^{Ala}$ differing by only a single base in the middle of the anticodon stem is uniquely expressed in the posterior silk gland.[225] This particular example of microheterogeneity might be considered as located in the extended anticodon and could destabilize the anticodon loop in translation. It is not known, however, whether the $tRNA^{Ala}$ isoacceptor unique to the silk gland has a unique role in translation of the silk proteins or whether the pattern of expression of the $tRNA^{Ala}$ genes is unrelated to the need for these tRNA gene products in translation.

b. tRNA CODING EFFICIENCY

Coding efficiency is defined as the preference for one tRNA isoacceptor over another in the translation of a specific codon.[226] As this review suggests, codon:anticodon interactions are only one aspect of tRNA function. Still, it is useful to recognize that in the translation of any given site, tRNA efficiency varies. Similarly, suppressor tRNAs are described as strong and weak, depending on how successful suppressor mutations are in providing for cell growth or how much gene product is synthesized.

c. tRNA ABUNDANCE

Like efficiency, the relative abundance of different cognate tRNA species clearly has an effect on their utilization. Less available tRNA species are used less frequently, all else being equal, as will be discussed below in Section I.D.3 on tRNA selection.[227] The relative abundance of the tRNA species differs in different kinds of cells, and the content of the tRNA species appears to be adapted or modulated or specialized for tRNA utilization in the synthesis of the major protein products of different kinds of cells.[99-101,227-231] Thus, cells that synthesize predominantly hemoglobin, silk proteins, or collagen have very different tRNA contents in which the isoacceptor levels reflect their requirements for incorporation of the relative amounts of amino acids in the corresponding proteins. tRNA adaptation assures a supply of the commonly used tRNA species. However, uncommonly used species are in low concentrations. Variations in the rate of elongation of nascent proteins have been correlated with the concentrations of required amino acids.[232-234] Thus, even an adapted tRNA content does not provide uniform adequacy for the translation of every site in every protein synthesized.

In suppression, tRNA abundance is sometimes a determinant of whether a tRNA can serve as a suppressor and/or of its efficiency as a suppressor. As described below, suppressor

tRNA at high copy number performs qualitatively and quantitatively differently than the same tRNA at a low copy number.[35,36]

D. tRNA AFFINITY FOR THE TRANSLATIONAL MACHINERY

There are studies showing variable affinity of different tRNA species for ribosomal proteins and for elongation factors, and this could affect the utilization of tRNA in translation.[235-237]

2. mRNA Characteristics Affecting Translation and Suppression

a. THE BASE SEQUENCE

The base sequence of mRNA specifies the point of initiation of translation, the reading frame by which the base sequence is organized into triplet codons specifying a meaningful amino acid sequence, and the termination codon. While codon:anticodon interactions are critical to the reading of the genetic code, they are not the only determinants of cognate tRNA utilization or even of the amino acid incorporated.

b. FURTHER CONSIDERATION OF CODON CONTEXT, READING CONTEXT, AND SITE-SPECIFIC EFFECTS ON TRANSLATION

Codon context may be defined more generally in protein synthesis as the adjacent bases in mRNA that have an effect on the translation of a codon. There are orders of magnitude more codon contexts than there are codons, thus providing for a great variety of site-specific effects on translation. As noted above, there are well-recognized codon context effects seen in the suppression in prokaryotes in which the nature of the base 3′ to the codon being translated is important.[103,104,238-243]

In our studies on the site-specific utilization of competing tRNA isoacceptor species with different levels of modification in eukaryotes,[183] we have found that utilization varies over nearly a twofold range from site to site in globin translation. There are sites in which the generalizations made above about general preferences in isoacceptor utilization are reversed, with the generally less preferred isoacceptor being the more utilized at certain sites. We were unable to relate the effects on utilization to adjacent bases. Studies described above on eukaryote suppression have similarly failed to demonstrate codon context effects, although much site specificity is seen in other studies on eukaryote suppression. In the prokaryote literature, it has been found that context effects are prominent with weak suppressors and are not seen with strong suppressors.[103,243] It is possible that the cognate tRNA species in our studies and the suppressor tRNAs investigated by other workers are more analogous to strong suppressors.

