Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 3607–3611, June 1984
Biochemistry

Identification of a site on 23S ribosomal RNA located at the peptidyl transferase center

(photoaffinity labeling/aromatic ketones/tRNA binding site/hybridization/reverse transcription)

ANDREA BARTA*, GÜNTER STEINER*, JÜRGEN BROSIUS†‡, HARRY F. NOLLER†, AND ERNST KUECHLER*

*Institut für Biochemie, Universität Wien, 1090 Wien, Währinger Strasse 17, Austria; and †Thimann Laboratories, University of California, Santa Cruz, CA 95064

Communicated by Alexander Rich, December 30, 1983

ABSTRACT **3-(4′-Benzoylphenyl)propionyl[^{3}H]Phe-tRNA bound to the peptidyl site of the ribosome is photocrosslinked exclusively to 23S RNA on irradiation at 320 nm. The site of reaction has been identified both by hybridization and primer-extension experiments as uridine-2584 and uridine-2585, located within the central loop of domain V according to the secondary structure model of 23S RNA. The fact that the covalently crosslinked tRNA retains its ability to form a peptide bond, together with the proximity of this site to the position of several mutations leading to chloramphenicol or erythromycin resistance strongly argue that this region of the 23S-like rRNAs is an integral component of the peptidyl transferase site. On the basis of these results, and from comparative analysis of the 16 available large subunit rRNA sequences, we propose a model for the functional organization of the peptidyl transferase site involving interaction of domains II and V of 23S rRNA.**

The three-dimensional structure of the ribosome still presents one of the most challenging problems in molecular biology. On the ribosome, a variety of events must take place in a coordinate fashion to translate the information provided by the mRNA into the correct amino acid sequence. To define the mechanism of translation, attention so far has mainly focused on the protein components of the ribosome, although from our current knowledge, it is difficult to describe the dynamics of the ribosomal machinery in terms of protein functions alone. There is, however, accumulating evidence that structural interactions between the RNA components—i.e., rRNAs, tRNAs, and mRNA—play an important role in ribosomal function (1, 2). It is generally assumed that the ribosome provides a favorable environment for peptide-bond formation, the prerequisite for this reaction being the correct alignment of the two tRNAs and the close proximity of their CCA-terminal moieties. Therefore, the elucidation of the architecture of the peptidyl transferase center is of utmost importance for the understanding of the mechanism of protein synthesis.

A variety of chemically or photochemically reactive derivatives of aminoacyl tRNA and antibiotics have been used to investigate the ribosomal peptidyl transferase site (for reviews see refs. 3–6). Depending on the derivative used, several ribosomal proteins and/or 23S RNA are affinity-labeled. However, it has not been possible until now to assign a specific peptidyl transferase function to one of the ribosomal proteins. Furthermore, no site of crosslinking of such probes to 23S RNA has been unambiguously positioned in the rRNA sequence.

In previous experiments, we used the 3-(4′-benzoylphenyl)propionyl derivative of Phe-tRNA (BP-Phe-tRNA; Fig. 1) as a probe of the peptidyl transferase region (7–9). The lone

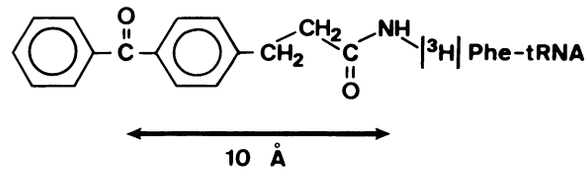

FIG. 1. Structure of 3-(4′-benzoylphenyl)propionyl[^{3}H]phenylalanyl-tRNA (BP-[^{3}H]Phe-tRNA).

electron pair of the carbonyl group of the benzophenone moiety can be activated by UV irradiation at 320 nm to react with the C_α–H bond of peptides as well as with nucleic acids (9, 10). BP-[^{3}H]Phe-tRNA bound to the peptidyl site (P site) of poly(U)-programmed ribosomes crosslinks in high yield to the 23S RNA. Limited hydrolysis of the photoaffinity-labeled 23S RNA with RNase A demonstrates that the crosslink occurs at a site in the 11S RNA fragment, which comprises the 3′-proximal 1100 nucleotides of 23S RNA. After photoreaction, this complex retains its ability to participate in peptide-bond formation (9). The labeled region of the 23S RNA must therefore reside within the peptidyl transferase center.

