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Mechanism of translocation: Relative arrangement of tRNA and mRNA on the ribosome

(wybutine/photocrosslinking/codon–anticodon interaction/acceptor site/peptidyl site)

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ABSTRACT **AcPhe-tRNA^{Phe} from yeast can be photocrosslinked to poly(U) on *Escherichia coli* ribosomes. The photoreaction occurs at the wybutine base situated next to the 3′ side of the anticodon. The kinetics and efficiency of crosslinking of AcPhe-Phe-tRNA are the same at both the acceptor site and the peptidyl site. Therefore, the orientation of wybutine with respect to the mRNA is similar in both the pretranslocational and posttranslocational states. AcPhe-Phe-tRNA crosslinked at the acceptor site can still be translocated to the peptidyl site, demonstrating that tRNA and mRNA are transported together. The experiments support a model of translocation in which the conformation of the anticodon loop of tRNA is similar in both the peptidyl site and the acceptor site.**

Among the many different reactions that take place on the ribosome during protein biosynthesis, translocation is one of the most complicated. During translocation the peptidyl-tRNA is moved from the acceptor site (A site) to the peptidyl site (P site) with concomitant release of the deacylated tRNA, thus enabling the ribosome to bind the aminoacyl-tRNA corresponding to the next codon. Under natural conditions, elongation factor G (EF-G) participates in translocation and each translocation step is accompanied by cleavage of one molecule of GTP. Under *in vitro* conditions, translocation also occurs spontaneously in the absence of EF-G and without GTP cleavage (1–4). The ability to translocate is thus an endogenous property of the ribosome. EF-G and GTP cleavage are required only to stimulate the rate of the reaction.

During translocation the mRNA is moved by the length of one codon with respect to the ribosome. The movement of the mRNA seems to be determined by the tRNA. Riddle and Carbon (5) have demonstrated that a frameshift mutation containing one additional nucleotide can be suppressed by a mutant tRNA^{Gly} having four bases in the anticodon rather than three. However, the mechanism by which the movement of tRNA and mRNA occurs during translocation is completely unknown. Woese (6) suggested that the tRNA undergoes a structural change in the anticodon loop from a 5′ stacked conformation (corresponding to the A site) to a 3′ stacked conformation (corresponding to the P site). This model was also used by Crick *et al.* (7) to explain protein synthesis on RNA templates under prebiotic conditions. In contrast, Rich (8) presented a model in which both tRNAs on the ribosome have the same conformation as in the crystal structure with the anticodon in the 3′ stacked form. During translocation, the tRNA rotates from the A site to the P site, opening the A site for binding of the incoming aminoacyl-tRNA. Because the tRNA molecules are L-shaped, the -CCA ends of the two tRNAs come close enough for peptide bond formation. An analogous model was later also suggested by Sundaralingam *et al.* (9).

In this paper we have studied photocrosslinking between tRNA^{Phe} and poly(U) on ribosomes. The experiment exploits the photoreactivity of wybutine (Y-Wye), which occurs in tRNA^{Phe} from yeast at the 3′ side of the anticodon (10). The results demonstrate that the orientation of Y-Wye with respect to the mRNA is the same, or at least very similar, before and after translocation. Because codon and anticodon are believed to interact at both the A site and the P site (11–13), the mutual arrangement of codon and anticodon loop must be similar at both sites.

