# Fidelity of Protein Synthesis with Chicken Embryo Mitochondrial and Cytoplasmic Ribosomes†

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ABSTRACT: A mitochondrial *in vitro* protein synthesizing system was characterized in order to determine the effects of aminoglycoside antibiotics and other agents on leucine–phenylalanine ambiguity with poly(uridylic acid) as messenger. Compared to postmitochondrial (cytoplasmic) ribosomes, mitoribosomes exhibited greater inhibition and higher levels of mistranslation when aminoglycosides which are known to cause mistranslation in bacterial systems but not in animal cell (postmitochondrial) systems were present. Ethanol and low temperature also promoted mitoribosomal mistranslation although high concentrations of magnesium were ineffective. The data indicate that the mitochondrial component of cell sap protein synthesis is prone to errors much in the same way as has been shown in bacterial systems.

Many investigators have shown similarities between mitochondrial and bacterial ribosomal function in a variety of organisms (for review, see Borst and Grivell (1971)). Reports of inhibition of mitochondrial protein synthesis by antibacterial antibiotics (Kuntzel, 1969a; Burton, 1972), some of which are known to promote mistranslation in bacteria (Scragg *et al.*, 1971; Schiff, 1970), have been made.

A high degree of fidelity *in vitro* with postmitochondrial ribosomes relative to bacterial systems has been shown (Friedman *et al.*, 1968). The investigation reported here was designed to characterize an *in vitro* system from chick embryo mitochondria in order to determine if the mitochondrial-bacterial simile could be extended to include inducible mistranslation of synthetic mRNA. Our results indicate that chick mitoribosomes are more likely to mistranslate than cytoribosomes, and behave very much like bacterial ribosomes in this regard.

## Experimental Section

*Materials and Methods.* Poly(uridylic acid)[1] (poly(U)) was obtained from Schwarz/Mann, Orangeburg, N. Y. Aminoglycoside antibiotics were from Mann Research Laboratories, New York, N. Y. Chloramphenicol and cycloheximide were obtained from Sigma Chemical Co., St. Louis, Mo. Fertilized chicken eggs were from Spafas, Inc., Norwich, Conn. *Escherichia coli* L-[$^{14}$C]phenylalanyl-tRNA (specific activity 0.154 $\mu$Ci/mg of tRNA) and L-[$^{14}$C]leucyl-tRNA (specific activity 0.357 $\mu$Ci/mg of tRNA) were obtained from NEN, Boston, Mass.

*Preparation of Ribosomes and Supernatant Fractions.* Mitochondria and mitochondrial ribosomes were prepared by a modification of the technique described by Kuntzel (1969a); 11-day old chicken embryos were decapitated and the bodies homogenized in 3 volumes (v/v) of buffer (10 mM magnesium acetate–50 mM KCl–3 mM mercaptoethanol–28 mM Tris-HCl (pH 7.6) and 0.32 M sucrose ("Special Density Gradient Grade," Schwarz/Mann, Orangeburg, N. Y.)) and strained through three layers of cheesecloth. The homogenate was centrifuged for 10 min at 700*g* and the supernatant three times at 7000*g* for 10 min. The pellet (crude mitochondria) was resuspended in the same buffer and centrifuged twice at 24,000*g* for 10 min. The final pellet was suspended in a small volume of the same buffer (except 0.25 M sucrose plus 1 mM EDTA) and layered on 25-ml discontinuous sucrose gradients (0.8, 1.1, 1.3, 1.5, 1.6, and 1.8 M sucrose plus 1 mM EDTA) and centrifuged in a swinging bucket rotor (SW 25.1) at 53,000*g* for 120 min. The mitochondrial fraction (1.5 M) was collected, spun down at 10,000 rpm for 10 min, and suspended by homogenization in 0.25 M sucrose buffer plus 2% Triton X-100. The suspension was stirred for 10 min and centrifuged at 105,000*g* for 120 min. The mitoribosome pellet was resuspended in 0.25 M sucrose buffer and centrifuged again at 105,000*g* for 120 min. The resulting pellet was suspended by homogenization in an appropriate volume of the same buffer, centrifuged at 2000 rpm for 5 min to remove aggregates, and frozen in small aliquots at −90°. The yield of mitoribosomes was of the order of 2–5% relative to total microsomes (based on $A_{260}$). Mitochondrial supernatant enzymes were prepared from purified mitochondria disrupted by sonication (Kuntzel, 1969b), and treated further as described for the cytoplasmic enzyme fraction.