In this paper we report the identification of the peptidyl transferase region by precise positioning of the photoaffinity-labeled nucleotides of 23S RNA. The results are discussed in light of the proposed secondary structure of 23S RNA. A model for the binding of the CCA terminus to the P site of the ribosome is proposed.

MATERIALS AND METHODS

Materials. [^{3}H]Phenylalanine (specific activity, 70 Ci/mmol; 1 Ci = 37 GBq) and [γ-^{32}P]ATP (specific activity, 5000 Ci/mmol) were obtained from Amersham, tRNA^{Phe} was purchased from Boehringer, Mannheim, and poly(U) was from Miles Seravac. Restriction enzymes were obtained either from Bethesda Research Laboratories or from New England Biolabs; T4 polynucleotide kinase and bacterial alkaline phosphatase were from P-L Biochemicals. Avian myeloblastosis virus reverse transcriptase was provided by J. Beard (Life Sciences, St. Petersburg, FL). Ribosomes from *Escherichia coli* MRE 600 were photoaffinity-labeled with BP-[^{3}H]Phe-tRNA as described (8).

Southern Blotting and Hybridization Conditions. Restriction digests were prepared as recommended by the suppliers. The fragments were separated on a 2% agarose gel, transferred to nitrocellulose, and hybridized with 80 μg (1.25 $\times$ 10^6 cpm) of 23S RNA photoaffinity-labeled with BP-[^{3}H]Phe-tRNA (11). Hybridization was carried out for 40 hr at 42°C. The filters were washed 3 times for 30 min each with

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: kb, kilobase(s); bp, base pair(s); BP-[^{3}H]Phe-tRNA, 3-(4′-benzoylphenyl)propionyl[^{3}H]Phe-tRNA; P site, peptidyl site.

‡Present address: Center for Neurobiology, Columbia University, 722 W. 168th Street, New York, NY 10032.

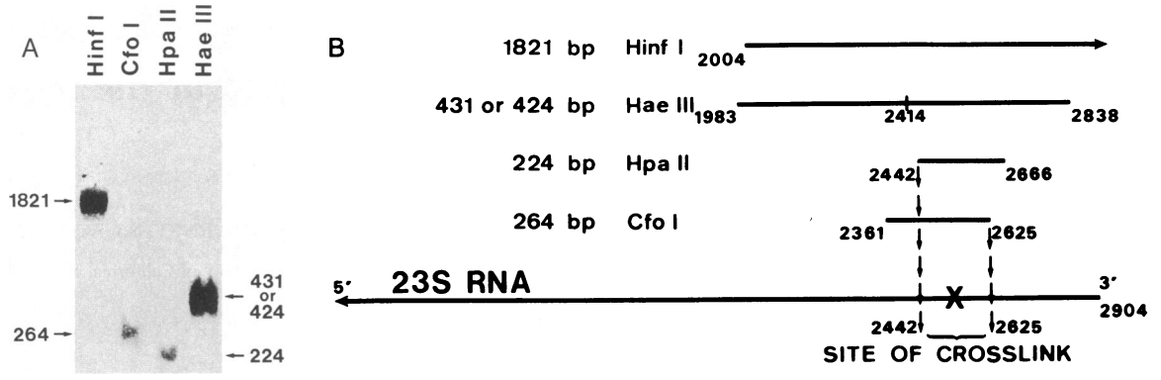

FIG. 2. (*A*) Southern blot of rDNA restriction fragments hybridized to crosslinked ^{3}H-labeled 23S RNA. The *Eco*RI/*Bam*HI insert of the plasmid pKK123 was digested with several restriction enzymes. The resulting fragments were separated on an agarose gel, transferred to nitrocellulose, and hybridized to photoaffinity-labeled ^{3}H-labeled 23S RNA. After digestion of the single-stranded RNA with RNase A, the nitrocellulose filter was soaked in 2,5-diphenyloxazole/toluene and exposed at −70°C to a preflashed x-ray film. (*B*) Localization of the ^{3}H-labeled RNA·DNA hybrid fragments of 23S RNA. This identifies the region between nucleotides 2442 and 2625 as the site of crosslink.

