Precise excision of bacteriophage Mu DNA

Chiraz Abbas, Guennadi Sezonov, Danièle Joseleau-Petit, Richard D'Ari, and Jean-Claude Liébart

Abstract: The temperate bacteriophage Mu is a transposable element that can integrate randomly into bacterial DNA, thereby creating mutations. Mutants due to an integrated Mu prophage do not give rise to revertants, as if Mu, unlike other transposable elements, were unable to excise precisely. In the present work, starting with a lacZ::Muc62(Ts) strain unable to form Lac⁺ colonies, we cloned a lacZ⁺ gene in vivo on a mini-Mu plasmid, under conditions of prophage induction. In all lac⁺ plasmids recovered, the wild-type sequence was restored in the region where the Mu prophage had been integrated. The recovery of lacZ⁺ genes shows that precise excision of Mu does indeed take place: the absence of Lac⁺ colonies suggests that precise excision events are systematically associated with loss of colony-forming ability.

Key words: transposable element, Escherichia coli, mini-Muduction.

Résumé : Le bactériophage tempéré Mu est un élément transposable qui s'intègre au hasard dans l'ADN bactérien, créant ainsi des mutations. Les mutants résultant d'un prophage Mu intégré ne réversent pas, comme si Mu, à la différence d'autres éléments transposables, était incapable de s'exciser précisément. Dans le présent travail, à partir d'une souche lacZ::Muc62(Ts) incapable de donner des colonies Lac⁺, nous avons cloné un gène lacZ⁺ en vivo sur un plasmide mini-Mu, dans des conditions d'induction du prophage. Dans tous les plasmides lac⁺ récupérés, la séquence du gène sauvage a été restaurée dans la région où le prophage Mu avait été intégré. L'obtention de gènes lacZ⁺ démontre que l'excision précise de Mu a effectivement lieu; l'absence de colonies Lac⁺ suggère que les événements d'excision précise sont systématiquement associés à la perte de la capacité à former une colonie.

Mots clés : élément transposable, Escherichia coli, mini-Muduction.

Introduction

Transposable genetic elements, through promiscuous recombination, are at the origin of major genetic changes in all organisms (Berg and Howe 1989). They can inactivate genes by interrupting coding sequences, they can activate genes by providing a promoter or enhancer sequence, and they can create deletions, inversions, and other rearrangements. The insertion of a transposable element into a DNA sequence requires the ends of the element and the corresponding transposase, but little or no sequence specificity in the target DNA. In general, transposable elements can also disappear from a given site. They then either leave a "scar" (imprecise excision) or restore the original DNA sequence (precise excision). These acts of precise excision are generally considered by-products of RecA-independent transposition events. In certain cases of mutant transposons or mutant bacteria, a RecA-dependence has also been reported (Bukhari 1975; Kleckner 1989).

Other genetic entities have acquired extended mobility by "hitchhiking" on transposable elements. Transposons are a well-studied example; the bacteriophage Mu is another. This temperate phage is a transposable element within which there is a full phage genome (Pato 1989). In the lytic mode, it replicates its genome by repeated replicative transposition, and in the lysogenic mode, it creates mutations by integrating within genes. Curiously, mutants that result from an integrated Mu prophage do not give rise to wild-type revertants (Bukhari 1975), as if Mu, unlike other transposable elements, were unable to promote precise excision.

Some insight as to why Mu-induced mutations do not seem to revert comes from studies of Mu mutants, which, when integrated in a gene, do give rise to revertants. The first, called Muc62(Ts) X (Bukhari 1975), was selected from a lacZ::Muc62(Ts) lysogen, whose repressor is temperature sensitive; the X mutations, which are insertions of an insertion sequence (IS) in the Mu B gene (Khatoon and Bukhari 1981), suppress the lethality associated with repressor inactivation and prophage induction at 42°C, and the Muc62(Ts) X phage is defective for plaque formation. The lacZ::Muc62(Ts) X strains give rise to Lac⁺ revertants at frequencies around 10⁻⁶, at 37°C. The Mu transposase, a product of the Mu A gene, is required for this precise excision; the A gene is partially derepressed at 37°C.
Mutants due to an integrated Mu3(Ts) prophage have also been reported to revert at frequencies around 10^{-6} (Ghelardini et al. 1994, 1995). This phage is mutated in the promoter region of the gemA-mor operon (also called gemA-gemB) (La Valle et al. 1996). The presence of a Mu3(Ts) prophage relaxes the superhelical density of DNA in lysogens (mentioned in Fabozzi et al. 1998). Reversion of a miniF::Mu3(Ts) mutation was observed at both 30°C and 42°C (Ghelardini et al. 1994), permisive and nonpermisive temperatures, respectively, for plaque formation of Mu3(Ts).

