Direction of Transcription in Bacteriophage T5 First-Step Transfer DNA

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The transcription of the T5 first-step transfer genes A2 and A3 has been shown to proceed from right to left (i.e., from A3 to A2) on the T5 DNA molecule.

The injection of bacteriophage T5 DNA into its host occurs in two steps. After adsorption of the phage particle to the bacterial cell wall, 8% of the left end of the genome penetrates the cell (FST, first-step transfer DNA) (9, 10, 16). RNA and protein syntheses then take place for 10 min before the remaining 92% of the chromosome is transferred (SST, second-step transfer DNA). Nine polypeptides are produced from the FST region immediately after infection (13), and three of them have been correlated to the known preearly genes A1, A2 (13), and A3 (3, 4). The A1 gene product is a pleiotropic membrane protein involved in host DNA degradation, shutoff of preearly gene expression, and SST (7). The A2 protein also participates in the transfer of SST DNA (11). The A3 gene product is known to interfere with phase growth in cells carrying plasmid ColIb, although its mechanism of action still has to be elucidated (12, 14).

The purpose of this work was to determine the direction of transcription of the A2 and A3 genes. These two genes (as well as part of gene A1) have previously been cloned in λgtWES::AB on a 1.8-kilobase (kb) EcoRI fragment (4, 6) which lies between the mutational induced ris1 and ris3 EcoRI sites (2). In the corresponding recombinant phage, λgtWES::T5-6, A2 and A3 are only expressed from the AP1 promoter, showing that the fragment itself does not contain any elements capable of initiating RNA synthesis (3). Thus, the direction of transcription of A2 and A3 would be known if the orientations of the 1.8-kb fragment in both the λ-T5 hybrid and phage T5 were determined.

Orienting the 1.8-kb fragment in λgtWES::T5-6 is complicated by the presence of the Aβ EcoRI fragment which serves as "stuffer" DNA to bring the recombinant genome to a size that can be packaged (6). The possible physical structures of λgtWES::T5-6 are shown in Fig. 1. There are four combinations of orientations of the Aβ fragment (Fig. 1A), each of which can be further subdivided according to the two possible orientations of the 1.8-kb T5 fragment. Locating Aβ relative to the insert can easily be achieved by performing a Sall-SacI double digestion on the recombinant

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![Diagram](http://jvi.asm.org/)

**FIG. 1.** Strategy employed to determine the direction of transcription in the FST region of T5 DNA. (A) Aβ fragment (hatched box) and the 1.8-kb T5 fragment (black box) in the four possible configurations they might have taken relative to the AP1 promoter during cloning into λgtWES::AB. (B) Possible orientations of the T5 insert. (C) Direction of transcription in the FST region as determined in this work. The restrictions sites are those used to differentiate between the following configurations: Ec, EcoRI; Ss, SacI; Sa, SalI, and Bs, BstElI. The distances are calculated, taking into account the known positions of the restriction sites on the λ DNA molecule (15) and the size of the T5 fragment (1.8 kb).
DNA, since the cleavage coordinates of these enzymes on λgrWES::AB DNA are known (15) and neither enzyme cuts the T5 insert (5). The result of such a double digestion (Fig. 2, lane c) is compatible only with the orientation of Fig. 1A, part a, in which the 28,900-base-pair (bp) Sall left arm of λgrWES::T5-6 is cut by Sall into three fragments of 24,750, 3,050, and 1,100 bp.

It was then necessary to find a unique, asymmetrically located restriction site on the T5 insert to distinguish between the two configurations of the 1.8-kb fragment (Fig. 1B). The FST region of T5" contains no EcoRI restriction sites and only one BstEII site, the latter being situated within the cloned fragment (D. Lafontaine, unpublished results). To locate it more precisely, use was made of the ris mutants (2). T5" DNA and the DNA from T5 ris1 and T5 ris3 were cleaved by BstEII and EcoRI (Fig. 3, lanes b, c, d, and e). The two 4.4- and 5.7-kb BstEII fragments (marked by arrows), which encompass the entire FST region, were not cleaved by EcoRI in T5" DNA (Fig. 3, lanes b and c).

However, the 4.4- and 5.7-kb fragments were cleaved by EcoRI in ris1 and ris3 DNA, respectively (Fig. 3, lanes d and e). Therefore, the BstEII site was to be found between the ris1 and ris3 mutations, 190 bp from ris3.

To orient the T5 fragment in λgrWES::T5-6, advantage was then taken of this BstEII site. The SalI-Sall 3,050-bp fragment of λgrWES::T5-6 DNA was cut by BstEII into two subfragments of 1,850 and 1,200 bp (Fig. 2, lane d). Therefore, the correct orientation for the 1.8-kb fragment in λgrWES::T5-6 is as depicted in Fig. 1B, part a. The orientation of the A1, A2, and A3 genes relative to the ris mutations has been determined previously (2). The transcription of this region is, therefore, from right to left and begins at a promoter located to the right of ris3, as shown in Fig. 1C. This result is consistent with previous data (8) that most preearly transcription comes from the H strand of T5 DNA. T5 A2 and A3 gene transcription proceeds in the same direction as that of gene PI of BF23 (1), which corresponds to the A1 gene of T5. Thus, it is possible that A1, A2, and A3 are transcribed from a common promoter.
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