Determinants in tRNA for Activation of Arginyl-tRNA Synthetase: Evidence that tRNA Flexibility Is Required for the Induced-Fit Mechanism†

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Received August 9, 2005; Revised Manuscript Received October 19, 2005

ABSTRACT: Arginyl-tRNA synthetase (ArgRS) catalyzes formation of arginyl-adenylate in a tRNA-dependent reaction. Previous studies have revealed that conformational changes occur upon tRNA binding. In this study, we analyzed the sequence and structural features of tRNA that are essential to activate the catalytic center of mammalian arginyl-tRNA synthetase. Here, tRNA variants with different activator potential are presented. The three regions that are crucial for activation of ArgRS are the terminal adenosine, the D-loop, and the anticodon stem−loop of tRNA. The Add-1 N-terminal domain of ArgRS, which has the very unique property among aminoacyl-tRNA synthetases to interact with the D-loop in the corner of the convex side of tRNA, has an essential role in anchoring tRNA and participating in tRNA-induced amino acid activation. The results suggest that locking the acceptor extremity, the anticodon loop, and the D-loop of tRNA on the catalytic, anticodon-binding, and Add-1 domains of ArgRS also requires some flexibility of the tRNA molecule, provided by G:U base pairs, to achieve the productive conformation of the active site of the enzyme by induced fit.

Aminoacyl-tRNA synthetases are responsible for translation of the genetic code in associating amino acids to trinucleotide sequences. This family of enzymes catalyzes the esterification of amino acid to the 3′-end of tRNA in a two-step reaction. In the first step the amino acid is activated to form an enzyme-bound aminoacyl-adenylate intermediate; the second step involves the transfer of the amino acid to the 3′-end of the tRNA molecule to give the aminoacyl-tRNA product (*1*). Generally, formation of the aminoacyl-adenylate does not require the presence of the cognate tRNA molecule. In some cases, the reaction may even be performed by the isolated catalytic domain of the synthetase, in the absence of the anticodon-binding domain. For instance, the N_{1-287} fragment of *Escherichia coli* CysRS (*2*), a class I enzyme, or the N_{1-461} fragment of *E. coli* AlaRS (*3*), a class II synthetase, are indistinguishable from wild-type for synthesis of aminoacyl-adenylate and for aminoacylation of RNA-minihelices. However, the three class I synthetases ArgRS, GlnRS, and GluRS, as well as the exceptional class I LysRS, do require tRNA to accomplish the first step of the reaction.

The absolute requirement for tRNA in the aminoacyl-adenylate formation has been described for ArgRS from different sources (from the bacteria *E. coli* (*4*), *Bacillus stearothermophilus* (*5*), or *Mycobacterium smegmatis* (*6*), from the lower eukaryotes *Saccharomyces cerevisiae* (*7*) or *Neurospora* crassa (*8*), and from mammals (*9*)) and should be a general feature of this enzyme. Thus, $tRNA^{Arg}$ serves as an activator of ArgRS in the first step of the reaction, and as the substrate in aminoacylation. Despite the fact that ArgRS is active only in the presence of the three substrates arginine, ATP, and tRNA, the aminoacylation is not a concerted mechanism but proceeds by the aminoacyl-adenylate route (*10*). The identity determinants of $tRNA^{Arg}$ for aminoacylation have been uncovered (*11*−*16*). Positions 35 and 36 in the anticodon are major identity elements in *E. coli* and *S. cerevisiae*. Adenosine at position 20 located in the D-loop in the corner of the convex side of tRNA is also a major identity element in most organisms (*11*, *16*) with the noticeable exception of lower eukaryotes (*15*, *17*) and of the mitochondria of higher eukaryotes that lack A20 in this position. In yeast, an organism that lacks A20, extensive genetic screens have detected a series of amino acid residues that are essential for aminoacylation within the arginine-binding pocket and the anticodon-binding domain of ArgRS (*18*, *19*).

Crystal structures are available for ArgRS from *S. cerevisiae* bound in complexes with arginine (*20*), with $tRNA^{Arg}$ or arginine and $tRNA^{Arg}$ (*21*), and for ligand-free ArgRS from *Thermus thermophilus* (*16*). On the basis of these crystal structures and of NMR spectroscopy experiments (*22*, *23*), extensive structural changes induced by substrate binding have been reported for the tRNA and the enzyme. It has been proposed that anticodon binding triggers conformational changes in the active site of the synthetase via communication between the catalytic center and the anticodon binding domain (*21*). Mutations located at the interface of the two domains impaired the catalytic activity of mammalian ArgRS (*9*).

In mammals, two cytoplasmic forms of ArgRS have been described (*24*, *25*). They differ by the presence of an additional 73-amino acid residue N-terminal sequence that

† This work was supported by grants from the "Centre National de la Recherche Scientifique", the "Association pour la Recherche sur le Cancer", and "La Ligue". L.G. is the recipient of a doctoral fellowship of "Ministère de l'éducation nationale, de l'enseignement supérieur et de la recherche" and of "Association pour la Recherche sur le Cancer".

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10.1021/bi051575h CCC: $30.25
Published on Web 11/19/2005

is required for assembly of ArgRS within the multisynthetase complex (*26*). The two mammalian ArgRS species synthesized arginyl-adenylate in a strictly tRNA-dependent manner (*9*, *27*). In this work, we investigated the structural determinants in tRNA required to promote activation of the catalytic center of mammalian ArgRS. We determined whether the complete $tRNA^{Arg}$ molecule is necessary for aminoacyl-adenylate formation by ArgRS and which sequence or structural feature of tRNA is essential in this reaction. Because yeast $tRNA^{Arg}$ is a poor activator of mammalian ArgRS we constructed chimeric tRNA molecules in which domains of the yeast and mammalian $tRNA^{Arg}$ were combined. We also examined the role of tRNA flexibility in activation of ArgRS by induced fit by introducing G:U wobble base pairs in various locations of the stems of tRNA.

MATERIALS AND METHODS

Enzyme Purification. Hamster arginyl-tRNA synthetase (hArgRS[1]) and a truncated derivative with a deletion of the 73 N-terminal amino acid residues (hArgRS-ΔN) were expressed in yeast and purified as described (*26*). Protein concentration was determined by using calculated absorption coefficients of 0.728 and 0.803 A_{280} units $\times$ mg^{-1} $\times$ cm^2, respectively, for hArgRS and hArgRS-ΔN.

tRNA Cloning and Synthesis. Synthetic tRNA genes were constructed in a manner similar to that described previously (*27*) by simultaneous ligation of 10 overlapping oligonucleotides. The genes, placed under the control of a T7 RNA polymerase promoter, were inserted between the *Hin*dIII and *Bam*HI sites of pUC18. Linearization of plasmids carrying tRNA genes was performed with *Bst*NI or *Fok*I. A *Fok*I site was used for tRNA genes possessing an internal *Bst*NI site. In the case of the A76-deleted tRNA (M-$tRNA^{Arg,\Delta A76}$), an HDV (hepatitis delta virus) ribozyme coding sequence was fused to the 3′-end of the deleted tRNA gene by performing a PCR reaction using the pUC18 plasmid containing the M-$tRNA^{Arg}$ gene as the template, with an appropriate primer carrying the deletion of A76. After digestion with *Hin*dIII/*Nco*I, the PCR product was inserted into the pRZ vector (*28*) containing the HDV ribozyme gene. The plasmids were produced in the JM101TR strain and checked by DNA sequencing.

