Volume 9 Number 19 1981 Nucleic Acids Research

The nucleotide sequence of the intergenic region between the 5.8S and 26S rRNA genes of the yeast ribosomal RNA operon. Possible implications for the interaction between 5.8S and 26S rRNA and the processing of the primary transcript

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Received 24 July 1981

ABSTRACT

We have determined the nucleotide sequence of part of a cloned yeast ribosomal RNA operon extending from the 5.8S RNA gene downstream into the 5'-terminal region of the 26S RNA gene. We mapped the pertinent processing sites, viz. the 5' end of 26S rRNA and the 3' ends of 5.8S rRNA and its immediate precursor, 7S RNA. At the 3' end of 7S RNA we find the sequence UCGUUU which is very similar to the type I consensus sequence UCAUU$^{A}/_{U}$ present at the 3' ends of 17S, 5.8S and 26S rRNA as well as 18S precursor rRNA in yeast. At the 5' end of the 26S RNA gene we find a sequence of thirteen nucleotides which is homologous to the type II sequence present at the 5' termini of both the 17S and the 5.8S RNA gene. These findings further support the suggestion put forward earlier (G.M. Veldman et al. (1980) Nucl. Acids Res. 8, 2907-2920) that both consensus sequences are involved in the recognition of precursor rRNA by the processing nuclease(s). We discuss a model for the processing of yeast rRNA in which a processing enzyme sequentially recognizes several combinations of a type I and a type II consensus sequence.

We also describe the existence of a significant base complementarity between sequences in the 5'-terminal region of 26S rRNA and the 3'-terminal region of 5.8S rRNA. We suggest that base pairing between these sequences contributes to the binding between 5.8S and 26S rRNA.

INTRODUCTION

The RNA constituents of the ribosome in yeast are all encoded in a repeating unit of about 9000 base pairs [1]. This repeating unit contains two separate transcription units, one for 5S rRNA and one for 17S, 5.8S and 26S rRNA. The primary transcript of the latter is a common 37S precursor RNA which is processed in a number of steps into the respective mature rRNAs [1]. This processing is carried out on nucleoprotein (pre-ribosomal) particles rather than on the naked RNA and includes both modification and nucleolytic cleavage at a number of precisely defined sites. We are interested in the molecular features defining such processing sites. In this paper we focus our attention on the specific interaction between ribonucleoprotein particles and the processing nuclease(s).

At least part of the information defining a nucleolytic processing site is likely to be contained in the nucleotide sequence in the immediate neighbourhood of the phosphodiester bond to be cleaved. Sequence analysis has already revealed certain features common to either the 5'- or the 3'-termini of several precursor and mature rRNA species in yeast [2]. In this paper we present the sequence of the intergenic spacer separating the 5.8S and 26S rRNA gene as well as the first 186 nucleotides of the 26S gene. Both the 3'-end of 7S precursor rRNA and the 5'-end of 26S rRNA were mapped in this sequence which completes the inventory of the flanking sequences of all known processing sites in yeast ribosomal precursor RNA. Thus, we can now establish definitively the common sequence features possibly involved in the recognition of nucleolytic processing sites in yeast precursor rRNA by the cleavage enzyme(s).

MATERIALS AND METHODS

Isolation of DNA and DNA fragments

We used the hybrid plasmids pMY1 and pMY60 which contain the large HindIII fragment (cf. Fig. 1) and the full repeating unit of S. carlsbergensis rDNA respectively, combined with pBR322 [3]. Plasmid DNA was isolated from E. coli K12 cells by the clear lysate method [4]. The procedures for the restriction of the DNAs and the isolation of the DNA fragments have been described previously [2].

Terminal labelling of DNA fragments

5'-End labelling was performed with [γ-^{32}P]ATP and polynucleotide kinase as described previously [2,5]. 3'-End labelling was performed by filling in the protruding ends with [α-^{32}P]dATP (400 Ci/mmol, the Radiochemical Centre, Amersham) as described by Levis et al. [6].

S_1 nuclease mapping

About 0.5 pmol of terminally labelled DNA fragment was hybridized with RNA in 10 µl of 80% deionized formamide, containing 400 mM NaCl, 1 mM EDTA and 40 mM Pipes, pH 6.4 [7] during 4 h at a temperature linearly decreasing from 52°C to 42°C. Hybridization was terminated by rapidly mixing with 9 volumes of ice-cold 0.03 M Na-acetate pH 4.5, containing 0.28 M NaCl, 4.5 mM Zn-acetate, 20 µg/ml thermally denatured calf thymus DNA and 10 units of endonuclease S_1 (Sigma). The mixture was subsequently incubated at 43°C for 30 min. The DNA was precipitated with ethanol using 8 µg of calf thymus DNA as carrier. The precipitate was dissolved in 90% formamide, heated for 3 min

at 100°C and loaded onto a 5% polyacrylamide gel (0.3 mm) in Tris-borate (pH 8.3) and 7 M urea.

