The EMBO Journal Vol.2 No.1 pp.77 – 80, 1983

Genetic study of a membrane protein: DNA sequence alterations due to 17 *lamB* point mutations affecting adsorption of phage lambda

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Communicated by M.Hofnung
Received in 19 November 1982

Gene *lamB* encodes the outer membrane receptor for phage lambda in *Escherichia coli* K12. We have determined the DNA sequence alterations of 17 *lamB* point mutations which result in resistance to phage lambda h$^+$. The mutations correspond to four phenotypic classes according to the pattern of growth of three phages which use the lambda receptor: lambda h (a one-step host-range derivative of lambda h$^+$), lambda hh* (a two-step host-range derivative of lambda h$^+$) and K10 (another lambdoid phage). Fourteen mutations are of the missense type and correspond to Gly to Asp changes distributed as follows. One class I mutation is at position 382 of the mature lambda receptor. Seven class I* mutations, four of which at least are independent, are at position 401. Six independent class II mutations are at position 151. The three other (class III) mutations are of the nonsense type. They change codons TGG (Trp) into TAG (amber) at positions 120 (two mutations) and 351 (one mutation). Implications of these results for the topological organization of the lambda receptor as well as possible reasons for the limited number of altered sites detected are discussed.

Key words: membrane protein/*lamB*/phage lambda adsorption mutations

Introduction

The LamB protein (the lambda receptor) in *Escherichia coli* K12 is a multifunctional outer membrane protein involved in the permeation of maltose and maltodextrins (Szmelcman and Hofnung, 1975; Ferenci and Boos, 1980). It is used as a cell surface receptor by several bacteriophages including lambda, K10 and TPI (Randall-Hazelbauer and Schwartz, 1973; Hofnung *et al.*, 1976; Roa, 1979; Wandersman and Schwartz, 1978). We have recently determined the complete DNA sequence of *lamB*, its structural gene (Clément and Hofnung, 1981). One approach to studying the relationships between the sequence of the protein, its structure and its functions, consists in isolating mutations affecting one or several functions and determining the corresponding DNA sequence alterations. This has already been carried out to some extent in two instances: mutations altering the export process and localized in the signal sequence (Emr and Silhavy, 1980; Emr *et al.*, 1980) and mutations impairing adsorption of phage K10 (Roa and Clément, 1980). We present here the DNA sequence alterations due to 17 *lamB* point mutations affecting the interactions of the LamB protein with phage lambda. The corresponding amino acid changes are discussed in terms of the topological organization of the LamB protein.

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Results

Sequencing strategy

The mutations sequenced are located in two regions of the genetic map namely interval V and interval X – XI (Figure 1). The exact correspondence between the genetic and the physical maps is not known except that a *Sal*I cut defines the border between intervals VI and VII (Raibaud *et al.*, 1979) and the end of *lamB* is known from the gene sequence (Clément and Hofnung, 1981).

Mutations localized in interval V were sequenced using a *Sal*I-*Sal*I DNA fragment of derivatives of phage lambda sigma 3h434, cut with *Hae*II, labelled and recut with *Hha*I. In the case of mutation *lamB* 206 am another strategy was used: the *Sal*I-*Sal*I fragment was cut with *Ava*II, labelled and recut with *Hin*fI and *Sma*I (details of the restriction map have been published by Clément and Hofnung (1981). In interval X – XI, *lamB* 511 am has been sequenced by cutting the *Sal*I-*Sac*I fragment with *Eco*RII, labelling, recutting with *Hin*fI and separating strands of the *Eco*RI-*Sac*I fragment. The distal missense mutations were sequenced by cutting the *Sal*I-*Sac*I fragment with *Hin*fI, labelling and recutting with

