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Initiation of *Escherichia coli* ribosomal RNA synthesis *in vivo*

(CTP starts/promoters P_1 and P_2/ribonuclease III/rRNA processing)

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ABSTRACT **The 5′-terminal sequences of *Escherichia coli* ribosomal RNA precursors (pre-rRNAs) synthesized *in vivo* were characterized by RNA oligonucleotide sequence analysis. The 60- to 170-nucleotide-long 5′-end-specific fragments were produced by RNase III treatment of 30S and 18S pre-rRNAs. Comparison of the RNA oligonucleotides of these fragments with known DNA sequences of the promoter regions of several ribosomal RNA operons allows us to determine the start points of transcription of each operon. We conclude that transcription of most (and perhaps all) rRNA operons is initiated *in vivo* at two tandem promoters, called P_1 and P_2, which have recently been identified by *in vitro* transcription studies of several groups. Depending on the transcription unit, the initiating nucleotide at P_1 promoters is either ATP or GTP, whereas at P_2 promoters it is either CTP or GTP.**

The ribosomal RNA (rRNA) genes of *Escherichia coli* are arranged in seven transcription units, each of which directs the synthesis of 16S, 23S, and 5S rRNAs as well as several associated transfer RNAs (tRNAs) (1–6,*). Initiation of rRNA transcription takes place at the 5′ side of the 16S rRNA gene, and the transcripts are terminated beyond the 3′ end of the 5S rRNA gene (or beyond the 3′-distal tRNA genes found in certain rRNA operons) (4–8). During synthesis the transcript is subject to several steps of endonucleolytic processing by a number of specific RNases (4, 9–17).

Recent studies of rRNA synthesis *in vitro* have indicated that several rRNA operons each contain two transcription initiation sites (18–20). These tandem promoters are about 110 base pairs apart and have been called P_1 and P_2. The P_1 promoters are farthest upstream, located about 285 base pairs from the 5′ ends of the structural genes for 16S rRNA (20, 21). Transcripts synthesized *in vitro* from P_1 promoters are initiated with ATP or GTP (18–20), whereas the RNAs transcribed from the P_2 promoters are reported to start with CTP (19, 20) or GTP (18).

We have studied the sites of initiation of rRNA synthesis *in vivo* by analyses of 5′-terminal fragments of rRNA transcripts. These fragments were obtained by RNase III cleavage of 30S and 18S rRNA precursors (pre-rRNAs) synthesized *in vivo;* they include the 5′ triphosphates and extend to the point(s) where RNase III cuts the transcripts to make the 5′ end of 17S pre-rRNA (11, 14, 17). The results show that rRNA transcription is initiated at *both* the P_1 (ATP and GTP starts) and the P_2 (CTP and GTP starts) promotors *in vivo*. Similar results have been obtained by deBoer and Nomura (22).

MATERIALS AND METHODS

RNase III was generously provided by R. Crouch. RNase T1 and RNase T2 were obtained from Calbiochem, and pancreatic RNase was from Worthington. Nuclease P1 was from P-L Biochemicals.

Preparation of ^{32}P-labeled pre-rRNAs from *E. coli* AB301/105, cleavage of pre-rRNAs with RNase III, and isolation of RNase III cleavage products have been described in detail (4, 16). The RNase III-generated RNA fragments were further characterized according to the standard procedures of RNase T1 fingerprinting (23, 24) and oligonucleotide redigestion analysis using pancreatic RNase (25) and RNase T2 (26).

Determination of 5′-terminal nucleotides of RNase III-generated fragments was done by digestion first with RNase T2 and then with nuclease P1 [1 unit/25μg of RNA for 30 min at 37°C and pH 7.0 (27)]. The digestion products were analyzed by one-dimensional chromatography on polyethyleneimine-cellulose, using 0.75 M NaH_2PO_4 (pH 3.4) as solvent. Only the 5′-terminal nucleotides can be distinguished in this double digest (cf. Fig. 4) because 3′-terminal phosphate groups, generated by RNase T2, are hydrolyzed to inorganic phosphate by nuclease P1.

