Navon, G., Shulman, R. G., Wyluda, B. J., & Yamane, T. (1970), *J. Mol. Biol. 51,* 15.
Reid, E. E. (1958), *Organic Chemistry of Divalent Sulfur,* Vol. 1, Chemical Publishing Co., New York, N.Y.
Rombauts, W. A. (1968), in *Physiology and Biochemistry of Haemocyanins* (Ghiretti, F., Ed.), pp 75–80, Academic Press, London.
Salvato, B., Ghiretti-Magaldi, A., & Ghiretti, F. (1974) *Biochemistry 13,* 4778.
van Holde, K. E., & van Bruggen, E. F. J. (1971) in *Subunits in Biological Systems,* (Timasheff, S. N., & Fasman, G. D., Eds.), Part A, pp 1–53, Marcel Dekker, New York, N.Y.
Walker, J. B., & Walker, M. S. (1960) *Arch. Biochem. Biophys. 86,* 80.
Wilkinson, G. N. (1964) *Biochem. J. 80,* 324.
Witters, R., & Lontie, R. (1975) *FEBS Lett. 60,* 400.

Heterogeneity in the 3′-Terminal Sequence of Ribosomal 5S RNA Synthesized by Isolated HeLa Cell Nuclei in Vitro†

Mikio Yamamoto‡ and Klaus H. Seifart*

ABSTRACT: Isolated HeLa cell nuclei synthesize ribosomal 5S RNA of very nearly correct sequence and size. The in vitro product was resolved according to size on formamide-containing polyacrylamide gels and the fractions were subsequently hybridized to recombinant DNA containing the 5S genes from *Xenopus mulleri.* It could be shown that the 5S RNA synthesized in vitro differed only very slightly in size from the mature species labeled in vivo and contained a few extra nucleotides in some of the molecules. Analysis of the 3′-terminal base of molecules synthesized independently with four different nucleotides showed that the chains were almost exclusively terminated with uridine. Digestion of the in vitro product with ribonuclease T_1 and analysis of the oligonucleotides on DEAE-Sephadex A-25 in the presence of 7 M urea revealed that the molar yield of the internal fragments agreed well with the expected theoretical values. The 3′-terminal fragments, however, were found to be present in three different species with the sequences CUU_{OH}, $CUUU_{OH}$, $CUUUU_{OH}$ which occurred with a frequency of about 60%, 20%, and 20%, respectively. From these data we conclude that 5S RNA synthesis in isolated HeLa cell nuclei was correctly initiated but that termination occurred with a slight ambiguity, adding either one or two uridine residues to some of the chains.

To analyze regulatory mechanisms of transcription, it is necessary to establish in vitro systems in which a given RNA product is synthesized with acceptable fidelity in primary sequence and size. Conventional sequencing and/or hybridization techniques can be used for these studies provided the exact size of the initial product (precursor molecule) is known for a given RNA species. In a previous report (Yamamoto and Seifart, 1977) we have shown that isolated HeLa cell nuclei provide a suitable system to study the transcription of ribosomal 5S RNA. Although the system itself is crude, it has been shown that 5S RNA is transcribed with high fidelity both in sequence and size.

Analysis of 5S rRNA synthesized in vivo has provided evidence that most of these molecules have retained the β or even γ-phosphate (Denis and Wegnez, 1973), indicating that the 5′ terminus of the mature molecule probably represents that of the initial transcription product. On the contrary, the 3′ end is heterogeneous both in sequence and in length (Denis and Wegnez, 1973; Forget and Weissmann, 1967), indicating that the 3′ terminus of the mature RNA is different from the primary transcript. It is not known, however, whether this end is generated by trimming of larger precursor molecules, or by the addition of nucleotides. Precursor molecules of 5S RNA were initially shown to occur in cells of *E. coli* (Freunteun et al., 1972) and bacilli (Pace et al., 1973; Stoof et al., 1974). The existence of precursor molecules to 5S rRNA has not definitively been shown for eukaryotic cells, although suggestive evidence to this extent has been put forward by Denis and Wegnez (1973) and Rubin and Hogness (1975) for *Xenopus* oocytes and cultured *Drosophila* cells, respectively. In both cases the molecules contained approximately 15 additional nucleotides at the 3′ end. Especially in *Drosophila* cells such molecules were accumulated following the exposure of the cells to higher than physiological temperatures, presumably due to a temperature-sensitive processing step (Rubin and Hogness, 1975). We have investigated this question by analyzing the 3′-terminal sequence of 5S rRNA synthesized by isolated nuclei from HeLa cells.

