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Precursor of C4 antisense RNA of bacteriophages P1 and P7 is a substrate for RNase P of *Escherichia coli*

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ABSTRACT **The C4 repressor of the temperate bacteriophages P1 and P7 inhibits antirepressor (Ant) synthesis and is essential for establishment and maintenance of lysogeny. C4 is an antisense RNA acting on a target, Ant mRNA, which is transcribed from the same promoter. The antisense–target RNA interaction requires processing of C4 RNA from a precursor RNA. Here we show that 5′ maturation of C4 RNA *in vivo* depends on RNase P. *In vitro*, *Escherichia coli* RNase P and its catalytic RNA subunit (M1 RNA) can generate the mature 5′ end of C4 RNA from P1 by a single endonucleolytic cut, whereas RNase P from the *E. coli rnpA49* mutant, carrying a missense mutation in the RNase P protein subunit, is defective in the 5′ maturation of C4 RNA. Primer extension analysis of RNA transcribed *in vivo* from a plasmid carrying the P1 *c4* gene revealed that 5′-mature C4 RNA was the predominant species in *rnpA*$^+$ bacteria, whereas virtually no mature C4 RNA was found in the temperature-sensitive *rnpA49* strain at the restrictive temperature. Instead, C4 RNA molecules carrying up to five extra nucleotides beyond the 5′ end accumulated. The same phenotype was observed in *rnpA*$^+$ bacteria which harbored a plasmid carrying a P7 *c4* mutant gene with a single C → G base substitution in the structural homologue to the CCA 3′ end of tRNAs. Implications of C4 RNA processing for the lysis/lysogeny decision process of bacteriophages P1 and P7 are discussed.**

Antisense inhibition of gene expression has been demonstrated in a variety of prokaryotic systems. Typically, antisense and target RNAs are transcribed convergently from two separate promoters, and the resulting transcripts are complementary along the entire overlapping region (for reviews, see refs. 1 and 2). A novel antisense system of bacteriophages P1 and P7 has been described (3, 4). Here, the C4 repressor is an antisense RNA which inhibits expression of the antirepressor gene (*ant*) by occlusion of a ribosome binding site involved in *ant* expression (Fig. 1). The novelty of the system is twofold: C4 and Ant mRNA are transcribed from the same promoter and the C4 RNA has to be processed from a precursor RNA. Mature C4 RNA is 77 ± 1 nt long (4). In the folded C4 RNA model (Fig. 2), the structure of stem I with G at its 5′ end and a single-stranded ACC protruding from the 3′ end is strikingly similar to the overall structure of acceptor stems in tRNAs and to the terminal stem region of *Escherichia coli* 4.5S RNA (6). Since the latter two classes of RNA molecules originate from precursor RNAs which are processed by RNase P, we suspected that the pC4 RNAs of P1 and P7 may also be matured by this enzyme. Here we show that RNase P from *E. coli* indeed generates the mature 5′ ends of C4 RNAs *in vitro* and *in vivo*. A possible involvement of the host RNase P in the lysis/lysogeny decision process of bacteriophages P1 and P7 is discussed.

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MATERIALS AND METHODS

Bacteria. The *E. coli* strains used were NHY312 [Δ(*proB–lac*) *ara gyrA thi zic-501*::Tn*10 rnpA*$^+$] and NHY322 [Δ(*proB–lac*) *ara gyrA thi zic-501*::Tn*10 rnpA49*] (7), abbreviated *rnpA*$^+$ and *rnpA49* bacteria, respectively.

Recombinant Plasmids Containing *c4* Genes. Plasmid pJM13Δ11 (P1 *c4*) contains the P1 sequence from the BAL-31 nuclease truncation at −75 to the *Eco*RI restriction site at +159 [inserted into pJF119EH (3, 8)] to allow inducible *c4* transcription from the *tac* promoter (Fig. 1). Plasmids pAH1034 (P7 *c4*) and pAH1054 (P7 *c4.76*) contain the P7 sequence from the BAL-31 truncation at −233 to the *Eco*RI site at +159 [inserted into pT7-6 (S. Tabor and C. C. Richardson, personal communication)], including the natural promoters P51a and P51b for constitutive expression of C4 RNA (Fig. 1). The *c4.76* mutant was constructed by using pAH1034 as template, the oligonucleotide 5′-CGTCACGTCCTGCCAGCTGTTTAG-CAGCGGG-3′ (nt 61–91, mutant base underlined), and the U.S.E. mutagenesis kit (Pharmacia). The mutation was confirmed by dideoxy sequencing. Plasmid pAH1008 (P1 sequence from the *Dra* I site at −250 to the *Eco*RI site at +159, inserted into pT7-6; Fig. 1 and ref. 5) was used as *c4* template for PCR amplification.

