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# A Factor from *Escherichia coli* Concerned with the Stimulation of Cell-Free Polypeptide Synthesis by Exogenous Ribonucleic Acid. I. Evidence for the Occurrence of a Stimulation Factor*

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ABSTRACT: The high-speed supernatant fraction of *Escherichia coli* contains a factor which enhances polypeptide synthesis by the *E. coli* cell-free system in the presence of $f_2$ ribonucleic acid (RNA), but has little effect on endogenous activity (activity without added RNA). The factor is nondialyzable and is rapidly inactivated at 37°. *E. coli* ribosomes are capable of endogenous polypeptide synthesis in the presence of *Euglena gracilis* high-speed supernatant fraction, but the mixed system was not stimulated by $f_2$ RNA. *Euglena* chloroplast ribosomes, which are markedly stimulated by viral RNA in the presence of *Euglena* supernatant fraction, were also unresponsive to $f_2$ RNA when incubated with *E. coli* supernatant. The mixed system of *E. coli* ribosomes plus *Euglena* supernatant responded to $f_2$ RNA when supplemented with the factor preparation.

The results indicate the existence of a factor concerned with polypeptide chain initiation in protein synthesis. The factor seems to be species specific, as indicated by the fact that it functions only with ribosomes from the same species.

The induction of protein synthesis by messenger ribonucleic acid (m-RNA) presumably involves some steps concerned with polypeptide chain initiation, distinct from the reactions which operate in the assembly and polymerization of amino acids. The occurrence of factors concerned with chain initiation is suggested by the study of Lamfrom and Knopf (1964) with the reticulocyte cell-free system. These authors found that reticulocyte ribosomes in the presence of crude supernatant promoted the synthesis of new hemoglobin chains, while with purified supernatant factors only completion of preexisting chains occurred. Cell-free extracts from *Escherichia coli* and *Euglena gracilis* can promote the synthesis of $f_2$ coat protein in the presence of $f_2$ RNA (Nathans *et al.*, 1962; Schwartz *et al.*, 1965). These systems, therefore, are capable of supporting chain initiation and must contain the factors presumably involved in this process.

The present study was prompted by the observation that *E. coli* ribosomes supplemented with *Euglena* high-speed supernatant fractions, although active in endogenous polypeptide synthesis, cannot be stimulated by $f_2$ RNA. *Euglena* chloroplast ribosomes are also active in the presence of *E. coli* high-speed supernatant, but again no stimulation by $f_2$ RNA is obtained with this mixed system. These results suggested that the supernatant fractions from *E. coli* and *E. gracilis* contain a factor (or factors) essential for the induction of polypeptide synthesis by exogenous RNA, and that this factor can operate only with ribosomes from the homologous species. In view of the possible relevance of this finding to the problem of chain initiation

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in protein synthesis, a search for the "stimulation" factor was undertaken. Fractions obtained from *E. coli* extracts were examined for their ability to enhance selectively the $f_2$ RNA stimulated amino acid incorporation by *E. coli* ribosomes. The present report deals with the evidence for the occurrence of such a "stimulation" factor in *E. coli*, and with its partial purification. In this report, the incorporation by ribosomes in the absence and in the presence of RNA will be referred to as endogenous and exogenous synthesis, respectively.

## Experimental Section

*Cell-Free Preparations.* The extract from *E. coli* (strain B) used for amino acid incorporation was prepared by preincubation of 30,000*g* supernatant fluid (S-30) by the procedure of Matthaei and Nirenberg (1961), modified somewhat as described previously (Brawerman *et al.*, 1963). Ribosomes were prepared from the preincubated S-30 fraction by centrifugation at 100,000*g* for 2 hr. The sedimented ribosomes were either washed by resuspension in the extracting medium (0.02 M Tris–HCl, pH 8.0; 0.1 M KCl; 5 mM $MgCl_2$; and 6 mM β-mercaptoethanol) and recentrifugation, or treated with sodium deoxycholate[1] by including it in the extracting medium at a concentration of 0.5% prior to centrifugation. The DOC-treated ribosomes were further washed in extracting medium. The final ribosomal pellets were suspended in a small volume of the same solution and stored in liquid $N_2$. The supernatant fraction was isolated from nonincubated S-30 by centrifuging at 100,000*g* for 2 hr, and using the upper two-thirds of the supernatant fluid. This was dialyzed overnight in the cold against 0.02 M Tris buffer, pH 7.6, and 6 mM β-mercaptoethanol, and stored in liquid $N_2$.