MATERIALS AND METHODS

Materials. 70S ribosomes that had been washed in a solution containing a high level of salt ("tight couples") from *Escherichia coli* strain MRE 600 were prepared according to Noll *et al.* (14). [^{32}P]tRNA^{Phe} (7×10^8 cpm/mg by Cerenkov radiation) was isolated by the procedure of Wimmer *et al.* (15) from yeast grown in $^{32}PO_4^{3-}$-containing medium (16). Phe-[^{32}P]tRNA^{Phe} was further purified by binding to *E. coli* ribosomes in the presence of poly(U) followed by chromatography on Sepharose 6B (17) and extraction with phenol. tRNA^{Phe} from yeast and tRNA^{Phe} from *E. coli* strain MRE 600 were purchased from Boehringer Mannheim; [^{3}H]phenylalanine (76 Ci mmol^{-1}; 1 Ci = 3.7×10^{10} becquerels) was from Amersham; oligo(dA)-cellulose was from Collaborative Research (Waltham, MA); ribonucleases T1 and T2 were from Sankyo (Tokyo, Japan); Polygram Cel 400 thin-layer sheets were from Macherey–Nagel (Dürren, W. Germany); and x-ray film (X-Omat R film XR 5) was from Kodak. Elongation factor Tu (EF-Tu) was prepared according to Arai *et al.* (18); EF-G was a kind gift from J. Bodley (University of Minnesota, Minneapolis, MN). tRNA was charged with phenylalanine as described (19); AcPhe-tRNA was prepared as described (20).

Buffers. *Buffer A*: 50 mM NH_4Cl/ 50 mM Hepes (Na salt), pH 7.4/6 mM 2-mercaptoethanol. *Buffer B*: 10 mM cacodylate (Na salt), pH 7/1 mM EDTA. *Buffer C*: 10 mM Tris·HCl, pH 7.4/10 mM EDTA.

Irradiation. A Philips 500-W high-pressure mercury lamp was used with a WG 320 cutoff filter (Schott, Mainz, W. Germany). The intensity of light at the site of irradiation was 2.8×10^{16} quanta/min in a volume of 1 ml, as determined by ferrioxalate actinometry (21).

Photochemical Reaction on Ribosomal Complexes. Ribosomal complexes containing Ac[^{3}H]Phe-tRNA at the A site (22, 23) were formed by incubation in 1 ml of 0.4 nmol of ri-

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Abbreviations: A site, acceptor site; P site, peptidyl site; EF-G, elongation factor G; EF-Tu, elongation factor Tu; Y-Wye, wybutine.

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bosomes, 0.8 nmol of tRNA from *E. coli*, and 30 μg of poly(U) in buffer A containing 10 mM $Mg(OAc)_2$. After a 3-min incubation at 37°C, 0.4 nmol of Ac[^{3}H]Phe-tRNA from yeast was added and the incubation was continued for 10 min. The aminoacyl-tRNA was translocated to the P site by addition of 50 μg of EF-G per ml and GTP to 0.3 mM. After incubation for 10 min at 20°C, samples were irradiated for 10 min at 0°C. Each sample was then divided in half, and one half was incubated with 2 mM puromycin for 10 min at 20°C. All incubations were carried out in parallel.

For the formation of ribosomal complexes containing AcPhe-[^{3}H]Phe-tRNA at the A site the method of Girbes *et al.* (4) was modified. Solution I contained in 1 ml 0.8 nmol of ribosomes, 1.6 nmol of AcPhe-tRNA from *E. coli*, 60 μg of poly(U) in buffer A, and 5 mM $Mg(OAc)_2$. It was incubated for 3 min at 37°C. Solution II contained in 1 ml 1 nmol of [^{3}H]-Phe-tRNA from yeast, 300 μg of EF-Tu in buffer A, 5 mM $Mg(OAc)_2$, and 0.02 mM GTP. Incubation was for 0.5 min at 37°C. Before mixing, GTP was added to solution I to a final concentration of 0.6 mM. After equal volumes of solutions I and II were mixed, the sample was allowed to stand for 10 min at 0°C. Ribosomal complexes were purified by chromatography on Sepharose 6B (17). Translocation was performed as above.

