Bacteriophage lambda and plasmid vectors, allowing fusion of cloned genes in each of the three translational phases

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ABSTRACT

We have constructed vectors from bacteriophage lambda and from plasmid pBR322 having a single EcoRI restriction site which is immediately downstream from the lac UV5 promoter. Each vector allows the fusion of a cloned gene to the lac Z gene in a different phase relative to the translation initiation codon of the lac Z gene. These vectors were constructed through modification of the initial EcoRI restriction site by S1 endonuclease treatment and then addition of octadeoxyribonucleotides (EcoRI linkers), which shifted the restriction site by 2 or 4 nucleotides. Used in combination these vectors should allow translation of a cloned gene in any one of the three coding phases. The bacteriophages vectors are certified as B2 (EK2) safety level vectors by the French "recombinaison génétique in vitro" committee (D.G.R.S.T.).

INTRODUCTION

The first instances of the expression of eukaryotic genes in E.coli were obtained after insertion of random DNA fragments into a vector molecule and a search for complementation of a bacterial mutation (1, 2, 3). Up to now, these results concern only lower eukaryotic species, saccharomyces cerevisiae and Neurospora crassa, and in all cases the enzyme synthesis corresponding to the cloned gene is low. This is thought to be due to a poor recognition of the eukaryotic initiation signals by bacterial enzymes.

Fusion of the cloned gene to a bacterial gene which is carried by the vector molecule, places the eukaryotic gene under the control of the bacterial initiation signals and seems to be more suitable for an efficient expression of the cloned gene. Indeed, by this method the synthesis in E.coli of somatostatin (4), proinsulin (5) and chicken ovalbumin (6) were achieved. In these three cases, the gene was obtained either by chemical synthesis of the DNA or from the corresponding mRNA. At the present time both these

methods of preparation of genes appear to be the only general ones to yield expression in E.coli, since introns have been shown to exist in many eukaryotic genes (7, 8, 9, 10) and since it seems very unlikely that mRNA from such spliced genes can be correctly translated in E.coli. This emphasizes the importance of the method of genetic fusion, since the expression of both a chemically synthesized gene and cDNA require bacterial initiation signals.

We have recently developed the $\lambda\Delta Z1$ and $\lambda Y1EQS$ vectors derived from $\lambda plac5$ which have only one EcoRI restriction site located next to the lac promoter (11). The advantage of such vectors is that they allow the fusion of a cloned gene at the very beginning of the β -galactosidase gene (6). However, since expression of the cloned gene requires correct translation phase, an adjustment has often to be achieved at the gene junction.

In order to avoid this adjustment for each particular case, we decided to construct a set of vectors, each having a single EcoRI restriction site in which the cloned gene can be placed in each of the three possible phases relative to the translation initiation site of the lac Z gene. A DNA fragment inserted in the EcoRI site of the bacteriophage $\lambda\Delta Z1$ is in the reading frame defined as $\phi 1^*$. To construct the $\lambda\Delta Z$ vectors able to position the inserted fragment in phases $\phi 2$ and $\phi 3$, we planned to modify chemically the EcoRI site of $\lambda\Delta Z1$. Direct chemical modification of this site would have been difficult because of the large size of the right half of the genome and because it would have been necessary to protect the right cohesive end of lambda from any chemical attack. Instead, we proceeded as follows (fig. 1) : 1) Chemical modification of the extremities of the EcoRI 203 (UV5) DNA fragment in order to add 2 base pairs at each end (this new fragment is called EcoRI 207 (UV5)). 2) In vitro insertion of the EcoRI 207 (UV5) DNA fragment into the EcoRI restriction site of $\lambda plac5-1$

* The vectors described in this paper present at their EcoRI sites different phases with respect to the translation initiation site of the lac Z gene. The 3 phases are numbered as follows : $\phi 1$ 5' $\overline{G^{\dagger}A A T T C}$ 3', $\phi 2$ 5' $\overline{G^{\dagger}A A T T C}$ 3', $\phi 3$ 5' $\overline{G^{\dagger}A A T T C}$ 3'. In the nomenclature of each vector, the figure refers to its phase.

genome. 3) In vivo intramolecular genetic recombination. This leads to bacteriophage $\lambda\Delta Z2$ with the phase $\Phi 2$. A similar procedure applied to the EcoRI 207 (UV5) DNA fragment gives the EcoRI 211 (UV5) fragment and bacteriophage $\lambda\Delta Z3$ with the phase $\Phi 3$.