Processing Assays. ^{32}P-labeled pC4 RNA or precursor to tRNA^{Gly} (ptRNA^{Gly}) (9) was incubated at 37°C with M1 RNA in buffer I [50 mM glycine/NaOH, pH 7.5/100 mM $MgCl_2$/100 mM NH_4Cl/4% (wt/vol) PEG 6000 (Merck)/0.5% (wt/vol) SDS] or with RNase P in buffer II (50 mM glycine/NaOH, pH 7.5/10 mM $MgCl_2$/100 mM NH_4Cl). Enzyme and substrate were preincubated separately for 1 hr under processing-assay conditions. Reactions were started by combining enzyme and substrate and were stopped by extraction with phenol/chloroform (1:1, vol/vol) followed by ethanol precipitation. For analysis of DEAE-Sepharose fractions from *rnpA*$^+$ and *rnpA49* bacteria (see Fig. 5), 5-μl samples of individual fractions, preincubated for 5 min at the respective assay temperature, were added to 1 μl of ^{32}P-labeled pC4 RNA or ptRNA^{Gly} (about 1 pmol) and assayed at 30°C or 43°C. Before processing, RNA substrates were heated at 70°C for 2 min in buffer II and then slowly cooled to room temperature. Reactions were stopped by adding 6 μl of loading buffer [67% formamide/0.3× TBE/2.7 M urea/0.1% bromophenol blue/0.1% xylene cyanol blue) (1× TBE is 90 mM boric acid, 90 mM Tris base, 2 mM EDTA, pH 8.3)]. Cleavage products were separated by 8 M urea/10% PAGE and visualized by autoradiography.

Identification of the RNase P Cleavage Site. pC4 RNA (5 μM) was incubated with M1 RNA (5 μM) in buffer I for 150 min at 37°C. After ethanol precipitation, the RNA was 3′-end labeled by using [5′-^{32}P]pCp and T4 RNA ligase (10), yielding labeled mature C4 RNA, M1 RNA, and the 5′-cleavage product. The 3′-terminal nucleotide of the latter product was determined as described (10).

Abbreviations: pC4 RNA, ptRNA, and p4.5S RNA, precursors to C4 RNA, tRNA, and 4.5S RNA, respectively.

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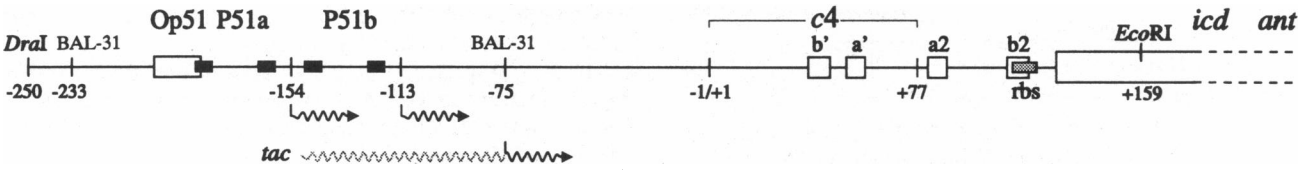

FIG. 1. The immunity operon *imm*I of bacteriophages P1 and P7. The *imm*I operon contains, in the following order, the C1-controlled operator Op51 (open box) overlapping the promoter P51a (−35 and −10 regions, black boxes), the constitutive promoter P51b, the *c*4 gene (bracket), and the ribosome binding site (rbs, stippled box) for the expression of the translationally coupled *icd* and *ant* genes (large open box) (3, 5). Transcription initiating at P51a and P51b is indicated by bold wavy arrows. Transcripts derived from the *tac* promoter are composed of vector- and P1-specific sequences and are shown by light and bold wavy lines. Following processing, the mature C4 RNA can interact with the target Icd–Ant mRNA via the complementary sequences a′/a2 and b′/b2 (open squares; see also Fig. 2). Nucleotide positions are indicated by numbers with positive and negative signs; the arbitrary numbering system starts with the first nucleotide (G^{+1}) of 5′-mature C4 RNA. Restriction sites and BAL-31 nuclease truncations that were used for the construction of recombinant plasmids containing P1 or P7 *c*4 are indicated.

