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tRNA hopping: enhancement by an expanded anticodon

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At a low level wild-type $tRNA^{Val}_1$ inserts a single amino acid (valine) for the five nucleotide sequence GUGUA which has overlapping valine codons. Mutants of $tRNA^{Val}_1$ with an insertion of A or U between positions 34 and 35 of their anticodons have enhanced reading of the quintuplet sequences. We propose that this decoding occurs by a hopping mechanism rather than by quintuplet pairing. Such hopping involves disengagement of the paired codon and anticodon with the mRNA slipping two (or more) bases along the ribosomal-peptidyl tRNA complex and subsequently re-pairing at a second codon—the landing site. The mutant with the anticodon sequence 3'CAAU5' 'hops' over the stop codon in the mRNA sequence GUG UAA GUU with the insertion of a single amino acid (valine). In contrast, in reading the same sequence, the mutant with the anticodon 3'CAUU5' hops onto the stop with the insertion of two valine residues. It is likely that in some instances of hopping alternate anticodon bases are used for the initial pairing and at the landing site.

Key words: frameshifting/protein synthesis/tRNA hopping

Introduction

Studies coming from three different directions have uncovered a new phenomenon that occurs during the elongation phase of protein synthesis. At certain mRNA sequences, the paired codon and anticodon can disengage, allowing the mRNA to slip along the ribosome-peptidyl tRNA complex. The complex may then recognize a second codon for this tRNA and re-engage, so that synthesis continues, decoding the next codon. One manifestation of this phenomenon is frameshifting on a run of identical bases of the mRNA. A second is where disengagement and re-engagement can occur over some distance. This has been termed 'tRNA hopping' to reflect the relative movement of the tRNA and mRNA (Weiss *et al.*, 1987).

The first demonstration of tRNA hopping was with wild-type *Escherichia coli* cells (Weiss *et al.*, 1987). The re-pairing was observed to occur up to 6 nucleotides along the mRNA without the possibility of re-pairing at intermediate steps. The efficiency of this hopping, which appears to be independent of distant mRNA sequence or structure, was up to 1%. In a second study, which was of the decoding of phage T4 gene 60 in wild-type *E.coli*, long-range mRNA sequences were found to be crucial for hopping (Huang *et al.*, 1988; R.B.Weiss, D.M.Dunn and W.M.Huang, in preparation) and the hop was 50 nucleotides. However, the analogy with shorter hops is preserved in that the codon at the jump 'take-off' site is repeated at the landing site immediately before the codon for the next encoded amino acid. In addition to the length of the 'ribosome jump' this case is also dramatic for the efficiency with which it occurs, approaching or at, 100%. The third study was with mutants of *E.coli* which were isolated by selection for the ability to cause ribosomal frameshifting at a particular sequence (i.e. frameshift mutant external suppressors) (Hughes *et al.*, 1989). A class of these mutants, *hopR*, causes tRNA hopping (Falahee *et al.*, 1988), and is analyzed in this paper.

The T4 gene 60 ribosome frameshift jump results in a 50 nucleotide jump, but in several cases of natural high-level ribosomal frameshifting a tRNA most likely disengages from the mRNA and re-pairs with a triplet codon just one or two bases removed from the original codon. Whether in these later examples of frameshifting (on runs of repeat bases), the disengagement is complete, and how distinct it is from the disengagement involved in tRNA hopping is not clear. With the retroviral examples of frameshifting, two tandem tRNAs shift in unison (Jacks *et al.*, 1988). In contrast, with the frameshifting for expression of the *E.coli* polypeptide chain release factor 2 gene only one tRNA shifts (Weiss *et al.*, 1987, 1988). In this case the next codon is a stop codon and so does not pair with a tRNA.

In this light, we examine the detailed sequence requirements for hopping, by taking advantage of the *hopR* mutants that increase the efficiency. We conclude that these *hopR* mutants are base additions to the anticodon loop of valine tRNA, that there is considerable latitude in permitted base pairing during hopping, that hopping efficiency declines with increasing distance of the hop, and that wild-type valine tRNA also causes hopping.

Results

All of the *hopR* alleles studied here were isolated as external suppressors of the -1 frameshift mutation, *trpE91* in *E.coli* (Atkins *et al.*, 1983; Hughes *et al.*, 1989). In order to study the decoding specificity of *hopR*, specific *lacZ* mutations have been constructed by cloning synthetic, complementary oligonucleotides into the 5' region of a plasmid-borne β-galactosidase gene (Weiss *et al.*, 1987). To a first approximation, the measured β-galactosidase activity reflects the number of ribosomes that traverse the test sequence to emerge in the correct reading frame to synthesize active monomer. N-terminal sequencing of purified β-galactosidase allows a determination of the manner in which ribosomes avoid stop codons within the inserted test sequence.

