Volume 10 Number 17 1982 Nucleic Acids Research

A yeast transcription system for the 5S rRNA gene

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Received 7 May 1982; Revised and Accepted 12 July 1982

ABSTRACT - A cell-free extract of yeast nuclei that can specifically transcribe cloned yeast 5S rRNA genes has been developed. Optima for transcription of 5S rDNA were determined and conditions of extract preparation leading to reproducible activities and specificities established. The major *in vitro* product has the same size and oligonucleotide composition as *in vivo* 5S rRNA. The *in vitro* transcription extract does not transcribe yeast tRNA genes. The extract does increase the transcription of tRNA genes packaged in chromatin.

INTRODUCTION

Insights into the mechanisms of gene expression have been derived from the study with *in vitro* systems that contain the components for gene transcription (1-5). We have developed an *in vitro* transcription system from yeast (*Saccharomyces carlsbergensis*) which specifically transcribes plasmids containing the 5S rRNA gene.

The transcription of endogenously added class III genes (transcribed by RNA polymerase III or C) by lysates of *Xenopus laevis* germinal vesicles, oocytes and Human KB cells has revealed some of the parameters for the lysates and template requirements for accurate *in vitro* transcription (1-3, 6-8). *In vitro* deletion of portions of the *X. borealis* somatic 5S rDNA has revealed a control region within the transcribed region (at position +50 to +80) (9,10). A similar internal region for the transcription of the VA I region of adenovirus (11) and the involvement of both 5' and 3' regions of tRNA genes in their transcription have been revealed (12-14). Factors involved in 5S rRNA gene transcription in *X. laevis* other than those with polymerization activity have also been identified (15-18). The *Xenopus* oocyte and oocyte-lysate

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0305-1048/82/1017-5233$2.00/0

transcription systems are permissive in that they transcribe class III genes from a variety of organisms (3, 19-21). However, there are indications that the transcription products of the heterologous templates are not completely identical with the _in vivo_ products of the same genes. The $tRNA^{ala}$ gene of _Bombyx mori_ is transcribed accurately in both the _Xenopus_ system and in a homologous _Bombyx_ ovary extract. However, the regulation of transcription differs in these extracts since deletion of the 5' flanking sequences completely abolishes transcription in the homologous extract only (22). A similar result is found for several yeast $tRNA^{tyr}$ mutant genes. Genes with point mutations (23) that were not transcribed or aberrantly transcribed in the _Xenopus_ system _in vitro_ (24) were accurately expressed _in vivo_ in yeast cells, even the mutant genes that gave prematurely termination in the _Xenopus_ extract (25). Thus, the use of a homologous transcription system will be an advantage over a ("permissive") heterologous system to study transcription and regulation of transcription of yeast genes.

A previous approach (26) to the study of transcription of yeast class III genes is the use of yeast chromatin and the endogenous or exogenous added RNA polymerase. This system gives faithful transcription of both tRNA and 5S rRNA genes (26,27). Transcription of chromatin fragments enriched in 5S rDNA showed accurate transcription of the 5S rRNA genes by exogenously added RNA polymerase III.

However, several read-through products up to ten nucleotides longer than the mature 5S rRNA were observed (27). Whether this is a true reflection of the _in vivo_ situation is not clear but this could be so as an enzyme activity was found that accurately trimmed the 5S rRNA precursors to mature 5S rRNA.

The development of a yeast transcriptional system that completely depends upon added DNA will enable us to use mutationally altered yeast DNA templates in a homologous system. Additionally the altered DNA can be tranformed into yeast (28) and assembled into a multicopy (up to 50 copies per cell) mini-chromosome (packaged as chromatin) (29), which can

be isolated from yeast and added to the transcriptional lysate instead of naked plasmid DNA. We have found that the yeast lysate has all the components necessary to transcribe the 5S rRNA gene but not tRNA genes, but that upon addition of total yeast chromatin to the system the tRNA genes of the chromatin are transcribed.

MATERIALS AND METHODS

Materials - Glusulase was obtained from Endo Laboratories, zymolyase from Seikgaku Kugyo Co. Ltd., Nucleoside triphosphates and α-amamitin were from Boehringer, α-^{32}P labelled UTP, CTP and ^{32}P orthophosphate from New England Nuclear (Canada Ltd.). T_1 ribonuclease from P-L-Biochemicals, cellulose acetate strips and PEI cellulose plates from Schleicher and Schuell.

