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Pseudouridylation of yeast ribosomal precursor RNA

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ABSTRACT

The pseudouridylation of ribosomal RNA of Saccharomyces carlsbergensis was investigated with respect to its timing during the maturation of rRNA and its sequence specificity.

Analysis of 37-S RNA, the common precursor to 17-S, 5.8-S and 26-S rRNA and most probably the primary ribosomal transcript, shows that this RNA molecule contains already most if not all of the 36-37 pseudouridine residues found in the mature rRNAs. Thus pseudouridylation is, like 2'-O-ribose-methylation, an early event in the maturation of rRNA, taking place immediately after, or even during, transcription.

The data presented show that the non-conserved sequences of 37-S precursor rRNA contain very few pseudouridine residues if any. The pseudouridine residues within the rRNA sequences are apparently clustered to a certain degree as can be inferred from the occurrence of a single oligonucleotide containing 3 pseudouridines, which was obtained by digestion of 26-S rRNA with ribonuclease T_1.

INTRODUCTION

In the regulation of rRNA formation, the processing of the primary transcript of the rRNA genes, - a long precursor molecule containing the sequences of 17/18-S, 5.8-S and 26/28-S rRNA and in addition non-ribosomal sequences -, is considered to be of particular importance (1). This processing of precursor rRNA comprises a very complex, though well-ordered series of events, involving nucleolytic cleavages and chemical modification of the polynucleotide chain by methylation and conversion of certain uridine residues into pseudouridines (2-5).

The precise biological function of the chemical modifications of the precursor rRNA is not yet clear. It is known that 2'-O ribose methylation, the predominant type of methylation of eukaryotic rRNA, is an early event taking place immediately after or even during transcription (6-8), suggesting that ribose methylation plays a role in the maturation process. And indeed, it has been shown that the introduction of methyl groups in the RNA

chain has a positive effect on the overall efficiency of the maturation process (9-11). Even less is known about the conversion of uridines into pseudouridines in the course of the maturation process. Only limited data on the specificity and the timing of pseudouridylation of HeLa cell rRNA are available (12, 13, 22).

Therefore, we decided to undertake a study on the timing and the specificity of the pseudouridylation of yeast precursor rRNA. Since yeast rRNA contains a relatively low number of pseudouridine residues (36-37 (14, 15)) compared with HeLa cell rRNA (98 (2)), the yeast system seems to be appropriate for answering the question whether or not individual pseudouridines are formed at an early stage of rRNA formation. By comparative analysis of the oligonucleotides derived from both mature rRNA components and 37-S precursor rRNA by enzymatic digestion, it was found that most if not all pseudouridines present in the mature rRNA components are already formed at the level of 37-S precursor rRNA. The pseudouridine formation within 37-S RNA appears to be confined to the rRNA sequences, and occurs at specific sites, which are clustered to some extent.

MATERIALS AND METHODS

Isolation of ^{32}P-labelled RNAs

^{32}P-labelled RNAs were isolated from *Saccharomyces carlsbergensis* strain N.C.Y.C. 74. For the isolation of 26-S *plus* 5.8-S and 17-S rRNA yeast cells were grown in a medium containing per litre 15 g glucose, 2 g yeast extract (Oxoid), 3 g malt extract (Oxoid), 1 g bactopeptone (Difco) and 0.1-0.2 Ci (^{32}P)orthophosphate (The Radiochemical Centre) and harvested in the late-logarithmic growth phase. Cells were suspended in 0.01 M Tris-HCl, 0.01 M $MgCl_2$ and 0.06 M KCl, pH 7.6 containing 1% (w/v) Brij-58 and shaken with glass beads (Ø 0.45-0.52 mm) in a Braun shaker for 2.5 min. Ribosomes were pelletted by differential centrifugation (16) and RNA was extracted by phenol-SDS. Individual rRNAs were isolated *via* sucrose gradient centrifugation (17).

18-S precursor rRNA was isolated from 43-S pre-ribosomal subunits and purified as described by De Jonge *et al.* (18). The isolation and purification of 29-S and 37-S precursor rRNA were performed as described previously (6).

Analysis of ψp in T_1 *plus* pancreatic ribonuclease digests of various yeast RNAs

RNA samples were digested to completion with T_1 *plus* pancreatic

ribonuclease (13) and the digestion products were separated by two-dimensional electrophoresis (19). Labelled oligonucleotides were located by radioautography and numbered according to Klootwijk et al. (14). Most ψ-containing products are resolved except for ψp and A-ψp (14). All spots of the 'fingerprint' pattern were excised from the paper and ^{32}P-radioactivity was counted after addition of a toluene-based scintillation liquid.

