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# Labeling the peptidyltransferase center of the *Escherichia coli* ribosome with photoreactive $tRNA^{Phe}$ derivatives containing azidoadenosine at the 3′ end of the acceptor arm: A model of the tRNA–ribosome complex

(photoaffinity labeling/tRNA-protein crosslink/tRNA-rRNA crosslink/peptidyl-tRNA site)

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ABSTRACT **Photoreactive derivatives of yeast $tRNA^{Phe}$ containing 2-azidoadenosine ($2N_3A$) at position 73 or 76 have been crosslinked to the peptidyl site of *Escherichia coli* ribosomes. Covalent tRNA-ribosome attachment was dependent upon the replacement of adenosine by $2N_3A$ in the tRNA, irradiation with 300-nm light, and the presence of poly(U). In all cases, the modified tRNAs became crosslinked exclusively to 50S ribosomal subunits. While the tRNA derivative containing $2N_3A$ at position 73 labeled only protein L27, that containing $2N_3A$ at position 76 labeled proteins L15, L16, and L27 as well as a segment of the 23S rRNA. The site of crosslinking in the rRNA was identified as guanosine-1945, which lies within a highly conserved sequence adjacent to a number of modified bases and has not until now been identified at the peptidyltransferase center. On the basis of these results, and previously reported crosslinks from tRNA containing 8-azidoadenosine in the 3′-terminal -A-C-C-A sequence [Wower, J., Hixson, S. S. & Zimmermann, R. A. (1988) *Biochemistry* 27, 8114–8121], we propose a model for the arrangement of tRNA molecules at the peptidyl and aminoacyl sites that is consistent with most of the information available about the location of the peptidyltransferase center and the decoding domain of the *E. coli* ribosome.**

The formation and analysis of intermolecular crosslinks provides an effective methodology for delineating the topography of tRNA binding sites on the ribosome (1, 2). The UV-induced attachment of $tRNA_1^{Val}$ to the peptidyl (P) site of *Escherichia coli* ribosomes (3), for instance, led to the demonstration that the 5-carboxymethyoxyuridine at position 34 ($cmo^5U^{34}$), the 5′ anticodon base of the tRNA, undergoes cycloaddition with, and is therefore within a few angstroms of, $C^{1400}$, a highly conserved nucleotide near the 3′ end of 16S rRNA (4). Further, through the use of immuno electron miroscopy, the site of interaction was shown to lie in the cleft between the head and platform of the 30S ribosomal subunit (5). Precise localization of the decoding site within the ribosome was feasible in large part because of the short length of this crosslink. In contrast, contacts between the 3′ end of tRNA and the ribosome at the peptidyltransferase center are still poorly defined. Thus, tRNAs containing chemically or photochemically reactive groups attached to the aminoacyl moiety have been reported to label proteins L2, L11, L14, L15, L16, L18, L23, and L27 of the 50S subunit, proteins S14 and S18 of the 30S subunit, and various segments of the 23S rRNA (2). However, immuno electron microscopic studies indicated that the proteins listed above are scattered over a large fraction of the ribosome surface (6) and clearly cannot all be in the vicinity of the tRNA 3′ terminus. The diversity of proteins labeled no doubt derives, at least in part, from the size of the reactive substituents, as the crosslinks they establish are typically 10–20 Å in length and may therefore extend to ribosomal components at a considerable distance from the site of tRNA binding.

To develop a strategy that would yield short-range crosslinks between tRNA and the peptidyltransferase center approximating that between $cmo^5U^{34}$ in $tRNA_1^{Val}$ and $C^{1400}$ in 16S rRNA, we have selectively incorporated photoreactive azidopurines into yeast $tRNA^{Phe}$ in place of their natural counterparts. In a previous report (7), we described the preparation of modified $tRNA^{Phe}$ molecules containing 8-azidoadenosine ($8N_3A$) at position 73 or 76 within the 3′-terminal -A-C-C-A sequence and characterized the ribosomal proteins labeled when these derivatives ($[8N_3A^{73}]tRNA^{Phe}$ and $[8N_3A^{76}]tRNA^{Phe}$) were photolyzed at the ribosomal P site. More recently, we demonstrated (8) that 2-azidoadenosine ($2N_3A$), an adenosine analog whose photoreactive group extends from the opposite side of the purine ring, can be introduced into position 76 of $tRNA^{Phe}$ ($[2N_3A^{76}]tRNA^{Phe}$) without significantly altering its amino acid acceptance and that the aminoacylated $tRNA^{Phe}$ derivative can be crosslinked to 70S ribosomes. In the present work, we describe the construction and crosslinking of a further variant of $tRNA^{Phe}$ ($[2N_3A^{73}]tRNA^{Phe}$) in which $A^{73}$ has been replaced by $2N_3A$ and identify the components of the 50S ribosomal subunit that become photochemically linked to the $2N_3A$-containing $tRNA^{Phe}$ derivatives. Using the results obtained with these tRNA probes together with other available data, we propose a model for the arrangement of the peptidyl- and aminoacyl-tRNAs at their respective sites on the ribosome.

