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Variation in the Proportion of 50*S* and 30*S* Ribosomal Subunits at Different Growth Rates

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The ratio of 50*S* to 30*S* native ribosomal subunits varies in a systematic way with the growth rate in a 15T$^-$ strain of *Escherichia coli.*

This study reports that the ratio of 50*S*/30*S* native ribosomal subunits, distinguished from subunits derived from dissociated 70*S* ribosomes, varies in a systematic way with the growth rate in a 15T$^-$ strain of *Escherichia coli.* An early study of Ecker and Schaechter (1) indicated a relatively constant ratio of 50*S*/30*S* particles in *Salmonella typhimurium* over a wide range of growth rates. However, the schlieren patterns used in their analysis did not permit precise quantitation of the 50 and 30*S* regions. More recently, Sykes and Young (6) reported that the 50 and 30*S* ribosomal subunits occurred at a mass ratio of 1.54 $\pm$ 0.08 when all of the ribosomal material of *Aerobacter aerogenes* was displayed as subunits (derived plus native). This ratio was shown to be relatively constant with changes in growth rate, and it falls close to that reported by Hill et al. (3), 1.6 to 1.8, for the 50*S*/30*S* mass ratio. In the studies of Sykes and Young, the free native subunits were viewed against a background of dissociated 70*S* particles (derived subunits) equal to about 80 to 90% of the total ribosomal material (polysomes plus 70*S* plus 50*S* plus 30*S*), precluding precise estimation of values for the native subunits.

Even when the native subunits are well resolved on sucrose gradients free from derived subunits, the picture is complicated by the fact that ribosomal precursor particles cosediment in both the 50 and 30*S* regions of the gradients (5). These precursors comprise a relatively small percentage of the total ribosomal material. However, they contribute significantly to the mass in the 50 and 30*S* regions when the subunits are displayed at high Mg^{2+} concentration (10^{-2} M) which leave 70*S* particles undissociated (3). Under these conditions, the material in the subunit region, native 50 and 30*S* particles, constitutes approximately 15% of the total ribosomal material (polysomes plus 70*S* plus 50*S* plus 30*S*). The precursors have been estimated to comprise an amount equal to approximately 10% of this subunit material (5).

The present study shows that the ratio of native 50*S*/30*S* ribosomal subunits varies in a systematic way in a 15T$^-$ strain of *E. coli* growing at different rates. Four different growth rates, determined by various carbon sources and amino acid supplements, were examined. Significant differences in the distribution of ribosomal material were observed (Fig. 1). At the Mg^{2+} and NH_4^+ concentrations used, the 70*S* particles remained intact and only the native 50 and 30*S* subunits were displayed. The areas under the appropriate peaks of optical density OD_{260nm}) recordings of sucrose density gradients were determined by weighing tracings. Table 1 shows the average 50*S*/30*S* mass ratios for at least five experiments at each of four different growth rates. With decreasing growth rate, the ratio of 50*S*/30*S* material dropped from about 1.65 to about 0.8. Lindahl and Forchhammer (J. Mol. Biol., *in press*) have mentioned similar observations in another 15T$^-$ strain, TAU bar.

The subunit peaks in the $OD_{260\ nm}$ tracings ot sucrose gradients include precursor particles as well as mature native 50 and 30*S* particles. To eliminate the participation of these precursors, measurements were conducted on pulse-chased cultures. Under the conditions of these experiments, all radioactivity was present in the mature native 50 and 30*S* particles. The label was chased out of the precursors by labeling with an amount of ^{14}C-uracil that was totally consumed by the culture long before sampling. The ^{14}C counts in the 50 and 30*S* regions are then representative of the amounts of the mature native particles (Fig. 2 and Table 1).

