J. Biosci., Vol.4, Number 2, June 1982, pp.239-244.

Evidence for non-coordinated synthesis of 5 S RNA in the thermophilic fungus, *Thermomyces lanuginosus*

S. NAGESWARA RAO and JOSEPH D. CHERAYIL*

Department of Biochemistry, Indian Institute of Science, Bangalore 560 012.

MS received 24 October 1981; revised 3 March 1982

Abstract. Analysis of ribosomes and the post ribosomal supernatant fraction of actively growing cells of *Thermomyces lanuginosus* showed the presence of free 5 S RNA in the supernatant fraction. This 5 S RNA was identical to the ribosomal 5 S RNA in its electrophoretic mobility on 10% Polyacrylamide gel and in its base composition. 5 S RNA from both the sources gave evidence for the presence of diphosphate at the 5' end. Most of the 5 S RNA that appeared in the cytoplasm was that transported from the nucleus during the isolation. This could be prevented by the use of a hexylene glycol-HEPES buffer.

Keywords. 5 S RNA; thermophilic fungus; ribosomal RNA; non-coordinated synthesis of PNA

Introduction

The genes for 5 S ribosomal RNA of prokaryotes are located on the chromosome as a cluster with other ribosomal RNA components and these RNA species are transcribed together in a unimolar ratio (Kossman *et al*., 1971; Hayes *et al*., 1975. On the other hand, eukaryotic 5 S RNA is a primary transcription product and often contains diphosphates or triphosphates at the 5′ terminus (Takai *et al*., 1975). Precursor 5 S RNA species when analysed have been found to have extra sequences at the 3′ end but not at the 5′ end (Rubin and Hogness, 1975). As the synthesis of eukaryotic 5 S RNA is independent of that of high molecular weight rRNA, an exact 1:1 molar ratio between the two types cannot be expected. In agreement with this, the kinetics of labelling of RNA with [^{3}H]-uridine has shown that 5 S RNA is synthesized in excess in HeLa cells and a free pool of 5 S RNA has been observed in the HeLa Cell nucleus (Knight and Darnell, 1967). It is reported that in exponentially growing HeLa cells the amount of 5 S RNA synthesized is four times greater than the 18 S and 28 S rRNA species (Leibowitz *et al.,* 1973). Seventy five per cent of the 5 S RNA synthesized is apparently degraded. In the ovaries of *Xenopus laevis* non-coordinated accumulation of 5 S RNA in early oogenesis has been observed (Ford, 1971). In this system the relative proportion of 5 S RNA has

* To whom communications should be sent.

been found to vary widely at different stages of oogenesis. Although there is an excess production of 5 S RNA during early oogensis it is compenstated by an excess production of 18 S and 28 S rRNA at later stages. The presence of free 5 S RNA has been found in the post-ribosomal supernatant fraction of rabbit reticulocytes (Zahavi-Willner and Danon, 1972), but it has been suggested that the soluble 5 S RNA present in reticulocytes arises by the degradation of ribosomes. Thus the production of 5 S RNA in various systems apparently does not follow a uniform pattern. Very little information is available regarding the biosynthesis and the nature of 5 S RNA in thermophilic fungi like *Thermomyces lanuginosus*. In this report we present evidence for the non-coordinated synthesis of 5 S RNA in logarithmically growing cells of *T. lanuginosus* and for the occurrence of a diphosphate at its 5′ end.

Materials and methods

Details regarding the organism and sources of radioactive materials and chemicals have been given earlier (Nageswara Rao and Cherayil, 1979).