The 7000*g* supernatant was adjusted to 0.5% deoxycholate and centrifuged at 105,000*g* for 120 min. The cytoribosome pellet was resuspended in 0.25 M sucrose buffer and centrifuged again as before. The resulting pellet was resuspended, spun at 2000 rpm to remove insoluble particles, and stored at −90°.

For the preparation of the postmitochondrial ribosome free supernatant fraction (S-105), the 7000*g* supernatant was centrifuged at 105,000*g* for 120 min (without deoxycholate). The resulting supernatant was routinely tested for optimal concentration in the *in vitro* system (mito- or cytoribosome), diluted with 0.25 M sucrose buffer if necessary, and frozen in small aliquots.

Figure 1 shows a typical mitoribosomal sucrose density centrifugation profile. The chicken embryo monosome appears to sediment at approximately 53 S at a magnesium concentration of 10 mM. The larger material sedimenting faster than 70 S has an *s* value of between 83 S and 95 S in different preparations (calculated by the method of McEwen (1967)). This material may contain some contaminating cytoribosomes as well as mitoribosome polymers.

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[1] Abbreviations used are: ssDNA, single-stranded DNA; poly(U), poly(uridylic acid).

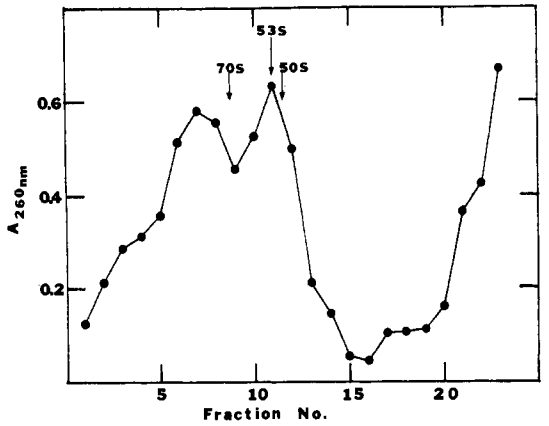


FIGURE 1: Sedimentation of chicken embryo mitochondrial ribosomes. Mitoribosomes were prepared as described in text. Suspensions of mitoribosomes in buffer (10 mM magnesium acetate–50 mM KCl–3 mM mercaptoethanol–28 mM Tris-HCl (pH 7.6)) were layered on 10-ml linear sucrose gradients (10–30%). Gradients were centrifuged for 6 hr at 23,000 rpm in a Beckman L275B ultracentrifuge (5°) in an SW41 rotor and 0.4-ml fractions were collected. *E. coli* 70S ribosomes and subunits (in low magnesium gradients) run in parallel gradients were used as references.

TABLE 1: Effect of Magnesium Concentration on Mitochondrial and Cytoplasmic Incorporation of Leucine in Response to Poly(uridylic acid).[a]

| | | | | + DHSm | |
|---|---|---|---|---|---|
| | | | Net Stimulation | | Net Stimulation |
| | | − Poly(U) | + Poly(U) | − Poly(U) | + Poly(U) |
| Ribosomes | $Mg^{2+}$ (mM) | Cpm of L-[$^{14}$C]Leucine Incorporated/ $A_{260}$ Unit | | | |
| Mito | 8 | 1047 | 113 | 959 | 748 |
| | 10 | 1045 | 366 | 841 | 981 |
| | 15 | 789 | 96 | 838 | 289 |
| | 20 | 635 | 20 | 726 | 73 |
| Cyto | 8 | 1144 | 36 | 811 | 31 |
| | 10 | 889 | 60 | 736 | 34 |
| | 15 | 686 | 6 | 583 | 17 |
| | 20 | 608 | 21 | 685 | −2 |

[a] Conditions were as described in the Experimental Section. Dihydrostreptomycin (DHSm) was added at a final concentration of 50 μM.

