The primary transcript of the ribosomal repeating unit in yeast

Jacobus Klootwijk, Petrus de Jonge and Rudi J.Planta

Biochemistry Laboratory, Vrije Universiteit, De Boelelaan 1085, Amsterdam-Buitenveldert, Netherlands

Received 22 November 1978

ABSTRACT

Endgroup analysis of 37S ribosomal precursor RNA from Saccharomyces carlsbergensis has revealed that the major 5' endgroup is pppA-Up, with a molar yield of 0.8. This shows that most, if not all, 37S RNA molecules have preserved a transcriptional initiation sequence. Analysis of the 3' terminus of 37S RNA has shown the presence of a uridine rich oligonucleotide, tentatively identified as Ur-G-A-NqH. This long stretch of uridines at the 3' end of 37S RNA may represent a transcriptional termination site. The two sets of data on the terminal sequences suggest that 37S ribosomal precursor RNA, if not already spliced, is a primary transcription product.

Since the 3' terminus of 26S rRNA, U-U-U-G-UqH, appears to be clearly different from the 3' end of 37S RNA, we conclude that 37S ribosomal precursor RNA contains additional nucleotides 3'-distal to the 26S rRNA sequence.

INTRODUCTION

Both in bacteria and eukaryotic cells ribosomal RNA (rRNA) components are synthesized as part of a common transcript, containing also non-ribosomal sequences. This so-called ribosomal precursor RNA (pre-rRNA) is processed in a number of steps into the mature rRNAs. Since processing takes place immediately after or even during transcription it is often difficult to say whether the largest pre-rRNA, which can be detected in pulse labelling experiments, is indeed the primary transcript of the ribosomal repeating unit.

Recently Batts-Young and Lodish (1) detected pppAp at the 5' end of the immediate precursor to 17S rRNA from Dictyostelium and this observation indicates that 37S pre-rRNA, from which the precursor to 17S rRNA originates, has preserved an initiation sequence at its 5' end. In addition, Reeder et al. (2) recently described an indirect method to detect 5' termini bearing a di- or triphosphate, using vaccinia capping enzymes. This assay clearly showed that such polyphosphate-containing ends
are present in 40S pre-rRNA of Xenopus laevis. These recent data suggest that in these two cases the largest and first-labelled pre-rRNA is likely to be a primary transcript.

For yeast, Retel and Planta (3) have described a 3.1x10^6 dalton molecule, the so-called 42S RNA, as the initial ribosomal RNA precursor; this RNA species was observed only after methionine deprivation of a methionine-requiring mutant of S. carlsbergensis. In wild type cells of S. carlsbergensis, however, the same group found a 37S RNA with a molecular weight of about 2.8x10^6 dalton as the first detectable pre-rRNA (4, 5), and an initial pre-rRNA of similar size was reported for S. cerevisiae (6). These data led Planta et al. (7) to the assumption that the 42S RNA is the primary transcript of the ribosomal repeating unit in yeast, which accumulates after a period of methionine starvation but is converted very rapidly into 37S pre-rRNA under normal conditions.

This hypothesis can be verified in principle by endgroup analysis to establish whether transcriptional initiation and termination sequences are present or not at the termini of both RNAs. As 37S RNA, in contrast with 42S RNA, can be labelled sufficiently high now, we decided to perform this type of analysis for 37S pre-rRNA.

In enzymatic digests of 37S RNA we detected pppA-Up as the major 5' endgroup with a molar yield of 0.8. Qualitative analysis of the 3' end revealed a uridine rich nucleotide sequence, which may represent a transcriptional termination site. We therefore conclude that 37S RNA is most likely the primary transcript of the ribosomal repeating unit in yeast under normal conditions.

Whether 42S RNA represents either an undermethylated conformomer of 37S pre-rRNA or an aberrant ribosomal transcription product remains to be established.