From each $\lambda\Delta Z$ vector, a lambda derivative carrying the lac Y gene and safety mutations, and a plasmid including the lac regulatory region and the EcoRI site corresponding to each phase was constructed.

MATERIALS AND METHODS

Bacterial and Bacteriophage Strains : All bacterial strains are derivatives of E.coli K12 : MM 294 carrying the pOP 203 (UV5) plasmid was a gift from W. Gilbert. C600 recBC $rk^{-}mk^{-}$ was a gift from J. Cameron. YMC (SupF) and 3000X74 (lac sup^o) were from the Institute Pasteur collection.

Bacteriophage strains are listed in table 1.

Chemicals : The following chemicals were used : Xgal = 5-bromo-4-chloro-3-indolyl-beta-D galactoside (Bachem). Melibiose (Bak $\bar{e}$ r). Agarose (Sigma). Acrylamide (Serva). 32P γ ATP (New England Nuclear). The octadeoxyribonucleotide 5'-OH-GGAATTCC-OH-3' (Collaborative Research). Dimethylsulfate (Aldrich Chemical). Hydrazine (Eastman Organic Chemicals). Piperidine (BDH Biochemicals).

Enzymatic Reactions : HindIII endonuclease (Biolabs) digestion was performed in Tris HCl : 6.6 mM pH 7.5, MgCl₂ : 6.6 mM, beta-mercaptoethanol : 6.6 mM, NaCl : 60 mM buffer. HpaII and AluI endonuclease (Biolabs) digestion were performed in Tris HCl : 6.6 mM, pH 7.5, MgCl₂ : 6.6 mM, beta-mercaptoethanol : 6.6 mM, NaCl : 6.6 mM buffer. EcoRI endonuclease (gift from A. Lariv $\acute{e}$) digestion and cohesive ends ligation with T4 polynucleotide ligase were performed as previously described (12). Alkaline phosphatase (PL Laboratories), DNase I electrophoretically purified (Worthington) and Snake venom phosphodiesterase (Worthington) were used in Tris HCl : 10 mM pH 8.5, MgCl₂ : 10 mM buffer (13). 5'phosphorylation with 32P γ ATP reaction using polynucleotide kinase (PL Laboratories) was performed as described by Maxam and Gilbert (14). S1 endonuclease (gift from F. Rougeon) reaction was performed in NaCOOCH₃ : 25 mM, pH 4.5, ZnSO₄ : 1 mM, NaCl : 125 mM

