Gene, 71 (1988) 177–186
Elsevier

GEN 02680

Transposition of λ*plac*Mu is mediated by the A protein altered at its carboxy-terminal end

(Mu transposase; *lacZ* fusions; bacteriophages λ and Mu; transducing phages; recombinant DNA)

Erhard Bremer[a], Thomas J. Silhavy[b] and George M. Weinstock[c]

[a] Department of Biology, University of Konstanz, D-7750 Konstanz (F.R.G.); [b] Department of Molecular Biology, Princeton University, Princeton, NJ 08544 (U.S.A.) Tel. (609)452-5889, and [c] Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston, TX 77225 (U.S.A.) Tel. (713)792-5600

Received 27 May 1988
Accepted 22 June 1988
Received by publisher 4 August 1988

SUMMARY

λ*plac*Mu phages are derivatives of bacteriophage λ that use the transposition machinery of phage Mu to insert into chromosomal and cloned genes. When inserted in the proper fashion, these phages yield stable fusions to the *Escherichia coli lac* operon in a single step. We have determined the amount of DNA from the *c* end of phage Mu present in one of these phages, λ*plac*Mu3, and have shown that this phage carries a 3137-bp fragment of Mu DNA. This DNA segment carries the Mu *c*-end attachment site and encodes the Mu genes *c*ts62, *ner*$^+$, and gene *A* lacking 179 bp at its 3′ end (*A*′). The product of this truncated gene *A*′ retains transposase activity and is sufficient for the transposition of λ*plac*Mu. This was demonstrated by showing that λ*plac*Mu derivatives carrying the *A*am1093 mutation in the *A*′ gene are unable to transpose by themselves in a Su$^-$ strain, but their transposition can be triggered by coinfection with λpMu507(*A*$^+$*B*$^+$). We have constructed several new λ*plac*Mu phages that carry the *A*′am1093 gene and the *kan* gene, which confers resistance to kanamycin. Chromosomal insertions of these new phages are even more stable than those of the previously reported λ*plac*Mu phages, which makes them useful tools for genetic analysis.

INTRODUCTION

Transposition is an integral part of the life cycle of the temperate bacteriophage Mu (for reviews see Toussaint and Resibois, 1983; Mizuuchi and Craigie, 1986). Both lytic and lysogenic pathways require the obligatory integration of the Mu DNA into the host chromosome. This integration occurs by a highly efficient transposition process with little site-specificity. Mu transposition requires both the *S* and *c* attachment sites located, respectively, at the right and left ends of the phage genome, and it is mediated by the Mu-encoded A and B proteins. The A protein, which is regarded as a transposase, is essential for transposition, whereas the B protein significantly (approx. 100-fold) enhances transposition efficiency but is not essential.

The ability of Mu to transpose into many genes

Correspondence to: Dr. E. Bremer, Department of Biology, University of Konstanz, P.O. Box 5560, D-7750 Konstanz (F.R.G.) Tel. (49)7531-882042.

Abbreviations: *A*′, the 3′ truncated gene *A* of phage Mu; aa, amino acid(s); bp, base pair(s); kb, 1000 bp; Km, kanamycin; LB, Luria broth; pfu, plaque-forming units; ^{R}, resistance; XGal, 5-bromo-4-chloro-indolyl-β-D-galactopyranoside; ::, novel joint.

(Taylor, 1963) and into many locations within a single gene (Bukhari and Zipser, 1972; Daniell et al., 1972) has been exploited to create *lac* fusions in vivo (Casadaban, 1975; 1976; Casadaban and Cohen, 1979; Casadaban and Chou,1984; Castilho et al., 1984). Such fusions have wide-ranging applications in studies of gene expression and function (for reviews see Weinstock et al., 1983; Silhavy and Beckwith, 1985). We previously described the construction of several λ-Mu hybrid phages (λ*plac*Mu) in which bacteriophage λ is endowed with the transposition properties of phage Mu (Bremer et al., 1984; 1985). These λ*plac*Mu phages are plaque-forming derivatives of phage λ that lack the phage sequences (*attP*) necessary for the efficient site-specific integration into the *E. coli* chromosome at *attB*. They carry both of the Mu attachment sites (*S* and *c*) and an appropriately constructed *lac* operon that can form stable protein (λ*plac*Mu9) or operon (λ*plac*Mu53) fusions in a single step when they are inserted into a chromosomal or cloned target gene. The structure of a λ*plac*Mu phage and the proposed scheme of λ*plac*Mu transposition is shown in Fig. 1. Like phage Mu, these phages create a 5-bp duplication at the point of insertion (Nag and Berg, 1987). The λ*plac*Mu phages also permit isolation of λ specialized transducing phages carrying various segments of the *E. coli* chromosome (Bremer et al., 1984; 1985). Such transducing phages have various applications in genetic analysis (Silhavy et al., 1984) and facilitate cloning of the *lac* fusion as well as intact genes adjacent to the λ*plac*Mu insertion (Bremer et al., 1984; Trun and Silhavy, 1987). The use of the λ*plac*Mu system was recently extended from its natural host *E. coli* to *Salmonella typhimurium* (Harkki et al., 1987).

The Mu *c* region (including the Mu *c*-end attachment site) present on the λ*plac*Mu phages was derived from λp1(209) by a series of in vivo homologous recombination events (Bremer et al., 1984; 1985; Casadaban, 1976). Its size was determined by electron microscopy to be about 2.8 kb (Leathers et al., 1979). Since 3316 bp are required to encode the Mu *c*-end attachment site and a complete *A* gene (Priess et al., 1987), a substantial segment of the 3′ end of *A* should be missing from the Mu *c*