RESULTS

The initiation points for rRNA synthesis *in vivo* were studied by analysis of the 5′ ends of the primary rRNA transcripts. 30S pre-rRNA, which accumulates in RNase III$^-$ strains of *E. coli*, was chosen as a likely candidate for containing the 5′ ends of the initial transcripts (11), as was 18S pre-rRNA (12), which contains the 5′ third of the 30S pre-rRNA.* Because of their complexity these large RNAs were treated *in vitro* with RNase III prior to further analysis. This enzyme cleaves both RNAs at several specific sites (4, 9–11, 16, 17,*). The relatively short fragments generated in this way, particularly those extending from the 5′ ends of the RNAs up to the 17S pre-rRNA sequences, were characterized in detail by oligonucleotide analysis.

The two pre-rRNAs (30S and 18S), isolated from total RNA of *E. coli* AB301/105 cells, labeled with $^{32}PO_4^{3-}$ in the presence of chloramphenicol, were purified by sucrose gradient centrifugation as illustrated in Fig. 1*A*. After an additional sucrose gradient centrifugation (not shown) the pre-rRNAs were digested with RNase III and the products obtained from 30S and 18S pre-rRNA were separated as shown in Fig. 1 *B* and *C*, respectively. The smaller fragments produced by RNase III cleavage of the pre-rRNAs were isolated as indicated from the "4S" and "8S" regions of the gradients. These mixtures of fragments were then separated by two-dimensional polyacrylamide gel electrophoresis (28) as shown in Fig. 2 *A–D*.

The RNAs present in each spot in the gels shown in Fig. 2 were eluted and characterized both by hybridization and by RNase T1 digestion and oligonucleotide fingerprinting (23, 24). Hybridization to separated *Eco*RI fragments of *rrn*B DNA on filters was used to determine the locations of the RNase III digestion products in the intact transcript (29,*). Similar conclusions were reached by comparison of the RNase T1 oligonucleotides and the DNA sequences of several *rrn* promoter (20, 21) and spacer (30, 31) regions.

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* E. Lund and J. E. Dahlberg, unpublished data.

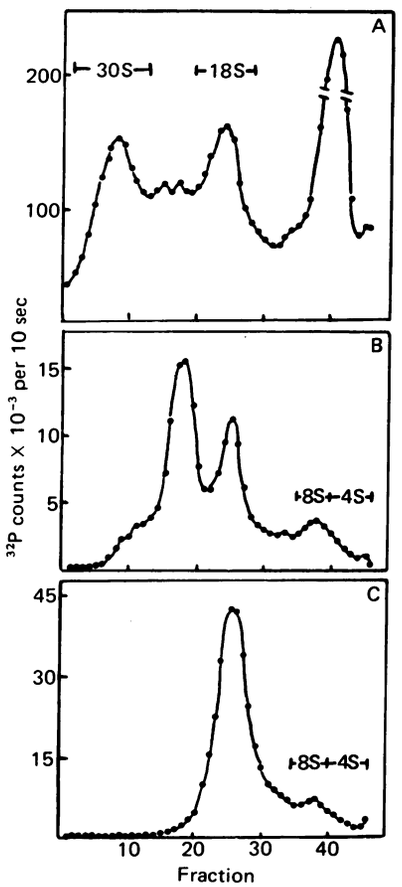

FIG. 1. Size fractionation of ribosomal RNA precursors before and after cleavage with RNase III. Sucrose gradient centrifugation of (*A*) total RNA isolated from chloramphenicol-treated cells of *E. coli* AB301/105, (*B*) 30S pre-rRNA, and (*C*) 18S pre-rRNA after treatment with RNase III. ^{32}P-Labeled RNAs were fractionated on 5–20% sucrose gradients containing 10 mM Tris·HCl at pH 7.6, 100 mM NaCl, 1 mM EDTA, and 0.5% Sarkosyl by centrifugation for 16 hr at 28,000 rpm and 4°C in a Beckman SW 40Ti rotor. The radioactivity of each fraction was determined by Cerenkov counting; centrifugation was from right to left. In order to ensure complete but not excessive digestion by RNase III, the amounts of enzyme used in preparative experiments (*B, C*) were determined by titration of each enzyme preparation in preliminary analytical experiments.