The uridine-2',3'-monophosphate (Up) fraction was eluted with a saturated solution of $(NH_4)_2CO_3$ and ψp was separated completely from Up by two-dimensional paperchromatography according to Hughes and Maden (2). Oligonucleotide material containing A-Up plus A-ψp was first digested with ribonuclease T_2 and the products were separated by electrophoresis at pH 3.5 (6). Then ψp and Up were separated by two-dimensional paperchromatography (2). A-A-ψp was screened for contamination with A-A-Up using the same procedure.

Characterization of ψp-containing oligonucleotides in ribonuclease T_1 digests of various yeast RNAs

^{32}P-labelled RNA preparations were digested with ribonuclease T_1 (Calbiochem) for 30 min at 37°C at an enzyme: substrate ratio of 1:20 (w/w). The digestion products were fractionated according to Brownlee and Sanger (20) as modified by Volckaert et al. (21). Samples containing more than 100 µg of RNA were applied onto strips of Cellogel (Chemotron, Italy) instead of cellulose acetate. After electrophoresis on this strips, the oligonucleotide material was transferred to a thin-layer plate (20x40 cm) of DEAE-cellulose (Polygram Cel 300 DEAE/HR-2/15). The plate was developed at 60°-65°C with a 3% homomixture 'C' buffered with 0.02 M Tris-HCl pH 7.5 until the blue marker dye (Xylene cyanol FF) had reached the top of the plate. The plate was dried, washed with ethanol, dried again and radioautographed (Medical X-ray film, Sakura, type QH). All uridine-containing products present in 'fingerprints' of either 26-S plus 5.8-S or 17-S rRNA were recovered by elution with 30% triethylamine carbonate pH 10 for 30 min (21). Oligonucleotide material was then digested with 25 mU ribonuclease T_2 (Calbiochem) in 10 µl 0.1 M ammonium acetate pH 4.5 for 16 h at 37°C. The digestion mixture was applied to Whatman 3 MM paper and subjected to electrophoresis for 1 h at 55 V/cm in 5% acetic acid, pH 3.5, containing 1 mM EDTA. The Up fraction of all oligonucleotides was screened for the presence of ψp by descending paperchromatography as described by Hughes et al. (22). Because of some trailing of Up, the ψp-containing area was excised and stitched onto another sheet of paper (Whatman 1) and rechromatographed using the same solvent to ensure

complete separation of ψp and Up. All spots on the various radioautographs were excised from the paper and counted after solubilization with 3% (v/v) Nuclear Chicago Solubilizer in a toluene-based scintillation liquid.

In a 'fingerprint' of 37-S precursor rRNA the spots, corresponding to ψp-containing oligonucleotides in 'fingerprints' of the mature rRNAs, were screened for the presence of ψp in the same way as described above.

A few ψp-containing oligonucleotides were further characterized by digestion with either pancreatic or U_2 ribonuclease (23) and fractionation of the products by electrophoresis on DEAE-cellulose paper at pH 3.5.

RESULTS

As a first step we estimated the number of pseudouridines in the various rRNAs and their precursors. To this end we digested the RNAs either with T_1 plus pancreatic ribonuclease or with alkali and after electrophoretic separation of the digestion products all uridine-containing (oligo)nucleotides were screened for the presence of ψp. The T_1 plus pancreatic ribonuclease 'fingerprints' of the various rRNA species are shown in Fig. 1. This procedure resolves a number of ψp-containing products, e.g. A-A-ψp and A-A-A-ψp; however the bulk of pseudouridines is present as free ψp and A-ψp and these products are not separated in this way from Up and A-Up, respectively. Quantitation of the amount of pseudouridine in these cases requires a subsequent two-dimensional paperchromatography (22) to ensure the complete separation of ψp from an excess of Up. All data are summarized in Table I. 17-S rRNA, on the average, contains 12 ψp residues per molecule including the ψp which is present as 3-(3-amino-3-carboxypropyl)-1-methylpseudouridine (25). 26-S rRNA contains 23-24 ψp residues plus one ψp derived from 5.8-S rRNA, which is present as A-A-ψp (26).