Chromatography on Oligo(dA)-Cellulose. RNA was prepared from the irradiated samples by extraction with phenol. It was chromatographed on an oligo(dA)-cellulose column (0.5 × 3 cm) kept at 20°C in buffer B containing 500 mM NaCl and 0.01% $NaDodSO_4$. After the column was washed with 30 column volumes of the same buffer, the poly(U)-containing fraction was eluted with buffer B and the radioactivity was determined. AcPhe-[^{3}H]Phe-tRNA was hydrolyzed in 0.35 M triethylamine for 2 hr at 37°C. The hydrolysate was subjected to descending chromatography on Whatman 3 MM paper in ethylmethylketone/pyridine/H_2O, 70:15:15 by vol. The paper was cut and radioactivity of the strips was determined in toluene scintillator. For AcPhe-Phe, R_F = 0.66; for Phe, R_F = 0.28.

Identification of Crosslinked [^{32}P]Oligonucleotide Fragment. Phe-[^{32}P]tRNA was bound enzymatically to ribosomes in the presence of poly(U) and was irradiated as described above. RNA was extracted and digested in 0.1 ml of buffer C with 400 units of ribonuclease T1. After incubation for 1 hr at 37°C, chromatography on oligo(dA)-cellulose was performed as described above. The poly(U)-containing fraction was eluted with buffer B; 40 μg of carrier rRNA per ml and 2 vol of ethanol were added. The precipitate was dissolved in 50 μl of 4 mM ammonium acetate (pH 4.6), 12 units of ribonuclease T2 were added, and the mixture was incubated for 4 hr at 37°C. The nucleotides were separated by electrophoresis on Polygram Cel 400 thin-layer sheets in acetic acid/pyridine/H_2O, 5:0.5:94.5 by vol, at pH 3.5 and 20 V/cm for 3.5 hr for the first dimension. Chromatography in the second dimension was in isobutyric acid/NH_3/H_2O, 57.7:3.8:38.5 by vol. Individual spots were identified by comparison with the standard two-dimensional chromatography system (ref 24; M. Sprinzl, personal communication). Y-Wye was identified by its fluorescence.

The ribonuclease T1-resistant dodecanucleotide from the anticodon region containing Y-Wye was isolated from the ribonuclease T1 digest of purified ^{32}P-labeled $tRNA^{Phe}$ from yeast by high-voltage electrophoresis on cellulose acetate strips at 70 V/cm and pH 3.5 for 30 min in acetic acid/pyridine/H_2O, 5:0.5:94.5 by vol, and, after transfer to Polygram Cel 300 DEAE-cellulose thin-layer sheets, by homochromatography in the second dimension (25). For autoradiography, a DuPont intensifier screen was used.

Table 1. Photocrosslinking between Ac[^{3}H]Phe-$tRNA^{Phe}$ from yeast and poly(U) on ribosomes

System	Irradiation	Radioactivity bound to oligo(dA)-cellulose, cpm
Complete	+	13,360
Complete	−	1,700
Without ribosomes	+	1,640
Without poly(U)	+	1,510

The experiments were carried out under conditions of nonenzymatic binding of Ac[^{3}H]Phe-tRNA to the A site; 0.2 ml of the incubation mixture was irradiated. After extraction with phenol, the RNA was chromatographed on an oligo(dA)-cellulose column in high-salt buffer. Poly(U) and material bound to poly(U) were adsorbed on oligo(dA)-cellulose. Material retained on the oligo(dA)-cellulose column was eluted with low-salt buffer.

