JOURNAL OF BACTERIOLOGY, Mar. 1979, p. 1219-1226
0021-9193/79/03-1219/08$02.00/0

Vol. 137, No. 3

Arrangement of the Genes Coding for Ribosomal Ribonucleic Acids in *Neurospora crassa*

STEPHEN J. FREE,* PHILIP W. RICE, AND ROBERT L. METZENBERG

Department of Physiological Chemistry, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 19 December 1978

We have cloned and characterized *Neurospora crassa* ribosomal deoxyribonucleic acid (rDNA). The rDNA is found as a tandemly repeated 6.0-megadalton sequence. We have mapped a portion of the rDNA repeat unit with respect to its sites for 13 restriction endonucleases and defined those regions coding for the 5.8S, 17S, and 26S ribosomal ribonucleic acids (rRNA's). We have also isolated several clones containing 5S rRNA sequences. The 5S rRNA coding sequences are not found within the rDNA repeat unit. We found that the sequences surrounding the 5S rRNA coding regions are highly heterogeneous.

To gain a better understanding of the organization of the eucaryotic genome, a number of investigators have studied the rRNA genes from several organisms. Most eucaryotic organisms examined have about 150 copies of a DNA sequence which contains the coding regions for the two large rRNA's as well as "spacer" regions. In all cases where it has been looked for, the sequence for the 5.8S rRNA has been located in the "transcribed spacer" between the 17S and 26S rRNA coding regions. The arrangement of "untranscribed spacer", 17S rRNA sequence, "transcribed spacer" (including the 5.8S rRNA sequence), and the 26S rRNA sequence has been called ribosomal DNA (rDNA). Most organisms examined have within their genome one or more clusters of tandemly repeated rDNA's ("head to tail" arrangement). However, some organisms have the rDNA's arranged as large extra chromosomal palindromes ("head to head" arrangement).

The size of the rDNA's varies from the 5.8-megadalton tandemly repeated unit of *Saccharomyces cerevisiae* DNA (3, 6, 21) to at least 28 megadaltons for the half-palindrome of *Dictyostelium discoideum* DNA (27). In those higher eucaryotes which have been studied carefully, the rDNA's have been found to be heterogeneous with respect to their size, even within a single individual or cell line. The heterogeneity is due to differences in the size of the "spacer regions" (5) and to the presence of intervening sequences within rRNA coding regions (12). The rDNA's of the lower eucaryotes examined to date seem homogeneous with respect to size.

The sequences coding for the 5S rRNA species are not located within the major rDNA repeat unit of most organisms, but are clustered at one or more sites elsewhere in the genome. However, there are at least two exceptions to this generalization. The yeast *S. cerevisiae* and the cellular slime mold *D. discoideum* both contain 5S rRNA sequences as part of their rDNA (3, 17). Even in these organisms, the 5S rRNA is transcribed separately from the large rRNA precursor which contains the 5.8S, 17S, and 26S rRNA's (1, 28).

To study the organization of the *Neurospora crassa* genome, we have cloned and characterized rDNA sequences. *N. crassa* contains 190 copies of rDNA per haploid genome (R. Krumlauf and G. Marzluf, personal communication). The rDNA has been found to consist of a tandemly repeated 6.0-megadalton sequence. A restriction map of most of the rDNA is presented. The major *Neurospora* rDNA unit does not contain the 5S rRNA sequence. These sequences are located elsewhere in the *Neurospora* genome. We have found the sequences surrounding the 5S rDNA genes to be heterogeneous.

MATERIALS AND METHODS

Materials. Bis(acrylyl)cystamine was bought from Bio-Rad Laboratories. Acrylamide was from the Eastman Chemical Co. and was recrystallized by R. E. Nelson. Seakem agarose (LE) was a product of Marine Colloids, Inc. *Escherichia coli* C600 SF8 was obtained from Kevin Bertrand and has been used previously for DNA cloning experiments (26). pBR322, a ColE1-derived plasmid (4) was obtained from Fred Blattner. Miles Laboratories, Inc., was the source of T4 ligase and the restriction endonucleases *Eco*RI, *Bam*HI, and *Hin*dIII. The restriction endonucleases *Sal*I, *Pst*I, *Xho*I, *Xba*I, *Sma*I, *Bgl*I, *Kpn*I, and *Pvu*II were bought from New England Biolabs, and *Bgl*II, *Hinc*II, and *Hpa*I were from Boehringer Mannheim. CsCl was purchased from Kawecki Berylco Industries Inc. Macaloid was kindly provided by N L Industries, Inc. Sodium dodecyl sulfate (SDS) was bought from Gallard-Schlesinger. Saline sodium citrate (SSC) is 150

mM NaCl and 15 mM Na_3 citrate.

