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Insertions in the anticodon loop of $tRNA_1^{Gln}$ (*sufG*) and $tRNA^{Lys}$ promote quadruplet decoding of CAAA

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ABSTRACT

Base insertion mutations in the anticodons of two different *Escherichia coli* tRNAs have been isolated that allow suppression of a series of +1 frameshift mutations. Insertion of a U between positions 34 and 35 of $tRNA_1^{Gln}$ or addition of a G between positions 36 and 37 of $tRNA^{Lys}$ expand the anticodons of both tRNAs similarly to 3′-GUUU-5′ and allow decoding of complementary 5′-CAAA-3′ quadruplets. Analysis of the suppressed mRNA sequences suggests that suppression occurs by pairing of the expanded anticodons to all four bases of the complementary, quadruplet codon. The $tRNA_1^{Gln}$ mutants are identical to the *sufG* class of frameshift suppressors isolated both in *Salmonella enterica* serovar Typhimurium and *E.coli* by Kohno and Roth and previously thought to affect $tRNA^{Lys}$.

INTRODUCTION

The mechanism of reading frame maintenance has been studied genetically in both bacteria and yeast through the isolation of mutations in components of the protein synthesis apparatus that promote or inhibit switches into alternate reading frames (reviewed in 1). Many of these mutants are altered tRNAs, and have typically been isolated as suppressors of frameshift (*suf*) mutations. Early analyses of tRNA mutants that promoted quadruplet decoding showed that these tRNAs had single base insertions in their anticodon loops. These results suggested that the translocation step size might be determined simply by the size of the anticodon loop. However, extensive analyses of the decoding properties of many different tRNA mutants indicates that a more complex picture obtains (1–3). In some instances, it is now apparent that it is not the mutant tRNA itself that causes frameshifting, but instead frameshifting occurs when a near-cognate tRNA first binds in-frame to the A site codon and subsequently shifts into the alternate reading frame (4,5). Presumably, the mutations in the cognate tRNA impair its activity and allow the near-cognate tRNA to compete effectively for A site binding. Further unexpected decoding possibilities of tRNAs with enlarged anticodon loops emerged from analysis of $tRNA_1^{Val}$ mutants that were isolated as suppressors of a –1 frameshift mutation (3). These tRNAs have single base insertions in the anticodon loop and decode quadruplets, but can also 'hop', that is they can become unpaired from the zero frame codon and re-pair on an overlapping cognate codon in an alternate reading frame. The unexpected decoding properties of these altered tRNAs have illuminated unanticipated potentials of the decoding process and have made it of interest to characterize more tRNA mutants and study their decoding properties.

In this study, *Escherichia coli* mutants that enhance frameshifting into the +1 reading frame at CAA AAA ACC have been isolated and characterized. These frameshift suppressor mutants consist of insertions in the anticodon loops of $tRNA^{Lys}$ and $tRNA_1^{Gln}$ so that the enlarged anticodon of both tRNAs is now 3′-GUUU-5′. These studies also prompted a re-examination of the *sufG* class of frameshift suppressors isolated by Kohno and Roth in both *Salmonella enterica* serovar Typhimurium and *E.coli* (6), that had been thought to cause frameshifting at AAAA and AAAU sequences. Sequencing of two of these *sufG* mutants showed that they also contained insertions of a single U in the anticodon of $tRNA_1^{Gln}$ and characterization of a series of *sufG*-suppressible frameshift mutations in the *his* operon of *Salmonella* showed that each contained a CAAA quadruplet. Together these data suggest that expansion of the anticodon allows the extended anticodon to decode CAAA quadruplets by pairing with all four bases of the complementary 5′-CAAA-3′ sequence in the mRNA.

Historically, most of the mutant tRNAs that can decode quadruplets were isolated genetically during the course of investigations into the mechanism of reading frame maintenance. Recently, novel approaches have been used to identify quadruplet- and quintuplet-decoding tRNAs. These tRNAs all contained expanded anticodons and were constructed with the aim of expanding the genetic code and directing the incorporation of non-natural amino acids (2,7,8). The two quadruplet-decoding tRNAs described here add to these examples of mutant tRNAs with expanded decoding possibilities.