*Bacterial contamination* of the mitoribosome preparations were monitored by plating aliquots on nutrient agar plates which were incubated at 38° for 3 days. The final suspension of mitoribosomes contained no more than 60 colony forming bacteria mg of $RNA^{-1}$ $ml^{-1}$. Experiments containing higher numbers of bacteria are not reported (Hernandez *et al.*, 1971).

*In Vitro Protein Synthesis Assay.* A modification of the *in vitro* assay as described by Soeiro and Amos (1966) was used. The incorporation of L-[$^{14}$C]amino acids into $Cl_3CCOOH$ precipitable material was effected in a total volume of 0.35 ml containing the following concentrations: 6 mM 2-mercaptoethanol, 1.4 mM ATP and GTP, 1.6 mg/ml of creatine phosphate, 71 μg/ml of creatine phosphokinase, 37 mM KCl, 140 μg/ml of L-[$^{14}$C]aminoacyl-tRNA, 0.08 μM each of 19 [$^{12}$C]amino acids, 0.1 M sucrose, 13.6 mM Tris-HCl (pH 7.6), magnesium acetate as indicated, 70 μg/ml of poly(uridylic acid), 1.0–1.5 mg/ml of S-105 protein, and approximately 1.0 $A_{260}$ units/0.35 ml of ribosomes. After moderate shaking for 30 min at 37°, the reaction was stopped with cold 6% $Cl_3CCOOH$ plus 1% Casamino acids. After heating at 90° for 30 min, the material was filtered through Whatman glass fiber paper filters (Maidstone, England), washed with 10% $Cl_3CCOOH$ plus 1% Casamino acids, dried, and counted in an Isocap (Nuclear-Chicago) liquid scintillation spectrophotometer. Counts were corrected for zero time controls (10–20 cpm/$A_{260}$ unit). The mean deviation of duplicate assays ranged from 1 to 5% of the average. Net stimulation plus poly(U) refers to the incorporation with poly(U) minus the endogenous (no poly(U)) incorporation. In their respective homologous *in vitro* system, cytoribosomes were, on the average, 52% more active in poly(U) directed phenylalanine incorporation than mitoribosomes. Mitoribosomes incorporated 6.1 ± 1.7 molecules of phenylalanine ribosome$^{-1}$ hr$^{-1}$ compared with 9.3 ± 1.8 molecules of phenylalanine ribosome$^{-1}$ hr$^{-1}$ for cytoribosomes at their respective magnesium optima. The endogenous incorporation rates (minus poly(U)) for phenylalanine and leucine were nearly equal when both mito- and cytoribosomes were measured at 6 mM magnesium.

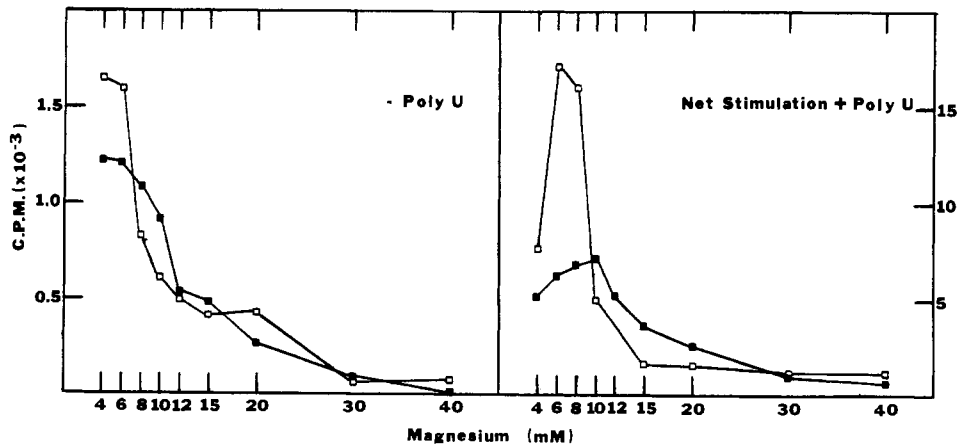


FIGURE 2: The effect of magnesium concentration on phenylalanine incorporation in a mitochondrial and cytopolasmic *in vitro* system: cytoplasmic ribosomes (□) and mitochondrial ribosomes (■). The composition of the reaction mixture is given in the Experimental Section.

