MAMMALIAN DNA-SRNA HYBRIDS

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In the past year it has been reported from this laboratory that either DNase or actinomycin inhibits the incorporation of uridine-2-^{14}C into amino acid acceptor RNA (SRNA) of nuclei fractions of mouse Ehrlich ascites carcinoma cells (Cook and Fraser, 1963; Cook, Bouchard and Fraser, 1964). These nuclei fractions also catalyze the incorporation of radioactivity from ^{14}C-CH_3-methionine into nuclear SRNA. Similar observations have been made independently by Chipchase and Birnstiel (1963). The reports of Sirlin, Jacob and Tandler (1963) and of Birnstiel, Fleissner and Borek (1963) further indicate that the site of methylation of SRNA is the nucleolus. The ability of E. Coli SRNA to form specific hybrids with homologous heat-denatured DNA (Giacomoni and Spiegelman, 1962; Goodman and Rich, 1962) strongly indicates that regions of the DNA have nucleotide sequences complementary to those of SRNA. Taken together these findings are consistent with the hypothesis that amino acid acceptor RNA is made on a DNA template in the nucleus of the cell, is released from that template, and methylated in the nucleolus before its release into the cytoplasm.

As a first step in the isolation of the system responsible for the biosynthesis of SRNA in mouse Ehrlich ascites carcinoma cells we have attempted to purify the DNA which serves as template for the synthesis of SRNA. It is reasoned that, if the template DNA could be recovered

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from DNA-SRNA hybrids and incubated with DNA-dependent RNA polymerase in the presence of the appropriate nucleoside triphosphates, then a precursor "methyl poor" SRNA might form. Since Starr (1963) has found that "methyl poor" RNA can accept amino acids, it might be possible to synthesize RNA with amino acid acceptor activity in the proposed system. We wish to report here for the first time evidence for the formation of specific DNA-SRNA hybrids in a mammalian system and the partial purification of these hybrids by chromatography on methylated-albumin-kieselguhr columns.

MATERIALS AND METHODS

The source and the growth of the mouse Ehrlich ascites carcinoma cells have been described previously (Fraser, 1962) as have also the methods of washing the cells, lysis and preparation of cell fractions (Littlefield and Keller, 1957; Fraser, 1962). DNA was prepared from ascites cells and from E. Coli B* by the method of Marmur (1961). SRNA was prepared from the "pH 5 enzymes" fraction of 105,000 x g supernatants of cell homogenates by a modification (Hoagland, Stephenson, Scott, Hecht and Zamecnik, 1958) of the phenol method of Kirby (1956). The SRNA was further purified by chromatography on methylated-albumin-kieselguhr (MAK) columns (Mandell and Hershey, 1960). The adsorbed RNA was eluted from the columns in a linear NaCl gradient (0·4 - 1·2 M) in 0·05 M sodium phosphate buffer, pH 6·5 (Cook, Bouchard and Fraser, 1964). For the preparation of ^{32}P-labelled SRNA a single tumor-bearing mouse was injected intraperitoneally (i.e., at the tumor site) with

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approximately 0·5 mc ^{32}P-orthophosphate solution (pH 7·4, 0·9% in NaCl) on the sixth day after transplantation of the tumor into the mouse. The mouse was sacrificied 20 hours later and the labelled SRNA isolated.

Hybridization and CsCl gradient centrifugation procedures have been carried out as described by Giacomoni and Spiegelman (1962). Denatured DNA (heated 15 minutes at 90°C and rapidly cooled) and ^{32}P-labelled SRNA were heated together at 72°C for 2 hours and then quickly cooled in an ice-bath. The samples were then diluted to 3·0 ml with a CsCl solution (final concentration 7·7 M, density 1·72) and centrifuged at 33,000 r.p.m. for 65 hours in an SW 39 swinging bucket rotor of the Model L Spinco ultracentrifuge. After centrifugation, 19 or 20 fractions (4 drops each) were collected from the bottom of the tube. Each fraction was diluted with 1·0 ml of H_2O, and its optical density measured at 260 mμ. Alternate fractions were treated with pancreatic RNase (20 μg/ml for 1 hour at 37°C) and the radioactivity in the acid-precipitable material of all fractions was determined according to the methods of Yankofsky and Spiegelman (1962) and Goodman and Rich (1962).

Other experiments were also carried out in which mixtures of heat-denatured ascites DNA and ^{32}P-SRNA were annealed at 72°C for 2 hours, cooled and then chromatographed on MAK columns. Before chromatography the mixtures were treated for 30 minutes at 37°C with phosphodiesterase of E. Coli B prepared by the method of Lehman (1960). This enzyme specifically attacks single-stranded (heat-denatured) DNA. The chromatography was carried out as described by Cook, Bouchard and Fraser (1964). Three ml fractions were collected and the optical density at 260 mμ and acid-precipitable radioactivity before and after RNase treatment were measured in each fraction as indicated above.

RESULTS AND DISCUSSION

When heat-denatured ascites DNA and ascites ^{32}P-SRNA were simply mixed at room temperature and sedimented in a CsCl gradient, the two

nucleic acids separated well. The ^{32}P-SRNA sedimented to the bottom quarter of the centrifuge tube and the DNA banded about two-thirds of the way up from the bottom (see Fig. 1A). However, when these two nucleic acid preparations were mixed and heated together for 2 hours at 72°C, some of the radioactive material was found to sediment with the DNA (see Fig. 1B). This material was not rendered acid-soluble by treatment with RNase. The results indicated that an association between the SRNA and DNA strands had occurred. That this was a very specific association and not simply a non-specific adsorption of the ^{32}P-SRNA on to the DNA, is further indicated by the observation that when heat-denatured DNA from a different source (E. coli) was heated together with ascites tumor ^{32}P-SRNA under identical conditions, none of the radioactivity sedimented with the DNA (see Fig. 1C). In this case, all of the ^{32}P-SRNA was rendered acid-soluble by treatment with RNase.