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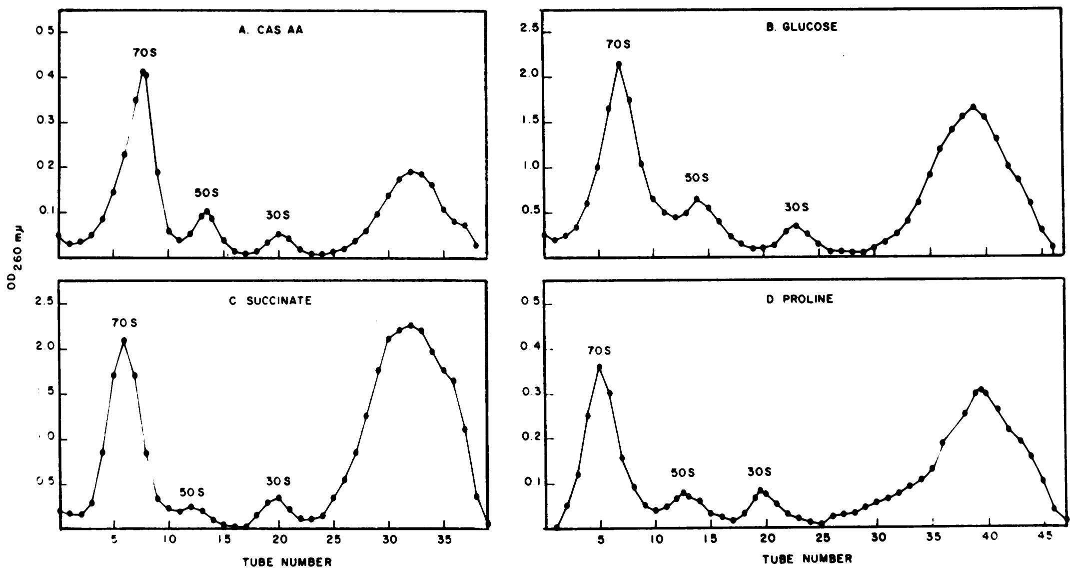

FIG. 1. *$OD_{260\,nm}$ recordings of sucrose density gradient analysis of sonically treated material of E. coli 15 $T^-H^-A^-L^-$. E. coli 15 $T^-H^-A^-L^-$ was grown under four different nutrient conditions in a tris(hydroxymethyl)aminomethane (Tris) minimal medium [100 mM Tris, 27 mM KCl, 37 mM NH_4Cl, in 1 mM Na_2HPO_4, 2.8 mM Na_2SO_4, 0.1 mM $FeCl_3$ (adjusted to pH 7.6 with HCl)]. Samples (200 ml) of cells growing exponentially on the various carbon sources and appropriate supplementation were poured over crushed (distilled water) ice including 0.01 M sodium azide and pelleted. After one wash in TMN (0.01 M Tris-hydrochloride, 0.01 M $MgSO_4$, 0.05 M NH_4Cl, pH 7.4) buffer, the pellets were suspended in 2.5 ml of TMN buffer containing 5 μg of electrophoretically purified deoxyribonuclease per ml and sonically treated. Subsequent to a clarifying centrifugation the sonically treated materials were applied to 15 to 35% (w/v) sucrose density gradients in TMN buffer and centrifuged for 14 hr at 25,000 rev/min in the SW-25.1 rotor of a Spinco L2-65B ultracentrifuge. The $OD_{260\,nm}$ was monitored and recorded on a Unican spectrophotometer equipped with a flow-through cuvette. (A) Glucose (0.2%) plus 0.5% Casamino Acids, (B) 0.2% glucose, (C) 0.4% succinate, and (D) 1.0% proline.*

TABLE 1. *Distribution of ribosomal material in the 50 and 30S regions of sucrose density gradients*[a]

Expt	Sample	50S/30S at $OD_{260\,nm}$	50S/30S counts/min[b]
A (0.2% glucose + 0.5% Casamino Acids, 2 doublings per hr)	1	1.599[c]	*
	2	1.559	*
	3	1.643	*
	4	1.773	*
	5	1.532	*
	6	1.825	*
	Avg	1.655 ± 0.170	
B (0.2% glucose, 1.25 doublings per hr)	1	1.731	1.524[c]
	2	1.597	1.578
	3	1.574	1.404
	4	1.624	1.603
	5	1.686	1.717
	6	*	1.762
	Avg	1.642 ± 0.089	1.596 ± 0.192
C (0.4% succinate, 0.6 doubling per hr)	1	0.782	*
	2	0.776	*
	3	0.801	*
	4	0.923	*
	5	0.850	*
	Avg	0.826 ± 0.097	
D (1% proline, 0.35 doubling per hr)	1	0.965	1.098
	2	0.976	0.907
	3	1.084	0.850
	4	0.974	1.019
	5	0.961	0.172
	6	*	0.893
	Avg	0.992 ± 0.092	0.990 ± 0.182

