AN ALTERED PATTERN OF RIBOSOME SYNTHESIS IN A MUTANT OF E. COLI

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It has recently become fashionable to study the formation of ribonucleoprotein particles in Escherichia coli whose metabolism has been altered either by conditions of unbalanced growth (Nakada, 1965; Sypherd, 1965; Nakada and Marquisee, 1965) or by addition of metabolic inhibitors (Hills and Horowitz, 1966; Hosokawa and Nomura, 1965). While such particles seem analogous to the transitory ribosomal precursors found in exponentially growing cells (McCarthy et al., 1962), the possibility exists that the products of metabolic manipulations and the normally occurring nucleoproteins are not identical. Approaching the study of ribosome synthesis from another direction, we considered that mutants might exist which, under balanced growth conditions, would accumulate ribosomal precursors in amounts large enough to make possible their isolation and characterization.

Recalling that sensitivity, dependence and resistance to streptomycin (SM) resides on the ribosomes (Flaks et al, 1962; Speyer et al., 1962), it seemed possible that mutants selected for the presence of a suppressor that changed the response of the bacterium to SM might display a change in the physical properties

of the ribosomes or in the kinetics of synthesis. Such has proven the case. This paper describes the sedimentation analyses of extracts of one of our mutants, K-106, which, under exponential growth conditions, accumulates particles with a sedimentation rate of approximately 43S.

MATERIALS AND METHODS

We have isolated from a wild-type SM-sensitive strain of E. coli K12 (laboratory number K-100) a SM-dependent mutant, and, utilizing the techniques of Hashimoto (1960), have selected from this strain a spontaneous revertant to SM-independence (K-106). A detailed description of the isolation, characterization and properties of this particular mutant is reported more fully elsewhere (Brownstein and Lewandowski, in preparation).

Cultures were grown in Tris-maleate minimal medium (Paranchych and Graham, 1962) supplemented with 20 μg methionine/ml and 0.2% glucose. All studies were performed with cultures in early log phase (5-40 x 10^7 cells/ml). Cell extracts were prepared at 4°C by suspending washed cells in Tris buffer (0.01 M, pH 7.4) containing 0.1 mM $MgCl_2$, and disrupting at 15000psi in a French pressure cell. DNase (5 μg/ml) was added, and debris and unbroken cells were removed by centrifugation. RNA was isolated from such extracts by extraction with sodium dodecyl sulfate-phenol (Sagik et al., 1962). RNA and ribosome fractions were analyzed by sedimentation through 5-20% sucrose gradients in an SW 39 rotor in a Beckman L-2 ultracentrifuge. Tubes were punctured and three-drop fractions collected directly on filter paper in vials. These filters were dried, covered with 2 ml of PPO-POPOP-toluene solution and counted in a Packard 4000 Tri-Carb liquid scintillation counter.

RESULTS AND CONCLUSIONS

Figure 1a shows the results of sucrose gradient centrifugation of extracts of exponentially growing cultures sampled at 15, 30 and 60 minutes after addition of H^3-uridine. It is clear that at 15 minutes, aside from the labeling of the 4S component, there is an almost equal distribution of isotope in three main areas. By 30 minutes these areas are more clearly differentiated into 50, 43 and 30S peaks; by 60 minutes the amount of label in the 50 and 30S peaks has markedly increased in proportion to the 43S material.

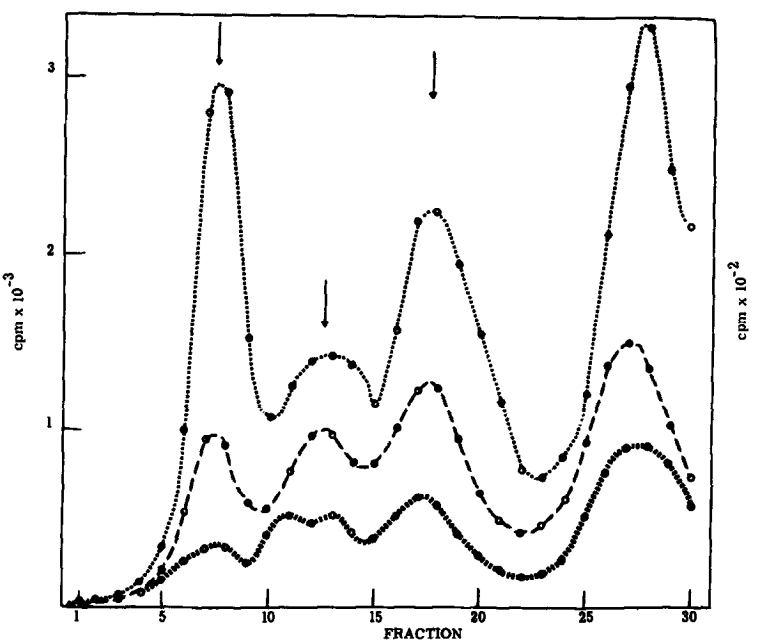
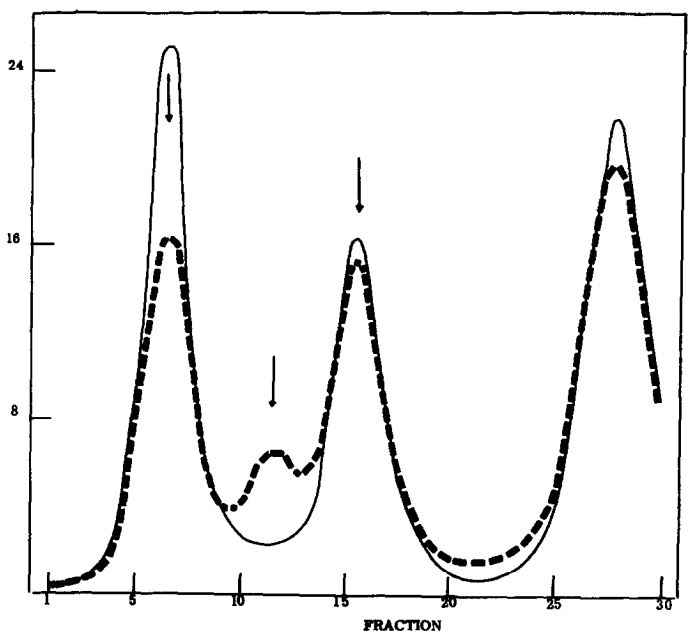


