—21°C, but a threefold higher yield in the *res*⁻ cells (versus *res*⁺) when not frozen. On reincubation of irradiated *res*⁻ cells in growth medium, the DNA chain breaks were not repaired and the cells showed extensive DNA degradation. The molecular weight of denatured (but not native) DNA decreased significantly. We have not observed this type of degradation in *E. coli* K-12 *pol A1*. Kato and Kondo offer two alternative hypotheses to account for the x-ray sensitivity of their mutant: (i) "excessive endonucleases" and (ii) "polymerase participates in the repair of X-ray damage." Our own data demonstrate that DNA polymerase is necessary for the fast repair of DNA single-strand breaks.

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14. Phosphate buffer, *p*H ~7, has been used in studies on *E. coli* which demonstrate a low yield of x-ray–induced DNA single-strand breaks. Barbital buffer, *p*H 8.6, was used in the work on *M. radiodurans* in which a higher number of chain breaks was found. These data suggested that a shift to *p*H 8 might be an important factor in controlling the rate of *pol* repair, particularly at 0°C. This proved to be so. We also observed that after irradiation in tris(hydroxymethyl)aminomethane (tris) and barbital buffers, inhibition was less reversible (by raising the temperature) than in phosphate buffer.
15. The mean energy requirement to produce one DNA single-strand break in *pol*⁻ and inhibited *pol*⁺ (W3110 *thy*) cells is 75 ev per break, and was determined from 22 measurements in 11 experiments.
16. The *pol-rec*⁻ double mutant (which would presumably be devoid of any repair capacity of the kind discussed here) is thought to be inviable (J. D. Gross, *Int. Congr. Microbiol. Rep. Proc. 10th, Mexico City, August 1970*).
17. S. Lehnert and H. Moroson have reported [*Radiat. Res.* **45**, 299 (1971)] a value of 87 ev per break for log phase *E. coli* B/r CSH irradiated in cold 0.01*M* tris buffer (*p*H 8.0) in the presence of 1 m*M* EDTA.

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Cross-Linked Transfer RNA Functions in All Steps of the Translation Process

Abstract. *A specific photochemical reaction between 4-thiouridine and cytosine cross-links two arms of transfer RNA. This cross-link, introduced into phenylalanine transfer RNA and arginine transfer RNA, limits the conformational freedom of the molecule. Both modified transfer RNA's are capable of functioning in all steps of protein synthesis with this restraint on allowable conformations.*

The role of tRNA (*1*) as an adaptor molecule for translation of a genetic message into a protein sequence requires highly specific interactions between tRNA and various enzymes, messenger RNA, and ribosomes. The steps necessary for the total translation process in which tRNA is involved include enzymatic aminoacylation of tRNA, binding of GTP and aminoacyl tRNA to T factor, binding to the ribosomal site for aminoacyl-tRNA, peptide bond formation, translocation of mRNA–polypeptidyl-tRNA complex to the peptidyl-tRNA binding site on the ribosome, and release of tRNA from the ribosome. This multitude of functional steps involving tRNA has led to proposals that changes in the macromolecular conformation of tRNA may be required for some of these steps. Through studies of the longitudinal relaxation of water protons enhanced by paramagnetic manganous ions bound to tRNA, Cohn *et al.* (*2*) suggested that tRNA conformation changes on aminoacylation. In proposing the reciprocating ratchet model for translation, Woese (*3*) suggests that tRNA may pass through several conformational states.

We have investigated the question of whether a chemical cross-link between the positions 8 and 13 in phenylalanine tRNA and arginine tRNA modifies the ability to function in a total protein synthesis system. Favre *et al.* (*4*) have reported for $tRNA^{Val}$, $tRNA_2^{Val}$, $tRNA^{Met}$, $tRNA^{fMet}$, and $tRNA^{Phe}$ a specific modification of 4-thiouridine in the position 8. The reaction requires a cytosine in position 13. This modification is unique in that it makes a cross-link between the amino acid acceptor stem and the dihydrouridine-containing loop of the cloverleaf model of tRNA. Chemical cross-linkage of nonadjacent nucleotides in tRNA can reduce the number of allowed conformational states in a tRNA molecule.