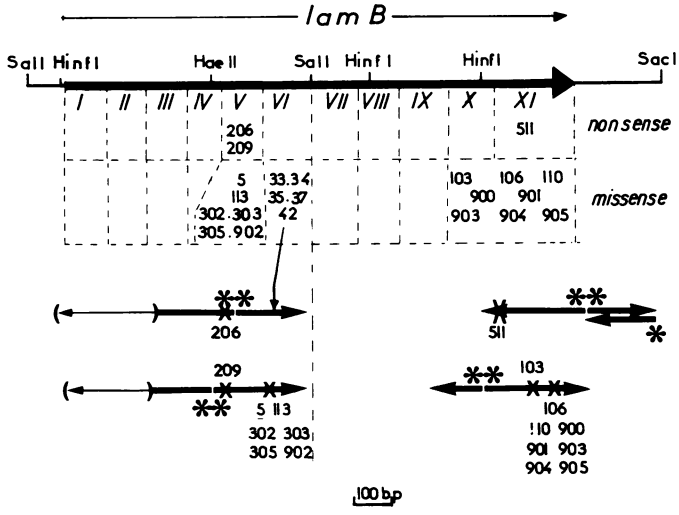

Fig. 1. Genetic map of *lamB* and sequencing strategy for *lamB* mutations. Gene *lamB* is divided in XI genetic intervals (Raibaud *et al.*, 1979) arbitrarily drawn of equal size. The genetic localization of nonsense and missense mutations are shown. The mutations sequenced in the present work yield resistance to phage lambda and map in intervals V and X – XI. The sequence changes for five mutations yielding resistance to phage K10 and mapping in interval VI have been published elsewhere (Roa and Clément, 1980, and Figure 2). At the bottom of the figure the labelled DNA fragments used are indicated. The labelled phosphate in 5′ is symbolized by an asterisk. The thick line on each arrow corresponds to the extent to which the sequence of each fragment was determined. See details in the text. The sequencing results presented show that mutations *lamB* 103, 106, 110, 900, 901, 903, 904, 905 which map in interval X or XI are distal to *lamB* 511 and are thus in interval XI. Mutations *lamB* 5, 113, 302, 304, 305 and 902 which affect residue 151 and mutations N33, 35, 37, 42 which affect residue 154 have been mapped in two different genetic intervals but by two different procedures which have not been directly compared. It is thus not possible to decide whether the distal end of the deletion which separates interval V and VI is between residues 151 and 154 or after residue 154.

*Eco*RII. For each mutation, a fragment of at least 400 nucleotides was analyzed (i.e., most or all of the corresponding genetic interval) without detecting any change other than the one reported. The extent of the region sequenced in each case is indicated on Figure 1.

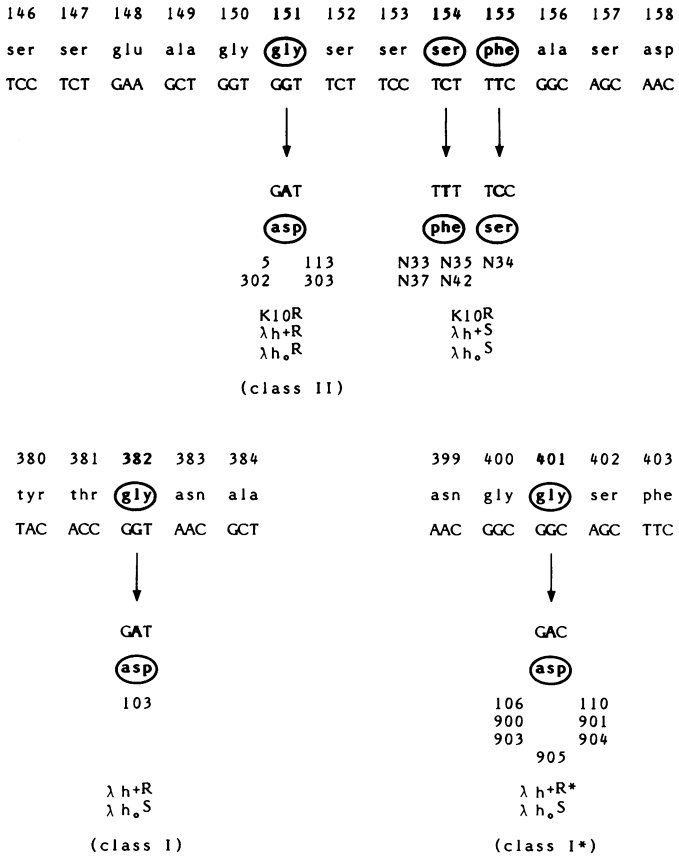

Fig. 2. DNA sequence of wild-type and missense mutations in *lamB*. The DNA sequence of coding strand with the corresponding amino acid sequence of three portions of the *lamB* gene are shown: amino acids 146–158 (upper part) 380–384 (bottom left) 399–403 (bottom right). The numbering of amino acids starts from the NH_2-terminal residue of the mature protein (Clément and Hofnung, 1982). The amino acid change related to the various mutations is indicated as well as its consequence on the growth of phages.