Estimation of the total number of ψp residues in the respective precursor rRNA species may in principle shed some light on the timing of the pseudouridylation as well as on the presence of pseudouridines in the non-conserved regions. Inspection of the data in Table I reveals that 18-S and 29-S precursor rRNA contain the same number of ψp residues as observed in their mature counterparts, 17-S and 26-S plus 5.8-S rRNA, respectively. In addition, the number of pseudouridines in the common 37-S precursor rRNA is nearly the same as the total number of pseudouridines in 29-S plus 18-S RNA. These data suggest that most if not all ψp residues found in the mature rRNAs are present already in 37-S precursor rRNA and, in addition that pseudouridylation is restricted to the ribosomal sequences of the precursor

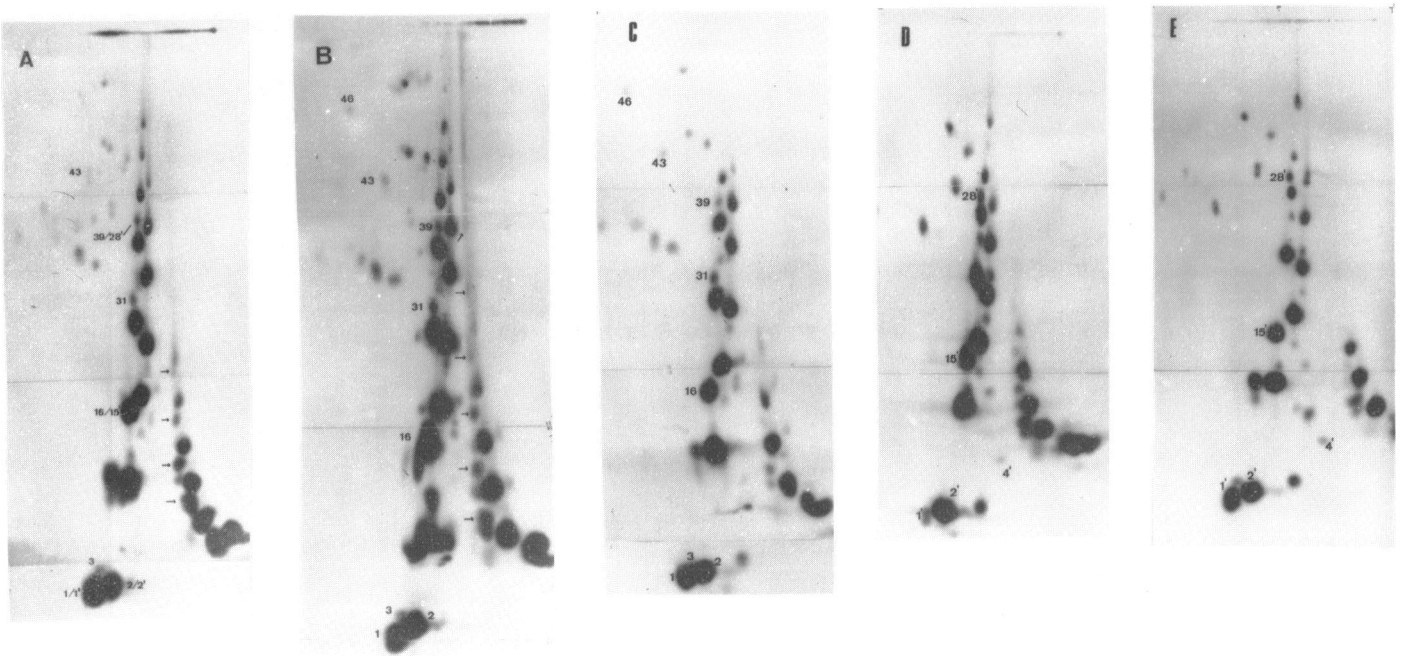

Fig. 1. Combined T_1 plus pancreatic ribonuclease 'fingerprints' of 37-S, 29-S, 26-S plus 5.8-S, 18-S and 17-S RNA. ^{32}P-labelled RNA was digested with T_1 plus pancreatic ribonuclease (13) and the products were fractionated according to Sanger et al. (19). (A) 33 µg 37-S precursor rRNA (4.0×10^6 cpm); (B) 70 µg 29-S precursor rRNA (2.4×10^6 cpm); 40 µg 26-S plus 5.8-S rRNA (6.0×10^6 cpm); (D) 16 µg 18-S precursor rRNA (7.1×10^6 cpm); (E) 9 µg 17-S rRNA (5.3×10^6 cpm). The numbers refer to Table I. In panel B some weak spots are visible, which are due to contamination with 37S precursor rRNA. The series of $(A_{>})_n$ products (n=2,3, etc), indicated by arrows, is derived from (precursor) mRNA contamination.

