ON THE BIOSYNTHESIS OF 5.8 S RIBOSOMAL RNA IN YEAST

J. TRAPMAN, P. DE JONGE and R. J. PLANTA
Biochemisch Laboratorium, Vrije Universiteit, De Boelelaan 1085, Amsterdam, The Netherlands

Received 16 June 1975

1. Introduction

Yeast ribosomes, like all other eukaryotic ribosomes, contain four stable RNA components, which in this case have sedimentation values of 26S, 17S, 5.8S and 5S, respectively [1,2]. The small ribosomal subunits contain only 17S rRNA, whereas the other three rRNA components are present in the large ribosomal subunits in a unimolar ratio [3–6]. The 5.8S rRNA appears to be noncovalently bound to the 26S rRNA [4–6].

The production of the larger rRNA molecules starts with the synthesis of a large common precursor RNA. This first transcription product is then converted into the mature rRNAs in a number of cleavage steps, during which stretches of so-called excess RNA are removed [1,2]. Both kinetic analysis [4] and recent structural studies for mammalian systems [7,8] indicate, that 5.8S rRNA originates from the direct precursor (29S RNA) of the largest rRNA component. In which way the 5.8S rRNA is produced during the conversion of 29S to 26S rRNA is largely unknown.

After pulse labelling of yeast protoplasts we observed a rapidly labelled 7S RNA of about 250 nucleotides in length. From its location, kinetics of synthesis and some structural properties it can be condluded that the 7S RNA is a precursor of 5.8S rRNA.

2. Materials and methods

2.1. *Conditions of growth and labelling*

Saccharomyces carlsbergensis (N.C.Y.C. S74) was grown and converted into protoplasts as described previously [9]. For labelling with [^{3}H]uridine (29 Ci/mmol, Radiochemical Centre, Amersham, England) protoplasts were suspended in a 'double medium' (see ref. [9]) at a cell density of 8×10^7 protoplasts/ml. In the present experiments the amount of yeast extract in the medium was reduced to 0.1 mg/ml. For labelling with ^{32}P-phosphate (carrier free, Philips Duphar, Petten, The Netherlands) the composition of the medium was per litre: 15 g glucose, 1 g peptone, 2 g yeast extract, 3 g maltextract, 120 g mannitol. Protoplasts were incubated for 1 hr at 29°C and subsequently pulse labelled at 15°C for an appropriate time. To stop labelling, protoplasts were rapidly chilled to 0°C and harvested by centrifugation.

2.2. *Isolation of RNA and ribonucleoprotein particles*

RNA was prepared from protoplasts by phenol-0.5% SDS extraction as described previously [9], except that the first extraction was carried out at room temperature. Extraction was repeated at 0°C until deproteinization was complete.

In some experiments RNA was fractionated on 5–20% linear sucrose gradients [10] and the low molecular weight RNAs (4–10S) were pooled. Sucrose gradient centrifugation was in a Spinco SW27 rotor for 16 hr at 24 000 rpm and 4°C.

Ribonucleoprotein particles were extracted and analyzed on 15–30% linear sucrose gradients essentially as described previously [11]. RNA was isolated from the pooled fractions by phenol-SDS extraction at 0°C.

3. Results and discussion

3.1. *Gel electrophoretic analysis of small pulse labelled RNAs*

Fig.1a shows a polyacrylamide gel electrophoretic analysis of low molecular weight RNAs after labelling with [^{3}H]uridine for 45 min at 15°C. RNA was isolated under conditions, which liberate 5.8S rRNA from 26S

