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In Vivo Transcription of Bacteriophage ϕ29 DNA: Transcription Termination

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The main early and late transcription termination sites in vivo in bacteriophage ϕ29 DNA were determined by nuclease S1 mapping. Transcription of the ϕ29 early genes located at the left end of the viral genome terminated at the very end of the DNA molecule and within the *Hin*dIII G fragment of the viral DNA. Transcription termination of the early genes located at the right end of the genome and that of the late viral genes overlapped in a specific region of the ϕ29 DNA within the *Eco*RI D fragment. Stem-loop structures followed by uridine-rich tails could be derived close to the 3′ ends of early and late mRNAs, suggesting Rho-independent transcription termination in ϕ29 DNA.

The transcription initiation sites of ϕ29 DNA in vivo have been accurately localized by nuclease S1 mapping experiments (1, 9; see Fig. 4C), and a very good correlation between the sites of initiation of transcription in vivo and in vitro has been found (10). Transcription of the ϕ29 early genes located at the left end of the viral genome initiates mainly at the A2b and A2c promoters, and transcription of the early genes located at the right end of the genome initiates at the C1 and C2 promoters. Late transcription initiates at the unique late promoter A3 and extends over the central part of the genome in which all the ϕ29 late genes are present. S1 mapping experiments were performed to locate precisely where the termination of the transcripts initiating at the main ϕ29 promoters occurs.

Previous studies on ϕ29 transcription (1, 9) showed the existence of a region of the viral DNA in which the transcription initiated at the early promoters C1 and C2 and at the late promoter A3 seemed to terminate. To locate these termination sites, the viral *Eco*RI D fragment was fractionated with the *Hin*fI endonuclease, the 383-base-pair (bp) subfragment (Fig. 1C) was 3′ end labeled, and the separated strands were hybridized with RNA extracted from *Bacillus subtilis* infected with ϕ29 in the presence (early strand) or absence (late strand) of chloramphenicol. In the presence of chloramphenicol only early viral transcription can take place since viral late transcription depends on the function of the product of the ϕ29 early gene 4, protein p4 (14); in the absence of chloramphenicol, both early and late RNA can be synthesized. After hybridization, the samples were subjected to nuclease S1 digestion, and the protected oligonucleotides were analyzed on denaturing polyacrylamide gels side by side with a set of sequencing reaction mixtures. Early transcription terminated mainly between nucleotides 107 and 112 from the right end of the *Eco*RI-*Hin*fI subfragment (Fig. 1A), whereas late transcription termination took place about 320 to 329 nucleotides from the left end of the subfragment (Fig. 1B). The sizes of the protected oligonucleotides in these experiments and those described below were all corrected for the 1.5-nucleotide difference in mobility between deoxynucleotides generated by S1 cleavage and those generated by chemical sequencing reactions (15). Stem-loop structures could be drawn by the rules of Tinoco et al. (16), with free energies of −14.8 kcal (ca. −61.9 kJ)/mol and −16.8 kcal (ca. −70.2 kJ)/mol for the early and late transcripts, respectively (Fig. 1D and E). The stem-loops are followed by uridine-rich tails, corresponding to the Rho-independent termination described for *Escherichia coli* (7). Even assuming a moderate S1 nibbling effect (9), these transcripts apparently did not terminate at a single base-pair position but rather ended heterogeneously, which is also consistent with a Rho-independent termination event (7). Therefore, the region of ϕ29 DNA in which both early and late transcription terminate (TD1 terminator) is included in both types of transcripts, this region acting as a transcription termination signal in either orientation (Fig. 1F), a phenomenon that has also been described for *E. coli* (7).

The ϕ29 DNA early transcription initiating at the A2b and A2c promoters apparently reaches the very left end of the viral DNA molecule in vivo, as suggested by previous studies (1). The S1 mapping done with the leftmost *Hin*fI subfragment from the *Hin*dIII B fragment (see Fig. 4B) confirmed this (results not shown), with the termination of transcription occurring as if the RNA polymerase would run off the template. Another termination site was located within the *Hin*dIII G fragment of the viral DNA (see Fig. 4B and C) for the transcripts initiated at the A2b and A2c promoters. The *Hin*dIII G fragment was 3′ end labeled, and the early strand was hybridized with RNA extracted from *B. subtilis* infected with ϕ29 in the presence of chloramphenicol. After hybridization, the samples were digested with S1, and the resulting protected oligonucleotides were fractionated on denaturing polyacrylamide gels side by side with a set of sequencing reaction mixtures (Fig. 2). Transcription terminated mainly between nucleotides 184 and 188 from the right end of the fragment (TA1 terminator). Again, a stem-loop structure could be drawn, with a free energy of −12.0 kcal (ca. −50.2 kJ)/mol, which was followed by a tail rich in uridine residues.

