Suppression of a double missense mutation by a mutant tRNA^{Phe} in *Escherichia coli*

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ABSTRACT

We report here the isolation of a mutant tRNA^{Phe} that suppresses a double missense auxotrophic mutation in *trpA* of *Escherichia coli*, *trpA218*. The doubly mutant protein product differs from wild-type TrpA by the replacements of Phe22 by Leu and Gly211 by Ser. A partial revertant TrpA phenotype can be obtained from *trpA218* by changing either Leu22 back to Phe or Ser211 back to Gly. Translational suppressors were previously obtained that act at codon 211, replacing the Ser211 in the TrpA218 protein, presumably with Gly. In the present study, we selected for *trpA218* suppressors caused by mutation of a cloned tRNA^{Phe} gene, *pheV*. DNA sequence analysis of the suppressor isolated reveals a singular structural alteration, changing the anticodon from 5'-GAA-3' to 5'-GAG-3'. Sequencing of *trpA218* confirmed the likely identity of Leu22 as CUC. The new missense suppressor, designated *pheV*(SuCUC), is lethal to the cell when highly expressed, as from a high copy number plasmid. This may be due to efficient replacement of Leu by Phe at CUC (and, probably, CUU) codons throughout the genome. We anticipate that *pheV*(SuCUC) will prove, like other missense suppressors, to be extremely useful in studies on the specificity and accuracy of decoding.

INTRODUCTION

In *Escherichia coli* the double mutant designated *trpA218* affects positions 22 and 211 of the *trpA* gene product, the α subunit of tryptophan synthetase (1). This double mutant was shown to be auxotrophic for tryptophan, in spite of the fact that neither mutation by itself results in auxotrophy. The wild type amino acid residues Phe22 and Ser211 are replaced by Leu22 and Ser211 in the protein product of *trpA218*, which can be represented as TrpA(Leu22-Ser211). It was previously demonstrated (1) that a partial revertant TrpA phenotype can be obtained from *trpA218* by changing either Leu22 back to Phe,

resulting in TrpA(Phe22-Ser211), or Ser211 back to Gly, resulting in TrpA(Leu22-Gly211). Furthermore, translational suppressors were obtained that act at codon 211, replacing the Ser211 in the TrpA protein, presumably with Gly (2). However, no suppressors acting at codon 22 were isolated in these studies. The identity of the Leu codon at position 22 in *trpA218* was unknown, but was presumably related to the wild type Phe codon UUC by a single base change, and could therefore be UUA, UUG or CUC. The availability of a cloned gene, *pheV*, coding for tRNA^{Phe} (3) presented the opportunity to search for suppressors of *trpA218* inserting Phe in response to the Leu codon at position 22. Here we describe the isolation and characterisation of such a suppressor, *pheV*(SuCUC), and identification of codon 22 in *trpA218*.

MATERIALS AND METHODS

Strains and standard techniques

The *E. coli* strains and plasmids used in this work are listed in Table 1. Bacteriological methods were as described by Miller (5). When maximum efficiency was important, transformation was performed as described by Hanahan (6). General cloning methods followed those described by Sambrook *et al.* (7)

Media

Media were as described by Miller (5), except for the minimal medium described by Vogel and Bonner (8). When required, tetracycline was added at a concentration of 12.5 μ g/ml in *pcnB*⁺ strains and 5 μ g/ml in *pcnB* strains. Supplements were added at the following concentrations: indole, 10 μ g/ml; Arg, 50 μ g/ml; Trp, 20 μ g/ml; low Trp, 40 ng/ml.

Mutagenesis and selection of mutants

Mutagenesis of pPP15 was performed by multiple generations of the plasmid in strain ES1578, carrying a *mutD5* mutation, grown in glucose minimal A medium. Extracted plasmid was used to transform about 10¹⁰ cells from a *trpA218* strain,

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FTP2443, made competent as described by Hanahan (6). After heat shock, cells were grown for 2 hr in LB broth, before spreading on minimal glucose medium A plus tetracycline, Arg and low Trp (5 plates). Low Trp accelerates the appearance of suppressed mutants, without allowing more than microscopic growth of Trp⁻ colonies.

