Specificity of Mitochondrial and Cytoplasmic Ribosomes and Elongation Factors from *Xenopus laevis*†

Ronald F. Swanson

ABSTRACT: Elongation factors and ribosomes were prepared from *Xenopus laevis* ovarian mitochondria and cytoplasm, rat liver cytoplasm, and *Escherichia coli*. Various combinations of elongation factors and ribosomes were tested for ability to catalyze poly(U)-dependent incorporation of phenylalanine into hot trichloroacetic acid insoluble peptides. It was found that rat and *Xenopus* cytoplasmic ribosomes are active with elongation factors from either cytoplasmic source but have little or no activity with factors prepared from mitochondria or *E. coli*. Furthermore, ribosomes from *Xenopus* mitochondria or *E. coli* are functionally compatible with mitochondrial or *E. coli* elongation factors but not with cytoplasmic elongation factors. Since mitochondrial ribosomes from vertebrates appear to be smaller than ribosomes from any other source, having sedimentation coefficients of 55–60 S, it is striking that these ribosomes are still compatible with *E. coli* elongation factors. These results are also significant in that they show that the mitochondrial and cytoplasmic ribosomes from *Xenopus* are not only different in size, but also are functionally distinguishable.

In recent years it has become clear that mitochondria contain their own DNA which codes for the synthesis of unique mitochondrial tRNAs and rRNAs (Borst, 1972). These RNAs function in a protein synthesizing system which differs from the cytoplasmic system with respect to responses to various inhibitors of protein synthesis and the sizes of the ribosomes and rRNAs (Ashwell and Work, 1970). For example, the cytoplasmic and mitochondrial ribosomes from *Xenopus laevis* have sedimentation coefficients of 87 and 60 S, respectively (Swanson and Dawid, 1970). The two large rRNAs from *Xenopus* mitochondria have molecular weights of 3.0×10^5 and 5.3×10^5 (Dawid and Chase, 1972), while the molecular weights of the cytoplasmic rRNAs are 0.7×10^6 and 1.5×10^6 (Loening, 1968).

Parisi *et al.* (1967) reported that ribosomes and elongation factors can be classified as either of a procaryote or eucaryote type. All ribosomes of one type cross react with elongation factors of the same type in poly(U)-dependent poly(phenylalanine) synthesis but do not react with enzymes of the other type. It has been shown that the ribosomes and elongation factors from the mitochondria and cytoplasm of fungi are of the procaryote and eucaryote type, respectively (Kuntzel, 1969; Richter and Lipmann, 1970; Scragg, 1971). In order to determine whether the same is true of mitochondrial and cytoplasmic protein synthesizing systems of a vertebrate, various combinations of ribosomes and elongation factors from *Xenopus* cytoplasm or mitochondria, rat liver cytoplasm, and *E. coli* were tested for ability to catalyze poly(U)-dependent phenylalanine incorporation into polypeptides. The results are the subject of this report.

Methods

Preparation of Ribosomes and Elongation Factors. Mitochondrial extract was prepared from the ovaries of *Xenopus laevis* as previously described (Swanson and Dawid, 1970). The extract was centrifuged at 100,000*g* for 2.5 hr. The resultant supernatant fraction (I) was removed and brought to pH 5 by addition of 0.5 M acetic acid. After standing in ice for 30 min, supernatant I was centrifuged at 20,000*g* for 15 min. The pellet was suspended in the hypotonic medium (0.5 ml/g of ovary) of Swanson and Dawid (1970), the pH was adjusted to 7.2 with 1 N KOH and the resultant solution was stored at −70°. Under these conditions the elongation factors had a half-life of about 1 week.

The 100,000*g* ribosomal pellet obtained from the mitochondrial extract (above) was suspended in hypotonic medium (0.1 ml/g of ovary) using a dounce-type homogenizer and stored at −70°.

