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The Nucleotide Sequence of *Saccharomyces cerevisiae* 5.8 S Ribosomal Ribonucleic Acid

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SUMMARY

The nucleotide sequence of *Saccharomyces cerevisiae* 5.8 S ribosomal RNA (also known as the 7 S or 1RNA species) has been determined to be pApApApCpUpUpUpCpApApCpA pApCpGpGpApUpCpUpCpUpUpGpGpUpUpCpUpCpGpC pApUpCpGpApUpGpApApGpApApCpGpCpApGpCpGpApA pApUpGpCpGpApUpApCpGpUpApApUpGpUpGpApApΨpUpG pCpApGpApApUpUpCpCpGpUpGpApApUpCpApUpCpGpA pApUpCpUpUpUpGpApApCpGpCpApCpApUpUpGpCpGpC pCpCpCpUpUpGpGpUpApUpUpCpCpApGpGpGpGpGpCpA pUpGpCpCpUpGpUpUpUpGpApGpCpGpUpCpApUpUpU.

Ribosomes from the cytoplasm of eukaryotic cells contain two low molecular weight RNA species: the 5 S and the 5.8 S RNA (1–9). Both RNA species are structural components of the large subunit and each is found in equimolar amount to the 28 S[1] ribosomal RNA (1, 5, 7, 9–11). The 5.8 S RNA is noncovalently bound to the large ribosomal RNA from which it can be dissociated by treatments known to disrupt hydrogen bonding (1–6, 8, 9). Prokaryotic organisms and chloroplasts have only one low molecular weight ribosomal RNA (1, 4, 5). Mitochondria appear to lack both low molecular weight RNAs (12, 13).

The yeast, *Saccharomyces cerevisiae*, is a highly suitable organism for studying the synthesis and processing of ribosomal RNA and the assembly of ribosomes in eukaryotic cells (14, 15). Yeasts are readily subjected to genetic manipulation, and several genes which control ribosome formation have been defined and characterized (16, 17). As these studies continue there will be a need for detailed structural knowledge of the many components involved. This will provide defined markers for following ribosome biogenesis and facilitate study of the relevant RNA-protein and RNA-RNA interactions. The determination of the nucleotide sequence of *S. cerevisiae* 5.8 S ribosomal RNA is described in this paper.

MATERIALS AND METHODS

Strains—Saccharomyces cerevisiae strain A364A *gal*-1 *ade*-1 *ade*-2 *ura*-1 *his*-7 *lys*-2 *tyr*-1 (ATCC no. 22244) was used as a source of 5.8 S RNA.

Low Phosphate Medium—Inorganic phosphate was precipitated (as $MgNH_4PO_4$) from 10% Bacto-yeast extract and 20% Bacto-peptone by the addition of 10 ml of 1 M $MgSO_4$ and 10 ml of concentrated aqueous ammonia per liter. The phosphates were allowed to precipitate at room temperature for 30 min, and the precipitate was removed by filtration through Whatman No. 1 filter paper. The filtrate was adjusted to pH 5.8 with HCl and autoclaved. Sterile glucose was added to a final concentration of 2%.

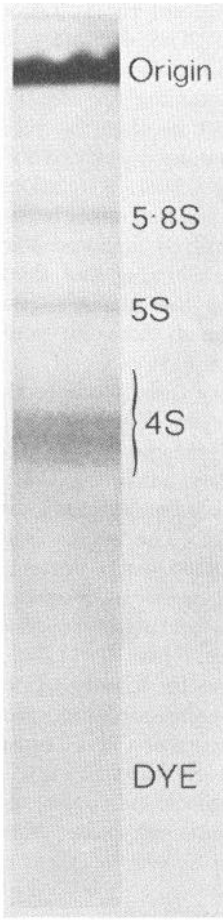

FIG. 1. Radioautograph of a portion of the preparative polyacrylamide gel. *DYE* marks the position of bromphenol blue. The material which remains at the origin consists mainly of the 28 S and 18 S rRNAs. The mobility of the 5.8 S RNA is consistent with its determined nucleotide length.

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[1] The terms 28 S RNA and 45 S RNA are used to denote the general class of RNA. The actual sedimentation constants of these RNAs as isolated from *S. cerevisiae* are 25 S and 35 S as determined by Udem and Warner (14).

Preparation of ^{32}P-Labeled RNA—Cells were grown in 140 ml of low phosphate medium at 28° with vigorous aeration. When the A_{550} of the culture reached 1.5 (approximately 10^7 cells per ml), 20 mCi of carrier-free [^{32}P]phosphoric acid were added. After 5 hours of further growth the cells were harvested by centrifuging for 5 min at 3000 × *g*.

RNA was prepared from both whole cells and partially purified ribosomes. When preparing RNA from whole cells the cell pellet was resuspended in 20 ml of 10 mM EDTA, 10 mM Tris-HCl, pH 7.4, 0.5% sodium lauryl sulfate. An equal volume of water-saturated phenol was added and the mixture was shaken vigorously for 15 min at 60° and then for 30 min at room temperature. The phases were separated by centrifugation and the aqueous phase was re-extracted twice at room temperature with equal volumes of water-saturated phenol. To the aqueous layer were added a 1/10 volume of 2 M sodium acetate, pH 5.0, and 2 volumes of ethanol. The RNA was allowed to precipitate overnight at −20°. The yield of RNA was 7 to 10 mg at a specific activity of 1 to 2 × 10^6 cpm per μg.

When preparing RNA from ribosomes the cell pellet was resuspended in 6 ml of Buffer A (50 mM NaCl, 30 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.4) containing 0.5% each of sodium deoxycholate and Brij 58. The cells were disrupted by blending with 13 ml of No. 8 glass beads (Jencons, Hertfordshire, England) in the 50 ml chamber of a Sorvall Omni-mixer for 2 min at half-speed while the chamber was immersed in ice water. Then 2 mg of bentonite were added to inhibit RNase activity. The glass beads and cell debris were removed by centrifugation at 20,000 rpm for 20 min at 4° in a Beckman 50Ti rotor. The ribosomes were sedimented from the 20,000 rpm supernatant by further centrifugation at 40,000 rpm for 90 min. The ribosomal pellet was resuspended in 5 ml of Buffer A containing 0.2% sodium lauryl sulfate and extracted three times at room temperature with equal volumes of water-saturated phenol. The RNA was allowed to precipitate from the aqueous layer overnight at −20° after the addition of 2 volumes of ethanol.

Purification of 5.8 S RNA—The precipitated RNA was dissolved in 4 M urea, made 20% in sucrose and 0.05% in bromphenol blue, heated 1 min at 65°, and applied to slabs of polyacrylamide gel (18). Each gel was prepared by mixing 0.2 ml of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, 1 ml of freshly prepared 10% ammonium persulfate, and 300 ml of an acrylamide solution (10% acrylamide, 0.5% bisacrylamide in 20 mM Tris-acetate, pH 8.0, 1 mM EDTA, 4 M urea). The electrode buffer was 20 mM Tris-acetate, pH 8.0, 1 mM EDTA, and 4 M urea. Each gel was prerun for 4 hours at 20 ma. Up to 5 mg of RNA could be applied per gel. Electrophoresis was carried out for 18 hours at 18 ma at 4°.

The gel was radioautographed and the band corresponding to 5.8 S RNA was cut out of the slab with a sterile scalpel. The gel band was homogenized in a Potter-Elvehjem homogenizer with 3 ml of 0.5 M NaCl, 0 1 M Tris-HCl, pH 9.1, 10 mM EDTA, containing 2 A_{260} units of *Escherichia coli* tRNA (gift of B. F. C. Clark), and 2 ml of phenol saturated with 0.01 M Tris-HCl, pH 7.6. The mixture was allowed to stand for 30 min on ice. The phases were separated by centrifugation for 15 min in a clinical

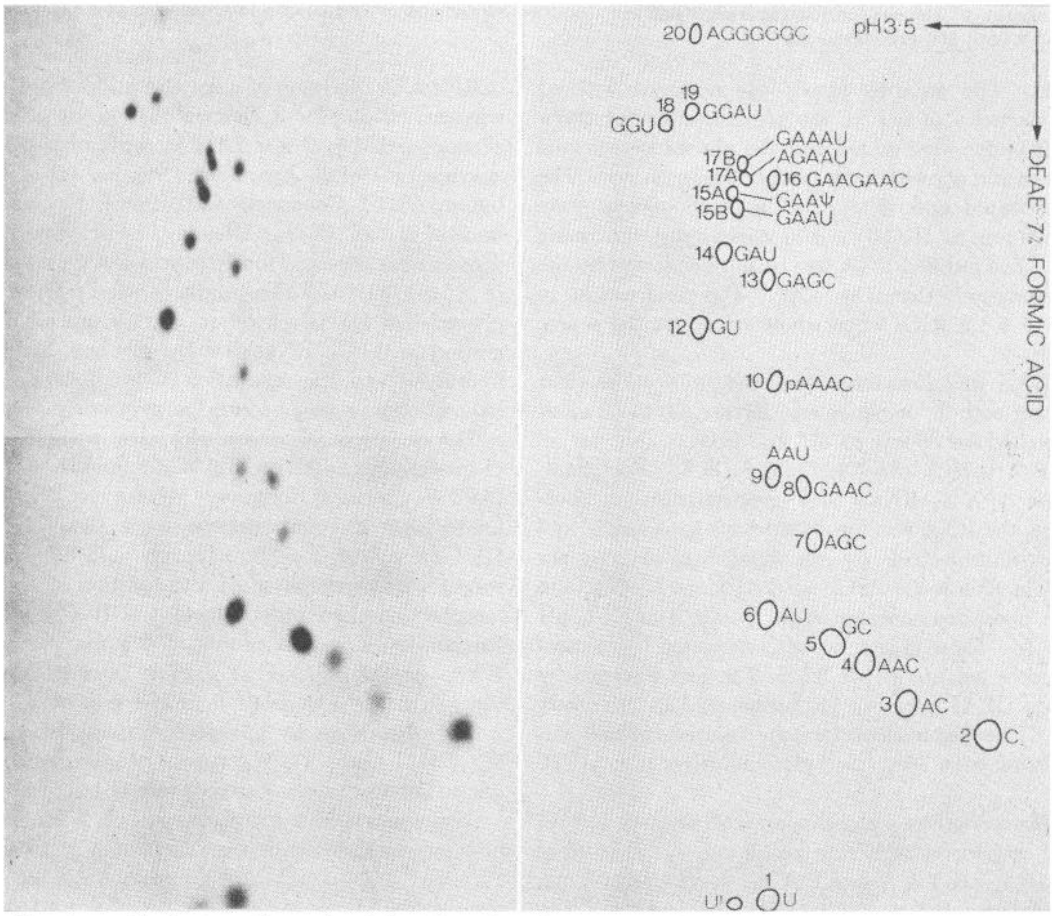

FIG. 2. *Left*, radioautograph of a two-dimensional fractionation of a pancreatic RNase digest of ^{32}P-labeled *Saccharomyces cerevisiae* 5.8 S RNA. Electrophoresis was from right to left on cellulose acetate, at pH 3.5, and from top to bottom on DEAE-paper, in 7% formic acid. *Right*, diagram of the radioautograph showing the deduced sequences of the oligonucleotides. The numbers correspond to those in the text which are prefixed with "P."