pUC18 derivatives were linearized using *Bst*NI or *Fok*I. pRZ derivatives were linearized with *Eco*RI. The linearized plasmids were then subjected to in vitro transcription with T7 RNA polymerase. The polymerase was purified from the strain BL21/pAR1219 generously provided by Prof. W. Studier (Brookhaven National Laboratory). The transcription reaction mixture typically contained 100 μg of linearized plasmid, 40 mM Tris-HCl (pH 8.0), 22 mM $MgCl_2$, 0.01% Triton X-100, 1 mM spermidine, 5 mM DTT, 4 mM each ATP, CTP, UTP and GTP, 16 mM GMP, 750 U (25 μg) T7 RNA polymerase, and 10 units of pyrophosphatase in a final volume of 1 mL. After incubation at 37 °C for 4 h, the reaction mixture was subjected to a double phenol/chloroform extraction, precipitated with ethanol and the pellet solubilized in 250 μL of formamide containing 10 mM EDTA. For M-$tRNA^{Arg,\Delta A76}$, cleavage by the HDV ribozyme appeared to be cotranscriptional, so that no additional heating−cooling cycle was required. The transcripts were purified on a 12% polyacrylamide gel containing 8 M urea. RNAs were visualized by UV exposure using a sheet of silica gel 60 F_{254} (Merck). Products of the expected size were cut out, and the tRNA was extracted by electroelution with a Bio-Trap apparatus (Schleicher & Schuell) followed by ethanol precipitation. The final pellet was solubilized in 5 mM Tris-HCl (pH 7.5) and 10 mM $MgCl_2$. tRNA concentration was estimated from absorption at 260 nm, using the theoretical absorption coefficient. Concentration of the tRNA solution was adjusted to 100 μM, and renaturation was performed by heating at 90 °C for 2 min followed by slow cooling (90 °C to 30 °C in 2 h). In these conditions, aminoacylation plateaus ranged from 300 to 600 pmol/A_{260}, corresponding to 20−40% of functional tRNA.

tRNA Dephosphorylation. The 2′,3′-cyclic phosphate created at the 3′-end of the tRNA molecule after cleavage by the HDV ribozyme was hydrolyzed with T4 polynucleotide kinase to yield an aminoacylatable tRNA, bearing 2′-OH and 3′-OH groups (*29*). The dephosphorylation reaction contained 100 mM imidazole-HCl (pH 6.0), 10 mM $MgCl_2$, 0.1 mM ATP, 0.1 mg/mL BSA, 10 mM 2-mercaptoethanol, and 0.5 unit of T4 polynucleotide kinase (BioLabs) per μg of tRNA. tRNA concentration was 100 μg/mL in the assay. After incubation for 12 h at 37 °C, tRNA was extracted with phenol/chloroform and precipitated with ethanol and the pellet solubilized in 5 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$. tRNA concentration was estimated from absorption at 260 nm. An aminoacylation plateau was then performed to estimate the percentage of functional tRNA.

Isotopic [^{32}P]PPi−ATP Exchange. The reaction mixture contained, in a final volume of 0.1 mL, 20 mM imidazole-HCl (pH 7.5), 10 mM $MgCl_2$, 0.1 mM EDTA, and 2 mM each of ATP, arginine, and [^{32}P]pyrophosphate (2.5 Ci/mol). The reaction was started by the addition of limiting amounts of enzyme (5−20 nM) appropriately diluted in 10 mM Tris-HCl (pH 7.5), containing 10 mM 2-mercaptoethanol and BSA at 4 mg/mL. After 10 min at 25 °C, the reaction was stopped by the addition of 2.5 mL of a solution containing 100 mM sodium pyrophosphate, 50 mM sodium acetate (pH 4.5), 0.35% perchloric acid, and 0.4% Norit to adsorb ATP. Samples were filtered through Whatman No.1 filters and washed three times with 12 mL of 100 mM sodium pyrophosphate. [^{32}P]-Labeled ATP adsorbed on Norit was quantified by liquid scintillation counting. Kinetic parameters in the ATP−PP_i exchange reaction were determined with tRNA concentrations ranging from 1 nM to 1 μM. Kinetic constants reported in this study are the mean values of at least three independent experiments, with a variation of less than 20%.

Aminoacylation Assay. Reactions were performed at 25 °C in 0.1 mL of 20 mM imidazole-HCl buffer (pH 7.5), 150 mM KCl, 0.5 mM DTT, 5 mM $MgCl_2$, 3 mM ATP, 20 μM ^{14}C-labeled arginine (Perkin-Elmer Life Sciences; 50 Ci/mol), variable amounts of tRNA, and catalytic amounts (1−10 nM) of enzyme appropriately diluted in 10 mM Tris-HCl (pH 7.5) and 10 mM 2-mercaptoethanol containing bovine serum albumin at 4 mg/mL. Reaction was stopped by adding 2.5 mL of cold (4 °C) trichloroacetic acid (TCA) at a concentration of 5%. Total RNA from yeast (0.2 mg) was then added to help precipitation. After filtration on GF/C filters and washing three times with 10 mL of 5% cold TCA, filters

[1] Abbreviations: hArgRS, hamster arginyl-tRNA synthetase.

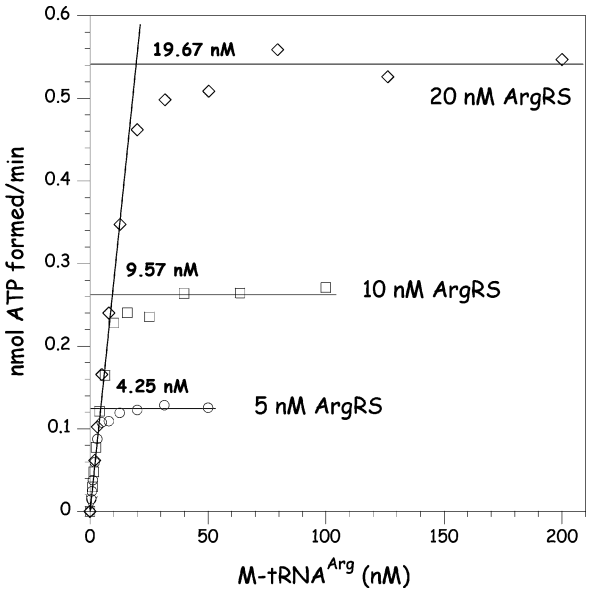

FIGURE 1: Titration of hArgRS with M-tRNA^{Arg}. The rate of the ATP−PP_i exchange reaction was followed at different enzyme concentrations (5 nM, 10 nM, or 20 nM) as a function of the concentration of M-tRNA^{Arg} added in the assay. The intersection of the slope of the increase in rate of the reaction with the maximum value gives the stoichiometry. Catalytic constants (k_{cat}) were 4.33 s^{-1}, 4.48 s^{-1}, and 4.58 s^{-1} in the presence of 5 nM, 10 nM, and 20 nM hArgRS, respectively.

were dried and counted in a liquid scintillator. For determination of kinetic parameters for M-tRNA^{Arg}, Y-tRNA^{Arg}, and derivatives, concentrations of tRNA were varied from 0.01 to 10 μM. When the initial rate of tRNA charging increased linearly with increasing tRNA up to 10 μM, indicating that this concentration is much lower than the Michaelis constant K_m for the tRNA, accurate values for the kinetic parameters could not be obtained, but the slope of the linear plot of initial rate versus tRNA concentration gave a good approximation of the catalytic efficiency (k_{cat}/K_m).

The K_i value for inhibition of M-tRNA^{Arg} aminoacylation by an RNA minihelix corresponding to the anticodon stem−loop (Ant^{Arg}) was deduced from a plot of $1/v$ versus concentration of Ant^{Arg} added in the assay.

