Evidence for Class-Specific Discrimination of a Semiconserved Base Pair by tRNA Synthetases†

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Received February 3, 1995; Revised Manuscript Received April 11, 1995⊗

ABSTRACT: Aminoacyl-tRNA synthetases have been divided into two classes based on the existence of two structurally distinct active sites. To date, few class-specific tRNA recognition features have been elucidated. High-resolution X-ray structures of representative class I and class II synthetases complexed to cognate tRNA substrates have been solved. In these structures, the class I enzyme approaches the end of the tRNA acceptor stem from the minor-groove side, while the class II synthetase approaches its cognate tRNA from the major-groove side. This distinction is reflected in the different initial sites (2′- or 3′-OH) of amino acid attachment. The role that the semiconserved G1•C72 terminal base pair plays in the aminoacylation of *Escherichia coli* tRNAs is probed in this *in vitro* study. We show here that class II alanyl-, prolyl-, and histidyl-tRNA synthetases are sensitive to changes at position 1•72. Previous work on class I synthetases and new data presented here with the valine-specific enzyme indicate that class I enzymes show little sensitivity to replacements of G1•C72. This work provides new evidence for class-specific differences in tRNA acceptor stem interactions that appear to be reflected not only in the initial site of aminoacylation but also in the mode of synthetase interaction with the semiconserved G1•C72 base pair proximal to the amino acid attachment site.

Aminoacyl-tRNA synthetases catalyze the specific attachment of amino acids onto the 3′-adenosine (A76) of their cognate tRNA substrates. The partition of synthetases into two classes of 10 each is based on sequence and structural similarities in the core catalytic domains of these enzymes (Cusack et al., 1990; Eriani et al., 1990). High-resolution crystal structures of representative members of both class I and class II synthetases complexed with their respective tRNA substrates have now been solved (Rould et al., 1989; Ruff et al., 1991; Biou et al., 1994). These structures help to explain the major known functional difference between the two classes of enzymes, namely, the initial site of amino acid attachment (Cavarelli et al., 1994). Class I synthetases initially attach the amino acid to the 2′-OH of A76, while class II synthetases aminoacylate the 3′-OH. In the *Escherichia coli* glutamyl-tRNA synthetase−$tRNA^{Gln}$ cocrystal structure (Rould et al., 1989), the class I enzyme approaches the tRNA acceptor stem from the minor-groove side and only the 2′-OH is correctly positioned for attack of the aminoacyl-adenylate. On the other hand, class II yeast aspartyl-tRNA synthetase (AspRS)[1] is seen to approach $tRNA^{Asp}$ from the major-groove side, and the 3′-hydroxyl of A76 is in position to attack the enzyme-bound aminoacyl-adenylate (Ruff et al., 1991; Cavarelli et al., 1994).

The mechanism that aminoacyl-tRNA synthetases use to discriminate among tRNAs involves specific interactions with structural features designated as positive and negative "identity elements" (Schimmel, 1989; Schulman, 1991; McClain, 1993; Saks et al., 1994). The amino acid acceptor stem domain proximal to the CCA-3′ end that is common to all tRNAs has been found to contain nucleotides that contribute to the specific recognition of tRNAs by both classes of aminoacyl-tRNA synthetases. In particular, the discriminator base (N73) and base pairs 2•71 and 3•70 are common locations for tRNA identity elements (Francklyn et al., 1992a). A G•C pair is the terminal (1•72) base pair of approximately 75% of all *E. coli* tRNA isoacceptors (Steinberg et al., 1993). This base pair has not been as extensively probed in tRNA identity studies, perhaps due to its semiconserved nature. Therefore, in the present work, we wanted to examine the role that the semiconserved G1•C72 base pair plays in tRNA discrimination by *E. coli* synthetases. Our results, combined with previous work, provide evidence for class-specific differences in the importance of this base pair in *E. coli* tRNA recognition. Furthermore, the results obtained correlate well with the apparent difference in how the two classes of synthetases are thought to approach the end of the tRNA acceptor stem (major groove for class II vs minor groove for class I).

