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Arthur L. Beaudet  
*Yale University*

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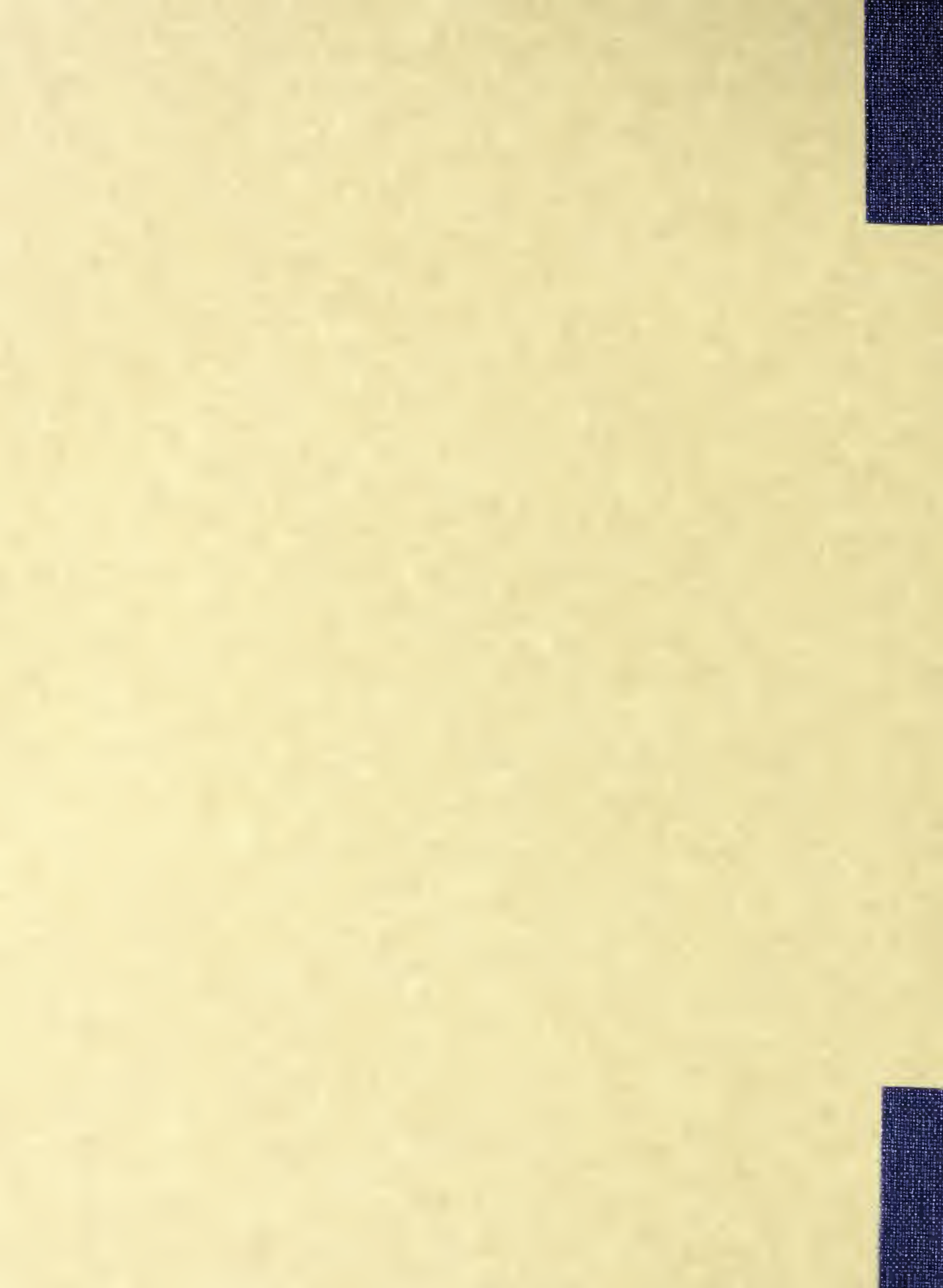
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DIFFERENCES IN RNA CODON RECOGNITION AS  
A FUNCTION OF CELLULAR tRNA CONTENT

Arthur L. Beaudet

B.S., College of the Holy Cross, 1963

Submitted to the Faculty  
of Yale University in partial  
fulfillment of the require-  
ments for the degree of  
Doctor of Medicine.

New Haven, Connecticut

Yale University School of Medicine

1967



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The first precise suggestions as to the nature of the genetic code were made by Gamow<sup>1</sup> in 1954 at a time when indirect evidence was mounting to indicate that the amino acid sequence of a particular protein is in some way determined by the sequence of bases in some length of nucleic acid. Both Gamow and Crick<sup>2</sup> favored a code in which a triplet of the nucleotide bases, U, C, A, and G<sup>3</sup>, coded for an amino acid. The 64 permutations allowed by a four lettered triplet code was more than adequate for the 20 known amino acids. On the basis of bacterial genetic experiments Crick<sup>4</sup> proposed a triplet, non-overlapping code which is read sequentially from a fixed starting point. This allows for degeneracy within the code; that is, a particular amino acid can be coded by one of several triplets of bases. Experimental evidence has accumulated in support of these proposals.

In 1961 Nirenberg and Matthaei<sup>5</sup> used a synthetic polynucleotide, polyuridylic acid, to direct the synthesis of poly-L-phenylalanine in a stable, cell free, protein synthesizing system isolated from E. coli W3100. Using other randomly ordered synthetic polynucleotides of varying base composition and ratio as messenger for in vitro polypeptide synthesis, the code words for the 20 amino acids could be determined as to base composition and ratio but not sequence.

Nirenberg and Leder<sup>6</sup>, in 1964, demonstrated that trinucleotide diphosphates of known composition and sequence would form stable complexes with H<sup>3</sup>- or C<sup>14</sup>-AA-tRNA and ribosomes under certain conditions. These H<sup>3</sup>- or C<sup>14</sup>-AA-tRNA-triplet-ribosome complexes were retained on cellulose nitrate filters and radioactivity measured. The stimulation of H<sup>3</sup>- or



$C^{14}$ -AA-tRNA binding to ribosomes by a particular triplet reflects its ability to code for the particular amino acid. The 64 triplets of known sequence were synthesized enzymatically using ribonuclease<sup>7</sup> or polynucleotide phosphorylase<sup>8</sup>, and each triplet was tested in the binding assay against each of the 20 amino acids. The codon assignments based on these methods have been substantiated by Khorana<sup>9</sup> in similar experiments and confirmed by protein incorporation experiments using synthetic repeating trinucleotides of known sequence<sup>10</sup> so that most codon assignments can now be regarded as certain. This in vitro data is supported by in vivo studies by Yanofsky<sup>11</sup> and Wittman<sup>12</sup> of amino acid replacement mutations in bacterial enzymes. Amino acid substitutions found are consistent with single nucleotide changes as expected from the mutagenic agent used; that is, there are codons identical in 2 of the 3 base positions for both amino acids involved in any substitution.

Table 1 represents the triplet assignments for E. coli AA-tRNAs based on binding assay and protein incorporation studies<sup>9,13</sup>. Trinucleotide diphosphates are conventionally abbreviated with the 3'-hydroxyl end to the right. Degeneracies--defined as multiple codon assignments for a single amino acid--are common as was suspected from the 64 possible triplets coding for 20 amino acids. That the 3'-base may vary without change in the amino acid translation in some cases is written as U = C = A = G for that position. In essentially all cases U = C and often A = G in the 3'-base position. UUG and AUG and possibly CUG and GUG represent the infrequent occurrence of a degeneracy in the 5'-base position when coding for N-formyl methionine. This probably occurs only when these codons are in the initial position of the mRNA



while their occurrence internally codes for leucine, methionine, or valine as appropriate. UAA and UAG are thought to represent special function codons since they do not stimulate binding of any of the 20 AA-tRNAs. Evidence that at least one of these codons functions as a chain terminator in E. coli is discussed below. AGU and AGC are codons which may stimulate the binding of CYS-tRNA in addition to SER-tRNA, giving rise to a little understood ambiguity in the code at this time.

The universality of this code for a bacterial, amphibian, and mammalian system has been investigated by Marshall et al.<sup>14</sup> Using C<sup>14</sup>- or H<sup>3</sup>-AA-tRNAs from Xenopus laevis and guinea pig liver the template activity of 51 codons was tested and compared to the codon recognition by E. coli AA-tRNA. Almost identical codon responses were observed, indicating the universality of the code. The differences observed involved the relative response of AA-tRNAs to synonym codons and the activity of particular triplets for AA-tRNAs in the higher species which were inactive in E. coli. Codon differences were only noted between E. coli and the two higher species, and those between E. coli and guinea pig liver are shown in Table 2

The occurrence of order in the genetic code has been a topic of some discussion<sup>15,16</sup>. There are at least two notable features. First, codons assigned to the same amino acid are similar; and second, amino acids related structurally and functionally often have similar codons. Whether these similarities result from amino acid-nucleotide interactions or are a result merely of the advantage offered by such a code in the evolutionary scheme is unresolved.



Holley and his co-workers<sup>17,18,19</sup> demonstrated the heterogeneity of amino acid specific tRNAs using countercurrent distribution. Zachu<sup>20</sup> using similar methods and Sueoka<sup>21</sup> using MAK column chromatography confirmed this heterogeneity which is now known to involve many specific tRNAs. In *E. coli* multiple tRNAs have been described for alanine<sup>22</sup>, arginine<sup>23</sup>, histidine<sup>18</sup>, leucine<sup>19,21,23</sup>, lysine<sup>24</sup>, methionine<sup>22,25</sup>, threonine<sup>21</sup>, serine<sup>21,23,26</sup>, and valine<sup>22</sup>. In yeast serine<sup>27,28</sup>, tryptophane<sup>29</sup>, and tyrosine<sup>30</sup> have been shown to have multiple tRNAs. Two leucine tRNAs have been demonstrated in rabbit reticulocytes<sup>31</sup>, and two lysine tRNAs have been described in rat liver.<sup>32</sup>

Purified tRNAs could be obtained by countercurrent distribution and in 1965 Holley et al<sup>33</sup> were able to determine the nucleotide base sequence of an ALA-tRNA from yeast. This purified ALA-tRNA when tested in the ribosomal binding assay with the ALA codons was found to respond to GCU, GCC, and GCA but not to GCG.<sup>13</sup> Thus degeneracy in the code is not solely on the basis of tRNA heterogeneity for a single ALA-tRNA could respond to three codons. Possible anticodon sites--defined as that portion of the tRNA base sequence which pairs with the mRNA--in Holley's ALA-tRNA are IGC MeI and DiHu-CGG-DiHU. The latter could involve parallel hydrogen bonding with GCU, GCC, and GCA, while the first allows anti-parallel Watson-Crick hydrogen bonding in two positions and alternate pairing of inosine in the anticodon site with U, C, or A in the codon. Subsequently the structures of two SER-tRNAs<sup>28</sup>, a TYR-tRNA<sup>34</sup>, and a VAL-tRNA<sup>35</sup>, all from yeast, have been described. The anticodon sites for these other purified tRNAs have been predicted and antiparallel Watson-Crick hydrogen bonding between codon and anticodon is most consistent with available evidence.





