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Photocross-linking Analysis of the Contact Surface of tRNA^{Met} in Complexes with *Escherichia coli* Methionine:tRNA Ligase†

Joseph J. Rosa,‡ Margaret D. Rosa,§ and Paul B. Sigler*

ABSTRACT: Photoinduced covalent cross-linking has been used to identify a common surface of four methionine-accepting tRNAs which interact specifically with the *Escherichia coli* methionine:tRNA ligase (EC 6.1.1.10). tRNA–ligase mixtures were irradiated, and the covalently linked complexes were isolated and digested with T1 RNase (Schimmel & Budzik, 1977). The fragments lost from the elution profile of the T1 RNase digest were considered to have been cross-linked to the protein and therefore in intimate contact with the enzyme. Only specific cognate tRNA–ligase pairs produce covalently linked complexes. The four substrate tRNAs used in this study have substantially different sequences, but all showed a common cross-linking pattern, supporting the view that the sites cross-linked to the enzyme reflect the functionally common contact surface rather than particularly photoreactive regions of tRNA. The cross-linked contact surface is comprised of three regions: (1) the narrow groove of the anticodon stem and its extension into the anticodon loop; (2) the 3′ terminal residues; and (3) the 3′ side of the "T arm". Unlike previous studies with other tRNAs, the D arm is not involved and significant radiation damage is suffered by the tRNA which must be taken into account in the analysis. The results are consistent with and complement chemical modification studies [Schulman, L. H., & Pelka, H. (1977) *Biochemistry 16*, 4256].

A large body of information has been accumulated concerning the specific recognition of tRNA by its cognate amino acid:tRNA ligase. It has become apparent that this interaction is a subtle chemical process which is sensitive to the ionic strength and character of the solvent and no doubt involves regions of tRNA structure that are spatially separated from the site of aminoacylation (see a review, Söll & Schimmel, 1974). A number of strategies have been used in attempts to identify the structural features of tRNA responsible for ligase recognition; they include (a) observing the effects of chemically modifying tRNA [e.g., Schulman & Pelka, 1977 (and references cited therein); Litt & Greenspan, 1972], (b) testing ligase recognition of tRNA fragments (e.g., Thiebe et al., 1972; Oda et al., 1969; Seno et al., 1969), (c) comparing the accessibility of nascent and complexed tRNA to various reagents ranging from the very small tritium nucleus (Schoemaker & Schimmel, 1976) to a very large probe such as a nuclease molecule (e.g., Horz & Zachau, 1973; Dube, 1973; Dickson & Schimmel, 1975), (d) the use of spectral probes such as fluorescence (Lam & Schimmel, 1975), (e) testing ligase interaction with mutant tRNA species (Smith et al., 1970; Celis et al., 1973), (f) comparing sequence homologies among tRNAs recognized by the same enzyme (Roe et al., 1973), and (g) competitive oligonucleotide binding to indicate shielding of complementary sequences by the bound ligase (Schimmel et al., 1972).

While all of these approaches are informative, they have serious limitations. For example, it is difficult to infer with any precision the details of a tRNA–ligase interaction from the inaccessibility of a particular region of tRNA to a chemical or enzymatic probe. Most probes used in analyzing tRNA–protein interactions interact preferentially with single stranded RNA and are therefore likely to point preferentially to contact regions in the tRNA that are devoid of significant secondary (or tertiary) structure. There is no reason to believe the same constraint applies to photocross-linking. Further, interpretations of the disruptive effect of either a chemical or genetic modification are generally compromised by the qualifying consideration of a secondary or indirect conformational effect. The strongest statement of a "protection" or "modification" experiment is therefore usually restricted to the null result; that is to say, if the chemical accessibility of a region in a molecule remains *un*changed in a complex, or if a structural alteration has *no* discernible effect on complex formation, then the reaction sites are considered *not* to be involved in the specific interactions of the complex.

An alternative approach was suggested by the work of Markovitz (1972), who reported that irradiating a complex between DNA and the *Escherichia coli* DNA polymerase I with UV light rendered the complex stable to high salt and denaturants, presumably through photoinduced covalent cross-links between the protein and nucleic acid. This finding was exploited by Schimmel and his co-workers (Schoemaker & Schimmel, 1974; Budzik et al., 1975; Schoemaker et al.,

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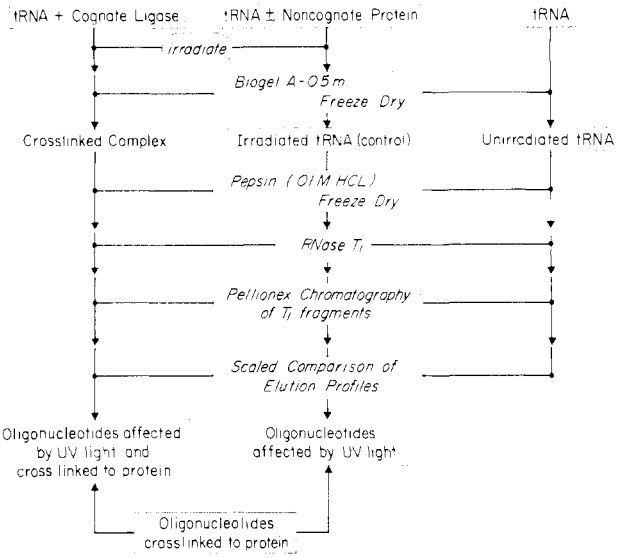

FIGURE 1: Overall scheme for analyzing the pattern of photoinduced cross-links in tRNA produced by UV irradiation of complexes of tRNA and tRNA:aminoacyl ligases.

1975) who studied complexes between various tRNAs and their cognate amino acid:tRNA ligases by photoinduced cross-linking of tRNA and protein. In this way, the regions of tRNA in contact with protein were mapped out without the use of extraneous reagents or functional groups alien to the molecular interface. The rationale of these experiments rests on the reasonable presumption that intimate contact is a necessary (but not necessarily sufficient) requirement for photoinduced cross-linking.

In this paper we describe the application of photoinduced cross-linking to the analysis of the molecular interface between the *E. coli* methionine:tRNA ligase (EcoMetRL)[1] and four tRNAs, all of which have been shown to be good substrates of this enzyme (Rosa, 1976). They are the following: *E. coli* $tRNA_f^{Met}$, *E. coli* $tRNA_m^{Met}$, yeast $tRNA_f^{Met}$, and wheat germ $tRNA_i^{Met}$. Four different methionine-accepting tRNAs were used in these experiments to minimize the likelihood of observing fortuitous sequence-dependent effects which would be unrelated to the mechanism of ligase recognition, thereby bringing into clearer relief the common structural features of the tRNA that mediate the formation of a productive complex. To further minimize the appearance of nonessential interactions preliminary photocross-linking experiments were also carried out with complexes formed between the four methionine-accepting tRNAs and an active tryptic fragment of EcoMetRL (EcoMetRL-TF) which has similar enzymatic properties but, being a 64 000-dalton monomer (Cassio & Waller, 1974; Fayat et al., 1973), has a rather different molecular architecture than the native enzyme which is a dimer composed of two identical 86 000-dalton protomers (Koch & Bruton, 1974).

[1] Abbreviations used: $tRNA_f^{Met}$, formylatable methionine-accepting transfer RNA from either yeast or *E. coli*; $tRNA_i^{Met}$, the initiator methionine-accepting tRNA from wheat germ cytoplasm, is designated with i rather than f since it is not formylatable with the *E. coli* transformylase; $tRNA_m^{Met}$, the species of methionine-accepting tRNA whose methionine adduct cannot enzymatically accept the formyl group and is responsible for chain elongation rather than initiation; Met:tRNA ligase (in running title) and EcoMetRL, the methionine:tRNA ligase (EC 6.1.1.10) from *E. coli*; EcoMetRL-TF, an active trypsin fragment of EcoMetRL; EcolleRL, the isoleucine:tRNA ligase (EC 6.1.1.5) from *E. coli*; A_{258}, that amount of tRNA which when dissolved in 1 mL of neutral dilute salt buffer produces an absorbance of 1 at 258 nm with a 1-cm light path.

Materials and Methods

Overall Experimental Scheme. The experimental protocol used to establish the extent of photocross-linking and the photocross-linked sites in the tRNA is schematically outlined in Figure 1.

