Experimental Cell Research 71 (1972) 33–40

PRECURSORS OF RIBOSOMAL RNA IN YEAST NUCLEUS

Biosynthesis and Relation to Cytoplasmic Ribosomal RNA

W. W. SILLEVIS SMITT, J. M. VLAK, R. SCHIPHOF and Th. H. ROZIJN

Laboratory for Physiological Chemistry, The State University, Utrecht, The Netherlands

SUMMARY

In vivo methylated precursors of ribosomal RNA in yeast have been characterized on acrylamide gels. The initial ribosomal precursor in the yeast nucleus is a 37S RNA component, which is processed to a nuclear 28S RNA. Both the 37S and the 28S RNA components are important constituents of the yeast nucleus. A possible 33S RNA intermediate has been observed. Newly formed 18S rRNA rapidly enters the cytoplasm, while newly formed 26S rRNA appears later. The 26S rRNA is most probably formed from the nuclear 28S RNA.

Evidence has come from several sources that in yeast, as in higher eukaryotes, ribosomal RNA is synthesized via large precursor molecules [1–3]. In a previous publication [3] we have established that the large precursors of ribosomal RNA are located exclusively within the yeast nucleus. Sucrose gradient analysis of nuclear RNA, labelled with radioactive uracil, has revealed that the initial step in the formation of ribosomal RNA is the formation of a 37S molecule which is converted into a nuclear 28S RNA. This 28S ribosomal precursor RNA is probably an intermediate in the formation of the 26S cytoplasmic ribosomal RNA [3]. Little or no newly formed 18S ribosomal RNA is associated with the nuclei. The cytoplasmic 18S RNA seems to be labelled earlier than the cytoplasmic 26S RNA. Apparently the 18S RNA leaves the nucleus almost as soon as it is formed.

In the experiments with radioactive uracil [3] all species of RNA become labelled. This interferes to a certain extent with the estimations of the radioactivity associated with ribosomal RNA (rRNA). The use of methyl-labelled methionine makes it possible to discriminate between rRNA and heterogeneous RNA, since rRNA, its nuclear precursors and tRNA are the only methylated species of RNA [2–7]. A kinetic study of the methylation in vivo of rRNA in yeast is presented in this paper. Acrylamide gel electrophoresis [8] has been used to study in more detail the relation between nuclear rRNA precursors and cytoplasmic rRNA.

MATERIALS AND METHODS

Organism and isotope

The experiments were performed with spheroplasts [9] of the yeast strain *Saccharomyces carlsbergensis*, no. 74 from the British National Collection of Yeast Cultures. The spheroplasts were incubated (27°C) at a cell density of 7×10^7/ml in a medium containing per ml 120 mg mannitol, 10 mg glucose, 0.2 μg of resp. thiamine HCl, riboflavin phosphate, nicotinamide, pyridoxal HCl, calcium pantothenate, biotin and inositol, 15 μM $(NH_4)_2SO_4$, 1 μM $MgCl_2$ and 20 μM sodium-potassium phosphate, pH 6.3. After preincubation for 15 min ^{3}H-Me-methionine (spec.

act. 5.4 Ci/mM; Philips Duphar) was added to make a final concentration as given in the legends of figs 2 and 3. Incubation was then continued for different periods of time. After the labelling, the spheroplast suspensions were rapidly chilled and the spheroplasts were then harvested by centrifugation at 3 000 g for 5 min, at 2°C.

Preparation of nuclei

The nuclei were isolated as described previously [9] with a few modifications. The Mg concentration in the homogenization medium was raised from 0.5 mM to 1.0 mM, because at the lower Mg concentration the nuclei showed a tendency to swell. In the later stages of the isolation procedure the Mg concentration of 0.5 mM could be maintained. The purification of the isolated nuclei was slightly modified. The discontinuous sucrose gradient consisted of the following layers from bottom to top: 2.0 ml 2.0 M, 1.0 ml 1.8 M and 1.0 ml 1.5 M sucrose, all in "PVP medium": 8 % polyvinylpyrrolidone (mol. wt 40 000)–0.5 mM $MgCl_2$-0.02 M potassium phosphate, pH 6.5. One ml of a suspension of crude nuclei in a 0.6 M solution of sucrose in PVP medium was layered on top of this gradient.

