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Transcriptional organization of the 5.8S ribosomal RNA cistron in Xenopus laevis ribosomal DNA

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ABSTRACT

Hybridization of purified, ^{32}P-labeled 5.8S ribosomal RNA from Xenopus laevis to fragments generated from X. laevis rDNA by the restriction endonuclease, EcoRI, demonstrates that the 5.8S rRNA cistron lies within the transcribed region that links the 18S and 28S rRNA cistrons.

INTRODUCTION

In prokaryotes, the cistrons specifying the three ribosomal RNA (rRNA) molecules are arranged in compound transcriptional units which are read by the RNA polymerase in the order 16S-23S-5S (1,2). Eukaryotic ribosomes contain four rather than three rRNA molecules, including 18S, 28S, 5S and 5.8S rRNA species. The 18S, 28S and 5.8S molecules are derived from common, compound transcriptional units (3, 4) with the 18S cistron being promoter proximal (5-7). Nucleolar DNA consists of multiple such units, individually separated by regions of DNA which apparently are not transcribed (8). The eukaryotic 5S rRNA genes are not part of these transcriptional units (9, 10).

The similarity in the transcriptional polarity of the larger rRNA cistrons in both prokaryotes and eukaryotes coupled with the observation that 5.8S rRNA is transcribed from the same unit as 18S and 28S rRNA suggests that 5.8S rRNA may be one evolutionary homolog of the bacterial 5S rRNA. If so, then the eukaryotic 5.8S and prokaryotic 5S genes might be expected to occupy the same relative positions in their respective transcriptional units. Maden and Robertson (11) have demonstrated through nucleotide sequence analysis that the 5.8S rRNA is one component of the 32S precursor of 28S rRNA in HeLa cells; so, the 5.8S rRNA gene certainly lies adjacent to the 28S rRNA gene. Recently, Speirs and Birnstiel (12) observed that 5.8S rRNA hybridized to sheared DNA enriched for sequences complementary to 18S rRNA as well as to DNA corresponding to the 28S rRNA. These data are consistent with, but do not prove, the location of the 5.8S rRNA gene in the region between the 18S

and 28S rRNA cistrons. However, in light of the possible evolutionary homology between 5.8S rRNA and the bacterial 5S rRNA, the rather low levels of observed 5.8S rRNA hybridization to enriched 18S-specific DNA, and the presence of 28S-specific sequences within the 18S-specific DNA, we undertook, and here report, a rigorous confirmation of the conclusion of Speirs and Birnstiel.

In these experiments we have used a definitive approach to gene mapping which involves hybridizing the purified, ^{32}P-labeled rRNA species to fragments generated from Xenopus laevis rDNA by the restriction endonuclease, EcoRI. Since the polarity of the 18S and 28S rRNA cistrons (5-7), and the positions of the EcoRI cleavage sites (13) have been established, it is possible to determine unambiguously the position of the 5.8S rRNA cistron in its transcriptional unit.

MATERIALS AND METHODS

Escherichia coli transformants CD18 and CD30 have been described by Morrow et al. (14) and were obtained from Drs. S.N. Cohen and H.W. Boyer, respectively. These strains each carry one of the EcoRI cleavage fragments of X. laevis rDNA integrated via in vitro ligation into the tetracycline resistance plasmid, pSC101. Covalently-closed-circular plasmid DNA was isolated from these strains essentially as described by Sharp et al. (15), with the exception that the DNA was not centrifuged onto a CsCl shelf but rather was precipitated with polyethylene glycol and NaCl as described by Humphreys et al. (16). The precipitated plasmid DNA was dissolved in 0.05 M Tris-HCl, pH 8.5, 0.05 M NaCl, 0.005 M disodium EDTA and centrifuged to equilibrium in a CsCl-ethidium bromide density gradient. Subsequent to centrifugation, the plasmid DNAs were collected from the bottom of the tube, extracted twice with an equal volume of isopropanol, and then dialyzed against the above buffer. The dialysate was adjusted to contain 0.5 M NaCl, and the DNA was precipitated by the addition of 2.5 volumes of ethanol.