RESULTS

Requirement of tRNA^{Arg} for Arginyl-Adenylate Synthesis. In the absence of tRNA, hamster ArgRS (hArgRS) is unable to catalyze arginyl-adenylate formation (Figure 1). When increasing amounts of a cognate mammalian tRNA^{Arg} (M-tRNA^{Arg}), tRNA^{Arg,CCU} from beef, were added to the reaction mixture, the formation of ATP in the reverse reaction was activated and reached a plateau value corresponding to the maximal activation state of the enzyme (Figure 1). The saturation curve was similar to a titration curve (Figure 1). The high affinity (K_{Act} < 3 nM) of the ligand allows an accurate estimation of the stoichiometry of the interaction, which corresponds to one mole of tRNA bound per mole of enzyme. Consequently, the activation constant (K_{Act}) is low and could not be determined with high accuracy. No ATP formation could be monitored in the presence of a noncognate tRNA. Two forms of ArgRS coexist in the cytoplasm of mammalian cells (*24*, *25*). The low-molecular-mass species lacks 73 amino acid residues at the N-terminus and is not associated within the multisynthetase complex (*26*). The two enzyme species have nondistinguishable kinetic parameters in aminoacylation and arginyl-adenylate formation (*9*, *27*). All the data reported below have been determined with the longest form of hArgRS.

Table 1: Kinetic Parameters of hArgRS in ATP−PP_i Exchange and tRNA Aminoacylation

	ATP−PP_i exchange			aminoacylation		
tRNA	k_{cat}, s^{-1}	k_{cat} relative	K_{Act}, nM	k_{cat}, s^{-1}	K_m, μM	k_{cat}/K_m relative
M-tRNA^{Arg}	4.5	1	<3.0	0.53	0.16	1
Y-tRNA^{Arg}	1.15	0.25	24	0.45	4.5	0.03
M-tRNA^{Arg,C35U}	nd[a]		nd	nd	nd	
M-tRNA$^{Arg,\Delta A76}$	nd		nd	nd	nd	
M-tRNA$^{Arg,2'3'P}$	nd		nd	nd	nd	
M-tRNA^{Arg,A20U}	0.91	0.20	25			0.016
M-tRNA$^{Arg,\Delta A20}$	1.0	0.22	77			0.016
M-tRNA$^{Arg,A20C,\Delta U20a}$	0.45	0.10	600			nt[b]

[a] Not detectable. [b] Not tested.

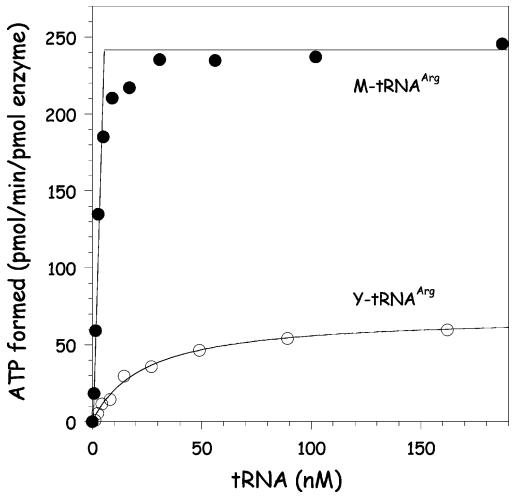

FIGURE 2: Activation of hArgRS by M-tRNA^{Arg} and Y-tRNA^{Arg}. The rate of the ATP−PP_i exchange reaction was followed in the presence of 5 nM hArgRS as a function of the concentration of M-tRNA^{Arg} or Y-tRNA^{Arg} added in the assay.

The Extent of hArgRS Activation Is Controlled by tRNA. To study the tRNA-dependent activation of the catalytic center of hArgRS, we determined whether arginine-specific tRNAs that are good or poor substrates in the aminoacylation reaction could act as potent and equal activators in the ATP−PP_i exchange reaction. Total yeast tRNA proved to be inefficiently aminoacylated by hamster ArgRS (*9*). tRNA^{Arg,UCU} from the yeast *S. cerevisiae* (Y-tRNA^{Arg}) was compared to M-tRNA^{Arg}. These two tRNAs were produced by in vitro transcription. hArgRS aminoacylated the yeast tRNA with a 33-fold lower efficiency (k_{cat}/K_m) as compared with the homologous tRNA (Table 1). This loss in catalytic efficiency resulted from an increase in K_M for tRNA. As expected, Y-tRNA^{Arg} also activated hArgRS but to a lower extent as compared with M-tRNA^{Arg} (Figure 2). In agreement with these data, hArgRS was not able to sustain growth of yeast cells carrying a null-allele of the *RRS1* gene encoding ArgRS from *S. cerevisiae* (result not shown).

The saturation curve obtained with Y-tRNA^{Arg} did not correspond to a titration curve of the enzyme, and a K_{Act} of 24 ± 2 nM could be determined (Table 1), a value at least

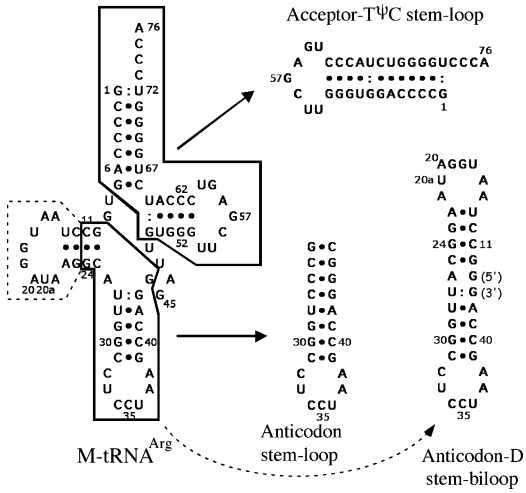

FIGURE 3: Schematic diagram of M-tRNA^{Arg} and related substrates. The sequence and cloverleaf structure of beef tRNA^{Arg,CCU} and sequence and hairpin structures of acceptor and anticodon minihelices and of anticodon-D stem−biloop. After synthesis, the 5′- and 3′-ends of the anticodon-D stem−biloop substrate were ligated with T4 RNA ligase.

10-fold higher as compared with M-tRNA^{Arg}. Interestingly, the k_{cat} obtained in the presence of saturating amounts of Y-tRNA^{Arg} was not optimal. At saturating concentrations of Y-tRNA^{Arg}, the maximal velocity of hArgRS for the formation of ATP (k_{cat} = 1.15 s^{-1}) was 4-fold lower as compared to that obtained with M-tRNA^{Arg} (k_{cat} = 4.5 s^{-1}). These results showed that tRNA^{Arg} is required for activation of the catalytic center of hArgRS and suggested that the extent of activation is a measure of the ability of the tRNA species to induce the catalytic site of the enzyme into an active conformation upon tRNA−enzyme interaction.

Arginine-Activation Requires the L-Shaped tRNA Molecule. Minihelices mimicking the acceptor-TΨC stem−loop structure of tRNA are substrates for many aminoacyl-tRNA synthetases (*30*). Thus, in some instances, the anticodon and D stem−loop domains are dispensable parts of the tRNA molecule in the aminoacylation reaction. We determined whether the addition of an RNA minihelix corresponding to the acceptor arm of M-tRNA^{Arg} could be sufficient to activate hArgRS. We prepared the RNA minihelix corresponding to the acceptor-TΨC stem−loop structure of M-tRNA^{Arg} (Figure 3) and tested it as an activator of hArgRS in the ATP−PP$_i$ exchange reaction and as a substrate in the aminoacylation assay. None of these two activities could be detected even when high concentrations of RNA (100 μM) and enzyme (0.1 μM) were added in the assays.

In yeast, binding of the anticodon of tRNA is believed to be an essential contribution to the activation of the catalytic center of ArgRS (*21*). We thus asked whether the sole addition of an RNA minihelix mimicking the anticodon stem−loop of M-tRNA^{Arg} (Figure 3) could be sufficient to activate hArgRS in the ATP−PP$_i$ exchange reaction. Although this RNA minihelix is an inhibitor of M-tRNA^{Arg} aminoacylation, with a K_i of 30 μM, we were unable to detect any activity in the ATP−PP$_i$ exchange reaction, even upon addition of 50 μM anticodon minihelix.

