Arrangement on the Chromosome of the Known Pre-Early Genes of Bacteriophages T5 and BF23

L. DAVID BECKMAN, GERRY C. ANDERSON, AND D. J. MCCORQUODALE

The University of Texas at Dallas, Dallas, Texas 75230

Received for publication 27 June 1973

Genetic crosses between mutants defective in the known pre-early genes of T5 and BF23 and the detection of putative N-terminal fragments have allowed the determination of the order of genes along the initially transferred 8% section of DNA of these phages.

Bacteriophages T5 and BF23 are closely related coliphages. Both have virtually identical gross morphology, both require calcium ions for growth, and both are arrested in their development in cells carrying colicinogenic factor Ib (Col Ib+ cells) (17, 19). They readily undergo phenotypic mixing, substitution of gene products, and genetic recombination, and their genetic maps are colinear (7, 16; G. C. Anderson and D. J. McCorquodale, manuscript in preparation). The DNA of phage T5+ is a double-stranded molecule with five constant interruptions (nicks) in the phosphodiester backbone that are located at specific, genetically determined points along one of its strands. These nicks delineate 4, 4, 10, 12, 35, and 35% sections of DNA in that order from one end of the molecule (1, 3, 4). The T5 DNA molecule is also terminally repetitious to the extent of 9% of its length (18). H. Bujard (personal communication) has observed that the thermal melting profiles of T5 and BF23 DNA are virtually identical, an indication that the base sequence of BF23 DNA is similar, but not necessarily identical, to that of T5. Phages T5 and BF23 both inject their DNA into host cells in a two-step process (9, 12, 14, D. J. McCorquodale and M. R. Stallcup, unpublished data). An initial 8% section is transferred first, and when proteins coded by genes in this section are synthesized, the remaining 92% of the phage DNA is transferred (10, 11). The initial 8% section of DNA of each phage contains the genetic information for its pre-early proteins (13, 15, 16, K. Mizobuchi and D. J. McCorquodale, manuscript in preparation).

Lanni (11) as well as Hendrickson and McCorquodale (7) have identified two genes (A1 and A2) in the initial 8% section that are required for transfer of T5 DNA beyond this initial section, and we have mapped a mutation (h20) in a gene (gene A3) responsible for the arrest of growth of T5+ in Col Ib+ cells (Fig. 1). Mizobuchi et al. (16) have identified a gene responsible for the arrest of growth of BF23+ in Col Ib+ cells (gene P3) as well as a gene required for the transfer of the BF23 DNA beyond the initial 8% section (gene P1, functionally identical to T5 gene A1). BF23 genes P1 and P3 are both in the initial 8% section of DNA (16). A list of the genes and the particular mutants of each phage with which we are concerned in this note is given in Table 1. We present here data that define the relative location of these pre-early genes on their respective chromosomes.

To determine the genetic order of these pre-early genes, homologous and heterologous two-factor crosses were performed. The resulting genetic maps of the initial 8% section of both phages is shown in Fig. 1. The order of the known pre-early genes is A1-A2-A3 for T5, and P1-P3 for BF23. We assume that these pre-early genes are linearly arranged along the DNA of both phages, and that their linear genetic maps (Fig. 1; see also reference 11) are not generated as a result of their location within the redundant region of their DNA (18). The orientation of these pre-early genes along the physical genome of each phage can be unequivocally deduced if we know (i) the polarity of the two strands in the phage DNA, (ii) the direction of transcription of at least one of the pre-early genes, and (iii) the sense strand for this pre-early gene.

The most recent physical model for T5 DNA (4) is shown in Fig. 2 where the polarity of the strands is taken from reference 18.

