In vitro transcription of the *Bacillus subtilis* phage φ29 DNA by *Bacillus subtilis* and *Escherichia coli* RNA polymerases

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ABSTRACT

The Escherichia coli RNA polymerase bound to phage Ø29 DNA has been visualized by electron microscopy. Thirteen specific binding sites have been observed at 1.7,2.6,5.5,10.4,13.7,25.2,25.7,26.3,33.5,59.5,69.2,91.7 and 99.6 DNA length units and they have been named A1,A1I,A1II,A1III,A1IV,A2,A2I,A3, A4,B1,B1I,C1 and C2, respectively. The binding sites A1,A2,A3,B1,C1 and C2 coincide with those found with Bacillus subtilis RNA polymerase. The transcription of phage Ø29 DNA with B. subtilis or E. coli RNA polymerases has been studied. With the B. subtilis RNA polymerase eight transcripts were found, starting at positions corresponding to the binding sites A1, A1III, A2,A3,B1I,B2,C1 and C2, respectively. With the E. coli RNA polymerase the same transcripts were found and a new one starting at position corresponding to the A4 binding site. The RNAs starting at binding sites A1,A1III,A2,B1I, B2,C1 and C2 are transcribed from right to left, as expected for early RNA. The RNAs which initiate at positions A3 and A4 are transcribed from left to right and probably correspond to late RNAs.

INTRODUCTION

B. subtilis RNA polymerase exists as multiple holoenzymes, each containing a different sigma subunit (1-4). The major form, containing a σ subunit of molar mass 55,000 g mol^{-1} (5,6), seems to recognize a promoter sequence similar to that established for the E. coli RNA polymerase, which contains a σ subunit of molar mass 84,000 g mol^{-1} (7,8). The consensus sequences at the -35 and -10 regions recognized by E. coli RNA polymerase, TTGACA and TATAAT, respectively (9,10) seem to be the preferred ones for the B. subtilis RNA polymerase containing the σ^{55} subunit (4,11,12).

To study further the specificity of promoter recognition of B. subtilis and E. coli RNA polymerases we have chosen the B. subtilis phage Ø29. The viral DNA has a molar mass of 11.8 x 10^6 g mol^{-1} (13) and contains a protein covalently linked to the two 5' ends (14-17). In vivo, early Ø29 RNA is synthesized from the light (L) DNA strand, from right to left, and late RNA is produced from the heavy (H) DNA strand, from left to right (18,19). The in vivo

transcription map determined by Sogo et al. (13) has shown that early transcription occurs at the two ends of Ø29 DNA and late transcription at the middle of the phage genome. A region of symmetric transcription exists in the middle of the Ø29 genome. Electron microscopy had revealed the existence on Ø29 DNA of seven specific binding sites for B. subtilis RNA polymerase containing the σ^{55} subunit (13). In this paper we show that the E. coli RNA polymerase binds to Ø29 DNA in thirteen specific sites; six of them coincide with those used by the B. subtilis enzyme. To correlate the binding sites with the initiation of transcription, the R-loop technique (20) has been used to analyze the in vitro transcripts using both the B. subtilis and the E.coli RNA polymerases.

MATERIALS AND METHODS

Reagents and enzymes

B. subtilis RNA polymerase was prepared as described (13). E. coli RNA polymerase was obtained from Dr. T. Trautner. Restriction endonuclease Eco RI was prepared as described (21) and Hpa I was obtained from New England Biolabs. Fungal proteinase K was from Merck. Ø29 DNA was prepared as described (21).

Binding of RNA polymerase to Ø29 DNA and formation of transcription R-loops

Binding of E. coli RNA polymerase to Ø29 DNA was carried out as described (13). The incubation mixture for transcription contained in 0.05 ml, 30 mM triethanolamine-HCl (pH 7.9), 8 mM magnesium acetate, 0.2 mM ATP, GTP, CTP and ^{3}H-UTP (15 µCi/µmol), 5.2 µg of either intact Ø29 DNA or restricted with Hpa I or Eco RI and either 5.2 µg of B. subtilis RNA polymerase or 4.5 µg of E. coli RNA polymerase. When indicated, 160 mM KCl was also added. The incubations were at 37°C for different time periods. Samples were diluted into R-loop buffer (20) and incubated for 2-3 h at 56°C. When indicated, the R-loop mixture, diluted to a final concentration of 50% formamide (vol/vol) and 3.5% formaldehyde, was treated for 11 min at 39°C and immediately quenched in ice.

Specimen preparation for electron microscopy

The RNA polymerase-DNA complexes were processed as described (13,22). The R-loop samples were chilled to 0°C and diluted up to a concentration of 50% formamide (vol/vol). The spreading was as described (23). In some experiments glyoxal to a final concentration of 0.35 M was added to the spreading solution.

The quantitative analysis was carried out as described (13).

