Insertion of a Tetracycline Resistance Marker into Bacteriophage Mu

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Introduction :

Like any other bacteriophage, Mu adsorbs to the outer surface of its host cell and injects its genetic material which will inserts in the host chromosome, and either stay there as a prophage or start transposing to replicate itself, leading to the lysis of the cell. This particular lytic mode, transposition as a way of replicating, is what mates Mu a peculiar virus. Its DNA behaves as a giant transposon (39 kb long) hoping from place to place in the host chromosome, leaving each time a copy of itself at every insertion site. This characteristic has made of phage Mu a focus of research interest for those who are studying the transposition phenomenon.

One of the problem in using Mu as a probe in studies of "jumping genes" is its lack of markers. Leach and Symonds(1) have already introduced a fragment of Tn 3 conferring Ampicillin resistance to Mu, but one marker is still not sufficient, if one is to work with Ampicillin resistant strain for instance. Another use for markers is for the detection of Mu lysogens, which are difficult to screen.

Material :

Transposon : Tetracycline resistance was the chosen marker because it is carried by a mini transposon, mini-Tn 10, only 2.3 kb long. Mini-Tn 10 is the result of deletions 16 and 17 in Tn 10, a transposon 9.3 kb long (2). Mini-Tn 10 is made of the Tetracycline resistance gene, flanked by two inverted insertion

sequences that allow it to hop. The hoping is under the control of a gene responsible for the synthesis of the enzyme transposase, which is itself under the control of tac operon, induced by isopropyl thiogalactopyranoside (IPTG). The transposase gene is found in Tn 10 but not in mini-Tn 10, where it has been excised and reinserted near the mini transposon, so that when jumping it does not take the gene with it, and therefore stays in the new insertion site.

Bacteria : Different strain of Escherischia coli were used : NK5830 and W1485 as recipients, mH4372 as the original Mu lysogen.

Phages : A coliphage Lambda carrying mini-Tn10, the gene responsible for tranposase synthesis, and the tac operon was available. The Mu used as a target was a thermoinducible mutant and had part of its G loop and the mom gene deleted.

Medium : Tryptone Broth medium was used throughout : 10 gr/l bacto-tryptone, 5 gr/l NaCl. Ten gamma of Tetracycline were added to T.B when required.

Methods : 3-4 drops of Lambda:mini-Tn 10 were applied to a lawn of NK5830 Tet^S and incubated at 37°c ; lysogens were picked up from the cloudy center of the plaques and found to be Tet^R, indicating that they were Lambda:mini-Tn10 lysogens. A Mu lysate was obtained from mH4372 by growing the strain up to $2x10^8$ cells per ml at 30°c and then exposing it to 42°c for 45 mn. 3-4 drops of Mu were applied to a lawn of the NK5830 Tet^R strain made up above, to obtain lysogens containing Mu and Lambda: mini-Tn10. This strain was then grown with IPTG up to 2×10^8 to induce the hoping of mini-Tn10 (fig 1).

Mu lysates were obtained from the strain by exposing it to 42°c (the Lambda used was not thermoinducible) ; 10 ml of W1485 grown up to 5×10^8 were centrifugated at 10 000 rpm for 10 mn

Lambda:mini-Tn10 lysogens. $CaCl_2$ was added up to 5 mM and the Mus were allowed to adsorb for 30 mn at 30°c. The cells were then spinned down, resuspended in 10 ml of 1.0 M sodium citrate, and plated on 10 gamma Tet T.B plates.

Results and discussion :

The goal of this experiment was to introduce the mini-Tn10 into Mu without damaging any of its "vital" functions. The probability of this happening was low, since in a cell there was one mini-Tn10 that would hop only once. Considering that Mu was a 39 kb target lost in the 3000 kb genome of E. Coli, it can be understood that it took quite a few plates to finally obtain the strain with the desired characteristics.

Such a strain was observed ; it was Tet^R, temperature sensitive, Lambda sensitive, and Mu immune. The fact that the strain was temperature sensitive and Mu immune indicates that the organism is a Mu lysogen ; the Lambda sensitivity shows that the Tetracycline resistance was not conferred by Lambda:mini-Tn10. To confirm that the Tetracycline resistance was actually due to a Mu:mini-Tn10 (named AMu), lysates were obtained from the AMu lysogen and used to infect a W1485 Tet^S strain : Tet^R was conferred.