TABLEAU 1
Bacteriophage strains

Usual name	Genotype	Reference or origin
λ plac5-1 UV5	λ plac5 c1857 Δ (shindIII λ 2-shindIII λ 3) SRI λ ³ SRI λ ⁴ SRI λ ⁵ UV5	P. Charnay et al. (11)
λ Δ Z1*	Δ (SRI lacZ-shaeIII lacZ) EcoRI- ϕ 1 derivative of λ plac5-1 UV5	P. Charnay et al. (11)
λ Δ Z2	Δ (SRI lacZ-shaeIII lacZ) EcoRI- ϕ 2 derivative of λ plac5-1 UV5	This paper
λ Δ Z3	Δ (SRI lacZ-shaeIII lacZ) EcoRI- ϕ 3 derivative of λ plac5-1 UV5	This paper
λ Y2	lacY derivative of λ Δ Z2	P. Charnay et al. (11)
λ Y3	lacY derivative of λ Δ Z3	P. Charnay et al. (11)
λ YIEQ5*	λ lacY (SRI lacZ-shaeIII lacZ) EcoRI- ϕ 1, UV5, Δ (shindIII λ 2-shindIII λ 3) KH54 SRI λ ³ , SRI λ ⁴ , SRI λ ⁵ , shindIII ϕ nin5, Qam73, Sam7, Eam1100. λ lacY Δ (SRI lacZ-shaeIII lacZ) EcoRI- ϕ 2 UV5, Δ (shindIII λ 2-shindIII λ 3) KH54 SRI λ ³ , SRI λ ⁴ , SRI λ ⁵ , shindIII ϕ nin5, Qam73, Sam7, Uam413	This paper
λ Y2UQ5	λ lacY (SRI lacZ-shaeIII lacZ) EcoRI- ϕ 3 UV5, Δ (shindIII λ 2-shindIII λ 3) KH54, SRI λ ³ , SRI λ ⁴ , SRI λ ⁵ , shindIII ϕ nin5, Qam73, Sam7, Zam 718.	This paper
λ Y3ZQ5	λ lacY (SRI lacZ-shaeIII lacZ) EcoRI- ϕ 3 UV5, Δ (shindIII λ 2-shindIII λ 3) KH54, SRI λ ³ , SRI λ ⁴ , SRI λ ⁵ , shindIII ϕ nin5, Qam73, Sam7, Zam 718.	This paper
λ Uam413	λ GT λ B λ C vir SRI λ ⁴ , SRI λ ⁵ nin5 Uam413.	D.J. Donoghue (21)
λ Zam718	λ GT λ B λ C, vir, SRI λ ⁴ , SRI λ ⁵ nin5 Zam718.	D.J. Donoghue (21)
λ ZEQ5	λ plac5 UV5, KH54, (shindIII λ 2-shindIII λ 3) SRI λ ³ , SRI λ ⁴ , SRI λ ⁵ , nin5, Qam73 Sam7 Eam1100. λ pl1-6 imm21 57.	C. Pourcel et al. (19)
λ pF'ZY57		M. Hofnung

* λ Δ Z1 and λ YIEQ5 were previously called λ Δ ZUV5 and λ YEQ5 (11).

buffer for one hour at 25°C. The suitable quantity of enzyme was determined from analysis of the 5' terminal nucleotide of the EcoRI 203 fragment treated with increasing amounts of enzyme. Blunt-end ligation of octadeoxyribonucleotide (EcoRI linkers) to DNA fragments : 1 µg of EcoRI linker was labelled at the 5' end with 25 µCi of 32P γ ATP (specific activity) : 3 mCi/mM) using 2 units of polynucleotide kinase in 150 µl Tris HCl : 50 mM, pH : 9.5, MgCl₂ : 10 mM, DTT : 5 mM buffer for one hour at 37°C. After addition of ATP 0.03 mM, 2 units of polynucleotide kinase were added and the incubation was continued for one hour at 37°C. The mixture was dialysed for 3 hours with Tris HCl : 1 mM, pH 7.4, EDTA : 0.01 mM buffer. The mixture was lyophilized and the solid residue was dissolved in 20 µl Tris HCl : 6.6 mM, pH 7.6, MgCl₂ : 6.6 mM, ATP : 1 mM, DTT : 10 mM buffer, containing approximately 100 ng of EcoRI 203 (UV5) DNA fragment previously treated with S1 endonuclease. After addition of 10 units of T4 polynucleotide ligase, the mixture was incubated for 48 hours at 14°C.

Other techniques : Analytical and preparative polyacrylamide - gel electrophoreses were performed according to ADAMS (15). DNA fragment elution was performed as described by Maxam and Gilbert (14). 3MM paper electrophoresis at pH 3.5 was accomplished as described by Barell (13). DNA sequencing was performed as described by Maxam and Gilbert (14). Sephadex G100 filtration was performed according to Sanger (16) in 1 ml serological Corning pipettes. Bacteriophage multiplication and genetic crosses in YMC bacterial strain, bacteriophage purifications, E.coli transfection in C600 rk⁻mk⁻recBC, and growth tests on melibiose, agarose gel electrophoresis were performed as described previously (11). Xgal, tetracycline and ampicillin in solid L medium were used respectively at the following concentration : 40 mg/litre, 15 mg/litre and 100 mg/litre.

Containment : Experiments were carried out in a L1 (P1) and B1 (EK1) facility.

RESULTS

I - Chemically modifications of the ends of the EcoRI 203 (UV5) DNA fragment (fig. 1).

Figure 1 : Schematic diagram for construction of $\lambda\Delta Z2$.