Preparation of (^{32}P)-labelled RNA from ribosomes and S_{100} fractions

The fungus was labelled with radioactive phosphorus as indicated earlier (Nageswara Rao and Cherayil, 1979). After 18-22 h of growth the mycelia were collected by filtration followed by washing with water and drying. The fungal cake was mixed with glass powder (1 g for 1 g of fungus) and ground for 10-15 minutes at 0°C. The finely ground mycelial paste was extracted with polysomal extraction buffer containing 0.025 M Tris-HCl, pH 7.4, 0.02 M NaCl and 0.005 M $MgCl_2$ (5 ml/g wet weight of the cells). The mixture was centrifuged at 12,000 g for 15 min to remove the glass powder and cell debris. The supernatant fraction was recentrifuged at 105,000 *g* for 1 h. RNA was prepared from the pellet and supernatant fractions separately.

Instead of Tris-HCl, a buffer consisting of hexylene glycol (0.5 M), HEPES (0.05 mM) and $CaCl_2$ (1.0 mM), pH 6.8 was also used for the preparation of ribosomes and post-ribosomal supernatant fractions.

RNA from the ribosomes and the supernatant fractions was prepared by repeated extraction with phenol. The ribosomal pellet was stirred with 1-2 ml of a buffer containing 0.14 M NaCl, 0.01 M Tris-HCl, pH 7.3, 0.001 M Na_2EDTA and, 0.5% SDS. An equal volume of water-saturated phenol was added and stirred for 1 h. The aqueous phase was collected by centrifugation and again extracted with phenol two more times. The RNA was precipitated with two volumes of ethanol and washed with 75% ethanol. RNA from post-ribosomal supernatant fraction was prepared in a similar manner by extraction with phenol. The RNA samples thus prepared were subjected to gel-electrophoresis on Polyacrylamide gels (Peacock and Dingman, 1967).

Results

^{32}P-Labelled RNA samples isolated from ribosomes as well as S_{100} fraction were subjected to electrophoresis on a 10% acrylamide slab gel (Peacock and Dingman,

1967). Upon autoradiography the RNA sample from ribosomes showed the presence of 5.8 S RNA and 5 S RNA well separated from each other in addition to some contaminating tRNA. There was a considerable amount of radioactivity at the origin due to the high molecular weight rRNA which did not enter the gel. The sample from the post-ribosomal supernatant fraction showed the presence of tRNA and 5 S RNA (figure 1). There was no evidence for the presence of 5.8 S RNA in this fraction and there was very little radioactivity at the origin indicating the absence of contaminating ribosomes. Thus the presence of free 5 S RNA was indicated in the post-ribosomal supernatant fraction. The RNA from both the fractions moved with the same mobility, which was slightly slower than that of xylene cyanol and was similar to that of 5 S RNA from other sources.

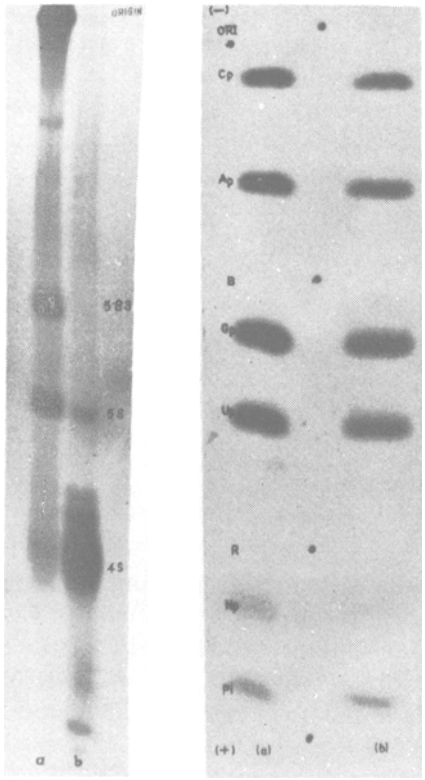

Figure 1. Autoradiogram of the separation of 5 S RNA on a 10% Polyacrylamide slab gel.

RNA samples prepared from ribosomes and post-ribosomal supernatant fractions were subjected to electrophoresis on 10% Polyacrylamide gels in Tris-borate buffer, pH 8.3 followed by autoradiography, (a) RNA from ribosomes, (b) RNA from post-ribosomal fraction.