Chloroplast ribosomes were prepared from highly purified chloroplasts derived from *E. gracilis* (strain Z) grown autotrophically (Eisenstadt and Brawerman, 1964a). The *Euglena* supernatant preparation was obtained by ammonium sulfate fractionation of 100,000*g* supernatant fluid of disrupted cells as described previously (Eisenstadt and Brawerman, 1964b).

*RNA Preparations.* Transfer ribonucleic acid (t-RNA) from *E. coli* and from *Euglena* was prepared by phenol extraction of disrupted cells as described previously (Eisenstadt and Brawerman, 1964b). Polysaccharides were removed from the *E. coli* preparations by partition between potassium phosphate buffer, pH 7.5, and 2-methoxyethanol, according to Kirby (1964).

$f_2$ RNA was prepared from the virus (a generous gift from Dr. N. Zinder) by the following procedure. The virus, suspended in 0.1 M Tris buffer, pH 7.6, was mixed with an equal volume of ice-cold 80% aqueous phenol, and the mixture stirred for 1 hr at room temperature. The RNA was precipitated from the aqueous phase by addition of 0.1 volume of 10% NaCl and 2.5 volumes of ethanol. The precipitate was washed several times with cold 66% aqueous ethanol containing 0.3% NaCl. All RNA preparations were dissolved in water and stored at −20°.

*Isolation of Stimulation Factor.* Washed *E. coli* cells (strain B), obtained from late log phase cultures (Klett reading 172 with no. 66 filter) in trypticase-soy broth (Difco), were used fresh or stored at −20° for up to several months before use. The cells, 40 g of wet weight, were suspended in 80 ml of 0.02 M Tris buffer (pH 8.0), 0.1 M KCl, 5 mM $MgCl_2$, and 6 mM β-mercaptoethanol, and passed through the french press at 15,000 psi. After incubation with 300 μg of DNAase (Worthington) for 10 min in the cold, the suspension was centrifuged at 23,000*g* for 1 hr, and the supernatant fluid was further centrifuged at 100,000*g* for 2 hr. An equal volume of ammonium sulfate solution (saturated at 4° and adjusted to pH 7.0) was added to the resulting supernatant. After 15 min in the cold, the precipitate was removed by centrifugation at 10,000*g* for 10 min, and saturated ammonium sulfate was added to the supernatant (4.3 ml/10 ml of supernatant). After 15 min, the pellet was collected by centrifugation and drained well to remove excess supernatant. The subsequent steps could be postponed until the next day and the pellet kept overnight in the cold. The pellet was next dissolved in 34 ml of 0.01 M Tris buffer, pH 7.6, and 0.006 M β-mercaptoethanol, and 11.5 ml of saturated ammonium sulfate was added per 10 ml of solution. The resulting precipitate was removed as above, and saturated ammonium sulfate was added to the supernatant (2 ml/10 ml of supernatant). The precipitate, which represents the "purified" factor, was collected by centrifugation. The pellet was dissolved in 2 ml of 0.01 M Tris buffer, pH 7.6, and 0.006 M β-mercaptoethanol, dialyzed overnight against the same solution, distributed in small vials, and stored in liquid $N_2$. The final protein concentration of the preparations was about 12 mg/ml.