We next determined whether simultaneous addition of the acceptor and anticodon domains of tRNA could activate hArgRS. Addition of the two minihelices could not activate hArgRS in the ATP−PP$_i$ exchange reaction. We also

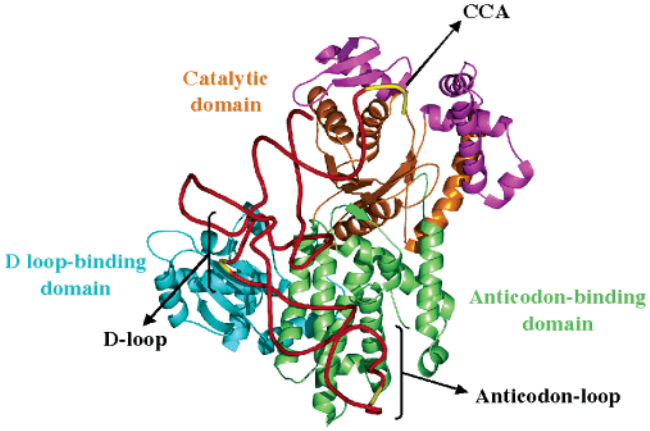

FIGURE 4: Crystal structure of the ArgRS:tRNA^{Arg} complex in yeast (*21*). The figure is drawn with PyMol (*45*).

synthesized an RNA molecule that corresponds to the anticodon-D stem−biloop structure of M-tRNA^{Arg} (Figure 3). Together, these two RNA moieties (acceptor-TΨC stem−loop and anticodon-D stem−biloop) recapitulate the complete tRNA molecule. Thus, upon addition of these two RNA domains, the full set of RNA determinants that interact with hArgRS in an ArgRS:tRNA^{Arg} complex are present but no physical link relates the two domains of the tRNA molecule. Nevertheless, we were unable to detect any activation of hArgRS even in the presence of 30 μM of the two RNA domains.

The tRNA−Protein Contact Areas Are Crucial for Arginine Activation. The crystal structure of a complex of ArgRS and tRNA^{Arg} from yeast revealed a particular feature of the ArgRS system (*21*). Recognition of tRNA involves three contact areas (Figure 4): the terminal CCA interacts with the catalytic center, the anticodon domain is bound to the α-helical C-terminal domain, and the D-loop region interacts with an additional N-terminal module. The later interaction, which involves the outer corner of the tRNA molecule as a contact area with the protein, is rather unusual among aaRSs.

To investigate the structural bases for tRNA-dependent activation of the catalytic center of hArgRS, we analyzed whether the three major contact areas are essential for tRNA aminoacylation as well as for arginyl-adenylate formation. All tRNA^{Arg} species possess a cytosine in position 35 of the anticodon loop, and C35 has been shown to be a major determinant for the identity of *E. coli* and *S. cerevisiae* tRNA^{Arg} in the aminoacylation reaction (*12*, *13*). When the mutation C35U was inserted in M-tRNA^{Arg}, the mutant tRNA (M-tRNA^{Arg,C35U}) could not be aminoacylated by hArgRS and was not able to promote activation of hArgRS as measured in the ATP−PP$_i$ exchange reaction (Table 1). We also deleted the terminal adenosine from the CCA end of M-tRNA^{Arg}. To overcome the problem of transcript heterogeneity at the 3′-end of the tRNA molecule, we introduced the hepatitis delta virus (HDV) ribozyme at the 3′-extremity of the tRNA sequence (*28*). As expected, the homogeneous M-tRNA$^{Arg,\Delta A76}$ could not be aminoacylated. Moreover, M-tRNA$^{Arg,\Delta A76}$ was also not able to promote activation of hArgRS (Table 1). To further analyze the importance of the 3′-terminal adenosine, we tested the ability of the complete tRNA^{Arg} molecule generated after transcription and cleavage with the HDV ribozyme to activate hArgRS. The tRNA^{Arg} obtained after enzymatic cleavage (M-tRNA$^{Arg,2'3'P}$) contains

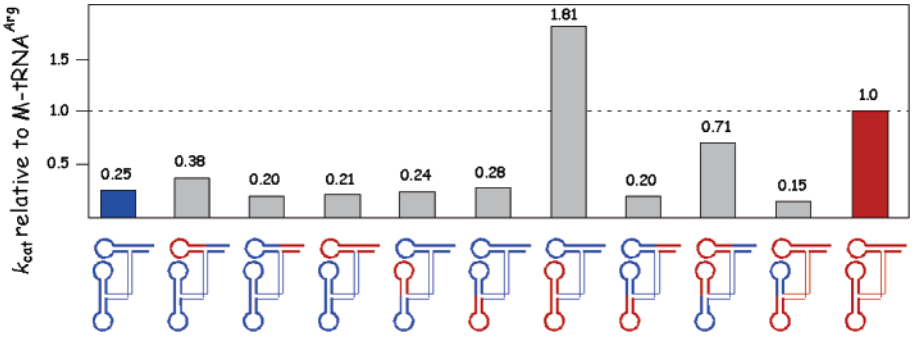

FIGURE 5: Comparison of rates of arginyl-adenylate synthesis triggered by chimeric tRNA molecules. Rates, indicated above bar graphs, are expressed relative to native M-tRNA^{Arg}. The chimeric tRNAs contain regions of M-tRNA^{Arg} (in red) and of Y-tRNA^{Arg} (in blue).

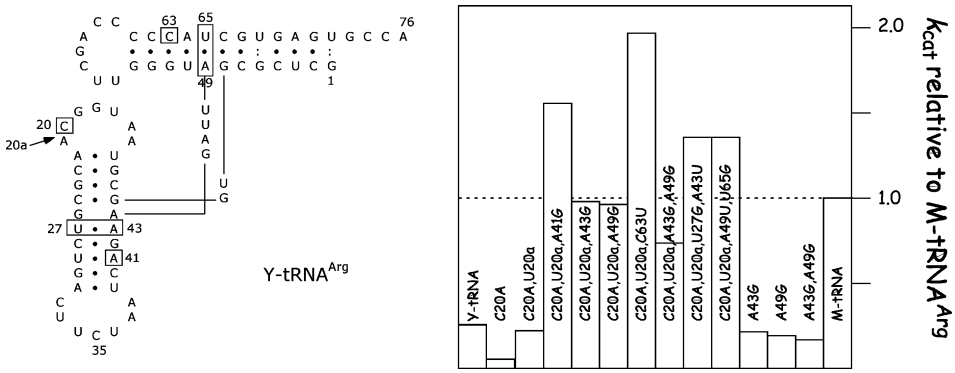

FIGURE 6: Sequence and L-shaped structure of Y-tRNA^{Arg}, and location of the mutations. The relative k_{cat} in arginyl-adenylate synthesis for various tRNA substrates are indicated.

a 2′,3′ cyclic phosphate group. It did not promote arginyl-adenylate formation (Table 1). Treatment of this tRNA with polynucleotide kinase, which restores the 2′- and 3′-OH groups of the ribose (*29*), also restored its activity. Thus, the two contact areas that are crucial for aminoacylation are also necessary to activate the catalytic center of the enzyme in the first step of the reaction.

The nucleotide A20 is a major identity element of the *E. coli* and *T. thermophilus* tRNA^{Arg} (*11*, *16*, *31*). Likewise, base substitution (A20U) or deletion (ΔA20) of the nucleotide A20 of M-tRNA^{Arg} also impaired the arginine charging activity of hArgRS by over 50-fold (Table 1). These mutations also had a deleterious effect on the ATP−PP$_i$ exchange reaction. The extent of hArgRS activation was reduced by 5-fold and attained the level observed with Y-tRNA^{Arg} (Table 1).

