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The Context Effect Does Not Require a Fourth Base Pair

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The translational activity of a transfer RNA at a codon varies at different message sites, although the codon does not vary. The source of this effect, which may help to determine the level of gene expression, is generally agreed to be in nearby message sequences. By making every possible nucleotide combination between position 33 of the transfer RNA and the major context nucleotide of the message, it was shown that base-pairing between the two nucleotides is not the source of this context effect on translation in vivo.

THE EFFICIENCY OF TRANSLATION OF polypeptide chain termination codons (UAA, called ochre; UAG, amber; and UGA, opal) by their transfer RNA (tRNA) suppressors depends on other message nucleotides near the nonsense codon (*1*). This is termed the context effect, and nucleotides 3′ to the codon are most strongly implicated. By selecting for increased activity of a weakened amber suppressor, Bossi and Roth (*2*) showed that *supE* in *Salmonella typhimurium* suppressed a particular UAG in *hisD* tenfold more efficiently when UAGC was mutated to UAGA. More recently, this pattern has been observed in several amber mutations (*1, 3*). In general, amber codons followed by pyrimidines are less efficiently translated than are amber codons followed by purines. Bossi and Roth (*2*) have adapted a hypothesis originally formed by Taniguchi and Weissman (*4*), which explains these observations by suggesting that a fourth base pair occurs between the tRNA's anticodon loop and the message.

All elongator tRNA's have a U at position 33 (just 5′ to the anticodon), which could base-pair with the nucleotide on the 3′ side of the codon, forming a U-A or U-G pair (Fig. 1). Such a fourth base pair would favor elongation of the nascent polypeptide chain, rather than termination at UAG purine.

In order to test this possibility, we used Su7 (the amber-suppressing variant of $tRNA^{Trp}$) and three amber-suppressing, nucleotide-33 derivatives of this tRNA to suppress amber codons in four contexts within *lacI-Z* fusion genes. The suppressor mutants used in the study are identical to Su7 except that U33 in Su7 has been replaced by C, G, or A (Fig. 1). These tRNA's will be called Su7 U33, Su7 C33, Su7 G33, and Su7 A33, respectively. The *lacI-Z* message sequences were chosen to complement nucleotide 33 of this set of tRNA's and to keep the remainder of the context of the UAG codon consistent. All four nucleotides occur 3′ to the UAG in this set of contexts (Table 1). Therefore, we made strains containing the *lacI-Z* fusions and the suppressors in all combinations. When all suppressor activities on all messages are determined (by measuring β-galactosidase activity), every possible combination of nucleotides at the position of the hypothetical fourth base pair can be compared.

The *lacI-Z* genes we used also conserve the nucleotide 5′ to the amber codon and the nucleotide that is the next-nearest 3′ neighbor of the codon (Table 1). This normalizes the effects of other nucleotides that might have a role in the context effect. Miller and Albertini (*3*) have shown that the next-nearest 3′ neighbor has an influence on the translation of amber codons. The 5′ neighbors of sense codons are correlated with the sequence of the codon, and therefore might have functional effects (*5*). In all measurements in Table 1, both potential "context" positions are always C.

The *lacI-Z* fusions were created by a deletion which fused the NH_2 terminus of *lacI*, where the amber resides, to the COOH-terminus of *lacZ* (*6*). There is no substantial level of reinitiation of translation after termination at the amber codon. Translation of the amber codon in *lacI* is required for active *lacI-Z* product (β-galactosidase), and enzyme levels should be proportional to the fraction of message transits in which the codon is translated by the suppressor tRNA. This fusion system for the measurement of suppressor efficiency (the fraction of transits in which the tRNA acts) has been extensively characterized by Miller and Albertini (*3*). The β-galactosidase was measured as described by Miller (*7*), with the modifications of Raftery *et al.* (*8*).