TABLE I. MOLAR YIELDS OF ψp-CONTAINING PRODUCTS OBTAINED AFTER T_1 PLUS PANCREATIC RIBONUCLEASE DIGESTION OF YEAST rRNAs AND THEIR PRECURSORS. The molar yields were calculated from the relative amount of ^{32}P-label in the ψp fraction and the chain lengths of the various rRNA species (37-S RNA: 8000 nucleotides, 29-S RNA: 4350, 26-S plus 5.8-S rRNA: 3550, 18-S RNA: 2350 and 17-S rRNA: 2000 (24)). A correction was made for contamination of the 29-S RNA preparations with 37-S RNA; the degree of contamination was inferred from gel electrophoretic analysis (6) and 'fingerprints' (cf. Fig. 1). The molar yields of ψp-containing products of 37-S and 29-S RNA will be slightly underestimated due to some (precursor) mRNA contamination in these preparations. The numbering refers to Fig. 1; n.d. stands for not detectable. The values given with their standard deviations are the average of 3-5 determinations.

Spot number		Product	Molar yield				
			37-S	29-S	26-S + 5.8-S	18-S	17-S
17-S	26-S+5.8-S						
1'+2'	1+2+3	ψp	?	13.9±1.4	14.5±1.4	6.9±0.6	7.2±0.8
1'+2'	-	$m^1ψp$[b]	0.9[c]	-	-	0.7[c]	-
15'	16	A-ψp	?	4.2±0.4	3.7±0.1	2.3±0.5	2.2±0.2
-	31	A-A-ψp	3.8±0.8	2.8±0.4	3.1±0.4	-	-
28'	39	A-A-A-ψp	3.4±0.6	1.1±0.1	1.2±0.1	2.0±0.2	1.9±0.2
4'	-	amψ-Cp[a]	n.d.	-	-	0.2±0.1	1.0±0.1
-	43	Um-A-ψp	0.7±0.1	0.8±0.2	0.9±0.1	-	-
-	46	Um-Gm-ψp	n.d.	0.3±0.1	1.0±0.1	-	-
		Total	?	23.1±1.5	24.4±1.5	12.1±0.8	12.3±0.9
		Total[d]	33.0±2.4	-	25.1±1.4[e]	-	11.8±0.5[f]

a amψ denotes the hypermodified nucleotide 3-(3-amino-3-carboxypropyl)-1-methyl-pseudouridine (25).
b $m^1ψp$, 1-methylpseudouridine-2',3'-monophosphate, is the precursor of amψp (25) and comigrates with ψp and Up during two-dimensional electrophoresis; during paperchromatography in isopropanol/HCl/H_2O (68:17.6:14.4 by vol) m'ψp migrates between Up and ψp.
c data taken from (6).
d estimated independently using complete alkaline digestion of the ^{32}P-labelled RNA and separation of ψp according to Hughes and Maden (2).
e the experimental value was augmented by 1.0 for the ψp residue within the alkali-stable product Um-Gm-ψp.
f this figure includes the amψ-residue.

RNA. However, on the basis of these figures we cannot rule out completely that some ψp residues in 37-S RNA are present in the non-conserved stretches of the precursor RNA and are removed during processing with the concomitant formation of an equal number of ψp residues in the ribosomal sequences. Therefore specific sequences of (precursor) rRNA have to be analyzed for the