MATERIALS AND METHODS

Isolation of labelled RNAs

Cells of Saccharomyces carlsbergensis,NCYC S74, were grown and converted into protoplasts as described by Retel and Planta (8). Protoplasts were suspended in a phosphate-free medium (8) containing 120 g/l of mannitol and incubated for 30 min at 29°C to permit recovery from the hemicellulase treatment. 10 min before label was added a shift-up situation was created (9) and pulse labelling for 15-25 min was then performed after cooling the suspension to 15°C (10) and adding (32p)orthophosphate (The Radiochemical Centre, Amersham, UK) to a final concentration
of 0.25 mCi/ml. The protoplast suspension was then poured into a two-fold volume of ice-cold citrate buffer (10 mM sodium citrate pH 7.5 containing 5 mM MgCl₂ and 120 g/l of mannitol), followed by centrifugation during 5 min at 4,000 x g at 2°C. A nuclear fraction was prepared (10) and RNA was extracted from the nuclei with phenol-sodium dodecyl sulphate at 35°C (8). 37S RNA was purified by two cycles of sucrose gradient centrifugation (11) and its purity was checked by electrophoresis on 2.6% polyacrylamide gels (10).

³²P-labelled 26S plus 5.8S rRNA was prepared from yeast ribosomes as described earlier (11).

Digestion of the RNA preparations and fractionation of the digests

For the analysis of 5'-terminal structures RNA was digested with T₁ plus pancreas RNase at an enzyme to substrate ratio of 1:10 (w/w) each, in 0.01 M Tris-HCl, pH 7.5, containing 1 mM EDTA, for 45 min at 37°C. The digest was then fractionated by two-dimensional high-voltage electrophoresis (12). The molar yield of spot X (cf. Fig. 1) was calculated after determination of the radioactivity per phosphate group averaged over a number of oligonucleotides of known sequence and stoechiometry. The yields of those oligonucleotides present in both 37S RNA and 29S RNA were corrected for the contamination with 29S RNA (up to 20%).

Analysis of 3' termini was performed by column chromatography of enzymatic digests on DBAE-cellulose as described (13,14). To this end the RNA's were digested with T₁ RNase at an enzyme to substrate ratio of 1:20 (w/w) during 30 min at 37°C. Modification by kethoxal and subsequent digestion with U₂ RNase was performed as follows: RNA was dissolved (100 μg/240 μl) in 0.42 M kethoxal (2-keto-3-ethoxy-n-butyraldehyde, Nutritional Biochem. Corp.), 0.1 M sodium acetate, 0.1 M EDTA (pH 5.0) and heated in a sealed tube for 5 min at 75°C, followed by an incubation for 1 h at 37°C. These conditions allow modification of at least 80% of all guanines. After two cycles of ethanol precipitation RNA was digested with 0.5 unit of U₂ RNase (Calbiochem) in 40 μl of 0.1 M sodium acetate, 1.5 mM EDTA (pH 4.5) for 2.5 h at 37°C. Removal of the substituted kethoxal groups was performed by adding 150 μl of a solution containing 0.125 M MgCl₂, 1.25 M NaCl and 10 ml morpholine per liter and incubating the mixture for 45 min at 37°C. After addition of 50 μl dimethylsulfoxide and 300 μl of solution A (13) the sample was applied to the column. The material of the so-called 'peak C' in the eluate (14) was pooled, dialyzed against distilled water, lyophilized and fractionated according to Brownlee and Sanger (15).
Nucleotide sequence analysis of oligonucleotides

Oligonucleotides obtained after electrophoresis on DEAE-cellulose paper were digested using standard procedures with T$_2$ RNase (25 mU in 10 µl of 0.1 M ammonium acetate, pH 4.5, during 2 h at 37°C), endonuclease P$_1$ (0.1 µg in 10 µl of 0.05 M ammonium acetate, pH 5.3, containing 0.1 mM ZnSO$_4$ for 2 h at 37°C) or 0.5 µl KOH (16 h, 37°C). The products were identified after paper electrophoresis in 5% (v/v) acetic acid, pH 3.5, either on DEAE-cellulose or on Whatman 3 MM. Oligonucleotides recovered from DEAE-cellulose thin-layer plates were digested either with 70 units of T$_2$ RNase, 2 µg of pancreas RNase or with 0.025 units of U$_2$ RNase per 200 µg of RNA for 7 h at 37°C in the appropriate buffers (16).