2× NaCl/Cit (1× NaCl/Cit is 0.15 M NaCl/0.015 M Na citrate), twice for 15 min each with 0.1× NaCl/Cit, incubated with RNase A (40 μg/ml) in 2× NaCl/Cit for 30 min at 37°C, washed twice for 15 min each with 0.1× NaCl/Cit/0.1% sodium dodecyl sulfate, and twice for 15 min each with 0.1× NaCl/Cit. After drying, the filters were soaked in toluene containing 10% 2,5-diphenyloxazole and exposed at −70°C to preflashed Kodak XR5 (X-Omat) film.

Preparation of the Labeled Primer and Reverse Transcription. A 124-base-pair (bp) *Hpa* II fragment and a 56-bp *Ava* II fragment of pKK123 (12) were labeled on their 5′ ends with ^{32}P and the strands were separated (13). The end-labeled primers (1–2 × 10^4 cpm) were annealed to 1 μg of 23S RNA in 20 μl of buffer (100 mM KCl/50 mM Tris·HCl, pH 7.5/10 mM $MgCl_2$/2 mM dithiothreitol) for 5 min at 65°C (14, 15). After cooling, the four dNTPs were added to a final concentration of 0.25 mM and the mixture was incubated with 8 units of avian myeloblastosis virus reverse transcriptase for 30 min at 37°C. For the sequencing reactions with dideoxynucleotides, the dNTP concentrations were decreased to 100 μM for each dNTP. The ddNTP/dNTP ratios were 1:4 for dGTP, dATP, and dTTP and 1:10 for dCTP (14). The reaction products were separated on a polyacrylamide/7 M urea sequencing gel (13).

RESULTS

Hybridization of Photoaffinity-Labeled 23S RNA to rDNA Fragments. The 3.16-kilobase (kb) *Eco*RI/*Bam*HI insert of the recombinant plasmid pKK 123 comprises part of the *E. coli rrnB* operon and contains 2061 bp from the 3′ end of the 23S RNA (12). This insert contains the DNA sequences corresponding to the 11S fragment previously identified as the site of the photoreaction on 23S RNA. The restriction enzymes *Hin*fI, *Hae* III, *Hpa* II, and *Cfo* I were chosen to digest the purified insert DNA. The resulting DNA fragments were separated on a 2% agarose gel and transferred to nitrocellulose. Fig. 2*A* shows a fluorograph of the filter after hybridization to 23S RNA ^{3}H-labeled by the photoaffinity probes. As the filter had been incubated with RNase A, the label could only be protected by hybridization to a DNA fragment, resulting in a positive hybridization signal. It should be noted that the efficiency of transfer to the nitrocellulose membrane is lower for smaller fragments, which explains the weaker hybridization signals in the *Cfo* I and *Hpa* II fragments. From the size of the hybridizing fragments and the known sequence, their positions on the 23S RNA can be deduced (Fig. 2*B*). This experiment demonstrates that the crosslink lies within a 183-nucleotide region between positions 2442 and 2625 in 23S RNA.

Primer Extension and Sequencing. To identify the precise position of the photoaffinity-labeled nucleotide, advantage was taken of the fact that reverse transcriptase pauses or halts at modified nucleotides (15, 16). A 124-bp *Hpa* II fragment (corresponding to positions 2715–2839) was chosen for priming reverse transcription on photoaffinity-labeled 23S RNA. This primer binds 90 bases to the 3′ side of the 183-nucleotide region identified in the previous experiment. Fig. 3 (lane 2) shows the elongation products of a primer-extension experiment using unmodified 23S RNA as template. The resulting fragment pattern is reproducible under the conditions used and is probably attributable to post-transcriptionally modified bases and/or higher order structure in the RNA. When the same experiment is carried out with 23S RNA photoaffinity-labeled with BP-[^{3}H]Phe-tRNA on poly(U)-programmed ribosomes, two additional strong bands appear, corresponding to fragments arising from extra