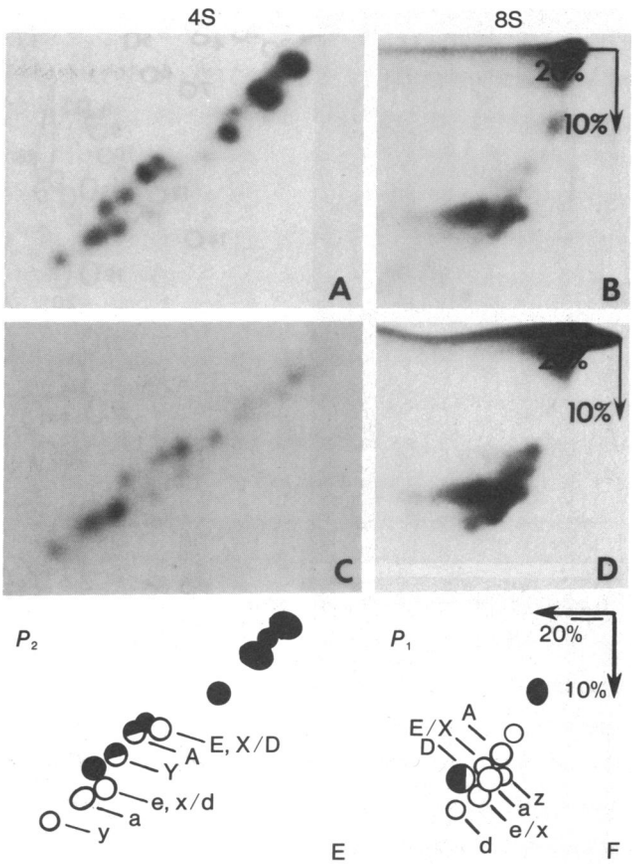

FIG. 2. Two-dimensional polyacrylamide gel analyses of RNase III-generated fragments of 30S and 18S pre-rRNAs. The 4S and 8S RNA fragments of Fig. 1 *B* and *C* were separated on two-dimensional polyacrylamide gels as described (28). Autoradiograms of the relevant sections of the gels are shown. (*A*) The 4S and (*B*) 8S RNase III-generated fragments of 30S pre-rRNAs; (*C*) 4S and (*D*) 8S RNase III-generated fragments of 18S pre-rRNA. (*E* and *F*) Schematic representations of the 4S and 8S gel patterns summarizing the assignments of RNAse III fragments to different regions of the rRNA transcripts as determined by hybridization and oligonucleotide analyses. Fragments arising from the spacer regions between 16S and 23S rRNAs (16, *) are represented by filled spots; fragments arising from the 5′ ends of the rRNA transcripts are designated by outlined spots and letters. Partially filled spots represent coincident fragments. The letters used (A, E, D, X, a, e, d, x) correspond to four *rrn* genes whose 5′ ends have been sequenced; z denotes the 5′ end fragment of a not-yet-identified *rrn* gene. Fragments Y and y are very similar to fragments A and a of *F* but have GTP rather than CTP at their 5′ ends (see *Discussion*). Upper-case letters denote fragments that extend up to the 5′ end of 17S pre-rRNA, whereas lower-case letters denote fragments that are shorter by 8 nucleotides (i.e., lack RNase T1 oligonucleotide no. 8 plus the 3′ phosphate of oligonucleotide no. 1*; cf. Tables 1 and 2). *E* and *F* show P_2- and P_1-specific 5′ end fragments, respectively.

Examples of the data used to assign fragments to the 5′ ends of different *rrn* operons are presented in Fig. 3 and in Tables 1 and 2. Fig. 3 *A* and *C* show RNase T1 fingerprints of two representative 5′-terminal RNase III-generated fragments. The oligonucleotides (shown schematically in Fig. 3 *B* and *D*) were redigested as illustrated in Tables 1 and 2. In these tables, the compositions of the RNase T1 oligonucleotides are compared to the RNase T1 oligonucleotides predicted from the DNA sequences of four *rrn* genes (20, 21).

