RELEASE OF NEWLY SYNTHESIZED NUCLEOPLASMIC RIBOSOMAL SUBUNITS OR THEIR PRECURSOR PARTICLES FROM ISOLATED NUCLEI OF REGENERATING RAT LIVER

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1. Introduction

When regenerating rat liver nuclei, labeled in vivo with [^{14}C]orotic acid were incubated in a medium containing ATP, only 40 S informosome-like particles were released [1]. However, when yeast RNA was added to the system, labeled 60 S and 40 S particles were released [1]. Since 60 S particles contain 28 S RNA, and 40 S particles both 18 S and heterogeneous RNA, it is suggested that the 60 S particles are the newly synthesized 60 S ribosomal subunits or its precursor [1–3], and that the 40 S particles are newly synthesized 40 S ribosomal subunits or its precursor and nuclear informosome-like particles [1,2]. The pattern of CsCl density-gradient centrifugation supported the above view [1,2].

We present here the time course of the labeling of the RNA and protein moieties of these particles in vivo as well as the pattern of one-dimensional acrylamide gel electrophoresis of their protein moieties labeled with [^{35}S]methionine in vivo, which shows that released 60 S particles are newly synthesized ribosomal large subunits or their precursor particles in the nucleoplasm on their way from the nucleolus to the cytoplasm. It appears likely that released 40 S particles contain newly synthesized ribosomal small subunits or their precursors in the nucleoplasm.

2. Materials and methods

The procedures for the preparation of nuclei from regenerating rat liver labeled in vivo were described in [1]. For the incubation of labeled nuclei, modified medium II [1] was used, which contained in a 3 ml final vol., 0.25 M sucrose, 25 mM KCl, 5 mM $MgCl_2$, 2 mM ATP, 4 mM phosphocreatine, 10 units creatine phosphokinase, 50 mM Tris–HCl (pH 7.6), dialyzed cytosol containing 9–10 mg proteins, 500 μg yeast RNA and 2.5 mM dithiothreitol and labeled nuclei (5×10^7/ml). After incubation at 20°C for 20 min, the released fraction was subjected to sucrose density-gradient centrifugation and the radioactive pattern was analyzed as in [1]. One-dimensional acrylamide gel electrophoresis (pH 8.6) of the protein moiety of 60 S and 40 S particles labeled with [^{35}S]methionine was done by a slight modification [4] of the method in [5] and acid-insoluble radio-activity of gel slices was measured as in [1].

3. Results and discussion

To characterize the 60 S and 40 S particles released from labeled regenerating rat liver nuclei by incubation in modified medium II, we examined the effects of treatment with a low dose of actinomycin D [6] in vivo on the labeling of their RNA and protein moieties. As shown in fig.1, the radioactivities of the RNA and protein moieties of 60 S particles released from isolated nuclei labeled in vivo for 5 h became negligible after actinomycin D treatment, while those of 40 S particles were partially inhibited. Similar results were obtained with labeling for 2 h. The results support the view that a greater part of labeled 60 S particles consist of large subunits or their precursor particles, and that part of 40 S particles may be small subunits or their precursor particles, in good agreement with [1].

On the basis of these findings, the time courses of the in vivo labeling of RNA and protein moieties of 60 S and 40 S particles released from isolated