## MATERIALS AND METHODS

The sources of enzymes, radioactively labeled compounds, and other biological materials are given in refs. 7 and 8. The preparation of $tRNA^{Phe}$ derivatives containing azidoadenosines within the 3′-terminal -A-C-C-A sequence and the formation of covalent tRNA-ribosome complexes have been described in detail (7, 8). The crosslinked complexes were separated into 30S and 50S subunits by sucrose gradient centrifugation in 0.25 mM $Mg^{2+}$, which also released noncovalently bound tRNA. Proteins crosslinked to $tRNA^{Phe}$ derivatives were identified by PAGE (9) and by the "agarose" immunological test (10). RNA sequences were determined by

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Abbreviations: $cmo^5U$, 5-carboxymethoxyuridine; $2N_3A$, 2-azidoadenosine; $8N_3A$, 8-azidoadenosine; $[2N_3A^{73}]tRNA^{Phe}$ and $[2N_3A^{76}]tRNA^{Phe}$, $tRNA^{Phe}$ derivatives containing $2N_3A$ at position 73 or 76; $[8N_3A^{73}]tRNA^{Phe}$ and $[8N_3A^{76}]tRNA^{Phe}$, $tRNA^{Phe}$ derivatives containing $8N_3A$ at position 73 or 76; P site, peptidyl site; A site, aminoacyl site.

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the enzymatic technique (11) using RNases T1 and U2 (Calbiochem–Behring), CL3 (Bethesda Research Laboratories), and Phy M (Pharmacia). Bases in the 23S rRNA are numbered according to ref. 12.

## RESULTS

**Crosslinking of [$2N_3A^{76}$]tRNA$^{Phe}$ to the 50S Ribosomal Subunit.** Yeast tRNA$^{Phe}$ containing the photoreactive nucleoside $2N_3A$ in place of $A^{76}$ was prepared by ligating [5′-$^{32}$P]p$2N_3$Ap to tRNA$^{Phe}$ molecules truncated by one residue at the 3′ terminus. The presence of the adenosine analog did not interfere with aminoacylation of the modified tRNA$^{Phe}$ by yeast phenylalanyl-tRNA synthetase. When bound to the P site of *E. coli* 70S ribosomes programmed with poly(U) and irradiated with 300-nm light, 13% of the noncovalently bound AcPhe-[$2N_3A^{76}$]tRNA$^{Phe}$ became crosslinked to the ribosome (8). Nonaminoacylated [$2N_3A^{76}$]tRNA$^{Phe}$ also crosslinked to 70S ribosomes under the same conditions, in a yield of 11%. In each case, covalent attachment required UV irradiation and poly(U), and both tRNA derivatives were linked exclusively to the 50S ribosomal subunit (8). Reconstructed tRNA$^{Phe}$ with $A^{76}$ instead of $2N_3A^{76}$ did not crosslink to ribosomes when similarly treated.

To determine the distribution of the crosslinked tRNA within the 50S subunit, protein and RNA components were dissociated in NaDodSO$_4$/LiCl solution and separated by sucrose gradient centrifugation. As indicated in Fig. 1*a* and Table 1, 55–60% of the $^{32}$P-labeled, crosslinked tRNA sedimented with ribosomal proteins in the 3–5S peak, while 40–45% was associated with 23S rRNA. The pattern of labeling was independent of whether or not the tRNA was aminoacylated. Treatment of covalent Ac[$^3$H]Phe-[$2N_3A^{76}$]-tRNA$^{Phe}$-ribosome complexes with puromycin resulted in the release of >95% of the aminoacyl moiety (8). When the 50S subunits from such complexes were fractionated in sucrose gradients with NaDodSO$_4$ and LiCl, neither the protein nor the 23S RNA peaks contained a significant amount of $^3$H (Fig. 1*b*). These results demonstrate that virtually all of the AcPhe-[$2N_3A^{76}$]tRNA$^{Phe}$ was crosslinked to the ribosomal P site.