Transcription initiating at the B2 promoter (see Fig. 4C) seemed to terminate in a scattered fashion, as determined by S1 mapping experiments. The 3′-end-labeled early strand

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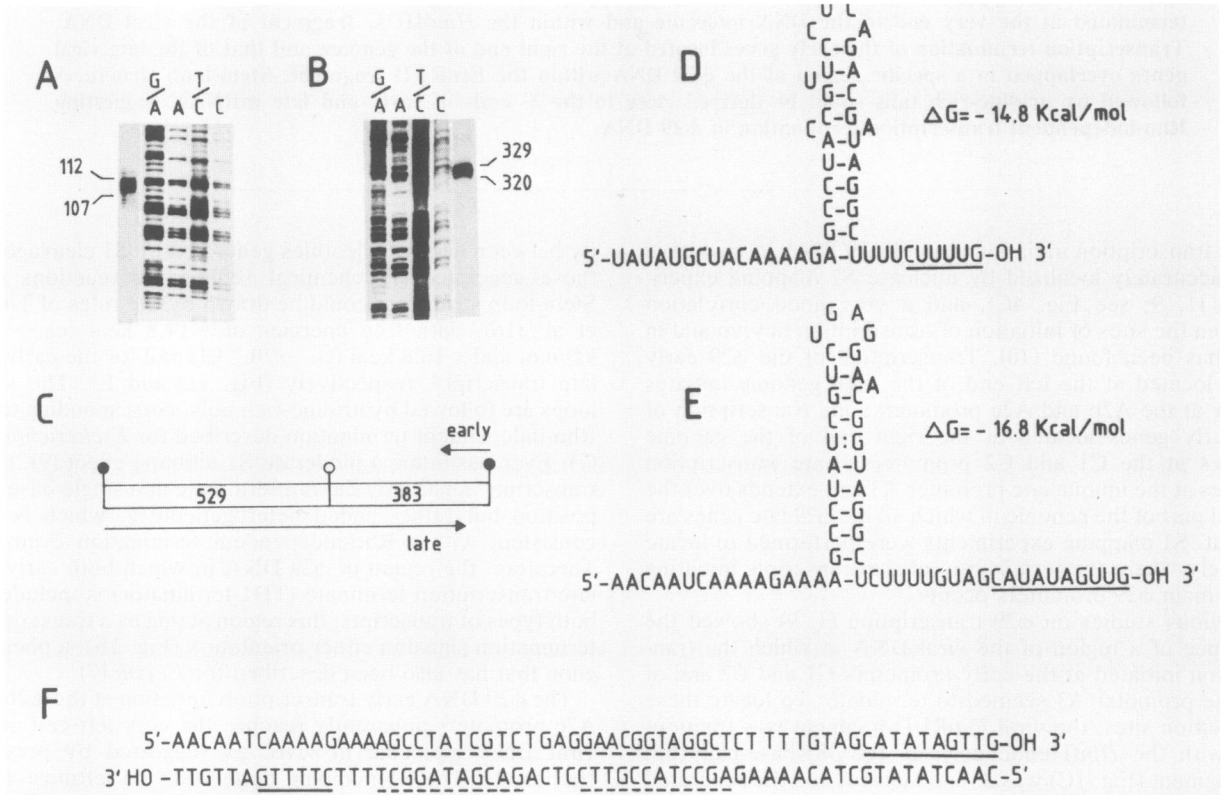


FIG. 1. Early and late transcription termination within the *Eco*RI D fragment, (A and B) Oligonucleotides protected from S1 digestion by the in vivo early (A) and late (B) RNA in the 383-bp *Eco*RI D-*Hin*fI subfragment. Sequencing reactions (8) were from a 672-bp *Hin*dIII-*Hin*fI subfragment from *Hin*dIII-B of known sequence (19). (C) Physical and transcriptional map of the *Eco*RI D fragment. Symbols: ♀ , *Eco*RI; ♀ , *Hin*fI. The arrows indicate the direction and extent of transcription. The numbers represent base pairs. (D and E) Stem-loop structures derived for early (D) and late (E) transcription termination. ΔG, Free-energy change. (F) DNA sequence of the TD1 terminator region. -----, Nucleotides forming part of the stem-loop structures; ——, sequence corresponding to the termination of transcription. Labeling of DNA fragments at their 3′ termini with the Klenow enzyme and [α-^{32}P]dATP was performed as described previously (5). Purification of double- and single-stranded DNA by diffusion from polyacrylamide gels, the S1 mapping conditions, and the preparation of the RNA made in vivo were as described previously (1, 3).