***In Vitro* Transcription of RNAs.** RNAs were synthesized as runoff transcripts by phage T7 RNA polymerase (9); $ptRNA^{Gly}$ and pC4 RNA were transcribed by using PCR-amplified templates carrying the T7 promoter sequence (9). The 5′ and 3′ primers for pAH1008 encoding the pC4 RNA were 5′-TAATACGACTCACTATAGGTTACGCCCGAATTATGGTG-3′ [T7 promoter sequence (underlined) followed by a G and nt −16 to +4 of pC4 RNA] and 5′-ACAGGTGGCAGGACGTGACA-3′ (nt 80 to 61; complementary sequence), respectively. The pC4 RNA used for *in vitro* processing contained the 77-nt-long C4 RNA and 16 and 3 nt of natural flanking sequences (Fig. 2). An additional G at the 5′ end served to enhance transcription efficiency. M1 RNA from *E. coli* was transcribed from the plasmid pJA2 (11) linearized with *Fok* I. Transcript preparations were treated with RNase-free DNase I (0.2 unit/μl) for 10 min at 37°C, extracted with phenol/chloroform (1:1), and precipitated by ethanol. RNAs were purified by 8 M urea/6% PAGE and eluted as described (9).

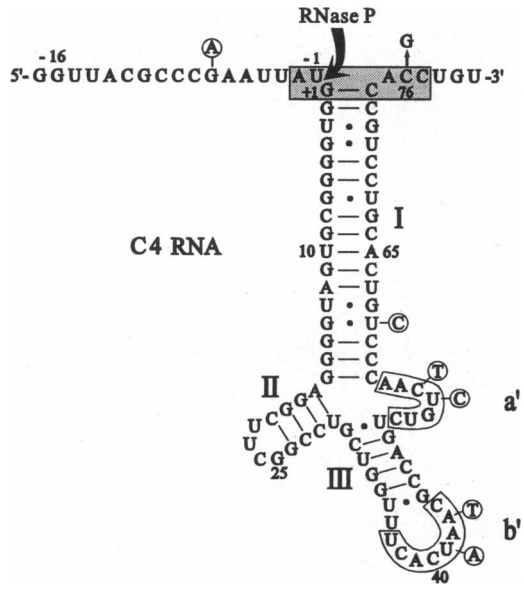

FIG. 2. Secondary structure of C4 RNA from P1 and P7. The sequence of the precursor to C4 RNA (pC4 RNA) used to study RNase P processing *in vitro* is shown. The 77-nt C4 RNA (nt 1–77) was folded as described (4). Stems are marked by Roman numerals. For P7, only differences from P1 are displayed, as circled nucleotides. The boxed sequences a′ and b′ are complementary to the a2 and b2 sequences in the target Icd–Ant mRNA of P1 and P7 (see Fig. 1). The RNase P cleavage site (arrow) and the P7 *c*4.76 base substitution (C → G at nt 76) are indicated. Nucleotides important for processing by *E. coli* RNase P and M1 RNA (see text) are contained within the shaded box. Nucleotides are numbered consecutively, starting with G^{+1} for nucleotides downstream of the RNase P cleavage site and with U^{-1} for those located upstream (see also Fig. 1).