Which tRNA-Determinants Are Crucial for Arginine-Activation? The finding that Y-tRNA^{Arg} is less efficient than M-tRNA^{Arg} at inducing activation of hArgRS prompted us to identify the nucleotide determinants that were responsible for optimal activation of the enzyme. To gain more insight into the structural bases for tRNA-dependent activation of the catalytic center of hArgRS, we constructed a series of chimeric tRNA molecules in which domains of Y-tRNA^{Arg} and M-tRNA^{Arg} were combined (Figure 5). When the TΨC stem−loop, the acceptor stem, the D stem−loop, or the anticodon stem−loop of M-tRNA^{Arg} were independently transferred into Y-tRNA^{Arg}, no enhancement of the level of hArgRS activation was detected as compared with the tRNA template, Y-tRNA^{Arg} (Figure 5). Then, two domains of Y-tRNA^{Arg} were replaced at a time by homologous domains of M-tRNA^{Arg}. Transplantation of the acceptor-TΨC stem−loop structure or of the anticodon stem−loop and acceptor stem of M-tRNA^{Arg} into Y-tRNA^{Arg} did not yield a tRNA molecule with a higher propensity to activate hArgRS. However, when the D stem−loop of M-tRNA^{Arg} was introduced into Y-tRNA^{Arg} either with the anticodon stem−loop or with the TΨC stem−loop of M-tRNA^{Arg}, the capacity of the resulting tRNA molecules in triggering activation of hArgRS was induced by over 7-fold and 3-fold, respectively. To assess the importance of the D stem−loop of M-tRNA^{Arg} in triggering activation of hArgRS, we introduced the D stem−loop of Y-tRNA^{Arg} into M-tRNA^{Arg} (Figure 5). The D stem−loop swap was sufficient to induce a 7-fold decrease in activation of hArgRS. Altogether, these results indicate that nucleotide sequences located within the D stem−loop of M-tRNA^{Arg} are necessary but not sufficient to trigger optimal activation of hArgRS. Discrete determinants must also be located within the anticodon or TΨC stem−loops of the mammalian tRNA.

Other constructs were made to pinpoint the nucleotide determinants required for the maximal activation of hArgRS. The D loop of Y-tRNA^{Arg} contains 7 nucleotides and a C at position 20. By contrast, M-tRNA^{Arg} has an 8-nucleotide long D loop with A at position 20 and with an additional U20a nucleotide. We constructed variants of Y-tRNA^{Arg} with the mutation C20A, with or without the addition of U at position 20a. These two mutations had little effect on the capacity of Y-tRNA^{Arg} to activate hArgRS (Figure 6; Table 2), as expected from the result of the sole transplantation of the D stem−loop of M-tRNA^{Arg} into Y-tRNA^{Arg} described above (Figure 5). However, we reported above that the simultaneous addition of the anticodon stem−loop or of the TΨC stem−loop of M-tRNA^{Arg} together with the D stem−loop is required to produce an active tRNA species. Whereas these two regions of the tRNA molecule display significant

Table 2: Kinetic Parameters of hArgRS in ATP−PP_i Exchange and tRNA Aminoacylation

	ATP−PP_i exchange			aminoacylation		
tRNA	k_{cat}, s^{-1}	k_{cat} relative	K_{Act}, nM	k_{cat}, s^{-1}	K_m, μM	k_{cat}/K_m relative
M-tRNA^{Arg}	4.5	1	<3.0	0.53	0.16	1
Y-tRNA^{Arg}	1.15	0.25	24	0.45	4.5	0.03
Derivatives of Y-tRNA^{Arg}						
C20A	0.15	0.03	425	nt[a]	nt	
C20A,U20a	1.0	0.22	525	nt	nt	
A43G	0.96	0.21	27			0.021
A49G	0.86	0.19	18			0.024
A43G,A49G	0.74	0.16	18			0.020
Derivatives of Y-tRNA^{Arg,C20A,U20a}						
A41G	7.0	1.55	45	2.90	4.48	0.19
A43G	4.4	0.98	15	nt	nt	
A49G	4.3	0.96	17	nt	nt	
C63U	8.9	1.98	52	1.79	2.16	0.25
A43G,A49G	3.1	0.69	15	nt	nt	
U27G,A43U	6.1	1.35	47	2.43	3.50	0.21
A49U,U65G	6.1	1.35	47	2.97	4.61	0.19
Derivatives of M-tRNA^{Arg}						
A20U	0.91	0.20	25			0.016
ΔA20	1.0	0.22	77			0.016
A20C,ΔU20a	0.45	0.10	600	nt	nt	
G43A	2.5	0.56	<3.0	0.65	1.1	0.18
G49A	3.46	0.77	<3.0	0.78	1.0	0.24
G43A,G49A	2.88	0.64	25	nt	nt	
Derivatives of M-tRNA$^{Arg,A20C,\Delta U20a}$						
G43A	1.07	0.24	37	0.51	4.4	0.035
G49A	0.73	0.16	67	0.40	8.0	0.015
G43A,G49A	0.81	0.18	55	0.53	8.5	0.019

[a]Not tested.

differences of sequence when Y-tRNA^{Arg} and M-tRNA^{Arg} are compared, none of these nucleotides seems to be involved in a specific interaction with the synthetase (*21*). Alternatively, the presence of G:U base pairs within the anticodon and TΨC stem of M-tRNA^{Arg} but not of Y-tRNA^{Arg} prompted us to consider the possibility that the unique properties of the G:U wobble base pair might be an important structural feature required for activation of hArgRS. To test this hypothesis, we introduced into Y-tRNA^{Arg} the mutations A41G or A43G in the anticodon stem and A49G or C63U in the TΨC stem, in addition to the mutation C20A and the insertion U20a in the D loop (Figure 6; Table 2). The four constructs were fully able to activate hArgRS. Addition of two G:U base pairs in the double mutant A43G and A49G had no further effect. The polarity of the wobble base pair (G:U or U:G) was not crucial for activation of hArgRS, as judged by the activity of Y-tRNA^{Arg,C20A,U20a} carrying the additional mutations U27G:A43U or A49U:U65G (Figure 6; Table 2). As a control, Y-tRNA^{Arg} carrying the mutations A43G, A49G, or A43G and A49G in the absence of the mutation C20A and the insertion U20a did not activate hArgRS to its maximal rate (Figure 6; Table 2). Therefore, the nucleotides A20 and U20a in the D loop and a G:U wobble base pair in either the anticodon or the TΨC stem are necessary to trigger maximal activation of hArgRS. The tRNA derivatives that triggered efficient activation of hArgRS were also significantly more active in aminoacylation (6-fold increase in k_{cat}/K_m; Table 2).

In line with these data, mutants of M-tRNA^{Arg} with a deletion of the nucleotide at position 20 (ΔA20), with the mutation A20U, or with the double modification A20C,ΔU20a lost their capacity to activate hArgRS to the maximal level and mimicked Y-tRNA^{Arg} (Table 1; Figure 7). When the G:U wobble base pairs U27:G43 and/or G49:U65 from M-tRNA^{Arg} are replaced by U·A Watson−Crick base pairs in addition to the mutations A20C,ΔU20a in the D loop, the capacity of the mutant tRNAs to activate hArgRS was not further affected (Figure 7; Table 2). It is noteworthy that within the framework of M-tRNA^{Arg} containing a wild-type D loop, mutation of the G:U wobble base pairs alone had only a moderate effect on the maximal level of activation of hArgRS as measured in the ATP−PP_i exchange reaction (Figure 7; Table 2). In aminoacylation, mutation of the G:U base pairs caused a 6-fold to 53-fold increase in K_m, but the k_{cat} remained unaffected (Table 2).

