Prevention of Translational Frameshifting by the Modified Nucleoside 1-Methylguanosine

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The methylated nucleoside 1-methylguanosine (m^1G) is present next to the 3′ end of the anticodon (position 37) in all transfer RNAs (tRNAs) that read codons starting with C except in those tRNAs that read CAN codons. All of the three proline tRNA species, which read CCN codons in *Salmonella typhimurium*, have been sequenced and shown to contain m^1G in position 37. A mutant of *S. typhimurium* that lacks m^1G in its tRNA when grown at temperatures above 37°C, has now been isolated. The mutation (*trmD3*) responsible for this methylation deficiency is in the structural gene (*trmD*) for the tRNA(m^1G37)methyltransferase. Therefore, the three proline tRNAs in the *trmD3* mutant have an unmodified guanosine at position 37. Furthermore, the *trmD3* mutation also causes at least one of the $tRNA^{Pro}$ species to frequently shift frame when C's are present successively in the message. Thus, m^1G appears to prevent frameshifting. The data from eubacteria apply to both eukaryotes and archaebacteria.

TRANSFER RNA FROM EUBACTERIA, eukaryotes, and archaebacteria contains modified nucleosides, which are derivatives of the four canonical nucleosides. Some of the modified nucleosides are specific to one of the above groups, but a few are conserved in tRNA from all (*1, 2*). One of these conserved modified nucleosides is 1-methylguanosine (m^1G), which is present next to the 3′ end of the anticodon (position 37) in tRNAs that read codons starting with C except in those tRNAs that read CAN codons. The fact that m^1G37 is present in the same subset of tRNAs from all organisms implies that the function of m^1G37 is the same regardless of the origin of the tRNA.

The function of modified nucleosides may be to improve or prevent certain interactions of the tRNA and therefore to fine-tune tRNA function. The importance of tRNA modification is reflected in the fact that a substantial part (more than 1%) of the genetic information in bacteria is devoted to this aspect of cellular metabolism (*2*). Although the cell expends a great deal of energy to synthesize modified nucleosides in tRNA, none has so far been shown to be essential for cell viability. However, the lack of certain modified nucleosides results in a dramatically lower growth rate, whereas lack of other modified nucleosides reduces the growth rate only moderately but still significantly from an evolutionary point of view (*2*). Various modified nucleosides have been identified at position 37, and a certain pattern of modified nucleosides and coding capacities of tRNA has been pointed out (*2, 3*). Codons that start with U are mostly read by tRNAs having a hydrophobic modification, such as isopentenyladenosine, whereas those that start with A are read by tRNAs with a hydrophilic modification, such as threonyladenosine. It has been established that such modifications at position 37 stabilize the anticodon-codon interaction and make the tRNA less sensitive to differences in the codon context (*2*). Transfer RNAs that read codons starting with C or G usually have an unmodified purine nucleoside or a simple modification, like m^1G, in position 37 (*2, 3*). The energetically more stable GC pair compared to an AU pair would have a lower requirement for stabilization of the anticodon-codon interaction. Very little information has been obtained about the function of the simple modified nucleoside m^1G (*2*). Moreover, its role in vivo has not been presented. We now describe a mutant of *Salmonella typhimurium* that is deficient in m^1G in its tRNA when grown at high temperatures.

