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INTERNAL ECORI-GENERATED DELETION IN PROPHAGE MU DNA

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Summary : The hybrid plasmid consisting of the plasmid pRP1.2 (derivative of RP4) genome and deleted prophage Mucts 62 genome which lost the central EcoRI fragment of DNA was constructed. The ability of deleted Mu phage to carry out E. coli chromosomal genes transposition was still retained.

Introduction

Bacteriophage Mu is known to have a unique property of integrating nonspecifically its DNA into genome of its host bacteria (1) being of particular interest for construction of gene-integrating vectors. However genetic engineering on Mu DNA is quite difficult because of 1) the low frequency of Mu DNA transfection (2), 2) the presence of more than one cleavage site for restriction endonucleases EcoRI, Hind III, BamHI and other (3), 3) the lack of suitable methods for selection of Mu particles carrying foreign DNA insertions, 4) the headful mechanism of Mu DNA packing (4). One approach allowing to overcome these difficulties is using prophage Mu DNA inserted into a plasmid (it is not difficult to obtain such hybrid in vivo (5)) instead of manipulating Mu DNA directly. This was applied to generate the internal EcoRI fragment deletion in Mu DNA.

Material and Methods

Bacterial and Mu phage strains utilized were : E. coli K12 JC411 (pRP1.2). The plasmid pRP1.2 (Tc^rTra^+) kindly given by V. N. Danilevich is a RP4 plasmid derivative (6). Phage Mu cts62, Mu amber mutants used for the marker rescue experiments Mucts 62 am A, B, C, lys and Muc^+ am H, M, R were received from A. L. Taylor, M. Howe and A. Toussaint, correspondingly. Mu phage mediated transposition of E. coli genes was carried out as it was described by Faelen and Toussaint (7). Plasmid DNA was prepared as described by Figurski et al. (8). Electron microscope heteroduplex analysis was carried out following Davis et al. (9).

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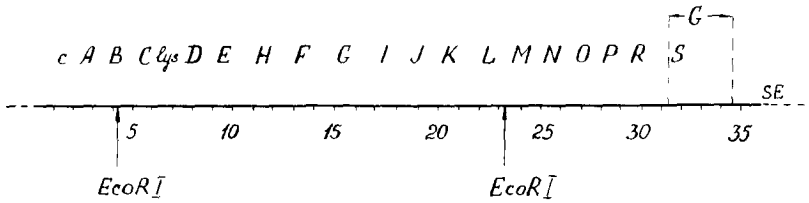

Fig. 1. Location of Eco RI endonuclease cleavage sites in Mu phage DNA (5). The letters indicate the corresponding Mu phage genes. G segment and bacterial DNA at Mu DNA ends are shown by dashed lines. The distances between the genes are not given to scale.

Results and discussion

Plasmid pRP1.2 was chosen as a "vector" because its DNA had no EcoRI cleavage sites which facilitated isolation of the internal EcoRI fragment deletion in Mu genome (as shown in fig. 1, EcoRI cuts Mu DNA in 2 sites between genes B and C and L and M). pRP1.2:: Mucts 62 plasmids were obtained in the following way. At first hybrid plasmids containing E. coli pro genes and two identically oriented Mu prophages were selected by Mu-mediated transposition of E. coli chromosomal genes onto plasmid pRP1.2. Plasmids pRP1.2:: Mucts 62 were obtained from these hybrid plasmids after their transfer into Rec^+ recipient (such hybrid are known to loose the transposed fragment and one of the Mu prophages in Rec^+ bacteria with a frequency of about 10% through recombination between two Mu genomes).

Then the pRP1.2:: Mucts 62 plasmid DNA was cleaved with EcoRI endonuclease and ligated using phage T4 polynucleotide ligase. The ligation was carried out at low DNA concentrations to increase the yield of circular EcoRI-fragments. Ligated DNA was transformed into E. coli K12 Mucts 62 am C lysogen MH 408 and clones not able to release phage particles on a lawn of Su^- bacteria at 42°C were picked out among the Tc^r transformants. Such clones were considered as possible candidates that would contain plasmids without prophage DNA internal EcoRI-fragment. Plasmids from these clones were tested in marker rescue experiments for the presence of Mu phage genes located within and outside the fragment after their transfer into appropriate recipients.

One of the clones which were supposed to contain no genes situated within the fragment, was further analysed. By electron microscopy of