DNA purification. DNA was purified from the slime strain of *N. crassa* (8). Slime cells were pelleted and washed with 10% sorbitol. The cells were lysed and the DNA was isolated as described by Firtel and Bonner (11), except that the slime cells were osmotically stabilized in 10% sorbitol prior to lysis.

Cloning. *N. crassa* DNA (40 μg) and pBR322 (4 μg) were digested to completion with the restriction endonuclease *PstI*. The digest was heated to 65°C for 30 min to inactivate the endonuclease. The DNA fragments were ligated in a volume of 1.2 ml (20 mM Tris, pH 7.4, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA, 0.1 mM ATP) with 2.5 U of T4 ligase at 4°C for 96 h. Transformation was done by the $CaCl_2$ shock method (18). The ligated DNA was used for 12 different transformation experiments in generating the 15,000 clones of *E. coli* containing recombinant DNA plasmids. Transformants containing *N. crassa* DNA were distinguished from transformants containing only pBR322 by their altered antibiotic resistance (4). Of the total transformants, 16% contained *Neurospora* DNA fragments inserted into the pBR322 plasmid. All cloning work was done in a certified P2 facility and in compliance with National Institutes of Health guidelines.

RNA purification. Wild-type *N. crassa* (strain 74-OR8-1a) was grown in 20 ml of Fries medium (2) containing 1.5 mM PO_4^{3-} and 1 mCi of $^{32}PO_4^{3-}$. Whole-cell RNA was isolated from late-log-phase cultures as described by Russell et al. (22). Macaloid (1 mg/ml) was included in the initial homogenization (P. J. Russell, personal communication). The 17S and 26S rRNA's were purified by electrophoresis in 3% acrylamide–bis(acrylyl)cystamine gels as described by Hansen (14). The positions of the 26S and 17S rRNA's were determined by autoradiography, and these regions were cut from the gel. The RNA was recovered as described by Hansen. To purify the RNAs further, each was hybridized to a limiting amount of the *N. crassa* rDNA-containing plasmid, pMF2 (enough to remove approximately 20% of the input radioactivity). This is designed to "hybridize out" any contaminating 26S sequences from the 17S rRNA preparation and vice versa.

The 5.8S and 5S rRNA's were isolated by the procedure of Dahlberg, Kintner, and Lund (7). The purity of the 5.8S and 5S species was assessed by fingerprinting an RNase T1 digest of the rRNA's (24).

Mapping of restriction sites within recombinant plasmids. The sites for different restriction endonucleases within the parent pBR322 plasmid have been well characterized (4). Restriction sites within pMF2 were determined by a series of single and double digests with endonucleases. The double digests were subjected to electrophoresis between two lanes containing the appropriate single digests to aid in the identification of restriction sites. Electrophoresis of DNA fragments, either from total *N. crassa* DNA or from plasmid molecules, was carried out in agarose slab gels as described by Sharp et al. (24). The gels contained either 1 or 1.5% agarose, depending upon the sizes of DNA fragments expected. Fragments produced by digestion of λcI1857 with *Eco*RI plus *Hin*dIII were used as marker DNA to determine the sizes of DNA fragments generated by endonuclease digestion of pMF2 (20). Using these markers, we obtained a size of 2.77 megadaltons for pBR322 as compared to the value of 2.6 megadaltons obtained by others using different DNA markers (4).

Hybridizations. The methods outlined by Grunstein and Hogness (13) were followed in fixing *E. coli* colonies containing recombinant DNA plasmids to nitrocellulose filters. These filters were hybridized to various labeled RNAs in 6× SSC–0.1% SDS for 20 h at 65°C. Nitrocellulose filters carrying pMF2 fragments generated by restriction enzymes were made by the procedure worked out by Southern (25). rRNA's were hybridized to these filters in 6× SSC at 68°C for 1.5 to 6 h.

RESULTS

We have isolated 15,000 *E. coli* clones containing *PstI*-generated fragments of *N. crassa* DNA inserted into the plasmid pBR322. Some 200 of these clones contained DNA sequences which hybridized to *N. crassa* RNA that had been labeled with ^{32}P in vivo. These 200 clones have been further screened with purified 26S, 17S, 5.8S, and 5S rRNA's. The results of this screening for some of these plasmids with 5.8S rRNA are shown in Fig. 1. We isolated 28 clones

