THE DEGRADATION OF RIBOSOMAL RNA DURING THE DEVELOPMENTAL STAGES IN *DROSOPHILA MELANOGASTER*

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The specific changes in the degradation of ribosomal RNA of *Drosophila melanogaster* were analyzed during the developmental stages. Severe degradation of rRNA was observed in the homogenates of the specific stages (72, 144, and 168 h old larvae) during the larval development from the results of polyacrylamide gel electrophoresis without purification of rRNA. It was suggested that this degradation of rRNA was induced by endogenous RNase contained in the larvae.

INTRODUCTION

A number of studies have been published on the synthesis and properties of the *Drosophila* ribosomal RNA (Greenberg 1969; Lengyel *et al.* 1975; Puckett and Snyder 1974, 1975a, 1975b; Wellauer and Dawid 1977). Greenberg suggested that the size of the rRNA of *Drosophila* was smaller than that of the mammalian rRNA. In 1975, Lengyel *et al.* estimated the size of the large rRNA of *Drosophila melanogaster* to be 26S and the small rRNA to be 19S from the comparison with the rRNA of HeLa cells.

The quantitative aspects of rRNA synthesis during ovarian development of *Drosophila melanogaster* was analyzed by using two mutant strains by Puckett and Snyder (1974). They thought that the rate of rRNA synthesis was highest at stages 9 and 10, because the greatest amplification of DNA was suggested to occur in ovarian chamber at these stages.

A position effect suppression of rRNA synthesis was also reported by Puckett and Snyder (1975a). The average rate of rRNA synthesis and the net rRNA content showed close association with an X-chromosome inversion. Their data indicated that a 15% reduction in rRNA content and in the rate of rRNA synthesis in X/O males carrying the inverted chromosome.

However, the dynamic changes of the degradation of rRNA during the developmental stages of *Drosophila* have not yet been studied. In the present experiment, it was found that a particular degradation of rRNA was observed at the specific stages during the development.

MATERIALS AND METHODS

A stock of *Drosophila melanogaster* having a genotype of *l(2)/Cy; spa^{pol}* was used in this experiment. This stock has been kept in the laboratory more than 15 years under mass mating at 25°C with standard culture medium.

The samples of eggs, larvae, pupae and adult flies were collected in every 24 h interval after oviposition according to the developmental stages. The developmental stages of the collected sample were shown in Table 1.

In most cases, three individuals except eggs and younger larvae, were homogenized in 0.1 ml of the homogenizing buffer A (Tomkins and Billington 1972) containing 0.04 M Tris-HCl (pH 7.2), 0.02 M sodium acetate, 2 mM EDTA, 10% sucrose and 1% sodium dodecyl sulfate under cold condition. In the case of eggs and young larvae, about 50-100 mg of sample were used. Fifty μ l of the homogenized sample were placed on the top of 2.5% polyacrylamide gel (0.5 $\times$ 8.0 cm) and electrophoresed for 1 h and 40 min (5 mA/tube) at 4°C.

After electrophoresis, the gels were removed from the column and fixed in the test tubes with 1:1 mixture of 2% acetic acid and 96% ethanol for 2.5 h. Then, the gels were stained with 0.3% toluidine blue O in 1% acetic acid for 5 min. After staining, the gels were destained overnight with 2% acetic acid containing 10% glycine. The stained rRNA bands were scanned with the densitometer (Toyo, Model DMU-33C).

The homogenate of three adult flies in 0.1 ml of homogenizing buffer A, showing no degradation of rRNA, was incubated with 5 μ l of pancreatic RNase (E.C.2.7.7.16; 1 mg/ml) for 1 h at 16°C or 37°C to know the sensitivity of *Drosophila* rRNA to the RNase.

