Mu DNA reintegration upon excision: evidence for a possible involvement of nucleoid folding

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Mutations induced by the integration of a *Mugem2ts* prophage can revert at frequencies around 1×10^{-6} . In these revertant clones, the prophage excised from its original localization is not lost but reintegrated elsewhere in the host genome. One of the most intriguing aspects of this process is that the prophage reintegration is not randomly distributed: there is a strong correlation between the original site of insertion (the donor site) and the target site of the phage DNA migration (the receptor site). In this paper, it is shown that in the excision–reintegration process mediated by *Mugem2ts*, the position of the initial prophage site strongly influences the location of the reintegration site. In addition, for each donor site, the receptor site is a discrete DNA region within which the excised Mu DNA can reintegrate and the two sites implicated in phage DNA migration must be located on the same DNA molecule. These data suggest the involvement of nucleoid folding in the excision–reintegration process.

Keywords: Bacteriophage Mu, excision–reintegration process, nucleoid folding

INTRODUCTION

The mobility of a transposable element from its original site to another on the same chromosome or on a different replicon does not require any specificity for the target site, with the exception of some transposons which recognize one or few sites along the host genome (for reviews see Kleckner, 1981; Grindley & Reed, 1985; Mahillon & Chandler, 1998). This property of the transposition process, however, does not exclude the existence of preferential targets which vary from one transposable element to another (i.e. hot spots, AT- or GC-rich regions) (for a review see Craig, 1997).

After infection, the DNA of the phage-transposon Mu integrates randomly in the host genome (for a review see Pato, 1989) although either hot spot or conditions are known which can bias the choice of the target site (Casadeus & Roth, 1989; Wang & Higgins, 1994). A peculiarity of Mu integration is the inability of the prophage to excise from the target site. In fact, during

the lytic cycle, free phage DNA is never found in the cytoplasm and phage DNA extracted from the virion particles is always covalently associated with host DNA (for a review see Pato, 1989). Therefore, after infection, the phage DNA transposes from the old integration target, i.e. from the DNA sequences flanking the virionic DNA, to a new one. Precise excision of the prophage, thereby restoring the original target sequence, has been reported with certain defective mutants or particular conditions (Bukhari, 1975; Shapiro, 1984) but, in all cases, the revertant strains examined lost the prophage.

In contrast with this classical description of the Mu DNA fate, we observed that mutant *Mugem2ts* carrying a mutation in the *gem* operon promoter, excises at a relatively high frequency (Ghelardini *et al.*, 1994, 1995). This phenomenon, besides representing a new aspect of Mu behaviour, is peculiar since the phage DNA excised is not lost but is reintegrated at a new site in the host genome. Moreover, the reintegration site depends strictly on the original prophage localization.

To ascertain the mechanism underlying this novel recombination event, we investigated the relationship between the donor (original integration site) and the receptor (new integration site) sites implicated in the phage DNA migration during the excision–reintegration process.

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Abbreviation: RAGE, rapid amplification of genomic DNA ends.

METHODS

Bacterial strains, phages and plasmids. Bacterial strains, all *Escherichia coli* K-12 derivatives, phages and plasmids used in this work are listed in Table 1.

Media and chemicals. LB broth, used for bacterial cultures and plating, MacConkey plates and SM, used for bacteria and phage dilutions, have been described by Miller (1972). Ampicillin, chloramphenicol, tetracycline, streptomycin and kanamycin (Sigma) were used at 50, 20, 50, 100 and 30 mg ml⁻¹, respectively.

General microbiological and DNA recombinant techniques. Standard microbiological and DNA recombinant techniques were as described by Miller (1972) and Sambrook *et al.* (1989). DNA sequences were performed with an ABI Prism 310 Sequencer (Applied Biosystems) as recommended by the manufacturer. The sequences of the synthetic oligonucleotides used in this work are as follows: 1 (target, Mu), 5'-TG GTGACAGCTTGGTTGTTATCGG-3' [used for RAGE (rapid amplification of genomic DNA ends)]; 2 (target, pUC19), 5'-GTTTCCAGTCACGAC-3' (RAGE); 3 (target, Mu), 5'-ATTTGCACTACAGGCTTGCAAGCC-3' (RAGE); 4 (target, pUC19), 5'-GTAAACGACGCCAGTGAATTTCG-3' (RAGE); 5 (target, Mu), 5'-CAAATCTAATCCCATCAGATCCCG-3' (sequencing).