presence of ψp. This was achieved by digesting 26-S plus 5.8-S and 17-S rRNA with ribonuclease T_1 followed by fractionation of the products by high-voltage electrophoresis and 'homochromatography'; the radioautographs are shown in Fig. 2. All uridine-containing oligonucleotides were recovered from the thin-layer plate and digested to completion by ribonuclease T_2. The products were fractionated by electrophoresis at pH 3.5, followed by screening of the Up fraction for the presence of ψp by two cycles of paperchromatography. All spots, corresponding to ψp-containing oligonucleotides, are marked in the keys to the 'fingerprints' of Fig. 2. The number of ψp residues in each product was roughly estimated by direct comparison of the amount of ^{32}P-label in the purified ψp fraction with the average amount of ^{32}P-radioactivity in the Gp fraction of several unimolar oligonucleotides (cf. Table II). Despite rather large standard deviations (up to 35%) the total numbers of ψp per RNA molecule in Table II matches rather well with those of Table I. Moreover, this analysis permits the conclusion that most of the pseudouridylated sites of yeast rRNA are within homogeneous parts of the rRNA molecules. The 'fingerprint' of 17-S rRNA revealed 12 ψp-containing products, amounting to 12-14 ψp residues per molecule. Some of them were characterized in more detail as is shown in Table III. Spot 46' represents an oligonucleotide with the sequence of U-U-U-A-ψ-U-U-Gp and spot 60' appeared to be A-A-A-A-A-A-U-ψ-A-Gp. These oligonucleotides represent examples of specific ψ-containing sequences, which can be isolated now from 37-S RNA to check for the presence of ψp. 'Fingerprints' of 26-S plus 5.8-S rRNA showed at least 19 different ψp-containing products, altogether corresponding to 24-25 pseudouridines per 26-S plus 5.8-S rRNA complex. The presence of 3 ψp-residues in oligonucleotide product 88 was confirmed by partial sequence analysis of this oligonucleotide (cf. Table III).

Next we screened the set of oligonucleotides represented by the spots summarized in Table II but derived now from 37-S RNA for the presence of ψp. The 'fingerprint' of 37-S RNA is shown in Fig. 3. Due to the relatively low yield of label in ψp after the screening procedure, only qualitative data could be collected (cf. Table II). All, except two, pseudouridylated oligonucleotides present within the mature species are also found in 37-S RNA. The bulk of these products displayed similar intensities in the ψp fraction after the screening procedure and therefore they are assumed to be present in unimolar yield. Spot 11'+22, 42, 82 and 88 clearly contained a higher number of pseudouridines. From this observation we draw the conclusion that most, if not all, conversions of U into ψ occur early during processing of

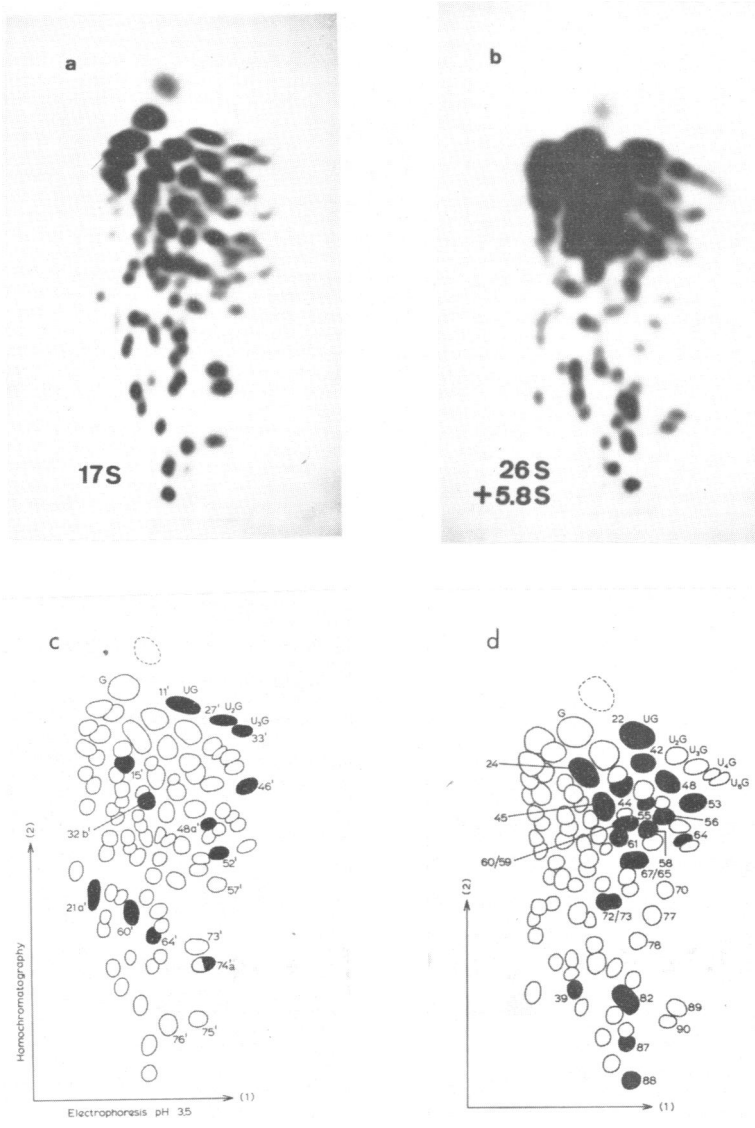

Fig. 2. Two-dimensional separation of the ribonuclease T_1 digestion products of ^{32}P-labelled 17-S and 26-S plus 5.8-S rRNA. (a) 24 µg 17-S rRNA (2×10^6 cpm); (b) 44 µg 26-S plus 5.8-S rRNA (3.8×10^6 cpm); (c) and (d) are keys to (a) and (b) respectively. ψp-containing products are represented by the closed circles; the numbering of the oligonucleotides corresponds to Table II.