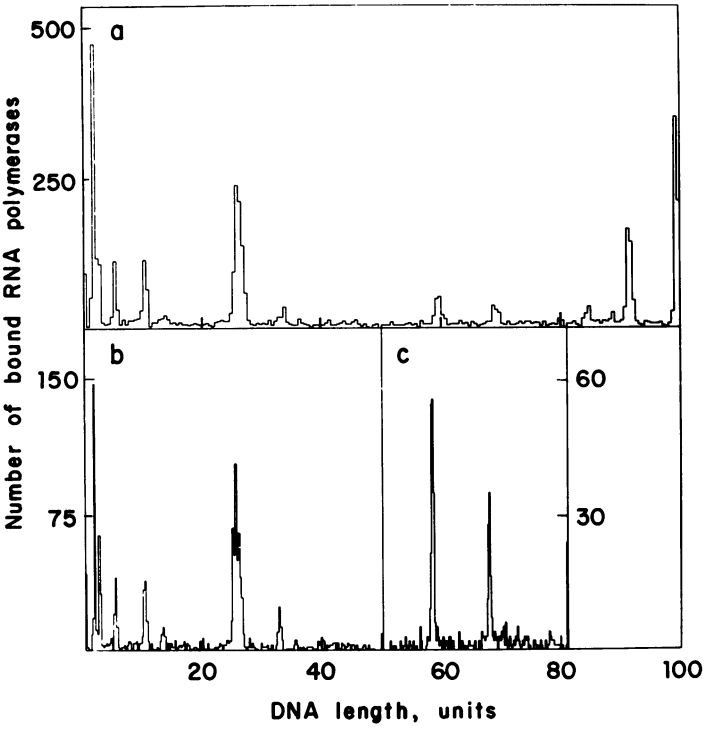

Fig.1. Histogram of E. coli RNA polymerase bound to Ø29 DNA or to fragments Eco RI A or B.(a). The histogram shown is the addition of three histograms obtained in the absence and presence of 50 mM KCl and in the presence of ATP, GTP and CTP. 570 DNA molecules with 4691 bound RNA polymerases were analyzed. The contour length of the DNA was 6.15 (±0.08)µm. (b) and (c). Eco RI fragments of Ø29 DNA were incubated with E. coli RNA polymerase in the presence of ATP, GTP and CTP. (b) 182 DNA molecules of fragment Eco RI A with 1368 bound RNA polymerases were analyzed. The contour length of fragment Eco RI A was 3.21 (±0.06) µm. (c) 225 molecules of fragment Eco RI B with 554 bound RNA polymerases were analyzed. The contour length of fragment Eco RI B was 1.89 (±0.03) µm.

RESULTS

Mapping of E. coli RNA polymerase binding sites on Ø29 DNA

Binding assays with the E. coli RNA polymerase carried out in the presence or in the absence of 50 mM KCl gave rise to six main peaks and several small ones along the Ø29 genome. The six main peaks (Fig. 1a) were located at 2.2, 5.5, 10.4, 26.3, 91.7 and 99.6 length units. Peaks at positions 2.2 and 26.3 resulted from clusters of 2 and 3 specific binding sites, respectively (see later). Fig. 2a shows a Ø29 DNA molecule to which twelve E. coli RNA po-

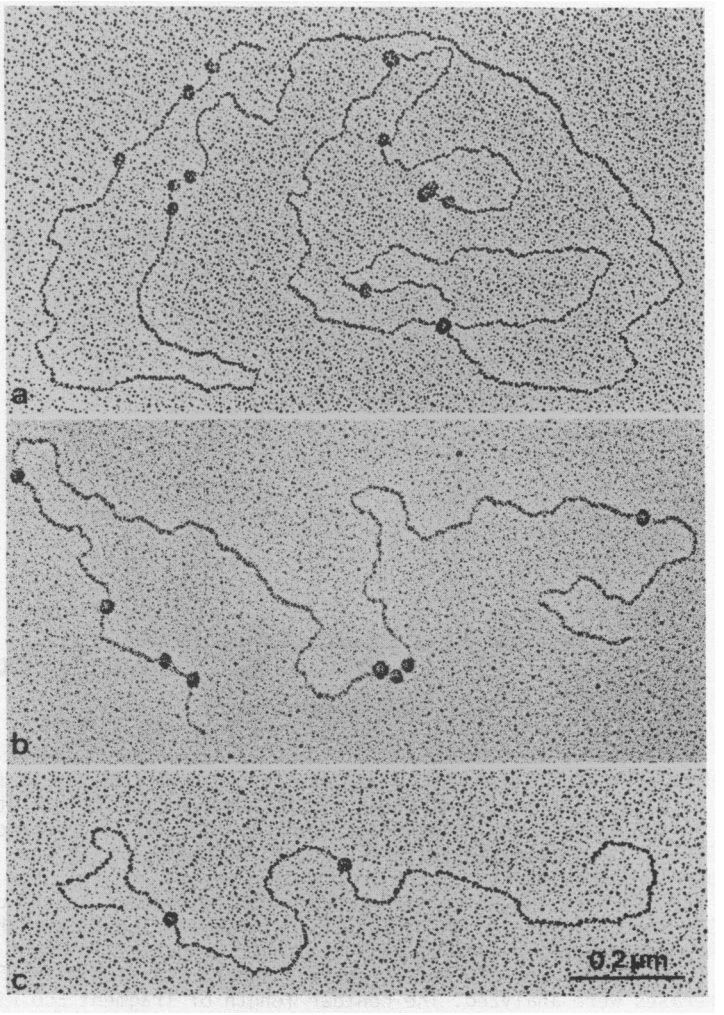

Fig.2. Electron micrographs of E. coli RNA polymerase bound to Ø29 DNA (a) and to Eco RI fragments A (b) and B (c). The scale line represents 0.2 µm.

lymerases were bound, ten of them to specific sites. The cluster of two RNA polymerases can be clearly distinguished at the left end as well as the cluster of three enzymes in the position around 26 length units. To determine whether the above clusters with two and three enzymes bound, respectively, represent individual peaks and whether the small peaks are specific sites, binding assays were carried out using the Ø29 DNA Eco RI A and B fragments. Fig. 2b shows an Eco RI A fragment containing the cluster of two and three

RNA polymerases. The histogram obtained (Fig. 1b) shows two very strong peaks. The first one is split into two at 1.7 and 2.6 length units. The other strong peak is split into three at 25.2, 25.7 and 26.3 length units. The other main peaks obtained with the whole genome were also found with the Eco RI A fragment as well as two weak ones at positions 13.7 and 33.5. Fragment Eco RI B contained two binding sites (Fig. 2c and Fig. 1c) at positions 59.9 and 69.3, very close to those obtained when the whole DNA was used as template (Table 1).

