Studies on the 5′ Termini of Novikoff Ascites Hepatoma Ribosomal Precursor RNA†

Ross N. Nazar‡

ABSTRACT: The 5′ termini of 18S and 28S rRNA and their 32S, 41S, and 45S nRNA precursors were analyzed by fractionating alkaline hydrolysates of the RNAs on columns of diethylaminoethyl-Sephadex A-25 at neutral pH followed by electrophoresis on Whatman 3MM paper at pH 3.5. The results indicate that each RNA begins with a mononucleoside diphosphate of heterogeneous composition. The principal nucleoside diphosphate was pCp in 28S, 32S, 41S, and 45S RNA and pUp in 18S RNA. The 18S rRNA also contained the most homogeneous composition with approximately 70% of the molecules beginning with pUp; the 41S and 45S nRNA termini were the most heterogeneous, each containing pCp in only one-third of the molecules. Each terminal composition was reproducible and characteristic for its RNA. The composition of 28S rRNA was very similar to that of 32S nRNA, but distinctly different from 5.8S or 18S rRNA or 45S nRNA. The isomeric forms of 45S nRNA contained no appreciable amounts of a terminal 5′-triphosphate, although they had similar nucleoside diphosphate compositions. These results are discussed in relation to the transcription of ribosomal genes and the arrangement of the 5.8S rRNA sequence within these genes.

The 5.8S, 18S, and 28S rRNAs of mammalian ribosomes are transcribed in the nucleolus as a single precursor molecule, 45S nRNA. Labeling kinetics, hybridization studies, and sequence analyses (see reviews by Grierson et al., 1970; Choi et al., 1975) have shown that the mature RNA species are derived from this 45S precursor through a series of intermediate molecules and cleavages (Figure 1). A significant portion of the precursor is lost during processing; in mammalian cells, about 2.3×10^6 daltons or 50% is eliminated as nonribosomal sequences (Weinberg et al., 1967). Studies utilizing labeling kinetics (Siev et al., 1969; Hackett and Sauerbier, 1975), RNA–DNA hybridization (Reeder and Brown, 1970), and in vitro synthesis (Hecht and Birnstiel, 1972; Liau and Hurlbert, 1975) have shown that the 18S rRNA sequence is proximal to the 5′ terminus of 45S RNA with the 28S sequence distal to it. Electron micrographs of partially RNase digested precursors (Wellauer and Dawid, 1973) previously suggested the opposite polarity but recently were reinterpreted (Dawid and Wellauer, 1976; Reeder et al., 1976) and now also support this topographic order. While sequence studies have unequivocably shown mammalian 5.8S rRNA to originate in 32S nRNA (Maden and Robertson, 1974; Nazar et al., 1975a), the arrangement of the 5.8S and 28S rRNA sequences in 32S nRNA is unknown. In *Xenopus laevis*, however, hybridization studies have suggested that 5.8S RNA cistrons are located internally between the 28S and 18S rRNA sequences (Speirs and Birnstiel, 1974).

Despite these advances, many aspects of rRNA maturation remain unclear. In the present study the 5′-termini of Novikoff ascites hepatoma ribosomal RNAs and their precursors were examined in considering the overall arrangement and processing of mammalian rRNA precursors. As previously reported for L-cell 18S and 28S rRNA (Lane and Tamaoki, 1967), both the mature and precursor RNA species from the Novikoff ascites hepatoma were found to contain characteristic but heterogeneous mononucleoside diphosphates at their 5′ ends and not 2′-O-methylated 5′ termini as has been reported (Choi and Busch, 1970).

Materials and Methods

Cells and Labeling Conditions. Novikoff ascites hepatoma cells were maintained in male Holtzman rats for 6 days and then labeled in vitro with [^{32}P]orthophosphate as described by Mauritzen et al. (1970). Two grams of packed cells was incubated with 100 to 200 mCi of carrier-free [^{32}P]orthophosphate for 5 h.