The EcoRI 203 (UV5) DNA fragment contains the operator and the promoter (carrying the L8 and UV5 mutations) of the *lac* operon and the beginning of the β -galactosidase gene (*Z'*). The *lac Z'* extremity of this DNA fragment possesses the $\phi 1$ phase defined in the Introduction. Chemical modifications of this DNA fragment leads to a *lac Z'* extremity corresponding to the $\phi 2$ phase. This modified DNA fragment is then inserted *in vitro* into the EcoRI restriction site of λ plac5-1 UV5. Finally, an *in vivo* intramolecular recombination was selected, giving rise to the deleted bacteriophage $\lambda\Delta Z2$.

The procedure of adding 2 base pairs at each end of the EcoRI 203 (UV5) DNA fragment was the following : 1) Treat this fragment with the S1 endonuclease in order to produce flush ends with $\text{G}_{5'}$ as the final base pair. 2) With T4 polynucleotide ligase attach the $\text{P5' GGAATTCC CCTTAAGG}_{5'}$ EcoRI linkers to the ends of the DNA fragment. 3) Digest the new DNA fragment with EcoRI endonuclease with the result of a DNA fragment with two additional GG_{CC} base pairs at each EcoRI end. This last DNA fragment is cal-

led EcoRI 207 (UV5) fragment. Using the same procedure with the EcoRI 207 (UV5) fragment, the EcoRI 211 (UV5) fragment was constructed.

125 µg of the pOP203 plasmid, which harbors the EcoRI 203 (UV5) fragment, was hydrolyzed with EcoRI endonuclease. The DNA was then treated with S1 endonuclease. After labelling at the 5' end with 32P γ ATP using polynucleotide kinase, the EcoRI 203 (UV5) fragment was prepared by electrophoresis on an 8 % polyacrylamide gel. In order to check the efficiency of the S1 endonuclease treatment, an aliquot of this DNA sample was used to determine the 5' terminal nucleotide. It was, as expected, mostly cytosine (results not shown).

The EcoRI linkers were then ligated to the DNA fragment (see Materials and Methods). Electrophoretic analysis of aliquots, taken before and after EcoRI treatment, showed that the linkers had attached to the DNA fragment (results not shown). EcoRI digested DNA fragment was separated from linkers by column chromatography on Sephadex G100. The fragment was eluted in total volume of 100 µl.

Part of the modified DNA fragment, in 15 µl, was then ligated in vitro with pBR322 (17) digested with EcoRI. This DNA was used to transform the bacterial strain MM294. About 8 % of the colonies were blue on L medium, Xgal, Tetracycline plates, which indicates that they harbor the pBR322 plasmid with the lac operator.

II - Analysis of the lac Z end of the EcoRI 207 (UV5) DNA fragment.

From the blue colonies described in the previous section, 4 clones were selected and amplified and the structure of their plasmids (pPC21, pPC22, pPC23 and pPC24) was analysed. The treatment of each plasmid with EcoRI endonuclease produced a DNA fragment whose electrophoretic mobility (on an 8 % polyacrylamide gel) is slightly less than the mobility of the initial EcoRI 203 (UV5) fragment (results not shown). This slower DNA band corresponds to the EcoRI 207 (UV5) fragment.

In order to ascertain that the EcoRI extremities of the EcoRI 207 fragment had been shifted by 2 base pairs, the nucleotide sequence of the lac Z end of the EcoRI 207 (UV5) DNA fragment was determined. 100 µg of the pOP203, pPC21 and pPC22 plasmids were

digested with EcoRI endonuclease. To follow the purification of the DNA fragment 5 µg of each plasmid was 5' labelled with ^{32}P γ ATP and added to the remainder of the sample. The DNAs were precipitated with ethanol and the pellet dissolved in 100 µl of 30 mM sodium acetate. The EcoRI 203 (UV5) and EcoRI 207 (UV5) fragments were purified through electrophoresis on an 8 % polyacrylamide gel. After elution from the gel the DNAs were 5' labelled with high specific activity ^{32}P γ ATP. After digestion with HpaII restriction endonuclease, the shorter radioactive HpaII DNA fragment which corresponds to the lac Z gene end was purified by electrophoresis on an 8 % polyacrylamide gel. The nucleotide sequence of these DNA fragments was established by the method of Maxam and Gilbert. As expected, the sequence of the pPC21 and pPC22 fragments shows that 2 GC base pairs had been inserted immediately before the EcoRI restriction site and, thus, that the lac Z end of the two EcoRI 207 (UV5) fragments has a translational phase which is the $\phi 2$ phase defined previously (fig. 2).