TABLE II: Effects of Antibiotics on Cell-free Mitochondrial Protein Synthesis.[a]

| Amino Acid | Addition | Concn ($\mu$M) | − Poly(U) | Net Stimulation + Poly(U) | % Control Phe Net Stimulation + Poly(U) | Leu/Phe Net Stimulation + Poly(U) |
|---|---|---|---|---|---|---|
| | | | Cpm of L-[$^{14}$C]Amino Acid Incorporated/$A_{260}$ Unit | | | |
| Phe | None | | 1225 | 7082 | (100) | 0.032 |
| Leu | None | | 1552 | 227 | | |
| Phe | CHI | 100 ($\mu$g/ml) | 1152 | 5496 | 77.6 | |
| Phe | CAP | 100 ($\mu$g/ml) | 964 | 3456 | 48.8 | |
| Phe | DHSm | 50 | 1151 | 4346 | 61.3 | 0.492 |
| Leu | DHSm | 50 | 1180 | 2136 | | |
| Phe | Km | 50 | 1068 | 4569 | 64.5 | 0.062 |
| Leu | Km | 50 | 884 | 281 | | |
| Phe | Sm | 50 | 739 | 2416 | 34.1 | 0.089 |
| Leu | Sm | 50 | 566 | 214 | | |
| Phe | Nm | 25 | 1123 | 2341 | 33.1 | 0.049 |
| Leu | Nm | 25 | 1274 | 115 | | |
| Phe | Nm | 50 | 944 | 1013 | 14.3 | 0.643 |
| Leu | Nm | 50 | 590 | 651 | | |
| Phe | Ery | 50 | 858 | 5605 | 79.3 | 0.044 |
| Leu | Ery | 50 | 1666 | 226 | | |

[a] Conditions were as described in the Experimental Section. The reaction mixture was assayed at 10 mM $Mg^{2+}$. Abbreviations used are: CHI, cycloheximide; CAP, chloramphenicol; DHSm, dihydrostreptomycin; Km, kanamycin; Sm, streptomycin; Nm, neomycin; Ery, erythromycin.

Ribosome concentration is given in $A_{260}$ units (20 $A_{260}$ units equal 1 mg of ribosomal RNA) and was routinely adjusted to deliver 40–60 $\mu$g of RNA per assay as determined by the orcinol method (Schneider, 1957). Protein was determined by the method of Lowry *et al.* (1951).

## Results

Figure 2 shows the effect of magnesium concentration on endogenous and poly(U)-stimulated phenylalanine incorporation with mitochondrial and cytoplasmic ribosomes. The mitochondrial and cytoplasmic endogenous incorporation show similar magnesium requirements (highest at 4 mM). Poly(U)-directed phenylalanine incorporation with mitoribosomes required a higher magnesium concentration (10 mM) than the cytoplasmic system (6–8 mM). These results are in agreement with those of other investigators (see for example Scragg *et al.*, 1971) who showed a 12 mM *vs.* 5 mM magnesium optima in the poly(U) system for mitoribosomes and cytoribosomes, respectively, isolated from yeast. This figure also agrees with the work of Kuntzel (1969b) on homologous systems from *Neurospora crassa* in that the mitochondrial ribosomes have a lower response to poly(U) than cytoplasmic ribosomes.

Table I shows the effect of magnesium concentration on leucine incorporation with poly(uridylic acid) added. In contrast to bacterial systems (Davies *et al.*, 1964), increasing the magnesium concentration beyond the optimum for phenylalanine incorporation does not stimulate leucine incorporation in either the cytoribosome or mitoribosome system. In contrast to the cytoplasmic system, the addition of 50 $\mu$M dihydrostreptomycin stimulates leucine incorporation in the mitoribosomal system over the entire magnesium range. In addition, magnesium concentrations above 8 mM appear to antagonize the misreading effect of the antibiotic.