The two frameshift suppressor mutations *sufA6* and *sufB2* are dominant and induce frameshifts when C's are present successively in the message. Such mutations change the chromatographic properties of the major and minor $tRNA^{Pro}$, respectively (*4*). The three tRNA chains specific for proline in *S. typhimurium* all contain m^1G37 (*5*). We have localized the structural gene (*trmD*) for the tRNA(m^1G37)methyltransferase responsible for the formation of m^1G37 close to the *tyrA* gene in *Escherichia coli* (*6*). Modification at position 37 increases the translational efficiency of tRNA during translation (*2*). Therefore, an m^1G37 deficiency in a *sufA6* strain would reduce the efficiency of the *sufA6* suppressor. Hence, a mutation in the *trmD* gene, which results in an m^1G deficiency, should act as an antisuppressor to *sufA6*. Using localized mutagenesis (*7*), we were unsuccessful in isolating antisuppressors to *sufA6* that were cotransducible with *tyrA*. However, several clones were found to be temperature-sensitive for growth even on rich medium. The mutation in one of those mutant strains was shown to be 52% cotransducible with *tyrA*, a frequency expected for a mutation in *trmD*. One of the temperature-sensitive transductants is hereafter referred to as strain GT546 (*sufA6, hisC3737, tyrA555::Tn10, xffE1*) (*8*). The mutation *xffE1*, which induces the temperature sensitivity of strain GT546, was shown to be in the *trmD* gene by complementation with different plasmids carrying the *trmD* operon from *E. coli* (*9*) (Fig. 1). The mutation *xffE1* was therefore designated *trmD3* (*10*).

Two isogenic strains [GT874 ($trmD^+$) and GT875 (*trmD3*)] were grown in 2-(*N*-morpholino)propanesulfonic acid (MOPS)–glucose medium at 30°, 37°, and 41°C. Transfer RNA was prepared, degraded to nucleosides, and subjected to high-performance liquid chromatography (HPLC). The tRNA from strain GT875 (*trmD3*) was deficient of m^1G at 30°C and almost devoid of m^1G at 37° and 41°C (Fig. 2). The spectrum of the small amount of ultraviolet light–absorbing material that migrated as m^1G

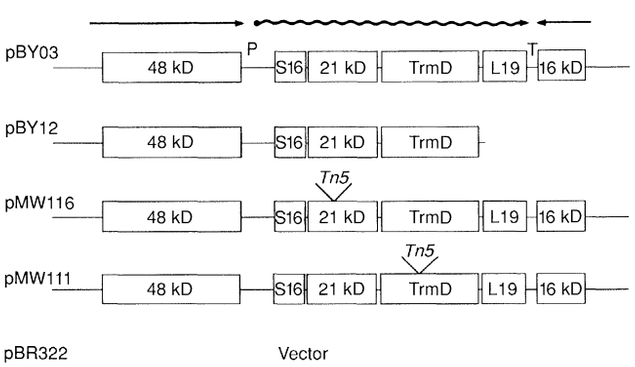

Fig. 1. Genetic organization of the chromosomal fragment carrying the *trmD* operon on plasmids used in this study. Plasmid pBY03 carries the whole *trmD* operon and two flanking genes encoding a 48-kD and a 16-kD protein. The transcript from the *trmD* operon is shown as a wavy arrow. Straight arrows indicate the direction of transcription of the genes for the 48-kD and 16-kD polypeptides. The first and the last gene in the *trmD* operon encode ribosomal proteins S16 and L19, respectively. The trmD denotes the tRNA(m^1G-37)methyltransferase. The function of the 48-kD, 16-kD, and 21-kD polypeptides is unknown. Plasmids pMW116 and pMW111 harbor Tn5 insertion (Tn5) in the 21-kD protein gene or in the *trmD* gene, respectively, and plasmid pBY12 is deleted for the genes for ribosomal protein L19 and 16-kD polypeptide.

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obtained from strain GT875 (*trmD3*) grown at 37°C was different from that of pure m^1G suggesting that not all of this material was m^1G (compare the inset in Fig. 2). Furthermore, no m^1G (less than 10% of that in wild type) was detected upon analysis of ^{14}C-methyl–labeled tRNA from the mutant grown at 37°C (*10*). Thus, tRNA from strain GT875 (*trmD3*) grown at 37° and 41°C seemed to be almost devoid of m^1G (less than 0.01 mole per mole of tRNA). However, at 30°C the amount of m^1G in the mutant was 39% of that in the wild type (Fig. 2). Analysis of in vivo ^{14}C-methyl–labeled tRNA from the mutant grown at 30°C showed that the level of m^1G was 46% of that in the wild type (*10*).