*Amino Acid Incorporations.* The incorporations with the *E. coli* cell-free system were carried out by the procedure of Matthaei and Nirenberg (1961), with some modifications. The reaction mixtures contained 0.1 M Tris–HCl buffer, pH 8.0; 32 mM KCl; 20 mM $NH_4Cl$; 10 mM $MgCl_2$; 4 mM phosphoenolpyruvate ($Na^+$ salt); 1 mM ATP ($K^+$ salt); 0.6 mM GTP; 1.8 mM β-mercaptoethanol; 40 μM each of a mixture of unlabeled amino acids (minus leucine or phenylalanine); 110 μg/ml of *E. coli* t-RNA; 20 μg/ml of phosphoenolpyruvate kinase (Boehringer Mannheim Corp.); 0.6 μc/ml of either L-[$^{14}$C]leucine (40 μc/μmole) or L-[$^{14}$C]phenylalanine (330 μc/mole). The reaction mixtures were incubated at 37° for 45 min. Samples were then placed on filter paper disks and processed for scintillation counting by the procedure of Mans and Novelli (1961).

For the incorporations with the mixed *Euglena* and *E. coli* systems, the concentrations of some of the components were altered in order to be closer to those

[1] Abbreviations used are: DOC, sodium deoxycholate; DNAase, deoxyribonucleate oligonucleotide-hydrolase; ATP, adenosine 5′-triphosphate; GTP, guanosine 5′-triphosphate.

optimal for the *Euglena* cell-free system (Eisenstadt and Brawerman, 1964b): 5 mM KCl, 4 mM phosphoenolpyruvate, 2 mM ATP. Glutathione, 15 mM, was used instead of β-mercaptoethanol. No *E. coli* t-RNA was included, unless stated otherwise in the individual experiments.

*Analytical Methods.* Protein and RNA were determined as described previously (Brawerman, 1963).

## Results

*Polypeptide Synthesis in Mixed E. coli and Euglena Cell-Free Systems.* Addition of $f_2$ RNA to the cell-free systems from *E. coli* and from *Euglena* chloroplasts produces a marked stimulation of polypeptide synthesis (Table I). When the ribosomes from each species are supplemented with high-speed supernatant from the heterologous species, the resulting mixed systems, although capable of polypeptide synthesis, are not stimulated by $f_2$ RNA.

TABLE I: Effect of $f_2$ RNA on Leucine Incorporation in Mixed *Euglena* and *E. coli* Systems.[a]

| Ribosomes | Supernatant Fraction (ml) | $f_2$ RNA None | 200 μg/ml | 400 μg/ml |
|---|---|---|---|---|
| *E. coli* | None | 16 | ... | ... |
| | *E. coli* (0.02) | 110 | 720 | 1500 |
| | *Euglena* (0.02) | 350 | 350 | 350 |
| | (0.04) | 420 | ... | ... |
| *Euglena* chloroplasts | None | 23 | ... | ... |
| | *Euglena* (0.02) | 360 | 700 | 1300 |
| | *E. coli* (0.02) | 540 | 490 | 560 |
| | (0.04) | 1140 | ... | ... |

[a] Ribosomes were treated with DOC. *E. coli* ribosomes derived from preincubated S-30. Amounts of protein in the *Euglena* and *E. coli* supernatant fractions were 8.0 and 5.5 mg/ml, respectively. Each supernatant fraction also contained 2 mg/ml of the homologous t-RNA. Values expressed as counts per minute per milliliter of reaction.

The capacity of the mixed cell-free systems for endogenous polypeptide synthesis is clearly indicated by the results shown in Tables I and II. The activity of *E. coli* ribosomes with 0.02 ml of *Euglena* supernatant appears to be near the maximum capacity of this system, since a doubling of the amount of supernatant produces little additional increase in activity (Table I). The smaller effect of *E. coli* supernatant is rather puzzling, but this was not observed in all instances. Moreover, the stimulation by *E. coli* supernatant can be further enhanced by increasing the amount of supernatant (see Figure 1).