On the basis of data from many investigators Crick<sup>36</sup> has proposed a mechanism of alternate base pairing referred to as the "Wobble Hypothesis" to explain 3<sup>rd</sup>-base degeneracy in the coding of certain AA-tRNAs. For the first two positions of the codon pairing is proposed to be strictly equivalent, i.e., A pairs with U and C with G. For the third base he proposes that pairing involves positions close to the standard one. Using the information available on the code he proposes which of the numerous possible close positions are used in pairing in addition to the standard position. The ability to pair in additional positions to the standard one is referred to as wobble, an example of which is the fact that I pairs primarily with C but by the wobble mechanism might pair in addition with alternate bases U and A. This would explain I in the anticodon site pairing with U, C, and A for a single tRNA. Table III lists the proposed codon-anticodon pairings according to the wobble mechanism. The typical amino acid coded by four codons differing only in the 3<sup>rd</sup>-base position will require at least two tRNA species with no theoretical maximum. If tRNAs for a particular amino acid are fractionated, the responses of a single species will include one to three codons depending on the anticodon base in position to pair with the 3<sup>rd</sup>- base in the codon.

Using the fractionation procedures described above, certain tRNAs have been shown to be of special interest as possible regulatory mechanisms. In fractionating E. coli MET-tRNA Clark and Marker<sup>25</sup> and others<sup>37,38,39</sup> found two tRNAs, the smaller fraction, MET-tRNA<sub>M</sub>, responding to AUG while the larger fraction, MET-tRNA<sub>F</sub>, responded to AUG > GUG > UUG. In addition MET-tRNA<sub>F</sub> could be formylated in the N position of methionine by a purified E. coli transformylase with N<sup>5,10</sup>-C<sup>14</sup>-methenyl-H<sub>4</sub>-folate. The formylated



methionine could then be incorporated into the N-terminal position of polypeptide chains indicating that N-formyl methionine-tRNA can function as a chain initiator in E. coli protein synthesis. It has been proposed that if AUG, UUG, or GUG occur in the 5'-base terminus of a mRNA, N-formyl methionine-tRNA is bound to the ribosomal site for initiation, while these same codons in internal mRNA positions lead to the binding of MET, LEU, and VAL-tRNAs, respectively, in the ribosomal site for AA-tRNAs. The 5'-base degenerate codon responses of MET-tRNA<sub>F</sub> represent another special feature which may be explained by alternate base pairing of the wobble mechanism type when the 5'-base is the terminal one in the mRNA.

A group of E. coli mutants, referred to as amber and ochre, are of special interest because they involve point mutations which result in premature termination of a polypeptide chain, and the mutations can be suppressed. The suppression is accomplished by the substitution of an amino acid at the site of premature termination allowing the synthesis of a complete but slightly altered protein. Brenner et al.<sup>40</sup> and Weigert and Garen<sup>41</sup> have used the knowledge of the genetic code and the specific substitutions to deduce that these mutations generated a UAG codon in amber mutants and a UAA codon in ochre mutants, both of which result in termination of the polypeptide chain. Suppression depends on a tRNA which will recognize this codon and substitute an amino acid. The nature of this alteration in tRNA is unclear although Bergquist and Capecchi<sup>42</sup> have demonstrated a suppressor tRNA which substitutes SER and chromatographs with a major SER-tRNA peak.

Another group of E. coli mutants referred to as missense suppressions are of special interest. A single amino acid substitution in the mutant



strain is partially corrected in the suppressed strain so that the latter produces proteins of both the wild type and mutant amino acid sequences<sup>43</sup>. In two cases<sup>44,45</sup> of missense suppression the correction of the amino acid sequence has been shown to be mediated through the appearance of a tRNA which substitutes the wild type amino acid at the site of the mutated codon. Whether this represents alteration of the anticodon site, the amino acid acceptor site, or both is uncertain, although there is some evidence to favor a change in codon recognition of a small portion of a given AA-tRNA. These suppressor mutations are excellent examples of tRNAs acting as regulators of protein synthesis.

Recently there is other more indirect evidence that tRNAs may function in regulatory capacities. Alterations in the chromatographic patterns of VAL-tRNA<sup>46</sup> and LYS-tRNA<sup>47</sup> have been observed with the change from spore to vegetative forms in Bacillus subtilis. The chromatographic pattern of E. coli LEU-tRNA has been shown by Sueoka<sup>48</sup> to change with infection by T-2 phage. The SER-tRNA chromatographic pattern has been shown to change with different growth conditions in Bacillus subtilis<sup>49</sup>. Whether these changes represent regulatory changes on the part of tRNAs or secondary changes in their amounts according to needs is unknown at present.

That differences in relative and absolute template activity between bacteria and mammals was based on tRNA differences appeared likely. This paper reports the use of Kelmer's reverse phase chromatographic system to fractionate E. coli and guinea pig liver tRNAs into their respective species. Ribosomal binding studies on fractions indicate that alterations in tRNAs are intimately linked to changes in template activity between





species. The regulatory possibilities of these differences between species is unresolved. The codon assignments to fractionated species of tRNA from both species is consistent with the alternate base pairing "Wobble Hypothesis" of Crick.

#### METHODS AND MATERIALS

Preparation of tRNA: E. coli B tRNA was purchased from General Biochemicals, Inc., (Chagrin Falls, Ohio) and was found to be equivalent to 20 A<sup>260</sup> units in a solution of 1 mg RNA/ml H<sub>2</sub>O.

The method of Brunngraber<sup>50</sup> was used to prepare tRNA from livers of adult guinea pigs of mixed sex, Hartley strain, average weight 250 gm. One hundred grams of liver were homogenized in 150 ml of water saturated phenol and 150 ml of 1.0 M NaCl, 0.005 M EDTA in 0.1 M tris-chloride buffer, pH 7.5. The upper aqueous layer was decanted and to this were added 3 volumes of 95% ethanol. The resultant precipitate was spun down, collected, and resuspended in 250 ml of 0.1 M tris-chloride buffer, pH 7.5. This was then passed over a 2 x 10 cm column of 2 g of DEAE-cellulose previously equilibrated with 0.1 M tris-chloride buffer, pH 7.5. Elution with 1.0 M NaCl continued until the optical density was less than 3 at 260 mμ. The eluate was precipitated with 0.1 volume of 20% potassium acetate and 3.0 volumes of absolute ethanol and stored overnight at -20°C. The precipitate was collected, lyophilized, and stored in liquid nitrogen. Approximately 40 mg of RNA were obtained from 100 gm of guinea pig liver.

Acylation: A 100,000 x g supernatant was obtained from E. coli B cells using the French press for rupture. Aminoacyl synthetases were precipitated by adding solid ammonium sulfate (neutralized with ammonium carbonate 2% by weight) until 70% saturation was reached. Ammonium





sulfate precipitates were dialyzed against 0.002 M tris-chloride, pH 7.5 with 0.006 M  $\beta$ -mercaptoethanol before storage in liquid nitrogen.

A 150,000 x g supernatant was prepared from guinea pig liver according to Moldave<sup>51</sup> and similarly precipitated with solid ammonium sulfate. Precipitates were dissolved in 0.35 M sucrose; 0.035 M potassium bicarbonate; 0.025 M potassium chloride; 0.004 M magnesium chloride; and 0.02 M potassium phosphate, pH 7.4 with a final protein concentration of 25-30 mg/ml and stored in liquid nitrogen. A portion of the guinea pig liver synthetase was used to acylate E. coli tRNA and was treated to remove guinea pig liver tRNA from the synthetase preparation. This was done by rapid passage through a DEAE cellulose column in 0.1 M tris-chloride, pH 7.4. The absorbance ratio 280 m $\mu$ /260 m $\mu$  was 1.2 before and 1.6 after column passage.

Acylation reactions contained 0.005 M sodium ATP; 0.01 M magnesium chloride; 0.1 M tris-chloride, pH 7.5;  $1 \times 10^{-4}$  to  $1 \times 10^{-5}$  M  $C^{14}$ - or  $H^3$ -amino acid;  $1 \times 10^{-4}$  to  $1 \times 10^{-5}$  M each of the other 19  $C^{12}$ ,  $H^3$ -amino acids; 1-2 mg/ml tRNA; and 1-2 mg protein/ml aminoacyl tRNA synthetases in reaction volumes from 2.5 to 10 ml. Reactions were incubated at 37°C for 20 min. at which time 0.1 volume of 20% potassium acetate, pH 5.5, was added. This mixture was immediately twice deproteinized with equal volumes of aqueous saturated phenol. The method of Marshall et al.<sup>14</sup> was used to pass the combined aqueous phases over a G-25 Sephadex column equilibrated with  $5 \times 10^{-4}$  M potassium cacodylate, pH 5.5. Fractions containing radioactive AA-tRNA were lyophilized and stored at -20°C.

Isotopes: From New England Nuclear were obtained 4, 5  $H^3$  DL-lysine, 2.95 c/ $\mu$ mole; U.L.  $C^{14}$  L-serine, 115  $\mu$ c/ $\mu$ mole; and UL  $C^{14}$  L-arginine



260  $\mu\text{c}/\mu\text{mole}$ . UL  $\text{H}^3$  L-serine 1.2  $\text{c}/\mu\text{mole}$  and UL  $\text{H}^3$  L-threonine 2.4  $\text{c}/\mu\text{mole}$  were obtained from Schwartz Biochemicals. Methyl  $\text{H}^3$  L-methionine, 1.36  $\text{c}/\mu\text{mole}$  and UL  $\text{H}^3$  40% D-arginine, 1.83  $\text{c}/\mu\text{mole}$  were obtained from Nuclear Chicago.

Column Fractionation of AA-tRNA: The reverse phase chromatographic column described by Kelmers<sup>23,52</sup> was used. A column (9 mm x 8 feet) was packed with one part organic phase (4% w/v dimethyldilaurylammonium chloride<sup>53</sup> in isoamyl acetate) thoroughly mixed with two parts (w/w) of hydrophobic diatomaceous earth<sup>54</sup>. The column was filled with a solution of 0.30 M sodium chloride; 0.01 M magnesium acetate; 0.01 M sodium acetate, pH 4.5; and 0.001 M sodium EDTA. The packing material was poured in slowly and allowed to settle. Elution gradients are described in figure legends. Ten ml fractions were collected every 4 min. and frozen after 0.05 ml aliquots were removed for radioactive counting. Samples were counted after 10% TCA precipitation, filtration on nitrocellulose filters, drying and placement in toluene-POPOP counting fluid. More simply samples would be counted directly in a naphthalene-dioxane counting solution<sup>55</sup>. Counting was in a Packard tricarb scintillation counter. Between 50 and 80% of  $\text{C}^{14}$ - or  $\text{H}^3$ -AA-tRNA added to the column was recovered as acylated tRNA.

Concentration and Desalting of tRNA Fractions: Two methods of desalting and concentrating were used. In the first, fractions were dialyzed under vacuum against  $5 \times 10^{-4}$  potassium cacodylate for 24 hours with two changes and lyophilized. An alternative preferred method resulting in less deacylation used a methylated albumin silicic acid column of high tRNA capacity.<sup>56</sup> Columns of 1-2 ml volume effectively retained acylated

The following table shows the results of the analysis of variance for the different treatments. The values in parentheses are the standard errors of the means.

