rDNA REPLICATION IN A SYNCHRONIZED CULTURE OF SACCHAROMYCES CEREVISIAE

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SUMMARY. From a synchronized culture of Saccharomyces cerevisiae cells were harvested at different intervals during S-phase. The purified DNA of each sample was challenged with ribosomal RNA by specific DNA/rRNA hybridization. From the constant rDNA/DNA ratio observed it is concluded that in yeast, the replication of the rRNA cistrons is not restricted to a distinct interval but proceeds at a constant rate throughout the entire S-period.

INTRODUCTION

A sequential and polarized manner of replication is well established for the single chromosome present in the bacterial cell (1-4). Bacterial division-synchronized cultures may therefore be used for marker sequence determination studies (5,6). This method proved especially useful in studying the position of rRNA and tRNA gene loci to which the usual methods of gene mapping can not be applied. However, much less is known on the mode of DNA replication in eucaryotes. For instance, the 16-18 different chromosomes present in the yeast, Saccharomyces cerevisiae (7), may replicate either simultaneously or sequentially. The study of gene replication times in synchronous yeast will therefore provide only limited information about the genetic loca-

tion of a specific gene. In the case of redundant genes, however, like those coding for ribosomal or transfer RNA (8), this study may help to discriminate between a clustered or scattered arrangement on the genome. In the present paper, the distribution of the 140 different rRNA cistrons on the yeast genome was investigated by following the course of rDNA replication during the DNA synthetic period of the cell cycle (= S-phase).

MATERIALS AND METHODS

Strains. The diploid strain Z 65 (lys2-1/lys2-2, tyr1-1/tyr1--2, his7-1/his7-2, ade2/+, ura1/+) was obtained from Dr. R.E. Esposito, the haploid wild type strain X 2180-1B from Dr.R.K. Mortimer.

Media. YEPD: 1% yeast extract, 2% peptone, 2% glucose; "chase" medium: YEPD + 0.1% K_2HPO_4 + 0.9% KH_2PO_4; starvation medium (9) and low phosphate medium (8) as described elsewhere.

DNA Determination. Cells were isolated by centrifugation from 10 ml aliquots of the culture. The DNA was extracted according to OGUR and ROSEN (12) and subsequently determined by a modification of BURTON's procedure (13) as described by GILES and MYERS (14).

Synchronization. Z 65 cells were grown in 1.5 liters of double-strength YEPD to which 1 mC/ltr of 8-^{3}H-adenine (Schwarz/Mann) were added. After reaching a density of 2.5 x 10^8 cells/ml the culture was subject to the feeding-starvation synchronization procedure described by WILLIAMSON and SCOPES (9). After 4 feeding-starvation cycles the cells were transferred to unlabeled YEPD medium and vigorously shaken at 30°C. Every 10 minutes an aliquot of 250 ml was removed for

DNA isolation. Other aliquots were taken for cell number and DNA determinations.

^{3}H-DNA Isolation. After withdrawal from the culture the 250 ml samples were instantaneously poured on ice, the cells collected by centrifugation at 0°C and subsequently kept at -20°C for a longer period of time. This period happened to be almost 1 year. After thawing the cells were converted into spheroplasts by treating them at 37°C for 3 hours with glusulase (0.3 ml/gr wet cells; Endo Laboratories) according to DUELL et al.(10). From the spheroplasts the DNA was isolated according to SMITH and HALVORSON (11). Final DNA purification was achieved by two subsequent CsCl equilibrium density gradient centrifugations as described previously (8). For each sample, 50-90 µg of pure DNA were obtained.

^{32}P-rRNA Isolation. ^{32}P-labeled rRNA was isolated from X 2180 -1B cells. These were grown in 500 ml of low phosphate medium containing 10 mC of ^{32}P-orthophosphate. Subsequently, the cells were grown for two generations in unlabeled "chase"-medium. After cell rupture in a French pressure cell, ribosomes were isolated by differential centrifugation and from them the ^{32}P-labeled rRNA was isolated by subsequent phenol extraction and sucrose density gradient centrifugation procedures.