We have shown that this photochemical reaction occurs readily in *Escherichia coli* $tRNA^{Arg}$ (*5*). The prog-

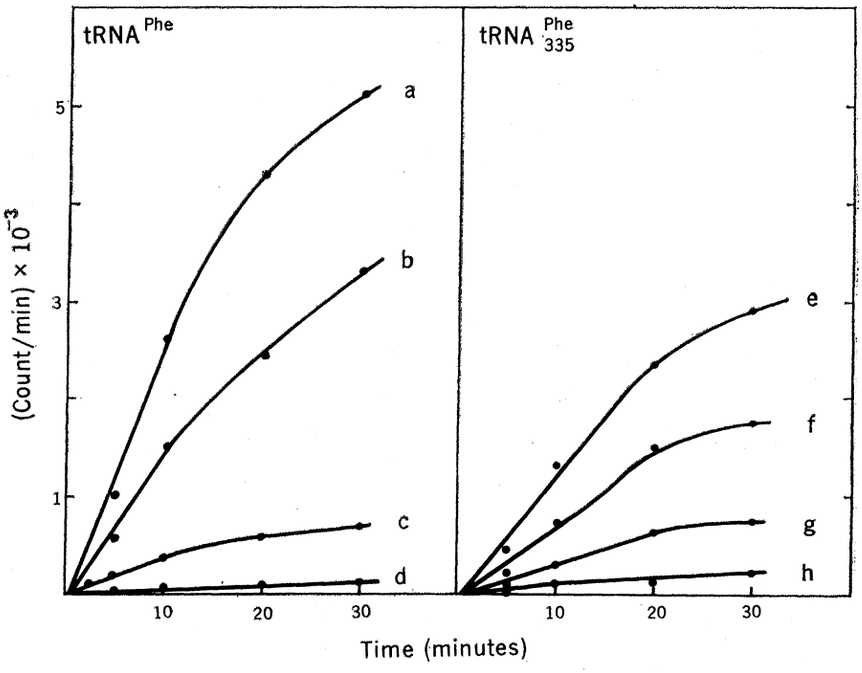

Fig. 1. [^{14}C]Phenylalanine incorporation in response to added $tRNA^{Phe}$ and $tRNA^{Phe}_{335}$. A 1-ml reaction mixture contained 50 μmole of tris buffer (*p*H 7.2), 15 μmole of $MgCl_2$, 30 μmole of NH_4Cl, 3 μmole of ATP, 0.2 μmole of GTP, 5 μmole of phosphoenolpyruvate, 20 μg of pyruvate kinase, 10 μmole of mercaptoethanol, 10 to 12 A_{260} units (absorbance at 260 nm) of ribosomes, and as much as 3 mg of S 100 protein (*10*). In addition, for phenylalanine incorporation the reaction mixture contained 100 μg of poly(U) and 17.4 nmole [^{14}C]phenylalanine (specific activity 28.6 μc/μmole). The incubations were at 37°C with variable amounts of $tRNA^{Phe}$ and $tRNA^{Phe}_{335}$ added. Incorporation of phenylalanine into polypeptide linkage was measured as product insoluble in 5 percent trichloroacetic acid after release from tRNA by incubation in alkali. Curves a, b, c, d represent 2.33, 1.16, 0.09, and 0.0 A_{260} units of $tRNA^{Phe}$ per milliliter added, respectively, to the incubation mixture. Curves e, f, g, and h represent 2.43, 1.22, 0.28, and 0.0 A_{260} units of $tRNA^{Phe}_{335}$ per milliliter added, respectively, to the reaction mixture.

ress of the cross-linkage was measured by following the spectral changes in the 335-nm absorption peak (*4*). The reaction was 95 percent or more complete after irradiation at 335 nm for 8 hours in the chamber of an Aminco-Bowman spectrophotofluorimeter. Diethylaminoethyl (DEAE)-cellulose chromatography (*6*) of a T_1 digest of $tRNA^{Arg}$ indicated that the starting material was a single arginine acceptor species, and that a new large oligonucleotide appeared as a result of the irradiation. No other new photoproducts were detected among the separated oligonucleotides (*7*). Two photochemically modified tRNA's, $tRNA^{Phe}_{335}$ and $tRNA^{Arg}_{335}$, were assayed in an *E. coli* in vitro protein synthesis system for their ability to support amino acid polymerization.