Treatment	Mean	Standard Error
Control	1.23	0.05
Treatment A	1.45	0.06
Treatment B	1.38	0.04
Treatment C	1.52	0.07
Treatment D	1.41	0.05
Treatment E	1.35	0.06
Treatment F	1.48	0.04
Treatment G	1.39	0.05
Treatment H	1.43	0.06
Treatment I	1.37	0.05
Treatment J	1.46	0.04
Treatment K	1.34	0.06
Treatment L	1.49	0.05
Treatment M	1.36	0.07
Treatment N	1.44	0.04
Treatment O	1.38	0.06
Treatment P	1.42	0.05
Treatment Q	1.35	0.07
Treatment R	1.47	0.04
Treatment S	1.39	0.06
Treatment T	1.45	0.05
Treatment U	1.37	0.07
Treatment V	1.43	0.04
Treatment W	1.36	0.06
Treatment X	1.48	0.05
Treatment Y	1.38	0.07
Treatment Z	1.44	0.04

The results of the analysis of variance are shown in the following table. The values in parentheses are the standard errors of the means.

tRNA from many 10 ml fractions of these were diluted to a maximum sodium chloride concentration of 0.2 M. Elution with 1.5 M sodium chloride and 0.02 M sodium acetate, pH 5.2, gave small volumes which could be desalted on G-25 Sephadex columns as described in acylation procedures. These were then lyophilized and stored at -20°C.

Synthesis and Characterization of Trinucleotides; The synthesis, isolation, purity, and nucleotide sequence analyses of each trinucleotide used in these studies have been described<sup>6,57-60</sup>. A 2% contamination by UV absorbing material could be detected. No contaminants were seen.

Ribosomal Binding Assays: The binding assay has been described<sup>6</sup> as has the preparation of *E. coli* W3100 ribosomes<sup>50,61</sup>. Buffered ribosomes were added to oligonucleotide at 0°C and allowed to remain 4 min. before addition of radioactive aminoacyl tRNA and incubation at 24°C for 15 min. The tRNA addition yielded a final volume of 55 µl with 0.05 M tris-acetate, pH 7.2; 0.05 M potassium acetate; and 0.01-0.02 M magnesium acetate. Amounts of oligonucleotide, aminoacyl tRNA, and ribosomes are indicated in table legends. Fifteen ml of buffer, ionic concentrations equal to the reaction mixture, was used to wash the reaction mixture over nitrocellulose filters (HA Millipore filters .25 mm diameter, 0.45 µ pore size) which were subsequently dried and counted in toluene POPPOP in a Packard Tricarb Scintillation counter. Tritium was counted at an efficiency of 10% and C<sup>14</sup> at 80% efficiency. All assays were performed in duplicate.

## RESULTS

Arginine: Fractionation of *E. coli* B C<sup>14</sup>-ARG-tRNA yielded two peaks ARG-tRNA<sub>1</sub><sup>\*</sup>, representing about 90% of the total radioactivity and ARG-tRNA<sub>2</sub> as

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\* AA-tRNA<sub>1</sub> refers here to a radioactive tRNA fraction likely to represent a single tRNA species (often contaminated) although the possibility of multiple species within a peak is recognized.





shown in Figure 1. The template specificity of these fractions is shown in Table 4. ARG-tRNA<sub>1</sub> responded in the ribosomal binding assay to CGU > CGA > CGC whereas ARG-tRNA<sub>2</sub> responded only to CCG. Fractionation of guinea pig liver H<sup>3</sup>-ARG-tRNA shown in Figure 2 indicates five possible species, some only partially separated from one another. Successive column fractionations were necessary before fractions could be selected to demonstrate the five codon specific species of ARG-tRNA. Template activity of guinea pig fractions as shown in Table 5 is summarized: ARG-tRNA<sub>1</sub>, AGG > CGG\*; ARG-tRNA<sub>2</sub>, CCG > AGG; ARG-tRNA<sub>3</sub>, CGA > AGG > CGG; ARG-tRNA<sub>4</sub>, CGU > CGA > CGC; and ARG-tRNA<sub>5</sub>, CGA > CCG. Figure 3 illustrates a double label study of E. coli H<sup>3</sup>-ARG-tRNA acylated with guinea pig liver synthetase free of endogenous tRNA versus to homologously charged E. coli C<sup>14</sup>-ARG-tRNA. There appear to be only two species of ARG-tRNA in E. coli B.

#### Serine:

The radioactive profile of E. coli B C<sup>14</sup>-SER-tRNA (Figure 4) reveals four well separated peaks. SER-tRNA<sub>3</sub> and SER-tRNA<sub>4</sub> have not been reproducible and may represent an artifactual separation due to gradient disruption and will be referred to as SER-tRNA<sub>3-4</sub>. This is consistent with the similar coding responses found for these fractions. A minimum of three species of SER-tRNA are present in E. coli. Template activity of fractions as shown in Table 6 is summarized: SER-tRNA<sub>1</sub>, UCU > UCC; SER-tRNA<sub>2</sub>, AGU = AGC; and SER-tRNA<sub>3-4</sub>, UCA > UCG > UCU. Guinea pig liver H<sup>3</sup>-SER-tRNA fractionation (Fig. 5) revealed the first and third fractions to be relatively large and the second and fourth quite small with good separation of fractions. Responses were (Table 7) SER-tRNA<sub>1</sub>, AGU = AGC; SER-tRNA<sub>2</sub>, UCG; SER-tRNA<sub>3</sub>,

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\* Underlined responses are those felt likely to represent contamination from other fractions.





UCU > UCA > UCC; and SER-tRNA<sub>4</sub>, poly UC slightly only. Results for SER-tRNA<sub>4</sub> have been reproducible.

#### Methionine:

Guinea pig liver H<sup>3</sup>-MET-tRNA fractionated as shown in Figure 6 yields two fractions, the major one having been shown by Caskey et al.<sup>62</sup> to be formylated by E. coli transformylase and N<sup>5,10</sup>-methylene-H<sub>4</sub>-folate. The suggestion of another fraction on the trailing edge of MET-tRNA<sub>1</sub> has not been reproducible. Template activity for guinea pig MET-tRNA is shown in Table 8 and summarized: MET-tRNA<sub>1</sub>, AUG > GUG; and MET-tRNA<sub>2</sub>, AUG > GUG.

#### Threonine:

Guinea pig liver H<sup>3</sup>-THR-tRNA fractionation (Fig. 7) reveals two radioactive peaks which are not completely separated. The second has been divided into two fractions for binding assay studies to allow better definition of its responses. Template activity shown in Table 9 reveals: THR-tRNA<sub>1</sub>, ACU > ACC = ACA; THR-tRNA<sub>2</sub>, ACA > ACU > ACG > ACC; and THR-tRNA<sub>3</sub>, ACG > ACA ≧ ACU > ACC. THR-tRNA<sub>2-3</sub> is interpreted as a single tRNA species responding to ACG > ACA > ACU.

#### Lysine:

Guinea pig liver H<sup>3</sup>-LYS-tRNA fractionation (Fig. 8) yielded two well-separated fractions of relatively equal size. Data shown in Table 10 indicate that LYS-tRNA<sub>1</sub> responds to AAA > AAG and LYS-tRNA<sub>2</sub> to AAG only.

#### Leucine:

Fractionation of guinea pig liver H<sup>3</sup>-LEU-tRNA revealed a rapid moving large fraction and a small well-separated fraction as shown in Figure 9. The large fraction was divided and rerun (not shown) to yield two well-separated fractions. Results indicate the presence of at least three



LEU-tRNAs, but fractions were lost in preparation for binding assays and coding data are not available.

#### DISCUSSION:

The reverse phase chromatographic system described by Kelmers has been found effective in fractionating radioactive AA-tRNA from E. coli and guinea pig liver on a preparative scale. Separations comparable to those using countercurrent distribution and MAK column chromatography were obtained rapidly without the necessity for separate acylation procedures subsequent to fractionation. The relative amounts of tRNAs for a single amino acid can be estimated if similar deacylation rates are assumed for the tRNAs binding that amino acid.

All tRNAs were acylated with homologous synthetases excepting the heterologous double labeled ARG-tRNA study. The use of E. coli ribosomes in all binding studies has the advantage of uniformity of binding conditions since these ribosomes bind heterologous tRNAs with an efficiency similar to that for homologous tRNA. Specific tRNAs might not be acylated with radioactive amino acid or might be lost in preparation but fractionation has been reproducible.

Despite the universality of the genetic language in these organisms demonstrated by Marshall et al.<sup>14</sup>, there is striking variability in tRNA patterns. The number, relative amount, column migration, and codon recognition of tRNAs have been variable between species. All codons recognized by unfractionated AA-tRNA were recognized by one or more fractions after separation, and unfractionated data often represented a summation of template activity of fractionated tRNA species.



Table 11 shows the codon assignments for all fractions studied correcting for contamination where possible. Proposed anticodons are also shown. Results are consistent with Crick's "Wobble Hypothesis" in that no unexpected combinations, e.g. U = G are found. As proposed by Crick<sup>36</sup>, the 3'-base degeneracy U = C = A = G appears never to occur for a single tRNA species although common for a single amino acid. Inosine in the 5'-position of the proposed anticodon site is quite common in guinea pig liver tRNA, occurring in the tRNAs for arginine, serine, and threonine of the five amino acids studied. The 5'- position is also frequently occupied by C in the anticodon, resulting in a G specific tRNA accompanying a tRNA with a U = C = A degeneracy. The 5'- position of the anticodon appears to always have significant effect on template activity.

Comparison of data available for ARG-tRNA<sub>1</sub>, SER-tRNA, and MET-tRNA in E. coli and guinea pig liver is revealing. E. coli ARG-tRNA<sub>2</sub> and guinea pig liver ARG-tRNA<sub>2</sub> both code for CGG and would be expected to have the same anticodon but the percentage of ARG-tRNA<sub>2</sub> in guinea pig is significantly higher than ARG-tRNA<sub>2</sub> in E. coli. This is an example of alteration in amount of a tRNA responding to a particular codon, and correlates with the higher relative template activity of CGG found by Marshall et al.<sup>14</sup> in guinea pig liver, (See Table 2).

Guinea pig liver ARG-tRNA<sub>1</sub> with codon responses AGG > CGG represents a tRNA not detectable in E. coli. This new tRNA adequately explains the finding by Marshall et al.<sup>14</sup> (Table 2) of an absolute difference in template activity of AGG in E. coli versus guinea pig.

Also found were multiple tRNAs responding to a single codon or codon group. Guinea pig liver ARG-tRNA<sub>3</sub> and ARG-tRNA<sub>5</sub> both respond to CGA > CGG. Possible explanation for multiple tRNAs with similar codon





responses are either structural differences in the tRNAs in other than the anticodon site or conformational changes, both of which might occur naturally or be artifactually induced in preparation or fractionation.

Alterations in codon groupings are also demonstrated with SER-tRNAs representing the best example. E. coli and guinea pig liver both have major fractions, SER-tRNA<sub>2</sub> and SER-tRNA<sub>1</sub>, respectively, which respond to AGU = AGC. The UCX series of codons, however, are divided differently in E. coli and guinea pig liver. In the former SER-tRNA<sub>1</sub> responds to UCU > UCC and SER-tRNA<sub>3-4</sub> to UCA > UCG > UCU. In the mammalian system, however, SER-tRNA<sub>2</sub> responds to UCG alone while SER-tRNA<sub>3</sub> responds to UCU > UCA > UCC. These findings could represent examples of mutations in the anticodon site of tRNAs. The finding of two such mutations together should not be surprising since the first might leave one codon unreadable and functioning as a chain terminator giving great evolutionary pressure for a second compensating mutation to make all codons readable. Alternative explanations would be a less specific structural or conformational change altering codon response, or the acceptance of serine by a tRNA which formerly was absent or not acylated with serine. In this regard Peterkofsky<sup>63,64</sup> has shown that methyl deficient LEU-tRNA has different coding responses than the usual tRNA in E. coli.