TABLE II. ANALYSIS OF ψp-CONTAINING PRODUCTS IN RIBONUCLEASE T_1 DIGESTS OF THE MATURE rRNAs AND OF 37-S PRECURSOR rRNA.
Products obtained by digestion of 26-S plus 5.8-S rRNA and 17-S rRNA with ribonuclease T_1 were fractionated as shown in Fig. 2. All Up-containing products were monitored for the presence of ψp. The number of residues per molecule was calculated from the amount of ^{32}P-radioactivity in ψp relative to the average amount of ^{32}P-label in Gp derived from a number of unimolar oligonucleotides (spots 57', 73', 75' and 76' for 17-S rRNA and spots 70, 77, 78, 89 and 90 for 26-S plus 5.8-S rRNA). All figures are the average of three separate experiments; the suggested number of ψp residues is given in parentheses. 37-S RNA was digested and 'fingerprinted' similarly as the mature rRNAs (Fig. 3). Those products, containing ψp in mature rRNA, were screened qualitatively for the presence of ψp in two separate experiments. The presence of ψp in an oligonucleotide derived from 37-S RNA is denoted by +; - stands for not detectable.

Spot number		Number of ψp residues per RNA molecule		
17-S	26-S plus 5.8-S	17-S	26-S plus 5.8-S	37-S
11'	22	1.5 (1-2)	1.2 (1)	+
15'		0.9 (1)		+
21a'+82'[a]		1.0 (1)		+
27'		1.6 (1-2)		+
32b'		1.2 (1)		+
33'		0.9 (1)		+
46'		1.0 (1)		+
48a'		1.0 (1)		+
52'		0.7 (1)		+
60'		0.7 (1)		+
64'		0.9 (1)		+
74a'		1.0 (1)		+
	24		0.9 (1)	+
	39		0.9 (1)	+
	42		1.9 (2)	+
	44		0.8 (1)	+
	45		1.0 (1)	+
	48		0.8 (1)	+
	53		1.2 (1)	+
	55		0.5 (0-1)	-
	56		1.1 (1)	+
	58		1.1 (1)	+
	59/60		1.2 (1)	+
	61		2.0 (2)	+
	64		0.9 (1)	+
	65/67		1.1 (1)	+
	72/73		2.0 (2)	+
	82		1.9 (2)	+
	87[b]		1.0 (1)	-
	88		2.7 (3)	+
	Total	12.4 (12-14)	24.2 (24-25)	

a the oligonucleotide from spot 21a' contains the hypermodified nucleotide amψp and spot 82a' contains its precursor m'ψp (25).
b spot 87 represents an oligonucleotide containing ..Um-Gm-ψ.. within its sequence (6) which is lacking in 37-S RNA due to a lack of methylation at the G-residue.

TABLE III. CHARACTERIZATION OF SOME ψp-CONTAINING OLIGONUCLEOTIDES IN YEAST rRNA.
Oligonucleotide material was recovered from the thin layer plates and digested with either pancreatic or U_2 ribonuclease (23), followed by electrophoresis on DEAE-cellulose paper at pH 3.5. Molar yields are given within parentheses using the underlined value as a reference.

