ON THE DISTRIBUTION OF 5S RNA CISTRONS ON THE GENOME OF *SACCHAROMYCES CEREVISIAE*

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1. Introduction

The use of DNA from aneuploid mutants for RNA–DNA hybridization studies has given valuable information about the distribution of rRNA cistrons on the genome of *Saccharomyces cerevisiae* [1–5]. A low rRNA hybridization level for DNA from chromosome I monosomic strains has been interpreted to mean that chromosome I normally comprises about 70% of the rRNA cistrons [3–5]. Recently, we have shown that the amount of rDNA* may vary also in different wild type strains, possibly as a result of unequal crossing over within rRNA cistron regions [6,7]. In the present report we have investigated the variation in number 5S RNA cistrons for strains differing in amounts of rDNA. Earlier, Retèl and Planta have shown that in *S. carlsbergensis*, the gene dosages for 5S RNA and for the larger rRNA species are approximately the same [8]. They found that the DNA segments specifying 5S RNA cobanded with rDNA when nuclear DNA was fractionated in density gradients, and suggested that all the ribosomal RNA species may be transcribed as parts of a common precursor. For *S. cerevisiae*, Rubin and Sulston have reported that the cistrons for 5S RNA and for 17S and 26S rRNA are physically linked and are present in equal numbers, possibly in an alternating arrangement [9]. Based on studies of monosomic strains, Cryer et al. [10] suggested that some of the 5S RNA cistrons as well may be located on chromosome I. Our present results also indicate a linking of the 5S RNA cistrons to the cistrons for 17S plus 26S rRNA. We find, however, that there are about twice as many 5S RNA cistrons, and that they may be arranged in separate transcriptional units.

*Abbreviation: rDNA, DNA segments carrying the genes for 17S and 26S ribosomal RNA.

2. Materials and methods

2.1. *Strains of S. cerevisiae*

Y-233, a haploid wild type with a high rDNA content [6,7] Y-242, an aneuploid with at least seven chromosomes in disomy, and a low rDNA level [1], Y-55 and X-951, diploids with normal rDNA contents [4,11] and X-951-13, a chromosome I monosomic segregant of X-951, earlier found to be low in rDNA [4].

2.2. *DNA isolation and fractionation*

Nuclear DNA, [^{3}H]-labelled or not, was isolated and purified in a dextran–polyethylene glycol two-phase system, as previously described [6], and was fractionated in Ag^+-Cs_2SO_4 density gradients [7].

2.3. *RNA isolation*

Stable RNA was labelled with $^{32}P_i$ as described earlier [6]. 17S and 26S rRNA were isolated from ribosomes and purified on sucrose gradients [1,6,11]. 5S RNA and tRNA were extracted from whole cells by phenol–SDS–EDTA treatment according to Hindley and Page [12] and were separated by preparative polyacrylamide gel electrophoresis (0.5-0.7 mg of crude RNA on each 7.5% gel in 10 × 120 mm tubes). Continuous elution (3 ml/hr) was obtained by fitting the anode end of the tube to a perforated adapter, connected to a peristaltic pump. Using a phos-

phate buffer [13] and 12.5 mA/tube, the tRNA eluted after 9–10 hr and 5S RNA after 11–12 hr.

2.4. *Hybridizations*

RNA–DNA hybridizations were carried out with denatured DNA fixed to membrane filters at conditions as described previously [4,6,7]. To estimate the hybridization level for 5S RNA, contaminated by labelled fragments from the larger rRNAs, the [^{3}H] DNA-filters were incubated with [^{32}P] 5S RNA (3 μg/ml) and varying amounts of nonlabelled 17S plus 26S rRNA (from 0 to 30 μg/ml). For the equilibrium reaction, where labelled fragments (r_2) are competed against by nonlabelled RNA (r), the following equation has been derived according to Bishop et al. [14]:

$$\frac{1}{q_2 - q} = \frac{K + r_2}{q_2}\,\frac{1}{r} + \frac{1}{q_2} = \frac{1}{Q_0 - Q}$$

where q_2 and q are the hybrid counts (cpm/μg DNA) from labelled fragments (r_2) in absence, and in presence, of competing 17S and 26S rRNA (r), respectively, and K is a constant. Setting q_1 for the hybrid counts from 5S RNA, then the observed hybrid counts are $Q_0 = q_1 + q_2$ and $Q = q_1 + q$. By plotting $1/Q_0 - Q$ against $1/r$ and extrapolating to infinite r, the hybrid counts due to 5S RNA (q_1) and hence the % of DNA in hybrid, can be calculated.