When poly(U) was introduced to stimulate [^{14}C]phenylalanine incorporation, both irradiated and nonirradiated tRNA's were active in all experiments. In Fig. 1, [^{14}C]phenylalanine incorporation in the presence of poly(U) shows dependence on the amount of tRNA added. In five experiments to determine initial rate and maximum extent, both tRNA's were always active, with the observed values variable. Figure 2 compares the two tRNA forms in one experiment for initial rate and maximum extent of [^{14}C]phenylalanine incorporation. The initial rate of $tRNA^{Phe}_{335}$ and maximum extent are 0.45 and 0.48 that of nonirradiated material. The initial rate of incorporation in independent experiments varied from 0.37 to 0.77 that of $tRNA^{Phe}$. The maximum extent ranged from 0.48 to 1.15 that of the nonirradiated material.

Poly(C,I) and poly(A,I) (*8*), which contain the two possible sets of arginine codons, were used to check the codon assignment of $tRNA^{Arg}$. Binding of $tRNA^{Arg}$ to ribosomes and incorporation of arginine in the presence of poly(C,I) and poly(A,I) indicated that poly(C,I) was the preferred polymer. The modified $tRNA^{Arg}_{335}$ is charged by partially purified arginyl-tRNA synthetase with a K_m five times that of the unmodified $tRNA^{Arg}$ (*9*). Favre *et al.* (*4*) reported a K_m for irradiated $tRNA^{Val}$ three times greater than that for nonirradiated material. The irradiated $tRNA^{Arg}$ interacts with *E. coli* T factor to a similar extent as does $tRNA^{Arg}$ (*9*). Incorporation of [^{14}C]-arginine in response to poly(C,I) with the use of $tRNA^{Arg}$ and $tRNA^{Arg}_{335}$ is shown in Table 1. Both irradiated and nonirradiated tRNA's were active

Table 1. Poly(C,I) directed [^{14}C]arginine incorporation with $tRNA^{Arg}$ and $tRNA^{Arg}_{335}$. The incubation conditions were the same as described in Fig. 1. For arginine incorporation, the reaction mixture contained 100 μg of poly(C,I), 3.16 nmole of [^{14}C]arginine (specific activity 158 μc/μmole), 3.3 nmole of the 19 other [^{12}C]amino acids, and oxidized tRNA ($tRNA_{ox}$) (*11*). Incubation was for 30 minutes at 37°C.

tRNA added (A_{260}/ml)	[^{14}C]Arginine incorporated (count/min)
None	70
6.05 $tRNA_{ox}$	98
2.42 $tRNA^{Arg}$ + 6.05 $tRNA_{ox}$	260
2.52 $tRNA^{Arg}_{335}$ + 6.05 $tRNA_{ox}$	250

in this system with $tRNA^{Arg}_{335}$ varying between 50 to 100 percent activity of $tRNA^{Arg}$. When incorporation stimulated by endogenous mRNA was followed, addition of purified arginyl tRNA at the concentration used here was inhibitory.

These results show that both $tRNA^{Phe}_{335}$ and $tRNA^{Arg}_{335}$ are capable of function in all of the steps of protein synthesis. The photochemical modification restricts the relative movement between the amino acid acceptor stem and the dihydrouridine loop. Conformational changes of other parts of the tRNA molecule in relation to each other or these two parts are not prohibited by this modification. For example, the conformational change proposed by Woese is in the anticodon loop, which may be remote from the cross-link tested by us. Other specific cross-links would have to be introduced between other loops or stems of the tRNA molecule to test the question of whether other degrees of conformational freedom are necessary for certain of the functional roles of tRNA.