FIG. 3. A 124-bp ^{32}P-labeled DNA fragment (P_{124}; position 2715–2839) was used to prime reverse transcription on 23S RNA. The resulting cDNA transcripts were separated on an 8% polyacrylamide/urea gel. Lane 1, reverse transcription of 23S RNA photoaffinity-labeled with BP-[^{3}H]Phe-tRNA; lane 2, reverse transcription of nonirradiated control 23S RNA; lane 3, reverse transcription of 23S RNA from ribosomes irradiated in the absence of photoaffinity label. Irradiation of ribosomes alone already generates band I, whereas band II only appears when photoaffinity-labeled 23S RNA is used as template.

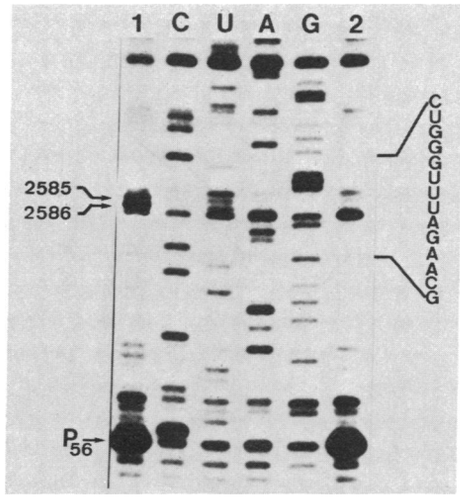

FIG. 4. Autoradiogram of a dideoxy sequencing gel using reverse transcriptase to elongate a 56-bp primer (P_{56}; position 2607–2663). Lanes labeled C, U, A, and G already refer to 23S RNA sequence and result from reactions containing the complementary ddNTP. Reverse transcription products generated on photoaffinity-labeled 23S RNA (lane 1) and on 23S RNA from nonirradiated ribosomes (lane 2) were run in parallel. Using P_{56} as primer, reverse transcription starts beyond the site of the internal crosslink (band I). Band II splits into two bands corresponding to positions U-2585 and U-2586.

stops of the reverse transcriptase (Fig. 3, lane 1, bands designated I and II). These bands are clearly resolved from similarly migrating control bands in high-resolution gels. As a control, an incubation mixture lacking the photoreactive BP-[^{3}H]Phe-tRNA was irradiated with UV light of 320 nm under identical conditions. If 23S RNA from the latter ribosomes is used as a template for reverse transcription (Fig. 3, lane 3), band I, but not band II, is observed. This shows that the stop corresponding to band I is due to the inherent photoreactivity of a nucleotide in 23S RNA. Therefore, only band II is generated by photoaffinity labeling with BP-[^{3}H]Phe-tRNA.

To identify the position corresponding to band II more precisely, a shorter *Ava* II fragment (positions 2607–2663) closer to the crosslink site was used to prime the reverse transcription. In parallel, sequencing reactions with dideoxynucleotides were carried out using 23S RNA as a template. The sequencing tracks in Fig. 4 (lanes C, U, A, and G) have been transposed to refer to the 23S RNA sequence. Lane 1 represents the reverse transcripts obtained with photoaffinity-labeled 23S RNA, and lane 2 is the nonirradiated control. As seen in Fig. 4 (lane 1), band II is split into two bands corresponding to uridine-2585 and uridine-2586. As Youvan and Hearst (16) have demonstrated that reverse transcription stops exactly at the nucleotide immediately 3′ to a hypermodified base, we assign the sites of photoaffinity labeling of the BP-[^{3}H]Phe-tRNA at the P site to positions uridine-2584 and uridine-2585 of 23S RNA. In another experiment, the site of the internal crosslink giving rise to band I has been identified as uridine-2613 (unpublished results).