"Mini-Mu" elements in which nearly all phage sequences have been deleted except those required for transposition, can excise precisely. Mini-Mu3A carries the c62(Ts) repressor gene, the A and B genes, and the left and right ends of Mu. It is deleted for 30 kb of phage DNA to the right of B. A lacZ::Mu3A mutant gives rise to Lac^+ revertants at a frequency of about 10^{-5} (Desmet et al. 1981).

The observations offer several hypotheses to explain the lack of revertants of mutants due to the integration of a wild-type Mu prophage. On the one hand, it is possible that precise excision is physically prevented, for example, by an unfavourable density of supercoiling. Alternatively, one can imagine that precise excision does in fact take place, but is systematically associated with loss of viability, possibly because of concomitant lytic growth of the phage. In the present work, we show that a Mu X^+ gem^+ prophage is in fact able to excise precisely, and our results strongly suggest that the cells in which this takes place systematically lose their colony-forming capacity.

Materials and methods

Strains and plasmids

The strains used in the present work, all *Escherichia coli* K-12 derivatives, are described in Table 1. The plasmids used were pEG5005 (Grosman and Casadaban 1986) and pCR-TOPO (Invitrogen, Paisley, Scotland); both confer resistance to kanamycin and ampicillin.

Media and growth conditions

Rich medium was Luria-Bertani (LB) broth and minimal medium was M63 (Miller 1992) supplemented with 1 μg/ml thiamine, 0.25% Casaminoacids, and 0.4% glucose. MacConkey lactose indicator plates (Miller 1992) containing kanamycin were used to detect Lac^+ colonies. Solid media contained 1.5% Difco agar. Kanamycin was used at 50 μg/mL. Cells were grown aerobically in shaking water baths.

Preparation of mini-Mu lysates and mini-Muduction

Strain JC355 was cultured overnight at 30°C in LB broth containing kanamycin, then diluted 100-fold in LB broth, and grown at 30°C to an OD_{600} of 0.6. The culture was heat-shocked by diluting twofold with 60°C LB and cultured at 42°C for 25 min. It was then shifted to 37°C for 20 min, at which time the culture was treated with chloroform and centrifuged at 4°C. The supernatant was concentrated about 100-fold by centrifugation (2 h at 35 000 x g), giving a final titre of 10^{11} phage/mL. Mini-Muduction was carried out as follows. The recipient strain was grown in LB broth to an OD_{600} of 1.0. It was then infected with the mini-Mu lysate at a multiplicity of about 5 phage/cell. The infected cells were plated at 30°C on MacConkey lactose kanamycin plates to isolate Lac^+ clones or at 37°C on M63 glucose Casaminoacids kanamycin plates lacking tryptophan to isolate Trp^+ clones; in the latter case, the frequency of Km^+ transductants was evaluated on LB kanamycin plates.

Determination of the point of insertion of a Mu prophage in the lacZ gene

Chromosomal DNA was extracted from strains JC354 and AT397, according to the methods of Pospiech and Neumann (1995), followed by phenol-chloroform extraction. DNA fragments spanning the Mu::lacZ junction were amplified by polymerase chain reaction (PCR), cloned in pCR2.1-TOPO and sequenced. From JC354, the joint on the 5' side was determined from a DNA fragment amplified using oligonucleotides in lacZ and in the right end of Mu, and the joint on the 3' side was determined using oligonucleotides in lacZ and in the left end of Mu. From AT397, the joint on the 3' side was determined using an oligonucleotide in lacZ and in the left end of Mu.