Assays. The capacity for tRNA to accept methionine was assayed by essentially the method of Hoskinson & Khorana (1965). $tRNA_f^{Met}$ was distinguished from $tRNA_m^{Met}$ by its capacity to accept formate using a purified preparation of *E. coli* transformylase (Dickerman, 1971) and [^{14}C]formyltetrahydrofolate prepared by the method of Samuels et al. (1970). Cl_3Ac-precipitable formate was determined by the same technique used for methionine.

E. coli $tRNA_f^{Met}$ and E. coli $tRNA_m^{Met}$. Crude *E. coli* B tRNA (30 000 A_{258}) was fractionated successively on DEAE-Sephadex A-50 and BD-cellulose according to Nishimura (1971). *E. coli* $tRNA_f^{Met}$ accepted ~1.8 nmol of methionine or formate/A_{258}; *E. coli* $tRNA_m^{Met}$ accepted ~1.5 nmol of methionine/A_{258}. Overall yield was 300 and 140 A_{258} for *E. coli* $tRNA_f^{Met}$ and $tRNA_m^{Met}$, respectively.

Yeast $tRNA_f^{Met}$. Bulk yeast tRNA (4.5 g) prepared according to Holley (1967) was dissolved in 30 mL of 1.3 M $(NH_4)_2SO_4$, 0.01 M $MgCl_2$, 0.01 M NaOAc (pH 4.5) and applied to a 4 × 100 cm column of Sepharose 4B (Holmes et al., 1975) equilibrated with the same solution at 4 °C. Fractions (15 mL) were collected every 10 min from an 8-L linear gradient extending between the equilibration solution and 0.01 M $MgCl_2$, 0.01 M NaOAc (pH 4.5). The peak of methionine acceptance (10-fold purified), which eluted first and contained almost 100% of both the initiator and elongator $tRNA^{Met}$, was dialyzed against 1.0 M NaCl, 0.01 M $MgCl_2$ and precipitated at 4 °C with two volumes of 95% ethanol. The precipitate was dissolved in 20 mL of 0.40 M NaCl, 0.01 M $MgCl_2$, 0.05 M NaOAc (pH 5.0) and applied to a 1.5 × 60 cm column of BD-cellulose (Gillam et al., 1967) equilibrated with the same solvent. Fractions (4.5 mL) were collected every 20 min from a 2-L gradient extending from the equilibration buffer to 1.0 M NaCl, 0.01 M $MgCl_2$. Yeast $tRNA_f^{Met}$ (50% pure), which eluted at ~280 mL and was separated from the $tRNA_m^{Met}$ (eluted at ~540 mL), was dialyzed and precipitated as above and brought to homogeneity (>1.8 nmol of Met/A_{258}) by chromatography on a 0.6 × 200 cm column of DEAE-Sephadex A-50 eluted at 2.0 mL/h with a 250-mL gradient from 0.40 to 0.45 M NaCl in 0.01 M $MgCl_2$, 0.02 M Tris-HCl (pH 7.3). Overall yield was 600 A_{258}.

Wheat Germ $tRNA_i^{Met}$. By use of the same protocol as that used to isolate yeast $tRNA_f^{Met}$, 4.5 g of bulk wheat germ tRNA (Dudock & Holley, 1969) yielded 500 A_{258} of wheat germ $tRNA_i^{Met}$ which accepted 1.8 nmol of Met/A_{258}.

Wheat Germ and Yeast $tRNA_m^{Met}$. These two tRNAs, which are not charged by EcoMetRL under conventional conditions, were purified to acceptance levels of 1.3 and 1.5 nmol/A_{258}, respectively, as described by Rosa (1976) and Rosa & Sigler (1977).

E. coli $tRNA^{Ile}$. *E. coli* $tRNA^{Ile}$ (1.3 mol/A_{258}) was isolated by BD-cellulose chromatography as the phenoxyacetyl derivative of the aminoacyl adduct as described by Gillam et al. (1968).

The identity of the isoacceptor species was confirmed in each case by comparing the composition of the T1 RNase fragments recovered from a Pellionex elution profile with the composition expected on the basis of the sequence (Yarus & Barrell, 1971; Dube et al., 1968; Corey et al., 1968; Simsek & RajBhandary, 1972; H. P. Ghosh, K. Ghosh, M. Simsek, and U. L.

RajBhandary, private communication).

E. coli Methionine:tRNA Ligase and the Tryptic Fragment. Mid log-phase *E. coli* B (Grain Processing Corp.) was disrupted by a Vibrogen cell mill (Rho Scientific) in the presence of protease inhibitors (10 μM each α-toluenesulfonyl fluoride and methanesulfonyl fluoride), and the lysate was subjected to the purification procedure of Lemoine et al. (1968). Enzyme (28 mg) was obtained from 1360 g of cells. Sucrose-gradient sedimentation and gel filtration indicated a molecular weight of 170000. Over 90% of the final product migrated as a single 86000-dalton band on standardized $NaDodSO_4$-polyacrylamide gel electrophoresis. The active tryptic fragment was prepared by trypsin treatment of the native enzyme according to Cassio & Waller (1971). Over 95% of the protein in the gel migrated as a single 64000-dalton band on $NaDodSO_4$-polyacrylamide gel electrophoresis.

The ligases were prepared for complexation and irradiation by passage through a Sephadex G-25 column equilibrated with 50 mM sodium cacodylate, 10 mM $MgCl_2$ (pH 6.0). This was done to remove β-mercaptoethanol from the sample since mercaptans were found to greatly augment the photolability of these enzymes.

Irradiation of Samples. Samples of either tRNA alone or tRNA–protein mixtures were irradiated under the conditions described by Schoemaker & Schimmel (1974). Solutions containing 1 μM tRNA and 2 μM protein in 10 mM $MgCl_2$, 50 mM sodium cacodylate (pH 5.5 or 6.0) were irradiated as 300-μL droplets on a Parafilm sheet placed on an ice–water bath. The total volume of the droplets was typically 4 mL. The droplets were irradiated from a distance of 7 cm with two low-pressure mercury lamps (G15T8, General Electric), each with a 14-in. arc length and a nominal energy input of 15 W and a total output of 3 W at wavelengths less than 380 nm. These lamps primarily emit two bands of radiation centered at 253.7 and 184.9 nm. While the radiation at very short wavelengths (185 nm) is probably effectively filtered by the glass envelope of these lamps (Calvert & Pitts, 1966), samples were also irradiated with radiation passed through a Corning 7-54 filter, which is opaque to wavelengths shorter than 235 nm (56% transmission at 254 nm and less than 10% transmission at 235 nm as determined with a Cary 14 spectrophotometer). The amount of radiation incident on the sample droplets was determined with a potassium ferrioxalate actinometer (Hatchard & Parker, 1956; Calvert & Pitts, 1966). In the absence of the filter, doses were found to be 0.57 μEinstein min^{-1} cm^{-2} ($\Phi_{Fe^{2+}}$, the product quantum yield, was assumed to be 1.25). This dose is very similar to that reported by Budzik et al. (1975). The Corning 7-54 filter reduced the incident intensity to 0.27 μEinstein min^{-1} cm^{-2}. Neither the effects of radiation on tRNA nor the cross-linking results were noticeably affected by the use of the filter. All of the results reported here were obtained with unfiltered radiation.

Analysis of Irradiated Samples. The amount of cross-linked product was determined by subjecting small aliquots of the irradiated mixture to either (a) $NaDodSO_4$-polyacrylamide gel electrophoresis by use of tRNA-C-C-[^{3}H]A or (b) gel filtration of the complex by use of either tRNA-C-C-[^{3}H]A or the UV absorption of the tRNA. Samples of tRNA labeled with [^{3}H]adenosine at the 3′-OH terminus were prepared by limited digestion with snake venom phosphodiesterase, followed by enzymatic rebuilding of the 3′ terminus with CTP and [^{3}H]ATP by use of either *E. coli* or yeast nucleotidyltransferase (Pasek et al., 1973).

