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Doublet Translocation at GGA Is Mediated Directly by Mutant $tRNA_2^{Gly}$

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Members of the *sufS* class of −1 frameshift suppressors have alterations of the GGA/G-decoding $tRNA_2^{Gly}$. Suppressor-promoted frameshifting at GGA was shown in this study to be directly mediated by the mutant $tRNA_2^{Gly}$. We disproved the possibility that, in the presence of the compromised mutant $tRNA_2^{Gly}$, either wild-type $tRNA_1^{Gly}$, wild-type $tRNA_3^{Gly}$, a GGA-reading mutant form of $tRNA_3^{Gly}$, or any other agent suppresses the frameshift mutation *trpE91*.

Translation component mutants that promote ribosomal frameshifting at specific sequences have been isolated as suppressors of frameshift mutations (2, 4). Most work has been done with +1 suppressors, although suppressors of the −1 frameshift mutation *trpE91* have been extensively characterized in *Salmonella typhimurium* and *Escherichia coli*. Suppressors of *trpE91* comprise several classes, including *sufS* (12), whose alleles are mutants of the single-copy gene *glyT* (11), which encodes $tRNA_2^{Gly}$. That tRNA has the anticodon 3′-CCU*-5′ and decodes GGA and GGG. In *E. coli*, $tRNA_2^{Gly}$ is the only isoacceptor that reads GGA, at least sufficiently well to permit cell growth (9). This is almost certainly also the case in *S. typhimurium*, as apart from two modification differences the sequences of $tRNA_1^{Gly}$ and $tRNA_2^{Gly}$ are the same in both organisms (6, 11). Furthermore, no mutant forms of the other glycine isoacceptors that might read GGA have been found in wild-type *Salmonella* cells (11). In contrast, GGG is decoded also by $tRNA_1^{Gly}$, which has the anticodon CCC. $tRNA_1^{Gly}$ is encoded by *glyU*, and $tRNA_3^{Gly}$, which reads GGU and GGC, is encoded by three identical, tandem *glyV* genes and an unlinked *glyW* gene (for a review, see reference 8).

With the normal balance of wild-type $tRNA_1^{Gly}$ and $tRNA_2^{Gly}$, doublet translocation at GGA codons is rare. This was determined directly with synthetic constructs having a *lacZ* reporter system (10). It can also be inferred from the absence of significant leakiness of *trpE91* (12), which has GGA adjacent to the site of its frameshift mutation (1). In contrast, in cells containing *glyT*(SufS)-encoded mutant $tRNA_2^{Gly}$, doublet translocation is readily detectable at GGA (5). In an earlier work (11), it was assumed that it was the mutant $tRNA_2^{Gly}$ that mediated the doublet translocation. However, as the SufS phenotype is recessive (10), it is possible that in *glyT*(SufS)-containing cells, with diminished competition from mutant $tRNA_2^{Gly}$, one of the other glycine-accepting tRNAs mediates the doublet translocation, despite its inability, or inadequacy, in triplet decoding of GGA. Perhaps the most likely alternative frameshift agent is $tRNA_1^{Gly}$. $tRNA_1^{Gly}$ and $tRNA_2^{Gly}$ are ≈30% nonhomologous, so even though the $tRNA_2^{Gly}$ encoded by the most efficient mutant allele, *glyT*(SufS601), has the same anticodon as wild-type $tRNA_1^{Gly}$ (CCC), they have different decoding properties. The CCC anticodon of the *glyT*(SufS601)-encoded tRNA results from substitution of the U* in the 5′ anticodon base by C, the sole change from wild-type $tRNA_2^{Gly}$. This change must be compatible with sufficient GGA triplet reading ability to permit cell growth. It is also conceivable that the doublet translocation is mediated by wild-type $tRNA_3^{Gly}$, an occasionally encountered GGA-reading mutant form of $tRNA_3^{Gly}$, namely, *glyV*(Ins) tRNA (3, 13), a cryptically mutant form of $tRNA_3^{Gly}$, or some other as yet undefined agent. These considerations prompted the various experiments reported here.

