DEFECTIVE BACTERIOPHAGE LAMBDA CHROMOSOME, POTENTIAL VECTOR FOR DNA FRAGMENTS OBTAINED AFTER CLEAVAGE BY BACILLUS *AMYLOLIQUEFACIENS* ENDONUCLEASE (BAMI)

Michel PERRICAUDET and Pierre TIOLLAIS

Unité de Physico-chimie des Macromolécules, Département de Biologie Moléculaire, Institut Pasteur, 25, rue du Docteur Roux, 75015 Paris, France

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1. Introduction

There are two essential steps involved in the multiplication of a foreign DNA fragment in bacteria. First, the covalent insertion of this fragment into a vector molecule, capable of self replication in *E. coli*, is accomplished through recombination in vitro. Second, the hybrid DNA molecule is introduced into the bacteria by transformation or transfection. The key enzymatic step in the in vitro recombination procedure is provided by a restriction endonuclease that creates cohesive ends that can be covalently rejoined with DNA ligase. At the present time, two types of vectors are used: plasmids such as psc 101 or colEl [1,2] and derivatives of bacteriophage lambda [3–5]. These phages have lost by mutation the Eco RI cleavage sites located in the essential region of the genome. A main advantage of the phage system, compared to the plasmid system, is that its propagation can be well controlled and therefore accidental dissemination can be more easily prevented. This is important because some genetic engeneering experiments could be potentially dangerous, and should therefore be very carefully controlled. Though the derivatives of phage lambda presently used as vectors are safe enough for most of the insertion experiments, it could be important to construct vectors which present higher security criteria.

In this work, we present the cleavage sites localization of *Bacillus amiloliquefaciens* restriction endonuclease (BamI) [6], in the bacteriophage lambda genome and we propose a derivative of phage lambda which could after having incorporated the foreign DNA fragment, lose the genes coding for the tail proteins and therefore become defective. The multiplication of such a phage would depend on the presence of a helper phage and could therefore be easily controlled.

2. Materials and methods

The localization of the targets for BamI in the phage lambda genome was determined by the following method. DNA fragments obtained after double hydrolysis with EcoRI and BamI of DNA from different derivatives of phage lambda having lost some EcoRI cleavage sites by mutation [4] were analysed by polyacrylamide gel electrophoresis. The bacteriophage strains used in this work are listed in fig.1. The mol. wt of the DNA fragments obtained after double hydrolysis were determined according to the reference curve constructed with EcoRI hydrolysates of lambda phage and adenovirus 2 DNA (fig.2).

3. Results and discussion

The j fragment is present in λ, λplac-*l*-2 and λplac-*l*-2-3 hydrolysed by EcoRI and BamI, and absent in λ hydrolysed by EcoRI or BamI (fig.3). The only EcoRI cleavage site present in the three phages is sRIλ2. The j fragment is then located between sRIλ2 and a BamI cleavage site. Moreover, the EcoRI - C fragment is absent in the double hydrolysates. There-