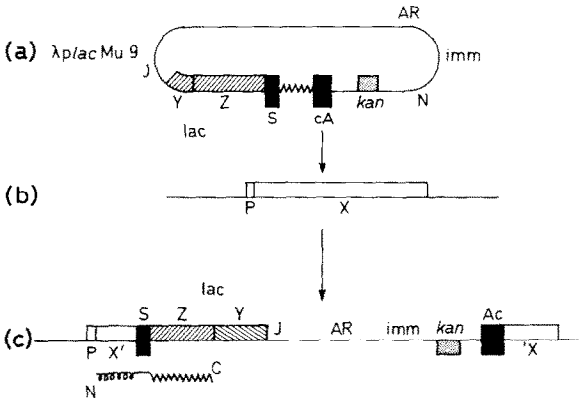

Fig. 1. Transposition of λ*plac*Mu. The proposed scheme of transposition for the phage λ*plac*Mu9, which forms *lacZ* protein fusions, is shown. Upon infection into *E. coli*, the circularized DNA of λ*plac*Mu9 (**a**) transposes into a target gene *X* (**b**) by means of the Mu attachment sites *S* and *c* to form a Lac$^+$ protein fusion between gene *X* and *lacZ* (**c**). The bacterial DNA (wavy line) present between the Mu ends (**a**) is lost during this process and the Mu *S* and *c* attachment sites are joined to sequences from gene *X* whose integrity is disrupted into *X′* and *'X*. The formation of a Lac$^+$ fusion requires the integration of the transposable phage into the target gene in the proper orientation and reading frame with respect to the fused gene. Expression of the hybrid gene depends on the target gene's transcriptional and translational initiation signals (P) and leads to the formation of a hybrid protein whose N terminus (N) is encoded by the target gene. This N-terminal region is attached to a large C-terminal segment (C) of β-galactosidase. The thin line represents λDNA. The positions of several genes from phage λ(*J*, *A*, *R*, *N*) and its immunity (*imm*) region are also indicated. The *'lacZ* and *lacY*$^+$ genes are shown by the cross-hatched areas, and the Mu material is symbolized by the blackened boxes. The *kan* gene, which confers Km resistance, is represented by the stippled box.

region. It was expected that a *A*$^+$*B*$^+$ helper phage supplying the A and B proteins in *trans* would be required for the transposition of λ*plac*Mu phages; however, these phages can transpose in the absence of such a helper phage, although at a 100-fold reduced level (Bremer et al., 1984; 1985). We show here that the *A* gene present in λ*plac*Mu has a deletion of 179 bp at its 3′ end (*A′*). However, the encoded *A′* protein still has transposase activity and is responsible for the helper-independent transposition of λ*plac*Mu. In addition, we report the isolation of several new transposition-defective λ*plac*Mu phages that yield chromosomal insertions with further enhanced genetic stability.

MATERIALS AND METHODS

(a) Bacterial strains, bacteriophages and plasmids

The bacterial strains, phages and plasmids used are listed in Table I.

(b) Media, chemicals and enzymes

LB, M63, MacConkey, and tetrazolium media were prepared as previously described (Miller, 1972; Silhavy et al., 1984). Liquid minimal medium containing M63 salts was supplemented with glycerol at 0.4%. When indicated, 0.1 ml of a 10 mg/ml solution of XGal in dimethlyformamide was spread on agar plates or added to L soft agar to detect colonies and phage plaques showing a LacZ$^+$ phenotype. Ampicillin, Km and tetracycline were added to media at 125 μg/ml, 30 μg/ml and 25 μg/ml, respectively. Deoxy- and dideoxyribonucleoside triphosphates, PolIk, and sequencing primer were from Bethesda Research Laboratories

TABLE I

Bacteria, bacteriophages and plasmids

Strain	Description[a]	Origin/reference
Bacteria derived from *E. coli* K-12		
MC4100	F$^-$ *araD*139 *Δ(argF-lac)* U169 *rpsL*150 *relA deoC*1 *ptsF*25 *rbsR flbB*5301	Casadaban (1976)
SE5000	MC4100 *recA*56	Silhavy et al. (1984)
MBM7007	F$^-$ *ara*Cam *araD Δ(argF-lac)*U169 *trp*am *malB*am *rpsL relA thi*	Berman and Beckwith (1979)
MBM7014	MBM7007 *supF*	Berman and Beckwith (1979)
MH219	*mel pro supF* (Mucts62*A*am1093)	M. Howe
TST3	MC4100 *malT*::Tn*10*	T.J. Silhavy
BRE1047	MC4100 *φ(malK-'lacZ)*hyb1002 (λ*plac*Mu1) *malT*::Tn*10*	Bremer et al. (1984)
BRE1167	MC4100 *φ(malK-lacZ$^+$)*1113 (λ*plac*Mu50) *malT*::Tn*10*	Bremer et al. (1985)
BRE1297	MC4100 *φ(malK-'lacZ)*hyb1238 (λ*plac*Mu5) *malT*::Tn*10*	This work
BRE1299	MC4100 *φ(malK-lacZ$^+$)*1263 (λ*plac*Mu52) *malT*::Tn*10*	This work
BRE1223[b]	MBM7014 (Mucts62*A*am1093)	This work
BRE2003[c]	MBM7007 *recA srl*::Tn*10*	This work
BRE2004[c]	MBM7014 *recA srl*::Tn*10*	This work
Bacteriophages		
λ*plac*Mu507	Mucts62*A*$^+$*B*$^+$ λcIts857 *S*am7	Magazin et al. (1977)
λRZ2	*cI*$^+$ *att*$^+$ *φ(tyrT-lacZ$^+$) kan*	R. Zagursky
λ*plac*Mu1	Mucts62 *ner*$^+$ *A' 'ara'* Mu*S' 'lacZ lacY*$^+$ *lacA' immλ*	Bremer et al. (1984)
λ*plac*Mu3	λ*plac*Mu1 *imm*21	Bremer et al. (1984)
λ*plac*Mu5	λ*plac*Mu1 *A'*am1093 *immλ*	This work
λ*plac*Mu13	λ*plac*Mu5 *imm*21	This work
λ*plac*Mu15	λ*plac*Mu5 *kan immλ*	This work
λ*plac*Mu50	Mucts62 *ner*$^+$ *A' 'uvrD'* Mu*S' 'trp' lacZ*$^+$ *lacY*$^+$ *lacA' immλ*	Bremer et al. (1985)
λ*plac*Mu52	λ*plac*Mu50 *A'*am1093 *immλ*	This work
λ*plac*Mu54	λ*plac*Mu52 *imm*21	This work
λ*plac*Mu55	λ*plac*Mu52 *kan immλ*	This work
Plasmids		
pGE172	pBR327::λ*plac*Mu3	Bremer et al. (1984)
pGE178	pGE172*Δ*(*Sma*I-*Sma*I)	This work
pFRV1	pBR322 (Mu *ner*$^+$ *A'*)	This work

[a] The symbol *φ* indicates the presence of a *lacZ* fusion, and the abbreviation hyb indicates that the gene fusion encodes a hybrid protein. The symbols *lacZ*$^+$ and *'lacZ* denote *lacZ* genes with or without translational initiation signals, respectively. Genes marked with a prime are truncated.

