STRUCTURAL COMPARISON OF 17 S RIBOSOMAL RNA OF YEAST AND ITS IMMEDIATE PRECURSOR, 18 S RNA

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1. Introduction

The synthesis and processing of ribosomal RNA (rRNA) in yeast has been the subject of considerable investigation [1–5]. The synthesis process appears to be very similar to that in other eukaryotes [5–7], and proceeds by the transcription of a large common precursor molecule which is subsequently converted in a number of steps into mature ribosomal RNA. As to the mode of processing the opinions of the various authors differ slightly. Some authors [5–7] think that at a certain stage of the process a common precursor is cleaved to give separate precursors for each of the two large rRNA components, which in addition to the rRNA segments contain a still considerable piece of excess RNA (about $0.2 - 0.5 \times 10^6$ daltons). The existence of such a separate precursor for yeast 26 S rRNA, a 29 S RNA component, has been well established [1–4]. However, the 20 S RNA, which was supposed to be a precursor to the 17 S rRNA by Grierson et al. [5], appeared to have no structural resemblance to this rRNA component [8]. On the basis of its labelling kinetics and its size it was suggested that 20 S RNA is rather a nonribosomal segment split off from the initial ribosomal transcription product [8].

In this communication we describe the isolation and structural analysis of an 18 S RNA found after pulse-labelling of yeast cells with (Me-^{3}H) methionine. As judged from a comparative analysis of the methylation patterns this 18 S RNA, which according to its sedimentation velocity and electrophoretic mobility is only slightly larger than 17 S rRNA (less than 0.1×10^6 daltons), is in all probability an immediate precursor of 17 S rRNA.

2. Materials and methods

2.1. *Isolation of yeast RNA pulse-labelled with (Me-^{3}H)methionine*

Saccharomyces carlsbergensis (Strain Sce/I/317, a methionine-requiring mutant) was grown at 29° in 200 ml of a synthetic medium (see [1]), but with a methionine concentration of 1 mg/ml. Cells were collected by centrifugation in the mid-logarithmic growth phase (corresponding to an A_{550} of 0.6), washed with water and resuspended into 200 ml of the same synthetic medium but now lacking methionine. Growth at 29° was continued until the culture had reached a turbidity corresponding to A_{550} of 1.0 (after 2–4 hr). Then 1.5 mg of each amino acid except methionine, 1 mg of glucose and 2.5 mg of each of the four ribonucleosides was added to the culture, and 15 min later 1 mCi (Me-^{3}H)methionine (6.9 Ci/mmole, Radiochemical Centre, Amersham, England). After 4 min labelling was rapidly stopped by chilling with ice and simultaneously filtering the culture through a Millipore filter [2]. RNA was extracted from the washed cells as described in a previous paper [8].

2.2. *Enzymatic digestion and fingerprinting of RNA*

Aliquots of 0.1 mg RNA in 0.01 ml 0.01 M Tris-HCl (pH 8.0) were digested with T_1-ribonuclease (enzyme:substrate ratio of 1:10) and alkaline phosphatase (enzyme:substrate ratio of 1:5) at 37° for 30 min. The digestion products were separated in two dimensions according to Brownlee and Sanger [9]. In the first dimension electrophoresis was done at pH 3.5 on cellulose acetate strips. In the second dimension homochromatography with 3% "homomixture C" on

20 X 40 cm DEAE-cellulose thin-layer plates was
applied. The thin-layers consisted of DEAE-cellulose
(Macherey and Nagel; MN 300 DEAE) and cellulose
(MN 300 HR) in a ratio of 1:12.5. ^{14}C-spots were
visualized by radioautography.