**Ribosomal Proteins Labeled by [$2N_3A^{76}$]tRNA$^{Phe}$.** $^{32}$P-labeled protein-tRNA complexes isolated as in Fig. 1*a* were digested with RNase T1 and subjected to NaDodSO$_4$/PAGE. Autoradiography of the gel indicated that both aminoacylated and nonaminoacylated [$2N_3A^{76}$]tRNA$^{Phe}$ crosslinked to proteins located in the same two bands (Fig. 2*a*). The proteins were identified immunologically as L15, L16, and L27 (Fig. 2*b*). From their relative electrophoretic mobilities, we concluded that the upper band in Fig. 2*a* corresponds to L15 and L16 whereas the lower band corresponds to L27. Densitometry of the autoradiogram of the gel showed that of the total $^{32}$P-labeled tRNA crosslinked to the 50S subunit, about 50% was associated with L27 and 5–10% with L15 plus L16. The distribution of the label among the proteins was similar for both AcPhe-[$2N_3A^{76}$]tRNA$^{Phe}$ and [$2N_3A^{76}$]tRNA$^{Phe}$. When similar experiments were performed without poly(U) or UV irradiation, no radioactive protein bands were observed.

Table 1. Ribosomal components labeled by tRNA$^{Phe}$ derivatives containing $2N_3A$ or $8N_3A$

| tRNA$^{Phe}$ derivative | % cross-linking* | % distribution (protein/RNA)† | Site of labeling: 50S-subunit proteins‡ | Site of labeling: 23S rRNA |
|---|---|---|---|---|
| [$2N_3A^{73}$] | 4.6 | 100:0 | L27 | – |
| AcPhe-[$2N_3A^{73}$] | 6.3 | 100:0 | L27 | – |
| [$2N_3A^{76}$]§ | 11.0 | 55:45 | L27, L15, L16 | G1945 |
| AcPhe-[$2N_3A^{76}$]§ | 13.0 | 60:40 | L27, L15, L16 | G1945 |
| [$8N_3A^{73}$]¶ | 12.4 | 100:0 | L27 | – |
| AcPhe-[$8N_3A^{73}$]¶ | 12.5 | 35:65 | L27, L2 | + |
| [$8N_3A^{76}$]¶ | 4.3 | 100:0 | L27 | – |

*Fraction of tRNA bound noncovalently to P site that became crosslinked to ribosomes upon UV irradiation; values are averages from 2–5 experiments; average deviation for each tRNA derivative was 0.3% or less.
†Percent of crosslinked tRNA associated with protein or RNA.
‡Main protein labeled is underlined.
§Crosslinking percent from Sylvers *et al.* (8).
¶All data from Wower *et al.* (7).

**Site in 23S rRNA Labeled by [$2N_3A^{76}$]tRNA$^{Phe}$.** To identify the segment of 23S rRNA to which [$2N_3A^{76}$]tRNA$^{Phe}$ became crosslinked, $^{32}$P-labeled tRNA-23S rRNA complexes were partially hydrolyzed with RNase T1 and the products were fractionated by denaturing PAGE. Two main radioactive fragments resulted under our digestion conditions (Fig. 3*a*). Sequence analysis demonstrated that these fragments, P1 and P2, encompassed nucleotides 1870–1950 and 1870–1945, respectively, of *E. coli* 23S rRNA. A third fragment, P3, was observed in some partial digests but not in others; its identity was not determined.

To further define the tRNA crosslinking site, covalent tRNA-23S rRNA complexes were completely digested with RNase T1 and the resulting oligonucleotides were isolated by PAGE. Autoradiography revealed the presence of three radioactive bands, designated C1, C2, and C3 in Fig. 3*b*. While the example presented in the figure derived from a complex that contained nonaminoacylated tRNA, the same three bands were obtained when a complex that contained aminoacylated tRNA was analyzed in an identical fashion.