Because the DNA sequences of the different rRNA operons vary from one another on the 5′ side of 17S pre-rRNA, it is possible to assign almost all of the individual 5′-terminal fragments to specific *rrn* genes. The predominant species in each spot is indicated by the letters in Fig. 2 *E* and *F*. In some cases a spot contained comparable amounts of transcripts from more than one *rrn* operon; for instance 4S RNA of spot E, X/D (Fig. 2*E* and Table 2) contained oligonucleotides characteristic of both *rrnE* and *rrnX/D* [the latter two genes being identical up to position −241 from the 5′ end of 16S RNA gene (20)]. Lower-case letters indicate that the fragments are shorter by eight nucleotides at their 3′ ends (data not shown) as mentioned in the *Discussion*. The RNase T1 oligonucleotides present in 8S 5′ end fragments (Fig. 2*F*) correspond to DNA sequences from the P_1 promoters up to the 5′ end of 17S pre-rRNA; oligonucleotides from 4S 5′-end fragments (Fig. 2*E*) make up a subset of these sequences extending from the P_2 promoters up to the 5′ end of 17S pre-rRNA.

Examples of the evidence used to deduce the structures of the 5′ terminal oligonucleotides of each fragment are presented in Fig. 4 (see also Tables 1 and 2). These data are summarized in Table 3. Thus, we conclude that all of the 8S 5′ fragments (i.e., those extending from the P_1 promoters to the 5′ end of 17S pre-rRNA) start with ATP or GTP, whereas all of the 4S fragments (i.e., those extending from the P_2 promoters) start with

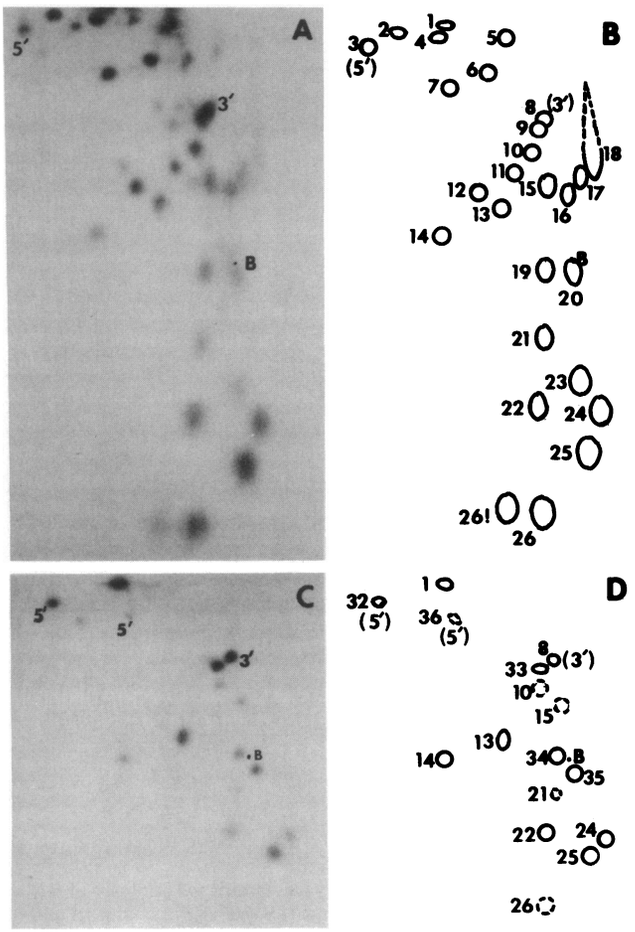

FIG. 3. RNase T1 fingerprint analyses of P_1- and P_2-specific 5′-end RNase III fragments. Autoradiograms of RNase T1 fingerprints are shown. (*A*) The 8S fragment A of Fig. 2*F* and (*C*) 4S fragment E, X/D of Fig. 2*E*. The 5′- and 3′-terminal oligonucleotides of the fragments are indicated. *B* and *D* are schematic representations of the fingerprints shown in *A* and *C*, respectively, indicating the numbering system of the T1 oligonucleotides used for the redigestion analyses presented in Tables 1 and 2.