The cleavage of CD18 and CD30 plasmid DNAs with the restriction endonuclease, EcoRI (Miles Research), was conducted in volumes of 25-50 µl containing 0.1 M Tris-HCl, pH 7.5, 0.05 M NaCl, 0.003 M $MgCl_2$, 0.002 M dithiothreitol and 10% (v/v) glycerol. The DNA concentration in the reaction mixtures was always 170 µg/ml or less, and 2 units of EcoRI were used per µg of DNA. Incubation was carried out for 60 min at 37° after which the reaction was terminated by the addition of disodium EDTA to 0.01 M and sodium dodecyl sulfate to 0.5%. Prior to electrophoresis, the samples were incubated at 65° for 5 min.

EcoRI-digested DNA was electrophoresed on a 12.5 x 18 x 0.3 cm vertical slab gel containing 0.7% agarose (Sigma Chemical Co.) in 0.04 M Tris-acetate, pH 7.8, 0.005 M sodium acetate and 0.001 M disodium EDTA (17) at 3 volts/cm for 15 hr. After electrophoresis, the gels were stained with ethidium bromide, photographed, and the DNA fragments were denatured in situ and transferred to a nitrocellulose membrane, all as described by Southern (18).

Membrane filters containing restriction fragments were immersed for 6 hr at 80° in 5ml of 6 x SSC containing either 0.1 µg/ml of 5.8S, 1.5 µg/ml of 18S, or 3 µg/ml of 28S rRNA, each with a specific activity of about 4 x 10^5 cpm/µg. The filters were washed with 2 X SSC, treated with ribonuclease A (25 µg/ml in 2 x SSC for 20 min at 22°), washed again with 2 x SSC, dried and autoradiographed.

Monolayers of X. laevis kidney cells (ATCC, CCL 102) were labeled for 24 hr at 22° with 100 µCi/ml of carrier-free $H_3{}^{32}PO_4$ in phosphate-free MEM containing 10% dialyzed fetal calf serum and 0.02 M HEPES. Cells were collected and total RNA purified by phenol extraction and precipitation from ethanol as described previously (19). Precipitates dissolved in 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.4, 0.001 M disodium EDTA were layered onto 5-20% sucrose gradients containing the same buffer, and following centrifugation in a Spinco SW41 rotor at 40,000 rpm for 7 hr at 4°, fractions were collected from the bottom of the tube and monitored for radioactivity. The 28S and 18S rRNAs were each submitted to a second sucrose gradient centrifugation as described above except the 28S rRNA was heated for 5 min at 65° in 0.01 M Tris-HCl, pH 7.4, 0.001 M disodium EDTA before centrifugation. The 5.8S rRNA released from the 28S rRNA during heat denaturation was collected from the top of the gradient and purified further by electrophoresis through 8% polyacrylamide gels (19); Figure 1 illustrates the radioactivity profile of such a preparative gel.

RESULTS AND DISCUSSION

The arrangement of known DNA segments in the repeating rDNA of Xenopus laevis, as reported by Dawid and Wellauer (6), is outlined in Figure 2. As indicated, the restriction endonuclease, EcoRI, cleaves the rDNA into two fragments of unequal size. The smaller of these fragments (3.0 x 10^6 daltons) contains the transcribed region connecting the 18S and 28S rRNA genes, whereas the larger(3.9 x 10^6 daltons) includes the DNA segment between the 28S rRNA gene and the termination site for transcription. The ability of purified 5.8S rRNA to hybridize with one or the

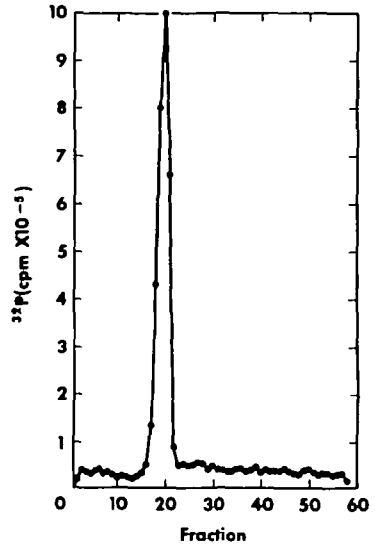

Figure 1. Electrophoretic profile of X. laevis 5.8S rRNA. Material released from heat-denatured 28S rRNA and recovered from a sucrose gradient was further purified by electrophoresis on an 8% polyacrylamide gel as described (19).