DISCUSSION

For hArgRS, as for a subset of aminoacyl-tRNA synthetases of class I, GluRS, GlnRS, and LysRS-I, synthesis of the arginyl-adenylate is absolutely conditioned by the realization of a proper enzyme:tRNA complex. Thus, upon tRNA binding, the active site of these enzymes undergoes structural rearrangements that contribute a switch from an inactive to an active state. As shown in this report, this switch does not necessarily imply a complete transition from an inactive state to a fully activated state, but may be modulated. Intermediate activation states were observed when heterologous or chimeric tRNA^{Arg} was used. It should be noticed that the k_{cat} of hArgRS for the formation of ATP was 3-fold lower with the M-tRNA^{Arg} transcript as compared to natural tRNA, which suggests that modified nucleotides also contribute to the capacity of tRNA^{Arg} to activate the catalytic center of ArgRS to its maximal level (results not shown).

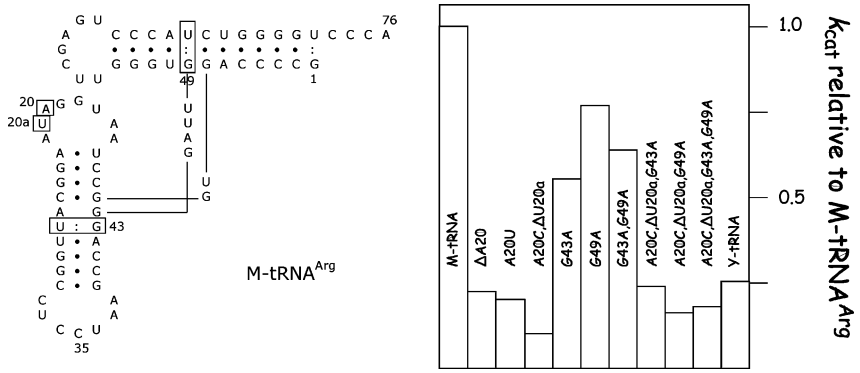

FIGURE 7: Sequence and L-shaped structure of M-tRNA^{Arg}, and location of the mutations. The relative k_{cat} in arginyl-adenylate synthesis for various tRNA substrates are indicated.

Several models should be considered to understand the role of tRNA in the tRNA-dependent activation of the catalytic center of hArgRS.

One of the first remarkable features reported here concerns the role of the terminal adenosine. A mutant tRNA with a deletion of A76 (M-tRNA$^{Arg,\Delta 76}$), or a complete tRNA molecule with A76 displaying a 2′,3′ cyclic phosphate group generated after cleavage by the HDV ribozyme, was not able to activate hArgRS. These data strongly suggest that the terminal adenosine is essential to switch the catalytic site of hArgRS to its productive mode. Previous studies have also stressed the importance of the terminal adenosine in activating *E. coli* ArgRS (*32*). Periodate-treated tRNA^{Arg} or phosphodiesterase-treated tRNA^{Arg} with a deletion of the −CCA or only of A76 failed to activate the enzyme. Crystal structures of ArgRS from *S. cerevisiae* have been obtained with L-arginine bound to the active site in the presence (*21*) or in the absence (*20*) of the tRNA molecule. Despite many attempts, cocrystals of ArgRS with ATP could not be obtained (*20*, *21*). L-Arginine is required for the correct positioning of the CCA-end of the tRNA^{Arg}, but the possible involvement of A76 in the binding of ATP remained elusive. Thus, although the binding of tRNA does induce important structural changes, the specific requirement for A76 in the control of yArgRS activation could not be explained.

In the case of GluRS, another synthetase that requires tRNA for aminoacyl-adenylate formation, cocrystals of the enzyme in the presence of ATP have been described. The comparison of the crystal structures of GluRS from *T. thermophilus* in the complex with ATP or in the complex with ATP and tRNA^{Glu} showed two distinct subsites for the binding of ATP (*33*). In the absence of tRNA^{Glu}, ATP and glutamate bind to GluRS in a nonproductive manner. In the presence of tRNA^{Glu}, the 2′-hydroxyl group of A76 directly interacts with the α- and γ-phosphates of ATP. Consequently, ATP binds to the productive subsite of the enzyme, which places the α-phosphate group of ATP in the vicinity of the α-carboxyl group of glutamate to facilitate the reaction.

Structural and mutational analyses also exemplified the crucial role of the 3′-terminal adenosine of tRNA^{Gln} in positioning ATP and glutamine in a productive conformation within the active site of *E. coli* GlnRS (*34*−*36*). GlnRS also requires tRNA for activation of glutamine. The crystal structure of *E. coli* GlnRS complexed with tRNA^{Gln} suggested that stacking of the terminal nucleotide, A76, against Tyr211 may orient the hydroxyl group in a conformation suited for binding glutamine (*36*).

Our results show that the 3′-terminal adenosine of tRNA^{Arg} also plays an active role in switching the catalytic center of hArgRS into a productive mode. A possible scenario would be that binding of L-arginine induces the correct positioning of the CCA-end of tRNA, thus enabling A76 to bind ATP in a productive manner, as observed for GluRS and GlnRS. However, despite the fact that A76 is necessary to activate hArgRS, it is not sufficient.

Several lines of evidence have suggested that binding of the complete tRNA molecule to the enzyme, and especially binding of the anticodon stem−loop to the anticodon binding domain of ArgRS and GlnRS, may produce a signal that propagates to the active site and induces its activation (*21*, *37*). It is noteworthy that *E. coli* GlnRS is able to aminoacylate a seven-base pair RNA microhelix mimicking the acceptor stem of tRNA^{Gln} (*38*) whereas hArgRS does not (this study). Furthermore, the acceptor stem of M-tRNA^{Arg} is not able to activate hArgRS in the ATP−PP$_i$ exchange reaction. Thus, even if the catalytic efficiency of GlnRS is severely impaired in the absence of the anticodon moiety of tRNA (k_{cat}/K_m reduced by over 10^7-fold), the enzyme is not completely inactive in aminoacylation. By contrast, our results suggest that binding of the full-length tRNA^{Arg} molecule is absolutely required to induce conformational changes that promote activation of ArgRS. Moreover, aminoacylation and aminoacyl adenylate formation do not seem to be strictly coupled in the GlnRS system. We showed previously that yeast tRNA and mammalian tRNA both efficiently activate human GlnRS in the ATP−PP$_i$ exchange reaction, but that yeast tRNA is a poor substrate in aminoacylation (*39*). It is remarkable that the tRNA mutants described in this study which were good activators of hArgRS were also good substrates of that enzyme in aminoacylation, suggesting that the two reactions are tightly coupled. Thus, in addition to the prerequisite of A76 in the active site of hArgRS, the building of an active enzyme also requires global conformational changes induced by binding of tRNA^{Arg}.

Comparison of the structures of unliganded enzymes and of tRNA-bound complexes revealed that tRNA binding generates significant conformational changes in ArgRS and GlnRS (*20*, *21*, *37*, *40*). It has been proposed that structural elements of ArgRS and GlnRS may transfer the signal of tRNA-binding from the anticodon to the catalytic site. Indeed, it is remarkable that the mutant tRNA^{Arg,C35U} not only abolished aminoacylation but also was unable to promote activation of hArgRS. However, it is noteworthy that the productive mode of hArgRS could not be attained by the sole addition of an RNA minihelix mimicking the anticodon stem−loop of M-tRNA^{Arg}, in the presence or in the absence of the acceptor-TΨC stem−loop structure of tRNA. Therefore, a simple tRNA:protein contact is not sufficient to trigger a conformation change to the active site. Somehow, the continuity of the tRNA molecule is a prerequisite. Similarly, *E. coli* GlnRS poorly aminoacylates an RNA microhelix mimicking the acceptor stem of tRNA^{Gln}, and addition of an anticodon microhelix did not enhance its aminoacylation (*38*).