Preparation of Yeast Nuclei - *S. carlsbergensis* YF-322 cells were grown at 30°C in YEPD medium (2% dextrose, 2% bactopeptone, 1% yeast extract), harvested at A_{600} = 0.6 - 0.8 by centrifugation, washed once with cold distilled water and once with cold 1M sorbitol. Spheroplasts were made in 1/10 th of the original culture volume of 1M sorbitol, 40 mM Tris.HCl pH 7.5, 0.1% β-mercaptoethanol and 1% glusulase or 200 μg/ml zymolyase at 30°C, in 45 to 90 minutes. The spheroplasts were centrifuged at 1500 x g for 5 minutes and washed by centrifugation three times with 1M sorbitol containing 1.7 mM PMSF (phenylmethylsulphonylfluoride). The spheroplast pellet was resuspended in 0.06 times the original cultures volume of buffer B + 18% w/v Ficoll (0.02 M potassiumphosphate pH 6.5,. 20 mM β-mercaptoethanol, 5 mM $MgCl_2$, 1.7 mM PMSF) by homogenization in 30 ml aliquots in a Dounce homogenizer (10 strokes of a 'small' clearance pestle). The lysate was layered above a cushion of 7 ml 70% sucrose in buffer B and centrifuged 45 minutes at 52,200 x g at 4°C in a Beckman SW-27 rotor. The upper layer was removed by suction and the nuclei at the Ficoll/sucrose interphase were mixed with the 70% sucrose and removed from the pellet of unlysed cells and cell debris. This suspension was diluted 1:1 with buffer B containing 25% glycerol. The nuclei were finally precipitated by centrifugation for 40 minutes at 13,200 x g in a Sorvall SS-

34 rotor. The nuclei were then stored at -70°C until use. Alternatively, the sucrose cushion centrifugation step was replaced by centrifugation 10 minutes at 2,310 x g in a Sorvall SS-34 rotor the resulting supernatant was then centrifuged at 13,200 x g for 20 minutes. This method was faster and no dilution of nuclei was necessary which resulted in higher activities of the preparations.

Preparation of Transcriptionally-Active Extracts from Nuclei

Nuclei were suspended in TEMMGA buffer (20 mM Tris.HCl pH 8.0. 0.1 mM EDTA, 5 mM $MgCl_2$, 20 mM β-mercaptoethanol, 20% glycerol and 0.2 M $(NH_4)_2SO_4$ or other concentrations of $(NH_4)_2$ SO_4 as indicated in the figure legends, in a total volume of 4 ml (for nuclei isolated from 1 L yeast culture). The suspension was kept on ice for 15 minutes and then centrifuged at 30,000 x g for 30 minutes. The supernatant fractions were used directly if prepared in 0.2 M $(NH_4)_2SO_4$ in TEMMGA buffer, or were dialyzed against buffer containing 20 mM Tris. HCl pH 8.0, 0.1 mM EDTA, 50mM $(NH_4)_2SO_4$, 1 mM DTT, 50% glycerol at 4°C for about 4 h. All preparations were frozen on dry ice and stored at -70°C.

In vitro Transcription - The *in vitro* transcription reaction contained in 50 µl 5-10 µl extract or chromatin (the pellet fraction of the nuclear lysates), 1.0-0.5 mM each of ATP, GTP and CTP, 0.1-0.05 mM UTP, 60 mM Tris.HCl pH 8.0, 5 mM $MgCl_2$, 1 mM DDT and 10 µCi α^{32}P-UTP (400-500 Ci/mmole). Incubation was 45-60 minutes at 24°C. Plasmid DNA was usually added at a concentration of 20 µg/ml. The reaction was stopped by adding 0.25% SDS and 200 µl H_2O, then phenol extracted and precipitated with ethanol.