CTP, with the exception of fragments Y/y, which start with GTP (see *Discussion*). These results are in excellent agreement with the *in vitro* transcription studies cited earlier (18–20), and they show that all of the rRNA operons studied utilize both their P_1 and P_2 promoters *in vivo*.

DISCUSSION

In vitro transcription studies of several rRNA operons of *E. coli* have revealed the presence of two promoters, called P_1 and P_2, which are located in tandem in each rRNA transcription unit (18–20). The results presented here demonstrate that both promoters function *in vivo*, at least in some transcription units. In addition, these results demonstrate that a significant number of *in vivo* rRNA transcripts are initiated with the pyrimidine nucleoside triphosphate CTP. Similar conclusions have been made by deBoer and Nomura (22).

In the present study, 5′-end fragments of rRNA transcripts were produced by RNase III cleavage of pre-rRNAs synthesized *in vivo* in the presence of chloramphenicol. The fragments obtained extended from the P_1 and P_2 promoters up to the 5′ side of 17S pre-rRNA (see also below). Because the total number of these different "long" and "short" 5′-end fragments (arising from P_1 and P_2 promoters, respectively) is greater than the number of rRNA operons in *E. coli*, we conclude that at least some of these operons, and most likely all of them, utilize both of their promoters *in vivo*. This conclusion is most compelling in the cases of those *rrn* genes whose 5′-terminal DNA sequences have been determined (20, 21). Both short and long 5′-end fragments corresponding to each of these rRNA operons were identified.

One long (P_1) RNA fragment, fragment z of Fig. 2*F*, contains oligonucleotides that only partially match promoters with known sequences. Presumably, this comes from a gene whose sequence has not yet been determined.

Interestingly, short (P_2) RNA fragments of one class start with GTP rather than CTP. These fragments, Y and y of Fig. 2*E*, are almost as abundant as *rrnA*-specific P_2 fragments (A and a of Fig. 2*E*) and resemble those fragments except for the presence of a pppGp instead of the pppCp-containing RNase T1 oligonucleotide. It is likely that *in vivo* initiation at the P_2 promoter

Table 1. Redigestion products of RNase T1 oligonucleotides of 8S fragment A

RNase T1 oligonucleotide no.	Pancreatic RNase redigestion products	Probable sequence
1	A-A-Up, A-A-Cp, A-Gp, A-Up, Cp, Up	C-U-C-U-U-U-A-A-C-A-A-U-U-U-A-U-C-A-Gp
2	A-Up, Gp, Up	U-A-U-U-A-U-Gp
3	pppA-Cp,* Gp, Up	pppA-C-U-Gp
4	Gp, Cp, Up	U-U-C-U-C-C-U-Gp
5	"Ori," A-A-A-Up, Gp, Cp	C-A-A-A-A-A-U-A-A-A-U-Gp
6	A-Cp, Gp, Cp, Up	A-C-U-C-U-Gp
7	Gp, Cp, Up	C-U-U-Gp
8	A-A-Up, A-Cp, Cp, Up	A-C-A-A-U-C-U-G_{OH}
9	A-A-Cp, Gp, Cp, Up	A-A-C-U-C-C-Gp
10	A-Cp, Gp, Cp, Up	C-A-C-U-Gp
11	A-Gp, Cp, Up	U-C-A-Gp
12	A-Gp, Up	U-A-Gp
13	Gp, Cp, Up	C-U-Gp
14	Gp, Up	U-Gp
15	"Ori"	A-A-A-A-A-Gp
16	A-A-Cp, Gp	A-A-C-A-A-C-Gp
17	A-A-A-Cp, A-Cp, Gp, Cp	C-A-A-A-C-A-C-Gp
18	["Ori," A-Cp, Gp, Cp]†	C-A-C-A-C-C-C-C-Gp
19	A-A-A-Gp	A-A-A-Gp
20	A-Cp, Gp	A-C-A-C-Gp
21	A-A-Gp	A-A-Gp
22	A-Gp	A-Gp
23	A-Gp, Cp	C-A-Gp
24	Gp, Cp	C-C-Gp
25	Gp, Cp	C-Gp
26 + 26!	Gp	Gp

