

Construction of a composite tRNA gene by anticodon loop transplant

(recombinant DNA/nucleic acid function/amber suppressor)

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ABSTRACT By using sites for the restriction nuclease *Hpa* II, the information for the anticodon stem and loop of an altered Su^{+2} amber suppressor tRNA (a mutant of $tRNA^{Gln}$) has been transplanted to a specially prepared $tRNA^{Trp}$ gene, which lacks its homologous anticodon stem and loop sequence. The resulting tRNA gene was cloned under *lac* operator-promoter control. The result is a functional, hybrid, amber-suppressor tRNA that can exhibit a moderately high efficiency in translation. It appears less efficient, however, than Su^{+7} tRNA, the amber suppressor that results from a direct anticodon mutation in $tRNA^{Trp}$. As judged by its suppressor spectrum, which is almost identical to the spectra of Su^{+2} and Su^{+7} , the recomposed tRNA inserts glutamine at amber sites. This experiment is the prototype of a series of constructions that examine the role of the nucleotides in the anticodon region.

The anticodon region of tRNA must be an optimal instrument of translation. Nevertheless, we currently know little of how tRNA collaborates in the surprisingly accurate and efficient ribosomal microcycle of protein synthesis. Accordingly, we introduce a method that allows synthesis of tRNAs for use in structure-function studies of the anticodon loop.

Fig. 1 illustrates our technique. A number of tRNAs, notably $tRNA^{Trp}$ (*Escherichia coli*) and $tRNA^{Gln}$ (*E. coli*), have the sequence C-C-G-G at the uppermost four base pairs of the anticodon stem (refs. 3 and 4; Fig. 1A). This implies that the anticodon region in the tRNA gene will be bounded by *Hpa* II restriction endonuclease sites (C¹C-G-G) (Fig. 1B). These anticodon stems and loops therefore may be interchanged or replaced by recombinant DNA techniques, and novel tRNA genes created.

In these experiments, we avoided synthesis of a potential missense translator, which might have been difficult to detect because of deleterious effects on the cell. We have used the normal $tRNA^{Trp}$ and the amber-suppressor anticodon of $tRNA^{Gln}$ (Su^{+2} ; refs. 1, 5, 6). Furthermore, we have controlled expression of the newly constructed tRNA gene by using the *lac* operator. In fact $tRNA^{Trp}$ mutated to an amber translator is already known (Su^{+7} ; ref. 7), and it inserts glutamine (8). It was therefore probable that an Su^{+2} $tRNA^{Trp}$ hybrid tRNA would be a glutamine-inserting amber suppressor, which would confer a phenotype we can easily recognize.

MATERIALS AND METHODS

Bacteria, Phage, and Plasmids. The usual host for tRNA gene-containing recombinants was *E. coli* LS286: W3110 $trpR^{-} trpA9605_{am} his-29_{am} ilv-1 recA56/F' i^Q$. When selection for the normal $tRNA^{Trp}$ was required, LS874 was used: W3110 $trpR^{-} trpA9605_{am} his-29_{am} metE^{-}$. These and the λ amber mutants in Table 2 were from the collection of Larry Soll. The *lac* operator-promoter plasmid pOP203-3, which has a 203-

