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Further evidence for two types of 18S rRNA in Neurospora

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Abstract

Evidence for two types of 18S rRNA

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Evidence for two types of 18S rRNA in N. crassa was first reported by Michelson and Suyama (1968 Biochim. Biophys. Acta 157: 200), who found that 18S rRNA, which had been isolated from sucrose gradients, yielded two peaks when analyzed by methylated albumin-coated Kieselguhr (MAK) column chromatography. 28S rRNA yielded only one peak. The difference between the two types of 18S was not due to artifacts resulting from the isolation procedure, extent of hydrogen bonding or amount of Mg^{+2} bound. The purpose of the present work has been to determine whether the two 18S species are due to differences in molecular weight or bore sequence, as revealed by polyacrylamide gel electrophoresis and RNA-DNA hybridization studies.

Wild type N. crassa 74A was grown and handled as in the paper cited above. To obtain [^{3}H]-labeled RNA, a pyrimidineless mutant, KS43, was grown in Vogel's medium containing 2% sucrose and 40µg/ml "cold" uridine plus IO &i/ml [^{3}H]-uridine. After a 16-hr growth period, 100 X the concentration of "cold" uridine was added as a chase and the culture was kept for 3 additional hours. The bulkwhole-cell or post-mitochondrial RNA was isolated as described [ibid.' and 28S and 18S ribosomal RNA's were obtained by sucrose gradient centrifugation. Two successive purifications through MAK chromatography (Sueoka and Chang 1962 J.Mol.Biol.4: 161) were employed to obtain chromatographically pure [^{3}H]-A peak RNA (see Fig. 1). DNA was isolated from lyophilized mycelium by the method of Marmur (1961 J. Mol. Biol. 3: 208) and mitochondrial DNA was *removed* by CsCl gradient centrifugation. The fractions containing nuclear DNA (density = 1. 712 g/cm^3) were collected.

Whole-cell RNA and post-mitochondrial RNAs were analyzed by MAK column chromatography, using salt concentrations from 0.75 to 1 .O M for elution. Peak C is 28S rRNA, while peaks A and B are both 18S rRNAs (Fig. 1). 18S RNA, first isolated by sucrose gradient centrifugation, gave rise to two peaks an MAK, corresponding to peaks A and B. Heating whole-cell RNA with or without 1 mM EDTA did not affect the elution pattern in MAK chromatography.

RNA-DNA hybridization-saturation experiments were performed by the method of Gillespie and Spiegelman (1965 J. Mol. Biol. 12: 829) to determine the maximum saturation levels for 28S, 18S (A + B) and A-peak RNAs. The 18S RNA was purified by two consecutive sucrose gradient centrifugations to obtain a purity that producer a saturation plateau. As shown in Fig. 2, saturation was achieved with 28S RNA at a level equal to 1. 74% of the membrane-bound DNA. The 18S species saturated at 0.93%, and A-peak RNA at 0.6%. approximately 65% of the 18S level. The fact that A-peak rRNA alone only hybridizer at 65% of the level of total 18S rRNA suggests that the A-peak and B-peak 18S rRNAs differ in bare sequence. The B-peak 18S rRNA would be predicted to hybridize with the remaining 35% of the DNA sequences which anneal with total 18S rRNA. The

difficulty in isolating sufficient pure B-peak RNA from the MAK column, due to its overlap with the A and C regions, has prevented us from carrying out hybridization experiments with B-peak RNA. These saturation values are higher than those reported earlier; perhaps difficulties in specific activity determinations of RNAs con account for these variations in hybridization levels.

Separations of rat liver 18S RNA and E. coli 16S RNA into doublet bands by ogarore-supported polyacrylamide gels have been reported by Peacock and Dingman (1968 Biochemistry 7: 668). In the present experiments, whole-cell RNA or 18S RNA was run on gels of various concentrations (2.3%, 3.0% and 5.0%), essentially as described by these authors. It was not possible to obtain separation of Neurospora 18S RNA into two distinguishable bands, suggesting a close similarity in molecular weights of A-peak and B-peak 18S RNAs. The molecular weights of 28S and 18S rRNA of Neurospora were estimated from 10 independent runs in 3.0% gel, using rat liver 30S and 18S and E. coli 23S and 16S RNAs as references. These esimated weights are $1.28 \pm 0.09 \times 10^6$ and $0.78 \pm 0.03 \times 10^6$ daltons for 28S and 18S RNAs, respectively.