Sequence alterations

The 17 mutations are due to GC–AT transitions and fall at only five different sites (Figure 2).

Mutations in interval V. (1) Two nonsense mutations (*lamB* 206 am and *lamB* 209 am) identically affect codon 120 of the mature lambda receptor (Clément and Hofnung, 1981) changing Trp to Amber. They may not be independent mutations. (2) Six missense mutations (*lamB* 5, *lamB* 113, *lamB* 302, *lamB* 303, *lamB* 305, *lamB* 902) obtained independently affect codon 151 changing Gly to Asp.

Mutations in interval XI. (1) *lamb* 511 am is a nonsense mutation corresponding to the change Trp to Amber at position 351. (2) *lamB* 103 is a missense mutation affecting codon 382 changing Gly to Asp. (3) Seven missense mutations identically affect codon 401: *lamB* 106, *lamB* 900, *lamB* 901 and *lamB* 904 are independent from each other, while *lamB* 110, *lamB* 903 and *lamB* 905 are possibly related to respectively *lamB* 106, *lamB* 901 and *lamB* 904 (Gly to Asp). Thus the 14 missense mutations analyzed fall in only three sites (residues 151, 382 and 401) and correspond to the same Gly to Asp change.

Discussion

We have sequenced 17 *lamB* point mutations located in two regions of the genetic map: intervals V and X–XI (Figure 1). The fact that they all correspond to a GC–AT transition could be expected since they were obtained after mutagenesis with ethyl methane sulfonate (EMS). Why only three sites have been altered in missense mutants can be explained in at least two ways. These sites may be hot spots for EMS mutagenesis. Another more likely explanation is the following. The selection for lambda resistance which was used is very stringent since a reduction of at least 10^3 in the activity of the lambda receptor *in vivo* is required (Colonna and Hofnung, 1981). Very few sites would thus allow such a reduction of activity by missense mutations.

The properties of the different mutants are presented in Table I. Four phenotypic classes can be distinguished. All the known functions of *lamB* are abolished by nonsense mutants including growth on dextrins (Dex^-). Missense mutants fall into three classes according to their behaviour against various phages. They all still allow growth on dextrins (Dex^+).

Table I. Properties of the *lamB* mutations sequenced

lamB mutation number	E.o.p. of phages[a]: λh⁺	λh	λhh*	K10	Growth on dextrins	Migration on gels[b]	Phenotypic class	Codon and amino acid change
206–209	0	0	0	0	–		III	TGG (Trp) 120 → TAG am
511								TGG (Trp) 351 → TAG am
113–902 302–303 305–5	0	0	1	0	+	slow	II	GGT (Gly) 151 → GAT (Asp)
106–110 901–903 904–905	0	0.1 to 0.001	1	1	+	slow	I*	GGC (Gly) 401 → GAC (Asp)
103	0	1	1	1	+	normal	I	GGT (Gly) 382 → GAT (Asp)

[a]The value is the ratio between the e.o.p. for the mutant and the e.o.p. for the wild-type. 0 means $<10^{-5}$ (e.o.p. = efficiency of plating).
[b]Migration on gel: all missense mutations sequenced correspond to Gly to Asp changes. Class II and Class I* LamB proteins have slower migrations in the gel system used, while the class I mutation examined shows normal migration (see Figure 3).

Class III nonsense mutants (three mutations): *lamB* 206 am and 209 am are at position 120. *lamB* 511 am, at position 351, is one of the most distal nonsense mutations mapped in *lamB*. It is located quite far (70 amino acids) from the carboxy end of the mature LamB protein which includes 421 residues (Clément and Hofnung, 1981). However, since missense mutations are found at positions 382 and 401, it seems that at least part of the protein located after amino acid 351 is involved in its activity.

Class I mutant (one mutation): in this case the binding of lambda h^+ is affected, which suggests that Gly 382 is involved in the binding of the phage to the lambda receptor.