The *Euglena* chloroplast ribosomes also appear to be more effective with the heterologous supernatant, and in this case the activity is doubled when twice as much supernatant is used (Table I). This would suggest that some *E. coli* factors are more effective with chloroplast ribosomes than are the *Euglena* factors. In this context, it may be significant that the *Euglena* supernatant used for incorporation by chloroplast ribosomes is derived from the total cell extract, rather than from the chloroplasts. It is possible that the chloroplast incorporation factors differ from those for the cytoplasmic ribosomal system in *Euglena*. The chloroplast incorporation factors would then be present in relatively small amounts in the *Euglena* supernatant used here.

TABLE II: Effect of t-RNA on Endogenous Incorporation of Leucine by *E. coli* Ribosomes in Mixed Systems.[a]

| Supernatant Fraction | t-RNA None | *E. coli* | *Euglena* |
|---|---|---|---|
| None | 12 | 160 | 100 |
| *E. coli* | 1302 | 2970 | 1130 |
| *Euglena* | 510 | 3080 | 970 |

[a] *E. coli* ribosomes, prepared from nonpreincubated S-30 and washed three times, were used at a concentration of 3 mg/ml; *E. coli* and *Euglena* supernatant protein, 0.55 and 1.4 mg/ml, respectively; added t-RNA from each species, 0.5 mg/ml. For other details see Table I.

*Species Specificity of Euglena and E. coli t-RNA.* In order to determine the requirements of the mixed cell-free systems, the species specificity of the t-RNA derived from each type of cells was investigated. The *E. coli* cell-free system (ribosomes and supernatant from *E. coli*) is greatly stimulated by the addition of homologous t-RNA (Table II). When *Euglena* transfer RNA is used, however, no stimulatory effect is observed. The activity of the *E. coli* system without added transfer RNA can be attributed to the RNA normally present in the *E. coli* supernatant fraction.

The experiments with the *Euglena* supernatant fraction show that *Euglena* t-RNA is capable of supporting the activity of *E. coli* ribosomes to a limited extent (Table II). In the mixed system, *E. coli* ribosomes and *Euglena* supernatant, no *E. coli* t-RNA is present, yet substantial activity is evident. Addition of *Euglena* t-RNA produces an increase in the activity. *E. coli* t-RNA with *Euglena* supernatant, however, is far more effective, and the level of activity achieved in this case is the same as that obtained with *E. coli* supernatant (Table II). It appears from these experiments that *Euglena* supernatant can effectively replace *E. coli* supernatant in promoting endogenous polypeptide synthesis by *E. coli* ribosomes, but that *Euglena* aminoacyl t-RNA serves as a rather poor donor of amino acids in this system.

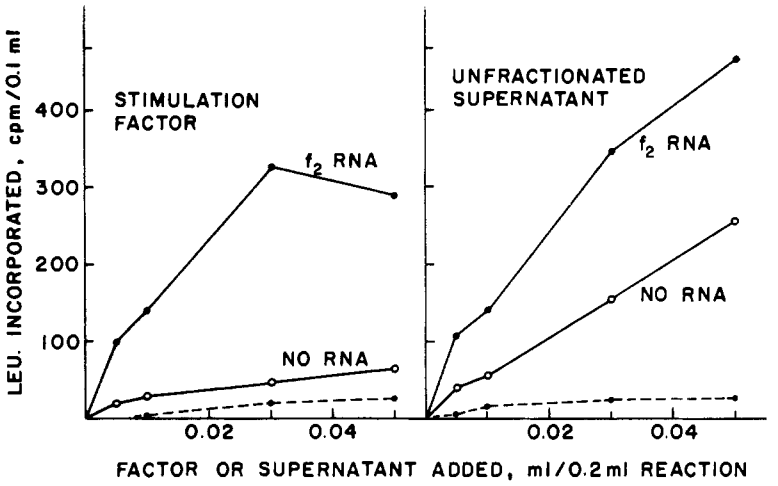


FIGURE 1: Preferential effect of factor on leucine incorporation in the presence of $f_2$ RNA. Amount of S-30 used, 0.1 ml/0.2 ml of reaction; amount of protein in *E. coli* supernatant was 11 mg/ml. Closed circles, incorporation in the presence of RNA; open circles, incorporation in the absence of RNA; dashed lines, radioactivity insoluble in hot acid obtained when factor or supernatant was incubated without S-30.