Reverse transcription extension

DNA-RNA hybrids (see above) were diluted with 9 volumes of 10 mM Tris-HCl (pH 7.5) and precipitated with ethanol. The precipitate was dissolved in 100 µl 50 mM Tris-HCl (pH 8.3) containing 40 mM KCl, 6 mM $MgCl_2$, 10 mM DTT, 1 mM of each of the four dNTP's, 0.2 mg/ml actinomycin D and 20 units of reverse transcriptase (kindly provided by Dr. J.W. Beard). The reaction was allowed to proceed for 2 h at 42°C. The RNA template was then hydrolyzed in 0.1 M NaOH by incubation for 3 min at 100°. After neutralization with HCl, tRNA was added as carrier and ethanol precipitation was carried out. Gel electrophoretic analysis was the same as described above.

Isolation of RNAs

Purified 26S rRNA was isolated from ribosomes prepared from logarithmically growing cells of S. carlsbergensis S74. The cells were homogenized by shaking with glass beads (0.45 -0.52 cm) in a Braun shaker in 50 mM Tris-HCl, pH 7.8, containing 10 mM $MgCl_2$, 60 mM KCl, 1% (w/v) Brij-58 and 0.5% (w/v) Macaloid. Ribosomes were collected by centrifugation [8]. RNA was extracted with phenol saturated with a solution containing 0.1 M NaCl and 0.01 M EDTA in the presence of 1% SDS and precipitated with ethanol. The RNA was dissolved in 0.1 M NaCl containing 0.01 M EDTA and heated at 53°C for 5 min, to dissociate 5.8S rRNA from 26S rRNA. After rapidly chilling the solution in ice the RNA was fractionated by sucrose gradient centrifugation [8].

Nuclear precursor rRNAs were enriched by isolating a crude nuclear pellet from yeast spheroplasts [9] in the presence of 50 µM aurintricarboxylic acid (E. Merck, Darmstadt) as a nuclease inhibitor. The RNA was extracted with phenol-SDS at 60°C and fractionated on a 15-30% (w/v) sucrose gradient in 0.1 M NaCl and 0.01 M EDTA after thermal dissociation of 5.8S and 7S RNA as described above. After centrifugation for 16 h at 24,000 rpm in an SW27 rotor, RNA material having an S-value of 6-8S was precipitated.

5'-end labelling of 26S rRNA and "wandering spot" analysis

26S rRNA was dephosphorylated and labelled at the 5'-end by polynucleotide kinase in the presence of [γ-^{32}P]ATP essentially according to Donis-Keller et al. [10] and the intact 26S rRNA was reisolated from 2.6% polyacrylamide gels [11]. The RNA was eluted from the gel with 1 M NaCl,

dialysed against distilled H_2O and lyophilized. Partial alkaline hydrolysis was effected by heating the RNA for 15 min at 90°C in 50 mM $NaHCO_3$(pH 9.0). The products were separated by high-voltage electrophoresis followed by homochromatography according to standard procedures [12].

R-looping and electron microscopy

The EcoRI-XbaI fragment (0.5 µg) was hybridized with purified 26S RNA (1 µg) in sealed capillaries in 0.1 M Pipes (pH 7.8) containing 10 mM EDTA, 0.4 M NaCl and 70% (v/v) formamide (final volume 25 µl) at a temperature linearly decreasing from 55°C to 45°C during 4 h. The hybrids were spread at a concentration of 1 µg DNA per ml in 100 mM Tris-HCl (pH 8.5) containing 10 mM EDTA, 40 mM NaCl, 65% (v/v) formamide and 100 µg/ml cytochrome c onto quartz double-distilled water. pBR322 (final concentration 0.5 µg DNA per ml) was added to the spreading mixture to serve as an internal length standard. The protein-nucleic acid film was picked up on 400 mesh copper grids covered with a supporting film of parlodion. After dehydration in 90% (v/v) ethanol and isopentane respectively the grids were rotary-shadowed with platinum at an angle of 6°C. Specimens were examined in a Zeiss EM 109 electron microscope. Contour lengths were measured on photographic prints with a Hewlett Packard 9874A digitizer coupled to a 9825A calculator.

RESULTS

The intergenic region between the 5.8S and 26S rRNA genes

Figure 1 shows a simplified restriction map of the ribosomal transcription unit of yeast. The 3'-end of the 5.8S rRNA gene has been mapped [19-21] on EcoRI fragment A at a position 80 base pairs downstream from the EcoRI site separating fragments D and A. The 5'-end of 26S rRNA has been mapped at about 300 to 400 base pairs downstream from the same EcoRI site, somewhere near the only KpnI site present in fragment A [3].