Table II shows the effect of several antibiotics on mitoribosomal protein synthesis *in vitro*. Cycloheximide inhibited mitoribosomes by 22%. This degree of sensitivity may be explained by some degree of contamination with cytoribosomes. Chloramphenicol is over 50% effective in inhibiting mitoribosomes in the poly(U)-directed system. This is a relatively high degree of *in vitro* poly(U) dependent inhibition for mitoribosomes (Lamb *et al.*, 1968), but is comparable to that observed by Allen and Suyama (1972) with *Tetrahymena* mitoribosomes and Scragg *et al.* (1971) with mitoribosomes from *Saccharomyces*.

Although leucine–phenylalanine ambiguity in postmitochondrial systems has been shown to be virtually unaffected by aminoglycosides, these drugs are inhibitory at high concentrations (Weinstein *et al.*, 1966). For the direct comparison of mito- and cytoribosomes, we have chosen to use a concentration of $5 \times 10^{-5}$ M at which we find little or no inhibition of cytoribosomes with most of the antibiotics. As shown in Table II, mitoribosomal leucine–phenylalanine ambiguity is increased by all the aminoglycosides tested. Erythromycin, a macrolide antibiotic (affecting the large bacterial subunit), is somewhat inhibitory at 50 $\mu$M but causes little change in the leucine/phenylalanine ratio.

Table III shows the effects of the same drugs on cytoribosomes. Cytoribosomes are inhibited by cycloheximide and resistant to chloramphenicol. Streptomycin is slightly effective in increasing leucine incorporation (to 2% of phenylalanine incorporation). Neomycin is strongly inhibitory at 50 $\mu$M to both mito- and cytoribosomes and causes marked increases in mistranslation in both systems although the cytoplasmic Leu/Phe was still below 10%. Dihydrostreptomycin and kanamycin do not stimulate leucine incorporation by cytoribosomes. Higher concentrations of any of the drugs (with the exception of neomycin) do not increase cytoribosome mistranslation (unpublished results).

TABLE III: Effects of Antibiotics on Cell-Free Cytoplasmic Protein Synthesis.[a]

| Amino Acid | Addition | Concn ($\mu$M) | − Poly(U) | Net Stimulation + Poly(U) | % Control Phe Net Stimulation + Poly(U) | Leu/Phe Net Stimulation + Poly(U) |
|---|---|---|---|---|---|---|
| | | | Cpm of L-[$^{14}$C]Amino Acid Incorporated/$A_{260}$ Unit | | | |
| Phe | None | | 2148 | 5990 | (100) | <0 |
| Leu | None | | 1193 | −19 | | |
| Phe | CHI | 100 ($\mu$g/ml) | 797 | 1102 | 18.4 | |
| Phe | CAP | 100 ($\mu$g/ml) | 2192 | 5960 | 99.5 | |
| Phe | DHSm | 50 | 2323 | 7058 | 117.8 | <0 |
| Leu | DHSm | 50 | 977 | −14 | | |
| Phe | Km | 50 | 2116 | 6613 | 110.4 | <0 |
| Leu | Km | 50 | 801 | −67 | | |
| Phe | Sm | 50 | 1696 | 5842 | 97.5 | 0.020 |
| Leu | Sm | 50 | 862 | 117 | | |
| Phe | Nm | 25 | 1227 | 4993 | 83.3 | 0.009 |
| Leu | Nm | 25 | 762 | 46 | | |
| Phe | Nm | 50 | 442 | 1286 | 21.5 | 0.082 |
| Leu | Nm | 50 | 140 | 105 | | |

[a] Conditions were as described in the Experimental Section. The reaction mixture was assayed at 6 mM $Mg^{2+}$.

Two other external factors that have been shown to encourage mistranslation in bacterial but not in postmitochondrial *in vitro* systems are organic solvents (Friedman and Weinstein, 1964) and low temperature (So and Davie, 1964).

Table IV shows the effects of both agents on mitochondrial and cytoplasmic protein synthesis. There is a four- to sixfold increase in leucine–phenylalanine ambiguity due to the presence of 0.5–1.5 M ethanol in the mitoribosome system with no comparable effect on cytoribosomes. Low temperature of incubation (18° *vs.* 37°) increases the ratio of leucine in the product from 10.2 to 23.2% with mitoribosomes whereas there is very little leucine incorporation by cytoribosomes in either case (0.3% *vs.* 0.1%).