In vitro transcription. R-loop mapping

To determine whether all the binding sites could act as promoters in vitro, their relative strength, as well as the direction and the extent of the transcription, Ø29 DNA was transcribed either with B. subtilis or E. coli RNA polymerase holoenzymes and the hybrid DNA-RNA molecules were analyzed by electron microscopy. The sharp side of the R-loop peaks was considered to be the initiation point of transcription, according to Brack (20).

Transcription of Ø29 DNA with B. subtilis RNA polymerase

After 0.75 and 1.5 min of transcription most of the DNA molecules had one R-loop, preferentially located at a unique position (Fig. 3a). The sharp side of the peak is located at position 25.8 and probably corresponds to the initiation of the transcription from the binding site A2 which takes place from right to left. Other small transcription peaks located towards the right end of the genome were also obtained. When the incubation was carried out for 2.5 or 5 minutes (Fig. 3b) the transcription pattern drastically changed with respect to that described above. The number as well as the extension of the loops per DNA molecule increased. Close to the peak starting at 25.8 units, a new one appeared initiating at position 28.0 and transcribing from left to right (see also Fig. 3c). At the right end of the DNA the extension of transcription greatly increased and at least three transcribed regions could be distinguished. The initiation of the RNA synthesis in two of them seems to be at 91.3 and 99.1 length units and the transcription goes from right to left. For the third peak it is not possible to determine the direction of the transcription. Fig. 4 a and b shows two micrographs of molecules transcribed for 5 min. In both of them, between positions 25.8 and 28.0, there was a small non transcribed DNA region between two loops. After 10 min of incubation (Fig. 3e) the transcription pattern was basically the same as that obtained at incubation times of 2.5 and 5 min (Fig. 3b). Additionally, a new R-loop appeared probably starting transcription at 10.3 length units and going from right to left.

To confirm both the correct orientation of the DNA-RNA hybrid molecules

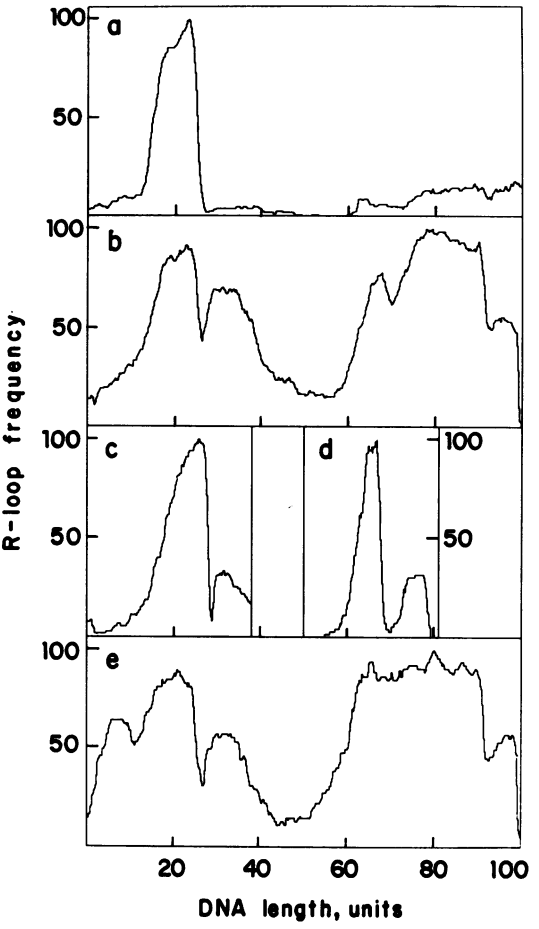

Fig.3. Histogram of transcription R-loop molecules obtained with B.subtilis RNA polymerase on Ø29 DNA transcribed for 0.75 and 1.5 min (a), 2.5 and 5 min (b) and 10 min (e). 193 DNA molecules with 269 R-loops were analyzed in (a), 190 DNA molecules with 497 R-loops in (b) and 74 DNA molecules with 206 R-loops in (e). The contour length of the DNA was 7.05 (±0.33) μm. As already reported (13,23), it can be seen that the length of the DNA is greater with the BAC spreading technique used here than with the ethidium bromide technique used in the experiments shown in Fig. 1. Ø29 DNA fragments Hpa I A (c) and Eco RI B (d) were transcribed with B. subtilis RNA polymerase for 2.5 min. 112 molecules with 146 R-loops were analyzed in (c) and 96 molecules with 101 R-loops in (d). The contour length of fragments Hpa I A and Eco RI B was 2.70 (± 0.15) and 2.35 (± 0.14) μm, respectively.

and that the transcription initiated at 25.8 and 28.0 length units takes place in opposite direction as well as to resolve possible overlapping transcription regions, the same kind of experiments described above were carried

