Mischarging mutants of Su\(^+\)2 glutamine tRNA in \textit{E. coli}.

1. Mutations near the anticodon cause mischarging

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ABSTRACT

In order to select the mischarging mutants of Su\(^+\)2 glutamine tRNA, auxotrophic amber mutants of \textit{E. coli} K12 which cannot be suppressed particularly by Su\(^+\)2 were screened. By utilizing these mutants, \textit{cys}_{am235} and \textit{met}_{am3}, several tens of mischarging mutants of Su\(^+\)2 were isolated, as those conferring altered suppression patterns for a set of tester amber mutants of bacteria and phages. Nucleotide sequence analysis revealed that the mutation sites were found to be exclusively at \(\gamma37\) residue located at the 3'-end of anticodon loop, changing it to either A\(_{37}\) or C\(_{37}\). These mutants were obtained as those suppressing \textit{cys}_{am235}, and not \textit{met}_{am3}. From these, secondary mutants were selected. In these mutants suppression patterns were further altered by the additional base substitutions, capable of suppressing \textit{met}_{am3}. Such mutants were obtained exclusively from A\(_{37}\) and not from C\(_{37}\) mutant tRNA. Additional mutations to A\(_{37}\) were found to be either A\(_{29}\) or C\(_{38}\), which are located at the lowermost two base pairs in anticodon stem. The mischarging sites in Su\(^+\)2 glutamine tRNA locate in the newly detected region of tRNA, differing from the previous case of Su\(^+\)3 tyrosine or Su\(^+\)7 tryptophan tRNAs. Implication of this finding is discussed on L-shaped tRNA molecule in relation to aminoacyl-tRNA synthetase recognition. Suppression patterns given by the double-mutants, A\(_{37}A_{29}\) and A\(_{37}C_{38}\), were consistent with the observation that the mutant tRNAs interact with tryptophanyl-tRNA synthetase.

1. INTRODUCTION

All tRNA molecules have a sufficiently similar shape to pass through the ribosomal machinery, yet they can be so distinct for aminoacyl-tRNA synthetase that each enzyme will act only on one type. This specific recognition of a tRNA\((s)\) by its cognate enzyme is primarily required to maintain the fidelity of genetic translation in protein biogenesis. Among the variety of experimental approaches to understand the molecular basis of this selective interaction, genetic trials have provided unique information to elucidate the sites of tRNA crucially involved in the enzyme recognition. Experimentally this was achieved by isolat-

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ing the mutants of a suppressor tRNA, capable of inserting an amino acid(s) different from the original one to the particular codon(s).

The mutants of this type were isolated first by Aono et al. (1969), by using an amber (UAG) suppressor of Su+3 tyrosine tRNA in E. coli, under the following principle. Among amber mutants of bacteria or phages, some of them may be suppressed, for instance, by Su+1 (serine-inserting) or Su+2 (glutamine) but not by Su+3 (tyrosine). Utilizing such amber markers, the mutants of altered amino acid acceptor specificity of Su+3 tRNA may be efficiently selected as those capable of suppressing the markers used. In these “mischarging” mutants, the limited base changes could alter the specificity towards aminoacyl-tRNA synthetase, keeping the decoding function in vivo. Thus, the key sites in a tRNA which may be involved in the synthetase recognition could be pertinently elucidated by identifying the mutant residues in mischarging tRNA. Under this strategy, many mischarging mutants of Su+3 tyrosine tRNA have been isolated and analyzed (Shimura et al., 1972; Hooper et al., 1972; Smith and Celis, 1973; Celis et al., 1973; Ghysen and Celis, 1974; Inokuchi et al., 1974). In the case of Su+3 tyrosine tRNA in E. coli, the mischarging mutations have been found exclusively at the terminal region of amino acid acceptor stem; namely either at the fourth residue from the 3'‐end or at the first or second base pairs in the amino acid acceptor stem. All of these mutations provided a new specificity towards a non-cognate enzyme, glutaminyl-tRNA synthetase, resulting in the misaminoacylation of the tyrosine tRNA with glutamine. These results implied an important role of the terminal part of amino acid acceptor stem of tRNA in the synthetase recognition. This also agreed with the discriminator hypothesis by Crothers et al. (1972). They proposed that the fourth residue from 3'-end of tRNA could be the first discriminator site in the synthetase recognition.