MATERIALS AND METHODS

Bacterial strains and plasmids

The *trpE9777*-containing strain (9), strain MC141 [F^- Δ(*lac-Pro*) *thi-1 trpE9777*], was used for the isolation and identification of suppressors of the *trpE9777* +1 frameshift mutation. A *recA*$^-$ derivative of this strain, designated MC223, was made

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by mating MC141 with JC10240 (*Hfr srlC300::Tn10 recA56 thr-300 ilv-318 rpsE300*). Strains TR4447 (*E.coli trpE9777 cysB met nag F'152 nag+ sufG100*) and TR4592 (*S.enterica* serovar Typhimurium *hisO1242 hisD6580 nag1 sufG70 F'152 nag*$^+$) containing *sufG* alleles (6) were obtained from Dr John Roth, University of Utah. A strain with *suf-3* linked to a kanamycin resistant transposon (10) was constructed to facilitate mapping of the *suf-3* suppressor. Hfr mapping was carried out using the Hfr::Tn10 strain kit obtained from the *E.coli* Genetic Stock Center, Yale University, and the methodology described in Wanner (11). Strains carrying genetic markers in the 10–30 min region of the chromosome were obtained from Dr Barbara Bachmann and Dr Mary Berlyn at the *E.coli* Genetic Stock Center. P1 transductions, transposon and chemical mutagenesis and other genetic procedures were performed as described (10).

The *Salmonella* strains TR2706 (*hisO1242 C6581 tyr545 sufA2 sufG70*), TR3683 (*hisO1242 B6480 nag-1*), TR2692 (*hisO1242 B6575 xyl-540 tyr-545*) and TR3696 (*hisO1242 F6527 proA622*) containing *sufG*-suppressible *his* mutations (6) were obtained from Dr John Roth, University of Utah. Because many of these strains contained additional undesired mutations, each *his* mutation was transferred to a clean genetic background by P22-mediated crosses with TT27 (*hisD::Tn10*) or TT10286 (*hisD9953::mudJ*).

Plasmids carrying *lacZ* frameshift mutations were constructed by ligating complementary oligonucleotides with *Hin*dIII and *Apa*I overhangs into *Apa*I–*Hin*dIII cleaved pLM90.91, a wild-type *lacZ* plasmid, as described previously (3). Cells to be assayed for β-galactosidase activity were grown to mid-logarithmic phase in minimal E medium (12) containing glucose (0.2%), thiamine, proline, tryptophan and casamino acids (0.2%), tetracycline (12.5 mg/l) and neomycin (50 mg/l), and assayed as described previously (3). Chromosomal and plasmid DNA isolations, construction of genomic libraries and transformations were carried out as described (13). The low copy number, pSC101-derived plasmid, pLG339 (14), was used as a cloning vector for the *suf-3* mutant. The kanamycin resistant, pLG339-derived plasmid, p815, contains the wild-type *valU* operon inserted in the *Bam*HI site of the tetracycline resistance gene of pLG339 (3).

Isolation and identification of frameshift suppressors

Suppressors of *trpE9777* were isolated by plating 0.1 ml of an overnight culture of MC141 on minimal (E) medium plates (12) containing thiamine and proline and incubating at 30°C. Intragenic and *trpE*$^+$ revertants were identified by preparing P1 lysates on each Trp$^+$ isolate, using this phage to transduce strain MC35 [F^- Δ(*lac-Pro*) *thi-1* Δ(*trpE-C*)8] to *trpC*$^+$ *trpD*$^+$ (growth on anthranilic acid) and screening for inheritance of tryptophan independence.

RESULTS AND DISCUSSION

Isolation, mapping and sequencing of suppressors of the +1 frameshift mutant *trpE9777*

