PRIMARY AND SECONDARY NICKS IN THE RIBOSOMAL RIBONUCLEIC ACID OF INSECTS

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Summary: In addition to the primary nick in the larger rRNA that leads to two 18-S fragments which had been reported, secondary nicks also can be introduced. These result in the formation of smaller polynucleotides that arise as a result of cleavage at specific points.

Introduction

A brief heating of a preparation of larger rRNA from insects leads to the breakdown of this molecule into two 18-S molecules (1--7). This also occurs in Protostomian animals (8) and Protozoa (9, 10). This process is not a cleavage of covalent bond, but a dissociation of chains in larger rRNA held together by non-covalent bonds (6). The monodispersity of the dissociation product indicated that the nick lies near or in the middle of the chain (6). In the course of studies on the mechanism of nick formation, it was discovered that other nicks can be introduced at specific points of the larger rRNA from insect tissue depending on experimental conditions.

Materials and Methods

The greater wax moth, Galleria mellonella (L.) was cultured as previously described (11, 12). The silkglands from last-instar larvae which had just started spinning were used as the source of RNA. The nucleic acids were extracted, and treated with DNase (5). The larger rRNA isolated by sucrose gradient centrifugation (6), was dissolved in a small volume of E-buffer (0.04M Tris-acetate--0.02M sodium acetate--1mM EDTA--0.2% SDS, pH 7.2), and treated as specified elsewhere. Ribosomes were isolated from the whole tissue of Galleria larvae. The frozen tissue was homogenized in 0.02M Tris-HCl

(pH 7.6) containing 0.4M sucrose, 0.02M KCl, 5mM $MgCl_2$ and 5mM phenylthiourea. The homogenate was filtered through two-layers of gauze and the filtrate centrifuged at 10,000xg for 120 min. The microsomal pellet was kept overnight in the freezer, and then resuspended in 0.05M Tris-HCl (pH 7.6) containing 1mM $MgCl_2$, 2mM phenylthiourea and 0.5% sodium deoxycholate, and spun down. The crude ribosomes were rinsed twice in the buffer containing deoxycholate, once in the buffer without deoxycholate, and kept frozen. The larger rRNA was extracted from the ribosomes, and isolated by sucrose density gradients. The method of Bishop *et al.* for polyacrylamide gel electrophoresis was followed with slight modifications (11). After electrophoresis, the gels were scanned for ultra-violet absorbancy with a Gilford recording spectrophotometer adapted for this purpose.

Results

When the larger rRNA was isolated from fresh silkglands and heated, a monodisperse dissociation product was observed (Fig. 1). Under these conditions an intermediary, transient product was not observed during the course of thermal dissociation of the RNA molecule.

In another experiment, dissected silkglands were frozen in liquid nitrogen, thawed, and frozen again prior to homogenization. As shown in Fig. 2a, the larger rRNA from tissue treated in this way appeared as a single peak. Upon heating at 45°C, several products were found indicating the RNA molecule contained several nick (Fig. 2b,c,d). Heating for 1 min at 45°C resulted in the formation of three components: A, B and M (main product) (Fig. 2b). A longer heating at 45°C (3 min) resulted in the formation of an additional product (C) (Fig. 2c). If the preparation was heated for 10 min at 45°C, the product A disappeared leaving components M, B and C (Fig. 2d). If the preparation was heated at 45°C for up to 30 min, the pattern was the same as for a 10 min incubation.