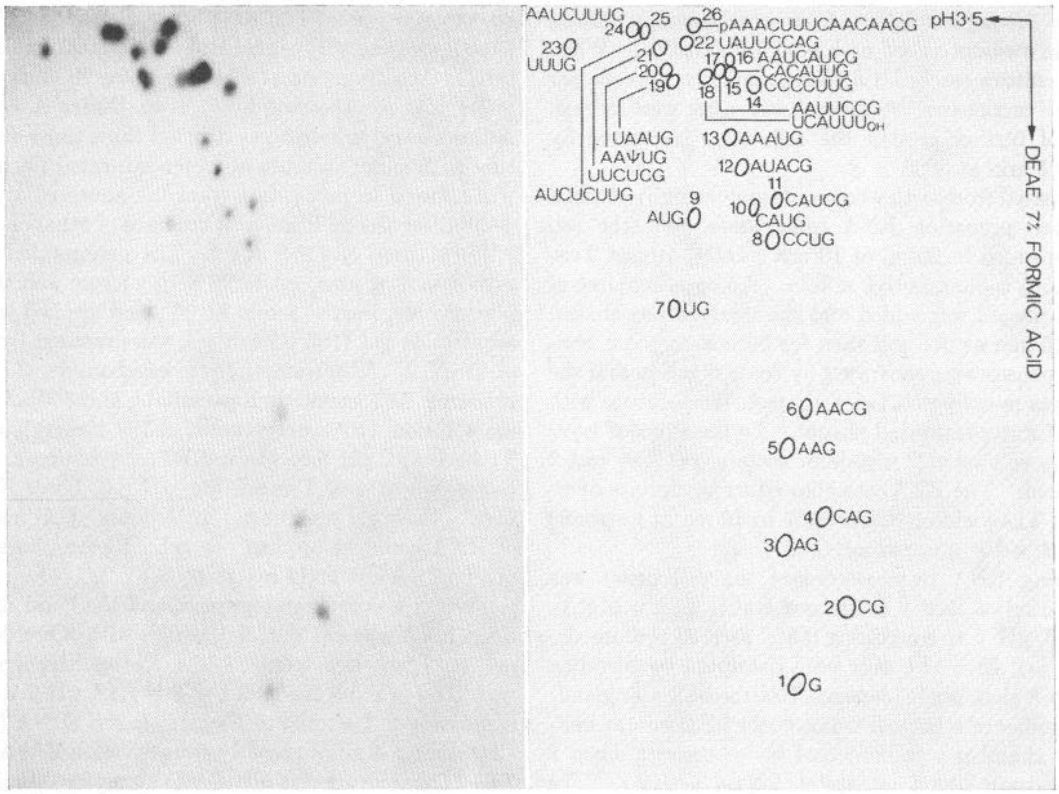

FIG. 3. *Left*, radioautograph of a two-dimensional fractionation of a T_1 RNase digest of ^{32}P-labeled *Saccharomyces cerevisiae* 5.8 S RNA. Electrophoresis was from left to right on cellulose acetate, at pH 3.5, and from top to bottom on DEAE-paper, in 7% formic acid. *Right*, diagram of the radioautograph showing the deduced sequences of the oligonucleotides. The numbers correspond to those in the text which are prefixed with "T."

centrifuge at 4°. The aqueous phase was removed without disturbing the particles of gel at the phenol-water interface. Another 3 ml of buffer were added and the phases were mixed on a Vortex mixer and allowed to stand for 10 min on ice. The phases were separated and the pooled aqueous phases were centrifuged for 20 min at 10,000 × *g* to remove any remaining particles of gel. The purified RNA was then precipitated by the addition of 2 volumes of ethanol at −20°. The yield was 80 to 120 × 10^6 cpm of 5.8 S RNA when whole cells were the source of crude RNA.

Digestion of RNA and Fractionation of Oligonucleotides—For complete digestion with T_1 or pancreatic RNase, 30 to 50 μg of RNA were incubated for 30 min at 37° in 2 μl of 0.5 mg per ml of enzyme, 10 M Tris-HCl, pH 7.4, 1 mM EDTA. For simultaneous digestion with T_1 RNase and bacterial alkaline phosphomonoesterase, the RNA was incubated for 1 hour at 37° in 3 μl of an enzyme solution made by combining 1 μl of 1 mg per ml of T_1 RNase in 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, and 2 μl of alkaline phosphomonoesterase in 50 mM Tris-HCl, pH 8.9, 10 mM $MgCl_2$. These digests were fractionated by electrophoresis on cellulose acetate at pH 3.5 in 7 M urea followed by electrophoresis on DEAE-paper in 7% formic acid as previously described (19). The oligonucleotides were located by radioautography and eluted with 30% triethylamine carbonate, pH 10 (19).

Analysis of Oligonucleotides—The oligonucleotides were further digested with T_1 or pancreatic RNase for 30 min at 37° in 10 μl of 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, containing 0.1 mg per ml of enzyme and 0.2 A_{260} units of *E. coli* tRNA. The products were fractionated by electrophoresis on DEAE-paper at pH 3.5. Oligonucleotides were digested with RNase T_2 and the products separated as previously described (19).

RNase U_2 digestion of oligonucleotides from T_1 RNase digests was carried out for 2 hours at 37° in 10 μl of 50 mM sodium acetate, pH 4.5, 2 mM EDTA, containing 0.1 unit per ml of enzyme (gift of F. Egami), 0.1 mg per ml of bovine serum albumin (BDH Chemicals Ltd., Poole, England), and 0.2 A_{260} units of *E. coli* tRNA. Oligonucleotides from pancreatic RNase digests were digested for 1 hour in the presence of 0.4 A_{260} unit of *E. coli* tRNA. The products were fractionated by electrophoresis on DEAE-paper at pH 1.9 and characterized by digestion with T_1 RNase or by alkaline hydrolysis. Alkaline hydrolysis and the separation of products in this and all subsequent steps were performed as previously described (19).

The reaction of oligonucleotides with *N*-cyclohexyl-*N'*-β-(4-methylmorpholinium)ethylcarbodiimide *p*-toluene sulfonate (Aldrich Chemical Company, Milwaukee, Wisc.) was carried out for 16 hours at room temperature in 10 μl of 100 mg per ml of CMCT[2] in 0.05 N sodium borate, pH 8.5. The modification reaction was terminated by the addition of 5 μl of 0.05 M 2-(*N*-morpholino)ethanesulfonic acid (BDH Chemicals Ltd., Poole, England).[3] Two microliters of 0.5 mg per ml of pancreatic RNase in 10 mM Tris-HCl, pH 7.4, 1 mM EDTA were added and the mixture was incubated for 90 min at 37°. The products were fractionated by electrophoresis at pH 3.5 on Whatman No. 3MM paper. The oligonucleotides were eluted with 5% triethylamine carbonate and characterized by alkaline hydrolysis.

Oligonucleotides from the combined T_1 RNase, alkaline phosphomonoesterase digest were incubated in 10 μl of 50 mM Tris-HCl, pH 8.9, 10 mM $MgCl_2$ containing 5 μg per ml of snake venom phosphodiesterase and 0.4 A_{260} unit of *E. coli* tRNA.

[2] The abbreviation used is: CMCT, *N*-cyclohexyl-*N'*-β-(4-methylmorpholinium) ethylcarbodiimide *p*-toluene sulfonate.

[3] H. L. Weith, personal communication.

Samples of the reaction mixture were taken after 5, 10, and 15 min of incubation at room temperature. These partial digestion products were fractionated by electrophoresis on DEAE-paper at pH 1.9. Snake venom phosphodiesterase was obtained from Worthington Biochemical Corporation, Freehold, New Jersey, and further purified by the procedure of Laskowski (20) (gift of E. Ziff).

For base composition analysis, oligonucleotides were incubated for 1 hour at 37° in 10 μl of 50 mM Tris-HCl, pH 8.9, 10 mM $MgCl_2$ containing 0.1 mg per ml of snake venom phosphodiesterase and 0.2 A_{260} unit of *E. coli* tRNA The products were fractionated by electrophoresis on Whatman 540 paper at pH 3.5.

Screening for Minor Nucleotides—T_1 and pancreatic RNase digestion products were digested to mononucleotides with RNase T_2. These digests were examined for the presence of minor nucleotides by electrophoresis at pH 3.5 on Whatman 540 paper and by ascending chromatography on cellulose thin layers (0.1 mm, E. Merck, Darmstadt, Germany) in the following solvent systems: isobutyric acid (100 ml), 0.5 N aqueous ammonia (60 ml); propan-2-ol (680 ml), HCl (176 ml), and H_2O to 1 liter; propan-2-ol (70 ml), aqueous ammonia (1 ml), H_2O (30 ml).