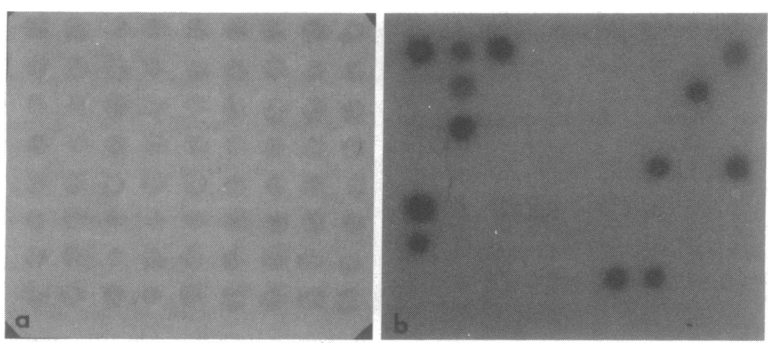

FIG. 1. *Screening for plasmids containing rDNA sequences. (a) A nitrocellulose filter containing bacterial colonies which have been fixed to the filter according to the method of Grunstein and Hogness (13). (b) An autoradiograph of a filter after hybridization with purified 5.8S rRNA.*

capable of hybridizing with 26S, 17S, and 5.8S rRNA's. Analysis of plasmid DNA digests from seven of these clones with the restriction endonucleases *Pst*I and *Eco*RI showed them to be identical with respect to DNA sites sensitive to these enzymes. One of these plasmids, pMF2, was chosen and used for all subsequent experiments. pMF2 has been subjected to endonuclease digestion with 13 restriction enzymes, and its cleavage sites for these enzymes have been mapped (see Fig. 3, 4, and 5 for examples of analysis of endonuclease digests). The restriction map of the rDNA contained in pMF2 is given in Fig. 2. One of the *Pst*I sites used to insert the rDNA into pBR322 was chosen to have an "address" of 0.00, and all other sites were given an "address" equal to the amount of DNA, in megadaltons, between them and the *Pst*I site.

We have transferred the DNA fragments from several agarose gels containing pMF2 restriction digests to nitrocellulose (25). These nitrocellulose filters were hybridized to purified ^{32}P-labeled 5.8S, 17S, and 26S rRNA's. Examples of such hybridizations are shown in Fig. 3, 4, and 5. From such data we have determined which regions of pMF2 code for the different rRNA species.

Hybridization of filter-bound DNA fragments with 5.8S rRNA showed that the region coding for this rRNA contains the *Eco*RI site at 1.46 megadaltons (see Fig. 2 and 3). The 5.8S rDNA is totally contained between the two *Bam*HI sites at 1.35 and 1.54 megadaltons.

Hybridization of 17S rRNA to restriction fragments (Fig. 4) indicated that the 17S rRNA coding region lies to the right of the *Xba*I site at 0.30 megadaltons, as shown in Fig. 2. The 17S coding sequence must stop before reaching the *Eco*RI site at 1.46 megadaltons, since the 5.8S coding sequence contains this *Eco*RI site. There-

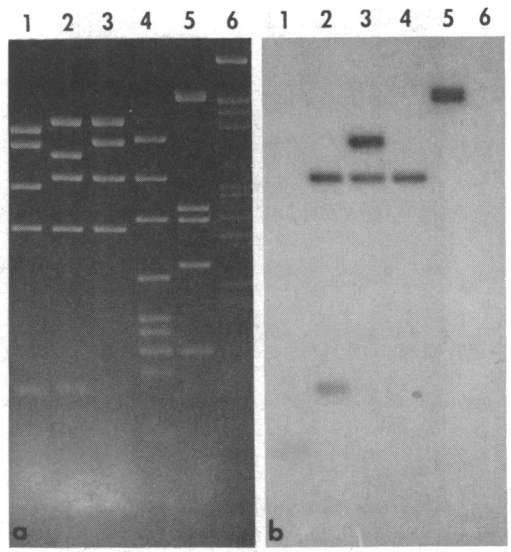

FIG. 3. *Localization of the 5.8S rRNA coding sequence. pMF2 was digested with endonucleases, and the digests were analyzed by electrophoresis in a 1.5% agarose gel. The DNA fragments were transferred to a nitrocellulose filter and hybridized with 5.8S rRNA. The digests are as follows: lane 1, EcoRI and BamHI; lane 2, EcoRI and HindIII; lane 3, EcoRI; lane 4, EcoRI and HincII; lane 5, HincII; lane 6, marker DNA fragments, λ DNA digested with EcoRI and HindIII (18). (a) Ethidium bromide-stained agarose gel. (b) Autoradiograph of the nitrocellulose filter.*