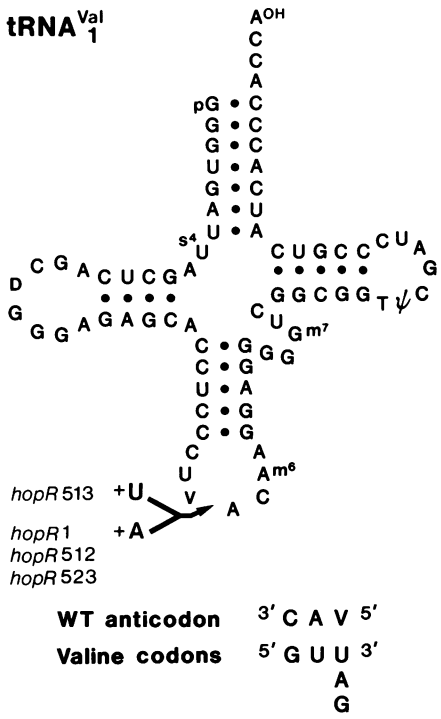

Fig. 1. Wild-type tRNA$^{Val}_1$ showing the insertions found in the two types of *hopR* mutants with enlarged anticodons. The sequence of the *hopR* mutants was determined at the DNA level only, and the modifications in the mutant tRNAs may not be the same as in wild-type tRNA$^{Val}_1$. V at anticodon position 34 is uridine-5-oxyacetic acid and its occurrence at the first anticodon position is thought to permit reading of U, A or G at the third codon base (Yokoyama *et al.*, 1985).

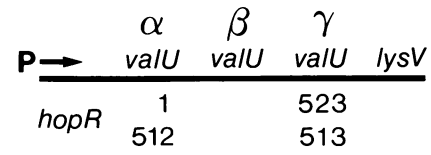

Fig. 2. Location of the *hopR* alleles with enlarged anticodons in the different copies of the *valU* gene for tRNA$^{Val}_1$. As indicated the *valU* α gene is the promoter proximal in the four tRNA operons.

Expanded anticodons of hopR alleles

We have characterized four *hopR* alleles that decode GUGUA with an efficiency of 2–4%. Each was cloned out of a genomic library of the suppressor-carrying strain by virtue of its ability to suppress the *trpE91* mutation. Initial mapping data located the mutants in the vicinity of the *valU* operon encoding one lysine tRNA (Uemura *et al.*, 1985) and three valine tRNAs (this work). Since the original *hopR1* allele was known to insert valine it was suspected that the *hopR* mutations might be in any one of the three identical valine tRNA genes of this operon. Therefore, this region was sequenced for each of the four alleles. Each of the four mutant alleles contained an extra nucleotide inserted between the bases corresponding to positions 34 and 35 of the mature tRNA (Figure 1) in one of the three valine tRNA genes of the operon (Figure 2). To confirm that the insertions were responsible for the altered decoding properties of these tRNAs, we sequenced a revertant of the cloned *hopR1* allele, obtained by subculturing in the absence of suppressor selection. The revertant lacked the extra base and had a wild-type sequence.

These anticodon insertion changes were of two types: insertion of an A (*hopR1, 512* and *523*) or a U residue (*hopR513*). *hopR1* [*hopR1*(3'CAAU5')] and *hopR513* [*hopR513*(3'CAUU5')] were studied in detail. The anticodon of wild-type tRNA$^{Val}_1$ is 3'CAV5' where V is a modified U (uridine 5-oxyacetic acid), capable of forming V-U as well as V-G and V-A pairs (Yokoyama *et al.*, 1985). Consequently, the wild-type tRNA is capable of decoding GUU, GUG and GUA valine codons. (GUC is decoded by two other valine tRNAs.) We have not determined whether the mutant tRNAs are similarly modified.

Predictions of hopping

A hopping mechanism requires two sites, the take-off site (the site at which the tRNA first pairs) and the landing site where the tRNA re-pairs following slippage of the message. The previous experiments led to the proposal that +2 frameshifting by *hopR1* occurring at GUGUG and GUGUA was by hopping of valine tRNA from GUG onto GUG or GUA with the take-off and landing sites overlapping by one nucleotide. A direct test of the hopping model is to separate the take-off and landing sites by, for instance, a stop codon (e.g. GUG UAA GUU). This would predict that the valine codons be recognized by the same tRNA and that the nine nucleotides would encode a single amino acid (valine). Enzyme levels from the GUG UAA GUU construct in strains with either of the two *hopR* mutant alleles are substantially higher than with the parental strain from which the *hopR* alleles were derived. [Table I, p100, *hopR1*(3'CAAU5'), 229 units and *hopR513*(3'CAUU5'), 1028 units versus 30 units with wild-type tRNA. Without the stop codon barrier, a value of 10 000 units is obtained.] Direct evidence that hopping is responsible for bypassing the stop codon is provided by amino-terminal sequencing of the β-galactosidase. *hopR1*(3'CAAU5') inserts a single valine for the GUG UAA GUU sequence (Figure 3). Absence of a second valine suggests that GUG is the take-off site and GUU is the landing site, thus hopping over the stop codon. In striking contrast, *hopR513*(3'CAUU5') inserts two valine residues at the same sequence (Figure 3). We propose that the valine tRNA in *hopR513*(3'CAUU5') hops from the initial GUG to land on the UAA stop codon, effectively decoding six nucleotides. The pairing with the stop codon could include the adjacent 5' G since there is complementarity [*hopR513*(3'CAUU5')/mRNA 5'GUAA3']. A second, presumably wild-type, tRNA$^{Val}_1$ can then decode the next codon—GUU. We conclude that both *hopR* mutants of val tRNA can cause hopping but use different landing sites.

The hopping mechanism predicts that there must be take-off and landing sites recognized by the same tRNA. Changing the GUG take-off site to an AUG methionine codon (Table I, p120) abolishes the effect of both *hopR* alleles.