DNA/rRNA Hybridization. DNA-rRNA hybridization was performed essentially as described by GILLESPIE and SPIEGELMAN (15). Each nitrocellulose filter paper (Sartorius Membranfilter GmbH, Göttingen, type SM 11306, 30 mm diameter, pore size 45 µ) contained 10 µg of ^{3}H-DNA. Before hybridization, the ^{32}P-rRNA was heat-treated for 5 min at 80°C in 4 x SSC, subsequently cooled in ice and passed through a nitrocellulose

filter which had been prewashed with 100 ml of 4 x SSC.

RESULTS AND DISCUSSION

By the feeding-starvation procedure applied, a good synchronization of the yeast culture was obtained (Fig.1). During the period studied, the cell number underwent one, the amount of DNA in the culture almost two distinct rounds of doubling. The timing of DNA replication with respect to cellular division as well as the relative length of the S-phase agrees with earlier reports from other laboratories (16-18).

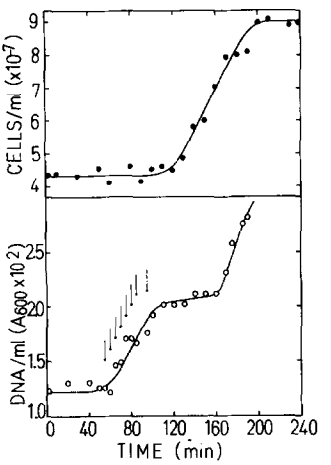

Fig.1. DNA replication and cellular division in a synchronized culture of S.cerevisiae. For details of synchronization, cultural conditions and DNA assay, see Materials and Methods. The arrows indicate time intervals at which 250 ml-samples were withdrawn for DNA isolation.

According to Fig.2, the proportion of rDNA to total cellular DNA remains constant throughout the entire period of DNA replication. The rDNA content of the genome being always about 2.2 percent is identical to that observed in non-synchronous yeast (8). Therefore, the 140 rRNA cistrons of yeast apparently replicate at a constant rate along with the

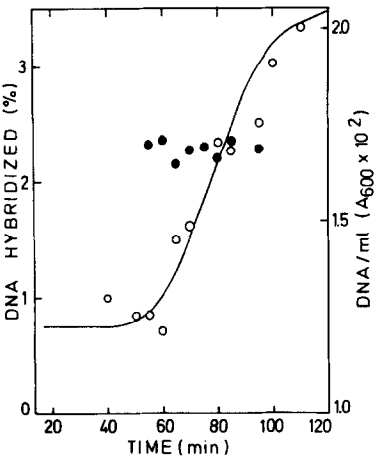

Fig.2. rDNA/DNA ratios (•) determined at different intervals during synchronous replication of S.cerevisiae DNA (o). The diagram represents the same experiment as depicted in Fig.1. The hybridization values are averaged from three, almost identical values obtained at different 32-P-rRNA concentrations (5,10 and 15 µg/ml, respectively). Each hybridization vial contained 10 ml of 4 x SSC, 32-P-rRNA (spec.act. 11 750 cpm/µg) as indicated above, 2 DNA filters and 2 controls without DNA. The specific activity of the 3-H-DNA continuously decreased from 295 cpm/µg in the first sample to 205 cpm/µg in the last one. The filters were incubated in the hybridization mixture for 12 hours at 64°C and subsequently treated as described earlier (8).

other cellular DNA. This constant rRNA gene dosis may explain the observation of TAURO et al.(19) that, in Sacch.lactis, the rate of rRNA synthesis is constant over the cell cycle.

The results obtained indicate that in yeast, the rRNA cistrons are either randomly scattered over the genome or combined in a large DNA segment the replication of which requires the entire S-period. A DNA molecule containing 140 rRNA cistrons should be somewhat larger than the average size of one of the other chromosomes (8). Supposed each chromosome represents an independent unit of replication, this chromosome consisting of mainly or exclusively rDNA would replicate

simultaneously along with the other chromosomes. Possibly, this DNA segment would be equivalent to the nucleolar organizer region of higher eucaryotes. This region is known to carry the rRNA genes, in these organisms (20). By cytological methods, the evidence for the existence of a nucleolus in yeast has been equivocal, so far (20,21). Although the nucleolus of higher cells is reported to replicate late during nuclear division (20) it has been recently shown by BALAZS and SCHILDKRAUT (22) that also in synchronized HeLa cells the 28 S rRNA cistrons replicate throughout the entire S-period. Future experiments must show if this correspondence between the two systems is based on a similar genetic organization of the rRNA cistrons.

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