Oligonucleotide numbers correspond to those indicated in Fig. 3*B*. The probable sequences of oligonucleotides were deduced from the DNA sequence of the 5′-terminal region of the *rrnA* gene (21), extending from the P_1 promoter to the RNase III cleavage site (17). "Ori" indicates that the redigestion product did not migrate from the origin during electrophoresis on DEAE-paper at pH 3.5. Underlining indicates more than 1 mole/mole of oligonucleotide, as judged by visual inspection of the x-ray film.

* The sequence of this pancreatic RNase product was determined by redigestion with RNase T2, which yielded pppAp and Cp. See also Fig. 4*A*, lane 2, for identification of the 5′-terminal nucleotide of 8S fragment a as ATP.

† The analysis indicated that redigestion was incomplete. However, the streaky appearance of this T1 oligonucleotide in the fingerprint (Fig. 3 *A* and *B*) is consistent with the probable sequence being very rich in Cp residues (cf. ref. 11).

Table 2. Analysis of RNase T1 oligonucleotides of 4S fragments E, X/D

RNase T1 oligonucleotide no.	Pancreatic RNase redigestion products	Probable sequence
1	A-A-Up, A-A-Cp, A-Gp, A-Up, Cp, Up	C-U-C-U-U-U-A-A-C-A-A-U-U-U-A-U-C-A-Gp
8	A-A-Up, A-Cp, Cp, Up	A-C-A-A-U-C-U-G_{OH}
10 (A)	A-Cp, Gp, Cp, Up	C-A-C-U-Gp
13 (X/D, A)	Gp, Cp, Up	C-U-Gp
(E, X/D)		U-C-Gp
14 (E, X/D)	ND	U-Gp
15 (A)	"Ori"	A-A-A-A-A-Gp
21 (A)	ND	A-A-Gp
22 (X/D, A)	ND	A-Gp
24 (X/D, A)	ND	C-C-Gp
25	ND	C-Gp
26 (A)	ND	Gp
32 (E, X/D)	pppCp,* Gp, Cp, Up	pppC-U-C-Gp
33a (E, X/D)	A-A-A-Gp, A-A-Cp,	C-A-A-C-U-Gp
33b (E)	Gp, Cp, Up	C-U-A-A-A-Gp
34 (X/D)	ND	A-A-A-Gp
35 (E, X/D)	A-Gp, A-Cp	A-C-A-Gp
36 (A)	pppCp,* Gp, Cp	pppC-C-C-Gp

Oligonucleotide numbers are those indicated in Fig. 3*D*; identical RNase T1 oligonucleotides in Fig. 3 *B* and *D* are given the same number. The letters in parentheses denote oligonucleotides specific for the transcript of the indicated *rrn* gene. Note that *rrnA*-specific oligonucleotides (indicated by broken circles in Fig. 3*D*) are present in much lower yield than *rrnE*- and *rrnX/D*-specific oligonucleotides. Probable sequences of oligonucleotides are deduced from the DNA sequences of the 5′ terminal regions of the *rrnA*, *rrnE* (21), and *rrnX/D* (20) genes, extending from the P_2 promoters to the RNase III cleavage site (17). "Ori" indicates that the redigestion product did not migrate from the origin during electrophoresis on DEAE-paper at pH 3.5. ND indicates not done; in these cases the probable sequence was deduced from the position of the RNase T1 oligonucleotide in the fingerprint (cf. Fig. 3 *C* and *D*). Underlining indicates more than 1 mole/mole of oligonucleotide, as judged by visual inspection of the x-ray film. Oligonucleotides 33a and 33b comigrated and were isolated as a mixture. The probable sequences were deduced from their mobilities and from the DNA sequences.