Xenopus cytoplasmic ribosomes and elongation factors were obtained from a postmitochondrial supernatant (Swanson and Dawid, 1970) by centrifugation at 150,000*g* for 1 hr. The ribosomal pellet was suspended in hypotonic medium (0.4 ml/g of ovary) and pH 5 enzymes were separated from the 150,000*g* supernatant by the method used for preparation of mitochondrial enzymes except that the final pellet was suspended in medium A′ (0.2 ml/g of ovary) of Maxwell (1962).

Rat liver and pH 5 enzymes were prepared by the method of Maxwell (1962).

E. coli cells were ground with alumina (2 g/g cells) and extracted twice with hypotonic medium (2 ml/g of cells). After each extraction alumina and cell debris were removed by centrifugation at 20,000*g* for 10 min. The pooled supernatants were centrifuged at 20,000*g* for 15 min and the resultant supernatant was centrifuged at 100,000*g* for 2 hr. This supernatant (II) was removed, mixed with 0.5 volume of hypotonic medium, and centrifuged at 150,000*g* for 1.5 hr. The top two-thirds of the 150,000*g* supernatant was removed and 1 ml was passed through a column of Sephadex G-50 (0.9 × 22 cm), eluting with hypotonic medium. The void volume was collected and used as a source of *E. coli* elongation factors. The final chromatography step is critical since it removes material which inhibits mitochondrial ribosomes.

E. coli ribosomes were prepared by a modification of the method used by Gordon *et al.* (1971). The ribosome suspension obtained by their method was mixed with 0.5 volume of hypotonic medium and centrifuged at 250,000*g* for 1.5 hr.

† From the Department of Biology, University of Virginia, Charlottesville, Virginia 22901. *Received January 15, 1973.* Supported by a grant from the National Science Foundation (GB23792).

The ribosomal pellet was suspended in a volume of hypotonic medium equal to the volume of the original suspension before dilution. When stored at −70° there was no detectable loss of activity of this preparation over a 7-month period.

Protein content of all ribosome and enzyme preparations was determined by the Biuret method (Layne, 1957).

Preparation of [^{3}H]Phe-tRNA. E. coli 100,000*g* supernatant II was prepared as described for preparations of elongation factors and dialyzed for 20 hr at 5° against a 100-fold excess of hypotonic medium. The reaction mixture for charging tRNA had the following composition (μmol/ml): $MgCl_2$ (13), KCl (20), Tris, pH 7.4 (110), mercaptoethanol (5.4), ATP (0.8), CTP (0.1), 19 amino acids minus phenylalanine (0.8 each), [^{3}H]phenylalanine (8 μCi; specific activity 6 Ci/mmol), yeast tRNA (0.3 mg), and dialyzed supernatant (II) protein (1.5 mg). Incubation was carried out at 32° and 20 μl aliquots were removed at 0, 10, and 20 min. Each aliquot was added to 0.1 ml of bovine serum albumin (1 mg/ml) and [^{3}H]Phe-tRNA was precipitated by addition of 1 ml of cold 5% trichloroacetic acid. The precipitate was collected by centrifugation at 6000*g* for 10 min, resuspended in 1 ml of cold 5% CCl_3COOH and filtered onto Gelman type E fiber glass filters. The filters were washed with 5 ml of 5% CCl_3COOH and dried in scintillation vials overnight at 60°, and radioactivity was determined by liquid scintillation counting.

Protein was extracted from the remainder of the reaction mixture with cold phenol and sodium dodecyl sulfate. The aqueous phase was collected by centrifugation and mixed with 2 volumes of ethanol. After standing at −20° overnight, the precipitate was collected by centrifugation at 6000*g* for 10 min and dissolved in 0.01 M sodium acetate (pH 5) to give a final [^{3}H]Phe-tRNA concentration of 0.3 μ*M*.