The gradient was centrifuged in a Spinco SW 39 rotor at 39 000 rpm for 90 min at 2°C. The chemical composition of the crude and purified nuclear preparations was the same as described previously [9, 10].

Preparation of RNA

RNA was extracted essentially as described by Parish & Kirby [11]. Unless mentioned otherwise all operations were carried out at 4°C. To extract RNA from spheroplasts and isolated nuclei, the pellets were suspended in 0.01 M sodium acetate buffer, pH 5.1, containing 0.02 % polyvinylsulfate. The suspension of nuclei (2×10^9) was treated with 100 μg DNase (Worthington, RNase free) for 12 min at 27°C. To extract cytoplasmic RNA an aliquot of the cytoplasmic fraction remaining after the isolation of nuclei was taken. All suspensions were made 1 % (w/v) in tri-isopropylnaphthalenesulphonate (TIPNS) (Kodak Ltd., Kirkby, Liverpool, T3513), 6 % (w/v) in 4-aminosalicylate (PAS), 6 % (v/v) in 2-butanol, and were then shaken with an equal volume of phenol-*m*-cresol mixture [11], for 10 min at 20°C. The phases were separated by centrifugation. The phenolphase and interphase were reextracted together with an equal volume of a solution containing 1 % TIPNS, 6 % PAS and 6 % 2-butanol. The combined waterphases were made 3 % with respect to NaCl and the phenol extraction was repeated as described above. RNA was then precipitated by the addition of 2.5 vol 96 % ethanol containing 2 % potassium acetate. After storage overnight at −20°C, the precipitated RNA was washed twice with 75 % ethanol containing 0.1 M NaCl [12]. The RNA from spheroplasts and cytoplasmic fractions was treated with LiCl according to Schweizer et al. [13] in order to remove tRNA, which was highly labelled.

RNA from spheroplasts and nuclei was prepared for gel electrophoresis according to Loening [14]. Cytoplasmic RNA was prepared for sucrose gradient centrifugation as described previously [3].

Electrophoresis

Acrylamide gel electrophoresis was performed according to Loening [15] and Bishop et al. [16]. The polymerizing mixture was made 2.4 % (w/v) in acrylamide and 0.12 % (v/v) in ethylene diacrylate [17]. The electrophoresis buffer (E buffer) contained 0.04 M Tris, 0.02 M sodium acetate and 0.001 M EDTA, adjusted to pH 7.4 with glacial acetic acid [16]. The gels (0.8 cm diameter) were treated with E buffer containing 0.2 % recrystallized sodium dodecylsulfate according to Bishop et al. [16]. The RNA sample contained 10–20 μg RNA dissolved in 10–20 μl 1/4 E buffer 5 % sucrose. Electrophoresis was performed in a Canalco apparatus at 20–25°C. Further conditions are given in the captions to the figures. After electrophoresis the gels were scanned at 266 nm in Zeiss spectrophotometer (slit 0.1 × 0.4 cm), that was adapted for this purpose [18] and combined with a Beckman recorder. The gel was then frozen on dry ice and was cut into 0.1 cm slices. These were solubilized in 0.5 ml 1 N NH_4OH in a scintillation vial for 2 h at 20–25°C [17]. For radioactivity measurements 14.5 ml of a 6:23 (v/v) mixture of Triton X-100 and a toluene solution of PPO and POPOP [19] was added. This mixture is homogeneous at 3°C and has a counting efficiency for ^3H of 33 % and for ^{14}C of 46 %. Radioactivity was measured in a Mark I liquid scintillation counter (Nuclear-Chicago).