Bacterial ribosomes can translate single-stranded DNA (ssDNA) extensively in the presence of neomycin (McCarthy and Holland, 1965). In the absence of neomycin, ssDNA can serve as a messenger in the GTP dependent binding of fMet-tRNA$^{fMet}$, and small peptides are synthesized (Bretscher, 1969).

Table V shows the relative translatability of ssDNA by mitochondrial and cytoplasmic ribosomes. There is a 24-fold increase in the mitochondrial incorporation of L-[$^{14}$C]phenylalanine into $Cl_3CCOOH$ precipitable material upon the addition of 7 $\mu$g of neomycin. A 40-fold increase is seen if the ssDNA is first dialyzed against a low concentration of neomycin and no further antibiotic is added to the assay. As shown in Table V, chick cytoplasmic ribosomes do not translate the natural heteropolynucleotide at all. Even though we have not yet looked for small peptide synthesis in the absence of neomycin, it is interesting to speculate that mito-

TABLE IV: Effect of Ethanol and Temperature on Mitochondrial and Cytoplasmic Cell-Free Protein Synthesis.[a]

| Ribosomes | $Mg^{2+}$ (mM) | Ethanol (M) | Poly(U) Phe | Poly(U) Leu | Net Stimulation + Poly(U) Phe | Net Stimulation + Poly(U) Leu | Leu/Phe Net Stimulation + Poly(U) |
|---|---|---|---|---|---|---|---|
| | | | Cpm of L-[$^{14}$C]Amino Acid Incorporated/$A_{260}$ Unit | | | | |
| Mitochondrial | 10 | 0 | 381 | 885 | 2,898 | 142 | 0.049 |
| | 10 | 0.5 | 115 | 493 | 870 | 271 | 0.311 |
| | 10 | 1.0 | 85 | 450 | 647 | 135 | 0.208 |
| | 10 | 1.5 | 83 | 446 | 627 | 140 | 0.223 |
| Cytoplasmic | 6 | 0 | 842 | 1270 | 2,946 | −127 | <0 |
| | 6 | 0.5 | 639 | 1056 | 1,674 | −161 | <0 |
| | 6 | 1.0 | 474 | 835 | 1,021 | −24 | <0 |
| | 6 | 1.5 | 364 | 674 | 1,016 | −48 | <0 |
| Mitochondrial | 10 | 18[b] | 399 | 333 | 440 | 102 | 0.232 |
| | 10 | 37[b] | 820 | 872 | 7,689 | 785 | 0.102 |
| Cytoplasmic | 6 | 18[b] | 526 | 318 | 4,689 | 32 | 0.001 |
| | 6 | 37[b] | 2090 | 1862 | 14,570 | 49 | 0.003 |

[a] Conditions were as described in the Experimental Section. [b] Values given are temperature, °C.

TABLE V: Translation of Single-Stranded DNA by Mitochondrial and Cytoplasmic Ribosomes.[a]

| Ribosomes | $Mg^{2+}$ (mM) | Additions | − DNA | + DNA | Net Stimulation | % Control Net Stimulation |
|---|---|---|---|---|---|---|
| | | | Cpm of L-[$^{14}$C]Phenylalanine Incorporated/$A_{260}$ Unit | | | |
| Mito | 10 | None | 1161 | 1190 | 29 | (100) |
| | 10 | 3.5 μg of Nm | 534 | 888 | 354 | 1221 |
| | 10 | 7.0 μg of Nm | 227 | 925 | 698 | 2405 |
| | 10 | None[b] | | 2315[b] | 1154 | 3980 |
| Cyto | 6 | None | 1854 | 629 | −1224 | |
| | 6 | 7.0 μg of Nm | 1166 | 465 | −701 | |

[a] Conditions were as described in the Experimental Section with the exception of a 15-min preincubation at 37° prior to the addition of isotope or 200 μg of DNA. Calf thymus DNA was heated for 2 hr at 60° in 0.3 N NaOH and then dialyzed against distilled water. [b] Base-treated DNA was dialyzed against 1 μg of neomycin/ml for 2 hr and then against distilled water.

ribosomes may be able to initiate translation of single-stranded DNA.