Preparation of [^{32}P]ΨMP—A mixture of several species of ^{32}P-labeled tRNA from *E. coli* (gift of D. Ish-Horowicz) was digested with T_1 RNase and the resulting oligonucleotides separated as described above. The spot corresponding to TpΨpCpGp, pres-

TABLE I

Analysis of oligonucleotides obtained by complete digestion with pancreatic RNase

Oligonucleotide (a)	RNase T_2 digestion products (e)					T_1 RNase digestion products (f)	Sequence deduced	Molar ratio (c)	
	(b)	Cp	Ap	Gp	Up			Experimental	Predicted
1		-	-	-	+		Up	21.9	22
2		+	-	-	-		Cp	18.0	17
3		1.0	1.0	-	-		ApCp	2.2	2
4		1.0	1.9	-	-		ApApCp	1.9	2
5		1.0	-	1.0	-		GpCp	9.2	9
6		-	1.0	-	1.0		ApUp	6.2	6
7		1.0	1.1	1.0	-	$(ApGp)_{1.1}$ $(Cp)_{1.0}$	ApGpCp	1.1	1
8		1.0	1.9	0.9	-	$(ApApCp)_{1.0}$ $(Gp)_{1.1}$	GpApApCp	0.9	1
9		-	2.1	-	1.0		ApApUp	1.1	1
10	$(pAp)_{0.9}$	1.0	2.0	-	-		pApApApCp	0.6	1
12		-	-	0.9	1.0		GpUp	5.3	5
13		1.0	1.0	1.7	-	$(ApGp)_{0.9}$ $(Gp)_{0.9}$ $(Cp)_{1.0}$	(ApGp, Gp) Cp	1.0	1
14		-	1.0	1.0	1.0	$(ApUp)_{1.0}$ $(Gp)_{0.9}$	GpApUp	2.4	2
15A	$(\Psi p)_{1.0}$	-	2.1	1.1	-	$(ApAp\Psi p)_{1.0}$ $(Gp)_{0.9}$	GpApApΨp	0.7	1
15B		-	1.8	0.9	1.0	$(ApApUp)_{1.0}$ $(Gp)_{0.9}$	GpApApUp	2.0	2
16		1.9	3.9	2.0	-	$(ApApGp)_{1.1}$ $(ApApCp)_{1.0}$ $(Gp)_{1.0}$	(Gp, ApApGp) ApApCp	0.7	1
17A		-	2.9	1.1	1.0	$(ApGp)_{0.9}$ $(ApApUp)_{1.0}$	ApGpApApUp	1.1	1
17B		-	3.0	1.1	1.0	$(ApApApUp)_{1.0}$ $(Gp)_{0.9}$	GpApApApUp	0.7	1
18		-	-	1.9	1.0		GpGpUp	2.2	2
19		-	1.0	1.7	1.0	$(ApUp)_{1.0}$ $(Gp)_{1.7}$	GpGpApUp	1.2	1
20		1.0	1.1	5.2	-	$(ApGp)_{1.2}$ $(Gp)_{3.8}$ $(Cp)_{1.0}$	(ApGp, Gp, Gp, Gp, Gp) Cp	0.4 (d)	1

(a) The numbers refer to the oligonucleotides shown in Figure 2.

(b) One minor nucleotide, Ψp, and one nucleoside diphosphate, pAp, were detected. Ψp was identified as described in the text. pAp was identified by its electrophoretic mobility.

(c) The experimental molar ratios were calculated from the percentage of the total radioactivity found in each spot, and the number of nucleotides in the oligonucleotide. The values are those obtained in a typical experiment. Predicted molar ratios refer to the moles of each oligonucleotide in the final structure.

(d) The efficiency of transfer of this oligonucleotide from the cellulose acetate strip to the DEAE-paper was low. This behavior has been observed previously with pancreatic RNase digestion products containing several consecutive G residues (personal communication, B.G. Barrell).

(e) The base compositions are expressed as the number of moles of each nucleotide relative to 1 mole of Cp or Up. Where yields were not determined quantitatively, the signs + or - indicate the presence or absence of the nucleotide as determined by visual inspection of the radioautograph.

(f) The yields of the T_1 RNase digestion products are expressed as the number of moles of each product relative to 1 mole of the product ending in Cp or Up.

ent in the T_1 RNase digests of most tRNAs (21), was identified by its position, its relative abundance, and its alkaline hydrolysis products. These alkaline hydrolysis products were separated and the fastest moving spot, containing a mixture Tp, Ψp, and a small amount of Up, was eluted with water. This mixture of nucleotides was concentrated by evaporation and resolved by ascending chromatography on cellulose thin layers with propan-2-ol:HCl as described above. The R_F values of Tp, Up, and Ψp in this system are 0.81, 0.71, and 0.51.[4] The Ψp was eluted with water.

[4] B. G. Barrell, personal communication.

Partial Digestion with T_1 and Pancreatic RNase—Limited T_1 and pancreatic RNase digestion products were prepared by incubating 5.8 S RNA for 30 min at 0° with 3 μl of 10 mM Tris-HCl, pH 7.4, 1 mM EDTA containing either 1, 5, or 20 μg per ml of enzyme and 1 A_{260} unit of *E. coli* tRNA. The products were fractionated by electrophoresis on cellulose acetate at pH 3.5 in 7 M urea, 5 mM EDTA followed by homochromatography (19) on thin layers of DEAE-cellulose (polygram CEL 300 DEAE, 0.1 mm, Macherey-Nagel & Co., Duren, Germany or made as described in Reference 19). Each oligonucleotide was eluted and divided into two equal aliquots, one of which was

TABLE II

Analysis of oligonucleotides obtained by complete digestion with T_1 RNase

Oligonucleotide (a)	RNase T_2 digestion products (e)					Pancreatic RNase digestion products (f)	Sequence deduced	Molar ratio (b)	
	(d)	Cp	Ap	Gp	Up			Experimental	Predicted
1		-	-	+	-		Gp	7.6	7
2		1.0	-	1.0	-		CpGp	4.5	4
3		-	1.0	1.0	-		ApGp	1.2	1
4		1.0	1.0	1.0	-	$(Cp)_{1.0}$ $(ApGp)_{1.0}$	CpApGp	1.9	2
5		-	1.9	1.0	-		ApApGp	0.9	1
6		1.0	1.9	1.0	-	$(ApApCp)_{1.0}$ $(Gp)_{1.0}$	ApApCpGp	1.4	2
7		-	-	1.0	1.0		UpGp	2.4	2
8		1.9	-	1.0	1.1		(Cp, Cp, Up) Gp	1.1	1
9		-	1.1	1.0	1.1	$(ApUp)_{1.0}$ $(Gp)_{1.0}$	ApUpGp	1.1	1
10		0.9	0.9	1.0	1.0	$(ApUp)_{1.1}$ $(Cp)_{1.0}$ $(Gp)_{1.0}$	(Cp, ApUp) Gp	1.0	1
11		2.0	1.0	1.0	1.1	$(ApUp)_{1.1}$ $(Cp)_{2.1}$ $(Gp)_{1.0}$	(Cp, Cp, ApUp) Gp	1.0	1
12		1.0	2.0	1.0	1.0	$(ApCp)_{1.2}$ $(ApUp)_{1.1}$ $(Gp)_{1.0}$	(ApUp, ApCp) Gp	1.0	1
13		-	2.8	1.0	1.0	$(ApApApUp)_{1.0}$ $(Gp)_{1.0}$	ApApApUpGp	1.0	1
14		4.0	-	1.0	2.0		(Cp, Cp, Cp, Cp, Up, Up) Gp	1.1	1
15		1.8	1.9	1.0	1.9	$(ApCp)_{0.9}$ $(ApUp)_{1.1}$ $(Cp)_{1.2}$ $(Up)_{1.0}$ $(Gp)_{1.0}$	(Cp, Up, ApCp, ApUp) Gp	0.8	1
16		1.8	2.8	1.0	2.0	$(ApApUp)_{1.1}$ $(ApUp)_{1.1}$ $(Cp)_{2.1}$ $(Gp)_{1.0}$	(ApApUp, ApUp, Cp, Cp) Gp	0.9	1
17		2.1	2.0	1.0	2.3	$(ApApUp)_{1.2}$ $(Cp)_{2.1}$ $(Up)_{1.4}$ $(Gp)_{1.0}$	(ApApUp, Cp, Cp, Up) Gp	1.0	1
18		1.0	1.1	-	2.8	$(ApUp)_{1.0}$ $(Cp)_{0.9}$ $(Up)_{2.0}$	(ApUp, Cp, Up, Up) N	0.9	1
19		-	1.8	1.0	1.8	$(ApApUp)_{1.1}$ $(Up)_{1.1}$ $(Gp)_{1.0}$	(ApApUp, Up) Gp	0.9	1
20	$(Ψp)_{1.0}$	-	1.7	1.0	1.9	$(ApApΨp)_{1.0}$ $(Up)_{1.0}$ $(Gp)_{1.0}$	(ApApΨp, Up) Gp	1.0	1
21		1.8	-	1.0	2.8		(Cp, Cp, Up, Up, Up) Gp	1.1	1
22		2.1	2.0	1.0	3.1	$(ApUp)_{1.0}$ $(Cp)_{2.1}$ $(Up)_{2.3}$ $(ApGp)_{1.0}$	(Cp, Cp, Up, Up, ApUp) ApGp	1.2	1
23		-	-	1.0	3.0		UpUpUpGp	1.1	1
24		1.1	2.0	1.0	4.1	$(ApApUp)_{1.2}$ $(Cp)_{1.1}$ $(Up)_{3.3}$ $(Gp)_{1.0}$	(ApApUp, Cp, Up, Up, Up) Gp	1.3	1
25		2.0	1.1	1.0	4.0	$(ApUp)_{1.1}$ $(Cp)_{2.2}$ $(Up)_{3.3}$ $(Gp)_{1.0}$	(ApUp, Cp, Cp, Up, Up, Up) Gp	0.8	1
26	pAp	4.0	6.1	1.0	(c)	$(pApApApCp)_{1.1}$ $(ApApCp)_{2.1}$ $(Cp)_{1.0}$ $(Up)_{3.0}$ $(Gp)_{1.0}$	pApApApCp(ApApCp, ApApCp, Cp, Up, Up, Up) Gp	0.9	1

Footnotes on the following page.