Figure 2. Autoradiogram of the separation of RNase T_2 digestion products of 5 S RNA.

The 5 S RNA bands from the ribosomal and post-ribosomal supernatant fractions were cut out from the gel, the radioactivity eluted from each, digested and subjected to electrophoresis on paper at pH 3.5. (a) Ribosomal, (b) Post-ribosomal.

The bands corresponding to 5 S RNA from the ribosomal and post-ribosomal supernatant fractions were cut out separately, and the RNA was eluted from the gel and digested with RNase T_2. The digest was then subjected to electrophoresis on paper at pH 3.5. The pattern of separation of radioactivity was identical in both the cases. In addition to the four major nucleotide species a minor species (denoted as N_p) moving faster than the red dye could be noted (figure 2). No other nucleotide spot could be observed in the autoradiogram. The spots were cut out and the radioactivity in each was determined. Base composition obtained from the data clearly indicated that both the 5 S RNA samples were identical (table 1).

Table 1. Base composition of 5 S RNA from *T. lanuginosus.*

Nucleotide	Ribosomal		Post-ribosomal	
	CPM	%	CPM	%
C_p	9,820	26.3	4,370	25.9
A_p	7,660	20.6	3,480	20.6
G_p	11,080	29.7	4,880	28.9
U_p	7,810	20.9	3,720	22.0
pp^Gp	940	2.5	440	2.6

The 5 S RNA bands were cut out from the gel and the radioactivity from each band was extracted. It was digested with RNase T_2 and nucleotides were separated by high-voltage paper electrophoresis at pH 3.5 in pyridine-acetate buffer. After auto-radiography (figure 2) the radioactive spots were cut out and counted in a liquid scintillation counter. The results of one of the representative experiments are presented.

The radioactivity in the unknown spot amounted to approximately 2.5% of the total, representing 3 phosphate groups for a chain of 120 nucleotides of the 5 S RNA (Erdmann, 1977). Therefore the minor spot could be a modified mononucleotide in the ratio of 3 mol per mol of 5 S RNA, an alkali resistant O'-methylated trinucleotide in unimolar ratio or a nucleotide with three phosphate groups on it. Since no 5 S RNA has so far been reported to contain modified nucleotides, except submolar amounts of pseudouridine, the possibility that the minor spot might be a nucleoside with three phosphate groups appeared more likely. Obviously, the nucleotide being an RNase T_2 digestion product, must be of the type ppNp, released from the 5' end. The spot moved faster than pGp and pppG which have 4 units of negative charge each, although pppG has an extra phosphate group. It moved very much faster than pAp and pCp· both of which have the same mobility as Up. pUp on the other hand, moved faster than the unknown spot. When considered among the various possible ppNp structures, ppGp was the most probable 5′ end of the 5 S RNA molecule. This type of 5′ end has been reported for 5 S RNA from a number of sources such as different strains of yeast (Erdmann, 1977).

It was not clear from the above studies whether the 5 S RNA observed in the cytoplasm was originally present there or whether it leached out into the cytoplasm from the nucleus during the isolation of the S_{100} fraction. A buffer system consisting of hexylene glycol and HEPES is known to prevent the leaching out of nuclear components into the cytoplasm (Wray *et al*., 1977). When post-ribosomal supernatant fraction was prepared using hexylene-glycol-HEPES buffer, pH 6.8, there was practically very little 5 S RNA in the supernatant fraction (Results not presented). This indicated that the free 5 S RNA largely remained in the nucleus during the growth of the cell.