North-Holland Publishing Company – Amsterdam

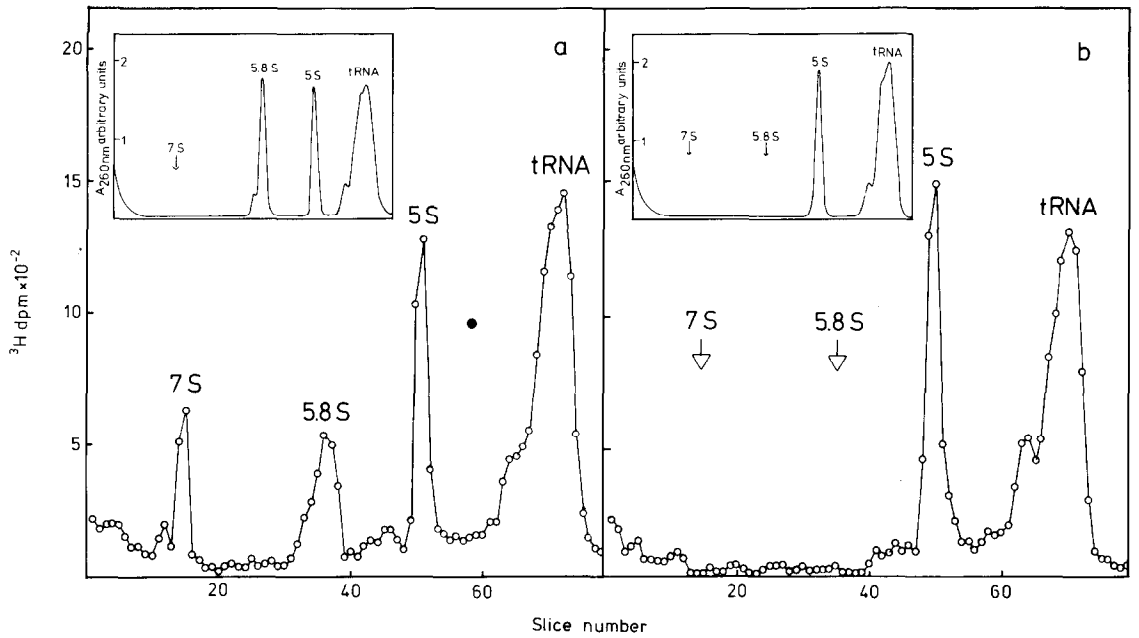

Fig.1. Polyacrylamide gel electrophoresis of pulse labelled low molecular weight RNA. RNA extracted from protoplasts, pulse labelled with [^{3}H]uridine (5 μCi/ml) was fractionated on sucrose gradients as described in the Methods section. RNA was pooled from the upper part of the gradient and analyzed by electrophoresis on 7.5% polyacrylamide gels according to Loening [12] using the Tris–phosphate buffer system [13] for 5 h at 3 mA/gel. The 7.5% gels were overlayered with 1 cm of a 2.6% gel made in the same buffer. After electrophoresis gels were cut into 1 mm slices and assayed for radioactivity [11]. The pattern shown in fig.1a was obtained after heating the RNA sample for 5 min at 60°C before layering on the sucrose gradient. The profile shown in fig.1b was obtained, if the sample was not heated.

rRNA. In addition to tRNA, 5S rRNA and 5.8S rRNA some other labelled RNA components can be detected the most prominent of them being a 7S RNA. This RNA component is absent in the optical density pattern (see inserts in fig.1). From its position in the gel a length of about 240–260 nucleotides can be deduced, assuming a length of 80 nucleotides for the bulk of the tRNA [14], 121 for 5S rRNA [15] and 158 for 5.8S rRNA [16].

If small RNAs are prepared without a denaturing step both 7S and 5.8S RNA are absent from the gel (cf. fig.1b). Therefore, 7S RNA appears to be non-covalently bound (like 5.8S rRNA) to a high molecular weight RNA.

To establish the location of 7S RNA, low molecular weight RNAs were prepared from the various ribosomal precursor particles [11] after pulse labelling. 7S RNA could only be detected in the 60–66S particles, in addition to 5S and 5.8S rRNA (fig.2). Furthermore