TABLE II—*continued*

(a) The numbers refer to the oligonucleotides shown in Figure 3.

(b) The experimental molar ratios were calculated from the percentage of the total radioactivity found in each spot, and the number of nucleotides in the oligonucleotide. The values are those obtained in a typical experiment. Predicted molar ratios refer to the moles of each oligonucleotide in the final structure.

(c) Separate quantitation of pAp and Up was not possible since these nucleotides are not well separated by electrophoresis at pH 3.5. The total radioactivity in the combined Up and pAp spot was equivalent to 4.8 moles of phosphate, which is consistent with the 5.0 moles predicted by the sequence deduced for this oligonucleotide.

(d) One minor nucleotide, Ψp, and one nucleoside diphosphate, pAp, were detected. Ψp was identified as described in the text. pAp was identified by its electrophoretic mobility.

(e) The base compositions are expressed as the number of moles of each nucleotide relative to 1 mole of Gp. Where yields were not determined quantitatively, the signs + or - indicate the presence or absence of the nucleotide as determined by visual inspection of the radioautograph.

(f) The yields of the pancreatic RNase digestion products are expressed as the number of moles of each product relative to 1 mole of the product ending in Gp, except for the 3'-terminal oligonucleotide, T18, in which case the yields are expressed relative to 1 mole of ApUp.

TABLE III

Further analysis of oligonucleotides obtained by complete digestion with T_1 RNase

Oligonucleotide	Sequence deduced from T_2 and pancreatic RNase digestion products (see Table 1)	Products of digestion with RNase U_2 (a)	Products of pancreatic RNase digestion of CMCT blocked oligonucleotide (b)	Sequence deduced
8	(Cp, Cp, Up) Gp		Cp, ÚpĠp	CpCpUpGp
10	(Cp, ApUp) Gp	CpAp, UpGp	Cp, ApÚpĠp	CpApUpGp
11	(Cp, Cp, ApUp) Gp	CpAp, (Cp, Up) Gp	Cp, Ġp, ApÚpCp	CpApUpCpGp
12	(ApUp, ApCp) Gp	CpGp, (Ap, Up) Ap	Ġp, (Ap, Ap, Úp) Cp	ApUpApCpGp
15	(Cp, Up, ApCp, ApUp) Gp	CpAp, UpUpGp	Cp, ApCp, streak (c)	CpApCpApUpUpGp
16	(ApApUp, ApUp, Cp, Cp) Gp	ApAp, (Up, Cp) Ap, (Up, Cp) Gp	Ġp, (Ap, Ap, Úp) Cp, (Ap, Úp) Cp	ApApUpCpApUpCpGp
17	(ApApUp, Cp, Cp, Up) Gp	ApAp, (Cp, Cp, Up, Up) Gp	Ġp, Cp, (Ap, Ap, Úp, Úp) Cp	ApApUpUpCpCpGp
18	(ApUp, Cp, Up, Up) N	(Up, Cp) Ap, UpUpN	UpCp, streak (c)	UpCpApUpUpN
19	(ApApUp, Up) Gp	UpApAp, UpGp		UpApApUpGp
20	(ApApΨp, Up) Gp	ApAp, (Up, Ψp) Gp		ApApΨpUpGp
21	(Cp, Cp, Up, Up, Up) Gp		Ġp, ÚpCp, ÚpÚpCp	(UpUpCp, UpCp) Gp
22	(Cp, Cp, Up, Up, ApUp) ApGp	UpAp, (Cp, Cp, Up, Up) Ap, Gp	(Ap, Úp, Úp, Úp) Cp, ApĠp, Cp	UpApUpUpCpCpApGp
24	(ApApUp, Cp, Up, Up, Up) Gp	ApAp, (Cp, Up, Up, Up, Up) Gp	(Ap, Ap, Úp) Cp, streak (c)	ApApUpCpUpUpUpGp
25	(ApUp, Cp, Cp, Up, Up, Up) Gp	Ap, (Cp, Cp, Up, Up, Up, Up) Gp	(Ap, Úp) Cp, ÚpCp, ÚpÚpĠp	ApUpCpUpCpUpUpGp
26	pApApApCp (ApApCp, ApApCp, Up, Up, Up) Gp	CpGp, CpApAp (d)	pApApApCp, ApApCp, ÚpÚpÚpCp Gp	pApApApCp (ApApCp, UpUpUpCp) ApApCpGp

(a) The digestion conditions were such as to preserve ApA bonds (19). Only the major products are shown. Products resulting from the cleavage of ApA bonds or from the incomplete cleavage of ApN bonds were often present in low yield. In all cases these products were consistent with the sequence deduced.

(b) Úp and Gp represent the CMCT-modified form of the nucleotides Up and Gp.

(c) In these cases a large fraction of the material migrated as a streak toward the cathode and thus was unsuitable for further analysis. Oligonucleotides with a high proportion of CMCT-modified bases, such as ÚpÚpÚpĠp, typically behave in this manner (personal communication, B.G. Barrell).

(d) Although other products were isolated, analysis of those containing Up or pAp was hindered by the fact that Up and pAp are not well separated by electrophoresis at pH 3.5.

digested with T_1 RNase and the other with pancreatic RNase. This digestion was carried out for 90 min at 37° in 10 µl of 10 mM Tris-HCl, pH 7.4, 1 mM EDTA containing 0.2 mg per ml of the appropriate enzyme. These digests were fractionated by electrophoresis of DEAE-paper in 7% formic acid alongside known markers. The identity of the products was verified by further enzymatic digestion. The limited pancreatic RNase product p55 (see below) also analyzed by partial digestion with spleen phosphodiesterase (19).

TABLE IV

Products obtained by partial degradation with snake venom phosphodiesterase of dephosphorylated oligonucleotides derived from simultaneous digestion of 5.8 S RNA with T_1 RNase plus alkaline phosphomonoesterase

Oligonucleotide (a)	Products Isolated (b)	Sequence Deduced
D14	CpCpCpCpU	CpCpCpCpUpUpG
	CpCpCpCpUpU	
	CpCpCpCpUpUpG	
D17	ApApUpU	ApApUpUpCpCpG
	ApApUpUpC	
	ApApUpUpCpC	
	ApApUpUpCpCpG	
D18	UpCpApU	UpCpApUpUpN
	UpCpApUpU	
	UpCpApUpUpN	
D21	UpU	UpUpCpUpCpG
	UpUpC	
	UpUpCpU	
	UpUpCpUpC	
	UpUpCpUpCpG	
D22	UpApUpUpC	UpApUpUpCpCpApG
	UpApUpUpCpC	
	UpApUpUpCpCpA	
	UpApUpUpCpCpApG	
D24	ApApUpCpU	ApApUpCpUpUpUpG
	ApApUpCpUpU	
	ApApUpCpUpUpU	
	ApApUpCpUpUpUpG	
D25	ApUpCpUpC	ApUpCpUpCpUpUpG
	ApUpCpUpCpU	
	ApUpCpUpCpUpU	
	ApUpCpUpCpUpUpG	

(a) The number of the oligonucleotide refers to the number of the corresponding product of digestion with T_1 RNase. The numbers are prefixed with 'D' to indicate that they refer to the dephosphorylated form of the oligonucleotide.

(b) The sequences of the products were determined by digestion with pancreatic RNase, by digestion with pancreatic RNase after modification with CMCT (in the case of D21 only), and from the data presented in Tables II and III.

RESULTS

Fig. 1 shows a radioautograph of a preparative polyacrylamide gel after electrophoresis of ^{32}P-labeled yeast RNA. The 5.8 S RNA produced a sharp band with mobility approximately three-fifths that of the 5 S RNA.

When 5.8 S RNA was digested with either pancreatic RNase (Fig. 2) or T_1 RNase (Fig. 3) and fractionated as described above, a consistent pattern of oligonucleotides was obtained. All of the digestion products could be resolved in this way. The same pattern was obtained whether the RNA was prepared from whole cells or from partially purified ribosomes. Subsequently, RNA was prepared from whole cells because of the higher yields and ease of this method.

Table I summarizes the results of base composition analysis and T_1 RNase digestion of the products from the pancreatic RNase digest of 5.8 S RNA and presents the relative molar yields of these products. The sequence of all of the pancreatic RNase digestion products was determined from the data in Table I,

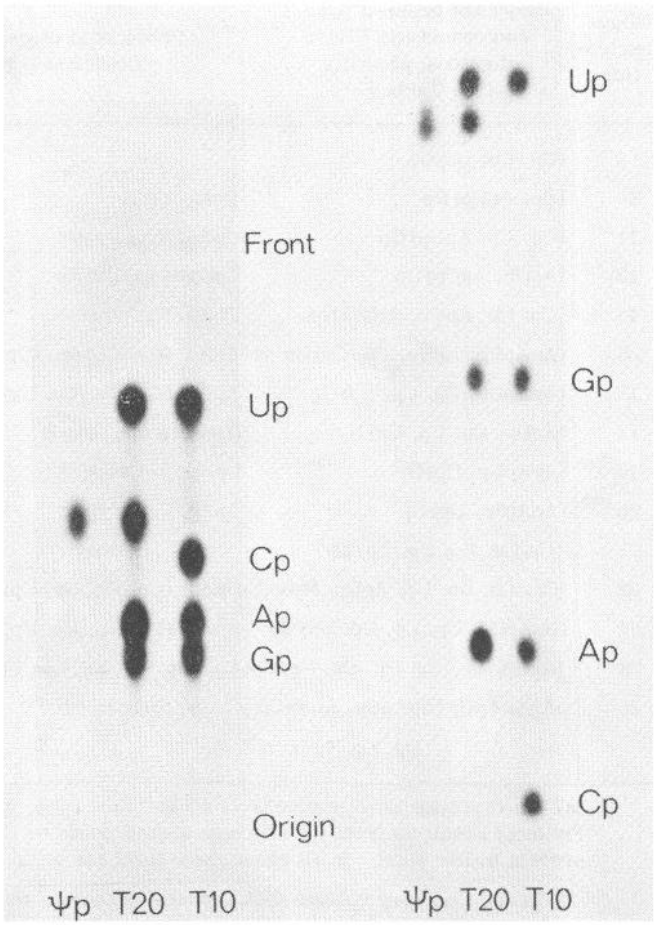

FIG. 4. Radioautographs of the products of RNase T_2 digestion of oligonucleotides T20 and T10 after fractionation alongside marker Ψp. *Left*, fractionation by chromatography on cellulose thin layers with propan-2-ol:HCl as described in the text. *Front* indicates the position of the solvent front. *Right*, fractionation by electrophoresis at pH 3.5 on Whatman 540 paper.

