Hybrid Ribosome Formation from Escherichia coli and Chloroplast Ribosome Subunits

Abstract. *A 70S ribosome was prepared from a 30S ribosome subunit from* Euglena gracilis *chloroplasts and a 50S ribosome subunit from* Escherichia coli. *This hybrid ribosome was active in polyuridylic acid–directed polyphenylalanine synthesis.*

Ribosomes from prokaryotes and chloroplasts have been shown to be similar in regard to the sedimentation coefficients of both the ribosomes and ribosomal RNA (*1*), the use of *N*-formylmethionine as the initiating amino acid in protein synthesis (*2*), and in the specific inhibition of in vitro synthesis of chloroplast protein by certain antibiotics which inhibit protein synthesis associated with ribosomes of the 70*S* type but not that associated with 80*S* ribosomes (*3*). Numerous reports (*4*) have indicated that 70*S* ribosomes are unable to catalyze one or more of the reactions required for protein synthesis in vitro when incubated with polymerizing enzymes obtained from the cytoplasm of eukaryotes, while the polymerizing enzymes from prokaryotes are unable to catalyze in vitro protein synthesis when combined with ribosomes of the 80*S* type. However, ribosomes from chloroplasts and mitochondria respond to the polymerizing enzymes of *Escherichia coli* (*5*, *6*). It has also been established that active hybrid ribosomes may be prepared from the 30*S* and 50*S* ribosome subunits from different prokaryotic organisms (*7*, *8*). However, to our knowledge there have been no reports of the preparation of active hybrid 70*S* ribosomes consisting of 30*S* and 50*S* subunits obtained from a prokaryotic organism and a cell organelle. We wish to report here the preparation of an active 70*S* ribosome composed of a 50*S* subunit from *E. coli* ribosomes and a 30*S* subunit from the chloroplast ribosomes of *Euglena gracilis*.

Escherichia coli, strain B, cells were suspended in ten times the cell volume of 0.01*M* tris buffer containing 0.01*M* $MgCl_2$ and 0.05*M* KCl and incubated with lysozyme for 15 minutes, after which the cells were broken with a French pressure cell at 1000 lb/in.^{2} (1 lb/in.^{2} = 70.31 g/cm^2). After reducing the viscosity of the lysate by incubation with deoxyribonuclease, the lysate was centrifuged at 30,000*g* for 15 minutes. The resulting supernatant was layered on 10 ml of 20 percent sucrose containing the above buffer salts, and the ribosomes were pelleted by cen-

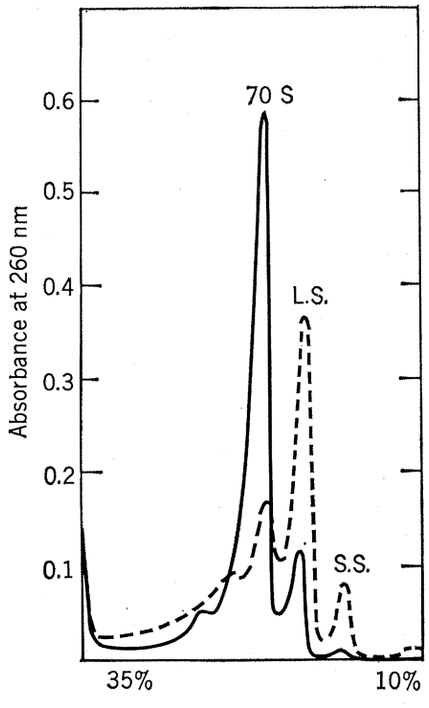

Fig. 1. Sucrose gradient analysis of *Escherichia coli* and *Euglena gracilis* chloroplast ribosomes. *Escherichia coli* ribosomes (1.2 mg) and chloroplast ribosomes (0.7 mg), both in 10 m*M* tris-HCl buffer (*p*H 7.6), 10 m*M* $MgCl_2$, and 50 m*M* KCl, were layered on 10 to 35 percent sucrose gradients and centrifuged for 5 hours at 27,000 rev/min with a Spinco SW-27 rotor. The gradients were analyzed by use of a flow cell having a 2-mm beam path. *L.S.*, large subunit; *S.S.*, small subunit; ———, *E.* coli ribosomes; - - - - -, *E. gracilis* chloroplast ribosomes.

