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# Location of the 5.8S rRNA Gene of *Saccharomyces cerevisiae*

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Direct DNA sequence analysis of *Saccharomyces cerevisiae* ribosomal DNA cloned in an *Escherichia coli* plasmid revealed part of the structural gene for 5.8S rRNA at one end of a 700-base-pair *Eco*RI fragment. Taken with the previously established *Eco*RI restriction map of the ribosomal repeat unit, this sequence establishes that the yeast 5.8S RNA segment is located between the 18S and 28S segments in the 42S rRNA precursor and in the DNA which codes for it.

5.8S rRNA is one of the two low-molecular-weight RNA components of the large ribosome subunit of eucaryotic cells (9, 13). Analysis of *Saccharomyces cerevisiae* rRNA processing intermediates has suggested that the 5.8S RNA is linked to the 38S RNA in a precursor (14). Similar observations have been made with HeLa cells (5) and *Xenopus laevis* (12). In this report, we investigate its position with respect to 28S and 18S rRNA's in the ribosomal DNA (rDNA) gene.

The *Eco*RI restriction maps of two types of yeast ribosomal genes are shown in the accompanying paper (10). The restriction pattern observed for the type I ribosomal gene has been seen by a number of workers (1, 2, 7; R. W. Davis, personal communication). The restriction fragments corresponding to fragments A and C have been shown to hybridize to the 28S rRNA and 18S rRNA species, respectively (6; K. G. Skryabin, unpublished data).

The *Eco*RI restriction maps of the ribosomal genes of *S. cerevisiae* (10) display small fragments lying around the larger fragments that hybridize to the 18S and 28S rRNA's. In addition, the sequence of yeast 5.8S RNA (11) indicates that the structural gene for this RNA must have one of the *Eco*RI cleavage sites within it. Thus, we examined DNA sequences at the ends of small *Eco*RI fragments from cloned yeast rDNA.

One end of the first fragment examined, the 700-base-pair *Eco*RI D fragment, contained DNA sequences homologous to those of the 5.8S rRNA. Since the D fragment is located between the two fragments that hybridize to the 28S and 18S rRNA species (1, 2, 7, 10), the 5.8S rRNA must be located between the 18S and 28S rRNA species in the precursor. Recently, several other groups have independently shown that the 5.8S rRNA gene is located in this position within the rDNA repeating unit (1, 8; P. Philippsen and R. Davis, personal communication).

## MATERIALS AND METHODS

**Isolation of *Eco*RI fragment D.** *Eco*RI fragment D was obtained from a composite *Escherichia coli* plasmid, pY1rA3 (10), containing part of the *S. cerevisiae* ribosomal repeat unit. DNA was isolated from *E. coli* cells containing the recombinant plasmids as described previously (10), and 0.5 mg was digested with 100 U of *Eco*RI in a solution containing tris(hydroxymethyl)aminomethane (Tris, pH 7.4), 50 mM NaCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, and 0.02% Triton X-100. The digest was then made 2 M in ammonium acetate. The DNA was precipitated with 2.5 volumes of ethanol, sedimented, dissolved in 0.3 M sodium acetate, precipitated with ethanol, sedimented, subjected to electrophoresis through 6% polyacrylamide in a solution of 50 mM Tris-borate (pH 8.3) and 1 mM ethylenediaminetetraacetate, and visualized by UV shadowing on a fluorescent thin-layer chromatography plate (3). Fragment D was eluted from an exised gel segment (6).

**Sequencing of D fragment.** After elution, the D fragment was dephosphorylated with 2 U of alkaline phosphatase, and the phosphatase was then removed by phenol extraction. After ethanol precipitation of the DNA, a portion was rephosphorylated with [$\gamma^{32}$P] ATP (900 Ci/mmol) and polynucleotide kinase (6) and digested with *Hae*III restriction nuclease in a solution of 10 mM Tris-hydrochloride (pH 7.4), 5 mM $MgCl_2$, and 1 mM dithiothreitol. The end-labeled products were then isolated from an 8% polyacrylamide gel (6), and each fragment was sequenced precisely as described previously (6).