[b] This strain is unable to grow on glycerol M63 minimal plates but can grow on glucose M63 plates, indicating that it carries a Mu insertion in a gene involved in glycerol transport or catabolism. Multiple Mucts62*A*am1093 prophages might be present in this strain.

[c] The Tn*10* insertion present in this strain is located at an undetermined position within the gene cluster encoding proteins for sorbitol (*srl*) transport and metabolism.

(BRL) and Boehringer (Mannheim); T4 ligase and restriction enzymes were purchased from several commercial sources.

(c) Methods used with nucleic acids

Isolation and transformation of plasmid DNA, digestion conditions for restriction enzymes, DNA ligation with T4 ligase and recovery of restriction fragments from agarose gels were all performed as previously described (Maniatis et al., 1982; Silhavy et al., 1984).

(d) Construction of plasmid pFRV1 and nucleotide sequence analysis of the *A'-λ* joint

Plasmid pEG172 (Bremer et al., 1984) contains the hybrid λ*plac*Mu3 phage transposed into plasmid pBR327 (Soberón et al., 1980). Most of the λ material was deleted from pEG172 by *Sma*I digestion followed by religation of the plasmid at two *Sma*I restriction sites occuring at bp 19399 and 31619 of phage λ (Sanger et al., 1982; Daniels et al., 1983). From the resulting plasmid, pEG178, a 4.74-kb *Hin*dIII-*Sma*I fragment was excised and then inserted into the *Hin*dIII and *Pvu*II sites of pBR322 (Bolivar et al., 1977), yielding plasmid pFRV1 (Fig. 3). A 1.44-kb *Pst*I-*Xmn*I restriction fragment (Fig. 3) was then isolated from pFRV1 and cloned into the phage vector M13mp19 (Norrander et al., 1983), which had been cleaved with *Pst*I and *Hin*dII. Analysis of the hybrid M13mp19 phages followed the procedures described in the BRL cloning and sequencing manual and those of Messing (1983). Nucleotide sequence determination was carried out using a modified version of the dideoxynucleotide chain-termination method of Sanger et al. (1977) as described in the BRL manual. Sequencing samples were run on a 14-cm 8% polyacrylamide gel with 8 M urea using the 'direct blotting' method and apparatus described by Beck and Pohl (1984). After the run the blotting membrane was exposed to Kodak XAR film at room temperature for 24 h.

(e) Genetic procedures

Standard techniques were used for the growth of bacteria and bacteriophages, titering bacteriophages, lysogen formation of phage λ, and generalized transduction with phage P1*vir* (Miller, 1972; Silhavy et al., 1984). Lysogens of phage Mu were isolated as described (Bukhari and Ljungquist, 1977). The *imm*21 region and the *kan* gene, which confers resistance to Km, were crossed into the λ*plac*Mu phages as described (Austin and Abeles, 1983; Bremer et al., 1985). Strains were made *recA* by P1 transduction using a *recA srl*::Tn*10* donor strain. Chromosomal insertions of λ*plac*Mu phages were isolated in strain MC4100 after coinfection with λ pMu507 and subsequent selection for Lac$^+$ colonies on lactose M63 minimal agar or for Km^R colonies on LB agar containing Km as described (Bremer et al., 1985). *lacZ* fusions to the *malK* gene were identified according to Bremer et al. (1984; 1985). To ensure that only one λ*plac*Mu insertion was present in the strain finally used, the *malK-lacZ* fusions were transduced with phage P1*vir* into strain MC4100.

(f) Isolation of λ*plac*Mu phages carrying the Mu*A*am1093 mutation

The λ*plac*Mu1 lysogens in strain BRE1223 (Mu*A*am1093) were isolated by exploiting the Mu *c* homology present on the Mu prophage and λ*plac*Mu1 (Fig. 2a,b). Homologous recombination between these sequences forms a λ*plac*Mu1-Mu*A*am1093 double lysogen in which the λ*plac*Mu1 prophage is flanked by Mu *c* homology (Fig. 2c). Recombination between these flanking sequences leads to spontaneous excision of the λ*plac*Mu1 prophage at various positions within the Mu *c* end. The excised λ*plac*Mu prophage will carry either the original *A'* gene or an *A'* gene harboring the *A*am1093 mutation (Fig. 2,c,d). The λ*plac*Mu phages carrying this mutation were detected by their inability to form Lac$^+$ lysogens with strain BRE2003 (Su$^-$ *Δ(lac) recA*) (Bremer et al., 1984). These phages could form Lac$^+$ lysogens by themselves with strain BRE2004 (*supF Δ(lac) recA*) and formed Lac$^+$ lysogens on both strains when coinfected with the λpMu507(A^+B^+) helper. The same strategy was used to isolate a derivative of λ*plac*Mu50 carrying the *A*am1093 mutation.