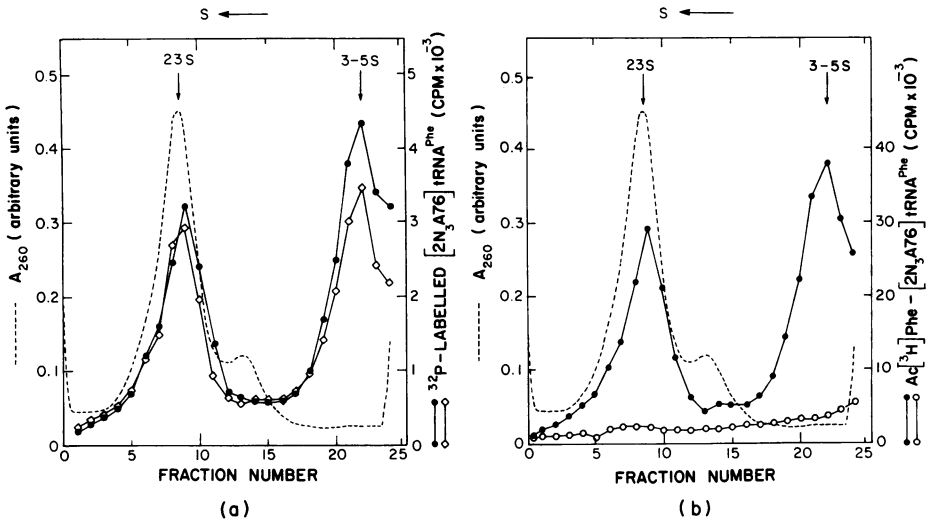


FIG. 1. Distribution of crosslinked [$2N_3A^{76}$]tRNA$^{Phe}$ between 23S rRNA and 50S subunit proteins: Effects of puromycin. (*a*) Covalent AcPhe-[$2N_3A^{76}$]-tRNA$^{Phe}$- and [$2N_3A^{76}$]tRNA$^{Phe}$-50S ribosomal subunit complexes, labeled in the tRNA moiety with $^{32}$P, were centrifuged through 5–20% sucrose gradients in 10 mM NaOAc, pH 5.0/100 mM LiCl/0.25 mM EDTA/0.5% (wt/vol) NaDodSO$_4$ at 40,000 rpm for 145 min at 10°C in a Beckman VTi50 rotor. ----, $A_{260}$; ●, AcPhe-[$2N_3A^{76}$]tRNA$^{Phe}$; ◇, [$2N_3A^{76}$]tRNA$^{Phe}$. (*b*) Crosslinked Ac[$^3$H]Phe-[$2N_3A^{76}$]-tRNA$^{Phe}$-70S ribosome complexes were treated with puromycin and then separated into subunits (8). The association of Ac[$^3$H]Phe with the 23S rRNA and 50S subunit protein fractions was analyzed as in *a*. ----, $A_{260}$; ○, complexes treated with puromycin; ●, untreated control.

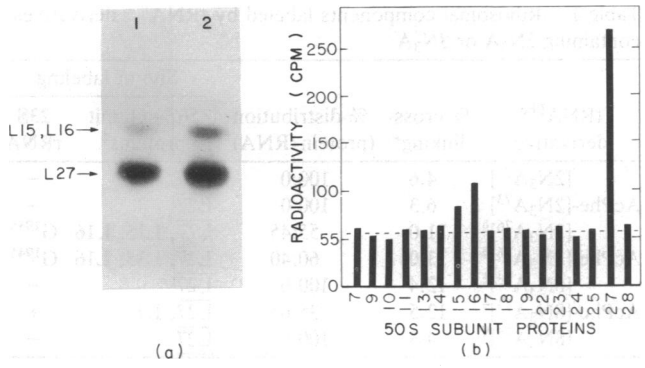


FIG. 2. Identification of ribosomal proteins crosslinked to $[2N_3A^{76}]tRNA^{Phe}$. Protein-tRNA complexes from fractions 18–24 of the sucrose gradients illustrated in Fig. 1*a* were pooled and digested with RNase T1. (*a*) One sample of each digest was subjected to $NaDodSO_4$/PAGE according to ref. 9. Autoradiogram depicts the locations of ribosomal proteins crosslinked to $^{32}P$-containing $[2N_3A^{76}]tRNA^{Phe}$ (lane 1) and AcPhe-$[2N_3A^{76}]tRNA^{Phe}$ (lane 2). (*b*) A second sample of each digest was analyzed immunologically by an agarose gel method (10). The histogram shows the proteins that were labeled with $^{32}P$ as a consequence of covalent attachment to $[2N_3A^{76}]tRNA^{Phe}$. The results for AcPhe-$[2N_3A^{76}]tRNA^{Phe}$-protein complexes were essentially the same. Dashed line indicates the level of background radioactivity.