Mixtures of heat-denatured ascites tumor DNA and ^{32}P-labelled ascites tumor SRNA were heated for 2 hours at 72°C and then treated with E. coli phosphodiesterase to destroy single-stranded DNA before chromatography on MAK columns. Two peaks of radioactivity were eluted by the NaCl gradient (see Fig. 2). The major one was eluted in the

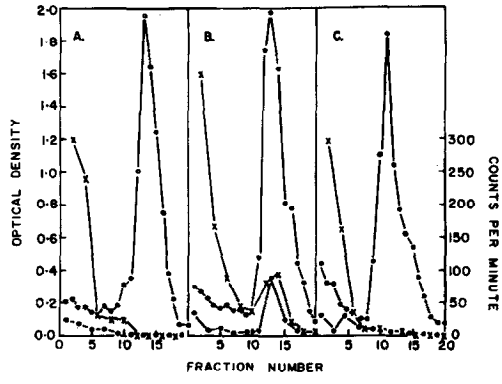

Fig. 1. CsCl gradient centrifugations. A. Mixture of 0·1 mg heat-denatured ascites DNA and 0·005 mg ascites ^{32}P-SRNA. B. Same as A, except annealed together then quickly cooled (see Materials and Methods). C. Same as B, except E. coli DNA used in place of ascites DNA.

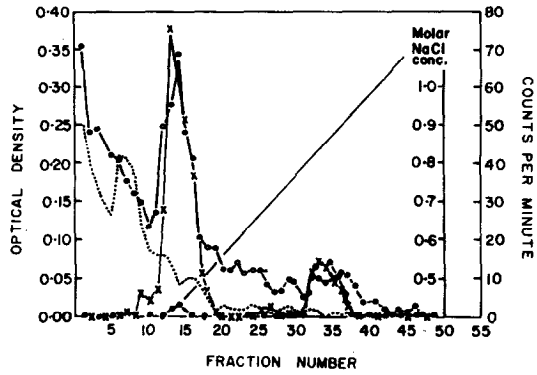

Fig. 2. Chromatography on MAK column. Mixture of 1·0 mg heat-denatured ascites DNA annealed together with 0·05 mg ascites ^{32}P-SRNA and then cooled quickly, treated with E. coli phosphodiesterase and placed on column. A control experiment was run in which 1·0 mg of ascites DNA, treated in the same way in the absence of ^{32}P-SRNA, was chromatographed on a second MAK column (dotted line).

Symbols for Figs. 1 and 2: O —— O optical density, X —— X radioactivity before RNase treatment, ● —— ● radioactivity after RNase treatment, ... optical density (control experiment).

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region 0·4 - 0·5 M NaCl and corresponds to ^{32}P-SRNA, since, in control experiments in which only ^{32}P-SRNA was adsorbed on to the column, all of the radioactivity was eluted in this region of the gradient. This elution pattern for SRNA has also been observed by Sueoka and Yamane (1962). The minor peak of radioactive material was eluted in the range 0·8 - 0·9 M NaCl. This is in the region of the gradient where DNA-SRNA hybrids might be expected to be eluted, since Hayashi, Hayashi and Spiegelman (1963) have found that hybrids of ϕX-174 DNA and ϕX-174-specific RNA were eluted from MAK columns in this range of salt concentration.

The large amount of U.V.-absorbing material which washed through the MAK column (see Fig. 2) corresponds to the degradation products resulting from the action of the phosphodiesterase on unhybridized heat-denatured and annealed DNA. In a control experiment (Fig. 2, dotted line) in which heat-denatured DNA only was heated and treated with phosphodiesterase and then chromatographed on an MAK column, most of the U.V.-

absorbing material was eluted from the column when the NaCl concentration had reached 0·5 M. Nevertheless, in the experiment described above, in which the DNA was annealed with ^{32}P-SRNA, an appreciable amount of U.V.-absorbing material was eluted above 0·5 M NaCl. Some of this may be hybrid material (0·8 - 0·9 M NaCl), but the major portion probably represents undegraded DNA (0·5 - 0·8 M NaCl), possibly because the presence of the SRNA partially inhibited the action of the phosphodiesterase. Despite this incomplete degradation, it may be concluded that a considerable purification of the "hybrid" DNA has been obtained in fractions 32 - 37. Presumably this DNA acts as template for SRNA biosynthesis.

Preliminary experiments indicate that the DNA of the "hybrid" material eluted from MAK columns is active as template for RNA synthesis. Small amounts of this "hybrid" have been "melted" by heating (15 minutes at 90°C, followed by rapid cooling) and then incubated with DNA-dependent RNA polymerase of E. coli in the presence of ^{14}C-UTP according to the methods of Chamberlin and Berg (1962). High incorporations of radioactivity into an "RNA-like" material (acid-precipitable, rendered acid-soluble by alkali and RNase) have been obtained. These incorporations were abolished when the hybrid material was first pre-incubated with DNase. The properties of this "RNA" are under examination.

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