Changing the last position of the take-off site has been tested with GUN UAA GUU (p100 and p150–p152). With *hopR1* all four constructs give substantial activity (2–10%) but which surprisingly is not correlated with the potential for base pairing. Pairing at the take-off site does not seem to need standard triplet interaction, perhaps employing only the first two codon–anticodon bases. However, the anomalously high level seen with GUC (Table I, p152) is difficult to understand.

Table I. In-frame stop hops

	mRNA	β-Galactosidase activity		
		hopRI 3′CAAU5′	wild-type 3′CAU5′	*hopR513* 3′CAUU5′
Single stops				
p100	GUG UAA GUU	229	30	1028
p110	UGA	556	351	522
p120	AUG UAA GUU	19	24	27
p130	GUG UAA CCC	23	11	107
	GUG UAA CUN			
p140	U	123	44	221
p141	G	331	96	443
p142	A	533	81	389
p143	C	67	26	212
	GUN UAA GUU			
p150	U	351	92	537
p151	A	203	46	153
p152	C	1174	133	515
Double stops				
p160	GUG UAA UGA GUU	84	15	85
p161	UAA UAA	90	17	99
Triple stops				
	GUG UAA NNA UAA GUU			
p170	UU	40	13	57
p171	GU	38	12	35
p172	GA	33	10	55
p173	UA	34	16	48
Two-stop codons				
	GUG NNA UAA GUU			
p180	UA	99	15	94
p181	AA	164	128	173
p182	GU	116	57	216
p183	GA	114	101	98
p184	CA	100	56	154

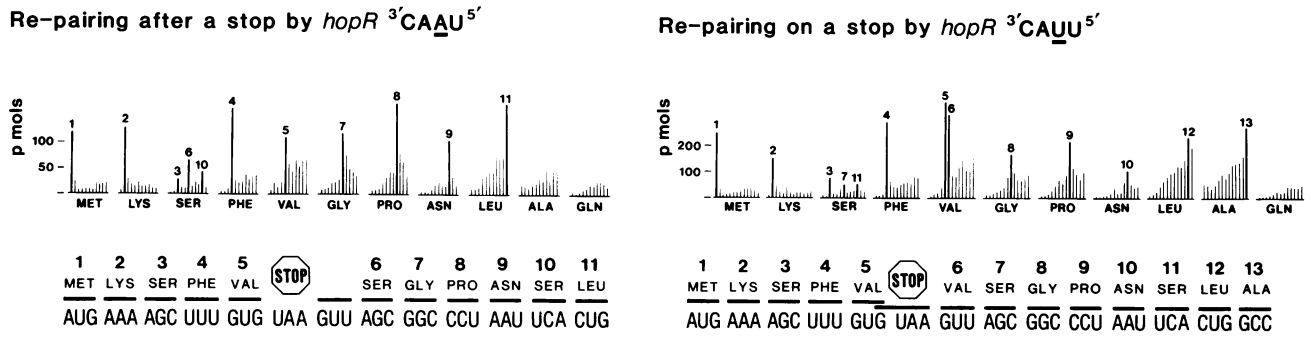

Fig. 3. Amino-terminal sequencing to examine hopping when valine codons flank a stop codon. Each panel shows the yield in picomoles of phenylthiodantoin-amino acids analyzed during each sequencing cycle. The anticodons of the $tRNA^{Val}_{I}$ mutants causing the hopping are shown above the panels, and underneath the panels the protein and mRNA sequences are aligned to show the deduced site of hopping. In the left-hand panel re-pairing is deduced to be with the overlined GUU without an amino acid indicated, and in the right-hand panel on the overlined GUAA under the stop sign.

The identity of the stop codon in 'stop hop' type constructs should be important for one *hopR* allele and not for the other on the basis of base pairing potential. When UGA is substituted for UAA to give GUG UGA GUU (p110) the efficiency of the *hopR1* (3′CAAU5′) allele is unchanged as expected. [The high background level with UGA (Table I, p110, 351 units) is due to the endogenous read-through of UGA by wild-type tryptophan tRNA.] In the case of *hopR513* changing the stop codon from UAA to UGA causes the level to drop, due either to the G−U pair if hopping is onto the stop, or to forcing hopping over the stop onto the GUU.

The possible involvement of the G of G UAA (Table I, p100) in a four base landing site for hopping onto the stop by *hopR513* (3′CAUU5′) was also tested with constructs p150−p152. A role is apparent but it is not easy to discern the contributions from the necessarily altered take-off site (considered further below).

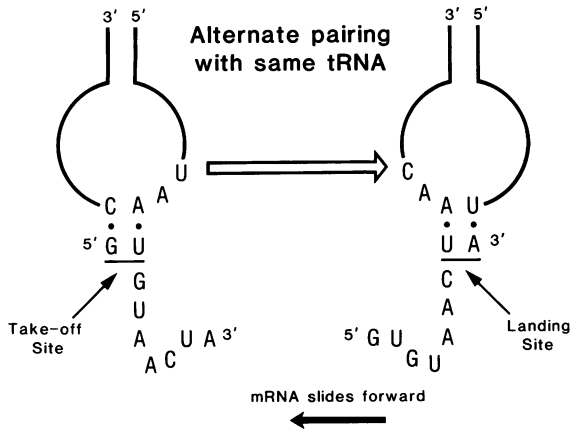

Fig. 4. Model for usage of different anticodon bases at take-off and landing sites. Disengagement from the take-off site (GU), is followed by mRNA slippage permitting re-pairing at the landing site (UA).