Excision experiments.

Construction of Mu *wt* and Mugem2ts lysogenic strains with the prophage inserted in a specific target gene. Strains were constructed and proved to be monolysogens as previously described (Ghelardini *et al.*, 1994).

Excision of Mugem2ts. Bacterial strains lysogenic for Mugem2ts, with the prophage inserted in a specific target gene (*lacY* or *malT*) were grown to early stationary phase, then

centrifuged, washed three times with SM, resuspended in SM and plated on M9 medium with lactose or maltose, or isolated on MacConkey plates with the appropriate sugar to select the Lac⁺ (or Mal⁺) revertants (excised clones). Clones, grown for 2–3 d on M9 medium, or red clones which appeared on MacConkey plates after 3–5 d, were collected, purified on MacConkey plates with the appropriate sugar and stored in stabs.

Prophage localization. The precise phage DNA insertion site was determined by RAGE (Mizobuchi & Frohman, 1993). Chromosomal DNA was extracted from the lysogenic strain, digested with *Hae*III and ligated to pUC19 DNA digested with *Hinc*II. Five microlitres of the ligation mixture were used to perform a PCR with synthetic oligonucleotides 1 and 2 as shown above. One tenth of the PCR product volume was reamplified with nested oligonucleotides 3 and 4. The products of the reaction were separated on an agarose gel, purified and sequenced using oligonucleotide 5 as the sequencing primer.

All sequence analyses were carried out using the Wisconsin Package version 9.1, from the Genetics Computer Group (Fuchs *et al.*, 1990) and FASTA.

Inversion of a large chromosomal region. The experiments were performed and the effective inversion of the genomic region was controlled by conjugation as described by Rebollo *et al.* (1988).

RESULTS

When the Mugem2ts prophage excises from its target sequence, its DNA is not lost, but is reintegrated elsewhere in the bacterial chromosome (Ghelardini *et*

Table 1. *E. coli* K-12 strains and phages used in this study

Relevant genotype		Source/reference
Bacterial strains		
RS54	su ⁰ <i>araD</i>	Paolozzi collection
R353	RS54 <i>lacZ</i> ::Mugem2ts	Ghelardini <i>et al.</i> (1995)
R354	RS54 <i>lacZ</i> ::Mugem2ts	Ghelardini <i>et al.</i> (1995)
R362, R363, R364, R365, R366	R354 Lac ⁺ derivatives <i>malT</i> ::Mugem2ts	Ghelardini <i>et al.</i> (1995)
R378, R379, R380	R353 Lac ⁺ derivatives <i>malT</i> ::Mugem2ts	Ghelardini <i>et al.</i> (1995)
R400	RS54 Δ <i>lac</i> F' _{ts} <i>lac</i>	This work
R410	RS54 Δ <i>lac</i> (Mu <i>cts</i> ₆₂)	This work
LN1883	<i>tna</i> ::Tn10-tet::kan <i>purE</i> ::Tn10-tet::sm P4x	Rebollo <i>et al.</i> (1988)
R420	LN1883 <i>malT</i> ::Mugem2ts	This work
R421	LN1883 tet ^R <i>malT</i> ::Mugem2ts	This work
R422-23	Mal ⁺ derivatives of R420	This work
R424-25	Mal ⁺ derivatives of R421	This work
R430	LN1883 <i>lacY</i> ::Mugem2ts	This work
R431	Tet ^R LN1883, <i>lacY</i> ::Mugem2ts	This work
R432-33	Lac ⁺ derivatives of R430	This work
R435-36	Lac ⁺ derivatives of R431	This work
R440	RS54 Δ <i>ara</i> , Tet ^R	This work
Phages		
Mu wild-type	<i>c</i> ⁺ <i>gem</i> ⁺	Paolozzi collection
Mugem2ts	<i>c</i> ⁺ <i>gem</i> 2ts	Paolozzi collection
Mu <i>cts</i> ₆₂	Repressor thermosensitive	Paolozzi collection