In contrast to tyrosine tRNA, a mutation in tryptophan tRNA to amber suppressor Su+7 in E. coli, having a change of middle base of anticodon C to U, is already sufficient to cause mischarging. This misaminoacylation has been shown to be catalyzed by glutaminyl-tRNA synthetase, and Su+7 tRNA translates the UAG codon as both glutamine and tryptophan in a ratio of 9:1 (Soll, 1974; Yaniv et al., 1974; Celis et al., 1976; Yarus et al., 1977). This suggests a possible involvement of anticodon in the synthetase recognition of tryptophan tRNA. Thus, it has been shown in two suppressor tRNAs of Su+3 and Su+7, that the most separated regions of L-shaped tRNA molecules may be involved in the recognition. Note that the Su+3 tyrosine tRNA also carries a mutation at its anticodon as an amber suppressor, although this mutation alone is not sufficient to cause detectable mischarging and its involvement in mischarging is not clear. In any case, the same glutaminyl-tRNA synthetase is concerned with the mischarging in both cases. This may mean that glutaminyl-tRNA synthetase has rather relaxed specificity to certain tRNAs in addition to glutamine tRNA. Indeed, a mutant of this enzyme which recognizes the several non-cognate tRNAs has been
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reported (Inokuchi et al., 1984).

The above observations raised a simple question whether glutamine tRNA can mutate so that it is mischarged with other amino acid, especially with tyrosine or tryptophan. If it be the case, then which part(s) of the tRNA confers the mischarging? In this paper, we describe the isolation and identification of mischarging mutants of $Su^+$ glutamine tRNA. A preliminary note of this work has been cited in a review by Ozeki et al. (1980).

2. MATERIALS AND METHODS

Bacterial and phage strains

Bacterial strains used were the derivatives of $E. coli$ 1000BT ($F^-$ lacZ$_{am1090}$ trp$_{am}$ str$^-$ su$^-$) which is described in Inokuchi et al. (1979). BT235 and BT3, cys$_{am235}$ and met$_{am3}$ derivatives of 1000BT, respectively, were isolated in this work. Five transducing phages were used, $\lambda$pSu$^+$1 (Yamao et al., 1975), $\lambda$pSu$^+$2 (Inokuchi et al., 1979), $\Phi$80pSu$^+$3 (Andoh and Ozeki, 1968), $\lambda$pSu$^+$β (Kimura et al., 1976) and $\lambda$pSu$^+$7 (Inokuchi et al., unpublished). T4 amber mutants, S116, NG187, Bu33, and C266 were described in Inokuchi et al. (1974), and N133, NG197, NG60, C74 and C216 were obtained from T. Minagawa. $\lambda$ phage amber mutants, A$_{am227}$, P$_{am3}$, P$_{am80}$ and S$_{am7}$ are from laboratory stock.

Media

$\lambda$ broth, M9 minimal medium and low phosphate medium used for $^{32}$P-labeling are described in Inokuchi et al. (1979).

Mutagenesis

Mutagenesis of phages was done in the phage-infected cells with N-methyl-N-nitroso-N-nitrosoguanidine as described by Abelson et al. (1970), or by ultraviolet light irradiation on the phage particles.

Selection of mischarging mutants

BT235 or BT3 was lysogenized with $\lambda$pSu$^+$2 or its derivatives, and Cys$^+$ or Met$^+$ revertants were selected. When mutagenesis was carried out on phage particles, the same cells were infected with the phages at a multiplicity of 10 and plated on the selective plates. All plates were incubated at 30°C for more than 3 days. Resulting colonies were phage-induced in liquid medium with mitomycin C at 1 μg/ml, then followed by re-transduction to BT235 or BT3 on the selective plates. All mutants were purified by single plaque isolation. Lysogenization was carried out by selecting Su$^+$ transductants showing Trp$^+$ phenotype. Suppression of T4 amber mutants was tested by spotting the $10^6$ phages and spreading them with paper strips on a lawn of lysogens with suppressors to be tested. Suppression of $\lambda$ amber markers were tested by the growth of recom-
binant phage carrying both suppressor and the amber marker on \( su^- \) bacteria.