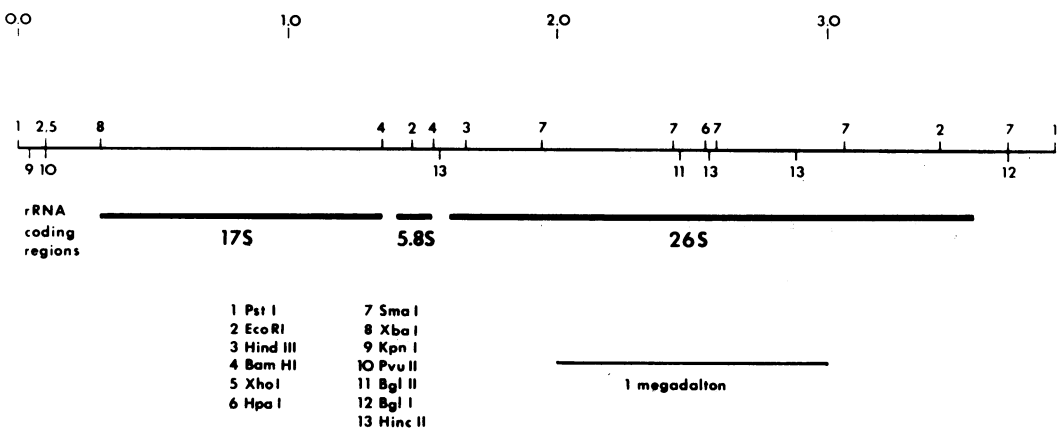

FIG. 2. *Restriction endonuclease map of Neurospora rDNA. pMF2 was subjected to a series of restriction endonuclease digestions, and the cleavage sites for these enzymes were mapped. Each site has an "address" equal to the amount of DNA, in megadaltons, between it and the PstI site at the left of the map (upper numbers). The EcoRI site of the parent plasmid, pBR322, lies 2.3 megadaltons to the left of the reference PstI site.*

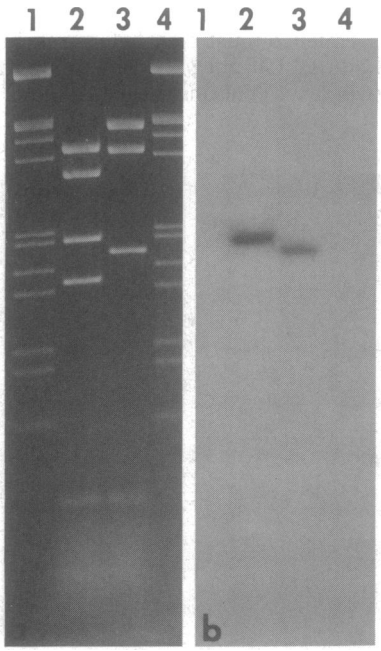

FIG. 4. *Localization of the 17S rRNA coding sequence. pMF2 was digested with restriction enzymes, and the DNA fragments were analyzed by electrophoresis in a 1.5% agarose gel. The DNA fragments were transferred to a nitrocellulose filter and hybridized with 17S rRNA. The digests used were: lanes 1 and 4, marker DNA fragments, λ DNA digested with EcoRI and HindIII; lane 2, XbaI and EcoRI; lane 3, XbaI and BamHI. (a) Ethidium bromide-stained gel. (b) Autoradiograph of the nitrocellulose filter.*

fore, the amount of DNA used in coding for the 17S rRNA is less than 1.16 megadaltons. This would yield an rRNA of less than 0.58 megadaltons in size (fewer than 1,770 bases). This is somewhat smaller than the estimated number of bases contained within the 17S rRNA's of eucaryotes (16) and only a little larger than the 1,550 bases in the *E. coli* 16S rRNA (10).

Hybridization of pMF2 fragments with the 26S rRNA (Fig. 5) indicated that the 26S rRNA coding region has one of its ends between the *Hinc*II site at 1.56 megadaltons and the *Hind*III site at 1.66 megadaltons. The other end of the 26S coding sequence lies between the *Eco*RI site at 3.41 megadaltons and the sites for *Sma*I and BglI at 3.66 megadaltons. The size of the 26S coding region can then be set as being between 1.75 and 2.10 megadaltons (between 2,650 and 3,200 base pairs). Again, this estimated size is smaller than has been given for eucaryotic 26S rRNA's (16). It is also smaller than most estimates of the size of *E. coli* 23S rRNA (10). However, recent evidence indicates that the *E. coli* 23S rDNA is only approximately 2,900 bases in length (E. Lund and J. E. Dahlberg, in preparation).

In mapping the regions coding for the 26S, 17S, and 5.8S rRNA's, we considered it important to use extremely pure rRNA probes. The 17S and 26S rRNA's were purified as explained in Materials and Methods. The hybridizations with the 26S rRNA allowed us to see partially digested fragments containing 26S sequences (many of which are not visible in the ethidium-stained gel in Fig. 5). Both probes showed only the faintest trace of hybridization to fragments which hybridize with the "opposite probe." We conclude that the probes were not mutually contaminated and showed specific hybridization to the 17S and 26S rRNA coding sequences.

The purity of the 5.8S and 5S species is critical in defining the structure of the rDNA's. The 5.8S species needed to be free from the degradation products of other rRNA's to localize the 5.8S coding region unambiguously within pMF2. Our analysis of the structure of the 5S rDNA sequences is dependent upon having a pure 5S rRNA probe. These rRNA's were purified by sequential electrophoresis of the RNA through three acrylamide gels, the first being a 10% gel, the second a 20% gel, and the third a 16% gel containing 7 M urea (7). The purity of the resulting RNAs was assessed by fingerprinting the oligonucleotides from RNase T1 digests (23). The fingerprints of the two RNAs are shown in Fig. 6. We conclude that the two RNAs are substantially free from contaminating species.