The direction of transcription of gene P1 of
Fig. 1. Genetic maps of the BF23 and T5 pre-early genes. The recombination values above the BF23 and T5 genetic maps were determined by two-factor homologous genetic crosses. The recombination values below the T5 genetic map were determined by two-factor heterologous genetic crosses. Recombination values for crosses between two amber mutants are expressed as $[(2 \times \text{titer on su}^+ \text{cells})/(\text{titer on su}^- \text{cells})] \times 100$, whereas recombination values for crosses between h mutants and amber mutants are expressed as $[(\text{titer on Col I}b^-\cdot \text{su}^-) + (\text{titer on Col I}b^-\cdot \text{su}^-) - 2(\text{titer on Col I}b^-\cdot \text{su}^-)]/(\text{titer on Col I}b^-\cdot \text{su}^-) \times 100$.

<table>
<thead>
<tr>
<th>Gene designation and corresponding phage</th>
<th>Mutant representatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 of phage T5</td>
<td>amH27</td>
</tr>
<tr>
<td>P1 of phage BF23</td>
<td>amA29, amM57</td>
</tr>
<tr>
<td>A2 of phage T5</td>
<td>amH231</td>
</tr>
<tr>
<td>A3 of phage T5</td>
<td>h20</td>
</tr>
<tr>
<td>P3 of phage BF23</td>
<td>h139</td>
</tr>
</tbody>
</table>

BF23 was deduced as follows. Cells infected with amM57 or amA29 are missing two polypeptides that are present in cells infected with BF23+ (bands 1a-1 and 1a-1') (Fig. 3). Cells infected with amM57 synthesize two polypeptides (bands 1a-1am and 1a-1am') that are not synthesized in cells infected with BF23+ or amA29, and these polypeptides have lower molecular weights than polypeptides 1a-1 or 1a-1'. It has previously been reported (2) that the polypeptide product of T5 gene A1 appears as a single band after extraction from cells of E. coli F, and, therefore, it seems reasonable to hypothesize that polypeptide 1a-1 is the product of gene P1, and that polypeptide 1a-1' is a host-modified form of the product of gene P1. From the data in Fig. 3, we conclude that polypeptide 1a-1am is a N-terminal amber fragment of the product of gene P1, and that polypeptide 1a-1am' is a host-modified form of this N-terminal amber fragment. Since we cannot detect any N-terminal amber fragment of the product of gene P1 in cells infected with amA29, we assume that it is too small to be detected in our gels. We conclude, therefore, that the direction

Fig. 2. Arrangement of BF23 and T5 pre-early genes on their respective physical chromosomes. The physical structure of T5+ DNA molecule is taken from reference 4. The physical structure of the BF23 DNA molecule is considered analogous to the T5 DNA molecule. The top line represents the uninterrupted strand of the double-stranded DNA of each phage, and the interrupted bottom line represents the complementary strand in which the relative locations of the single-strand nicks are indicated by the interruptions in the line. The placement of the 8% FST and 9% redundant sections is from reference 18.
of transcription of BF23 gene P1 is from its promoter toward site A29 followed by site M57. This direction is away from gene P3.

We now need only to determine the sense strand for gene P1 of BF23 in order to arrange the two pre-early genes of BF23 on its DNA, and, on the basis of the colinearity of the genetic maps of BF23 and T5 (Fig. 1), to arrange the three pre-early genes of T5 on its DNA. Hendrickson and Bujard (6) interpret their data to mean that genes A1 and A2 of T5 are transcribed from the intact strand of T5. We argue by analogy that if the intact strand of T5 is the sense strand for gene A1 of T5, then the intact strand of BF23 is the sense strand for gene P1 of BF23. If this is so, the arrangement of the pre-early genes of BF23 and T5 must be as shown in Fig. 2. This arrangement is the reverse of that given by Hendrickson and Bujard (6). Their order would be correct only if the interrupted strand were the sense strand for gene A1 of T5 (and, by analogy, for gene P1 of BF23).

We thank Shirley Nichols for technical assistance and Ruthann Ziegler (a Clark Foundation high school graduate research trainee), G. Chinnadurai, and S. Mahlum for help in performing the gel electrophoresis.

This work was supported by Public Health Service Research Grant GM-16212 to D.J.M. and by a Program in Molecular Biology grant GM-13234, both from the National Institute of General Medical Sciences, and by postdoctoral fellowship CA-08689-02 to L.D.B. from the National Cancer Institute.

LITERATURE CITED