A lysate could be obtained by exposing AMu lysogens to 42°c, but it took a longer time to have lysis compared to the original thermoinducible mutant. When streaked on plate and incubated at 42°c, a few AMu were able to grow. This phenomenon was observed from every "second generation" AMu lysogen : when a single colony was picked up and incubated at 42°c, a few colonies were growing.

This might be a hint as to where the mini-Tn10 is inserted : near the gne responsible for the thermoinducibility.

I have tried to extract Mu DNA to use restriction endonucleases

(EcoRI and BamHI) so as to determine the exact site of insertion (fig 2). Running out of time I have used a "rapid small-scale procedure for isolation of phage Lambda DNA"(3). Unfortunately this method did not seem to work, probably because of an insufficient phage titer (Mu is particularly difficult to titer).

In conclusion, a Tetracycline marker has been inserted in bacteriophage Mu, but further work is needed to determine where in Mu mini-Tn10 is.

REFERENCES

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(3) Benson, Spencer A. , and Taylor, Ronald K. : A Rapid Small-Scale Procedure for Isolation of Phage Lambda DNA. Bio Techniques. May/June 1984.

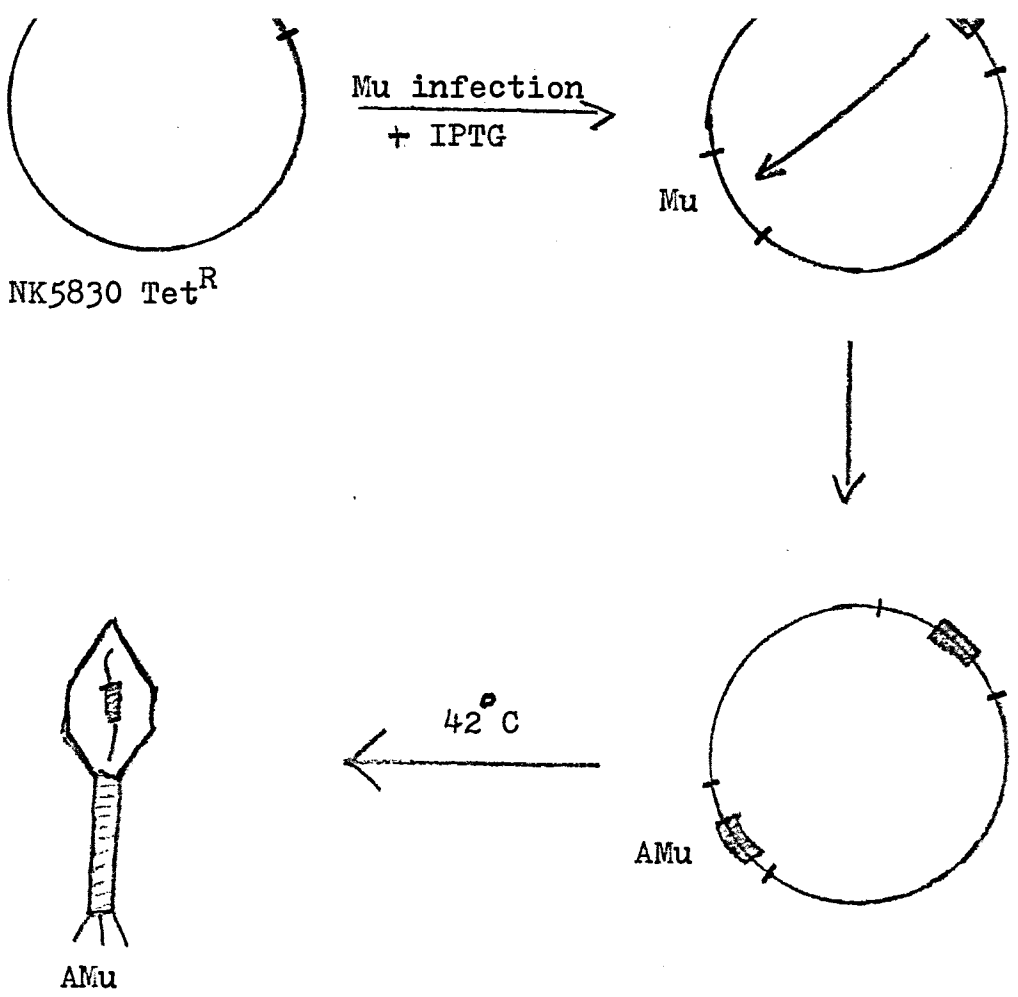

Figure 1: schematic representation of bacterial chromosomal DNA (although Lambda:mini-Tn10 might as well have inserted in a plasmid) showing the incorporation of the Tet^R from Lambda into Mu.