PCR amplification of plasmid and *trpA218* sequences

Plasmid preparations from PcnB⁻ cells yielded too little material for sequencing. The insert between the *Pst*I and *Pvu*I sites was therefore amplified using two synthetic oligonucleotides, complementary to plasmid sequences, 5'-TGCCATAACCATG-AGTGATAA-3' and 5'-GCCGGAAGCTAGAGTAAG-3', near the *Pvu*I and *Pst*I sites respectively. A symmetric amplification was performed, followed by asymmetric amplification and sequencing essentially as described by Gyllenstein & Erlich (9), except that the Centricon micro-concentrator step was repeated twice, and dITP replaced dGTP. An internal oligonucleotide, 5'-GACGAGGCGAATCAGG-3' was used for sequencing. Almost all of the *trpA* gene was sequenced by PCR amplification, using two oligonucleotides outside but close to the *trpA* coding sequence, 5'-ACTAGTGG-ATCCAAAGAGCAGCTACTGGTG-3' and 5'-GATATC GAATTCGGGTAAGCGAAACGGTAA-3'. Amplification was performed in two steps to yield single-stranded DNA. Internal oligonucleotides, 5'-GATACCTAACTCCAGCG-3' and 5'-AATGCTGGCACTGATTTCG-3', were used for sequencing in addition to those employed for amplification.

Test for missense and frameshift suppression

Strains IB226 and IB252, carrying different *trpA* alleles, were transformed with mutant *pheV*-carrying plasmid; transformants were picked and patched on minimum glucose plates including indole and a double series of replica-plates prepared as described by Tucker *et al.* (10). Strains IB256 and IB257 were constructed for use as positive controls in the tests for frameshift suppression (10).

RESULTS

Mutagenesis of plasmid-carried *pheV* *in vivo*; selection for missense suppressors

Mutant tRNAs able to insert Phe in response to the Leu codon at position 22 in *trpA218* would be expected to be able to suppress the Trp auxotrophy of this *trpA* mutant. In order to select for such missense suppressors derived from tRNA^{Phe}, a plasmid pPP15 carrying the *pheV* gene was mutagenised *in vivo* by replication over many generations in strain ES1578, which carries a *mutD5* mutation (Table 1). This mutator has been reported to permit transitions, transversions, frameshift mutations and deletions at an overall frequency several thousand times higher than normal (11). Mutagenised plasmid was then used to transform a *trpA218* strain, FTP2443, and Trp⁺ transformants were selected on plates containing tetracycline, Arg and a low concentration of Trp. Among about 3 × 10⁵ plated transformants, eight Trp⁺ revertants were recovered. Plasmid-carried suppressors were distinguished from *trpA* revertants and chromosomal suppressors by preparing plasmid from the Trp⁺ revertants, re-transforming FTP2443 and observing the frequency of Trp⁺ transformants. Only one of the initial Trp⁺ revertants yielded plasmid that led to Trp⁺ transformants on re-transformation. Furthermore, only about 0.3% of the second

Table 1. Bacterial strains and plasmids used in this work.

Strain	Genotype	Source
ES1578	<i>thr1 leuB6 mutD5 hisG4 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 ml-1 rpsL31 tsx-33 λ⁻ supE44</i>	B. Bachmann
FTP2443	<i>trpA218 argE(Am) glyV55</i>	Murgola & Yanofsky (1)
IB220	<i>trpA218 pcnB glyV55</i>	this work
IB226	<i>trpA46^{Asp}PR3 pcnB glyV55</i>	this work
IB252	<i>trpA8 pcnB glyV55</i>	this work
IB256	<i>trpA8 glyT(SuAAA/G)</i>	this work
IB257	<i>trpA46^{Asp}PR3 glyT(SuAAA/G)</i>	this work
MDA6371	<i>glyV55 Δ(tonB-trpAB17) pcnB Tn10 (10% linked to pcnB)</i>	Lopilato <i>et al.</i> (4)
Plasmids	Scorable markers	Source
pPP15	Tet ^R derivative of pBR322 carrying <i>pheV</i>	Caillet <i>et al.</i> (3)
pPP15(Su218)	point mutant of pPP15	this work

round transformants were Trp⁺. This behaviour was repeated in subsequent rounds of plasmid preparation and re-transformation. Transformants resulting from plasmid from Trp⁻ colonies remained Trp⁻. It thus appeared that plasmid prepared from suppressed *trpA218* transformants was always a mixture of suppressor-carrying and non-suppressor-carrying species.

Purification of suppressor-carrying plasmid in a *pcnB* strain

A likely explanation for the difficulty in obtaining homogeneous suppressor-carrying plasmid lay in the possibility that expression of a missense suppressor from a pBR322 derived plasmid was lethal or at least highly detrimental to cell growth. Propagation of the mutant plasmid might, however, be possible as a mixed population containing wild type plasmid as well as suppressor carrying plasmid. One approach to this problem was to introduce a *pcnB* mutation into strain FTP2443. This mutation results in a large reduction (about 15-fold) in the copy number of pBR322-like plasmids (4). It was thereby possible to prepare plasmid that resulted in 100% Trp⁺ transformants in subsequent rounds of transformation of a *trpA218*, *pcnB* strain (IB220), supporting the explanation offered above.