Preparative amounts of irradiated samples (either tRNA alone or tRNA–protein mixtures) were analyzed according to the scheme in Figure 1. Typically, after 20 min of irradiation,[2] the 4-mL sample solution was loaded onto a 2.5 × 25 cm column of Bio-Gel A-0.5m (Bio-Rad) equilibrated with 0.30 M NH_4HCO_3. The column effluent was continuously monitored at 254 nm with an UV absorbance detector (Chromatronix Model 220) (Figure 5). Peaks were collected and lyophilized. In order to avoid the effect of simple entrainment of tRNA in a protein aggregate, each lyophilizate was suspended in 1.0 mL of 0.01 N HCl, treated with 2 μg of freshly prepared pepsinogen (Worthington) for 1 h at room temperature and again lyophilized. The pepsin-digest samples were then treated with T1 RNase and chromatographed on A1-Pellionex-WAX (Whatman) essentially as described by Rosa & Sigler (1974). Repeated Pellionex chromatography of samples taken from the same digest produced elution profiles which were virtually superimposable. Control experiments demonstrated that the acid–pepsin treatment had no effect whatever on the T1 RNase products of *E. coli* $tRNA^{Ile}$ or any of the methionine-accepting tRNAs used in this study. Each tRNA species was analyzed in this manner following each of the three types of treatment outlined in Figure 1, i.e., (a) after irradiation with the cognate ligase, (b) after irradiation alone (or with a noncognate protein), and (c) after no irradiation (Figure 5).

Peaks in the Pellionex elution profile were assigned to specific T1 RNase fragments as follows. Oligonucleotides expected to be formed by T1 RNase cleavage were deduced from an examination of the sequences: *E. coli* $tRNA_f^{Met}$ (Dube et al., 1968), *E. coli* $tRNA_m^{Met}$ (Cory et al., 1968), yeast $tRNA_f^{Met}$ (Simsek & RajBhandary, 1972), and wheat germ $tRNA_i^{Met}$ (H. P. Ghosh, K. Ghosh, M. Simsek, and U. L. RajBhandary, personal communication). Each of the expected fragments was matched with a peak in the elution profile by reducing the isolated fraction to nucleosides with a mixed nuclease–phosphodiesterase–phosphophatase digestion and quantitating the nucleoside composition by Aminex chromatography as described by Rosa & Sigler (1974). Serious ambiguities did not arise since fragments having different sequences but the same composition did not occur. During the course of these and other experiments, occasional examination of unique features of an oligonucleotide, such as an unusual modified base, pancreatic RNase subfragmentation, or the uniquely terminated 3′-terminal oligonucleotide, always confirmed and never contradicted the assignment based on nucleoside composition.

In order to determine the changes in structure due to photodamage or photoinduced cross-linking, the elution profiles of the RNase T1 digests of the two irradiated samples were normalized to that of the unirradiated sample by applying a scale factor which minimized the total difference between the peak heights of the two chromatographs. Loss in a normalized peak height due to irradiation in the absence of ligase was ascribed to direct photodamage. The degree of photocross-linking was determined by comparing the normalized elution profiles of samples irradiated in the presence and absence of the cognate ligase. Losses in the normalized peak heights of fragments from samples irradiated in the presence of the ligase beyond that due to direct photodamage were attributed to photocross-linking and quantitated as in Figure 7.

Results

The Four Methionine-Accepting tRNAs Are Good Substrates for EcoMetRL. Implicit in the rationale of these

[2] Irradiation for periods longer than 20 min produced noticeable precipitation of the protein.

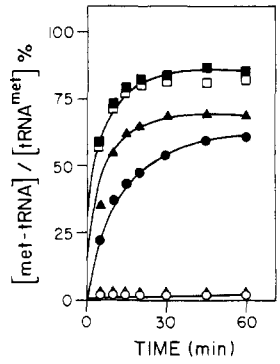

FIGURE 2: Time course for the aminoacylation of methionine-specific tRNAs by EcoMetRL. A 20-μL aliquot of EcoMetRL in 10 mM Tris-HCl, 1 mM dithiothreitol, 20% glycerol, pH 8.0, was added to 450 μL of 50 mM Tris-HCl, 4 mM $MgCl_2$, 1 mM dithiothreitol, 3 mM ATP, 0.2 mg/mL of BSA, 5×10^{-6} M $tRNA^{Met}$, 1×10^{-4} M [^{14}C]methionine (12.5 mCi/nmol) and incubated at 24 °C. At the times indicated the concentration of methionyl-$tRNA^{Met}$ was determined as described under Materials and Methods on 50-μL aliquots.[3] The final concentration of EcoMetRL was 2.2 nM. *E. coli* $tRNA_f^{Met}$ (■), *E. coli* $tRNA_m^{Met}$ (□), yeast $tRNA_f^{Met}$ (▲), wheat germ $tRNA_i^{Met}$ (●), yeast $tRNA_m^{Met}$ (△), wheat germ $tRNA_m^{Met}$ (○).

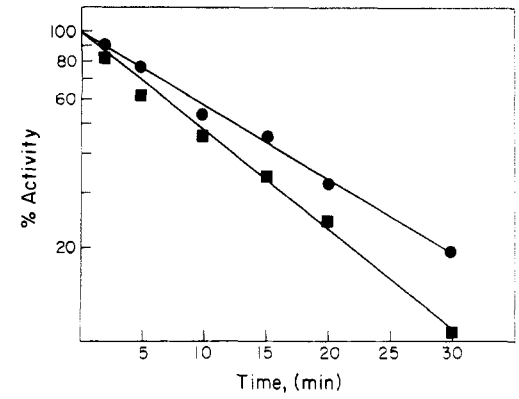

FIGURE 3: The effect of UV radiation on the methionine (●) and formate (■) acceptor activities of yeast $tRNA_f^{Met}$. Yeast $tRNA_f^{Met}$ was irradiated (0.57 μEinstein cm^{-2} min^{-1}) under the conditions used for cross-linking. At various times aliquots were removed and assayed for methionine- or formate-acceptor activity and compared with corresponding activities in aliquots taken from the same experimental setup which had not been irradiated.

cross-linking experiments is the capacity of all four methionine-accepting tRNAs to produce a stable, catalytically productive complex with the EcoMetRL. Figure 2[3] shows the time course for the EcoMetRL-catalyzed acceptance of methionine by the four tRNAs used in this study and two other methionine-specific tRNAs (wheat germ and yeast $tRNA_m^{Met}$) which are not substrates for the EcoMetRL. V_{max} for aminoacylation of the four substrate tRNAs under the conditions described in the legend to Figure 1 ranged from 172 (*E. coli* $tRNA_f^{Met}$) to 107 min^{-1} (wheat germ $tRNA_i^{Met}$) and showed similar Mg^{2+}-dependence profiles with an optimal concentration between 6 and 10 mM. The K_m values for aminoacylation were so low as to be difficult to measure accurately by initial rate experiments, but all were lower than 100 nM. These values are lower than those reported by Lawrence et al. (1973) but not unreasonably small in view of the value of 10 nM reported by Bonnet & Ebel (1972) for aminoacylation in the valine system from yeast. In any case, there is no question that the four methionine-accepting tRNAs used in this study exhibit similar kinetic behavior and are bona fide substrates for EcoMetRL.

EcoMetRL appears to be a rather selective enzyme. Although heterologous noncognate tRNAs are known to bind in a productive mode to certain amino acid:tRNA ligases (e.g., Roe et al., 1973), this does not appear to be a characteristic of the EcoMetRL system. A typical aminoacylation reaction mixture containing yeast bulk tRNA from which the two methionine-accepting species had been removed showed no EcoMetRL-catalyzed methionine acceptance, even when the total tRNA concentration was raised to 2.5×10^{-4} M, i.e., 50 times the usual level. Moreover, the concentrated bulk yeast tRNA did not competitively depress the methionine acceptance of yeast or *E. coli* $tRNA_f^{Met}$ when charged by the EcoMetRL. Thus, no other yeast tRNA species, representing more than 1% of the total tRNA, can compete effectively for the enzyme's active site with the four methionine-accepting tRNAs used in this study.

UV Light Alters the Structure and Function of tRNA. In order to properly interpret the photoproducts formed by the irradiation of tRNA in the presence of proteins, it was necessary to determine the effects of UV radiation on tRNA alone. This was done by irradiating tRNA in the absence of protein and analyzing both the loss of substrate capacity and changes in the covalent structure.