RESULTS

The photoreaction was first tested on ribosomal complexes formed by nonenzymatic binding of Ac[^{3}H]Phe-$tRNA^{Phe}$ from yeast to the A site. When ribosomes are preincubated with uncharged tRNA, AcPhe-tRNA added subsequently will bind mostly to the A site (22, 23). Only 5% of the bound AcPhe-tRNA reacts with puromycin (26). Preincubation was performed with $tRNA^{Phe}$ from *E. coli*, which lacks Y-Wye. After irradiation, the ribosomes were denatured and the components were tested for incorporation of radioactivity. Reaction took place exclusively with poly(U). No incorporation into RNA or ribosomal proteins was observed. Ac[^{3}H]Phe-$tRNA^{Phe}$ photocrosslinked to poly(U) was isolated by chromatography on oligo(dA)-cellulose. At high ionic strength, poly(U) and any material attached to poly(U) were specifically retained on the column. At low ionic strength, the oligo(dA)·poly(U) complex dissociated and the retained material was released from the column. Table 1 shows that crosslinking of Ac[^{3}H]Phe-tRNA and poly(U) depended on irradiation. Only background radioactivity was found upon omission of ribosomes or poly(U) from the incubation mixture, indicating that photocrosslinking took place on poly(U)-containing ribosomal complexes. Similar results were obtained when [^{3}H]Phe-tRNA or Phe-[^{32}P]tRNA from yeast was bound to ribosomes in the presence of EF-Tu. When the same experiments were performed with Phe-$tRNA^{Phe}$ from *E. coli*, which does not contain Y-Wye, no crosslinking to poly(U) was observed.

In order to identify the site of the reaction, we bound Phe-[^{32}P]$tRNA^{Phe}$ from yeast enzymatically to ribosomes in the presence of poly(U). The complexes were irradiated and RNA was extracted and treated with ribonuclease T1 before fractionation by oligo(dA)-cellulose chromatography. Ribonuclease T1 cleaves after G residues and produces a series of well-defined oligonucleotides from $tRNA^{Phe}$ from yeast (27). Upon chromatography, the oligo(dA)-cellulose column will retain only that oligonucleotide that is crosslinked to poly(U). If the crosslinking takes place at Y-Wye, the dodecanucleotide from the anticodon region, A-Cm-U-Gm-A-A-YWye*-A-Ψ-m^5C-U-G, is expected to be retained on oligo(dA)-cellulose (YWye* indicates Y-Wye modified by the crosslink to a U residue). The material retained on the oligo(dA)-cellulose column was subjected to digestion with ribonuclease T2, which cleaves all phosphodiester bonds in the tRNA except those at 2′-*O*-methylated ribonucleotides. Fig. 1*A* presents the pattern of the ribonuclease T2 digest of the ribonuclease T1-resistant oligonucleotide fragment crosslinked to poly(U). The chromatogram indeed shows a nucleotide composition characteristic of the dodecanucleotide from the anticodon region of $tRNA^{Phe}$ from yeast. All bases could be identified except for Y-Wye. As a