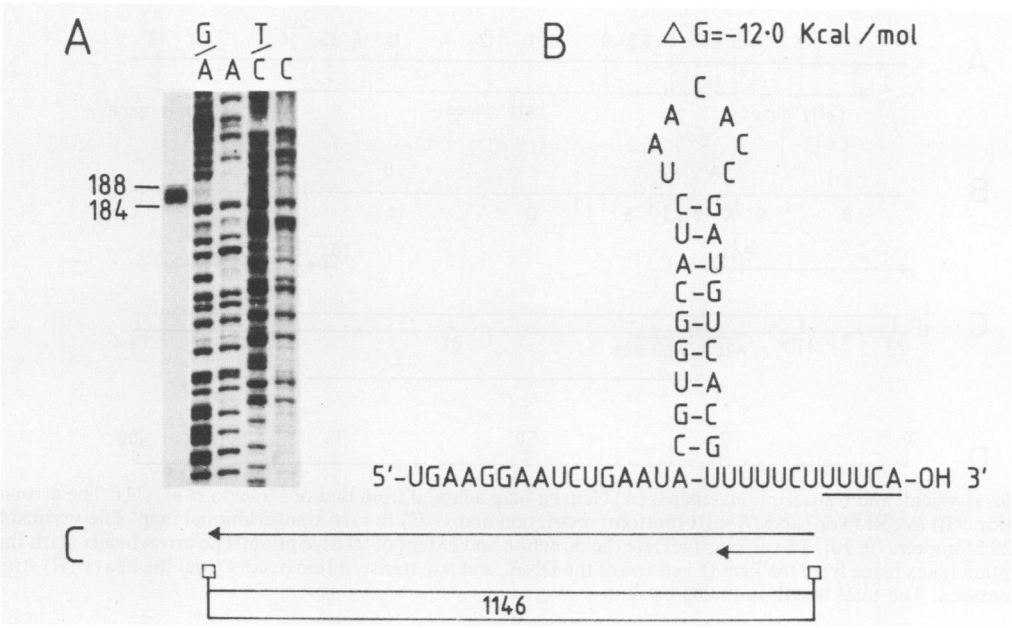
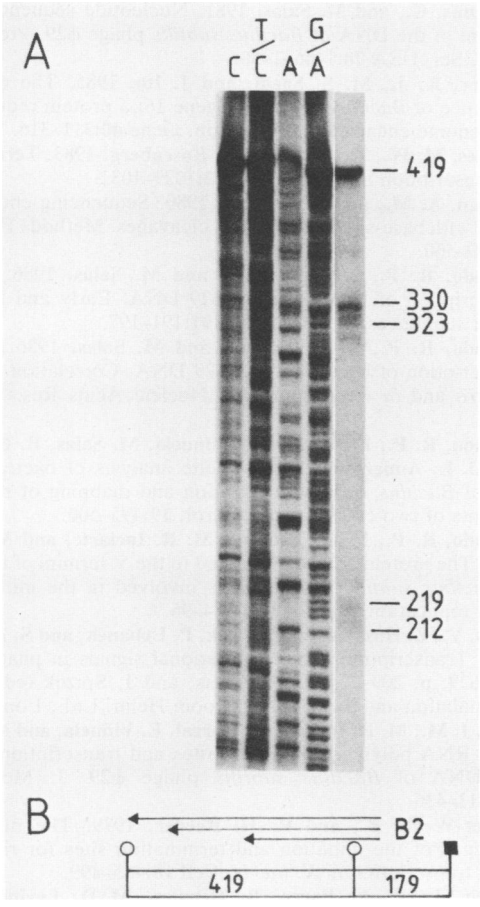


FIG. 2. Early transcription termination within the *Hin*dIII G fragment. (A) Oligonucleotides protected from S1 digestion by the in vivo early RNA in the 1,146-bp *Hin*dIII G fragment. Sequencing reactions (8) were from the late strand of the same fragment of known sequence (19). (B) Stem-loop structure derived for transcription termination. ΔG, Free-energy change. (C) Direction and extent of transcription (indicated by arrows) within the *Hin*dIII G fragment. Symbol: ⚲, *Hin*dIII.



from a 419-bp *Hin*fI subfragment spanning positions 14835 to 15254 of the viral genome (18; Fig. 3) was hybridized with RNA extracted from ϕ29-infected cells in the presence of chloramphenicol. The oligonucleotides protected from S1 digestion were analyzed on denaturing polyacrylamide gels side by side with a set of sequencing reaction mixtures (Fig. 3). Two transcription termination sites were located between nucleotides 212 and 219 (TB1) and 323 and 330 (TB2) from the right end of the DNA fragment, but in this instance no stem-loop structures could be drawn, although termination at the TB1 site occurred close to uridine-rich tails, CUUUUCUAUGC (nucleotides 209 to 219) being the sequence at the TB2 terminator, CAAUCUCUAGU (nucleotides 320 to 330). It is difficult to ascertain whether these sites are due to specific RNA cleavage events or to Rho-dependent termination. No clear transcription termination sites were found for the transcription initiated at the B1 promoter, which seems to proceed weakly in a leftward direction on the ϕ29 genome (results not shown).

