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Extrachromosomal circular ribosomal DNA in the yeast Saccharomyces carlsbergensis

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ABSTRACT

Purified ribosomal DNA from Saccharomyces carlsbergensis contains a small proportion of circular DNA molecules with a contour length of 3 µm or integral multiples thereof. Hybridization of yeast ribosomal DNA with 26 S rRNA, using the R-loop technique, reveals that these circular molecules contain sequences complementary to yeast ribosomal RNA. We suggest that these extrachromosomal rRNA genes may be intermediates in the amplification of rRNA genes in yeast.

INTRODUCTION

Genes coding for ribosomal RNA (rRNA) in eukaryotes generally exist as multiple copies which are tandemly arranged at one or more locations on the chromosomes. However, in a number of organisms rRNA genes are also present as extrachromosomal DNA molecules. Well-known examples are the oocytes of many amphibia. In these oocytes extrachromosomal rDNA occurs which comprises integral multiples of a basic repeating unit consisting of a non-transcribed spacer region plus the DNA coding for the primary rRNA transcript (1). The extrachromosomal rDNA is extensively replicated during the amplification stage of the rRNA genes.

Extrachromosomal rDNA has also been observed in a number of lower eukaryotes. Two basically different types of gene organization have been described. First, the macronuclear rDNA of Paramecium exists as relatively small molecules with a tandemly repeated basic unit, in both linear and circular forms (2). On the other hand, the free rRNA genes in Physarum, Dictyostelium and Tetrahymena exist as giant palindromes (3, 4, 5). The free macronuclear rDNA of Tetrahymena appears to arise from a single chromosomal rRNA gene in the micronucleus (6).

rDNA in yeast comprises 100-140 copies of a basic repeating unit, which are organized into long tandem arrays on the chromosome (7-12). We

wanted to establish whether or not additional extrachromosomal rDNA is present in yeast. By careful electron microscopic analysis of the rDNA preparations of the yeast Saccharomyces carlsbergensis we found a small proportion of circular molecules having the same contour length (or integral multiples thereof) as the basic ribosomal repeat within the chromosome and which form R-loops upon hybridization with homologous 26 S rRNA.

MATERIALS AND METHODS

Isolation of DNA: high molecular weight yeast nuclear DNA from S. carlsbergensis strain S74 was isolated as described previously (5). Ribosomal DNA was separated from the rest of the nuclear DNA by preparative Hg^{2+}/Cs_2SO_4 density gradient centrifugation (13).

Isolation of RNA: unlabeled 26 S rRNA was prepared from yeast ribosomes by phenol extraction and further purified by sucrose gradient centrifugation (14).

R-loop formation and specimen preparation: R-loops were formed as described previously (7) according to the method of Thomas et al. (15). The R-loop mixture as well as the purified rDNA were spread and mounted on grids essentially in the same way as earlier described (7).

Electron microscopy: Specimens were examined in a Philips E.M. 200 or 201 electron microscope. Photography and length determination were carried out as described previously (7).

RESULTS

Yeast rDNA, purified by Hg^{2+}/Cs_2SO_4 density gradients, was spread from 70% formamide onto water at room temperature. Plasmid pBR322 was spread under the same conditions in order to enable us to convert measured lengths into molecular weights. Most rDNA molecules appeared to be linear with a length up to 30 µm, corresponding to a molecular weight of 50-60 x 10^6 D. In addition a small

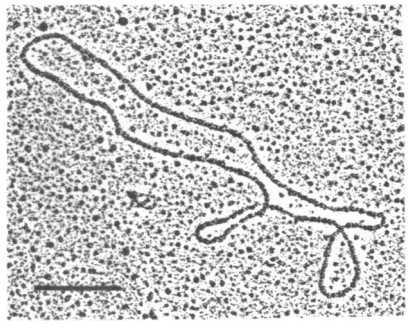

Fig. 1. Electron micrograph of a representative open circular DNA molecule found in yeast rDNA. The bar corresponds to 0.25 µm.

proportion of the molecules appeared to be circular. Figure 1 shows an example of this class of molecules whereas Figure 2 shows the contour length distribution of these open circular molecules (no supercoiled molecules were observed). They apparently constitute a homogeneous size class having a length of about 3 µm; the average contour length is 3.28 ± 0.16 µm (N=13, see Table I). This length is identical to the length of 3.28 µm earlier reported for the ribosomal repeating unit on yeast chromosomes, which suggests that these circular molecules represent extrachromosomal copies of rRNA genes. Molecules having two or three times this length were observed also (cf. Figure 2).