Isolation and Purification of ^{32}P-Labeled RNAs. After labeling, the cells were collected by centrifugation at 7000*g* for 10 min at 0 °C; RNA was extracted directly from the cell pellet with 100 mL of 0.3% (w/v) sodium dodecyl sulfate–0.14 M sodium chloride–0.05 M sodium acetate (pH 5.1) and 100 mL of phenol solution (Steele et al., 1965) at 65 °C. In some experiments RNA was extracted from ribosomal subunits as previously described (Nazar and Busch, 1974). The RNA was dissolved in 2 mL of 50% formamide, incubated at 65 °C for 5 min to dissociate all low-molecular-weight components, and fractionated by electrophoresis on a 1.5% agarose gel slab (5 mm × 17 cm × 12 cm) in a vertical gel apparatus (E-C Apparatus Corp., St Petersburg, Fla.) using a Tris[1]–boric acid–EDTA buffer at pH 8.3 (Peacock and Dingman, 1968). The gel was run at 3–6 °C with a 25–50 mA current for 6–12 h or until a bromophenol blue dye marker completely migrated from the gel. Individual RNA species were detected by autoradiography (Figure 2a) and recovered from the gel by homogenization in water followed by centrifugation (30 000*g*, 1 h) to remove the gel particles.

Hydrolysis of ^{32}P-Labeled RNAs and Analyses of Fragments. Purified RNA was precipitated at −20 °C with 2 volumes of ethanol (2% potassium acetate) and hydrolyzed with 0.5 mL of 0.3 N sodium hydroxide for 72 h at 37 °C. The hy-

† From the Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77025. *Received September 10, 1976; revised manuscript received April 6, 1977.* These studies were supported by the Cancer Center Research Grant CA-10893, P12 from the National Cancer Institute.

‡ Present address: Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada.

[1] Abbreviations used: Tris, tris(hydroxymethyl)aminomethane; DEAE, diethylaminoethyl; EDTA, ethylenediaminetetraacetic acid; TEA, triethylamine.

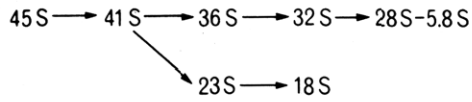

FIGURE 1: Proposed maturation pathway for rRNA in Novikoff ascites hepatoma cells. This maturation pathway is a summary of reports based on labeling kinetics, competitive hybridization, and partial sequence analyses (see reviews by Grierson et al., 1970; Choi et al., 1975).

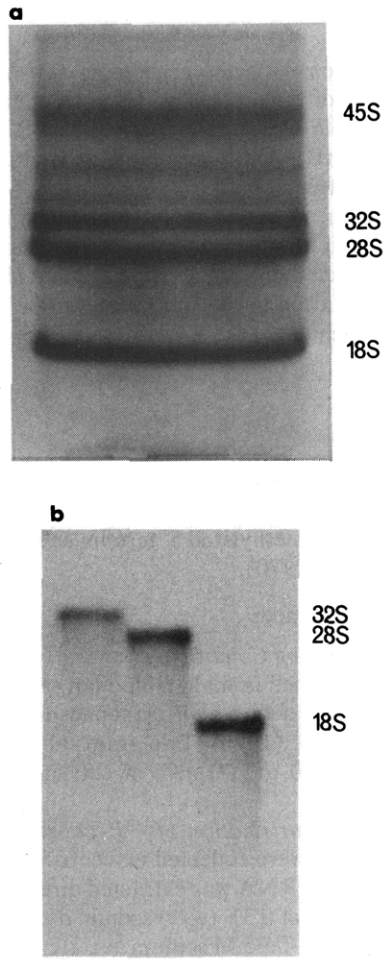

FIGURE 2: Autoradiographs of ^{32}P-labeled Novikoff ascites hepatoma ribosomal RNA and precursors fractionated on 1.5% agarose gels by electrophoresis at pH 8.3. (a) Whole cell RNA fractionated on a preparative slab gel. (b) Purified RNAs rerun on an analytical gel. The major RNA components are identified in the margins; low-molecular-weight components and the bromophenol blue dye markers migrated off the gel.