Oligonucleotide C1, which migrated as a dodecanucleotide according to a sequence ladder in an adjacent gel lane, was extracted, labeled to high specific activity with $^{32}P$ at the 5′ end, and sequenced enzymatically. Surprisingly, the resulting sequence corresponded to that of a unique *deca*nucleotide in the 23S rRNA, A-A-A-T-U-C-C-U-U-G, which spans positions 1936–1945 (Fig. 3*c*). The anomalous electrophoretic mobility of C1 indicates that one or more nucleotides of the crosslinked tRNA remained attached to this segment of the 23S rRNA. Moreover, the large gap between $U^{1944}$ and the undigested oligonucleotide band, x, suggests that the tRNA was crosslinked to $G^{1945}$ (see Fig. 3*c*). We have recently found that the bond between $[2N_3A^{76}]tRNA^{Phe}$ and 23S rRNA is thermolabile, and band y in Fig. 3*c* may represent the unmodified decanucleotide. These inferences are supported by the fact that [5′-$^{32}P$]pCp could not be ligated to the 3′ terminus of fragment C1. Finally, complete hydrolysis of each of the partial digestion products, P1 and P2, yielded a $^{32}P$-labeled oligonucleotide identical in mobility and sequence to C1. Although we have not been able to positively identify the sequences present in fragments C2 and C3, the bulk of the evidence favors the view that a principal site of tRNA linkage in 23S rRNA lies between residues 1936 and 1945 and that the crosslinked base is most likely $G^{1945}$.

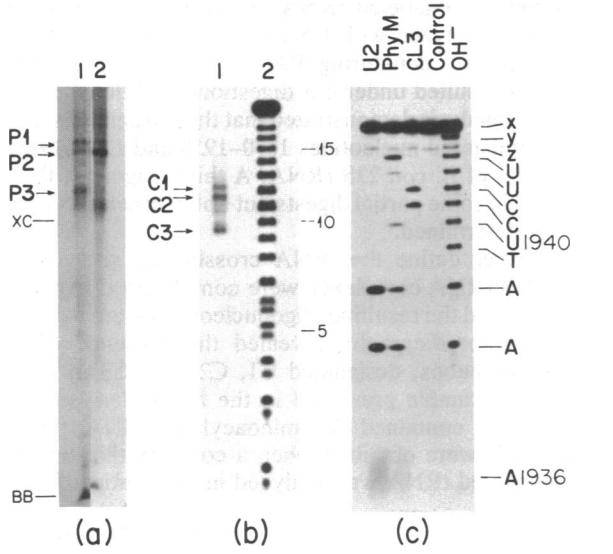


FIG. 3. Localization of the site of crosslinking in the 23S rRNA. (*a*) Covalent complexes of $^{32}P$-containing $[2N_3A^{76}]tRNA^{Phe}$ (lane 1) or AcPhe-$[2N_3A^{76}]tRNA^{Phe}$ (lane 2) with 23S rRNA from fractions 5–11 in Fig. 1*a* were partially digested with RNase T1 at an enzyme/substrate ratio of 1:170 and electrophoresed in a 10% polyacrylamide gel in 100 mM Tris borate, pH 8.3/8 M urea. XC and BB mark the final positions of the xylene cyanole and bromophenol blue dyes. (*b*) The $[2N_3A^{76}]tRNA^{Phe}$-23S rRNA complex was digested completely with RNase T1 and electrophoresed in a 20% polyacrylamide gel as in *a* (lane 1). The octadecanucleotide isoplith from the 23S rRNA was subjected to alkaline hydrolysis at 95°C and electrophoresed alongside as a size standard (lane 2). (*c*) The crosslinked oligonucleotide C1 from the gel in *b* was eluted, relabeled with $^{32}P$ at the 5′ end by the use of T4 polynucleotide kinase, and subjected to sequence analysis by the use of partial digestion with RNases U2, Phy M, and CL3. Lane $OH^-$ represents an alkaline hydrolyzate of the oligonucleotide. Samples were electrophoresed in a 20% polyacrylamide gel as in *a* and the gel pattern was visualized by autoradiography.