RESULTS

Analysis of the 5' terminus of 37S RNA

We applied the pancreatic plus T$_1$ RNase 'fingerprint' procedure which easily separates most oligonucleotides with 5'-[(poly)phosphate ends from oligonucleotides derived from internal positions. Fig. 1 shows such a 'fingerprint' of yeast 37S pre-rRNA. The pattern comprises three series of standard products, (Ap)$_n$ Up, (Ap)$_n$ Gp and (Ap)$_n$ Cp (n being 0,1,2,...), in addition to an array of non-standard products. Most of these non-standard products comprise 2'-0 methylated oligonucleotides, already present in 37S RNA within the sequences of mature rRNA (17,18).

Inspection of Fig. 1 reveals one significant non-standard product, spot X, which is definitely lacking in the corresponding 'fingerprints' of mature 26S and 17S rRNA (17), and therefore, most likely, belongs to the non-conserved sequences in 37S pre-rRNA. Its position in the 'fingerprint' indicates a highly negative charge, suggesting a high phosphate content. Digestion of the oligonucleotide material of spot X by T$_2$ RNase and subsequent electrophoresis on Whatman 3MM at pH 3.5 reveals 2 products, $X_1$ and $X_2$ (cf. Fig. 2A). $X_1$ coelectrophoreses with uridine-2',3'-monophosphate, whereas $X_2$ migrates rather fast and streaky. This latter behaviour is supposed to be characteristic for a polyphosphorylated nucleotide (16). All $^{32}$P-label present within $X_1$ and $X_2$ can be converted into $^{32}$P$_1$ by treatment with alkaline phosphatase (cf. Fig. 2B). This shows that no internucleotide phosphate is present either in $X_2$ or in $X_1$ and therefore both $X_1$ and $X_2$ are single nucleotides. $X_2$ is most likely an adenosine derivative since T$_2$ RNase apparently has cleaved a phosphodiester bond which was not cleaved by T$_1$ plus pancreatic RNase. Its electrophoretic
Fig. 1. Fractionation of a pancreatic plus T$_1$ RNase digest of yeast 37S pre-rRNA.

123 µg RNA containing $12.6 \times 10^6$ cpm $^{32}$P 37S RNA was digested to completion with pancreatic plus T$_1$ RNase and subjected to two-dimensional electrophoresis (a) on Cellogel (Chemotron, Italy) from right to left and (b) on DEAE-cellulose paper (downwards).

mobility (see Fig. 2A) as well as its relative amount (in terms of radioactivity in comparison with X$_1$, viz. 3.5:1) suggest that product X$_2$ is identical with (p)ppAp (16), and therefore the sequence (p)ppA-Up can be proposed for product X. From the data of Jordan et al. (19) it can be calculated that ppA-Up and pppA-Up have an electrophoretic mobility on DEAE-paper in 7% formic acid of 0.23 and 0.10, respectively (relative to the blue marker dye). As product X appears to have an electrophoretic mobility of 0.10 in this system (cf. the second dimension in Fig. 1) its sequence is most likely pppA-Up.

To confirm the assignment of pppA-Up to product X we applied digestion with endonuclease P$_1$. We expect as digestion products ppA, pU and P and indeed all three products are found after electrophoresis on DEAE-paper at pH 3.5 (cf. Fig. 2C), on which ($\gamma^{32}$P)labelled ppA was
Fig. 2. Analysis of the oligonucleotide corresponding to spot X in Fig. 1. (A) Material from spot X was digested to completion by T₂ RNase and the digest was fractionated by electrophoresis on Whatman 3MM at pH 3.5. Spot X₁ and X₂ contained 70 cpm and 248 cpm, respectively. (B) Spot X₁ and X₂ were recovered from the paper and incubated with alkaline phosphatase and reelectrophoresed under the same conditions as in (A). (C) Digests of the material from spot X obtained by incubation with endonuclease P₁ and T₂ RNase, respectively, were fractionated by electrophoresis on DEAE-cellulose at pH 3.5; (γ³²P)-labelled ATP (The Radiochemical Centre) was coelectrophoresed as a marker.

coelectrophoresed as a marker. A T₂ RNase digest of product X was coelectrophoresed as well and the spot close to the origin has an electrophoretic mobility, which may be expected for pppAp (1). This set of data shows that product X has the sequence pppA-Up.