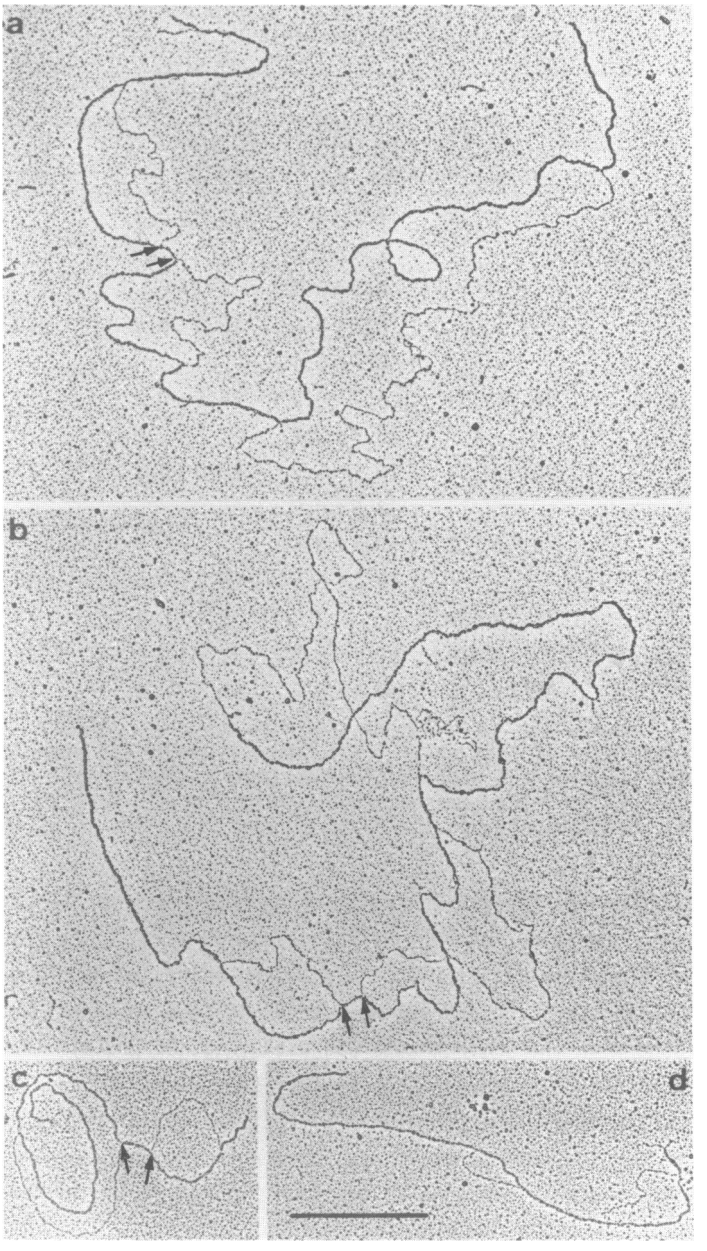

Fig.4. Electron micrographs of transcription R-loop molecules obtained with B.subtilis RNA polymerase on Ø29 DNA (a and b) and fragments Hpa I A (c) and Eco RI B (d). The arrows indicate the position of the untranscribed region between 25.8 and 28.0 length units. The scale line represents 0.5 µm.

Table 1. Position of binding and initiation of transcription in Ø29 DNA by *B. subtilis* and *E. coli* RNA polymerases

	Binding, *B.subtilis* RNA pol.[a]	Binding, *E. coli* RNA pol.[b]	Initiation of transcription, *B. subtilis* RNA pol.[c]	Initiation of transcription, *E. coli* RNA pol.[d]
A1(A1a/A1b;P_E1)[e]	1.7 ± 0.4	1.7 ± 0.2	1.6 ± 0.3	1.2 ± 0.3
A1I(G4a/G4b;$P_{(EC)}$1)	-	2.6 ± 0.2	-	-
A1II	-	5.5 ± 0.3	-	-
A1III	-	10.4 ± 0.4	10.3 ± 0.4	10.2 ± 0.4
A1IV	-	13.7 ± 0.4	-	-
A2(G1b/G3b;P_E2)	25.5 ± 0.5	25.2 ± 0.2	25.8 ± 0.6	25.2 ± 0.6
A2I(G1a/G3a;$P_{(EC)}$2)	-	25.7 ± 0.2	-	-
A3	26.7 ± 0.4	26.3 ± 0.3	28.0 ± 0.5	27.8 ± 0.6
A4	-	33.5 ± 0.5	-	33.2 ± 0.7
B1	59.4 ± 1.2	59.5 ± 0.5	-	-
B1I	-	69.2 ± 0.5	68.3 ± 0.5	68.9 ± 1.0
B2	79.3 ± 0.9	-	79.1 ± 0.4	79.2 ± 1.0
C1(G5;$P_{(EC)}$3)	91.3 ± 0.6	91.7 ± 0.5	91.3 ± 0.5	91.2 ± 0.7
C2(G2;P_E3)	99.3 ± 0.4	99.6 ± 0.3	99.1 ± 0.3	99.1 ± 0.4

[a]Taken from the work of Sogo et al. (13). [b]Mean value of the experiments from Fig. 1. [c]Mean value of the experiments from Fig. 3. [d]Mean value of the experiments from Fig. 5. [e]The names given by Davison et al. (24) and Yoshikawa and Ito (25), respectively, to the Ø29 promoters are given in parenthesis.

out using as templates the Ø29 DNA restriction fragments Hpa I A and Eco RI B. When fragment Hpa I A (see Fig. 8) was used, two large loops separated by a small non-transcribed region with the starting points at 27.5 and 29.0 genome length units (Figs. 3c and 4c) were seen, in good agreement with the positions found when the whole genome was used (Fig. 3b and e). The frequency of the first peak was greater than that of the second one and the transcription clearly takes place from right to left for the first one and from left to right for the second, as it was found when the whole genome was used. A small R-loop, probably starting at position 1.6 and moving to the left was also observed.

When fragment Eco RI B was used, two loops were seen, probably starting transcription at positions 68.3 and 79.1 and going from right to left (Figs. 3d and 4d). Comparison of the histograms shown in Figs. 3b and d gives a good correlation for the peak starting at position 68.3 whereas the small peak

starting at 79.1 length units is overlapped when the whole genome is used as template.

From all the above results we can conclude that there are at least eight transcription starting points in Ø29 DNA for the B. subtilis RNA polymerase at positions 1.6, 10.3, 25.8, 28.0, 68.3, 79.1, 91.3 and 99.1 (see Table 1).