Preliminary observations had indicated that the *trmD3* mutation induced frameshifting. To investigate this phenomenon, we transferred the *trmD3* mutation from strain GT620 (*tyrA555::Tn10, trmD3*) to strain TR674 (*hisO1242, hisC3737*). Transductants that carried the *trmD3* mutation showed a weak His^+ phenotype at 37°C and a reduced ability to grow on rich medium agar plates at high temperature (42.5°C). To establish firmly that these two phenotypes were due to a mutation in the *trmD* gene, we introduced various plasmids, containing the *trmD* operon or parts thereof, into strain GT818 (*hisC3737, trmD3, tyrA555::Tn10*). The two phenotypes were trans-complemented by plasmids that expressed the $trmD^+$ gene but not by plasmid pMW111, which is defective in the *trmD* gene (Table 1).

Since we wanted to examine the specificity of the *trmD3*-mediated frameshift suppression, we combined the *trmD3* mutation with any of several known frameshift mutations in the *his* operon. Several such *his* mutations were suppressed by the *trmD3* mutation (Table 2). All except one (*hisF2439*) *his* mutation suppressed by *trmD3* were also suppressed by *sufA6*. Furthermore, mutations not suppressed by *trmD3* were not suppressed by *sufA6*. Thus, the suppression mediated by *trmD3* seemed similar to that of *sufA6*, which is known to act at CCCC/U quadruplets (*11*). In addition, we scored the ability of the *trmD3* mutation to suppress some *hisD* mutations with known DNA sequences (Table 3). The *trmD3*-mediated suppression was almost as strong as that of *sufA6* at the *hisD3749*, *hisD3749-S7*, and *hisD3749-S15* sites. Although several base alterations upstream from the insertion site did not affect the *trmD3*- and the *sufA6*-mediated suppression, one change (*hisD3749-S11*), just two bases 5′ to the insertion, completely eliminated the suppression. Thus, the *trmD3*-mediated suppression must occur at the CCCU sequence, just upstream from the UGA codon, since the suppression was eliminated when this CCCU sequence was changed to ACCU. This specificity of suppression was similar to that of *sufA6* (*11*). The CCCU sequence present just upstream of the UGA codon in both *hisD3018* and *hisD6610* was also suppressed by both *sufA6* and *trmD3*. Recent results have shown that the presence of UGA next to the frameshifting site is not a prerequisite for the observed suppression (*10*). Since proline tRNAs read CCN codons, it is likely that a m^1G-deficient $tRNA^{Pro}$ is able to read CCCU but not ACCU quadruplets. This suggests that the nature of the base 5′ of this quadruplet base sequence is critical for the *trmD3*-mediated suppression.

A *sufA6*, *sufB2* double mutant is nonviable, whereas a *sufA6*, *sufG70* double mutant is viable (*12*). The *sufG70* mutation is dominant and affects a $tRNA^{Lys}$ and acts in runs of A, which means that it does not act at the same site as does *sufA6*. Since the *sufA6* and *sufB2* mutations both affect $tRNA^{Pro}$, it was suggested that the cell cannot tolerate changes in two of the three $tRNA^{Pro}$ species (*12*). However, *sufA6* and *sufG70* affect distinct tRNA species, and the growth rate of

