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Covalent crosslinking of $tRNA_1^{Val}$ to 16S RNA at the ribosomal P site: Identification of crosslinked residues

(tRNA/rRNA/RNA-RNA crosslink/rRNA function/cyclobutane dimer)

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ABSTRACT *N*-Acetylvalyl-$tRNA_1^{Val}$ (AcVal-$tRNA_1^{Val}$) was bound to the P site of uniformly ^{32}P-labeled 70S ribosomes from *Escherichia coli* and crosslinked to 16S RNA in the 30S ribosomal subunit by irradiation with light of 300–400 nm. To identify the crosslinked nucleotide in 16S RNA, AcVal-$tRNA_1^{Val}$-16S [^{32}P]RNA was digested completely with RNase T1 and the band containing the covalently attached oligonucleotides from tRNA and rRNA was isolated by polyacrylamide gel electrophoresis. The crosslinked oligonucleotide, and the ^{32}P-labeled rRNA moiety released from it by photoreversal of the crosslink at 254 nm, were then analyzed by secondary hydrolysis with pancreatic RNase A and RNase U2. The oligonucleotide derived from 16S RNA was found to be the evolutionarily conserved sequence, U-A-C-A-C-A-C-C-G_{1401}, and the nucleotide crosslinked to $tRNA_1^{Val}$, C_{1400}. The identity of the covalently attached residue in the tRNA was established by using AcVal-$tRNA_1^{Val}$-16S RNA prepared from unlabeled ribosomes. This complex was digested to completion with RNase T1 and the resulting RNA fragments were labeled at the 3′ end with [5′-^{32}P]pCp. The crosslinked T1 oligonucleotide isolated from the mixture yielded one major end-labeled component upon photoreversal. Chemical sequence analysis demonstrated that this product was derived from the anticodon-containing pentadecanucleotide of $tRNA_1^{Val}$, C-A-C-C-U-C-C-C-U-cmo^5U-A-C-m^6A-A-G_{39} (cmo^5U, 5-carboxymethoxyuridine). A similar study of the crosslinked oligonucleotide revealed that the residue covalently bound to 16S was cmo^5U_{34}, the 5′ or wobble base of the anticodon. The adduct is believed to result from formation of a cyclobutane dimer between cmo^5U_{34} of $tRNA_1^{Val}$ and C_{1400} of the 16S RNA.

When peptidyl-tRNA is bound to the P site of *Escherichia coli* ribosomes, the anticodon loop of the tRNA is in close proximity to a segment of the 16S rRNA. This assertion stems from the observation that several different acetylaminoacyl-tRNAs (AcAA-tRNAs) can be crosslinked to 16S RNA in the 30S subunit by irradiation of noncovalent AcAA-tRNA·ribosome complexes with light of 300–400 nm (1–3). Crosslinking occurs only when the tRNAs are positioned at the P site in response to an appropriate polynucleotide and, under these conditions, the covalently bound AcAA-tRNAs still react with puromycin (2, 3). Furthermore, all such adducts can be cleaved by photolysis at 254 nm (2, 3). In the case of *E. coli* $tRNA_1^{Val}$, 254-nm irradiation resulted in true photoreversal of the RNA-RNA bond formation (4). On the basis of this and other photochemical properties, crosslinking was judged to occur via pyrimidine-pyrimidine cyclobutane dimer formation (4). We earlier showed that $tRNA_1^{Val}$ becomes attached to a fragment of about 125 nucleotides within the 3′ portion of the 16S RNA (5). More recently, we determined that this fragment encompasses residues 1,362–1,497 of the rRNA molecule and we identified the RNase T1-derived oligonucleotide containing the crosslinked residue as U-A-C-A-C-A-C-C-G_{1401} (6).

Those tRNAs that can be crosslinked to the P site are distinguished from unreactive tRNAs by the presence of a modified uridine residue in the 5′ or wobble position of the anticodon. In view of this correlation, we suggested that the 5′ anticodon base is the photoreactive moiety in the tRNA (2, 3). Moreover, the efficiency of crosslinking was found to vary widely, depending on the residue in the wobble position (2, 3) and its ability to base-pair with the corresponding nucleotide in the codon (7). Despite such strong circumstantial evidence, it has not yet been demonstrated directly that the modified uridine of the anticodon is the crosslinked residue. In the present report, we confirm this hypothesis for the case of $tRNA_1^{Val}$ and, in addition, we establish the identity of the nucleotide in 16S RNA that becomes crosslinked to the tRNA anticodon.