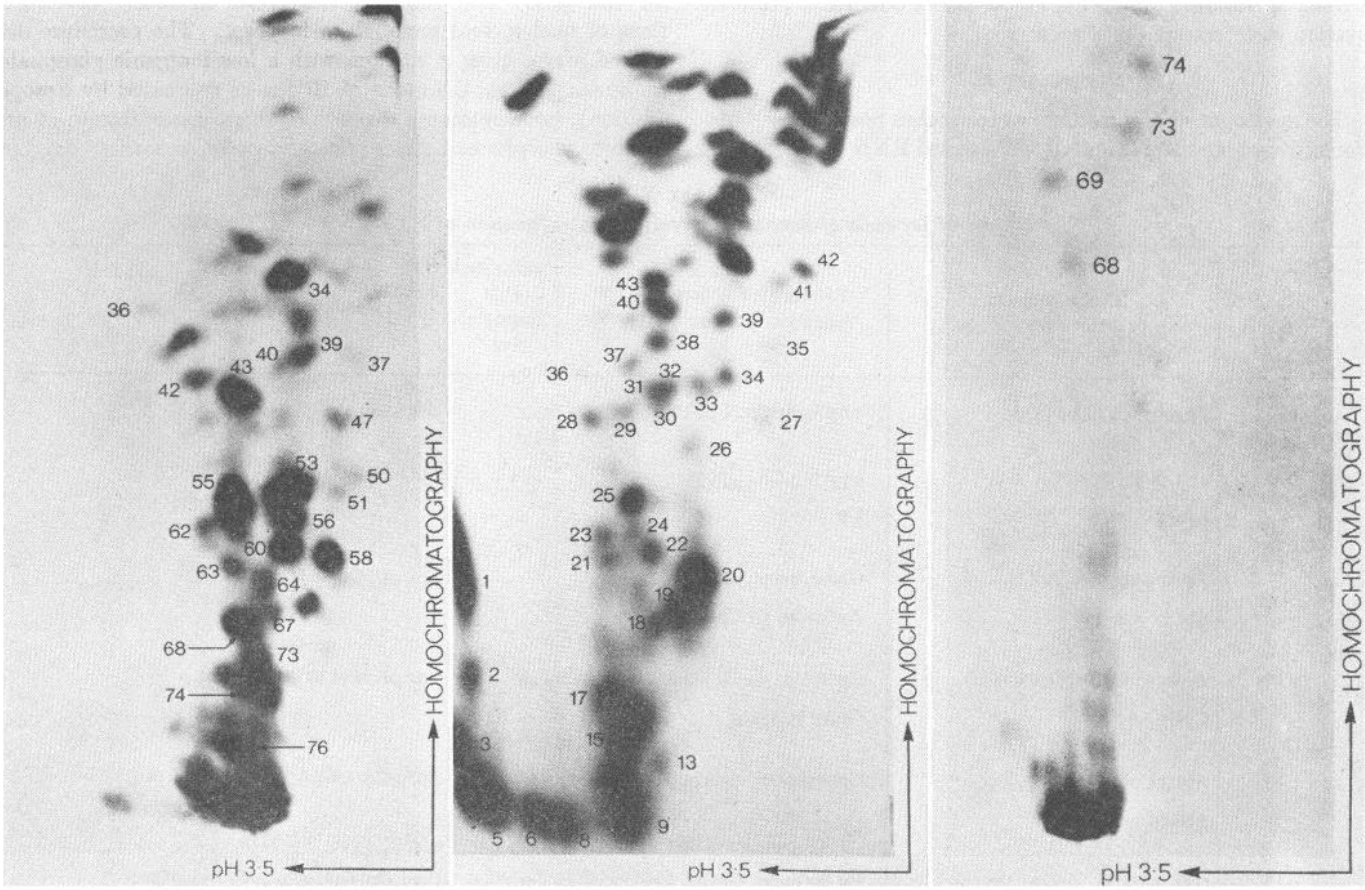

FIG. 5 (*left*). Radioautograph of a two-dimensional fractionation of a limited pancreatic RNase digest of *Saccharomyces cerevisiae* 5.8 S RNA. The digest was performed as described in the text using 5 μg per ml of pancreatic RNase. The *numbered spots* were eluted for further analysis (see Table V). In cases where two spots were not completely resolved only the outer edge of the spot was eluted in order to obtain a pure fragment.

FIG. 6 (*center*). Radioautograph of a two-dimensional fractionation of a limited T_1 RNase digest of *Saccharomyces cerevisiae* 5.8 S RNA. The digest was performed as described in the text using 5 μg per ml of T_1 RNase. The *numbered spots* were eluted for further analysis (see Table VI). In cases where two spots were not completely resolved only the outer edge of the spot was eluted in order to obtain a pure fragment.

FIG. 7 (*right*). Radioautograph of a two-dimensional fractionation of a limited T_1 RNase digest of *Saccharomyces cerevisiae* 5.8 S RNA. The digest was performed as described in the text using 1 μg per ml of T_1 RNase. The *numbered spots* were eluted for further analysis (see Table VI).

except for oligonucleotides P13, P16, and P20. P13 yielded GpAp and GpCp when partially digested with RNase U_2, thus establishing its sequence as GpApGpCp. The limited T_1 RNase digestion product t41 (ApApGpApApCpGp) determines the sequence of P16 as GpApApGpApApCp. The limited pancreatic RNase digestion product p73 (see below) establishes the sequence of P20 as ApGpGpGpGpGpCp.

Table II summarizes the results of base composition analysis and pancreatic RNase digestion of the products from the T_1 RNase digest of 5.8 S RNA and presents the relative molar yield of these products. Many products required further analysis in order to establish their sequence. Table III summarizes the results of digestion of some of these oligonucleotides with RNase U_2 and with pancreatic RNase after the reaction of the oligonucleotide with CMCT. The sequence of all of the T_1 RNase digestion products can be determined from the combined data presented in Tables II and III, except for oligonucleotides T14, T18, T21, and T26.

Extensive digestion of oligonucleotide T18 with snake venom phosphodiesterase released pA, pU, and pC in the relative molar yields of 1.0, 3.1, and 1.1. Comparison of this result with the base composition analysis presented in Table II identifies the 3′-terminal nucleoside as U and thereby establishes the sequence of T18 as UpCpApUpUpU. The limited pancreatic digestion product p34 (see below) determines the sequence of T26 as pApApApCpUpUpU CpApApCpApApCpGp.

Table IV presents the results obtained by partial degradation with snake venom phosphodiesterase of oligonucleotides derived from simultaneous digestion of 5.8 S RNA with T_1 RNase and alkaline phosphomonoesterase. These results establish the sequence of oligonucleotides T14 and T21 and confirm the sequences deduced above for oligonucleotides T17, T18, T22, T24, and T25.

Screening for minor nucleotides revealed one nucleotide present in oligonucleotides T20 and P15A with electrophoretic and chromatographic mobilities different from Up, Ap, Cp, and Gp. The behavior of this nucleotide is consistent with that of Ψp (Fig. 4).

Radioautographs of fractionated partial T_1 and pancreatic RNase digests are shown in Figs. 5, 6, 7. All of the partial digestion products necessary to determine a unique sequence for 5.8 S RNA were obtained from these digests except p5 and p15. These were obtained from another partial pancreatic RNase digest. The analysis of the partial digestion products is de-

scribed in Tables V and VI. Fig. 8 shows how these products overlap to determine the sequence.

DISCUSSION

The development of a medium which makes possible the isolation of very high specific activity ^{32}P-labeled RNA was necessary for this study and should prove useful in future investigations of nucleic acid metabolism in yeast. The procedure described above gives a medium with a low inorganic phosphate concentration (on the order of 10^{-4} M as estimated by isotope dilution), but containing a relatively high concentration of organic phosphates. Since *Saccharomyces cerevisiae* has an