Determination of Poly(U)-Dependent [^{3}H]Phenylalanine Incorporation. The reaction mixture used to assay the activity of the various enzyme and ribosome combinations had the following composition (μmol/ml): spermidine (1.6), ATP (1), GTP (0.25), phosphoenolpyruvate (4.4), $MgCl_2$ (10), mercaptoethanol (6.7), Tris, pH 7.4 (20), KCl (50), pyruvate kinase (90 μg), poly(U) (300 μg), [^{3}H]phenylalanine in the form of Phe-tRNA (24 μmol), sucrose (250), potassium bicarbonate (25), ribosomes, and enzymes as indicated in the legends of tables and figures. The reaction mixtures were incubated at 32° and 50 μl aliquots were removed at 0, 10, 20, and 30 min. Each aliquot was added to 0.1 ml of bovine serum albumin (1 mg/ml) and hot CCl_3COOH-insoluble radioactive material was determined as previously described (Swanson and Dawid, 1970). Incorporation is linear for 20–30 min and results expressed in the tables and figures represent the maximum reaction rates obtained during this time.

Sucrose Gradient Identification of Ribosomes Active in Phenylalanine Incorporation. E. coli or mitochondrial enzymes and ribosomes, as indicated in the legends to Figures 2 and 3, were incubated in 0.4 ml of the reaction mixture described for assay of phenylalanine incorporation. Incubation was carried out at 32° and 50-μl aliquots were removed after 0 and 20 min. Hot CCl_3COOH-precipitable radioactivity was then determined in order to estimate the total amount of poly(phenylalanine) synthesized. The remainder of each reaction mixture was chilled in ice and layered on a 15–30% sucrose gradient made with hypotonic medium. The gradients were centrifuged at 5° in an SW 27.1 Beckman rotor for 17 hr at 27,000 rpm and 20 drop fractions were collected. After addition of 0.1 ml of bovine serum albumin (1 mg/ml) to each fraction, hot CCl_3COOH-insoluble radioactivity was determined in the usual manner. The pellets on the tube bottoms were suspended in 1 ml of water, bovine serum albumin was added and radioactive material in the resultant suspension was also determined. Sedimentation coefficients were determined by the method of McEwen (1967). The accuracy of this method was determined with intact *E. coli* ribosomes which were found to have a sedimentation coefficient of 70–71 S.

Results

Tables I, II, and III summarize the results of experiments designed to test the ability of various combinations of elongation factors and ribosomes to catalyze poly(U)-dependent phenylalanine incorporation. In all cases incorporation has an absolute dependence on poly(U).

Before considering the results presented in Tables I, II, and III, it should be noted that parts a and b of each table differ in that the former presents activities in terms of the concentration of elongation factors while the latter gives activities relative to the concentration of ribosomes. Unless otherwise indicated, conditions were chosen such that the rates of incorporation are dependent on the concentration of elongation factors. Therefore, rates of incorporation presented in part b of each table give a minimum estimate of the potential activity of each ribosome preparation.

It is clear from the results in Table Ia that *Xenopus* and rat cytoplasmic elongation factors function well with both types of cytoplasmic ribosomes. Neither enzyme preparation is active with ribosomes from *Xenopus* mitochondria or *E. coli*. Since the ribosomes from *E. coli* and mitochondria are quite active with the homologous enzymes (Table Ib), it is apparent that the lack of activity of the cytoplasmic enzymes with those ribosomes is not due to inactive ribosome preparations.

The elongation factors from *Xenopus* mitochondria were also tested with various types of ribosomes (Table IIa). Mitochondrial elongation factors are most active with ribosomes from mitochondria and *E. coli*. In order to eliminate the possibility that the low rates of phenylalanine incorporation with cytoplasmic ribosomes might be due to rate-limiting concentrations of these ribosomes, the rates obtained with the homologous enzymes are compared to those obtained with the mitochondrial enzymes (Table IIb). Since the rates of incorporation per milligram of cytoplasmic ribosomal protein are much greater with the cytoplasmic elongation factors than with the mitochondrial elongation factors, it appears that the relatively low activity of the latter combination is not due to defective ribosomes.