Our current knowledge of ϕ29 transcription termination in vivo is shown in Fig. 4C. Early transcription from the left part of the genome terminates mainly at the far left end of the viral DNA, so that transcription of early genes 1 to 6 is ensured. The TA1 terminator within the *Hin*dIII G fragment would favor more frequent transcription of genes 5 and 6, probably ensuring higher levels of synthesis of protein p6,

FIG. 3. Termination of the early transcripts initiating at the B2 promoter. (A) Oligonucleotides protected from S1 digestion by the in vivo early RNA in the 419-bp *Hin*fI fragment of the ϕ29 DNA region close to the B2 early promoter. Sequencing reactions (8) were from the late strand of the 880-bp *Eco*RI-*Hin*dIII subfragment from the *Hin*dIII D fragment of known sequence (18). (B) Physical and transcriptional map of the region of viral DNA including the B2 promoter and the two termination signals. Symbols: ⚑, *Hpa*II; ⚲, *Hin*fI. The arrows indicate the direction and extent of transcription within the DNA fragment. The numbers represent base pairs.

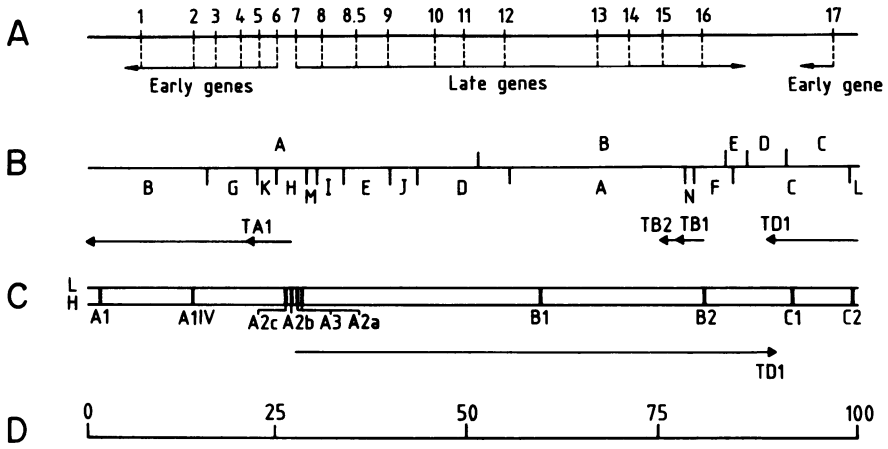


FIG. 4. ϕ29 genetic, physical, and transcriptional maps. (A) Genetic map adapted from that of Mellado et al. (11). The arrows indicate the direction of transcription. (B) *Eco*RI (Top) and *Hin*dIII (bottom) restriction maps. (C) In vivo transcriptional map. The vertical lines indicate the positions of the ϕ29 promoters (9, 10). The arrows indicate the direction and extent of transcription. The arrowheads mark the termination points. Early transcription takes place from the light (L) strand of the DNA, and late transcription occurs from the heavy (H) strand. (D) DNA length shown in percentages. The total length is 19,285 bp (18).

the product of gene 6, which is known to be made in high amounts in *B. subtilis* infected with ϕ29 (2) and is needed in DNA replication (12). The existence of the TA1 terminator in this particular region of ϕ29 DNA is in agreement with predictions made on the basis of DNA sequencing data (19) based on previous in vitro transcription studies (4). The bidirectional terminator TD1, whose existence could be derived from DNA sequencing data (6, 13), could eliminate the overlapping of the right early and late transcriptions.

Symmetrical transcription could play a regulatory role in ϕ29 gene expression, negatively modulating the levels of synthesis of particular gene products in a posttranscriptional step. This could be the role played by the transcripts starting at the B1 and B2 promoters, since no sufficiently long open reading frames are present in the neighborhood of the B1 and B2 promoters. In addition, the two short transcripts from the B2 promoter, terminating at the TB1 and TB2 sites, include a region of RNA complementary to the DNA sequences coding for the beginning of the viral protein p14 (18), which is needed for lysis of the host cell upon ϕ29 infection (2). Transcripts from the B1 promoter may act in a similar manner, regulating the levels of synthesis of protein p12, which forms the 12 appendages of the viral capsid (2) involved in the process of adsorption of the phage particles to the cell wall (17). Experiments to determine whether this mechanism of negative regulation is acting in the control of ϕ29 gene expression are under way.

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