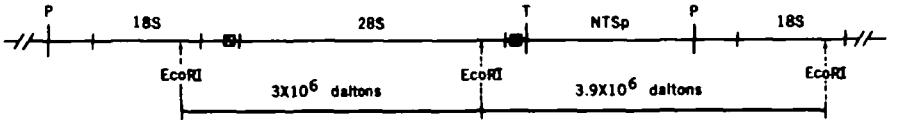

Figure 2. A diagrammatic representation of the repeating unit of X. laevis rDNA and the fragments generated by cleavage of the rDNA with the restriction endonuclease, EcoRI. The vertical arrows denote the positions of the EcoRI cleavage sites; the vertical lines labeled P and T represent, respectively, the RNA polymerase promoter and terminator sites on the transcriptional unit. The hatched rectangles indicate the possible positions of the 5.8S rRNA cistron based on the observation that the 28S and 5.8S rRNAs are both derived from a common 32S precursor rRNA molecule (11).

other of these DNA fragments therefore orients its gene, relative to the 28S rRNA gene, in the transcriptional unit.

Each of the X. laevis rDNA fragments has been cloned in E. coli by Morrow et al. (14), using the tetracycline resistance plasmid, pSC101. The plasmids CD18 (containing the 3 x 10^6 d. fragment) and CD30 (containing the 3.9 x 10^6 d. fragment), isolated as detailed in Materials and Methods, served as sources of DNA for these experiments. As illustrated in Fig. 3A, cleavage of the isolated plasmids with EcoRI, followed by electrophoresis through

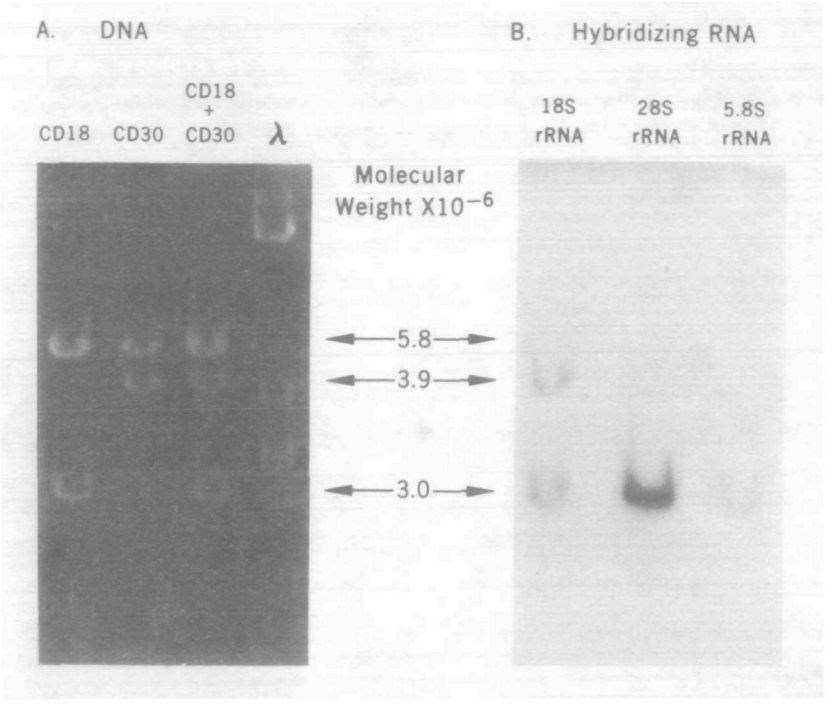

Figure 3. A. Agarose gel electrophoresis of EcoRI cleavage products of CD18 and CD30 plasmid DNAs. Bacteriophage lambda DNA was also cleaved by the nuclease to provide molecular weight markers.