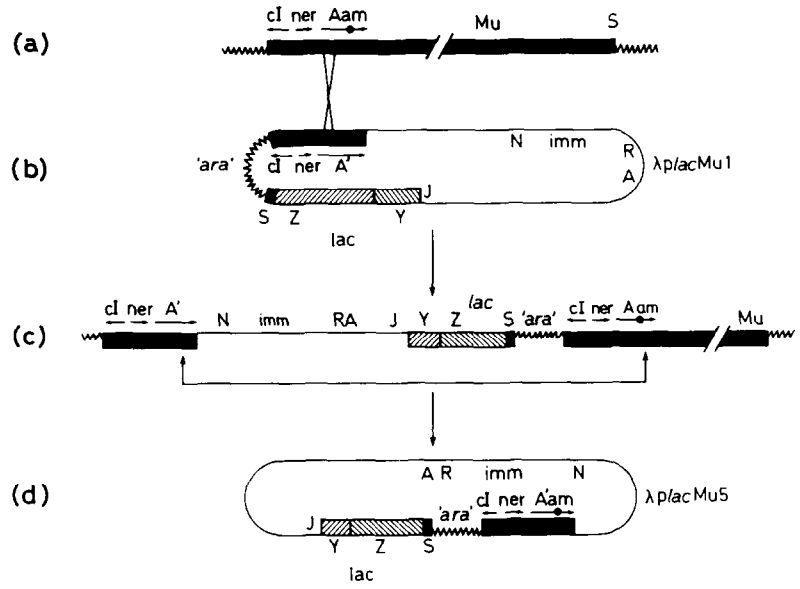

Fig. 2. Construction of λ*plac*Mu5. Strain BRE1223, which contains a Mu*A*am1093 prophage (**a**), was lysogenized with λ*plac*Mu1 (**b**). Integration of λ*plac*Mu1 occurred through *A* homology, leading to the formation of a λ*plac*Mu1-Mu*A*am1093 double lysogen (**c**). Excision of the λ*plac*Mu1 prophage by homologous recombination involving *A* sequences (**c**) results in the formation of λ*plac*Mu5, which carries the *A*am1093 mutation (**d**). The genetic organization of the Mu *c* region for the genes cts62, *ner*$^+$ and *A* is indicated by arrows showing the direction of transcription for these genes. The approximate position of the *A*am1093 mutation is represented by a black dot. Bacterial DNA is shown by a wavy line. All other symbols are as in Fig. 1.

RESULTS AND DISCUSSION

(a) Physical analysis of the Mu *c* region from λ*plac*Mu3

To determine the amount of Mu *c* DNA present in the λ*plac*Mu phages, we constructed a small plasmid that contained the joint between the Mu *c* and λ sequences from λ*plac*Mu3 (Bremer et al., 1984; see MATERIALS AND METHODS, section **d**). This plasmid, pFRV1 (Fig. 3), contains a 4.74-kb *Hin*dIII-*Sma*I fragment from λ*plac*Mu3 that is inserted into pBR322 (Bolivar et al., 1977). Restriction analysis of pFRV1 indicated that the joint between the Mu and λ sequences in λ*plac*Mu3 was in a 50-bp segment between the *Bgl*I site at position 3095 bp in the *A* gene (Harshey et al., 1985; Priess et al., 1987) and an *Xmn*I site at position 29993 bp of phage λ (Sanger et al., 1982) (Fig. 3). The *Bgl*I restriction site in the *A* gene is 221 bp 5′ to its translation stop codon. Hence, the Mu *c* region from λ*plac*Mu3 is between 3095 bp and 3145 bp long and the Mu*A* gene of λ*plac*Mu3 lacks a 171- to 221-bp segment at its 3′ end (Fig. 3).

(b) Nucleotide sequence of the Mu *c*-λ joint

The exact extent of the deletion at the end of the *A* gene was determined by nucleotide sequence analysis. For this purpose, a 1.44-kb *Pst*I-*Xmn*I restriction fragment containing the *A*′-λ joint from plasmid pFRV1 (Fig. 3) was cloned into the phage vector M13mp19. The nucleotide sequence for the segment between the *Xmn*I site in phage λ and the *Bgl*I site in gene *A* was determined (Fig. 4A). Comparison of this sequence with those of the corresponding regions from phage λ (Sanger et al., 1982) and the *A* gene (Harshey et al., 1985; Priess et al., 1987) allowed us to deduce where the *A* and λ sequences were joined: bp 29989 of phage λ was adjacent to bp 3137 of phage Mu (Fig. 4A). Thus, the Mu *c* region of λ*plac*Mu3 is 3137 bp long. The *A* gene is located between bp 1328 and 3316 and codes for a protein of 663 aa (Harshey et al., 1985; Priess et al., 1987). Therefore, the *A* gene located in the Mu *c* region of λ*plac*Mu3 contains a deletion of 179 bp at its 3′ end (*A*′) and its gene product should consequently lack the natural 60 C-terminal aa of the Mu transposase. Inspection of the λ DNA sequence (as

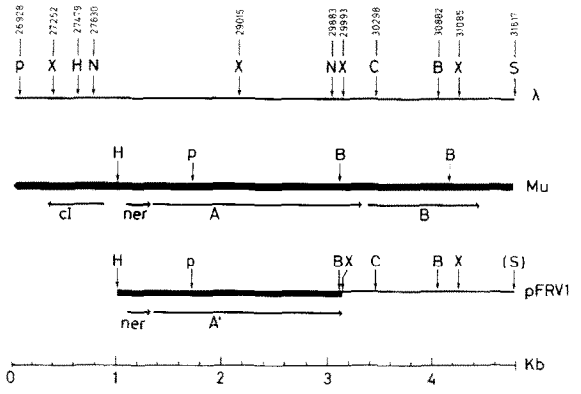

Fig. 3. Physical and genetic organization of the Mu *c*-λ joint in pFRV1. Restriction maps of segments from phage λ and Mu are compared with the cloned Mu *c*-λ fragment present in pFRV1. The position of the restriction sites shown for λ and Mu were compiled from the literature (Sanger et al., 1982; Priess et al., 1987). Those present in pFRV1 were determined by suitable single and multiple digests. The location and direction of transcription of the Mu genes *c*ts62, *ner*, *A* and *B* (Priess et al., 1987) are shown by horizontal arrows. The Mu material to the left of the unique *Hin*dIII site in the Mu *c* region is also present in λ*plac*Mu3 but was not cloned. The *Sma*I restriction site (position 31619 bp in phage λ) used for the cloning of the Mu *c*-λ fragment was destroyed during the blunt-end ligation into the *Pvu*II site of pBR322 and is therefore shown in parentheses. DNA from phage λ is indicated by a thin line; that of phage Mu by a thick line. B, *Bgl*I; C, *Cla*I; H, *Hin*dIII; N, *Nde*I; P, *Pst*I; S, *Sma*I; X, *Xmn*I.