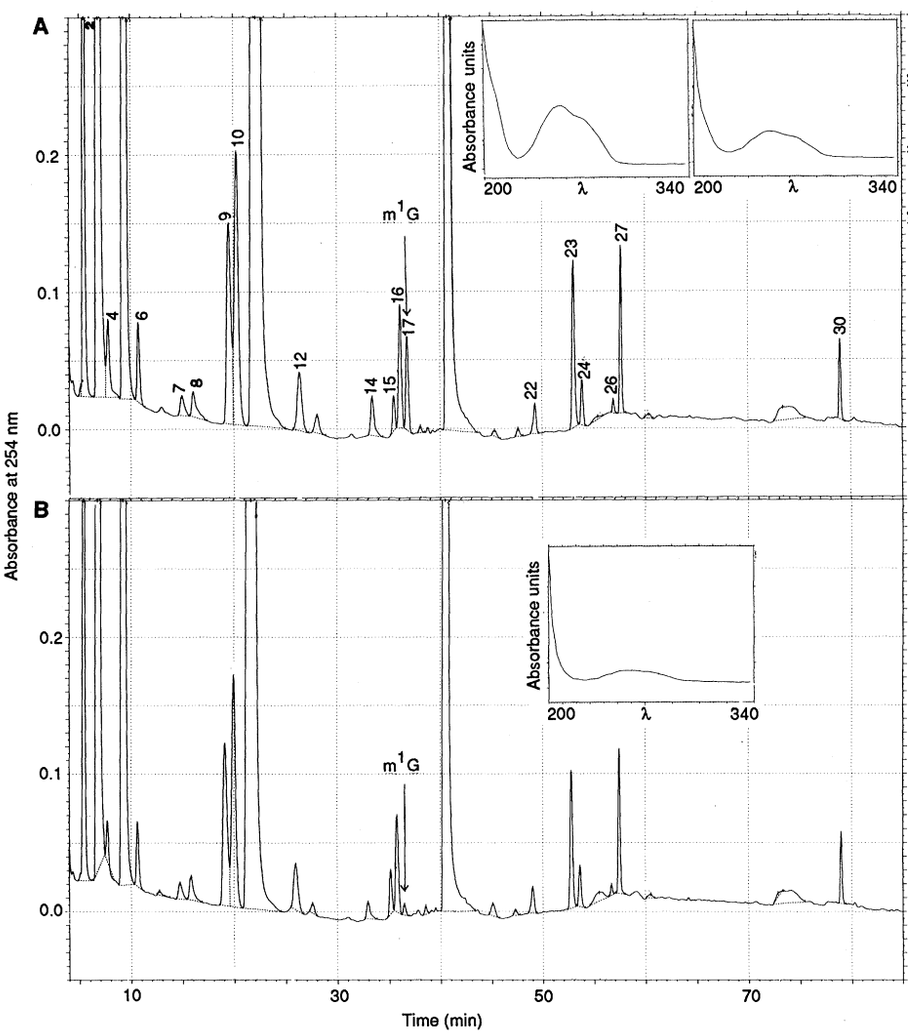

Fig. 2. Analysis of modified nucleosides of tRNA from strain GT874 [$trmD^+$ (**A**)] and strain GT875 [*trmD3* (**B**)] grown in 250 ml of MOPS-glucose medium (*22*) at 37°C. Transfer RNA was purified (*23*) and digested with nuclease P1 and alkaline phosphatase (*24*). Such a hydrolysate was analyzed by HPLC (*25*). The insets (A) show the spectra of pure m^1G (left) and of peak 17 (right). In (B), the inset shows the spectrum of peak 17. Areas of peaks were converted to mole per mole of tRNA, using m^5U as an internal standard and the relative molar response determined by Gehrke and Kuo (*25*). In eight (for m^1G four) independent determinations, the ranges (within parentheses) for different nucleosides were: pseudouridine, ψ (±9%); 7-methylguanosine, m^7G (±30%); 2-*O*′-methylguanosine, Gm (±9%); 1-methylguanosine, m^1G (±12%); and 2-methyladenosine, m^2A (±16%). The values for 37°C were the average of two independent experiments. The level of m^1G was 0.13, 0.11, and 0.13 mole per mole tRNA in $trmD^+$ grown at 30°, 37°, and 41°C, respectively. The corresponding value for m^1G in *trmD3* cells were 0.049, 0.014, and <0.01 mole of m^1G per mole of tRNA. The peaks are numbered as follows: 2, ψ; 6, 2-thiocytidine; 7, 5-methylaminomethyl-2-thiouridin; 8, 2′-*O*-methylcytidine; 9, m^7G; 10, 5-methyluridine; 12, 4-thiouridine; 15, queuosine; 16, Gm; 17, m^1G; 22, N^6-threonyladenosine; 23, m^2A; 24, 6-methyladenosine; 27, unknown; 30, 2-methylthio-N^6-isopentenylhydroxyadenosine.