TABLE V

Analysis of products of partial pancreatic RNase digestion of 5.8 S RNA

Fragment No. (a)	T_1 RNase digestion products (b)	Pancreatic RNase digestion products (b)	Additional data required (c)	Sequence deduced
p34	pApApApCpUpUpUpCp	pApApApCp, Cp, Up	-	pApApApCpUpUpUpCp
p36	UpUpUpGp, ApGp, Gp, Up, CpGp	GpApGpCp, GpUp, Up	t74	GpUpUpUpGpApGpCpGpUp
p37	ApApApUpGp, ApGp, CpGp, Cp	GpApApApUp, ApGpCp, GpCp	-	ApGpCpGpApApApUpGpCp
p39	ApApApUpGp, Gp, CpGp, ApUp	GpApUp, GpCp GpApApApUp	p37	GpApApApUpGpCpGpApUp
p40	ApUpCpUpCpUpUp, Gp, ApApCpGp	GpGpApUp, ApApCp, Up, Cp	-	ApApCpGpGpApUpCpUpCpUpUp
p42	(Up, Cp) Gp, ApGp, Up, UpUpUpGp, CpGp	GpApGpCp, GpUp, Up, Cp	p36	(Up, Cp) GpUpUpUpGpApGpCp GpUp
p43	ApApΨpUpGp, UpGp, Cp, ApApUpGp	GpApApΨp, GpUp, Up, ApApUp, GpCp	p5	ApApUpGpUpGpApApΨpUpGpCp
p47	pApApApCpUpUpUpCpApApCp	pApApApCp, ApApCp, Up, Cp	p34	pApApApCpUpUpUpCpApApCp
p50	ApApCpGp, ApApGp, ApUpGp, CpGp, Cp	GpApApGpApApCp, GpApUp, GpCp, Cp	p51	CpGpApUpGpApApGpApApCpGp Cp
p51	ApUpCpGp, ApUpGp, ApApGp, ApApCp	ApUp, GpApUp, Cp, GpApApGpApApCp	-	ApUpCpGpApUpGpApApGpApApCp
p53	ApApApUpGp, ApGp, ApUp, CpGp	GpApApApUp, GpCp, GpApUp, ApGpCp	p37	ApGpCpGpApApApUpGpCpGpApUp
p55	ApUpUpGp, CpGp, Gp, Up CpCpCpCpUpUpGp	GpGpUp, ApUp, GpCp, Up, Cp	(d)	ApUpUpGpCpGpCpCpCpCpUpUpGpGpUp
p56	ApApUpUpCpCpGp, UpGp, ApGp, ApApUpCp	ApGpApApUp, Up, GpUp, GpApApUp, Cp		ApGpApApUpUpCpCpGpUpGpApApUpCp
p58	ApUpCpGp, ApUpGp, Cp, ApApCpGp, ApApGp	GpApApGpApApCp, GpCp, GpApUp, ApUp, Cp	p51	ApUpCpGpApUpGpApApGpApApCpGpCp
p60	ApApUpCpUpUpUpGp, Cp ApUpCpGp, ApApCpGp	GpApApUp, ApUp, Cp, Up GpApApCp, GpCp	t31	ApUpCpGpApApUpCpUpUpUpGpApAp CpGpCp

TABLE V—*continued*

Fragment No. (a)	T_1 RNase digestion products (b)	Pancreatic RNase digestion products (b)	Additional data required (c)	Sequence deduced
p62	UpUpUpGp, ApUpGp, Up, CpCpUpGp, ApGp, CpGp	GpApGpCp, ApUp, GpCp, GpUp, Cp, Up	p36	ApUpGpCpCpUpGpUpUpUpGpApGpCp GpUp
p63	ApUpCpUpCpUpUpGp, Gp, UpUpCpUpCp, ApApCpGp	GpGpApUp, GpGpUp, ApApCp, $(Cp)_4$ $(Up)_5$	-	ApApCpGpGpApUpCpUpCpUpUpGpGp UpUpCpUpCp
p64	CpCpCpCpUpUpGp, Gp, Up, ApCpApUpUpGp, CpGp	GpCp, GpGpUp, ApUp, Cp, Up, ApCp	p55	ApCpApUpUpGpCpGpCpCpCpCpUpUpGp GpUp
p67	ApApApUpGp, ApGp, Up, ApUpApCpGp, CpGp	GpApApApUp, GpUp, GpApUp, ApGpCp, ApCp, GpCp	p53	ApGpCpGpApApApUpGpCpGpApUpApCp GpUp
p68	ApUpCpUpCpUpUpGp, Cp, Gp, UpUpCpUpCpGp, ApApCpGp	GpGpUp, GpGpApUp, GpCp, ApApCp, $(Up)_5$, $(Cp)_4$	p63	ApApCpGpGpApUpCpUpCpUpUp GpGpUpUpCpUpCpGpCp
p73	ApUpUpCpCpApGp, Cp, $(Gp)_4$ CpApUpGp	ApGpGpGpGpGpCp, Up, Cp, ApUp, GpCp	-	ApUpUpCpCpApGpGpGpGpGpCp ApUpGpCp
p74	ApUpUpCpCpApGp, $(Gp)_4$, CpApUpGp, CpCpUp	ApGpGpGpGpGpCp, Up, Cp, ApUp, GpCp	-	ApUpUpCpCpApGpGpGpGpGpCp ApUpGpCpCpUp
p76	ApApUpUpCpCpGp, UpGp, ApApUpGp, CpApGp, ApApUpCp, ApApΨpUpGp	ApGpApApUp, GpApApUp, Up ApApUp, GpApApΨp, GpUp, GpCp, Cp	t32 p43	ApApUpGpUpGpApApΨpUpGpCp ApGpApApUpUpCpCpGpUpGpAp ApUpCp
p5	ApApUpGp, Up	ApApUp, GpUp	-	ApApUpGpUp
p15	ApApΨpUpGp, Gp, Cp	GpApApΨp, GpCp, Up	-	GpApApΨpUpGpCp

(a) The numbers refer to the partial digestion products shown in Figure 5.

(b) The products of complete T_1 or pancreatic RNase digestion of the partial digestion products were fractionated and identified as described in the text. The notation for the relative molar yields of the products is no underline, one underline, two underlines, and subscript numbers 4-9 for relative molar yields of 1, 2, 3, and 4-9, respectively. Relative molar yields of 1 and 2 were estimated by visual inspection of the radioautograph. Relative molar yields of greater than 2 of pancreatic RNase digestion products were calculated from the sequences of the products obtained by T_1 RNase digestion of the fragment. Relative molar yields of greater than 2 of T_1 RNase digestion products were calculated from the sequences of the products obtained by pancreatic RNase digestion of the fragment.

(c) In some cases, more than one sequence for the fragment was consistent with its T_1 and pancreatic RNase digestion products. In these cases, it was necessary to refer to the sequence of additional fragments to determine a unique sequence for the fragment in question.

(d) The results of partial digestion with spleen phosphodiesterase were also used in the determination of a unique sequence for this fragment.

TABLE VI

Analysis of products of partial T_1 RNase digestion of 5.8 S RNA

Fragment No. (a)	T_1 RNase digestion products (b)	Pancreatic RNase digestion products (b)	Additional data required (c)	Sequence deduced
t74	UpCpApUpUpU, CpGp	GpUp, ApUp, Cp, Up	-	CpGpUpCpApUpUpU
t73	ApGp, UpCpApUpUpU, CpGp	ApGpCp, GpUp, ApUp, Cp, Up	-	ApGpCpGpUpCpApUpUpU
t69	UpUpUpGp, ApGp, CpGp, UpCpApUpUpU	GpApGpCp, GpUp, Cp, ApUp, $(Up)_4$	-	UpUpUpGpApGpCpGpUpCpAp UpUpU
t68	CpCpUpGp, UpUpUpGp, CpGp, ApGp, UpCpApUpUpU	GpApGpCp, ApUp, GpUp, Cp, $(Up)_4$	t74	CpCpUpGpUpUpUpGpApGpCp GpUpCpApUpUpU
t43	ApApUpUpCpCpGp, UpGp	ApApUp, GpUp, Gp, Up, Cp	-	ApApUpUpCpCpGpUpGp
t42	ApApCpGp, CpApGp	GpCp, ApApCp, ApGp	-	ApApCpGpCpApGp
t41	ApApCpGp, ApApGp	ApApGpApApCp, Gp	-	ApApGpApApCpGp
t40	UpUpCpUpCpGp, CpApUpCpGp	GpCp, ApUp, Gp, Cp, Up,	-	UpUpCpUpCpGpCpApUpCpGp
t39	ApApUpUpCpCpGp, CpApGp	ApGpApApUp, Gp, Cp, Up	-	CpApGpApApUpUpCpCpGp
t38	UpApApUpGp, ApUpApCpGp	ApApUp, GpUp, ApUp, ApCp, Gp	-	ApUpApCpGpUpApApUpGp
t37	UpUpCpUpCpGp, Gp, CpApUpCpGp	GpUp, ApUp, GpCp, Gp, Up, Cp	t40	GpUpUpCpUpCpGpCpAp UpCpGp
t36	UpApApUpGp, UpGp, ApApΨpUpGp	GpApApΨp, ApApUp, GpUp, Up, Gp	p5, p15	UpApApUpGpUpGpApApΨp UpGp
t35	CpGp, CpApGp, ApApCpGp	ApGpCp, GpCp, Gp, ApApCp	-	ApApCpGpCpApGpCpGp
t34	CpApCpApUpUpGp, ApApCpGp	ApApCp, ApUp, ApCp, GpCp, Up, Gp	-	ApApCpGpCpApCpApUpUpGp
t32	ApApUpUpCpCpGp, UpGp, CpApGp	ApGpApApUp, GpUp, Up, Gp, Cp	-	CpApGpApApUpUpCpCpGpUp Gp
t31	ApApUpCpUpUpUpGp, ApApCpGp	ApApUp, GpApApCp, Gp, Cp, Up	-	ApApUpCpUpUpUpGpApApCp Gp
t30	UpApApUpGp, CpGp, ApUpApCpGp	GpApUp, ApApUp, Gp, ApCp, GpUp, Cp	t38	CpGpApUpApCpGpUpApApUp Gp
t29	UpUpCpUpCpGp, ApUpGp, CpApUpCpGp	GpApUp, ApUp, GpCp, Up, Cp, Gp	t40	UpUpCpUpCpGpCpApUpCpGp ApUpGp