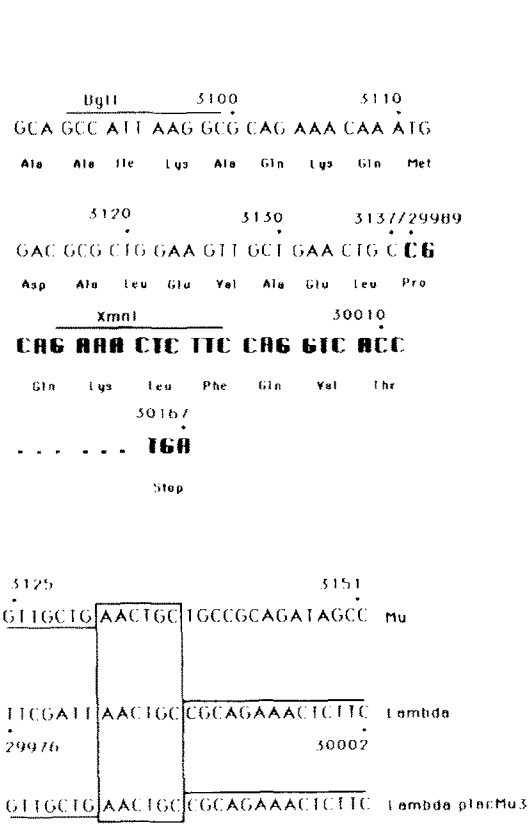

Fig. 4. Nucleotide sequences at the *A'*-λ joint in pFRV1. (**A**) The nucleotide sequence at the *A'*-λ joint in pFRV1, originating from λ*plac*Mu3, is shown. The nucleotide sequence between the *Xmn*I and *Bgl*I sites was determined; the sequence of DNA 3' to the *Xmn* site was taken from the literature (Sanger et al., 1982). Numbering of gene *A* sequence and the indicated reading frame of the A protein is according to Priess et al. (1987); λ DNA shown in bold print is numbered according to Sanger et al. (1982). (**B**) The nucleotide sequence at the *A'*-λ joint from λ*plac*Mu3 is compared to the corresponding regions of phages Mu and λ. The sequence shared with phage Mu is underlined, and that shared with phage λ is overlined. The 6-bp homology (boxed) is present in all three sequences. The nucleotide sequences are written in a 5'-to-3' direction.

deduced from the published nucleotide sequence of phage λ; Sanger et al., 1982) at the fusion joint revealed a short open reading frame for 59 aa adjacent to and in frame with *A'* (Fig. 4A). There is no apparent homology between these amino acids and the natural C-terminal end of the A protein (not shown).

It has been reported that λp1(209), from which the Mu *c* region of λ*plac*Mu3 was derived (Bremer et al., 1984), was generated by an illegitimate recombination event between a λ prophage and an adjacent Mu prophage (Casadaban, 1976). The Mu *c*-λ joint of λ*plac*Mu3 contains a 6-bp segment (Fig. 4B) present both in phage λ (Sanger at al., 1982) and phage Mu (Priess et al., 1987). This is probably the site of the recombination event that generated λp1(209). DNA sequences have become available from two other specialized transducing phages (λ*plac*5 and λ*gal*8) which have been reported to result from illegitimate recombination events (Shpakovski and Berlin, 1984; Debouck et al., 1985). Homologous stretches of 19 and 9 bp, respectively, were at the junction of the bacterial and phage λ DNA. These results, and the data presented here, suggest that such short segments of homology might be of general importance for the generation of specialized transducing phages.

The physical analysis of the Mu *c* region reported here involved a cloned segment derived from λ*plac*Mu3. However, the conclusions drawn about the size of its Mu *c* material are not limited to this particular phage, since all transposable λ*plac*Mu phages isolated so far (Bremer et al., 1984; 1985) were derived by an identical series of in vivo homologous recombination events. This 3137-bp

Mu *c* segment includes the Mu *c*-end attachment site and encodes the Mu genes *c*ts62, *ner*$^+$ and a partially deleted *A* gene (*A'*).

(c) Genetic analysis of the *A'* gene

Because the A protein is absolutely required for Mu transposition (O'Day et al., 1978), a functional form of this transposase is apparently encoded by the *A'* gene of λp*lac*Mu phages. To rule out the possibility that other factors might be responsible for λp*lac*Mu transposition in the absence of the λpMu507(*A*$^+$*B*$^+$) helper phage, we introduced an amber mutation into the *A'* gene. We then tested whether the resultant phages could transpose and give rise to Lac$^+$ lysogens in the absence or presence of a *supF* allele. Strain BRE1223 carries a Mu prophage with the well-defined *A*am1093 mutation that is located approximately in the middle of the *A* gene (Magazin et al., 1977; 1978; Toussaint et al., 1987). This mutation was crossed into λp*lac*Mu1 (Bremer et al., 1984) (see MATERIALS AND METHODS, section **f**; Fig. 2). When the resulting phage (λp*lac*Mu5) was spotted onto strain MBM7007 (Su$^-$), no Lac$^+$ lysogens were found, whereas Lac$^+$ colonies did appear when the *supF* strain MBM7014 was employed for this test (Fig. 5). The number of Lac$^+$ lysogens formed by λp*lac*Mu5 in strain MBM7014 was reduced, however, compared to λp*lac*Mu1. Thus, either the efficiency of suppression was limiting or the incorporated amino acid (tyrosine) did not fully restore transposase activity. The ability to transpose and give Lac$^+$ fusions was not impaired in this test when either MBM7007 or MBM7014 was coinfected with λp*lac*Mu5 and the *A*$^+$*B*$^+$ helper phage λpMu507 (Fig. 5). Thus, λp*lac*Mu1 derivatives carrying the *A*am1093 mutation in the *A'* gene undergo *supF*-dependent transposition.

We also isolated a transposition-defective derivative of phage λp*lac*Mu50 (which is used to obtain *lacZ* operon fusions; Bremer et al., 1985). Like λp*lac*Mu5, the derivative of λp*lac*Mu50 carrying the *A*am1093 mutation within the *A'* gene (λp*lac*Mu52) is unable to transpose by itself unless the amber mutation is suppressed (data not shown). Taken together, these data conclusively show that an A protein lacking its natural 60 aa at the C-terminal end still has transposase activity.