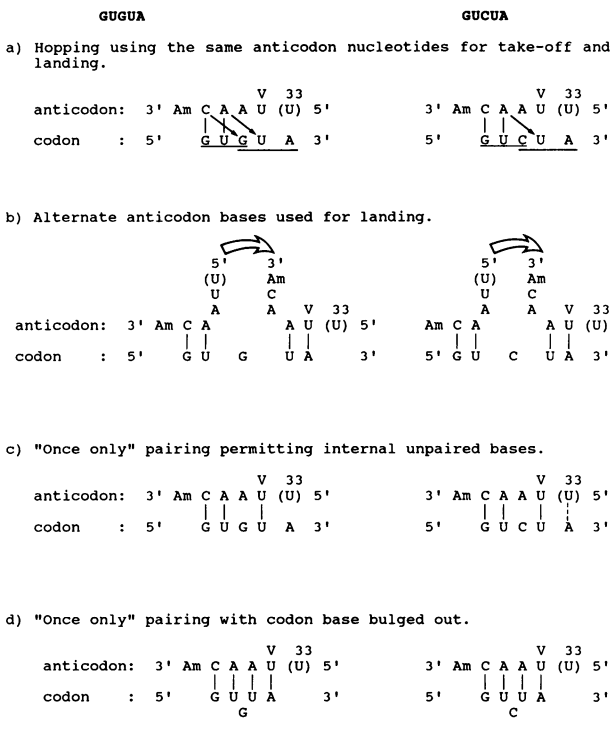

Fig. 5. Models for decoding the quintuplets GUGUA and GUCUA by *hopR1*.

Changing the GUU landing site to a CCC proline codon greatly decreases hopping by the *hopR1* (3′CAAU5′) allele [23 units versus 229 units (p130)]. With hopping onto the stop by *hopR513* (3′CAUU5′) the identity of the next codon should make little difference. Consistent with this, CCC still allows hopping onto the stop with a level of activity 10-fold greater than with wild-type. The 10-fold decrease over a GUU 3′ codon is difficult to explain without invoking local context effects in addition to codon–anticodon interactions.

Can different nucleotides of the anticodon be used for take-off and landing?

The extra nucleotides in the anticodons of *hopR1* and *hopR513* raise the possibility that alternate sets of bases of the anticodon might be used for landing site pairing. This was tested by measuring the activity of *hopR1* in GUG UAA CUN. Surprisingly, *hopR1* (3′CAAU5′) is active with GUG UAA CUN (p140–p143) where base pairing with the first nucleotide (C) of the landing site should not be possible. What is more, if the third codon base cannot pair (A or G with A), activity is increased (p142 and p141, 533 and 331 units respectively) but if it can (U with A) activity is down (p140, 123 units). This does not fit with equivalent pairing at the take-off and landing site sites. We will consider the possibility that different sets of consecutive bases of an expanded anticodon can be used at the take-off and landing sites (Figure 4). In this model take-off uses the two 3′ bases of the anticodon (CA) and landing uses the two 5′ bases (AU or AV). The anticodon base available for pairing with the third base of the CUN landing site in the p140–p143 series of constructs is U (or its modified form V). The activity seen with the constructs varies with the identity of the last codon base in the order A > G > U > C, consistent with the alternate anticodon model. Figure 4 depicts the use of alternative anticodon bases as the tRNA moves forward from the take-off site onto the landing site. Results described below with quintuplet reading are relevant to this interpretation.

To explain the results with the GUG UAA GUU construct it is not necessary to invoke the alternate anticodon model as the same anticodon bases could read the take-off and landing sites.

The activity of *hopR513* (CAUU) on the four GUG UAA CUN constructs is most easily explained by proposing that this tRNA hops from the initial GUG take-off triplet onto the overlapping GUAA quadruplet landing site, as is observed in the GUG UAA GUU construct, p100 (Figure 3).

Hopping over double and triple stops

The distance between the take-off and landing site is increased in the p160 and p170 series of constructs by one or two additional stop codons. *hopR1* (3′CAAU5′) and *hopR513* (3′CAUU5′) still cause the stop codons to be bypassed, but with reduced efficiency. With the double stop codon, the reduction is 3- and 12-fold respectively, and with the triple stop it is 10- and 30-fold. Replacing the central stop codon of the triple stop with either a valine codon (a potential intermediate landing site) or another sense codon had no effect on the efficiency. With hopping over a stop, the distance between take-off and landing sites appears to be the principal factor governing the efficiency.

Interestingly, if the upstream stop of the double stop hop is changed to certain sense codons, a low level of hopping remains (Table I, p180–p184).