MATERIALS AND METHODS

Materials. *E. coli* $tRNA_1^{Val}$ from Boehringer Mannheim was aminoacylated and *N*-acetylated as described in ref. 1. Poly(U_3,G), bacterial alkaline phosphatase, and bacteriophage T4 RNA ligase were purchased from P-L Biochemicals, pancreatic RNase A from Worthington, and RNases T1 and U2 from Calbiochem-Behring. [^{3}H]Valine (50–60 Ci/mmol), [^{32}P]orthophosphoric acid (carrier-free), and [5′-^{32}P]pCp (2,100 Ci/mmol) were obtained from New England Nuclear (1 Ci = 3.7×10^{10} becquerels). Unlabeled and uniformly ^{32}P-labeled tight-couple ribosomes were prepared from *E. coli* MRE600 cells grown in the absence or presence of [^{32}P]orthophosphoric acid (6, 8).

Isolation of Covalent tRNA-16S RNA Complex. Nonenzymatic binding of Ac[^{3}H]Val-$tRNA_1^{Val}$ to the P site of poly(U_3,G)-programmed 70S tight couples, irradiation of noncovalent AcVal-$tRNA_1^{Val}$·ribosome complexes with 350-nm light, and isolation of covalent AcVal-$tRNA_1^{Val}$-16S RNA complexes were carried out as reported (5, 6). Under these conditions, 30–40% of the tRNA initially bound to ribosomes became covalently crosslinked to 16S RNA (6).

Preparation of RNase T1 Digests. Covalent AcVal-$tRNA_1^{Val}$-16S RNA complexes were suspended in 5 mM Tris·HCl,

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Abbreviations: AcAA-tRNA, *N*-acetylaminoacyl-tRNA; AcVal-$tRNA_1^{Val}$, *N*-acetylvalyl-$tRNA_1^{Val}$; cmo^5U, 5-carboxymethoxyuridine.

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pH 7.4/0.5 mM Na_2EDTA, digested with RNase T1 at an enzyme-to-substrate ratio of 1:2 (wt/wt) for 1 hr at 37°C, and dried under reduced pressure. When the oligonucleotides were to be end-labeled, bacterial alkaline phosphatase was also added to the incubation mixture at a final concentration of 5 units/ml and RNA was recovered by extraction with phenol and precipitation with ethanol. The dephosphorylated oligonucleotides were then labeled with [5′-^{32}P]pCp at their 3′ termini by using T4 RNA ligase (9).

Isolation of Crosslinked Oligonucleotides. ^{32}P-Labeled oligonucleotides were fractionated on 20% polyacrylamide gels containing a 30:1 (wt/wt) mixture of acrylamide and *N,N′*-methylenebisacrylamide in 50 mM Tris·50 mM H_3BO_3, pH 8.3/1 mM Na_2EDTA/7 M urea. Prior to sample application, an aliquot of the RNA was dissolved in 30 μl of distilled water, spotted onto Parafilm, and irradiated for 15 min at 4°C in a Rayonet photochemical reactor equipped with four 254-nm lamps. Both irradiated and unirradiated samples were then resuspended in 7 M urea/0.02% bromophenol blue/0.02% xylene cyanole and subjected to electrophoresis at 1800 V and at 20°C. The appropriate bands were located by autoradiography, excised from the gel, and eluted with distilled water (uniformly labeled samples) or 50 mM Tris borate, pH 8.3/1 mM Na_2EDTA/500 mM KCl (end-labeled samples) as described in ref. 6.

Analysis of Nucleotide Sequences. The structures of uniformly ^{32}P-labeled T1 oligonucleotides were established by secondary hydrolysis with pancreatic RNase, RNase U2, and alkali (6). The sequences of 3′-^{32}P-labeled T1 oligonucleotides were determined by the chemical method of Peattie (10). Photoreversal of the crosslinking of adducts was performed as in the preceding section.