The denomination of the different RNA species by S values has been maintained in acrylamide gel electrophoresis. The relationship between S values and migration distance of RNA on acrylamide gels, as given by Lewicki & Sinskey [20], has been applied to our gels. The two species of rRNA, i.e. 26S and 18S, have been used as internal standards (see also next section). The S values of the nuclear ribosomal precursors, found by gel electrophoresis, were the same as those found by sucrose gradient analysis [3] i.e. 37S and 28S.

Sucrose density gradient centrifugation

The procedure has been described previously [3]. One modification was introduced. After centrifugation the gradient was displaced from below and the extinction was continuously monitored at 260 nm in a Zeiss spectrophotometer combined with a Beckman recorder. Fractions of 0.65 ml were collected in liquid scintillation vials. Radioactivity was measured as described above.

The sedimentation coefficients of RNA for use as internal standards in sucrose gradient and electrophoretic analyses, were determined in a Spinco model E analytical ultracentrifuge equipped with an ultraviolet optical system with photo-electric scanner. The analyses were carried out using a double sector cell at 44 770 rpm in an AN-D rotor. Concentrations of RNA used for sedimentation were within the range of 35–40 μg/ml in a 0.01 M sodium acetate

buffer, pH 5.1, containing 0.1 M NaCl and 0.001 % polyvinylsulfate. The sedimentation coefficients were corrected in the usual way to obtain $S_{20,w}$ values.

Determination of the specific activity of the extracted RNA

The procedure for the determination of the specific activity was as described previously [3] with one modification: the radioactivity measurements were carried out as described above.

RESULTS

Electrophoretic analysis of nuclear and cytoplasmic RNA

The results of an electrophoretic analysis on acrylamide gels of the RNA from the cytoplasmic and the purified nuclear fractions are depicted in fig. 1. The optical density (OD) profile of the cytoplasmic RNA (fig. 1*B*) shows the two normal classes of rRNA as two narrow peaks fully separated from each other. The ratio of total OD of 26S RNA to that of 18S RNA is 2.0. The OD profile of the nuclear RNA shows, in better resolution, the same RNA components as found in sucrose gradient analysis [3]: a 37S, a 26S and an 18S peak. It has been shown previously [3] that the mature rRNA that is present in the nuclear preparations originates mainly from perinuclear ribosomes. It is clear, however, from fig. 1*A*, that the 26S peak is disproportionately large compared with the 18S peak. The ratio of total OD of 26S RNA to that of 18S RNA in fig. 1*A* is 3.8. This indicates that about 50 % of the OD in the 26S region does not originate in the perinuclear ribosomes. Previously we have shown [3] that there is a relatively large pool of nuclear 28S RNA. It is reasonable to assume that in our nuclear preparations this 28S RNA species accounts for most of the RNA in the '26S' peak that does not originate in perinuclear ribosomes. Attempts to resolve this composite '26S' peak into more components by extending the time of electrophoresis were unsuccessful.

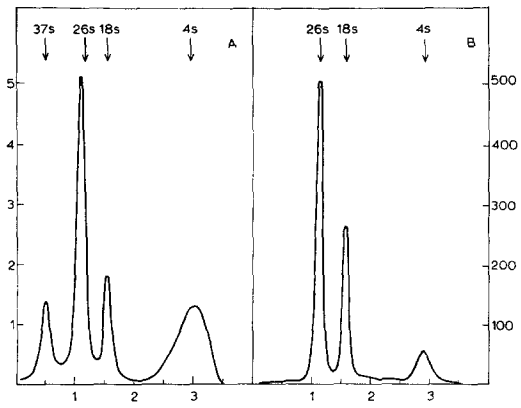

Fig. 1. Abscissa: cm travelled; *ordinate:* A_{266}.
Acrylamide gel electrophoretic analysis of nuclear RNA (*A*) and cytoplasmic RNA (*B*). Electrophoresis was for 90 min at 5 mA per gel. The OD in the 4S region (*A*) is due to degraded DNA. The OD measured in the analysis of the nuclear and cytoplasmic RNA are converted to values such that both diagrams (*A*, *B*) represent the same number of spheroplasts (1.4×10^9).