Quintuplet translocation

The activity of the *hopR* mutants initially detected in the strain in which they were isolated was quintuplet translocation of GUGUG. However, there are multiple possibilities for pairing that arise because of the uncertainty about the occurrence of the modification of either U to give uridine 5-oxyacetic acid (V), and about what the decoding properties of V might be in an eight-membered anticodon loop. As an alternative to hopping on GUGUN with *hopR513* (3′CAUU5′) there is the possibility of four base expanded pairing in a 'once only' manner with occlusion of the fifth base.

anticodon: 3′-CAUU-5′ (where either U might be V)
codon: 5′-GUGUC-3′

Table II. +2 frameshifting

								β-Galactosidase activity		
								hopRI 3′CAAU5′	wild-type 3′CAU5′	*hopR513* 3′CAUU5′
Codon effects										
	AAC	GUGUN	CUA							
p200		C						165	27	196
p201		A						350	32	488
p202		U						123	34	189
p203		G						824	864	878
	AAC	GUNUC	CUA							
p210		U						25	23	34
p211		A						30	44	43
p212		C						60	19	27
	AAC	GUCUN	CUA							
p220		A						257	44	46
p221		U						151	37	32
p222		G						325	153	116
3′ context effects										
	AAC	GUCUA	NAA							
p230			A					436	35	75
p231			C					110	85	64
p232			G					1402	25	168
p240	AAC	GUGUA	GGU					1676	161	942
5′ context effects										
	AAC	UUN	GUGUA	CUA						
p250		G						1916	58	1860
p251		A						1502	51	2158
p252		U						1609	76	1754
p253		C						1472	48	1952
Doublet decoding										
p260	AGC	UUU	AAG	UAC	GU	AUA	GAA	802	311	725

With *hopR1* (3′CAAU5′) the inserted base is A rather than U, consequently the latitude of pairing possibilities due to uncertainty of the V modification is greatly reduced. To examine GUGUG decoding the last nucleotide was first varied (p200–p203). [GUGUG itself is very leaky in the wild-type background (p203) due to internal initiation at the GUG in the +2 frame.] GUGUA (3%), GUGUU (1%) and GUGUC (1.4%) are all efficiently decoded. The product from GUGUA was sequenced previously (Falahee *et al.*, 1988) and showed a single valine inserted for the quintuplet. Based on the results presented above showing that *hopR1* (3′CAAU5′) does cause hopping, the simplest interpretation for the quintuplet reading is that the mutant tRNA hops from the GUG to the overlapping GUA using the same anticodon bases for the re-pairing (Figure 5a). However, other pairing possibilities must be considered. The central G could also be ignored if the tRNA first paired transiently with only GU then with the A using alternate anticodon bases, skipping the central G (Figure 5b). There could be 'once only' pairing between the expanded anticodon and the quintuplet codon (Figure 5c). This would require that the fifth codon base (A) either be occluded or perhaps paired with U33. Another alternative is that the central codon base (G) is not part of the codon. It could be bulged out of the mRNA allowing, in this case, two base pairs on either side of the bulge (Figure 5d) which would avoid postulating occlusion or involvement of U33.

As concluded above, when hopping over stop codons, the identities of the third base of the take-off site and the third base of the landing site are not crucial. This should also be the case if quintuplet reading of GUGUA by *hopR1* (3′CAAU5′) is mediated by hopping using the same anticodon bases for take-off and landing (Figure 5a and p201, Table II). It would then be expected that pairing at the first position of the landing site would be essential if hopping is involved. *hopR1* on GUCUA (p220, Table II) gives 2% activity compared to 3% with GUGUA. The protein sequence from this construct (GUCUA CUA, data not shown) or a related construct (p232 GUCUA GAA, Figure 6) shows again that a single valine was inserted at the GUCUA quintuplet by *hopR1*. As shown in Figure 5(a) there is potential for only one conventional base pair at the landing site, making this form of a hopping model questionable. The results rather support one of the other three models. For the alternate anticodon model (Figure 5b) the fifth base of the codon should be paired by the anticodon base U (or V). Consistent with this possibility, activity seen with the constructs varies with the identity of the last codon base in the order A > G > U > C. The values found were A (p220), 213 units over background; G (p222), 172 units over a high background presumably due to initiation at CUG; or U (p221) 114 units; C (p212) 41 units.

Both *hopR* mutants can decode quintuplets by hopping from a take-off triplet onto an overlapping, triplet landing

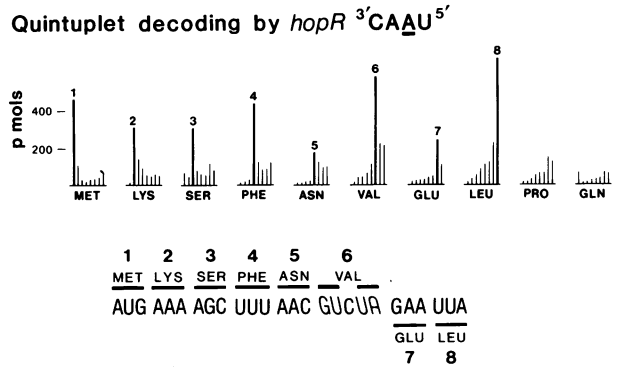

Fig. 6. Amino-terminal sequencing to test if pairing of any two consecutive bases of the anticodon and triplet landing site will suffice for hopping. The central base C of the GUCUA quintuplet is inferred not to form the first base of the landing site by standard pairing with an anticodon base.