(d) Genetic stability of λp*lac*Mu insertions carrying the *A'*am1093 allele

Fusions to the *lacZ* gene can be used to isolate mutations affecting expression of the fused gene by selecting or screening for an altered Lac phenotype

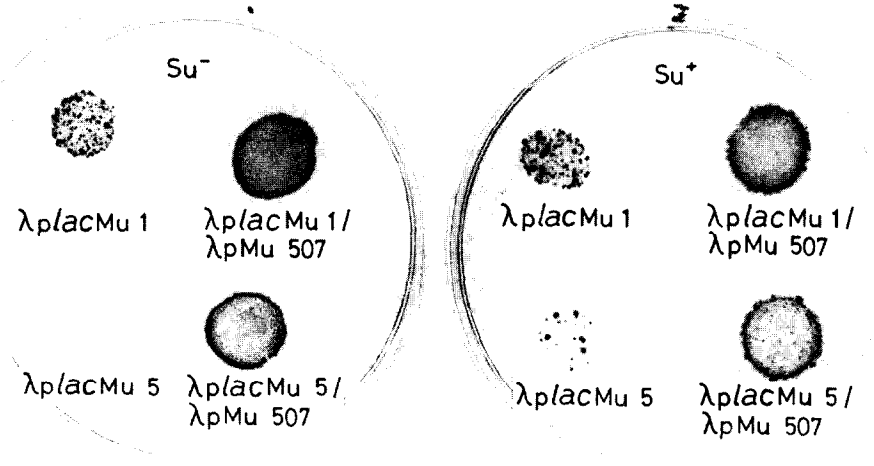

Fig. 5. Transposition of λp*lac*Mu1 and λp*lac*Mu5. Approximately 10^7 pfu of λp*lac*Mu1(*A'*) and λp*lac*Mu5(*A'*am1093) were spotted onto lawns of strains MBM7007 (Su$^-$) (left plate) and MBM7014 (*sup*F) (right plate) on lactose MacConkey agar. When strains were coinfected with λpMu507, approx. 10^8 pfu of λpMu507 were added. The plates were incubated for two days at 37°C. The Lac$^+$ lysogens (red colonies) appear on the photograph as shaded areas against a white (Lac$^-$) background.

in *lac* fusion strains (Weinstock et al., 1983; Silhavy and Beckwith, 1985). Such applications can be complicated if the *lac* genes transpose at high frequency from their original position within a fused gene to create additional *lacZ* fusions. We have previously shown that λp*lac*Mu insertions in the *E. coli* chromosome are very stable and rarely give rise to secondary transposition events, although these phages can transpose at an appreciable frequency upon infection into their host (Bremer et al., 1984; 1985).

The finding that λp*lac*Mu phages carrying the Mu*A'*am1093 allele no longer transpose by themselves upon infection in a Su$^-$ strain suggested that insertions of these phages should exhibit even greater genetic stability. We tested this by measuring the frequency of Lac$^+$ mutants arising from Lac$^-$ strains harboring *malK-lacZ* fusions. First, Lac$^+$ insertions of λp*lac*Mu5 (protein fusion) and λp*lac*Mu52 (operon fusion) were isolated in the *malK* gene in a Su$^-$ host using the λpMu507 helper phage. Expression of the fused genes in these strains was prevented by introducing with P1*vir* a *malT*::Tn*10* insertion; *malT* is the positive regulatory gene of the maltose regulon (Schwartz, 1987). As expected, the resulting Lac$^-$ strains gave rise to Lac$^+$ mutants at a lower frequency than their parent *A'* phages λp*lac*Mu1 and λp*lac*Mu50 (Table II). We suspect that the Lac$^+$ mutants found with the operon fusion phage in strain BRE1299 are not caused by transposition events but arise from other types of mutation. The higher frequency of Lac$^+$ mutants in the operon fusion strain is expected, since a larger category of events can lead to expression of the fused *lac* genes. The enhanced stability of λp*lac*Mu insertions containing the *A'*am1093 mutation should make them even more suitable than insertions of the parental phages for the isolation of mutants affecting the expression of the fused gene.

(e) Derivatives of λp*lac*Mu5 and λp*lac*Mu52 carrying a gene for kanamycin resistance

Since the λp*lac*Mu phages carrying the *A'*am1093 allele yield very stable *lacZ* fusions to chromosomal genes, they should be valuable genetic tools. To facilitate the isolation of λp*lac*Mu insertions, we introduced a gene (*kan*) which confers Km resistance into λp*lac*Mu5 and λp*lac*Mu52 (see MATERIALS AND METHODS, section **e**). This construction yielded λp*lac*Mu15 and λp*lac*Mu55, respectively. Both phages are unable to transpose by themselves in a Su$^-$ host but can insert into the *E. coli* chromosome upon coinfection with the λpMu507(*A*$^+$*B*$^+$) helper phage. When Km^R insertions of these new λp*lac*Mu phages were isolated in strain MC4100, λp*lac*Mu15 gave Lac$^+$ colonies with a frequency of 10–15%, while approx. 50% Lac$^+$ colonies were found with λp*lac*Mu55. Thus, as expected, a higher frequency of operon fusions than of protein fusions was found. These frequencies probably overestimate the true frequency of Lac$^+$ fusions since multiple insertions can occur under these conditions (Bremer et al., 1984; 1985). The different levels of *lac* expression found among fusion strains when they were streaked on several lactose indicator media strongly suggest that the *lac* operon was fused to different genes.

TABLE II

Stability of λp*lac*Mu prophages carrying the *A'* or the *A'*am1093 genes

Strain	Prophage[a]	Frequency of Lac$^+$ colonies[b]
BRE1047	φ(*malK-'lacZ*)hyb1002 (λp*lac*Mu1) *malT*::Tn*10*	3.5×10^{-8}
BRE1297	φ(*malK-'lacZ*)hyb1283 (λp*lac*Mu5) *malT*::Tn*10*	3.2×10^{-10}
BRE1167	φ(*malK-lacZ*$^+$)1113 (λp*lac*Mu50) *malT*::Tn*10*	1.7×10^{-7}
BRE1299	φ(*malK-lacZ*$^+$)1263 (λp*lac*Mu52) *malT*::Tn*10*	5.6×10^{-8}

[a] The λp*lac*Mu1 and λp*lac*Mu50 prophages carry the *A'* gene, while the λp*lac*Mu5 and λp*lac*Mu52 prophages encode the *A'*am1093 allele. These lysogens were originally isolated as Lac$^+$ protein (BRE1047, BRE1297) or operon (BRE1167, BRE1299) fusion strains. Expression of the *lac* fusions in all strains is prevented by a Tn*10* insertion in the positive regulatory gene (*malT*) of the maltose regulon.