If the larger rRNA preparation was frozen and thawed more than ten times extending over ten days, the results shown in Fig. 3 were obtained. The thermal treatment of this RNA gave rise

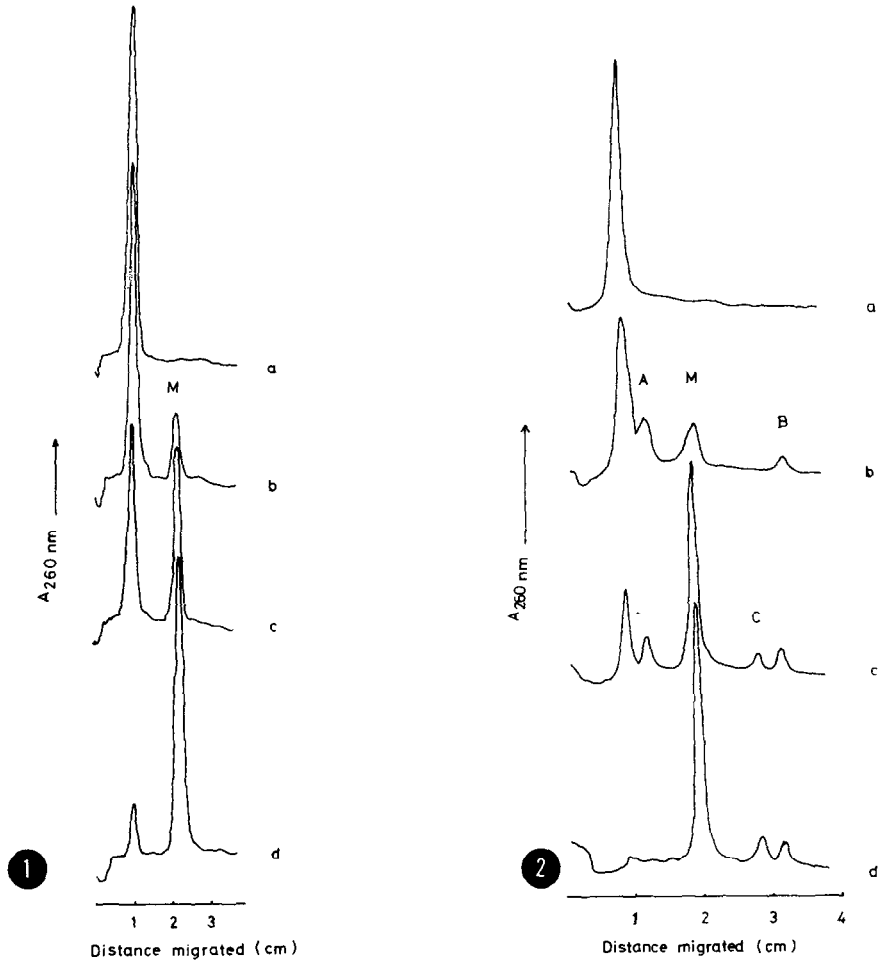

Fig. 1. Effect of heating on the "intact" RNA.

The larger rRNA was extracted and isolated from fresh silkglands. (a) incubated at 0°C, (b) 45°C for 30 sec, (c) 45°C for 1 min, (d) 45°C for 3 min in a small volume of E-buffer. The RNA's were subjected to polyacrylamide gel (3%, methylenebisacrylamide cross linked) electrophoreses for 180 min.

Fig. 2. Effect of heating on the "slightly-nicked" RNA.

The larger rRNA was isolated from silkglands which underwent freezing-thawing twice within an hour after being dissected. The RNA was incubated at 0°C (a), 45°C for 1 min (b), 45°C for 3 min (c), or 45°C for 10 min (d), and subjected to gel electrophoreses.