TABLE VI—*continued*

Fragment No. (a)	T_1 RNase digestion products (b)	Pancreatic RNase digestion products (b)	Additional data required (c)	Sequence deduced
t28	ApUpCpUpCpUpUpGp, Gp, UpUpCpUpCpGp	GpGpUp, ApUp, Gp, $(Cp)_4$, $(Up)_5$	-	ApUpCpUpCpUpUpGpGpUpUp CpUpCpGp
t27	ApApCpGp, ApApGp, CpApGp	ApApGpApApCp, ApGp, GpCp	-	ApApGpApApCpGpCpApGp
t26	ApUpApCpGp, CpGp, ApApApUpGp	ApApApUp, GpApUp, GpCp, ApCp, Gp	t38	ApApApUpGpCpGpApUpApCp Gp
t25	ApApUpCpUpUpUpGp, ApApUpCpApUpCpGp	GpApApUp, Gp, ApUp, ApApUp, Cp, Up	t22	ApApUpCpApUpCpGpApApUp CpUpUpUpGp
t24	UpUpCpUpCpGp, ApUpGp, CpApUpCpGp, ApApGp	GpApApGp, GpApUp, Gp, Up, GpCp, ApUp, Cp	t40	UpUpCpUpCpGpCpApUpCpGp ApUpGpApApGp
t23	ApApUpCpUpUpUpGp, UpGp, ApApUpCpApUpCpGp	GpApApUp, ApUp, Gp, Cp, $(Up)_4$	t22	UpGpApApUpCpApUpCpGpAp ApUpCpUpUpUpGp
t22	ApApUpCpApUpCpGp, UpGp, ApApUpUpCpCpGp	GpApApUp, ApApUp, Up, GpUp, ApUp, Gp, $(Cp)_4$	t32	ApApUpUpCpCpGpUpGpApAp UpCpApUpCpGp
t21	ApUpCpUpCpUpUpGp, Gp, UpUpCpUpCpGp, CpApUpCpGp	GpGpUp, GpCp, ApUp, Gp, $(Up)_5$, $(Cp)_5$	t28	ApUpCpUpCpUpUpGpGpUpUp CpUpCpGpCpApUpCpGp
t20	pApApApCpUpUpUpCpAp ApCpApApCpGp	pApApApCp, ApApCp, Gp, Up, Cp	-	pApApApCpUpUpUpCpApApCp ApApCpGp
t19	pApApApCpUpUpUpCpAp ApCpApApCpGp, Gp	pApApApCp, ApApCp, GpGp, Up, Cp	-	pApApApCpUpUpUpCpApApCp ApApCpGpGp
t18	ApApUpCpApUpCpGp, UpGp, ApApUpUpCpCpGp, CpApGp	ApGpApApUp, ApUp, Up, Gp, GpApApUp, GpUp, $(Cp)_5$	t22	CpApGpApApUpUpCpCpGpUp GpApApUpCpApUpCpGp
t17	ApApUpCpUpUpUpGp, UpGp, ApApUpUpCpCpGp, ApApUpCpApUpCpGp	GpApApUp, GpUp, ApApUp, ApUp, $(Up)_4$, $(Cp)_5$	t18	ApApUpUpCpCpGpUpGpApAp UpCpApUpCpGpApApUpCpUp UpUpGp
t15	ApUpCpUpCpUpUpGp, Gp, pApApApCpUpUpUpCpAp ApCpApApCpGp	GpGpApUp, ApApCp, $(Cp)_3$ pApApApCp, $(Up)_6$	-	pApApApCpUpUpUpCpApApCp ApApCpGpGpApUpCpUpCpUp UpGp
t13	ApUpApCpGp, CpApGp, CpGp, ApApApUpGp, ApApGp, ApApCpGp	GpApApApUp, GpApUp, ApCp, ApApGpApApCp, ApGpCp, GpCp, Gp	t35, t26	ApApGpApApCpGpCpApGpCp GpApApApUpGpCpGpApUpAp CpGp

TABLE VI—*continued*

Fragment No. (a)	T_1 RNase digestion products (b)	Pancreatic RNase digestion products (b)	Additional data required (c)	Sequence deduced
t9	pApApApCpUpUpUpCpAp ApCpApApCpGp, ApUpGp, CpApUpCpGp, ApApCpGp, UpUpCpUpCpGp, ApApGp, ApUpCpUpCpUpUpGp, Gp, CpGp, CpApGp	ApGpCp, GpGpUp, GpGpApUp, GpApUp, GpApApGpApApCp, pApApApCp, ApApCp, GpCp, ApUp, $(Cp)_6$, $(Up)_8$, Gp	t15, t21 t24, t35	pApApApCpUpUpUpCpApApCp ApApCpGpGpApUpCpUpCpUp UpGpGpUpUpCpUpCpGpCpAp UpCpGpApUpGpApApGpApAp CpGpCpApGpCpGp
t8	UpApUpUpCpCpApGp, ApApUpCpUpUpUpGp, CpCpCpCpUpUpGp, ApApUpCpApUpUpCpGp, CpApCpApUpUpGp, UpGp, CpApUpGp, CpCpUpGp, ApApCpGp, CpGp, $(Gp)_5$	ApGpGpGpGpGpCp, GpGpUp, GpApApUp, GpUp, GpApApCp, ApCp, Gp, $(ApUp)_4$ $(GpCp)_4$, $(Cp)_9$, $(Up)_8$	t5, t23	UpGpApApUpCpApUpCpGpAp ApUpCpUpUpUpGpApApCpGp CpApCpApUpUpGpCpGpCpCp CpCpUpUpGpGpUpApUpUpCp CpApGpGpGpGpGpCpApUpGp CpCpUpGp
t6	ApApUpCpUpUpUpGp, UpApUpUpCpCpApGp, CpApCpApUpUpGp, CpGp, CpCpCpCpUpUpGp, $(Gp)_4$, CpApUpGp, ApApCpGp,	ApGpGpGpGpGpCp, ApCp, GpGpUp, ApApUp, ApUp, GpApApCp, GpCp, Gp, $(Cp)_6$, $(Up)_7$	t5, t31	ApApUpCpUpUpUpGpApApCp GpCpApCpApUpUpGpCpGpCp CpCpCpUpUpGpGpUpApUpUp CpCpApGpGpGpGpGpCpApUpGp
t5	UpApUpUpCpCpApGp, $(Gp)_5$, CpApCpApUpUpGp, CpGp, CpCpCpCpUpUpGp, CpApUpGp, ApApCpGp, CpCpUpGp	ApGpGpGpGpGpCp, ApCp, GpGpUp, ApApCp, Gp, $(Up)_5$, $(ApUp)_3$, $(GpCp)_4$, $(Cp)_6$	t2, t34	ApApCpGpCpApCpApUpUpGp CpGpCpCpCpCpUpUpGpGpUp ApUpUpCpCpApGpGpGpGpGp CpApUpGpCpCpUpGp
t3	UpApUpUpCpCpApGp, $(Gp)_5$ CpApCpApUpUpGp, CpGp, CpCpCpCpUpUpGp, CpApUpGp	ApGpGpGpGpGpCp, $(Up)_4$, GpGpUp, ApCp, Gp, ApUp, GpCp, $(Cp)_6$	t2	CpApCpApUpUpGpCpGpCpCp CpCpUpUpGpGpUpApUpUpCp CpApGpGpGpGpGpCpApUpGp
t2	UpApUpUpCpCpApGp, CpGp, CpCpCpCpUpUpGp, $(Gp)_5$ CpApUpGp, CpCpUpGp	ApGpGpGpGpGpCp, GpGpUp, ApUp, Gp, $(Up)_4$, $(Cp)_6$, GpCp	t1, p62	CpGpCpCpCpCpUpUpGpGpUp ApUpUpCpCpApGpGpGpGpGp CpApUpGpCpCpUpGp
t1	UpApUpUpCpCpApGp, $(Gp)_5$, CpCpCpCpUpUpGp, CpApUpGp, CpGp	ApGpGpGpGpGpCp, ApUp, GpGpUp, Gp, GpCp, $(Cp)_6$, $(Up)_3$	p55	CpGpCpCpCpCpUpUpGpGpUp ApUpUpCpCpApGpGpGpGpGp CpApUpGp

(a) The numbers refer to the partial digestion products shown in Figures 6 and 7.

(b) The products of complete T_1 or pancreatic RNase digestion of the partial digestion products were fractionated and identified as described in the text. The notation for the relative molar yields of the products is no underline, one underline, two underlines, and subscript numbers 4-9 for relative molar yields of 1, 2, 3, and 4-9, respectively. Relative molar yields of 1 and 2 were estimated by visual inspection of the radioautograph. Relative molar yields of greater than 2 of pancreatic RNase digestion products were calculated from the sequences of the products obtained by T_1 RNase digestion of the fragment. Relative molar yields of greater than 2 of T_1 RNase digestion products were calculated from the sequences of the products obtained by pancreatic RNase digestion of the fragment.

(c) In some cases, more than one sequence for the fragment was consistent with its T_1 and pancreatic RNase digestion products. In these cases, it was necessary to refer to the sequence of additional fragments to determine a unique sequence for the fragment in question.