RESULTS

Identification of Crosslinked Nucleotide in 16S RNA. After photochemical crosslinking of AcVal-$tRNA_1^{Val}$ to the P site of uniformly ^{32}P-labeled 70S ribosomes, covalent AcVal-$tRNA_1^{Val}$-16S [^{32}P]RNA was extracted from the 30S subunits and hydrolyzed completely with RNase T1. The crosslinked oligonucleotide, which contains components from both rRNA and tRNA, was isolated from the digest by gel electrophoresis under denaturing conditions (6). This product, the largest prominent T1 oligonucleotide recovered, is designated band 1_U in Fig. 1*a*, lane 1. The susceptibility of the adduct to photoreversal is illustrated by the absence of a corresponding band when the digest was irradiated at 254 nm prior to electrophoresis (Fig. 1*a*, band 2). Band 1_U was eluted from the first gel and divided into two portions. One sample was exposed to light of 254 nm and then analyzed together with the unirradiated sample on a second gel. As depicted in Fig. 1*b*, photoreversal resulted in release from the covalent complex of a ^{32}P-labeled component, band 2_U, which has been identified as a nonanucleotide from the 16S RNA (6). The oligonucleotide derived from $tRNA_1^{Val}$ was unlabeled and hence did not appear in the autoradiograph.

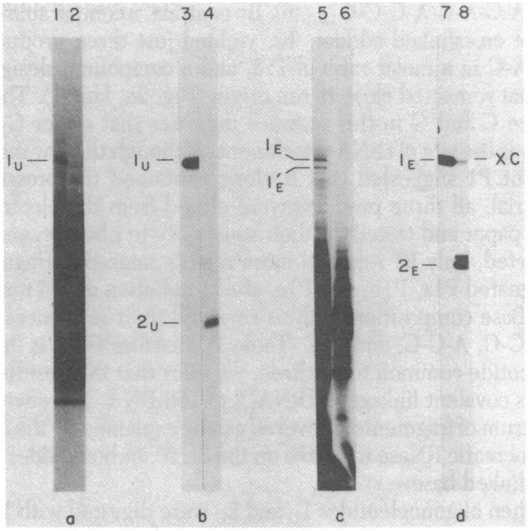

FIG. 1. Analysis of crosslinked oligonucleotides from complete RNase T1 digests of AcVal-$tRNA_1^{Val}$-16S RNA by polyacrylamide gel electrophoresis. (*a*) Digest of AcVal-$tRNA_1^{Val}$-16S [^{32}P]RNA was electrophoresed before (lane 1) and after (lane 2) irradiation at 254 nm. (*b*) RNA from band 1_U in *a* was electrophoresed before (lane 3) and after (lane 4) irradiation at 254 nm. (*c*) Digest of AcVal-$tRNA_1^{Val}$-16S RNA was 3′-end-labeled with [5′-^{32}P]pCp and electrophoresed before (lane 5) and after (lane 6) irradiation at 254 nm. (*d*) RNA from band 1_E in *c* was electrophoresed before (lane 7) and after (lane 8) irradiation at 254 nm. Results with RNA from band $1'_E$ were similar (not shown). The fraction of the total radioactivity present in bands 1_U and $1_E + 1'_E$ can account for all of the crosslink formed. Moreover, none of the other components in lanes 1 and 5 yielded smaller products after elution, 254-nm irradiation, and reelectrophoresis. XC indicates final position of xylene cyanole dye.

The crosslinked oligonucleotide, 1_U, and the rRNA component released from it, 2_U, were eluted from gels similar to the gel in Fig. 1*b* and characterized by secondary hydrolysis with pancreatic RNase (Fig. 2*a*). The products obtained from the nonanucleotide, 2_U, were U, C, G, and A-C in a molar ratio of 1:1:1:3 (Fig. 2*a*, lane 2). The only T1 oligonucleotide of this size and composition in the relevant segment of the 16S RNA