The results in Table IIIa indicate that *E. coli* elongation factors function only with ribosomes from *E. coli* or mitochondria despite the fact that the cytoplasmic ribosomes used in this experiment are quite active with the homologous cytoplasmic elongation factors (Table IIIb). Furthermore, Table IIIb shows that the mitochondria ribosomes are four times as active with mitochondrial enzymes as they are with the amount of *E. coli* enzymes used in Table IIIa.

In Table IIIb the rate of incorporation by the homologous mitochondrial system is that obtained in the presence of excess elongation factors. Therefore, it represents the maximum rate of incorporation obtainable with this preparation of mitochondrial ribosomes. In order to determine the maximum rate of incorporation which can be supported by these ribosomes in the presence of *E. coli* elongation factors, increasing amounts of *E. coli* factors were assayed for ability to catalyze phenylalanine incorporation in the presence of a fixed amount of mitochondrial ribosomes. For comparison, *E. coli* ribo-

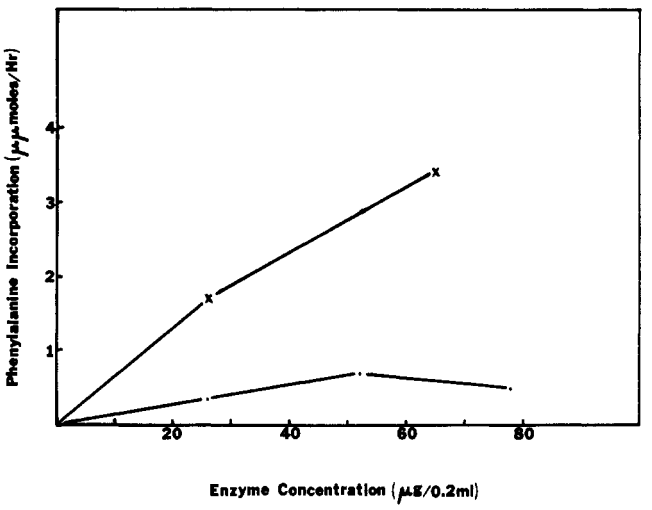

FIGURE 1: Incorporation of phenylalanine into peptides in the presence of *E. coli* elongation factors and mitochondrial or *E. coli* ribosomes. Variable amounts of *E. coli* elongation factors were mixed with *Xenopus* mitochondrial ribosomes (387 μg) or *E. coli* ribosomes (68 μg) in a final volume of 0.2 ml and assayed for ability to catalyze phenylalanine incorporation (see Methods). Mitochondrial ribosomes (·) and *E. coli* ribosomes (X).

TABLE I: [^{3}H]Phenylalanine Incorporation into Poly(phenylalanine) by Cytoplasmic Elongation Factors Combined with Ribosomes from Various Sources.[a]

Ribosome Source	Enzyme Source	pmol/mg of Enzyme Protein per hr	%
	Part a		
Xenopus cyto	*Xenopus* cyto	1.2	100
Rat cyto	*Xenopus* cyto	2.7	225
E. coli	*Xenopus* cyto	<0.01	<1
Xenopus mito	*Xenopus* cyto	<0.01	<1
Xenopus cyto	Rat cyto	0.7	44
Rat cyto	Rat cyto	1.6	100
E. coli	Rat cyto	<0.01	<1
Xenopus mito	Rat cyto	<0.01	<1
		pmol/mg of Ribosome Protein per hr	
	Part b		
Xenopus mito	Rat or *Xenopus* cyto	<0.01	
Xenopus mito	*Xenopus* mito	1.4	
E. coli	Rat or *Xenopus* cyto	<0.01	
E. coli	*E. coli*	77.7	

[a] The following amounts of ribosomes and elongation factors were assayed for ability to incorporate phenylalanine into CCl_3COOH-insoluble protein as described in Methods (μg of protein/0.2 ml of reaction mixture): *Xenopus* cytoplasmic enzymes (550); rat cytoplasmic enzymes (520); *Xenopus* cytoplasmic ribosomes (425); rat cytoplasmic ribosomes (285); *E. coli* ribosomes (42); *E. coli* enzymes (26); *Xenopus* mitochondrial ribosomes (390); *Xenopus* mitochondrial enzymes (170).