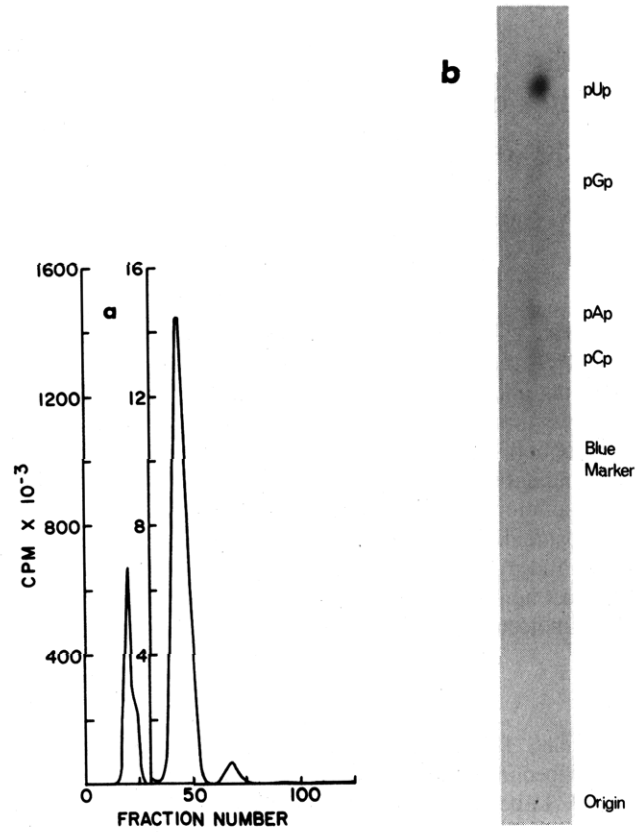

FIGURE 3: Patterns of separation for an alkaline hydrolysate of ^{32}P-labeled Novikoff hepatoma 18S rRNA. (a) Fractionation by DEAE-Sephadex A-25 column chromatography at neutral pH. The cpm scale is increased 100× beginning at fraction 30. (b) Fractionation of peak 3 (from a) by electrophoresis on Whatman 3MM paper at pH 3.5. The positions of the nucleoside diphosphates, xylene cyanol FF dye marker, and the origins are identified in the margins.

drolysate was neutralized with hydrochloric acid and diluted five- to tenfold with 7 M urea before analysis by chromatography on DEAE-Sephadex A-25 (Pharmacia Fine Chemicals, Uppsala) at neutral pH (Tener, 1967) followed by electrophoresis on Whatman 3MM paper at pH 3.5 (Sanger et al., 1965). For chromatography, the digest was applied to a 0.7 × 20 cm column and eluted with a 500-mL gradient (total volume) of 0.0 to 0.25 M sodium chloride in 7 M urea and 0.05 M Tris-HCl (pH 7.5). Four-milliliter fractions were collected every 10 min and counted to determine their radioactivity. Fractions comprising the various peaks were pooled and desalted on 0.5 × 1 cm columns of DEAE-Sephadex A-25, eluted with 30% TEA-carbonate, dried, dissolved in water, and spotted for electrophoresis. The 5′ termini and alkali resistant components were identified by electrophoresis on Whatman 3MM paper at pH 3.5 and further characterized by digestion with alkaline phosphatase or venom phosphodiesterase (Worthington Biochemicals Corp. Freehold, N.J.) and electrophoresis under the same conditions (Sanger et. al., 1965).

Results

Two prerequisites were considered essential for terminal analyses; the purified RNA molecules had to be undegraded, particularly with respect to their termini, and not be contaminated with low-molecular-weight components. To avoid degradation the RNAs were generally extracted directly from cells; this avoided lengthy preparative procedures which might allow nucleolytic degradation. As a control RNA was also prepared from ribosomal subunits and compared with whole cell RNA. To remove partially degraded and low-molecular-weight components, the RNA was heated in 50% formamide prior to gel electrophoresis; this treatment also significantly reduced the background in the preparative gels. When whole cell RNA was purified on 1.5% agarose slabs (Figure 2a), the four major high-molecular-weight components, the nucleolar 45S and 32S precursors and the mature 28S and 18S rRNAs, were completely fractionated. In addition, two minor precursor intermediates, 41S and 36S nRNA, were present between 32S and 45S nRNA and two very minor components, possibly direct precursors to 18S rRNA, were observed between the 18S and 28S rRNA bands. As previously reported by Tiollais et al. (1971), 45S nRNA was present as a broad heterogeneous