The strategy employed was fourfold: (i) to monitor the frameshift suppression properties of *sufS* in the presence of a mutant $tRNA_1^{Gly}$, disabled in its ability to read glycine codons; (ii) to monitor the frameshifting after replacing the *glyV* region of the chromosome with a known wild-type *glyV* region or with a *glyV* region in which one of the *glyV* genes had mutated to *glyV*(Ins); (iii) to determine whether the *glyW* sequence is wild type; and (iv) to determine whether frameshift suppression is lost when *glyT*(SufS) is replaced by a *glyT* mutant allele whose tRNA product is virtually devoid of its GGA-reading ability, in the presence of wild-type $tRNA_1^{Gly}$, wild-type $tRNA_3^{Gly}$, and *glyV*(Ins) tRNA. The *E. coli* strains used are described in Table 1.

In the first approach, we needed to replace wild-type *glyU* with a mutant gene whose product does not read glycine codons. For ease of detection in the replacement, a *glyU* missense or nonsense suppressor was desired. Such *glyU* suppressors, however, are currently available only in *E. coli*, and SufS suppressors have been characterized only in *S. typhimurium*. However, as *trpE91* had previously been introduced into *E. coli* and suppressors of several classes had been isolated (7), the first step undertaken was to characterize a SufS suppressor in *E. coli*.

One suppressor, *suf-519*, had been mapped by P1 transduction to the *glyT* region, and nine others were candidate SufS suppressors (9a). By using the asymmetric polymerase chain reaction (PCR), the *glyT* region of MC194, which contains *suf-519*, was amplified with 24-mer primers beginning 42 and 70 bases, 5′ and 3′, respectively, to the *glyT* gene. Sequencing was performed with internal primers, as well as with the amplification primers. *suf-519* was found to be a *glyT* allele with a sequence identical to that of the

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TABLE 1. *E. coli* strains used in this study

Strain	Genotype	Source or reference
MC194	Δ(*lac-pro*) *thi galE* Δ*trpEC8 trpE91*[a] *glyT*(SufS519)	M. O'Connor
KL1801	Δ(*tonB-trpAB*)*17 lysA zgb-224*::Tn*10 glyV55*/F'*trpA*(UAG211)	
KL2231	Δ(*tonB-trpAB*)*17 glyU*(SuUAG) *glyV55*/F'*trpA*(UAG211)	
FTP2783	*btuB460*::Tn*10* Δ(*argF-lac*)*205 flbB5301 non-9 gyrA219 relA1 rpsL150 metE70* [*araD139*] *glyV*$^+$ *purA*	B. Bachmann
FTP4808	Tn*10 purA argE*(Am) *supG* Δ(*tonB-trpAB*)*17*/F'*trpA*(AAG211)	Tn*10* obtained from C. Yanofsky
FTP5326	Tn*10 purA* Δ(*lac-pro*) *thi galE* Δ*trpEC8 trpE91 glyT*(SufS519)	MC194 transduced to Tet^r(Pur$^-$) with P1 on FTP4808
FTP5355	*glyT*(SuUGA/G) *btuB*::Tn*10 glyV55* (*purA*$^+$) Δ(*tonB-trpAB*)*17*/ F'*trpA*(UGA211)	
FTP5361	(*glyV*$^+$) Δ(*lac-pro*) *thi galE* Δ*trpEC8 trpE91 glyT*(SufS519)	FTP5326 transduced to Pur$^+$(Tet^s) with P1 on FTP2783
FTP5362	*glyV55* Δ(*lac-pro*) *thi galE* Δ*trpEC8 trpE91 glyT*(SufS519)	FTP5326 transduced to Pur$^+$(Tet^s) with P1 on FTP5355

[a] From *S. typhimurium*; chromosomally integrated.

previously described *glyT*(SufS601) of *S. typhimurium*. The sole change from the wild type was a C substitution at the position of the 5′ anticodon base (Fig. 1). The nine other candidate suppressors were examined in a similar way, but none of these was of the SufS type.

