SHORT COMMUNICATION

The Genome of *Bacillus subtilis* Phage SPP1: Structure of an Early Promoter

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The strongest of five 'early' promoters of *Bacillus subtilis* phage SPP1 was localized in a DNA restriction fragment by analysis of RNA polymerase binding and R-loop formation. The nucleotide sequence of the promoter region was established. The signal structures identified were similar to those recognized by the σ^55^ RNA polymerase of *B. subtilis*. The promoter precedes an open reading frame with 51 codons. A protein with the *M_, predicted from the nucleotide sequence was identified in minicells.

INTRODUCTION

'Early' genes of the *Bacillus subtilis* phage SPP1 are transcribed by the vegetative host RNA polymerase, 'late' genes by a phage-modulated host RNA polymerase (Esche, 1975; Montenegro & Trautner, 1981). This finding raises the question why the nonmodulated host RNA polymerase is diverted to preferentially transcribe the genome of phage SPP1 after infection. As a first step to analyse this problem the nucleotide sequence of early phage promoters must be compared with *B. subtilis* promoter structures recognized by the σ^55^ RNA polymerase of *B. subtilis*. Previous studies (Montenegro & Trautner, 1981; Stüber et al., 1981) identified and localized five promoters of RNA transcription in the genome of phage SPP1, which regulate the expression of early phage genes and also of foreign genes cloned into this region (Behrens et al., 1983). The strongest of these promoters (number 3, localized in EcoRI fragment 10) has been used in a cloning vector (Flock et al., 1984). This use and our interest in comparing the structure of SPP1 promoters with other known promoters has prompted us to determine the DNA sequence of SPP1 promoter 3.

METHODS

*Bacterial strain and plasmid*. *Escherichia coli* strain DS410 (Dougan & Sherratt, 1977) was used as the minicell-producing strain. Plasmid pBT20 is a pBR322 derivative carrying the SPP1 EcoRI fragment 10.

*DNA sequencing*. DNA sequencing followed the procedure of Maxam & Gilbert (1977).

*Electron microscopy*. *E. coli* RNA polymerase binding to isolated EcoRI fragment 10 was carried out essentially as described by Morelli *et al.* (1981) at 37 °C with a DNA/RNA polymerase ratio of 1:2. Conditions for transcription and for R-loop formation were essentially as described by Bruck (1979). Spreading of R-loop molecules followed the procedure of Morelli *et al.* (1978). Micrographs of DNA/RNA polymerase complexes and R-loop molecules were taken in a Philips EM301, and the positions of RNA polymerase binding sites and/or R-loop were measured with a Nomonics digitizer. Data were processed by computer.

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Fig. 1. Electron micrographs of SPP1 EcoRI fragment 10: (a) with bound RNA polymerase; (b–e) R-loop molecules showing progressive stages of transcription. Bar, 0.5 μm.

Fig. 2. (a) Distribution of RNA polymerase molecules bound to SPP1 EcoRI fragment 10. (b) Position and length of transcriptional R-loops. A restriction map of part of the SPP1 EcoRI fragment 10 is shown below (b).

Minicell analysis. Minicells with and without plasmid pBT20 were purified according to the procedure described by Mertens & Reeve (1977). Purified minicells were incubated for 1 h in the presence of 25 μCi [35S]methionine (Amersham; 1450 Ci mmol⁻¹, 53.65 GBq mmol⁻¹). Labelled proteins were separated on a 15% (w/v) polyacrylamide gel as described by Laemmli (1970).

RESULTS AND DISCUSSION

Promoter 3 had previously been localized towards the left end of the EcoRI fragment 10 of SPP1 DNA, using the published convention of representing the genome of bacteriophage SPP1 (Stüber et al., 1981). A restriction map of part of this fragment is shown in Fig. 2. Transcription from this promoter proceeds from right to left. The SPP1 H-strand is used as a template (Montenegro & Trautner, 1981; Stüber et al., 1981). With the purified EcoRI fragment 10 (1352 bp) we used RNA polymerase binding and R-loop formation to determine the precise location of the promoter within this fragment and the direction of transcription (Fig. 1). Evaluation of 81 DNA molecules, each of which had bound only one RNA polymerase molecule, indicated that RNA polymerase binds with a high degree of specificity at position 430 ± 30 bp (Fig. 2a). An evaluation of 36 molecules with R-loops (Fig. 2b) showed that all R-loops have a common start in a region between 380 and 480 bp, that R-loops extend from here only towards the short end of the molecule, i.e. transcription proceeds only from right to left, and that the length of the transcripts shows a rather broad distribution extending from 119 bp to the left end of the molecule (430 bp). Hence in vitro there is no active transcriptional termination signal between promoter and the left EcoRI end of fragment 10.

EcoRI fragment 10 has only one PvuII site, located at coordinate 496. This small EcoRI/PvuII fragment, to which RNA polymerase binds efficiently, was purified and sequenced. The
Fig. 3. Nucleotide sequence of part of SPPl EcoRI fragment 10, extending between coordinates 1 and 517 of Fig. 2. The predicted amino acid sequence of the expected polypeptide is shown. Signal sequences characterizing the promoter are indicated by boxes.

sequence is shown in Fig. 3. Reading the sequence in the previously determined direction of transcription, we recognize at a location anticipated on the basis of the electron microscopic work the characteristic elements of a promoter structure: a −35 sequence: TTGCCT, a −10 sequence: TTTAAT, and a ribosome-binding site AGAGAGG. This promoter is similar to the vegetative B. subtilis and E. coli promoters, which have been analysed by Moran et al. (1982).

The most often verified B. subtilis −35 sequence is TTGACA, with its second triplet variable. The most frequent B. subtilis −10 sequence is TATAAT. Here the second triplet is very common, whereas the first triplet is variable. In SPPl DNA there is a T at position −17, which has also been found in seven out of nine bacterial sequences analysed. We also find an AT-rich region around coordinates −40/−60. The comparison thus indicates that the preferred use of SPPl phage promoters after phage infection cannot solely be attributed to the nucleotide sequence of the phage promoter.

Beginning at codon ATG (402) we find an open reading frame with 51 codons. The anticipated size of this highly basic protein is 6.3 kDal. A protein corresponding to this size has previously been detected (Amann & Reeve, 1981; Montenegro & Trautner, 1981) both in a DNA-directed cell-free protein synthesis reaction (with SPPl EcoRI fragment 10 as a primer) and after infection of E. coli minicells with λ/SSP1 hybrid phages containing this EcoRI fragment. The same protein is also seen in minicells of E. coli containing plasmid pBT20, which is a pBR322 derivative with SPPl EcoRI fragment 10 cloned into its EcoRI site (Fig. 4). As in preceding experiments, we also see in this case an additional protein of 9.4 kDal, which is probably encoded in the larger EcoRI/PvuII subfragment. The function of the 6.3 kDal protein is unknown. Because of its predicted high content of basic amino acids one might speculate that it is involved in DNA binding.
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Fig. 4. Autoradiogram of gene products synthesized in minicells. Lane 1, molecular size standards (kDal); lanes 2 and 3, 35S-labelled protein extracts from minicells with pBR322 (2) and pBT20 (3). Proteins A and B encoded by fragment 10, ~9.4 and ~6.3 kDal in size, are indicated.

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REFERENCES