Partial Purification of RNase P. Bacteria of the *rnpA*$^+$ and *rnpA49* strains were grown at 30°C and harvested at an OD_{600} of 0.7; cells (wet weight, 2 g) were ground with 4 g of Alcoa A-305 (Serva) on ice until a paste had formed. The lysates were suspended in 8 ml (*rnpA*$^+$) or 6 ml (*rnpA49*) of buffer A [50 mM glycine/NaOH, pH 7.5/10 mM $MgCl_2$/100 mM NH_4Cl/0.1% (vol/vol) 2-mercaptoethanol/4 μM phenylmethanesulfonyl fluoride] and centrifuged at 2500 × *g* for 30 min at 4°C. The supernatants were centrifuged at 30,000 × *g* for 2 hr at 4°C, yielding 6.2 ml (60 mg of protein) and 4.2 ml (112 mg of protein) of S30 supernatants from *rnpA*$^+$ and *rnpA49* bacteria, respectively. Protein concentrations were determined by the protein microassay procedure of Bio-Rad (based on the Bradford method) with bovine serum albumin as standard. The S30 supernatants were applied to a DEAE-Sepharose Fast Flow column (Pharmacia; 10-ml bed volume) equilibrated with buffer A. The column was washed with 10 ml of buffer A and 1.5-ml fractions were eluted successively with 0.19, 0.28, 0.37, 0.46, and 1 M NH_4Cl in buffer A.

Primer Extension Analysis. Total cellular RNA was extracted with hot phenol (4) and then treated with DNase I and proteinase K. C4 RNA-specific primer extension was performed (4) with the 5′-^{32}P end-labeled primer 5′-CAGACTGGCGTTAGTGAAAC-3′ (nt 53–34, P1 *c*4 complementary sequence). The same primer was used for dideoxy sequencing of pAH1054 (see above). Samples were analyzed in an 8 M urea/6% polyacrylamide sequencing gel and visualized with a PhosphorImager (Molecular Dynamics).

RESULTS

Maturation of the 5′ End of pC4 RNA by RNase P and M1 RNA. In a P1 lysogen, promoter P51a is blocked by binding of C1 repressor to the operator Op51. C4 RNA is transcribed from the constitutive promoter P51b, whose start of transcription is located 113 nt upstream of *c*4 (Fig. 1). For the *in vitro* processing reaction we used a pC4 RNA which carried only 16 and 3 additional nucleotides of the natural sequence at the 5′ and 3′ ends, respectively (Fig. 2). A $tRNA^{Gly}$ from *Thermus thermophilus*, carrying 14 and 3 additional nucleotides at the 5′ and 3′ ends of the 76-nt tRNA, served as a control (9). These ^{32}P-labeled RNAs were treated with either M1 RNA, a partially purified RNase P, or an S30 extract from *rnpA*$^+$ bacteria. Reactions catalyzed by M1 RNA and RNase P yielded 5′-mature C4 RNA and $tRNA^{Gly}$ and the corresponding 5′-cleavage products. The size of the 5′-cleavage product is 14 nt for $ptRNA^{Gly}$ (ref. 9 and Fig. 3) and 17 nt for pC4 RNA (as inferred from its relative mobility in denaturing polyacrylamide gels, enzymatic sequencing, and determination of the 3′-terminal nucleotide; see below). A 17-nt 5′-cleavage product is exactly what is expected from the endonucleolytic cleavage by RNase P at the first G of the terminal helix I in C4 RNA. S30 extracts yielded somewhat smaller maturation products, most likely due to additional trimming at the dangling 3′

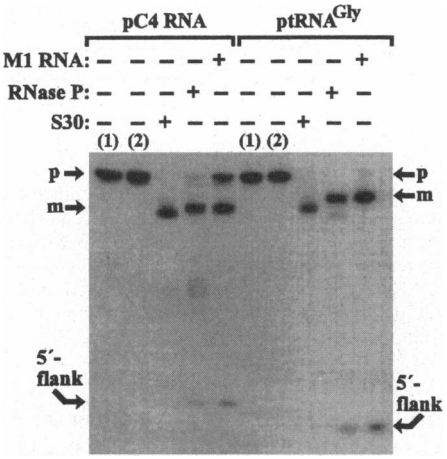

FIG. 3. Processing of pC4 RNA and ptRNA^{Gly} by M1 RNA, RNase P, and an *rnpA*$^+$ S30 extract. Assay mixtures (10 μl) contained either 10 nM ^{32}P-labeled pC4 RNA or 10 nM ptRNA^{Gly} and 1 μM M1 RNA, partially purified RNase P (prepared basically as described in *Materials and Methods*), or 3 μl of S30 extract as indicated. Incubation was for 2 hr at 37°C. For lanes 1 and 2, substrates were incubated in the absence of enzyme in buffer I and buffer II, respectively. p, Precursor RNA; m, mature RNA.

ends of C4 RNA and tRNA^{Gly}. The 5′-cleavage products could not be detected, probably due to degradation by other nucleases present in the S30 extract. In the experiment shown in Fig. 3, >95% of ptRNA^{Gly}, but only ≈70% of pC4 RNA, was converted to the 5′-mature form. More detailed experiments revealed that *E. coli* M1 RNA cleaves the ptRNA^{Gly} about 100 times more efficiently than pC4 RNA (data not shown).