B. Hybridization of purified, ^{32}P-labeled rRNA species to EcoRI cleavage products of CD18 and CD30 plasmid DNAs. Equal quantities of CD18 and CD30 plasmid DNAs were mixed, digested with EcoRI and electrophoresed on 0.7% agarose gels, all as detailed in Materials and Methods. After denaturation of the fragments in situ and their transfer to nitrocellulose filters, the fragments were hybridized to either 5.8S, 18S or 28S rRNA as described by Southern (18).

agarose gels and staining with ethidium bromide, yields the expected X. laevis rDNA fragments plus the pSC101 vehicle (5.8 x 10^6 d.). The molecular weights of these fragments were assessed by comparison of their electrophoretic mobilities with those fragments generated by cleavage of lambda DNA with the EcoRI endonuclease (14). Following alkali denaturation of DNA residing in the agarose gel tracts and elution onto a cellulose acetate sheet, hybridization with purified 18S, 28S or 5.8S ^{32}P-labeled rRNA was carried out. Figure 3B is an autoradiograph of the membrane filter following hybridization. X. laevis 28S rRNA is seen to hybridize predominantly with the 3 x 10^6 d. rDNA fragment. A much smaller amount hybridized to the

3.9 x 10^6 d. fragment, which contains only about 10% of the mature 28S rRNA sequences (13). Substantial X. laevis 18S rRNA was associated with both rDNA fragments; Wellauer et al. (13) have reported that about 20% of the mature 18S sequence is associated with the 3 x 10^6 d. fragment whereas the residuum is contained within the 3.9 x 10^6 d. component. The most important observation, however, is that the 5.8S rRNA hybridized exclusively with the 3 x 10^6 d. fragment; no association of the 5.8S rRNA with the 3.9 x 10^6 d. fragment could be detected. Therefore the 5.8S rRNA cistron must lie within the transcribed region which links the cistrons for 18S and 28S rRNA.

These results confirm the earlier findings of Speirs and Birnstiel (12) regarding the placement of the 5.8S rRNA gene in the rRNA transcriptional unit. Since the eukaryotic 5.8S rRNA cistron and the prokaryotic 5S rRNA cistron do not occupy the same relative positions in their respective transcriptional units, the 5.8S rRNA of eukaryotes probably is not an immediate evolutionary homolog of the bacterial 5S rRNA. A comparative computer analysis of the available nucleotide sequences of 5.8S rRNAs and the bacterial 5S rRNAs has led Cedergren and Sankoff (20) to the same conclusion. However, there are other, perhaps equally compelling, reasons for suggesting that these two molecules may share certain functions during protein synthesis.

Fox and Woese (21), when comparing the nucleotide sequence data on 5S rRNA from prokaryotes and eukaryotes, noted that the two molecules differ significantly in their secondary structural features. Most notable is the absence in the eukaryotic 5S rRNA of a helical region common to all prokaryotic 5S rRNAs and referred to as the "prokaryotic loop". However, a feature equivalent to this "prokaryotic loop" is present in the secondary structural model of Novikoff ascites hepatoma 5.8S rRNA presented by Nazar, Sitz, and Busch (22). That these differences in secondary structure are indeed meaningful is suggested by experiments (23) in which several structurally disparate prokaryotic 5S rRNAs, but no eukaryotic 5S rRNAs, are active in reconstituted Bacillus stearothermophilus 50S ribosomal subunits. Moreover, the 5.8S rRNA (4) and the prokaryotic 5S rRNA (24, J.E. Dahlberg, personal communication) are capable of forming hydrogen-bonded complexes with, respectively, 28S and 23S rRNA, whereas the eukaryotic (yeast) 5S rRNA apparently will associate with only 18S rRNA (25). These points raise the possibility that during the course of evolution the eukaryotic 5.8S rRNA may have assumed part of the role of

the prokaryotic 5S rRNA.

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