MATERIALS AND METHODS

Protein Purification and Assays. E. coli alanyl-tRNA synthetase (AlaRS) was purified according to Miller et al. (Miller et al., 1991). Protein concentrations were based on active-site titrations using the adenylate burst assay (Fersht et al., 1975). Prior to aminoacylation assays, duplex substrates were annealed by heating complementary strands to 80 °C for 3 min in 50 mM HEPES (pH 8.0) and then cooling rapidly to 4 °C. Assays were conducted with 8−50

† This work was funded by Grant GM49928 from the National Institutes of Health. Acknowledgment is also made to the donors of The Petroleum Research Fund, administered by the American Chemical Society, and to the American Cancer Society (Grant IN-13-33-15) for partial support of this research.

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⊗ Abstract published in *Advance ACS Abstracts,* July 15, 1995.

[1] Abbreviations: AspRS, aspartyl-tRNA synthetase; AlaRS, alanyl-tRNA synthetase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ValRS, valyl-tRNA synthetase; HisRS, histidyl-tRNA synthetase; ProRS, prolyl-tRNA synthetase.

0006-2960/95/0434-9795$09.00/0

μM annealed duplex and 0.2−0.8 μM AlaRS as described (Musier-Forsyth et al., 1991). Relative rates of aminoacylation are averages of 2−4 determinations using conditions where the slope of the initial rate is proportional to V_{max}/K_M. All standard errors were calculated as described (Strobel, 1960). Kinetic parameters for full-length tRNAs were derived from Lineweaver−Burk plots using tRNA concentrations ranging from 0.1 to 4 μM for wild-type $tRNA^{Ala}$ and from 2 to 24 μM for N72 mutant $tRNA^{Ala}$ transcripts. AlaRS concentrations of 5 and 50 nM were used for the wild-type transcript and the N72 variants, respectively. Aminoacylation assays with purified *E. coli* valyl-tRNA synthetase (ValRS), which was a gift of Dr. Jack Horowitz, were done essentially as described (Chu & Horowitz, 1989). Assays with *E. coli* histidyl-tRNA synthetase (HisRS) were conducted as described (Francklyn & Schimmel, 1990) using 10 μM microhelix^{His} and 50−250 nM purified HisRS, which was a gift of Dr. Christopher Francklyn. Conditions were employed such that the slope of the initial rate of aminoacylation is proportional to V_{max}/K_M. T7 RNA polymerase was purified according to Grodberg and Dunn (1988) from *E. coli* strain BL-21/pAR 1219, which was a gift of F. William Studier.

Ribonucleic Acids. RNA oligonucleotides used to prepare duplex and microhelix substrates were synthesized on a Gene Assembler Plus (Pharmacia) using the phosphoramidite method (Scaringe et al., 1990). Oligonucleotides were purified on 16% denaturing polyacrylamide gels. For the determination of RNA concentrations, the following extinction coefficients were employed: 13-mer, 10.7×10^4 M^{-1}; 9-mer, 8.9×10^4 M^{-1}; 25-mer, 18.1×10^4 M^{-1} (Musier-Forsyth et al., 1991). Plasmids encoding the genes for *E. coli* $tRNA^{Ala}$ and *E. coli* $tRNA^{Val}$ in front of a T7 promoter were a gift of Dr. Jack Horowitz. Following plasmid linearization with either *Bst*NI or *Fok*I, full-length tRNA molecules were prepared by *in vitro* transcription as described (Sampson & Uhlenbeck, 1988). Full-length tRNAs were purified on 12% denaturing polyacrylamide gels. Site-directed *in vitro* mutagenesis using the Kunkel method (Kunkel, 1985) was used to generate the N72 variant tRNAs.

RESULTS

In vitro transcription using T7 RNA polymerase is one of the most widely used methods of preparing RNAs for biochemical studies (Milligan & Uhlenbeck, 1989). However, since T7 RNA polymerase requires a guanosine at position 1 for efficient template transcription, C1-containing RNAs are typically prepared by priming transcription with CpG (Milligan & Uhlenbeck, 1989). Alternatively, chemical methods of RNA synthesis can by employed (Scaringe et al., 1990). To facilitate part of this work, therefore, chemically synthesized RNA minihelices and duplexes derived from the acceptor stems of *E. coli* tRNAs were prepared. Class II AlaRS and HisRS are able to efficiently aminoacylate minihelices (Francklyn & Schimmel, 1989, 1990) and duplexes (Musier-Forsyth et al., 1991; Francklyn et al., 1992a) derived from the acceptor stems of their respective tRNAs. The acceptor helix, in fact, makes the largest contribution to the V_{max}/K_M parameter for recognition of these tRNAs by their cognate synthetases (Francklyn et al., 1992b). These synthetases were, therefore, chosen for this study.