To establish the identity of these circular DNA molecules, we hybridized the yeast rDNA fraction to 26 S rRNA under conditions favouring the formation of R-loops (15). Figure 3 shows some examples of circular DNA molecules which were scored after this procedure. They display a single R-loop per unit length of about 3.2 µm. The average length of these molecules measured along the DNA-RNA hybrid strand and the DNA-DNA double strand is 3.21 ± 0.21 µm (N=7, see Table I). Figure 3d shows an additional ciruclar molecule of 6.30 µm which contains one intact R-loop and one broken R-loop. This DNA molecule apparently contains two basic units; whether they are arranged head-to-head or head-to-tail cannot be concluded unambiguously from the positions of the R-loop.

The average length of the R-loops themselves is about 1.10 ± 0.08 µm (N=6), which is virtually identical to the mean length of 1.06 µm found for the corresponding R-loops in the chromosomal genes. This observation confirms that we are dealing with genuine 26 S rRNA loops.

The circular rDNA molecules are apparently replicated by a rolling circle mechanism. Figure 4A shows an example of this type of molecule as observed after direct spreading of rDNA: in this molecule the linear DNA tail

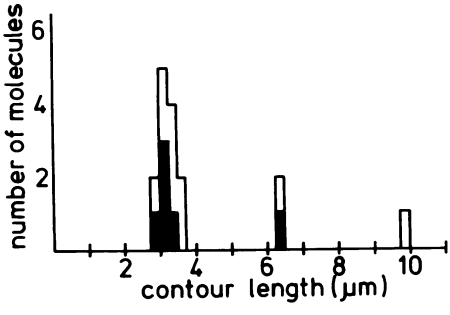

Fig. 2. Contour length distribution of circular DNA molecules present in yeast ribosomal DNA either after direct spreading (open bars) or after hybridization with 26 S rRNA (filled bars).

TABLE I. CONTOUR LENGTH OF PARTIAL HYBRIDS, CIRCULAR DNA MOLECULES AND pBR322

	Length (μm)	S.D. (μm)	N	Calculated M.W. (x 10^{-6} D)
pBR322	1.37	0.07	63	2.60*
chromosomal ribosomal repeat	3.28	0.19	26	6.23 ± 0.23**
circular DNA molecules	3.28	0.16	13	6.23 ± 0.20
circular DNA molecules with R-loops	3.21	0.21	7	6.10 ± 0.25

* This value was taken from ref. 23

** For details see ref. 7

is equivalent to nearly two repeating units.

Figure 4B shows a rolling circle-like molecule with two partial R-loops as a result of the hybridization with 26 S rRNA, one in the circular part of the molecule and the other at about 0.9 μm from the free end of the DNA tail.

DISCUSSION

The ribosomal DNA fraction isolated from the yeast *Saccharomyces carlsbergensis* contains circular DNA molecules, most of which have a contour length of approximately 3 μm while some have two or three times that length. These molecules appear to be extrachromosomal copies of rRNA genes since they are capable of forming R-loops with homologous 26 S rRNA. The homogeneity in size of the circular molecules makes it highly unlikely that they are artefacts of an isolation procedure, *e.g.* generated by a mechanism suggested by Thomas *et al.* (16). The much higher molecular weight of the remainder of the rDNA - 30-40 x 10^6 D *versus* 6 x 10^6 D for the circles - supports this conclusion.