Next we calculated the molar yield of spot X relative to a set of oligonucleotides of known sequence and molar yield (17). We obtained an apparent molar yield of 0.8 in two independent 'fingerprints' of 37S RNA preparations, which had been pulse-labelled for 25 min. This value of 0.8 may be considered as the true molar yield of product X since the specific activities of the phosphates in the respective positions are close to equilibration after this period of labelling; this latter conclusion has been drawn from nucleotide sequence analysis of oligonucleotides in 'fingerprints' of these 37S RNA preparations (18,20) and from the ratio of

The data presented so far show that pppA-Up is the major 5' terminus of 37S pre-rRNA. A few minor spots have been observed close to spot X which could be minor 5' termini but their low yield have precluded further analysis so far. In addition some pUp is always present in the T₁ plus pancreatic RNase 'fingerprints' of 37S RNA. This is predominantly caused by contamination with 29S pre-rRNA which has pUp as the major 5' endgroup (20).

Analysis of the 3' end of 37S RNA

A successful identification of a 3'-terminal oligonucleotide from a 32P-labelled RNA digest depends both on the choice of the correct ribonuclease and on the method for its selective isolation. Only after digestion of 37S pre-rRNA with T₁ RNase and subsequent chromatography of the digest over DBAE-cellulose columns we were able to locate and identify a 3'-terminal fragment. Fig. 3 shows a 'homochromatogram' of the oligonucleotide material in the so-called 'peak C' of the eluate, which is supposed to contain the 3'-terminal oligonucleotides (cf. Fig. 1 of ref. 14 for a typical elution profile of a DBAE-column). The autoradiogram reveals one intense spot, indicated by the arrow, superimposed on a background of spots. This background can also be observed in the pattern obtained for an unfractionated T₁ RNase digest of 37S RNA (20), and, therefore, is most probably caused by contamination of the sample with material from the preceding peak 'B' in the eluate. Nevertheless, all predominant spots of the background were analysed and all appeared to

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Fig. 3. Two-dimensional fractionation of material possibly containing the 3'-terminal oligonucleotide of 37S pre-rRNA. 37S RNA (27x10⁶ cpm 32P) was digested to completion with T₁ RNase and fractionated on a DBAE-cellulose column (13,14). Material of the so-called 'peak C' (see text) was subjected to high-voltage electrophoresis on cellulose acetate at pH 3.5 in 7 M urea (1) and then to homochromatography (2) using a homomix C hydrolyzed for 15 min (15). The dark spot marked by the arrow has been further analyzed as described in Table 1.
contain a 3'-terminal Gp and are, therefore, no 3' termini.

The results of the sequence analysis of the indicated intense spot of Fig. 3 are summarized in Table 1. Pancreatic RNase produced mainly uridine residues whereas after U₂ RNase digestion only one oligonucleotide was observed terminating in an adenosine residue. This adenosine residue must therefore be present in the other pancreatic RNase digestion product, tentatively identified as A-N₉ OH (cf. Table 1). After re-electrophoresis of this latter product on Whatman 3 MM paper at pH 3.5 it was found between the positions of Ap and Gp. At this stage of the analysis the radioactivity in this product was too low to perform further identification. The data in Table 1 support the deduction of the sequence U₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈. Although the identity of N₁₀₂₅₃ is unclear as yet, we conclude that this T₁ RNase generated oligonucleotide must have been derived from the 3' end of 37S RNA. This conclusion is based upon the pertinent absence of a guanosine-3'-phosphate in this product and further supported by its relative blackening in Fig. 3. Although we do not know its molar yield, we think that U₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈ represents the predominant 3' end of 37S RNA as no other 3'-terminal product could be found neither in Fig. 3 nor after other digestion procedures, i.e. with pancreatic RNase or U₂ RNase after modification of the RNA by kethoxal (21).

**Analysis of the 3' end of 26S rRNA**

To establish the presence of additional nucleotides in 37S pre-rRNA 3'-distal to the 26S rRNA sequence we also analyzed the 3' end of mature 37S pre-rRNA.