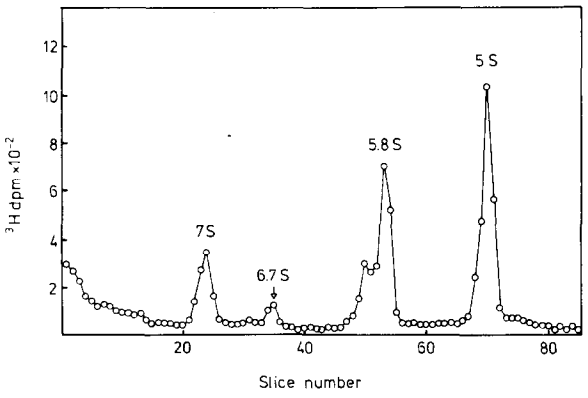

Fig.2. Polyacrylamide gel electrophoresis of pulse labelled small RNAs isolated from 66–60S ribonucleoprotein particles. Protoplasts were labelled with [^{3}H]uridine (10 μCi/ml) for 60 min at 15°C. Small RNAs from 66–60S particles [11] were analyzed on 7.5% polyacrylamide gels as described in the legend of fig.1. Electrophoresis was for 7 hr at 3 mA/gel.

these particles contain 26S rRNA and its immediate precursor, 29S RNA [11]. 7S RNA and 5.8S rRNA could also be recovered from the 25–30S region of an RNA gradient, on which total RNA was fractionated without a prior denaturing step (data not shown). Fig.2 shows the presence of an extra RNA species (6.7S) present in variable amounts in the preribosomes. This 6.7S RNA has probably arisen in vitro during extraction and sucrose gradient analysis of the ribonucleoprotein particles, since it could not be demonstrated in RNA extracted directly from the protoplasts.

3.2. *Kinetics of synthesis of 7S RNA*

By labelling protoplasts for various times with [^{3}H]uridine the labelling kinetics of 7S RNA and 5.8S rRNA were investigated. The results are summarized in fig.3. The labelling kinetics of 7S RNA show that this RNA species is unstable, in contrast to 5.8S rRNA. The first labelling of 7S RNA is detected after 14–16 min, but still about 3 min prior to the first labelling (at 17–19 min) of 5.8S rRNA. Labelling of 5S rRNA and tRNA under these conditions can already be detected in the first minute (J. Trapman and R. J. Planta to be published). The inset in fig.3 shows, that 26S rRNA (originating from 29S RNA) and 7S RNA are produced at about the same time. These

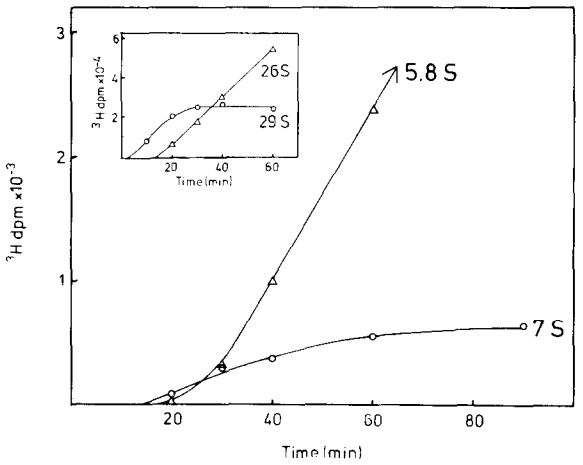

Fig.3. Labelling kinetics of 7S and 5.8S rRNA. Protoplasts were labelled with [^{3}H]uridine (10 μCi/ml) for 10, 20, 30, 40, 60 and 90 min at 15°C. At these samples were taken, RNA extracted and analyzed by electrophoresis as described in the legend of fig.1. The amount of radioactivity in each RNA species was determined by summing up the radioactivity of all gel slices containing that RNA species.

observations suggest, that 7S RNA also derives from 29S RNA, either as a stretch of excess RNA or as a precursor of 5.8S rRNA.

3.3. *Structural analysis of 7S RNA*

To establish the relationship between 7S RNA and 5.8S rRNA, both RNA species were labelled with ^{32}P-phosphate and subjected to fingerprint analysis. Fig.4 shows the ribonuclease T_1-fingerprints of 7S and 5.8S rRNA, respectively. The fingerprint of 7S RNA contains all the separate spots of 5.8S rRNA and in addition a number of extra spots. This shows that 7S RNA is structurally related to 5.8S rRNA, and does not represent a stretch of excess RNA.

In a recent paper Helser and McLaughlin [19] describe a 7S RNA species isolated from *S. cerevisiae.* Their results suggest that this RNA species is either a precursor of 5.8S RNA or a piece of excess RNA produced during the final maturation step of 26S rRNA. Although their results indicate that 7S RNA is produced coincidentally with 5.8S rRNA, in contrast to what we found, the 7S RNA observed in *S. cerevisiae* may be similar to the 7S RNA component described in the present study.