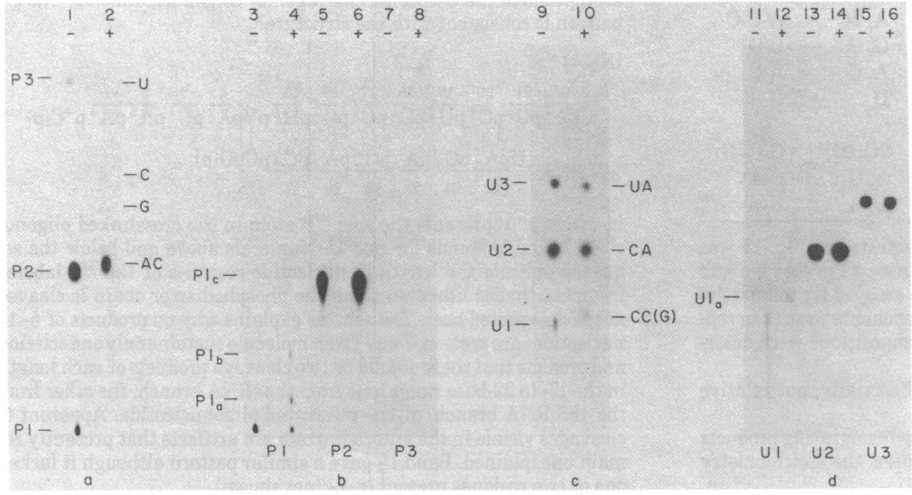

FIG. 2. Secondary digestion of the crosslinked T1 oligonucleotide 1_U and its rRNA component 2_U. (*a*) Pancreatic RNase digests of 1_U (lane 1) and 2_U (lane 2) were electrophoresed on DEAE-paper at pH 3.5. (*b*) Products from spots P1, P2, and P3 in *a* were electrophoresed at pH 3.5 before (lanes 3, 5, and 7) and after (lanes 4, 6, and 8) photoreversal at 254 nm. (*c*) RNase U_2 digests of 1_U (lane 9) and 2_U (lane 10) were electrophoresed on DEAE-paper at pH 2.3. (*d*) Products from spots U1, U2, and U3 in *c* were electrophoresed at pH 2.3 before (lanes 11, 13, and 15) and after (lanes 12, 14, and 16) photoreversal at 254 nm. A summary of the results is provided in Table 1.

is U-A-C-A-C-A-C-C-G_{1401} (6). By contrast, a comparable digest of the crosslinked adduct, 1_U, yielded just three products, U and A-C in a molar ratio of 1:3, and a component designated P1 that remained close to the origin (Fig. 2*a*, lane 1). The lack of free C and G in this instance indicates that either C_{1400} or G_{1401} is the site of tRNA attachment. Although the low mobility of spot P1 suggested that it alone contained the crosslinked material, all three products were eluted from the electrophoresis paper and tested for their sensitivity to photoreversal. As expected, only P1 released more rapidly migrating fragments, designated P1a, P1b, and P1c, after irradiation at 254 nm (Fig. 2*b*). Base composition analysis revealed their sequences to be A-C-C-G, A-C-C, and C-G (Table 1). Because C_{1400} is the sole nucleotide common to all three, we infer that this residue mediates covalent linkage of $tRNA_1^{Val}$ to 16S RNA. Moreover, the spectrum of fragments recovered can be explained by the failure of pancreatic RNase to cleave on the 3′, 5′, or both, sides of the crosslinked base.

When oligonucleotides 1_U and 2_U were digested with RNase U_2, three products were obtained in each case (Fig. 2*c*). The composition of the rRNA derivative, 2_U, which was determined to be U-A, C-A, and C-C in a molar ratio of 1:2:1 (Fig. 2*c*, lane 10), is consistent with the sequence of the nonanucleotide already described (6). Although the material used in this experiment appears to have lost the 3′-terminal G, it may only lack the 3′-[^{32}P]phosphate group. The crosslinked oligonucleotide, 1_U, also yielded U-A and C-A in a molar ratio of 1:2, but the putative C-C-(G) was replaced by a streak of lower mobility designated U1 in Fig. 2*c*, lane 9. This result suggests that the site of crosslinking is C_{1399} or C_{1400} (or, possibly, G_{1401}). As above, the digestion products from the adduct were tested for photoreversibility by exposure to 254-nm light. Whereas U2 and U3 were unaltered by irradiation, U1 released the fragment U1a, which migrated to the position of C-C-(G) and yielded Cp but not Gp upon alkaline hydrolysis (Fig. 2*d*; Table 1). On the basis of all of the data presented in this section, we conclude that the main site to which $tRNA_1^{Val}$ crosslinks in the 16S RNA is C_{1400}.

Identification of Crosslinked Nucleotide in $tRNA_1^{Val}$. Covalent AcVal-$tRNA_1^{Val}$-16S RNA prepared from unlabeled ribosomes was digested to completion with RNase T1 in the presence of bacterial alkaline phosphatase. The resulting oligonucleotides were then labeled with [5′-^{32}P]pCp at the 3′ end by using RNA ligase and fractionated by gel electrophoresis. In this case, two crosslinked T1 products, bands 1_E and $1'_E$, were identified by their size and susceptibility to photoreversal (Fig. 1*c*; compare lanes 5 and 6). After elution, a sample of RNA from band 1_E was irradiated at 254 nm and subjected to electrophoresis alongside the unirradiated portion (Fig. 1*d*). Here again, photoreversal released only one major radioactive component, band 2_E. As we demonstrate below, the ^{32}P label was associated exclusively with material derived from $tRNA_1^{Val}$. The rRNA moiety of the complex remained unlabeled in this instance, most likely because the position of the crosslink rendered it an unsuitable substrate for RNA ligase.