TABLE III: Attachment of Leucine to t-RNA by Homologous and Heterologous Supernatants.[a]

| Supernatant Fraction | t-RNA[b] None | t-RNA[b] *E. coli* (215 μg/ml) | t-RNA[b] *Euglena* (175 μg/ml) | Incorpn/mg of RNA *E. coli* | Incorpn/mg of RNA *Euglena* |
|---|---|---|---|---|---|
| *Euglena* | 16 | 6530 | 3560 | 30,300 | 20,200 |
| *E. coli* | 425 | 7240 | 1510 | 31,700 | 6,800 |

[a] t-RNA incubated for 10 min with *Euglena* supernatant fraction (1.5 mg/ml) or *E. coli* supernatant fraction (0.3 mg/ml) under the same conditions as for polypeptide synthesis, but without ribosomes. Samples were placed on filter paper disks, washed extensively with cold trichloroacetic acid, and counted as described in the Experimental Section. [b] Values expressed as counts per minute per milliliter of reaction.

The charging of t-RNA by the heterologous supernatant fractions was examined in the case of leucine (Table III). The RNA from *E. coli* is charged equally well by the supernatant fractions derived from *E. coli* and from *Euglena*. t-RNA from *Euglena*, however, is charged relatively poorly by the supernatant fraction from *E. coli*.

*Stimulation of Exogenous Incorporation by Fractions from E. coli High-Speed Supernatant.* Since the above results suggested that the high-speed supernatant contains a factor required for stimulation of amino acid incorporation by viral RNA, a search for such a factor was undertaken in *E. coli* supernatant. The criterion used for the detection of this factor was the ability to stimulate polypeptide synthesis in the presence of $f_2$ RNA, but not endogenous synthesis. The test system consisted of *E. coli* S-30 incubated in the presence and in the absence of $f_2$ RNA. Since the S-30 preparation should normally contain the presumed factor, it was used in small amounts in the hope that the factor would then be limiting, and that the response to added factor would be more apparent.

Fractions obtained by adsorption of *E. coli* supernatant on DEAE-cellulose and stepwise elution at different salt concentrations showed a differential effect on endogenous and exogenous incorporation of leucine (Table IV). The fractions obtained with 0.1 and 0.3 M NaCl showed a marked stimulation of incorporation in the presence of RNA, while having relatively little effect in its absence. A fraction which precipitated between 41 and 63% saturation with ammonium sulfate was also relatively more effective in the presence of $f_2$ RNA (Table IV). When this fraction was subjected to stepwise precipitation with ammonium sulfate, the bulk of the stimulation activity was concentrated in the fractions precipitated at 55 and 60% saturation (Table V). These fractions also showed the lowest effect on endogenous activity.

In the course of these experiments, it was noted that the unfractionated *E. coli* supernatant and some of the fractions, after dialysis, produced a considerable incorporation of leucine in the absence of ribosomes. This probably represents terminal addition to soluble proteins, as described by Kaji *et al.* (1965). This effect

TABLE IV: Stimulation Factor Activity in Fractions from *E. coli* High-Speed Supernatant.[a]

| Fractions Added | Endogenous | +$f_2$ RNA | Stimulation by $f_2$ RNA[b] |
|---|---|---|---|
| None | 110 | 300 | 2.8 |
| DEAE, not adsorbed | 940 | 1960 | 2.1 |
| Eluted with 0.1 M NaCl | 130 | 530 | 4.2 |
| 0.3 M NaCl | 190 | 1050 | 5.6 |
| 0.5 M NaCl | 190 | 500 | 2.7 |
| 0.7 M NaCl | 110 | 340 | 3.0 |
| 0.9 M NaCl | 170 | 290 | 1.7 |
| $(NH_4)_2SO_4$ precipitates | | | |
| 41% saturation | 1330 | 1830 | 1.4 |
| 41–63% saturation | 340 | 1540 | 4.6 |
| 63–79% saturation | 230 | 280 | 1.2 |