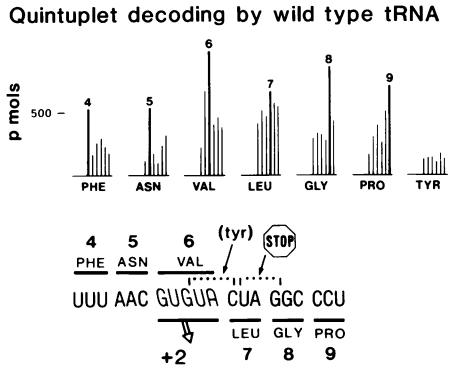

Fig. 7. Amino-terminal sequencing determination of +2 frameshifting by wild-type cells. The results are deduced to be due to hopping from GUG to the overlapping GUA. As shown in the mRNA sequence under the panels a continuation of normal triplet reading after the GUG valine codon would be expected to result in the insertion of Tyr at position 7. This is not seen.

site and can bypass in-frame terminators by hopping from a take-off triplet 5′ to the stop codon onto a landing site which either overlaps or flanks the stop. In addition, *hopR1* (3′CAAU5′), but not *hopR513* (3′CAUU5′), appears capable of using alternate sets of anticodon bases to pair with a variety of landing sites.

Unexpectedly, the identity of the codon 5′ to the take-off site has a surprisingly large effect on the level of quintuplet decoding (compare p201 with the p250 series), for reasons that remain obscure.

Stops enhance quintuplet decoding

When the first base of the codon 3′ of GUCUA was A or G (p230 and p232), the activity with *hopR1* was greatly increased to 4 and 14% respectively over the level with C of 0.25% (p231). A similar high level of 15% was seen when GUGUA was followed by G (p240). When written in the original frame these sequences are GUC stop (UAA or UAG), or GUG stop (UAG). Whether the effect of the stop codon could be due to lack of any tRNA or to some indirect interaction between the stop codon and some ribosomal component is unknown. In any case, a stop codon in this position is not essential for quintuplet translocation.

Quintuplet decoding by wild-type tRNA

Previous work had shown that the *hopR1* allele results in ribosomes inserting a single valine residue at the GUGUA

Table III. +1 frameshifting

		β-Galactosidase activity		
		hopR1 3′CAAU5′	wild-type 3′CAU5′	*hopR513* 3′CAUU5′
	AAC GUNA CAA			
p300	G	18	18	46
p301	A	16	19	238
p302	U	843	23	41
p303	C	90	24	28

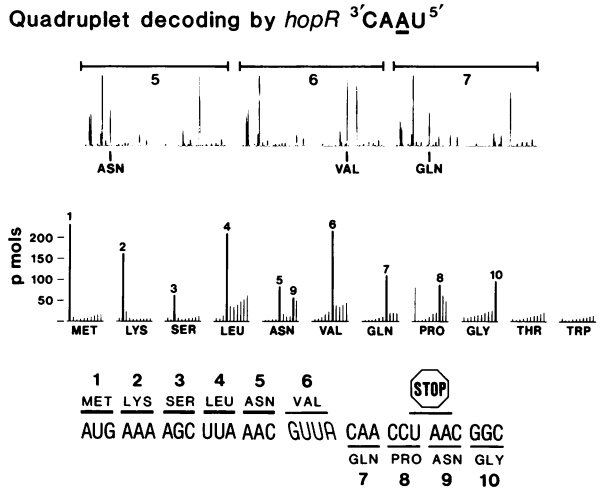

Fig. 8. Amino-terminal sequencing determination of the site of *hopR*-mediated frameshifting without hopping. The complete HPLC profile for selected cycles is shown in the upper panel, and selected phenylthiodantoin-amino acids analyzed during all the sequencing cycles in the lower panel. The anticodon of the mutant $tRNA^{Val}_{I}$ is shown above the upper panel, and the mRNA and protein sequences are aligned below the lower panel to show the site of frameshifting.

quintuplet, emerging in the +2 reading frame to make active monomer (Falahee *et al.*, 1988). This construct (p201) also gives a low level of enzyme in the isogenic, wild-type strain [32 units (0.3%) versus 350 units (3.5%) with *hopR1* (3′CAAU5′) compared to 10 000 units (100%) for an in-frame control construct], which suggests that wild-type valine tRNA might also be capable of the same reaction. The amino-terminal sequence of the polypeptide shows that here too, a single valine residue is decoded from the GUGUA quintuplet (Figure 7) as if the *hopR* mutation is enhancing a normal cellular reaction.

Quadruplet decoding by quadruplet pairing

Other tRNA mutants that have an additional base inserted in the anticodon loop result in quintuplet reading and have been isolated on the basis of their ability to decode four bases. When either *hopR* mutant tRNA is given the opportunity to read four bases with pairing potential in all four positions, a four base translocation is apparent. The efficiency is 8% for *hopR1* on GUUA and 2% for *hopR513* with GUAA (Table III, p302 and p301). Interrupting the base pairing potential greatly decreases activity, but the residual activity with some constructs, notably p303 (GUCA) with *hopR1*, is above background suggesting that there is some latitude in pairing. (It is noteworthy that U−G pairing at the third position is not sufficient, at least with *hopR513*.) Protein sequence of the product of *hopR1* acting on the GUUA

(p302) shows (Figure 8) that there must be a four base translocation since Val is found at position six (from GUUA) and Gln at seven (from the adjacent CAA). This can be explained using simple base pairing and without invoking hopping.