In the next experiment, the ¹⁴C-labeled rRNA from rat liver was used as a substrate to compare the RNase activities of the 144 h old larvae and adult flies. The homogenate of three 144 h old larvae or three adult flies in 0.3 ml of homogenizing buffer A was added to ¹⁴C-labeled rat liver rRNA solution (12 μ g in 15 μ l solution; 2470

Table 1. Developmental stages of the collected samples

A	0- 24 h	eggs
B	48 h	larvae
C	72 h	larvae
D	96 h	larvae
E	120 h	larvae
F	144 h	3rd instar larvae
G	168 h	puparium formation
H	168 h	pre-pupae
	172 h	
I	192 h	pupae
J	216 h	pupae (eyes slightly colored)
K	240 h	pupae (eyes, wings, etc. visible)
L	264 h	adults

cpm). After specified incubation time at 37°C, the samples precipitated with cold 6% TCA were collected on GFC filter and washed several times with cold 6% TCA. Radioactivities of these samples were counted with liquid scintillation spectrometer (Packard Tri-Carb 3375) using toluene scintillator cocktail.

In order to know the degradation of the rRNA of the 144 h old larvae, 5 μ l (5 μ Ci) of 3 H-labeled uridine (specific activity; 26 Ci/mM) per one larva were fed and the larvae were collected at the specified time and homogenized with cold 6% TCA and the precipitates were collected on the filter, and the radioactivity was measured. At the same time, the labeled larvae were homogenized with the buffer A as mentioned above, and electrophoresed in 2.5% polyacrylamide gel. The gel was stained with toluidine blue O and the stained bands were scanned. Then the gels were sliced to 1 mm thick and

Fig. 1. The profiles of rRNA electrophoresed 1 h after homogenization of samples which were collected according to the developmental stages. Each letter corresponds to the stages described in Table 1.

dissolved with 0.2 ml of hydroperoxide at 40°C and counted the radioactivity using toluene Triton X-100 scintillation cocktail.

RESULTS

Figure 1 shows the electrophoretic profile of rRNA in each sample collected at the specified developmental stages and electrophoresed 1 h after homogenization. In this figure, degradation of rRNA was observed at three stages, namely C (72 h old), F (144 h old) and G (168 h old). On the other hand, when these samples were electrophoresed immediately after homogenization, as shown in Fig. 2, degradation of the rRNA was not so severe when compared to the profiles shown in Fig. 1.

The adult flies and 144 h old larvae were homogenized together and a part of it

Fig. 2. The profiles of rRNA electrophoresed immediately after homogenization. Each letter corresponds to the letter in Fig. 1.

was electrophoresed immediately after homogenization and the other was electrophoresed after 1 h standing at 16°C to know the existence of a specific substance in 144 h old larvae to degrade the rRNA of the adult flies. In both cases, the rRNA of adult flies was degraded and the degree of degradation was high in the sample which had been kept for 1 h after homogenization (Fig. 3).

A similar degradation profile of the adult rRNA was observed when the homogenate of the adult flies was incubated with pancreatic RNase for 1 h either at 16°C or 37°C (Fig. 4).

These results suggested that the degradation observed in the larval rRNA might be caused by RNase which was present in the larvae. In order to know the activity of RNase contained in the larvae, ^{14}C -labeled rRNA prepared from rat liver was used as a substrate. As seen in Fig. 5, the homogenate of 144 h old larvae digested more than 90% of ^{14}C -labeled rRNA within 20 min. On the other hand, the adult homogenate digested only about 20% of the substrate during the same time.

The larvae of 144 h old were labeled with ^3H -labeled uridine to use in the experi-

Fig. 3. The profiles of adult flies homogenized together with 144 h old larvae (adult + 144 h old larvae).
a: Kept at 16°C for 1 h after homogenization.
b: Electrophoresed immediately after homogenization.

Fig. 4. The profiles of rRNA of adult flies.
1: Adult flies immediately after homogenization.
2: Adult + RNase; incubated at 37°C for 1 h.
3: Adult + RNase; incubated at 16°C for 1 h.

Fig. 5. Digestion of ¹⁴C-labeled rRNA from rat liver by the homogenates of 144 h old larvae or adult flies.
Closed circle: 144 h old larvae.
Open circle: adults.

Fig. 6. A labeling profile of 144 h old larvae according to the feeding time.