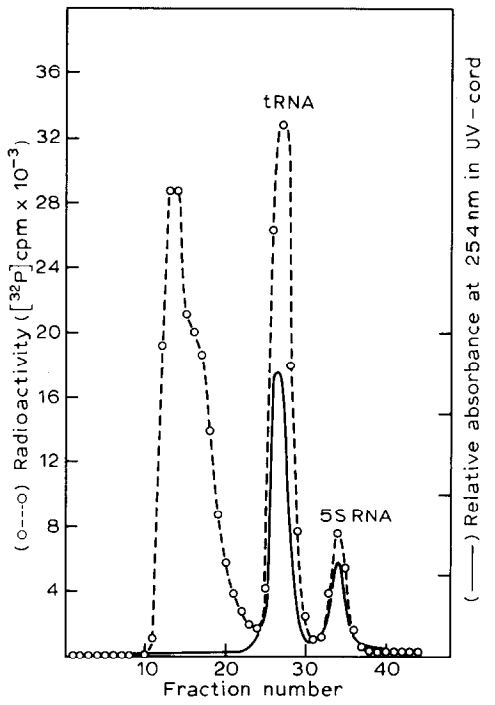

Fig.1. Elution pattern by preparative polyacrylamide gel electrophoresis of crude tRNA plus 5S RNA extracted from [^{32}P] labelled yeast cells by phenol-SDS-EDTA treatment. Electrophoresis was performed at room temperature with 12.5 mA/tube in 50 mM sodium phosphate buffer, 1 mM EDTA, pH 7. The absorbance during elution was monitored in a Uvicord. The radioactivity in each fraction (1 ml) was estimated by Cerenkov counting.

3. Results and discussion

The simplest procedure for isolation of 5S RNA is a direct extraction from the cells by phenol treatment, combined with polyacrylamide gel electrophoresis for separation of 5S RNA and tRNA. In fig.1 is shown the elution pattern upon electrophoresis of the crude tRNA plus 5S RNA extracted from [^{32}P] labelled cells. A large proportion of the radioactivity, possibly polyphosphates, eluted in front of the tRNA and was hence removed. When cells grown in presence of [^{14}C methyl] methionine had been extracted, only the tRNA peak became labelled. Although, hybridization of the purified [^{32}P] 5S RNA preparation to DNA revealed a 5 to 10% contamination from labelled fragments of the larger rRNA types. To estimate the amount of DNA complementary to 5S RNA then, the fragments had to be competed against. Such competition hybridization curves are shown in fig.2. The DNA used for hybridization had been isolated from strains that contained different amounts of rDNA. Evidently, these strains also contained different amounts of 5S RNA genes. The hybridization values with 5S RNA, obtained by extrapolating to infinite concentration of competing RNA (see Materials and methods), are summarized in table 1 together with the maximum hybridization of the same DNA preparations with 17S plus 26S rRNA. From the ratios between the number of 5S RNA cistrons and the number of 17S and 26S rRNA cistrons, it is seen that the two diploid strains, taken to have normal rDNA contents, contained about twice as many 5S RNA genes. This was also true for strain Y-233, which has been found to carry about 60% more rDNA than the diploids [6,7]. Hence,

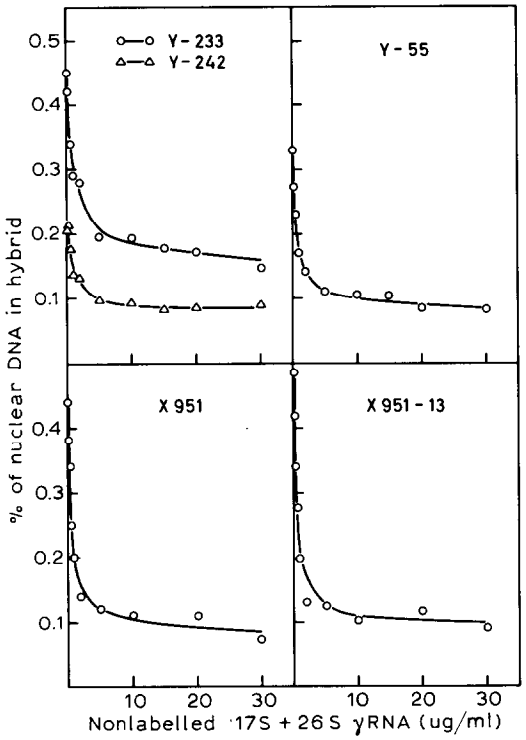

Fig.2. Hybridization of nuclear DNA, from different yeast strains, with [^{32}P] 5S RNA (3 μg/ml) in presence of increasing amounts of nonlabelled 17S plus 26S rRNA (0 to 30 μg/ml).

this strain has gained the same proportion of 5S RNA genes, indicating that the cistrons for 5S RNA and for 17S plus 26S rRNA are somehow genetically linked.