The *trpE9777* frameshift mutation contains an insertion of a single A residue at codon 4 or 5 of the *E.coli trpE* gene (Table 1) which abolishes anthranilate synthetase activity. Frameshift suppressor mutations that promoted frameshifting in the *trpE9777* mRNA were isolated by selecting for tryptophan-independent derivatives of MC141 (a *trpE9777*-containing strain). Approximately 50 tryptophan-independent isolates were obtained in this way and five isolates that grew at varying rates on tryptophan-free medium were chosen for further study. Four of these isolates were shown by co-transduction experiments to map close to, or within, the tryptophan operon. These presumptive intragenic suppressors were not studied further. One suppressor, *suf-3*, was not co-transducible with the *trp* region of the chromosome and a linked kanamycin resistant Tn10 element, *zbe2::Tn10Kan*, was isolated to facilitate mapping of this new suppressor. The *zbe2::Tn10Kan* element was mapped to the 10–30 min region of the chromosome by Hfr crosses. Previous work by Kohno and Roth (6) had suggested that mutants of $tRNA^{Lys}$ could suppress *trpE9777*, however, *suf-3* showed no linkage by P1 transduction to *nadA::Tn10*, which is close to the $tRNA^{Lys}$-encoded by *lysT* and *lysW* genes at 17 min (15). Further transductional mapping with markers in the 10–30 min region showed that *zbe2::Tn10Kan* was 91% co-transducible with *supE44*. The UAG suppressor, *supE44*, is an allele of *glnV*, encoding a CAG-decoding $tRNA_2^{Gln}$ and is located in an operon containing seven tRNA genes, including duplicate genes for $tRNA_1^{Gln}$ (*glnU* and *glnW*) and $tRNA_2^{Gln}$ (*glnV* and *glnX*). The close linkage of *zbe2::Tn10Kan* to *supE44* suggested that the *suf-3* might be a mutant form of one of these seven tRNAs.

A plasmid carrying the *suf-3* suppressor was isolated from a genomic library and the suppressor was localized to a 3.6 kb *Eco*RI–*Bam*HI DNA fragment. Sequence analysis indicated that the insert was derived from the *glnV* region of the chromosome. Sequencing of the *glnU*, *V*, *W* and *X* genes showed that *suf-3* contained an insertion of a single thymine residue in the *glnU* gene between the bases corresponding to U33 and U34 of wild-type $tRNA_1^{Gln}$. This insertion is predicted to enlarge the anticodon loop to eight bases and, according to the classical model for frameshift suppressor action, is predicted to allow the tRNA to decode 5′-CAAA-3′ (Fig. 1).

Analysis of *sufG* frameshift suppressors from *Salmonella* and *E.coli*

Kohno and Roth (6) have reported the isolation and partial characterization of frameshift suppressors, designated *sufG*, that were believed to act at runs of A residues. *sufG* mutations were isolated both in *Salmonella* and *E.coli* and *sufG* alleles from both organisms suppressed *trpE9777*. Mapping experiments had shown that *sufG* was 80% co-transducible (by P22-mediated transduction) with the *nag* locus and, based on the reported localization of $tRNA^{Lys}$-encoding genes to this region of the chromosome, it was proposed that *sufG* was a mutant form of $tRNA^{Lys}$ that allowed it to decode AAAA quadruplets. Both the *Salmonella* and *E.coli sufG* alleles also suppress the *hisD6580* +1 frameshift mutation (Table 1). This *hisD* mutation has since been sequenced (16) and, based on the presence of an in-frame AAAU sequence, it was inferred that *sufG* suppressed both AAAA and AAAU quadruplets. However, a comparison of the sequences of both *sufG*-suppressible frameshift mutations shows that *trpE9777* and *hisD6580* each contain an in-frame CAAA quadruplet (Table 1), raising the possibility that *sufG* mutants might be altered forms of a CAA-decoding $tRNA_1^{Gln}$ rather than mutants of $tRNA^{Lys}$, as had originally been proposed.

Table 1. Suppression of *hisB/C/D/F* and *trpE* frameshifts by mutant $tRNA_1^{Gln}$ and $tRNA^{Lys}$