Materials and Methods

Isolation of Nuclei and Synthesis of RNA. HeLa cells were grown in suspension culture and nuclei were isolated by the procedure described previously. They were suspended as described (Yamamoto and Seifart, 1977) at a concentration of about 1×10^8/mL and subsequently incubated at 25 °C for 20 min in a volume of 120 μL containing: 0.5 μg of α-amanitin/mL, 50 mM NH_4Cl, 5 mM $MgCl_2$, 12.5% glycerol, 25 mM Tris-HCl, pH 7.9, 2.5 mM dithiothreitol, 0.1 mM EDTA, 1 mM each of ATP, GTP, CTP, 0.044 mM and 100 μCi of [^{3}H]UTP (Radiochemical Centre, Amersham). The reaction was terminated by the addition of 10 μL of a solution of *E. coli*

† From Institut für Physiologische Chemie, Universität Marburg, 355 Marburg/Lahn, BRD, Lahnberge, West Germany. *Received July 7, 1977; revised manuscript received October 28, 1977.* This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 103). Financial support (to M.Y.) was provided by the Alexander von Humboldt Stiftung.
‡ Recipient of a Fellowship from the Alexander von Humboldt Stiftung.

0006-2960/78/0417-0457$01.00/0

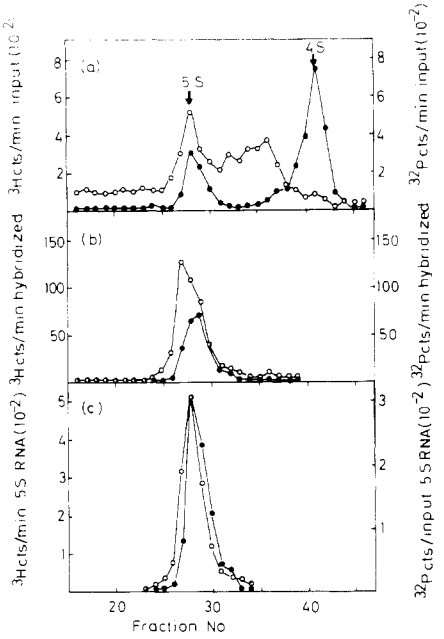

FIGURE 1: Co-electrophoresis of low molecular weight RNA species synthesized by nuclei in vitro and cells in vivo on formamide containing polyacrylamide gels. RNA was synthesized under standard conditions and electrophoresed for 9 h at room temperature. Gel fractions were solubilized and hybridized to plasmid DNA containing 5S DNA. (a) Gel electropherogram; (b) hybridization profile; (c) hybridization profile normalized for efficiency within each fraction; (○—○) ^{3}H-labeled in vitro RNA; (●—●) ^{32}P-labeled in vivo 5S and transfer RNA.

tRNA (2.5 mg/mL in water) and 20 μL of DNase (RNase-free Worthington, 375 μg/mL in 3 mM $MgCl_2$). The incubation was subsequently prolonged for 10 min at 0 °C and DNase digestion was stopped by the addition of 150 μL of 1% sodium dodecyl sulfate. RNA was subsequently extracted with phenol and chloroform and precipitated with ethanol at −20 °C. It was dissolved in 0.5% sodium dodecyl sulfate and used as such for hybridization and size analyses.