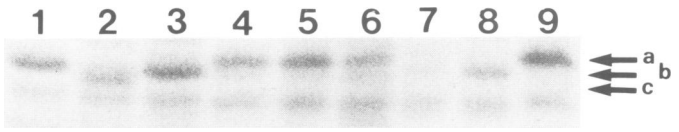

Fig. 3. Relative migrations of LamB proteins affected by class I or class II mutations. Crude extracts (Braun-Breton and M.Hofnung, 1981) were run for 15 h on a 30 cm long SDS 10% polyacrylamide gel. Only the relevant portion of the gel is shown here. The LamB protein is at the upper part. Its migration can be compared to that of an unknown protein present in the lower part of the picture. **a**: slow migration (**lanes 4, 5, 6** and **9**); **b**: normal migration (**lanes 1, 3** and **8**); **c**: fast migration (**lane 2**). **Lanes 1–3**: respectively, class I mutations *lamB* 63, *lamB* 112 and *lamB* 103. **Lanes 4–6** and **lane 9**: respectively, class II mutations *lamB* 5, *lamB* 302, *lamB* 305 and *lamB* 902. **Lanes 7** and **8**: wild-type *lamB* uninduced and induced. Derivatives of strain P4x8 were grown in 63 B_1 minimal medium supplemented with methionine and the adequate carbon source. The *lamB* allele examined was either chromosomal (maltose inducible) or carried by a transducing phage (IPTG inducible).

Class I* mutants (seven mutations) (Braun-Breton and Hofnung *et al.*, 1981; Marchal and Hofnung, 1983): their phenotype is similar to class I mutants except that the efficiency of plating of lambda h is reduced by a factor of $10-10^3$ and that the binding of lambda h^+ is not affected (Braun-Breton and Hofnung, 1981). They are therefore affected in a subsequent step of phage infection which is believed to be the irreversible binding of the phage to the lambda receptor (Braun-Breton and Hofnung, 1981). All the class I* mutations change Gly 401 into Asp.

Class II mutants (six mutations) (Hofnung *et al.*, 1976, 1981; Marchal and Hofnung, 1983): they are all resistant to lambda h^+, lambda h and K10 and correspond to a replacement of Gly 151 by Asp. This residue lies very close to the mutations at sites 154 and 155 which yield resistance to phage K10 but not to lambda (Roa, 1979; Roa and Clément, 1980). The region of the protein comprised between amino acids 151 and 155 is thus probably involved in the binding of phage K10. Within this region, residue 151 is essential for the binding of lambda h^+ and lambda h.

As already noted, all the missense mutations sequenced correspond to a Gly to Asp change at three different sites. However, the class I* and class II LamB proteins have slower migrations than the class I LamB 103 and $LamB^+$ proteins on SDS-polyacrylamide gel (Marchal and Hofnung, 1983 and