III - Construction of the EcoRI 211 (UV5) DNA fragment and verification of its structure.

The EcoRI 211 (UV5) DNA fragment has two additional GC base pairs at each end relative to the EcoRI 207 (UV5) fragment. This DNA fragment was obtained from the EcoRI 207 (UV5) fragment in a series of steps identical to those which gave the latter from the EcoRI 203 (UV5) DNA fragment. The nucleotide sequence of the lac Z end of EcoRI 211 (UV5) was determined from three plasmids (pPC31, pPC32 and pPC33). The EcoRI extremity has 4 additional GC base pairs as compared to the initial EcoRI 203 (UV5) fragment and correspond to the $\phi 3$ phase (fig. 2).

IV - Construction of bacteriophages $\lambda\Delta Z2$, $\lambda Y2UQS$ (phase $\phi 2$), $\lambda\Delta Z3$ and $\lambda Y3ZQS$ (phase $\phi 3$).

Bacteriophage $\lambda\Delta Z2$ has been constructed according to the procedure used for $\lambda\Delta Z1$ (11), as presented in figure 1 : 1) in vitro insertion of the EcoRI 207 (UV5) DNA fragment into the lac Z EcoRI site of the genome of $\lambda\text{plac5-1}$ UV5 and 2) in vivo intramolecular genetic recombination. The first step gave lac⁻ bacterio-

These EcoRI DNA fragments were prepared as described in the text. After labelling of their 5' ends, they were digested with HpaII endonuclease. The lac Z subfragments were purified by electrophoresis in a 8 % polyacrylamide slab gel. These subfragments were eluted from the gel, and sequence nucleotide was determined by the Maxam and Gilbert procedure. A : EcoRI 203 (UV5). B : EcoRI 207 (UV5). C : EcoRI 211 (UV5).

Inasmuch as transcription and translation are linked, introducing a gene whose expression can be easily identified beyond the EcoRI site should facilitate the study of the transcription

and possibly of the translation of a gene inserted into this site. With this aim we constructed bacteriophage λ Y2 which is homologous to λ Y1 (11). The lac Y gene was introduced through in vitro ligation of the EcoRI A fragment (left arm of the genome) from λ pF'ZY57 with the EcoRI B fragment (right arm of the genome) from $\lambda\Delta$ Z2. The lac Y⁺ character of the recombinants was checked by growth on melibiose medium at 42°C, of doubly lysogenic 3000X74 (λ ⁺, λ Y2) bacterial strain.

In order to give to the vectors the biological B2 (EK2) safety level (18), Uam413, Qam73 and Sam7 mutations were introduced as well as the KH54 and nin5 deletions (19). This was obtained through two genetic crosses : 1) λ Y2 was crossed with λ GT λ BC vir Uam413 nin5 giving λ Uam413Y2 and 2) λ Uam413Y2 was crossed with λ ZEQS giving rise to λ Y2UQS. The Qam73 and Sam7 mutations have the additional property that one obtains a large amplification of the expression of genes placed under the control of a bacterial promotor, particularly of the lac promotor (19, 20).

Bacteriophages $\lambda\Delta$ Z3, λ Y3 and λ Y3ZQS were constructed in a similar manner. $\lambda\Delta$ Z3 was obtained through in vitro insertion of the EcoRI 211 (UV5) DNA fragment into the EcoRI restriction site of λ plac5-1 genome followed by intramolecular genetic recombination. Phage λ Y3 is the result of an in vitro recombination between the right and left arms of phages $\lambda\Delta$ Z3 and λ pF'ZY57 genomes respectively. Phage λ Y3ZQS results from successive genetic crosses between phages λ Y3, λ GT λ BC vir Zam718 nin5 and λ ZEQS. In the λ Y2 and λ Y3 derivatives the Eam1100 mutation was replaced by the Uam413 and Zam718 mutations respectively, in order to have an easy recognition of the bacteriophages corresponding to the three translational phases. The structure of the λ Y2UQS and λ Y3ZQS was verified by electrophoretic analysis of restriction fragments (fig. 3), and is presented in figure 4.