Using these methods of fractionation Caskey et al.<sup>62</sup> have reported the formation of N-formyl-MET-tRNA<sub>1</sub> but MET-tRNA<sub>2</sub> could not be formylated in the presence of a purified E. coli transformylase. Caskey<sup>65</sup> has demonstrated markedly stimulated puromycin dependent deacylation of MET-tRNA, bound to E. coli ribosomes in response to AUG at 0.01 M Mg<sup>++</sup> while deacylation of MET-tRNA<sub>2</sub> is not stimulated. This is evidence for structural differences between the two MET-tRNAs and evidence that MET-tRNA<sub>1</sub> preferentially enters



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the initiator site. Although Noll<sup>66</sup> has reported finding N-formyl-MET-tRNA in rabbit reticulocytes, attempts to demonstrate its presence in guinea pig liver<sup>67</sup>, rat liver<sup>68</sup>, and oviduct extracts<sup>68</sup> have been unsuccessful. The preferential puromycin deacylation and the ability to accept formyl groups suggest that guinea pig liver MET-tRNA<sub>1</sub> may be similar to the formylatable MET-tRNA<sub>F</sub> which has been demonstrated to function as a chain initiator in E. coli<sup>25</sup>, although codon responses are slightly different. In vitro chain initiation in E. coli has been demonstrated to be independent of formylation of the MET-tRNA<sup>69</sup> leaving the role of MET-tRNA as a chain initiator in mammals an open question despite difficulties in demonstrating N-formyl-MET-tRNA.

Finding that E. coli SER-tRNA<sub>1</sub>, the largest fraction, responds to AGU = AGC is of interest since data from unfractionated material (Table 6) indicate only a questionable response to these codons. This may be intimately involved with the cysteine and serine ambiguity that exists for these codons. A competitive effect is likely with either two tRNAs binding to the same codon or the two amino acids binding to a single tRNA. Since the mammalian serine response is increased over E. coli while the cysteine response is less, a single tRNA is unlikely. That the column fractionation effectively eliminates the proposed competition supports the idea of a CYS-tRNA and a SER-tRNA responding to the same codons. The lower response of guinea pig liver CYS-tRNA to AGU and AGC<sup>14</sup> might reflect a lesser amount of a particular CYS-tRNA thus allowing the detection of the response in unfractionated guinea pig liver SER-tRNA to AGU and AGC. Interconversion and contamination as explanations for the ambiguity have been disproven by Marshall et al.<sup>14</sup>



In mammalian tissue there is always an equal or greater number of tRNAs for a particular amino acid than are present in E. coli. Since protein synthesis can occur in rather compartmentalized systems such as the nucleus or mitochondria in higher organisms, the possibility of tRNA variation between cellular compartments exists. The complexity of mammalian tRNAs might indicate the presence of a number of special function tRNAs. The variability of tRNA content is striking but its significance is unresolved. One hypothesis is that the availability of a tRNA species may limit the rate of mRNA translation. Hence species of mRNA differing in codon content may be translated at different rates.

I thank Dr. Marshall Nirenberg for encouragement and advice as well as the opportunity to perform this work; Dr. C. Thomas Caskey for daily assistance and valuable criticism of the manuscript; and Dr. Louis Gluck for support and advice throughout. Thanks are due to Theresa Caryk, Norma Zabriskie, and Anne Baur for expert technical assistance.



TABLE 1

NUCLEOTIDE SEQUENCES OF RNA CODONS FOR E. COLI tRNA

UUU PHE	UCU SER	UAU TYR	UGU CYS
UUC PHE	UCC SER	UAC TYR	UGC CYS
UUA <u>LEU</u>	UCA SER	UAA (OCHRE TERM?)	UGA <u>CYS</u>
UUG LEU (F-MET)	UCG SER	UAG (AMBER TERM?)	UGG TRP
CUU LEU	CCU PRO	CAU HIS	CGU ARG
CUC LEU	CCC PRO	CAC HIS	CGC ARG
CUA LEU	CCA PRO	CAA GLN	CGA ARG
CUG LEU (F-MET?)	CCG PRO	CAG GLN	CGG ARG
AUU ILE	ACU THR	AAU ASN	AGU SER (CYS)
AUC ILE	ACC THR	AAC ASN	AGC SER (CYS)
AUA <u>ILE</u>	ACA THR	AAA LYS	AGA <u>ARG</u>
AUG MET (F-MET)	ACG THR	AAG LYS	AGG <u>ARG</u>
GUU VAL	GCU ALA	GAU ASP	GGU GLY
GUC VAL	GCC ALA	GAC ASP	GGC GLY
GUA VAL	GCA ALA	GAA GLU	GGA GLY
GUG VAL (F-MET?)	GCG ALA	GAG GLU	GGG GLY

TABLE 1. Nucleotide sequences of RNA codons as determined by stimulating binding of E. coli AA-tRNA to E. coli ribosomes with trinucleotide templates. Parentheses indicate additional codon function. Question marks(?) indicate uncertain codon function. F-MET represents N-formyl- MET-tRNA functioning as chain initiator if that codon is in the initial position, while the codon will code for the amino acid listed first if in an internal position. TERM represents a terminator codon. Underlined responses may occur in certain species of E. coli, but not all. Data from Nirenberg et al<sup>13,58</sup>, Brimacombe et al<sup>59</sup>, and Khorana et al<sup>9</sup>.





TABLE 2

SPECIES DEPENDENT DIFFERENCES IN RESPONSE OF AA-tRNA TO TRINUCLEOTIDE

CODONS

AMINO ACID	CODON	tRNA	
		<u>E. COLI</u>	<u>G. PIG LIVER</u>
ARG	AGG	±	++++
	CGG	±	++++
MET	UUG	++	±
ALA	GCG	++++	++
ILE	AUA	±	++
LYS	AAG	±	++++
SER	UCG	++++	++
	AGU	±	+++
	AGC	+	+++
CYS	UGA	±	+++

TABLE 2. The following scale indicates the approximate response of AA-tRNA to a trinucleotide relative to the responses of the same AA-tRNA preparation to every other trinucleotide for that amino acid.

++++	70-100%
+++	50-70%
++	20-50%
±	0-20%

Adapted from Marshall et al <sup>14</sup>.



TABLE 3

ALTERNATE BASE PAIRING OF CRICK "WOBBLE" HYPOTHESIS

tRNA ANTICODON	mRNA CODON
U	A G
C	G
A	U
G	C U
I	U C A
rT	A G
$\Psi$	A G (U)
DHU	(U) (C)

TABLE 3. The base in a tRNA anticodon shown in the left-hand column forms antiparallel hydrogen bonds with the base(s) shown in the right hand column, which usually occupy the third position of degenerate mRNA codons. Relationships for U, C, A, G, and I of anticodons are "wobble" hydrogen bonds suggested by Crick<sup>36</sup>. Adapted from Nirenberg et al<sup>13</sup>.



TABLE 4

TEMPLATE SPECIFICITY OF TRINUCLEOTIDE FOR FRACTIONATED E. COLI C<sup>14</sup>-ARG-tRNA

<u>TRINUCLEOTIDE</u>	<u>Δ uuMOLES C<sup>14</sup>-ARG-tRNA BOUND TO RIBOSOMES</u>		
	Unfractionated*	F-1	F-2
CGU	0.64	0.65	0.03
CGC	0.05	0.08	0.03
CGA	0.26	0.25	0.01
CGG	0.11	-0.06	0.91
AGA	0.01	-0.01	
AGG	0.04	-0.03	-0.01
None (uuMole)	(0.43)	(0.21)	(0.10)

TABLE 4. The effect of trinucleotide upon the binding of E. coli C<sup>14</sup>-ARG-tRNA fractions obtained by fractionation on the Kelmers column<sup>23,52</sup> to E. coli ribosomes. Reactions, carried out as described in methods, contained 0.04 and 0.52 A<sup>260</sup> units tRNA and 1.0 and 2.0 A<sup>260</sup> units ribosomes for fractions 1 and 2, respectively. Reactions carried out in 0.01 M Mg<sup>++</sup>. Unfractionated data from Marshall et al<sup>14</sup>, performed in 0.01 M Mg<sup>++</sup>.



TABLE 5

TEMPLATE SPECIFICITY OF TRINUCLEOTIDES FOR FRACTIONATED G. PIG C<sup>14</sup>

ARG-tRNA

<u>TRINUCLEOTIDE</u>	<u>uuMOLES C<sup>14</sup>-ARG-tRNA BOUND TO RIBOSOMES</u>					
	Unfractionated*	F-1	F-2	F-3	F-4	F-5
CGU	0.81	0.01	0.09	0.03	0.43	0.09
CGC	0.67	0.00	0.07	0.03	0.23	0.03
CGA	1.28	0.01	0.07	0.67	0.38	1.00
CGG	0.97	0.29	1.66	0.21	0.02	0.24
AGA	0.12	0.01	0.03	0.00	0.05	0.04
AGG	0.63	1.68	0.47	0.48	0.05	0.04
None (uuMole)	(1.23)	(0.12)	(0.29)	(0.14)	(0.44)	(0.29)

TABLE 5. The effect of trinucleotide upon the binding of guinea pig liver C<sup>14</sup>-ARG-tRNA fractions obtained by fractionation on the Kelmers column<sup>23,52</sup> to *E. coli* ribosomes. Reactions, carried out as described in methods, contained 0.03, 0.08, 0.31, 0.05, and 0.03 A<sup>260</sup> units tRNA and 1.5, 1.5, 2.0, 1.5, and 1.5 A<sup>260</sup> units ribosomes for fractions 1, 2, 3, 4, and 5, respectively. Reactions performed in 0.01 M Mg<sup>++</sup>. Unfractionated data from Marshall et al<sup>14</sup>, performed at 0.02 M Mg<sup>++</sup>.





TABLE 6

TEMPLATE SPECIFICITY OF TRINUCLEOTIDES FOR FRACTIONATED *E. COLI* C<sup>14</sup>-

SER-tRNA

TRINUCLEOTIDE	$\Delta$ uuMOLES C <sup>14</sup> -SER-tRNA BOUND TO RIBOSOMES				
	Unfractionated*	F-1	F-2	F-3	F-4
UCU	1.27	2.10	0.04	0.37	0.69
UCC	0.54	1.47	-0.03	0.03	-0.18
UCA	1.56	0.08	0.05	1.96	2.10
UCG	1.09	0.16	-0.05	1.03	1.82
AGU	0.21	0.06	2.54	0.02	0.04
AGC	0.26	0.14	2.75	0.03	0.01
None (uuMole)	(0.43)	(2.97)	(0.56)	(1.32)	(1.64)

TABLE 6. The effect of trinucleotide upon the binding of *E. coli* C<sup>14</sup>-SER-tRNA fractions obtained by fractionation on the Kelmers column<sup>23,52</sup> to *E. coli* ribosomes. Reactions, carried out as described in methods, contained 0.10, 0.08, 0.08, and 0.09 A<sup>260</sup> units tRNA and for fractions 1, 2, 3, and 4, respectively. All reactions contained 2.0 A<sup>260</sup> units of ribosomes and were performed at 0.01 M Mg<sup>++</sup>. Unfractionated data from Marshall et al<sup>14</sup>, performed at 0.01 M Mg<sup>++</sup>.