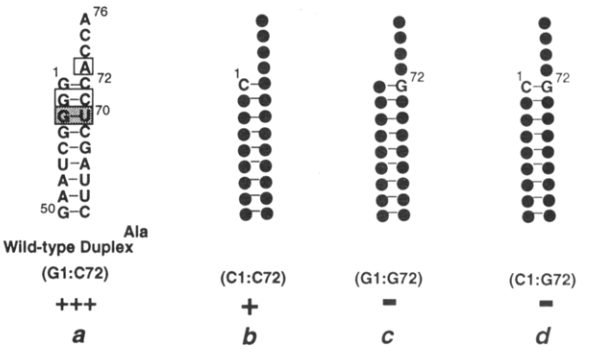

FIGURE 1: Sequences of chemically synthesized RNA duplex^{Ala} variants prepared and assayed for alanine acceptance activity using purified AlaRS. (a) The wild-type duplex^{Ala} sequence is based on the acceptor stem and part of the TΨC stem of *E. coli* $tRNA^{Ala}$. Open boxes are placed around nucleotides corresponding to minor alanine recognition elements, and shaded boxes indicate major alanine recognition elements (Francklyn et al., 1992b). (b−d) Duplex^{Ala} variants. Only the bases that differ from the wild-type duplex shown in panel a are explicitly shown. The − indicates that the decrease in aminoacylation relative to the wild-type duplex was >1000-fold and, therefore, no aminoacylation with alanine could be detected. The + indicates a 40-fold reduction in aminoacylation with alanine relative to the wild-type duplex^{Ala} substrate. The relative activity of the latter is indicated by +++. The estimated error of these measurements is ±6%.

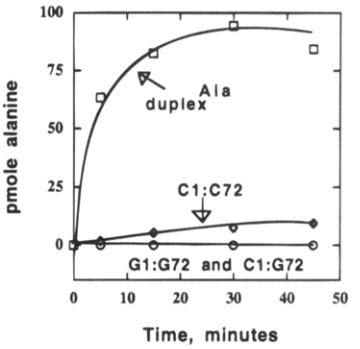

FIGURE 2: Aminoacylation of duplex^{Ala} substrates that differ at position 1•72. The assays were carried out as described in Materials and Methods using 8 μM duplex substrate and 0.43 μM AlaRS. Each time point represents the incorporation of alanine per 15-μL reaction aliquot. The curve labeled duplex^{Ala} corresponds to the G1•C72-containing wild-type duplex^{Ala} shown in Figure 1a. The other two curves were generated with duplex^{Ala} variants containing the indicated base changes.

A 9-base-pair RNA duplex previously proven to be an efficient substrate for *E. coli* AlaRS is shown in Figure 1a. We prepared and tested three variants of this duplex, which were designed to probe the role of the G1•C72 base pair. A G1 → C1 change creating a C1•C72 mismatch (Figure 1b) resulted in a significant reduction in aminoacylation (40-fold) relative to the wild-type duplex^{Ala} (Figure 2). Aminoacylation of the duplex substrate, however, is completely eliminated if a single C72 → G change is made (Figures 1c and 2) or if the double mutant is made, creating a C1•G72 base pair (Figures 1d and 2). No charging was detected even when experiments were conducted using 50 μM duplex substrate and 0.8 μM AlaRS (data not shown). On the basis of the sensitivity of our experiments, we estimate that the alanine charging capability of these duplexes is reduced at least 1000-fold relative to the wild-type duplex^{Ala}. The C1•G72 variant recreates a Watson−Crick base pair, and the results suggest that the defect in aminoacylation of the G1•G72 variant is not due to the introduction of a mismatch