Size Analyses of the RNA Products. The RNA synthesized in vitro was separated on formamide-containing polyacrylamide gels (12%; cross-linked with 0.25% ethylene diacrylate) or by gel filtration through Sephadex G-100 columns. The RNA was hybridized to recombinant plasmid DNA containing 5S DNA from *Xenopus* toads (Carroll and Brown, 1976). Growth of *E. coli* cells, purification of plasmid DNA, and hybridization were conducted as described previously (Yamamoto and Seifart, 1977).

Analyses of Oligonucleotides. In vitro RNA was synthesized in the presence of either [^{3}H]UTP or [^{3}H]CTP. It was purified through Sephadex G-100 and the 5S region was subsequently hybridized to plasmid DNA. The 5S RNA was then recovered from the DNA filters by heating at 96 °C in 0.1 SSC[1] for 5 min. It was digested by ribonuclease T_1 (500 units) in the presence of carrier tRNA from *E. coli* (200 μg) and a mixture of ^{32}P-labeled 28S and 18S ribosomal RNA from HeLa cells in 500 μL of 0.05 M Tris, pH 7.4, containing 0.002 M EDTA. The solution was incubated for 6 h at 37 °C and subsequently diluted tenfold with buffer containing 0.02 M Tris, pH 7.9, and 7 M urea. This sample was applied to a column of DEAE-Sephadex A-25 which was equilibrated in the same buffer and eluted with a linear gradient of NaCl from 0.066 to 0.40 M (150 mL each) according to the procedure of

[1] Abbreviations used: SSC, standard saline citrate; PEI, polyethylenimine.

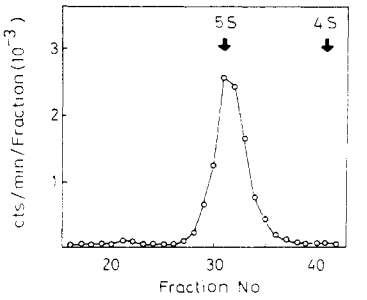

FIGURE 2: Size analysis of purified 5S RNA by gel filtration through a Sephadex G-100 column (1.5 × 60 cm). The 5S RNA was synthesized in vitro and purified as described in Materials and Methods. Hybridized RNA was eluted from the filter discs by heating at 96 °C for 5 min in 0.1 × SSC, precipitated with ethanol, and subsequently subjected to gel filtration.

Suzuki and Brown (1972) and described previously for this system (Yamamoto and Seifart, 1977).

Determination of 3'-Terminal Bases. RNA was synthesized in vitro in the presence of four different labeled nucleoside triphosphates. The 5S RNA was purified by gel filtration and hybridization as described above. After hydrolysis by alkali, neutralization by perchloric acid, lyophilization, and resuspension of the sample in distilled water, the ratio of nucleoside to nucleoside monophosphate was determined for the four different nucleotides by thin-layer chromatography on polyethylenimine plates (Randerath and Randerath, 1967). The areas on the plates containing the nucleosides and their monophosphates were localized by appropriate markers and subsequently excised. The radioactivity was solubilized in 0.2 mL of NCS (Amersham/Searle) and 10 mL of toluene based scintillation fluid by shaking overnight at 37 °C.

Results

(a) *Size of 5S RNA Synthesized in Vitro.* It was previously shown that isolated HeLa cell nuclei are capable of synthesizing ribosomal 5S RNA of very nearly correct sequence and length (Yamamoto and Seifart, 1977), although minor differences in size would not have been detected by the methodology employed. Two major classes of small molecular weight RNAs are synthesized by this nuclear system in the presence of low concentrations of α-amanitin, namely, 5S and 4.5S RNA (Figure 1a). Ribosomal 5S RNA sequences were analyzed by hybridizing the appropriate fractions to plasmid DNA. It was repeatedly observed previously (Yamamoto and Seifart, 1977), and is confirmed in Figure 1b, that the in vitro 5S RNA appeared slightly larger than mature in vivo 5S RNA, provided the double-labeled samples are coelectrophoresed in the same gel slot. This apparent difference in length does not necessarily reflect an actual size difference, since the hybridization efficiency could vary from fraction to fraction due to the presence of different amounts of cold endogenous 5S RNA. The correct hybridization profile for the in vitro product can, however, be depicted since the in vivo 5S RNA included in the same sample provides an ideal internal standard for the calculation of the hybridization efficiency within each fraction. Figure 1c represents data which were normalized in this fashion and it shows that the peak position is the same for in vitro and in vivo 5S RNA. The in vitro product possibly does contain a component, however, which migrates slightly slower than mature 5S RNA. This difference can obviously only be detected by the double-labeling and hybridization technique employed.