Gel electrophoresis - Samples were analysed on 10% polyacrylamide gels containing 7 M urea in Tris-borate buffer pH 8.3 (90 mM Tris-borate, 0.1 mM EDTA). Thin gels of either 0.8 or 1.5 mm were run in a Bethesda Research Laboratories model V 16 or SO gel electrophoresis apparatus. Samples were loaded in 8 M urea, 2% Ficoll, 0.5% SDS, 0.02% Bromephenolblue, 0.02% xylene cyanol FF. Electrophoresis was at 20 V/cm. After electrophoresis the lanes containing marker RNA were cut out and stained in 1 µg/ml ethidiumbromide and photographed under

UV illumination. The rest of the gel was covered with Saran wrap and exposed to Kodak X-omat RP X-ray film with a Kodak intensifying screen at 4°C for 16 hours.

Fingerprinting - *In vitro* synthesized RNA was detected by autoradiography (usually 8 hours exposure), RNA bands were excised from the acrylamide gel and the gel pieces were crushed by forcing them through a 5 ml syringe and eluted overnight at 37°C in 1 M NaCl, 1% phenol. The RNA was purified by centrifugation through a siliconized glasswool-stopped Eppendorf tip, phenol extracted, ether extracted and precipitated twice with ethanol. Digestion with T_1 ribonuclease was for 30 minutes at 37°C with 0.4 U/μg RNA, followed by 1 h at room temperature in 0.1 M HCl. Electrophoresis was on cellulose-acetate strips at pH 3.5 in the first dimension and homochromatography was on PEI-cellulose plates in the second dimension using homomix β according to Volckaert et al. (30). PEI cellulose plates were washed with water, dried and autoradiographed with Kodak X-omat RP films and Kodak intensifying screens at -70°C for about 7 days. Marker *in vivo* ^{32}P-labelled 5S rRNA was isolated according to the method of Holley (31) from a 200 ml yeast culture grown for 6 h in dephosphorylated YEPD medium (31) with 3 mCi ^{32}P-orthosphosphate and separated on a 10% polyacrylamide slabgel with 7 M urea.

Plasmid DNA

The plasmids used for *in vitro* transcription were pBR 322 (32) and pSZr1 which was constructed from pSZ53. pSZ53 was obtained from J.W. Szostak (33) and contains in the Eco RI - Hind III site of pBR322 a 2250 basepair Eco RI - Hind III yeast rDNA fragment with the 5S rRNA gene (see Fig. 1A). This plasmid also contains in the Sal I site of pBR322 a Sal I - Xho I yeast *LEU-2* gene. The Eco RI - Hind III rDNA 2250 basepair fragment was isolated by agarose gel electrophoresis and inserted into Eco RI - Hind III digested pBR322 in order to remove the yeast *LEU-2* sequence. Also used was plasmid pSS7-3.7 which was constructed by inserting in the pBR322 Hind III - Bam HI site a partial Hind III - Sau 3A fragment containing the yeast $tRNA^{tyr}$ gene from the plasmid pSU 4-A (35) which contains

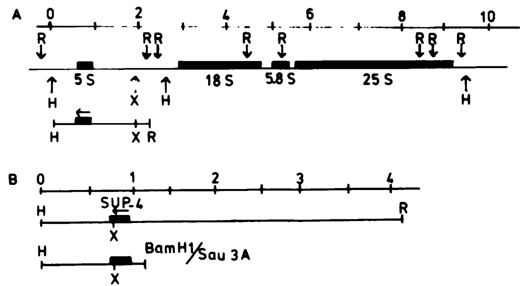

Figure 1. Restriction enzyme maps of yeast rDNA and plasmid DNAs. A. Restriction enzyme map of the yeast rDNA according to Bell et al. (34). R indicates the position of restriction endonuclease Eco R1; H: Hind III; X: Xma I (Sma I). pSZr1 was constructed by inserting the indicated 2250 basepair Eco RI - Hind III fragment containing the 5S rRNA gene into pBR322 in the Eco RI - Hind III sites.
B. Plasmid pSS7-3.7 contains in the Hind III - Bam HI sites of pBR322 a partial Hind III -Sau 3A fragment from pSU 4-A. The size of the DNA is given in kilobasepairs.

this gene on a 4.1 kilobasepair Eco RI - Hind III fragment (see Fig. 1B). Plasmid isolation was by the cleared-lysate method of Clewell and Helinski (36) CsCl-ethidium bromide gradients were spun for 18 - 20 hours in a Beckman type 60 rotor at 50 krpm. Plasmid DNA was precipitated twice with ethanol and stored in 5 mM Tris-HCl pH 7.6, 1 mM EDTA at -20°C.