**TABLE 1. SEQUENCE ANALYSIS OF THE OLIGONUCLEOTIDE POSSIBLY CONTAINING THE 3' END OF 37S PRE-rRNA FROM YEAST.** The oligonucleotide material from the spot indicated by the arrow in Fig. 3 was recovered from the thin layer plate and treated with pancreatic or U₂ RNase. The molar ratio of the two pancreatic RNase digestion products was determined from their ratio of radioactivity, taking the underlined product as a reference (2 determinations).

<table>
<thead>
<tr>
<th>Enzyme used for digestion</th>
<th>Products obtained</th>
<th>Molar ratio</th>
<th>Sequence deduced</th>
</tr>
</thead>
<tbody>
<tr>
<td>pancreatic RNase</td>
<td>A-N₉ OH</td>
<td>1.0</td>
<td>U₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈</td>
</tr>
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| U₂ RNase | Y-U₅₋₇₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋₆₋₈₋₁₋
26S rRNA. Shine et al. (22) have reported \((Y)\)-G-LI as the 3' end of 26S rRNA of *S. cerevisiae* using in vitro labelling with \(^{3}H\) isoniazid. We have confirmed this sequence for 26S rRNA of *S. carlsbergensis* using the 'diagonal procedure' of Dahlberg (23) applied to a pancreatic RNase digest of 26S rRNA (unpublished results). To obtain a longer 3'-terminal nucleotide sequence we performed specific cleavage behind adenosine residues after modification of guanosines by kethoxal and subsequent digestion with \(U_2\) RNase (21). Since the 'diagonal procedure' failed to reveal a 3'-terminal product we applied DBAE-column chromatography for the enrichment of 3'-OH containing oligonucleotides. To circumvent partly the disadvantage of the column procedure that no molar yield of the 3' terminal fragments can be calculated we used the homogeneous 3' end of 5.8S rRNA as an internal reference. The material in the so-called peak 'C' (14) was fractionated as described above for the \(T_1\) RNase digest of 37S RNA by electrophoresis and 'homochromatography' (cf. Fig. 4). The pattern reveals two clear spots (1 and 2); the two spots were subjected to further analysis as summarized in Table 2. These results allow us to deduce the sequences U-U-U-G-LI and U-U-U-LH for spot 1 and 2, respectively. As 5.8S rRNA is expected to have the 3'-terminal sequence U-U-U-\(OH\) (24), the product U-U-U-G-\(OH\) must represent the 3' end of 26S rRNA. The relative intensities of spot 1 and 2 strongly suggest that U-U-U-G-\(OH\) is the predominant 3' endgroup of 26S rRNA. This result clearly shows that the 3' termini of 37S pre-rRNA and 26S rRNA are different and therefore the
TABLE 2. SEQUENCE ANALYSIS OF THE 3'-TERMINAL OLIGONUCLEOTIDES PRESENT IN YEAST 26S PLUS 5.8S rRNA. The material from spots 1 and 2 (Fig. 4) was analyzed by digestion with T<sub>1</sub> RNase or pancreatic RNase. The molar ratios were determined using the underlined product as a reference.

<table>
<thead>
<tr>
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<th>Products obtained</th>
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<tbody>
<tr>
<td>1</td>
<td>pancreatic RNase</td>
<td>G-U&lt;sub&gt;a&lt;/sub&gt;&lt;sup&gt;OH&lt;/sup&gt;</td>
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<td>U-U-U-G-U&lt;sub&gt;OH&lt;/sub&gt;</td>
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<td></td>
<td>T&lt;sub&gt;1&lt;/sub&gt; RNase</td>
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<tr>
<td>2</td>
<td>pancreatic RNase</td>
<td>Up</td>
<td>-</td>
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<tr>
<td></td>
<td>T&lt;sub&gt;1&lt;/sub&gt; RNase</td>
<td>U-U-U-G&lt;sub&gt;a,b&lt;/sub&gt;&lt;sup&gt;OH&lt;/sup&gt;</td>
<td>-</td>
<td>U-U-U&lt;sub&gt;OH&lt;/sub&gt;</td>
</tr>
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</table>

a identified by digestion with T<sub>2</sub> RNase and snake venom phosphodiesterase.
b identified by its electrophoretic mobility on DEAE-cellulose paper at pH 3.5 relative to Gp.

determination of 37S RNA into 26S rRNA must involve the removal of sequences present at the 3' terminus of 37S pre-rRNA.