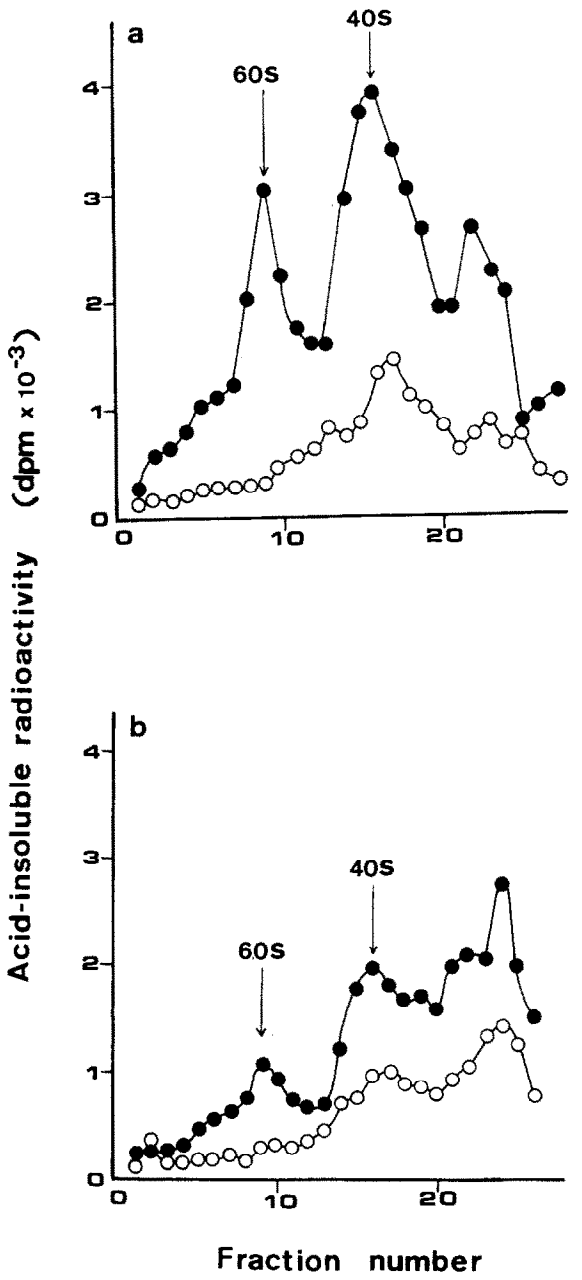

Fig.1. Effects of actinomycin D treatment on labeling of RNA (a) and protein moieties (b) of released 60 S and 40 S particles. Actinomycin D (550 μg/kg body wt) was injected intraperitoneally into partially hepatectomized rats 1 h before the administration of labeled compounds. The fraction released from isolated rat liver nuclei labeled in vivo with [^{14}C]orotic acid and [^{3}H]leucine (5 μCi and 100 μCi per 100 g body wt, respectively) for 5 h were subjected to sucrose density-gradient centrifugation. The acid-insoluble radioactivity of each fraction was measured. These procedures have been described in [1]. (○——○) actinomycin D-treated rat; (●——●) normal rat.

nuclei were examined. The radioactive patterns of the sucrose density-gradient centrifugation of the released fraction labeled with [^{14}C]orotic acid and [^{3}H]leucine for various time periods are shown in fig.2. The time courses of the in vivo labeling of RNA and protein moieties of the released fractions thus obtained are summarized in fig.3.

The radioactivity of the RNA moiety of 60 S particles increased gradually, depending on in vivo labeling time periods, up to 5 h, while that of 40 S particles reached a maximal value at ~3 h of labeling, then decreased. At 11 h after labeling, the radioactivities of both 60 S and 40 S subunits became very low. The radioactivity of the protein moiety of 60 S particles was rather low up to 3 h, and then increased and exhibited a distinct peak at 5 h. Labeling of the protein moiety of 40 S particles was more rapid and became maximal at 3–5 h (fig.2). At 11 h after labeling the radioactivities of protein moieties of both 60 S and 40 S particles became very low. The time courses of labeling of RNA and protein moieties of 60 S particles ran parallel with each other, while that of the RNA moiety was somewhat different from that of the protein moiety in the case of 40 S particles. The results may be explained by the heterogeneity of 40 S particles as mentioned above.

The labeling pattern of one-dimensional acrylamide gel electrophoresis of the protein moieties of both 60 S and 40 S particles released from regenerating rat liver nuclei labeled with [^{35}S]methionine in vivo are shown in fig.4. In the case of 60 S particles, most of radioactivity distributes in the fast moving area, in which ribosomal proteins and histones were shown to migrate specifically [7,8]. Furthermore, the main peaks of radioactivity coincide generally with the staining pattern of marker 60 S ribosomal proteins. In the case of 40 S particles, radioactivity is present not only in the 40 S protein area, but also in other areas, especially near the origin, suggesting the heterogeneity of the protein moieties.

Our previous experiments showed that the in vivo labeling of the RNA and protein moieties of nucleolar 60 S particles were very rapid in regenerating rat liver, reaching a maximal value at 20 min [9]. On the other hand, the labeling of RNA and protein moieties of cytoplasmic ribosomes in rat liver was very slow, increasing gradually up to 25 h and 12 h, respectively [10]. From the results of the present experiments and previous reports we conclude that the main labeled component of the released 60 S particles are