For a more precise mapping of the 5'-end of the 26S rRNA gene we performed R-loop hybridization between purified 26S rRNA and the appropriate EcoRI-XbaI fragment (cf. Fig. 1) and inspected the partial hybrids by electron microscopy. An example of such a partial hybrid is presented in Fig. 2. Using pBR322 as an internal standard, we determined the length for the double-stranded DNA from the EcoRI site down to the 5'-end of the 26S rRNA gene to be 289 ± 31 base pairs (N = 58). The 5'-end of 26S rRNA, thus, must be located upstream from the KpnI site in fragment A.

This result allowed us to use the EcoRI-KpnI fragment for S_1-nuclease

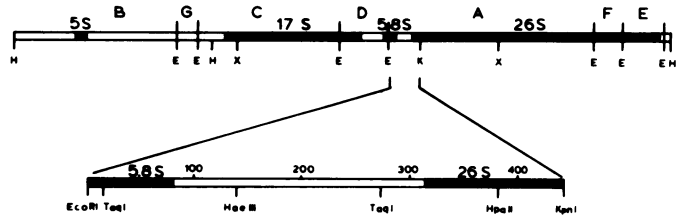


Fig. 1. Simplified restriction map of the ribosomal repeating unit in S. carlsbergensis. The lettering above the map refers to the EcoRI fragments. The small EcoRI-KpnI fragment is enlarged. E stands for EcoRI cleavage sites, X for XbaI, H for HindIII and K for KpnI.

mapping of the 5'-end of 26S rRNA. Figure 3A shows the DNA fragment which remains after digesting the hybrid between the [5'-^{32}P] labelled EcoRI-KpnI fragment and unlabelled pure 26S rRNA with S_1-nuclease. The size of the DNA fragment is about 125 nucleotides. This result is in excellent agreement with the "reverse transcription extension" experiment in Fig. 3B: reverse transcriptase extends the [5'-^{32}P] labelled HpaII-KpnI fragment (cf. Fig. 1), when hybridized to 26S rRNA, to a length of about 125 nucleotides as well.

Next we sequenced the small EcoRI-KpnI fragment using the Maxam and Gilbert technique [14]. Figure 4 shows the full sequence of this fragment as well as 57 nucleotides of the sequence downstream from the KpnI site as present in the hybrid plasmid pMY1 [3]. The EcoRI-KpnI fragment consists of 443 base pairs. The sequence from position 120 to position 400 was confirmed by analysis of a second, independent, clone of S. carlsbergensis rDNA (pMY60 [3]).

In order to establish the exact position of the 5'-end of 26S rRNA we

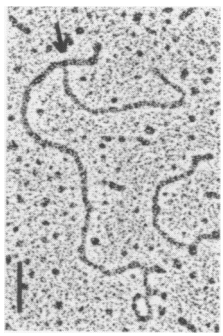


Fig. 2. Electron micrograph of a partial hybrid formed between the EcoRI-XbaI fragment (containing the single KpnI site, cf. Fig. 1) and purified 26S rRNA. The arrow points to the 5'-end of 26S rRNA. The length of the double-stranded DNA up to the 5'-end of 26S rRNA is 289 bp ± 31 (N = 58). The bar represents a length of 0.1 μm.

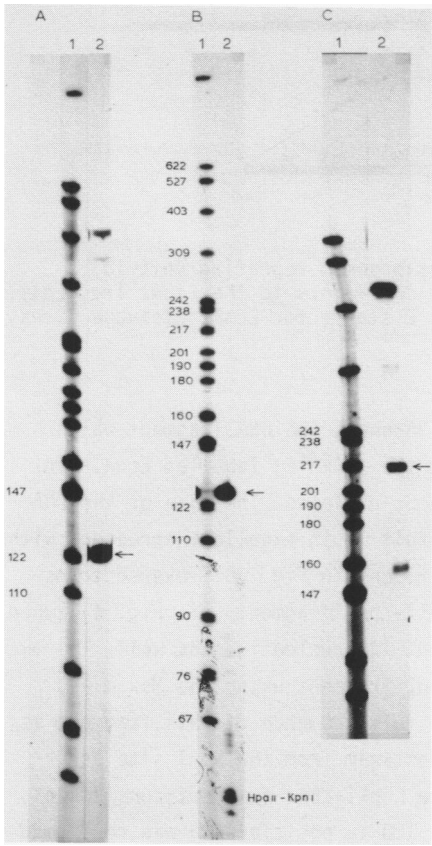
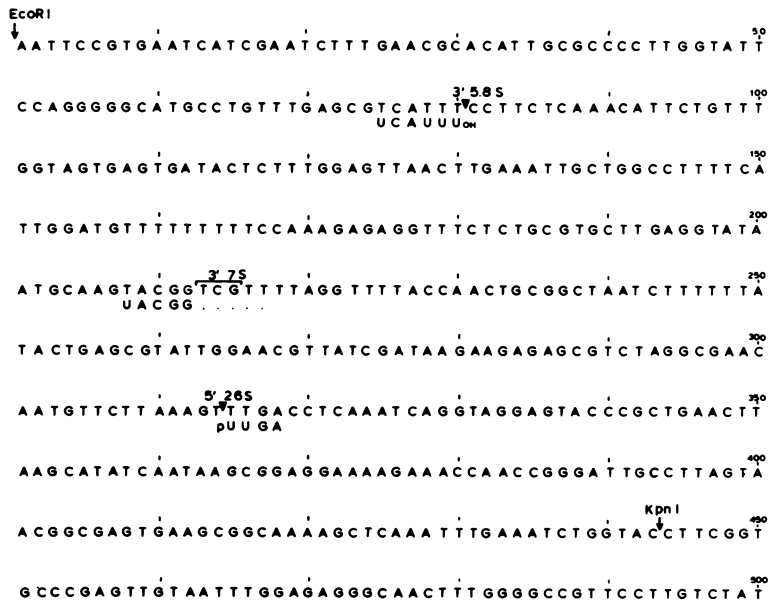
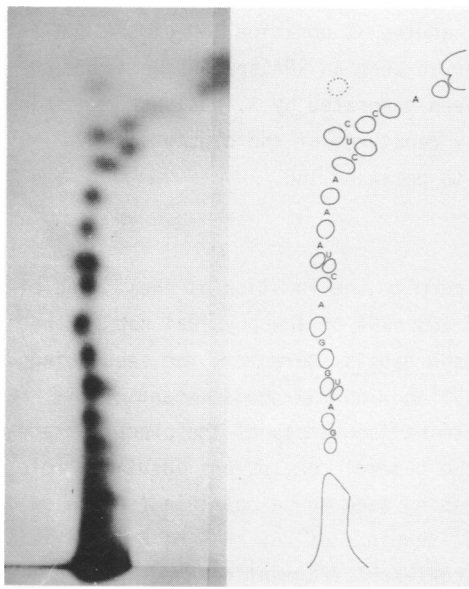