Mutation	Sequence of suppression window	pLG339 vector	pLG31 $tRNA_1^{Gln}$	p815 wt tRNAs	p911 $tRNA^{Lys}$
hisD6580	AUG ACC AGC UGC CGU **CAA**	ND	ND	ND	ND
	AAA UAU UGA				
hisD3068	CUG AAUGUG ACC AAA GAU	-	-	-	-
	UUA GCG CAG UGC GUC GCC AUC				
	UCU AAU CAG UCU GGG GCC				
	GGA ACA CUU AAU CAU CCA				
	GAC GCG **CAA** UGC GCG CGA				
	UUU GGU GGA UGC GAU UAC				
	CAG CGC AGG CUC GGU AUU				
	UCU CGG CGA CUG GUC GCC GGA				
	AUC CGC CGG UGA				
hisD85	CUG ACG CCU GAU GCU GAC	-	-	-	-
	AUU GCC CCG **CAA** GGU GGC GGA				
	GGC GGU AGA ACG UCA ACU				
	GGC GGA ACU GCC GCG CGC GGA				
	CAC CGC CCG GCA GGC CCU GAG				
	CGC CAG UCG UCU GAU UGU				
	GAC **CAA** AGA UUU AGC GCA				
	GUG CGU CGC CAU CUC UAA				
hisC6581	GUA GAG CGC CGG ACG GUU CCC	-	+++	-	+
	GCG CUU GAA AAC UGG CAG				
	CUG GAU CUA CAG GGG AUU				
	UCC GAC AAC CUU GAC GGC ACA				
	AAA GUG GUG UUC GUU UGU				
	AGC CCC AAU AAU CCU ACC				
	GGA **CAA** ACU UAU **CAA** CCC				
	GCA GGA UCU GCG CAC GCU GCU				
	GGA GUU GAC ACG CGG UAA				
hisB6480	.....CGC AAG CCC **CAA** AGU GAA	+	+++	+	++++
	GCU GGU GGA GCG UUA UCU				
	UGC GGA ACA AGC GAU GGA				
	UAG				
hisF6527	UUG AAA GAA AGU CCG UGA	-	-	-	-
trpE9777	AUG **CAA** ACA **CAA** AAA ACC	-	+++	-	++
	GAC UCU CGA ACU GCU AAC				
	CUG CGA AGG CGC UUA UCG				
	CGA CAA UCC CAC CGC GCU UUU				
	UCA CCA GUU GUG UGG GGA				
	UCG UCC GGC AAC GCU GCU GCU				
	GGA AUC CGC AGA UAU CGA				
	CAG CAA AGA UGA				

Suppression of *his* mutants was assessed by growth on minimal, histidine-free media. +, weak suppression; ++, +++ and ++++, increasingly efficient suppression; –, no suppression. *hisD6580* was shown to be suppressed by the *E.coli sufG100* and *Salmonella sufG70* mutants (6) that have the same alteration in $tRNA_1^{Gln}$ as the pLG31-encoded mutant analyzed here. CAAN quadruplets are indicated in bold type.

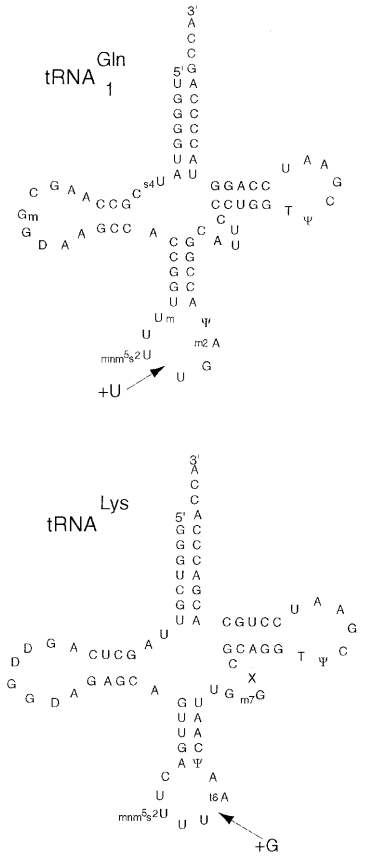

Figure 1. Secondary structure of $tRNA_1^{Gln}$ and $tRNA^{Lys}$ showing the base insertions found in the frameshift suppressor derivatives described in the text. The base modifications indicated are those found in the respective wild-type tRNAs, the modification status of the mutant tRNAs has not been determined.

Fragments of 200 bp (*glnU/W* genes) were amplified by PCR from both the *E.coli sufG100* and the *Salmonella sufG70*-containing strains, cloned into the high copy plasmid, pGEM-T, and shown to suppress the *trpE9777* frameshift mutation. Nucleotide sequencing of the *glnU* and *W* genes showed that *sufG100* and *sufG70* each had an insertion of a single U between the bases corresponding to U33 and U34 of the mature $tRNA_1^{Gln}$. Thus, the *sufG100* and *sufG70* mutations are both identical to the *suf-3* suppressor isolated in this study, and each suppressor is predicted to have an enlarged 3′-GUUU-5′ anticodon that can pair with the 5′-CAAA-3′ quadruplet found in both *trpE9777* and *hisD6580* frameshift mutations.