Ribosomal precursor RNAs in yeast

In order to obtain a complete picture of the ribosomal precursors in yeast an experiment was designed in which the RNA was isolated directly from the spheroplasts, thus avoiding degradation that might occur during cell fractionation. After short periods of incorporation of ^{3}H-Me-methionine, RNA was extracted as described in Materials and Methods. tRNA was removed from the isolated RNA by means of a LiCl fractionation. The specific radioactivity of the resulting RNA preparations was at least 1 000 dpm/μg, which is sufficient to permit analysis of the labelled RNA by means of acrylamide gel electrophoresis.

Within the time course of the experiments the total radioactivity in the RNA increased linearly with an initial delay of $\frac{1}{2}$–1 min, suggesting that the equilibration of the methyl donor pool is very fast. The radioactivity profile of a 5 min pulse (fig. 2*A*) shows a prominent 37S peak and a 28S peak with a shoulder in the 26–28S region. A small

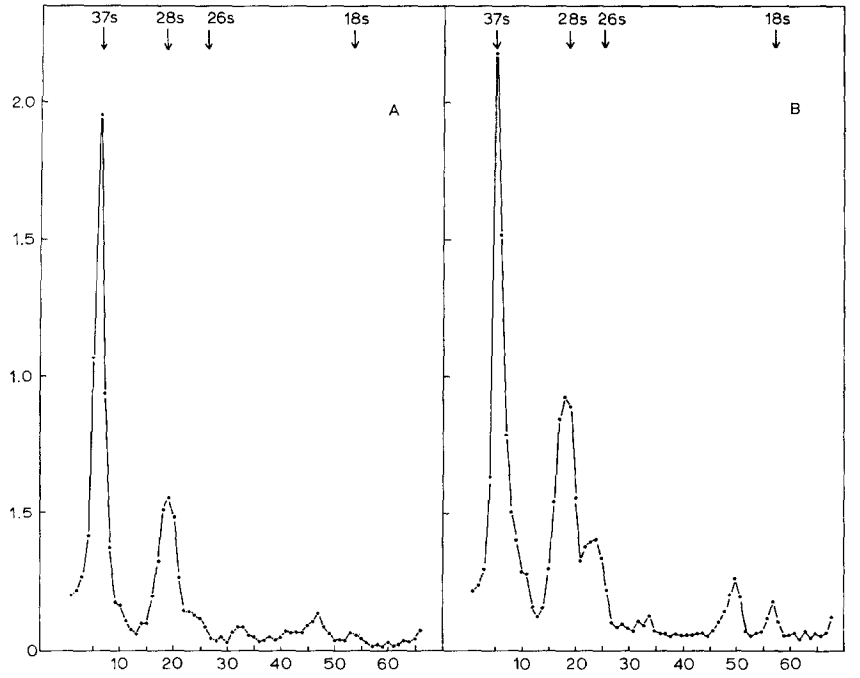

Fig. 2. Abscissa: slice number; *ordinate* dpm × 10^{-3}.
Acrylamide gel electrophoretic analysis of spheroplast RNA after a 5 min pulse (*A*) and a 10 min pulse (*B*) with ^{3}H-Me-methionine (final conc. 40 μCi/ml). Electrophoresis was for 6 h at 5 mA per gel. One μg of spheroplast RNA, labelled with ^{14}C-uracil to a spec. act. of 10 000 dpm/μg, was added as a marker to determine the position of 26S and 18S RNA in the gels. The radioactivities measured in the analysis of the spheroplast RNA are converted to such values that both diagrams (*A*, *B*) represent the same number of spheroplasts (0.7×10^{7}). Due to a new batch of ethylene diacrylate, the concentration of ethylene diacrylate in this experiment had to be increased to 0.22 % (v/v) to ensure sufficient rigidity of the gels.

peak of 18S RNA can also be observed. In between the 26S and the 18S RNA a species of methylated RNA migrates to which an approximate S value of 20S can be assigned.