(b) *Purification of 5S RNA Synthesized in Vitro.* To elucidate the above mentioned minor difference in size, it is es-

TABLE I: ^{3}H-Labeled Terminal Base Analysis.[a]

Labeled with	cpm in Nucleotide	cpm in Nucleoside	Nucleotide/ nucleoside
U	7336	262	28
G	2924	12	243
A	1752	5	351
C	4623	1	∞

[a] RNA was synthesized in vitro with [^{3}H]UTP, -GTP, -ATP, or -CTP as a sole radioactive precursor. The 5S RNA was purified as described, hydrolyzed with alkali (Randerath and Randerath, 1967), and chromatographed on a polyethylenimine thin-layer plate. The plates (20 × 20 cm) were developed in distilled water until the solvent front had migrated approximately 12 cm from the start. Radioactivity in the appropriate areas was assessed as described in Materials and Methods.

sential to purify in vitro 5S RNA from nonribosomal but 5S-sized RNA. As a first step, the in vitro product was therefore subjected to gel filtration on Sephadex G-100 (Yamamoto and Seifart, 1977). It was previously shown that hybridizable sequences could only be detected in the 5S-sized region. Fractions coeluting with 5S ribosomal RNA were therefore pooled, concentrated by ethanol precipitation, and subsequently hybridized to plasmid DNA. The radioactivity was recovered from the hybrid by heating at 96 °C for 5 min in 0.1 × SSC. This material was rechromatographed on Sephadex G-100 to validate its correct size. The results are presented in Figure 2 and they show that virtually all the radioactivity coelutes with 5S RNA on this column. The size homogeneity of the 5S RNA thus purified was also confirmed by electrophoresis on formamide containing polyacrylamide gels (data not shown).

(c) *3′-Terminal Bases.* It was shown (Figure 1) that 5S RNA synthesized by nuclei in vitro is possibly of slightly larger size than in vivo 5S RNA. Therefore 3′ termini were determined by synthesizing RNA with four different labeled nucleotides. In vitro 5S RNA samples were purified as described in Results (section b), hydrolyzed by alkali, and chromatographed on PEI plates as described in Materials and Methods. As summarized in Table I, the 3′ termini of 5S RNA contained predominantly uridylic acid and the ratio of uridine to UMP was estimated to be about 28. This ratio is in good agreement with the number derived from the reported sequence of mature human 5S RNA (Forget and Weissmann, 1967; Hatlen et al., 1969) and it indicates that the 5S RNA synthesized in vitro is uniformly labeled. Moreover, these data reinforce previously drawn conclusions concerning the efficient reinitiation of this class of RNA in isolated nuclei (Yamamoto and Seifart, 1977).

(d) *Analysis of Oligonucleotides.* 5S RNA labeled with [^{3}H]UTP and purified as described in section b was digested with ribonuclease T_1 in the presence of carrier tRNA from *E. coli* and ^{32}P-labeled 28S and 18S ribosomal marker RNA from HeLa cells. Oligonucleotides were separated on the basis of their length by chromatography on DEAE-Sephadex A-25 in the presence of 7 M urea. The results are shown in Figure 3 in which the roman numerals I to XIII denote the number of nucleotides contained in the respective fragments. Separation occurs according to net negative charge and, therefore, peak no. I contains the sequences X_P as well as $X_PX_PX_{OH}$. T_1 ribonuclease digestion of 5S RNA does not yield uridine mononucleotides and, therefore, the ^{3}H radioactivity in peak no. I should represent the 3′-terminal fragment $-CUU_{OH}$ according to its net negative charge (−2) derived from the phosphate groups (Denis and Wegnez, 1973). To substantiate