Spot number	digestion products				Sequence deduced or composition
	panc.		U_2		
46'	A-ψp	(1.0)	(U_2,ψ)Gp	(1)	
	Gp	(1)	U-U-U-Ap	(1.0)	
	Up	(5.2)			U-U-U-A-ψ-U-U-Gp
52'	A-Gp	(1)	U-U-U-Ap	(1.0)	
	A-ψp	(1.0)	(U,ψ)Ap	(1)	
	A-Up	(1.0)	U-Ap	(1.0)	
	Up	(4.4)	Gp	(0.7)	(U-U-U-A,ψ-U-A,U-A)Gp
60'	A_6-Up	(1.0)	(U,ψ)Ap	(1)	
	A-Gp	(1)	Gp	(0.7)	
	ψp	(1.1)	Ap	(5.3)	A-A-A-A-A-A-U-ψ-A-Gp
74a'	A_3-ψp	(1)	(C,U_2,ψ)Gp*	(1)	
	A-Up	(1.2)	(C,U_2)Ap*	(1.6)	
	Gp	(0.8)	Ap	(2.0)	
	Cp	(3.2)			
	Up	(5.3)			C_3,A_4,U_6,ψ,G
88	A_3-ψp	(1.3)	(C_2,U)Ap	(0.9)	
	A_3-Cp	(1.2)	U-Gp	(1.0)	
	A-Up	(1.8)	ψ-Ap	(1)	
	A-Cp	(1.0)	Ap	(4.2)	
	Gp	(1)	(C_2,U,ψ)Ap*	(0.8)	
	Cp	(2.7)	(C,U_2,ψ)Ap*	(0.8)	
	Up	(2.9)			
	ψp	(1.9)			C_5,A_9,U_5,$ψ_3$,G

* This product was not resolved after electrophoresis at pH 3.5 and therefore subjected to another run on DEAE-cellulose paper in 0.1 M ammonium formate buffer, pH 2.3 (27).

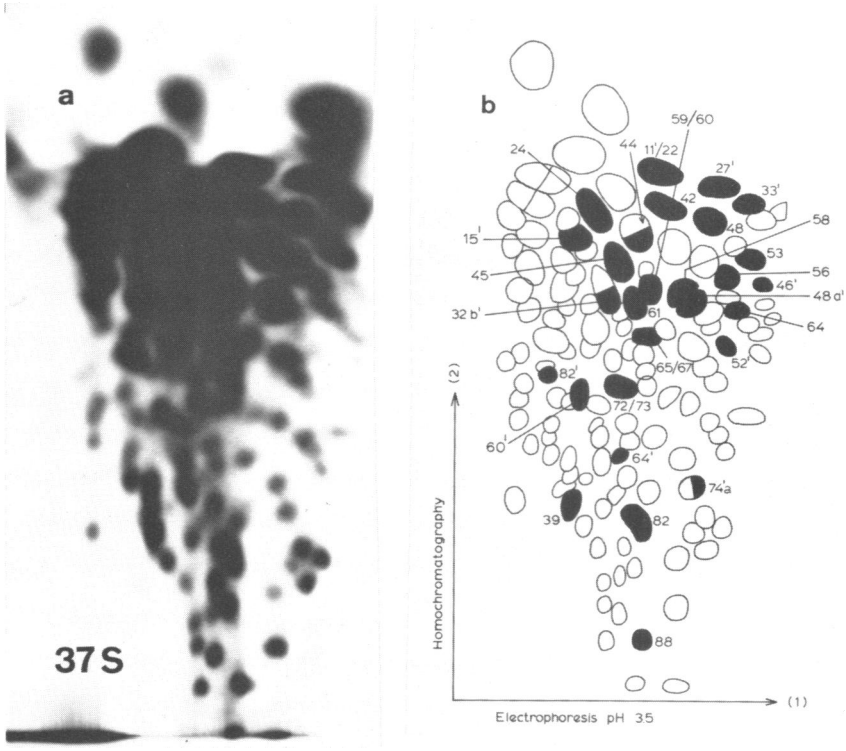

Fig. 3. Two-dimensional separation of the ribonuclease T_1 digestion products of 37-S precursor rRNA. 122 µg ^{32}P-labelled 37-S precursor RNA (2.1×10^6 cpm) was digested with ribonuclease T_1. ψ-containing products are represented as closed circles in the key (b) to the radioautograph (a) and numbered according to Table II.

precursor rRNA. Spot 55 in a digest of 37-S RNA did not show up to contain ψp whereas ψp in spot 55 of 26-S rRNA is apparently present in submolar amounts. Therefore this site might be pseudouridylated at a late stage of the maturation of 26-S rRNA. Spot 87 is missing in 'fingerprints' of 37-S RNA due to a lack of methylation at the Gp residue of ...Um-Gm-ψ..., which takes place at the level of 29-S precursor RNA (6), therefore the corresponding ψp might be present at the 5' end of a different oligonucleotide after ribonuclease T_1 digestion. With the method used here, the presence of such a ψp-containing oligonucleotide could not be established.