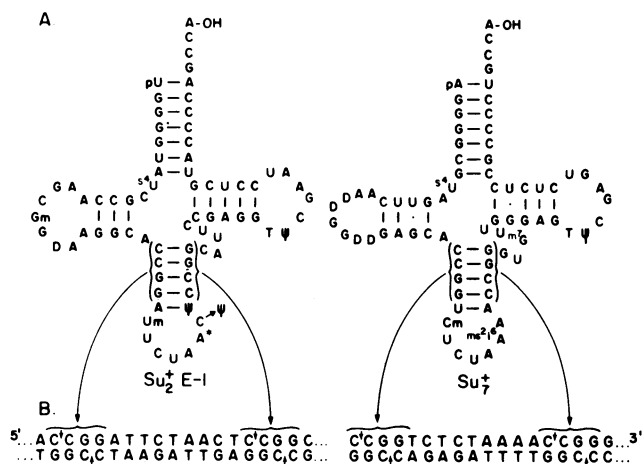


FIG. 1. (A) Nucleotide sequences of Su^{+2} E-1 tRNA and Su^{+7} tRNA (Su^{+} , suppressor). An arrow points to the nucleotide in the original Su^{+2} sequence (1). Braces indicate the areas in the anticodon stem that, when unfolded, imply the sequences shown in B. (B) An anticodon segment in the genes for the above tRNAs; the segment is bounded by *Hpa* II sites (C¹C-G-G). A* in the Su^{+2} anticodon loop indicates an uncertain modification of A; the irradiated cells used in sequencing do not modify tRNA fully (see, e.g., ref. 1). However, Carbon and Fleck (2) found that conversion of the 3' anticodon nucleotide to A causes modification of the adjacent A to $ms^{216}A$, just as in $tRNA^{Trp}$.

base-pair *Hae* III fragment of $\lambda dlac$ UV5 inserted at the *Eco*RI site of pMB9, was constructed and donated by Forest Fuller.

Enzymes. Polynucleotide kinase, phage T4 DNA ligase, *Hpa* II (*Msp* I), and *Taq* I were from New England BioLabs, and bacterial alkaline phosphatase was from Bethesda Research Laboratories (Rockville, MD).

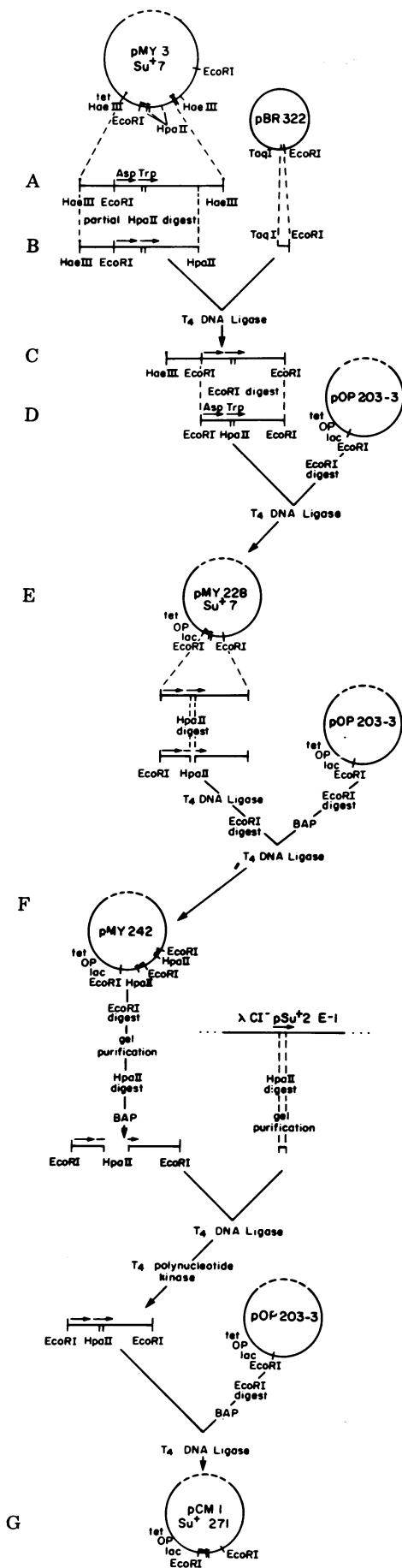
Restriction digests have been described (9, 10). Phosphatase treatment was in 50 μ l of 50 mM Tris-HCl, pH 8, with 10 units alkaline phosphatase for 60 min at 60°C. Ligations (15°C) and phosphorylation (37°C) were performed in the reaction mixture of Sugino *et al.* (11), using 0.01–0.1 μ M complementary ends for ligation at *Eco*RI sites, and 0.5–1 μ M ends for ligation at *Hpa* II sites. Reactions were terminated by addition of phenol equilibrated with 10 mM Tris-HCl, pH 8/1 mM EDTA and mixing at 60°C.