Doublet decoding

The construct AGC UUU AAG UAC **GUA** UAG (p260) encoding Ser Phe Lys Tyr Val stop, was designed to test backward hopping from the GUA in bold to the underlined GUA. Activity was seen with both *hopR* alleles that was 2- to 3-fold over the high background level of 3%. Amino acid sequencing showed, however, that backwards hopping could not be detected. The background without *hopR* has the sequence Ser Phe Lys Val Arg Ile with a −1 shift occurring at the AAG lysine codon. [The high level is surprising and is most likely due to the upstream C UUU codon where a Phe tRNA (anticodon 3′GAA5′) could shift back by one base to pair with CUU. This double shift sequence C UUU GAA is very similar to frameshifting sequences used for decoding of the *gap-pro-pol* genes in retroviruses (Jacks *et al.*, 1988) that are known also to shift efficiently in *E.coli* (Weiss *et al.*, 1989).] With *hopR513* the same construct gave the protein sequence Ser Phe Lys Tyr Val Ile with the Ile encoded by AUA. This is interpreted to mean that *hopR513* inserted a Val at the GU doublet implying that pairing in the third position is impaired in the mutant tRNA. This contrasts with our inability to observe doublet decoding with any GU NAU construct, indicating an influence of the identity of the codon 3′ to the GU doublet on this type of decoding.

Discussion

The analyses of the decoding specificities of *hopR* mutants presented here show that valine tRNA mutants with enlarged anticodons are capable of at least three distinct types of non-standard decoding. In the most straightforward case, the four bases of the anticodon can align with a complementary sequence on the message and read a quintuplet. Alternatively, depending on the identity of the downstream codon, the same mutant tRNA can decode a GU doublet. The third type of decoding necessitates that the tRNA and message become dissociated and that the tRNA re-pairs downstream in the message.

Current models of translocation envisage the size of the anticodon loop as being the main determinant of translocation step size. Early studies on mutant tRNAs which decoded quadruplets showed that the enlarged translocation step size was the result of a correspondingly larger anticodon loop. This finding was interpreted as indicating that the tRNA movement from A to P sites was responsible for passage of mRNA through the ribosome. More recent work with suppressor tRNAs has shown that some mutant tRNAs with eight base loops can decode only triplets (Murgola *et al.*, 1983) and, conversely, mutant tRNAs with seven-membered anticodon loops have been isolated which can decode a quadruplet (Tucker *et al.*, 1989), or in another case decode a GG doublet (O'Mahony *et al.*, 1989). The findings presented here demonstrate in an obvious manner the lack of strict correspondence between the number of nucleotides in the anticodon loop and the size of the translocation step. Furthermore, the demonstration that both wild-type and mutant tRNAs can dissociate from and re-pair with the message further downstream, indicates that mRNA can move through an actively translating ribosome independently of tRNA movement. Genetic studies on T4 mutants suggested a similar possibility, although the extent and sequence requirements necessary for this 'phaseless wandering' of mRNA through the ribosome are unknown (Sarabhai and Brenner, 1967).

Comparison of the effectiveness with which each of the four valine codons can serve as take-off (GUN UAA GUU) or landing sites (GUGUN) indicates that the variation in the efficiencies with which these variant sequences are decoded by the mutant tRNAs is not a simple reflection of the number and stability of possible codon−anticodon base pairs and suggests that only two base pairs might be formed at both the take-off and landing stages of hopping. This interpretation is consistent with our observation of doublet decoding in certain 3′ contexts by these same mutant tRNAs. One of the mutant valine tRNAs studied here, *hopR1*, appears to be capable of using alternate sets of consecutive anticodon bases to pair with CUN landing sites. By contrast, Murgola *et al.* (1983) have demonstrated that insertion of an extra base into the anticodon of $tRNA_2^{Gly}$ caused this tRNA to utilize only a new triplet anticodon. The other mutant, *hopR513*, appears incapable of 'anticodon shuttle', suggesting that UUAC and UAAC anticodons do not adopt equivalent base stacking conformations. Both wild-type and mutant tRNAs are capable of dissociation and re-pairing on the mRNA. One possible consequence of the anticodon insertions may be to favor two base codon−anticodon interactions; such an interaction is expected to be less stable than the normal tRNA−mRNA interaction and consequently should be more readily dissociable in the ribosome.

The observation that wild-type tRNAs can also hop (Weiss *et al.*, 1987; this work), suggests that the interaction between wild-type tRNAs and mRNAs is a dynamic rather than a static association. According to this view, wild-type tRNAs continually dissociate from and re-pair with a single codon on the message (the take-off site). Perhaps the mutant tRNAs can dissociate more readily and completely from the message and re-pair elsewhere in the mRNA (the landing site). It would not be surprising if some of the types of hopping, such as the use of alternate anticodon bases, exhibited by the mutant tRNAs were a consequence of eight-membered anticodon loops. Perhaps this is part of the reason why, with rare exceptions (Li and Tzagoloff, 1979; Sumner-Smith *et al.*, 1984), all normal tRNAs have seven-membered anticodon loops. This does not mean, however, that short distance hopping by wild-type tRNAs is not utilized to bypass gene termination signals. Possible examples of this have been discussed (Weiss *et al.*, 1987).

The demonstration that the mRNA of T4 gene 60 contains a 50 nucleotide interruption (Huang *et al.*, 1988) suggests that certain mRNA sequences can somehow be bypassed by the translational machinery. The T4 gene 60 interruption may assume an elaborate secondary structure. In contrast, the mRNA sequences bypassed by *hopR* appear to contain little potential for secondary structure as they are so short and the efficiency of *hopR*-mediated hopping is severely constrained by the distance separating take-off and landing sites.