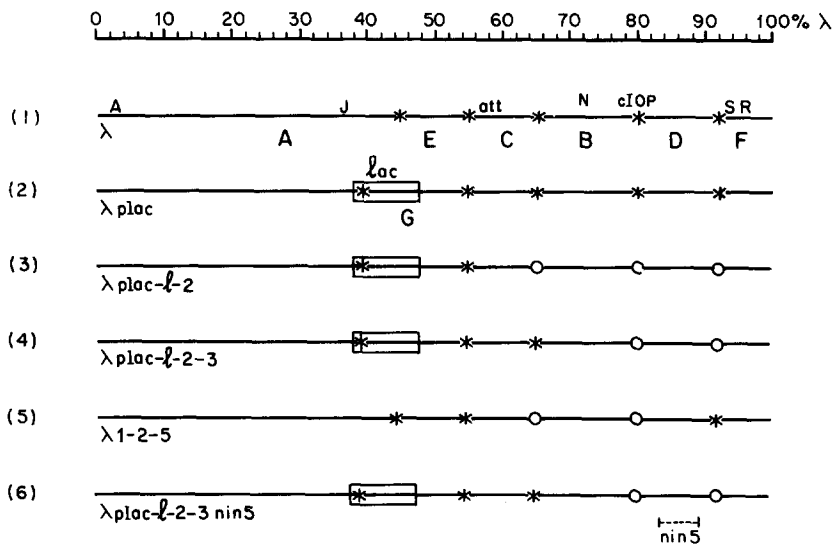
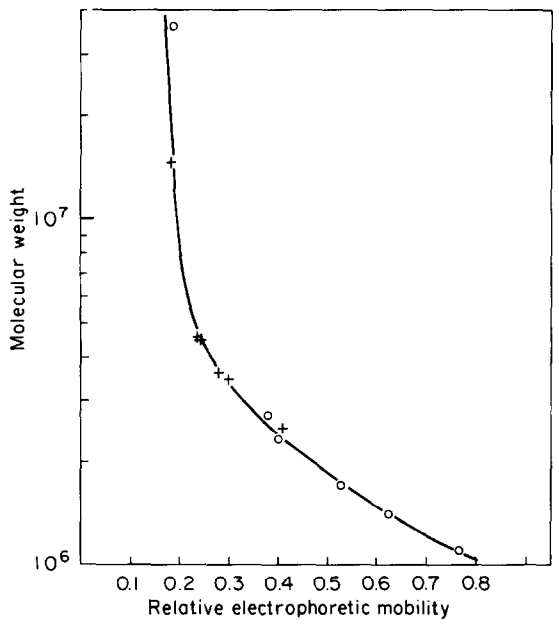


Fig.1. λ derivatives used to locate the BamI endonuclease cleavage sites. The bacteriophage strains are (1) λCI857S7 referred to as λ. (2) λplac5CI857S7 referred to as λplac. (3) λplac5CI857sRIλ$_3^\circ$ sRIλ$_4^\circ$ sRIλ$_5^\circ$, referred to as λplac-*l*-2 [4]. sRIλ refers to the EcoRI cleavage sites in λ, numbered 1 to 5 from the left to the right of the genome. The symbol sRIλ° indicates the loss, by mutation, of an EcoRI site. The letter in λplac-*l*-2 corresponds to the EcoRI cleavage site in the lac operon. The number 2 corresponds to the presence of the 2nd EcoRI cleavage site. (4) λplac5CI857 sRIλ$_4^\circ$ sRIλ$_5^\circ$ referred to as λplac-*l*-2-3. (5) λCI857 sRIλ$_3^\circ$ sRIλ$_4^\circ$, referred to as λ-1-2-5. This phage is a double recombinant of λplac-*l*-2 and λimm434 obtained after infecting the bacterial strain AR1002 (λ imm434) with λplac-*l*-2. (5) λplac5CI857 sRIλ$_4^\circ$ sRIλ$_5^\circ$ nin5, referred to as λplac-*l*-2-3 nin5. This phage was obtained by genetic cross between the λplac5 and the λgtλBC [5] which was a generous gift from R. Davis. The letters A to G correspond to the EcoRI fragments. * refers to the EcoRI cleavage sites. (○) Refers to the loss by mutation of the EcoRI cleavage sites.



fore the j fragment is located in the C fragment. The mol. wt of the j fragment (1.3×10^6) permits the location of this BamI cleavage site at 4.2% on the right of sRIλ2. This site referred to as sBamIλ3 is thus located at 58.8% (fig.4).