To determine whether or not the salt affects the in vitro transcription of Ø29 DNA by the B. subtilis RNA polymerase, the transcription was carried out in the presence of 160 mM KCl. After 5 or 10 min of incubation all the transcripts were present except the one starting at position 28.0 (data not shown).

Transcription of Ø29 DNA with E. coli RNA polymerase

Transcription of Ø29 DNA by the E. coli RNA polymerase was carried out as described before for the B. subtilis enzyme. At 0.75 min of incubation, a main peak was obtained (Fig. 5a). At this time, a peak at position 1.2 is clearly seen. After 2.5 and 5 min, eight peaks appeared, with transcription starting most likely at 10.2, 25.2, 27.8, 33.2, 68.9, 79.2, 91.2 and 99.1 length units (Fig. 5b and c). When the incubation was carried out for 10 min, the positions of the starting points were the same as before (Fig. 5d).

From the above results we can conclude that at least 9 of the 13 specific binding sites for E. coli RNA polymerase are initiation sites of transcription. In seven of them the transcription is probably from right to left beginning at 1.2, 10.2, 25.2, 68.9, 79.2, 91.2 and 99.1 length units, whereas in the other two transcription is from left to right starting at 27.8 and 33.2 length units (see Table 1).

Orientation of the transcripts obtained with the E. coli RNA polymerase

When the B. subtilis RNA polymerase was used in the in vitro transcription assay it was not difficult to orient the hybrid DNA-RNA molecules. The orientation was shown to be correct by using the restriction fragments Hpa I A and Eco RI B. With the DNA transcribed by the E. coli RNA polymerase the orientation was more difficult, on one hand because the number of specific binding sites was higher than with the B. subtilis enzyme, and on the other because only one or two R-loops (rarely more) were found per DNA molecule.The location of the loops varied along the DNA molecules, giving rise to symmetry problems. To orientate the hybrid molecules, the loop positions at each DNA molecule were considered in their direct and inverted measurement and the values which best fitted to the RNA polymerase binding sites were chosen to obtain the histograms shown in Fig. 5. To confirm the above orientation, the hybrid molecules were partially denatured. Under certain conditions of dena-

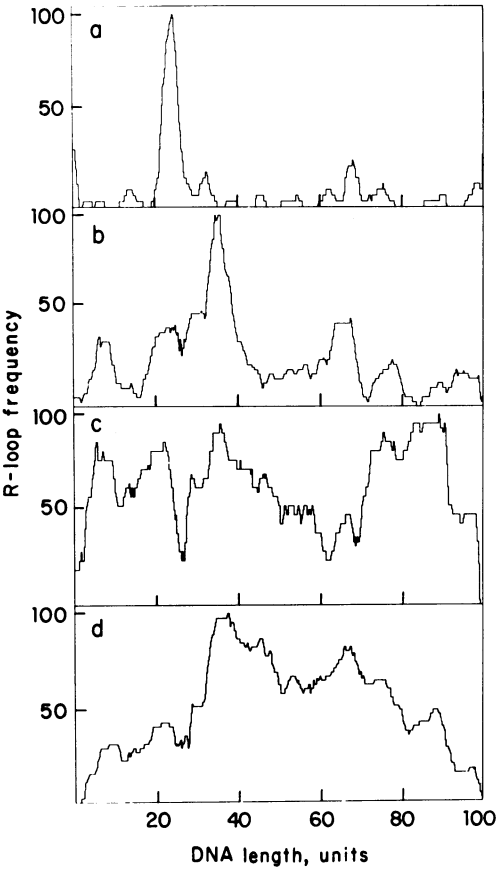

Fig.5. Histogram of transcription R-loop molecules obtained with E. coli RNA polymerase on Ø29 DNA transcribed for 0.75 min (a), 2.5 min (b), 5 min (c) and 10 min (d). The 0.75, 2.5 and 10 min samples were taken from the same experiment and the 5 min sample from a different experiment. 69 DNA molecules wiht 92 R-loops were analyzed in (a), 113 DNA molecules with 132 R-loops in (b), 79 DNA molecules with 97 R-loops in (c) and 105 DNA molecules with 126 R-loops in (d). The contour length of the DNA was 7.35 (± 0.32) μm.

turation the Ø29 DNA right end is always open whereas the left end remains closed (23), thus, the orientation would not depend on the location of the loops. Fig. 6 shows two partially denatured DNA molecules with one and two R-loops, respectively. Fig. 7a shows the histogram corresponding to the location of the R-loops in DNA molecules transcribed by the E. coli RNA polymerase during 2.5 or 5 min and Fig. 7b shows the partial denaturation map of the same hybrid molecules. The histogram of Fig. 7a correlates well with the one