3. Results and discussion

The total RNA synthesis of yeast cells was anal-
yzed using a short pulse of (Me-^{3}H)methionine
(fig. 1). Examination of the sedimentation pattern
reveals that the newly-synthesized, ^{3}H-labelled RNA
consists of the high molecular weight RNA species
which have been described in detail before [1, 3, 4],
and the recently described 20 S RNA [8]. In addition,
it can be seen that in the region of 17 S rRNA the
profiles of radioactivity and optical density do not
completely coincide, suggesting the presence of a
rapidly labelled RNA with an approximate sedimenta-
tion value of 18 S. A similar pattern was observed
after polyacrylamide gel electrophoresis of total yeast
RNA pulse-labelled for instance with ^{32}P-orthophos-
phate (see fig. 1 in [8]). After longer periods of
labelling this rapidly labelled 18 S peak is not visible,
since its relative amount with respect to the label in
17 S rRNA will become vanishingly small.

The 18 S RNA component was purified by pooling
the leading edge of the relevant peak (as indicated in
fig. 1) from a number of gradients, and recentrifuging
the combined RNA fractions through a sucrose gra-
dient as described in fig. 2. This procedure was
repeated until the 18 S RNA sedimented as a single
symmetrical band. Usually three subsequent centri-
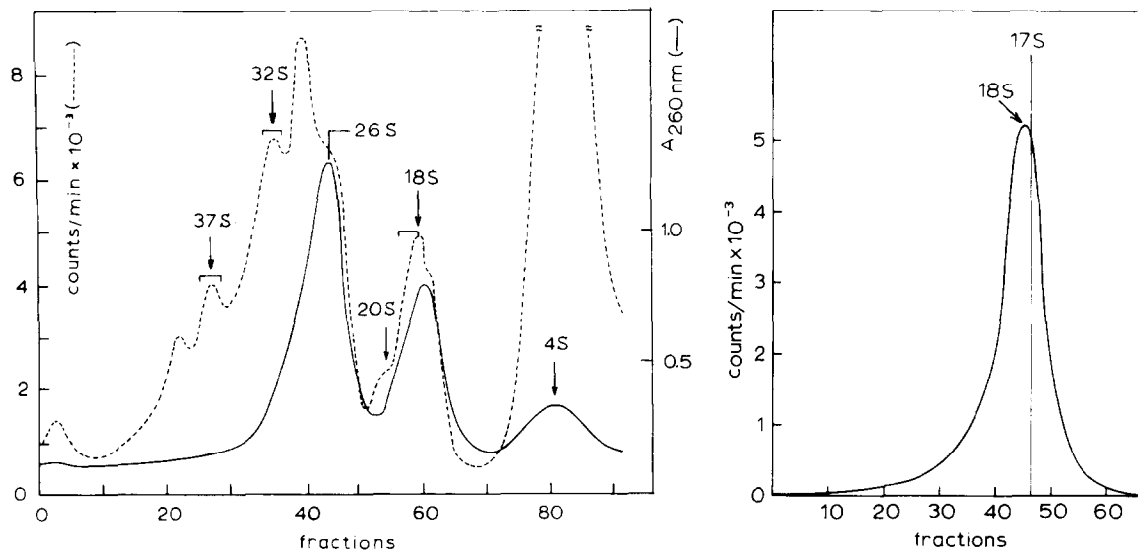fugation runs were required to attain this degree of
purity.

Fig. 1. Sedimentation analysis of rapidly methyl-labelled
yeast RNA. RNA, pulse-labelled for 4 min with (Me-^{3}H)-
methionine, was prepared from whole yeast cells as described
in Materials and methods. The RNA was fractionated by cen-
trifugation through a linear 5–20% sucrose gradient made up
in 0.1 M NaCl, 0.01 M disodium-EDTA (pH 5.2) in a SW 27-1
rotor of the Spinco ultracentrifuge for 16 hr at 23,000 rpm.
Fractions were collected, and aliquots of each fraction were
used for the determination of A$_{260}$ (———) and radioactivity
(-----). For the latter purpose 20 μl aliquots were mixed with
10 ml toluene based scintillation fluid containing 30 ml
Nuclear Chicago Solubilizer (NCS) per litre and counted in a
scintillation counter.