## Discussion

We have shown that a 53S mitochondrial ribosome contains some of the functional properties of the larger bacterial ribosome that allow for a considerable degree of mistranslation. The relaxation of restriction at the codon–anticodon site by aminoglycosides that allows for mistranslation with bacterial ribosomes does not appear to occur with animal cell (postmitochondrial) ribosomes. Zimmermann *et al.* (1971) have shown that the structural gene products defined by the *strA* (P10) and *ram* (P4a) genes in *E. coli* exert respectively stimulatory or restrictive control over fidelity. They further suggest that the mutant *ram* ribosomal protein may exert its antagonistic effect on the mutant *strA* protein by interfering with the proper positioning of P10 into the 30S subunit. Thus, alterations in the fidelity of protein synthesis with *E. coli* ribosomes can involve both the codon–anticodon site and protein–protein interaction. It is difficult to say from our data at present whether the sensitivity to aminoglycosides, ethanol, and temperature shown with the small mitoribosomes reflects a similar degree of sophistication or a more primitive state of ribosomal evolution.

As our data show, the analogy between bacterial and mitochondrial ribosomes is not exact. For example, in *E. coli*, the introduction of aminoglycosides *in vitro* depresses poly(U)-directed phenylalanine incorporation while increasing that of leucine (Davies *et al.*, 1964). We find with mitoribosomes that although the ratio of leucine to phenylalanine in the product is increased by all the aminoglycosides tested, the absolute leucine incorporation does not necessarily increase (Table II). Also, increasing the magnesium concentration in our mitoribosomal system (Table I) does not have the effect of increasing ambiguity that it has in bacterial systems (Davies *et al.*, 1964).

One of the problems that prompted this study of mitochondrial fidelity in protein synthesis was the increasing interest in the literature of the breakdown of fidelity in animal cell protein synthesis as a prelude to senescence, as first postulated by Orgel (1963). Evidence has begun to emerge that faulty proteins are produced in an aging mutant of *Neurospora* (Lewis and Holliday, 1970) and human fibroblasts (Holliday and Tarrant, 1972) although fidelity in animal cell (postmitochondrial) protein synthesis has been difficult to alter experimentally by most investigators. A complex interdependence between mitochondria and cytoplasmic protein synthesis has been demonstrated (Barath and Kuntzel, 1972) and it is reasonable to assume that errors made in the mitochondrion would have serious effects elsewhere in the cell.

## Acknowledgments

The author is indebted to Drs. Anthony P. Russell and F. Marott Sinex for their continued interest in this work and review of the manuscript.

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# Metal(II) Ion Catalyzed Transphosphorylation of Four Homodinucleotides and Five Pairs of Dinucleotide Sequence Isomers†

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ABSTRACT: The great rate enhancement in $Zn^{2+}$-catalyzed transphosphorylation was observed for four homodinucleotides, XpXp(3′) in which X represents adenosine (A), cytidine (C), guanosine (G), or uridine (U) relative to XpXp(2′). The relative reactivity has been found to decrease in the order: UpUp(3′) > ApAp(3′) > CpCp(3′) > GpGp(3′), which is the reverse of the order of the affinity of the base moiety toward the metal ion (G > C > A ≥ U). The base-specific nature of the $Zn^{2+}$-catalyzed degradation of dinucleotides has been further examined with five pairs of sequence isomers, such as ApUp(3′) and UpAp(3′). The present results provide new evidence regarding the origins of the base and nucleotide-sequence specificity in the zinc-cleavage reaction at a neutral pH; *i.e.*, the base binding of $Zn^{2+}$ as well as the stacking interaction between bases plays a controlling role in the observed differential rate enhancement. The effects of the pH and of the $Zn^{2+}$/P level on the rate of the $Zn^{2+}$-catalyzed transphosphorylation of ApUp(3′) have been interpreted in terms of a mechanism in which the dinucleotide, which is coordinated to $Zn^{2+}$ to form a reactive 1:1 complex through the *bridging* of $Zn^{2+}$ in a prior equilibrium, undergoes a cleavage reaction. Supporting evidence for this interpretation has been provided by observing the change in the optical properties of selected dinucleotides during $Zn^{2+}$ titration. For comparison, the rates of the $Cu^{2+}$-, $Co^{2+}$-, and $Ni^{2+}$-catalyzed cleavage reactions of four homodinucleotides have also been determined.

