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Biochemistry

Chemical probing of the tRNA–ribosome complex

(peptidyl site/dimethyl sulfate/diethyl pyrocarbonate/site-specific protection-enhancement/site-specific inactivation)

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ABSTRACT **We probed the (*Escherichia coli*) $tRNA^{Phe}$–ribosome interaction with the chemical reagents dimethyl sulfate and diethyl pyrocarbonate. This monitored the higher-order structure of the tRNA in this biological complex and identified critical sites in the tRNA molecule involved in binding to the ribosome. The methylation of the N-7 position of guanosine and the N-3 position of cytidine as well as diethyl pyrocarbonate attack on adenosines are sensitive to secondary and tertiary interactions. Here we identify specific bases in *E. coli* Phe-$tRNA^{Phe}$ affected by the interaction with the ribosome. The 70S ribosome protects the N-3 position of cytidine-74 and 75 in the 3′-terminal C-C-A, suggesting a strong, possibly base pairing, interaction between the ribosome and that universal sequence. The ribosome also induces strong reactivities at the N-7 positions of G-24 and G-46 in the central region of the tRNA molecule near the variable-loop domain as well as less significant reactivities at 11 other guanosines. Two of these, G-10 and G-44, are close to G-24 and G-46 in the center of the molecule; the others (guanosines 1, 5, 6, 18, 19, 63, 65, 69, and 71) are in the coaxial acceptor stem-T stem helix. All of the effects are ribosome induced and occur in the presence or absence of the messenger poly(U). Prior chemical modification of the anticodon bases as well as the two adjacent 3′ purines and, less effectively, four purines in the anticodon stem prevent stable poly(U)-directed ribosome binding. Thus, we identify the 3′ terminal C-C-A sequence, near the peptidyl transferase site, and the anticodon stem and loop of $tRNA^{Phe}$ as forming critical contacts with the ribosome. Other regions of the molecule become reactive on ribosome binding, but these do not suggest a significant conformational change being more likely due to a change of environment.**

To function as the universal adaptor molecule, tRNA must interact faithfully and specifically with the ribosome as well as with the mRNA. However, we do not yet understand how the ribosome both discriminates among and binds many different tRNA molecules at common sites. The process of discrimination is the codon–anticodon interaction, but the process of general recognition at common sites is less clear.

Here we examine (*Escherichia coli*) $tRNA^{Phe}$ with chemical probes and look for changes in reactivities when it binds nonenzymatically to the ribosomal peptidyl site under the direction of poly(U) (1, 2). We probe this interaction in two ways with the base-specific chemical reagents dimethyl sulfate and diethyl pyrocarbonate (3). First, we use these reagents to monitor site-specific chemical reactivity of the tRNA bound to the ribosome. Dimethyl sulfate monitors tertiary interactions at the N-7 position of guanosines and both secondary (base pairing) and tertiary interactions at the N-3 position of cytidines. Diethyl pyrocarbonate detects the stacking of adenosines resulting from base pairing or tertiary interactions. Thus, we can observe shielding and perturbation of these sites when the tRNA is bound to the ribosome. Alternatively, we can lightly damage the tRNA chemically (4) and then select those molecules that can still bind stably to the ribosome in the presence of poly(U).

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In both cases, the chemical reactions weaken or rupture the glycosyl bond between the base and the ribosyl moiety of the polynucleotide, allowing subsequent strand scission only at the modified sites. Thus, using tRNA terminally labeled with ^{32}P, we fractionate the structure-specific fragments by size on a polyacrylamide gel and display them by autoradiography. In this way, we have detected effects of the ribosome on a bound tRNA and have located bases crucial to a stable tRNA–mRNA–ribosome interaction.

MATERIALS AND METHODS

$tRNA^{Phe}$. (*E. coli*) $tRNA^{Phe}$ from Boehringer Mannheim was repurified and radioactively labeled as described (3). The $tRNA^{Phe}$ was acylated in 100 mM Tris·HCl, pH 7.2/100 mM KCl/20 mM $MgCl_2$/15 mM 2-mercaptoethanol/5 mM ATP (5), and, when desired, the Phe-$tRNA^{Phe}$ was acetylated with acetic anhydride (6). $tRNA^{Phe}$ was eluted from benzoylated DEAE-cellulose (Boehringer Mannheim) with buffer A (50 mM NaOAc, pH 5.0/10 mM $MgCl_2$/1.0 M NaCl); Phe-$tRNA^{Phe}$ was eluted with buffer A/7% ethanol; and acetylated Phe-$tRNA^{Phe}$ was eluted with buffer A/15% ethanol (7, 8). The A_{260} was measured, and the tRNA was stored at −20°C in 25 mM NaOAc, pH 5.0/10 mM $MgCl_2$.