There is a mutant of *glyU*, *glyU*(SuUAG), whose $tRNA_1^{Gly}$ product reads the UAG termination codon instead of the glycine codon GGG (8). The *glyU*$^+$ gene in *glyT*(SufS519)-containing strain MC194 was replaced with the *glyU* (SuUAG) mutant allele by P1 transduction in two consecutive steps. An auxotrophic marker, *lysA*, located close to *glyU*, was transduced from KL1801 into the MC194 recipient by way of transposon Tn*10* linked to *lysA*. Of 70 Tet^r transductants selected, 16 were Lys$^-$. Two of these were used as recipients in a second transduction in which the *glyU* mutant allele from strain KL2231 could be introduced nonselectively in a selection for Lys$^+$. This was performed in the presence of tryptophan so that there was no selective pressure for maintenance of suppression of *trpE91*, and exclusion of the *glyU* mutation could occur if it was detrimental or lethal to the cell. Use of the amber suppressor derivative of *glyU* made it possible to detect the introduced allele by spot testing the transductants with a bacteriophage T4 mutant known to contain an amber mutation. Suppression of this mutation results in lysis of bacteria otherwise unaffected by this bacteriophage. All of the transductants tested were sensitive to wild-type T4, and 60 to 70% were lysed by the amber mutant, thereby appearing to have received *glyU*(SuUAG). This was further verified by *glyU* retrieval experiments in which four amber phage-sensitive transductants (1A, 1E, 3B, and 3C) were used as donors in a transduction with KL1801 as the recipient (Table 2). Suppression of a *trpA* amber mutation in KL1801 by the expected proportion of transductants confirmed the presence of *glyU*(SuUAG) in the donors.

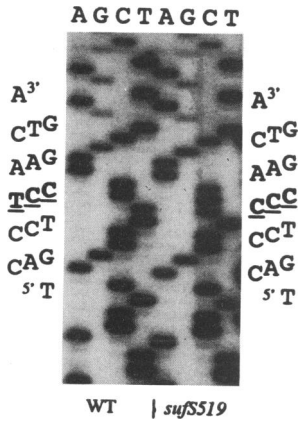

FIG. 1. Excerpts from sequences of *glyT* from asymmetric PCRs of wild-type (WT) and *suf-519*-containing strains of *E. coli*. Anticodon bases are underlined.

Having replaced the wild-type *glyU* gene, the key question was whether the *glyU*(SuUAG) transductant derivatives of MC194 retained the ability to suppress the *trpE91* frameshift mutation as a consequence of the direct action of *glyT* (SufS519) or became tryptophan auxotrophs owing to the absence of wild-type $tRNA_1^{Gly}$. Approximately 120 such transductants from each of the two final construction crosses (and about 80 of the *glyU*$^+$ category, to give 200 in total) were examined for growth on minimal medium and minimal medium supplemented with tryptophan. All were Trp$^+$. Proof that the transduction event that introduced *glyU* (SuUAG) into the chromosome did not simultaneously induce a duplication that allowed retention of wild-type *glyU* was provided by PCR sequence analysis of the *glyU* region. Such a duplication would be expected to give rise to marked heterogeneity in the anticodon-encoding sequence of a mixture of the genes for both *glyU*$^+$ (wild type) and *glyU* (SuUAG). Primers homologous to the 5′ and 3′ ends of the *glyU* gene were used in asymmetric amplification of two *glyU*(SuUAG) transduction derivatives of MC194 (1A and 3B; see above). The resulting single-stranded DNA was sequenced with these same primers. The result showed no

TABLE 2. P1-mediated transductions

Recipient	Donor	Selection	No. examined	Result
KL1801	1A[a]	Lys$^+$	54	47 (87%) Trp$^+$
KL1801	1E[a]	Lys$^+$	25	16 (64%) Trp$^+$
KL1801	3B[a]	Lys$^+$	33	22 (67%) Trp$^+$
KL1801	3C[a]	Lys$^+$	28	20 (71%) Trp$^+$
1A[a]	FTP2783	Tet^r	50	41 (82%) Trp$^-$
3B[a]	FTP2783	Tet^r	50	41 (82%) Trp$^-$

[a] The relevant genotype of this strain is (*lysA*$^+$) *glyT*(SufS519) *trpE91*, and it should have *glyU*(SuUAG) in place of wild-type *glyU*, as judged from its sensitivity to phage T4 amber mutants (see text).

such heterogeneity in the sequence of the gene, specifically, through the region corresponding to the anticodon loop 5′-TTCTAAA-3′, demonstrating the single-copy nature of the *glyU* gene.