Extension of the labelling period to 10 min results in an increase in the radioactivity of the 18S, 20S, 26S and 28S RNA species. The radioactivity in the 37S peak does not increase appreciably, however. The 20S RNA could be an intermediate in the formation of 18S RNA. Such an intermediate in the processing of rRNA has been described in HeLa cells [8]. There may be still another intermediate RNA component in the 33S region that presents itself as a small shoulder.

It is clear from fig. 2 that the main rRNA precursors in yeast are 37S and 28S RNA molecules. This outcome is in complete agreement with the results of the sucrose gradient analysis of RNA from isolated nuclei [3].

A kinetic study of the methylation of ribosomal RNA

In order to study the kinetics of methylation of nuclear and cytoplasmic RNA, spheroplasts were labelled with ^{3}H-Me-methionine. Short pulses were chosen as it is clear from our data presented in the preceding section that radioactivity appears very early in 26S and 18S rRNA. The results are given in the following.

Labelling kinetics of ribosomal RNA in the nucleus

The nuclear RNA has been analysed on acrylamide gels. The results of short periods

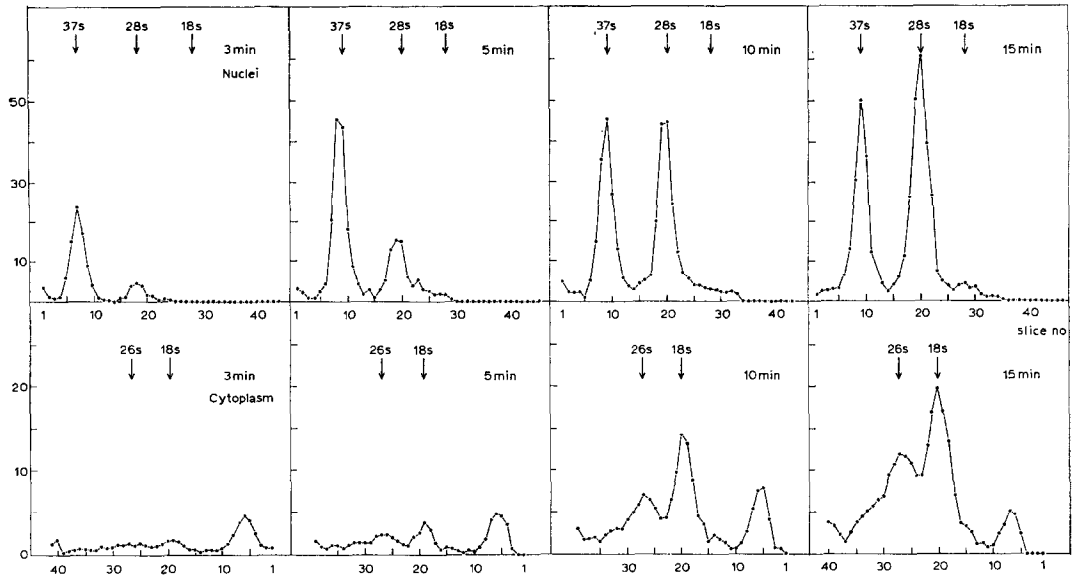

Fig. 3. Abscissa: (top) slice number; *(bottom)* fraction number; *ordinate:* dpm $\times 10^{-3}$.
Acrylamide gel electrophoretic analysis of nuclear RNA *(top)* and sucrose gradient analysis of cytoplasmic RNA *(bottom)* after labelling for various times with ^{3}H-Me-methionine (final conc. 7.5 μCi/ml). Electrophoresis was for 3 h at 5 mA per gel. Centrifugation was for 22 h at 20 000 rpm at 2°C. The radioactivities measured in the analysis of nuclear and cytoplasmic RNA are converted to such values that all diagrams represent the same number of spheroplasts (1.4×10^{9}). The S-values were deduced from the OD profiles (not shown).

