Transcription of the *Bacillus subtilis* Bacteriophage φ3T in vitro

BY ENDA KENNY,*† TONY ATKINSON† AND BRIAN S. HARTLEY
Centre for Biotechnology, Imperial College, London SW7 2AZ and †Microbial Technology Laboratory, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 0JG, U.K.

(Accepted 30 May 1985)

SUMMARY

The in vitro transcription pattern of *BglII*-digested φ3T DNA is described. Eight *BglII* fragments that hybridized to in vitro transcription products were unequivocally identified. A further hybridizing region corresponding to a *BglII* triplet was also revealed, giving a total of nine to 11 *BglII* fragments. These represent 47 to 53% of the φ3T genome. Transcription was shown to initiate within *BglII* fragments B, G, C, H, I, F and J, implying that all of these contain at least one promoter. The relevance of these data to the construction of a cloning vector based on φ3T is discussed.

φ3T is a large temperate *Bacillus subtilis* phage and together with the related phages ρ11 and SPβ forms a specific group in the subdivision of *B. subtilis* lysogenic phages (Rutberg, 1982). This phage is of primary interest since it encodes a thymidylate synthetase, the product of the thyP3 gene, and is capable of the lysogenic conversion of Thy- auxotrophic mutants (Tucker, 1969). The *thyP3* gene has been isolated on a variety of restriction fragments (Ehrlich *et al.*, 1976; Duncan *et al.*, 1977; Barstow *et al.*, 1983; Kenny *et al.*, 1985) and has recently been sequenced and mapped (Kenny *et al.*, 1985). φ3T also encodes a methyltransferase which renders its DNA resistant to host restriction by *BsuR* (Noyer-Weidner *et al.*, 1983). A detailed physical map of the φ3T genome has been constructed but there are no published studies on the molecular genetics of this phage.

In order to initiate such studies we describe here the pattern of transcription in vitro using *Escherichia coli* RNA polymerase (RPase). *E. coli* RPase was suitable for this study since it recognizes promoter sequences essentially identical to those recognized by the principal form of RPase in vegetative *B. subtilis* cells, Eσ55 (Moran *et al.*, 1982; Johnson *et al.*, 1983).

Following electrophoresis and Southern blotting (Southern, 1975), *BglII*-digested φ3T DNA was hybridized with RNA prepared by transcription of the φ3T genome. The resultant autoradiograph is shown in Fig. 1. *BglII* fragments B and C are of similar size and partially co-migrate in agarose. Both fragments were shown to hybridize to the transcription products by repeating the hybridization experiment with *BamHI*-cleaved DNA in the blot. *BamHI* fragments D and E are contained within *BglII* fragments B and C respectively (Fig. 2). In this latter experiment both *BamHI* fragments D and E were shown to hybridize to transcripts (data not shown) implying that *BglII* fragments B and C do likewise. *BglII* fragments L, M and N are similarly co-migratory. Accordingly, it was not possible to assign the hybridization signal at this position to a specific *BglII* fragment. However, from the *BamHI* hybridization experiment described above we showed that *BamHI* fragment B is transcribed, implying that *BglII* M, N or both are transcribed also. We were unable to determine whether or not *BglII* fragment L was transcribed.

The hybridization results derived from Fig. 1 are aligned with the physical map of φ3T (Cregg & Ito, 1979) in Fig. 2. Although *BglII* fragment W was also shown to hybridize to the in vitro transcription products this fragment was not mapped by restriction analysis by Cregg & Ito.

† Present address: Delta Biotechnology Ltd, 137 High Street, Burton-on-Trent DE14 1JZ, U.K.
Fig. 1. Hybridization of Bg/II-digested φ3T DNA with in vitro transcripts. (a) and (b) are 0.8% and 1.5% agarose gels, respectively. L and R contain end-labelled and unlabelled Bg/II-digested φ3T DNA, respectively, and the positions of these letters define the origins of electrophoresis. Labelled RNA was prepared in vitro using E. coli R Pase (Boehringer) with φ3T DNA as template incorporating [α-32P]CTP (Amersham) essentially by the method of Davison et al. (1980). After polymerization, the reaction mix was treated with RNase-free DNase (Worthington) and precipitated with ethanol. Hybridization conditions were as described by Wahl et al. (1979), using dextran sulphate. The Bg/II fragments hybridizing to RNA are indicated in the R lanes of each gel. Fragments S and T in gel (b) are seen to produce faint hybridization signals which we consider to be background since such bands are not evident in gel (a). We also consider the faint signals from other bands to be background due to non-specific initiation of transcription.

(1979). Eight fragments were unequivocally shown to be transcribed (B, G, C, J, H, I, F, W) plus additionally either M, N or both and possibly L (in all, nine to 11 Bg/II fragments from a total of 26), representing 47 to 53% of the φ3T genome. Approximately 50% of the genome is, therefore, apparently not transcribed in vitro. It is possible that these regions are transcribed in vivo and that such transcription requires additional regulatory factors, perhaps phage-encoded, not present in vitro. Analogous experiments using mRNA labelled in vivo would reveal whether or not these regions remain silent throughout phage development.
We next addressed the question of the origin of transcription of the contiguous Bg/II fragments. It was possible, for example, that transcription of Bg/II fragments B, G and C is initiated from a single promoter. Accordingly, we hybridized RNA derived from the transcription of a Bg/II digest of φ3T DNA to a Bg/II digest, as in Fig. 1. In this latter case, however, only those Bg/II fragments containing internal promoters should be revealed. An autoradiograph identical to that shown in Fig. 1 was obtained in this latter experiment but fragments L/M/N and W did not hybridize. This implies that Bg/II cuts at or near the promoters for these transcripts and/or cuts the transcribed region itself. It is evident, however, that Bg/II fragments B, G, C, J, H, I and F contain functional promoters.

The data presented here indicate a grouping of transcription within the genome; however, the extent of transcription within these regions is unknown. It was hoped that the intensity of the hybridization signal would indicate the relative strength of the promoter or extent of transcription within the fragment. Only Bg/II fragment F was observed to produce a signal significantly lower than that of the other bands. This weaker signal could be due to either an inefficient promoter or production of a short transcript.

Bg/II fragments J and H, both of which are transcribed in vitro, have been shown to encode the methyltransferase and thymidylate synthetase, respectively (Noyer-Weidner et al., 1983; Kenny et al., 1985). These gene products are not essential and it may prove possible to delete Bg/II fragments A, J, D and H (36 kb, Fig. 2) and construct an insertional cloning vector for large DNA fragments. We are also analysing promoters isolated from within the transcribed regions and hope that this will facilitate the study of gene regulation in φ3T.

REFERENCES

Short communication


(Received 28 January 1985)