TABLE 7

TEMPLATE SPECIFICITY OF TRI- OR POLYNUCLEOTIDES FOR FRACTIONATED G. PIG

H<sup>3</sup>-SER-tRNA

TRINUCLEOTIDE	<u>Δ uuMOLES H<sup>3</sup>-SER-tRNA BOUND TO RIBOSOMES</u>					
	Unfractionated*	F-1	F-2	F-3	F-3'	F-4
UCU	1.21	0.06	-0.04	0.42	3.38	-0.08
UCC	0.13	0.04	-0.06	-0.01	0.94	-0.09
UCA	0.77	0.03	-0.06	0.12	1.95	-0.08
UCG	0.50	0.04	0.73	0.03	0.09	-0.06
AGU	0.77	1.10	0.03	0.05	0.03	-0.04
AGC	0.83	1.06	0.02	0.01	-0.18	-0.08
Poly UC						0.11
None (uuMole)	(0.68)	(0.29)	(0.13)	(0.34)	(1.38)	(0.18)

TABLE 7. The effect of trinucleotide upon the binding of guinea pig liver H<sup>3</sup>-SER-tRNA fractions obtained by fractionation on the Kelmers column<sup>23,52</sup> to *E. coli* ribosomes. Reactions, carried out as described in methods, contained 0.054, 0.056, 0.030, 0.098, and 0.021 A<sup>260</sup> units tRNA for fractions 1, 2, 3, 3', and 4, respectively. Reactions contained 2.0 A<sup>260</sup> units ribosomes and were performed in 0.01 M Mg<sup>++</sup> except fraction 3' which represents fraction 3 repeated at 0.02 M Mg<sup>++</sup> to demonstrate the U = A = C degeneracy. Unfractionated data from Marshall et al<sup>14</sup>, performed at 0.02 M Mg<sup>++</sup>.



TABLE 8

TEMPLATE SPECIFICITY OF TRINUCLEOTIDES FOR FRACTIONATED G. PIG H<sup>3</sup>-

MET-tRNA

<u>TRINUCLEOTIDE</u>	<u>Δ uuMOLES H<sup>3</sup>-MET-tRNA BOUND TO RIBOSOMES</u>		
	Unfractionated*	F-1	F-2
UUG	0.17	0.12	0.07
CUG	0.08	0.01	0.02
AUG	1.67	3.47	4.65
GUG	0.47	0.51	0.27
None (uuMole)	(0.15)	(0.31)	(0.19)

TABLE 8. The effect of trinucleotide upon the binding of guinea pig liver H<sup>3</sup>-MET-tRNA fractions obtained by fractionation on the Kelmers column<sup>23,52</sup> to *E. coli* ribosomes. Reactions, carried out as described in methods, contained 0.02 and 0.03 A<sup>260</sup> units tRNA for fractions 1 and 2, respectively. Reactions contained 1.0 A<sup>260</sup> units of ribosomes and were performed at 0.01 M Mg<sup>++</sup>. Unfractionated data from Marshall et al.<sup>14</sup>, performed at 0.02 M Mg<sup>++</sup>.





TABLE 9

TEMPLATE SPECIFICITY OF TRINUCLEOTIDES FOR FRACTIONATED G. PIG H<sup>3</sup> -

THR-tRNA

<u>TRINUCLEOTIDE</u>	<u>Δ uuMOLES H<sup>3</sup>-THR-tRNA BOUND TO RIBOSOMES</u>			
	Unfractionated*	F-1	F-2	F-3
ACU	1.33	1.53	0.63	0.44
ACC	0.98	0.78	0.20	0.17
ACA	0.94	0.73	0.83	0.55
ACG	0.46	0.02	0.47	0.75
None (uuMole)	(0.37)	(0.15)	(0.02)	(0.10)

TABLE 9. The effect of trinucleotide upon the binding of guinea pig liver H<sup>3</sup>-THR-tRNA fractions obtained by fractionation on the Kelmers column<sup>23,52</sup> to E. coli<sup>11</sup> ribosomes. Reactions, carried out as described in methods, contained 0.096, 0.160, and 0.130 A<sup>260</sup> units tRNA for fractions 1, 2, and 3, respectively. Reactions contained 2.0 A<sup>260</sup> units of ribosomes and were performed at 0.01 M Mg<sup>++</sup>. Unfractionated data from Marshall et al<sup>14</sup>, performed at 0.02 M Mg<sup>++</sup>.



TABLE 10

TEMPLATE SPECIFICITY OF TRINUCLEOTIDES FOR FRACTIONATED G. PIG H<sup>3</sup>-

LYS-tRNA

<u>TRINUCLEOTIDE</u>	<u>Δ uuMOLES H<sup>3</sup>-LYS-tRNA BOUND TO RIBOSOMES</u>		
	Unfractionated*	F-1	F-2
AAA	0.33	1.62	-0.05
AAG	0.50	0.30	0.82
None (uuMole)	(0.69)	(0.72)	(0.27)

TABLE 10. The effect of trinucleotide upon the binding of guinea pig liver H<sup>3</sup>-LYS-tRNA fractions obtained by fractionation on the Kelmers column<sup>23,52</sup> to E. coli ribosomes. Reactions, carried out as described in methods, contained 0.02 A<sup>260</sup> units of tRNA and 0.25 A<sup>260</sup> units ribosomes and were performed at 0.01 M Mg<sup>++</sup>. Unfractionated data from Marshall et al<sup>14</sup>; performed at 0.01 M Mg<sup>++</sup>.



TABLE 11

## PATTERNS OF 3' TRINUCLEOTIDE DEGENERACY

Species and AA	Unfractionated Codon Pattern	3' Degenerate Codons for AA-tRNA Fractions (Proposed Anticodon in Parentheses)				
		U>C	U>A>C	A>G	A>G>U	G
<i>E. coli</i> ARG-tRNA	CGU, CGC, CGA, CGG		1(ICG)			2(CCG)
Guinea Pig ARG-tRNA	CGU, CGC, CGA, CGG AGG		4(ICG)	3,5(UCG)		2(CCG) 1(CCU)
Guinea Pig LYS-tRNA	AAA, AAG			1(UUU)		2(CCU)
Guinea Pig MET-tRNA	*AUG, GUG			1,2(CAU)		
<i>E. coli</i> SER-tRNA	UCU, UCC, UCA, UCG AGU, AGC	1(GGA) 2(GCU)			3,4( $\sqrt{GA}$ )	
Guinea Pig SER-tRNA	UCU, UCC, UCA, UCG AGU, AGC		3(IGA) 1(GCU)			2(CGA)
Guinea Pig THR-tRNA	ACU, ACC, ACA, ACG		1(IGU)		2( $\sqrt{GU}$ )?	

TABLE 11. Patterns of 3' trinucleoside diphosphate degeneracy for AA-tRNA fractions as determined from data of Tables 4-10.

\* MET-tRNA fractions are of the 5' degenerate type, A > G in the 5' base position. ? The pattern of degeneracy for THR-tRNA<sub>2</sub> cannot be resolved between A > G and A > G > U with the present data, although A > G > U is preferred. The anticodon in parentheses (XXX) is that expected according to the Crick "wobble" hypothesis<sup>36</sup>.



FIGURE 1

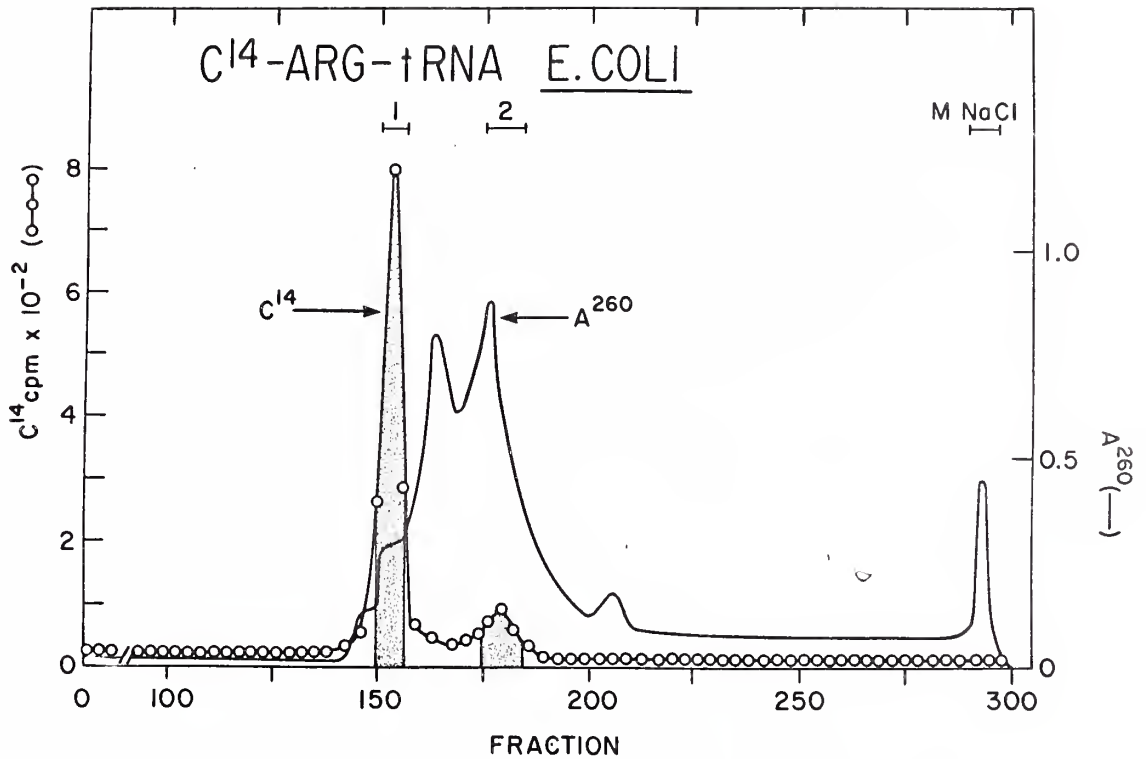


FIGURE 1. Radioactive profile (o—o) and U.V. absorbancy (—) for fractionation of *E. coli* C<sup>14</sup>-ARG-tRNA on Kelmers column<sup>23,52</sup>. Tubes 150-156 and 177-183 were combined for fractions 1 and 2 respectively as shown by shaded areas. Radioactivity is as TCA precipitable counts. A 3000 ml sodium chloride gradient from 0.3M to 0.75M was used.