Tight-Couple 70S Ribosomes. Freeze-thaw-lysozyme lysis (9) was used to disrupt 2 g of *E. coli* MRE600 cells (MRE600 strain was kindly provided by R. Reed), except that 20 μg of DNase (Worthington; deoxyribonuclease I, RNase-free) was added with the sodium deoxycholate. Tight-couple 70S ribosomes were isolated on 5–20% sucrose gradients in 100 mM NH_4Cl/10 mM 2-mercaptoethanol/20 mM Tris·HCl, pH 7.5 (buffer B)/6 mM $MgCl_2$ (10), pelleted in buffer B/10 mM $MgCl_2$, and divided into aliquots and stored at −70°C in 100 mM potassium cacodylate, pH 7.2/10 mM $MgCl_2$. The final concentration of the ribosomes was 262 A_{260} units/ml. To thaw before use, the ribosomes were incubated at 37°C for 15 min.

Chemical Probing Experiments. Both acetylated and nonacetylated Phe-$tRNA^{Phe}$ were used for the probing experiments. tRNA–ribosome binding reactions were done in 25–50 μl of buffer C (100 mM potassium cacodylate, pH 7.2/50 mM NH_4Cl/10 mM $MgCl_2$) using 8 pmol of Phe-$tRNA^{Phe}$ and 20 pmol of tight-couple 70S ribosomes. If poly(U) was present, 20 μg (potassium salt, M_r >100,000) was used. After binding for 15 min at 37°C, 100 mM potassium cacodylate, pH 7.2/10 mM $MgCl_2$ was added, and then the bound tRNA–complexes were modified with 1 μl of dimethyl sulfate or 10 μl of diethyl pyrocarbonate. The final volume of the dimethyl sulfate reaction mixture (37°C, 5 min) was 200 μl; the final volume of the diethyl pyrocarbonate reaction mixture (37°C, 10 min) was 100 μl. The modification reactions were terminated by ethanol precipitation and phenol extraction, and then strand scission was induced (3). The samples were subjected to electrophoresis on denaturing gels, and relative reactivities were determined by scanning the autoradiographs with an Ortec 4310 densitometer.

Abbreviation: ms^2i^6A37, 2-methylthio-N^6-isopentenyl A-37.

Damaged tRNA Selection Experiments. The purines of the acetylated Phe-tRNA^{Phe} (6) were carbethoxylated under denaturing conditions (4). The tRNA was incubated at 90°C for 2 min in 200 μl of 50 mM NaOAc, pH 5.3/1 mM EDTA and then chilled on ice. Then, 1 μl of diethyl pyrocarbonate was added, the reaction mixture was incubated at 90°C for 5 min, and the RNA was precipitated with ethanol. This treatment did not significantly remove tritiated phenylalanine from Phe-tRNA^{Phe} as assayed by Cl_3CCOOH precipitation. The modified tRNA was renatured in buffer C for 20 min at 37°C, and then 70S ribosomes and poly(U) were added. After 15 min at 37°C, the stable tRNA–ribosome complexes were selected by nitrocellulose filtration (11). The 13-mm filters were prewashed with buffer C and rinsed twice with 500-μl aliquots of buffer C after filtering the reaction mixture. The tRNA was eluted from the filters by washing them in 400 μl of 0.5 M NH_4OAc/0.1 mM EDTA/0.1% $NaDodSO_4$. Ribosomal proteins were removed by phenol extraction, and the tRNA was precipitated with 3 vol of cold ethanol before strand scission (4) was induced.

RESULTS

tRNA–Ribosome Complex Formation. To compare the chemical reactivity of free tRNA with that of tRNA bound to ribosomes, we must be sure that the tRNA binds quantitatively to the ribosomes. In initial experiments, we used acetylated Phe-tRNA^{Phe} to ensure binding to the peptidyl site. However, this had two drawbacks: the acetylation with acetic anhydride (6) consistently (*i*) prevented 25–30% of the tRNA molecules from forming stable tRNA–mRNA–ribosome complexes and (*ii*) modified G-34, making it sensitive to the strand-scission reaction. Further experiments with nonacetylated Phe-tRNA^{Phe}, which binds preferentially to the peptidyl site in the presence of excess ribosomes (1, 2), were clearer. Qualitatively, the ribosome-induced chemical modification pattern is the same for acetylated and nonacetylated Phe-tRNA^{Phe}.