RESULTS AND DISCUSSION

a. Preparation of a Nuclear Extract that Transcribes the 5S rRNA Gene - Nuclei were isolated as described in Materials and Methods and lysed in TEMMG buffer containing 0, 0.1, 0.2, 0.3 and 0.4 M $(NH_4)_2SO_4$. The lysates were centrifuged for 30 minutes at 30,000 x g. The supernatants were dialysed and tested for class III transcription activity by using alternatively pSS 7-3.7 plasmid DNA (containing the yeast $tRNA^{tyr}$ gene) (Fig. 2A lanes 1, 3, 5, 7 and 9) or pSZ53 plasmid DNA (containing the yeast 5S rRNA gene) (Fig. 2A lanes 2, 4, 6, 8 and 10) as templates. Fig. 2 shows that only the pSZ53 plasmid DNA is transcribed in lysates made in 0.2 M - 0.4 M $(NH_4)_2SO_4$ and gives a discrete RNA product of the size of 5S rRNA. When the lysates are made in 0.2 or 0.3 M $(NH_4)_2SO_4$ a

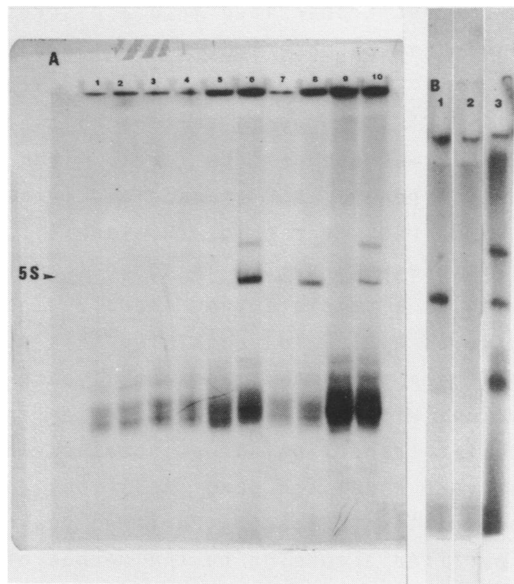

Figure 2. A. Transcription activity of different yeast nuclear extracts. Yeast nuclei prepared from a 2 litre yeast culture were lysed in 4 equal portions in 2 ml of TEMMG buffer containing zero, 0.1 M, 0.2 M, 0.3 M or 0.4 M $(NH_4)_2SO_4$. The lysed nuclei were centrifuged and the supernatants were dialyzed against buffer without $(NH_4)_2SO_4$ but containing 50% glycerol. The transcription assay contained in a total volume of 100 µl: 50 µl extract, 10 µg/ml plasmid DNA and 10 µCi of α-^{32}P UTP + α-^{32}P CTP. The transcription assays were phenol extracted, ethanol precipitated and loaded in 8 M urea on a 10% polyacrylamide gel (34 x 40 x 0.08 cm) with 7 M urea. Lane 1 and 2 is the extract without $(NH_4)_2SO_4$, lane 3 and 4 0.1 M, lane 5 and 6 0.2 M, lane 7 and 8 0.4 M and lane 9 and 10 0.3 M $(NH_4)_2SO_4$. The templates were pSS7-3.7 (tDNA) lanes 1, 3, 5, 7 and 9 and pSZ53 (5S rDNA) lanes 2, 4, 6, 8 and 10.
B. Transcription specificity of the yeast nuclear extract. A 0.2 M $(NH_4)_2SO_4$ nuclear extract was used for transcription of pSZr1 plasmid DNA (lane 1), pSS7-3.7 plasmid DNA (lane 2). Conditions and gel are the same as in Figure 2A, except only α-^{32}P UTP was used. Lane 3 contains marker ^{32}P RNAs 5.8S, 5S and tRNAs.

