

Schuppe, N.G., T.V. Syrota and V.T. Kakpakov. Institute of General Genetics, Moscow 117312, USSR. Turnover of ribosomal RNA in the course of *D. melanogaster* development.

were grown on labelled medium for 6 h. RNA was isolated from part of these larvae and the remaining larvae were thoroughly washed and transferred to non-radioactive medium. RNA was isolated in every other 12 h after transfer of the larvae up to the white prepupal stages. To determine specific activity of ribosomal RNA, isolated RNA was fractionated by sucrose gradient centrifugation or by polyacrilamide gel electrophoresis. The structural integrity of ribosomal RNA was also studied. It was found that during the development of larvae active synthesis of ribosomal RNA occurs so that specific activity RNA decreased proportionally with increase of ribosomal RNA content per larvae. Total amount of label per larvae practically does not change.

The second stage was the study of the fate of ribosomal RNA in the course of metamorphosis. For that, larvae were labelled as above on the fifth day after oviposition. RNA was isolated just after transfer of larvae to non-radioactive medium (third instar larvae), at 24 h (white prepupal stage), at 48 h (pupal stage) and at 120 h (flies) after transfer. Data obtained show that ribosomal RNA synthesis by third instar larvae was preserved at the late stage of development. During the transition from third instar larvae to white prepupae the specific activity of RNA decreased two times due to increasing RNA content per larva, then the specific activity of RNA remained constant. RNA characteristics obtained by sucrose gradient centrifugation or gel electrophoresis do not change at these stages of development. Our data can be explained by the fact that at the stage of white prepupae active synthesis of RNA and ribosomes occurs. Thereafter synthesis of new ribosomal RNA and ribosomes is virtually absent. This indicated that protein synthesis in pupae and flies is accomplished by ribosomes synthesized at the early stages of *Drosophila* development.

The question about structural changes of protein synthesizing apparatus in the course of insect development and metamorphosis remains completely obscure. To study the turnover of ribosomal RNA during the development of *D. melanogaster* larvae, 15 h old larvae were collected and placed on medium containing 50  $\mu$ Ci/ml of 2-[ $^{14}$ C]-uracil and

Schuppe, N.G., T.V. Syrota, N.G. Nosova and V.T. Kakpakov. Institute of General Genetics, Moscow 117312, USSR. Protein-synthesizing activity of *Drosophila* ribosomes containing rRNA with hidden breaks.

and small subunits of ribosomal RNA although ribosomes remain structurally intact (1). This allows us to study the significance of rRNA integrity within ribosomes for their functions. In this paper we present data about protein synthesizing activity of *Drosophila* ribosomes in a cell-free system. Ribosomes were isolated by the method of Wettstein et al. (2) from flies, larvae at second and third instars, eggs and established cell lines 67j25D (3).

Standard assays (final volume 0.5 ml) were incubated at 30°C and contained the following: 35 mM Tris-HCl (pH 7.6), 60 mM KCl, 5 mM MgCl<sub>2</sub>, 1 mM ATP, 0.6 mM GTP, 20 mM creatine phosphate, 15  $\mu$ g creatine phosphokinase, 4 mM dithiothreitol, 3  $\mu$ Ci of 1-[ $^{14}$ C]-phenylalanine, 0.05 mM of each of the 19 unlabelled l-amino acids, 1 mg ribosomes, 0.2 ml postribosomal supernatant (approximately 0.5 mg protein). This system was a slightly modified cell-free system reported by Ilan for *Tenebrio* pupae (4).

The kinetics of  $^{14}$ C-phenylalanine incorporation in hot acid-insoluble fraction are presented in Table 1. For comparison the ribosomes from rat liver were taken.

A mature large subunit of *Drosophila* ribosomal RNA contains a central hidden break when RNA was isolated from whole cells. The small subunit under these conditions remains covalently continuous. At the same time when RNA was isolated from previously isolated ribosomes the breaks become plural and appear on the large