Sequence characterisation of the *pheV*-derived missense suppressor

In view of the limited amount of suppressor-carrying plasmid that could be prepared from *pcnB* cells, a sequencing strategy was adopted that involved *in vitro* amplification of the plasmid insert by PCR, including asymmetric transcription to generate single-stranded DNA for dideoxy sequencing. Agarose gel electrophoresis indicated that the amplified fragment containing the *pheV* gene, somewhat larger than the insert between the plasmid *Pst*I and *Pvu*I sites, remained similar to that of the parent plasmid pPP15. Sequencing indicated a single base change in the *pheV* sequence, at the third position of the anticodon, corresponding to the change A36 to G36. The new anticodon, 5'-GAG-3', corresponds to a tRNA that might be expected to decode the Leu codons CUU and CUC.

Sequence analysis of codon 22 in *trpA218*

Since the wild type codon in position 22 is UUC, we deduced the likelihood that codon 22 in *trpA218* is CUC, which could probably be read by the new anticodon and is related to the wild

type codon by a single base change. This hypothesis was confirmed by asymmetric PCR amplification of the appropriate chromosomal *trpA218* and dideoxy sequence analysis. The codon at 211 was the Ser codon AGC, as previously deduced from mutational studies with 211 auxotrophic mutants. No other departures from the wild type *trpA* sequence were detected.

Search for frameshift suppression by *pheV*(SuCUC)

It has been shown recently that many missense suppressors in *E. coli* are also capable of correcting certain frameshift mutations, suggesting that subtle conformational features of the tRNA molecule are involved in the maintenance of translational accuracy (10). Frameshift suppressor activity was therefore looked for with two *trpA* frameshift mutants, *trpA8* and *trpA46^{ASP}PR3*, employing the same approach as before. In neither case was any significant suppressor activity detected in *pheV*(SuCUC).

DISCUSSION

The first characterised missense suppressors were isolated as revertants of *trpA* mutants and were derived from glycine-accepting tRNAs (12, 13 for reviews). The relative ease with which missense suppressors can be obtained from the glycine family of tRNAs has led to the suggestion that the poor aminoacylation that characterises many mutants in these tRNAs keeps mistranslation to an acceptably low level. All missense suppressors obtained from glycine tRNAs show alterations in the anticodon loop, now believed to be an important site of tRNA:aminoacyl-tRNA synthetase interaction for many tRNA species. However, outside the glycine isoacceptors, three examples exist of tRNA mutants that correct missense mutations. Two of these are mutants of *serU*, enabling this tRNA to read UGG and UUG respectively (14–17). The remaining example, *lysT*(SuAAA/G) does not alter the codon specificity of the tRNA, (although it does introduce translocation errors) but arises from the partial misacylation of the lysine tRNA by alanine as a result of a base change in the amino acid acceptor stem of the molecule (18, 19). The new base pair, G3·U70, appears to be an important element in the identity of alanine-isoaccepting species of tRNA (20, 21).

In a previous study, selection for Trp⁺ revertants of *trpA218* resulted in the isolation of eight translational suppressors (2), all of which acted at codon 211 in the *trpA218* gene, since they were unable to suppress a 'substitute *trpA218*' in which Ser211 was changed from AGU to UCG. Thus, no suppressors were isolated by selection for Trp⁺ revertants that acted at codon 22 of *trpA218*. There is no obvious reason why a chromosomal *pheV*(SuCUC) suppressor should not have been found in these studies, except that the frequency of occurrence may be very low compared to that of suppressors acting at site 211. Two genes code for tRNA^{Phe} in *E. coli* (22), and the loss of either one has no apparent effect on growth in rich or minimal media (23). We have no information concerning the amount or level of charging of the suppressor tRNA in *pcnB* cells when expressed from the mutant plasmid. If the anticodon of *E. coli* tRNA^{Phe} is an important identity element, as in many tRNAs, the change A36 to G36 might lead to a considerable decrease in chargeability with Phe, explaining why the presence of the suppressor, at least in a *pcnB* background, is not highly deleterious to cell growth.

Other translational suppressors have been, and continue to be, valuable tools in elucidating the intricacies of codon recognition.

This new missense suppressor should extend the range of problems amenable to this approach.

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