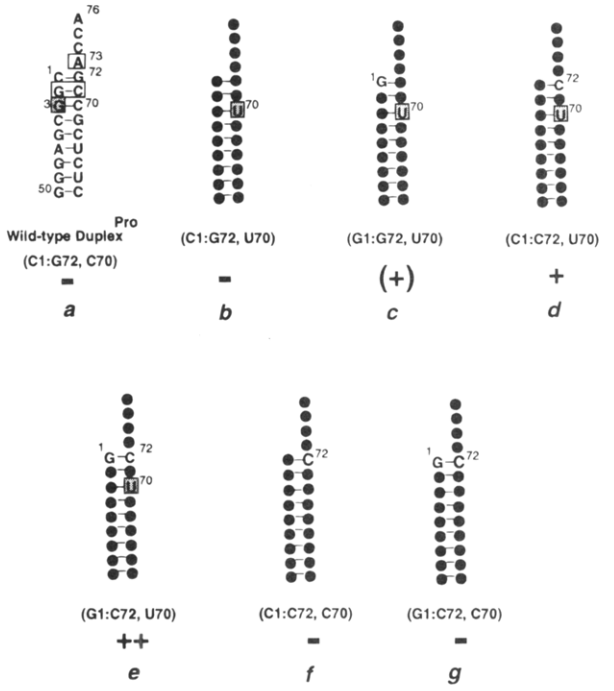

FIGURE 3: Sequences of chemically synthesized RNA duplex^{Pro} variants prepared and assayed for alanine acceptance activity using purified AlaRS. (a) The wild-type duplex^{Pro} sequence is based on the acceptor stem and part of the TΨC stem of *E. coli* tRNA$^{Pro/UGG}$. Open and shaded boxes have the same meaning as in Figure 1. (b−g) Duplex^{Pro} variants. Only the bases that differ from the wild-type duplex shown in panel a are explicitly shown. The − indicates that no aminoacylation with alanine could be detected (>1000-fold decrease). The (+), +, and ++, indicate 900-, 74-, and 2.7-fold reduction in aminoacylation with alanine relative to a wild-type duplex^{Ala} substrate, respectively. The estimated error of these measurements is ±17%.

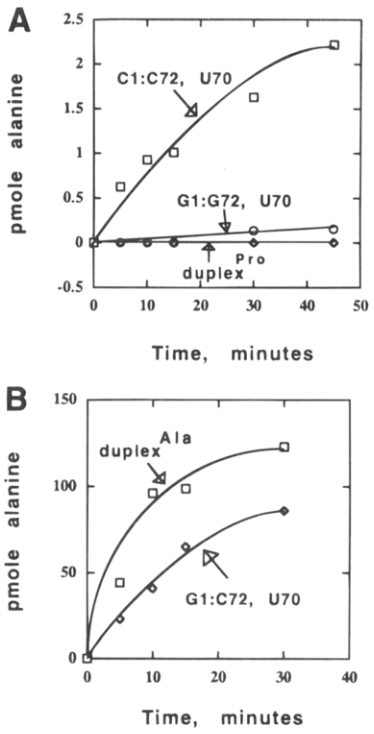

FIGURE 4: Aminoacylation of duplex substrates with alanine. The assays were carried out as described in Materials and Methods using 10 μM duplex substrate and 0.80 μM AlaRS. Each time point represents the incorporation of alanine per 15-μL reaction aliquot. (A) The curve labeled duplex^{Pro} corresponds to the wild-type duplex^{Pro} shown in Figure 3a. The other two curves in this graph are duplex^{Pro} variants with the indicated base substitutions at positions 70 and 1•72. (B) Comparison of the aminoacylation efficiency of wild-type duplex^{Ala} and a duplex^{Pro} variant with the indicated base substitutions at positions 70 and 1•72.

at the terminal position of the helix.