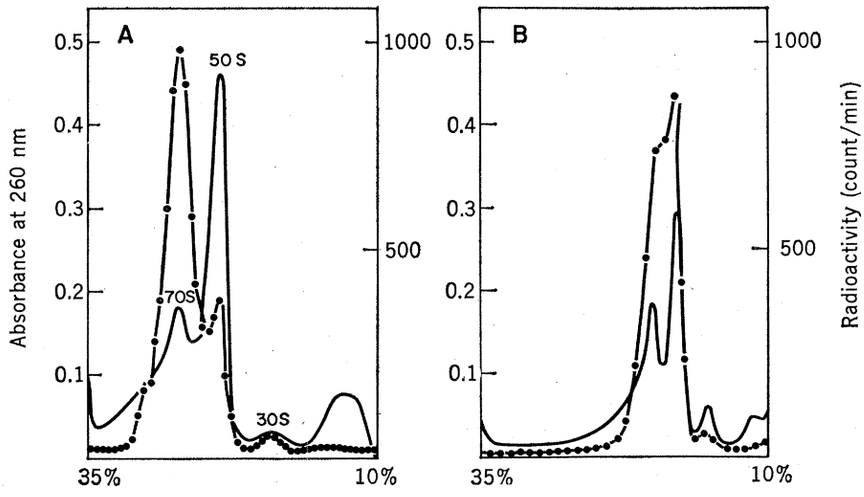

Fig. 2. (A) Sucrose gradient analysis of the interaction between crude *Euglena* chloroplast ribosomes and *Escherichia coli* 50*S* ribosome subunits. A mixture of 1.2 mg of crude chloroplast ribosomes and 0.2 mg of ^{14}C-labeled *E. coli* 50*S* ribosome subunits (9200 count/min) in 1 ml of 10 m*M* tris-HCl buffer (*p*H 7.6), 10 m*M* $MgCl_2$, and 50 m*M* KCl was layered on 36 ml of a 10 to 35 percent sucrose gradient in the same buffer salts, and the ribosomes and subunits were separated by centrifugation for 7 hours at 27,000 rev/min with a Spinco SW-27 rotor. The gradient was analyzed by use of a flow cell having a 2-mm beam path. Trichloroacetic acid was added to the fractions to a final concentration of 5 percent, the resulting precipitates were collected on Millipore filters (pore size, 0.8 μm in diameter), and the radioactivity was counted with a gas flow Geiger-Müller counter. The radioactive 50*S* *E. coli* ribosome subunits were obtained from Miles Laboratories and the specific activity was diluted with nonradioactive *E. coli* 50*S* subunits. ———, Absorbance at 260 nm; •—•, radioactivity. (B) Sucrose gradient analysis of the interaction between purified *Euglena* chloroplast ribosomes and *E. coli* 50*S* ribosome subunits. A mixture of 0.5 mg of purified chloroplast ribosomes and 0.2 mg of ^{14}C-labeled *E. coli* 50*S* ribosome subunits (4200 count/min) in 1 ml of 10 m*M* tris-HCl buffer (*p*H 7.6), 10 m*M* $MgCl_2$, and 50 m*M* KCl was layered on 36 ml of a 10 to 35 percent sucrose gradient in the above buffer salts, and the ribosomes and subunits were separated by centrifugation for 4½ hours at 27,000 rev/min with a Spinco SW-27 rotor. The gradient was analyzed and radioactivity in various fractions was counted in a manner described in (A). ———, Absorbance; •—•, radioactivity.

trifugation for 4 hours at 27,000 rev/min with the SW-27 rotor. Chloroplast ribosomes were prepared from phototrophically grown *E. gracilis*, strain Z, by a method similar to that reported by Rawson and Stutz (*1*). Various preparations of chloroplast ribosomes contained a large amount of free 50*S* subunits but few 30*S* subunits, probably due to the preferential retention of 30*S* subunits in the membrane fraction of chloroplasts. Subunits were prepared from *E. coli* and chloroplast ribosomes by incubation in 0.01*M* tris buffer (*p*H 7.6) containing 1 m*M* $MgCl_2$ and 0.1*M* KCl and then sedimenting through sucrose gradients containing the above buffer salts.

The ribosomes from chloroplasts of *E. gracilis* require 20 m*M* Mg^{2+} in the presence of 50 m*M* KCl to maintain ribosome integrity. At 10 m*M* Mg^{2+}, the chloroplast ribosomes dissociate into subunits, while the ribosomes from *E. coli* do not (Fig. 1). After incubation of chloroplast ribosomes with ^{14}C-labeled *E. coli* 50*S* subunits in the presence of 10 m*M* $MgCl_2$ and 50 m*M* KCl, analysis of the ribosome profile showed the formation of radioactive 70*S* ribosomes, which indicates the formation of hybrid monosomes resulting from the association of 30*S* chloroplast ribosome subunits and 50*S* *E. coli* ribosome subunits (Fig. 2). The formation of hybrid ribosomes was more extensive when a crude preparation of chloroplast ribosomes was used (Fig. 2A) than when purified chloroplast ribosomes were utilized (Fig. 2B) in the experimental protocol. When ^{14}C-labeled *E. coli* 70*S* monosomes were incubated with 50*S* chloroplast ribosome subunits and analyzed similarly, no radioactivity appeared in the 50*S* region.