To form the (*E. coli*) Phe-tRNA^{Phe}–ribosome complexes, we incubated ^{32}P-terminally-labeled aminoacylated tRNA^{Phe} with a 2- or 3-fold excess of 70S tight-couple ribosomes (10) and poly(U). To probe these complexes chemically, we then added dimethyl sulfate or diethyl pyrocarbonate. We obtained sucrose sedimentation profiles showing that the stable phe-tRNA^{Phe}–ribosome complex requires poly(U) (Fig. 1 *A* and *B*) and that the chemical modifications do not disrupt this complex (Fig. 1*C*). Virtually all of the tRNA is ribosome bound and polyacrylamide gel electrophoresis (data not shown) shows that it is not crosslinked to the ribosomes by the chemical modifications. Although this experiment shows only that the tRNA is bound to a site on the ribosome and that poly(U) is required to form a stable complex, the Phe-tRNA^{Phe} most likely binds to the ribosomal peptidyl site under these nonenzymatic binding conditions in the presence of 10 mM Mg^{2+} (1, 2). After limited chemical modifications, we terminated the reactions by precipitating the tRNA–ribosome complexes with ethanol, extracted each sample with neutralized phenol, reprecipitated the RNA, and then induced strand scission at the modified bases (3). We located and identified the sites of strand scission by polyacrylamide gel electrophoresis and autoradiography (Fig. 2).

Bases Affected by the tRNA–Ribosome Interaction. Free in solution, Phe-tRNA^{Phe} is remarkably resistant to chemical modification (see Fig. 2*a*). We detected no modification at adenosine or guanosine residues and only two modifications at cytidine residues: cytidine-75 and 74 in the single-stranded C-C-A terminus are methylated at their free N-3 positions when the tRNA is free in solution. We obtained an identical pattern in the presence of poly(U) alone (data not shown).

The cytidine lanes (Fig. 2 *b* and *c*) show that the tRNA-ribosome interaction protects C-75 and, less effectively (80%), C-74 from chemical modification. No cytidines become reactive to dimethyl sulfate; thus, none becomes unpaired and accessible as a result of the interaction.

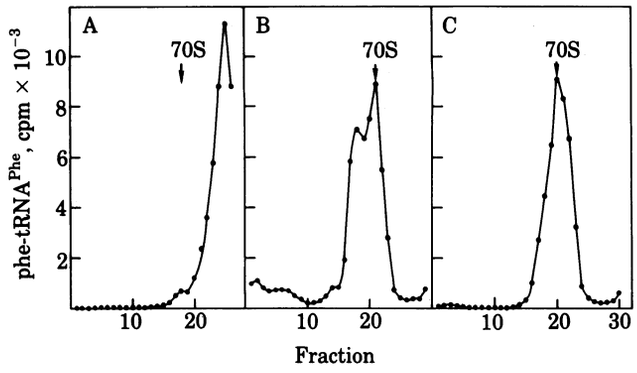

FIG. 1. Sucrose sedimentation profiles of ^{32}P-labeled (*E. coli*) Phe-tRNA^{Phe} and 70S ribosomes. The Phe-tRNA^{Phe}–ribosome complex dissociates in the absence of poly(U) (*A*) but is stable in its presence (*B*). Alkylation with dimethyl sulfate (*C*) does not disrupt the stable Phe-tRNA^{Phe} complex; identical results were obtained when stable tRNA–ribosome complexes were modified with diethyl pyrocarbonate. Disomes sedimented slightly faster than the 70S monosomes (*B*). The disappearance of the disomes in the dimethyl sulfate-treated sample is most likely due to slight ribonuclease activity during the reaction. The sucrose gradients (3.8 ml of 5–20% in buffer B/10 mM $MgCl_2$) were run at 4°C for 3 hr at 23,000 rpm in a Beckman model L2-65B ultracentrifuge using an SW 60 rotor. The fractions were ≈140 μl. The 70S ribosomes were positioned by their A_{260} profile.