of incorporation of the labelled methyl-moiety into nuclear RNA are shown in fig. 3. After a 3 min pulse a prominent 37S peak has appeared. The 37S RNA is apparently the first methyl-labelled species in the nucleus. Some radioactivity is associated with the 28S RNA. After 5 min of incorporation radioactivity has increased in both components. From 5 min on the labelling of the 37S RNA precursor does not increase appreciably, while there is still a progressive accumulation of radioactivity in the 28S RNA. The sequential labelling of 37S and 28S RNA is clearly shown in fig. 4. No appreciable radioactivity is observed in the 18S region in any of the radioactivity profiles of nuclear RNA presented in fig. 3. This indicates that, at least within the time of measurement, no newly formed 18S rRNA is associated with the nucleus.

Labelling kinetics of ribosomal RNA in the cytoplasm

The cytoplasmic RNA has been analysed on sucrose gradients. The low specific activity of the cytoplasmic RNA, after the short pulses applied, makes an analysis on acrylamide gels impossible. The results of the incorporation of the labelled methyl-moiety into cytoplasmic rRNA is shown in fig. 3. After a 3 min pulse the sedimentation profile shows some remnants of labelled tRNA, not removed by the LiCl fractionation. Some radioactivity is already associated with the 18S region of the gradient. There is virtually no radioactivity associated with the 26 S region of the gradient. From 5 min on radioactivity accumulates in both the 18S and the 26S region of the sedimentation profiles. It is clear that during the time of measurement there is more radioactivity associated with

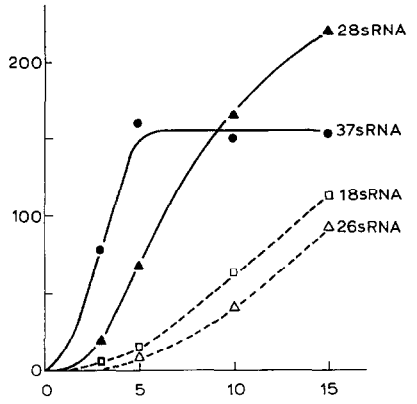

Fig. 4. Abscissa: min; *ordinate:* dpm × 10^{-3}.
Kinetics of labelling of nuclear and cytoplasmic rRNA with ^{3}H-Me-methionine. The total radioactivity of the nuclear 37S and 28S as determined on the electrophoretic profiles (fig. 3) and of the cytoplasmic 26S and 18S RNA as determined on the sedimentation profiles (fig. 3) is plotted against incorporation time.

the 18S rRNA than with the 26S rRNA. The accumulation of radioactivity in both rRNA species in the cytoplasm is depicted in fig. 4.

This figure clearly shows that after short pulses with methyl-labelled methionine more radioactivity is associated with 18S rRNA than with 26S rRNA.

DISCUSSION

Several authors [21; 22 for review] have shown that in mammalian nuclei the initial event in the synthesis of ribosomal RNA is the formation of a large 45S RNA molecule. This molecule is present within the nucleus in fairly large amounts. Subsequent work has shown that this 45S rRNA precursor is located in the nucleolus and forms an important RNA constituent of this organelle [8, 23].

The optical density profile of nuclear RNA from yeast, analysed on acrylamide gel (fig. 1*A*), shows the presence of several RNA components. The component with the greatest molecular weight is the 37S RNA species. It is present in relatively large amount. Kinetic studies on the labelling of nuclear RNA with ^{14}C-uracil led us to the view that the initial event in the biosynthesis of ribosomal RNA is the formation of a 37S precursor molecule [3]. Since only rRNA and its precursors, and tRNA are methylated [2–7], labelling experiments with ^{3}H-methyl-methionine enabled us to study this question more accurately. Such experiments (figs 2, 3) show that the 37S RNA is indeed the earliest labelled species of RNA. The kinetics of incorporation, more extensively shown in fig. 4, clearly indicate a precursor-product relationship between 37S and 28S RNA. There exists a relatively large nuclear pool of 28S RNA, while no newly formed 18S RNA can be found in the nucleus (fig. 3). The relatively short times of labelling enabled us to observe the appearance of radioactivity in the two cytoplasmic rRNAs. The results presented in figs 3 and 4 show that more radioactivity was associated with the 18S rRNA than with the 26S rRNA.