**Nucleotide sequencing**

Preparation and purification of \( ^{32}P \)-labeled glutamine tRNA\(_2\) synthesized after infection of phage strains were carried out according to Inokuchi et al. (1979). Sequence analysis of \( ^{32}P \)-labeled tRNA was according to Sanger et al. (1965), and Brownlee et al. (1968).

### 3. RESULTS

**Isolation of mischarging Su\(^+2\) mutants**

Among the auxotrophic amber mutants isolated from strain 1000BT, two, \( cyg_{am235} \) and \( met_{am3} \), were found to fulfill the minimum requirements to select mischarging mutants from Su\(^+2\) glutamine tRNA. The \( cyg_{am235} \) mutation was covered with F'143, suggesting that it belongs to the \( cyg \) operon at 59 min on the \( E. coli \) genetic map. Since \( met_{am3} \) mutation was covered with F'14 and co-transducible with \( argH1 \) by P1 phage, it is likely to be in one of the clustered \( met \) genes at 87 min. The patterns of suppression to \( cyg_{am235} \) and \( met_{am3} \) by the known amber or ocher suppressors are shown in Table 1. Both mutations are suppressed by Su\(^+3\) (at low temperature, 32°C) and Su\(^+7\), but not by Su\(^+1\), Su\(^+2\), and Su\(^+\beta\), suggesting that these amber mutants are not suppressed by the insertion of glutamine, serine or lysine at the UAG sites. Although Su\(^+7\) tryptophan tRNA translates UAG codons as both tryptophan and glutamine, the effective suppression of \( cyg_{am235} \) and \( met_{am3} \) by this suppressor should be referred to the insertion of tryptophan at the UAG site. Major difference between \( cyg_{am235} \) and \( met_{am3} \) in the suppression pattern is the response to leucine-inserting suppressor Su\(^+6\).

After mutagenesis of \( \lambda \) phages carrying Su\(^+2\) tRNA gene, some sixty mutants which suppressed \( cyg_{am235} \) were isolated, while no mutants suppressing \( met_{am3} \) were obtained. After the sequence analysis of suppressor tRNA from about 30 independent isolates (see below), the two types of mutation, A37 and C37 (shown by the altered base with the residue number from the 5'-end of the tRNA; see Fig. 1), were identified with relative frequencies shown in Table 2. The mutant A37 or C37 in the mutagenized stocks of \( \lambda p Su^-2 \) appeared at the frequencies of \( 10^{-7} \) to \( 10^{-8} \). Spontaneous mutants were not obtained by this selective condition, since true revertants of \( cyg_{am235} \) were found at a rather high frequency of \( 10^{-5} \) to \( 10^{-6} \). The results indicated that the \( cyg_{am235} \) suppressing mutants were the result of a single mutation at a particular site of Su\(^+2\) glutamine tRNA. On the other hand, no mutants that could suppress \( met_{am3} \) were found at least with a frequency of \( 10^{-2} \) lower than that of \( cyg_{am235} \) suppressing mutants. This suggested that \( met_{am3} \) suppressing mutant would not be resulted by a single mutational event. This prompted us to select successive mutants that can suppress
Mischarging mutants of Su^+2 glutamine tRNA. I.

Table 1. Suppression patterns

<table>
<thead>
<tr>
<th>Su^+2 mutant</th>
<th>Su^+1</th>
<th>Su^+2</th>
<th>Su^+3</th>
<th>Su^+β</th>
<th>Su^+6</th>
<th>Su^+7</th>
<th>A37</th>
<th>C37</th>
<th>A37A29</th>
<th>A37C38</th>
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<td></td>
<td>Ser</td>
<td>Gln</td>
<td>Tyr</td>
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<tr>
<td></td>
<td>42°C</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>met^+sam3</td>
<td>32°C</td>
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<tr>
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<td>-</td>
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<td>-</td>
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<td>32°C</td>
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<tr>
<td></td>
<td>42°C</td>
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<tr>
<td></td>
<td>42°C</td>
<td>+</td>
<td>+</td>
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<td>A^+sam227</td>
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<tr>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Suppression of bacterial markers was determined by the growth of mutant bacteria on minimal plates, which were lysogenized with the transducing phage carrying the indicated suppressor. In the case of Su^+6, the suppressor was transduced by P1vir to the indicated bacterial strains. + indicates normal growth; --, no growth. The plates were incubated for 2 days at the indicated temperature. T4 markers were tested by spotting the lysate (10^8 P.F.U./ml) and spreading with paper strip on a lawn of bacteria. λ phage markers were tested by the growth of derivative carrying suppressor and the indicated markers on su^- bacteria. + indicates confluent growth; ±, partial; --, no growth of phage. When S116 (rII) were tested, lysogenized cells with λ C+ were used. The plate were incubated at 32°C overnight.