FIGURE 2

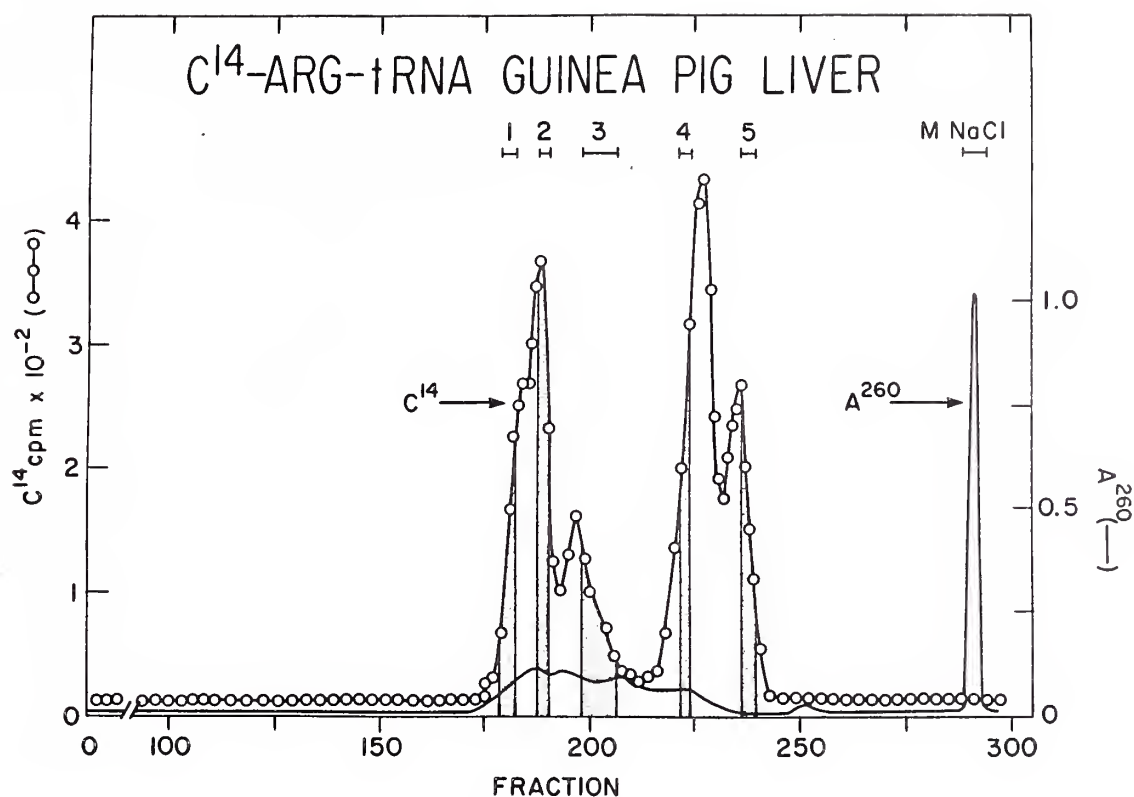


FIGURE 2: Radioactive profile (o—o) and U.V. absorbancy (—) for fractionation of guinea pig liver  $C^{14}$ -ARG-tRNA on Kelmers column<sup>23,52</sup>. Tubes 179-182, 188-190, 198-206, 222-224, and 236-239 were combined for fractions 1, 2, 3, 4, and 5, respectively as shown by shaded areas. Radioactivity was measured directly in naphthalene-dioxane counting solution<sup>55</sup>. A 3000 ml sodium chloride gradient from 0.3M to 0.65M was used.



FIGURE 3

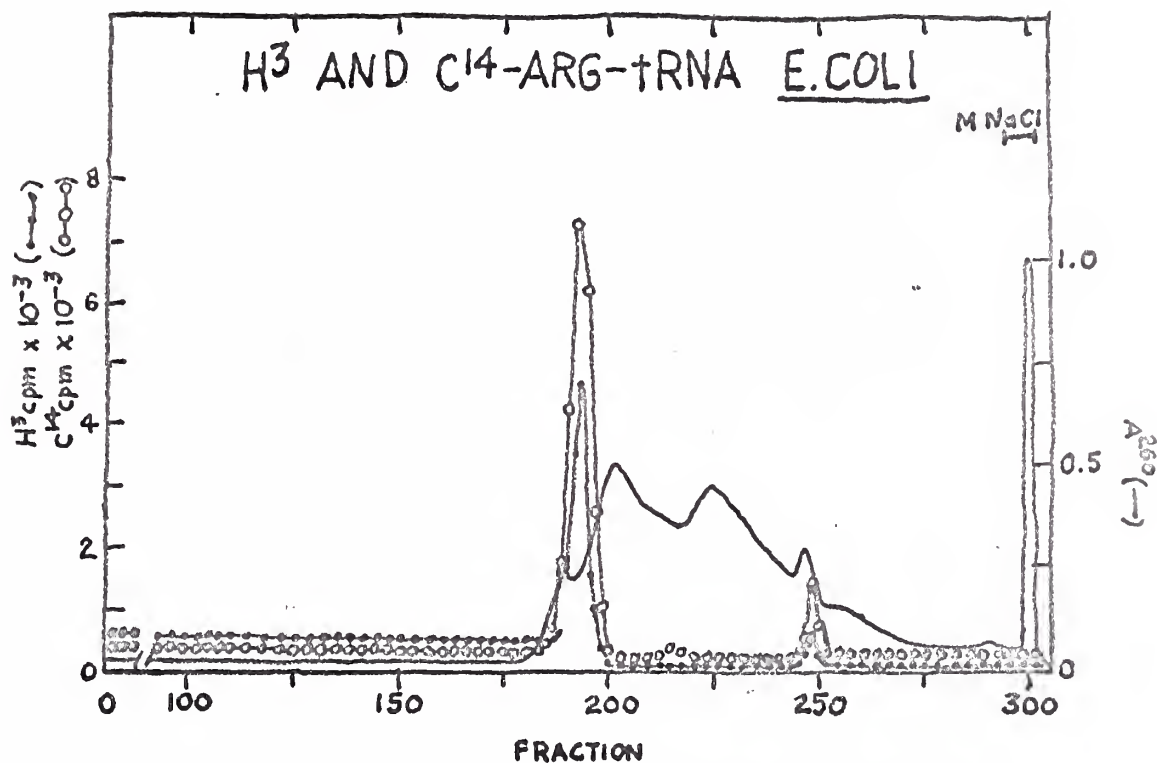


FIGURE 3. Radioactive profile of *E. coli* C<sup>14</sup>-ARG-tRNA charged with homologous aminoacyl synthetase (o—o) is compared with the radioactive profile of *E. coli* H<sup>3</sup>-ARG-tRNA charged with guinea pig liver aminoacyl synthetase (•—•—•). U.V. absorbancy (—). Radioactivity was measured as TCA precipitable counts. A sodium chloride gradient from 0.3M to 0.75M in 3000 ml was used.



FIGURE 4

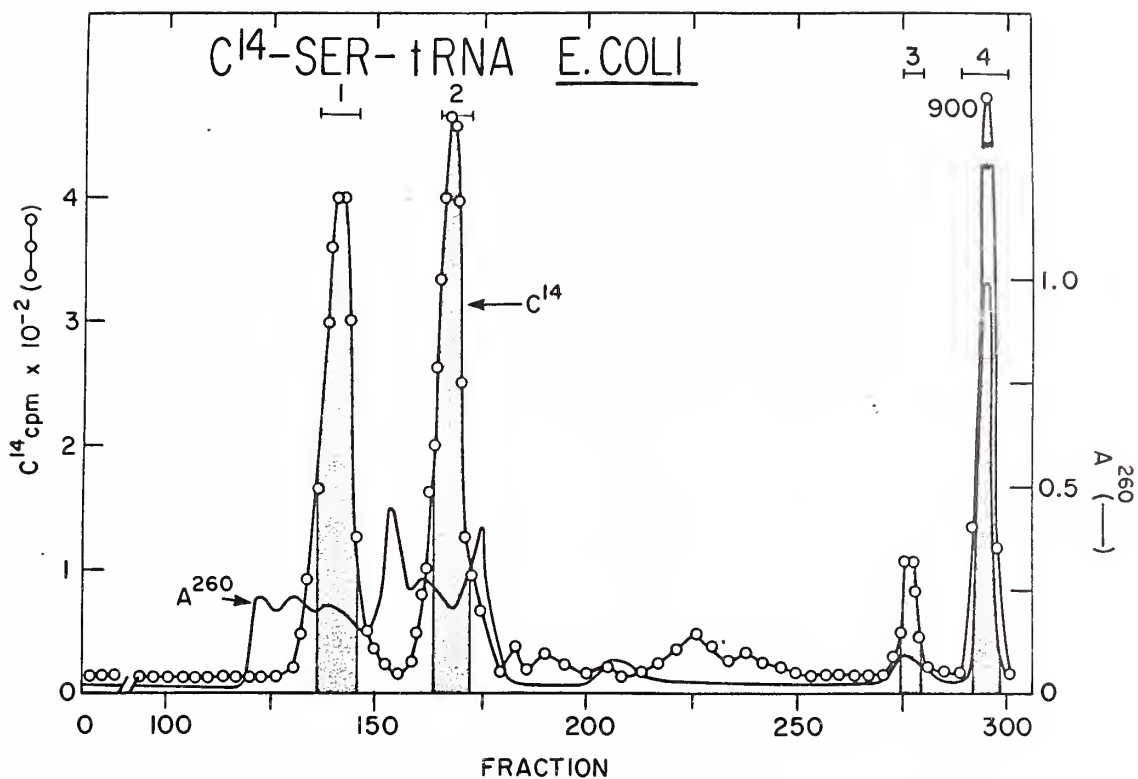


FIGURE 4. Radioactive profile (o—o) and U.V. absorbancy (—) for fractionation of *E. coli* C<sup>14</sup>-SER-tRNA on Kelmers column<sup>23,52</sup>. Tubes 137-143, 164-171, 277-283, and 296 were combined for fractions 1, 2, 3, and 4, respectively as shown by shaded areas. Radioactivity was measured as TCA precipitable counts. A 3000 ml sodium chloride gradient from 0.3M to 0.75M was used.



FIGURE 5

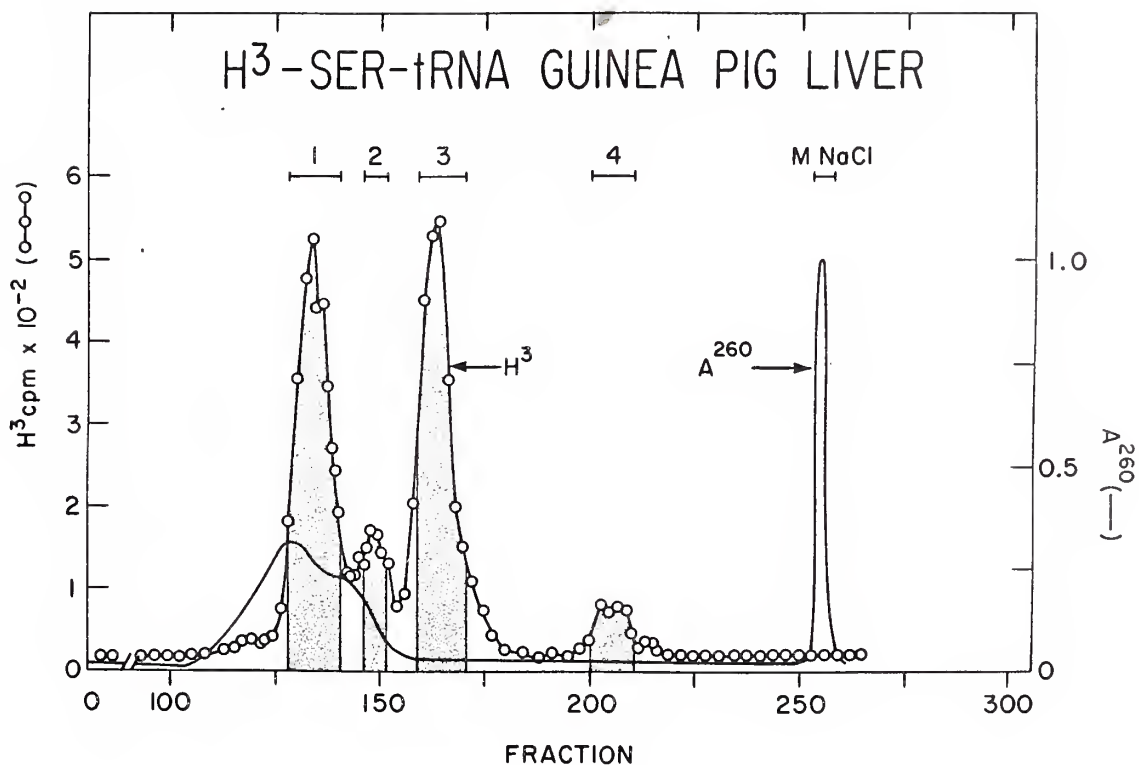


FIGURE 5. Radioactive profile (o—o) and U.V. absorbancy (—) for fractionation of guinea pig liver H<sup>3</sup>-SER-tRNA on Kelmers column<sup>23,52</sup>. Tubes 155-157, 166-169, 179-181, and 208-211 were combined for fractions 1, 2, 3, and 4, respectively as shown by shaded areas. Radioactivity was measured directly in naphthalene-dioxane counting solution<sup>55</sup>. A 3000 ml sodium chloride gradient from 0.3M to 0.75M was used.