Figure 3 shows the photoinduced loss of methionine- and formate-accepting activities of yeast $tRNA_f^{Met}$ when the tRNA alone was irradiated under the condition used to form the cross-linked tRNA–ligase complexes. Similar results were obtained for the amino acid accepting activity of *E. coli* $tRNA^{Ile}$ and the other three methionine-accepting tRNAs used in this study. The first-order inactivation kinetics seen here was also observed by Schulman & Chambers (1968) with similar doses of radiation. As noted by these authors, this type of response to irradiation indicates that a single photochemical event is sufficient to cause inactivation.

When tRNA alone was irradiated under the conditions used to form cross-linked tRNA–ligase complexes, its covalent structure was changed noticeably. After 20 min of irradiation (corresponding to approximately 12 μEinstein cm^{-2}, the same dose used for the irradiation of tRNA *plus* protein), the tRNA samples were desalted on the Bio-Gel A-0.5m column and digested with T1 RNase. While no cross-links between tRNA molecules were detected by gel filtration, all tRNA samples showed a 5–10% decrease in absorption at 254 nm. The extent of the UV-induced changes is evident from the comparison of the Pellionex chromatography of the T1 RNAse fragments from irradiated and nonirradiated tRNA. Figures 6b and 11b (middle panels) show the effects of irradiation on the elution profile of a T1 RNase digest of *E. coli* $tRNA_f^{Met}$ and *E. coli* $tRNA^{Ile}$. Similar changes were observed for all tRNAs used in this study. It is not clear why this degree of structural damage was not detected by Schimmel and co-workers (e.g., Budzik et al., 1975)[4] in their studies of the *E. coli* isoleucine and tyrosine systems. Using approximately 5 μEinstein cm^{-2} [as compared to 12 μEinstein cm^{-2} used by Budzik et al. (1975) and in this work], Chambers and Schulman (Schulman &

[3] The plateau below 100% aminoacylation is a common finding in the aminoacylation of tRNA and is discussed by Bonnet & Ebel (1972) and Rosa (1976). It is caused by competing spontaneous and enzyme-catalyzed deacylation reactions. Rosa (1976) has shown the effect here to be almost totally due to spontaneous hydrolysis and the plateau increased to 100% in the case of the *E. coli* $tRNA_f^{Met}$ when the enzyme level was raised to 40 nM.

[4] It should be noted that Schimmel and his co-workers used two-dimensional thin-layer chromatography for the separation of RNase T1 fragments and purines which were tritium-labeled at the C-8 position for their quantitation (Schoemaker & Schimmel, 1974).

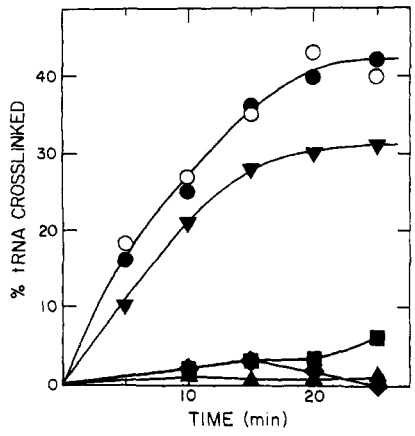

FIGURE 4: Time course for photoinduced cross-linking of tRNA to cognate and noncognate proteins. Mixtures of tRNA and proteins were prepared and irradiated as described under Materials and Methods except that the tRNAs were radioactively labeled with [^{3}H]A at the 3′ terminus by rebuilding venom diesterase-treated tRNA with CTP, [^{3}H]ATP, and partially purified *E. coli* nucleotidyltransferase as described by Pasek et al. (1973). Covalently cross-linked complexes were separated from the lower molecular weight tRNA either by gel filtration on Sephadex G-200 columns equilibrated with 1.0 M KCl, 0.005 M EDTA, and 0.01 M Tris-HCl (pH 8.7) or by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. The fraction of tRNA cross-linked to the protein was determined from either the relative distribution of radioactivity in the peaks of the elution profile of the Sephadex G-200 chromatographs or the loss of radioactivity from the tRNA band in the gel electrophoresis. The following irradiated mixtures were analyzed by gel filtration chromatography: yeast tRNA$_f^{Met}$ plus EcoMetRL (▼), yeast tRNA$_f^{Met}$ plus alcohol dehydrogenase (▲), yeast tRNA$_f^{Met}$ plus bovine serum albumin (◆), yeast tRNA$_f^{Met}$ plus EcoIleRL (■), *E. coli* tRNA^{Ile} plus EcoIleRL (●); irradiated mixture analyzed by polyacrylamide gel electrophoresis, *E. coli* tRNA^{Ile} plus EcoIleRL (○).

Chambers, 1968; Schulman, 1970; Chambers, 1971) also found similar levels of photoinduced modification of yeast tRNA^{Ala}, *E. coli* tRNA$_f^{Met}$, and yeast tRNA^{Tyr}.

UV Light Cross-links Complexes of tRNA and Cognate Ligase. Despite the UV-induced structural alterations and inactivation of tRNA, specific, nondissociable complexes between tRNAs and their cognate ligases were formed in good yield by the action of ultraviolet radiation. NaDodSO$_4$–polyacrylamide gel electrophoresis and analytical gel chromatography were used for the detection of cross-link formation. Figure 4 illustrates the time dependence of the percent of yeast tRNA$_f^{Met}$ cross-linked to EcoMetRL and *E. coli* tRNA^{Ile} cross-linked to EcoIleRL. Both of these methods indicated the cross-linking of tRNA to its cognate ligase followed a hyperbolic dose dependence until a plateau was reached. These results are very similar to those reported by Schimmel and collaborators for the *E. coli* isoleucine system (Budzik et al., 1975).

UV Light Does Not Cross-link tRNA and Noncognate Proteins. In order to determine that the photoinduced aggregates isolated after irradiation were representative of specific tRNA–ligase complex formation, yeast tRNA$_f^{Met}$ was irradiated in the presence of bovine serum albumin or yeast alcohol dehydrogenase. Figure 4 shows that no appreciable complex was detected. Figure 4 shows that irradiation of yeast tRNA$_f^{Met}$ with the *noncognate* EcoIleRL also produced no detectable cross-link formation. These necessarily limited results suggest that the UV-induced cross-linking phenomeon requires a proper tRNA–ligase complex.

Cross-linked Complexes between Substrate tRNAs and EcoMetRL Show a Consistent Pattern of Cross-linking. The methionine:tRNA ligase from *E. coli* was irradiated with tRNA$_f^{Met}$ and tRNA$_m^{Met}$ from *E. coli*, tRNA$_f^{Met}$ from yeast,

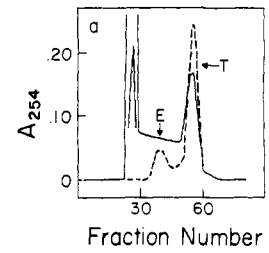

FIGURE 5: Preparative Bio-Gel A-0.5m chromatography of an irradiated complex of EcoMetRL and *E. coli* tRNA$_f^{Met}$. A 4-mL sample, irradiated for 20 min(12 μEinstein cm^{-2}) was applied directly to a 2.5 × 25 cm column in 0.3 M NH_4HCO_3 and eluted in with the same solution. The dashed line shows the chromatography of a nonirradiated mixture. The cross-linked tRNA–ligase complex elutes at the front of this column with an anomalously large apparent molecular weight and absorbance, both of which are due to aggregation of the irradiated enzyme. Aggregation is minimal when EcoMetRL-TF is used. E and T indicate the position at which the native enzyme and tRNA elute, respectively.