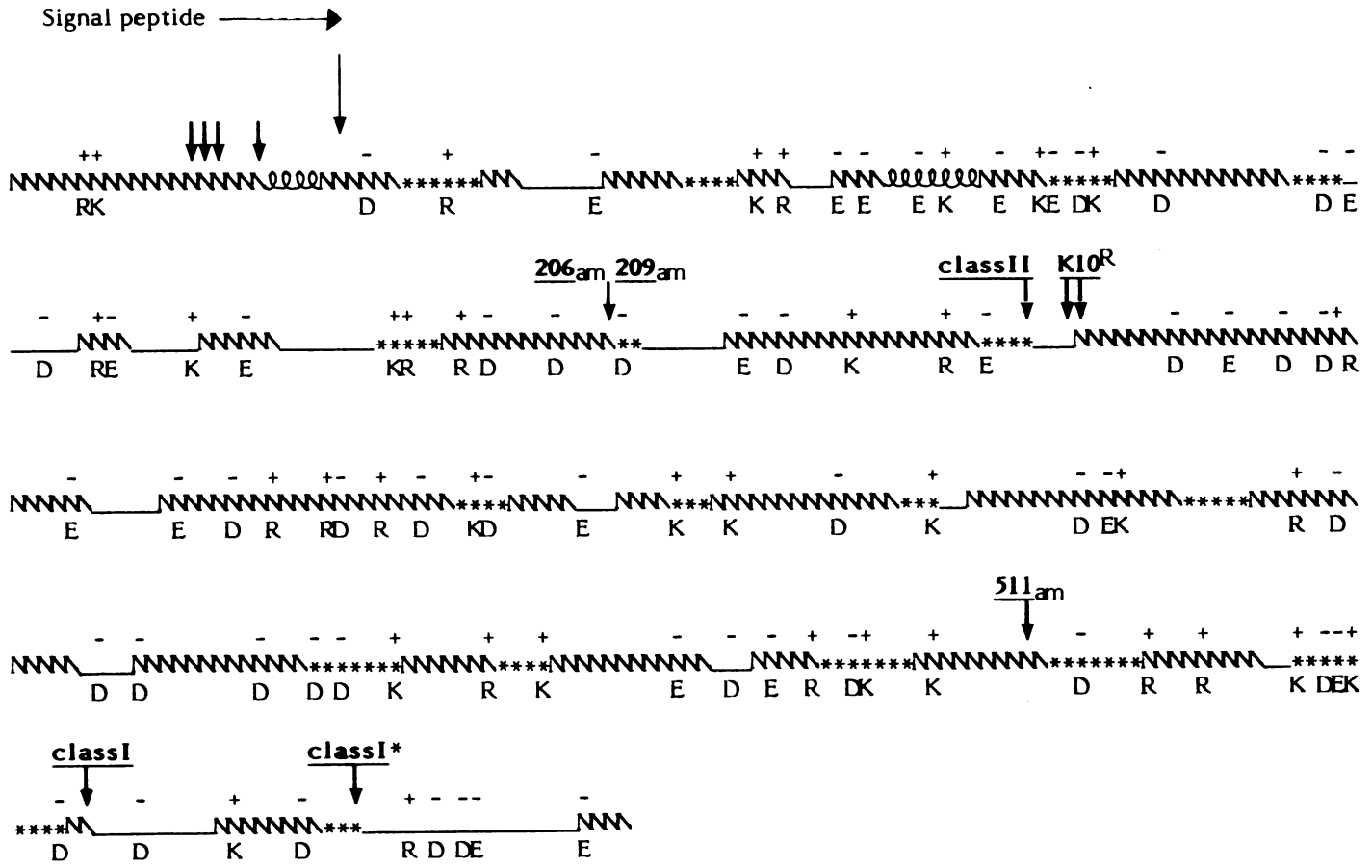

Fig. 4. Secondary structure predictions of LamB protein. The predictions are somewhat different from previous ones (Clément and Hofnung, 1982) since measurements of c.d. indicate that the proportion of α-helix in the LamB protein is <5% (J.M.Neuhaus and J.Rosenbush, personal communication). This value has now been taken into account in the computer analysis procedure developed by Garnier *et al.* (1978). ᔓ: α-helix, NNN: β-sheet, ****: β-turn, ——: random coil. R: arginine (+), K: lysine (+), D: aspartate (−), E: glutamate (−). The localization of the mutations which have been sequenced is indicated, including signal sequence mutations (Emr *et al.*, 1980) and $K10^R$ mutations (Roa and Clément, 1980).

Figure 3). Thus, substitution of a glycine at different locations of the protein by the same negatively charged amino acid (aspartate) may result in different migration properties (Noël *et al.*, 1979).

A similarity exists between the two sites 151 and 401 since in both cases the sequence Gly, Gly, Ser is found, the DNA sequence is however different (Figure 2). This structure is possibly involved in the recognition of the phage tail. However, one should note that in mutants affected at site 151, no binding of the phage occurs, while in mutants affected at site 401 the binding of lambda h^+ still occurs, but without phage inactivation (Braun-Breton and Hofnung, 1981). Thus, these identical sequences may not play the same role in the process of phage infection. One should also bear in mind that repetitions of a tripeptide within *lamB* are rather frequent (13 tripeptides, one tetrapeptide and one pentapeptide are found twice (Clément and Hofnung, 1981) and one tripeptide is found three times). Therefore, the occurrence of a repetition at sites 151 and 401 could be fortuitous. At any rate, we do not know if the important factor is the loss of a Gly residue or the acquisition of an Asp residue.

A secondary structure prediction (Garnier *et al.*, 1978) for the LamB protein is shown on Figure 4. All the missense mutations we describe here are located in regions predicted as beta turns (Clément and Hofnung, 1981). This is compatible with the idea that at least amino acids 151 – 155 and 382 belong to bends of the protein which are exposed to the exterior of the cell and are accessible to the phage tail, while the bulk of the protein is embedded in the membrane, creating a hydrophilic pore for the permeation of maltose and maltodextrins (Nikaido *et al.*, 1980). Using such secondary structure predictions, models can be made where the lambda receptor peptide chain crosses the membrane a number of times and the sites corresponding to regions 151 – 155 and 382 are at the outer surface of the cell (H.Nikaido, personal communication, J.M.Neuhaus, personal communication). Since a mutation at residue 401 does not affect the binding of phage lambda h^+, hypotheses about the location of this site with respect to the cell surface cannot be made so readily.