Identification of the RNase P Cleavage Site in pC4 RNA. Since cleavage by RNase P generates 3′-hydroxyl and 5′-phosphate termini, 5′-cleavage products can be labeled at their 3′ ends. Unlabeled pC4 RNA was cleaved with M1 RNA and 3′-end labeled with [5′-^{32}P]pCp (see *Materials and Methods*). A labeled RNA of the size expected for the 17-nt 5′ flank was obtained. This species was absent when unprocessed pC4 RNA was subjected to the same procedure. After elution from the gel, the identity of the 5′ flank (Fig. 2) was verified by enzymatic sequencing (ref. 10; data not shown). Analysis of the 3′-terminal nucleotide revealed exclusively U (Fig. 4), thus

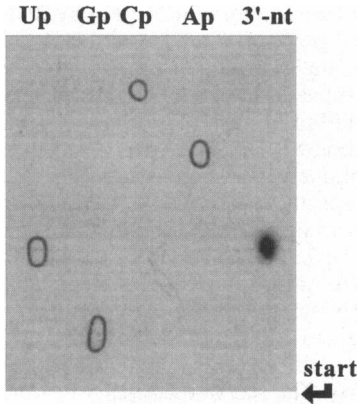

FIG. 4. Identification of the 3′-terminal nucleotide of the 5′-cleavage product generated by M1 RNA cleavage of pC4 RNA. The 5′-cleavage product, labeled at its 3′ end with [5′-^{32}P]pCp, was digested with RNase T2 and analyzed by thin-layer chromatography on PEI-cellulose. The unlabeled nucleoside 3′-monophosphates Up, Gp, Cp, and Ap used as standards were detected by UV shadowing (encircled spots), and the ^{32}P-labeled nucleoside 3′-monophosphate was visualized by autoradiography.

confirming a single RNase P processing site between U^{-1} and G^{+1} of pC4 RNA (Figs. 2 and 3).

The 5′ Maturation of pC4 RNA Depends on RNase P from *rnpA*$^+$ Bacteria. RNase P from isogenic *rnpA*$^+$ and *rnpA49* bacteria was partially purified by DEAE-Sepharose chromatography. The peaks of the 5′-maturation activities for pC4 RNA and ptRNA^{Gly} were eluted with 0.37 M NH_4Cl. The processing of pC4 RNA by the *rnpA*$^+$ RNase P holoenzyme appeared to be somewhat less efficient than with ptRNA^{Gly}, similar to the cleavage reaction with M1 RNA (see above). The activity of the enzyme in the corresponding fractions from *rnpA49* bacteria was strongly impaired for ptRNA^{Gly} processing (Fig. 5*A*), and the activity was too low to detect processing of pC4 RNA (Fig. 5*B*). Impaired processing in extracts from *rnpA49* bacteria was already manifest at 30°C, in agreement with earlier studies (12, 13).

The 5′ Maturation of pC4 RNA Depends on RNase P *in Vivo*. To study pC4 RNA processing *in vivo*, we used the same isogenic *rnpA*$^+$ and *rnpA49* bacterial strains from which RNase P was purified (see above). These strains were transformed with plasmid pJM13Δ11, from which P1 pC4 RNA can be induced via the *tac* promoter. Here, pC4 RNA contains 75 nt of the natural P1 sequence upstream of *c*4, preceded by a further 75 nt of vector-derived RNA (Fig. 1). After induction

