General Method for the Isolation of Conditional Lethal Mutants in Any Required Region of the Virus Genome: Its Application to the 'Semi-essential' Region of Phage Mu

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A general method is presented that enables viral mutants to be isolated in any required region of their genome. An application of the method is reported relating to the isolation of conditional lethal mutants in the 'semi-essential' region of phage Mu. Some properties are described of one mutant found in this way, Mu_{cts₆₂SEEam8Apl}, which is typical of a class of mutants that were isolated.

INTRODUCTION

It is often required to isolate viral mutants with mutations in a particular genome region. Here we describe a method which allows this to be done. The only requirement is that the desired region can be cloned into a plasmid and that the viral genes it contains are expressed in cells that are hosts for the growth of the virus. The method consists of mutagenizing a stock of the virus and then infecting host cells that contain the relevant plasmid. Only mutations in those viral genes expressed by the viral fragment on the plasmid will be complemented – other viral mutations will not, and hence upon infection the only mutants affected in essential genes that will be able to multiply will be those with mutations in the required region. Subsequently, these mutants can be distinguished from the wild-type virus by testing the released virus on indicator strains with and without the particular plasmid.

We describe the application of this method to the selection of phage mutants in the 'semi-essential' region of coliphage Mu (for a review see Toussaint & Resibois, 1983). This region lies between 5 and 10 kb at the left or *c* end of the genome (Fig. 1) and plays an intriguing role in the Mu life cycle. The 'semi-essential' genes are read from the same early transcript as the early genes *A* and *B*, but are not absolutely necessary for Mu transposition, even though they modify the synthesis of Mu DNA in different ways (Goosen *et al.*, 1982; Waggoner *et al.*, 1981). Little is known about the genes of this region. Previously only one report has been published of conditional lethal mutants mapping in this region. This refers to a class of temperature-sensitive mutants in the so-called *lig* gene, whose product is expressed constitutively and is able to complement the *lig* gene of *Escherichia coli* K12 (Paolozzi *et al.*, 1980). The Mu *lig* protein is apparently involved at some stage in the integration step which precedes Mu replication.

METHODS

Bacteria and phages. These are listed in Table 1.

Mutant isolation procedure. A 50 ml Luria broth (LB) culture of an *E. coli* K12 strain that is (a) Sup⁺ not permissive for amber mutations, (b) lysogenic for Mu_{cts₆₂Apl}, and (c) carries the plasmid pKN165 (see Fig. 1), was grown at 30 °C to a concentration of 2×10^8 ml⁻¹ and then treated with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (Sigma) as described previously (Paolozzi *et al.*, 1980). The culture was then diluted 100-fold into LB and divided into five parts; these five cultures were allowed to grow at 30 °C to stationary phase. Each culture was then diluted and plated onto L-plates at 30 °C to give approximately 100 colonies per plate. The colonies were

Fig. 1. Simplified map of the Mu genome. The 'semi-essential' early region (SEE) lies between genes *B* and *C*. The pKN plasmids (which were gifts of W. Schumann) are derivatives of pBR325 (Bolivar *et al.*, 1977). pKN50 contains the Mu fragment lying between the left-end *EcoRI* and *BamHI* sites, indicated in the figure (Schumann *et al.*, 1980). pKN165 (constructed by T. Baron) was derived from pKN50 by deleting the internal *HindIII* fragment. pKN165 does not complement mutations in either the *C* or *lys* genes of Mu (contrary to pKN50), but it does complement the Mu *ligts2* mutation.

Table 1. *Bacteria and phages*

<i>E. coli</i> strain	Source or reference
EB 105 Sup ⁺	Schumann <i>et al.</i> (1980)
EB 105 Sup ⁺ /pKN165	Provided by W. Schumann
EB 105 (Mu <i>cts</i> ₆₂ Ap1)/pKN165	This work
RS 54 Sup ⁺	Calef collection
U706 Sup ⁺	
U707 <i>supD</i>	
U708 <i>supE</i>	
U709 <i>supF</i>	
U710 <i>supC</i>	
U711 <i>supG</i>	
U712 <i>supP</i>	
U713 <i>supU</i>	
C600 <i>supE</i>	
U706/pKN165	This work
GC 375 Sup ⁻ RecA ⁻	Provided by J. C. Liebart
GC 375 Sup ⁻ RecA ⁻ /pKN165	This work
Phage	
Mucts ₆₂ Ap1	Leach & Symonds (1979)
Mucts ₆₂ SEEam8 Ap1	This work

replica-plated in duplicate: (i) to preserve the colonies, onto L-plates incubated at 30 °C; (ii) to test for the phage produced by the lysogenic colonies, onto L-plates incubated at 42 °C and seeded with mixed indicator containing Sup⁺ and Sup⁺/pKN165 bacteria, in a ratio of 1 : 2. Any Mu mutants in the region covered by pKN165 could easily be identified as they give turbid plaques on the mixed indicator. Colonies from the master plate corresponding to the turbid plaques were resuspended in 2 ml LB and grown to 5×10^7 ml⁻¹ at 30 °C, and then the cultures were shifted to 42 °C to obtain phage lysates. These lysates were retested by spotting directly onto plates seeded with either Sup⁺ or Sup⁺/pKN165 bacteria, and a further distinction between amber and non-amber mutants was made by spotting on Sup⁻ and Sup⁻/pKN165 bacteria.