Enzyme levels for the 16 strains in this experiment are shown in Table 1. Pyrimidines at tRNA position 33 resulted in more efficient suppressors, and U33, which is always found in natural sequences, is the most efficient pyrimidine (*9, 10*). This pyrimidine preference was clear and similar in all contexts. In comparisons of different contexts, we observed that the sequence GAC UAG ACA contains the most efficiently translated amber codon for all tRNA's, in agreement with the expected

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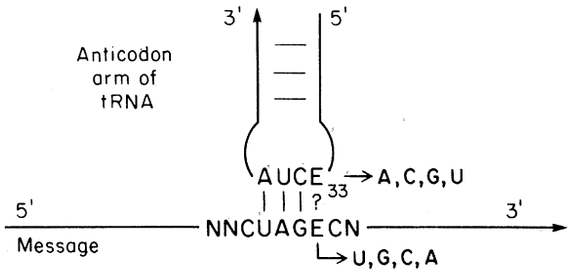

Fig. 1. The interaction of the amber anticodon and the message. Standard base pairs are indicated with solid lines; the hypothetical base pair is shown as ?; E indicates an experimentally varied nucleotide; and N indicates an arbitrary nucleotide.

preference for a 3′ purine neighbor.

The samples in which there could potentially be base pairing between the tRNA and the message are aligned along the principal diagonal of Table 1, from the upper left. These combinations were not unique, showing neither exceptionally high or low translational efficiencies. The absence of an apparent effect of base-pairing potential on translation argues strongly that no base pair forms between nucleotide 33 of the tRNA and the 3′ neighbor of the codon.

It seems unlikely that this conclusion is due to a particular choice of reactants. Transfer RNA's may vary in efficiency for reasons other than a fourth base pair; for example, because of differential modification of anticodon loop nucleotides (*11–13*). However, such effects, even if they exist, are unlikely to be concealing an effect of a fourth base pair. There are four independent columns in Table 1 in which no effect of a potential base pair was seen with the same tRNA occurring throughout the column. By a similar argument, masking of base pairs is not occurring because of varied tRNA levels or differential aminoacylation of the tRNA's.

Adventitious effects might obscure a pairing effect when the message is varied. For example, translation by a nonsense suppressor tRNA is always in competition with chain termination by peptide release factors. Although the release factor may also vary in its action in different nucleotide contexts, it is also unlikely that such an effect obscures the action of a fourth base pair. In the four independent rows of Table 1, no special qualities of base-pairing combinations appeared, although in each row the message (and presumably release factor action) was constant and the tRNA was varied.

What might determine the context effect? The data in Table 1 show several patterns which may be related to the nature of context action. Stronger suppressors were less subject to the effect. Su7 U33, the most efficient tRNA, showed no significant dependence on the selected set of contexts. This is in accord with the data of Miller and Albertini (*3*), in which the relatively efficient suppressors Su3 and Su6 also show little difference between these contexts. However, less efficient suppressors are context-dependent. This trend is visible in Table 1 for the homologous Su7 suppressors, and the unrelated weaker suppressor, Su2. Context effects of more than threefold were observed for Su2 (Table 1), which is similar to Su7 mutants of the same level of efficiency. But the Su2 tRNA showed a different pattern of context sensitivity. For example, A and G 3′-neighbors were equivalent, which is not true of the similar Su7 suppressors. Therefore, (i) the context effect is more significant for weaker suppressors, and (ii) the pattern of the effect is characteristic for each tRNA. Su7 A33, Su7 G33, and Su7 C33 showed differences in pattern, and differed in primary structure only in the anticodon loop at position 33. Therefore, the context patterns of tRNA's may originate in the differences in sequence, and therefore in the microstructure, of the anticodon loops.