Tryptophan synthase A and B activities were measured by the conversion of indole to tryptophan, as described by Smith and Yanofsky (12). Bacterial extracts were made by sonic disruption of cells grown at 37°C in M9 glucose minimal medium plus histidine, tryptophan, isoleucine, and valine at 50 μ g/ml each. Isopropyl β -D-thiogalactoside (IPTG), when present, was added to 1 mM.

Procedures. Transformations by the $CaCl_2$ method were as modified by Wensink *et al.* (13), and DNA nucleotide se-

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Abbreviations: Su^{+} , suppressor; IPTG, isopropyl β -D-thiogalactoside.



quences were determined by a modification of the methods of Maxam and Gilbert (ref. 14 and modified protocol received from Allan Maxam). Analytical and preparative gel electrophoresis in a Tris/acetate buffer have been described (10).

A Special Substrate for Transplantation. A specialized vehicle was created to facilitate the transplant. This sequence of operations is shown in Fig. 2, which also shows the origin of the tRNA genes. Plasmid pMY3 (15) contains an insert of 1.8 kilobases, including the tRNA^{Asp} and Su⁺7 tRNA genes. These tRNA genes are the distal part of the *rrnC* ribosomal transcription unit, near 83 min in the *E. coli* chromosome (9).

The topmost set of operations in Fig. 2 extracts the tRNA^{Asp} and tRNA genes from pMY3 and incorporates them into a small fragment bounded by *EcoRI* sites.

First, a suitable *Hae* III digest product was purified by electrophoresis on acrylamide (Fig. 2A; a detailed restriction map may be found in ref. 10). It includes the tRNA genes, and four *Hpa* II sites. One, at the extreme left, is not shown in Fig. 2. A partial *Hpa* II digest reveals a *Hpa* II site that must be removed in order to make transplantation convenient (Fig. 2B).

Taq I (T¹C-G-A) generates the same sticky end as *Hpa* II. Therefore when we joined a short linker [25 base pairs, from pBR322 (16)] terminated by *Taq* I and *EcoRI* sites to the *Hpa* II partial digest, the only possible result involving the tRNA fragment is that shown in Fig. 2C. *EcoRI* now releases the protected left-hand *EcoRI* site, as well as the right-hand one, if linkers have polymerized onto it. The resulting fragment (Fig. 2D) contains little more than the two tRNA genes, followed by the *rrnC* transcription terminator (see below), and has *Hpa* II sites *only* at the tRNA^{Trp} anticodon. This fragment is cloned in pOP203-3, a pMB9 derivative with one *EcoRI* site adjacent to the *lac* operator and UV5-*lac* promoter (17). In order to control the large number of *lac* promoters, which result from the high copy number of this plasmid, an *i*^Q gene in the host cell is used to overproduce the *lac* repressor (18). The result to this point is a plasmid, pMY228, that expresses the tRNA^{Asp} gene and Su⁺7 amber suppressor under IPTG control (Fig. 2E). Ref. 19 presents some expression data on a similar plasmid.

The Su⁺7 gene is convenient during constructions because we can apply a potent selection for plasmids containing it. We select tetracycline resistance from pOP203-3 and simultaneous suppression of two amber mutations in essential genes of the host by the tRNA insert. However, construction of new Su⁺ tRNAs cannot begin with Su⁺7; rare Su⁺ recombinants may be confused with Su⁺ survivors of the original gene. Therefore, we made a series of plasmids containing oligomers of the tRNA^{Trp} gene fragment, without an anticodon.