met^+sam3, starting from these single-mutants, A37 and C37. In the mutagenized stocks of λpSu^+2A37 strain, met^+sam3 suppressing mutants were detected at frequencies of 10^-7, while no such mutants were found in 10^10 mutagenized phage particles of λpSu^+2C37 strain. Spontaneous Met^+ revertants of lysogenic strain of met^+sam3 with λpSu^+2A37 appeared at a frequency of 10^-7, while that with λpSu^+2 or λpSu^+2C37 was less than 10^-8. These results showed that met^+sam3
suppressing mutants appeared exclusively in Su+2A37 via an additional single-base mutation. This was confirmed by the sequence analysis described below.

The double-mutants were classified into two groups as to the efficiency of mischarging suppression to metam3, which corresponded to two types of muta-

Table 2. The relative frequencies of mutant types

<table>
<thead>
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<th>Mutagenesis</th>
<th>Selective marker</th>
<th>frequency</th>
<th>Mutant type</th>
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<td></td>
<td></td>
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<td>A37</td>
</tr>
<tr>
<td>NTG on λ pSu+2</td>
<td>cφf5am295</td>
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<td>1</td>
</tr>
<tr>
<td>UV on λ pSu+2</td>
<td>cφf5am295</td>
<td>10⁻⁷⁻¹₀⁻⁸</td>
<td>6</td>
</tr>
<tr>
<td>NTG on λ pSu+2A37</td>
<td>metf3am3</td>
<td>10⁻⁷</td>
<td>1</td>
</tr>
</tbody>
</table>

Mutant frequency is shown per phage particles. Mutant type is according to results from sequence analysis.

suppressing mutants appeared exclusively in Su+2A37 via an additional single-base mutation. This was confirmed by the sequence analysis described below. The double-mutants were classified into two groups as to the efficiency of mischarging suppression to metf3am3, which corresponded to two types of muta-
Mischarging mutants of Su⁺2 glutamine tRNA. I.

In addition to the bacterial mutants used to select the mischarging mutants, some phage amber mutants also exhibited specific spectra when suppressed by the known suppressors in E. coli. Such nine mutants of phage T4 and four of λ phage were used to characterize the Su⁺2 mutants. Since the suppression pattern on bacterial markers was often affected at high temperature (Table 1), the phage test was performed at 32°C. The suppression patterns given by the Su⁺2 mutant suppressors, together with those by Su⁺1 (serine inserting), Su⁺2 (glutamine), Su⁺3 (tyrosine), Su⁺6 (leucine), Su⁺7 (glutamine and tryptophan) and an ocher suppressor Su⁺β (lysine) are shown in Table 1.

The mutant suppressors acquired a new ability to suppress T4 N133, NG187, NG197 and NG60, and A Aam227 that are not suppressed by Su⁺2. None of the mutants was able to suppress T4 Bu33 and 5116, or A Sam7. Two single-mutants gave a similar pattern, and similarly two double ones. There were, however, some evident differences between the single- and double-mutants, and it appeared that an additional mutation conferred new suppressor activity additively, while retaining the parental spectrum.

Both of the single-mutants did not seem to insert serine, tyrosine, lysine, leucine or tryptophan at UAG site so far as judged from the suppression patterns. The A37 and C37 mutations in Su⁺2 tRNA were reported to increase the suppression efficiencies, which may result in the some changes in the suppression patterns (see DISCUSSION). While the double-mutant suppressors appeared to accept tryptophan in vivo (Yamao et al., 1988). This was consistent with the results of the patterns on the defined E. coli amber markers, but some discrepancies existed in the suppression of T4 amber markers when their patterns were compared to those of Su⁺7. This may be explained by the different efficiencies or frequencies of tryptophan-inserting suppression in Su⁺2 double-mutant tRNAs and Su⁺7 tRNA.