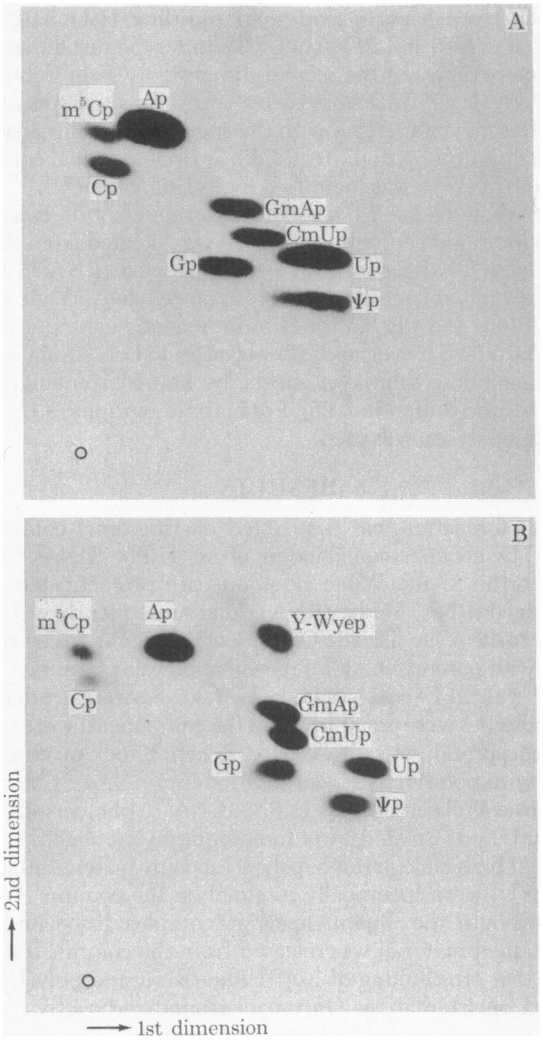

FIG. 1. Autoradiography of the ribonuclease T2 digest of the crosslinked [^{32}P]oligonucleotide fragment and the nonirradiated dodecanucleotide. Phe-[^{32}P]tRNA from yeast was enzymatically bound to ribosomes in the presence of poly(U) in a total volume of 1 ml. The complex was irradiated and the RNA was extracted. (*A*) RNA was treated with ribonuclease T1 before chromatography on oligo-(dA)-cellulose. The low-salt eluate was digested with ribonuclease T2; 1.5×10^3 cpm of [^{32}P]nucleotide digest was applied to the thin-layer sheet. First dimension, electrophoresis; second dimension, chromatography. (*B*) As a control, purified ribonuclease T1-resistant dodecanucleotide from the anticodon region of nonirradiated [^{32}P]-tRNA^{Phe} from yeast was isolated and digested with ribonuclease T2 as in *A*. Exposure was for 100 hr.

control, the pattern of the ribonuclease T2 digest of the dodecanucleotide isolated by homochromatography of ribonuclease T1-treated nonirradiated [^{32}P]tRNA^{Phe} is also shown (Fig. 1*B*). Comparison of Fig. 1 *A* and *B* clearly demonstrates that the Y-Wye present in the hydrolysate of the dodecanucleotide from nonirradiated tRNA^{Phe} (Fig. 1*B*) was absent in the crosslinked material (Fig. 1*A*). Aside from this, no significant difference could be detected. The presence of a small amount of C may be due to an undermethylation of Cm or m^{5}C (or both). This was also supported by quantitative determination of the nucleotides obtained, which indicated that all other bases were present in the expected stoichiometric ratio. It has not been possible to identify the photoproduct of Y-Wye. Partial overlapping with other nucleotides cannot be excluded.

Next, photocrosslinking was compared in the pretranslocational and posttranslocational states. Ribosomal complexes carrying Ac[^{3}H]Phe-tRNA^{Phe} from yeast in the A site were produced by binding of Ac[^{3}H]Phe-tRNA^{Phe} from yeast to ribosomes that had been incubated with tRNA^{Phe} from *E. coli*. After incubation, the sample was split. To one aliquot EF-G and GTP were added to promote translocation of the Ac[^{3}H]Phe-tRNA; the other aliquot was incubated in parallel. After irradiation, samples were again split and aliquots were incubated with puromycin. Puromycin reacts with Ac[^{3}H]Phe-tRNA at the P site, thus reducing the amount of radioactivity attached to poly(U). Control experiments demonstrated that the photocrosslinking between tRNA and poly(U) did not affect the puromycin reaction. Ac[^{3}H]Phe-tRNA^{Phe} from yeast crosslinked to poly(U) was isolated by chromatography on oligo(dA)-cellulose and the radioactivity was determined. The results, presented in Table 2, show that Ac[^{3}H]Phe-tRNA was photocrosslinked to poly(U) both before and after translocation. The puromycin controls indicate that under conditions of nonenzymatic A-site binding a fraction of Ac[^{3}H]Phe-tRNA either was spontaneously translocated or was bound directly to the P site. On the other hand, the P-site complexes contained material that did not react with puromycin. This mutual contamination can easily be corrected for, as shown in the last column of Table 2. A-site complexes should be resistant to incubation with puromycin. The fraction of P-site complexes can be calculated as the difference between the radioactivity obtained in the absence of puromycin and the radioacitvity recovered after incubation of ribosomal complexes with puromycin. A comparison of the corrected data shows that the efficiency of photocrosslinking was similar after both nonenzymatic binding to the A site and EF-G-dependent translocation to the P site.