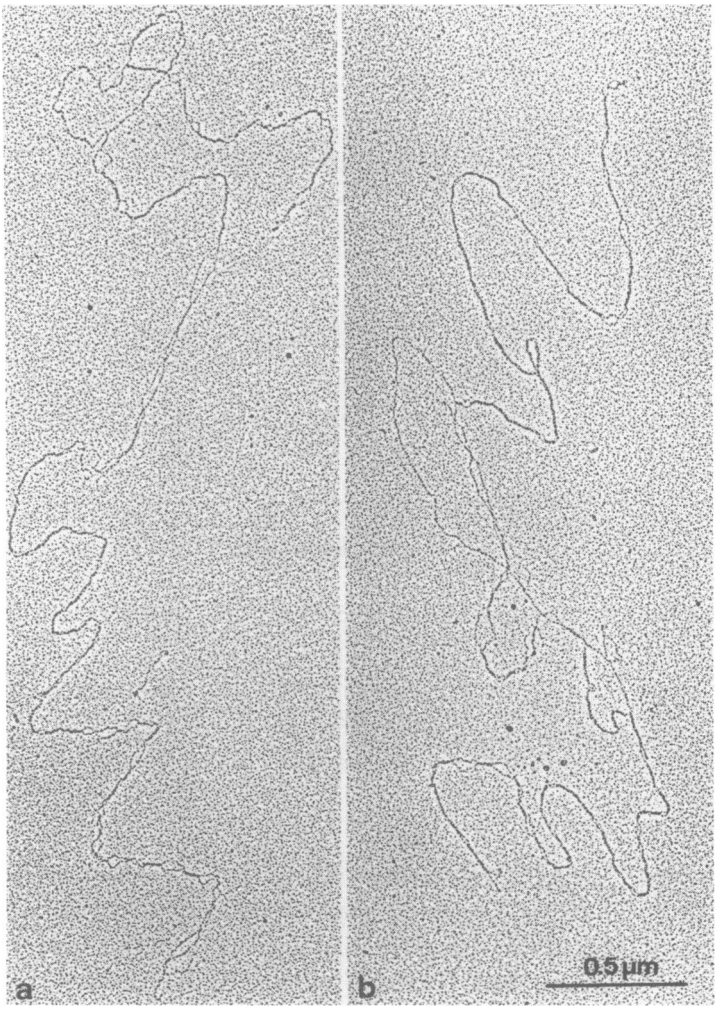

Fig.6. Electron micrographs of transcription R-loop molecules, partially denatured, obtained after 5 min incubation of Ø29 DNA with E. coli RNA polymerase. (a) Partially denatured molecule with one R-loop towards the left of Ø29 DNA. (b) Partially denatured molecule with two R-loops, one in the middle and one towards the right end of Ø29 DNA. The scale line represents 0.5 µm.

obtained without partial denaturation (Fig. 5b and c) although some of the R-loops, specially those at the right end, are lost probably due to the partial denaturation. Therefore, the orientation of the hybrid molecules seemed to be correct in most of the cases.

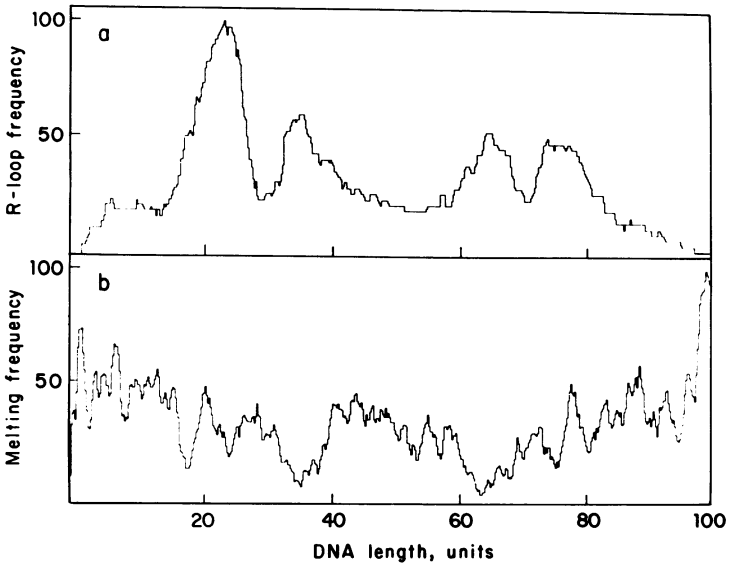

Fig.7. Histogram of transcription R-loop molecules, partially denatured, obtained with E. coli RNA polymerase on Ø29 DNA transcribed for 2.5 or 5 min. After incubation for R-loop formation, the samples were partially denatured. (a) 96 DNA molecules with 251 R-loops were analyzed. The contour length of the DNA was 8.66 (±0.49) μm. (b). Denaturation map of the same 96 DNA molecules with a denaturation degree of 30%. As already reported (23), the length of the DNA partially denatured is greater than that of native DNA.

DISCUSSION

In vivo and in vitro transcription maps of the Ø29 genome have been determined (13,18,24,26). It is generally accepted that the transcription of the early genes takes place from right to left from the DNA L strand, and the late transcription takes place from left to right from the DNA H strand. Symmetric transcription of a region of Ø29 DNA has also been found (13).

E. coli RNA polymerase binding sites in Ø29 DNA

By analysis with the electron microscope the complexes between Ø29 DNA and E. coli RNA polymerase we have found thirteen specific binding sites (see Table 1). Six of these binding sites, A1, A2, A3, B1, C1 and C2 coincide with those found with the B. subtilis RNA polymerase (13; see Table 1) and the other seven binding sites are new ones, not recognized by the B. subtilis enzyme. The binding sites A1, A1I, A1II, A1III, A1IV, A2, A2I, A3 and C2 are within A-T rich regions, whereas the remaining binding sites are in regions of a clearly lower A-T content (see Fig. 8). The six E. coli initiation sites described by Davison et al. (24) coincide with six of the seven strongest