FIGURE 6

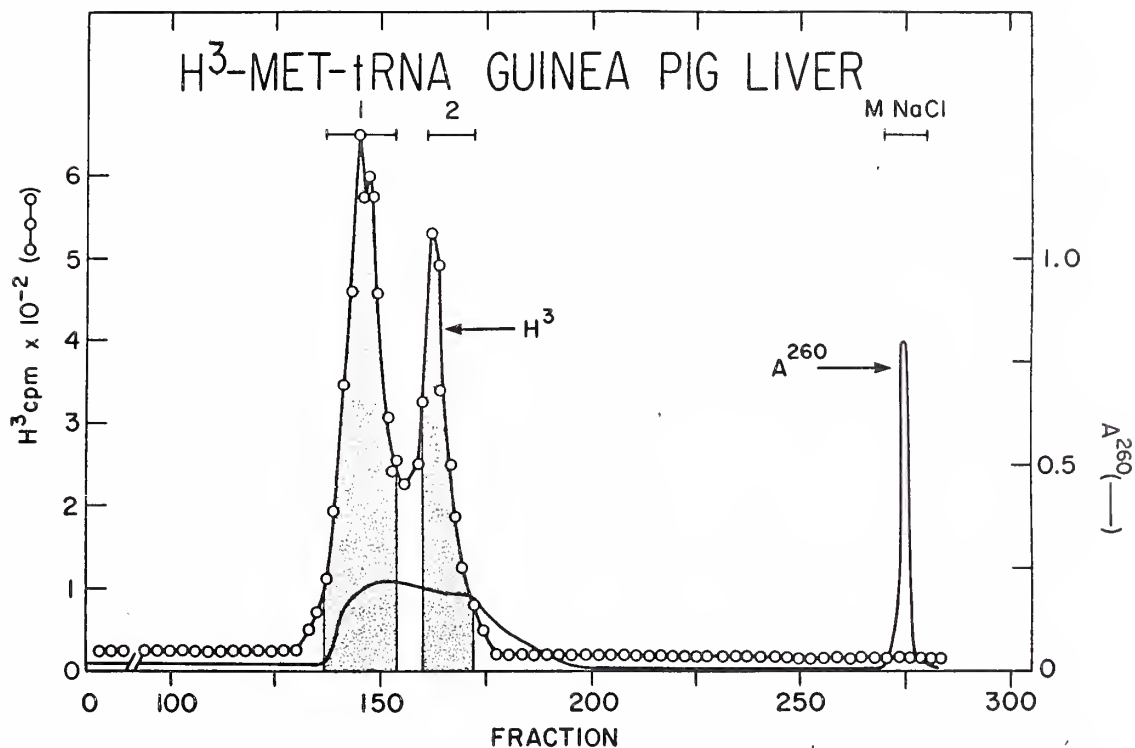


FIGURE 6. Radioactive profile (o—o) and U.V. absorbancy (—) for fractionation of guinea pig liver  $H^3$ -MET-tRNA on Kelmers column<sup>23,52</sup>. Tubes 196 and 214-215 went to make up fractions 1 and 2, respectively as shown by shaded areas. Radioactivity was measured directly in naphthalene-dioxane counting solution<sup>55</sup>. A 3000 ml sodium chloride gradient from 0.3M to 0.65M was used.



FIGURE 7

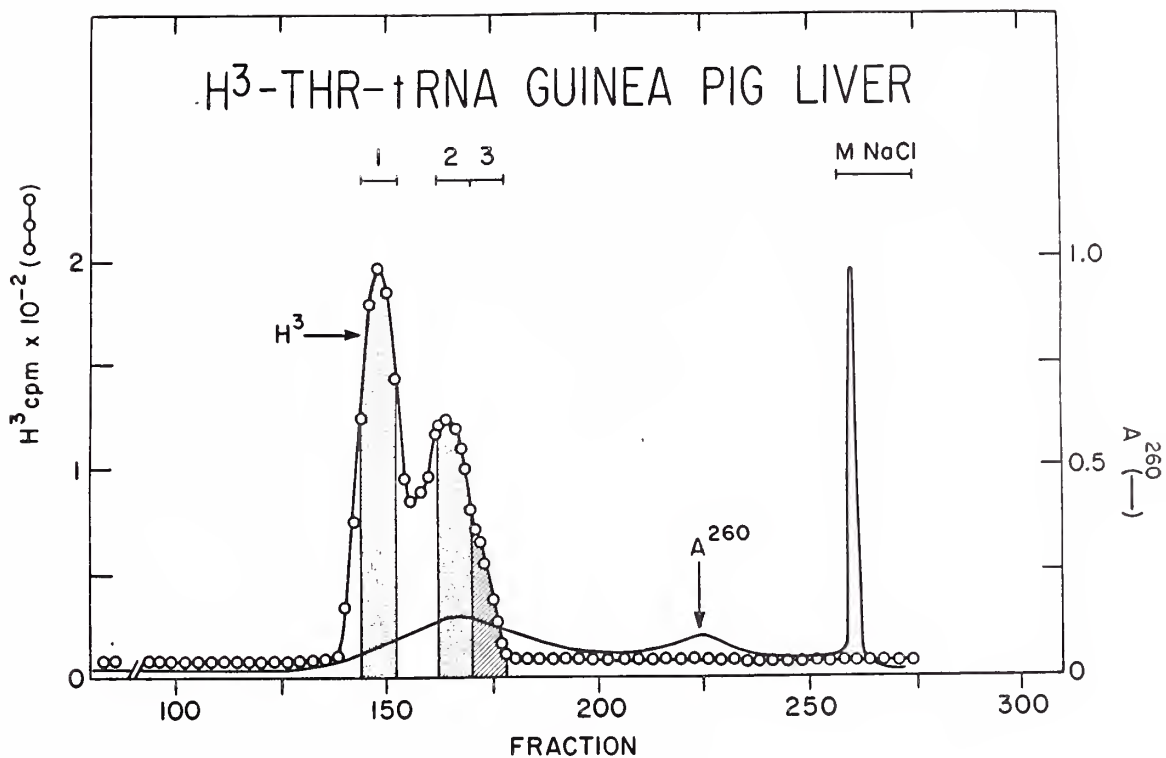


FIGURE 7. Radioactive profile (O—O) and U.V. absorbancy (—) for fractionation of guinea pig liver H<sup>3</sup>-THR-tRNA on Kelmers column<sup>23,52</sup>. Tubes 144-153, 161-169, and 170-173 were combined for fractions 1, 2, and 3, respectively as shown by shaded areas. Radioactivity was measured directly in naphthalene-dioxane counting solution<sup>55</sup>. A 3000 ml sodium chloride gradient from 0.4M to 0.65M was used.



FIGURE 8

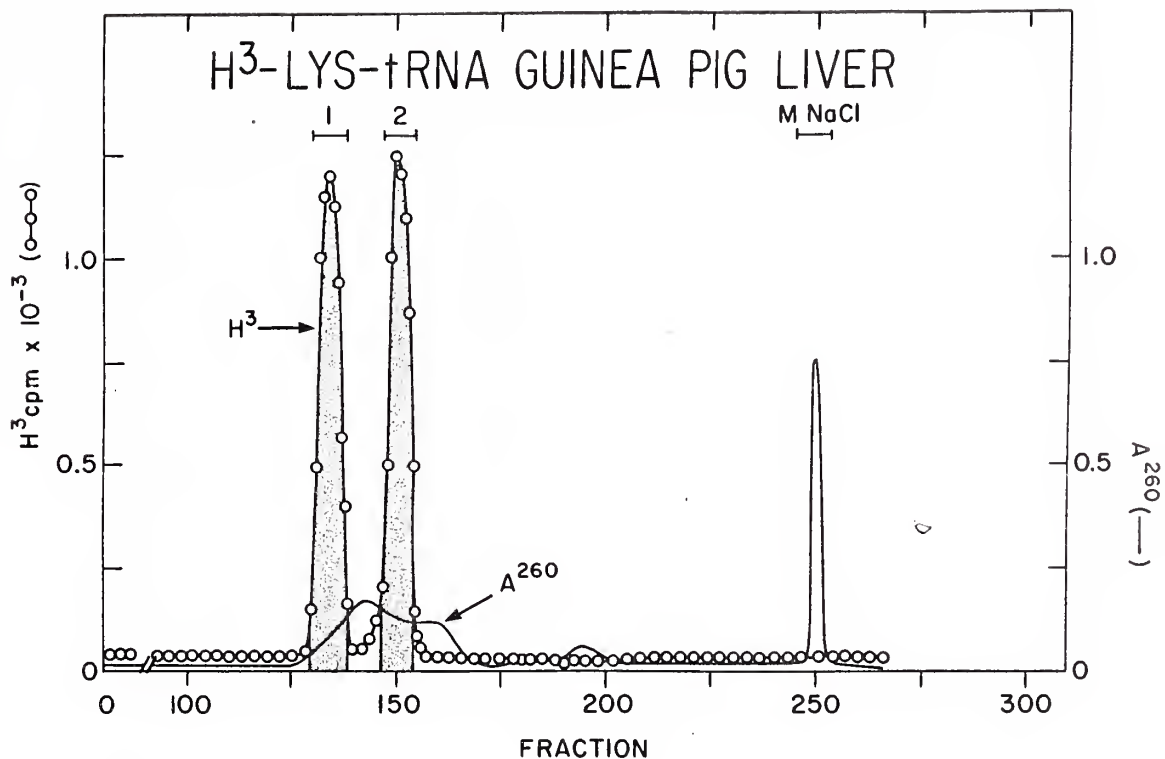


FIGURE 8: Radioactive profile (o—o) and U.V. absorbancy (—) for fractionation of guinea pig liver  $H^3$ -LYS-tRNA on Kelmers column<sup>23,52</sup>. Tubes 132-139 and 148-155 were combined for fractions 1 and 2, respectively, as shown by shaded areas. Radioactivity was counted directly in naphthalene-dioxane counting solution<sup>55</sup>. A 3000 ml sodium chloride gradient from 0.35M to 0.7M was used.