[a] Ratios of the amounts of material present in the 50 and 30S regions of sucrose density gradients as estimated by $OD_{260\,nm}$ recordings and ^{14}C-uracil incorporation. The amount of material in the 50 and 30S regions of the sucrose density gradients of sonically treated material of *E. coli* 15 $T^-H^-A^-L^-$, grown at four different growth conditions, was determined by the weight of tracings of the $OD_{260\,nm}$ recordings of the particular peaks and the ratios of the amounts calculated. In addition, the ratio of ^{14}C-uracil counts found in 50 and 30S subunits was determined for 0.2% glucose and 1% proline.

[b] Samples for the counts per minute do *not* correspond to those used for the $OD_{260\,nm}$ results.

[c] Ratio of weight (grams) of cut-out tracing of $OD_{260\,nm}$ recording and of ^{14}C-uracil counts of the 50 and 30S regions.

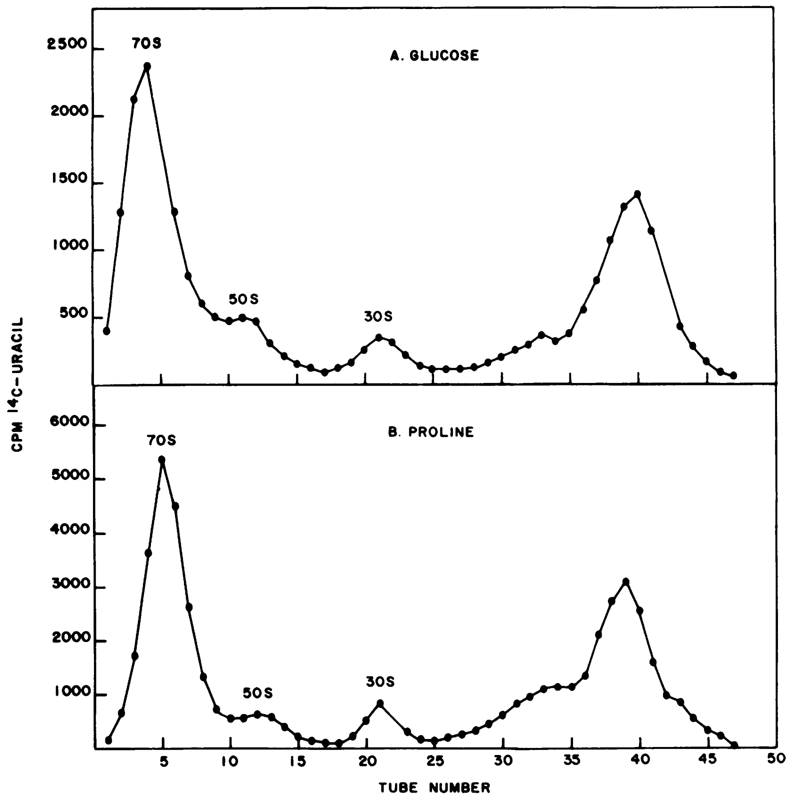

FIG. 2. Counts per minute of ^{14}C-uracil in sucrose density gradients of sonically treated material of E. coli 15 $T^-H^-A^-L^-$. Sonically treated materials of exponentially growing cells labeled with ^{14}C-uracil (specific activity, 55 mCi/mmole) at $OD_{450\,nm}$ = 0.1, chased by endogenous uracil synthesis, and harvested at $OD_{450\,nm}$ = 0.6. The cells were prepared, centrifuged, and analyzed as in Fig. 1, except that the gradients were fractionated into 0.7-ml samples. These fractions were treated with 10% trichloroacetic acid for 1 hr at 0 C, collected on Sartorius membrane filters, and counted in a toluene-based scintillation fluid.

It is concluded that in the glucose plus amino acids and in the glucose cultures, the native 50 and 30*S* subunits are present in approximately equal numbers. With either succinate or proline as the carbon source, there is a definite surplus of 30*S* particles. The significance of this finding to the ribosome functional cycle could be explored by examining the rate of exchange of the individual subunits labeled with heavy isotopes (4) at different growth conditions.

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