From their studies on the in vivo methylation of rRNA in yeast Retèl et al. [7] concluded that the degree of methylation is very similar for 18S and for 26S rRNA, being about 1 methyl group per 70 nucleotides for both rRNAs. These authors have also shown that in addition to the ribose methylation, which takes place at the level of the first ribosomal precursor, an additional methylation occurs at later stages of the processing to mature rRNA. This additional methylation, which comprises about 20% of the total methylation, is predominantly base methylation. It may thus be expected that after very short periods of labelling an important part of the methyl label in cytoplasmic rRNA represents base methylation. Taking into account that the degree of methylation is similar for 18S and 26S

rRNA, our results (figs 3, 4) can be interpreted as showing that the methyl-labelled 18S rRNA enters the cytoplasm earlier than the methyl-labelled 26S rRNA. These data supplement and support our previous observations on the incorporation of ^{14}C-uracil [3] from which we tentatively concluded that the newly formed 26S rRNA enters the cytoplasm later than the newly formed 18S rRNA. The accumulation of label in nuclear 28S RNA and the late appearance of label in the cytoplasmic 26S rRNA strongly suggest that these RNA species are related. It is reasonable to assume that the nuclear 28S RNA is a precursor of cytoplasmic 26S rRNA.

It is worthwhile mentioning our observation of a rapidly labelled RNA component in the 20S region (fig. 2). This could be an intermediate in the formation of 18S rRNA. The presence of such a '20S' intermediate has been demonstrated in HeLa cells [8], in the salivary glands of the insect *Chironomus tentans* [24] in pea root and artichoke [25] and in the cellular slime mold *Dictyostelium discoideum* [26]. We do not observe the '20S' component in any of our nuclear preparations, however (see fig. 3). Due to the poor resolving power of sucrose gradient analysis it is impossible to distinguish 20S RNA from 18S RNA in preparations of cytoplasmic RNA.

Leaver & Key [27] and Rogers et al. [25] have shown that there exists a common precursor of the two mature rRNAs in plants. This precursor molecule is, just as in yeast, smaller than the corresponding precursor in mammalian cells. The authors [25, 27] suggest that this precursor or a smaller common intermediate is cleaved into the direct precursor molecules of the two mature rRNAs. The data of fig. 2 suggest that there exists such a smaller common intermediate ('33S') molecule in yeast. The '33S' and '20S' RNA species (fig. 2), which may be intermediate in the formation of rRNA, are, however, quantitatively minor components. From the optical density and radioactivity patterns of nuclear RNA presented in the present paper and in a previous one [3], we can conclude that the '37S' and '28S' species of rRNA are the ones that are most abundant in the nucleus. This is in good agreement with the data of others who describe the presence of at least two rRNA precursors in yeast, namely '40S' and '30S' [7], or '38S' and '30S' [2]. The existence of a '32S' intermediate in the formation of rRNA from a '37S' precursor in yeast has been demonstrated in a recent publication of Retèl & Planta [28]. Their data also indicate the presence of a ribosomal precursor of still higher molecular weight, designated '42S', than we have found in our nuclear preparation.

Our data, together with the results of Taber & Vincent [2] and Retèl [1, 7, 28] give a good picture of the synthesis and the processing of ribosomal RNA in yeast. Two major species of rRNA precursor molecules, 37S and 28S, are located exclusively in the nucleus. The 37S RNA is a common precursor of both 28S and 18S RNA. While the 18S RNA rapidly leaves the nucleus, the 28S RNA accumulates inside the nucleus and only later becomes converted into 26S rRNA, which then enters the cytoplasm. An earlier entrance of 18S rRNA in the cytoplasm is also observed in HeLa cells [21], in rat liver [29], in the protozoan *Tetrahymena pyriformis* [30] and in plants [25, 27, 31]. A question which is still unanswered concerns the site of synthesis of the ribosomal RNA precursors within the yeast nucleus. Inside the nucleus a structure called 'dense crescent' can be visualized which resembles the nucleolonema of the mammalian nucleolus in morphology and chemical nature [10]. The nucleolus is the site of synthesis of ribosomal

RNA [22]. It is therefore tempting to believe that the dense crescent of the yeast nucleus is involved likewise in the synthesis of rRNA in yeast. Experiments, directed to answer this question, will be presented elsewhere [32, 33].