V - Construction of the pPC ϕ 1, pPC ϕ 2 and pPC ϕ 3 plasmids

These plasmids were constructed in order to extend the three phases system of the phage vectors to plasmid vectors. The three plasmids were obtained by substitution of the small EcoRI-HindIII fragment of the pBR322 plasmid by the EcoRI-HindIII fragment from $\lambda\Delta$ Z1, $\lambda\Delta$ Z2 and $\lambda\Delta$ Z3 genomes respectively.

Figure 3 : Agarose gel electrophoresis analysis of EcoRI-HindIII restriction DNA fragments of $\lambda\Delta Z1$, $\lambda\Delta Z2$, $\lambda\Delta Z3$, pPC ϕ 2 and pPC ϕ 3.

Each DNA sample was digested with EcoRI and HindIII endonucleases. The electrophoresis was performed at 100 V (8V/cm) for 3 hours in a slab gel containing 0.9 % agarose. 1 μ g of DNA fragments was used in each sample. The gel was stained with ethidium bromide. (1) : $\lambda Y1EQS$ DNA fragments. (2) : $\lambda Y2UQS$ DNA fragments. (3) : $\lambda Y3ZQS$ DNA fragments. (4) : pPC ϕ 1 DNA fragments. (5) : pPC ϕ 2 DNA fragments. (6) : pPC ϕ 3 DNA fragments. (7) : pBR322 DNA fragments.

The experimental procedure was the following. 500 μ g of each $\lambda\Delta Z1$, $\lambda\Delta Z2$ and $\lambda\Delta Z3$ DNA was hydrolyzed by EcoRI and HindIII restriction endonucleases mixed with aliquots of 100 μ g of pBR322 DNA, similarly digested. After ligase treatment the three DNA samples were used to transform the bacterial MM294 strain. Blue colonies on L medium, Xgal, ampicillin plates were isolated. For each vector type, the plasmids from three clones were analyzed. After digestion by EcoRI and HindIII restriction enzymes, the electrophoretic analysis gave in all cases two DNA bands, corresponding to the EcoRI-HindIII fragment originating from the phages, and to the large plasmid EcoRI-HindIII fragment.

Figure 4 : The three lambda and three plasmid vectors and their sequences at the EcoRI site.

Lac Z' corresponds to the sequence of the beginning of the lac Z gene between the translation initiation site and the EcoRI restriction site. Addition of two GC base pairs to this sequence transforms the EcoRI- Φ_1 site to EcoRI- Φ_2 site as defined in the Introduction. Addition of two GC base pairs more leads to the EcoRI- Φ_3 site. The amino acid sequences were deduced from the nucleotide sequences. The series of three bacteriophages, corresponding respectively to these phases are called λ Y1EQS, λ Y2UQS and λ Y3ZQS. Ψ represents the lac promoter and the lac Y gene which are separated by the EcoRI site. UV5, Q, S, HindIII, KH54 and nin5 represent amber mutations and deletions present on the three bacteriophage genomes. E, U and Z represent amber mutations carried respectively by λ Y1EQS, λ Y2UQS and λ Y3ZQS. The series of the three homologous plasmids are pPC Φ_1 , pPC Φ_2 and pPC Φ_3 . Φ represents lac promoter and the arrow indicates the direction of transcription. The EcoRI-HindIII fragments (thick lines) are identical to the EcoRI-HindIII DNA fragments of the homologous bacteriophages. ApR represents the ampicillin resistance character carried by the three plasmids.