Results

We reasoned that if precise excision of Mu actually occurs, but is associated with loss of colony-forming ability, then it might be possible to detect revertant genes, even if no revertant colonies are found. We used mini-Muduction (Faelen et al. 1979) to see whether, in a strain carrying a Mu prophage inserted in the lacZ gene, rare lac^+ alleles could be picked up. This technique, ingeniously exploiting the ability of Mu to transpose randomly in the bacterial chromosome during prophage replication, provides a method of in vivo cloning, using a mini-Mu Km^+ vector, such as pEG5005 (Grosman and Casadaban 1986) (Fig. 1). The mini-Mu moiety of this plasmid carries the left and right ends of Mu, a kanamycin resistance gene, and a replication origin. The left end of Mu includes the c gene and the A and B genes and thus provides transposase, product of the A gene. The repressor is temperature sensitive. After thermoinduction, the mini-Mu undergoes rapid replicative transposition, so that it is constantly associated with novel bacterial sequences. In the presence of a Mu62(Ts) helper phage, present on the chromosome, about 39 kb of DNA associated with the left end of Mu is encapsulated in phage heads, producing particles with about 30 kb of random bacterial DNA associated with the mini-Mu. When a mini-Mu lysate of this sort is used to infect an appropriate host, these plasmids are injected. Many of them contain a second copy of the mini-

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Fig. 1. In vivo cloning by mini-Muduction. (a) The strain from which the mini-Mu lysate is made carries a Muc62(Ts) phage inserted in the chromosome, in our case in the lacZ gene, and the 7.9-kb mini-Mu plasmid pEG5005. The mini-Mu part lies between (and including) the phage ends, L and R, indicated by open triangles; it includes the Mu genes c, A and B, a plasmid replication origin (rep), and a kanamycin resistance gene (Km'). The remainder of the plasmid carries an ampicillin resistance gene (Amp') and a second copy of the replication origin. (b) Upon thermoinduction, the mini-Mu undergoes frequent replicative transposition in the chromosome. The left end of Mu (L) triggers the encapsulation process, by which about 39 kb of DNA are encapsulated in a phage head. If any lacZ* genes are formed and if a mini-Mu has transposed to a nearby location, the lacZ* gene will be included in a phage particle. (c) When such phages infect recipient bacteria, they will inject this DNA. If the DNA carries two parallel copies of the mini-Mu, homologous recombination can take place between them. (d) This will generate a new mini-Mu plasmid carrying the Km' marker and a fragment of bacterial DNA. The lacZ* gene can then be screened for among these Km' transductants.

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Mu. If the two copies are in the same orientation, by homologous recombination they can generate a mini-Mu plasmid containing up to 30 kb of bacterial DNA. One can then select, from this Km' population, those carrying the gene to be cloned.

We used mini-Mu in vivo cloning to see whether, in a strain carrying a Mu prophage inserted in the lacZ gene, rare lac' alleles could be picked up. We first isolated strain JC354, with a Muc62(Ts) prophage in the lacZ gene. To determine the precise point of insertion of the Muc62(Ts) prophage, we used oligonucleotides in lacZ and in the ends of Mu to amplify by PCR the joints between Mu and lacZ DNA, which were then cloned and sequenced. The prophage was inserted at base pair 2357 of the lacZ coding sequence, with the Mu prophage oriented opposite to the lac operon (i.e., the c gene is promoter distal); the 5-bp repeat typical of Mu integration lies at base pairs 2357–2361 of lacZ.

We introduced the mini-Mu plasmid pEG5005 into strain JC354 to make strain JC355, of genotype lacZ::Muc62(Ts)/pEG5005. This strain, as expected, did not revert to Lac' (<2 × 10^-10 after a week at 30°C). We prepared a mini-Mu lysate from strain JC355 and used it to infect the parental lacZ::Muc62(Ts) strain JC354. Km' transductants were obtained at a frequency of about 10^-3 per active phage particle, and about 2 × 10^-6 of the Km' colonies were Lac'. Since the recipient strain carries a polar insertion in the lacZ gene, both lacZ* and lacY* should be expressed from these plasmids. As a control, we selected for trp' transductants. We used the same mini-Mu lysate to infect the trpC strain JC117; again, about 10^-3 Km' transductants were obtained per active phage particle, and 5 × 10^-3 of the Km' colonies were Trp'.