Table 1. Complementation of the His^+ phenotype and slow growth on rich medium of strain GT818 (*hisO1242*, *hisC3737*, *trmD3*, *tyrA555::Tn10*). Different plasmids were introduced, and their physical structure was analyzed by digestion with restriction enzymes. The isogenic strain GT819 (as strain GT818 but *trmD*$^+$) was unable to grow without added histidine at any temperature. +, Growth scored after 2 to 3 days; ++, growth equal to strain LT2; (−), poor growth; −, no growth.

Plasmid	Growth on medium lacking His			Growth on rich medium
	30°C	37°C	41°C	42.5°C
None	−	+	+	(−)
pBY03*	−	−	−	++
pMW111†	−	+	+	(−)
pBY12‡	−	−	−	++
pBR322§	−	+	+	(−)

*Entire *trmD* operon. †Tn5 in the *trmD* gene. ‡*rplS* and "*16-kD*" genes deleted. §Vector.

the double mutant was equal to that of a strain that contained either of the two mutations (*12*). The *trmD3* mutation in combination with *sufA6* or *sufB2* also led to a nonviable cell at 42.5°C. In contrast, this was not the case when the *trmD3* mutation was combined with *sufJ*, which is a frameshift suppressor that reads ACCN (*13*), or with *suf*$^+$. These results suggest that the *trmD3* mutation affects all or at least two of the three $tRNA^{Pro}$ species. This is consistent with the fact that all three $tRNA^{Pro}$ species in wild-type cells contain m^1G37 (*5*). Apparently, the cell does not tolerate one of the $tRNA^{Pro}$ species being inactivated by a *suf* mutation in combination with a frameshifting ability of the other two tRNA species due to the lack of m^1G. Alternatively, the frameshifting ability of the altered $tRNA^{Pro}$, for example, the major $tRNA^{Pro}$ in the case of *sufA6*, becomes too strong because of the lack of m^1G. These results together with the specificity of suppression (Tables 2 and 3) strongly suggest that a m^1G-deficient $tRNA^{Pro}$ species is the frameshift-promoting molecule in the *trmD3* mutant.

On the basis of the following considerations and the results presented in this paper, we propose a model for the function of m^1G (Fig. 3). The methyl group in position 1 of guanine prevents the formation of one of the three hydrogen bonds that are formed between a guanine and cytidine in a Watson-Crick base pair. In fact, the presence of a methyl group at this position of G destabilizes a double-helix structure by 1.0 to 1.8 kcal/mole of methyl substituent (*14*). From theoretical considerations Pieczenik (*15*) has argued that some modified nucleosides adjacent to the 3′ end of the anticodon could have evolved to limit an overlapping interaction on the 5′ side of the codon and to make the codons less context sensitive. Thus, a possible function of the methyl group of m^1G would be to prevent base pairing between position 37 and the nucleotide 5′ to the codon. Conceivably, an unmodified G37 could interact with the last nucleotide (if C) of the upstream codon (Fig. 3B). If an anticodon shift occurs (Fig. 3C), it would induce a −1 type of frameshifting. These two examples require that the m^1G-deficient tRNA in the A-site interferes with the third base of the upstream codon. Since this base is also involved in pairing with the wobble base of the tRNA in the P-site, such an interaction might be unlikely. However, backward steps and forward hops have been observed as well as anticodon shifts (*16*). Aberrant anticodon-codon interactions as such may also induce frameshifting (*17*). Interactions as depicted in Fig. 3, B, C, and D, should be subjected to proofreading. In fact, the lower polypeptide step time observed (*10*) in strains containing the *trmD3* mutation is consistent with aberrant anticodon-codon interactions occurring in the *trmD3* mutant.