What are the consequences of these findings on our understanding of the tRNA-induced activation of ArgRS? The 3′-terminal adenosine of tRNA and the cytosine at position 35 of the anticodon are both crucial for aminoacylation and activation of hArgRS. However, these two bases, located at the two extremities of the tRNA molecule, have to be physically linked to promote activation of the enzyme. A possible explanation would be that interaction of the anticodon of tRNA with the anticodon-binding domain of the enzyme may propagate a signal to the active site via the tRNA molecule. However, this simple model does not account for all the data available to date. In this connection, it is remarkable that mutations located at the interface of the anticodon-binding domain and the catalytic domain of hArgRS impair the catalytic parameters of the enzyme (*9*). The mutation Cys599 to Tyr is located at the N-terminal extremity of helix H22 that makes contact with the floor of the active site. This mutant showed a reduced activity in ATP−PP$_i$ exchange and tRNA aminoacylation reactions.

Consequently, the mutant strain showed a marked hyperauxotrophy for arginine (*41*). Strikingly, hArgRS^{C599Y} displayed two distinct K_m for L-arginine in aminoacylation (1.8 μM and 141 μM at 37 °C, as compared to 2.4 μM for the wild-type enzyme). One possible explanation to this finding could be that the population of hArgRS^{C599Y} is heterogeneous, displaying two activation states of the catalytic center. These results suggested that the conformational changes induced in the active site by binding of the tRNA to the anticodon-binding domain of the synthetase may be rate limiting in the mutant enzyme. These results also suggested that the building of a productive active site involves interdomain communication in hArgRS.

The requirement for a complete tRNA molecule, as well as the suggestion that transduction of the signal of tRNA-binding to the active site rests on the ability of the enzyme to achieve a proper conformational change that implies interdomain communication, supports an induced fit model for hArgRS activation. This model assumes that the substrate and the enzyme both undergo conformational changes following the initial recognition step. In the case of the tRNA^{Arg}:ArgRS complex, this model would require that the two partners are able to undergo concerted conformational changes to reach the most favorable final activation state. In that connection, it is worth mentioning that the interaction between nucleotide at position 20 and the N-terminal module of the protein is not essential to produce an active enzyme but is important to modulate the efficiency of activation of hArgRS. Our data are consistent with the interpretation according to which activation of hArgRS is a two step process: (i) initial pairing of the tRNA and the protein and (ii) conformational adjustment of the two partners via an induced fit mechanism. The three major points of contact between tRNA^{Arg} and hArgRS (A20, C35, and A76) are involved in building a productive active site, but the efficiency of this process rests on the accurate presentation of these three determinants to the enzyme, that is on the geometry of the tRNA molecule, but also seems to require some flexibility of the tRNA molecule and of the protein to achieve the correct final adjustment leading to the productive conformation of the active site of the enzyme. The flexibility of the tRNA molecule may be correlated with the occurrence of U:G base pairs. The anticodon and TΨC stems of tRNA^{Arg} with a conserved A_{20} nucleotide invariably possess a U:G base pair at variable positions. Because this base pair is found at variable positions, its conservation is probably not related to a specific RNA−protein interaction, but may be the consequence of an important structural role. Accordingly, no specific contact between the enzyme and the TΨC and anticodon stems of tRNA^{Arg} were depicted within the crystal structure of the complex formed by Y-ArgRS with its cognate tRNA (*20*). A G:U base pair induces an overwinding of the helix and provides more conformational flexibility to the RNA double helix (*42*). Because G:U and U:G base pairs are equally efficient to promote hArgRS activation, it seems unlikely that overwinding of the acceptor-TΨC stem or of the anticodon-D stem helices of M-tRNA^{Arg} contributes a pivotal element of ArgRS activation. However, increased flexibility of the RNA helix associated with the presence of a G:U or U:G base pair may allow for a better recognition by induced fit.

Finally, it is worth mentioning that tRNA-dependent amino acid activation is not a prerequisite for catalysis by active sites of paralog enzymes. The *E. coli* YadB gene product aminoacylates queuosine at position 34 of the anticodon of tRNA^{Asp} with glutamate (*43*, *44*). This protein, a paralog of GluRS deprived of the anticodon binding domain characteristic of all GluRSs, was shown to be active in amino acid activation and aminoacylation. The first step of the reaction can be accomplished in the absence of tRNA. Further understanding of the atomic features involved in generating the catalytically productive active site of GluRS, and of the other tRNA-dependent enzymes GlnRS and ArgRS, would benefit from the knowledge of the mutations that have been introduced into a GluRS-like domain to switch the Rossman fold of YadB into a constitutively activated conformation.

ACKNOWLEDGMENT

We thank G. L. Conn (UMIST, Manchester) for the gift of the pRZ vector, G. Eriani (IBMC, Strasbourg) for the diploid yeast strain with a null-allele of *RRS1*, and W. Studier (Brookhaven National Laboratory) for the BL21/pAR1219 strain.

REFERENCES

1. Ibba, M., and Söll, D. (2000) Aminoacyl-tRNA synthesis, *Annu. Rev. Biochem. 69*, 617−650.
2. Zhang, C. M., and Hou, Y. M. (2005) Domain-domain communication for tRNA aminoacylation: The importance of covalent connectivity, *Biochemistry 44*, 7240−7249.
3. Buechter, D. D., and Schimmel, P. (1993) Dissection of a Class-II tRNA synthetase−Determinants for minihelix recognition are tightly associated with domain for amino acid activation, *Biochemistry 32*, 5267−5272.
4. Mitra, K., and Mehler, A. H. (1966) The role of transfer ribonucleic acid in the pyrophosphate exchange reaction of arginine-transfer ribonucleic acid synthetase, *J. Biol. Chem. 241*, 5161−5162.
5. Parfait, R., and Grosjean, H. (1972) Arginyl-transfer ribonucleic-acid synthetase from *Bacillus stearothermophilus*. Purification, properties and mechanism of action, *Eur. J. Biochem. 30*, 242−249.
6. Char, S., and Gopinathan, K. P. (1986) Arginyl-tRNA synthetase from *Mycobacterium smegmatis* SN2: purification and kinetic mechanism, *J. Biochem. 100*, 349−357.
7. Gangloff, J., Schutz, A., and Dirheimer, G. (1976) Arginyl-tRNA synthetase from baker's yeast. Purification and some properties, *Eur. J. Biochem. 65*, 177−182.
8. Nazario, M., and Evans, J. A. (1974) Physical and kinetic studies of arginyl transfer ribonucleic acid ligase of Neurospora. A sequential ordered mechanism, *J. Biol. Chem. 249*, 4934−4936.
9. Lazard, M., Kerjan, P., Agou, F., and Mirande, M. (2000) The tRNA-dependent activation of arginine by arginyl-tRNA synthetase requires inter-domain communication, *J. Mol. Biol. 302*, 991−1004.
10. Fersht, A. R., Gangloff, J., and Dirheimer, G. (1978) Reaction pathway and rate-determining step in the aminoacylation of tRNA^{Arg} catalyzed by arginyl-tRNA synthetase from yeast, *Biochemistry 17*, 3740−3746.
11. McClain, W. H., and Foss, K. (1988) Changing the acceptor identity of a transfer RNA by altering nucleotides in a "variable pocket", *Science 241*, 1804−1807.
12. Schulman, L. H., and Pelka, H. (1989) The anticodon contains a major element of the identity of arginine transfer RNAs, *Science 246*, 1595−1597.
13. Sissler, M., Giegé, R., and Florentz, C. (1996) Arginine aminoacylation identity is context-dependent and ensured by alternate recognition sets in the anticodon loop of accepting tRNA transcripts, *EMBO J. 15*, 5069−5076.
14. Sissler, M., Giegé, R., and Florentz, C. (1998) The RNA sequence context defines the mechanistic routes by which yeast arginyl-tRNA synthetase charges tRNA, *RNA 4*, 647−657.