second transcript is also observed which is about 10 bases longer than the major 5S rRNA-sized product. This longer product is hardly detectable if the lysates are made in 0.4 M $(NH_4)_2SO_4$. Comparison with transcription reactions containing pSS7-3.7 shows that there is no endogenous synthesis of 5S rRNA

and that the tRNA is not transcribed into a discrete product since no extra bands can be observed. The high background of labelled tRNAs in this experiment is caused by exchange labelling with α-^{32}P CTP. To avoid this background in later experiments α-^{32}P UTP was used as the label. Then it is clearly visible that the tRNA gene is not transcribed (Fig. 2B, lane 2) in contrast to the 5S rRNA gene (Fig. 2B, lane 1). No transcripts are visible when no DNA is added (Fig. 4, lane 1) or when pBR322 is used as a template (not shown). The gene is biologically active as a plasmid containing this suppressor tRNA gene (SUP 4.0) has been shown to transform a yeast host which has 5 ochre mutations with a high transformation frequency (37).

No specific transcription was obtained when whole yeast cells were lysed under the same conditions and used as a source for a transcription extract. Purified yeast RNA polymerase III (purified according to the method of Valenzuela et al. (38)) plus template gave no specific transcript (data not shown).

The activities of several preparations of nuclear lysate were tested by incubation without any added DNA and with 125 µg/ml salmon sperm DNA as a non-specific template under standard incubation conditions. The results of time-course experiments with three different preparations are shown in Fig. 3. Usually the activity of the extract with added DNA under these conditions lies between 60 and 100 pMol UMP per 30 minutes incorporated in TCA precipitable RNA. The activity of the extracts is almost completely dependent upon external DNA although endogenous RNA synthesizing activities were found without added DNA which ranged from 2 to 10 pMol UMP incorporated. Centrifugation at higher x g values does not reduce this endogenous transcription. Lysis of nuclei at higher $(NH_4)_2SO_4$ concentration (0.5 - 1.0 M) increases the endogenous transcription activity. Lysis of nuclei up to 0.6 M $(NH_4)_2SO_4$ does not give any α-amanitin sensitive transcription activity. Finally a reactivation step of the spheroplasts in rich medium after the zymolyase treatment, followed by isolation of nuclei, did not increase the activity of the transcription extract significantly (data not shown).

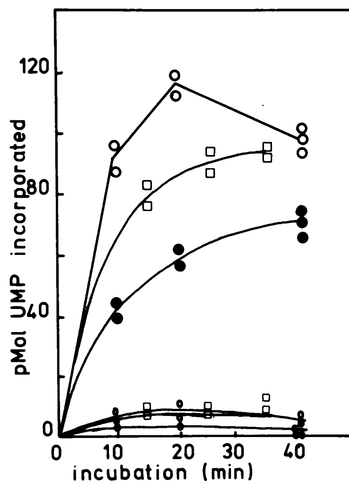

Figure 3. Time-course of transcription. Three independently prepared samples of yeast nuclei were extracted with 0.3 M $(NH_4)_2SO_4$ and used for transcription of salmon sperm DNA (125 µg/ml) in 200 µl assays containing 100 µl extract and 50 µCi 3H UTP. At the times indicated 20 µl samples were spotted on Whatman #1 filter paper. The filters were washed in 10% TCA, then with ethanol and with ether and counted in a toluene-based scintillation liquid. The zero time counts were subtracted as blanks. The upper three curves represent the transcription activity of the three extracts with added DNA, the lower three curves represent the endogenous transcription activity.

b. The Optimal Conditions for Transcription. The optimal DNA concentration was determined by incubating an increasing amount of plasmid DNA with a constant amount of extract (0.2 M $(NH_4)_2SO_4$ extract). As shown in Fig. 4 a broad optimum can be seen when the DNA concentration is increased from 15 to 60 µg/ml. At higher DNA concentrations (20 - 160 µg/ml) transcripts appear in the upper part of the gel and at the origin.