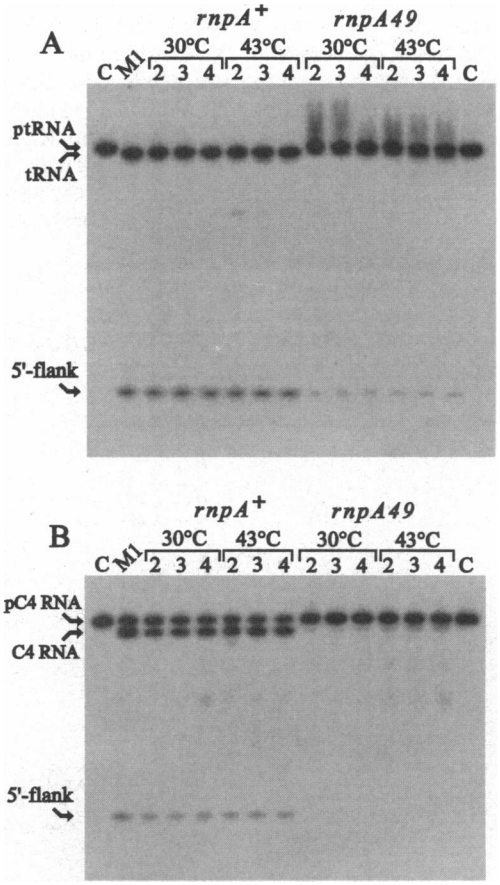

FIG. 5. Processing of ptRNA^{Gly} (*A*) and pC4 RNA (*B*) by RNase P derived from *rnpA*$^+$ and *rnpA49* bacteria. RNase P was partially purified by DEAE-Sepharose chromatography. Aliquots (5 μl) of the indicated fractions from *rnpA*$^+$ and *rnpA49* bacteria were incubated with ≈1 pmol of ^{32}P-labeled pC4 RNA or ptRNA^{Gly} in a total volume of 6 μl for 10 min (ptRNA^{Gly}) or 30 min (pC4 RNA) at 30°C or 43°C. Processing reactions were analyzed by 15% PAGE in the presence of 8 M urea. Fractions 2, 3, and 4 from *rnpA*$^+$ and *rnpA49* bacteria, each containing on the average 85 and 190 ng of protein per μl, respectively, represent three equivalent fractions of RNase P activity eluted at 0.37 M NH_4Cl. Lane C, uncleaved substrates; lane M1, processing of ptRNA^{Gly} or pC4 RNA by *E. coli* M1 RNA.

of the *tac* promoter for 10 min at 42°C, RNA was isolated and the 5′ termini of C4 RNAs were determined by primer extension analysis (Fig. 6). More than 95% of C4 RNA from *rnpA*$^+$ bacteria was present in its 5′-mature form, starting with G^{+1} (Fig. 6, lane 1), but only trace amounts of mature C4 RNA were found in *rnpA49* bacteria. Instead, C4 RNA with five extra nucleotides at the 5′ end prevailed, starting with A^{-5}, and a minor fraction of molecules had 2–4 extra nucleotides (Fig. 6, lane 2). Even in *rnpA49* bacteria grown at 30°C, considerable amounts of immature C4 RNA in addition to mature C4 RNA were observed (data not shown). These results clearly show that the 5′ maturation of C4 RNA *in vivo* depends on RNase P.

The 3′ end, which is NCCA in tRNAs and ACCC in *E. coli* 4.5S RNA (6), is a crucial recognition element for the cleavage of non-tRNA substrates by *E. coli* M1 RNA and RNase P (refs. 6, 14, and 15 and references therein; ref. 16). Moreover, the two central C residues were suggested to base pair with G^{292} and G^{293} of M1 RNA (16). Since we expected a base change at one of the cytidines in the 3′-end ACC of C4 RNAs from P1 and P7 to also interfere with RNase P processing, a plasmid containing the P7 *c*4.76 mutation was constructed (Figs. 1 and 2). *rnpA*$^+$ bacteria were then transformed with the isogenic plasmids pAH1034 (P7 *c*4) and pAH1054 (P7 *c*4.76). Transcription from these plasmids is initiated at promoters P51a and P51b, yielding the natural pC4 RNAs with 154 and 113 additional nucleotides at the 5′ end of C4 (Fig. 1). RNA was isolated from plasmid-carrying *rnpA*$^+$ bacteria grown at 37°C, and the 5′ termini of C4 RNAs were determined by primer extension analysis. Maturation of the pC4 RNA from P7 yielded the same G^{+1} at the 5′ end as was seen with the pC4 RNA of P1 (Fig. 6, compare lane 6 with lane 1). However, due to the P7 *c*4.76 mutation, we observed the same phenotype as with the wild-type C4 RNA of P1 in *rnpA49* bacteria at the restrictive temperature—namely, the accumulation of immature C4.76 RNA with 2–5 extra nucleotides at the 5′ end. Thus, maturation of C4 RNA by RNase P is prevented when either the enzyme is defective or the C4 substrate is altered in a region which is crucial for recognition by the enzyme.