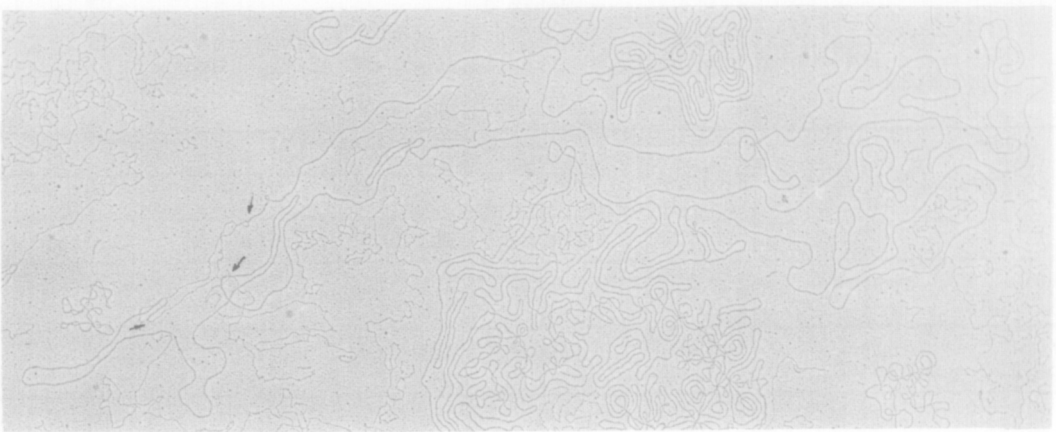

Fig. 2. Heteroduplex DNA molecule with G-bubble. The places where the single strands go away are marked with arrows.

heteroduplexes between supercoiled DNA from this clone and of plasmid pRP1.2:: Mucts 62 (fig. 2) it was shown that the deletion size was $18,9 \pm 1,7$ kb and the distance from the deletion site to G-segment was $8,2 \pm 0,1$ kb. Also, molecules with G-bubbles were found in the population of deleted homoduplex DNA molecules.

To test the ability of the deleted Mu phage to integrate into bacterial DNA we determined the frequency of Mu-mediated transposition of E. coli chromosomal genes onto plasmid Flac. For this purpose the E. coli KMBL 1910 (Flac) strain was transformed by DNA of the plasmid obtained. (The frequency of Tc^r transformants was about 10^4-fold lower than that in case of lysogenic recipients, apparently due to the presence of Mu kil gene (10)). This strain was used as the donor for transposition experiments (Table 1). The transposition frequencies were the same for deleted and intact phage as evident from table 1. It indicates that phage Mu lacking internal EcoRI-fragment is still able to carry out (at least partially) its integrative functions.

We suppose that the obtained plasmid might be suitable for studying "vector" properties of Mu phage taking the following into account : 1) the location of a single EcoRI-cleavage site within Mu-component of the plasmid allows one to use plasmid DNA for the insertion of foreign DNA fragment in Mu phage genome, 2) the introduction of Mu DNA carrying foreign insertion into recipient cells is facilitated by the possible use of a bacterial conjugation

Table 1

Recombinant's frequency in crosses between the derivatives of KMBL 1910 strain after heat induction of the prophage and AB2463 (Mucts 62) recipient

Donor	Transfer frequency of F'lac plasmid per a donor cell	Recombinant's frequency per a transferred F'lac plasmid			
		after prophage heat induction		without prophage heat induction	
		His^+Lac^+	Thr^+Lac^+	His^+Lac^+	Thr^+Lac^+
KMBL 1910(Mucts 62) (F'lac)	$1\text{-}5 \times 10^{-1}$	$3\text{-}4\times10^{-4}$	$1,5\text{-}3\times10^{-4}$	$1\text{-}5\times10^{-7}$	$5\times10^{-7}\text{-}10^{-6}$
KMBL 1910 (F'lac, pRPl.2:: Mucts 62)	$1\text{-}5 \times 10^{-1}$	$3\text{-}4\times10^{-4}$	$1,5\text{-}3\times10^{-4}$	5×10^{-7}	5×10^{-7}

and plasmid DNA transformation procedure, 3) it may be supposed that phage Mu will integrate into host DNA after substitution of the internal EcoRI DNA fragment by foreign DNA because phage Mu without the fragment still showed this feature.

REFERENCES

1. Howe,M.M. and Bade, E.G.(1975) Science 190, 624-633.
2. Kahmann, R., Kamp, D. and Zipser, D.(1976) Molec. Gen.Genet. 149, 323-328.
3. Allet, P., Blatner , F., Howe, M.M., Magazin, M., Moor, D., O'Day, K., Schultz, D. and Schumm, J. (1977) In DNA Insertion Elements, Plasmids and Episomes, Bukhari. A.I., Shapiro, J.A. and Adhya S.L., eds. pp. 745-748, Cold Spring Harbor Laboratory,N.Y.
4. Bukhari, A.I. and Taylor, A.L. (1975) Proc. Nat.Acad. Sci. USA 72, 4399-4403.
5. Denarie,J., Rosenberg, C., Bergeron, B., Boucher, C., Michel,M. and Barate de Bertalmio,M.(1977) in DNA Insertion Elements, Plasmids and Episomes, Bukhari A.I., Shapiro, J.A. and Adhya, S.L., eds. pp. 507-521, Cold Spring Harbor Laboratory, N.Y.
6. Danilevich, V.N., Safarjan,V.S. and Amosencko, F.A. (1980) Genetika in press.
7. Faelen, M. and Toussaint, A.(1976) J.Mol.Biol. 104, 525-539.
8. Figurski, D., Meyer, R., Miller, D.S. and Helinski, D.R. (1976) Gene 1, 107-119.
9. Davis, R.W., Simon,M. and Davidson,N. (1971) in Meth. in Enzymol vol. 21, Grossman,L. and Moldave,K., eds. pp. 413-428, Academic Press, N.Y.
10. Schumann,W., Bade, E.G., Delius, H. and Hübert,P. (1978) Mol. Gen. Gent. 160, 115-118.