AcPhe-tRNA nonenzymatically bound to the A site mimics a pretranslocational state, yet the conditions of its formation are purely artificial. For this reason pretranslocational complexes containing AcPhe-[^{3}H]Phe-tRNA were formed by binding [^{3}H]Phe-tRNA^{Phe} from yeast in the presence of EF-Tu and GTP to ribosomes that had been incubated with AcPhe-tRNA^{Phe} from *E. coli*. Transpeptidation will then yield AcPhe-[^{3}H]Phe-tRNA in the A site. The incubation mixture was then passed through Sepharose 6B (17) in order to remove unbound EF-Tu and [^{3}H]Phe-tRNA. Subsequently, half of the sample was incubated with EF-G and GTP to generate AcPhe-[^{3}H]Phe-tRNA complexes at the P site. After incubation the samples were divided in half, and one half was treated with puromycin. Samples were taken after different times of irradiation. After phenol extraction, the RNA was chromato-

Table 2. Photocrosslinking of Ac[^{3}H]Phe-tRNA^{Phe} in the A site and the P site

System	cpm Without puromycin	cpm With puromycin	Puromycin sensitivity, %	Corrected cpm
A-site complex	13,590	9750	28	9750
P-site complex	12,590	3250	74	9340

The A-site complex (1 ml) was formed under conditions of nonenzymatic binding as in Table 1. The P-site complex was formed by incubation with EF-G and GTP. Samples were subsequently irradiated and incubated with puromycin. The corrected value for the A-site complex corresponds to the radioactivity attached to poly(U) after treatment of ribosomal complexes with puromycin. The corrected value for the P-site complex corresponds to the radioactivity that can be released with puromycin. It was calculated as the difference between the radioactivity obtained in the absence of puromycin and that obtained after incubation of ribosomal complexes with puromycin. Background values of nonirradiated samples were subtracted.

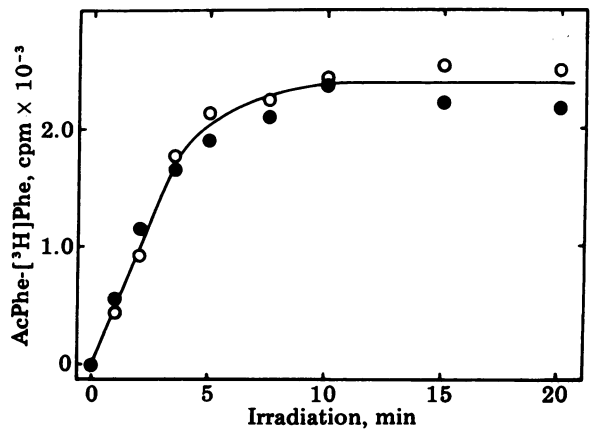

FIG. 2. Kinetics of the photoreaction at the A and the P sites. [³H]Phe-tRNA from yeast was enzymatically bound to ribosomes that had been incubated with AcPhe-tRNA from *E. coli* and with poly(U). After chromatography on Sepharose-6B, half of the sample was incubated with EF-G and GTP. Samples were again split, and one part of each was incubated with puromycin. During irradiation, samples were removed at time intervals indicated. AcPhe-[³H]Phe-tRNA bound to poly(U) was isolated on oligo(dA)-cellulose. After hydrolysis, AcPhe-[³H]Phe was purified by paper chromatography. The corrected values for A-site and P-site complexes were calculated as described in Table 2. ○, A-site complex; ●, P-site complex.

graphed on oligo(dA)-cellulose. The poly(U)-bound fraction was collected and treated with triethylamine to hydrolyze the peptide off the tRNA. AcPhe-[³H]Phe was then determined by paper chromatography. Fig. 2 presents the kinetics of the crosslinking of AcPhe-[³H]Phe-tRNA from yeast at the A site and the P site. The data show that the kinetics of the photocrosslinking of AcPhe-[³H]Phe-tRNA^{Phe} from yeast was essentially identical at both the A site and the P site, reaching a plateau after about 10 min of irradiation. The data presented demonstrate that the reactivity of Y-Wye towards poly(U) was similar in the pretranslocational and posttranslocational states.