The present investigations were 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.).

REFERENCES

1. Retèl, J & Planta, R J, Eur j biochem 3 (1967) 248.
2. Taber, R L & Vincent, W S, Biochim biophys acta 186 (1969) 317.
3. Sillevis Smitt, W W, Nanni, G, Rozijn, T H & Tonino, G J M, Exptl cell res 59 (1970) 440.
4. Moore, P B, J mol biol 18 (1966) 38.
5. Wagner, E, Penman, S & Ingram, U M, J mol biol 29 (1967) 371.
6. Isaksson, L A & Phillips, J H, Biochim biophys acta 155 (1968) 63.
7. Retèl, J, Bos, R C van den & Planta, R J, Biochim biophys acta 195 (1969) 370.
8. Weinberg, R A, Loening, U, Willems, M & Penman, S, Proc natl acad sci US 58 (1967) 1088.
9. Rozijn, T H & Tonino, G J M, Biochim biophys acta 91 (1964) 105.
10. Molenaar, I, Sillevis Smitt, W W, Rozijn, T H & Tonino, G J M , Exptl cell res 60 (1970) 148.
11. Parish, J H & Kirby, K S, Biochim biophys acta 129 (1966) 554.
12. Loening, U E, Jones, K W & Birnstiel, M L, J mol biol 45 (1969) 353.
13. Schweizer, E, MacKechnie, C & Halvorson, H O J mol biol 40 (1969) 261.
14. Loening, U E, Biochem j 113 (1969) 131.
15. — Ibid 102 (1967) 251.
16. Bishop, D H L, Claybrook J R & Spiegelman, S, J mol biol 26 (1967) 373.
17. Choules, G L & Zimm, B H, Anal biochem 13 (1965) 236.
18. Koch, P, Zeiss Mitt 4 (1968) 397.
19. Laarse, J D van der, Int j appl rad isot 18 (1967) 485.
20. Lewicki, P P & Sinskey, A J, Anal biochem 33 (1970) 273.
21. Penman, S, J mol biol 17 (1966) 117.
22. Attardi, G & Amaldi, P, Ann rev biochem 39 (1970) 183.
23. Muramatsu, M, Hodnett, J L, Steele, W J & Busch, H, Biochim biophys acta 123 (1966) 116.
24. Ringberg, U, Daneholt, B, Edström, J E, Egyházi, E & Lambert, B, J mol biol 51 (1970) 327.
25. Rogers, M E, Loening, U E & Fraser, R S S, J mol biol 49 (1970) 681.
26. Iwabuchi, M, Mizukami, Y & Sameshima, M, Biochim biophys acta 228 (1971) 693.
27. Leaver, C J & Key, J L, J mol biol 49 (1970) 671.
28. Retèl, J & Planta, R J, Biochim biophys acta 224 (1970) 458.
29. Willèn, R, Hoppe-Seyler's Z physiol Chem 351 (1970) 1141.
30. Kumar, A, J cell biol 45 (1970) 623.
31. Tanifuji, S, Higo, M, Shimada, T & Higo, S, Biochim biophys acta 217 (1970) 418.
32. Sillevis Smitt, W W, Vermeulen, C A, Vlak, J M, Rozijn, T H & Molenaar, I, Exptl cell res 70 (1972) 140.
33. Sillevis Smitt, W W, Vlak, J M, Molenaar, I & Rozijn, T H. Submitted for publication.

Received July 20, 1971
Revised version received October 1, 1971