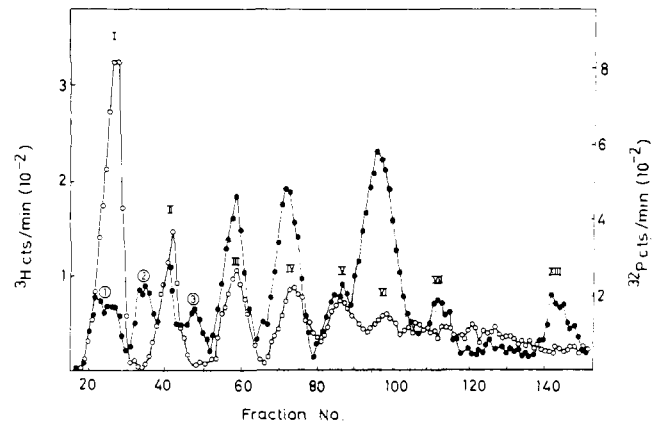

FIGURE 3: Chromatography of T_1 ribonuclease digests of 5S RNA on DEAE Sephadex A-25. The 5S RNA, labeled in vitro with [^{3}H]UTP, was digested with RNase T_1 in the presence of ribosomal 18 and 28S RNA labeled with ^{32}P in vivo and used as marker. RNA fragments were applied to the column (0.6 × 23 cm) and eluted with a linear gradient of NaCl (0.06 to 0.4 M; 150 mL each) in the presence of 7.0 M urea. (●—●) 5S [^{3}H]RNA; (○—○) high molecular weight ribosomal [^{32}P]RNA.

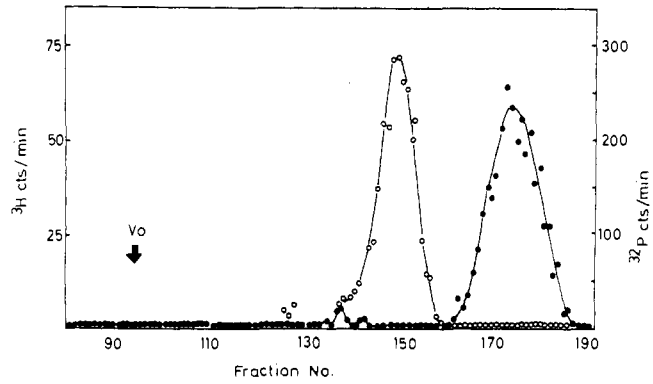

FIGURE 4: Separation of oligonucleotides contained in peak I from Figure 3 on Sephadex G-25. Radioactive material in peak I from Figure 3 was pooled, desalted according to the procedure of Suzuki and Brown (1972), and applied to a column of Sephadex G-25 (1.5 × 120 cm). The chromatography was conducted exactly as described by Denis and Wegnez (1973). (○—○) ^{3}H; (●—●) ^{32}P.

this point, the material in peak no. I of Figure 3, containing [^{32}P]GMP derived from high molecular weight ribosomal RNA labeled in vivo, and ^{3}H counts, derived from 5S rRNA labeled in vitro, was subjected to gel filtration on Sephadex G-25. The results in Figure 4 show that the ^{32}P counts associated with GMP are clearly separated from the ^{3}H counts derived from UTP labeling of 5S rRNA in vitro. Therefore the ^{3}H counts in peak I are contained in molecules of distinctly larger than mononucleotide size, probably representing the sequence CUU_{OH}.