Fig. 3. Mapping of the 5'-end of 26S rRNA and the 3'-end of 7S RNA.
A. [5'-^{32}P] labelled EcoRI-KpnI fragment was hybridized to an equimolar amount of 26S rRNA. The arrow points to the S_1 nuclease resistant DNA fragment from the hybrid.
B. [5'-^{32}P] labelled HpaII-KpnI fragment was hybridized to an equimolar amount of 26S rRNA and extended by reverse transcriptase. The arrow points to the main extension product.
C. [3'-^{32}P] labelled EcoRI-KpnI fragment was hybridized to 100 times excess (w/w) of an RNA sample containing 7S RNA. The arrow points to the S_1 nuclease resistant DNA fragment due to hybridization with 7S RNA.
Molecular weight markers are [5'-^{32}P] labelled HpaII fragments from pBR322 [13].

determined the 5'-terminal sequence of the RNA. Sugiura and Takanami [22] found pU-U-Gp as the 5'-terminal sequence in a ribonuclease T1 digest of 26S rRNA of S. cerevisiae. We obtained the same product in a nearly unimolar yield upon ribonuclease T1 digestion of S. carlsbergensis 26S rRNA and we found pU-U-G-Ap upon digestion with ribonuclease U_2 of kethoxal modified RNA (this modification blocks the G-residues; data not shown). In order to extend this sequence we applied the "wandering spot" method [16] to [5'-^{32}P] labelled 26S rRNA. From the autoradiogram (Fig. 5) we deduce the sequence pX_{2-3}-A-C-C-U-C-A-A-A-U-C-A-G-G-U-A-G-. The identified nucleotides fully match the DNA sequence in Fig. 4 from position 318 to position 333. Since this sequence is prec-ded by T-T-G, we conclude that the 5'-end of 26S rRNA maps at position 315.



(↑) Fig. 4. Nucleotide sequence of the non-coding strand from the intergenic region between the 5.8S and 26S rRNA genes and its adjacent sequences extending from the EcoRI site downstream past the KpnI site. Sequencing was performed according to Maxam and Gilbert [14] and the reaction products were fractionated on thin 8% or 12% polyacrylamide gels containing 7 M urea and either borate or citrate buffer [15].



(←) Fig. 5. Determination of the 5'-terminal nucleotide sequence of yeast 26S rRNA. Purified 26S rRNA was dephosphorylated and labelled at its 5'-end using polynucleotide kinase and [γ-^{32}P] ATP. The RNA was subjected to partial alkaline digestion and the sequence was determined from the mobilities of the successive digestion products ("wandering spot" procedure [16]).