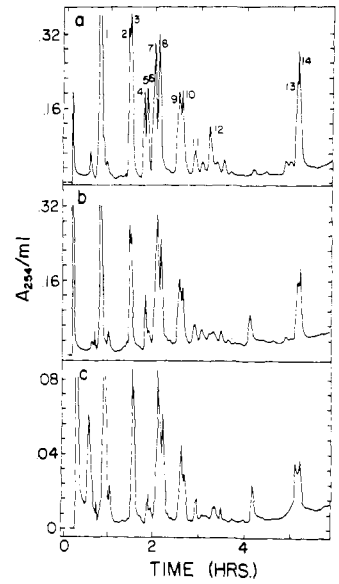

FIGURE 6: Pellionex chromatography of T1 RNase fragments from digests of (a) nonirradiated *E. coli* tRNA$_f^{Met}$, (b) *E. coli* tRNA$_f^{Met}$ irradiated in the absence of ligase, and (c) *E. coli* tRNA$_f^{Met}$ cross-linked to EcoMetRL. Similar sets of elution profiles for *E. coli* tRNA$_m^{Met}$, yeast tRNA$_f^{Met}$, and wheat germ tRNA$_i^{Met}$ are presented (see paragraph at end of paper regarding Supplementary Material).

and tRNA$_i^{Met}$ from wheat germ. Figure 5 shows the Bio-Gel A-0.5m chromatograph of an irradiated complex carried out under conditions in which the unirradiated complex dissociates. In all cases, 25–40% of the tRNA species was incorporated into a nondissociable complex with the cognate ligase, which eluted at the "front" of the Bio-Gel A-0.5m column. Each of these complexes was analyzed by T1 RNase digestion and Pellionex chromatography, as described above, to determine the regions of tRNA cross-linked to protein.

Figure 6 shows the Pellionex chromatography of the T1 RNase fragments from (a) nonirradiated *E. coli* tRNA$_f^{Met}$, (b) *E. coli* tRNA$_f^{Met}$ irradiated in the absence of the ligase, and (c) irradiation of the *E. coli* tRNA$_f^{Met}$–ligase complex. In order to quantitate the difference in these chromatograms, the profiles of irradiated tRNA$_f^{Met}$ (in the presence or absence of protein) were scaled to the nonirradiated profile. The percentage change for each fragment over and above that caused by radiation damage alone was then calculated and is shown in the histograms of Figure 7a. Similar analyses were done for *E. coli* tRNA$_m^{Met}$, yeast tRNA$_f^{Met}$, and wheat germ tRNA$_i^{Met}$. Experiments were repeated at least three times for

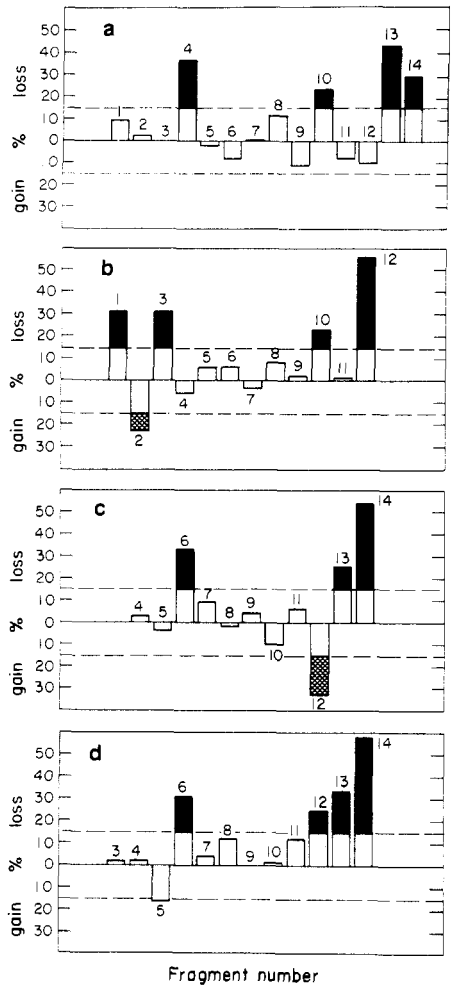

FIGURE 7: Changes in Pellionex elution profiles of T1 RNase fragments of methionine-accepting tRNAs attributable to photoinduced covalent cross-linking to EcoMetRL. Each bar represents the normalized change in the amount of the numbered fragment recovered from T1 RNase digests of the covalently cross-linked complex (e.g., panel c of Figure 6) compared to the amount recovered from a T1 RNase digest of tRNA irradiated in the absence of EcoMetRL (e.g., panel b of Figure 6). The numbering of the fragments corresponds to Figure 8. Losses above 15% are considered significant and are shaded black. (a) *E. coli* $tRNA_f^{Met}$, (b) *E. coli* $tRNA_m^{Met}$, (c) yeast $tRNA_f^{Met}$, (d) wheat germ $tRNA_i^{Met}$.

each cognate pair, and the *net* result is considered to be accurate within 10%. The averaged results for the four cases are shown in the histograms of Figure 7.

A Consistent Sequence Pattern of Cross-linking Sites Was Observed for All Four Substrate tRNAs. Figure 8 presents a composite view of the T1 RNase fragments which appear to be in most intimate contact with EcoMetRL on the basis of their tendency to cross-link when the complex was irradiated with UV light. The one exception is fragment 12 of yeast $tRNA_f^{Met}$, which is apparently not cross-linked but is considered to be in contact with the ligase in the complex since the presence of EcoMetRL causes a dramatic (>30%) protection against radiation damage. Because of a possible secondary structural adjustment, which could be responsible for this protective effect, this fragment's contribution to the composite analysis is weighed down by half. Subject to this adjustment a consistent pattern of sequence positions was observed for the four methionine-accepting tRNAs.

Figure 9 represents a composite histogram for the four methionine-accepting tRNAs in which every sequence position is scored for its inclusion in a cross-linked T1 RNase fragment. In this figure no account is taken of some obvious experimental biases. For example, it is more likely that a large oligonucleotide will appear cross-linked than a smaller one: first, because the probability it contains a contact and/or a photoreactable site is greater, and second, because the chromatographic analysis leads to more visible and unambiguous results for a larger oligonucleotide. Attempts to correct these biases by suitably weighting the elements of the composite histogram do not significantly alter the picture. Preliminary cross-linking experiments employing the enzymatically active monomeric 64000-dalton tryptic fragment of the EcoMetRL (Cassio & Waller, 1971) give results similar to those described here for the native enzyme.

Figure 10 indicates the regions of the three-dimensional structure of crystalline yeast $tRNA^{Phe}$ (Kim et al., 1974; Robertus et al., 1974), which correspond to the sequence positions of $tRNA^{Met}$ implicated by the composite histogram (Figure 9) as being in close contact with the ligase.

Cross-linking Pattern of E. coli $tRNA^{Ile}$ to EcoIleRL Differs from the Methionine System. There are several noteworthy points about the spatial distribution of contact sites in the $tRNA^{Met}$/EcoMetRL system which will be discussed below, but most striking is the lack of involvement of the D arm which was implicated by Schimmel and his colleagues in the cross-linking studies with yeast $tRNA^{Phe}$, *E. coli* $tRNA^{Ile}$, and $tRNA^{Tyr}$ (Schoemaker & Schimmel, 1974; Schoemaker et al., 1975; Budzik et al., 1975). Since our analytical approach is significantly different, we felt obliged to apply our analytical techniques to the pattern of photoinduced cross-links formed between $tRNA^{Ile}$ and EcoIleRL:tRNA ligase under the irradiation conditions described by Budzik et al. (1975) and those which were adopted for this study.

The results of the photocross-linking experiments using *E. coli* $tRNA^{Ile}$ and the cognate *E. coli* enzyme are analyzed and displayed in exactly the same manner as those for the $tRNA^{Met}$/EcoMetRL system (Figure 11). As expected, the 3′-terminal oligonucleotide was cross-linked to the enzyme but, unlike the $tRNA^{Met}$/EcoMetRL system, the D arm of the $tRNA^{Ile}$ was cross-linked and the Tψ arm was not. These results agree with those of Budzik et al. (1975) in that the acceptor and D arms are involved in the photocross-linked contact surface. However, Budzik et al. found that in addition to the fragment CUCAG, the sequence DDAG of the D arm also cross-links strongly, while our analysis showed no photoreactivity of this fragment in either the presence or absence of the cognate ligase. We can offer no obvious explanation for this discrepancy short of either a flaw in one or both of the analyses or an unnoticed difference in the procedure. Our analysis also implicates the anticodon arm, which is not observed by Budzik et al., to cross-link to the cognate synthetase even though the anticodon region had been implicated in cognate (and noncognate) ligase interactions by protection studies (Dickson & Schimmel, 1975; Schoemaker & Schimmel, 1976) and by photoinduced cross-linking in the productive complex with the noncognate yeast valine:tRNA ligase (Budzik et al., 1975). In any case, it is clear that the analysis employed here gives results which are quite consistent with all the previous studies of the interaction of $tRNA^{Ile}$ with EcoIleRL and indicates that the unique but self-consistent cross-linking pattern reported here for the interaction of the four methionine-accepting tRNAs with EcoMetRL is characteristic of the specific protein–nucleic acid interface rather than the analytical techniques.