Such context effect patterns might originate in contacts between the anticodon loops of neighboring tRNA's, and/or in differential effects of the 3′ neighboring nucleotides of the message on the stability of codon-anticodon complexes. The latter would be analogous to the "dangling end effect" (*14, 15*) on the stabilities of pairing of nucleotides in solution. Either mechanism would be consistent with the correlations we have found for the nucleotides of neighboring codons in weakly expressed genes (*5*), which we believe are related to context effects. The wobble nucleotide of the codon is correlated with all nucleotides of the 3′ neighboring codon. Statistical effects cease 3′ to this codon. The codon-sized extent of this interaction argues for a tRNA-based effect, most consistent with loop-loop interactions. But the dangling-end effect, strikingly, shows the same nucleotide specificity (purines stabilize more than pyrimidines) and positional specificity (3′ neighbors are most effective) (*15*) as does the context effect, and this second effect could exist concurrently. A dangling-end effect could also affect the tRNA entering the ribosomal A-site. The 3′ locus of the effective context nucleotides otherwise suggests that context affects survival of the nascent peptide attached to the suppressor tRNA in the P site (*5*).

By analogy with current understanding of ribosomal tRNA selection, we suggest that the context mechanism modulates the rate of dissociation of the tRNA from the ribosome. Thompson and Dix (*16*) and Thompson and Karim (*17*) have argued that the distinction between a tRNA well-matched to its codon and one that is mismatched

Table 1. β-Galactosidase activity produced by the suppressors. All suppressors were cloned on the same colE1 replicons and promoted by *lac* UV5 (*19*). The construction of Su7 U33 (*19*), Su7 C33 (*9*), as well as Su7 A33, Su7 G33, and Su2 clones (*10*) have been described. The cultures to be assayed for β-galactosidase activity were grown in Vogel-Bonner salts (*20*) supplemented with 0.2 percent glucose, 1 m*M* isopropyl-β-D-galactopyranoside (IPTG), B1 (4 μg/ml) and tetracycline (5 μg/ml). The assays were performed at 20°C and were repeated for eight independent cultures. The background level of transmission through the nonsense codons was determined in the presence of pMY231, which carries the nonsuppressing wild-type $tRNA^{Trp}$ gene. In all cases, the background β-galactosidase activity was undetectable. The plasmids carrying the suppressors and the F′ *lacproB* episome carrying the amber mutations were introduced into MY600, which has the genotype *ara (lacproB) thi* str^s.

Amber site	Messenger RNA sequence	Activity (% of wild type)*				
		Su7 A33	Su7 G33	Su7 C33	Su7 U33	Su2
A27	ACC UAG UCC	1.6 ± 0.2	2.1 ± 0.2	9.0 ± 0.9	60.0 ± 3.0	14.6 ± 0.4
018	ACC UAG CCG	1.7 ± 0.08	2.1 ± 0.1	7.9 ± 0.4	60.7 ± 4.2	3.9 ± 0.4
015	GUC UAG GCC	1.4 ± 0.08	4.7 ± 0.7	7.2 ± 0.4	64.8 ± 3.6	12.7 ± 0.5
A16	GAC UAG ACA	4.7 ± 0.3	6.4 ± 0.7	16.5 ± 1.1	57.3 ± 4.0	10.7 ± 0.4

*The percentage of the wild-type fusion activity (no amber mutation in the *lacI* reaction of the hybrid gene) ± the standard error of the mean. The wild-type activity was determined for strains carrying the corresponding suppressor. The wild-type values, in Miller units, for Su7 A33, Su7 G33, Su7 C33, Su7 U33, and Su2 are 145 ± 4, 143 ± 2, 126 ± 6, 121 ± 4, and 137 ± 3, respectively. Miller units (*7*) are calculated from the following equation: $(1000 \times OD_{420})/(OD_{550} \times \text{time} \times \text{mls culture})$.

resides in the higher velocity with which the latter dissociates from the decoding site by reversal of the binding reaction or proofreading (*18*). We suggest that efficient suppressors are slow to dissociate so that modulation of their back-reactions has a negligible effect on the probability that they will donate their amino acids. Inefficient suppressors, like those created in these experiments by 5′ anticodon loop substitutions, may have fast reverse reactions that compete with guanosine triphosphate hydrolysis by translational elongation factor Tu and with formation of peptide bonds. The efficiency of this type of weak suppressor will be altered when these back-reactions are modulated.