From the fingerprints shown in fig.4 no conclusions can be drawn about the position of the nonconserved regions in 7S RNA. The 5′-end of 5.8S rRNA, represented by spot 22 [17] seems also to be present in 7S RNA. It cannot be excluded, however, that this very long oligonucleotide contains some additional nucleotides in the case of 7S RNA. The oligonucleotide containing the 3′-OH end of 5.8S rRNA is located in spot 17 [17]. Due to the complexity of this spot it cannot be established with certainty whether this oligonucleotide is missing from the 7S RNA fingerprint.

Acknowledgements

The present study was supported in part by the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.). The authors are indebted to Miss I. Klein, Mr P. H. A. Grotendorst and Mr F. van Bemmelen for skilful technical assistance during part of this work and to Dr J. Klootwijk for stimulating discussions and critically reading the manuscript.

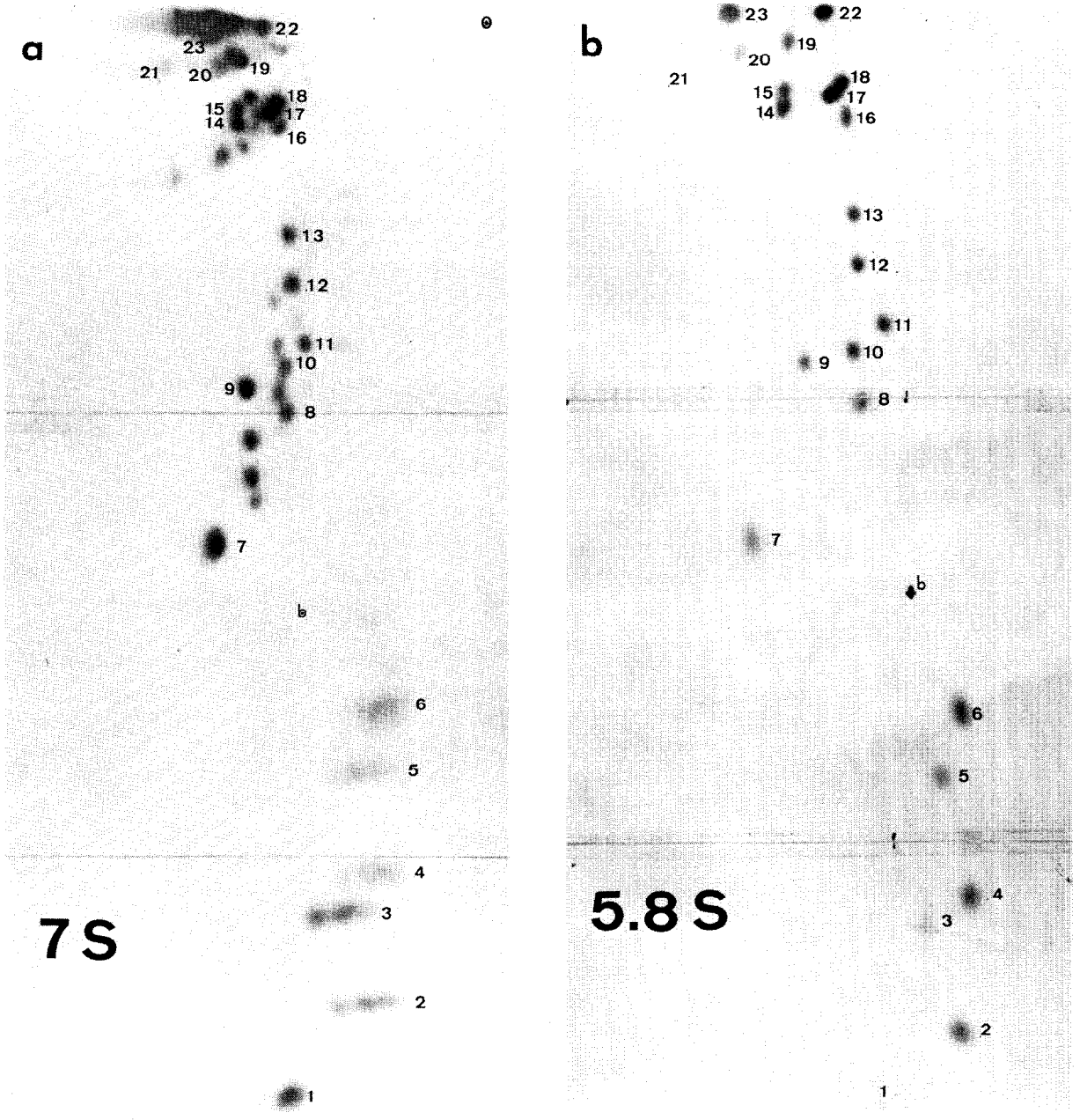

Fig.4. Two-dimensional separation of oligonucleotides obtained after digestion of ^{32}P-labelled 7S RNA (a) and 5.8S rRNA (b) with ribonuclease T_1. Preparation of 7S RNA: Protoplasts were labelled with ^{32}P-phosphate (0.3 mCi/ml) for 1 h at 15°C. Ribonucleoprotein particles were extracted and analyzed by sucrose gradient centrifugation as described previously [11]. RNA was extracted from the 66–60S fraction of the gradient, heated for 5 min at 60°C, separated on 7.5% polyacrylamide gels and recovered from the gel as described in the legend of fig.1 and in ref. [10]. 5.8S RNA was obtained from steady state ^{32}P-labelled 26S rRNA by heat treatment as described above and in ref. 17. RNA was digested with T_1-ribonuclease (Sankyo Co. Ltd., Tokyo, Japan) as described before [10,18]. The digestion products were separated by two-dimensional electrophoresis according to Sanger [18].