TABLE I: Isotope Distribution in Products of Alkaline Hydrolysis of ^{32}P-Labeled Novikoff Hepatoma rRNA and Precursors.[a]

RNA	Peak 3 (pXp + Um-Gm-Up)	Terminal composition				Peak 4 (Am-Gm-Cm-Ap)	Peak 5 (pppXp)
		pCp	pAp	pGp	pUp		
5.8S rRNA		41	4	51	4		
18S rRNA	0.120 (2.3)	13.0	8.2	8.3	70.5 ± 3.3		
28S rRNA	0.170 (6.6)	46.2 ± 2.5	16.9	17.3	19.6	0.081 (3.2)	
32S nRNA	0.110 (5.5)	47.8 ± 3.8	16.9	16.6	18.7	0.050 (2.5)	
41S nRNA	0.079 (5.9)	35.3 ± 2.8	17.8	20.1	26.0	0.033 (2.5)	0.014 (1.0)
45S nRNA	0.054 (5.4)	35.6 ± 1.5	20.3	22.4	21.6	0.026 (2.6)	0.010 (1.0)

[a] Fractions eluted from DEAE-Sephadex A-25 (e.g., Figure 3a) were pooled and the radioactivity in each peak is expressed as a percentage of the total radioactivity applied to the column. The terminal compositions of each RNA, determined by further electrophoresis on Whatman 3MM paper (e.g., Figure 3b), are expressed as percentages of the total mononucleoside diphosphate content in peak 3. The values are averages for three to six determinations ± SE for the principal mononucleoside diphosphate of each RNA. The molar yield of each peak, indicated in parentheses, is expressed as the total number of phosphate residues in each peak assuming chain lengths of 1900, 3900, 5000, 7500, and 10 000 for 18S, 28S, 32S, 41S, and 45S RNA, respectively (Grierson et al., 1970; Choi et al., 1975). Values for 5.8S rRNA were determined earlier (Nazar et al., 1975b).

band with at least two components. When aliquots of the purified RNAs were rerun on analytical gels (Figure 2b), they were found to be virtually undegraded[2] and free from all other RNA species.

When RNA hydrolysates were analyzed by chromatography on DEAE-Sephadex A-25 as many as five peaks were observed. The 18S hydrolysate contained three major peaks (Figure 3a) with −2, −3, and −4 charges, respectively. Further analysis by electrophoresis at pH 3.5 indicated that the first peak contained only mononucleotides, the second contained 2′-O-methylated dinucleotides which are resistant to alkaline hydrolysis and the third contained 5′-terminal mononucleoside diphosphates. As shown in Figure 3b, this terminus is heterogeneous; approximately 70% of the molecules have pUp at their 5′ end (Table I), while the others began with either pCp, pAp, or pGp. rRNA (18S) from either whole cell RNA or the 40S subunit had essentially identical terminal compositions.

Both 28S and 32S RNA hydrolysates contained four major components after chromatography at neutral pH with −2, −3, −4, and −5 charges, respectively. Further analysis by electrophoresis at pH 3.5 again indicated that the first two peaks contained mononucleotides and alkali stable dinucleotides, respectively. Peak 3 contained 5′-terminal mononucleoside diphosphates (Table I) and a 2′-O-methylated trinucleotide (Um-Gm-Up) which has previously been described (Nazar and Busch, 1974). The termini of both molecules were also heterogeneous but very similar; pCp was the major 5′ end, beginning approximately 50% of the molecules but others contained pAp, pGp, or pUp. The fourth peak contained an alkali stable tetranucleotide, Am-Gm-Cm-Ap, which has previously been described in 28S rRNA (Nazar and Busch, 1974).