Although the tRNA molecules of the mutants described here were thermostable, their suppression by mischarging to cy5am235 and metam3 showed a distinct temperature sensitivity at 42°C. Two single-mutants were not distinguishable as to the efficiency of mischarging suppression. One of the two double-mutants, A37A29, showed a rather weaker suppression by mischarging to metam3 than the other (A37C38), while both showed a strong and indistinguishable suppression to cy3am235.

Nucleotide sequence of mutant tRNAs

Fingerprint pattern after RNase T1 digestion of Su⁺2 tRNA21n is shown in Fig. 2a. Since tRNAs were labeled in the cells which were heavily irradiated by
ultraviolet light, modification of minor bases was incomplete. Assignment of each spot, based on the sequence of Su+2 tRNA21n shown in Fig. 1, was described by Inokuchi et al. (1979).

(a) Mutant C37

Fingerprint of RNase T1 digests of Su+2 tRNA21n synthesized by λpSu+2C37 mutant is shown in Fig. 2b. The resulting pattern was different from that of Su+2 wild type (Fig. 2a) only in mobility of anticodon-containing fragment in the first dimension. This spot was eluted and further digested with RNase A, producing a new trinucleotide AAC instead of trinucleotide AAA (Fig. 3d). The fingerprint by RNase A digestion of this mutant tRNA (Fig. 4a) also differed from that of Su+2 wild type by replacing AA with AAC. These results indicated that residue at the thirty-seventh position from the 5'-end was substituted by C in this mutant. All other spots were further digested and found to be identical to those of Su+2 wild type.

(b) Mutant A37

In the fingerprint pattern of RNase T1 digests of tRNA21n from Su+2A37 mutant, the mobility of anticodon-containing fragment on the first dimension differed from that of Su+2 wild type, which was similar to that of C37 mutant.
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The pancreatic digestion of this spot yielded a new tetranucleotide AAAF instead of AAF (Fig. 3c). In the fingerprint by pancreatic digestion of this mutant tRNA, spot AAF was also missing and a new spot AAAF appeared (Fig. 4b). These results showed that A37 mutant was resulted by base substitution of F with A at the thirty-seventh position from the 5'-end. In addition to this base substitution, 2'-O-methyl modification at the thirty-first residue was missing in this mutant tRNA. Although 2'-O-methyl uridine (Um) is resistant to RNase A or alkaline hydrolysis, trinucleotide AUmU or dinucleotide UmU were not produced by PNase A digestion or alkaline hydrolysis of anticodon-containing RNase T1 fragment, respectively. In pancreatic fingerprint, spot of GGAUmU also disappeared, while spot GGAU was present in full content. All other spots were found to be identical to those of wild type after analysis by further digestion.

(c) Mutant A37A29

RNase T1 digests of tRNA2'nm from mutant A37A29 yielded a fingerprint pattern which differed only in the migration of anticodon-containing fragment from that of Su+2 wild type (data not shown). Further digestion of this spot by RNase A yielded two new oligonucleotides, AAAF and AAU, with two oligonucleotides, AUmU and AU missing (Fig. 3e). The fingerprint of this mutant tRNA by RNase A digestion (Fig. 4c) also differed from that of wild type in the following two ways, (1) AAF spot was replaced by AAAF, which was specific to A37.
mutation, and (2) two spots, GGAU_{m}U and its unmodified and further cleaved form GGAU, were missing, accompanying a two molar yield of the spot GAAU (data not shown). These results showed that mutant A37A29 had two base substitutions, \( \Psi \) to A at the thirty-seventh and G to A at the twenty-ninth positions from the 5'-end of the tRNA. 2'-O-methyl uridine of the thirty-first residue was unmodified in this mutant tRNA, because \( U_{m}U \) dinucleotide was not produced by alkaline hydrolysis of anticodon-containing RNase T1 fragment.
Spots other than those mentioned above were subjected to further analysis and found to be identical to those of Su\(^+\)2 wild type.