Table 1. Secondary analysis of crosslinked oligonucleotide from band 1_U

Enzyme	Digestion product	Relative abundance*	Photoreversal product	Base composition	Deduced sequence
RNase A	P1	1.7	P1a	G, A, 2C	A-C-C-G
			P1b	A, 2C	A-C-C
			P1c	G, C	C-G
	P2	6.3		A, C	A-C
	P3	1.0		U	U
RNase U2	U1	0.7	U1a	2C, (G)†	C-C-(G)†
	U2	2.3		A, C	C-A
	U3	1.0		U, A	U-A

RNA extracted from band 1_U of the gel illustrated in Fig. 1*b* was digested and fractionated by paper electrophoresis as in Fig. 2 *a* and *c*. Products from the unirradiated digests were assayed for radioactivity and irradiated at 254 nm. The resulting components were then separated as in Fig. 2 *b* and *d*, and their base compositions were determined after alkaline hydrolysis.

* Relative abundance represents total ^{32}P radioactivity, not relative stoichiometry, of each product.

† Gp was recovered from alkaline digests of slowly migrating products such as U1 in some experiments; in these cases, the stoichiometry was 2C:1G.

The primary structure of the end-labeled RNA eluted from band 2_E was determined by the chemical procedure (Fig. 3*a*). Comparison of the sequence deduced with the complete sequence of $tRNA_1^{Val}$ provided clear evidence that component 2_E

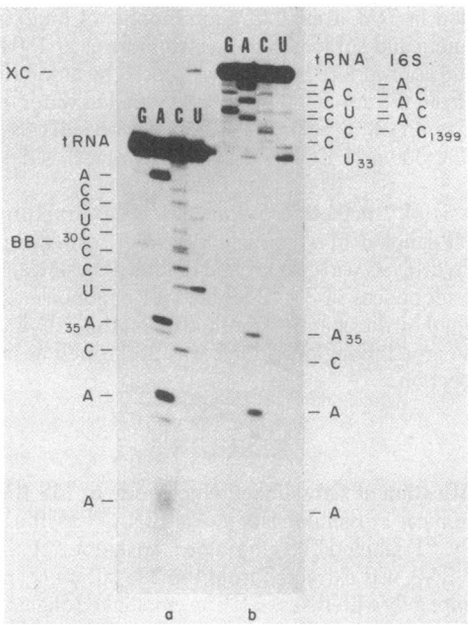

FIG. 3. Sequence analysis of the crosslinked T1 oligonucleotide 1_E and its tRNA component 2_E. Oligonucleotides from bands 1_E and 2_E in Fig. 1*d* were subjected to the base-specific cleavage reactions described by Peattie (10) and electrophoresed on a 20% polyacrylamide gel in 90 mM Tris·90 mM H_3BO_3, pH 8.3/2.5 mM EDTA/7 M urea. XC and BB mark the final positions of the xylene cyanole and bromophenol blue dyes. (*a*) Oligonucleotide 2_E. The deduced sequence corresponds to the anticodon-containing oligonucleotide from *E. coli* $tRNA_1^{Val}$ (11). Note that 5-carboxymethoxyuridine (cmo^5U) was not susceptible to the U-specific cleavage. (*b*) Oligonucleotide 1_E. The gel pattern is consistent with the structure

$tRNA_1^{Val}$

$$\overset{22}{Cp\overline{A}}\downarrow\overset{21}{p\overline{C}}\downarrow\overset{20}{p\overline{C}}\downarrow\overset{19}{p\overline{U}}\downarrow\overset{18}{p\overline{C}}\downarrow\overset{17}{p\overline{C}}\downarrow\overset{16}{p\overline{C}}\downarrow\overset{15}{p\overline{U}}\downarrow pV\overset{4}{p\overline{A}}\downarrow\overset{3}{p\overline{C}}\downarrow\overset{2}{p\overline{A}}\downarrow\overset{1}{p\overline{A}}\downarrow p^*C(p)$$

16S RNA $\overset{22}{Up\underline{A}}\uparrow\overset{21}{p\underline{C}}\uparrow\overset{20}{p\underline{A}}\uparrow\overset{19}{p\underline{C}}\uparrow\overset{18}{p\underline{A}}\uparrow\overset{17}{p\underline{C}}\uparrow pCpG(p),$

in which p* represents the sole ^{32}P atom in the crosslinked oligonucleotide and V stands for cmo^5U. Numerals above and below the sequence indicate the length in nucleotide residues of the ^{32}P-labeled fragment that is liberated when the phosphodiester chain is cleaved at the designated base. The scheme explains why no products of 5–14 nucleotides are present if any given molecule sustains only one scission and predicts that there should be two cleavage products of each length in the 17- to 22-base range, one from the tRNA branch, the other from the 16S RNA branch, of the bifurcated oligonucleotide. Apparent G cleavages visible in the autoradiogram are artifacts that presently remain unexplained. Band $1'_E$ gave a similar pattern although it lacked one or two residues present in 1_E (not shown).