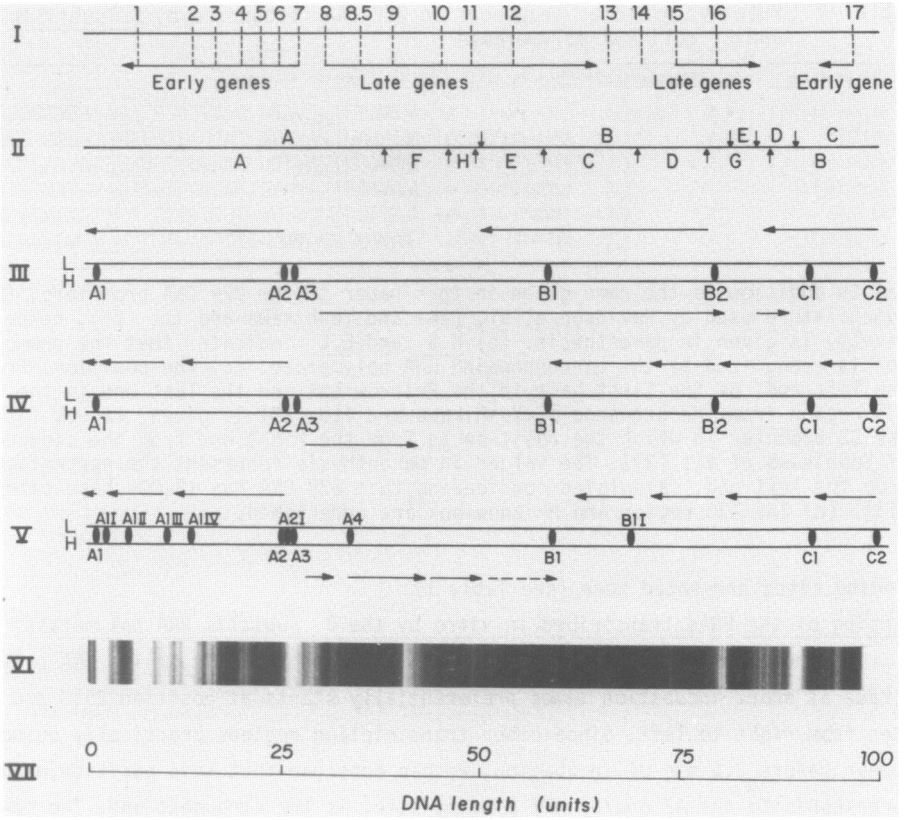

Fig. 8. Transcription map of phage Ø29 DNA by B. subtilis and E. coli RNA polymerases. I. Genetic map of Ø29 DNA adapted from Sogo et al. (13) taking into account, to locate genes 2, 3, 4, 6 and 17, data from Escarmís and Salas (28), Mellado and Salas (29), Murray and Rabinowitz (12), Yoshikawa et al. (27) and Yoshikawa and Ito (25). The numbers indicate genes and the lines with the arrows below the genes indicate direction of transcription. II. Upper part, Eco RI map cleavage sites taken from Inciarte et al. (21); lower part, Hpa I map cleavage sites taken from Yoshikawa and Ito (25). III. In vivo transcription map of Ø29 DNA taken from Sogo et al. (13). The lines in the middle represent the L and H DNA strands, and the binding sites for B. subtilis RNA polymerase are indicated in the middle of the DNA strands. In all cases the solid lines with arrows indicate direction and extent of early or late transcription from the L or H DNA strand, respectively. IV. In vitro transcription map of Ø29 DNA with B. subtilis RNA polymerase. As in III, in the middle is shown the DNA with the B. subtilis RNA polymerase binding sites. V. Binding sites and in vitro transcription map of Ø29 DNA with E. coli RNA polymerase. The DNA with the E. coli RNA polymerase binding sites are shown in the middle; the binding sites which are the same as those found with B.subtilis RNA polymerase are indicated in the lower part and those which are new are shown in the upper part. The broken line indicates transcription whose direction could not be determined. IV. Densitometry of the partial denaturation map of Ø29 DNA taken from Sogo et al. (23).

Table 2. Putative promoter sequences in Ø29 DNA recognized by B. subtilis and E. coli RNA polymerases

(a) Promoter	(b) RNA polymerase		(c) Position	(d) Promoter sequence
A1(A1a/A1b;P_E1)	B.S.	E.C.	328-356 (1.9%)	ATTAATGTTTGACAACTAT TACAGAGTATGCTATAATGGTAGTATCAAT
A1III	B.S.	E.C	1965-1993(10.9%)	AGTTCGAATTGAAAGAGGG CTATATACCCACTATACAGATAAAAAGAAG
A2(G1b/G3b;P_E2)	B.S.	E.C	4978-5006(27.7%)	GAAAAGTGTTGAAAATTGT CGAACAGGGTGATATAATAAAAGAGTAGAA
A2I(G1a/G3a)	-	E.C.	5075-5104(28.3%)	AAAAAGTCTTGCAAAAAGTTATACAGGTGTGGTTAAATAGAGAACGTAGA
A3	B.S.	E.C.	5183-5155(28.7%)	ACAAATCCTTATGTATCAA GGGTTCACGTGGTATAATTAAGTAGTACTA
C2(G2;P_E3)	B.S.	E.C.	154-125 (99.2%)	GAAAAGGGTAGACAAACTATCGTTTAACATGTTATACTATAATAGAAGTA

(a) In addition to the name given in this paper to the Ø29 DNA promoters, the nomenclature used by Davidson et al. (24) and Yoshikawa and Ito (25), respectively, is given in parenthesis. (b) B.S. and E.C. indicate that the promoter is recognized by the corresponding RNA polymerase. (c) The position, from the left end, of the first base in the Pribnow box and the last one in the -35 region from the sequence of Yoshikawa and Ito (25) is given, except for the C2 promoter in which the position is from the right end from the sequence of Yoshikawa et al. (27). The values in parenthesis represent the percentage from the left end, calculated considering that Ø29 DNA has 18,000 base pairs (13). (d) The -35 region and Pribnow-box are underlined.

binding sites presented here (see Table 1).