Even though the low hybridization values for 5S RNA are connected with relatively large experimental variations, it seemed significant that the two strains low in rDNA carried as many 5S RNA genes as the diploids. For strain X951-13, this could mean that a less proportion of the 5S RNA genes than of the 17S-26S genes are located on chromosome I. The same interpretation might hold for Y-242, as this strain behaves more like a monosome than a disome [1]. However, by repeated experiments with chromosome I monosomic strains, we have, like others, observed a tendency for the rDNA level to increase against the diploid level upon subculturing. This phenomenon was recently reported on by Kaback and Halvorson [15] as being due to a magnification mechanism, independent of chromosome I, rather than to a diploidization. The role of chromosome I as the main carrier of rRNA cistrons might hence be disputed. Compared to earlier results [4], the rDNA in strain X951-13 seemed to be partially magnified, an increase from 1.6% to 1.9% of the DNA in hybrid with 17S plus 26S rRNA. The data in table 1 could then mean that the 5S RNA cistrons had already magnified to the diploid level. This question could possibly be answered by following the 5S RNA hybridization level throughout the rDNA magnification process. In any case, our data seem to be inconsistent with an alternating arrangement of the 5S RNA cistrons and the 17S–26S rRNA cistrons, even though the genes may be linked.

As shown by Finkelstein et al. [3], the rDNA in *S. cerevisiae* is distributed on DNA molecules of two different size groups, possibly originating from two chromosomes. Nevertheless, the rDNA bands as one

Table 1
The maximum % of nuclear DNA in hybrid with 5S RNA, and with 17S plus 26S rRNA. The ratio in number of genes were calculated from the hybridization values by assuming a mol. wt of 4×10^4 for 5S RNA and 2.1×10^6 for 17S plus 26S rRNA

Strain	% of nuclear DNA in hybrid with,		number of 5S RNA genes number of 17S-26S rRNA genes
	5S RNA	17S plus 26S rRNA	
X-55	0.08	2.2	1.9
X951	0.09	2.2	2.1
Y-233	0.14	3.7	2.0
Y-242	0.08	1.7	2.5
X951-13	0.09	1.9	2.5

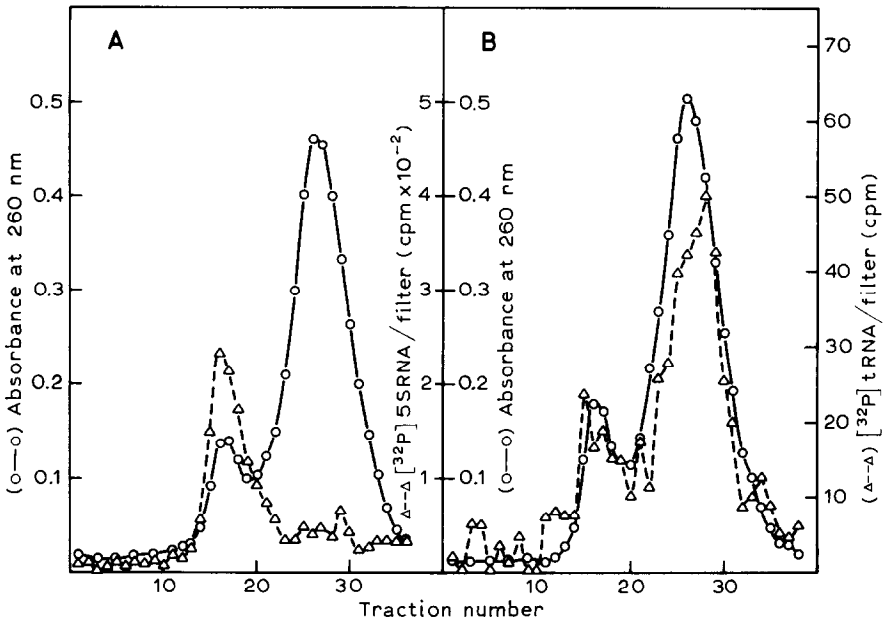

Fig.3. Banding of 5S RNA and tRNA cistrons upon fractionation of nuclear DNA (150–170 μg) in preparative Ag^+-Cs_2SO_4 density gradients (7.5 ml) at pH 7 with Ag^+/DNA-P = 0.24. Fractions of 15 drops were collected, denatured and fixed to membrane filters, and annealed with: A) [^{32}P] 5S RNA (3 μg/ml) in presence of nonlabelled 17S plus 26S rRNA (40 μg/ml), and B) [^{32}P] tRNA (5 μg/ml) in presence of nonlabelled 5S RNA (3 μg/ml) and 17S plus 26S rRNA (40 μg/ml).