Figure 1. Sedimentation pattern of K-106 ribosomes. (1a) At time 0, 150 μc H^3-uridine (1 mc/mg) was added to 100 ml of an exponentially growing culture of K-106. Twenty-ml samples were harvested and cell extracts obtained as described in Methods. Centrifugation was for 190 minutes at 38,000 rpm. Thirty fractions were collected from each gradient. Arrows from left to right mark the positions of 50, 43 and 30S respectively. Fifteen minute samples ▪▪▪▪▪▪▪▪▪▪▪▪▪; 30 minute — — — — ; 60 minute ..... .

In the next experiment a culture of K-106 was labeled for 120 minutes with H^3-uridine, divided, and one-half was incubated for an additional 90 minutes in the presence of excess unlabeled uridine (100 μg/ml) as a chase. Further incorporation of the label into RNA ceased immediately after addition of the unlabeled uridine. Sucrose gradient sedimentation patterns of chased and unchased samples are shown superimposed in Figure 1b. The distribution of label after the chase shows that approximately 37% of the total label in the gradient is under the 50S peak. The possibility that the 43S component is a precursor of the 50S particle is suggested by the observation that the sum of the label in the 50 and 43S peaks in Figure 1b (dashed line) and for each of the sedimentation patterns of Figure 1a is also 37% of the total label in the respective gradients. A detailed kinetic



(1b) K-106 incubated for 120 minutes with H^3-uridine, one-half the culture was analyzed immediately ▬▬▬; the remainder of culture was incubated for an additional 90 minutes with excess uridine as described in text ————. For convenience, points are omitted from figures; conditions are the same as above.

evaluation is now in progress to corroborate what seems probable from these results -- that the material in the 43S region is later incorporated into 50S ribosomes.

The 43S particles which accumulate in K-106 are not identical to the "core particle" resulting from CsCl treatment of 50S ribosomes (Hosokawa et al, 1966). When a mixture of 50S and 43S particles are exposed to CsCl, the majority of the 50S material is converted to 43S particles while the initial 43S particles sediment at 37S.

To determine whether the 50 and 30S ribosomes of the mutant, K-106, and the wild-type, K-100, have similar sedimentation properties, we analyzed an extract prepared from a mixture of C^{14}-

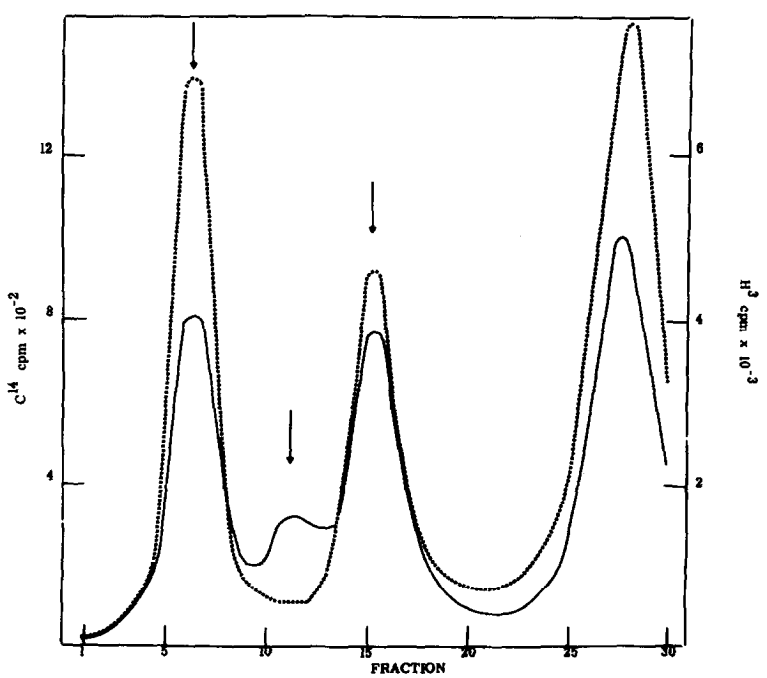


Figure 2. Comparison of ribosomal sedimentation pattern of K-106 and wild-type, K-100. A sample of K-106 labeled with H^3-uridine for 120 minutes was mixed with a sample of K-100 labeled with C^{14}-uridine for 120 minutes. Cells were lysed and extracts sedimented for 190 minutes at 38000 rpm. ——— H^3; ..... , C^{14}.