The g fragment is present in λ, and λ-1-2-5 hydrolysed by EcoRI and BamI, and absent in λplac-*l*-2

Fig.2. Reference curve used to determine the mol. wts of λ derivative fragments obtained after double hydrolysis with EcoRI and BamI endonucleases. This curve was constructed according to the EcoRI λ fragments (+) and the EcoRI adenovirus 2 fragments (0). The adenovirus 2 was a gift from P. Boulanger. The reference molecular weight values of the EcoRI λ fragments are given according to B. Allet et al. [9] and the reference molecular weight values of the Eco RI adenovirus 2 fragments are given according to Petterson et al. [10]. The DNA fragments were analysed by electrophoresis in a polyacrylamide gel slab (gradient of 2.5% to 7.5% acrylamide concentration).

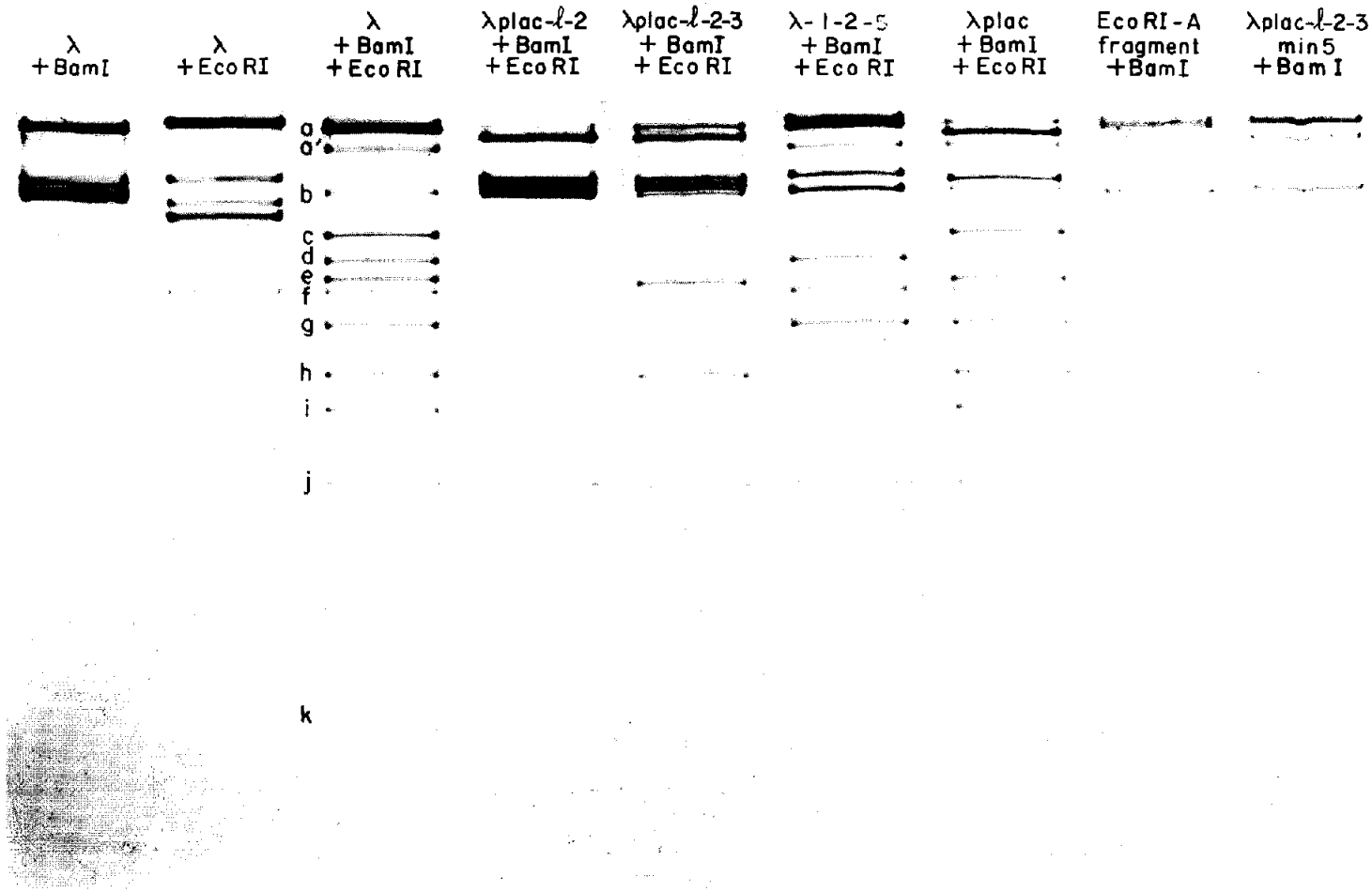


Fig.3. Electrophoretic separation of DNA fragments from λ and various derivatives obtained after hydrolysis by EcoRI, BamI, and EcoRI + BamI. The location of EcoRI cleavage sites on the chromosome of λ and the various derivatives analysed are shown in fig.1. The bacteriophage propagation, DNA extraction and hydrolysis by EcoRI were performed as previously described [11]. The EcoRI - A fragment from λ plac5 (fragment 0 to 40%) was prepared by cesium sulfate + silver nitrate isopicnic centrifugation as previously described [12]. (The EcoRI enzyme was purified from the bacterial strain