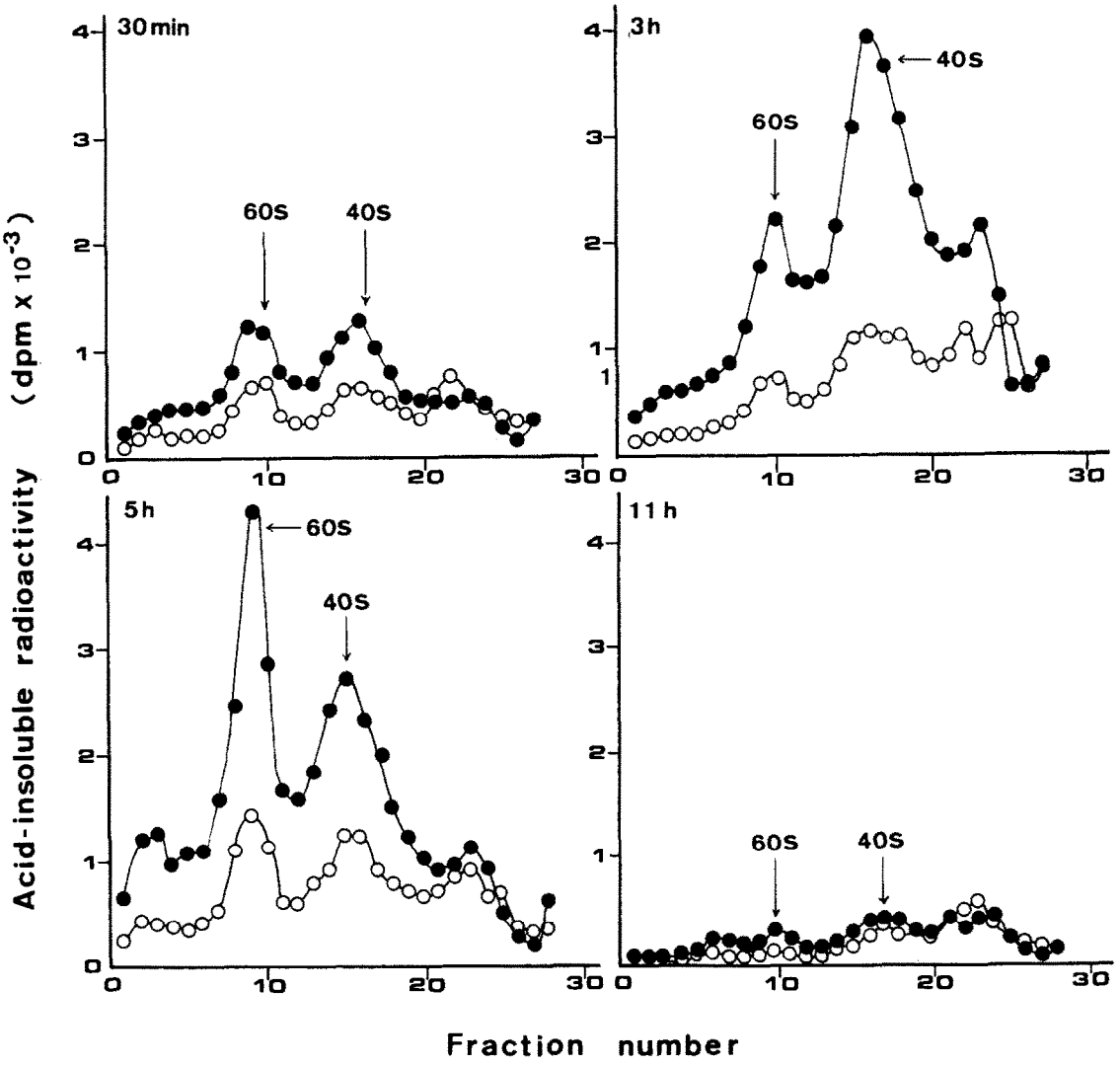

Fig.2. Sedimentation profiles of the released fractions from regenerating rat liver nuclei labeled in vivo. After labeling with [^{14}C]-orotic acid and [^{3}H]leucine, the amounts of which are shown in fig.1, for various time periods liver nuclei were incubated and the released fractions were analyzed by sucrose density-gradient centrifugation as in fig.1. (●——●) ^{14}C radioactivity; (○——○) ^{3}H radioactivity.