The methods we used do not allow us to determine accurately the number of circular rDNA molecules per cell. They do comprise only a minor fraction of the total yeast rDNA, however. Other authors (17, 18) have described the occurrence of 3 μm DNA circles in *S. cerevisiae* although the genetic properties of these circles were not determined. *S. cerevisiae*, in contrast to our *S. carlsbergensis* strain, also contains the 2 μm plasmids. The ratio of 3 μm

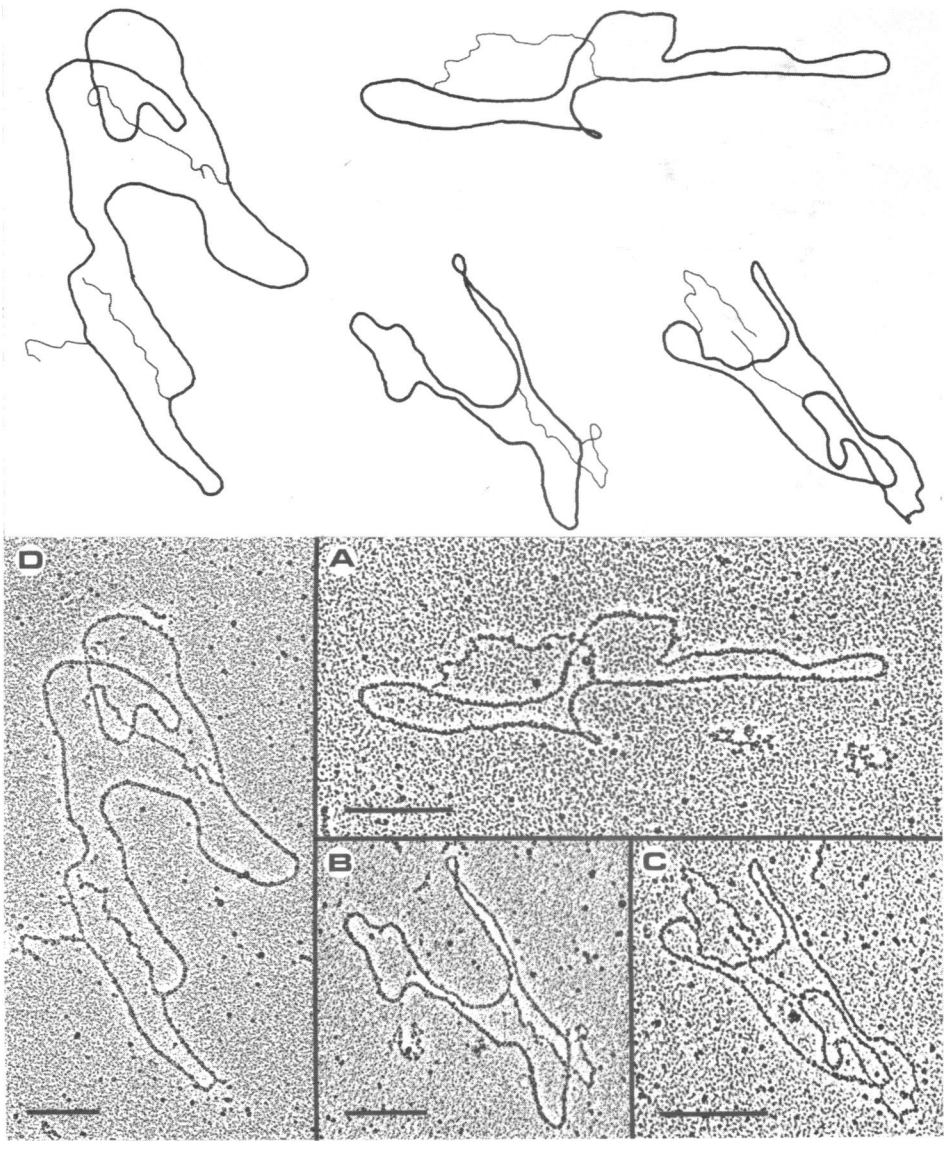

Fig. 3. Electron micrographs of circular DNA molecules containing one or more R-loops after hybridization of yeast ribosomal DNA with 26 S rRNA. The bar corresponds to 0.25 µm.

circles to 2 µm plasmids observed in S. cerevisiae suggests an average copy number between 0.5 and 2.0 3 µm circles per cell. We conclude that the per-