RY13 according to the method of Yoshimuri [13]). The BamI enzyme was a generous gift from R. Roberts. DNA hydrolysis by BamI and the double hydrolysis by EcoRI + BamI were performed for 12 hrs at 37°C in a 6.6 mM Tris–HCl, pH 7.5, 6.6 mM $MgCl_2$, 6.6 mM mercapthoethanol, 50 mM NaCl buffer. The analytical polyacrylamide slab gel electrophoresis (2.5% to 7.5% concentration gradient) was performed as previously described [4].

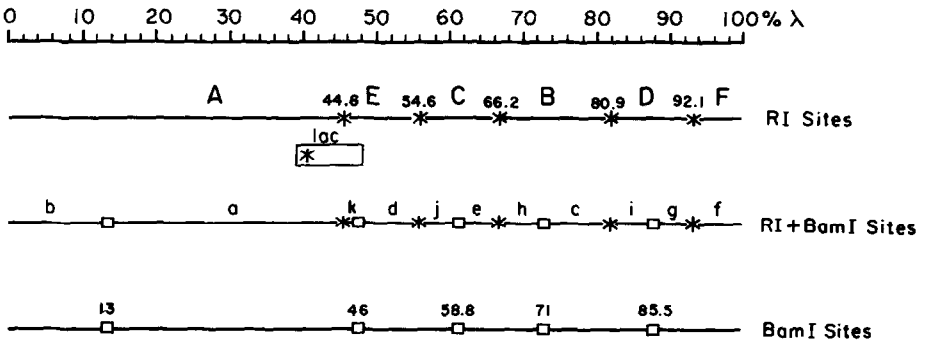


Fig.4. Location of the EcoRI and BamI cleavage sites on the chromosome of λ. The capital letters A to F correspond to the EcoRI fragments of λ. Small letters a to k correspond to the EcoRI + BamI fragments of λ. (*) EcoRI cleavage sites. (□) BamI cleavage sites. The location of the EcoRI cleavage sites are given according to B. Allet et al. [9].

and λplac-*l*-2-3 hydrolysed by EcoRI and BamI (fig.3). The only EcoRI cleavage site present in λ-1-2-5 and absent in plac-*l*-2, and plac-*l*-2-3 is srIλ5. The g fragment is then located between sRIλ5 and a BamI cleavage site. Moreover the EcoRI-D fragment is absent in the double hydrolysis, whereas the EcoRI-F fragment is present. Therefore the g fragment is located in the EcoRI-D fragment. The mol. wt of the g fragment (2×10^6) permits the location of this BamI cleavage site at 6.45% on the left of sRIλ5. This site referred to as sBamIλ5 is thus located at 85.5% (fig.4).