FIGURE 9

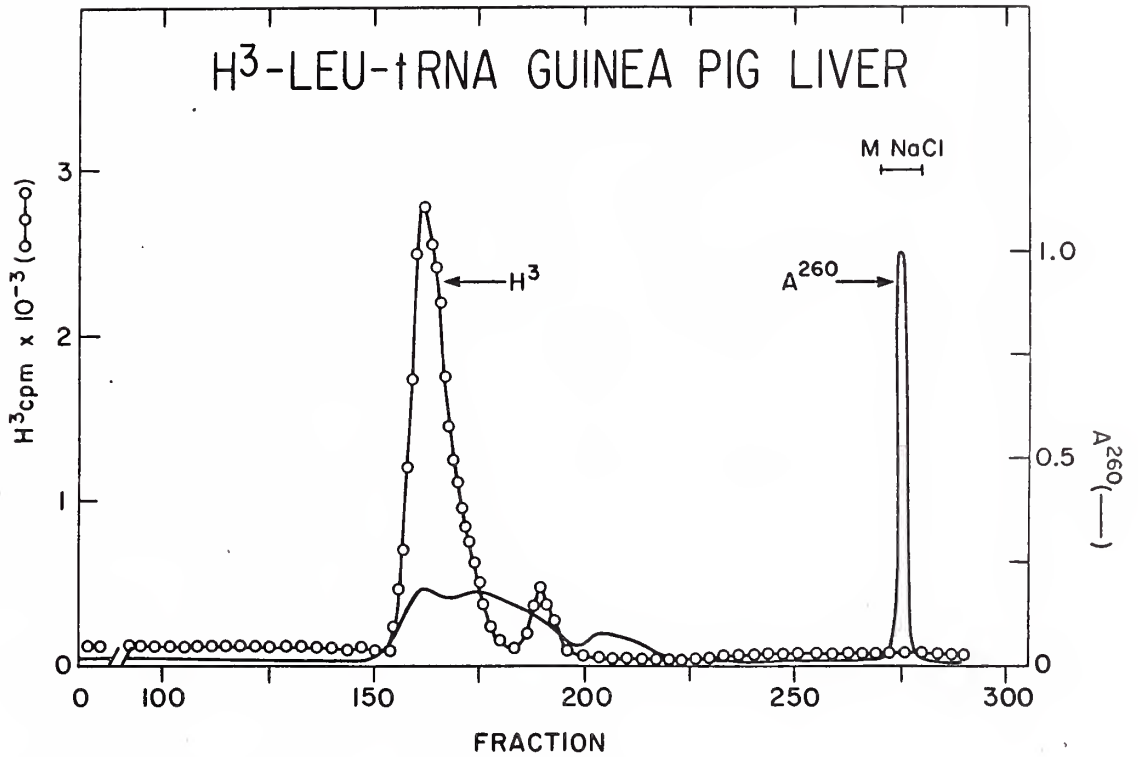


FIGURE 9. Radioactive profile (o---o) and U.V. absorbancy (---) for fractionation of guinea pig liver  $H^3$ -LEU-tRNA on Kelmers column<sup>23,52</sup>. The larger peak was rerun revealing two peaks but results are not shown here. A 3000 ml sodium chloride gradient from 0.35M to 0.7M was used.





## BIBLIOGRAPHY

1. G. Gamow, Nature 173, 318 (1954).
2. F. H. C. Crick, Brookhaven Sympos. Biol. 12, 35 (1959).
3. The following abbreviations and symbols are used: ALA, alanine; ARG, arginine; ASN, asparagine; ASP, aspartic acid; CYS, cysteine; GLN, glutamine; GLU, glutamic acid; GLY, glycine; HIS, histidine; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; PHE, phenylalanine; PRO, proline; SER, serine; TRP, tryptophane; THR, threonine; TYR, tyrosine; VAL, valine; F-MET, N-formyl methionine; tRNA, transfer RNA; AA-tRNA, aminoacyl-tRNA; mRNA, messenger RNA; U, uracil; C, cytidine; A, adenine; G, guanine; I, inosine; rT, ribothymidine;  $\psi$ , pseudouracil; DHU, dihydrouracil; ATP, adenosine triphosphate; trinucleotide refers to trinucleoside diphosphate; ACU or ApCpU represents a trinucleoside diphosphate with 5'-terminal hydroxyl attached to A and 2',3'-terminal hydroxyls attached to U; codon, codeword; poly UC, randomly ordered copolymer of uridylic and cytidylic acid;  $A^{260}$ , absorbancy at 260 m $\mu$ ; DEAE, O-diethylaminoethyl-cellulose; MAK, methylated albumin kieselguhr; MET-tRNA<sub>M</sub>, non-formylatable E. coli MET-tRNA; MET-tRNA<sub>F</sub>, formylatable E. coli MET-tRNA.
4. F. H. C. Crick, L. Barnett, S. Brenner, R. J. Watts-Tobin, Nature 192, 1227 (1961).
5. M. W. Nirenberg and J. H. Matthaei, Proc. Nat. Acad. Sci. U.S. 47, 1558 (1961).
6. M. W. Nirenberg and P. Leder, Science 145, 1399 (1964).
7. M. R. Bernfield, J. Biol. Chem. 240, 4753 (1965).
8. P. Leder, M. F. Singer, R. L. C. Brimacombe, Biochemistry 4, 1561 (1965).



9. D. Söll, E. Ohtsuka, D. S. Jones, R. Lohrmann, H. Hayatsu, S. Nishimura, H. G. Khorana, Proc. Nat. Acad. Sci. U.S. 54, 1378 (1965).
10. A. R. Morgan, R. D. Wells, H. G. Khorana, ibid 56, 1899 (1966).
11. C. Yanofsky, Cold Spring Harbor Symp. Quant. Biol. 31 (1966) in press.
12. H. G. Wittmann and B. Wittmann-Liebold, ibid.
13. M. Nirenberg, T. Caskey, R. Marshall, D. Kellogg, D. Doctor, D. Hatfield, J. Levin, W. Anderson, ibid.
14. R. Marshall, C. T. Caskey, M. Nirenberg, Science 155, 820 (1967).
15. T. M. Sonnenborn, in Evolving Genes and Proteins, Rutgers Symposium 1964, ed. H. Vogel (New York: Academic Press, 1965).
16. C. R. Woese, Proc. Nat. Acad. Sci. U.S. 54, 71 (1965).
17. B. P. Doctor, J. Apgar, R. W. Holley, J. Biol. Chem. 236, 1117 (1961).
18. J. Apgar, R. W. Holley, S. H. Merrill, Biochim. Biophys. Acta 53, 220 (1961).
19. B. Weisblum, S. Benzer, R. W. Holley, Proc. Nat. Acad. Sci. U.S. 48, 1449 (1962).
20. H. G. Zachau, M. Tada, W. B. Lawson, M. Schweiger, Biochim. Biophys. Acta 53, 221 (1961).
21. N. Sueoka and T. Yamane, Proc. Nat. Acad. Sci. U.S. 48, 1454 (1962).
22. D. A. Kellogg, B. P. Doctor, J. E. Loebel, M. W. Nirenberg, ibid 55, 912 (1966).
23. A. D. Kelmers, G. D. Novelli, M. P. Stulberg, J. Biol. Chem. 240, 3979 (1965).
24. S. Nishimura, D. S. Jones, E. Ohtsuka, H. Hayatsu, T. M. Jacob, H. G. Khorana, J. Mol. Biol. 13, 283 (1965).
25. B. F. C. Clark and K. A. Marker, ibid 17, 394 (1966).
26. D. Söll, D. S. Jones, E. Ohtsuka, R. D. Falkner, R. Lohman, H. Hayatsu, H. G. Khorana, J. D. Cherayil, A. Hampel, R. M. Bock, ibid 19, 556 (1966).
27. C. M. Connelly and B. P. Doctor, J. Biol. Chem. 241, 715 (1966).
28. H. Zachau, D. Dutting, H. Feldman, Angew. Chem. 5, 422 (1966).
29. J. T. Madison, G. A. Everett, H. Kung, Science 153, 531 (1966).



30. R. W. Holley, J. Apgar, G. A. Everett, J. T. Madison, S. H. Merrill, A. Zamir, Cold Spring Harbor Symp. Quant. Biol. 28, 117 (1963)
31. B. Weisblum, F. Gonano, G. von Ehrenstein, S. Benzer, Proc. Nat. Acad. Sci. U.S. 53, 328 (1965).
32. J. Apgar and R. W. Holley, in preparation.
33. R. W. Holley, J. Apgar, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J. R. Penswick, A. Zamir, Science 147, 1462 (1965).
34. J. T. Madison, G. A. Everett, H. Kung, ibid 153, 531 (1966).
35. V. M. Ingram and J. A. Sjoqvist, Cold Spring Harbor Symp. Quant. Biol. 28, 133 (1963).
36. F. H. C. Crick, J. Mol. Biol. 19, 548 (1966).
37. J. M. Adams and M. R. Capecchi, Proc. Nat. Acad. Sci. U.S. 55, 147 (1966).
38. R. E. Thach, K. F. Dewey, J. C. Brown, P. Doty, Science 153, 416 (1966).
39. R. E. Webster, D. L. Engelhardt, N. D. Zinder, Proc. Nat. Acad. Sci. U.S. 55, 155 (1966).
40. S. Brenner, A. W. Stretton, S. Kaplan, Nature 206, 994 (1965).
41. M. Weigert and A. Garen, ibid p. 992.
42. P. L. Bergquist, and M. R. Capecchi, J. Mol. Biol. 19, 202 (1966).
43. C. Yanofsky, D. R. Helinski, B. D. Maling, Cold Spring Harbor Symp. Quant. Biol. 26, 11 (1961).
44. J. Carbon, P. Berg, C. Yanofsky, Proc. Nat. Acad. Sci. U.S. 56, 764 (1966).
45. N. K. Gupta and H. G. Khorana, ibid p. 772.
46. I. Kaneko and R. H. Doi, ibid 55, 564 (1966).
47. R. A. Lazzarini, ibid 56, 185 (1966).
48. T. Kano-Sueoka and N. Sueoka, J. Mol. Biol. 20, 183 (1966).
49. R. H. Doi, I. Kaneko, B. Goehler, Proc. Nat. Acad. Sci. U.S. 56, 1548 (1966).
50. E. F. Brunngraber, Biochem. Biophys. Res. Commun. 8, 1 (1962).

10. K. G. ... ...

11. G. ... ...

12. J. ... ...

13. R. ... ...

14. T. ... ...

15. ... ...

16. ... ...

17. ... ...

18. ... ...

19. ... ...

20. ... ...

21. ... ...

22. ... ...

23. ... ...

51. K. Moldave, Methods Enzymol. 6, 757 (1963).
52. A. D. Kelmers, J. Biol. Chem. 241, 3540 (1966).
53. Aliquat 204, General Mills.
54. Chromosorb W (acid washed, DMCS treated, 100-120 mesh), Johns Manville.
55. G. Bray, Anal. Biochem. 1, 279 (1960).
56. T. Okamoto and Y. Kawade, Biochem. Biophys. Res. Commun. 13, 324 (1963).
57. P. Leder and M. Nirenberg, Proc. Nat. Acad. Sci. U.S. 52, 420 (1964).
58. M. Nirenberg, P. Leder, M. Bernfield, R. Brimacombe, J. Trupin, F. Rottman, C. O'Neal, ibid 53, 1161 (1965).
59. R. Brimacombe, J. Trupin, M. Nirenberg, P. Leder, M. Bernfield, T. Jaouni, ibid 54, 594 (1965).
60. M. Bernfield, J. Biol. Chem. 241, 2014 (1966).
61. M. Nirenberg, Methods Enzymol. 6, 17 (1963).
62. C. T. Caskey, B. Redfield, H. Weissbach, Archiv. Biochem. Biophys. in press.
63. J. D. Capra and A. Peterkofsky, J. Mol. Biol. 21, 455 (1966).
64. A. Peterkofsky, Proc. Nat. Acad. Sci. U.S. 52, 1233 (1964).
65. C. T. Caskey, A. Beaudet, M. Wilcox, M. Nirenberg, Fed. Proc. in press.
66. H. Noll, Science 151, 1241 (1966).
67. C. T. Caskey, unpublished data.
68. K. A. Marker and F. Sanger, J. Mol. Biol. 8, 835 (1964).
69. P. Leder and H. Bursztyn, Proc. Nat. Acad. Sci. U.S. 56, 1579 (1966).













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