The final $(NH_4)_2SO_4$ concentration in the transcription assay is 20 - 40 mM. Increasing the $(NH_4)_2SO_4$ concentration rapidly decreases the production of 5S rRNA (Fig. 5A). The insert in Fig. 5A shows the dependence of the total transcription activity of this extract on the $(NH_4)_2SO_4$ concentration measured with salmon sperm DNA as a template. The increase of activity at high $(NH_4)_2SO_4$ concentrations is a

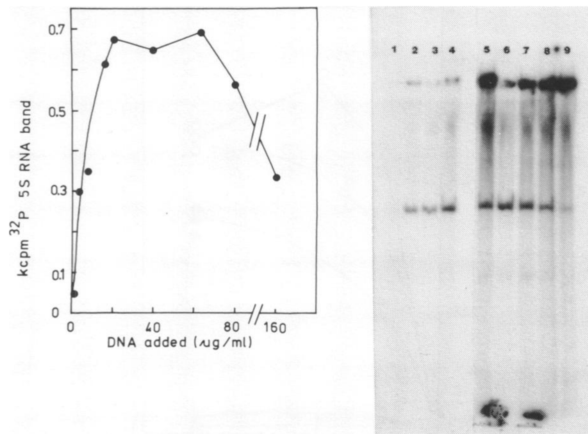

Figure 4. The optimum plasmid concentration for 5S rRNA synthesis. Yeast nuclei were extracted in 0.2 M $(NH_4)_2SO_4$ and 10 µl was used in 50 µl assays each containing 10 µCi α-^{32}P UTP and increasing concentrations of plasmid pSZrl. Lanes 1-9, respectively, no DNA added, 4, 8, 16, 20, 40, 60, 80 and 160 µg/ml DNA added. After autoradiography, the 5S rRNA bands were excised and counted in a liquid scintillation counter.

typical feature of RNA polymerase III activity (38) and does not result from an increase in transcription specificity. The optimum Mg^{++} concentration for this extract was between 9 and 13 mM (Fig. 5B).

The synthesis of the 5S rRNA is not sensitive to α-amanitin. Up to 1 mg/ml α-amanitin was used in the transcription assay without effect on the 5S rRNA synthesis (data not shown). This excludes the possibility that RNA polymerase II or I are responsible for the *in vitro* synthesis of the 5S-sized RNA since polymerase II is inhibited 50% at 1 µg/ml α-amanitin and polymerase I at 300-600 µg/ml (38,39). The activity and specificity of the lysate is not sensitive to repeated freezing and thawing of the preparation.

c. Characterization of the in vitro Transcripts by Fingerprint Analysis - Transcription products were prepared for fingerprinting by incubating the nuclear extract and optimal amounts of pSZrl DNA with α-^{32}P UTP as the only labelled nucleoside triphosphate. Separation of the RNA products on the gels was the same as for the analytical gels except that several (up to

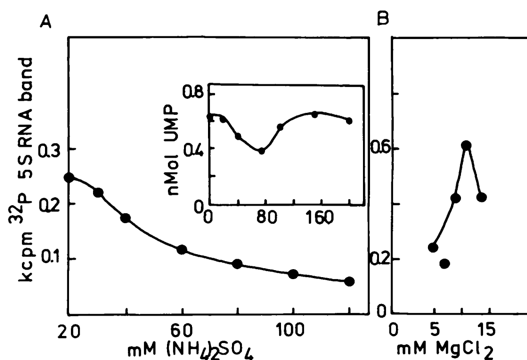

Figure 5. Optimal conditions for in vitro transcription.
A. The dependence upon $(NH_4)_2SO_4$ concentration. 5 µl extract was tested in 50 µl assays containing 20 µg/ml pSZr1 DNA. The $(NH_4)_2SO_4$ concentration is 20 mM in this assay and $(NH_4)_2SO_4$ was added to give the indicated concentrations. The 5S rRNA bands were excised from the gel and counted. The insert shows the dependence of the total transcription activity on the $(NH_4)_2SO_4$ concentration determined with salmon sperm DNA as template in the same manner as described in Fig. 3.
B. The dependence upon the $MgCl_2$ concentration. 10 µl extract was tested in 50 µl assays containing 20 µg/ml pSZr1 DNA. The $MgCl_2$ concentration is 5 mM, $MgCl_2$ was added to give final concentrations of 7, 9, 11 and 13 mM.