The capacities of the heterologous combinations of subunits to carry out polyuridylic acid–directed incorporation of phenylalanine into products insoluble in hot 5 percent trichloroacetic acid (TCA) were examined in the presence of 10 m*M* $MgCl_2$. The purity of the subunits may be seen from the lack of phenylalanine incorporation when the subunits were tested alone (Table 1, lines 1 to 4). The mixture of 50*S* subunits of chloroplast ribosomes and 30*S* subunits of *E. coli* ribosomes failed to incorporate phenylalanine, but the mixture of 50*S* subunits of *E. coli* ribosomes and 30*S* subunits of chloroplast ribosomes incorporated an appreciable amount of phenylalanine. The inability of the homologous mixture of chloroplast ribosome subunits to incorporate phenylalanine is not surprising in view of the fact that these ribosomes dissociate into subunits in the presence of 10 m*M* $MgCl_2$.

Table 1. Polyuridylic acid–directed incorporation of phenylalanine into polyphenylalanine with hybrid ribosomes of *Escherichia coli* and *Euglena* chloroplast ribosomes. The reaction mixtures, in a volume of 0.5 ml, contained 1 m*M* adenosine triphosphate, 0.3 m*M* guanosine triphosphate, Mg^{2+} (as $MgCl_2$) as indicated, 50 m*M* KCl, 20 m*M* NH_4Cl, 20 m*M* tris buffer, 0.08 μc of [^{14}C]phenylalanine (175 count/min per picomole), 80 μg of polyuridylic acid, 50 μg of transfer RNA from dark-grown *Euglena*, 0.32 mg of *E. coli* enzyme mixture obtained in ammonium sulfate (between 22 and 50 percent) from postribosomal supernatant, 18 μg of protein from *E. coli* ribosome washing (the proteins that were released when ribosomes were allowed to dissociate; these proteins stayed at the top of the sucrose gradient when centrifuged, while ribosome subunits sedimented), and 90 or 180 μg, or both, of 30*S* and 50*S* subunits, respectively. The *p*H of the reaction mixture was 7.6. After 30 minutes of reaction at room temperature, TCA was added and the mixtures were heated to 90°C for 10 minutes. The precipitates were collected on Millipore filters, washed with four 3-ml portions of 5 percent TCA containing 0.1 percent cold phenylalanine. The filters were dried and radioactivity was counted with a gas flow Geiger-Müller counter.

Mg^{2+} (conc.)	Ribosome subunits	Phenylalanine incorporated (count/min)
10 m*M*	*E. coli* 30*S*	23
10 m*M*	*E. coli* 50*S*	42
10 m*M*	Chloroplast 30*S*	21
10 m*M*	Chloroplast 50*S*	12
10 m*M*	*E. coli* 30*S* + chloroplast 50*S*	12
10 m*M*	Chloroplast 30*S* + *E. coli* 50*S*	910
10 m*M*	*E. coli* 30*S* + *E. coli* 50*S*	4030
10 m*M*	Chloroplast 30*S* + chloroplast 50*S*	16
10 m*M*	None	22
20 m*M*	Chloroplast 30*S* + chloroplast 50*S*	230

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Blowflies: Alteration of Adult Taste Responses by Chemicals Present during Development

Abstract. *The addition of certain sugars to the food consumed during larval development increases the taste sensitivity of adult blowflies to some sugars, decreases it to others, and is without effect on the sensitivity to still others. No correlation with metabolic phenomena is apparent. The hypothesis that repression of inductible enzyme synthesis by glucose is a relevant model is not supported.*

The chemical nature of food ingested by insects during larval life apparently can influence various behavioral responses of the adults (*1*). Of particular interest is the report of Evans (*2*) describing experiments in which the addition of specific sugars to a basal larval diet lacking all sugars except lactose reduced the gustatory sensitivity of adult blowflies (*Phormia regina* Meigen) to the particular supplementary sugar. For example, in flies reared in the presence of fructose the mean behavioral response to tarsal stimulation was 46 times higher than that of control flies reared in the absence of fructose. The sucrose threshold of these flies also was elevated. Evans suggested that the effective sugars acted by depressing sensitivity specifically to themselves, and to other sugars that act at the same site, because of a decrease in the number or affinity of their combining sites on the sugar-sensitive neuron. A possible parallel was drawn between this action and the depressive effect of regulator genes in *Escherichia coli* (*3*).