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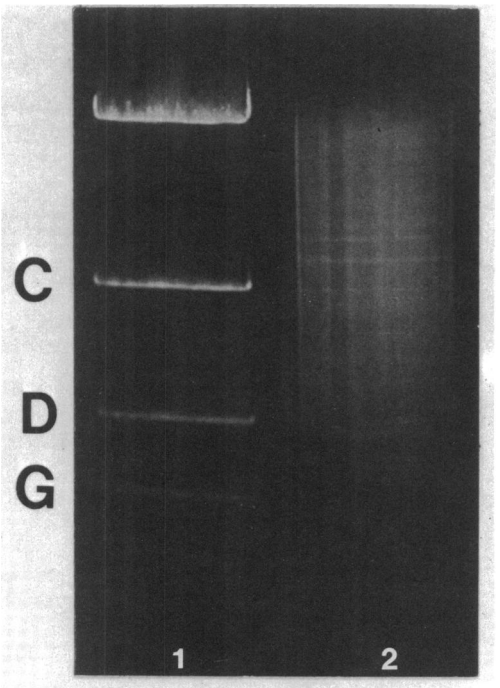



FIG. 1. *Separation of EcoRI restriction fragments of the composite E. coli plasmid pY1rA3 containing S. cerevisiae rDNA (1) and S. cerevisiae nuclear DNA (2). The DNA was subjected to electrophoresis through a 1% agarose gel (1.5 by 200 by 400 mm) at 100 V for 4 h, stained with ethidium bromide, irradiated with UV light, and photographed.*

## RESULTS AND DISCUSSION

Segments of yeast rDNA were generated by cleaving, with restriction endonuclease *Eco*RI, chimeric *E. coli* plasmid DNA (pY1rA3 [10]) containing part of the *S. cerevisiae* ribosomal repeat unit. Three fragments separated from the vector DNA on an analytical agarose gel (Fig. 1): the *Eco*RI C, D, and G fragments (10). After preparative electrophoresis of these *Eco*RI digestion products on a polyacrylamide gel, the D fragment was extracted, dephosphorylated, rephosphorylated with [$\gamma$-$^{32}$P]ATP and polynucleotide kinase, and cleaved with the restriction endonuclease *Hae*III (from *Haemophilus aegyptius*). Two singly labeled end pieces were then isolated, after autoradiography, from the polyacrylamide gel shown in Fig. 2. Each of these terminally labeled fragments was subjected to sequence analysis (6), and base-specific, partial chemical cleavage patterns from one are reproduced in Fig. 3.

Although only 33 bases of the sequence are identified as adenine (A), cytosine (C), thymine (T), or guanine (G) in Fig. 3, in the original autoradiogram about 70 nucleotides proximal to