We detected no increased reactivities at internal adenosines on ribosome-bound tRNA; thus, there is no obvious unstacking of the adenosines. We could not monitor any possible protection at A-76 because the label is 5′ to the 3′ terminal adenosine; no short fragment with the 3′ terminal ^{32}P label was released.

The binding to 70S ribosomes greatly increased the reactivities of guanosines 24 and 46 in Phe-tRNA^{Phe} (see Fig. 2 *b* and *c*). The increase in reactivity at G46 was surprising because the (*E. coli*) tRNA^{Phe} sequence (12) identifies residue 46 as a 7-methylguanosine, and this would preclude enhancement due to chemical methylation. In fact, this site is only partially methylated *in vivo* (13). We found, by successive chemical strand scission of unmodified tRNA^{Phe}, that, in our sample, only ≈30% of the tRNA molecules were methylated at G-46. We also found that the sodium borohydride-aniline strand-scission reaction is ≈70% effective. After dimethyl sulfate modification of ribosome-bound tRNA, 75–85% of the N-7 positions of G-46 were methylated. (This is not due to selection of molecules with 7-methylguanosines because all the tRNA is bound and examined.) Thirty–35% of the ribosome-bound tRNA molecules were methylated at G-24.

Several other guanosines were also affected by interaction with the ribosome. Fig. 2, longer polyacrylamide gel runs (not shown), and studies with 5′-end-labeled material (not shown) reveal 11 additional reactive guanosines. Of these, G-1, G-18, and G-71 are the most reactive. Guanosines 5, 6, 10, 19, 44, 63, 65, and 69 are only slightly reactive. Because 3′-end-labeled and 5′-end-labeled material produce complementary modification patterns, we conclude that modification at one site is independent of modification at any other site.

Sites Required for Stable tRNA-Ribosome Binding. To locate sites on the tRNA crucial for functional interaction with the ribosome, we (*i*) modified adenosines and guanosines of denatured *N*-acetyl-Phe-tRNA^{Phe} with diethyl pyrocarbonate at 90°C, (*ii*) renatured the *N*-acetyl-Phe-tRNA^{Phe} and then bound it to poly(U)-directed ribosomes, and (*iii*) selected stable tRNA–mRNA–ribosome complexes by nitrocellulose filtration