We continued to test the importance of G1•C72 in aminoacylation by class II AlaRS in the context of a duplex^{Pro} framework because *E. coli* tRNA^{Pro} contains a unique C1•G72 base pair. Of the 22 nucleotides constituting the wild-type duplex^{Pro} molecule (Figure 3a), 10 are identical to those found in duplex^{Ala} (Figure 1a). The common nucleotides include the CCA-3′ end that all tRNAs share, three previously identified minor alanine recognition elements (Figure 3a, open boxes), and G3 (Figure 3a, shaded), which is part of the critical G3•U70 base pair. This base pair has been shown to be the major recognition element for aminoacylation by AlaRS both *in vivo* (Hou & Schimmel, 1988; McClain & Foss, 1988) and *in vitro* (Park et al., 1989). Furthermore, in previous *in vitro* experiments, incorporation of the G3•U70 base pair into heterologous tRNA or minihelix frameworks resulted in efficient aminoacylation by AlaRS (Hou & Schimmel, 1988; Francklyn & Schimmel, 1989). It is not surprising that duplex^{Pro}, which lacks U70, is not aminoacylated by AlaRS (Figure 4A). We next prepared a U70 duplex^{Pro} that now contains the major determinant for aminoacylation with alanine (Figure 3b). Surprisingly, we could not detect any aminoacylation of this substrate with alanine by purified AlaRS (data not shown). This suggested that an element was present that functions to block the strong positive effect of G3•U70 and the three other minor determinants. On the basis of the results shown in Figure 2, it is likely that the unique C1•G72 base pair of tRNA^{Pro} indeed blocks aminoacylation by AlaRS. To test this, we prepared single and double mutations at this base pair in the context of the U70 duplex^{Pro} variant. We observed that the C1 → G change conferred slight alanine acceptance activity on this duplex (Figures 3c and 4A), whereas the G72 → C mutant was a much better substrate for AlaRS (Figures 3d and 4A). The double mutation C1•G72 → G1•C72 resulted in a U70 duplex^{Pro} that was aminoacylated almost as efficiently as the wild-type duplex^{Ala} substrate (Figures 3e and 4B). Moreover, removal of the putative blocking elements (G72 or the C1•G72 base pair) from the wild-type duplex^{Pro} did not confer alanine acceptance on the duplex (Figure 3f,g; data not shown). These results suggest that the G3•U70 base pair is necessary but not sufficient for conversion of a duplex^{Pro} molecule into a substrate for AlaRS. Removal of the C1•G72 element is a prerequisite for efficient aminoacylation of duplex^{Pro} with alanine. These results also show that the presence of G72 has a stronger blocking effect than C1, which is in agreement with the duplex^{Ala} experiments described above (Figure 2). Interestingly, slight aminoacylation of the G1•G72, U70 duplex^{Pro} variant could be detected (Figure 4B), whereas the G72 duplex^{Ala} variant was not charged (Figure 2).

An additional experiment to examine the role of C72 in aminoacylation by class II synthetases was performed with *E. coli* HisRS. A major determinant for tRNA^{His} aminoacylation is the unique G_{-1}•C73 base pair in the acceptor stem (Himeno et al., 1989; Francklyn & Schimmel, 1990). The G1•C72 base pair of this tRNA is, therefore, not in the terminal position of the helix. As mentioned above, HisRS can also efficiently aminoacylate minisubstrates derived from

FIGURE 5: Sequence of a chemically synthesized microhelix^{His}. The duplex region of this substrate is based on the acceptor stem of *E. coli* tRNA^{His}. The loop region is a nonspecific sequence (Francklyn et al., 1992b). Open boxes are placed around nucleotides corresponding to minor histidine recognition elements, and shaded boxes indicate major histidine recognition elements (Francklyn et al., 1992b). The fold-reduction in k_{cat}/K_M as a result of a single C72 → G mutation at position 72 is indicated in parentheses.

the acceptor stem alone. We prepared the wild-type microhelix^{His} substrate shown in Figure 5, as well as a C72 → G variant. This single base change reduced the overall rate of aminoacylation with histidine by approximately 13-fold (data not shown). While this reduction is much less than the decrease seen in the alanine system, it is nevertheless significant.