Fig. 2. Purification of 18 S RNA by repeated sucrose density
gradient centrifugation. The leading edge of the radioactive
peak corresponding to 18 S in fig. 1 was pooled from a number
of gradients. To the combined fractions an appropriate
amount of unlabelled 17 S rRNA was added as a carrier, and
subsequently the RNA was precipitated with two volumes of
ethanol at −20°. The pellet was redissolved into 0.25 ml 0.1
M NaCl, 0.01 M disodium-EDTA (pH 5.2) and centrifuged
again through a linear 5–20% sucrose gradient in a Spinco
SW 41 rotor for 5 hr at 35,000 rpm. Fractions were collected
and assayed for A$_{260}$ and radioactivity as described under
fig. 1. The profile shown here was obtained after three subse-
quent runs.

For a structural comparison of 18 S RNA and 17 S rRNA the distribution of the methyl groups along
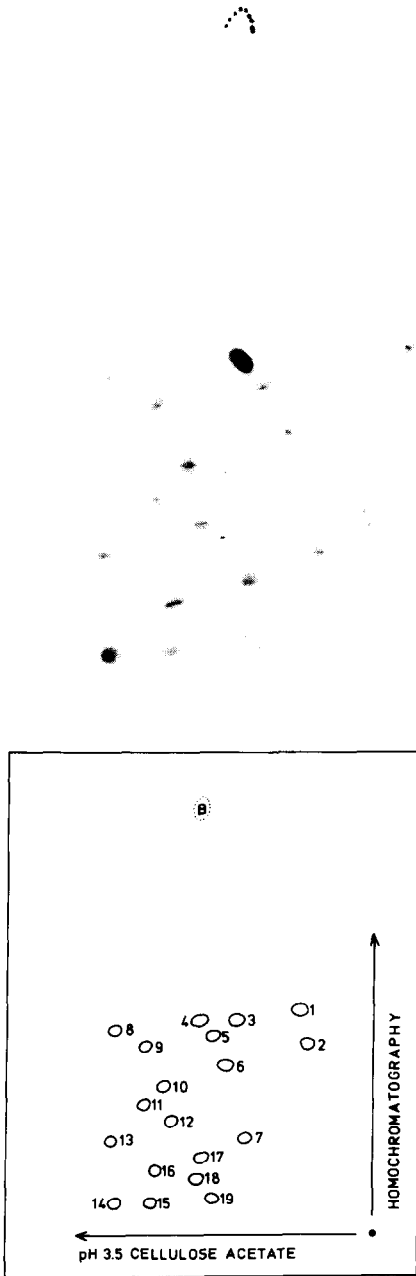

Fig. 3. Two-dimensional separation of the products obtained by digestion of a mixture of ^{14}C-methyl-labelled 17 S rRNA and ^{3}H-methyl-labelled 18 S RNA with T_1-ribonuclease and alkaline phosphatase. ^{3}H-methyl-labelled 18 S RNA was obtained as described under fig. 2. ^{14}C-methyl-labelled 17 S rRNA was isolated from yeast cells labelled for more than 10 generations with (Me-^{14}C)methionine (59 mCi/mmole, The Radiochemical Centre, Amersham, England) in essentially the same way as described in a previous paper [13]. A mixture of 60 μg 18 S RNA and 40 μg (11,000 dpm) 17 S rRNA was digested with T_1-ribonuclease and alkaline phosphatase, and the products were separated in two dimensions as described in Materials and methods. With the radioautograph (fig. 3A) as a guide the material, corresponding to each spot, was scraped from the glass-plate and treated overnight at 37° with 0.5 ml Nuclear Chicago Solubilizer in scintillation vials. After addition of 10 ml toluene based scintillation mixture to each vial radioactivity was determined in a liquid scintillation counter. A correction was made for spill-over of ^{14}C-counts in the ^{3}H-channel. The amounts of [3]H- and ^{14}C-radioactivity in the various spots (see diagram in fig. 3B) are recorded in table 1.