The protein sequence data in Figure 3 show that in a *hopR1* strain, only two amino acids (phenylalanine and valine) are

inserted at the sequence UUU GUG UAA GUU. Therefore, a peptide bond must be formed between a peptidyl tRNA Phe (decoding UUU) and a Val tRNA bound either to the take-off (GUG) or landing site (GUU). Transpeptidation requires that the 3′ termini of both A and P site tRNAs can be brought into close proximity while the anticodons of these tRNAs interact with adjacent codons on the template mRNA. These considerations, together with the finding that codon–anticodon pairings are maintained in both A and P sites of the ribosome (Ofengand and Liou, 1981; Bergemann and Nierhaus, 1984), render improbable the possibility of forming a peptide bond between a UUU-decoding phenylalanine tRNA in the P site and a valine tRNA bound to the GUU landing triplet in the A site. Consequently, it is unlikely that valyl $tRNA^{Val}_1$ located in the A site can dissociate and re-pair with a downstream codon as this would necessitate bulging out the intervening six nucleotides. It is also unlikely that the six nucleotides spanning take-off and landing sites can bulge out between a deacylated phenylalanine tRNA in the P site and a peptidyl valine tRNA in the A site, bound to the GUU landing site.

One possible model is that peptidyl $tRNA^{Val}$ in the P site of a ribosome with an unoccupied A site dissociates from the GUG take-off triplet, allowing free movement of the mRNA through the ribosome, and re-pairs with the GUU landing site downstream. An AGC serine codon is then present in the A site and transpeptidation can occur between the peptidyl $tRNA^{Val}_1$ and an A site serine tRNA. This model can be accommodated by either the classical two-site (Watson, 1964), or the more recent three-site model (Rheinberger *et al.*, 1981) that includes an E site for the tRNA exiting the P site. Although controversial, it has been proposed that strong codon–anticodon interactions are not maintained in the ribosomal E site (Wintermeyer *et al.*, 1986). The stimulatory effects of stop codons and inhibitory effects of certain sense codons located 3′ to the take-off site are consistent with the exposure of these codons in the A site while a peptidyl $tRNA^{Val}_1$ is bound to a take-off triplet in the P site of the ribosome. This model is appealingly simple, but caution is warranted since the paths and interactions of mRNA and tRNA with each other and with ribosomal RNA are only now being worked out. Studies on 'hopping' by wild-type and mutant tRNAs are likely to reveal constraints that possible models will have to accommodate.

Materials and methods

Bacterial strains

All suppressor-containing strains and their isogenic, suppressor-free progenitors are F^- Gal^+ trp deletion derivatives of CSH41 (Miller, 1972), carrying a chromosomal copy of the *Salmonella typhimurium trpE91* mutation (Hughes *et al.*, 1989). Strain 71-18 (Messing *et al.*, 1977) was used in all oligonucleotide cloning experiments.

Cloning and sequencing of suppressors

A cosmid library was prepared from the *hopR1 cysA* strain MC13 using the low copy number cosmid, pREG153 (Isberg and Falkow, 1985) essentially as described by Maniatis *et al.* (1982). A lambda NK561 lysate was prepared on 900 independent ampicillin-resistant cosmid clones and used to transduce a suppressor-free *trpE91* strain to ampicillin resistance and tryptophan independence. One suppressor-containing cosmid, p837, was chosen and the suppressor was located in subsequent subcloning steps to a 2 kb *Xho*II fragment. All other alleles were cloned directly onto the low copy number inc W vector pUB5572 (Chopra *et al.*, 1981; Ward and Grinsted, 1982) or pLG339 (Stoker *et al.*, 1982), using *Xho*II or *Hin*dIII digests, selecting transformants for drug resistance and suppressor activity. Oligonucleotide primers were synthesized on an ABI 380A or 380B machine. The dideoxy chain termination method of sequencing double-stranded DNA (Chen and Seeburg, 1985) was employed throughout.

Oligonucleotide cloning

Annealed mixtures of complementary oligonucleotides containing *Apa*I and *Hin*dIII overhangs were ligated to the *Apa*I–*Hin*dIII-restricted *lacZ* vector, p90.91 (Weiss *et al.*, 1987), and the inserts were verified by DNA sequencing. All *lacZ* constructs were transformed into isogenic strains which contained a chromosomal copy of the suppressor of interest or its wild-type allele.

β-Galactosidase assays

The assays were performed basically according to Miller (1972) as modified by Weiss (*et al.* 1987), and the inserts were verified by DNA sequencing. Strains to be assayed were grown in minimal (E) medium (Davis *et al.*, 1980) supplemented with anthranilic acid and containing ampicillin. Each assay value represents the mean of assays of at least four independent cultures. In all cases, standard errors were always <20%.

Protein extraction and sequencing

Cells were grown in superbroth (Davis *et al.*, 1980), harvested, resuspended in PBST (50 mM KPO_4, pH 7.4, 150 mM NaCl, 0.1% Tween-20, 10 mM β-mercaptoethanol) and disrupted by sonication. Cell debris was removed by centrifugation; β-galactosidase was removed from the supernatant by serial passage over an immunoaffinity column (Protosorb, Promega Biotech) and eluted with 0.1 M Na_2CO_3. Protein sequence analysis of the purified protein was carried out as described previously (Weiss *et al.*, 1987; Falahee *et al.*, 1988), using an Applied Biosystems model 470A gas-phase sequencer fitted with a liquid pulse attachment and data analysis module.

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