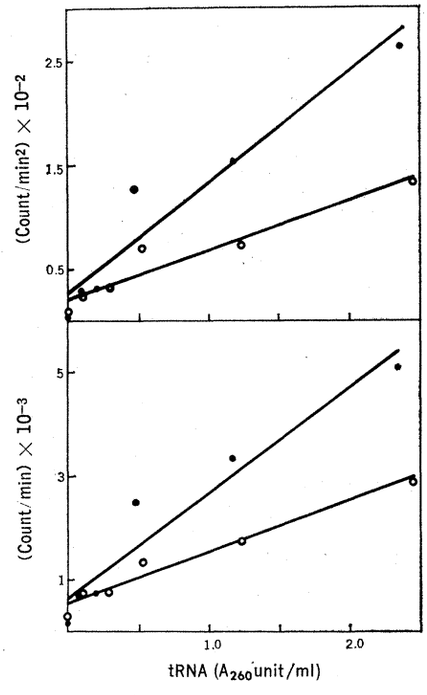

Fig. 2. Comparison of $tRNA^{Phe}$ and $tRNA^{Phe}_{335}$ with respect to intial rate and maximum extent of [^{14}C]phenylalanine incorporation. The intial rates of reaction (top) from the curves in Fig. 1 are dependent on the amount of $tRNA^{Phe}$ (closed circles) and $tRNA^{Phe}_{335}$ (open circles) added. The maximum extent of reaction (bottom) after 30 minutes of incubation is dependent on the amount of $tRNA^{Phe}$ (closed circles) and $tRNA^{Phe}_{335}$ (open circles) added.

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References and Notes

1. Abbreviations are as follows: tRNA, transfer RNA; mRNA, messenger RNA; DEAE, diethylaminoethyl; poly(U), polyuridylic acid; poly(C,I), random polymer of cytidylic and inosinic acids; poly(A,I), random polymer of adenylic and inosinic acids; K_m, Michaelis constant; GTP, guanosine triphosphate; $tRNA^{Phe}$, $tRNA^{Arg}$, $tRNA^{Val}$, $tRNA^{Met}$, and $tRNA^{fMet}$ are phenylalanine, arginine, valine, methionine, and formylmethionine transfer RNA's, respectively.
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5. Purified tRNA's were obtained from Oak Ridge National Laboratories: $tRNA^{Phe}$, *E. coli* K-12 MO7, lot 2, 78 percent pure; $tRNA^{Arg}$, *E. coli* K-12 MO7, lot 15-141, 70 percent pure and lot 15-143, 100 percent pure.
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8. Both polymers were obtained from P-L Biochemicals, and had a base ratio of **1 : 1.**
9. T factor preparation was supplied by Dr. Alan Scragg and assayed with $tRNA^{Arg}$ and $tRNA^{Arg}_{335}$ by the procedure of R. Ertel, B. Redfield, N. Brot, H. Weissbach [*Arch. Biochem. Biophys.* **128**, 331 (1968)].
10. Ribosomes were prepared by the method of R. W. Erbe, M. M. Nav, P. Leder [*J. Mol. Biol.* **38**, 441 (1969)]; and enzymes were prepared by the procedure of M. R. Capecchi [*Proc. Nat. Acad. Sci. U.S.* **58**, 1144 (1967).
11. Incorporation of [^{14}C]arginine directed by poly(C,I) requires tRNA's other than arginine. In order to supply these tRNA's with minimum addition of functional arginine acceptor, crude tRNA was aminoacylated with the 19 other amino acids and then treated with $NaIO_4$ to oxidize the unacylated $tRNA^{Arg}$ and removed its ability to be charged. The tRNA retains the capacity to accept other amino acids and was added to the reaction mixture in addition to the purified $tRNA^{Arg}$. The periodate oxidation procedure is described by S. L. Leppla, B. Bjoraker, R. M. Bock [*Methods Enzymol.* **12B**, 236 (1968)].
12. We thank Drs. K. Chakraburrty and A. H. Mehler of Medical College of Wisconsin, Milwaukee, for determining the K_m of $tRNA^{Arg}_{335}$.
13. This research was supported by NIH grant GM-12395 and NSF grant GB-6993.

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