After *Hpa* II digestion of the Su⁺7-containing, 264-base-pair *EcoRI* fragment, the two halves of the tRNA^{Trp} gene were purified on an acrylamide gel. These were ligated, cut with *EcoRI*, and then further ligated with *EcoRI*-linearized, dephosphorylated pOP203-3. The phosphatase-treated pOP203 gives little infectivity unless it circularizes by including an insert. The ultimate result was pMY242 (Fig. 2F), which has two *EcoRI* fragments, head to tail, in backward orientation to the *lac* promoter. The DNA sequence of these fragments (14) is that of tRNA^{Trp} genes that lack anticodon regions (Fig. 3). *EcoRI* digestion of this plasmid, followed by *Hpa* II digestion of the small gel-purified *EcoRI* fragments, is used to generate the flanking sequences for interjected anticodon DNA.

FIG. 2. Origin, structure, and construction of the transplant materials. Small arrows mark tRNA genes; see text for discussion. BAP, bacterial alkaline phosphatase.

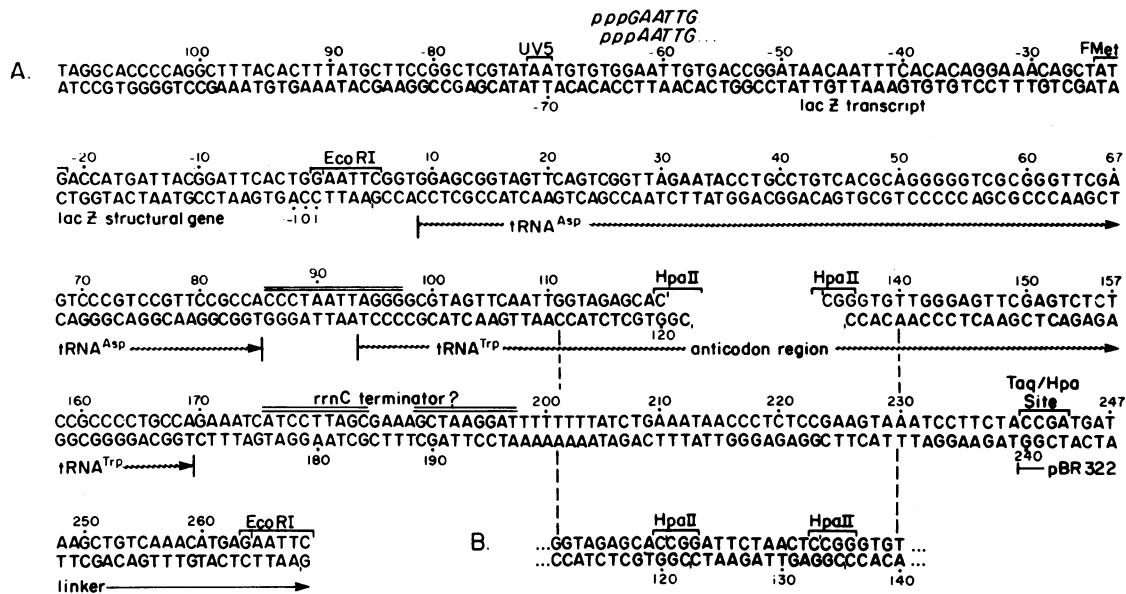


FIG. 3. (A) Nucleotide sequence of the proximal *lac* OP region and of the small *Eco*RI fragment from pMY242. The sequence of this latter fragment has been determined by labeling at the *Eco*RI sites with polynucleotide kinase, separating the strands, and sequencing both of them (14). Double overlines indicate symmetries referred to in the text. A gap in the sequence (at positions 121–135) is intercalated at the position of the anticodon fragment, even though the sequence of the pMY242 RI fragment (Fig. 2) is continuous here. The numbering of the sequence is therefore the same as that of a complete tRNA gene. (B) The altered anticodon region sequence in the *Eco*RI fragment from pCM1 Su⁺271.

RESULTS

Testing the Method by Reconstruction of a Known Gene.

We have shown that our techniques construct a functional wild-type (Su⁻⁷) tRNA^{Trp} gene. This gene can be selected decisively in a host strain that contains the *trpT*_{ts90} locus (20, 21), which codes for a mutant tRNA^{Trp} that is abnormally temperature sensitive and is destroyed *in vivo* at 42°C (22). Thus, this host cannot survive at high temperature unless tRNA^{Trp} is supplied by the recombinant gene.