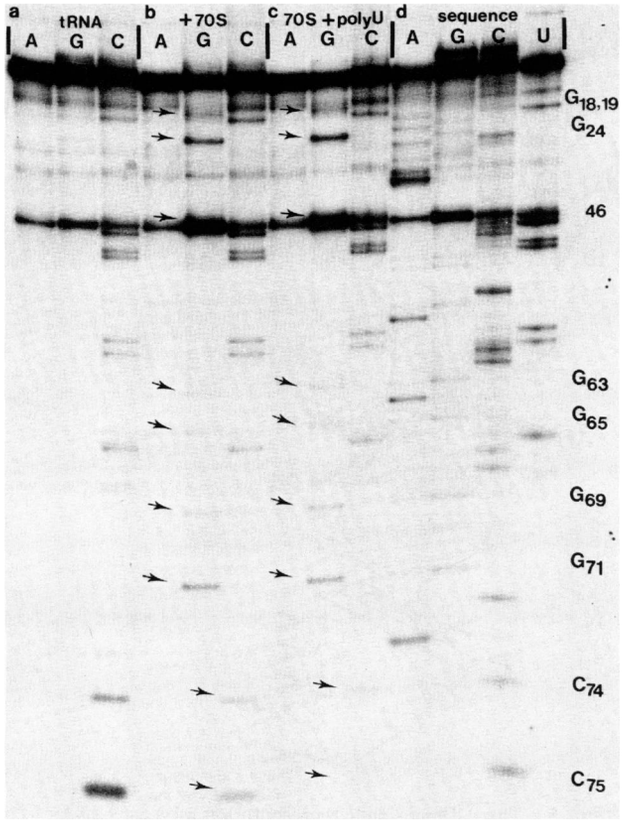

FIG. 2. Chemical probing shows that tRNA sites are affected by the tRNA–ribosome interaction. Reactivities of the adenosine, guanosine, and cytidine residues of (*E. coli*) Phe-tRNA^{Phe} are shown under three different conditions: (*a*) free in solution, (*b*) in the presence of 70S tight-couple ribosomes, and (*c*) in the presence of 70S tight-couple ribosomes and poly(U). The chemical sequence-determination reactions (3) locate each of these residues within the tRNA^{Phe} nucleotide sequence. The greatest effects are the ribosome-induced protection of C-75 and C-74 and enhanced reactivity of G-46 and G-24. Increased reactivities are also seen at G-71, G-69, G-65, G-63, and G-18. Guanosines 44, 19, 10, 6, 5, and 1 also have enhanced reactivities and are resolved more clearly by other gel runs (not shown). The uridine-specific bands in the cytidine lanes are due to the hydrazine treatment and not to the preliminary modification with dimethyl sulfate. The Phe-tRNA^{Phe} was probed free in solution or in the presence of ribosomes at 37°C in 100 mM potassium cacodylate, pH 7.2/12.5 mM NH_4Cl/10 mM $MgCl_2$. The Cerenkov radioactivity of each sample was equalized before electrophoresis on 20% polyacrylamide gels (3); the gel for this autoradiograph was subjected to electrophoresis at 2 kV for ≈2.5 hr. The nucleotides are numbered in the 5′ to 3′ direction.

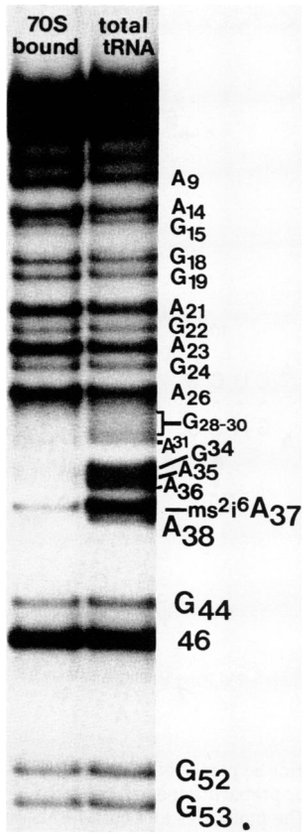

FIG. 3. A ribosome-binding-selection experiment shows sites that are crucial for a stable poly(U)-directed tRNA–ribosome interaction. If A-38, ms^{2}i^{6}A-37, A-36, or A-35 is damaged by diethyl pyrocarbonate or if G-34 is damaged by the acetic anhydride treatment prior to ribosome binding, *N*-acetyl-Phe-tRNA^{Phe} does not form a stable tRNA–ribosome complex. The modification of G-28–30 or A-31 also appears to affect the binding to the ribosome, but these sites are very resistant to modification and the effect is less dramatic. Other chemically modified adenosines and guanosines do not affect the stability of the complex as detected by nitrocellulose filtration (11). The filter-bound tRNA was eluted and cleaved at its damaged sites before polyacrylamide gel electrophoresis as described in the legend to Fig. 2. The nucleotides are numbered in the 5′ to 3′ direction.

(11). The chemical modification was limited; on average, fewer than one purine per tRNA molecule was damaged. We then dissociated the tRNA from the tRNA–mRNA–ribosome complexes and induced chemical strand scission at the damaged bases.

We found that most of the adenosines and guanosines in tRNA^{Phe} could be modified with diethyl pyrocarbonate without blocking the formation of stable poly(U)-directed tRNA–ribosome complexes (Fig. 3). However, modification of A-38, 2-methylthio-N^6-isopentenyl A-37 (ms^{2}i^{6}A-37), A-36, A-35, or G-34 prevented a stable tRNA–mRNA–ribosome interaction. In the course of these experiments, we observed that the acetic anhydride reaction (6) modifies G-34 and renders this position sensitive to aniline-induced strand scission. Thus, the strong cleavage at G-34 in the control (total tRNA) lane in Fig. 3 results largely from the acetylation reaction rather than from the diethyl pyrocarbonate modification. Nonetheless, the experiment shows that damage to G-34 blocks the poly(U)-dependent binding. Were the adenosine modifications dependent on the modification of G-34? A separate experiment with *nonacetylated* Phe-tRNA^{Phe}, 5′ terminally labeled, showed that it is the independent diethyl pyrocarbonate modification of residues A-38, ms^{2}i^{6}A-37, A-36, and A-35 that inactivates the tRNA.

Carbethoxylation of A-31 and G-28–30 also prevents formation of a stable tRNA–mRNA–ribosome complex. This inactivation is less dramatic than that seen for the anticodon loop purines. These stem residues were only slightly modified under our conditions; thus, it is more difficult to observe this inactivation. No other modified purines inactivate the binding of tRNA to the ribosomes under our conditions.