12) lanes were run. The 5S-sized bands were excised using the autoradiogram as a template and the RNA was extracted and purified as described in materials and methods. The RNAs were digested with T_1 ribonuclease and fingerprinted according to standard procedures using homochromatography on PEI-cellulose thin layer plates. T_1- oligonucleotides were compared with those obtained from in vivo ^{32}P-labelled 5S rRNA (Fig. 6). Fig. 6B shows the T_1-oligonucleotide fingerprint of the 5S-sized product.

Table I gives the catalogue of the T_1-oligonucleotides and the molar ratios in which they occur in the fingerprint. All the oligonucleotides predicted from the 5S-RNA sequence to be labelled with α-^{32}P UTP were found in the in vitro transcript at the expected position in the fingerprint and with a molar ratio which is in good agreement with the theoretical values. The 3'-end group CAAUCU is present in the fingerprint at the expected position. The 5' end group pppGp is not visible in the

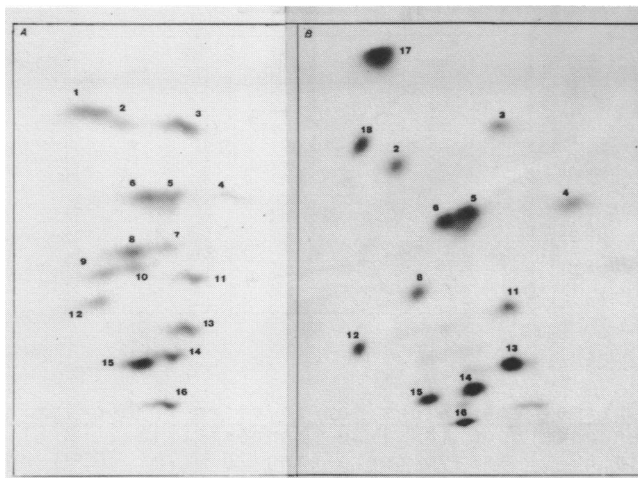

Figure. 6. Fingerprint analysis of 5S rRNAs. A. *In vivo* ^{32}P-labelled 5S rRNA. B. *In vitro* synthesized 5S-sized RNA. The RNAs were extracted from the gels purified and T_1 nuclease digested. The electrophoresis was from left to right on cellulose-acetate strips at 2.5 kV for 50 min. Homochromatography was on PEI-cellulose plates with homomix β. Oligonucleotides were identified from their position on the plates and the composition of some verified by comparing the fingerprints with those made of 5S rRNA purified from yeast nuclei labelled *in vitro* with either α-^{32}P UTP or α-^{32}P ATP. With α-^{32}P UTP-labelling spots 1, 7, 9 and 10 are missing, with α-^{32}P ATP-labelling spots 2, 4, 6 and 13 are missing in agreement with the expected identity of the oligonucleotides. The numbering is given in Table I. Gp (#17) ran off the plate (Fig. 6A), the 3' end group (#18) is not well resolved in Fig. 6A.

fingerprint of the *in vitro* transcript since it is followed by a G. No attempts were made to ascertain the 5' end of the transcript, since the second G must be present as all four of the four predicted G's were labelled . This result proves that the 5S rRNA gene is correctly or almost correctly transcribed *in vitro*.

Under some conditions a longer transcript is visible which is about 7-10 nucleotides larger than the mature 5S rRNA. This longer transcript is not always present and appeared to be dependent on small differences in concentration of template or extract in the transcription assays since even identical transcription assays show different, but low amounts of this

Table I: T_1-oligonucleotides of 5S rRNA

Spot number	T_1 oligonucleotide	theoretical	Yield *in vitro* 5S rRNA experiment 1	experiment 2
1	CG (G/A)	0		
2	AG (C/U)	1	1.4	1.2
3	UG (G/A/C/U)	1	0.8	1.2
4	UUG (C)	1	0.9	1.3
5	UAG (U/U/U)	3	2.7	3.0
6	CUG (G/C)	2	2.7	2.1
7	UAAG (A)	0		
8	CCUG (A), UCCG (A)	1	1.5	1.2
9	ACCG (A)	0		
10	AAAG (C)	0		
11	UUAAG (C)	1	1.2	1.2
12	CACCG (U)	1	1.1	1.2
13	C_3U_3G (U)	3	2.8	2.5
14	$C_2A_3U_2G$ (C)	2	2.5	3.0
15	C_3A_3UG (U) plus C_2A_4UG (U)	2	1.7	1.2
16	$C_5A_4U_3G$ (G)	3	1.7	3.0
17	Gp (U/C/U/U/U/G)	4	4.3	3.8
18	$CAAUCU_{OH}$	2	1.7	1.1