TABLE II: [^{3}H]Phenylalanine Incorporation into Poly(phenylalanine) by *Xenopus* Mitochondrial Elongation Factors Combined with Ribosomes from Various Sources.[a]

Ribosome Source	Enzyme Source	pmol/mg of Enzyme Protein per hr	%
	Part a		
Xenopus cyto	*Xenopus* mito	1.1	14
Rat cyto	*Xenopus* mito	2.2	28
E. coli	*Xenopus* mito	13.0	160
Xenopus mito	*Xenopus* mito	8.0	100
		pmol/mg of Ribosome Protein per hr	
	Part b		
Xenopus cyto	*Xenopus* mito	0.4	
Xenopus cyto	*Xenopus* cyto	1.5	
Rat cyto	*Xenopus* mito	0.7	
Rat cyto	Rat cyto	2.7	

[a] [^{3}H]Phenylalanine incorporation was assayed as described in the legend of Table I. The following amounts of ribosomes and elongation factors were used (μg of protein/0.2 ml of reaction mixture): *Xenopus* mitochondrial enzymes (75); *Xenopus* cytoplasmic enzymes (550); *Xenopus* cytoplasmic ribosomes (220); rat cytoplasmic ribosomes (240); rat cytoplasmic enzymes (520); *E. coli* ribosomes (100); *Xenopus* mitochondrial ribosomes (530).

somes were also assayed for activity with variable amounts of *E. coli* elongation factors. The results presented in Figure 1 indicate that the maximum rate of incorporation obtained with 387 μg of mitochondrial ribosomal protein is 0.65 pmol/hr or 1.68 pmol/hr per mg of ribosomal protein. This rate is half the rate obtained with the homologous mitochondrial system (Table IIIb). It is apparent from these results that the low rate of incorporation in the presence of *E. coli* elongation factors and mitochondrial ribosomes (Table IIIa) is not due to a rate limiting ribosome concentration.

The mitochondrial and *E. coli* ribosomes have sedimentation coefficients of 60 and 70 S, respectively, making possible the resolution of these ribosomes by sucrose gradient centrifugation. When mitochondrial elongation factors are incubated with mitochondrial or *E. coli* ribosomes, radioactive polypeptides are found in the 60 S or 70 S regions of the gradients, respectively (Figure 2). Similar results are obtained when *E. coli* elongation factors are substituted for mitochondrial factors (Figure 3): in the presence of mitochondrial ribosomes nascent polypeptides sediment at 60 S and in the presence of *E. coli* ribosomes labeled polypeptides are in the 70 S region of the gradient. These results eliminate the possibility that activity obtained with mitochondrial ribosomes and *E. coli* elongation factors may be due to bacterial contamination of the mitochondrial preparations. The resolution of the two ribosome types is quite reproducible when both gradients are centrifuged simultaneously. It can be seen that the positions of the 60 S and 70 S peaks of radioactivity in Figure 2 differ slightly from the positions of the corresponding peaks in Figure 3. This is due to the fact that the analyses reported