The presence of *glyT*(SufS519) in two of the *glyU* (SuUAG) transductants that were Tet^s (1A and 3B) was tested by introducing wild-type *glyT* from strain FTP2783 by selecting for the closely linked transposon Tn*10* in *btuB* and monitoring for the consequent lack of tryptophan prototrophy in a high proportion of the transductants: 82% were Trp$^-$ (Table 2), confirming the dependence on *glyT*(SufS519). Results similar to those obtained with *glyU*(SuUAG), described above, were obtained when wild-type *glyU* was replaced with missense suppressor *glyU*(SuUGG).

In our second approach, we wished to rule out the presence (even though we did not expect it) in one of the three *glyV* genes of a cryptic alteration that might allow the *glyV* product to cause doublet translocation in the presence of a mutant *glyT* tRNA that is deficient in GGA reading. The potential cryptic mutation could be a totally unprecedented type, or it could be a *glyV*(Ins) mutation. The latter type, also designated *glyV55* (8), enables the mutant $tRNA_3^{Gly}$ to decode GGA and GGG instead of GGU and GGC (3, 13). There is no reason to suspect that MC194 or the derivative strains constructed here contain such an allele. *glyT* (SufS519) and the *Salmonella glyT*(SufS) alleles previously characterized were of spontaneous origin and arose with the frequency expected of a single mutation in a strain without prior selection for alleles such as *glyV55*. Furthermore, such a mutant $tRNA_3^{Gly}$ was shown not to be present in the *Salmonella* SufS strains previously examined (11). Nevertheless, we replaced the *glyV* region of MC194 in two steps. With a P1 lysate made on FTP4808, we transduced MC194 to Tet^r and screened for cotransduction of *purA* with the transposon. The gene order in this region is *purA* . . . *glyV* . . . Tn*10* (11a). This step sufficed to replace the *glyV* region. But to introduce two different *glyV* regions in parallel and be able to cross out the transposon, we proceeded to the second step. With phage lysates on FTP2783 and FTP5355, we transduced one of the Tet^r *purA* derivatives of MC194 to Pur$^+$ and screened for Tet^s, that is, tetracycline sensitivity resulting from loss of the transposon. The selected recombinational events involved the entire *purA* to Tn*10* region. Consequently, in the two kinds of resulting transductants we had introduced either a wild-type *glyV* region (FTP2783) or a *glyV*(Ins)-containing region (FTP5355). We then tested both types of transductant for retention of the ability to suppress *trpE91*. All such transductants, of either type, were still Trp$^+$.

In our third approach, we wished to confirm that *glyW* was wild type in strains 1A and 3B to rule out the possibility that it contained a mutation that would allow the product tRNA to promote doublet translocation at GGA. Primers beginning 63 bases upstream and 31 bases downstream, respectively, of *glyW* were used in asymmetric PCR amplification of the single gene for *glyW* in each of these strains. The PCR products were resolved on a 1.5% low-melting-point agarose gel (Seaplaque GTG; FMC) and further purified through Spin-X filter units (Costar) before sequencing with reverse transcriptase (Life Sciences). The sequence showed that the *glyW* gene of both strains was wild type (data not shown).

In our fourth approach, we sought to replace *glyT* (SufS519) with a mutant *glyT* allele that is much more deficient in GGA reading. We reasoned that if the hypothesized GGA-reading deficiency of *glyT*(SufS519)-encoded tRNA allows other tRNAs (glycine accepting or otherwise) to shift frame, then replacing it with a *glyT* allele that is more deficient should enhance the frameshifting or, at least, not cancel it. Such an allele was available, namely, *glyT* (SuUGA). This *glyT* mutant forms colonies only in the presence of *glyV55* (9). Consequently, we transduced FTP5362 to Tet^r by using a P1 lysate on *glyT*(SuUGA)-containing strain FTP5355. The *btu* transposon is highly linked to *glyT*(SuUGA). High-frequency cotransduction of *glyT*(SuUGA) with the transposon was verified by spot testing the Tet^r transductants with phage T4 UGA mutants as described above. Single-colony isolates of such *glyT* (SuUGA) transductants were then examined for suppression of *trpE91*. In all of the cases examined, the transductants were Trp$^-$.

The experiments reported here demonstrate that the doublet translocation at GGA in *glyT*(SufS) strains is caused not by $tRNA_1^{Gly}$, wild $tRNA_3^{Gly}$, *glyV*(Ins) tRNA, or some unidentified agent but rather directly by the mutant $tRNA_2^{Gly}$.

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