Both 41S and 45S RNA hydrolysates also contained four major peaks which corresponded to those found in 28S and 32S RNA and an additional minor peak (fractions 95–103) with a net charge of −6 (Figure 4a). As found with other RNAs, the first two peaks contained mononucleotides and alkali stable dinucleotides. As shown in Figure 4b, the third peak also contained 5′-terminal mononucleoside diphosphates (Table I) and a 2′-O-methylated trinucleotide, Um-Gm-Up (Nazar and Busch, 1974). The terminal compositions were very heterogeneous, similar to each other, but distinct from those found in 18S, 28S, or 32S RNA (Table I). In some instances pAp ran

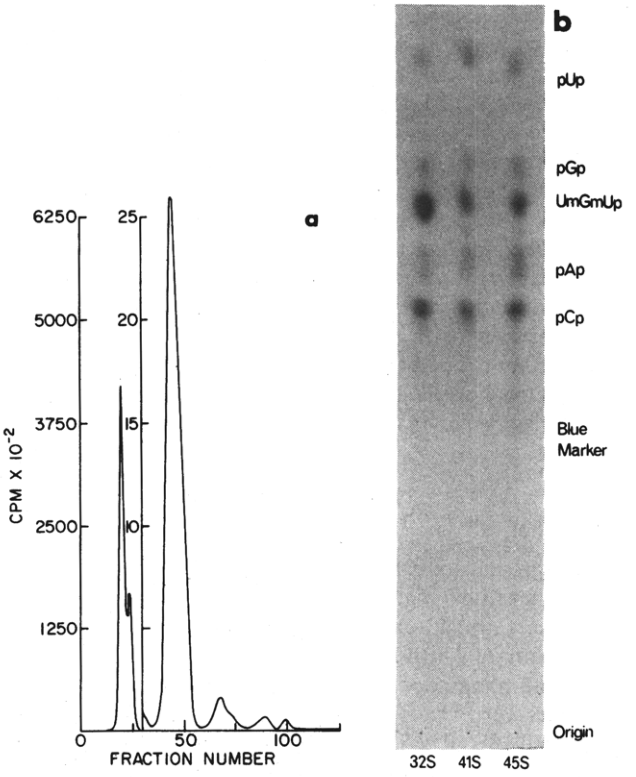

FIGURE 4: Patterns of separation for alkaline hydrolysates of ^{32}P-labeled Novikoff hepatoma ribosomal precursors. (a) Fractionation of 45S nRNA hydrolysate by DEAE-column chromatography at neutral pH. The cpm scale is increased 250× beginning at fraction 30. (b) Fractionation (peak 3, Figure 3a) by electrophoresis on Whatman 3MM paper at pH 3.5. The positions of the xylene cyanol FF dye marker and origin are identified in the margin.

as two spots (Figure 4b); further complete and partial digestion with alkaline phosphatase confirmed that this was adenosine diphosphate and indicated that the two spots probably represent 2′ and 3′ isomers. This behavior has previously been observed with oligonucleotides terminating in Ap (Nazar and Busch, 1974). As in 28S and 32S RNA, the fourth peaks contained the alkali stable tetranucleotide, Am-Gm-Cm-Ap (Table I). The −6 charge of peak 5 was consistent with a terminal tetraphosphate of the type pppXp which would be expected if 45S nRNA is the initial ribosomal gene product. An almost complete conversion to inorganic phosphate after al-

[2] When the autoradiographs were scanned, 88–92% of the radioactivity was found in the major bands shown in Figure 2b.