**Labeling of the 50S Ribosomal Subunit by $[2N_3A^{73}]tRNA^{Phe}$.** Substitution of $2N_3A$ for $A^{73}$ of $tRNA^{Phe}$ was accomplished by first removing four residues from the 3′ end of the tRNA molecule (13). [5′-$^{32}P$]p$2N_3AP$ was then added to the truncated tRNA by using T4 RNA ligase and the -C-C-A terminus was regenerated through the action of nucleotidyltransferase. The amino acid acceptance of the resulting $[2N_3A^{73}]tRNA^{Phe}$ was 960 pmol/$A_{260}$ unit, or ≈85% that of the initial, intact $tRNA^{Phe}$. When complexes containing Ac[$^3H$]Phe-$[2N_3A^{73}]$-$tRNA^{Phe}$, 70S ribosomes, and poly(U) were irradiated with 300-nm light, 6–7% of the noncovalently bound tRNA was crosslinked to the ribosomal particles. The sensitivity of the covalently attached AcPhe-$[2N_3A^{73}]tRNA^{Phe}$ to reaction with puromycin verified its location in the P site. Under identical conditions, nonaminoacylated $[2N_3A^{73}]tRNA^{Phe}$ crosslinked to ribosomes with a yield of 4–5%. Here again, formation of the covalent tRNA-ribosome crosslinks depended upon the presence of $2N_3A$ in the tRNA, UV irradiation, and the presence of poly(U).

Fractionation of covalent ribosomal complexes containing $^{32}P$-labeled $[2N_3A^{73}]tRNA^{Phe}$ showed that only the 50S subunit was labeled and, in experiments similar to that depicted in Fig. 1*a*, it was found that the crosslinked tRNA was associated only with the 3–5S protein fraction (data not shown). Analysis of this fraction by PAGE after RNase treatment revealed that all of the radioactivity migrated as a single band which corresponded in mobility to protein L27. Immunological assays confirmed that this protein was the sole target of labeling by both aminoacylated and nonaminoacylated $[2N_3A^{73}]tRNA^{Phe}$.

## DISCUSSION

The portion of the 50S subunit that is in contact with the aminoacyl moiety and the acceptor end of tRNA corresponds to the peptidyltransferase center of the ribosome. To identify the ribosomal components in the immediate vicinity of the 3′ end, we replaced $A^{73}$ and $A^{76}$ of $tRNA^{Phe}$ with the photoreactive analogs $2N_3A$ and $8N_3A$ and crosslinked the tRNA derivatives to the P site of *E. coli* ribosomes with UV light in the presence of poly(U) (refs. 7 and 8 and this work). As summarized in Table 1, the modified tRNAs labeled 50S-subunit proteins L2, L15, L16, and L27 as well as nucleotide $G^{1945}$ of the 23S rRNA. Although none of the four proteins alone is capable of catalyzing peptide bond formation, all have been implicated in peptidyltransferase activity by partial reconstitution or single-protein omission studies (14), by their involvement in the binding of chloramphenicol, puromycin, and a variety of macrolide antibiotics (15–20), and by affinity labeling with a number of aminoacyl-tRNA deriva-