To determine whether the 3.83-megadalton piece of DNA found in pMF2 contains the entire rDNA repeat unit or only a fraction of it, we digested total *Neurospora* DNA with *Xba*I, *Xho*I, *Kpn*I, and *Hind*III and subjected the digests to electrophoresis in a 1% agarose gel. These endonucleases were chosen because the rDNA insert of pMF2 either lacks sites for them or has only one site sensitive to them. We transferred the DNA from the gels to a nitrocellulose filter and hybridized it with ^{32}P-labeled DNA from pMF2. The autoradiogram showed only one band of hybridization with DNA cut with *Xho*I, *Xba*I, and *Kpn*I. In each case the DNA fragments which hybridized with pMF2 had a size of 6.0 megadaltons. The *Hind*III-digested DNA fragments showed two bands of hybridization. The sum of the sizes of the two bands was 6.0 megadaltons. Thus, we conclude that pMF2 does not contain the entire rDNA repeat unit. We also conclude that *Neurospora* rDNA consists of a tandemly repeated 6.0-megadalton sequence, since no other arrangement would give the observed results. The *N. crassa* 6.0-megadalton repeat unit is just slightly larger than the 5.8-megadalton repeat unit of yeast (3, 6) and

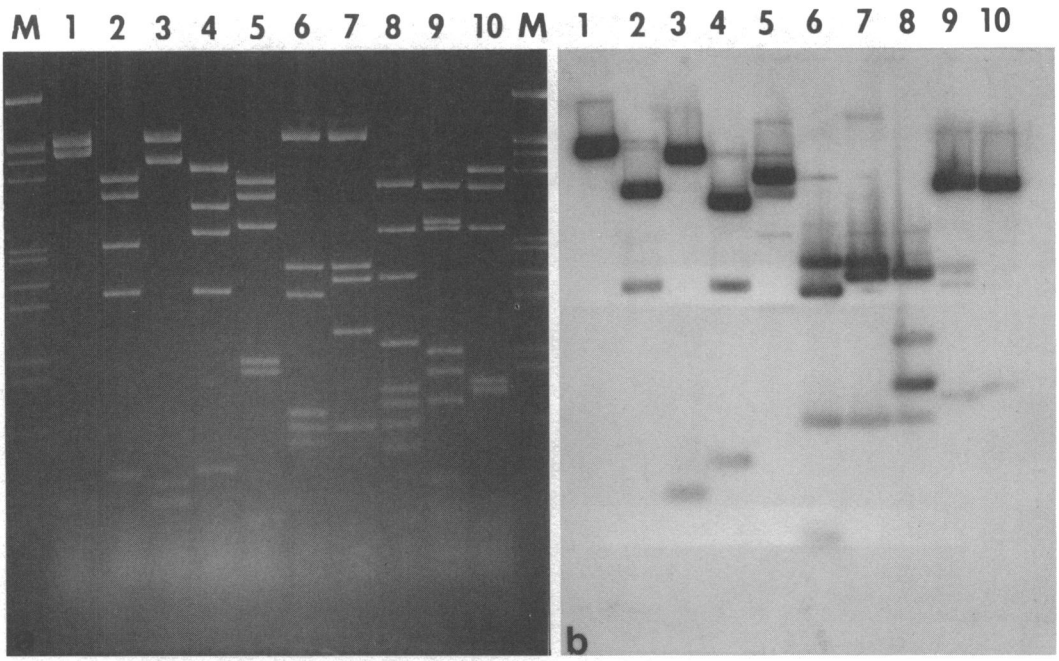

FIG. 5. *Localization of the 26S rRNA coding sequence. pMF2 was digested with endonucleases, and the DNA fragments were analyzed by electrophoresis in a 1.5% agarose gel. The DNA fragments were transferred to a nitrocellulose filter and hybridized with 26S rRNA. The digests used were: lane 1, BamHI; lane 2, BamHI and EcoRI; lane 3, BamHI and HindIII; lane 4, HindIII and EcoRI; lane 5, HindIII and BglI; lane 6, HindIII and HincII; lane 7, HincII; lane 8, HincII and EcoRI; lane 9, EcoRI and BglI; lane 10, EcoRI and PstI; lanes M, marker DNA, λ DNA digested with EcoRI and HindIII. (a) The DNA fragments seen in the ethidium bromide-stained gel. (b) Autoradiograph of the nitrocellulose filter. The BglI preparation was contaminated with a trace of BglII. Some of the light bands of hybridization in lanes 5 and 9 are due to digestion products of BglII. The other extra bands of light hybridization in panel b which do not correspond to DNA bands in panel a are due to incomplete digestion of pMF2 DNA.*

smaller than the rDNA repeat unit of the other eucaryotes so far characterized. We found no evidence for any heterogeneity within the *N. crassa* rDNA.