* RNase T2 redigestion products are listed (compare Fig. 4*B* for T1 oligonucleotide no. 32). See also Fig. 4*A*, lane 1, for identification of the 5′-terminal nucleotide of 4S fragment E, X/D as CTP.

of *rrnA* or a similar *rrn* gene (e.g., *rrnB*†) can also take place at the G-located 3 or 4 nucleotides downstream from the CTP start point; initiation with either CTP† or GTP (18) has been observed *in vitro* at the P_2 promoter of *rrnB*. Because the DNA sequence of the promoter region of *rrnB*† is identical to that of *rrnA* (21), we refer to the sequences of these regions as *rrnA*-type.

Because of the number of manipulations of the RNAs in these experiments, it is difficult to be precise about the relative numbers of P_1 versus P_2 starts. Furthermore, relating yields of fragments to the frequency of initiation assumes that all transcripts are equally stable. We consistently find that the major 5′-terminal P_1 fragment of 30S pre-rRNA corresponds to the sequence of *rrnA* (or *rrnB;* see above), whereas the 5′-terminal P_1 fragments of 18S pre-rRNA do not show this strong bias toward *rrnA*-type sequences. Thus, at least some *rrnA*-type transcripts appear to be less susceptible than other *rrn* transcripts to the cell nucleases that cleave 30S pre-rRNAs in RNase III⁻ cells.

A comparison of the yields of P_1 and P_2 fragments made from *rrnA*-type 30S transcripts shows a 4- to 5-fold higher yield of P_1 fragments over P_2 fragments (CTP plus GTP starts; data not shown). An overall comparison of total P_1 fragments to P_2 fragments (from all operons in 30S pre-rRNA) puts this ratio at about 3:1 for P_1:P_2 starts. Analyses of the yields of 5′ ends of 18S pre-rRNA are consistent with these ratios. Thus, given the assumption that yields are indicative of initiations, we conclude that P_1 promoters are used about 3- to 5-fold more frequently than P_2 promoters under the labeling conditions used here.

† P. Earl and M. Cashel, personal communication.

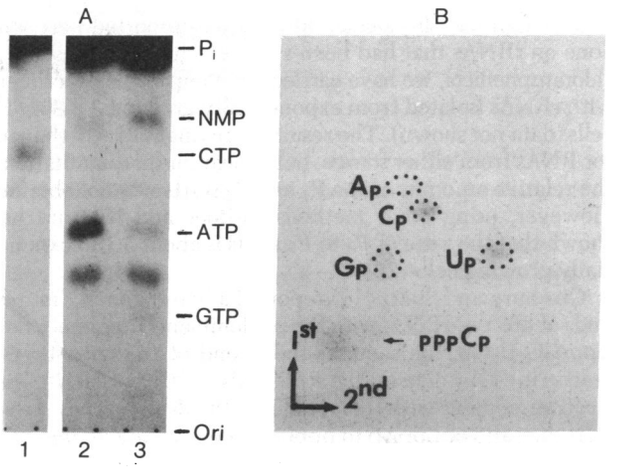

FIG. 4. Identification of 5′-terminal nucleotides of RNase III-generated 5′-end fragments. (*A*) Analyses of the 5′-terminal nucleotides of P_2 fragment E, X/D (lane 1), P_1 fragment a (lane 2), and a mixture of P_1 fragments a and D (lane 3). RNase III-generated 5′-end fragments were digested with RNase T2 plus nuclease P1, and the digestion products were analyzed by one-dimensional polyethyleneimine-cellulose chromatography. An autoradiogram is shown and positions of marker GTP, ATP, and CTP are indicated. The appearance of a mononucleotide, NMP, in lane 3 is due to the contamination by the RNase III-generated spacer fragment, pre-Glu_{III}, which contains pG at its 5′ end (16) (cf. Fig. 2*F*). The radioactive spot between GTP and ATP (in lanes 2 and 3) does not correspond to a known nucleoside triphosphate and has not been identified. (*B*) RNase T2 redigestion analyses of the 5′-terminal RNase T1 oligonucleotide of P_2 fragment E, X/D (no. 32 of Fig. 3*D* and Table 2). An autoradiogram is shown of the two-dimensional chromatogram (26) and the positions of marker nucleoside 3′-monophosphates are indicated. From this analysis and the results shown in *A*, lane 1, the sequence of oligonucleotide no. 32 was deduced to be pppC(C,U)-Gp.