Alternatively, lack of m^1G37 would induce a +1 type of frameshifting if the undermodified tRNA reads four bases including the first base of the downstream codon (Fig. 3D). We emphasize that nucleotide X in the mRNA would only be a C, since it should base pair with G36, which is present in all

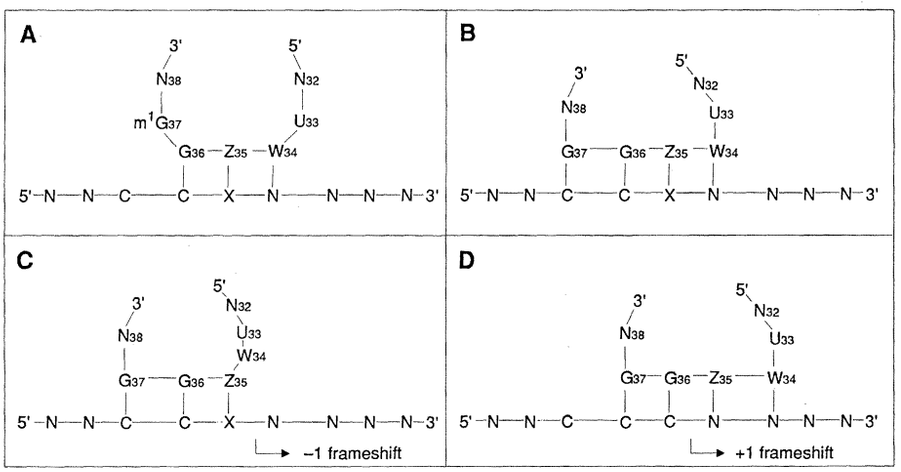

Fig. 3. Proposed molecular mechanisms for the action of m^1G to prevent frameshifting. (**A**) The methyl group at position 1 of guanosine prevents the formation of a Watson-Crick base pair and, thus, lowers the probability of base pairing to the C 5′ of the codon. Z_{35} can be A, G, or C since m^1G is present in $tRNA^{Leu}_{CUN}$, $tRNA^{Pro}_{CCN}$, and $tRNA^{Arg}_{CGN}$. X is complementary to Z, that is U, C, or G. W_{34} is the wobble nucleoside and N can be any of the four nucleosides. (**B**) Lack of the methyl group allows G_{37} to interfere with the last nucleoside (if C) of the upstream codon. (**C**) Lack of the methyl group induces an anticodon shift that results in a −1 type of frameshifting. (**D**) Lack of methyl group induces a +1 type of frameshifting that results from a four-base anticodon.

Table 2. Frameshift mutations in the *his* operon tested for suppression by *trmD3*. Strains were constructed and tested as described in (*19*). All strains contain the *hisO1242* mutation except for *his* alleles *hisA2770*, *hisC3060*, *hisC3072*, *hisD3040*, *hisD3702*, *hisF6527*, and *hisG3037*. The *hisO1242* mutation is a deletion in the attenuator region, which derepresses the histidine operon. In *trmD*$^+$ background no growth was observed after more than 5 days on medium lacking histidine except for strains containing the *hisB6480*, *hisD6610*, and *hisD2780* mutations, which showed some growth after 4 days at 37°C. Growth was observed after 1 day (+++), 2 days (++), and after 3 days (+); −, no growth after 4 days. The *hisC3060*, *hisC3072*, *hisD3068*, *hisD3702*, *hisD6580*, *hisF6527*, and *hisG3037*, which are suppressed by *sufD*, *E*, *G*, or *J* (*20*), were not suppressed by *trmD3*.

his allele	Growth on medium lacking His at		Suppressible by	Reference
	30°C	37°C		
hisA2770	−	++		
hisB6480	+++	+++	*sufA, J*	(*20*)
hisC3734	+	++	*sufA, B, C*	(*4*)
hisC3737	−	+	*sufA, B, C*	(*4*)
hisD2780	+++	+++		
hisD3018	+	+++	*sufA, B, J, M*	(*20*)
hisD3040	−	++		
hisD3749	+	+++	*sufA, B, C*	(*4, 20*)
hisD6610	−	+	*sufA, B, M*	(*20*)
hisF2439	+++	+++	*sufD*	(*4*)

Table 3. Suppression mediated by *trmD3* and *sufA6* of *hisD* frameshift mutations. Strains were constructed and tested as described in (*20*). Growth was observed after 12 hours (++++), 24 hours (+++), 36 hours (++), 48 hours (+), or 73 hours (−); −, no growth after 73 hours. The numbers above the DNA sequence indicate the nucleotide number in the *hisD* sequence. Bold letters indicate the primary insertion, and the underlined bases are the second site changes isolated by Bossi and Roth (*13*). Sequences of *hisD3018* and *hisD6610* are from (*21*).