TABLE II: Isotope Distribution in Products of Alkaline Hydrolysis of ^{32}P-Labeled Novikoff Hepatoma 45S RNA Fractions.[a]

45S fraction	"Slow"	"Fast"
Peak 3 (pXp + Um-Gm-Up)	0.056	0.060
Terminal composition: pCp	35.3	38.5
pAp	22.0	24.2
pGp	22.3	18.8
pUp	20.2	18.5
Peak 4 (Am-Gm-Cm-Ap)	0.022	0.024
Peak 5 (pppXp)	0.009	0.008

[a] The broad 45S RNA band (Figure 2a) was divided into two fractions according to electrophoretic mobility and each fraction was analyzed separately as described in Figure 4. The distribution of radioactivity was determined as a percentage of the total radioactivity as described in Table I.

kaline phosphatase digestion was also consistent with such a structure. However, further analysis by paper electrophoresis suggested that this component was also heterogeneous, and, since the molar yield (Table I) was very low (<0.1 mol for each molecular species), this peak probably represents minor terminal contaminants from nuclear heterogeneous RNA.

As shown in Figure 2a, 45S nRNA behaves as a heterogeneous band during gel electrophoresis with at least two components. When separate analyses were carried out on the faster and slower moving regions of the 45S band (Table II), no significant differences were observed in the terminal compositions or in the relative areas between the peaks. These data are consistent with a recent report by Slack and Loening (1974) that the heterogeneity in ribosomal 45S precursors on gel electrophoresis is due to multiple conformations of the molecules rather than actual differences in the primary sequences.

Discussion

The present study has shown that the 5′ termini of Novikoff ascites hepatoma high-molecular-weight ribosomal RNAs and precursors are heterogeneous mononucleoside diphosphates. Approximately 70% of the 18S rRNA molecules begin with pUp; the remainder begin with pCp, pAp, or pGp. Similar analyses were reported earlier by Lane and Tamaoki (1967) for L-cell 18S rRNA and more recently the major 5′-terminal sequence of mouse cell (LS178Y) 18S rRNA has been determined to be pU-A-C-C-U-Gp (Eladari and Galibert, 1975). In this laboratory the same sequence has also been demonstrated in the Novikoff hepatoma (R. G. Williams, unpublished results). These results do not support the earlier report by Egawa et al. (1971) that 18S rRNA from this hepatoma begins with pGp.

The terminal composition was more heterogeneous in the other RNAs. Both 28S and 32S RNA had very similar compositions. Almost half the molecules began with pCp; the remainder contained pAp, pGp, or pUp in near equimolar amounts, similar to that reported by Lane and Tamaoki (1967) for L-cell 28S rRNA. In a recent study on the conservation of 5′-terminal sequences in ribosomal RNA, Sakuma et al. (1976) reported that the principal terminus of 28S rRNA in a number of high eukaryotes was pCp present in 49–78% of the RNA molecules depending on the cell species. The most heterogeneous termini were found in 41S and 45S nRNA where the principal terminus, pCp, was only present in one-third of the molecules. In contrast, earlier studies by Choi and Busch (1970) on these same RNA molecules have suggested a common pCmpUp 5′ sequence, for 45S, 32S, and 28S RNA, but this was subsequently shown to be an alkali stable tetranucleotide which originates internally in a pentanucleotide fragment (Nazar and Busch, 1974).

The 45S nRNA is thought to be the initial ribosomal gene transcript (see review by Grierson et al., 1970) and as such is expected to contain a terminal 5′-triphosphate. The heterogeneous mononucleoside diphosphates found in this study are not consistent with this generally accepted view. No other mammalian 45S terminus has been reported; however, in *Xenopus laevis* the 40S equivalent also begins with a mononucleoside diphosphate, pGp, rather than a terminal 5′-triphosphate (Slack and Loening, 1974) which differs from the mature ribosomal RNA species. Apparently some dephosphorylation or trimming process must occur before the 45S molecule is completely transcribed or shortly after. Alternatively 45S RNA may be a stable intermediate in the processing of a very labile polycistronic transcript (Hidvegi et al., 1971). It will be important to test these possibilities and to amend the present processing scheme accordingly.