The results of the probing and selection experiments are summarized in Figs. 4 and 5. Fig. 4 depicts the protected cytidines, the reactive guanosines, and the crucial anticodon loop and stem purines within the nucleotide sequence of (*E. coli*) tRNA^{Phe} (13). These bases are located within the three-dimensional structure of yeast tRNA^{Phe} shown in Fig. 5 (15).

DISCUSSION

In the presence of excess ribosomes and poly(U), Phe-tRNA^{Phe} is quantitatively and tightly bound to the 70S ribosome nonenzymatically, most likely at the peptidyl site (1, 2). We have analyzed this interaction with two types of experiments: chemical

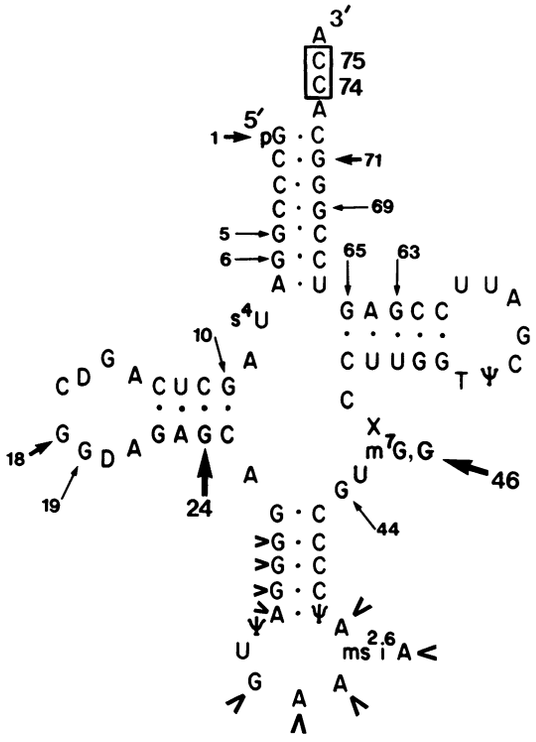

FIG. 4. Nucleotide sequence of (*E. coli*) tRNA^{Phe} (12) in the clover leaf form (14). In our preparation of (*E. coli*) tRNA^{Phe}, G-46 was methylated in ≈30% of the molecules and both forms are shown in the figure. Arrows denote those bases whose reactivity is increased when the tRNA binds to the ribosome. The two boxed cytosines are protected by the ribosome. If any of the purines identified in the anticodon loop or stem are chemically modified, the tRNA is not able to bind stably to poly(U)-programmed ribosomes. □, ribosome protected; ➡, strongly enhanced by the ribosome; →, moderately enhanced by the ribosome; ⟶, weakly enhanced by the ribosome; >, modification inactivates tRNA binding.

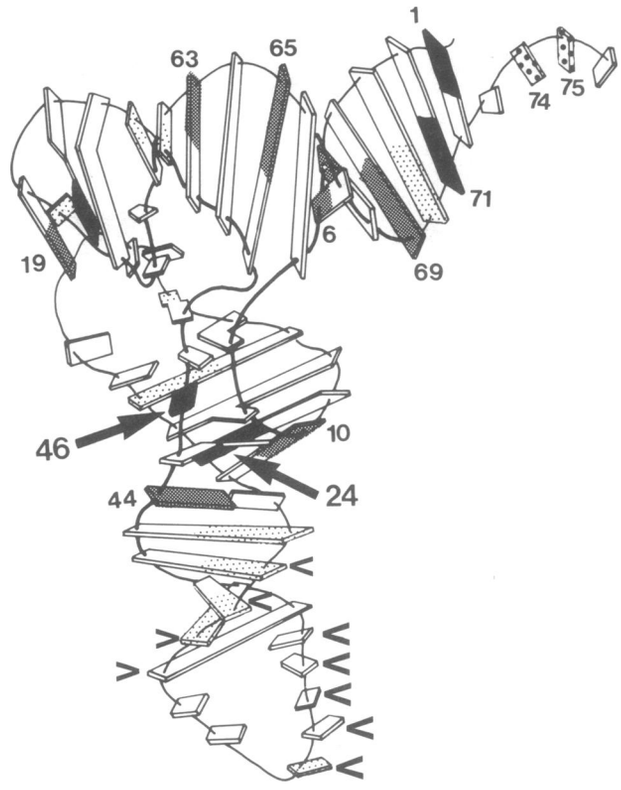

FIG. 5. Three-dimensional representation of yeast tRNA^{Phe} (15) showing analogous sites affected by, or which when modified affect, the (*E. coli*) tRNA^{Phe}–ribosome interaction. The bases denoted in the figure are from the (*E. coli*) tRNA^{Phe} nucleotide sequence (12). Cytidines: ⁙, methylation protection. Guanosines: ■, strong to moderate methylation enhancement; ■, weak methylation enhancement; ⁘, no methylation enhancement. Purines: >, modification inactivates tRNA binding.