Hybridization of the purified 5S rRNA to the recombinant plasmid-containing *E. coli* clones indicated that several clones contained 5S rRNA sequences. The plasmids from some of these clones have been examined. What is clear from the analysis is that, although these plasmids contain the 5S rRNA coding region, the "flanking sequences" are highly heterogeneous (unpublished data).

In trying to estimate the number of different types of "flanking sequences" in which 5S rRNA coding sequences reside, we digested total *Neurospora* DNA with several different endonucleases and subjected the digests to electrophoresis in a 1% agarose gel. The DNA fragments were transferred to a nitrocellulose filter. The filter-bound DNA was then hybridized with the purified 5S rRNA. The results are shown in Fig. 7. We would hesitate to estimate how many different types of "flanking sequences" exist for the 5S rDNA genes, except to say that there must be many. We conclude that the 5S rRNA coding regions do not exist as a simple repeating unit, either as part of the rDNA unit described above or elsewhere. It seems perfectly possible that they are physically dispersed throughout the genome.

DISCUSSION

The arrangement of the rDNAs in the genomes of five of the lower eucaryotes has now been examined. These organisms present several types of rDNA organization. *S. cerevisiae* has a small tandemly repeated rDNA (5.8 megadaltons) which contains the sequences for all four (5S, 5.8S, 17S, and 26S) rRNA's (3, 6). *D. discoideum* has all four of the rRNA coding regions contained within a large extrachromosomal palindrome (27). The rDNA of the true slime mold *Physarum polycephalum* has also been found to be organized as an extrachromosomal palindrome (19, 29). The macronuclear rDNA in *Tetrahymena pyriformis* is palindromic and lacks the 5S rRNA coding sequence (9, 15). Presum-

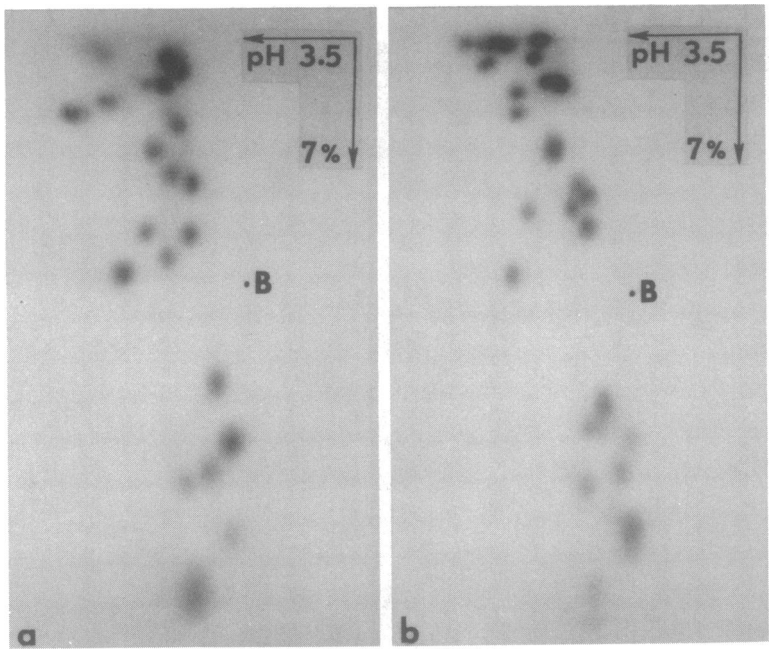

FIG. 6. *RNAse T1 digestion of rRNA's. RNA's were digested with RNase T1 and analyzed as described by Sanger et al. (23). Panel a: 5S rRNA. Panel b: 5.8S rRNA.*