An experiment was then performed to test the effect of the photocrosslinking on translocation. For this purpose the order of the experiment was simply reversed. Ribosomal complexes carrying AcPhe-[³H]Phe-tRNA^{Phe} in the A site were prepared as above and the mixture was irradiated. Subsequent to the irradiation, EF-G and GTP were added and the incubation was continued. Samples were then incubated with puromycin to check for translocation. Table 3 shows the results. At the time of irradiation, AcPhe-[³H]Phe-tRNA was clearly in the pretranslocational state (A site). Only a very small amount of the total radioactivity could be released by puromycin. Addition of EF-G and GTP to the crosslinked complex converted it to a puromycin-sensitive state, indicating that the photocrosslinked complex could still be translocated efficiently.

Table 3. Translocation of the photocrosslinked AcPhe-[³H]Phe-tRNA–poly(U) complex

System	cpm		Puromycin sensitivity, %
	Without puromycin	With puromycin	
Complete	3500	3210	8
Complete + EF-G + GTP	3580	730	80

The ribosomal A-site complex (1 ml) was formed as in the legend of Fig. 2. After irradiation, the mixture was split and EF-G and GTP was added to one half of the sample. Both samples were then incubated in parallel. After incubation, the samples were again split and half of each was incubated with puromycin. The amount of AcPhe-[³H]Phe-tRNA crosslinked to poly(U) was determined as in the legend of Fig. 2.

DISCUSSION

The experiments presented in this study demonstrate that the Y-Wye base in tRNA^{Phe} from yeast undergoes a photoreaction with poly(U) on ribosomes. Photocrosslinking at Y-Wye is shown by isolation of the dodecanucleotide from the anticodon region of tRNA^{Phe} attached to poly(U). Upon hydrolysis, all nucleotides are found except for Y-Wye. The system used to separate the ribonuclease T2 hydrolysate uses electrophoresis for the first dimension and chromatography in isobutyric acid/ammonia/H_2O for the second dimension. The reason for choosing this system rather than the conventional two-dimensional chromatography system of Nishimura (24) was that Y-Wye was found to undergo partial hydrolysis during chromatography in the acidic solvent used for the second dimension of the Nishimura system (unpublished data).

The irradiated complexes are still active in transpeptidation with puromycin and in translocation. Thus, neither the conditions of the irradiation nor the formation of the crosslink affects the ribosomal activity. The site of the reaction on the Y-Wye molecule is not known. Recently Paszyc and Rafalska (28) have studied the photoreactivity of synthetic Y-Wye in aqueous solution. They found that photoreaction with oxygen results in cleavage of the ring structure. In other studies the photocrosslinking on ribosomal complexes of tRNA^{Phe} from brewer's yeast has been compared with that of tRNA^{Phe} from *Torulopsis utilis*, which carries a Y-Wye lacking the side chain attached to the C-10 position (29). Both tRNA^{Phe} species were equally efficient in crosslinking, indicating that the photoreaction with poly(U) presumably also occurs in the ring structure of the Y-Wye molecule (unpublished data).