Discussion

Limitations of the Technique. The validity of the results presented here rests primarily on the self-consistency of the

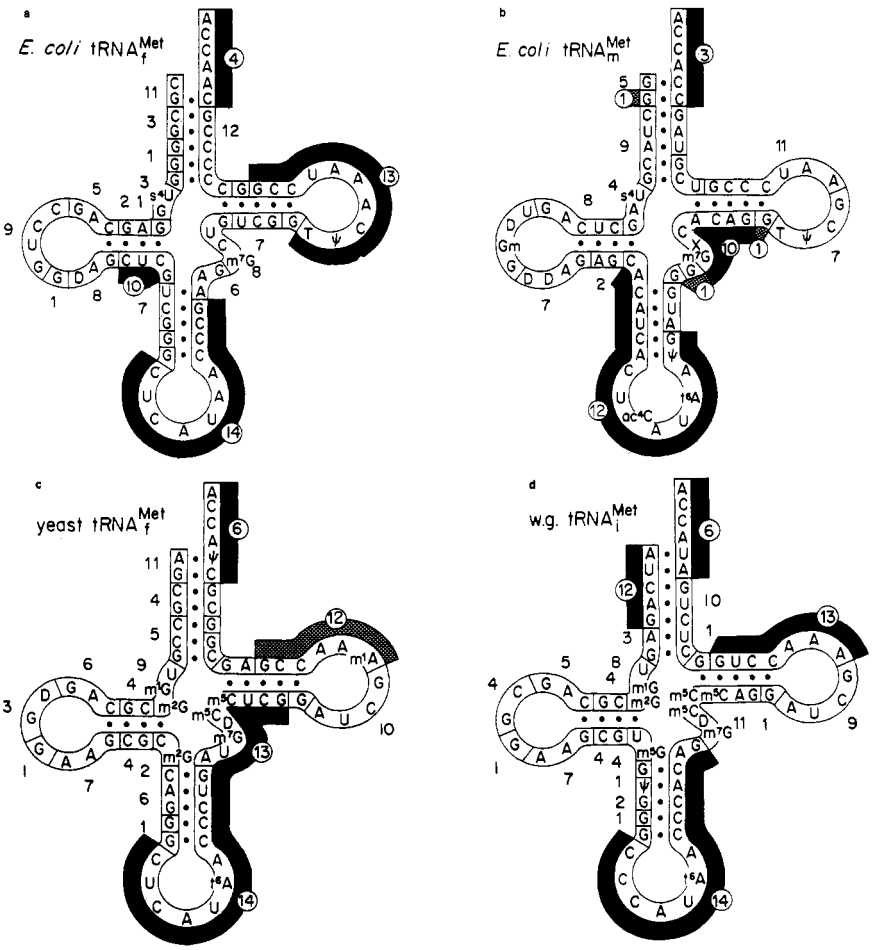

FIGURE 8: Photocross-linked regions of tRNA^{Met} substrates considered to interact with EcoMetRL. Four methionine-accepting tRNAs drawn with their sequences in the cloverleaf representation. T1 RNase fragments are labeled with the same numbers used in Figure 7, which indicate the order in which the oligonucleotides elute from a Pellionex. The solid black bars correspond to the bars on the histograms of Figure 7 that exceed 15% loss; crosshatched regions imply uncertainty in the interpretation. In (c) it reflects an anomolously large protective effect (30% gain); in (b) it indicates that the peak is composed of three fragments. Oligonucleotides were assigned to specific peaks in the elution profile as described under Materials and Methods. (a) *E. coli* tRNA$_f^{Met}$, (b) *E. coli* tRNA$_m^{Met}$, (c) yeast tRNA$_f^{Met}$, (d) wheat germ tRNA$_i^{Met}$

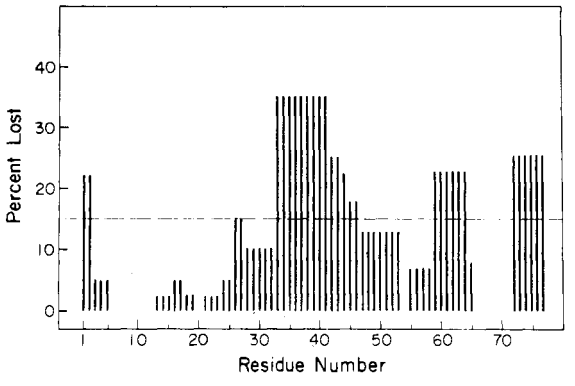

FIGURE 9: Composite histogram showing photocross-linking of each sequence position in the four substrate tRNAs of EcoMetRL. The histogram was calculated by averaging the percentage change in each of the four T1 RNase fragments containing the residue in question. The numbering corresponds to *E. coli* tRNA$_f^{Met}$ and *E. coli* tRNA$_m^{Met}$ which have two more residues in the D loop than the two eukaryotic initiators. The dashed line at 15% change indicates an arbitrary threshold of significance.

findings and points convincingly to the regions indicated in Figure 9 and Figure 10 as participating in the interface of a productive complex involving methionine-accepting tRNAs and EcoMetRL (and probably its active tryptic fragment). However, these and other cross-linking experiments must be cautiously interpreted for the following reasons. (1) Although it can be reasonably assumed that intimate contact between the protein and nucleic acid is a necessary requirement for effective photocross-linking (Sperling & Havron, 1976; Havron & Sperling, 1977), it is unlikely to be sufficient since it is probable that there is a great deal of variation in the photoexcitability of the various bases and/or amino acid residues and the capacity of functional groups at the interface to react with the photoexcited species (McLaren & Shugar, 1964; Ben-Ishai et al., 1973). In these experiments we used radiation having a wide range of wavelengths and four different tRNAs in an effort to help suppress wavelength and sequence-dependent effects on photoreactability. (2) As mentioned above, our analytical techniques impose a bias that focuses attention on the large oligonucleotides. It is quite possible that we have missed interactions involving residues in small RNase T1 fragments, which must be corrected by using other fragmentation and isolation techniques in future experiments. Moreover, large oligonucleotides tend to blur the resolution of the analysis, necessitating further work to identify exactly which residues within the large oligonucleotides represent the point of contact. (3) UV light has a disruptive effect on tRNA (Schulman & Chambers, 1968) and on amino acid:tRNA ligases (Schoemaker & Schimmel, 1974; Budzik et al., 1975), which might lead to cross-linking of damaged moieties, thus giving a distorted impression of the contact surface in the complex. This possibility has again been diminished by the consistent results observed for four methionine-accepting

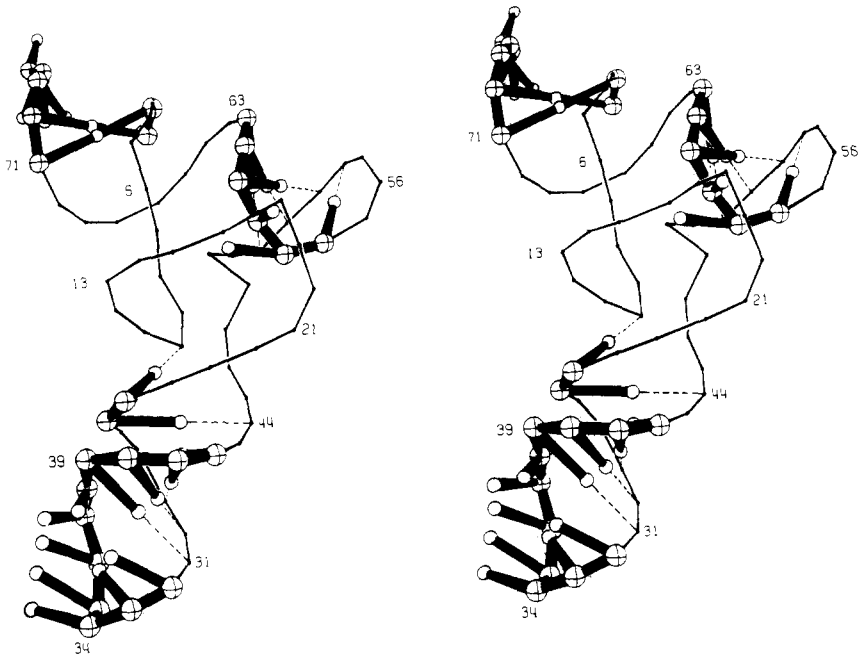

FIGURE 10: Stereoscopic rendering of the photocross-linked contact surface of $tRNA^{Met}$ substrates for EcoMetRL as it would appear in the canonical form of $tRNA^{Phe}$ deduced from the crystal structure. The accentuated regions are those that show 15% or greater cross-linking on the composite histograms of Figure 9. Numbering corresponds to yeast $tRNA^{Phe}$.