The 13-nucleotide-long *Hpa* II fragment containing the Su⁻⁷ anticodon was isolated by gel electrophoresis on 20% acrylamide, after complete digestion of pMY352 Su⁻⁷ (19). Purified halves of the tRNA^{Trp} gene, without anticodon, were combined with the Su⁻⁷ anticodon fragment and *Eco*RI-cut pOP203-3. After ligation and transformation, controls containing Su⁺, or no anticodons at all, gave no growth at 42°C, but 1/600 tetracycline-resistant transformants grew at 42°C when the Su⁻⁷ anticodon had been supplied. A monomer plasmid insert with the tRNA genes oriented productively to the *lac* promoter was purified (called pMY231, it is homologous to pMY228 in Fig. 2). Its DNA sequence (not shown) contains a normal tRNA^{Trp} gene.

A New Amber-Suppressor Gene. The Su⁺² anticodon fragment was purified, as described above, from a transducing phage, λ CI-pSu⁺² E-1 (D. Bradley and L. Soll, unpublished). The nucleotide sequence of Su⁺² E-1 tRNA is shown in Fig. 1. It is in agreement with the sequence of Su⁺² tRNA determined by Inokuchi *et al.* (1), except that the anticodon loop contains a second mutation, besides the amber change in the anticodon itself. The second alteration (Fig. 1) is the result of selection for more efficient amber suppression under constraint by *rpsL* (*strA*) ribosomes (23).

Anticodon-free tRNA^{Trp} gene fragments from pMY242 (Fig. 2F) were digested with *Hpa* II and dephosphorylated. After phenol treatment, the tRNA^{Trp} gene fragments were combined with the Su⁺² E-1 anticodon and ligated, treated with phenol again, then rephosphorylated with polynucleotide kinase. This sequence of operations was intended to produce a high yield of complete tRNA genes. In fact, ligated products are pre-

dominantly the three combinations of two rejoined tRNA gene fragments with one anticodon sandwiched between (data not shown). The rephosphorylated tRNA genes were combined with pOP203-3 that had been treated with *Eco*RI, dephosphorylated, and ligated again. On transformation of our *his*_{am} *trp*_{am}/F'ⁱQ strain and selection in the presence of mM IPTG, 1/4700 tetracycline-resistant transformants was also histidine- and tryptophan-independent and therefore contained an amber suppressor. The resulting plasmid (Fig. 2G) is homologous to pMY228 (Fig. 2E); it has a single tRNA gene insert, productively oriented with respect to *lac* OP.

Table 1. Transmission of the nascent chain in strains containing an amber suppressor

Strain	IPTG during growth	Tryptophan	
		A activity/B activity	% efficiency
<i>trp</i> ⁺		1.30 (3)	100
<i>trpA9605</i> , Su ⁺⁷	–	0.096 (3)	7.4 ± 1.9
<i>trpA9605</i> , Su ⁺⁷	+	1.23 (2)	94 + 6, –10
<i>trpA9605</i> , Su ⁺²⁷¹	–	0.009 (3)	0.7 ± 0.05
<i>trpA9605</i> , Su ⁺²⁷¹	+	0.59 (3)	45 ± 5.3

Su⁺⁷ implies pMY228 resident in LS286/F'ⁱQ; Su⁺²⁷¹ implies pCM1 in the same strain. This bacterium is constitutive for *trp* expression (*trpR*⁻). It contains an amber mutation (*trpA9605*) in the most operator-distal *trp* cistron. The *trpB* cistron, just operator proximal to *trpA*, is intact, and the two are usually expressed coordinately and equally from the same message (24). Therefore, a comparison of the number of ribosomes that translate *trpB* (giving the B protein) with those that continue through *trpA* (giving the A protein) determines the transmission efficiency. Measurement of the *trpB* (or *trpA*) product is performed enzymatically in extracts of log-phase cells by fully complementing it with exogenous *trpA* (or *trpB*) product (12) to generate tryptophan synthase. Various altered proteins all bind to the B protein and activate it (for synthesis of tryptophan from indole) to the same extent (25). Therefore, the ratio of enzymatic activities, A/B, in a suppressor strain, normalized to the same ratio from wild type, is a plausible estimator of the absolute transmission efficiency. Numbers in parentheses are the number of independent measurements averaged to get the number displayed. The range supplied for the efficiency is the SEM.