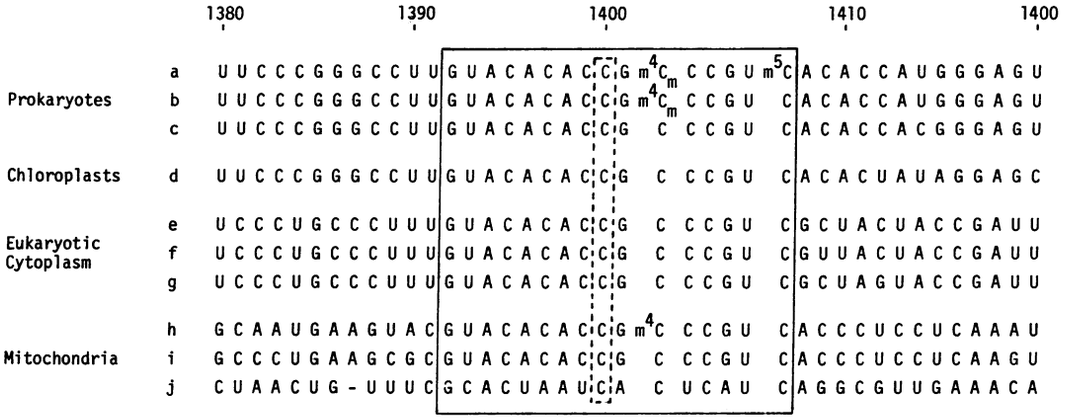

FIG. 4. Conserved sequence in the 3′ region of small-subunit rRNAs from phylogenetically diverse organisms. Numbering system is that for *E. coli* 16S RNA. Solid box delineates the highly conserved sequence (residues 1,392–1,407). Dashed box encloses the invariant cytidine at position 1,400. Primary structures are from the following: a, 16S RNA of *E. coli* (12, 13); b, 16S RNA of *Proteus vulgaris* (14); c, 16S RNA of *Bacillus brevis* (J. Kop, A. M. Kopylov, H. F. Noller, R. Siegel, R. Gupta, and C. R. Woese, personal communication); d, 16S RNA of *Zea mays* chloroplasts (15); e, 18S RNA of *Drosophila melanogaster* (16); f, 18S RNA of *Xenopus laevis* (17); g, 18S RNA of *Saccharomyces cerevisiae* (18); h, 13S RNA of hamster mitochondria (19); i, 12S RNA of human mitochondria (20); and j, 15S RNA of *S. cerevisiae* mitochondria (21).

was the anticodon-containing oligonucleotide, C-A-C-C-U-C-C-C-U-cmo^{5}U-A-C-m^{6}A-A-G_{38} (11). Although this product lacked the expected 3′-terminal G residue, a related fragment encompassing the sequence C-A-C-C-U-C-C-C-U-cmo^{5}U-A-C-m^{6}A-A-G_{39} was recovered in another experiment. The doublets visible in the lower part of the gel were probably caused by the absence of 3′-terminal phosphates from some of the molecules because this feature was eliminated by treatment of the labeled RNA with bacterial alkaline phosphatase prior to sequence analysis (not shown).