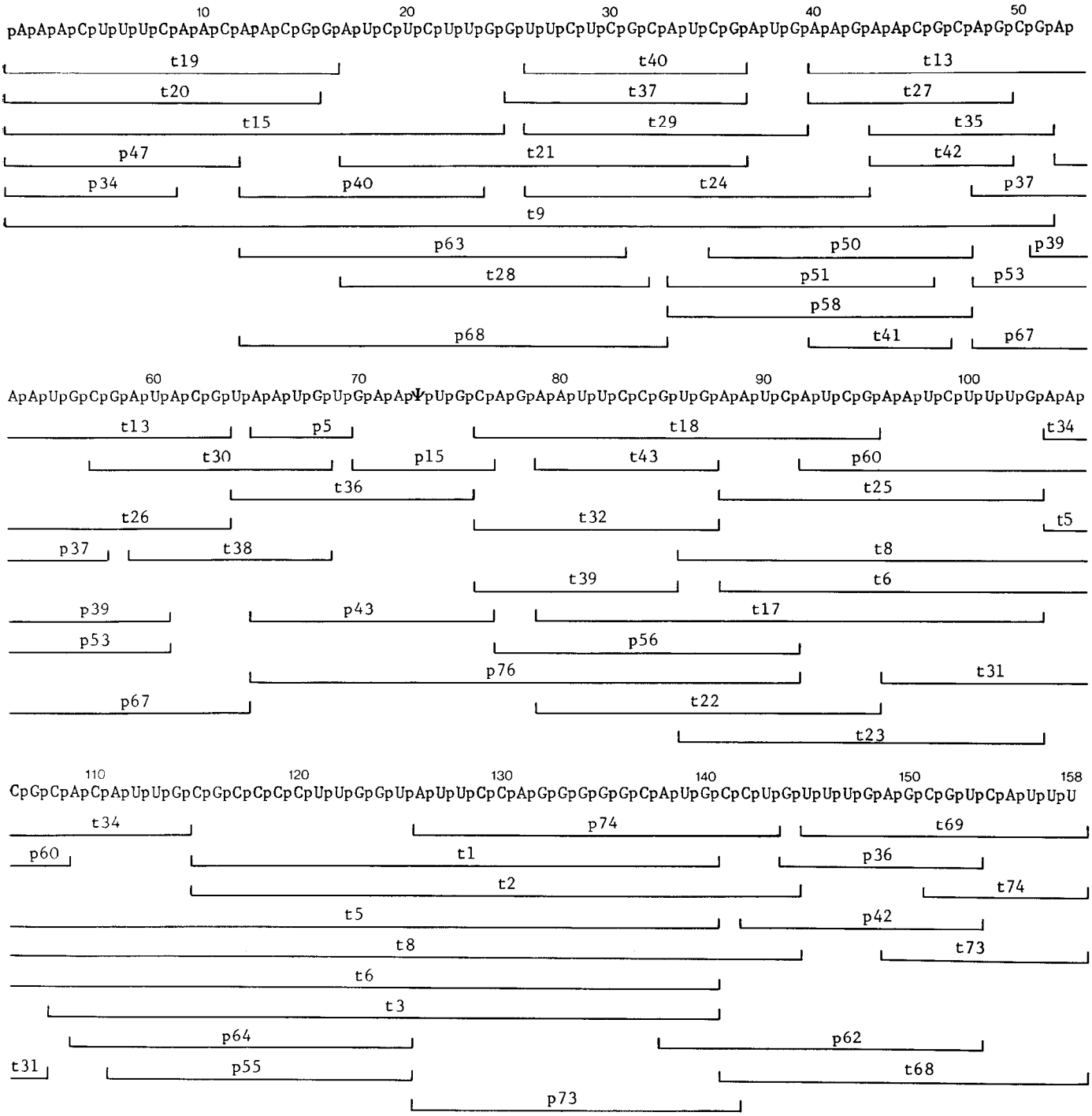

FIG. 8. Overlaps between the sequences of partial digestion products.

inducible acid phosphomonoesterase (22) it can readily grow in this medium. Nevertheless, inorganic phosphate is used preferentially as a source of phosphate; over 90% of carrier-free [^{32}P]phosphoric acid added to the medium at a concentration of 1 mCi/20 ml is found to be precipitable with the cells in less than half of the generation time. Of practical importance is my observation that when yeast is grown in this medium inorganic polyphosphates do not accumulate within the cells. This contrasts with media previously used for ^{32}P-labeling of yeast which contain only inorganic phosphates as a source of phosphate (23, 24).

There are 140 copies of the DNA homologous to 28 S ribosomal RNA in the haploid yeast (23, 25) and presumably the same number of copies for 5.8 S RNA, since both RNAs are cleavage products of the 45 S precursor (14). However, no heterogeneity was detected in the sequence of 5.8 S RNA. The pancreatic and T_1 RNase fingerprints of 5.8 S RNA isolated from *Saccharomyces italicus* (NRRL Y-1434), *Saccharomyces uvarum* (NRRL Y-969), *Saccharomyces chevalieri* (NRRL Y-2045), and *Saccharomyces carlsbergensis* (D. 1006, a derivative of N.C.Y.C. 74 obtained from A.M.A. ten Berge) appear to be the same as those obtained from the *S. cerevisiae* 5.8 S RNA, suggesting that the nucleotide sequence of 5.8 S RNA in all these strains is identical.

While it is possible to deduce secondary structures for 5.8 S RNA, such as shown in Fig. 9, it is unclear at this time what relation these models have to the actual secondary and tertiary

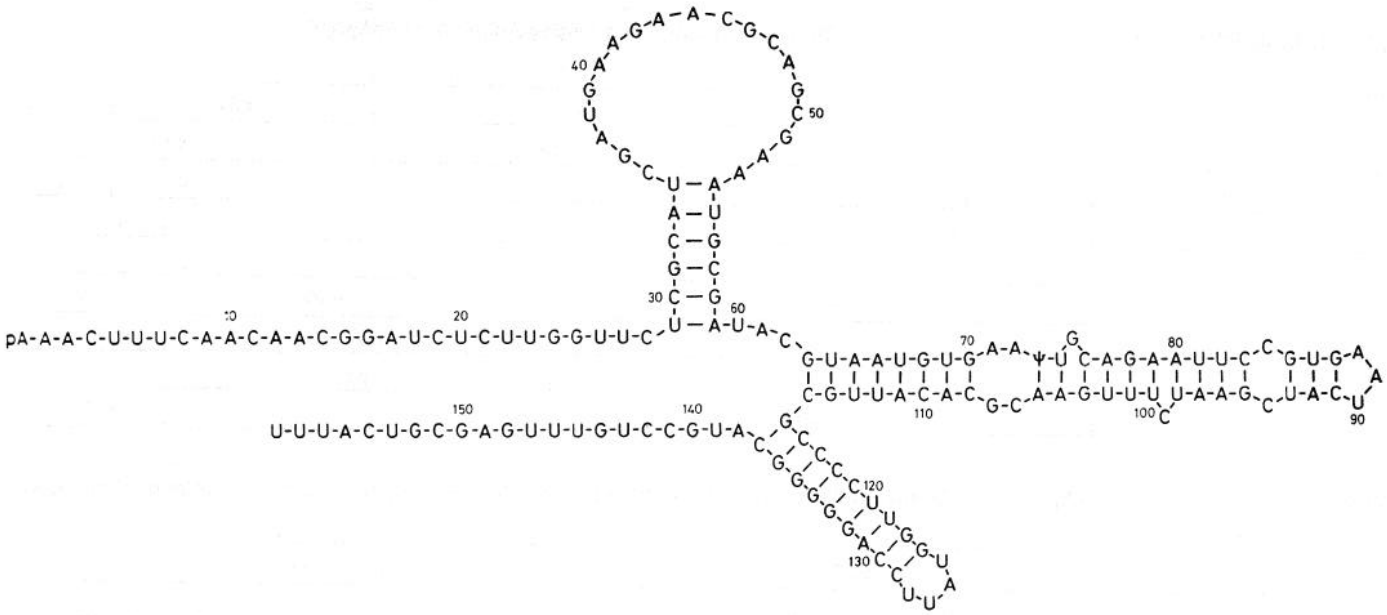

FIG. 9. A possible secondary structure for *Saccharomyces cerevisiae* 5.8 S RNA. The three loops have stability numbers, as defined by Tinoco *et al.* (26), of +2 (nucleotides 29 to 59), +5 (nucleotides 64 to 115), and +9 (nucleotides 115 to 137).

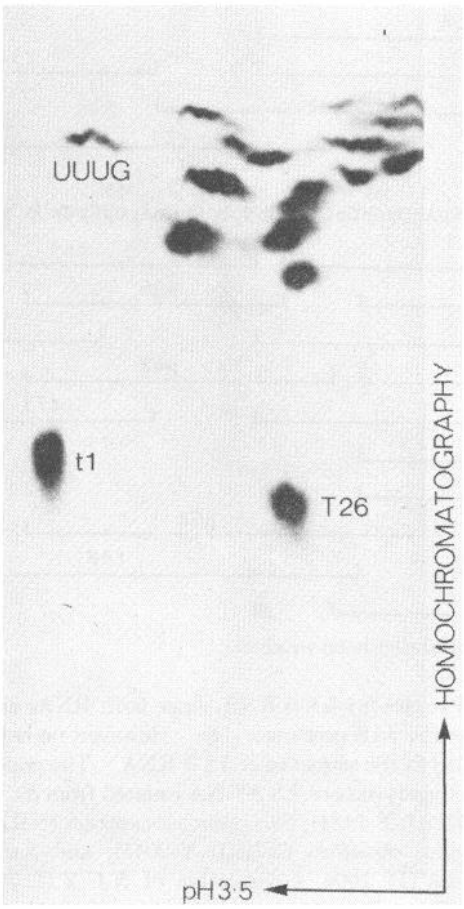

FIG. 10. Radioautograph of a two-dimensional fractionation of a limited T_1 RNase digest of *Saccharomyces cerevisiae* 5.8 S RNA. The digest was performed as described in the text using 20 μg per ml of T_1 RNase. The molecule, except for Fragment t1, has been completely digested to its normal T_1 RNase digestion products. These products are represented by *T26*, *UUUG*, and the *unnumbered spots*.

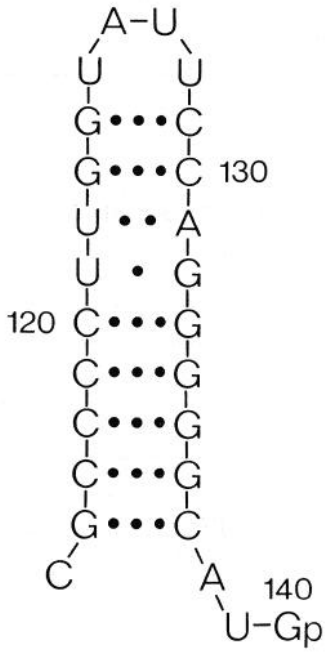

FIG. 11. A possible structure for Fragment t1. This structure has a stability number of +9 as defined by Tinoco *et al.* (26).

structure of this RNA as it exists either in solution or complexed with the 28 S RNA in the ribosome. There is, however, one very stable hairpin loop which almost certainly exists as part of the structure. The sequence of this loop, which can be isolated in molar yield following partial RNase T_1 digestion (see Fig. 10), is shown in Fig. 11. This oligonucleotide has abnormally fast mobilities in both dimensions of the fractionation procedure (see Fig. 10), suggesting base pairing even in the presence of 7 M urea at elevated temperatures.

The nucleotides near the 3′ end of 5.8 S RNA are the most susceptible to nuclease attack (see Fig. 7). It is likely that the

3′ and 5′ ends of the molecule, which seem not to be involved in internal hydrogen bonding, are responsible for the interaction with the 28 S RNA. The single-stranded nature of the ends of this molecule is a distinct structural difference from other stable RNAs in which the regions at the ends of the RNA are base-paired to each other (21, 27–33). As no other 5.8 S RNA has as yet been sequenced, it is not possible to say whether the above structural features, or the presence of pseudouridine, are specific for the yeasts studied or general properties of all 5.8 S RNAs.

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