Mapping of the RNAs transcribed in vitro by the B. subtilis RNA polymerase

In the absence of salt, the transcription with the B. subtilis RNA polymerase at short incubation times preferentially starts at position 25.8 and runs from right to left. Since other transcription regions practically do not appear before 2.5 min of incubation, we can conclude that this position,which corresponds to the A2 and/or A2I binding site, is the strongest one. The sequences in the DNA L strand which correspond to the A2 promoter have been assigned (12, 25; see Table 2). A possible sequence for the A2I binding site is given in Table 2.

Between 2.5 and 5 minutes additional transcribed regions appear. At the right end, transcription starts at 91.3 and 99.1 length units, which correspond to the strong C1 and C2 binding sites, being the transcription from the C1 promoter stronger than from the C2 promoter. The most likely sequence of the Pribnow-box and -35 region for the C2 promoter is given in Table 2 (27). No sequence data for the DNA region corresponding to the C1 promoter is presently available. The initiation of the transcription at 68.3 length units does not correspond to any previously described B. subtilis RNA polymerase binding site, but to the B1I site described here for the binding of the E.coli enzyme. It is possible that, although the binding of B. subtilis RNA polymerase was made in the presence of three nucleoside triphosphates (13), binding at the B1I site was not strong enough to remain attached to the DNA after all

the preparation steps.All the above transcripts,starting at the binding sites A2(A2I), B1I,C1 and C2 go from right to left and,therefore, correspond to early RNA transcribed from the DNA L strand.

The RNA starting at 28 length units is transcribed from left to right,in the direction of late transcription from the DNA H strand. This suggests that there is a late promoter at position A3 recognized to a certain extent by the B. subtilis RNA polymerase. When the incubation is carried out in the presence of 160 mM KCl, transcription does not take place in this region and probably for this reason Davison et al. (24) did not find this transcript.By looking at the DNA sequence (12,25) a perfect Pribnow box with a possible -35 region is found in the complementary DNA H strand (see Table 2). The sequence at the -35 region (TTATGT) differs greatly from the one used by the E. coli and B. subtilis RNA polymerases but this is not surprising taking into account that A3 is probably a late promoter. Why the above enzymes recognize to some extent this promoter is an open question.

Two additional small transcripts were detected with the B. subtilis RNA polymerase, one of them corresponding to the B2 promoter. The other small transcript is localized at the left end of the genome but it does not appear frequently, although its position corresponds to the strong A1 promoter (13). The great difference between promoter strength and frequency of the R-loop in this case could be explained assuming that the probability that the small transcribed RNA is still attached to the template at the time of hybridization is very low. The location of promoter A1 agrees with the sequence given by Yoshikawa and Ito (25) for the P_E1 promoter (see Tables 1 and 2).

When the incubation with B. subtilis RNA polymerase was carried out for 10 min, an additional transcript initiating at 10.3 length units was found. This position agrees well with the A1III binding site (10.4 length units) found with the E. coli RNA polymerase. No binding site at this position was found with the B. subtilis RNA polymerase (13), similarly to what happens for the B1I binding site. At 10.9% from the left end of the Ø29 genome there is a possible Pribnow-box and -35 region which could correspond to the A1III promoter (see Table 2).

Mapping of the RNAs transcribed in vitro with E. coli RNA polymerase

The initiation of the transcription at promoters A1, A1III, A2, A3, B1I, C1 and C2 (see Table 1) seems to be the same as for the B. subtilis RNA polymerase, and again the C1 promoter is stronger than C2. At a short transcription time, the strongest promoter with E. coli RNA polymerase is also A2, but later on the strongest peak is the one starting at 33.2 length units. The

latter transcript runs from left to right and probably starts at the A4 binding site. Although the B2 binding site is not found with the E. coli RNA polymerase we have detected transcription starting at this position. This situation is similar to that described with the B. subtilis RNA polymerase for the A1III and B1I transcripts. In addition, at late times of transcription, the E. coli RNA polymerase, contrary to the B. subtilis polymerase, transcribes a region extending from 42 to 60 length units (see Fig. 8).

We do not know if transcription with the E. coli RNA polymerase takes place in the binding sites A1II and A1IV, but if this is the case, the promoters are relatively weak and their products are overlapping with the A1III and A2 RNAs. Also, we have not detected transcription from the binding site A1I.

In vitro transcription map of Ø29 DNA

Figure 8 shows a summary of the transcription map of Ø29 DNA by B. subtilis and E. coli RNA polymerases. Seven transcripts, read from the DNA L strand from right to left, appear with the two enzymes, with similar initiation points. These transcripts start at promoters A1, A1III, A2, B1I, B2, C1 and C2. In addition, one transcript read from the DNA H strand from left to right, starting at the A3 promoter is observed with the B. subtilis RNA polymerase, the region between 42 and 60 length units remaining essentially untranscribed. In the case of the E. coli enzyme, in addition to the A3 promoter, transcription occurs from the A4 promoter. In this case, the position between 42 and 60 length units is transcribed at long incubation times. If the transcription between 28 and 60 length units corresponds to late RNA,then both B. subtilis and E. coli RNA polymerases would recognize in vitro some late promoters, A3 and A3 + A4, respectively.

It has been shown that, in vivo, the control of late transcription is carried out by the product of the early gene 4 (13). However, a small amount of late transcription and of late protein synthesis occurs after infection with a sus4-mutant (13,19). These results could be due either to leakiness of the mutant or, more likely, taking into account the results shown in this paper, to the possibility that the host RNA polymerase can recognize and read to a certain extent some late promoters. This ability to recognize and read late promoters on Ø29 DNA is even greater in the case of the E. coli RNA polymerase. A region of the Ø29 genome containing gene 4 has been sequenced (28) and placed under the control of bacteriophage λP_L promoter, and protein p4, which is overproduced in E. coli, has been characterized (29). Purification of protein p4 to study its action in the control of late Ø29 transcrip-

tion is in progress.

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