the polynucleotide chain was used as a criterion. We have demonstrated earlier [10] that 17 S and 26 S rRNA from yeast have highly characteristic patterns of methylation. Two-dimensional separation of T_1-ribonuclease digests of 17 S and 26 S rRNA, labelled with ^{14}C-methyl groups, demonstrated that the two RNA species have very few, if any, methylated sequences in common [11]. The best way to compare the patterns of methylation of 18 S and 17 S RNA would be a comparative analysis of the two "fingerprints" of the individual ^{14}C-methyl-labelled RNA components. This approach has also been applied by Salim et al. [12] for the analysis of the ribosomal RNA precursors in HeLa cells. In yeast, however, it appeared difficult to incorporate in the various labelled RNA species sufficient amounts of ^{14}C-methyl label to carry out such an experiment. Therefore, in the present experiments a more indirect method was used. A mixture of appropriate amounts of ^{3}H-methyl-labelled 18 S RNA and ^{14}C-methyl-labelled 17 S rRNA was digested completely with T_1-ribonuclease and alkaline phosphatase. The digest was separated in two dimensions of DEAE-cellulose thin-layer plates as illustrated in fig. 3. In this fingerprint only the ^{14}C-labelled oligonucleotides derived from 17 S rRNA are detected by radioautography. All these spots were assayed for both [14]C- and ^{3}H-radioactivity (table 1).

Although the total recoveries of [3]H- and ^{14}C-radio-

Table 1

Amounts of ^{3}H-radioactivity (from 18 S RNA) and ^{14}C-radioactivity (from 17 S rRNA) found in ^{14}C-marked spots of fig. 3

Spot no.	^{14}C-dpm (17 S rRNA)	^{3}H-dpm (18 S RNA)	^{3}H/^{14}C ratio
1	176	42	< 1
2	196	288	1.5
3	222	764	3.4
4	818	21	< 1
5	248	699	2.8
6	172	103	0.6
7	224	492	2.2
8	188	51	< 1
9	204	602	2.9
10	264	79	< 1
11	162	321	2.0
12	162	490	3.0
13	192	555	2.9
14	414	816	2.0
15	202	402	2.0
16	210	628	3.0
17	200	664	3.3
18	32	119	3.7
19	240	741	3.1

The numbers of the spots correspond to those in fig. 3B.

activities were not very satisfactory (both about 50%), presumably the recoveries of the two radioactivities are equally affected by losses during blotting and elution of the spots. For, the great majority of the spots contained both ^{3}H- and ^{14}C-radioactivity in a roughly constant ratio (see table 1), indicating that the methylation patterns of 18 S and 17 S RNA are very similar. Only a few methylated oligonucleotides present in 17 S rRNA (corresponding to the spot nos. 1, 4, 8 and 10) appear to be nearly absent in the digest of ^{3}H-methyl-labelled 18 S RNA. A possible explanation for the lack of these few methylated sequences in 18 S RNA may be that some sequences, which contain methyl groups in 17 S rRNA, are not yet methylated in 18 S RNA. Another consideration is that pulse-labelling may cause a non-proportional labelling of the various methyl groups in 18 S RNA. However, although this effect may well be responsible for the relatively large variability of ^{3}H-activity in the joint methylated oligonucleotides, it seems less likely that it can explain fully the dramatic differences between the two labels in spot nos. 1, 4, 8 and 10.

Finally, if the terminal fragments of 17 S rRNA are methylated, and if 18 S RNA is actually an extended 17 S polynucleotide chain, one or two methylated oligonucleotides of the 17 S rRNA digest may not be found in the 18 S RNA digest. In this respect it is a disadvantage that the radioautograph does not show whether there are additional ^{3}H-labelled spots which are specific for 18 S RNA.