his-allele	DNA sequence	*trmD3*-mediated suppression at		*sufA6*-mediated suppression at
		30°C	37°C	37°C
	25 48			
Wild type	UUG AAC AGC UGU AGC CCU GAA CAG			
3749	UGG AAC AGC UGU AGC CCC UGA ACA	(−)	+++	++++
3749-S6	UGG A**C**C AGC UGU AGC CCC UGA ACA	(−)	++	++++
3749-S7	UGG A**A**C A**C**C UGU AGC CCC UGA ACA	(−)	+++	++++
3749-S15	UGG AAC A**G**C UGU A**C**C CCC UGA ACA	(−)	+++	++++
3749-S11	UGG AAC AGC UGU A**G**C A**C**C UGA ACA	−	−	−
	196 219			
Wild type	CUA CGC GUC ACC CCU GAA GAG AUC			
6610	CUA CGC GUC **ACA** CCC **CCC** UGA AGA	−	+++	++++
3018	CUA CGC GUC ACC CCC UGA AGA	(−)	+++	++++

tRNAs that normally contain m^1G37. Therefore, if G · U base pairing is not considered, this mechanism predicts that +1 type of frameshifting can only occur at CCN (Pro) codons. The fact that the *trmD3* mutation mediates suppression of *hisD3018, hisD6610*, and *hisD3749* and its derivatives except *hisD3749-S11*, which has an A 5′ to the suggested frameshifting site (Table 3), is consistent with such a mechanism. Although our results do suggest that a $tRNA^{Pro}$ is the frameshift-promoting molecule at CCN (Pro) codons in the *trmD3* mutant, the model does not exclude other tRNAs that normally contain m^1G from also causing a frameshift at CCN codons. If G · U base pairing is considered, CUN (Leu), UCN (Ser), and UUN (Phe) codons can also be frameshifting sites. However, no CGN (Arg) codons are likely to be a +1 frameshifting site, which would require a G36-G base pairing to take place. Thus, the model (Fig. 3) is partly supported by our result and allows some specific and testable predictions. The conservation of m^1G37 in $tRNA^{Leu}_{CUN}$, $tRNA^{Pro}_{CCN}$, and $tRNA^{Arg}_{CGN}$ during evolution strongly suggests that whatever the organism, the function of m^1G is to prevent frameshifting. The lack of m^1G in the tRNA also influences the growth rate (reduced by more than 40% at 41°C in glucose minimal media) of the bacteria, which demonstrates the importance of this simple but ubiquitously present modified nucleoside on cell physiology.