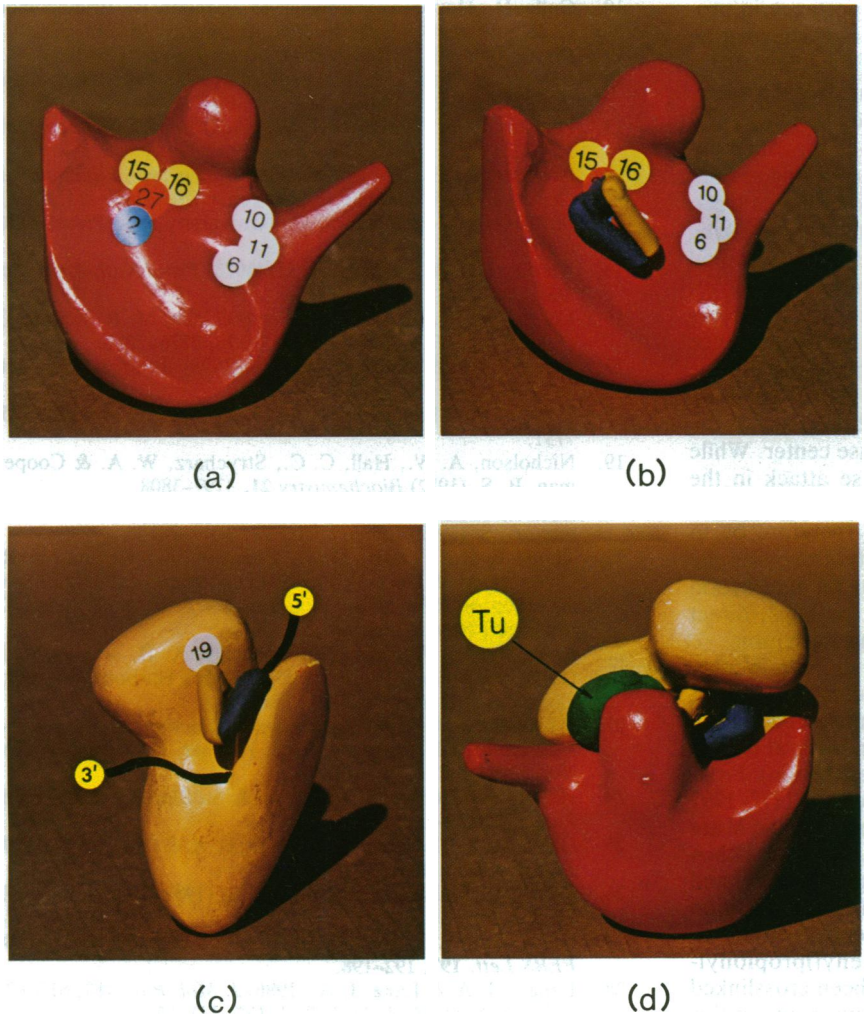


FIG. 4. Model for the arrangement of tRNAs in the peptidyl (P) and aminoacyl (A) sites of the *E. coli* ribosome. (*a*) A 50S subunit showing placement of proteins L2, L15, L16, and L27, which have been labeled at the P site by tRNA derivatives containing $2N_3A$ and $8N_3A$ at positions 73 and 76 (ref. 7 and this work), and of proteins L6, L10, and L11, which lie within the elongation-factor domain (see ref. 23). L2, L6, L10, L11, L15, and L27 have all been located by immuno electron microscopy (6, 24); the position of L16 is constrained by crosslinks to L15 and L27 (6, 21). (*b*) A 50S subunit with P-site-bound tRNA (blue) at left and A-site-bound tRNA (yellow) at right. L-shaped tRNA molecules are in correct scale relative to the ribosomal subunit. (*c*) A 30S subunit with tRNAs in P (right) and A (left) sites. The tRNA anticodons are located in the cleft between the head and platform (5), and the direction of the mRNA has recently been defined as 5′ (right) → 3′ (left) (25). Protein S19 has been crosslinked to A-site-bound tRNAs derivatized at position 8 (26). (*d*) A 70S ribosome. The position of elongation factor Tu (green) is consistent with immuno electron microscopy results (27, 28). The relative orientation of the two subunits is as described in ref. 29. Subunit models were used with the permission of J. A. Lake.

tives containing reactive groups attached to the aminoacyl moiety (2). In contrast, $G^{1945}$ lies within a highly conserved and relatively highly modified portion of the 23S rRNA whose role in ribosome function has not been clearly defined.

There is ample evidence that proteins L2, L15, L16, and L27 are close neighbors in the 50S subunit. Treatment of intact 50S particles with a variety of bifunctional reagents, for example, resulted in the formation of L2–L15, L2–L16, L15–L16, and L16–L27 crosslinks (6, 21), and recent neutron scattering studies indicated that the centers of mass of L2, L15, and L16 are in close proximity (22). Moreover, as depicted in Fig. 4*a*, mapping of 50S-subunit proteins by immuno electron microscopy has shown that three of the four proteins, L2, L15, and L27, are clustered on the subunit interface in the valley between the L1 ridge and the central protuberance (6, 24). Our results show that this cluster is at the site of contact of the aminoacyl end of tRNA with the ribosomal P site. Furthermore, the pattern of crosslinking from positions 73 and 76 of the tRNA (see Table 1), together with the nearly linear disposition of antibody binding sites for L2, L27, and L15, permits us to propose a likely orientation for the aminoacyl arm on the 50S subunit (Fig. 4*b*).