[a] High-speed supernatant fluid from *E. coli* subjected to fractionation procedures in the presence of 10 mM Tris buffer, pH 7.6, and 6 mM β-mercaptoethanol. DEAE fractionation done by batch procedure. All fractions dialyzed overnight. Amount of $f_2$ RNA, 100 μg/ml. Results expressed as amount of leucine (counts per minute per milliliter) incorporated by *E. coli* S-30 (0.005 ml/0.2 ml of reaction). [b] Stimulation by $f_2$ RNA represents ratio of incorporation in the presence and absence of RNA.

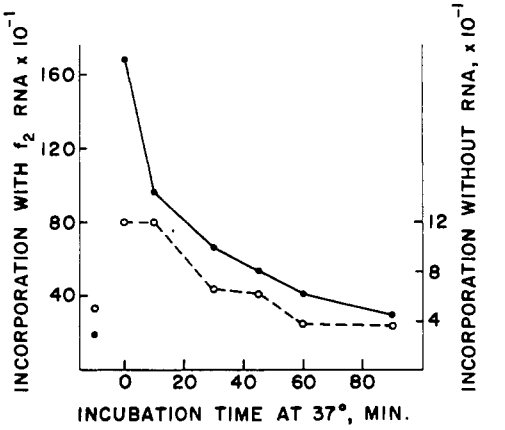


FIGURE 2: Effect of incubation at 37° on activity of stimulation factor. Samples of factor preparation (0.02 ml) kept at 37° for varying time intervals, then added to assay system. S-30, 0.005 ml/0.2 ml of reaction, and labeled phenylalanine used in this experiment. Activities expressed as cpm/0.1 ml. Solid lines and full circles, incorporation in presence of $f_2$ RNA (100 μg/ml); dashed lines and open circles, incorporation in absence of RNA; points at the extreme left of diagram represent levels of activity without factor.

accounts in part for the high values for endogenous incorporation in the presence of some of the fractions (Table IV). This activity was reduced to a low level in the fractions rich in stimulation factor (Table V).

The results in Table V served as a basis for the final isolation procedure. Attempts at further purification of the factor, such as DEAE chromatography or additional ammonium sulfate fractionation, led to a complete loss of activity.

*Stability of Stimulation Factor.* The factor preparations retained their activity for several months when stored in liquid $N_2$. They also remained active after freezing and thawing once. The factor, however, is heat labile as indicated by the rapid loss of activity upon incubation at 37° (Figure 2). It may be noted that approximately 50% of the stimulation activity is lost after 10 min of incubation, while the ability to enhance the endogenous activity is hardly affected at that time.

*Effect of Factor Concentration on Activity.* The effect of factor on the stimulation of $f_2$ RNA increases with concentration up to about 0.03 ml/0.2 ml of reaction (Figure 1). Beyond this it becomes inhibitory. The endogenous incorporation also rises with factor concentration, but to a much smaller extent. In contrast, addition of unfractionated supernatant raises both the endogenous and exogenous activities to about the same extent (Figure 1).

TABLE V: Activity of Ammonium Sulfate Fractions from *E. coli* High-Speed Supernatant.

| | Leucine Incorpn | | |
|---|---|---|---|
| | No S-30 | S-30 | S-30 $f_2$ RNA |
| No addition | ... | 120 | 175 |
| Ammonium sulfate fractions[a] | | | |
| 45% | 164 | 774 | 1470 |
| 50% | 196 | 586 | 1970 |
| 55% | 90 | 490 | 2140 |
| 60% | 144 | 446 | 3180 |
| 65% | 146 | 518 | 702 |
| Unfractionated supernatant | 254 | 800 | 1350 |

[a] Ammonium sulfate (50–65%) fraction from *E. coli* high-speed supernatant fluid was dissolved in buffer (10 mM Tris, pH 7.6, 6 mM β-mercaptoethanol) and subjected to a second fractionation as follows. Ammonium sulfate first added to 45% saturation; resulting precipitate represents 45% fraction. Same procedure continued up to 65% saturation. All precipitates dialyzed overnight against buffer. Amount of S-30 used in reactions, 0.01 ml/0.2 ml. For other details see Table IV.