REFERENCES AND NOTES

1. G. R. Björk, *Chem. Scripta* **26B**, 91 (1986).
2. G. R. Björk *et al.*, *Annu. Rev. Biochem.* **56**, 263 (1987); G. R. Björk, in *Escherichia coli* and *Salmonella typhimurium: Cellular and Molecular Biology*, F. C. Neidhardt *et al.*, Eds. (American Society for Microbiology, Washington, DC, 1987), pp. 719–731.
3. Reviewed in S. Nishimura, in *Transfer RNA: Structure, Properties and Recognition*, P. R. Schimmel, D. Söll, J. N. Abelson, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1979), pp. 59–79.
4. D. L. Riddle and J. R. Roth, *J. Mol. Biol.* 54, 131 (1970); *ibid.* **66**, 483 (1972); *ibid.*, p. 495.
5. Y. Kuchino, Y. Yabusaki, F. Mori, S. Nishimura, *Nucleic Acids Res.* **12**, 1559 (1984).
6. A. S. Byström and G. R. Björk, *Mol. Gen. Genet.* **188**, 440 (1982).
7. J.-S. Hong and B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 3158 (1971).
8. The bacterial strains used are all derivatives of *S. typhimurium* strain LT2. All genetic procedures used have been described (*18*).
9. A. S. Byström and G. R. Björk, *Mol. Gen. Genet.* **188**, 447 (1982); A. S. Byström, K. J. Hjalmarsson, P. M. Wikström, G. R. Björk, *EMBO J.* **2**, 899 (1983).
10. G. R. Björk and T. G. Hagervall, unpublished observations.
11. J. Yourno and T. Kohno, *Science* **175**, 650 (1972).
12. T. Kohno and J. R. Roth, *J. Mol. Biol.* **126**, 37 (1978).
13. L. Bossi and J. R. Roth, *Cell* **25**, 489 (1981).
14. J. D. Engel and P. H. von Hippel, *Biochemistry* **13**, 4143 (1974).
15. G. Pieczenik, *Proc. Natl. Acad. Sci. U.S.A.* 77, 3539 (1980).
16. E. J. Murgola, N. E. Prather, B. H. Mims, F. T. Pagel, K. A. Hijazi, *ibid.* **80**, 4936 (1983); R. B. Weiss, D. M. Dunn, J. F. Atkins, R. F. Gesteland, *Cold Spring Harbor Symp. Quant. Biol.* **52**, 687 (1987).
17. C. G. Kurland, in *Nonsense Mutations and tRNA Suppressors*, J. E. Celis and J. D. Smith, Eds. (Academic Press, New York, 1979), pp. 97–108.
18. R. W. Davis, D. Botstein, J. R. Roth, *A Manual for Genetic Engineering: Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1980).
19. The *trmD3* mutation was introduced to recipient cells harboring the indicated *his*-alleles by means of its cotransducibility with *tyrA555::Tn10*. If no His^+ clones were observed among 40 Tet^R clones, the *his* allele was scored as not suppressible by *trmD3* (*tyrA* and *trmD3* is about 60% cotransducible). To verify that the observed His^+ phenotype was due to *trmD3*-mediated suppression, a cross was performed for each His^+ derivative, using strain LT2 as donor and selecting for Tyr^+. Expected frequency among 50 Tyr^+ of His^+/His^- was observed. Furthermore, the slow growth on rich medium at 42.5°C characteristic for *trmD3* cells, was 100% linked to the ability to suppress the *his*-mutation (12 Tyr^+ clones were tested for this phenotype for each individual cross). In addition, plasmid pBY03 (*trmD*$^+$) complemented the His^+-phenotype, while plasmid pMW111 (*trmD111::Tn5*) did not. Thus, linkage to *tyrA*, slow growth on rich medium at 42.5°C and complementation by plasmid pBY03 (*trmD*$^+$) but not by plasmid pMW111 (*trmD111::Tn5*), demonstrate that the observed His^+ phenotype was due to *trmD3*-mediated suppression.
20. T. Kohno, L. Bossi, J. R. Roth, *Genetics* **103**, 23 (1983).
21. D. E. Levin, E. Yamasaki, B. N. Ames, *Mutat. Res.* **94**, 315 (1982).
22. F. C. Neidhardt, P. L. Bloch, D. F. Smith, *J. Bacteriol.* **119**, 736 (1974).
23. M. Buck, M. Cornick, B. N. Ames, *Anal. Biochem.* **129**, 1 (1983).
24. C. W. Gehrke, K. C. Kuo, R. A. McCune, K. O. Gerhardt, *J. Chromatog.* **230**, 297 (1982).
25. C. W. Gehrke and K. C. Kuo, in preparation.

26. We thank D. Andersson, J. Atkins, M. Mahan, J. Roth, and L. Isaksson for critical reading of the manuscript; A. Olsén and K. Jacobsson for technical assistance; J. R. Roth and K. E. Sanderson for providing numerous bacterial strains; and G. Nyberg, who isolated the *trmD3* mutation as part of a course experiment. Supported by the Swedish Cancer Society (project 680), the Swedish Natural Science Council (project B-Bu 2930), the Swedish Technical Board of Development, and the Knut and Alice Wallenberg Foundation (A.S.B.).

31 October 1988; accepted 2 March 1989