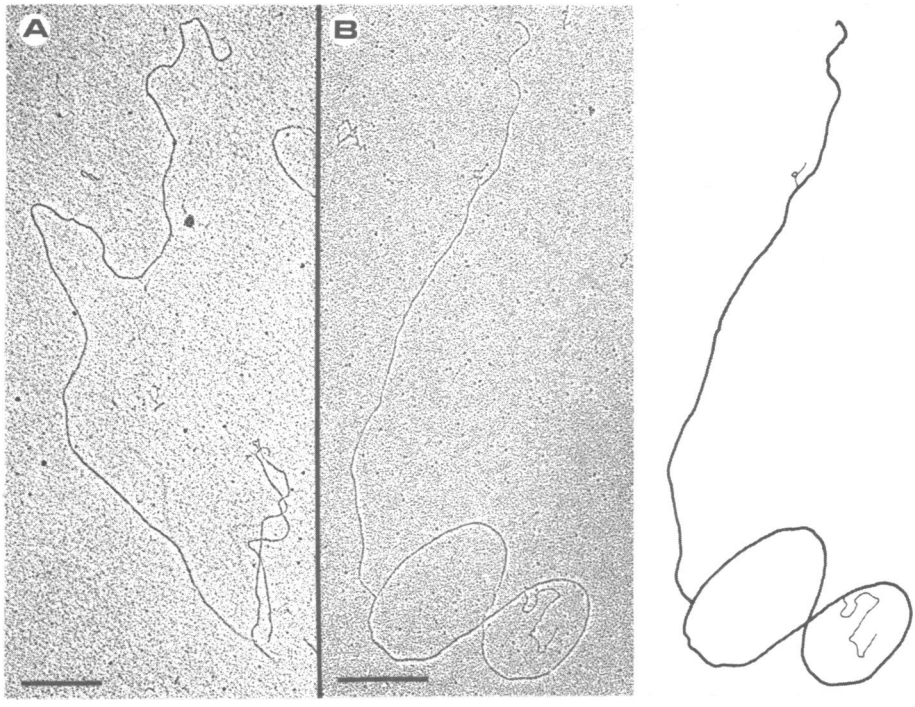

Fig. 4. Electron micrographs of replicative intermediates of circular DNA molecules in yeast ribosomal DNA; (A) after direct spreading, (B) after hybridization with 26 S rRNA. The bar corresponds to 0.40 µm.

centage of rRNA genes present on extrachromosomal DNA is very low indeed. The circular rDNA molecules in yeast, therefore, seem to have a rather transient function. For instance one can envisage a mechanism whereby extrachromosomal copies of rDNA are involved in the regulation of rRNA gene dosage. Øyen et al. (19) and Kaback and Halvorson (20) have described yeast strains which have amplified their number of rRNA genes. The number of rRNA genes increased with 60-70 over a period of about 300 generations (19, 21). The extra rRNA genes were present on chromosomal DNA. It is conceivable that the vegetative yeast cell controls its number of chromosomal rRNA genes by excision and integration of circular repeating units, to wit the 3 µm rDNA molecules. Excised molecules could either be degraded or replicated by a rolling circle mechanism followed by integration into the chromosome. Normally these two processes would be in balance whereas in the strains exhibiting

the amplification phenomenon this balance would be disturbed in favour of replication of the 3 μm circles. It will be necessary to develop a sensitive assay for the average number of 3 μm circles per cell in order to monitor both the wild-type yeast cell under various growth conditions (e.g. logarithmic growth, sporulation etc.) and the mutants that amplify their number of rRNA genes. The data should indicate whether or not the 3 μm circles are involved in regulating the gene dosage for rRNA. If the extrachromosomal copies of rDNA in yeast indeed can replicate autonomically then an origin of replication must of course be present on each ribosomal repeating unit in yeast.

The presence of a small amount of circular rDNA in Xenopus tissue cells and blood cells (22) suggests that the mechanism postulated for amplification of yeast rRNA genes may also apply to other eukaryotes.

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