probing experiments to locate sites on the tRNA affected by ribosome binding and chemical modification-selection experiments to identify bases required for a stable tRNA–mRNA–ribosome complex.

In native Phe-tRNA^{Phe}, the only sites reactive to our chemical probes are the N-3 positions of cytidine-75 and 74 in the universal 3′ C-C-A sequence. When the tRNA binds to the ribosome, these sites are protected from dimethyl sulfate attack. As the N-3 position of cytidine is hydrogen bonded in a Watson–Crick G·C base pair, the protection we observe is consistent with a base pairing of the single-stranded C-C-A sequence to a ribosomal RNA near the peptidyl transferase center of the 50S subunit. The complete protection of C-75 indicates a strong interaction affecting the N-3 position; the partial protection we observe at C-74 could reflect structural breathing of a base pair. Both properties could, of course, reflect another type of structure. Slow tritium-exchange experiments (16) indicate ribosomal protection of the purines in the 3′-terminal A-C-C-A sequence.

None of the guanosine N-7 positions is reactive in native (*E. coli*) tRNA^{Phe} free in solution. [This contrasts with (yeast) tRNA^{Phe} in which the N-7 positions of eight guanosines react with dimethyl sulfate (3) and could reflect a different structure or ionic differences in the reaction buffers used. It is not due to acylation of the tRNA.] Yet many guanosines become reactive when (*E. coli*) tRNA binds to the ribosome (see Fig. 2). This reactivity varies in degree from the hyperreactive G-46 and G-24 to the only slightly reactive G-65 and G-63 (see Fig. 2) and could reflect ribosome-induced deshielding of these N-7 atoms (signifying localized perturbations at these sites) or the creation of hydrophobic pockets that increase the local concentration of dimethyl sulfate.

If the (*E. coli*) tRNA^{Phe} structure is analogous to that determined for (yeast) tRNA^{Phe} in a crystal (17) (see Fig. 5), then the 13 reactive N-7 atoms are not involved in any intramolecular tertiary interaction that might be disrupted as the ribosome binds. In addition, the N-7 positions of G-22 in the D stem and G-57 in the T loop are involved in intramolecular hydrogen bonds that stabilize the tRNA^{Phe} conformation, and neither reacts with dimethyl sulfate in the tRNA–ribosome complex, even though both are near reactive guanosine residues (G-46 and G-18, respectively; see Fig. 5). This suggests that these tertiary interactions remain intact or, less likely, break and reform. Thus, we do not detect any gross conformational changes in the tRNA molecule induced by ribosome binding. This is also suggested by the fact that none of the stacked adenosines or base-paired cytidines becomes reactive on binding to the ribosome.

The most reactive guanosines, G-24 and G-46, are proximately located within the central region of the tRNA molecule (see Fig. 5). Curiously, G-46, within the variable domain, is only partially methylated at its N-7 position *in vivo* and becomes hyperreactive when the tRNA^{Phe} is ribosome bound; perhaps the ribosome induces such methylation at G-46 *in vivo*. The hyperreactivity of G-24 could also reflect a particular role of this residue in protein synthesis. There is an (*E. coli*) tRNA^{Trp} U-G-A-suppressor that differs from its nonsuppressing cognate only at position 24 (adenosine in place of guanosine) and not in the anticodon (18). [The interaction of tRNA with eukaryotic ribo-

somes may be different at this region because this suppressor tRNA does not function in a eukaryotic translation system (A. Geller and A. Rich, personal communication)]. Thus, G-24, G-46, and two other proximate less-reactive guanosines (G-10 and G-44) could represent an important localized configuration of both the ribosome and the bound tRNA molecule. This region may reflect the interface between the 30S and 50S ribosomal subunits because the anticodon binds on the 30S subunit and the C-C-A end binds on the 50S subunit.