References

[1] Planta, R. J., van den Bos, R. C. and Klootwijk, J. (1972) in: Ribosomes: Structure, function and biogenesis (Bloemendal, H. and Planta, R. J., eds.) pp. 183–196, North Holland, Amsterdam.
[2] Maden, B. E. H. (1971) Progr. Biophys. Mol. Biol. 22, 127–177.
[3] Knight, E. and Darnell, J. (1967) J. Mol. Biol. 28, 491–502.
[4] Pene, J., Knight, E. and Darnell, J. (1968) J. Mol. Biol. 38, 608–623.
[5] Sy, J. and McCarty, K. S. (1970) Biochim. Biophys. Acta 199, 86–94.
[6] Udem, S. A., Kaufman, K. and Warner, J. R. (1971) J. Bact. 105, 101–106.
[7] Maden, B. E. H. and Robertson, J. S. (1974) J. Mol. Biol. 87, 227–255.
[8] Nazar, R. N., Owens, T. W., Sitz, T. O. and Busch, H. (1975) J. Biol. Chem. 250, 2475–2481.
[9] Retèl, J. and Planta, R. J. (1967) Eur. J. Biochem. 3, 248–258.
[10] Stoof, T. J. and Planta, R. J. (1973) Mol. Biol. Rep. 1, 243–249.
[11] Trapman, J., Retèl, J. and Planta, R. J. (1975) Exp. Cell Res. 90, 95–104.
[12] Loening, U. E. (1967) Biochem. J. 102, 251–257.
[13] Loening, U. E. (1969) Biochem. J. 113, 131–138.
[14] Miura, K. I. (1967) in: Progr. Nucl. Ac. Res. Mol. Biol. (Davidson, J. N. and Cohn, W. E., eds.) Vol. 6, pp. 39–83, Acad. Press, New York.
[15] Hindley, J. and Page, S. M. (1972) FEBS Lett. 26, 157–160.
[16] Rubin, G. M. (1973) J. Biol. Chem. 248, 3860–3875.
[17] Klootwijk, J., van den Bos, R. C. and Planta, R. J. (1972) FEBS Lett. 27, 102–106.
[18] Sanger, F., Brownlee, G. G. and Barrell, B. G. (1965) J. Mol. Biol. 13, 373–398.
[19] Helser, T. L. and McLaughlin, C. S. (1975) J. Biol. Chem. 250, 2003–2007.