[b] Strains carrying Lac$^-$ λp*lac*Mu insertions were grown in 0.4% glycerol-minimal medium overnight at 37°C. These cultures were titered on M63 glycerol plates for colony-forming units and on M63 lactose plates for Lac$^+$ derivatives. The plates were incubated for two days at 37°C. The values given are the mean values from two independent experiments.

Indeed, we have already used λ*plac*Mu55 to isolate *tsx-lacZ*$^+$ operon fusions (Bremer et al., 1988).

(f) Conclusions

Transposition of Mu DNA to random chromosomal sites is a multistep process that is dependent on the activity of the 663-aa A transposase. The data presented here show that the transposase function is retained by a mutant A protein (A′) missing its natural 60 C-terminal aa. Initiation of the transposition process requires the binding of the A protein to specific sequences in the *S*- and *c*-end attachment sites (Mizuuchi and Craigie, 1986), and the DNA binding domain has been localized to the N-terminal end of A (Nakayama et al., 1987; Betermier et al., 1987). Obviously, DNA binding activity is not abolished in the A′ protein but we do not know whether the overall activity of the A′ transposase is reduced since no λ*plac*Mu phage carrying an intact *A* gene is available. However, an analysis of the properties of several A proteins with deletions at their C-terminal ends has shown that a deletion of 47 aa reduces Mu transposition about ten-to 20-fold compared to wild-type A protein (Harshey and Cuneo, 1986). In the same study it was also shown that transposase activity was abolished by the removal of approx. 75 aa but not of 55 aa. Thus the λ*plac*Mu phages contain the shortest functional *A* segment yet reported. As previously mentioned, the *A′* gene is fused to a DNA segment from phage λ containing an open reading frame for 59 aa. These aa are not homologous to the natural C-terminal end of the Mu transposase but possibly could contribute to the stability of the A′ protein.

When the *A*am1093 mutation was crossed into the *A′* gene a series of new λ*plac*Mu phages was obtained that cannot transpose by themselves in a Su$^-$ strain; however, efficient transposition of these phages can be conveniently triggered by coinfection with the *A*$^+$*B*$^+$ λpMu507 helper phage. The enhanced genetic stability of *lac* fusions isolated with these new λ*plac*Mu phages is particularly important when selections are applied to isolate mutants with altered regulation of the fused gene.

ACKNOWLEDGEMENTS

We thank M. Berman, M. Howe and R. Zagursky for providing strains and bacteriophages and S. Beck for advice on DNA sequencing. We are grateful to D. Kamp for the communication of data on the Mu*A* sequence prior to publication. We thank M. Manson and G. Sweet for critical reading of the manuscript, V. Koogle for help in preparing the manuscript, and A. Middendorf for technical assistance. E.B. thanks W. Boos for his support. This work was supported in part by the National Cancer Institute, Department of Health and Human Services, under Contract No. N01-CO-23909 with Litton Bionetics, and in part by the Deutsche Forschungsgemeinschaft through SFB 156. E.B. was the recipient of a fellowship from the Deutsche Akademische Austauschdienst.

REFERENCES

Austin, S. and Abeles, A.: Partion of unit-copy mini plasmids to daughter cells. J. Mol. Biol. 163 (1983) 353–372.

Beck, S. and Pohl, F.M.: DNA sequencing with direct blotting electrophoresis. EMBO J. 3 (1984) 2905–2909.

Berman, M.L. and Beckwith, J.: Fusions of the lac operon to the transfer RNA gene *tyrT* of *Escherichia coli*. J. Mol. Biol. 130 (1979) 285–301.

Betermier, M., Alazard, R., Ragueh, F., Roulet, E., Toussaint, A. and Chandler, M.: Phage Mu transposase: deletion of the carboxy-terminal end does not abolish DNA-binding activity. Mol. Gen. Genet 210 (1987) 77–85.

Bremer, E., Silhavy, T.J., Weisemann, J.M. and Weinstock, G.M.: λ*plac*Mu: a transposable derivative of phage lambda for creating *lacZ* protein fusions in a single step. J. Bacteriol. 158 (1984) 1084–1093.

Bremer, E., Silhavy, T.J. and Weinstock, G.M.: Transposable λ*plac*Mu phages for creating *lacZ* operon fusions and kanamycin-resistance insertions in *Escherichia coli*. J. Bacteriol. 162 (1985) 1092–1099.

Bremer, E., Gerlach, P. and Middendorf, A.: Double negative and positive control of *tsx* expression in *Escherichia coli*. J. Bacteriol. 170 (1988) 108–116.

Bolivar, F., Rodriguez, R.L., Green, P.J., Betlach, M., Heyneker, H.L., Boyer, H.W., Crosa, J.H. and Falkow, S.: Construction and characterization of new cloning vehicles, II. A multipurpose cloning system. Gene 2 (1977) 95–113.

Bukhari, A.I. and Ljungquist, E.: Bacteriophage Mu: methods for cultivation and use. In Bukhari, A.I., Shapiro, J.A. and

Adhya S.L. (Eds.), DNA Insertion Elements, Plasmids, and Episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1977, pp. 749–756.

Bukhari, A.I. and Zipser, D.: Random insertion of Mu-1 DNA within a single gene. Nature New Biol. 136 (1972) 240–243.

Casadaban, M.J.: Fusion of the *Escherichia coli lac* genes to the *ara* promoter: a general technique using bacteriophage Mu.1 insertions. Proc. Natl. Acad. Sci. USA 72 (1975) 809–813.

Casadaban, M.J.: Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol 104 (1976) 541–555.

Casadaban, M.J. and Chou, J.: In vivo formation of gene fusions encoding hybrid β-galactosidase proteins in one step with transposable Mu-*lac* transducing phage. Proc. Natl. Acad. Sci. USA 81 (1984) 535–539.

Casadaban, M.J. and Cohen, S.N.: Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: in vivo probe for transcriptional control sequences. Proc. Natl. Acad. Sci USA 76 (1979) 4530–4533.