(d) Mutant A37C38

In fingerprint pattern of RNase T1 digests of tRNA\(_{21}^{\text{Su2}}\) from mutant A37C38, spot of the anticodon-containing fragment migrated more slowly than that of wild type (data not shown). This spot was eluted and digested with pancreatic RNase, producing tetranucleotide AAAC instead of AAA\(^F\) in Su\(^+\)2 wild type or AAA\(^F\) in Su\(^+\)2A37 mutant (Fig. 3b). In the fingerprint by pancreatic digestion (Fig. 4d), AAA\(^F\) or AAA\(^F\) spot was missing and a new spot of AAAC was found. All other spots were eluted and subjected to further digestion by pancreatic, U2 or T1 RNase. No differences from those of Su\(^+\)2 wild type were not found. From these results, we concluded that mutant A37C38 had two base changes, \(F\) to A at the thirty-seventh and \(F\) to C at the thirty-eighth positions from the 5’-end of the tRNA.

4. DISCUSSION

In the present paper, we have selected the mutants of Su\(^+\)2 suppressor as those exhibiting the suppression activity to certain amber markers which are otherwise not suppressed by the original Su\(^+\)2. Four types of such mutants have been identified and designated as mischarging mutants of Su\(^+\)2 glutamine tRNA, namely A37, C37, A37C38 and A37A29. As mentioned above, Bradley et al. (1981) have independently isolated the mutants of Su\(^+\)2 which acquired the increased suppression efficiency, resulting in the same mutations with our single-base mutants of A37 and C37. It seems therefore that an alteration in suppression patterns by tRNA mutation could be motivated not only by inserting a non-cognate amino acid at the UAG site, but also, in some cases, by increasing the efficiency of suppression with cognate amino acid. These two possibilities could not be distinguished by the suppression patterns alone, but in the present case the followings might favor the latter, although the former possibility still remains. Among the 17 amber markers used to characterize the mutants, only three showed difference between the original Su\(^+\)2 and A37 or C37 mutants, i.e. E. coli cys\(^{am235}\), T4 NG60 and \(\lambda\) A\(^{am237}\) (Table 1), and at least two of them, cys\(^{am235}\) and NG60 have been known to be suppressed with Su\(^+\)2, very weakly though.

Although it is not clear whether the base changes at the thirty-seventh position of this tRNA result in the mischarging by itself, the participation of this residue in the synthetase recognition would be implied by the appearance of secondary mischarging mutants. The A37 and C37 mutants are indistinguishable in their suppression patterns, but the double-mutants are obtained exclusively only in one of them, A37 strain, so far searched as those capable of suppressing met\(^{am3}\). The double-mutant tRNAs seem to be mischarged with tryptophan in the cell, since their suppression to met\(^{am3}\) is affected by mutations in trpS gene (Yamao et al.,
Thus, a base substitution of U37 to A but not to C is apparently required for the recognition of this glutamine tRNA by tryptophanyl-tRNA synthetase.

In the mutants, Su+2A37 and A37A29, the modification of 2'-O-methyl uridine at the thirty-first position, that definitely exists in wild type and other mutant tRNAs in our experimental condition, were absent. The absence of the modification, however, does not seem to correlate with mischarging, because 2'-O-methyl uridine is present in other mischarging mutant tRNAs. A A37 mutation in Su+2 tRNA would prevent the tRNA from the modification enzyme for 2'-O-methyl uridine. Such a modification has been restored by the secondary mutation in A37C38 but not in A37A29 double-mutant.

As shown in Fig. 1, all of the mutation sites in Su+2 mischarging tRNA are in the limited regions, anticodon loop and stem, which are in striking contrast with those of Su+3 mischarging mutants in the terminal part of amino acid acceptor stem. On the other hand, the mutation site of Su+7 and the sites of Su+2 mischarging mutants are in the similar region but not at the identical site, though. Thus, the sites of mischarging mutation recognized by non-cognate enzyme are idiosyncratic. However, a general interpretation of these results can be drawn when viewed on a term of L-shaped tertiary structure of tRNA molecule (Kim et al., 1972). Although the mutation sites are different in each tRNA, all of them locate within the diagonal side of the folded tRNA molecule, which could provide an incremental interaction with certain non-cognate synthetase without affecting the folded structure of tRNA. Thus, these would imply some features of tRNA and synthetase interaction, as also discussed by Rich and Schimmel (1977) and Ozeki et al. (1980), that tRNAs may interact with synthetases in their diagonal side. The specificity of aminoacylation, therefore, should be accounted for by additional fine interactions which would be provided by some specific sites locating within the diagonal side of the tRNA molecules. It may be reasonable to assume that the residue in both terminal parts of L-shaped tRNA molecule would serve as determinants for such critical interaction.

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