When the chemical sequence analysis procedure was applied to the crosslinked oligonucleotide, 1_E, an unusual and highly characteristic gel profile was obtained (Fig. 3*b*). To interpret the fragmentation pattern, it must be recalled that the tRNA component alone bears a ^{32}P label at its 3′ terminus and that the base-specific sequencing reactions used here introduce no more than one scission per molecule. Thus, if cleavage occurs on the 3′ side of the crosslink, the tRNA sequence A-C-m^{6}A-A_{38} can be read in the usual way between the 3′ terminus and the site of attachment to rRNA. Scission on the 5′ side of the crosslink, however, leads to cleavage of *either* the tRNA strand *or* the rRNA strand, but not both. The resulting fragments therefore retained one of the 5′-proximal strands intact, causing a long gap in the pattern from which products in the 5- to 14-nucleotide size range were absent (Fig. 3*b*). Among the products above the gap, two superimposed sets of oligonucleotides could be discerned, one corresponding to the rRNA segment, U-A-C-A-C-A-C_{1399}, the other to the tRNA segment, C-A-C-C-U-C-C-C-U_{33}, which arise from cleavages between the crosslinking site and the 5′ termini of the two components. Both the size and location of the gap indicate that $tRNA_1^{Val}$ is attached to 16S RNA via cmo$^5U_{34}$. Moreover, the appearance of the rRNA sequence at the expected position above the gap corroborates our proposal that C_{1400} participates in crosslink formation. We therefore conclude that the complete structure of the covalent oligonucleotide is

$tRNA_1^{Val}$ C-A-C-C-U-C-C-C-U-cmo^{5}U-A-C-m^{6}A-A-G_{39}
16S RNA U-A-C-A-C-A-C-C-G_{1401}.

DISCUSSION

Our previous studies on the photochemically induced crosslinking of underivatized AcAA-tRNAs to the ribosomal P site demonstrated that 16S RNA is an integral part of this important functional domain (1–3). Specifically, we have shown that AcVal-$tRNA_1^{Val}$ can be covalently attached to the nonanucleotide U-A-C-A-C-A-C-C-G encompassing residues 1,393–1,401 of the 16S RNA molecule from *E. coli* (6). The nonanucleotide lies within a longer, highly conserved sequence, which has been found to occur in small-subunit rRNAs from prokaryotes, eukaryotes, chloroplasts, and mitochondria (12–21). The relevant sequences and the sections adjacent to them are presented for comparison in Fig. 4. Owing to its exceptional evolutionary

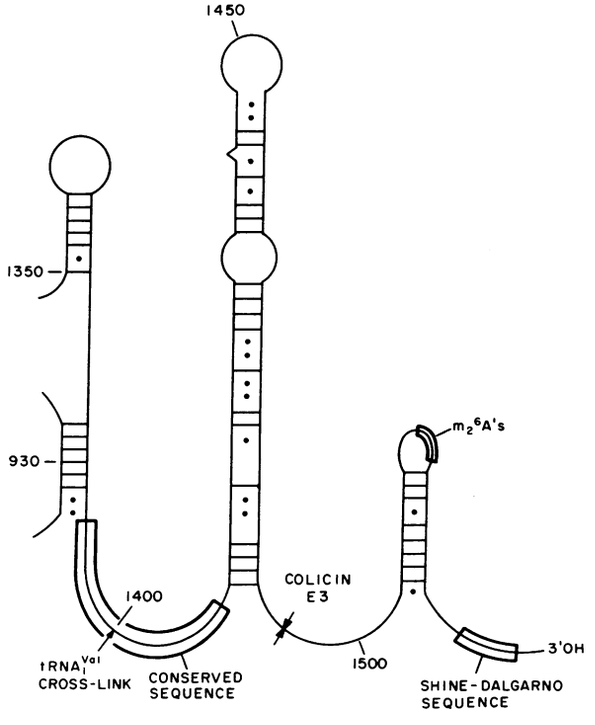

FIG. 5. Location of functional sites in the 3′-terminal segment of *E. coli* 16S RNA. Large box encloses highly conserved sequence (residues 1,392–1,407; see Fig. 4). Single arrow points to residue C_{1400}, which can be crosslinked to the 5′ anticodon base of $tRNA_1^{Val}$. Double arrow indicates site of cleavage by colicin E3 (24). Small boxes enclose the two adjacent m_2^6A residues that mediate ribosomal sensitivity to kasugamycin (25) and the Shine–Dalgarno sequence, which is complementary to the preinitiation signal in mRNA (26). Horizontal bars represent G-C or A-U base pairs and dots indicate G-U base pairs. Secondary-structure model was adapted from Woese *et al.* (23).

persistence and its physical proximity to peptidyl-tRNA, we infer that the conserved sequence is an essential component of the ribosomal P site.