Isolation of CAAA-decoding mutants of $tRNA^{Lys}$

$tRNA^{Lys}$ is involved in several instances of ribosomal frameshifting and its ability to shift frames has been proposed to be due, in part, to an unusual anticodon loop conformation (17,18). The ability of altered forms of this tRNA to promote +1 frameshifting was explored by selecting for $tRNA^{Lys}$ mutants that could suppress the *trpE9777* frameshift mutation. The *trpE9777* strain MC141 containing plasmid p815 (3), which carries genes for both valine and lysine tRNAs, was mutagenized with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and tryptophan-independent colonies were selected on

minimal medium containing kanamycin. Plasmid DNA was extracted from pooled Trp+ colonies, MC141 was transformed with this DNA and Trp+ transformants were again selected on minimal medium. In this way, plasmid-borne suppressors were separated from chromosomal suppressors and *trpE* revertants. Two plasmid associated suppressors (designated *suf-11* and *suf-13*) were recovered and nucleotide sequencing revealed that each of these plasmids contained an insertion of a single G residue between the bases corresponding to U36 and t^6A37 in the anticodon loop of the $tRNA^{Lys}$ (Fig. 1). The simplest interpretation of this result is that, as is seen with the $tRNA_1^{Gln}$ -derived *sufG* mutants described above, the insertion in the anticodon of $tRNA^{Lys}$ generates a four base 3′-GUUU-5′ anticodon that can decode 5′-CAAA-3′ quadruplets.

Decoding properties of the mutant tRNAs

Analysis of the suppression spectrum of *sufG70* by Kohno and Roth (6) identified several mutations in the *his* operon of *Salmonella* that were suppressed by the *E.coli sufG100* and *Salmonella sufG70* alleles. Since the $tRNA_1^{Gln}$ mutant isolated in this study is identical to *sufG100* and *sufG70*, several of these *his* mutations were sequenced and tested for their ability to be suppressed by the $tRNA_1^{Gln}$ - and $tRNA^{Lys}$-derived tRNAs. The sequences of *hisB6480* and *hisB6575* mutations were identical; insertion of a C residue generated a CAAA quadruplet that was suppressed by both mutant tRNAs. Curiously, it had been reported previously (6,19) that while *hisB6575* was suppressed by *sufB*, *hisB6480* was not. When the *hisB6480* and *hisB6575* mutations were transferred to a clean genetic background by P22-mediated transductions with TT27 (strains MS44 and MS50, respectively) or TT10286 (strains MS52 and MS53, respectively), all the resultant strains were detectably leaky on histidine-free media, particularly at 30°C. However, of the original *hisB6575*/*hisB6480*-containing strains, only TR2692 (*hisO1242 B6575 tyr545 xyl*–) was leaky. Conceivably, additional mutations are present in the original *hisB6480*-containing strain TR3683 (which had been subjected to proflavin mutagenesis) that restrict both its leakiness and its ability to be suppressed by certain mutant tRNAs. Sequencing of the *hisC6581* mutant indicated that it also contained a CAAA quadruplet in its frameshift window (Table 1). This mutant was strongly suppressed by the altered $tRNA_1^{Gln}$ but only poorly suppressed by $tRNA^{Lys}$. It had been reported that *hisF6527* was suppressed by *sufG70* (6). However, neither the $tRNA_1^{Gln}$ mutant analyzed here (which is identical in primary sequence to the *Salmonella sufG70* allele) or the $tRNA^{Lys}$ mutant suppressed *hisF6527*. Suppression was not observed either in the original TR3696 strain or in MS51, a reconstructed version of this *hisF* mutant. The reason for this discrepancy is unclear.

Several other frameshift mutations of known sequence and containing CAAN sequences in their frameshifting windows were also tested for suppression by the mutant tRNAs. The *hisD3068* mutation contains a CAAU quadruplet 10 codons downstream of the +G insertion but is not suppressed. The *hisD85* mutation contains a CAAG quadruplet 2 codons downstream of the +C insertion, however, no suppression by either tRNA mutant was observed. A CAAA quadruplet 33 codons downstream of the frameshift is also found in *hisD85* but even if suppression occurs at this site, it is unlikely to yield an active protein given the number of miscoded amino acids that would be incorporated between the potentially suppressible CAAA quadruplet and the site of the insertion mutation. Thus, suppression by mutant *sufG*/$tRNA_1^{Gln}$ and mutant $tRNA^{Lys}$ appears to be limited to CAAA quadruplets located close to the sites of base insertion in +1 frameshift mutations.