The identity of the base at position 72 is clearly critical for conferring alanine aminoacylation on duplex substrates (Figures 1−4) and also contributes to the efficiency of aminoacylation of microhelix^{His} by class II HisRS (Figure 5). Full-length tRNAs are generally aminoacylated more efficiently than minisubstrates, especially in the case of substrates for Class I synthetases (Musier-Forsyth & Schimmel, 1992a). In order to more accurately determine the magnitude of the observed effect of changes at position 72 of duplex^{Ala} and to investigate the role of this base in aminoacylation by a class I enzyme, we performed aminoacylation assays of full-length tRNA$^{Ala/UGC}$ and tRNA$^{Val/UAC}$ transcripts (Figure 6). These tRNAs are aminoacylated by class II and class I synthetases, respectively. The kinetic parameters for aminoacylation of the tRNA$^{Ala/UGC}$ *in vitro* transcript with AlaRS were very similar to those previously reported for *E. coli* tRNA$^{Ala/GGC}$ (Francklyn & Schimmel, 1989). We measured a k_{cat} of 2.5 s^{-1} and a K_M of 2.1 μM for the unmodified transcript. The G72 variant transcript, however, had a greatly reduced k_{cat}/K_M of 2.1 × 10^{-5} s^{-1} μM^{-1}. This corresponds to an overall rate reduction of 6.2 × 10^4-fold for the G72-containing transcript (Figure 6A). Due to the extremely low levels of charging obtained for this mutant, reliable k_{cat} and K_M parameters could not be determined. However, we wanted to determine whether other mutations at this position had similar effects. An A72 tRNA^{Ala} variant also displayed extremely low levels of aminoacylation, with a k_{cat}/K_M of 1.3 × 10^{-4} s^{-1} μM^{-1}, corresponding to an overall rate reduction of 1.0 × 10^4 (Figure 6A). Of the three mutants tested, a U72 substitution had the smallest but still significant effect, with a 4.1 × 10^3-fold reduction in aminoacylation (Figure 6A). This mutation creates a purine•pyrimidine wobble base pair at position 1•72. Taken together, the duplex results and the full-length tRNA^{Ala} mutagenesis studies demonstrate that the identity of the nucleotide at position 72 plays an important role in aminoacylation by class II AlaRS.

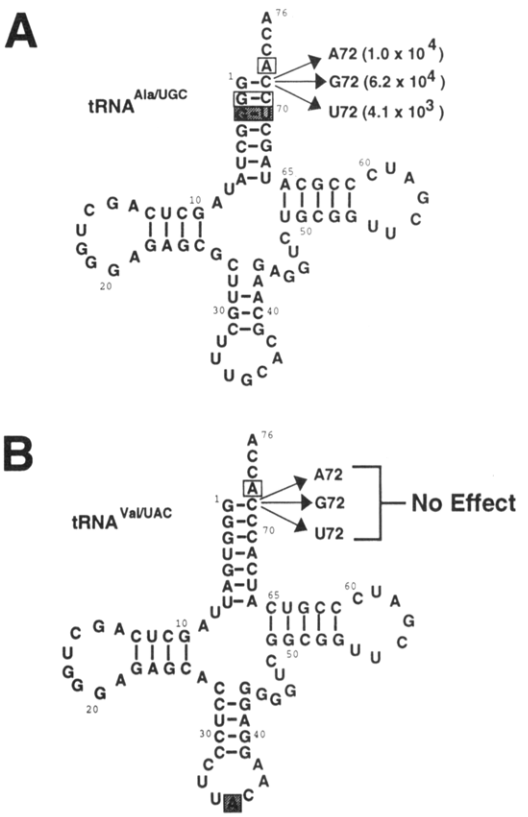

FIGURE 6: (A) Sequence of an unmodified full-length tRNA^{Ala} transcript used in this study. Open and closed boxes have the same meaning as in Figure 1. The sequence is based on the *E. coli* tRNA$^{Ala/UGC}$ isoacceptor. The fold-reductions in k_{cat}/K_M as a result of single C72 → N mutations are indicated in parentheses. The estimated error of these measurements is ±29%. (B) Sequence of an unmodified tRNA^{Val} transcript used in this study. Open and closed circles indicate minor and major determinants for valine aminoacylation, respectively. The sequence is based on the *E. coli* tRNA$^{Val/UAC}$ isoacceptor. As indicated in this figure, within the experimental error of these measurements, no reduction was observed in k_{cat}/K_M as a result of single C72 → N mutations.