The position of the 3' end of 7S pre-rRNA

The immediate precursor of 5.8S rRNA is a 7S RNA which contains about 150 additional nucleotides at its 3' end and none at its 5' end [23]. We have published an oligonucleotide catalogue of the non-conserved region in 7S RNA [23]. However, this catalogue lacks the 3'-terminal sequence, since we could not detect this sequence in the nuclease digests of 7S RNA. Comparison of the sequence in Fig. 4 with the oligonucleotide catalogue reveals that the DNA sequence from position 80, the 3' end of 5.8S rRNA, to position 212 can encode all oligonucleotides derived from the non-conserved portion of 7S RNA. From the lack of U-C-Gp, corresponding to position 213-215, from the digests of 7S RNA we infer that the 3' end of 7S RNA is located somewhere between position 212 and 215. This deduction is supported by the "S_1-nuclease mapping" experiment described below. An RNA preparation enriched in 7S RNA was isolated from a nuclear fraction from yeast spheroplasts [9] as described in Materials and Methods. We hybridized this RNA with the [3'-^{32}P] labelled EcoRI-KpnI fragment. Fig. 3C reveals a DNA fragment of about 215 nucleotides which is protected when the hybrid is digested with S_1 nuclease. Consequently the 3' end of 7S RNA is apparently located at about position 215 (Fig. 4), a conclusion which is very well compatible with the RNA sequence data. A protected DNA fragment of about 80 nucleotides (not shown in Fig. 3C) represents the hybridization with 5.8S rRNA, which is also present in the RNA preparation. A further DNA fragment of about 160 nucleotides (Fig. 3C) suggests the presence of an RNA species ending at position 160 of the EcoRI-KpnI fragment. Since we have never observed such an RNA species we assume that the DNA fragment in question has been generated by S_1-nuclease digestion of DNA-RNA hybrids, which were partially denatured at the cluster of T residues extending from position 157 to position 166.

DISCUSSION

The data presented in this paper permit the location of the 3'-end of the 7S precursor rRNA and the 5'-end of 26S rRNA on the physical map of the ribosomal transcription unit of *S. carlsbergensis*. Moreover, the sequencing of the intergenic spacer between the 5.8S and 26S RNA genes extends the information already available on the terminal sequences of the precursor and mature rRNA species to a point where some general conclusions about the role of these sequences in nucleolytic processing seem to be possible (see below). Bayev *et al.* [24] recently mapped the 5'-terminus of 26S rRNA of *S. cerevisiae* at positions 313 and 314 of the small EcoRI-KpnI fragment derived from the

EcoRI fragment A (cf. Figs. 1 and 4), using "S_1-nuclease mapping". Our data on the 5'-terminal sequence of 26S rRNA from S. carlsbergensis (Fig. 5), however, allow an unequivocal mapping of the 5' end of this RNA at position 315. Since both S. cerevisiae and S. carlsbergensis 26S rRNA have at least the sequence p-U-U-Gp at their 5' ends in common it seems highly probable that the positions of the 5' ends on the physical map are identical in both strains. Moreover, we did not notice any sequence heterogeneity at the 5' end of 26S rRNA.

The spacer separating the 5.8S and 26S rRNA genes has a length of 234 nucleotides, is relatively rich in uridines (37%) and poor in cytidines (16%). In this respect there is a marked difference with the corresponding spacer in Xenopus laevis ribosomal DNA which is similar in length (262 nucleotides) but extremely rich in cytidines (53%) and guanosines (35%) [17]. This sequence divergence contrasts strongly with the homology of about 80% in the adjoining 5'-terminal 116 nucleotides of 26/28S rRNA of both species (shown in Fig. 6). This strong sequence conservation suggests that the 5'-terminal region of 26/28S rRNA plays an important role in ribosome structure and/or function.

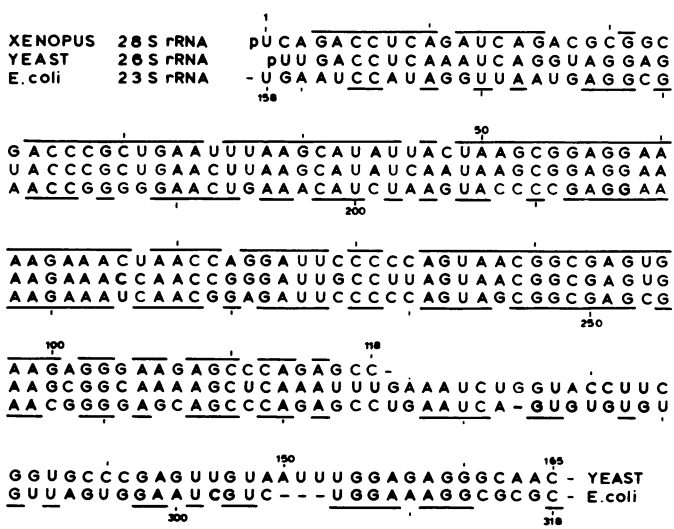


Fig. 6. Homology between the 5'-terminal regions of X. laevis 28S rRNA [17], yeast 26S rRNA and E. coli 23S rRNA [18]. Homologies between yeast and Xenopus are overlined and between yeast and E. coli underlined. The upper numbering refers to the sequence in Xenopus and yeast and the lower one to the E. coli sequence according to Refs. 17 and 18.

