The Ribosomes of *Drosophila*

I. Subunit and Protein Composition*

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**Summary.** Functional ribosomes from *Drosophila virilis* dissociate to form 40S and 55S subunits. One-dimensional gel electrophoresis in either dodecyl sulfate-acrylamide gels (Fig. 1) or urea-acrylamide gels (Fig. 2) reveal complex protein patterns. Increased resolution in two-dimensional acrylamide gels (Figs. 3 and 4) indicate the existence of at least 28 proteins in the small subunit, and 39 proteins in the large subunit. Molecular weight estimates indicate a molecular weight range (and average) of 16500-40000 (25800) for the 40S subunit proteins, and 12500-45000 (24300) for the 55S subunit proteins. Comparison of larval and adult ribosomal protein patterns did not reveal qualitative differences between them.

1. Introduction

It is known that in *E. coli* most, if not all, of the proteins contained in ribosomes are genetically clustered (Dekio, Takata and Osawa, 1970) and under coordinate synthetic control (Nomura and Engback, 1972). Studies on anucleolate mutants of *Xenopus* (Hallberg and Brown, 1969) reveal that in the absence of ribosomal RNA genes the normal onset of ribosomal protein synthesis, during gastrulation, fails to occur suggesting that a coordinate mechanism regulating ribosome biogenesis may exist in eucaryotes as well. A report has appeared describing non-coordinate control of ribosomal RNA and ribosomal protein synthesis in human L-cells (Craig and Perry, 1971), however the authors point out that because of numerous differences between the two experimental systems the results are not comparable.

In an effort to further explore the question of coordinate control in higher eucaryotes a detailed structural and genetic analysis of *Drosophila* ribosomes was begun. The purpose of this report is to describe the methodology of ribosome isolation, dissociation, and protein extraction, for *Drosophila*, and provide a preliminary physiochemical description of the subunit proteins by electrophoretic procedures.

2. Methods and Materials

a) Ribosome Isolation and Dissociation

*Drosophila virilis* adults or third instar larvae grown on banana-agar medium were homogenized in a buffer containing 50 mM Tris-HCl, 250 mM sucrose, 1 mM MgCl₂, 25 mM KCl, 1 mM dithiothreitol, pH 7.8, at 0° C. The slurry was centrifuged at 18000 × g, for 15 minutes at 0° C.

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4°C in a Sorval RC-2B centrifuge and the supernatant filtered through cotton gauze to remove lipid aggregates. The centrifugation was repeated to completely remove nuclei and mitochondria. The supernatant was then made 0.5% with respect to deoxycholate (sodium salt) and centrifuged at 27000 × g for 20 minutes and the pellet discarded. The clear supernatant was next centrifuged at 109000 × g for 2 hours at 4°C, in an International B-60 ultracentrifuge. The crude ribosomal pellet was gently resuspended in the homogenizing buffer modified to contain 10 mM MgCl₂ and clarified by centrifugation at 27000 × g for 10 minutes. The supernatant was next layered on a discontinuous sucrose gradient (2 ml of 30% sucrose under 3 ml of 10% sucrose) containing the modified homogenizing buffer, and centrifuged at 109000 × g for 3 hours at 4°C. The washed ribosomal pellet was finally resuspended in a dissociation buffer containing 20 mM Tris-Cl, 100 mM KCl, pH 7.4, layered on a 35 ml continuous sucrose gradient (10% to 30%) containing dissociation buffer, and centrifuged at 42000 × g 15.5 hours, at 4°C, in an International SB-110 swinging bucket rotor.

b) Ribosomal Protein Isolation

Following centrifugation gradients were fractionized in an Isco Model 640 instrument and the appropriate fractions pooled. An equal volume of ice cold 20% trichloroacetic acid (TCA) was added and incubated on ice for an hour. The tubes were then centrifuged at 18000 × g for 20 minutes, and the subunit pellets washed in acetone, 95% ethanol, and anhydrous ether, and finally dehydrated by evacuation.

To extract the ribosomal proteins the pellets were resuspended overnight in a buffer containing 10 mM Tris-Cl, pH 7.4, and next treated overnight with two volumes of glacial acetic acid containing 100 mM MgCl₂. All operations were carried out at 4°C. The preparation was then centrifuged at 18000 × g for 30 minutes and the RNA pellet discarded. Protein was precipitated by the addition of 5 volumes of acetone, and after an hour on ice the suspension was centrifuged at 18000 × g for 30 minutes. The protein pellet was dehydrated by successive washing in acetone, 95% ethanol, and anhydrous ether, and stored at −20°C.

c) Electrophoresis

Protein pellets were resuspended in 4 M urea (deionized) and the protein concentration determined by the Folin procedure (Lowry, Rosebrough, Farr, and Randall, 1951) using recrystallized bovine serum albumin as a standard. For one-dimensional electrophoresis in dodecylsulfate acrylamide slab gels, a modification of the technique of Blattler, Garner, Slyke, and Bradley (1972) was employed. For one-dimensional electrophoresis in urea-acrylamide disc gels the procedure of Lambertsson, Rasmuson, and Bloom (1970) was used. For two-dimensional electrophoresis proteins were first separated in urea-acrylamide cylinders, and the disc polymerized on to a dodecylsulfate-acrylamide slab gel prepared according to the procedure of Weber and Osborn (1969). In all cases proteins were visualized, following electrophoresis, by staining in a 0.1% solution of Coomassie Brilliant Blue dissolved in 50% TCA, and destained in a 5:5:1 mixture of methanol, water, acetic acid. Exact conditions of electrophoresis are described in individual figure legends.

3. Results

a) Properties of Monosomes and Subunits

Ribosomes isolated from adult Drosophila virilis were found to contain 50% protein, by weight. Optical density determinations revealed an OD₉₀₀/OD₂₅₀ ratio of 1.87 and OD₉₀₀/OD₂₃₅ ratio of 1.50, well within the accepted limits of purity for eucaryotic ribosomes (Martin, Rolleston, Low, and Wool, 1969). Utilizing E. coli 70 S monosomes, and 30 S and 50 S ribosomal subunits as parallel standards, Drosophila monosomes were found to have a sedimentation coefficient of 80 S. Studies on the biosynthetic activity of our 80 S preparations were carried