Table 2. Effects of mutant tRNAs on +1 frameshifting

lacZ plasmid	*lacZ* Frameshift Window	pLG339 Vector	pLG31 $tRNA_1^{Gln}$ +U33.1	p815 wt $tRNA^{Lys}$	p911 $tRNA^{Lys}$ +G36.1
		β-galactosidase activity‡			
trpE9777 frameshift windows					
9777-9	UUA ACA CAAA AAU CCU AAG	27 ± 5	347 ± 23	28 ± 5	324 ± 71
9777-29	UUA ACA CAAA UAC CCU AAG	225 ± 33	1874 ± 117	280 ± 41	2327 ± 115
9777-6	UUA ACA CAAA AAA CCU AAG	11 ± 3	329 ± 34	9 ± 2	438 ± 18
9777-59	UUA ACA CAAA UAA CCU AAG	9 ± 1	11 ± 3	8 ± 1	8 ± 2
9777-62	UUA ACA CAAA GAA CCU AAG	8 ± 7	981 ± 190	5 ± 1	1035 ± 11
9777-8	UUA ACA CAAA UAU CCU AAG	16 ± 2	1270 ± 54	14 ± 3	1829 ± 383
CAAN & AAAC windows					
CAAG -3	UUA AUC CAAG GCU	113 ± 6	148 ± 10	89 ± 8	157 ± 17
CAAU -8	UUA AUC CAAU GCU	27 ± 4	36 ± 3	24 ± 3	23 ± 4
CAAC -6	UUA AUC CAAC GCU	137 ± 5	103 ± 4	89 ± 14	72 ± 4
AAAC-17	UUA AUC AAAC GCU	70 ± 7	56 ± 1	57 ± 2	61 ± 8
CAAA -14	UUA AUC CAAA GCU	99 ± 4	983 ± 102	100 ± 23	756 ± 237

‡Numbers represent Miller units of β-galactosidase activity and each is the mean ± SE of at least three independent determinations.

Quadruplet decoding of CAAA quadruplets and related sequences was analyzed further by constructing *lacZ* frameshift mutants containing the desired target sequences within a frameshifting window and assaying the effects of mutant tRNAs on β-galactosidase activity. In the first series of synthetic *lacZ* mutants (*trpE9777* windows), the CAAA target sequence was embedded in the sequence context found in the *trpE9777* frameshift and the 3′ codon was varied. The data presented in Table 2 show that almost all CAAA-containing sequences were well suppressed by both mutant tRNAs. The sole exception occurred when a CAAA quadruplet was followed by a UAA stop codon. The efficiency of suppression varied considerably, depending on the identity of the sense codon 3′ to the CAAA quadruplet. A further series of constructs (CAAN and AAAC windows) indicated that AAAC, CAAC or CAAU quadruplets were not suppressed, suggesting that suppression required codon–anticodon complementarity at the first and fourth positions. The single species of wild-type $tRNA^{Lys}$ in *E.coli* decodes AAG as well as AAA and wild-type $tRNA_1^{Gln}$ may decode CAG as well as CAA (another isoacceptor, $tRNA_2^{Gln}$, exists that decodes CAG). Surprisingly, however, the CAAG quadruplet was only poorly suppressed by either mutant tRNA, consistent with the failure to observe suppression of the CAAG-containing *hisD85* frameshift described above. In both wild-type $tRNA^{Lys}$ and $tRNA_1^{Gln}$, position U34 is similarly modified with a mnm^5s^2 modification (20). This modification has been proposed to limit wobble pairing, however, recent experimental results with unmodified tRNAs are inconsistent with this proposal (21,22).

Recent analyses of random libraries of $tRNA^{Ser}$ mutants that can suppress four base codons in the β-lactamase mRNA indicated that the highest suppression efficiency was obtained with four base codon–anticodon interactions involving Watson–Crick pairings at all positions. In this system, G–U wobble pairings at the fourth codon position led to 10–15-fold decreases in four base reading. It may be that in contrast to triplet decoding, efficient four base reading requires that the extended codon–anticodon helix be stabilized by canonical, Watson–Crick pairings. In conclusion, only 5′-CAAA-3′ quadruplets are decoded efficiently by mutant tRNAs that have complementary 3′-GUUU-5′ anticodons.