The recovery of a low frequency of Lac' clones suggested that lacZ* genes are occasionally formed in cells of strain JC355, although this mutant produces no Lac' revertants on plates at 30°C. Three independent clones, obtained in two experiments, were characterized at the molecular level. These three plasmids, extracted from the transductants and introduced into strain C600 (lacYI), again conferred a Lac' phenotype, showing that all the genetic information is carried by the plasmids. The plasmids carried inserts of approximately 6.7, 12, and 20 kb. The restriction map of the inserts was consistent with that of the chromosomal lac region: in particular, two of the plasmids carried an 11-kb PstI fragment containing a rare AscI site, resembling the PstI fragment containing the lacZ gene. We then used PCR to amplify either the left or the right half of the lacZ gene. In the parental strain, the right half could not be amplified, as expected, since the Muc62(Ts) prophage is inserted there. From all three lac' plasmids, in contrast, both halves of the lacZ gene could be amplified.

To see whether these functionally lacZ' genes had in fact restored the wild-type sequence, we used PCR to amplify the region corresponding to base pairs 1666–2483 of lacZ, covering the Muc62(Ts) integration site, using chromosomal DNA extracted from the three Lac' plasmids. The resulting 818-bp fragments were sequenced. In all cases, the exact wild-type sequence was found, showing that the original Muc62(Ts) prophage had been excised precisely.

We conclude that the cloned lac' genes arose in cells unable to form colonies, presumably because of prophage induction. The lower frequency of Lac' clones, as compared with Trp' clones, presumably reflects the low frequency of precise excision.

**Discussion**

The phage Mu was known from its discovery to have the ability to create mutations by integrating within different genes. Such mutants do not give rise to revertants. This apparent lack of precise excision of Mu was paradoxical, in view of the existence of precise excision of virtually all other transposable elements.

In the present work, we resolved the puzzle: Mu, like other transposable elements, is able to excise precisely, restoring the wild-type sequence, and the wild-type gene thus formed can be cloned in vivo in a mini-Mu plasmid. This shows that the lack of reversion of Mu-induced mutations is not because precise excision cannot take place. We propose a simple explanation of this apparent paradox. If precise excision requires prophage induction to express transposase, then it will systematically be associated with cell killing (loss of colony-forming ability) because of the ensuing lytic development of Mu. Thus, the sequence of events (i) spontaneous induction, (ii) precise excision, and (iii) lytic phage growth would give rise to revertant (lacZ') genes but, not to revertant (Lac') colonies.

The formation of functional lac' genes that do not give rise to Lac' revertant colonies is reminiscent of the formation, in a recB mutant, of functional lacZ' recombinant genes that do not give rise to Lac' recombinant colonies, presumably because the recombination event itself is lethal in the absence of RecB function (Birge and Low 1974). These lacZ' recombinant genes were detected by their ability to synthesize active β-galactosidase. Our lacZ' revertant genes were cloned in vivo in a mini-Mu plasmid.

The reversion of lacZ::Muc62(Ts) X and lacZ::Muc3A (mini-Mu) mutations probably reflects the fact that these defective prophages do not kill their host upon induction, the X mutant because of a polar IS in the B gene and the mini-Mu because of an IS just downstream of B and the deletion of 30 kb of phage sequences. The reversion of Mugeum2(Ts)-induced mutants (Ghelardini et al. 1994, 1995), however, cannot be attributed to lack of killing, even at a nonpermissive temperature (Buø et al. 1987). In view of our results, it also does not imply a newly acquired ability of the mutant prophage to excise precisely. It is as though the gme2(Ts) mutation somehow allows the cell to escape the loss of colony-forming ability normally associated with Mu excision. A plausible scenario for this is that the gme2(Ts) promoter mutation delays the expression of Mor (or GemB), thereby delaying the expression of all Mu late genes; thus, after induction there is perhaps sufficient time between the precise excision event and lethal phage development to allow cell division, occasionally giving rise to a viable segregant with a revertant (lacZ') gene.

Mu is known to have two modes of transposition. The first, the replicative mode, does not result in the excision of Mu from its initial site, but inserts a semiconservatively replicated copy in a target site. The other is conservative, integrating both strands of the initial phage into a new target site; this mode is used immediately after infection (Liebart © 2001 NRC Canada
et al. 1982; Akroyd and Symonds 1983; Chaconas et al. 1983; Harshay 1984). It is presumably the conservative mode of transposition that is responsible for precise excision. An interesting and completely unresolved problem in Mu biology is the question of how Mu regulates the relative frequency of these two modes of transposition.

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References


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