To confirm the results obtained by UTP labeling, 5S rRNA was labeled with [^{3}H]CTP and subsequently purified and analyzed as described. A quantitative assessment of the internal oligonucleotides obtained after in vitro labeling of 5S rRNA with either UTP or CTP is summarized in Table II. The molar yields of internal fragments demonstrate an almost complete stoichiometric agreement with that theoretically deduced from the sequence of in vivo 5S rRNA from KB cells. The correlation coefficient between the experimentally observed and the theoretical yields was 0.97^{+++}. It can therefore be concluded that the 5S rRNA purified by this method is transcribed from the initiation site to the termination signal on the 5S DNA. It was observed, however, that a certain degree of heterogeneity was observed in the 3′-terminal oligonucleo-

TABLE II: Analysis of Molar Yields of Oligonucleotides Obtained after T_1 RNase Digestion of 5S rRNA Synthesized in Vitro.

(a) Internal Oligonucleotides[a]

Oligonucleotide fragment	Labeling of RNA with [^{3}H]UTP: Radioact. in fragment (cpm)	[^{3}H]UTP: Molar yield Obsd	[^{3}H]UTP: Molar yield Theor	Labeling of RNA with [^{3}H]CTP: Radioact. in fragment (cpm)	[^{3}H]CTP: Molar yield Obsd	[^{3}H]CTP: Molar yield Theor
II	324	1.3	1.0	475	1.9	1.0
III	944	4.1	4.0	1115	4.4	4.0
IV	1228	5.3	6.0	2749	11.0	11.0
V	296	1.3	1.0	438	1.7	1.0
VI	2092	9.0	9.0	1532	6.2	7.0
VII	228	1.0	1.0	588	2.4	2.0
XIII	380	1.6	2.0	1420	5.7	7.0

(b) 3′-Terminal Fragments after [^{3}H]UTP Labeling.

Peak	Radioact. (cpm)	Supposed sequence	Molar yield	% of total
1	356	CUU	0.76	58.0
2	208	CUUU	0.29	22.1
3	248	CUUUU	0.26	19.8

[a] The radioactivity in the individual fragments after UTP and CTP labeling was compiled from Figures 3 and 5, respectively. To assess the relative yield of the individual fragments, the radioactivity in the most predominant peak was correlated with the number of labeled residues expected in that particular T_1 fragment as deduced from the known sequence of 5S RNA. The hexanucleotide fragments (VI) are known to contain 9 residues of uridine. Since these are associated with 2092 cpm, a specific activity of 232 cpm per uridine residue was used to calculate the yield of the other fragments. Similarly the tetranucleotide fragments (IV), containing 11 residues of cytidine, were employed to calculate the observed yields in the other fragments after CTP labeling. The theoretical yields of the fragments, deduced from the sequence, are given for comparison. Correlation coefficient between predicted and experimentally observed yields was calculated to be 0.97^{+++}.

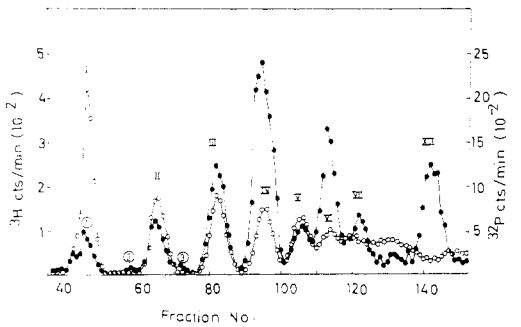

FIGURE 5: Analysis of T_1 ribonuclease digests of 5S RNA on DEAE-Sephadex A-25. The 5S RNA was labeled with [^{3}H]CTP, digested with RNase T_1, and chromatographed as described in Figure 3. (●—●) The 5S [^{3}H]RNA labeled in vitro; (○—○) ^{32}P-labeled ribosomal RNA, used as marker.