Fig. 3A shows the sequence of the anticodonless tRNA^{Trp} gene fragment, as derived by chemical sequencing methods (14). Fig. 3B shows the altered anticodon sequence of the Su⁺ tRNA gene whose synthesis is described above. The new tRNA^{Trp} gene sequence is unaltered, except that an anticodon sequence which corresponds to that expected from Su⁺² E-1 has been inserted at the *Hpa* II site of the tRNA gene (Figs. 1 and 3B). This plasmid will be called pCM1, and the tRNA gene Su⁺²⁷¹. The latter nomenclature suggests the tRNA's ancestry.

Efficiency of the Hybrid tRNA Gene. The new tRNA gene was selected for suppressor function, and so must be aminoacylated and competent in chain extension at the ribosome. To get a quantitative measure of its function, we determined the fraction of transits of an amber codon that yields insertion of an amino acid, rather than termination (the efficiency). Table 1 presents these data for cells with pCM1 Su⁺²⁷¹ and pMY228 Su⁺⁷ grown in the presence or absence of IPTG.

Enhanced transcription of both tRNA genes dramatically raises the fraction of completed amber transits. A 66-fold increase in Su⁺²⁷¹ activity and a 13-fold increase for Su⁺⁷ (to 94% transmission) occur after IPTG induction (Table 1). The difference between these numbers presumably reflects a monotonic asymptotic approach to 100% transmission. That is, one cannot have more than 100% transmission, and Su⁺⁷ is more efficient to begin with.

Such increases are to be expected, because transmission and release are in balance: increases in Su⁺ tRNA can increase transmission (26) and decreases in release factor can have the same effect (27).

However, these data show that, in detailed comparisons, transmission must be related to quantity of mature tRNA. The two tRNA genes for Su⁺²⁷¹ and Su⁺⁷ are under the same promoter, in the same genetic context, and differ in sequence at only four nucleotides (Fig. 1). Therefore, making the plausible assumption that they are matured in similar amounts, the 10-fold (uninduced) superiority of Su⁺⁷ means that this tRNA is the more efficient suppressor. We hope to learn what process has been affected by the differences in Su⁺⁷ and Su⁺²⁷¹. It is apparent, however, that an anticodon loop sequence, selected for function on one tRNA, can function acceptably when transposed to another.

Table 2. Action of various suppressors on λ amber mutations

Phage	Su ⁺⁷				Su ⁺²⁷¹			
	Su ⁻⁷	Su ⁺¹	Su ⁺²	Su ⁺³	No IPTG	Plus IPTG	No IPTG	Plus IPTG
λ susA ₃₂	0	±	+	+	0	+	0	+
λ susB ₁₀	0	+	+	+	+	+	±	+
λ susE ₁₃	0	±	+	0	0	+	0	+
λ susJ ₂₇	0	0	0	+	0	+	0	0
λ susN ₇	0	0	±	±	0	+	0	0
λ susO ₂₉	0	0	+	+	0	+	0	+
λ susP ₃	0	0	+	0	+	+	0	+
λ susP ₈₀	0	0	+	0	+	+	+	+
λ susQ _{t57}	0	0	0	+	0	0	0	0
λ susR ₆₀	0	±	+	+	+	+	+	+
λ susR ₂₂₁	0	0	+	0	+	+	+	+
λ susS ₇	0	0	0	+	0	0	0	0
λ CI ⁺	+	+	+	+	+	+	+	+

These are spot tests performed on H plates, with late-log-phase cultures of the strains in H-top agar (28). About 10⁵ phage were spotted in 10 μ l and the plate was incubated overnight at 42°C before reading. +, Complete clearing; ±, partial clearing; 0, no clearing. Su⁺¹, Su⁺², and Su⁺³ were transduced into the chromosome of LS270, which is a *recA*⁺ derivative of LS286. Su⁺⁷ is LS286/F⁺i^Q/pMY228 and Su⁺²⁷¹ is LS286/F⁺i^Q/pCM1. Su⁻⁷ is LS286/F⁻i^Q.