DISCUSSION

The results presented in this paper report the precise identification of a site in 23S RNA at the peptidyl transferase region using photoaffinity-labeling techniques. Previously, a number of studies demonstrating affinity labeling of 23S RNA at the peptidyl transferase center have been published (3–6), but no site of crosslinking has been localized unambiguously within the 23S RNA sequence.

The photoaffinity reagent BP-[^{3}H]Phe-tRNA fulfills the criteria for a specific probe of the peptidyl transferase center. Labeling of 23S RNA occurs with a yield of 20–30% when calculated on the basis of bound BP-[^{3}H]Phe-tRNA, a value that compares favorably with that of other affinity probes. Reaction with 23S RNA depends on the presence of poly(U) and is specifically inhibited by puromycin (7, 8). Furthermore, the covalently attached BP-[^{3}H]Phe-tRNA retains its ability to form a peptide bond with acceptor-site-bound Phe-tRNA (9). This indicates that the photoreaction occurs at the P site in such a way that the ribosomal complex remains in a functionally active state. Omission of poly(U) from the incubation mixture results in photoaffinity labeling of 30S ribosomal proteins (unpublished results). Reaction with 23S RNA is thus due to close proximity of the affinity probe rather than to a limited chemical specificity of the photoreagent. This demonstrates that BP-[^{3}H]Phe-tRNA probes the actual topography of the ribosomal complex before transpeptidation. Identification of the precise site of affinity labeling is therefore of considerable interest.

Previously, we were unable to identify the site of reaction by conventional RNA sequencing methods because of the intractable properties of the resulting hypermodified nucleotide. However, hybridization experiments of BP-[^{3}H]Phe-tRNA-labeled 23S RNA with various 23S rDNA restriction fragments localized the site of reaction to a 183-nucleotide sequence (position 2442–2625). Precise determination of the labeled site was achieved by primer-extension experiments using reverse transcriptase. Such experiments have previously been performed on 16S and 18S RNA (15, 16), showing that reverse transcriptase pauses at a N^2-methylguanosine on 16S RNA from *E. coli* and halts before a hypermodified nucleotide on 18S RNA from *Drosophila*. Therefore, reverse transcriptase would similarly be expected to pause or stop at nucleotides modified by photoreaction with BP-[^{3}H]Phe-tRNA. Bands due to stops at two different sites have been observed, designated I and II, respectively (Fig. 3). Band I can be attributed to an internal crosslink (involving uridine-2613; unpublished data), because it results from the irradiation process alone, whereas band II is brought about by photoreaction with BP-Phe-tRNA (Fig. 3, lanes 1 and 3).

Band II caused by photoreaction with BP-Phe-tRNA actually consists of two bands as seen in Fig. 4. These bands can be attributed to stops of reverse transcription at uridine-2585 and uridine-2586. The sites of photoreaction can therefore be assigned to positions uridine-2584 and uridine-2585. The involvement of uridine residues is in agreement with our previous identification of the modified nucleotide from alkaline hydrolysis of photoaffinity-labeled 23S RNA (9). The photoaffinity-labeled uridine residues are located in the central loop of domain V of 23S rRNA (Fig. 5*A*). It is remarkable that this region is structurally extremely conserved throughout evolution (17–19). In addition, most of the unpaired bases in the loop are conserved in systems as different as eubacteria, mitochondria, chloroplasts, and eukaryotes, as might be expected for a region involved in an essential ribosomal function (Fig. 5*A*). Further support for the importance of this loop region in ribosomal function is provided by genetic studies of resistance to chloramphenicol and erythromycin. Both antibiotics are inhibitors of peptide bond formation. Chloramphenicol acts specifically on the peptidyl transferase, whereas the mode of action of erythromycin is less well-defined (20). Several mutants found in mammalian and yeast mitochondrial rRNA conferring resistance to chloramphenicol (21–24) and erythromycin (25) exhibit single base changes in the central-loop region of domain V (Fig. 5*A*). This provides additional evidence for the functional role of this region in the peptidyl transferase reaction.