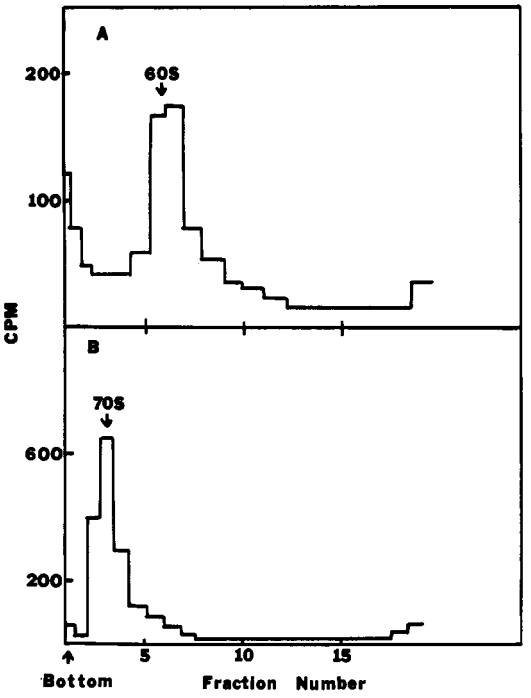

FIGURE 2: Sedimentation characteristics of ribosomes labeled with nascent poly(phenylalanine) chains in the presence of *E. coli* elongation factors. (A) Mitochondrial ribosomes (264 μg) or (B) *E. coli* ribosomes (63 μg) were incubated with *E. coli* elongation factors (52 μg) under conditions required for poly(phenylalanine) synthesis and the reaction mixtures were simultaneously analyzed by sucrose gradient centrifugation (see Methods).

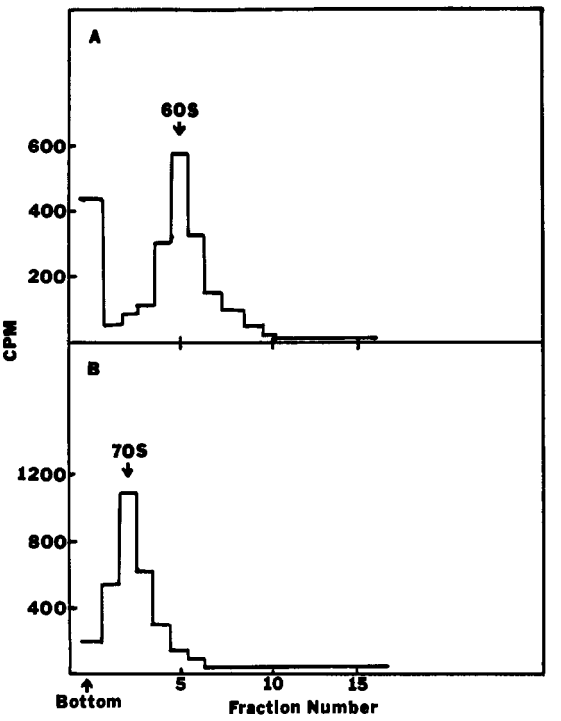

FIGURE 3: Sedimentation characteristics of ribosomes labeled with nascent poly(phenylalanine) chains in the presence of mitochondrial elongation factors. (A) Mitochondrial ribosomes (264 μg) or (B) *E. coli* ribosomes (126 μg) were incubated with mitochondrial elongation factors (450 μg) and analyzed as described in the legend to Figure 2.

in Figure 2 were not performed at the same time as those reported in Figure 3.

The results in Table IV indicate that 71–93% of the radioactive protein applied to the gradients was recovered after centrifugation. Therefore, the gradient analyses give an accurate representation of the type of ribosome carrying out polypeptide synthesis in each case. These experiments demonstrate that the activity observed in each case is actually dependent upon the particular ribosome preparation used and is not due to contamination of elongation factor preparations with ribosomes.

Discussion

The results presented in this report indicate that the properties of the *Xenopus* cytoplasmic and mitochondrial protein synthesizing systems are similar to those from yeast and *Neurospora* (Kuntzel, 1969; Richter and Lipmann, 1970; Scragg, 1971). The *Xenopus* cytoplasmic ribosomes and elongation factors freely interchange with the corresponding fractions from rat liver cytoplasm but show little or no ability to substitute for those from mitochondria or *E. coli*. Furthermore, *Xenopus* mitochondrial ribosomes and elongation factors are functionally compatible with the appropriate *E. coli* constituents.