Results are shown for one plasmid of each type, called respectively pPC Φ_1 , pPC Φ_2 and pPC Φ_3 (fig. 3). To check that these three plasmids actually correspond to the three phases the following analysis was made. DNA from each of the three plasmids was digested by EcoRI endonuclease, the ends were dephosphorylated and

then labelled with ^{32}P γ ATP and finally digested with AluI restriction endonuclease which cleaves the EcoRI lac promoter fragment very near to its lac Z extremity. The electrophoretic analysis of the fragments on a 20 % polyacrylamide gel showed that they had the expected relative size (results not shown). The three plasmids pPC Φ 1, pPC Φ 2 and pPC Φ 3 have a single EcoRI restriction site located at the beginning of the lac Z gene and allow the in vitro insertion of a gene into this site in one of the three phases relative to the translation initiation site of the lac Z gene (fig. 4).

DISCUSSION

By successive use of S1 endonuclease and chemically synthesized octadeoxyribonucleotides, the EcoRI 203 (UV5) DNA fragment was elongated by the addition of 2 or 4 base pairs at each end. Bacteriophage lambda vectors were constructed by insertion of these modified fragments into the EcoRI lac Z restriction site of λ plac5-1 followed by in vivo intramolecular recombination between the inserted fragment and the regulatory homologous region from the lac operon. Plasmid vectors homologous to the phage vectors were obtained from pPB322. The general structure of these vectors is shown in figure 4.

All these vectors have a single EcoRI restriction site, located next to the lac promoter. They allow the in vitro fusion of a foreign gene to the beginning of the lac Z gene in either one of the three possible phases, the correct reading frame at the junction being a necessary condition for expression of the fused gene.

The three vectors λ Y1EQS, λ Y2QS and λ Y3ZQS have a 17 % deletion of their genomes, thus allowing the insertion of a DNA fragment up to 10, 000 base pairs long. These phage vectors also have 1) the lac Y gene beyond the EcoRI restriction site. The expression of this gene which can be easily detected, can yield information about the transcriptional and/or translational expression of the inserted gene. 2) Qam73 and Sam7 mutations which can help in increasing the protein synthesis corresponding to the inserted gene (19, 20). 3) Safety mutations which set the vectors to the B2 (EK2) biological safety level, in as far as

the multiplication of the phages is carried out in the E.coli K12 DP50 supF bacterial strain or in an E.coli K12 strain completely deleted for the lac operon (X74 deletion) (19).

In these three phage vectors the expression of the lac Y gene is controlled by the lac promotor. In two of them, λ Y2UQS and λ Y3ZQS, the beginning and the end of the lac Z gene are out of phase with respect to the EcoRI restriction site (11). The absence of polar effects on the expression of lac Y is probably due to the shortness of the distal end of lac Z.

The three plasmid vectors pPC ϕ 1, pPC ϕ 2 and pPC ϕ 3 were obtained from plasmid pBR322 into which the EcoRI-HindIII DNA fragment from the respective $\lambda\Delta$ Z1, $\lambda\Delta$ Z2 and $\lambda\Delta$ Z3 phage genomes was inserted. They carry the ampicillin resistance character. The pPC ϕ 1 plasmid is identical to the pOMPO plasmid which was constructed in parallel and used for the fusion of chicken ovalbumin gene with the beginning of lac Z. The hybrid protein was synthesized in large quantity in E.coli, and appeared to be stable (6). The utilization of the vectors corresponding to the phases ϕ 2 and ϕ 3, for gene fusions would implicate the introduction of new amino acids at the peptide junction. It is difficult to predict a priori, the influence of such modification on the stability of the hybrid protein.

Up to now the correct reading frame of a eukaryotic gene which has been fused in vitro to a prokaryotic gene has been obtained in two ways. When the primary structures, i.e. the reading phases of the two genes were known, the adjustment of the two phases at their junctions was obtained through chemical modification of the end of one of the genes (4, 6). When the translational phase of one of the two genes was unknown, the fusion had to be carried out by a random method which gave the three possible phases. This is how Villa-Komaroff et al. obtained the synthesis of proinsulin after insertion of its gene with an oligo (dC) oligo (dG) joining procedure into the β -lactamase gene (5). With the phage and plasmid vectors described in this article, genetic fusions can be achieved simply in either situation.

These vectors have been constructed essentially for the fusion of eukaryotic genes, but obviously they can also be used

to place bacterial genes under the control of the lac promotor. In particular the bacteriophages allow the insertion of the genes between the lac promotor and the lac Y gene.

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