Anyhow, it seems to be justified to conclude that 18 S RNA is an immediate precursor of 17 S rRNA. Estimated by both its sedimentation value (fig. 2) and its electrophoretic mobility in polyacrylamide gels (see fig. 1 in [8]) 18 S RNA seems to be about 200 nucleotides longer than 17 S rRNA (consisting of 1600 nucleotides [14]). The conversion of 18 S RNA into 17 S rRNA might then be caused by the splitting off of a nonconserved segment comprising these 200 nucleotides. Another possibility, which cannot be excluded at present, is that the length of 18 S RNA is not different from that of 17 S rRNA, and that the conversion is brought about by a final methylation step with a concomitant change in secondary structure of the RNA.

Finally, we want to draw attention to the fact that in most spots of fig. 3 the amount of ^{14}C-label is nearly the same (with an average of about 200 dpm), except for the spot nos. 4, 14 and 18. This indicates that all these spots represent an oligonucleotide carrying one methyl group, and that all these oligonucleotides are present in approximately equimolar amounts. Similar findings were obtained earlier by Fellner and Sanger [15] for the methylated oligonucleotides in T_1-ribonuclease digests of the rRNA's of *E. coli*. Spot no. 4 might correspond to 4 methyl groups, spot no. 14 to 2 methyl groups, whereas minor spot no. 18 might represent a microheterogeneity or might be due to an aberrant enzymatic splitting. If these assumptions are correct, it can be calculated that the total number of methyl groups per 17 S rRNA molecule equals 22, in good agreement with results obtained by more direct methods (J. Klootwijk and R.J. Planta, manuscript in preparation).

After this manuscript had been completed, a paper by Udem and Warner [16] appeared, in which on the basis of pulse-chase kinetics the occurrence of a precursor to the smaller rRNA components in *S. cerevisiae* was suggested. To this precursor an S-value of 20 S was assigned, and to the smaller rRNA compo-

nents an S-value of 18 S. If we substitute the S-values of the rRNA components used by these authors (25 S and 18 S, respectively) by the ones we used (26 S and 17 S, respectively) the rapidly labelled 20 S RNA described by Udem and Warner might well correspond to the 18 S RNA described in this paper.

Acknowledgements

References

[1] J. Retèl and R.J. Planta, European J. Biochem. 3 (1967) 248.

[2] R.L. Taber and W.S. Vincent, Biochim. Biophys. Acta 186 (1969) 317.

[3] J. Retèl and R.J. Planta, Biochim. Biophys. Acta 224 (1970) 458.

[4] J. Retèl, Ph.D. Thesis, Vrije Universiteit, Amsterdam, 1970.

[5] D. Grierson, M.E. Rogers, M.L. Sartirana and U.E. Loening, Cold Spring Harbor Symp. Quant. Biol. 35 (1970) 589.

[6] S. Penman, H. Fan, S. Perlman, M. Rosbash, R. Weinberg and E. Zylber, Cold Spring Harbor Symp. Quant. Biol. 35 (1970) 561.

[7] R.P. Perry, J.R. Greenberg and K.D. Tartof, Cold Spring Harbor Symp. Quant. Biol. 35 (1970) 577.

[8] R.C. van den Bos and R.J. Planta, Biochim. Biophys. Acta 247 (1971) 175.

[9] G.G. Brownlee and F. Sanger, European J. Biochem. 11 (1969) 395.

[10] R.C. van den Bos and R.J. Planta, Nature 225 (1970) 183.

[11] R.C. van den Bos, Ph.D. Thesis, Vrije Universiteit, Amsterdam, 1972.

[12] M. Salim, R. Williamson and B.E.H. Maden, FEBS Letters 12 (1970) 109.

[13] J. Retèl, R.C. van den Bos and R.J. Planta, Biochim. Biophys. Acta 195 (1969) 370.

[14] R.C. van den Bos, J. Retèl and R.J. Planta, Biochim. Biophys. Acta 232 (1971) 494.

[15] P. Fellner and F. Sanger, Nature 219 (1968) 236.

[16] S.A. Udem and J.R. Warner, J. Mol. Biol. 65 (1972) 227.