The present experiments suggest that two types of 18S RNA separable by MAK chromatography differ in base sequence rather than in molecular weight or gross conformation. We also hove evidence which suggests that there two species of 18S RNA are equally methylated. It is still conceivable that the two peaks of 18S RNA ore actually due to artifacts inherent in the techniques employed. To truly substantiate rRNA heterogeneity, it seems necessary to undertake sequence analysis of these RNAs.

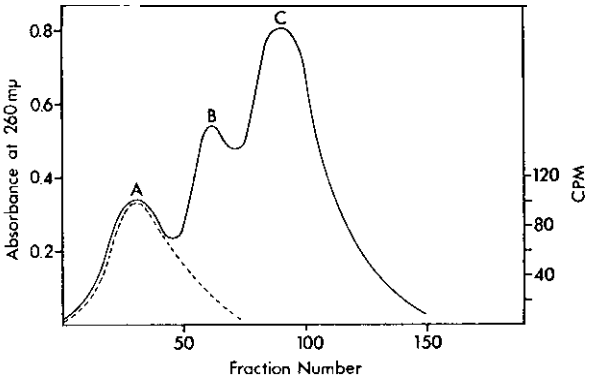

Figure 1. MAK column chromatography of purified whale cell RNA and [^{3}H] A-peak RNA.

Whole-cell RNA was diluted to 1 A260 with 0.3N NaCl, 0.05M sodium phosphate buffer (pH 6.7), mixed with [^{3}H] A-peak RNA, and added to the column. The column was then washed with 50 ml of the same buffer. RNA was eluted from the column with a linear salt gradient made with 130 ml each of 0.3N NaCl and 1.35 N NaCl, 0.05M sodium phosphate buffer (pH 6.7). When the salt concentration of the effluent reached 0.7M MaCl, fractions were collected. A260 measurements were made with a Beckman DB spectrophotometer. To measure radioactivity, fractions were precipitated with 10% cold TCA and filtered onto Boc-T-Flex membrane filters. After drying, IX spectrafluor (Amersham and Searle) was added and counts were read. Counting efficiency was ca. 25%. A_{260} (——); [^{3}H] cpm (----).

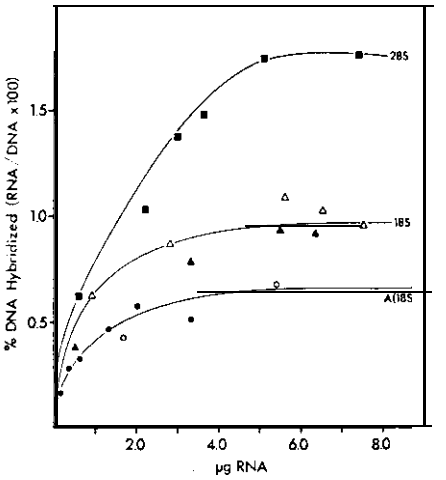

Figure 2. Hybridization of rRNA components with nuclear DNA.

Nuclear DNA (10μg) was immobilized on nitrocellulose membrane filters. The filters were incubated at 64°C far 22-24 hrs in vials containing various concentrations of labeled RNA in 5 ml of 2XSSC (1XSSC = 0.5M NaCl, 0.015M sodium citrate, pH 7.0). A blank filter was added to each viol to correct for background adsorption. After incubation, the filters were rinsed briefly in 2XSSC, incubated for one hour at room temp. in 5 ml 2XSSC containing 50 μg/ml of ribonuclease, and washed on both sides with 50 ml 2XSSC. They were finally dried and counted in a liquid scintillation spectrometer. Solid and open points denote independent experiments run with RNAs isolated at different times. Specific radioactivities 28S RNA 3,936 cpm/μg; 18S RNA 3,936 cpm/μg for solid points and 3,846 cpm/μg for open points.

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