DISCUSSION

Analysis of the ψp-residues in 37-S precursor rRNA has shown not only that 37-S RNA and the mature yeast rRNAs contain a nearly identical number of ψp residues, but also that these ψp residues are present in the same set of oligonucleotides in the two cases. Although this method does not strictly prove that each individual ψp site in 37-S RNA corresponds to an identical ψp site in the mature rRNAs, it is very likely that most, if not all, ψp residues in the mature rRNA are present already in the primary transcript. This means that pseudouridylation is an early event in the maturation of a ribosome, taking place immediately after or even during transcription.

Analysis of the ψp-containing oligonucleotides obtained after ribonuclease T_1 digestion of the mature rRNAs shows that only a limited number of sites in the RNA is subjected to this type of modification and that most of the pseudouridines are in homogeneous parts of the rRNA molecules. A similar conclusion was recently drawn by Choi and Busch (28) who sequenced all ribonuclease T_1-generated oligonucleotides of 18-S rRNA of Novikoff hepatoma cells. At least some of the pseudouridines are clustered within yeast 26-S rRNA: we found one oligonucleotide (spot no. 88) which contains 3 pseudouridines within a stretch of 22 contiguous nucleotides, whereas the average degree of pseudouridylation in yeast rRNA is one ψp per 150 nucleotides. Also in Novikoff hepatoma 18-S rRNA several closely spaced ψp's have been observed (28).

Comparison of ψp-containing sites in yeast 17-S and Novikoff hepatoma 18-S rRNA reveals one significant homology so far:

(G)-A-A-A-A-A-A-U-ψ-A-Gp (yeast)

and (G)-A-A-A-A-A-A-ψ-ψ-A-Gp (Novikoff hepatoma (28)).

This sequence has clearly been conserved in eukaryotic small subunit rRNA but the number of pseudouridines differs. On the other hand, for neither U-U-U-A-ψ-U-U-G nor (U-U-U-A,ψ-U-A,U-A)G present in yeast 17-S rRNA a comparable oligonucleotide was found in the 'catalogue' of Novikoff hepatoma 18-S rRNA.

Our data suggest that the non-conserved sequences of 37-S RNA contain very few ψp residues if any. However, an extra sequence of A-A-ψp might be present within these non-conserved regions since the molar yield of A-A-ψp in 37-S RNA is somewhat higher than in 29-S precursor rRNA (cf. Table I).

The general characteristics of pseudouridylation of yeast precursor rRNA appear to be similar to those of 2'-O ribosemethylation: a) both are early modifications, b) both are relatively abundant, c) they occur mainly

within the ribosomal sequences and hardly, if at all, in the non-conserved sequences of precursor rRNA and d) neither exhibits simple sequence specificity. These joint characteristics may suggest a relationship between the functions of both types of modification. This idea is supported by the observation that several sites in mature rRNA contain both types of modifications e.g. -Um-Gm-ψ- and -Um-A-ψ- in yeast 26-S rRNA, -ψm-, -Am-C-ψ and -Um-Gm-ψ- in HeLa cell 28-S rRNA (8) and -Um-U-ψ-,-ψ-Gm- and -ψ-U-Gm- in 18-S rRNA of Novikoff hepatoma cells (28). How are all these sites recognized by the modifying enzymes? If we assume that there is only one (or only a few) ψ-forming enzyme(s) rather than one for each site, the structural feature to be recognized must be present a large number of times within the ribosomal sequence, and virtually absent within the non-conserved stretches of the precursor RNA. It is likely that a specific conformational element is recognized rather than a simple sequence around the uridine to be modified (28, this paper). It may be relevant to know how pseudouridylation and 2'-O ribosemethylation are timed relative to the assembly of 37-S precursor rRNA with ribosomal proteins into the 90-S preribosome. This is difficult to establish because all these events occur within a short period of time between the initiation of transcription and the first nucleolytic cleavage of 37-S RNA. If these modifications take place after assembly of the 37-S RNA with a number of proteins, these proteins may play a role in the recognition as well. One may envisage that the conversions of U into ψ cause subtle changes in the RNA conformation which may favour the assembly of a number of ribosomal proteins and subsequent processing of the preribosomal particles. A similar role may be proposed for 2'-O ribosemethylation. This idea is compatible with the observation that during inhibition of the methylation still functional ribosomes are synthesized, containing undermethylated rRNA, but that the overall efficiency of the whole maturation process is slowed down (16).

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