The fact that the endogenous activity is increased considerably by addition of *E. coli* supernatant indicates that the assay system is deficient in the soluble enzymes

TABLE VI: Effect of Factor on Leucine Incorporation by Mixed System.[a]

| Supernatant Fraction | No Factor | | | Factor Added | | |
|---|---|---|---|---|---|---|
| | No RNA | $f_2$ RNA | Stimulation | No RNA | $f_2$ RNA | Stimulation |
| None | 0 | 20 | ... | 280 | 410 | 1.5 |
| *Euglena* | 280 | 280 | 1.0 | 470 | 1620 | 3.5 |
| *E. coli* | 1870 | 2880 | 1.5 | 1940 | 4000 | 2.1 |

[a] *E. coli* ribosomes, derived from preincubated S-30 and washed three times, 3.6 mg/ml of reaction; *Euglena* supernatant fraction, 1.5 mg of protein/ml; *E. coli* supernatant fraction, 1.7 mg of protein/ml; factor, 0.02 ml/0.2 ml; $f_2$ RNA, 100 μg/ml. *E. coli* t-RNA (100 μg/ml) included in all reactions. Values expressed as counts per minute per milliliter of reaction. Value for factor incubated without ribosomes, 130 substracted from all results with factor.

required for polypeptide synthesis. These enzymes appear to be present to a limited extent in the factor preparation, since the endogenous activity is raised somewhat in its presence.

The interpretation of the experiment described in Figure 1 is complicated by the possibility that the S-30 preparation might have a limited capacity for polypeptide synthesis because of lack of template RNA. The relatively small effect of the factor preparation on the endogenous activity could then be attributed to this limited capacity. It is difficult to assess unequivocally the maximal capacity of a ribosomal preparation. Addition of unfractionated supernatant to the S-30 preparation raises the endogenous activity considerably (Figure 1). This would suggest that the endogenous capacity of this system is far higher than the level reached with the factor. It is conceivable, however, that the effect of supernatant is due in part to some template RNA possibly present in this material. We consider this unlikely because supernatant prepared from preincubated S-30, which has lost most of its template RNA (Brawerman and Eisenstadt, 1964), was found to enhance endogenous incorporation of S-30 preparations to an extent similar to that obtained with untreated supernatant.

*Effect of Factor on the Incorporation with Euglena Supernatant Fraction.* Since *Euglena* supernatant is capable of supporting polypeptide synthesis by *E. coli* ribosomes (Table I), but is presumably lacking a factor necessary for the stimulation of these ribosomes by exogenous RNA, addition of the *E. coli* factor to the mixed system should now permit the RNA-stimulated synthesis to take place. This effect is clearly shown in Table VI. Addition of the factor preparation had a relatively small effect on the endogenous incorporation in the presence of *Euglena* supernatant, but a strong stimulation by $f_2$ RNA was now evident. This exogenous incorporation was far higher than that obtained by the factor alone, although not as high as that reached with the factor in conjunction with *E. coli* supernatant.