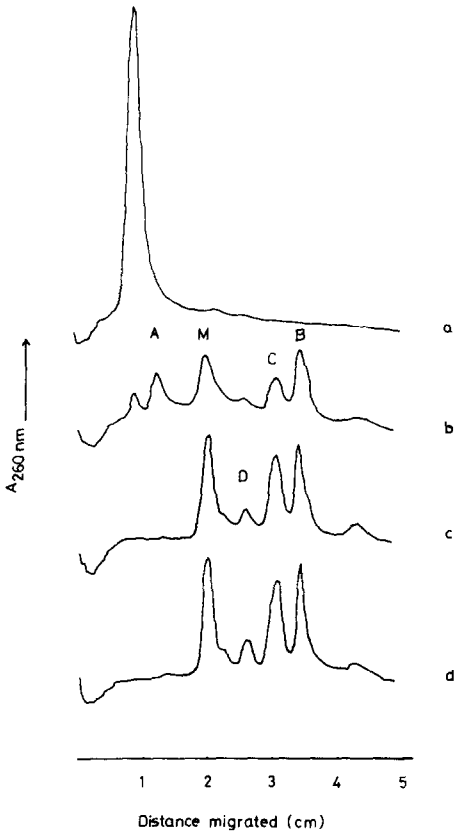

Fig. 3. Effect of heating on the "considerably-nicked" RNA. The larger rRNA was isolated from silkglands which underwent freezing-thawing more than ten times extending over ten days. The sample was incubated at 0°C (a), 45°C for 1 min (b), 45°C for 3 min (c) or 45°C for 10 min (d), and subjected to gel electrophoreses.

to the three components: M, B and C, while the product A was transient as in the previous case. The final dissociation pattern was not exactly the same as that of the previous case in that both the amounts of components B and C were greater and a new component D appeared.

The larger rRNA from isolated ribosomes was more labile, in that some degradation occurred at 35°C (Fig. 4). In addition at 45°C for 1 min, the rRNA molecule dissociated nearly completely into components M, C, B and D. In these experiments, component B and transient product A arose first as discrete peaks(Fig. 4b), and this followed by components M and C (Fig.

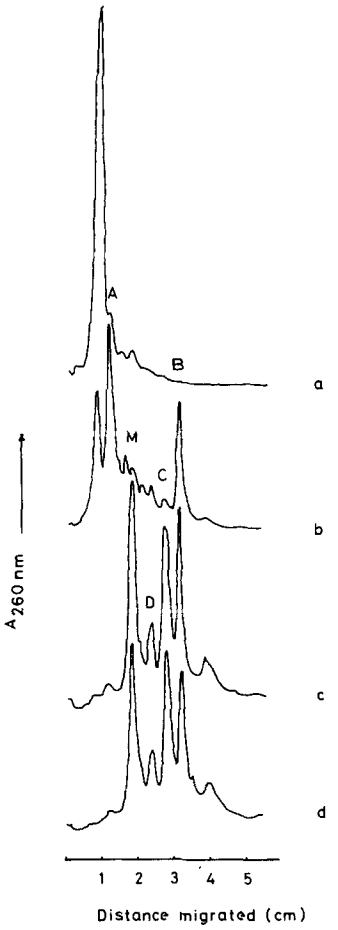

Fig. 4. Effect of heating on the larger rRNA from isolated ribosomes.

The sample was incubated at 0°C (a), 35°C for 5 min (b), 45°C for 1 min (c) or 45°C for 10 min (d), and subjected to gel electrophoreses.

4c). The final dissociation pattern (Fig. 4d) was basically the same as those observed in the previous experiments (Fig. 2d and 3d).

Discussion

Due to a primary nick, the larger rRNA molecule dissociates into two components that are similar to one another (Fig. 1)[6]. It does appear on the basis of current information that the primary nick is due to an intrinsic structural ribosomal

factor, rather than to exogenous nucleases, since the nick is always observed regardless of the method employed for extracting RNA (cf. 13).

In contrast, the secondary nicks, which were demonstrated for the first time in the present communication, can be introduced into the molecule of the larger rRNA depending on the experimental procedure for its isolation. It is likely that nucleases present in the preparation are involved in the formation of these nicks. It is significant that each product due to the secondary nicks was limited in both size and amount. This means that the secondary nicks are limited in number as is the primary one, and are introduced into a specific location in the RNA molecule. On the basis of mobility on the polyacrylamide gels[15], molecular weights of 1.6×10^6, 1.2×10^6, 0.7×10^6, 0.4×10^6, 0.3×10^6, and 0.2×10^6 daltons were tentatively calculated for the larger rRNA, product A, M, D, C and B, respectively. Such high-molecular-weight constituents as the products A, B, C and D reproducibly obtained from the larger rRNA should ultimately provide some insight into the topography of RNA molecule in the larger subunit of the ribosomal particle.

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