DISCUSSION

The results described in this paper provide strong evidence that yeast 37S pre-rRNA is a primary transcript. The finding of pppA-Up in a close-to-one molar yield shows the preservation of the transcriptional initiation sequence in this RNA species. So far 5'-polyphosphate termini of eukaryotic ribosomal precursor RNA have only been reported by Reeder et al. (2) for the 40S pre-rRNA of Xenopus laevis, using an indirect method, and by Bates-Young and Lodish (1) for the direct precursor to 17S rRNA of Dictyostelium. In all three cases the primary transcript of the ribosomal repeating unit appears to start with pppA-, suggesting that the eukaryotic RNA polymerase A uses an adenosine triphosphate rather than guanosine triphosphate for the initiation of RNA synthesis. There is some evidence that the 5' terminus of the primary ribosomal transcription product is heterogeneous (see Ref. 2 and this paper). Fig. 1 reveals a few weak spots close to product X which could be minor 5' termini but their low yield of radioactivity has precluded further analysis so far. Only pUp can be found as an additional 5' endgroup in our 37S RNA preparations (cf. Fig. 1) with a molar yield up to 0.2. However we cannot
distinguish as yet whether pUp represents a genuine minor 5' endgroup of 37S pre-rRNA or an artefact caused by hidden breaks in the RNA chain.

The finding of $U_g$-$A$-$N_{OH}$ in 37S RNA suggests the preservation of a transcriptional termination sequence. This suggestion is based upon the detection of stretches of 5 to 8 uridines at the 3' termini of a number of bacterial primary transcripts (25). In addition, DNA sequencing data of the genes coding for yeast 5S rRNA (26,27), yeast tRNA$^{pHe}$ (28) and yeast tRNA$^{tryp}$ (28) have revealed significant stretches of deoxyadenosine residues downstream the coding sequences and these stretches have been designated as putative transcription termination sites.

The conclusion that yeast 37S RNA represents a primary transcript presupposes that this RNA is not a "splicing" product of an initial transcript. So far we cannot exclude completely that "splicing" of the primary transcript of the ribosomal repeating unit in yeast is part of the rRNA maturation process. However, the absence of intervening sequences in at least the rRNA regions of the yeast ribosomal repeat seems to be well established (30,33). Furthermore, our observations rather suggest that the first nucleolytic event in the maturation of yeast rRNA is the conversion of 37S pre-rRNA into 29S and 18S pre-rRNA, a step which occurs relatively late after transcription and which is preceded by the bulk of the 2'-O-methylations (18), pseudouridylations (20) and the assembly with a large number of proteins into a 90S preribosomal particle (10).

The nature of the previously found, rapidly labelled 42S RNA (3) is uncertain. This RNA has been observed only by pulse-labelling after methionine starvation of a methionine-requiring mutant (3). In addition, in vitro transcription of total yeast nuclear DNA with purified yeast RNA polymerase A produces ribosomal transcript with molecular weights up to about $3 \times 10^6$ dalton (i.e. equal to the size of a 42S RNA). Furthermore, it was found that RNA polymerase A, before reading into the 17S rRNA sequences, transcribes a stretch of DNA corresponding with a RNA leader sequence of about $1 \times 10^6$ dalton (31,32). Therefore, it remains possible that 42S RNA is an aberrant ribosomal transcription product which arises by initiation of transcription at a position upstream of the initiation site for 37S pre-rRNA. Another possibility is that the 42S RNA is an undermethylated conformomer of 37S pre-rRNA.
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 Pure Research (Z.W.O.). The authors are grateful to Dr. R.C. Brand, Miss I. Klein and Mr. C.P. Sibum for their respective important contributions to parts of this study.

REFERENCES

24 Rubin, G.M. (1973) J.Biol.Chem. 248, 3860-3875