In order to see if a similar role is played by C72 in the context of a G1•C72-containing tRNA aminoacylated by a class I synthetase, we substituted all three bases for C72 in tRNA^{Val} (Figure 6B). The k_{cat}/K_M for the wild-type transcript was determined to be 3.8 s^{-1} μM^{-1}. As indicated in Figure 6B, no reduction in the overall rate of aminoacylation by *E. coli* ValRS was seen for any of the N72 mutants.

DISCUSSION

A G1•C72 base pair is found at the end of the acceptor stem of most prokaryotic tRNAs (Steinberg et al., 1993). Interestingly, this base pair is more highly conserved among tRNAs aminoacylated by class II synthetases than those charged by class I enzymes (Steinberg et al., 1993). Within the subset of 10 tRNA isoacceptor groups aminoacylated by class II enzymes, only *E. coli* tRNA^{Asn} and all isoacceptors of *E. coli* and phages T4 and T5 tRNA^{Pro} contain a nucleotide combination other than G•C at the 1•72 position (Steinberg et al., 1993). In contrast, approximately 50% of all tRNAs aminoacylated by class I *E. coli* synthetases contain non-G1•C72 base pairs (Steinberg et al., 1993). It has been shown previously that G72 is important for positive tRNA^{Pro} recognition by *E. coli* ProRS *in vitro* (Liu et al., 1995) and *in vivo* (McClain et al., 1994) but that C1 is dispensable for aminoacylation (Liu & Musier-Forsyth, 1994; Liu et al.,

1995). The work reported here suggests that, in addition to its role as a positive $tRNA^{Pro}$ recognition element, G72 also functions to block aminoacylation of $tRNA^{Pro}$ by noncognate class II synthetases. In particular, the presence of the C1•G72 base pair prevents U70 $duplex^{Pro}$ aminoacylation by AlaRS (Figure 3b). Furthermore, C72 → G72 and G1•C72 → C1•G72 substitutions eliminate $duplex^{Ala}$ aminoacylation by AlaRS (Figure 2). The major groove of RNA is accessible at duplex termini (Weeks & Crothers, 1993) and presents more potential recognition sites than the minor groove (Seeman et al., 1976). Moreover, Watson–Crick G•C and C•G base pairs present a similar pattern of hydrogen-bond donors and acceptors in approximately the same location in the minor groove but look significantly different in the major groove (Seeman et al., 1976). Previously, incorporation of inosine at position 1 of $duplex^{Ala}$ substrates was shown to have little effect on aminoacylation efficiency (Musier-Forsyth & Schimmel, 1992b). Inosine is a modified guanosine lacking the exocyclic 2-amino group that is normally located in the minor groove. This result suggests that AlaRS does not make a minor-groove functional contact with the G1 amino group. Substitutions that will change specific atomic groups in the major groove of the terminal 1•72 base pair of $duplex^{Ala}$ substrates will be necessary to test whether major-groove discrimination is indeed occurring at this site.

Previous *in vivo* studies also support the notion that G1•C72 contributes to the identity of *E. coli* $tRNA^{Ala}$ (McClain et al., 1991a), *E. coli* $tRNA^{Gly}$ (McClain et al., 1991b), and *E. coli* $tRNA^{Ser}$ (Normanly et al., 1992), all of which are aminoacylated by class II synthetases. Furthermore, an *in vitro* study to investigate the recognition elements of class II threonyl-tRNA synthetase showed that a G1•C72 → C1•G72 base pair change resulted in a 500-fold reduction in aminoacylation of $tRNA^{Thr}$ (Hasegawa et al., 1992). Therefore, substitutions in the 1•72 position result in significant decreases in the efficiency of aminoacylation of at least six out of the nine G1•C72/C1•G72-containing tRNAs aminoacylated by class II *E. coli* synthetases.