Placement of the P-site-bound tRNA so that its aminoacyl stem is directed toward the 50S subunit, as shown in Fig. 4*b*, requires that tRNA in the ribosomal A site be similarly oriented since, to be able to participate in peptide bond synthesis, the 3′ termini of the two tRNAs must be closely juxtaposed while their anticodons interact with adjacent triplets in the mRNA. Based on the position of the binding site for elongation factor Tu (27, 28), we conclude that A-site-bound tRNA is located to the right of P-site-bound tRNA in the view of the 50S subunit presented in Fig. 4*b*. The predicted position of A-site-bound tRNA is consistent with the finding that $tRNA^{Phe}$ crosslinked via position 47 to elongation factor Tu can bind to the A site when the P site is occupied by nonaminoacylated tRNA (30).

The arrangement of the two tRNAs on the 50S subunit (Fig. 4 *b* and *d*) fixes their relative positions on the 30S subunit subject to the condition that the tRNA anticodons lie in the cleft between the platform and the head (Fig. 4 *c* and *d*). Placement of the P- and A-site-bound tRNAs so that their anticodon arms contact only the 30S subunit is consistent with the observation that the anticodon stem–loop portion of the tRNA alone is sufficient for specific, codon-dependent association with the small ribosomal particle (31, 32). Although different conclusions about the relative positions of the P and A sites have been reached by others (refs. 33–35; see also ref. 2), only our model is consistent with new immuno electron microscopy studies that define the 3′ → 5′ direction of the mRNA through the 30S subunit cleft (ref. 25; see Fig. 4*c*). Owing to the paucity of information on the location of tRNA segments other than the 3′ end and the anticodon, the ribosomal components that neighbor the central region of the tRNA molecule are rather poorly defined. In our model, we have placed the corners of the L-shaped tRNAs so that their D loops are separated by 35 Å, the distance derived from fluorescence energy-transfer measurements (36).

Our finding that $[2N_3A^{76}]tRNA^{Phe}$ crosslinks to $G^{1945}$ at the ribosomal P site is of considerable interest, as this base lies within domain IV (residues 1648–2010) of the 23S rRNA, a

highly conserved region whose possible importance in protein synthesis has been until now a matter of speculation (37–39). The present results provide direct evidence that a portion of this domain is at the peptidyltransferase center. The crosslinked base, $G^{1945}$, lies at one end of a nearly invariant sequence spanning residues 1925–1945, which appears to be predominantly single-stranded according to both phylogenetic criteria and chemical probe experiments (refs. 39 and 40; see also ref. 41). In addition, the site of crosslinking is adjacent to one of the few posttranscriptionally modified bases in 23S rRNA, $T^{1939}$, and close to three others, $\psi^{1911}$, $mU^{1915}$, and $\psi^{1917}$. The high level of evolutionary conservation, the lack of conventional base pairing, and the presence of modified bases all support the idea that this portion of the 23S rRNA is involved in ribosome function (42). Domain IV also contains the binding site for protein L2 (38), one of the protein components of the peptidyltransferase center. While much of domain IV is resistant to nuclease attack in the presence of L2, nucleotides in the vicinity of $G^{1945}$ remain accessible (38). The main role of L2 may therefore be to stabilize a conformation of the 23S rRNA that exposes the sequence surrounding the crosslinked base for interaction with the 3′ end of P-site-bound tRNA or, perhaps, direct participation in the catalysis of peptide bond formation.

The notion that certain sequences within domain IV play an active role at the peptidyltransferase center is not in conflict with evidence indicating that the conserved central loop of domain V (residues 2011–2630) is an integral part of this functional site (refs. 43–45 and references therein). First, a UV-induced crosslink between oligonucleotides 1777–1792 and 2584–2588 showed that segments of domain IV and V are closely juxtaposed in the 50S subunit (46). Second, tRNA bound to the ribosomal P site was found to protect $A^{1916}$ and $A^{1918}$ in the highly methylated loop of domain IV, in addition to several residues in domain V, against chemical modification (47). Third, while 3-(4′-benzoylphenyl)propionyl-Phe-$tRNA^{Phe}$ at both the P and A sites has been crosslinked to bases within domain V (48, 49), the reactive group of this derivative extends >10 Å from the aminoacyl moiety and may therefore label the peptidyltransferase center at some distance from the 3′-terminal adenosine. Crosslinking of the $2N_3A$- and $8N_3A$-containing derivatives of $tRNA^{Phe}$ to the A site should aid in further delineating the fine structure of this region of the ribosome.

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