The context effect does not depend on a fourth base pair. When we understand the biochemistry of the actions of context, then perhaps we will also be able to judge whether these effects have developed during the process of evolution to vary gene expression.

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A Balanced Translocation in Mice with a Neurological Defect

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A semisterile male translocation heterozygote [t(2; 14) 1Gso] that exhibited neurological symptoms and an inability to swim (diver) was found among the offspring of male mice treated with triethylenemelamine. All breeding and cytogenetic data showed a complete concordance between translocation heterozygosity and the neurological disorders. Homozygosity for the translocation seemed to be lethal at an early embryonic stage. Despite the distinctive neurologic symptoms, no anatomic or histological defects in either the ear or in the central nervous system were observed. Thus, a balanced chromosomal translocation can produce disease with an inheritance pattern that mimics a single dominant gene defect.

MOST TRANSMITTED BALANCED, reciprocal translocations cause no ill effects in heterozygous carriers, except for a reduced chance to reproduce (death of aneusomic offspring). In humans, however, certain apparently balanced translocations may have deleterious effects on carriers. Among mentally handicapped individuals, the incidence of carriers of balanced reciprocal translocations is higher than normal (*1*). Studies of several family pedigrees for syndromes with multiple skeletal abnormalities (*2*) and the Greig polysyndactyly-craniofacial anomaly syndrome (*3*) have shown that some of these morphological defects were associated with an inherited balanced translocation. The de novo occurrence of X-linked Duchenne muscular dystrophy in seven females is due to a balanced translocation involving one of the X chromosomes (*4*).

In the mouse, cases of association between balanced translocations and congenital defects have also been rare. For example, in heritable translocation induction studies with chemical mutagens and ionizing radiation, no abnormalities except semisterility or sterility were found in a large number of translocation carriers screened (*5–8*). On the other hand, among 37 independently induced (by ionizing radiation) dominant skeletal mutations, three were associated with balanced reciprocal translocations (*9*). Furthermore, several types of balanced reciprocal translocations can cause spermatogenic blockage that leads to complete sterility.

We have determined that one of the offspring of males treated with triethylenemelamine during a mutagenesis experiment was a semisterile male translocation heterozygote with obvious neurological symptoms and an inability to swim normally ("diver"). The translocation altered gene expression so as to mimic dominant Mendelian inheritance.

The original male translocation heterozygote was derived from a triethylenemelamine–treated male (SEC × C57BL) F_1 that had been mated to a (C3H × C57BL) F_1 female. The translocation heterozygote was mated to (SEC × C57BL) F_1 females to produce the second generation. Subsequent generations were produced from mating second generation males with (C3H × C57BL) F_1 females, and most of the pathology specimens came from this cross. Chromosomal analysis of the translocation was conducted on the meiocytes and on cultured kidney cells of neurologically abnormal males according to the methods of Evans *et al.* (*10*) and Cacheiro and Russell (*11*).

Systemic autopsies with examinations of histopathology and the central nervous system were performed on ten diver and ten normal mice, (ages 3 months to 1 year), with multiple levels of the central nervous system examined by light microscopy after staining with hematoxylin and eosin and the Golgi stains. The sizes of the vestibular nuclei were compared in serial sections of the brain stem from one diver mouse and one control mouse. Electron microscopy of the cerebellar myelinated fibers was performed on two diver mice. The middle and inner ears of five diver mice were examined by microdissection, temporal bone histology, and scanning electron microscopy. Multiple histologic levels were examined from the brains of fetuses of diver male mice crossed with normal (C3H × C57BL) F_1 hybrid females at gestational ages 12, 14, 16, and 18 days as well as newborns and mice at 3, 12, 18, and 24 days of age (ten at each day). Twenty-eight fetuses from 13

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