tRNAs and two different forms of the enzyme, which presumably share the same interactive surface but differ substantially in sequence and molecular architecture, respectively. (4) It is also possible that in the formation of a tight specific complex the ligase may alter the direct photoreactivity of the tRNA. Indeed, such a possibility is suggested by the fact that the EcoMetRL apparently protects fragment 12 of the yeast $tRNA_f^{Met}$ from photodamage. It follows that a ligase-induced increase in photodamage of a particular region of the tRNA could be misinterpreted as a photoinduced cross-link.

The D Arm and Variable Loop Are Not Involved. It was stressed earlier that the failure of the D arm of $tRNA^{Met}$ to cross-link to EcoMetRL represents a distinct departure from the results obtained by others with yeast $tRNA^{Phe}$ and *E. coli* $tRNA^{Tyr}$ and $tRNA^{Ile}$ and our own analysis of the *E. coli* isoleucine system. The conclusion that the D arm of $tRNA^{Met}$ is *not* involved in the interaction with EcoMetRL is compatible with findings of Seno et al. (1969), who excised residues G_{15}-C-C-U-G-G_{20} from the D loop of *E. coli* $tRNA_f^{Met}$ with little loss of acceptor activity. Moreover, as Schulman & Pelka (1977) point out, none of the seven chemically modified residues of the D arm (C_{12}, C_{13}, C_{16}, C_{17}, U_{18}, G_{19}, and G_{20}) or the two nucleotides which connect it to the acceptor stem (s^4U_8 and G_9) interfere with the substrate capacity of *E. coli* $tRNA_f^{Met}$.

It is noteworthy that the photoinduced cross-linking pattern does not strongly implicate the variable loop in the ligase contact surface of methionine-accepting tRNAs. At first glance this might be considered inconsistent with Schulman's (1971) observation that photooxidation of G_{46} in *E. coli* $tRNA_f^{Met}$ destroys methionine-acceptor activity and its capacity to bind to the EcoMetRL. However, Schulman & Pelka (1977) cite inferential evidence to suggest that the disruptive effect of photooxidizing G_{46} is most likely due to an alteration in the local conformation rather than destruction of a residue directly involved in the ligase contact surface. Their assertion that the guanine moiety of G_{46} is not directly involved in the ligase interaction is further supported by the fact that in yeast $tRNA_f^{Met}$, an excellent substrate for EcoMetRL (RajBhandary & Ghosh, 1969; Rosa & Sigler, 1977), the position homologous to G_{46} is U_{44} (Simsek & RajBhandary, 1972).

The 3′-Terminal Residues Interact with EcoMetRL. It is gratifying to see that the 3′-terminal oligonucleotides are cross-linked consistently since it is difficult to envisage a productive mode of binding in which the site of aminoacylation is not stabilized by direct contact with the ligase. There is evidence in at least three crystal structures that the non-hydrogen-bonded 3′ terminus of tRNA is more flexible and its conformation is less well defined than the rest of the molecule (Kim et al., 1974; Robertus et al., 1974; Schevitz et al., 1975). It appears, then, that the conformation of the 3′ terminus, which is appropriate for productive interaction with the ligase, is imposed to some extent by interaction of the C-C-A residues with the ligase. In the case of the EcoMetRL the base most involved in properly orienting the acceptor end in a productive mode is the penultimate C since it is only the C_{76} of *E. coli* $tRNA_f^{Met}$ which when modified completely destroys acceptor activity (Schulman, 1970). Positioning the 3′ terminus in a productive mode evidently does not affect the stability of the enzyme–substrate complex since conversion of C_{76} to U_{76} while destroying acceptor activity does not alter its capacity to compete with the binding of unmodified substrate (Stern & Schulman, 1977).

The Anticodon Arm Is Involved but Not the Major Groove. The composite representations in Figures 9, 10, and 11 indicate that the substrate's anticodon arm and particularly its distal end are in intimate contact with the EcoMetRL. This conclusion is compatible with the finding that both the bisulfite-mediated conversion of C to U in the first letter of the anticodon and chloroacetaldehyde modification of A in the second letter of the anticodon abolishes acceptor activity in *E. coli* $tRNA_f^{Met}$ (Schulman & Goddard, 1973; Schulman & Pelka, 1977). Dube (1973) also found the anticodon to be involved in ligase interaction since it was protected from hydrolysis when the *E. coli* $tRNA_f^{Met}$–EcoMetRL complex was subjected to T1 RNase digestion. On the other hand, we know from the early study of RajBhandary and Kumar (1970) that certain portions of the anticodon loop do not interact with the EcoMetRL since the aminoacylation reaction is insensitive

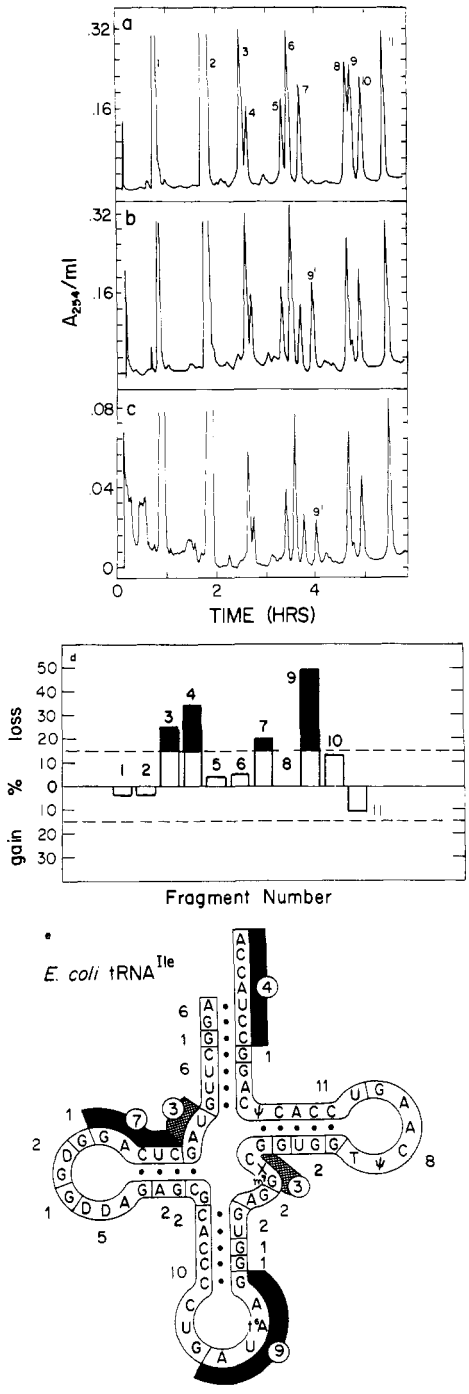

FIGURE 11: Photoinduced cross-linking pattern obtained for complexes of *E. coli* tRNA^{Ile} and EcoIleRL. Details given in legends to Figures 6a, 6b, 6c, 7, and 8 apply to (a), (b), (c), (d), and (e), respectively. Peak 9′ in elution profiles (b) and (c) is assigned to a photoproduct of peak 9 in profile (a) since it arose comcomitantly with and in proportion to the substantial radiation-induced diminution of peak 9. The sequence was determined by Yarus & Barrell (1971).