Fig. 7. Degradation profile of newly synthesized rRNA in 144 h old larvae (limited partial digestion).

ment to know which portion of rRNA could be degraded at first. A labeling profile of the larvae according to time is shown in Fig. 6. As seen in this Fig. 6, these larvae incorporated ^3H -labeled uridine almost linearly with feeding time. The homogenates of these samples were analyzed by electrophoresis after 1 h standing under cold condition to make a partial degradation of rRNA. The result of the distribution of the radioactivities after gel electrophoresis of this RNA is shown in Fig. 7. The large rRNA in newly synthesized rRNA showed more degradation than a small one and the ratio of the large and small rRNA showed by the arrows in Fig. 7 was 1.78: 1.00.

DISCUSSION

The ribosomal RNA of *Drosophila melanogaster* differs from a mammalian rRNA in several respects. Two ribosomal species have a sedimentation coefficient of 26S and 19S, when determined with the 28S and 18S ribosomal species of HeLa cells (Lengyel *et al.* 1975). Another interesting property of *Drosophila* rRNA and of an insect RNA in general, is that the 26S species are dissociable by heat into two species which cosediment with the 19S rRNA. Lengyel *et al.* (1975) reported that the degradation of 26S rRNA occasionally occurred during extraction procedure. Therefore, the ratio of the rRNA species is 1:1 in *Drosophila* instead of the expected value (2:1) after extraction and analysis by sucrose gradient centrifugation.

In order to avoid the degradation of rRNA during extraction and purification, a direct analysis of the rRNA was made by using a polyacrylamide gel electrophoresis in this experiment. From the preliminary experiment, at least three individuals of the larvae and adult flies, except eggs and larvae at younger stages were enough to compare the rRNA profiles by this method.

The distinct differences were observed in the profiles of rRNA after electrophoresis in different developmental stages. In the eggs, pupae and adult flies, no apparent degradation of the rRNA was observed in the samples which had been kept for 1 h after homogenization (cf. Fig. 1). From the results of the comparison of the rRNA profiles, the 144 h old larvae were chosen in the following experiment to study the nature of the degradation, because the larvae at this stage showed completely degraded profiles.

It was suggested that a specific substance to degrade the rRNA might be contained in 144 h old larvae. When the adults were homogenized together with 144 h old larvae, complete degradation of the adult rRNA occurred, if the homogenate was kept for 1 h at 16°C. In the case of immediate analysis after homogenization, some degree of the degradation was also observed. Therefore, it was suggested that the RNase-like substance might be present in the larvae. Then, the pancreatic RNase was added to the homogenate of the adult flies which did not show severe degradation of rRNA in the previous experiment, and incubated for 1 h at 16°C or 37°C to compare the profiles after electrophoresis. At either incubation temperature, almost the same type of degradation profiles were observed.

The RNase activities in the homogenate of 144 h old larvae and adult flies were

tested using ^{14}C -labeled rRNA prepared from rat liver. In the case of the homogenate of 144 h old larvae, more than 90% of the ^{14}C -labeled rat liver rRNA was digested within 20 min. On the other hand, the homogenate of adult flies digested only about 20% of the substrate during the same time. This means that the activity of RNase in the homogenate of 144 h old larvae is much higher than in the homogenate of the adults.

Incorporation of ^3H -labeled uridine into larvae was almost linear after 2 h according to feeding time. From the result of the distribution of radioactivities in the rRNA after electrophoresis, a newly synthesized 26S rRNA was degraded more specifically than the 19S rRNA after partial degradation.

Inoue *et al.* (1974, 1975) analyzed the degradation of rRNA of the rat liver and found that the autodegradation of the 28S rRNA into six minor components and also that the autodegradation was age dependent. They suggested that ribosome possessed a mechanism by which their own rRNA was degraded.

From the results of the present experiment, it was shown that the stable electrophoretic profiles of rRNA were observed in the case of the eggs, pupae and adult flies, but the degradation of the rRNA existed at mature larval stage (144 h old) during the larval development. This suggests that a difference in RNase activity existed *in vivo* during the developmental stages of *Drosophila melanogaster*. This change of RNase activity may play an important role in the larval development, but further extensive study has to be made to elucidate its relationship to precise developmental stages and to gene expression which related to the rRNA synthesis during the development of larvae of *Drosophila*.

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