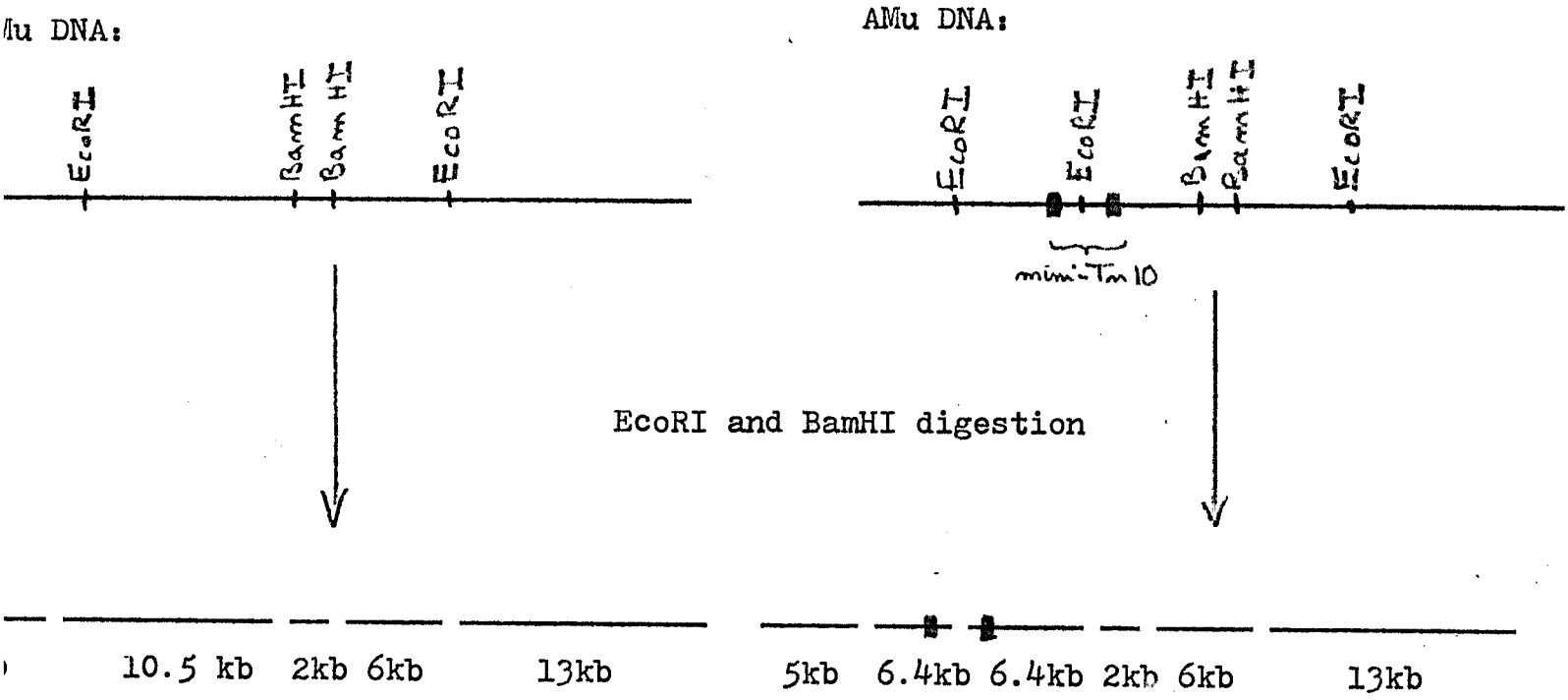

Figure 2:Mu DNA has two digestion sites for EcoRI and two for BamHI;AMu DNA has one more for EcoRI ,which is found in mini-Tn10.One should therefore be able to determine the insertion site of mini-Tn10 by running the samples of digested DNA through a gel (on this figure an arbitrary site has been chosen for the mini-transposon).