Fig. 3 presents a schematic drawing of the anticodon loop interacting with the mRNA in both the 5′ stacked (Fig. 3*A*) and the 3′ stacked (Fig. 3*B*) conformation (6, 30). In the 3′ stacked conformation, which is the form found in the tRNA^{Phe} crystal structure, Y-Wye is stacked onto the anticodon. It is therefore located very close to the mRNA. In the 5′ stacked form, the stack is broken between the anticodon and Y-Wye due to the turn of the loop. As a consequence, the Y-Wye base points away

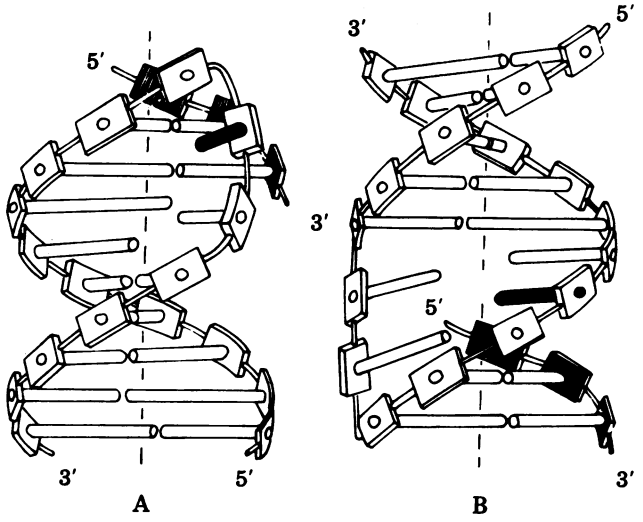

FIG. 3. Diagram of the anticodon loop during interaction with the codon. (*A*) Anticodon loop in the 5′ stacked conformation. (*B*) Anticodon loop in the 3′ stacked conformation. The mRNA is shown hatched. Y-Wye is drawn in black. The representations are redrawn from Woese (6) and Lake (30).

from the mRNA (Fig. 3*A*). The orientation of the Y-Wye base with respect to the mRNA is therefore an indicator of the conformation of the anticodon loop. If the 5′ stacked and the 3′ stacked conformations indeed represent the pretranslocational and posttranslocational states, respectively, steric hindrance in one of the conformations should cause a large difference in the kinetics and the efficiency of the photoreaction. This was clearly not observed when the photoreaction of A-site and P-site complexes was compared. On the contrary, the photocrosslinking at both sites was identical within the resolution of the experiment, indicating that the orientation of the Y-Wye base with respect to the mRNA is similar in both the A site and the P site. The ribosomal capability for translocating the photocrosslinked tRNA–mRNA complex furthermore demonstrates that during translocation tRNA and mRNA move together without dramatic alteration in the relative arrangement of codon and anticodon loop. However, photocrosslinking will detect only changes that affect the relative position of Y-Wye and mRNA. Subtle differences in the anticodon loop structure such as that observed by Wrede *et al.* (31) between initiator and elongator tRNAs may not be revealed in this type of photocrosslinking experiment.

When the models of the 5′ stacked (Fig. 3*A*) and the 3′ stacked (Fig. 3*B*) conformations of the anticodon loop are compared, it appears more likely that photocrosslinking of Y-Wye can occur in the 3′ stacked form. The 3′ stacked form of the anticodon loop of tRNA on the ribosome is supported by the fluorescence measurements of Odom *et al.* (32). The tilt of the base pairs in the helical axis of the anticodon stem also favors the 3′ stacked conformation (33). The formation of a 5′ stacked anticodon loop would require a complete rearrangement of the helical array in the anticodon stem (34).

The photocrosslinking experiments presented agree with the model of translocation suggested by Rich (8) in which the conformation of the anticodon loop is preserved during movement of the tRNA from the A site to the P site. However, they cannot be taken as a proof because some basic assumptions in this model have not been tested experimentally. One of these assumptions is that the tRNAs at both the A site and the P site interact simultaneously with the respective codons on the mRNA. The photocrosslinking system described should allow investigation of this problem.

Note Added in Proof. Recent experiments have shown that photocrosslinking with $tRNA^{Phe}$ from yeast can be performed with hexanucleotides as mRNA (G. Steiner and E. Kuechler, unpublished data). This indicates that the crosslinking at the mRNA occurs close to the site of codon–anticodon interaction.

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