Table 2. *E.o.p.* and burst size of *Mucts*₆₂*SEEm8Apl* on strains with different amber or ochre suppressors

The e.o.p. was tested by plating 0.1 ml from a dilution series of *Mucts*₆₂*SEEm8Apl* onto plates seeded with different indicators. The best of the suppressors is *supF*, on the basis of both the number of plaques observed and the sizes of the plaques, which were comparable to those observed on U706/pKN165. To measure burst size, bacteria (1×10^8 ml⁻¹) were infected at an m.o.i. of 0.25. After 13 min at 37 °C, non-adsorbed phage were eliminated by two successive centrifugations and the pellet resuspended at 10^8 cells ml⁻¹ and treated for 2 min with anti-Mu serum (fractional rate of inactivation, $K = 300$). The culture was then diluted 10^4 -fold into LB at 37 °C. A sample was tested immediately on strain U706/pKN165 to determine the number of infective centres (*A*). The rest of the culture was aerated at 37 °C for a further 60 min and then lysed with CHCl₃. The lysate was plated on L-plates seeded with U706/pKN165 (*B*). The burst size is given by the ratio *B/A*. The results are means of three independent experiments. NT, Not tested.

<i>E. coli</i> strain	E.o.p.	Burst size
U706 Sup ⁺	5×10^{-5}	0.8
U706/pKN165	1	200
U707 <i>supD</i>	8×10^{-1}	NT
U708 <i>supE</i>	7×10^{-1}	NT
U709 <i>supF</i>	1	23
U710 <i>supC</i>	$< 10^{-4}$	< 1
U711 <i>supG</i>	$< 10^{-3}$	< 1
U712 <i>supP</i>	7×10^{-1}	NT
U713 <i>supU</i>	$< 10^{-3}$	< 1

RESULTS AND DISCUSSION

Results from the isolation procedure described showed that approximately 0.5% of the total colonies examined after mutagenesis yielded mutants that were complemented by pKN165. About 20% of these mutants were stringent, in that they only produced plaques on bacteria carrying plasmid pKN165; the other 80% were leaky and made tiny plaques on the plasmid-free indicator. Among the 64 mutants isolated in this series, 50 were amber mutants; some preliminary results concerning these are reported below.

In order to eliminate any effect of recombination between the Mu lysogens and the Mu region of pKN165, the amber mutants were first repurified by growing lysates in a RecA⁻Sup⁻/pKN165 host. The presence of the plasmid is necessary due to the poor suppression of the RecA⁻Sup⁻ strain. Of the 50 mutants, eight were stringent, their e.o.p. being reduced by a factor of 10^4 to 10^5 in the absence of the plasmid (see results for *Mucts*₆₂*SEEm8Apl* in Table 2), and the others were leaky, with an e.o.p. close to 1. The method itself should not favour the selection of leaky mutants; the results obtained are probably due to the 'semi-essential' character of some of the genes in this region (Waggoner *et al.*, 1981; Goosen *et al.*, 1982). The eight stringent amber mutants (of which at least five must be independent) were shown to be in a single complementation group. Complementation tests between the leaky and stringent mutants are difficult to perform and it is not known whether these leaky mutants fall into other complementation groups.

One of the stringent amber mutants, *Mucts*₆₂*SEEm8Apl*, was tested in more detail (Table 2). This mutant had a latent period of 60 min and a burst size of about 200 in the host Sup⁺/pKN165, and made normal plaques on this strain. Its efficiency of plating on the *supD*, *supE*, *supF* and *supP* strains was the same as that on the Sup⁺/pKN165 host, although on all these Sup⁻ strains the plaques were smaller. On the *supC*, *supG* and *supU* strains the burst size was less than 1, as it was on the plasmid-free Sup⁺ strain. Among the Sup⁻ set of strains the largest plaques were found on the *supF* host, on which the burst size in lytic infection was reduced to about 25. Under conditions where *Mucts*₆₂*Apl* gave a lysogenization frequency of 10^{-1} , the lysogenization frequency with *Mucts*₆₂*SEEm8Apl* was only 10^{-4} (tested on strains C600, RS 54, U709 and U706). Interestingly, this frequency was unaffected by whether the host strain was Sup⁻ or Sup⁺ (for technical reasons the lysogeny experiments could not be performed on strains carrying plasmid pKN165).

As the only type of conditional lethal mutation that has been described previously in this region of the Mu genome is located in the *lig* gene, we did complementation tests between Mu_{cts62}SEEam8Apl and the mutant Mu_{ligts2} which lies within the *lig* gene. No complementation was observed in these tests, although normal complementation was found between am8 and phage having an amber mutation in gene *C*. Work is in progress to confirm that the am8 mutation is in fact in the *lig* gene.

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