Decoding by the mutant tRNAs was further characterized by peptide sequencing of β-galactosidase isolated from strains expressing a *lacZ* frameshift reporter gene construct and a mutant tRNA. N-terminal sequencing of β-galactosidase isolated from MC141 carrying the p9777–29 *lacZ* frameshift construct (Table 2) and plasmid pLG31 encoding the mutant $tRNA_1^{Gln}$ showed that the sequence ACA CAAA UAC was decoded as threonine, glutamine, tyrosine. Similarly, sequencing of β-galactosidase isolated from MC141 carrying the p9777–8 *lacZ* frameshift construct (Table 2) and plasmid p911 encoding the mutant $tRNA^{Lys}$ showed that the sequence ACA CAAA UAU was decoded as threonine, lysine, tyrosine. These data indicate that single amino acids, glutamine and lysine, are inserted by the mutant $tRNA_1^{Gln}$ and $tRNA^{Lys}$, respectively, at the CAAA quadruplet. Insertion of lysine at CAAA by the mutant $tRNA^{Lys}$ provides an explanation for the weak suppression of *hisC6581* by this tRNA; presumably substitution of lysine for glutamine at this position in the polypeptide adversely affects the folding and/or enzymic activity of the HisC polypeptide.

Mechanism of frameshifting: dual error versus quadruplet decoding models

Early studies on altered tRNAs that suppressed +1 frameshift mutants suggested that four base translocations were effected by tRNAs with correspondingly larger anticodons (1), leading to the proposal of a simple relationship between anticodon loop size and translocation step size. However, the available evidence indicates that the situation is considerably more complex. Rare wild-type tRNAs exist with eight-membered anticodon loops that presumably do not cause rampant frameshifting (23). Some of the characterized tRNA mutants that effect doublet or quadruplet decoding have normal-sized anticodon loops and yeast tRNA mutants with nine-membered loops have been isolated that can decode quadruplets (1,2,24). Moreover, at least some of the tRNA suppressor mutations (including the classical *sufB* class derived from $tRNA_2^{Pro}$) cause frameshifting indirectly, by allowing a near-cognate tRNA to decode an in-frame triplet in the A site. However, this partially mismatched codon–anticodon helix is unstable and the tRNA is prone to slippage into alternate reading frames. The availability of a cognate codon in an alternate reading frame stabilizes the shifted tRNA, thereby re-phasing translation. According to this mechanism, two sequential events bring about shifts in reading frame. This 'dual error' model has since been expanded to explain the action of all frameshift suppressor tRNAs; in the case of the *sufA* and *sufJ* type suppressors, base insertions in the anticodon are proposed to promote slippage of the tRNA or isomerization of the codon–anticodon complex, again leading to re-phasing of the reading frame.

Several considerations argue against the universal applicability of such slippage models. In many instances, either a suitable near-cognate tRNA does not exist, the possibility of a mutant tRNA or a near-cognate tRNA binding stably in an alternate reading frame cannot be easily envisioned, and/or there is a strict requirement for Watson–Crick base pairing at each of the four codon–anticodon pairs (2,25). The work described here indicates that the 5′-CAAA-3′ quadruplet can be decoded by either of two different tRNAs with complementary 3′-GUUU-5′ anticodons, and quadruplets that are not perfectly complementary are not decoded by the mutant tRNAs. Moreover, peptide sequencing shows that glutamine and lysine, respectively, are inserted by these mutant tRNAs at CAAA quadruplets, indicating that the mutant tRNAs themselves (and not some near-cognate species) are responsible for suppression. Suitable near-cognate tRNAs do not exist that can cause suppression of CAAA by a dual error mechanism and the possibility of either mutant tRNA binding, and subsequently re-pairing in the +1 reading frame is extremely unlikely, given the number of codon–anticodon mismatches required.

The example of four base decoding described here is similar to quadruplet decoding of 5′-CCGU-3′ by either mutant $tRNA_2^{Arg}$ (*sufT*) or $tRNA_1^{Pro}$ with enlarged 3′-GGCI-5′ and 3′-GGCA-5′ anticodons, respectively (25). In both cases, the decoded quadruplet is precisely complementary to the enlarged anticodon and, where tested, there is the necessity for Watson–Crick pairing at the first and fourth codon–anticodon pairs. Both sets of suppressors are dominant and the possibility of pairing and subsequent slippage by the mutant (or a near-cognate) tRNA does not exist. Together, these data argue in favor of four base decoding by direct pairing of the quadruplet codon with the enlarged anticodon.

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