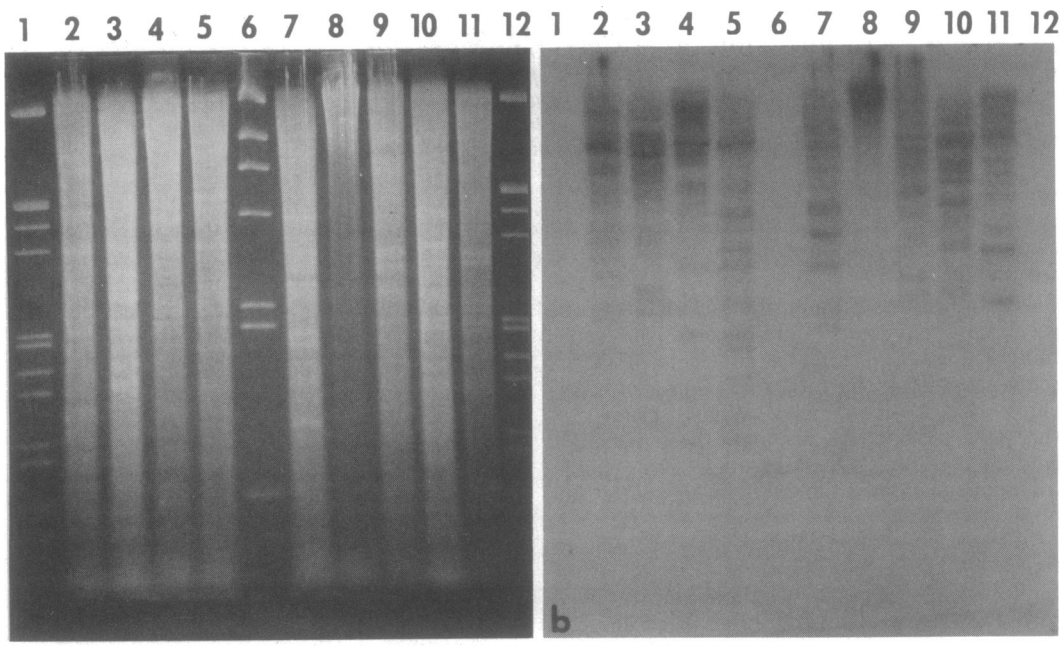

FIG. 7. *Analysis of 5S rRNA sequences in Neurospora DNA. Total Neurospora DNA was digested with endonucleases, and the DNA fragments were subjected to electrophoresis in a 1% agarose gel. The DNA fragments were transferred to a nitrocellulose filter and hybridized with 5S rRNA. The digests used were: lanes 1 and 12, marker DNA, λ DNA digested with EcoRI and HindIII; lane 2, XhoI; lane 3, KpnI; lane 4, XbaI; lane 5, BamHI; lane 6, marker DNA, λ DNA digested with HindIII; lane 7, HindIII; lane 8, SalI; lane 9, PvuII; lane 10, BglI; lane 11, EcoRI. (a) Ethidium bromide-stained gel. (b) Autoradiograph of nitrocellulose filter. The SalI digestion in lane 8 may be a partial digestion.*

ably, the macronuclear rDNA reflects the situation within the genomic micronuclear DNA since the former is derived from the latter.

We found the *N. crassa* rDNA to consist of a tandemly repeated 6.0-megadalton sequence. We found no evidence for any heterogeneity with respect to length or restriction endonuclease cleavage sites. We estimate that we could have easily detected a second type of rDNA if it represented 5% of the rDNA. *Neurospora* rDNA does not contain the 5S rRNA coding sequence. Instead, the 5S rDNA genes are found within a large number of different types of "flanking sequences." Two lines of evidence support this conclusion: (i) the location of sites recognized by restriction endonucleases in plasmids containing 5S rDNA sequences (unpublished data), and (ii) the size heterogeneity of DNA fragments containing the 5S rDNA genes that are obtained when total *Neurospora* DNA is digested with restriction endonucleases (Fig. 7).

In all other systems where the 5S rRNA coding sequences have been studied, these sequences either are part of the rDNA or are found clustered at a small number of sites within the genome. Our data suggest that the 5S rDNA genes of *N. crassa* are not physically tightly clustered. The arrangement of the *N. crassa* 5S rDNA genes presents the organism with two potential problems. One is that of maintaining sequence homogeneity of the 5S rRNA coding regions while the "flanking sequences" differ and have presumably been under quite different selective pressures. The other potential problem is that of regulating transcription of 5S genes, which are found in several different environments, coordinately with the major rDNA repeat unit.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant GM-08995-17 from the National Institutes of Health.

We thank Elsebet Lund for encouragement and helpful discussion.

