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_r 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 Brack (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 Numonics digitizer. Data were processed by computer.

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Fig. 1

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

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 [^{35}S]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

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 had previously been detected (Amann & Reeve, 1981; Montenegro & Trautner, 1981) both in a DNA-directed cell-free protein synthesis reaction (with SPP1 *Eco*RI fragment 10 as a primer) and after infection of *E. coli* minicells with λ /SSP1 hybrid phages containing this *Eco*RI fragment. The same protein is also seen in minicells of *E. coli* containing plasmid pBT20, which is a pBR322 derivative with SPP1 *Eco*RI fragment 10 cloned into its *Eco*RI 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 *Eco*RI/*Pvu*II 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.

Fig. 4. Autoradiogram of gene products synthesized in minicells. Lane 1, molecular size standards (kDa); lanes 2 and 3, ^{35}S -labelled protein extracts from minicells with pBR322 (2) and pBT20 (3). Proteins A and B encoded by fragment 10, ~ 9.4 and ~ 6.3 kDa in size, are indicated.

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