Throughout this report it has been assumed that the rate limiting step in poly(U)-dependent phenylalanine incorporation is elongation rather than initiation or termination. This assumption appears reasonable for two reasons. First, the results presented in Figures 2 and 3 indicate that essentially all radioactive polypeptides formed in the presence of mitochondrial or bacterial ribosomes are associated with the intact ribosomes. Thus, termination does not occur in this

TABLE III: [^{3}H]Phenylalanine Incorporation into Poly(phenylalanine) by *E. coli* Elongation Factors Combined with Ribosomes from Various Sources.[a]

Ribosome Source	Enzyme Source	pmol/mg of Enzyme Protein per hr	%
	Part a		
Xenopus cyto	*E. coli*	<0.1	<1
Rat cyto	*E. coli*	<0.1	<1
E. coli	*E. coli*	58	100
Xenopus mito	*E. coli*	12.3	21
		pmol/mg of Ribosome Protein per hr	
	Part b		
Xenopus cyto	*Xenopus* cyto	5.4	
Rat cyto	Rat cyto	5.4	
Rat or *Xenopus* cyto	*E. coli*	<0.01	
Xenopus mito	*E. coli*	0.82	
Xenopus mito	*Xenopus* mito	3.5	

[a] [^{3}H]Phenylalanine incorporation was assayed as described in the legend to Table I. The following amounts of ribosomes and elongation factors were used (μg of protein/0.2 ml of reaction mixture): *E. coli* enzymes (26); *Xenopus* cytoplasmic ribosomes (840); rat cytoplasmic ribosomes (380); *E. coli* ribosomes (68); *Xenopus* cytoplasmic enzymes (1440); rat cytoplasmic enzymes (1290); *Xenopus* mitochondrial enzymes (220); *Xenopus* mitochondrial ribosomes (387).

TABLE IV: Recovery of [^{3}H]Phenylalanine from Sucrose Gradients.

Enzyme Source	Ribosome Source	Cpm Applied[a]	Cpm Recovd[b]	%
E. coli	*Xenopus* mito	648	600	93
E. coli	*E. coli*	2520	1800	71
Xenopus mito	*Xenopus* mito	2500	2253	90
Xenopus mito	*E. coli*	3798	3460	91

[a] Radioactivity applied to each gradient was determined by multiplying the amount of [^{3}H]phenylalanine incorporated in 50 μl of the reaction mixture by 6 (see Methods). [b] Cpm recovered was corrected for a background of 26 cpm/fraction.

system and cannot be the rate limiting step in the reaction. Second, it has been shown in yeast and *Neurospora* that poly(U)-dependent poly(phenylalanine) synthesis on mitochondrial ribosomes occurs in the presence of highly purified elongation factors; *i.e.*, soluble initiation factors are not required in this reaction (Scragg, 1971; Richter and Lipmann, 1970).

When considering the results presented in Tables I, II, and III one should keep in mind the fact that the absolute rate of incorporation for any combination of ribosomes and elongation factors in part a of any table is determined by the purity of that particular preparation of elongation factors. Although the absolute activity of various preparations of elongation factors from the same source varied when tested with a given type of ribosome (results not shown), the relative ability of each elongation factor preparation to interact with various types of ribosomes remained constant. In other words, comparisons of absolute activities presented in one table with those presented in another are meaningless: only comparisons of relative activities within the same part of the same table are useful.

The results in Table IIa indicate that there is a low level of interaction between mitochondrial elongation factors and cytoplasmic ribosomes. This may have been due to either contamination of the mitochondrial preparations with cytoplasmic enzymes or relatively low specificity of the mitochondrial enzymes. It is also possible that the cytoplasmic ribosomes were contaminated by G factor and the mitochondrial preparation served as a T factor source. This combination might have activity since it was shown that fungi mitochondrial or cytoplasmic T factor in combination with cytoplasmic G factor catalyze poly(U)-dependent poly(phenylalanine) synthesis on cytoplasmic ribosomes (Richter and Lipmann, 1970). However, this explanation is unlikely since *E. coli* enzymes were not active with cytoplasmic ribosomes (Table III); *E. coli* T factor would also be expected to function with cytoplasmic G factor on cytoplasmic ribosomes (Richter, 1970). Although an explanation of these results will require further purification of the *Xenopus* elongation factors from both sources, the existence of the cross reaction does not significantly affect the conclusions drawn from the experiment. It is evident that the mitochondrial enzymes are much more active with mitochondrial or *E. coli* ribosomes than with either cytoplasmic ribosome preparation.