15. Liu, W., Huang, Y. W., Eriani, G., Gangloff, J., Wang, E. D., and Wang, Y. L. (1999) A single base substitution in the variable pocket of yeast $tRNA^{Arg}$ eliminates species-specific aminoacylation, *Biochim. Biophys. Acta 1473*, 356−362.
16. Shimada, A., Nureki, O., Goto, M., Takahashi, S., and Yokoyama, S. (2001) Structural and mutational studies of the recognition of the arginine tRNA-specific major identity element, A20, by arginyl-tRNA synthetase, *Proc. Natl Acad. Sci. U.S.A. 98*, 13537−13542.
17. Geslain, R., Martin, F., Camasses, A., and Eriani, G. (2003) A yeast knockout strain to discriminate between active and inactive tRNA molecules, *Nucleic Acids Res. 31*, 4729−4737.
18. Geslain, R., Martin, F., Delagoutte, B., Cavarelli, J., Gangloff, J., and Eriani, G. (2000) In vivo selection of lethal mutations reveals two functional domains in arginyl-tRNA synthetase, *RNA 6*, 434−448.
19. Geslain, R., Bey, G., Cavarelli, J., and Eriani, G. (2003) Limited set of amino acid residues in a class Ia aminoacyl-tRNA synthetase is crucial for tRNA binding, *Biochemistry 42*, 15092−15101.
20. Cavarelli, J., Delagoutte, B., Eriani, G., Gangloff, J., and Moras, D. (1998) L-Arginine recognition by yeast arginyl-tRNA synthetase, *EMBO J. 17*, 5438−5448.
21. Delagoutte, B., Moras, D., and Cavarelli, J. (2000) tRNA aminoacylation by arginyl-tRNA synthetase: induced conformations during substrates binding, *EMBO J. 19*, 5599−5610.
22. Yao, Y. N., Zhang, Q. S., Yan, X. Z., Zhu, G., and Wang, E. D. (2003) Substrate-induced conformational changes in *Escherichia coli* arginyl-tRNA synthetase observed by F-19 NMR spectroscopy, *FEBS Lett. 547*, 197−200.
23. Yao, Y. N., Zhang, Q. S., Yan, X. Z., Zhu, G., and Wang, E. D. (2004) *Escherichia coli* tRNA(4)(Arg)(UCU) induces a constrained conformation of the crucial Omega-loop of arginyl-tRNA synthetase, *Biochem. Biophys. Res. Commun. 313*, 129−134.
24. Vellekamp, G., Sihag, R. K., and Deutscher, M. P. (1985) Comparison of the complexed and free forms of rat liver arginyl-tRNA synthetase and origin of the free form, *J. Biol. Chem. 260*, 9843−9847.
25. Cirakoglu, B., and Waller, J. P. (1985) Multiple forms of arginyl- and lysyl-tRNA synthetases in rat liver: a re-evaluation, *Biochim. Biophys. Acta 829*, 173−179.
26. Lazard, M., and Mirande, M. (1993) Cloning and analysis of a cDNA encoding mammalian arginyl-tRNA synthetase, a component of the multisynthetase complex with a hydrophobic N-terminal extension, *Gene 132*, 237−245.
27. Guigou, L., Shalak, V., and Mirande, M. (2004) The tRNA-interacting factor p43 associates with mammalian arginyl-tRNA synthetase but does not modify its tRNA aminoacylation properties, *Biochemistry 43*, 4592−4600.
28. Walker, S. C., Avis, J. M., and Conn, G. L. (2003) General plasmids for producing RNA in vitro transcripts with homogeneous ends, *Nucleic Acids Res. 31*, e82.
29. Schürer, H., Lang, K., Schuster, J., and Mörl, M. (2002) A universal method to produce *in vitro* transcripts with homogeneous 3′ ends, *Nucleic Acids Res. 30*, e56.
30. Beuning, P. J., and Musier-Forsyth, K. (1999) Transfer RNA recognition by aminoacyl-tRNA synthetases, *Biopolymers 52*, 1−28.
31. Tamura, K., Himeno, H., Asahara, H., Hasegawa, T., and Shimizu, M. (1992) *In vitro* study of *E. coli* $tRNA^{Arg}$ and $tRNA^{Lys}$ identity elements, *Nucleic Acids Res. 20*, 2335−2339.
32. Mehler, A. H., and Mitra, S. K. (1967) The activation of arginyl transfer ribonucleic acid synthetase by transfer ribonucleic acid, *J. Biol. Chem. 242*, 5495−5499.
33. Sekine, S., Nureki, O., Dubois, D. Y., Bernier, S., Chenevert, R., Lapointe, J., Vassylyev, D. G., and Yokoyama, S. (2003) ATP binding by glutamyl-tRNA synthetase is switched to the productive mode by tRNA binding, *EMBO J. 22*, 676−688.
34. Perona, J. J., Rould, M. A., and Steitz, T. A. (1993) Structural basis for tRNA aminoacylation by *Escherichia coli* glutaminyl-tRNA synthetase, *Biochemistry 32*, 8758−8771.
35. Liu, J. H., Ibba, M., Hong, K. W., and Söll, D. (1998) The terminal adenosine of $tRNA^{Gln}$ mediates tRNA-dependent amino acid recognition by glutaminyl-tRNA synthetase, *Biochemistry 37*, 9836−9842.
36. Rath, V. L., Silvian, L. F., Beijer, B., Sproat, B. S., and Steitz, T. A. (1998) How glutaminyl-tRNA synthetase selects glutamine, *Structure 6*, 439−449.
37. Sherlin, L. D., and Perona, J. J. (2003) tRNA-dependent active site assembly in a class I aminoacyl-tRNA synthetase, *Structure 11*, 591−603.
38. Wright, D. J., Martinis, S. A., Jahn, M., Söll, D., and Schimmel, P. (1993) Acceptor stem and anticodon RNA hairpin helix interactions with glutamine tRNA synthetase, *Biochimie 75*, 1041−1049.
39. Agou, F., Quevillon, S., Kerjan, P., and Mirande, M. (1998) Switching the amino acid specificity of an aminoacyl-tRNA synthetase, *Biochemistry 37*, 11309−11314.
40. Rould, M. A., Perona, J. J., Söll, D., and Steitz, T. A. (1989) Structure of *E. coli* glutaminyl-tRNA synthetase complexed with $tRNA^{Gln}$ and ATP at 2.8 Å resolution, *Science 246*, 1135−1142.
41. Thompson, L. H., Lofgren, D. J., and Adair, G. M. (1977) CHO cell mutants for arginyl-, asparaginyl-, glutaminyl-, histidyl- and methionyl-tRNA synthetases: identification and initial characterization, *Cell 11*, 157−168.
42. Varani, G., and McClain, W. H. (2000) The G·U wobble base pair. A fundamental building block of RNA structure crucial to RNA function in diverse biological systems, *EMBO Rep. 1*, 18−23.
43. Salazar, J. C., Ambrogelly, A., Crain, P. F., McCloskey, J. A., and Söll, D. (2004) A truncated aminoacyl-tRNA synthetase modifies RNA, *Proc. Natl Acad. Sci. U.S.A. 101*, 7536−7541.
44. Blaise, M., Becker, H. D., Keith, G., Cambillau, C., Lapointe, J., Giegé, R., and Kern, D. (2004) A minimalist glutamyl-tRNA synthetase dedicated to aminoacylation of the $tRNA^{Asp}$ QUC anticodon, *Nucleic Acids Res. 32*, 2768−2775.
45. DeLano, W. L. (2002) *ThePyMOL Molecular Graphics System*, DeLano Scientific, San Carlos, CA, http://www.pymol.org., accessed July 2005.

BI051575H