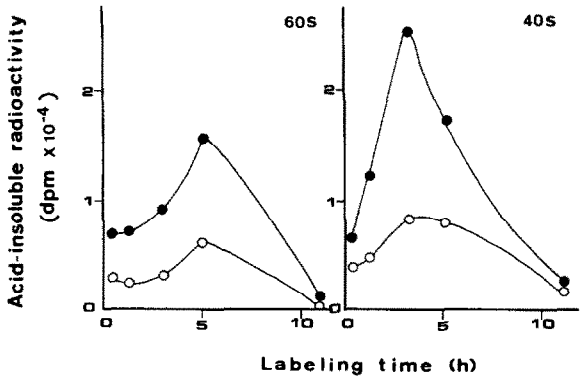

Fig.3. Time courses of the in vivo labeling of RNA and protein moieties of released 60 S and 40 S particles. Radioactivities of 60 S and 40 S particles shown in fig.2 and of both particles released from nuclei labeled for 1.5 h were plotted. (●——●) ^{14}C radioactivity; (○——○) ^{3}H radioactivity.

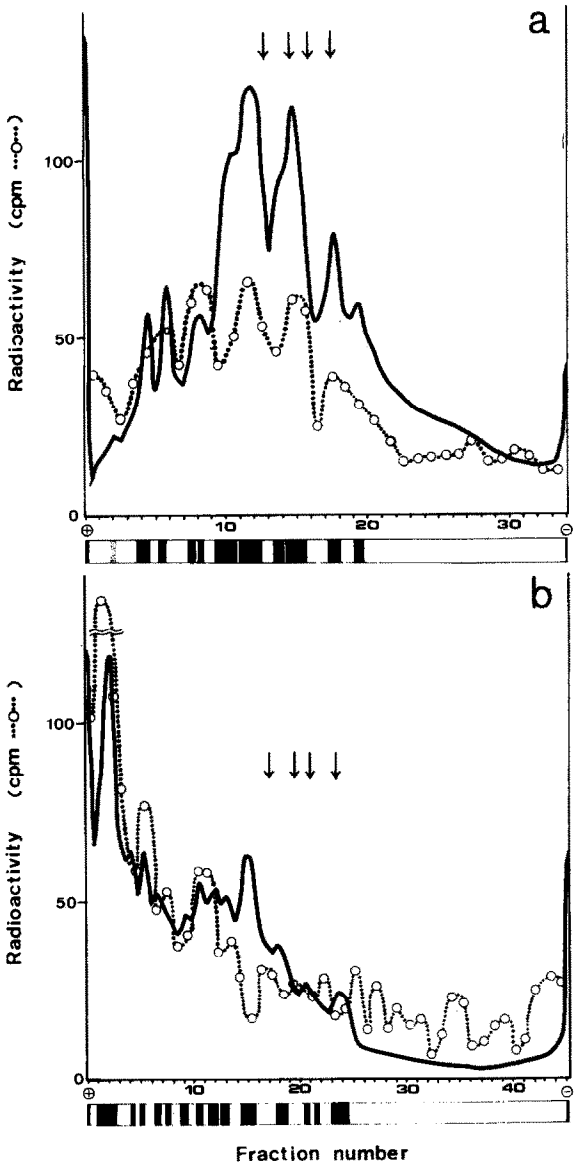

Fig.4. One-dimensional acrylamide gel electrophoresis of labeled proteins of 60 S (a) and 40 S particles (b) released from regenerating rat liver nuclei. [^{35}S]Methionine and [^{3}H]-orotic acid (25 μCi and 12.5 μCi per 100 g body wt, respectively) were intraperitoneally injected into rats at 3 h and 4.5 h before sacrifice. The released fractions from liver nuclei were subjected to sucrose gradient centrifugation, and 60 S and 40 S particles were isolated. After addition of 100 μg protein of rat liver 60 S or 40 S subunits as a carrier and marker, the 60 S and 40 S particles were diluted with sucrose-free medium A [7] and then sedimented at 105 000 × *g* for 12 h. The precipitate was extracted with 66% acetic acid [4]. The acid-soluble proteins were then precipitated with 10 vol. acetone at −20°C and subjected to one-dimensional acrylamide gel electrophoresis. The gel was stained with amido black 10B and traced with a densitometer. The gel was sliced into 4 mm discs and their radioactivities were measured. The staining patterns of the electrophoresis are also shown. Arrows indicate the migrating positions of histones. (——) Densitometry; (o———o) ^{35}S radioactivity.

the large subunits of cytoplasmic ribosomes or their precursor particles in the nucleoplasm on their way from the nucleolus to the cytoplasm. It appears likely that released 40 S particles contain newly synthesized small subunits of cytoplasmic ribosomes or their precursor particles in the nucleoplasm.

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