tides. In addition to peak no. 1, representing the terminal oligonucleotide CUU_{OH}, two additional components, denoted by encircled arabic numerals 2 and 3, appeared in Figure 3. They carry a net negative charge of −3 ($-C_pU_pU_pU_{OH}$) and −4 ($-C_pU_pU_pU_pU_{OH}$), respectively. The shift in the chromatogram relative to peaks no. II (−3) and III (−4) is probably due to the different purine-pyrimidine composition which results in an earlier elution from the column. These results are in good agreement with those reported by Denis and Wegnez (1973). The sum of the terminal nucleotides is slightly in excess of the expected value (1.3, Table IIb, vs. 1.0). This could be due to experimental error, or to the fact that some of the 5S molecules were obviously initiated in vivo and only terminally labeled in vitro. The relative stoichiometry of peaks 1, 2, and 3, analyzed in Table IIb, indicates that approximately 60% of the newly synthesized 5S rRNA was terminated with $-CUU_{OH}$, whereas 40% had either one or two excess uridylic acid residues, considered to be $-CUUU_{OH}$ and $-CUUUU_{OH}$, respectively (Denis and Wegnez, 1973). These results support the findings obtained from polyacrylamide gel electrophoresis (Figure 1) showing that in vitro 5S RNA is slightly longer, at least in a certain proportion of the molecules. The 3′-terminal sequences after CTP labeling are present in one major peak 1 as CUU_{OH} whereas the two other components 2 and 3 (Figure 5) are merely present as a shoulder and presumably represent the sequence $CUUU_{OH}$ and $CUUUU_{OH}$, respectively. The radioactivity associated with these components 2 and 3 after CTP labeling is too small to allow a quantitative assessment because they represent minor components and each terminal fragment contains only one cytidylic residue (Tables I and IIb). Additionally added terminal nucleotides are clearly detectable by UTP but not by CTP labeling.

Discussion

Isolated nuclei have recently been shown to synthesize 5S rRNA molecules in vitro which closely resemble mature 5S rRNA with respect to sequence and size (Weinmann et al., 1976; Yamamoto and Seifart, 1977). The primary transcript for ribosomal 5S RNA in higher eukaryotic cells has, however, not yet been definitely identified. It has previously been observed in organ culture cells from *X. laevis* ovaries (Denis and Wegnez, 1973) and in heat-shocked cultured cells from *D. melanogaster* (Rubin and Hogness, 1975) that 5S RNA species could be isolated which contained approximately 15 extra nucleotides at the 3′ terminus of the molecule. Based on sequence analyses of 5S DNA from two closely related animals *X. laevis* and *X. mulleri*, Brownlee et al. (1974) have suggested that the termination signal for ribosomal 5S RNA could reside at an AT-rich stretch with the sequence

...C T T T T...
...G A A A A...

Those 5S RNA molecules containing 15 extra nucleotides could possibly result from a partial skipping of the normal

termination signal and continuation of transcription to a second site

...C T T T A...
...G A A A T...

in the spacer with a related sequence (Fedoroff and Brown, 1977).

We have examined the question concerning the general synthesis of a precursor molecule to 5S RNA in extensive studies in which HeLa cells were heat shocked at 43 °C for 2 h. Subsequent synthesis of RNA by isolated nuclei from such cells has not yielded any evidence for a larger precursor to 5S rRNA (Yamamoto and Seifart, unpublished). Additional studies using isolated chromatin from HeLa cells have likewise failed to document such a precursor (Yamamoto et al., 1977). These in vitro transcription systems from HeLa cells contain a very low quantity of ribonuclease activity (Udvardy and Seifart, 1976) and, although a putative pre-5S RNA processing enzyme was not extensively screened for, we would like to conclude that the primary transcription product of ribosomal 5S RNA in HeLa cells is almost equivalent in size to that of the mature molecule.