Little is known about the molecular mechanism of peptide bond formation. Models have been proposed suggesting that either histidine residues from ribosomal proteins (26) or the 5′-terminal phosphate group of tRNA participate in the pep-

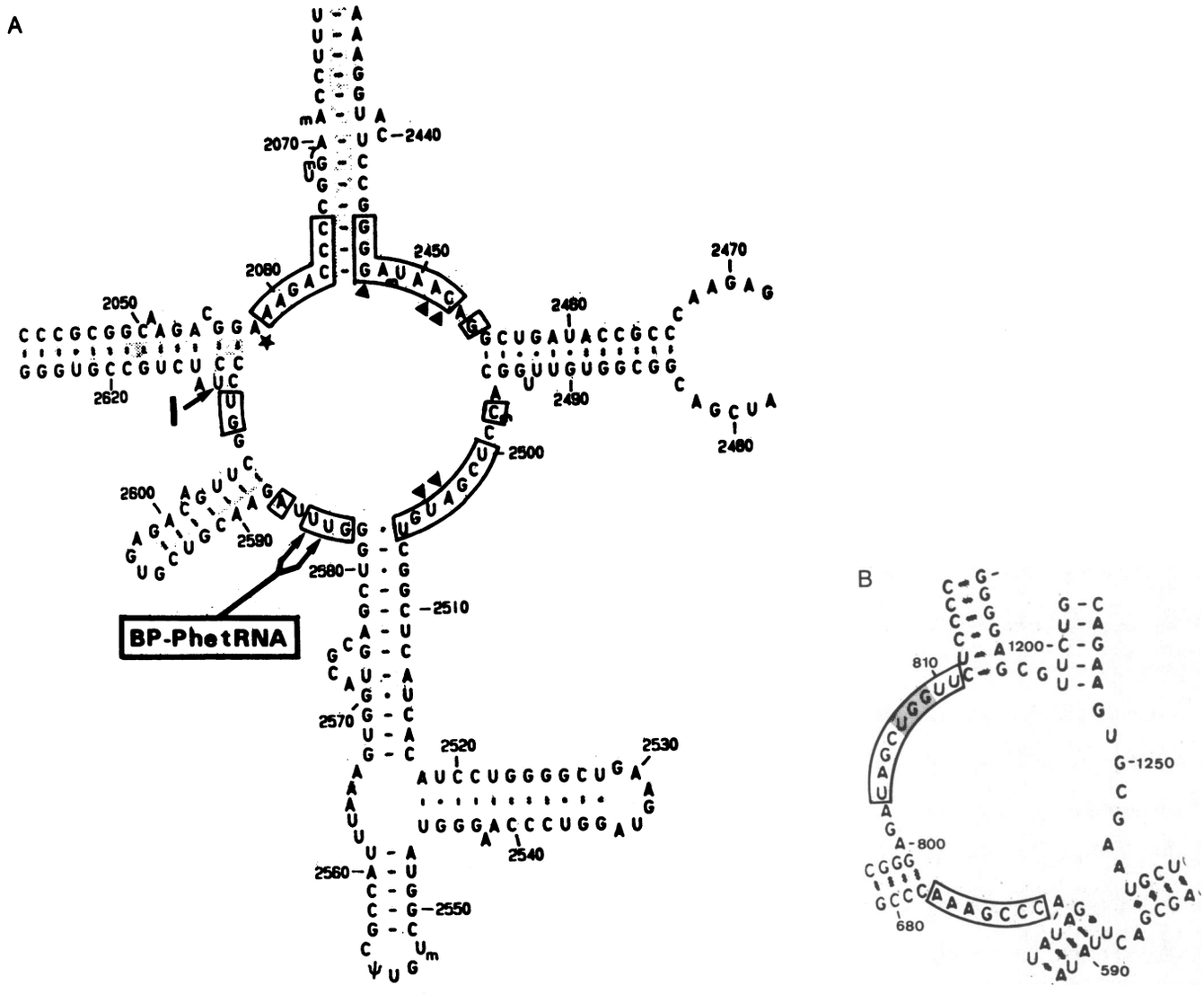

FIG. 5. (*A*) Secondary structure of domain V of the 23S RNA according to the model of Noller *et al.* (17, 19). U-2584 and U-2585 are photoaffinity-labeled by BP-Phe-tRNA at the ribosomal P site. Boxed sequences are highly conserved throughout evolution; sites of base changes resulting in resistance to chloramphenicol (▲) and erythromycin (★) in mitochondrial RNAs from yeast and mammals (21–25). (▮) indicates the site of the internal crosslink obtained by irradiation of ribosomes at 320 nm; the same region was found to be photocrosslinked at 254 nm to region 739–748 of domain II (34). (*B*) Secondary structure of a loop region in domain II. Again, sequences highly conserved throughout evolution are boxed (19). The shaded nucleotides UGG are proposed to base pair with the CCA terminus of tRNA as discussed in the text.

tidyl transfer reaction (27). Independent of the mechanism, the precise positioning of the two CCA termini is a prerequisite for the transfer reaction. The available evidence supports specific interaction of the CCA sequence with the ribosome (28–33). If binding is caused by conventional base pairing, a UGG sequence in 23S rRNA is required, whose evolutionary conservation is comparable with the conservation of the CCA sequence of tRNA. Inspection of the available 16 23S-like rRNA sequences (19) shows that only one universally conserved UGG exists. It is found in the invariant U-A-G-C-U-G-G-U-U-811 sequence in a loop region of domain II (Fig. 5*B*), present in all eubacterial, chloroplast, mitochondrial, and eukaryotic cytoplasmic ribosomes in which large subunit RNA sequences are presently known. If UGG-809 is in fact involved in binding the 3′ CCA of tRNA, this region of domain II must be in close proximity to the central loop of domain V. Specifically, the distance between the affinity-labeled residues 2584 and 2585 and uridine-807 would have to be within 14 Å. Indeed, experimental evidence obtained by UV-induced crosslinking of 23S rRNA places the 575 and 745 regions of domain II in contact with the 2030 and 2615 regions, respectively, in domain V (34). The above structural constraints on the folding of 23S rRNA place the conserved U-A-G-C-U-G-G-U-U-811 sequence in the general vicinity of the affinity-labeled site. This is consistent with a model whereby the CCA terminus of tRNA binds to a site in domain II that is spatially juxtaposed with the peptidyl transferase region in the central loop of domain V. Partitioning of the CCA-binding and peptide bond-forming functions between two structural domains could imply that these functions may be coupled via a dynamic interaction between these domains. As a general principle, uncoupling of tRNA binding from peptide bond formation could, for example, form the basis of a molecular mechanism for translational proofreading.

The studies presented here identify the peptidyl transferase region on 23S RNA. The methods developed for this investigation can be extended to studies of different functional states in the ribosomal cycle. Future experimentation should provide further insight into the architecture and dynamics of 23S rRNA and its relationship to peptidyl transferase function.

We wish to thank R. Gutell for alignment of 23S rRNA sequences, A. Gupta for technical assistance, T. Skern for critical reading, and B. Gamperl for typing the manuscript. This work was supported by a grant from the Austrian Fonds zur Förderung der wissenschaftlichen Forschung (to E.K.) and by National Institutes of Health Grant GM-17129 (to H.F.N.). J.B. was a postdoctoral fellow of the Fogarty International Center of the National Institutes of Health.