labeled K-100 and H^3-labeled K-106, each exposed to labeled uridine for 120 minutes. The 30 and 50S peaks coincide; however, K-100 lacks any distinguishable accumulation of 43S material. Although the generation time of K-106 is 1.5 times that of the wild type, the accumulation of 43S material in one and not the other does not seem to be merely a function of the slower growth rate. No

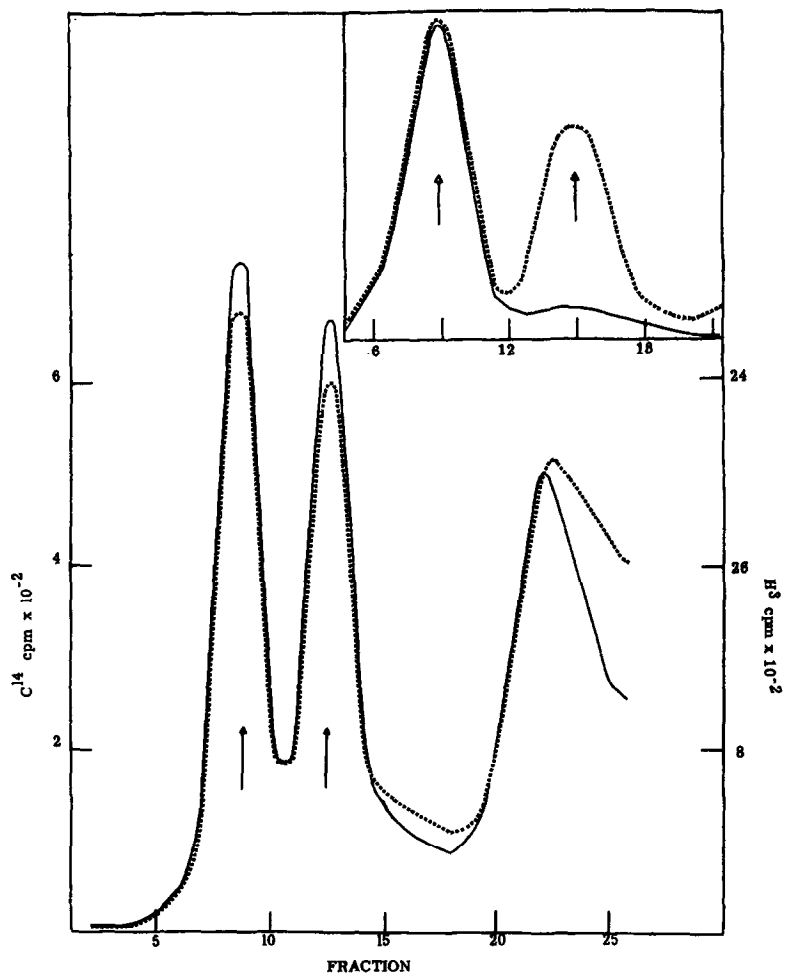


Figure 3. Comparison of RNA extracted from K-106 and wild-type. An aliquot of the mixture of K-106 labeled with H^3, and K-100 labeled with C^{14}, described in Figure 2, was treated with phenol-SDS and sedimented for 6.5 hrs. at 39000 rpm. Arrows mark the position of 23 and 16S RNA. ——— H^3; ..... C^{14}. Insert: RNA extracted from purified 43S particles of K-106 ——— and from crude extract of K-100 ..... .

comparable 43S peak accumulates in K-100 at any time point between 5 and 120 minutes after addition of labeled uridine.

As a further control a phenol extract was prepared from a second sample of the material analyzed in Figure 2. The sedimentation profiles (Figure 3) of the resulting material show that the RNA contained in the 43S particle does not cause any visible change in the overall sedimentation pattern for RNA of K-106. The distribution of label between the 23 and 16S RNAs of the mutant coincides exactly with that of the control. More than 90% of the RNA extracted from purified 43S particles sediments with 23S RNA (Insert, Figure 3).

It seems therefore that in K-106 we may be observing a large pool of neosome-like particles, less transitory than those of McCarthy et al. (1962), which represents a step in the production of 50S ribosomes. The final step(s) in the assembly of the ribosome may be the addition of proteins to the 43S particle or a molecular rearrangement of an otherwise completed particle. We are now analyzing the proteins of the 43 and 50S components by acrylamide gel electrophoresis to determine whether specific proteins are missing from the 43S particle.

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