The first to investigate the catalytic effect of metal ions on the degradative reactions of RNA were Dimroth *et al.*, who found that nucleosides and nucleotides could be obtained by the degradation of RNA in the presence of $Pb^{2+}$ (Dimroth *et al.*, 1950) and $Zn^{2+}$ (Dimroth *et al.*, 1959; Dimroth and Witzel, 1959), respectively. It has since been shown that the increase in rate of the RNA and synthetic homopolyribonucleotide degradations is caused by the presence of $Cd^{2+}$, $Bi^{3+}$, $Al^{3+}$, $La^{3+}$, $Mn^{2+}$, $Cu^{2+}$, $Ni^{2+}$, and $Co^{2+}$ (Witzel, 1960; Eichhorn and Butzow, 1965; Farkas, 1968; Eichhorn *et al.*, 1971). However, until recently no detailed kinetic study has been made with defined oligoribonucleotides (Butzow and Eichhorn, 1971). Butzow and Eichhorn analyzed their data by considering the effects of the charge of an adjacent phosphate group and the nature of the nearest-neighbor base; the rates of the zinc-cleavage of the internal phosphodiester bonds of adenosine dimers and a trimer were found to decrease in this order: ApAp(3′) (100 ~ 150) ≫ Ap|ApA(~7) > ApAp(2′) (~3) ≥ ApAp > p(2 ~ 3) ≥ ApAp|A(~2) > ApA(~1).

With a view to studying the structure–reactivity correlation in oligonucleotides, a preliminary report on the base-catalyzed transphosphorylation of adenylate dinucleotides has appeared (Koike and Inoue, 1972); this work forms an extension of similar studies of divalent metal ion (mainly $Zn^{2+}$)-catalyzed reactions of various dinucleotides. We have thus examined: (a) the effect of the terminal phosphate group, (b) the effect of the nature of the neighboring base, and (c) the base sequence dependence, if any, on the rate of zinc ion-catalyzed transphosphorylation by using XpX, XpXp(2′), XpXp(3′), and five pairs of sequence isomers, represented by XpYp(3′) and YpXp(3′).

## Experimental Section

*Materials.* Poly(rA) was purchased from Boehringer, Mannheim. Dinucleotides, ApCp(3′), ApUp(3′), GpCp(3′), and GpUp(3′), were obtained from pancreatic ribonuclease digest of yeast RNA in a manner similar to that described by Aoyagi and Inoue (1968a). ApGp(3′), CpGp(3′), and UpGp(3′) were also prepared by the ribonuclease $T_1$ catalyzed degradation of RNA (Aoyagi and Inoue, 1968b). The other dinucleotides used in this study, ApAp(2′), ApAp(3′), CpCp(2′), CpCp(3′), GpGp(2′), GpGp(3′), UpUp(2′), UpUp(3′), CpAp(3′), GpAp(3′), and UpAp(3′), were prepared by a controlled alkaline hydrolysis of RNA in 0.2 N NaOH, followed by column chromatographic separations with DEAE-Sephadex A-25 and Dowex 1-X2 anion exchangers. The details of the procedures are substantially the same as those reported in a previous paper (Satoh and Inoue, 1969). Dinucleoside monophosphates, ApA and UpU, were prepared by the enzymatic dephosphorylation of ApAp and UpUp with *Escherichia coli* alkaline phosphatase at 36° and at a pH of about 8. Ribonuclease $T_1$ and $T_2$ were gifts from Dr. H. Okazaki (Sankyo Co. Ltd.), while ribonuclease IA and alkaline phosphatase were from Boehringer, Mannheim. All the other chemicals, including $Zn(NO_3)_2$, were analytical grade.

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