In the present report, the site at which 16S RNA is crosslinked to $tRNA_1^{Val}$ has been identified as C_{1400}. Although a small amount of crosslinking to C_{1399} or G_{1401} cannot be entirely excluded, the latter possibility is clearly incompatible with cyclobutane dimer formation (4). Moreover, it seems likely that covalent attachment is restricted to a unique site in both rRNA and tRNA, given the exacting stereochemical requirements for such reactions (22). In this regard, we note that the nucleotide corresponding to C_{1400} in *E. coli* 16S RNA is strictly preserved as a C residue in all other small-subunit rRNAs whose sequences have been determined to date, including that from yeast mitochondria, which exhibits substantial variation in primary structure upstream and downstream from the site in question (Fig. 4). It is also gratifying that C_{1400} and the surrounding bases have been judged to lie in a single-stranded region of the 16S RNA and are presumably accessible to ligands even in the 30S subunit (23). This aspect, as well as the relationship of the crosslinking site to other features of interest near the 3′ end of the rRNA molecule, is illustrated in Fig. 5.

Although the identity of the crosslinked residue in 16S RNA has been determined only for *E. coli* $tRNA_1^{Val}$, we believe it likely that other competent tRNAs react at or near the same site (2, 3, 7). At the very least, $tRNA_1^{Ser}$, $tRNA^{Lys}$, and $tRNA_2^{Glu}$ from *E. coli* and $tRNA^{Val}$ from *Bacillus subtilis* have been shown to attach to a 125-nucleotide subfragment from the 3′ third of the 16S RNA (3, 5). The position of the photoreactive base in the crosslinked tRNA molecules is probably identical in all cases as well. While various lines of evidence indicated that this residue was a modified uridine in the 5′ position of the anticodon (2–4, 7), definitive proof was lacking. The work described here provides direct evidence that the site of crosslinking in $tRNA_1^{Val}$ is indeed the 5′ anticodon base, cmo^5U.

The $tRNA_1^{Val}$-16S RNA adduct is believed to result from pyrimidine-pyrimidine cyclobutane dimerization as indicated by the properties of the crosslinking reaction and its characteristic photoreversibility (4). Cyclobutane dimerization would be facilitated if cmo^5U_{34} of $tRNA_1^{Val}$ were stacked with C_{1400} of the 16S RNA at the P site prior to irradiation (22). We have previously suggested that stacking of the 5′ anticodon base on an exposed pyrimidine residue of 16S RNA might increase the stability of tRNA–ribosome interaction without impeding codon–anticodon pairing (2). A model of possible structural relationships among mRNA, tRNA, and rRNA that is consistent with these postulates is presented in Fig. 6. Although it is still not clear to what extent the invariant C residue at position 1,400 of *E. coli* 16S RNA contributes to tRNA-ribosome interaction under conditions of normal protein synthesis, our studies conclusively demonstrate that the 5′ base of the tRNA anticodon is closely juxtaposed to this nucleotide when functionally bound to the ribosomal P site.

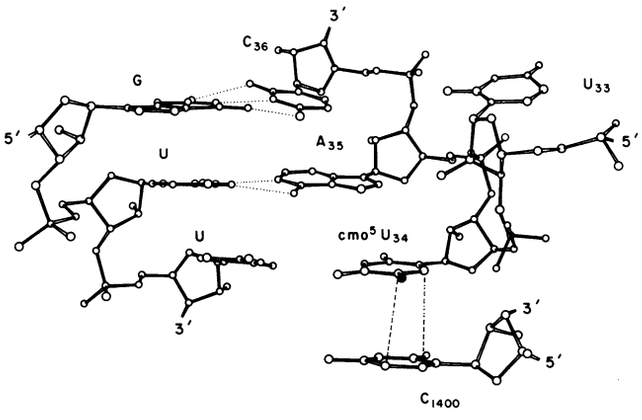

FIG. 6. Model of relationship among mRNA codon, $tRNA_1^{Val}$ anticodon, and 16S RNA nucleotide C_{1400} at the ribosomal P site. In this speculative scheme, the mRNA codon G-U-U is base-paired with the tRNA anticodon cmo^5U-A-C through its first two residues. Hydrogen bonds are indicated by dotted lines. The wobble base cmo^5U, which remains unpaired, is stacked upon $C_{1,400}$ in a configuration that meets the stereochemical requirements for cyclobutane dimerization (22). Dashed lines connect reactive atoms. The alignment shown would lead to formation of the *cis,syn* dimer, although the actual structure of the adduct is not known. Note that the sharp bend in the phosphodiester backbone between cmo^5U_{34} and U_{33} leaves the 5′ anticodon base unusually exposed. The substituent at position 5 of cmo^5U_{34} is represented by a filled circle corresponding to the atom closest to the ring. Perspective view was generated on the basis of molecular parameters given in refs. 27–29.

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