Despite the apparent high purity and homogeneity of the analyzed RNA (Figure 2), the 5′ termini were markedly heterogeneous. After being heated in 50% formamide, it was unlikely that low-molecular-weight contaminants were associated with the RNA; in a previous study on the origin of 5.8S rRNA (Nazar et al., 1975a), this strongly hydrogen-bonded component was not detected in 28S rRNA which had been prepared in the same way. Furthermore, since RNA, either rapidly extracted directly from whole cells or more indirectly from ribosomal subunits, had the same characteristic terminal compositions, it is also unlikely that these heterogeneous termini result from degradation during isolation. The 5.8S rRNA from different mammalian species prepared by the same procedure were previously found to differ in their degree of terminal heterogeneity; Novikoff hepatoma 5.8S rRNA, for example, contained significant amounts of pCp and pGp, while HeLa cell RNA contained only pCp (Nazar et al., 1976). Therefore, the heterogeneity must arise from degradation in vivo or differences in processing. Both the 5′ termini of 5.8S rRNA from yeast (Rubin, 1974) and the Novikoff hepatoma (Nazar et al., 1975b) have previously been shown to contain heterogeneous 5′ termini which result from differences in chain length of the RNA molecules. In the Novikoff hepatoma, for example, about half the molecules begin with pG while many of the others contain an additional pC residue at the 5′ end. The nature of the heterogeneity in the high-molecular-weight RNAs is unclear but it is attractive to postulate similar differences in chain length until further sequence analyses are completed.

Since the termini were reproducible and characteristic for each RNA molecule, they may be useful markers in considering the topographical arrangement of ribosomal sequences within the precursor molecules. Mature 5.8S and 28S rRNA are directly derived from 32S nRNA (Pene et al., 1968; Maden and Robertson, 1974; Nazar et al., 1975a). Previously, Novikoff hepatoma 5.8S rRNA was shown to contain two major 5′ termini, pCp and pGp, in near equimolar amounts (Nazar et al., 1975b). In the present study 32S nRNA and 28S rRNA had a very similar terminal composition, 47% pCp, 17% pAp, 17% pGp, and 19% pUp, quite different from 5.8S rRNA. Assuming that this similarity is not fortuitous due, for example, to a common cleavage specificity in 32S and 28S RNA production, these termini suggest that 32S nRNA contains the 28S sequence at its 5′ end and, therefore, the 5.8S sequence is closer to the 3′ end. Two different models may be proposed

$$5'\ \underline{28S}\ \ldots\ \underline{5.8S}\ 3' \text{ or } 5'\ \underline{28S}\ \ldots\ \underline{5.8S}\ \ldots\ 3'$$

With a single nonconserved region, a minimum of two cleavage steps would be required for maturation; with two nonconserved regions, at least three steps would be involved. Recently, low-molecular-weight precursors have been reported for yeast 5.8S rRNA (Helser and McLaughlin, 1975; Trapman et al., 1975) and, if these exist in mammalian cells, a more complex scheme may have to be evolved.

In contrast to the present models, hybridization studies in *Xenopus laevis* (Speirs and Birnstiel, 1974) indicate that the 5.8S RNA sequence is proximal to the 5′ end of the 32S precursor molecule. These studies suggest that the common terminal composition of 28 and 32S RNA is coincidental. Furthermore, analyses of the secondary structure of ribosomal precursors indicate that a nonconserved sequence is present at the 5′ end of 45S nRNA and that this is lost during processing to form the 41S nRNA intermediate (Wellauer and Dawid, 1973; Dawid and Wellauer, 1976). The similarity in the terminal composition of 45S and 41S nRNA may, therefore, also be coincidental. Such an interpretation would suggest that the processing of ribosomal precursors is sequence specific and that the sequences which specify cleavage points in the formation of 32S and 28S RNA or 45S and 41S RNA are very similar. Extensive studies on the 5′-terminal sequences of these molecules will be required to test these alternatives. Nevertheless, the present data unequivocally show that none of the ribosomal sequences are located at the 5′ end of the 45S or 41S nRNA precursors, and that the 5.8S rRNA sequence is located internally in its 32S nRNA precursor.

Acknowledgments

The author wishes to thank Dr. H. Busch for his encouragement and support of these studies and Mrs. Rose K. Busch for supplying the Novikoff hepatoma bearing rats. The author is also indebted to Dr. B. G. Lane for helpful discussions during the course of this work.