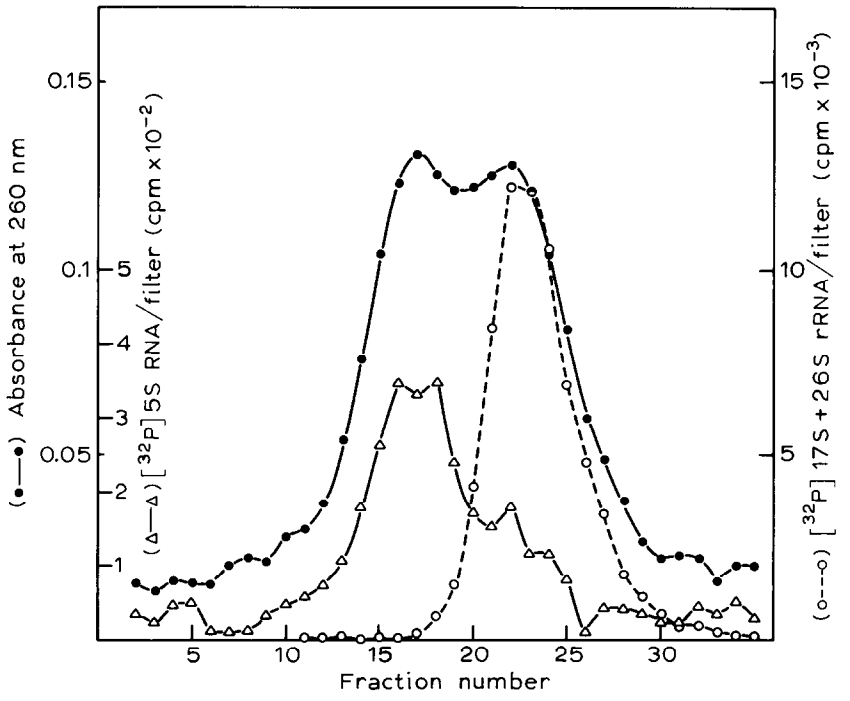

Fig.4

component, the γ-DNA, in cesium salt density gradients [7,11,16]. The γ-DNA can be separated out as a defined satellite by fractionation of nuclear DNA in Ag^+-Cs_2SO_4 density gradients [7]. As appears from fig.3, the 5S RNA cistrons cobanded with γ-DNA in such gradients, while the cistrons for tRNA were found distributed throughout both the γ-DNA and the main DNA fraction. The same conclusion was reached by Retèl and Planta for *S. carlsbergensis* DNA, using different fractionation methods [8]. None of the fractionation methods for native DNA, hence, seems to resolve the 5S RNA cistrons from the γ-DNA. However, when the γ-DNA is isolated and centrifuged in alkaline CsCl density gradients, two bands of single stranded DNA are formed [7,16,17], and from fig.4 it is seen that most of the regions complementary to 5S RNA then were found in the heavy band, while 17S plus 26S rRNA exclusively hybridized to the light band. Therefore, although the 5S RNA cistrons are located on DNA segments with the same average base composition as the γ-DNA, as revealed by fractionation of native DNA, the single stranded segments complementary to 5S RNA contains more G+T than the regions complementary to 17S plus 26S rRNA.

According to the observed linking then, we conclude that the genes for the larger rRNA species and for 5S RNA may be located on the same DNA molecules, but arranged in separate transcriptional units. These could either be on opposite strands and hence be transcribed in an anti-parallel manner, or they could represent clusters different in G+T contents on the same DNA strands. In both cases, it seems likely that the 5S RNA and the precursor rRNA are synthesized by different RNA polymerases. This situation would be in accordance both with the results of Hindley and Page [12], saying that 5S RNA is a primary gene product, and with those of Udem and Warner [18] showing that 5S RNA is synthesized independently of the precursor rRNA. Furthermore, from the differential effect of 8-hydroxyquinoline on the synthesis of the ribosomal species in fission yeast, Fraser and Creanor [19] concluded that different RNA polymerases are engaged in the syntheses, with polymerase C as a likely candidate for transcription of the 5S RNA cistrons. Cramer et al. [17] have found that in *S. cerevisiae* RNA polymerase A (I) is responsible for the synthesis of the precursor rRNA.

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Fig.4. Banding of the regions complementary to the ribosomal RNA species upon centrifugation of isolated γ-DNA in alkaline CsCl density gradients. Parts of each fraction were fixed to membrane filters and annealed with [^{32}P] 5S RNA (3 μg/ml) in presence of non-labelled 17S plus 26S rRNA (30 μg/ml) (△———△), or with [^{32}P] 17S plus 26S rRNA (5 μg/ml) (○———○).