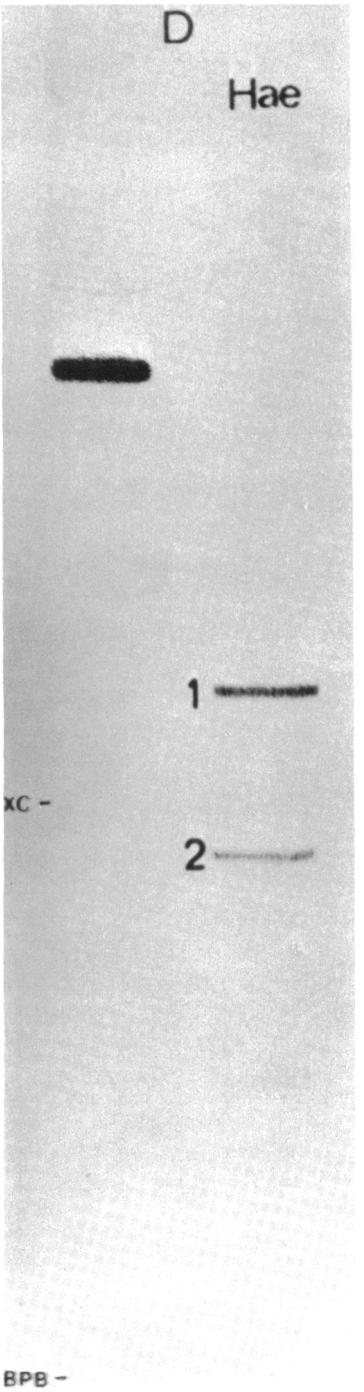
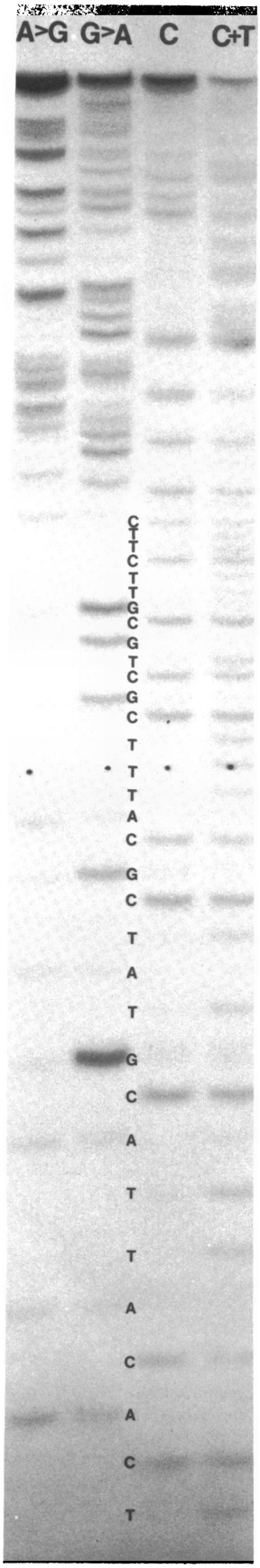



FIG. 2. *Autoradiograph of the HaeIII digestion products of $^{32}$P-end-labeled EcoRI fragment D after electrophoresis on 8% polyacrylamide in a solution of 50 mM Tris-borate and 1 mM ethylenediaminetetraacetate (pH 8.3). Electrophoresis marker dyes: XC, xylene cyanol; BPB, bromophenol blue.*




10 20 30 40 50 60 70 80 90

*pAAACUUUCAACAACGGAUCUCUUGGUUCUCGCAUCGAUGAAGAACGCAGCGAAAUGCGAUACGUAAUGUGAAψUGCAGAAAUUCCGUGAAUCAUC...*

....AAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGA.....GAATT
....TTTGAAAGTTGTTGCCTAGAGAACCAAGAGCGTAGCTACTTCTTGCGTCGCTTTACGCTATGCATTACACT.....CTTAA$_p$

FIG. 4. *Sequences of S. cerevisiae 5.8S rRNA (for explanation of italics, see reference 11) and the end of EcoRI fragment D from S. cerevisiae DNA, aligned. All nucleotides in the DNA sequence corresponding to 1 to 71 of the 5.8S RNA sequence were read from the gel shown in Fig. 3. Nucleotides in sequencing positions 72 to 77 could not be resolved on the gel. Nucleotides in positions 78 to 82 represent the EcoRI cleavage site (4).*

FIG. 3. *Autoradiograph of a sequencing gel of the fragment D1 (see Fig. 2). Only 33 of the 71 nucleotides that can be read from this gel have been given letters.*

one of the labeled ends of fragment D (D1) could be read. This sequence is complementary to nucleotides 71 through 1 of *S. cerevisiae* 5.8S rRNA (11). The DNA and RNA sequences are aligned in Fig. 4. The restriction maps of the *S. cerevisiae* ribosomal repeat unit presented in the accompanying paper (10) and these sequences determine that the 5′ half of 5.8S rRNA is encoded by *Eco*RI fragment D and suggests that the order of rRNA's in the 42S primary transcript is 18S-5.8S-28S. This result is in agreement with those of Bell et al. (1) and Nath and Bollon (8).

An alternative approach to locating RNA transcripts on DNA restriction fragments is to hybridize labeled RNA to purified fragments. One disadvantage of this approach is that RNA may hybridize to DNA sequences that are partially homologous but nonidentical. An advantage of DNA sequence analysis for the location of known RNA species is that flanking nontranscribed regions can also be identified in some cases.

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