The sites for the 5.8S-26S rRNA interaction

Studies on the association between 5.8S and 26/28S rRNA in yeast, Xenopus, Drosophila and mouse have provided evidence that both the 5'- and the 3'-terminal sequences of 5.8S rRNA are involved in this binding [25-28]. No information as to the sites on 26S rRNA participating in this association was available until recently, when Kelly and Cox postulated a binding site for 5.8S rRNA near the 3' end of 26S rRNA of Neurospora crassa [29].

Inspection of the sequence in Fig. 4 reveals an extended complementarity between sequences at the 5' end of 26S rRNA and the 3' end of 5.8S rRNA. The possible base pairing between the RNA molecules is shown in Fig. 7B. We propose that this base pairing is involved in the association of the 5.8S/26S rRNA complex in the mature ribosome. In Fig. 7C we show that a similar base pairing is possible between the corresponding regions in Xenopus laevis rRNA; in spite of a few base substitutions, the overall base pairing scheme is maintained. The proposed hydrogen bonding between the 3' end of 5.8S rRNA and the 5' end of 26S rRNA is fully compatible with the recent observations made by Nazar [30] and Walker [31] that the 5'-terminal region of prokaryotic 23S rRNA begins with a 5.8S rRNA-like sequence immediately followed by a sequence homologous with the 5'-terminal region of eukaryotic 28S rRNA. Fig. 6 shows the homology (about 60%) between the 5'-terminal region of yeast 26S rRNA and

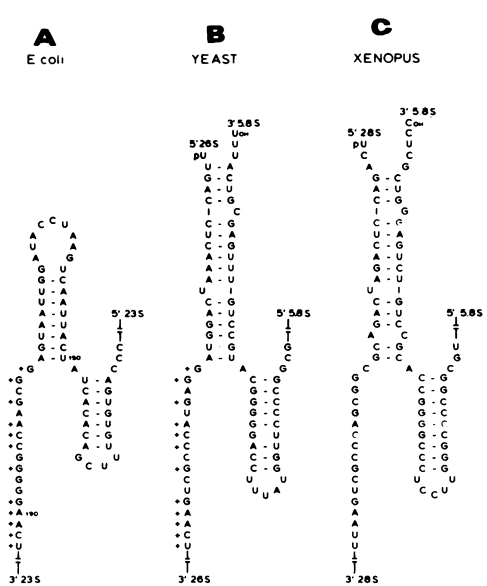


Fig. 7. Possible base pairing between the 3'-terminal region of 5.8S rRNA and the 5'-terminal region of 26/28S rRNA in yeast (B) and X. laevis (C; Ref. 17). A similar structure possible at the 5'-end of E. coli 23S rRNA [18] is shown in Fig. 7A. Nucleotides in the unpaired sequences, which are homologous in yeast and E. coli, are marked with a '+'.

the sequence of E. coli 23S rRNA starting at position 158. Apparently, sequences homologous with the 3' end of 5.8S rRNA and with the 5' end of of 26S/28S rRNA are physically linked in the bacterial RNA. Inspection of the nucleotide sequence of E. coli 23S rRNA [18] at this "junction" reveals a dyad symmetry of eight consecutive nucleotides. Figure 7A shows the possible base pairing between these nucleotides (the upper stem). The overall structure of this stem and its adjoining sequences is very similar to the corresponding structures proposed for yeast and Xenopus (Fig. 7B and C). It should be noted that the homologous nucleotides in the adjoining 5.8S rRNA sequence of yeast and in the 5.8S RNA like sequence of E. coli (marked by a '+') are located in exactly the same position relative to the upper stem. The structural similarity between bacterial 23S rRNA on the one hand and eukaryotic 5.8S + 26/28S rRNA on the other may extend even further. Evidence has been obtained that during processing of E. coli 30S pre-rRNA the 5'- and 3'-terminal sequences of 23S rRNA are juxtaposed in a long stem structure [32]. It is conceivable that this interaction is maintained in the mature 50S subunit. If we assume that only the 5'-terminal part of 5.8S rRNA is responsable for the recently proposed interaction between 5.8S rRNA and the 3' end of 26S rRNA of N. crassa [29], an analogous structure would be present in the eukaryotic 60S ribosomal subunit. We therefore propose the 5.8S rRNA functions as a bridge between the two termini of the large rRNA in the 60S ribosomal subunit. In view of the analogies between 23S rRNA and 5.8S + 26/28S rRNA discussed above we would like to consider the intergenic spacer between the 5.8S and 26S rRNA genes as a 'pseudo-intron' which is spliced out during processing, without subsequent ligation, despite the relatively short distance in space between the two termini of both 'exons'.

A structural model for the processing of yeast rRNA

In Fig. 8A the processing of yeast rRNA is depicted schematically. Before discussing a possible model for this processing we have to make the following comments with respect to the timing of the various cleavage events.