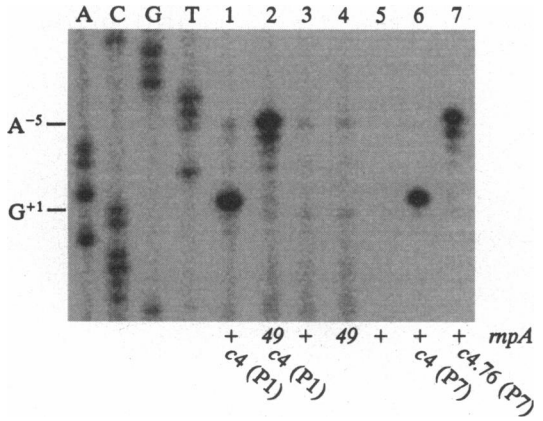

FIG. 6. Primer extension analysis of C4 RNAs from bacteriophages P1 and P7 in *rnpA*$^+$ (+) and *rnpA49* (*49*) bacteria. For lanes 1–4, bacteria carrying plasmid pJM13Δ11 (P1 *c*4, lanes 1 and 2) or vector pJF119EH (lanes 3 and 4) were grown at 28°C to 2×10^8 cells per ml, and the temperature was shifted to 42°C by adding an equal volume of 54°C prewarmed medium. After 15 min, isopropyl β-D-thiogalactopyranoside (1 mM) was added to induce transcription from the *tac* promoter and incubation at 42°C was continued for 10 min. Cells were harvested and total RNA was isolated. For lanes 5–7, *rnpA*$^+$ (+) bacteria harboring the vector pT7-6 (lane 5), plasmid pAH1034 (P7 *c*4, lane 6) or plasmid pAH1054 (mutant P7 *c*4.76, lane 7) were grown at 37°C to 1.5×10^8 cells per ml. Cells were harvested and total RNA was isolated. Sequencing ladders are shown for plasmid pAH1054 (lanes A, C, T, and G). The indicated positions G^{+1} (mature C4 RNA) and A^{-5} (immature C4 RNA) correspond to the complementary bases C and T in the sequencing ladders.

DISCUSSION

We have found that pC4 RNAs of bacteriophages P1 and P7 are processed by *E. coli* RNase P *in vivo* and that immature C4 RNA with 2–5 extra nucleotides at the 5′ end accumulates when RNase P processing is defective (Fig. 6). These results are in line with earlier observations that in RNase P temperature-sensitive mutants of *E. coli*—whose mutations map to the *rnpB* or *rnpA* gene encoding the RNA and C5 protein subunit, respectively—incompletely processed tRNA transcripts accumulate at restrictive temperatures. In *rnp* mutant strains, small monomeric precursors accumulate which carry extra nucleotides at their 5′ termini. Similarly, a precursor to 4.5S RNA (p4.5S RNA) carrying 22 extra nucleotides at the 5′ end accumulated in an *rnp* temperature-sensitive strain (ref. 17 and references therein). The immature 4.5S RNA could be converted to mature 4.5S RNA by RNase P *in vitro* (18).

The natural substrates of *E. coli* RNase P known so far are precursor molecules of tRNAs, 4.5S RNA, 10Sa RNA and species 1 RNA, an RNA of unknown function derived from bacteriophage T4 (ref. 15 and references therein; refs. 19 and 20). The bacteriophage φ80-induced M3 RNA may be another substrate. *E. coli* RNase P cleaves M3 RNA *in vitro*, but processing was not shown to take place *in vivo* (21). Recently, it has been found that RNase P is involved in the processing of the polycistronic mRNA of the histidine operon of *Salmonella typhimurium*. Here, the substrate for RNase P is an intermediate processing product generated by RNase E. Cleavage of this product by RNase P stabilizes the distal part of the polycistronic transcript (22). As demonstrated in this paper, the pC4 RNAs of P1 and P7 can be added to this list of natural substrates for *E. coli* RNase P.