T_1 - oligonucleotides were taken from the 5S rDNA sequence (40), the nucleotide(s) following each T_1 oligonucleotide are indicated in parentheses. The labelling was with α-^{32}P UTP. Spot 5 UAG contains possibly ΨAG in the *in vivo* and the *in vitro* rDNA.

longer transcript. It was not possible for this reason to prepare enough RNA for fingerprint analysis. It is possible that this evanescent RNA species is the readthrough or precursor RNA observed by Tekamp et al. (27).

d. Transcription of tRNA genes - No transcription of tRNA genes was observed with this extract. However, the chromatin fraction recovered as a pellet after the final centrifugation in the preparation of the transcriptional extracts still shows transcription activity, resulting in synthesis of 5S-sized RNA and tRNA-sized products (Fig. 7, lane 1). When the transcriptional extract containing the rest of the RNA polymerase III activity (Fig. 7, lane 3) is added back to the

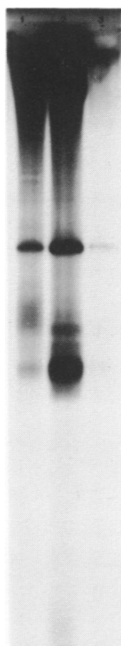

Figure 7. Low molecular weight RNAs synthesized using yeastchromatin. A. 0.3 M $(NH_4)_2SO_4$ yeast nuclear extract was prepared by lysis of nuclei for 15 minutes at 0°C followed by centrifugation at 30,000 x g. The pellet was solubilized in TEMMG buffer and is now called chromatin. Both chromatin fraction and supernatant fraction were dialyzed against buffer containing 50% glycerol. The assays contain in 100 µl: 25 µl chromatin (lane 1), 25 µl supernatant (transcriptional extract) (lane 3) and the reconstituted system of 25 µl chromatin plus 25 µl transcriptional extract (lane 2). The 5S rRNA band and the major tRNA band were cut out and counted in a liquid scintillation counter. cpm 5S band: lane 1: 900, lane 2: 2390 and lane 3: 250. cpm major tRNA band: lane 1: 460, lane 2: 1260, and lane 3: 200.

chromatin fraction, the synthesis of 5S rRNA and tRNA is greatly stimulated (Fig. 7, lane 3). The amount of 5S rRNA synthesis in this reconstituted system is more than the sum of the activities when chromatin is used (lane 1) or the extract alone is used (lane 3).

FINAL DISCUSSION

This *in vitro* transcription system is of value for several developments. Mutants of the 5S rRNA gene can be constructed *in vitro* (9,11,14) and used as a template to define the sites involved in transcriptional control. Additionally, the wild type and mutant genes can be isolated from yeast transformants as chromatin in a high copy number plasmid. These "mini- chromosomes" can be separated from bulk chromatin and have been shown to be packed into nucleosomes (29). The results show that although the cell free transcription system we have described can transcribe 5S rRNA genes it cannot transcribe the other abundant class of class III genes, the tRNA genes. The transcription of tRNA genes in the reconstituted system with

chromatin is greatly stimulated by addition of the transcriptional extract. Apparently the factors involved with tDNA transcription are still present in the chromatin fraction, and afford an opportunity for their isolation and assay. Klekamp and Weil (pers. comm.) using an in vitro transcription system from whole yeast cells find that in this system both the 5S rRNA and tRNA genes are specifically transcribed when added to this extract. They also suggest the presence of separate factors for tDNA and 5S rDNA transcription specificity.

ACKNOWLEDGEMENTS

We thank Dr. J.W. Szostak for providing us with plasmid DNA containing the yeast 5S rRNA gene. We are grateful to Dr. P.A. Weil for communicating results before publication and Dr. J. Klootwijk on his comments on the fingerprints.

This is N.R.C.C. publication number 20483.

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