1. 37S pre-rRNA is first cleaved virtually simultaneously at positions A_1 and A_2 generating 18S RNA and 29S(a) RNA without loss of any internal nucleotides. Subsequent cleavage at position B_1 shortens 29S(a) to 29S(b) RNA by removing a stretch at the 5'-end. This generates the 5'-terminus of 7S (and 5.8S) RNA. This sequence of events is deduced from "reverse transcription extension" and "S_1 nuclease mapping" experiments (not shown) using an RNA fraction enriched in 29S RNA, but feed of 7S and 5.8S RNA. These

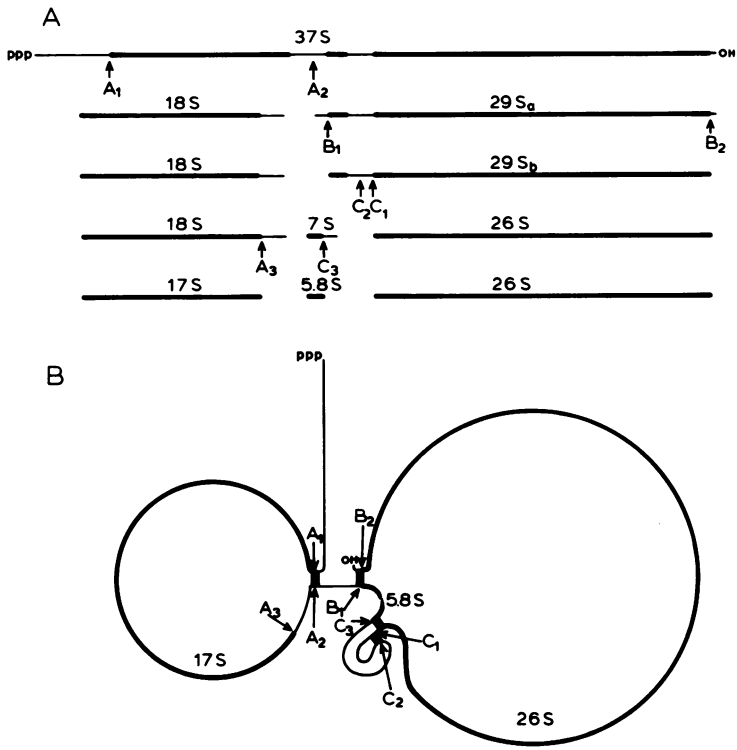


Fig. 8. Processing of yeast precursor rRNA. A. Processing scheme; B. Structural model. The lettering A, B and C corresponds to the structures depicted in Fig. 9A, 9B and 9C, respectively.

experiments suggest the presence of two 29S RNA species: the 5'-terminus of one of these maps immediately adjacent to the 3'-terminus of 18S RNA (site A_2); the 5'-terminus of the other coincides with that of 5.8S RNA (site B_1). The separation of glyoxylated 29S RNA into two closely spaced bands upon gel electrophoresis [33] is further evidence for the existence of two 29S RNA species having slightly different lengths.

Previously, we postulated a 32S RNA as an intermediate after cleavage at A_1 only [1]; this was based upon labelling experiments of a methionine requiring mutant after methionine deprivation and subsequent analysis of the RNA on non-denaturing gels [35]. However we cannot detect such an RNA species under normal conditions and on denaturing gels. Neither can we detect an RNA precursor to 17S rRNA larger than 18S RNA; the apparent absence of both possible intermediates indicates that site A_1 and A_2 are

cleaved virtually simultaneously.

2. Cleavage at site B_1 occurs virtually simultaneous with cleavage at site B_2 which generates the 3'-end of 26S RNA. This is inferred from qualitative end-group analysis of 29S(a+b) RNA which reveals the presence of the 3'-terminal sequences of both 37S and 26S RNA [36]. The presence of some contaminating 37S as well as 26S RNA in the 29S preparation analysed complicates the interpretation of these experiments to some extent. Nevertheless we deem it likely that cleavage at site B_2 precedes the formation of the 5'-end of 26S RNA (site C_2).
3. 29S(b) RNA again is cleaved virtually simultaneously at sites C_1 and C_2 generating 7S and 26S RNA. "S_1-nuclease mapping" experiments did not reveal any precursor intermediate between 29S(b) and 26S RNA having extra nucleotides at the 5'-end of 26S rRNA (experiments not shown).
4. Cleavages at positions A_3 and C_3 are separate, late, events. The former even occurs in the cytoplasm [34].

The occurrence of several pairs of virtually simultaneous processing cleavages in yeast rRNA deduced above shows some analogy to the action of RNAase III on E. coli 30S pre-rRNA [37] and also to the generation of 5S RNA from its immediate precursor by RNAase M5 in Bacilli [1,38]. In these cases the two cleavage sites are put into close proximity by base-pairing between the surrounding sequences. The analogy leads us to propose a similar structural arrangement of the yeast rRNA processing sites.