References

Choi, Y. C., and Busch, H. (1970), *J. Biol. Chem. 245,* 1954-1961.

Choi, Y. C., Nazar, R. N., and Busch, H. (1975), in the Cell Nucleus, Vol. III, Busch, H., Ed., New York, N.Y., Academic Press, p 109.

Dawid, I. B., and Wellauer, P. K. (1976), *Cell 8,* 443-448.

Egawa, K., Choi, Y. C., and Busch, H. (1971), *J. Mol. Biol. 56,* 565-577.

Eladari, M.-E., and Galibert, F. (1975), *Eur. J. Biochem. 55,* 247-255.

Grierson, D., Rogers, M. E., Sartirana, M. L., and Loening, V. E. (1970), *Cold Spring Harbor Symp. Quant. Biol. 35,* 589-597.

Hackett, P. B., and Sauerbier, W. (1975), *J. Mol. Biol. 91,* 235-256.

Hecht, R. M., and Birnstiel, M. L. (1972), *Eur. J. Biochem. 29,* 489-499.

Helser, T. L., and McLaughlin, C. S. (1975), *J. Biol. Chem. 250,* 2003-2007.

Hidvegi, E. J., Prestayko, A. W., and Busch, H. (1971), *Physiol. Chem. Phys. 3,* 17-35.

Lane, B. G., and Tamaoki, T. (1967), *J. Mol. Biol. 27,* 335-348.

Liau, M. C., and Hurlbert, R. B. (1975), *J. Mol. Biol. 98,* 321-332.

Maden, B. E. H., and Robertson, J. S. (1974), *J. Mol. Biol. 87,* 227-255.

Mauritzen, C. M., Choi, Y. C., and Busch, H. (1970), *Methods Cancer Res. 6,* 253.

Nazar, R. N., and Busch, H. (1974), *J. Biol. Chem. 249,* 919-929.

Nazar, R. N., Owens, T. W., Sitz, T. O., and Busch, H. (1975a), *J. Biol. Chem. 250,* 2475-2481.

Nazar, R. N., Sitz, T. O., and Busch, H. (1975b), *J. Biol. Chem. 250,* 8591-8597.

Nazar, R. N., Sitz, T. O., and Busch, H. (1976), *Biochemistry 15,* 505-508.

Peacock, A. C., and Dingman, C. W. (1968), *Biochemistry 7,* 668-674.

Pene, J. J., Knight, J. E., and Darnell, J. (1968), *J. Mol. Biol. 33,* 609-623.

Reeder, R. H., and Brown, D. D. (1970), *J. Mol. Biol. 51,* 361-377.

Reeder, R. H., Higashinakagawa, T., and Miller, O. L., Jr. (1976), *Cell 8,* 449-454.

Rubin, G. M. (1974), *Eur. J. Biochem. 41,* 197-202.

Sakuma, K., Kominami, R., and Muamatsu, M. (1976), *Eur. J. Biochem. 63,* 339-350.

Sanger, F., Brownlee, G. G., and Barrell, B. G. (1965), *J. Mol. Biol. 91,* 235-256.

Siev, M., Weinberg, R., and Penman, S. (1969), *J. Cell Biol. 41,* 510-520.

Slack, J. M. W., and Loening, V. L. (1974), *Eur. J. Biochem. 43,* 59-67.

Spiers, J., and Birnstiel, M. (1974), *J. Mol. Biol. 87,* 237-256.

Steele, W. J., Okamwa, N., and Busch, H. (1965), *J. Biol. Chem. 240,* 1742-1749.

Tener, G. M. (1967), *Methods Enzymol. 12,* 398-404.

Tiollais, P., Galibert, F., and Borian, M. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 1117-1120.

Trapman, J., Delonge, P., and Planta, R. J. (1975), *FEBS Lett. 57,* 26-30.

Weinberg, R. A., Loening, V. L., Willems, M., and Penman, S. (1967), *Proc. Natl. Acad. Sci. U.S.A. 58,* 1088-1095.

Wellauer, P. K., and Dawid, I. B. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 2827-2831.