One site-specific effect we have observed is in the translation of tandem lysine and phenylalanine residues that are encoded by identical tandem codons.[183] In each of three cases, tRNA utilization in the translation of the first of the residues reflects the generally observed isoacceptor preference, while the alternative isoacceptor is favored in the translation of the second residue. It is as if the selection of a preferred isoacceptor for the translation of the first residue militates against the selection of the same isoacceptor for the translation of the second residue. The A- and P-sites on the ribosome are in close proximity, and it is likely that the tRNA in the P-site or that being translocated would affect the properties of the A-site and thereby the selection of the tRNA to occupy the A-site.[242,245,246] A similar phenomenon may be seen in prokaryotes in the translation of tandem codons in the process of attenuation,[247,248] in which there are fastidious requirements for tRNA modification. The same requirements are not seen in tRNA in most other amino acid incorporation in which the unmodified isoacceptor performs nearly as well as the modified isoacceptor.[249] The effect of the previous tRNA probably applies not only to the translation of the tandem codons we have observed, but also to other sequences of codons. The term "reading context" is sometimes used synonymously with "codon context" and may be more descriptive of the reality of site-specific translation, which includes not only the bases around the codon being translated, but also the involvement of the translational machinery in the reading of the adjacent codons. Like codon context, reading context provides for a multitude of site-specific effects on translation. The effect of the previously selected tRNA on translation involves the translation of the 5′ codon and is to be distinguished from the codon context effect on suppression noted above, as seen widely in prokaryotes, which involves the base in the 3′ direction. The effect we have observed is more likely to involve tRNA-tRNA interactions and to be affected by tRNA posttranscriptional base modifications.

3. tRNA Selection and Postselectional tRNA Function

Typically 50 to 100 tRNA species are resolved from eukaryotic cells by chromatography and two-dimensional gel electrophoresis.[101,228,250,251] As described above, the heterogeneity results from extensive base sequence differences, from small differences involving single bases and base pairs (microheterogeneity), and from differences in posttranscriptional modifications. This variety of tRNA species considerably exceeds the 32 species that would be needed to translate the genetic code under the rules of the wobble hypothesis[41] and, as noted above, it has been shown that as few as 24 tRNA species can translate the genetic code.[222] The consequence is that there is a choice of cognate tRNA species to incorporate amino acids in response to most codons. Several cognate tRNA species can be thought of as competing, with preference in utilization being determined by the characteristics of tRNA and of the coding site. Selection probably occurs not from the pool of free aminoacyl-tRNA molecules, but from the pool of aminoacyl-tRNAs in ternary complexes with GTP and elongation factor EF-1.[252] tRNA selection is a process of trial and error, with studies of GTP consumption indicating that numerous noncognate and abortive interactions occur for each tRNA ul-

timately selected in a process that reduces error in translation.[253-255] The result of tRNA selection is a quaternary complex that includes the selected aminoacyl-tRNA in the ribosomal A site, mRNA with the codon to be selected in alignment with the anticodon on the tRNA, elongation factor EF-1, and GTP.[252]

Postselectional tRNA function consists of the reactions of protein synthesis, including peptide bond formation, translocation of tRNA from the A- to the P-site on the ribosome, release of the nascent peptide following peptide bond formation to the next tRNA, and release of the tRNA from the ribosome. There is evidence that tRNA selection is the more time-consuming process in amino acid incorporation.[233]

4. Consequences of the Variability in tRNA Function

The consequence of variability in tRNA function is variability in the rate of incorporation of amino acids into nascent peptides, and therefore variation in the rate of elongation of the nascent peptides has been described many times.[232-234,256-259] In general, the tRNA content may be considered as "globally attuned" to the utilization of the preferred species in translation so that, while the rate of peptide elongation is irregular, translation is not a major consideration in the control of gene expression. Special situations can occur, however, when the tRNA content is not attuned to the translation of a particular protein because of a failure of tRNA gene expression or tRNA postsynthetic modification or because of the need to translate in a reading context that has unusual tRNA requirements. Large effects on gene expression can occur if points of translational delay occur several times in the synthesis of a protein[232] or if a single base sequence (e.g., Reference 260) cannot be translated at the required rate.

Suppressor tRNAs must compete with other tRNAs and, in the case of nonsense suppression, with the termination process.[261] The variable efficiency and the site specificity of suppression are consequences of this competition and the factors involved in its resolution.

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