## Discussion

The evidence presented in this report indicates that the amino acid incorporation promoted by exogenous RNA requires a factor distinct from those concerned with polypeptide synthesis. This evidence rests essentially on the following two findings: (1) mixed *E. coli* and *Euglena* cell-free systems, while capable of endogenous polypeptide synthesis, cannot be stimulated by $f_2$ RNA; (2) the factor preparation from *E. coli* supernatant enhances preferentially the RNA-stimulated amino acid incorporation. The first finding is unambiguous. It also indicates that the presumed factor is effective only with ribosomes from the same species. Moreover, the system consisting of *E. coli* ribosomes and *Euglena* supernatant becomes responsive to $f_2$ RNA when supplemented with the *E. coli* factor preparation. The second finding is more difficult to interpret, because of the possibility that the factor preparation may contain some of the soluble enzymes for polypeptide synthesis. The S-30 preparation used for the assay of the factor is clearly deficient in these enzymes, since addition of *E. coli* supernatant greatly enhances the endogenous amino acid incorporation. Thus the factor preparation could conceivably act by providing some of these soluble enzymes. If this were the case, however, it should be reasonable to expect that both the endogenous and exogenous activities be enhanced to the same extent. The possibility that the factor effect is due to one of the transfer enzymes of Nishizuka and Lipmann (1966) was examined. Preparations of these enzymes, obtained through the courtesy of Dr. Lipmann, were tested in our assay system, but no stimulation of the RNA-dependent polypeptide synthesis was observed.

The assay system for the factor requires some comments. An ideal system should consist of all the components of protein synthesis in saturating amounts, except for the factor under study. The system used here contains limiting amounts of the soluble enzymes, and must also have some of the factor. The amounts of S-30 used in these studies are two to four times

lower than that usually used in amino acid incorporation experiments. The factor effect was also evident with the higher amount of S-30, but the most consistent results were obtained with the reduced amounts. As is shown in a following publication, *E. coli* supernatant appears to contain an agent which counteracts the effect of the factor.

The extent of the factor effect tended to be variable, but we attribute this to variations in the S-30 preparations rather than to differences in the factor preparations. Although no systematic study of these variations was made, it is our impression that the best S-30 preparations were obtained from cells grown in broth and harvested around the middle of the exponential phase of growth.

The stimulation factor is heat sensitive, and appears to be a protein. This is indicated by the procedure used for its isolation. The present data provide no information on the nature of its action. This problem is explored in a following publication, where it is shown that the reaction stimulated by polyuridylic acid does not require the factor. It also appears that the factor interacts with the ribosomes and leads to a more rapid response to exogenous RNA. This behavior suggests that the factor may play a role in the interaction of ribosomes with template RNA or in the initiation of polypeptide chains, but the nature of the reaction still remains to be determined.

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References

Brawerman, G. (1963), *Biochim. Biophys. Acta 72*, 317.

Brawerman, G., and Eisenstadt, J. M. (1964), *J. Mol. Biol. 10*, 403.

Brawerman, G., Gold, L., and Eisenstadt, J. (1963), *Proc. Natl. Acad. Sci. U. S. 50*, 630.

Eisenstadt, J. M., and Brawerman, G. (1964a), *J. Mol. Biol. 10*, 392.

Eisenstadt, J. M., and Brawerman, G. (1964b), *Biochim. Biophys. Acta 80*, 463.

Kaji, A., Kaji, H., and Novelli, G. D. (1965), *J. Biol. Chem. 240*, 1185.

Kirby, K. S. (1964), *Progr. Nucleic Acid Res. 3*, 1.

Lamfrom, H., and Knopf, P. (1964), *J. Mol. Biol. 9*, 558.

Mans, R. J., and Novelli, G. D. (1961), *Arch. Biochem. Biophys. 94*, 48.

Matthaei, J. H., and Nirenberg, M. W. (1961), *Proc. Natl. Acad. Sci. U. S. 47*, 1580.

Nathans, D., Notani, G., Schwartz, J. H., and Zinder, N. D. (1962), *Proc. Natl. Acad. Sci. U. S. 48*, 1424.

Nishizuka, Y., and Lipmann, F. (1966), *Proc. Natl. Acad. Sci. U. S. 55*, 212.

Schwartz, J. H., Eisenstadt, J. M., Brawerman, G., and Zinder, N. D. (1965), *Proc. Natl. Acad. Sci. U. S. 53*, 195.