Table 3. Initiating nucleoside triphosphates of *rrn* transcripts *in vivo*

Gel spot (*rrn* locus)	Nucleoside 5′-triphosphate	Location of start site
P_1		
A, a	pppA-	−291
E, e/X, x	pppA-	−283/−285
D, d	pppG-	−284
z	pppA-	?
P_2		
A, a	pppC-	−173 or −174
E, e; X, x/D, d	pppC-	−174; −176/−176
Y, y	pppG-	−171 or −169

The gel spots are indicated in Fig. 2; letters refer to *rrn* operons. The transcripts were identified and the start sites (upstream from the 5′ end of 16S rRNA gene) were located by comparison of oligonucleotide analyses (e.g., Fig. 3) and DNA sequences (20, 21). Initiating nucleoside triphosphates were determined as shown in Fig. 4. The location of the start site of transcript z is unknown because the DNA sequence of this operon has not yet been determined; the start site location of transcripts Y and y is based on the close similarity of these transcripts and P_2 transcripts A and a.

Although the oligonucleotide analyses reported here were done on rRNAs that had been synthesized in the presence of chloramphenicol, we have carried out comparable experiments with rRNAs isolated from exponentially growing AB 301/105 cells (data not shown). The results were qualitatively the same for RNAs from either source, but a meaningful quantitation of the relative amounts of the P_1 and P_2 starts was not obtained. However, using other methods, deBoer and Nomura have shown that the ratio of P_1 to P_2 starts is about 5:1 in exponentially growing cells (22).

Ginsburg and Steitz (11) reported a heterogeneity in the 5′ ends of 30S pre-rRNA, with the predominant 5′ sequence being pppA-C-U-Gp, the same as the 5′ end of the *rrnA*-type P_1 transcript. Our data on the P_1 5′ ends of 30S pre-rRNA are in good agreement with their findings. In addition, they showed that the ratio of pppAp to pppGp 5′ ends changed depending on the growth medium of the cultures. Although that change could have resulted from an alteration in the relative stabilities of ATP vs. GTP initiated transcripts, it is equally plausible that the variation resulted from a change in the frequency of initiation at different promoters. If that were the case, the GTP-initiated transcripts might well have arisen from the GTP start site of *rrnA*-type P_2 promoters—i.e., corresponding to fragments Y, y of Fig. 2*E*. Likewise, the failure of Ginsburg and Steitz to detect significant levels of pppCp 5′ ends might have resulted from a decrease in the number of CTP starts. This could be due to a reduction of the intracellular CTP concentration (see below) under their conditions of labeling (11), which appeared to be similar to a nutritional "down shift."

The advantage of having two tandem promoters in individual rRNA operons is unknown. Obviously, this organization might be responsible for the very efficient transcription of rRNA genes that is observed *in vivo* (cf. ref. 32). Additionally, such sets of dual promoters could permit a differential control of rRNA synthesis. For example, the utilization of P_2 promoters might be regulated by the level of CTP, as suggested by the *in vitro* transcription studies (19,†).

As indicated in Fig. 2, all of the classes of RNase III-generated 5′-end fragments studied here exhibited an interesting kind of heterogeneity. Only about half of each class of fragments extended up to the nucleotide immediately adjacent to the 5′ end of 17S pre-rRNA. The other half of the fragments were precisely 8 nucleotides shorter at the 3′ end (see legend to Fig. 2). However, other studies of RNase III-generated 17S pre-rRNA (11,*) or of 17S pre-rRNA isolated from chloramphenicol-treated cells (33) gave no indication of a corresponding heterogeneity of 5′ ends of 17S pre-rRNA. A more detailed discussion of these and other RNase III cleavage sites in 30S pre-rRNA will be presented elsewhere (16,*).

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