Castilho, B.A., Olfson, A. and Casadaban, M.J.: Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol 158 (1984) 488–495.

Daniell, E., Roberts, R. and Abelson, J.: Mutations in the lactose operon caused by bacteriophage Mu. J. Mol. Biol. 69 (1972) 1–8.

Daniels, D.L., Schroeder, J.L., Szybalski, W., Sanger, S., Coulson, A.R., Hong, G.F., Hill, D.F., Petersen, G.B. and Blattner, F.R.: Complete annotated lambda sequence. In Hendrix, R.W., Roberts, J.W., Stahl, F.W. and Weisberg, R.A. (Eds.), Lambda II, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1983, pp. 519–576.

Debouck, C., Riccio, A., Schumperli, D., McKenny, K., Jeffers, J., Hughes, C., Rosenberg, M., Heusterspreute, M., Brunel, F. and Davison J.: Structure of the galactokinase gene of *Escherichia coli*, the last (?) gene of the *gal* operon. Nucl. Acids Res. 13 (1985) 1841–1853.

Harkki, H., Karkku, H. and Palva, E.T.: Use of λ vehicles to isolate *ompC-lacZ* gene fusions in *Salmonella typhimurium* LT2. Mol. Gen. Genet. 209 (1987) 607–611.

Harshey, R.M. and Cuneo, S.D.: Carboxyl-terminal mutants of phage Mu transposase. J. Genet. 65 (1986) 159–174.

Harshey, R.M., Getzoff, E.D., Baldwin, D.L., Miller, J.L. and Chaconas, G.: Primary structure of phage Mu transposase: homology to Mu repressor. Proc. Natl. Acad. Sci. USA 82 (1985) 7676–7680.

Leathers, T.D., Noti, J. and Umbarger, H.E.: Physical characterization of *ilv-lac* fusions. J. Bacteriol. 140 (1979) 251–260.

Magazin, M., Howe, M. and Allet, B. Partial correlation of the genetic and physical maps of bacteriophage Mu. Virology 77 (1977) 677–688.

Magazin, M., Reeve, J.N., Maynard-Smith, S. and Symonds, N. Bacteriophage Mu encoded polypeptides synthesised in infected minicells. FEMS Microbiol. Lett. 4 (1978) 5–9.

Maniatis, T., Fritsch, E.F. and Sambrook, J.: Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982.

Messing, J.: New M13 vectors for cloning. Methods Enzymol. 101 (1983) 20–78.

Miller, J.H.: Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1972.

Mizuuchi, K. and Craigie, R.: Mechanisms of bacteriophage Mu transposition. Annu. Rev. Genet. 20 (1986) 385–429.

Nag, D.K. and Berg, D.E.: Specificity of bacteriophage Mu excision. Mol. Gen. Genet. 207 (1987) 395–401.

Nakayama, C., Teplow, D.B. and Harshey, R.M.: Structural domains in phage Mu transposase: identification of the site-specific DNA-binding domain. Proc. Natl. Acad. Sci. USA 84 (1987) 1809–1813.

Norrander, J., Kempe, T. and Messing, J.: Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene 26 (1983) 101–106.

O'Day, K.J., Schultz, D.W. and Howe, M.M.: Search for integration-deficient mutants of bacteriophage Mu. In Schlessinger, D. (Ed.) Microbiology—1978. American Society for Microbiology, Washington, DC, 1978, pp. 48–51.

Priess, H., Kamp, D., Kahmann, R., Bräuer, B. and Delious, H.: Nucleotide sequence of the immunity region of bacteriophage Mu. Mol. Gen. Genet. 186 (1982) 315–321.

Priess, H., Bräuer, B., Schmidt, C. and Kamp, D.: Sequence of the left end of Mu. In Symonds, N., Toussaint, A., Van de Putte, P. and Howe, M. (Eds.), Phage Mu. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1987, pp. 277–296.

Sanger, F., Nicklen, S. and Coulson, A.R.: DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74 (1977) 5463–5467.

Sanger, F., Coulson A.R., Hong, G.F., Hill, D.F. and Petersen, G.B.: Nucleotide sequence of bacteriophage λDNA. J. Mol. Biol. 162 (1982) 729–773.

Schwartz, M.: The maltose regulon. In Neidhardt, F.C. (Ed.), *Escherichia coli* and *Salmonella typhimurium*. American Society for Microbiology, Washington, DC, 1987, pp. 1482–1502.

Shpakovski, G.V. and Berlin, Y.: Site-specificity of abnormal excision: the mechanism of formation of a specialized transducing bacteriophage λ*plac*5. Nucleic Acids Res. 12 (1984) 6779–6795.

Silhavy, T.J. and Beckwith, J.R.: Uses of *lac* fusions for the study of biological problems. Microbiol. Rev. 49 (1985) 398–418.

Silhavy, T.J., Berman, M.L. and Enquist, L.W.: Experiments with Gene Fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1984.

Soberón, X., Covarrubias, L. and Bolivar, F.: Construction and characterization of new cloning vehicles. Gene 9 (1980) 287–305.

Taylor, A.L.: Bacteriophage-induced mutation in *E. coli*. Proc. Natl. Acad. Sci. USA 50 (1963) 1043–1051.

Toussaint, A. and Resibois, A.: Phage Mu: transposition as a life style. In Shapiro, A. (Ed.), Mobile Genetic Elements. Academic Press, New York, 1983, pp. 105–158.

Toussaint, A., Desmet, L., Faelen, M., Alazard, R., Chandler, M. and Pato, M.: In vivo mutagenesis of bacteriophage Mu transposase. J. Bacteriol. 169 (1987) 5700–5707.

Trun, N.J. and Silhavy, T.J.: Characterization and in vivo cloning of *prlC*, a suppressor of signal sequence mutations in *Escherichia coli* K12. Genetics 116 (1987) 513–521.

Weinstock, G.M., Berman, M.L. and Silhavy, T.J.: Chimeric genetics with β-galactosidase. In: Papas, T.S., Rosenberg, M. and Chirikjian (Eds.), Gene Amplification and Analysis, Vol. 3. Elsevier, New York, 1983, pp. 23–64.

Communicated by J. Davison.