1. Woese, C. R. (1980) in *Ribosomes: Structure, Function, and Genetics*, eds. Chambliss, G., Craven, G., Davies, J., Davis, K., Kahan, L. & Nomura, M. (Univ. Park Press, Baltimore, MD), pp. 357–373.
2. Steitz, J. A. (1980) in *Ribosomes: Structure, Function, and Genetics*, eds. Chambliss, G., Craven, G., Davies, J., Davis, K., Kahan, L. & Nomura, M. (Univ. Park Press, Baltimore, MD), pp. 479–495.
3. Kuechler, E. & Ofengand, J. (1979) in *Transfer RNA: Structure, Properties, and Recognition*, eds. Schimmel, P. R., Söll, D. & Abelson, J. N. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 413–444.
4. Cooperman, B. S. (1980) in *Ribosomes: Structure, Function, and Genetics*, eds. Chambliss, G., Craven, G., Davies, J., Davis, K., Kahan, L. & Nomura, M. (Univ. Park Press, Baltimore, MD), pp. 531–554.
5. Ofengand, J. (1980) in *Ribosomes: Structure, Function, and Genetics*, eds. Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L. & Nomura, M. (Univ. Park Press, Baltimore, MD), pp. 497–529.
6. Leitner, M., Wilchek, M. & Zamir, A. (1982) *Eur. J. Biochem.* **125**, 49–55.
7. Barta, A., Kuechler, E., Branlant, C., SriWidada, J., Krol, A. & Ebel, J. P. (1975) *FEBS Lett.* **56**, 170–174.
8. Kuechler, E. & Barta, A. (1977) *Methods Enzymol.* **46**, 676–683.
9. Barta, A. & Kuechler, E. (1983) *FEBS Lett.* **163**, 319–323.
10. Galardy, R. D., Printz, M. P. & Craig, L. C. (1973) *Nature (London) New Biol.* **242**, 127–128.
11. Thomas, P. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
12. Brosius, J., Dull, T. J. & Noller, H. F. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 201–204.
13. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
14. Sanger, F., Nicklen, S. & Coulsen, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
15. Youvan, D. C. & Hearst, J. E. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3751–3754.
16. Youvan, D. C. & Hearst, J. E. (1981) *Nucleic Acids Res.* **9**, 1723–1741.
17. Noller, H. F., Kop, J. A., Wheaton, V., Brosius, J., Gutell, R. R., Kopylov, A. M., Dohme, F., Herr, W., Stahl, D. A., Gupta, R. & Woese, C. R. (1981) *Nucleic Acids Res.* **9**, 6167–6189.
18. Branlant, C., Krol, A., Machatt, M. A., Poyet, J., Ebel, J. P., Edwards, K. & Kössel, H. (1981) *Nucleic Acids Res.* **9**, 4303–4324.
19. Noller, H. F. (1984) *Annu. Rev. Biochem.*, in press.
20. Cundliffe, E. (1980) in *Ribosomes: Structure, Function, and Genetics*, eds. Chambliss, G., Craven, G., Davies, J., Davis, K., Kahan, L. & Nomura, M. (Univ. Park Press, Baltimore, MD), pp. 555–581.
21. Dujon, B. (1980) *Cell* **20**, 185–197.
22. Kearsey, S. E. & Craig, I. W. (1981) *Nature (London)* **290**, 607–608.
23. Blanc, H., Adams, C. W. & Wallace, D. C. (1983) *Nucleic Acids Res.* **9**, 5785–5795.
24. Blanc, H., Wright, C. T., Bibb, M. J., Wallace, D. C. & Clayton, D. A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3789–3793.
25. Sor, F. & Fukuhara, H. (1982) *Nucleic Acids Res.* **10**, 6571–6577.
26. Nierhaus, K. H. (1982) *Curr. Top. Microbiol. Immunol.* **97**, 81–155.
27. Garrett, R. A. & Woolley, P. (1982) *Trends in Biochem. Sci.* **7**, 385–386.
28. Monro, R. E., Cerna, J. & Marcker, K. A. (1968) *Proc. Natl. Acad. Sci. USA* **61**, 1042–1049.
29. Cerna, J. (1975) *FEBS Lett.* **58**, 94–98.
30. Sundari, R. M., Pelka, H. & Schulman, L. H. (1977) *J. Biol. Chem.* **252**, 3941–3944.
31. Peattie, D. A. & Herr, W. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2273–2277.
32. Douthwaite, S., Garrett, R. A. & Wagner, R. (1983) *Eur. J. Biochem.* **131**, 261–269.
33. Farber, N. & Cantor, C. R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5135–5139.
34. Stiege, W., Glotz, C. & Brimacombe, R. (1983) *Nucleic Acids Res.* **11**, 1687–1706.