LITERATURE CITED

1. **Batts-Young, B., and H. F. Lodish.** 1978. Triphosphate residues at the 5′ ends of rRNA precursor and 5S RNA from *Dictyostelium discoideum*. Proc. Natl. Acad. Sci. U.S.A. **75**:740–744.
2. **Beadle, G. W., and E. L. Tatum.** 1945. Neurospora. II. Methods of producing and detecting mutations concerned with nutritional requirements. Am. J. Bot. **32**: 678–686.
3. **Bell, G. I., L. J. DeGennaro, D. H. Gelfand, R. J. Bishop, P. Valenzuela, and W. J. Rutter.** 1977. Ribosomal RNA genes of *Saccharomyces cerevisiae*. I. Physical map of the repeating unit and location of the regions coding for 5S, 5.8S, 18S, and 25S ribosomal RNAs. J. Biol. Chem. **252**:8118–8125.
4. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, and H. W. Boyer.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
5. **Botchan, P., R. H. Reeder, and I. B. Dawid.** 1977. Restriction analysis of the nontranscribed spacers of *Xenopus laevis* ribosomal DNA. Cell **11**:599–607.
6. **Cramer, J. H., F. W. Farrelly, and R. H. Rownd.** 1976. Restriction endonuclease analysis of ribosomal DNA from *Saccharomyces cerevisiae*. Mol. Gen. Genet. **148**: 233–241.
7. **Dahlberg, J. E., C. Kintner, and E. Lund.** 1978. Specific binding of $tRNA_f^{met}$ to 23S rRNA of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **75**:1071–1075.
8. **Emerson, S.** 1963. Slime, a plasmodoid variant of *Neurospora crassa*. Genetica (the Hague) **34**:162–182.
9. **Engberg, J., P. Andersson, V. Leick, and J. Collins.** 1976. Free ribosomal DNA molecules from *Tetrahymena pyriformis* GL are giant palindromes. J. Mol. Biol. **104**:455–470.
10. **Fellner, P.** 1974. Structure of the 16S and 23S ribosomal RNAs, p. 169–191. *In* M. Nomura, A. Tissieres, and P. Lengyel (ed.), Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Firtel, R. A., and J. Bonner.** 1972. Characterization of the genome of the cellular slime mold *Dictyostelium discoideum*. J. Mol. Biol. **66**:339–361.
12. **Glover, D. M., and D. S. Hogness.** 1977. A novel arrangement of the 18S and 28S sequences in a repeating unit of *Drosophila melanogaster* rDNA. Cell **10**:167–176.
13. **Grunstein, M., and D. S. Hogness.** 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. U.S.A. **72**:3961–3965.
14. **Hansen, J. N.** 1976. Electrophoresis of ribonucleic acid on a polyacrylamide gel which contains disulfide crosslinkages. Anal. Biochem. **76**:37–44.
15. **Karrer, K. M., and J. G. Gall.** 1976. The macronuclear ribosomal DNA of *Tetrahymena pyriformis* is a palindrome. J. Mol. Biol. **104**:421–453.
16. **Loening, U. E.** 1968. Molecular weights of ribosomal RNA in relation to evolution. J. Mol. Biol. **38**:355–365.
17. **Maizels, N.** 1976. Dictyostelium 17S, 25S and 5S rDNAs lie within a 38,000 base pair repeated unit. Cell **9**:431–438.
18. **Mandel, M., and A. Higa.** 1970. Calcium-dependent bacteriophage DNA infection. J. Mol. Biol. **53**:159–162.
19. **Molgaard, H. V., H. R. Matthews, and E. M. Bradbury.** 1976. Organization of genes for ribosomal RNA in *Physarum polycephalum*. Eur. J. Biochem. **68**:541–549.
20. **Murray, K., and N. E. Murray.** 1975. Phage lambda receptor chromosomes for DNA fragments made with restriction endonuclease III of *Haemophilus influenzae* and restriction endonuclease I of *Escherichia coli*. J. Mol. Biol. **98**:551–564.
21. **Petes, T. D., L. M. Hereford, and K. G. Skryabin.** 1978. Characterization of two types of yeast ribosomal DNA genes. J. Bacteriol. **134**:295–305.
22. **Russell, P. J., J. R. Hammett, and E. U. Selker.** 1976. *Neurospora crassa* cytoplasmic ribosomes: cold-sensitive mutant defective in ribosomal ribonucleic acid synthesis. J. Bacteriol. **125**:1112–1119.
23. **Sanger, F., G. G. Brownlee, and B. G. Barrell.** 1965. A two-dimensional fractionation procedure for radioactive nucleotides. J. Mol. Biol. **13**:373–398.
24. **Sharp, P. A., B. Sugden, and J. Sambrook.** 1973. Detection of two restriction endonuclease activities in *Haemophilus parainfluenzae* using analytical agarose-ethidium bromide electrophoresis. Biochemistry **12**: 3055–3063.
25. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.

26. **Struhl, K., J. R. Cameron, and R. W. Davis.** 1976. Functional genetic expression of eukaryotic DNA in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **73**:1471–1475.
27. **Taylor, W. C., A. F. Cockburn, G. A. Frankel, M. J. Newkirk, and R. A. Firtel.** 1977. Organization of ribosomal and 5S coding regions in *Dictyostelium discoideum*, p. 309–313. *In* G. Wilcox, J. Abelson, and C. F. Fox (ed.), Molecular approaches to eukaryotic systems, vol. 6. ICN-UCLA Symposia on Molecular and Cellular Biology. Academic Press Inc., New York.
28. **Valenzuela, P., G. I. Bell, A. Venegas, E. T. Sewell, F. R. Masiarz, L. J. DeGennaro, F. Weinberg, and W. J. Rutter.** 1977. Ribosomal RNA genes of *Saccharomyces cerevisiae*. II. Physical map and nucleotide sequence of the 5S ribosomal RNA gene and adjacent intergenic regions. J. Biol. Chem. **252**:8126–8135.
29. **Vogt, V. M., and R. Braun.** 1976. Structure of ribosomal DNA in *Physarum polycephalum*. J. Mol. Biol. **106**: 567–587.