The other reactive guanosines lie within the coaxial acceptor stem–T stem helix along the top of the molecule depicted in Fig. 5. The guanosine N-7 atoms lie within the large major groove of this RNA-A helix, and the most reactive guanosines, G-1, G-18, and G-71, lie at the ends of this helix. Guanosine-5, -6, -19, -63, -65, and -69 are only slightly reactive but are also localized within this coaxial helix. These reactivities probably reflect only minor changes between free and bound Phe-tRNA^{Phe} and were most likely detected in these experiments because the free tRNA was resistant to chemical modification at these sites. The nonreactive residue G-70, which lies between the reactive residues G-69 and G-71, resists methylation even when the tRNA is denatured (see Fig. 2*d*) and could reflect sequence-induced methylation inhibition such as that seen in other RNA molecules (3).

The five guanosines within the anticodon stem and loop do not react with dimethyl sulfate when the tRNA is free or ribosome bound. This may reflect the inherent inaccessibility of this region of the native tRNA molecule. The chemical selection experiments show the importance of this region for a stable tRNA–mRNA–ribosome interaction. As might be expected, chemical damage to any of the three anticodon bases (GAA) prevents stable poly(U)-directed binding of the tRNA (see Figs. 3 and 5). In addition, however, the two proximal 3′ residues, A-38 and ms^{2}i^{6}A-37, are also critical for a stable tRNA–mRNA–ribosome complex. Chemical damage at these sites could preclude a specific anticodon loop conformation required for ribosomal binding or could locate crucial points of contact between the tRNA, ribosome, and message. Decreased messenger-dependent ribosome binding of (yeast) tRNA^{Ser} with an iodinated ms^{2}i^{6}A-37 (19) and of (yeast) tRNA^{Phe} with an acid-released Y37 residue (20) also indicate the importance of the hypermodified base in the anticodon loop for formation of a stable tRNA–mRNA–ribosome complex. The decreased ribosome binding of tRNA modified at anticodon stem residues G-28–30 and A-31 is less effective than modification of the anticodon loop purines but indicates the importance of the conformation of this region for messenger-directed binding.

We observe the same qualitative ribosome-induced changes when the tRNA binds in the presence or absence of messenger RNA (see Fig. 2). However, we can isolate stable tRNA–ribosome complexes on a sucrose gradient (see Fig. 1) only when the messenger is present. Therefore, the similar chemical modification patterns generated with and without mRNA probably reflect a highly reversible interaction between the tRNA and the ribosome in the absence of poly(U). This has been suggested by fluorescence (21, 22) and tritium-exchange (16) studies. Indeed, these experiments show that the chemical reactivity of ribosome-bound tRNA is unaffected by the codon–anticodon interaction, suggesting that this interaction does not affect the conformation of the tRNA in the peptidyl site. In contrast, oligonucleotide-binding studies (23) and kethoxal-modification experiments (24) have suggested that high codon concentrations induce conformational changes in tRNA in the absence of ribosomes. The changes we observe are induced only by the ribosome.

In conclusion, these experiments identify the cytidines in the 3′-terminal sequence as exposed residues that interact strongly with the ribosome when the tRNA is bound. This interaction, probably base pairing, is most likely common to all tRNA–ribosome complexes because the terminal C-C-A sequence is universal in all known tRNA molecules; in addition, it is adjacent to the peptidyl transferase site on the ribosome. tRNA molecules damaged chemically in the anticodon stem-and-loop region do not bind stably to messenger-directed ribosomes; therefore, as expected, the tRNA appears to form an intimate structure with the messenger and ribosome in this region. Two guanosines in the central variable-loop region of the tRNA^{Phe} become hyperreactive to dimethyl sulfate in the tRNA–ribosome complex. This may result from a critical interaction with the ribosome in this region but may also be the result of a pocket that accommodates the variable loop, formed, perhaps, by the interface between the two subunits. The other reactive guanosines in the ribosome-bound tRNA^{Phe} appear to represent a slightly altered environment surrounding the coaxial acceptor stem–T stem helix. We observe chemical reactivity only at sites expected to be accessible as defined by crystal studies; therefore, we do not observe any gross conformational changes in the tRNA on binding to the ribosome. Overall, we observe a relatively static ribosome-bound tRNA structure in which the 3′-C-C-A sequence and the anticodon loop form strong, intimate interactions with the ribosomal peptidyl site.

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