pC4 RNA and p4.5S RNA bear striking similarities: (*i*) both RNAs have extended terminal stem regions (17 and 16 bp, respectively), (*ii*) RNase P cleavage occurs between U^{-1} and G^{+1}, (*iii*) the nucleotide at position −2 is A, and (*iv*) both RNAs share the 3′-terminal sequence ACC (ref. 6 and Fig. 2). In conclusion, pC4 RNAs of P1 and P7 carry several identity elements near the cleavage site (Fig. 2, shaded box) which were found to be important for cleavage-site recognition in $tRNA^{Tyr}Su3$ (ref. 15 and references therein; refs. 23 and 24) or derivatives of p4.5S RNA (6). For example, nucleotide G^{+1} was proposed to function as a context-dependent "guiding nucleotide" in some natural substrates of *E. coli* RNase P (15, 24).

Changing the ACC at the 3′ terminus to AGC in the C4.76 RNA of P7 (Fig. 2) virtually abolished processing by RNase P *in vivo* (Fig. 6). This finding adds further support to the notion that the two cytidines at this location are essential for efficient processing of non-tRNA substrates by *E. coli* RNase P, as suggested by previous data obtained *in vitro* (refs. 6, 14, and 15 and references therein; ref. 16).

The pC4 RNA of P1 (Fig. 2) was cleaved less efficiently by M1 RNA *in vitro* than the $ptRNA^{Gly}$ (Fig. 3). This is in line with studies of p4.5S RNAs (6), which revealed a large increase in K_m for the p4.5S RNA but not for a $ptRNA^{Tyr}Su3$ in reactions catalyzed by M1 RNA alone. However, in the RNase P holoenzyme reaction, Michaelis–Menten kinetic constants of p4.5S RNA and a shortened derivative thereof were very similar to those obtained with the $ptRNA^{Tyr}Su3$ (6), supporting the notion that the protein subunit (C5) of *E. coli* RNase P is particularly crucial for the processing of non-tRNA substrates. There is evidence that non-tRNA substrates may interact somewhat differently with the enzyme. For example, some deletions of M1 RNA abolished processing of $ptRNA^{Tyr}Su3$ but still allowed cleavage of the p4.5S RNA, and vice versa (25). By *in vitro* evolution of RNase P substrates, only tRNA-like substrates were selected with the RNA subunit alone, whereas non-tRNA substrates of the 4.5S RNA type were additionally selected in the presence of the protein subunit C5 (26).

The amount of plasmid-derived immature P1 C4 RNA which accumulated in *rnpA49* bacteria at 42°C, as inferred from Northern blot analysis, was not reduced when compared with the mature C4 RNA found in *rnpA*$^+$ bacteria (data not shown). Obviously, the extra nucleotides at the 5′ end of immature C4 RNA do not affect the stability of the molecule. However, compared to mature wild-type C4 RNA of P7, only about 60% of the immature C4.76 RNA was found (data not shown). The *c*4.76 mutation either affects the stability of the immature RNA or may give rise to aberrant processing reactions.

Because *c*4 and *ant* share the same promoter(s), transcription of *c*4 always precedes that of *ant* and, therefore, is a prerequisite for *ant* expression (Fig. 1). How then is *ant* expression ever accomplished? In a P1 or P7 lysogen, translation of Ant mRNA is blocked because C4 RNA occludes the corresponding ribosome binding site. As a consequence, transcription of *ant* is prematurely terminated via a Rho-dependent terminator and no *ant*-specific RNA is found in the lysogen (27). However, when bacteria are infected by P1 or P7, Ant mRNA is found in the bacterial population. Its 5′ end is identical to the +1 position of the promoter P51b. We assume that in an infected cell the decision for synthesis of either C4 or Ant mRNA is determined by the outcome of a race between pC4 RNA processing from the primary transcript by RNase P and other, unknown enzymes and the ongoing transcription which leads to the completion of Ant mRNA synthesis. If processing is not accomplished in time, a signal may be triggered to the cellular RNA polymerase to continue transcription which then, in turn, might no longer allow the pC4 RNA to be processed. As a consequence Ant would be synthesized to force lytic growth of the phage.

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