Discussion

It is evident from the present studies that there is a pool of soluble 5 S RNA (unassociated with proteins) in the nucleus of *T. lanuginosus* at the log phase of its growth. This free 5 S RNA could leach out during extraction with aqueous buffers normally used. The pool size in the present case is not known. The results clearly indicate that in *T. lanuginosus* 5 S RNA is synthesized in amounts larger than that required for the maintenance of a stoichiometric ratio with other ribosomal species. Although an excess of 5 S RNA is produced in early oogenesis in *Xenopus,* it is compensated by an excess production of 18 S and 28 S RNAs later (Ford, 1971). In HeLa cells the excess 5 S RNA produced is degraded (Leibowitze *et al.*, 1973). As there is an excess production of 5 S RNA in actively growing *T. lanuginosus* cells, in which ribosome synthesis is expected to take place at a very fast rate, it must be assumed that there is no compensation. The excess of 5 S RNA produced may be degraded.

Transport of soluble nuclear components into the cytoplasm from the nucleus during extraction has been observed in a number of cases. This is restricted mainly to low molecular weight components. Presence of precursors of tRNA in the cytoplasm has been noted, although the splicing and processing enzymes are present in the nucleus only (Melton *et al*., 1980). The present studies have shown that migration of 5 S RNA from the nucleus to the cytoplasm may be prevented by the use of the hexylene-glycol-HEPES buffer. This may apply to precursors of tRNA also. Under the conditions employed the nucleus apparently is not disrupted. No RNA, other than 5 S RNA and tRNA is observed in the cytoplasm.

The presence of a small amount of 5 S RNA even when hexylene-glycol-HEPES buffer is used has been noted. It is not clear whether a small amount of 5 S RNA was transported into the cytoplasm during the isolation or whether it was originally present there *in vivo*. It has been reported by Wreschner (1978) that 5 S RNA in nanogram quantities inhibits the translation of globin mRNA in wheat germ system whereas other RNAs tested do not. It is possible that the free 5 S RNA acts as a modulator of protein synthesis. It is conceivable that the free 5 S RNA present in the nucleus can, under certain metabolic conditions, cross over into the cytoplasm and exert its inhibitory influence on protein synthesis and thus control translation.

Eukaryotic 5 S RNA is a primary transcription product and in most cases it has a triphosphate at the 5′ end (Erdmann, 1977). The 5 S RNA of *T. lanuginosus,* on the

other hand, has a diphosphate at the 5′ end. In this respect it resembles yeast, to which it is related. The mechanism of processing of the triphosphate to diphosphate and the significance of the presence of the diphosphate at the 5′ end of the mature 5 S RNA is not yet clear.

Acknowledgement

This research project is supported in part from funds obtained from the Indian Council of Medical Research, New Delhi.

References

Erdmann, V. A. (1977) *Nucleic Acid Res.,* **4,** rl.
Ford, P. J. (1971) *Nature,* **233,** 561.
Hayes, F., Vasseur, M., Nikolaev, N., Schlessinger, D., Sriwidada, J., Krol, A. and Branlant, C. (1975) *FEBS Lett.,* **56,** 85.
Knight, E. Jr. and Darnell, J. E. (1967) *J. Mol. Biol*., **28,** 491.
Kossman, C. R., Stamato, T. D. and Pettijohn, D. E. (1971) *Nature New Biol.*, **234,**102.
Leibowitz, R. D., Weinberg, R. A. and Penman, S. (1973)*J. 'Mol. Biol.,* **73,** 139.
Melton, D. A., DeRobertis, E. M. and Cortese, R. (1980) *Nature,* **284,**143.
Nageswara Rao, J. S. and Cherayil, J. D. (1979) *Curr. Sci*., **48,** 983.
Peacock, A. C. and Dingman, C. W. (1967) *Biochemistry,* **6,**1818.
Rubin, G. M. and Hogness, D. S. (1975) *Cell,* **6,** 207.
Takai, K., Hashimoto, S. and Muramatsu, M. (1975) *Biochemistry,* **14,** 536.
Wray, W., Conn, P. M. and Wray, V. P. (1977) *Methods Cell Biol.,* **16,** 69.
Wreschner, D. H. (1978) *FEBS Lett.,* **94,** 145.
Zahavi-Willner, T. and Danon, D. (1972) *FEBS Lett.,* **26,**151.