The transmission efficiencies in Table 1 are consistent with the growth of the plasmid-carrying *his*_{am} *trp*_{am} strains on a minimal medium, which requires suppressor. Growth of Su⁺²⁷¹ without IPTG is very slow, Su⁺⁷ without IPTG is faster but less rapid than normal, and Su⁺²⁷¹ plus IPTG and Su⁺⁷ plus IPTG are normal, about the same as when the medium is fully supplemented with histidine and tryptophan. Thus, all strains show amber-suppressor phenotype with or without IPTG, though to various degrees.

The Amino Acid Inserted by Su⁺²⁷¹. We expected Su⁺²⁷¹ tRNA to insert glutamine. As a first test of this expectation, Table 2 presents the suppressor spectrum of Su⁺¹, Su⁺², Su⁺³, Su⁺⁷ with or without IPTG, and Su⁺²⁷¹ with or without IPTG. Various λ amber mutants were spotted on a lawn of Su⁺ bacteria. The λ mutants have been selected because only certain amino acids work well at these amber sites. Therefore different suppressors allow the growth of different subsets of phage, and this quality can be used to classify Su⁺ tRNAs.

Inspection of Table 2 confirms that these spot tests are distinctive. For example, Su⁺² (inserts glutamine) differs from Su⁺³ (inserts tyrosine) in 7 entries of 12, Su⁺¹ (inserts serine) differs from Su⁺² in 5 entries.

However, the table also demonstrates that the same suppressor can score differently at different transmission efficiencies; 5 of 12 ambers are not suppressed by Su⁺⁷ in the absence of IPTG but respond when inducer is included in the top agar.

For the purpose of classification, we emphasize that, with efficient promotion, Su⁺², Su⁺⁷, and Su⁺²⁷¹ give similar patterns. There are only two differences between Su⁺² and Su⁺²⁷¹, or Su⁺⁷ and Su⁺²⁷¹. The differences occur for ambers that are shown in the table itself to be responsive to IPTG. They may therefore be attributed to differences in transmission coefficient. Thus, because Su⁺²⁷¹ is similar to Su⁺² and Su⁺⁷, two amber suppressors known to insert glutamine (5, 6, 29), it is probably also a tRNA^{Gln}. This is not necessarily to say that Su⁺²⁷¹ tRNA accepts only glutamine. Consider Su⁺⁷ tRNA, which accepts both tryptophan and glutamine with equal frequency *in vivo* (30) and *in vitro* (30, 31), yet usually inserts glutamine (29, 32). The detailed biochemistry of the new suppressor tRNA must therefore be investigated.

DISCUSSION

We have constructed a functional tRNA gene by combining sequences from two preexisting tRNAs. The anticodon region is that of a mutant amber suppressor derived from Su⁺², a tRNA^{Gln}, and the rest is tRNA^{Trp}. This Su⁺ tRNA gene is carried under *lac* operator control in a cell that overproduces *lac* repressor, though enough suppressor tRNA is produced to confer a weak amber suppressor phenotype even under maximal repression. Control of suppressor tRNA levels is evident, however, because IPTG produces dramatic increases in transmission of the nascent polypeptide at an amber codon (Table 1) and also produces suppression of ambers unsuppressed in the absence of effective transcription from the *lac* promoter (Table 2).