All three *E. coli* isoacceptors of $tRNA^{Val}$ contain a G1•C72 base pair. The present study shows that class I ValRS is not affected by substitutions at position C72 of *E. coli* $tRNA^{Val}$. Furthermore, recent studies by other researchers showed that substitution of the G1•C72 of $tRNA^{Val}$ with A1•U72 or A1•C72 also had no effect on aminoacylation (J. Horowitz, personal communication). In previous work, the role of the 1•72 pair in the aminoacylation of several other G1•C72-containing tRNA isoacceptors aminoacylated by class I synthetases has been probed. Changes at position 72 in an *E. coli* $tRNA^{Tyr}$ amber suppressor, for example, resulted in a tRNA that could be aminoacylated by class I tyrosyl-tRNA synthetase both *in vivo* and *in vitro* (Celis et al., 1973). While a G1 → A change in this same amber suppressor resulted in glutamine identity *in vivo*, using high levels of TyrRS, this mutant could still be aminoacylated with tyrosine *in vitro*. A single C72 → G mutation in $tRNA^{Met}$ resulted in only a 2.3-fold decrease in aminoacylation by class I *E. coli* methionyl-tRNA synthetase *in vitro* (Lee et al., 1992). Finally, while the role of G1•C72 in the acceptor stem of $tRNA^{Cys}$ has not been explicitly tested, a mutant of *E. coli* initiator tRNA ($tRNA^{fmet}$) containing a C1/A72 mismatched pair along with the discriminator base (U73) and the anticodon of $tRNA^{Cys}$ was a good substrate for class I cysteinyl-tRNA synthetase *in vivo* (Pallanck et al., 1992). This result suggests that the G1•C72 pair is unlikely to be important for $tRNA^{Cys}$ identity.

In the limited studies that have been done to date, it is clear that at least some of the recognition features used by prokaryotic synthetases have been conserved in eukaryotes. For example, the importance of the G3•U70 base pair in $tRNA^{Ala}$ aminoacylation appears to be conserved in evolution (Hou & Schimmel, 1989). Likewise, the extra G_{-1} nucleotide is important for the recognition of both yeast and *E. coli* $tRNA^{His}$ by their cognate synthetases (Himeno et al., 1989; Francklyn & Schimmel, 1990; Nameki et al., 1995). Our experiments address the role of the 1•72 base pair in *E. coli* tRNAs. According to the cocrystal structure of class II yeast AspRS, this eukaryotic synthetase approaches the top of the acceptor stem from the major-groove side (Ruff et al., 1991). Our results are consistent with major-groove discrimination of the 1•72 base pair by class II *E. coli* synthetases specific for G1•C72/C1•G72-containing tRNAs. Yeast $tRNA^{Asp}$ contains a U1•A72 base pair. However, yeast AspRS will recognize a G1•C72 mutant $tRNA^{Asp}$ transcript with equal efficiency (Pütz et al., 1991). As previously noted (Pütz et al., 1991), this result is not inconsistent with major-groove discrimination at this site because a U:A pair presents similar atomic groups in the central region of the major groove as a G•C pair (Seeman et al., 1976; Pütz et al., 1991).

In conclusion, in the case of most G1•C72/C1•G72-containing tRNAs from *E. coli* examined to date, the different orientations of the two classes of synthetases with respect to the end of the tRNA acceptor helix appear to correlate with differences observed in the way that these enzymes interact with the 1•72 base pair. Several results are presented here that are consistent with major-groove discrimination at this site by class II synthetases. In contrast, discrimination of the G1•C72 base pair is less likely for class I synthetases, which are thought to approach the end of the acceptor stem from the minor-groove side. The results presented here for $tRNA^{Val}$, along with earlier data (Celis et al., 1973; Lee et al., 1992; Pallanck et al., 1992), are also consistent with this prediction. Clearly, additional experiments need to be performed with tRNAs aminoacylated by both classes of synthetases before class-specific functional distinctions in tRNA recognition are fully understood. Experiments such as those presented in this paper should also lead to a better understanding of the evolutionary role of two classes of synthetases.

ACKNOWLEDGMENT

We thank Dr. Jack Horowitz for the plasmids encoding the $tRNA^{Ala}$ and the $tRNA^{Val}$ genes and for purified ValRS, Dr. Christopher Francklyn for purified HisRS, and Dr. Paul Schimmel for the overexpression plasmid for AlaRS. We are also grateful to Drs. Schimmel, Francklyn and Horowitz and to Dr. Uttam RajBhandary for helpful comments on the manuscript.

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BI950252Q