We have previously reported that the extended base complementarity observed between the terminal sequences of E. coli pre-rRNAs does not exist in yeast 18S RNA [2]. Neither does it in yeast 29S RNA [2,5]. However, rRNA in yeast takes place at the level of nucleoprotein particles whereas neither RNAase III nor RNAase M5 action requires additional proteins [38,39]. We therefore suggest that in yeast much shorter complementarities should be taken into account since these might well be stabilized by additional protein-RNA interactions. Such complementarities indeed are present (i) between the 5'- and 3'-terminal sequences of 18S RNA (covering sites A_1 and A_2); (ii) between the 5'- and 3'-ends of 29S(b) RNA (sites B_1 and B_2; see discussion on the interaction between 5.8S and 26S rRNA) and (iii) between the 5'-end of 26S rRNA and the 3'-end of 7S RNA (site C_1 and C_2).

Figure 9 shows a possible structure for each of these three pairs of processing sites. When alternative base-paired structures were possible the choice was guided by a second feature common to the three pairs of sites, to wit the presence of a concensus sequence at both the 3'- (type I) and the 5'-

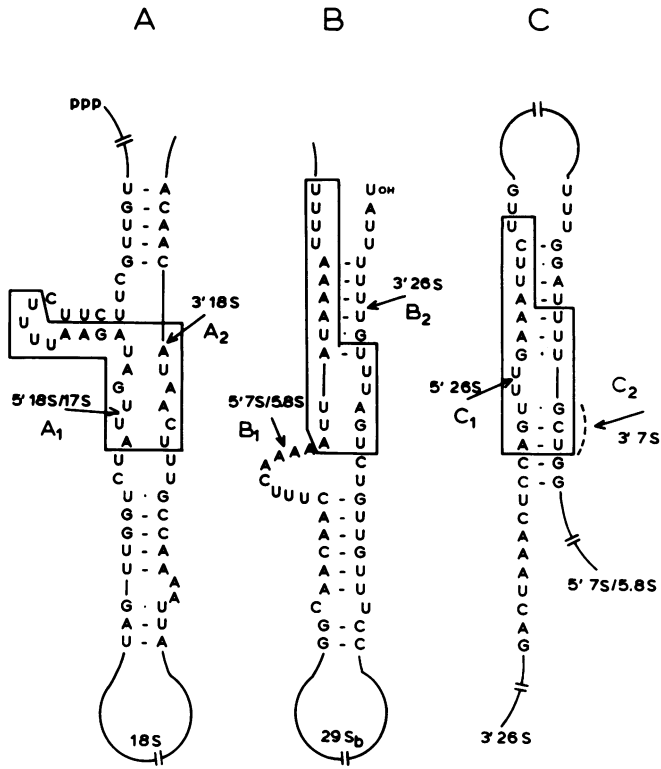


Fig. 9. Possible secondary structure formed between A: the 5'- and 3' end of 18S pre-rRNA; B: the 5' end of 5.8S rRNA and the 3' end of 26S rRNA; C: the 5' end of 26S rRNA and the 3' end of 7S pre-rRNA.

TABLE I.

3'-processing sites			5'-processing sites		
site	sequence	ref.	site	sequence	ref.
3' 18S	UCAAUA	[40]	5' 7/5.8S	UUUUAAAAUA-UUA	[2]
3' 17S	UCAUUA	[41]	5' 18/17S	UUUUAAGAUAGUUA(UCU)	[43]
3' 5.8S	UCAUUU	[42]	5' 26S	UCUUAAAGUU-UGA(CCU)	[this paper]
3' 7S	UCGUUU	[this paper]			
3' 26S	UGAUUU	[5]			

(type II) termini of the RNA molecule generated by the two cleavages. Table I lists these sequences.

The structures drawn in Fig. 9 all have a similar configuration consisting of a combination of a type I and a type II concensus sequence flanked by short base-paired regions. In all cases the two cleavage sites are close to each other. We would like to suggest that this configuration represents an important, though maybe not the only, feature for recognition of the processing sites by the nuclease(s). An additional trait of our model is that it places the regions A, B and C into close proximity (Fig. 8B). This would even allow one single processing enzyme molecule to cleave the three regions in rapid succession.

Although the model agrees with the known data on yeast rRNA processing no direct evidence supporting it is as yet available. Moreover, it remains to be seen whether it can be extended to cover rRNA processing in other, especially higher, eukaryotes. We offer the model as a working hypothesis useful in guiding, for instance, crosslinking experiments on precursor rRNA which could verify, or otherwise, the proposed interactions.

ACKNOWLEDGEMENTS

This work 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 grateful to Dr. P. de Jonge, Miss I. Klein, Mrs. R. de Leede-Twente, Mr. C.P. Sibum and Mr. T.C. Bootsman for their participation in some of the experiments. We thank Dr. H.A. Raué for critically reading the manuscript and Mrs. P.G. Brink for preparation of the manuscript.

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