Fig. 3 exhibits the sequence of the proximal part of the *lac* OP region (ref. 17, and references therein) and our fragment in a productive orientation with respect to this promoter. The *Eco*RI site used for cloning has been created at a *Hae* III site that occurs in the ninth amino acid of the β -galactosidase sequence. The 5' end of the tRNA gene transcript thus is that of normal UV5 *lacZ* mRNA (33), beginning at position -62 or -63 (G-A-A-T-T-G . . . or A-A-T-T-G . . .) in Fig. 3. It continues through the normal initiation region for the *lacZ* protein but switches to the tRNA fragment sequence at position 1. Position 8 is the first base of tRNA^{Asp}; therefore the tRNA sequences

presumably begin 71 nucleotides from the 5' end. Of these nucleotides, only the proximal 9 are the same as in the original location of the tRNAs, at the 5' end of *rrnC* in the *E. coli* chromosome (9, 34). Nevertheless some transcripts are matured into functional tRNA molecules. There cannot therefore be an absolute requirement for the large amount of 5'-ward information during processing. But we believe the efficiency of maturation to be small; therefore this information may stimulate maturation, or the attachment of the *lacZ* message terminus (including, e.g., a ribosomal binding site) depresses maturation, or both.

The sequence of the tRNAs agrees in detail with that expected from the published tRNA sequences (1, 3, 4). This includes the two C-C-A-OH 3' tRNA termini, which exist in the DNA sequence. There is an interesting 12-base-pair-long perfect symmetry precisely at the end of the tRNA^{Asp} gene (shown in Fig. 3), which may mark the boundary between tRNA^{Asp} and tRNA^{Trp} during the early history of the transcript.

Young (35) has determined the sequence of a region from *rrnC* that covers our fragment. Our sequences agree perfectly in the region held in common (1-241 in Fig. 3). He has also pointed out the extensive symmetry around our position 185, which is followed by a string of A·T base pairs. This strongly suggests a transcription terminator (36), and indeed, *in vitro* transcription of a restriction fragment does terminate in the string of As near position 200 (35). We therefore surmise that the transcript of our tRNA genes probably also ends here, at the *rrnC* terminator, 30 nucleotides beyond tRNA^{Trp}.

At position 238 are the remains of the *Hpa* II site destroyed when the "linker" fragment of pBR322 was added during construction (Fig. 2). The sequence from this site to the end is in agreement with this section of pBR322 (16).

The finding that Su⁺²⁷¹ inserts glutamine (Table 2) is not surprising, at first sight. Both ancestral tRNAs, in their amber suppressor forms, also insert this amino acid (Table 2). Their recombined product, in particular, possesses the features of the C-C-A stem and central U of the anticodon that appear essential for glutamyl-tRNA synthetase action (31). However, Su^{+2 E-1} is a mutation of Su⁺², which has been selected to overcome the constraint on suppression imposed by *rpsL* (*strA*) ribosomes (ref. 23; D. Bradley and L. Soll, unpublished). The mutation therefore might have conferred a new aminoacylation specificity and thereby made an improved polypeptide. These current data suggest that a Ψ → C change at the 3' side of the anticodon loop (Fig. 1) is consistent with continuing acylation by glutamyl-tRNA synthetase and therefore favor a direct effect on transmission, though they do not exclude an effect on aminoacylation.

The Su⁺⁷ and Su⁺²⁷¹ sequences differ in only four positions at the top of the anticodon loop (Fig. 1). The data of Table 1 on transmission therefore suggest that changes in these nucleotides affect chain propagation on the ribosome. While this impression is subject to modification if glutamyl Su⁺⁷ tRNA and glutamyl Su⁺²⁷¹ tRNA exist *in vivo* at very different levels, it is consistent with the selection of Su^{+2 E-1} from Su⁺², which implicates one of the same nucleotides in ribosomal function. We can continue to construct a series of anticodon progressively differing transplants and hope to parse the anticodon loop and stem nucleotides, evaluating their function *in vivo* and *in vitro*. Biochemical studies may assign the influential nucleotides to roles at the individual steps in the ribosomal cycle of tRNA. More generally, study of such a series may reveal unsuspected tRNA structure-function relationships. Genetic selection of altered molecules requires foreknowledge of the phenotype, and that requirement is relaxed by synthetic approaches.

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