Under conditions where the concentration of elongation factors is rate limiting the activity of the *E. coli* elongation factors with mitochondrial ribosomes is only 21% of the activity obtained with the homologous *E. coli* system (Table IIIa). These results differ from those of Kuntzel (1969) who found that *E. coli* elongation factors interact with the mitochondrial ribosomes of *Neurospora* almost as well as with *E. coli* ribosomes. Even under conditions where the concentration of *Xenopus* mitochondrial ribosomes determines the rate of phenylalanine incorporation, the maximum activity obtained with *E. coli* elongation factors (Figure 1) is only half that obtained with mitochondrial elongation factors (Table IIIb). Once again quite different results were obtained with fungi mitochondrial ribosomes which have greater levels of activity with *E. coli* elongation factors than with mitochondrial factors (Richter and Lipmann, 1970; Scragg, 1971). The relatively low interaction of the *Xenopus* mitochondrial and *E. coli* systems may be due to incomplete removal of an inhibitor of mitochondrial ribosomes present in the *E. coli* enzyme preparations (see Methods) or to functional differences between ribosomes of *Xenopus* mitochondria and those of fungi mitochondria and bacteria. In favor of the latter possibility it appears that the mitochondrial ribosomes of *Xenopus* are much smaller than the ribosomes of fungi mitochondria or bacteria (Borst, 1972) and would therefore be expected to have different functional characteristics. If such differences do exist it seems likely that further comparisons of the physical and catalytic properties of vertebrate mitochondria ribosomes and ribosomes from other sources may provide information on the relationship between ribosomal structure and function. Such information may explain, for example, why cytoplasmic ribosomes of eucaryotes are so much larger than those from mitochondria and procaryotes; *i.e.*, it may be possible to identify requirements of eucaryotic cells which can only be satisfied by the larger ribosomes.

Acknowledgment

I thank Mrs. Nancy McFarlane for excellent technical assistance and Dr. Elizabeth Kutter for providing *E. coli* cells. I am also grateful to Mr. D. Beebe, Dr. I. B. Dawid, Mr. R. Devlin, Dr. C. Emerson, Mr. G. Koch, and Dr. L. Rebhun for help in preparation of the manuscript.

References

Ashwell, M., and Work, T. S. (1970), *Annu. Rev. Biochem. 39*, 251.

Borst, P. (1972), *Annu. Rev. Biochem. 41*, 333.

Dawid, I. B., and Chase, J. W. (1972), *J. Mol. Biol. 63*, 217.

Gordon, J., Lucas-Lenard, J., and Lipmann, F. (1971), *Method Enzymol. 20*, 281.

Layne, E. (1957), *Method Enzymol. 3*, 447.

Kuntzel, H. (1969), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 4*, 140.

Loening, U. E. (1968), *J. Mol. Biol. 38*, 355.

Maxwell, E. S. (1962), *Proc. Nat. Acad. Sci. U. S. 48*, 1639.

McEwen, C. R. (1967), *Anal. Biochem. 20*, 114.

Parisi, B., Milanesi, G., van Etten, J. L., Perani, A., and Ciferri, O. (1967), *J. Mol. Biol. 28*, 295.

Richter, D. (1970), *Biochem. Biophys. Res. Commun. 38*, 864.

Richter, D., and Lipmann, F. (1970), *Biochemistry 9*, 5065.

Scragg, A. H. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 17*, 111.

Swanson, R. F., and Dawid, I. B. (1970), *Proc. Nat. Acad. Sci. U. S. 66*, 117.