A more comprehensive understanding of the genetic determination of the various properties of the lambda receptor will be obtained through an extension of the approach presented here, by the use of other mutagens, of other phages using the lambda receptor for adsorption, and of different mutant selection procedures. Such approaches are in progress in our laboratory.

Materials and methods

Phages and bacterial strains

The virulent phages used were lambda b_2vh^+ (denoted lambda h^+) and its one-step lambda vh_0 (denoted lambda h) and two-step lambda vh_0h^*16 (denoted lambda hh*) host-range derivatives.

All the *lamB* mutations analyzed are on derivatives of phage lambda sigma 3h434 which carries the gene *lamB* under *lac* promoter control (Hofnung *et al.*, 1981). The lysogens used carried either a *lamB* deletion (derivatives of pop 725 F^- *thr leu lacy malB delta* 12 (Hofnung *et al.*, 1981) or the same mutation on the chromosomal *lamB* copy (derivatives of pop 1021 HfG_6 *met* A *trp* E9780 am *gal* E *gal* z *rpo* B500 (Braun-Breton and Hofnung, 1981)). These lysogens were made resistant to phage lambda vh434 to increase the yield of phage preparation.

The derivatives of pop 725 were: pop 6450 (*lamB* 117), pop 6451 (*lamB* 901), pop 6452 (*lamB* 902), pop 6453 (*lamB* 903), pop 6454 (*lamB* 904), pop 6455 (*lamB* 905), pop 6456 (*lamB* 209 am).

The derivatives of pop 1021 were: pop 5532 (*lamB* 302), pop 5533 (*lamB* 305), pop 5536 (*lamB* 113), pop 5537 (*lamB* 106), pop 5538 (*lamB* 5), pop 5539 (*lamB* 110), pop 5540 (*lamB* 206 am), pop 5541 (*lamB* 511 am), pop 5542 (*lamB* 103).

Gel electrophoresis of proteins

Gel electrophoresis of proteins in the presence of SDS was performed as described in Laemmli (1970).

Phage production and DNA extraction

Lysogens were grown in 750 ml of 4YT medium at 32°C to an O.D. 600 = 1.5. Rapid temperature shift was achieved by adding 750 ml of preheated (56°C) 4YT medium. 20 min later the temperature was lowered to 37°C and maintained until lysis (usually 1.5 h after induction). The lysate was cleared from cell debris by short centrifugation (15 min at 7000 r.p.m.) and phages were pelleted overnight (15 000 r.p.m.). The pellet was resuspended in Tris 10 mM pH 7.4, NaCl 100 mM, $MgSO_4$ 1 mM and submitted to a CsCl block gradient followed by an equilibrium gradient. DNA was extracted twice with phenol, five times with chloroform, precipitated twice with ethanol and resuspended in H_2O at a concentration of 1 mg/ml.

DNA hydrolysis and fragment isolation

400 μg DNA were hydrolyzed by *Sal*I and/or *Sac*I (Biolabs 20 units) in the appropriate buffer overnight. After complete digestion (checked with quick 1% agarose electrophoresis), the preparation was submitted to a preparative 3 mm thick 5% polyacrylamide gel electrophoresis at 2.5 V/cm (usually 400 μg were loaded into a 60 mm x 3 mm well) in Tris-borate EDTA buffer. Once the bands were clearly separated, the gel was stained with ethidium bromide and bands were cut off under u.v. light. Polyacrylamide fragments were placed into dialysis bags and DNA was electroeluted. The eluate was retained on small DEAE-cellulose columns (Wattman DE52 in Eppendorf blue cones), extensively washed with Tris 10 mM pH 8, EDTA 1 mM, NaCl 0.3 M. Finally the DNA was eluted with Tris 10 mM pH 8, EDTA 1 mM, NACl 1.5 M and precipitated with ethanol. This DNA preparation was well suited for proper cutting with restriction endonucleases and labelling with [γ-^{32}P]ATP and polynucleotide kinase.

DNA sequencing

We used five reactions from the chemical technique (Maxam and Gilbert, 1980) i.e., G, A + G, C + T, C, A > C. Currently 5–10 μg of a 1000 bp fragment were sufficient for the sequencing experiments.

Acknowledgements

We thank C. Braun-Breton who provided us with strains and unpublished results, J.Garnier for performing the computer analysis of secondary structure predictions and A.Ceccarelli for typing the manuscript. This work was supported by grants from the Centre National de la Recherche Scientifique (C.P. 960002), North Atlantic Treaty Organization (grant 1297) and the Fondation pour la Recherche Médicale.

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