The c and i fragments are present in λplac hydrolysed by EcoRI and BamI and absent in λplac-*l*-2, λplac-*l*-2-3 and λ-1-2-5 hydrolysed by EcoRI and BamI (fig.3). The only EcoRI cleavage site present in λplac and absent in λplac-*l*-2, λplac-*l*-2-3 and λ-1-2-5 is sRIλ4. The c and i fragments are therefore located between srIλ4 and a BamI cleavage site. The comparison of the mol. wt of the c fragment (3.15×10^6) and the EcoRI D fragment (3.2×10^6) shows that the c fragment cannot be located in the D fragment. Therefore the c fragment is located in the EcoRI-B fragment, and the i fragment (mol wt = 1.6×10^6) is located in the D fragment. The molecular weight of the c fragment (3.15×10^6) permits the location of BamI cleavage site at 10% on the left of sRIλ4. This site referred to as BamIλ4 is thus located at 71% (fig.4).

The d and k fragments are present in λ hydrolysed by EcoRI and BamI and absent in λplac hydrolysed by EcoRI and BamI (fig.3). Therefore the *lac* insertion suppresses a BamI cleavage site and this site is located in the EcoRI-E fragment. The lac insertion being located between 39.6% and 48.1% [7], and considering the molecular weights of the d (2.7×10^6) and the k (0.4×10^6) fragments, the d fragment must be located between the BamI cleavage site (of the E fragment) and sRIλ2 and the k fragment must be located between the BamI cleavage site (of the E fragment) and sRIλ1. The molecular weight of the d fragment (2.7×10^6) permits the location of the BamI cleavage site at 8.7% on the left of sRI2. This site referred to as sBamIλ2 is thus located at 46% (fig.4).

The b fragment is present in λ,λplac hydrolysed by EcoRI and BamI and the EcoRI-A fragment purified by cesium sulfate + silver nitrate isopicnic centrifugation [10] and hydrolysed by BamI (fig.3). The b fragment is then located in the A fragment. The sRIλ1 being absent in λplac, the b fragment is then located between the left end of λ and a BamI cleavage site. The mol. wt of the b fragment (4×10^6) permits the location of the BamI cleavage site at 13% in the right of the left end of lambda. This site is referred to as sBamIλ1. (fig.4).

Therefore, there are 5 BamI cleavage sites in the phage lambda genome. Three sites, BamIλ2, BamIλ3 and BamIλ4 are located in the non essential region of the genome (fig.4). One site, BamIλ1 is located in a head protein gene, which is probably the E gene. One site BamIλ5 is located between the replication and the lysis gene (fig.4). In order to use the phage lambda as a vector for BamI DNA fragments the cleavage site BamIλ5 must be deleted. This can be done by

Fig.5. Possible model for a hybrid molecule constructed with the lambda vector BamI and a foreign DNA fragment. (——) lambda DNA, (▬) foreign DNA fragment.

introducing the nin5 deletion into the lambda genome. Effectively the two fragments which are located between sBamIλ4 and sBamIλ5, and between sBamIλ5 and the right end of lambda are absent in λplac-*l*-2-3 nin5 (fig.3). Consequently the λplac-*l*-2-3 nin5 DNA fragment located between sBamIλ4 and the right end of lambda contains all the early genes essential in vegetative growth (N, O and P), the control gene of late functions (Q) and the lysis genes (S and R). BamI restriction enzyme recognizes 6 base pairs and creates cohesive ends (R. Roberts personal communication). Consequently, a hybrid molecule could be constructed in vitro, by introducing a foreign DNA fragment between sBamIλ1 and sBamIλ4 (fig.5). The structure of such a phage would be comparable to a phage λgalbio [8]. This hybrid molecule could be replicated but not be encapsulated in the absence of a helper phage. The propagation of this defective phage could be controlled and its accidental dissemination easily prevented. Moreover such a vector molecule would be able to insert BamI DNA fragments of mol. wts up to 18×10^6. This could not be possible with the lambda vectors constructed for EcoRI DNA fragments.

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