to the t^6A modification of the adenine base immediately to the 3′ side of the anticodon. The crystal structures of t^6A (Parthasarathy et al., 1974a,b) and yeast tRNA^{Phe} (Kim et al., 1974; Robertus et al., 1974) indicate that this substantial base modification is likely to project well into the region of the anticodon loop which extends from the deep groove of the anticodon stem. Assuming that carbamoylthreonyl modification of the t^6A assumes a conformation within canonical limits reported by Parthasarathy (1974a,b) and that the adenine moiety does not differ significantly from the stacking exhibited in the crystal structure of yeast tRNA^{Phe}, it is unlikely that the extension into the anticodon loop of the deep or major groove of anticodon stem forms part of the contact surface. The deep groove is further ruled out by the finding that the bulky bis(pyridylosmium) adduct of C_{38} of yeast tRNA$_f^{Met}$, the first base-paired residue to the 3′ side of the anticodon loop, also does not interfere with the enzymatic aminoacylation by EcoMetRL or the yeast methionine:tRNA ligase (Rosa & Sigler, 1974). Behrman's studies of osmium reactions (Subbaraman et al., 1971, 1972, 1973; Daniel & Behrman, 1975, 1976) conclusively point to the 5–6 double bond as the likely site of addition causing the bulky adduct to protrude down into what would be an extension of the deep groove of the anticodon stem. In summary, it is clear from both the cross-linking experiments and chemical modification studies that some region in the distal part of the anticodon arm interacts strongly with EcoMetRL, but indifference of the EcoMetRL to certain bulky modifications rules out the deep groove and its extension into the loop.

The 3′-Terminal Portion of the Tψ Arm Interacts with EcoMetRL. Although not as strongly implicated as the molecular 3′ terminus and the anticodon arm, Figure 9 suggests that the 3′-terminal residues of the Tψ loop, or more properly loop IV (Barrell & Clark, 1974), are also important in the interaction of substrate tRNAs with EcoMetRL. This is noteworthy in that previous cross-linking studies of Schimmel and his colleagues have not implicated this part of the tRNA molecule.

Possible Conformational Changes in tRNA^{Met} Associated with Ligase Interaction. Figure 10 shows that, in the canonical form of class I tRNA structure represented by the crystal structure of yeast tRNA^{Phe}, the distal portion of the Tψ arm is "covered" to some degree by the D loop. It has been pointed out (Sigler, 1975) that the tertiary interactions which link the D and Tψ loops together are likely to be less stable and/or permanent on structural considerations alone. Indeed, Schimmel and his colleagues (Gamble & Schimmel, 1974; Gamble et al., 1976; Schoemaker et al., 1976) have shown that G_{18} of yeast tRNA^{Phe}, which the crystal structure shows to be hydrogen bonded to the D-loop backbone, does not have the sluggish tritium exchange rate in solution expected of a purine residue which is involved in a tertiary structural interaction. Moreover, it is apparently necessary to disrupt the D–Tψ loop interactions in order for the Tψ loop to take part in the reaction purported to take place between the Tψ loop and a complementary sequence of 5S RNA during interaction of aminoacyl tRNA with the ribosome (Erdmann et al., 1973; Richter et al., 1973; for reviews see Sigler, 1975; Rich & RajBhandary, 1976). We suggest here that, if necessary, a similar disruption of intramolecular organization could take place on the interaction of tRNA$_f^{Met}$ with EcoMetRL and that the position of the D loop shown in Figure 8 need not be considered a serious impediment to the participation of the Tψ loop in the molecular interface between tRNA^{Met} and the EcoMetRL.

Added in Proof

The crystal structure of yeast tRNA$_f^{Met}$ has now been solved, and the general conformation was found to be similar to that of yeast tRNA^{Phe} (Schevitz et al., 1979). Although there are small but significant differences between the crystal structure of yeast tRNA$_f^{Met}$ and yeast tRNA^{Phe}, Figure 10 is an adequate rendering for the purpose of this paper.

Acknowledgments

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germ $tRNA_i^{Met}$, Professor Kan Agarwal for his useful advice, and Eric Ackerman for help with model building and graphics.

Supplementary Material Available

Supplement Figures 1 (*E. coli* $tRNA_m^{Met}$), 2 (yeast $tRNA_f^{Met}$), and 3 (wheat germ $tRNA_i^{Met}$) containing Pellionex elution profiles of RNase T1 digests comparing (a) unirradiated tRNA, (b) tRNA irradiated in the absence of ligase, and (c) tRNA irradiated in the presence of ligase (each presenting the comparative profiles for a methionine-accepting tRNA used in this study as a substrate for EcoMetRL) (3 pages). Ordering information is given on any current masthead page.

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Control of Embryonic Development: Isolation and Purification of Chick Heart Myosin Light Chain mRNA and Quantitation with a cDNA Probe†

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ABSTRACT: Myosin light chain mRNAs were isolated from the myosin light chain synthesizing polysomes of the 16-day-old chick embryonic heart tissue by immunoadsorption of total polysomes to myosin light chain specific antibodies. The mRNA, purified by successive sucrose gradient centrifugations and oligo(dT)-cellulose chromatography, was assessed for purity by translation in mRNA-dependent rabbit reticulocyte lysate, electrophoretic separation on denaturing gels, and hybridization assays. Proteins synthesized in response to the RNA in a cell-free system were two myosin subunits of 24 000 and 18 000 molecular weight, identical with those of authentic myosin light chain subunits obtained from the homologous chick heart tissue. The RNA resolved on a denaturing polyacrylamide gel into two RNA bands of approximately 1090 and 980 nucleosides in chain length, the putative mRNAs for the myosin light chain subunits LCM_1 and LCM_2. Additional evidence for purity comes from hybridization kinetics of the mRNA with the complementary DNA (cDNA) synthesized with avian myeloblastosis virus reverse transcriptase. The RNA sequence complexity based on the hybridization assay was in excellent agreement with the combined molecular sizes of the two mRNAs. The observed $R_0t_{1/2}$ values for cDNA hybridization with the purified mRNAs, poly(A)-containing RNA, and total polysomal RNA indicated that the LCM mRNAs comprise 2.0 and 0.02% of poly(A) RNA and polysomal RNA, respectively. The availability of pure myosin light chain mRNAs and the cDNA probes should facilitate the analysis of the mechanism(s) underlying embryonic heart induction and differentiation during chick development.

The phenomena of morphogenesis and cell differentiation during early embryonic development are poorly understood at the molecular level. Heart muscle differentiation, which is an early event in chick embryonic development (Romanoff, 1960), provides a model system to investigate the mechanism(s) controlling the transition of presumptive heart cells to well defined, highly differentiated myocytes. The transition is characterized by several biochemical parameters, including the synthesis of muscle specific proteins, light and heavy chain myosin and actin, the major constituents of muscle proteins. Myosin consists of a large molecular weight (200 000) heavy chain myosin polypeptide (HCM)[1] and two to three small polypeptides, the light chain myosin subunits (LCM) (Taylor, 1972). The molecular weights of LCM subunits range between 15 000 and 27 000. While actin is synthesized in appreciable amounts in various cell types, the occurrence of specific differences in both composition and content of LCM subunits (Lowey & Risby, 1971; Sarkar et al., 1971; Yaffe & Dym, 1972; Sarkar, 1972; Low et al., 1971; Sreter et al., 1975) makes the latter a suitable marker for monitoring changes during muscle development. Also, a particular advantage of the chick embryo heart muscle system is the availability of a fate map of embryonic cells which facilitates the identification and isolation of the presumptive heart-forming cells from the very early stages of developing embryo (DeHaan et al., 1970; Rosenquist, 1970). These cells, when grown in culture, differentiate predominantly into well-defined heart muscle tissue.

According to the current concepts, changes in early morphogenetic pattern and cell differentiation, which are caused by differential expression of gene activity, are controlled in

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[1] Abbreviations used: HCM and LCM, heavy and light chain myosin, respectively; AMV, avian myeloblastosis viral; $NaDodSO_4$, sodium dodecyl sulfate; Hepes, *N*-2-hydroxyethylpiperazine-N^1-2-ethanesulfonic acid.

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