Our results do show, however, that the termination site for 5S RNA is not quite rigid in isolated nuclei since at least three different 3′-terminal oligonucleotides were observed after ribonuclease T_1 digestion of the product (Figures 3 and 5). Previous data from sequence analyses of 5S RNA obtained from mature ribosomal particles of KB cells have shown that the 3′ terminus was ambiguous and could either be $-CUU_{OH}$ or $-CUUU_{OH}$ (Forget and Weissmann, 1967). At that time it was, however, impossible to determine whether this ambiguity is derived from termination of transcription, posttranscriptional processing, or the nontranscriptive addition of one uridylic acid residue. In this report we present evidence showing that the terminal ambiguity might occur through the transcription process itself since the yield of 3′-terminal fragments was similar to that of the internal oligonucleotides (Table II). The sequence of the spacer adjacent to the 5S gene is not known for HeLa cells and hence it is difficult to prove that the additional residues of UMP are added transcriptionally. Assuming that this is the case, however, the spacer would contain at least two residues of A in the codogenic strand immediately adjacent to the 5S gene in HeLa cells. This would agree with the known sequence for *Xenopus* (Fedoroff and Brown, 1977) and yeast (Gilbert et al., 1977) spacer regions adjacent to the 5S gene. It is unlikely that the ambiguity observed occurs by nontranscriptive addition of uridylic residues in vitro (Yamamoto and Roeder, unpublished data), since the molar yield of 3′ termini should then be much higher due to large amounts of unlabeled endogenous 5S RNA contained in nuclei which would serve as a ready substrate for such a mechanism. Uridylation of 5S RNA is furthermore rendered unlikely by the ratio of uridine to UMP in alkaline hydrolysates which is very close to the figure expected for in vivo RNA (Table I). Moreover, the synthesis of 5S rRNA in HeLa nuclei is completely sensitive to high concentrations of α-amanitin (Yamamoto and Seifart, 1977).

It is completely unknown whether ambiguous termination can be generalized to the transcription of other products by RNA polymerase C (III) such as pre-tRNA or to the syntheses catalyzed by enzymes A (I) and B (II). Sequence heterogeneity at the 3′ terminus has been observed in reconstituted transcriptional systems from λ DNA and *E. coli* RNA polymerase, in which λ-specific small molecular weight RNA species (4S and 6S) were terminated at related sequences, regardless of whether termination factor rho was present (Rosenberg et al., 1975). Although two different forms of 5S RNA could be recovered from mature ribosomal particles, it is impossible to functionally separate such particles and the significance of ambiguous termination of 5S RNA transcription therefore remains to be established.

Acknowledgments

We gratefully acknowledge expert technical assistance of Doris Schwarz and Frauke Seifart.

References

Brownlee, G. G., Cartright, E. M., and Brown, D. D. (1974), *J. Mol. Biol. 89,* 703–718.
Carroll, D., and Brown, D. D. (1976), *Cell 7,* 467–475.
Denis, H., and Wegnez, M. (1973), *Biochimie 55,* 1137–1151.
Federoff, N. V., and Brown, D. D. (1977), personal communication.
Feunteun, J., Jordan, B. R., and Monier, R. (1972), *J. Mol. Biol. 70,* 465–474.
Forget, B. G., and Weissmann, S. M. (1967), *Science 158,* 1695–1699.
Gilbert, W., Maxam, A. M., Tizard, R., and Skryabin, K. (1977), personal communication.
Hatlan, L. E., Amaldi, F., and Attardi, G. (1969), *Biochemistry 8,* 4989–5005.
Pace, N. R., Pato, M. L., Mc Kibbin, J., and Radcliffe, C. W. (1973), *J. Mol. Biol. 75,* 619–631.
Randerath, K., and Randerath, E. (1967), *Methods Enzymol. 12A,* 323–347.
Rosenberg, M., Weissmann, S., and De Combrugghe, B. (1975), *J. Biol. Chem. 250,* 4755–4764.
Rubin, G. M., and Hogness, D. S. (1975), *Cell 6,* 207–213.
Stoof, J. J., de Regt, V. C. H. F., Raue, H. A., and Planta, R. J. (1974), *FEBS Lett. 49,* 237–241.
Suzuki, Y., and Brown, D. D. (1972), *J. Mol. Biol. 63,* 409–429.
Udvardy, A., and Seifart, K. H. (1976), *Eur. J. Biochem. 62,* 353–363.
Weinmann, R., Brendler, T. G., Raskas, H. J., and Roeder, R. G. (1976), *Cell 7,* 557–566.
Yamamoto, M., and Seifart, K. H. (1977), *Biochemistry 16,* 3201–3209.
Yamamoto, M., Jonas, D., and Seifart, K. H. (1977), *Eur. J. Biochem. 80,* 243–253.