Fig. 1. Location of the Mugem2ts prophage excised from *lacZ*. (a) Location of Mugem2ts in *lacZ*. (b) Location of Mugem2ts in *malt*, after excision from *lacZ*. Strains R362–R366 are derivatives of R354 and strains R378–R380 are derivatives of R353. The arrows indicate the orientation of the prophage inside the target gene.

al., 1994). The localization of the reintegrated DNA [receptor site(s)] depends on the site in which the prophage was originally inserted (donor site). The receptor site was genetically determined by conjugation and transduction experiments and could, therefore, be either a specific sequence or a short chromosomal region where the excised phage DNA could reintegrate. In any case, this kind of recombination seems to need the recognition of particular pairs of donor/receptor sites. One way to explain the strong relationship between the two sites is to hypothesize the existence, between the two sites, of sequence similarities that could promote this kind of recombination.

Search for sequence similarities in pairs of donor/receptor sites

To test if donor and receptor sites are related in primary sequence, we analysed four receptor sites for the Mugem2ts element inserted at two donor sites within *lacZ*. In one case (strain R354) the phage was inserted at base 4154 with the Mu *c* end promoter-proximal, while in the other (strain R353), the phage was at base 3788 with the Mu *S* end promoter-proximal (Fig. 1a). From both strains, Lac⁺ revertants were obtained with frequencies around 1×10^{-6} and, in these clones, the prophage moved from *lacZ* (located at 8 min) to about 74/75 min on the *E. coli* genetic map (Ghelardini *et al.*, 1995). In particular, 21 clones, obtained from independent excision experiments with both the *lacZ*::Mugem2ts lysogens, resulted in a Mal[−] phenotype and were located in the *malt* gene. In these clones, the precise point of prophage insertion was determined using the method described previously (Ghelardini *et al.*, 1994) and the localization of eight of these Lac⁺ revertants, namely R362 to R366 and R378 to R380, is shown in Fig. 1b. The data reported in Fig. 1(b) indicate that the DNA excised from a donor site reintegrates at a cluster of points that we define as the acceptor site.

Since the prophage excised from an identical donor site integrates at different points along the receptor site, the hypothesis of sequence similarities is already weakened. In fact it is difficult to imagine that the same sequence (with a significant degree of similarity to a sequence of *lacZ*) could be interspersed along the whole *malt* gene. Nevertheless, we have sequenced the integration sites of Mugem2ts in the *malt* gene of strains R362 and R366 and compared the sequences obtained with each other and with the integration site of the parental strain (R354). For each pair of sequences, we analysed 50 bp upstream and 50 bp downstream from the junction between Mu and the bacterial chromosome, using the program BLASTN 2.0.9. As shown in Fig. 2, no significant similarity was found. Therefore, we can conclude that, in the excision–reintegration process mediated by Mugem2ts, the results do not support the hypothesis invoking sequence similarities between the sites. In

Fig. 2. Sequence of the DNA region upstream and downstream of the junction between Mugem2ts and the bacterial chromosome. The box indicates the 5 bp duplicated after the Mu insertion.

addition, for each donor site, the receptor site is a discrete DNA region within which the excised Mu DNA can reintegrate.

Does site-selection involve *cis* interactions between donor and receptor sites?

Since the donor/receptor site sequence analysis failed to reveal any similarity, we hypothesized the existence of some topological constraint(s) between the two sites involved in phage DNA migration. The topological model postulates that the donor and receptor sites, although genetically far apart, could be bound to each other by some topological constrictions. A consequence of this model is that, in the excision–reintegration process of Mu DNA, the receptor site should always be located on the same DNA molecule as the donor site (*cis*-dependence of the process). To verify this hypothesis, we studied the reintegration of Mugem2ts DNA when the receptor site is *in trans* with respect to the donor site.

The Mugem2ts Ap1 prophage was integrated in the *lacZ* gene carried by the $F'_{ts}lac$ episome of the bacterial strain R400 Δlac to give the strain R400 $\Delta lac/F'_{ts}lac::Mugem2ts$ Ap1 and Lac⁺ revertants were selected at 30 °C. As we have previously shown (Ghelardini *et al.*, 1995), the prophage excised from *lacZ* is usually reintegrated at about 74 min on the *E. coli* map. In this case, however, where the two sites implicated in the excision–reintegration process are *in trans*, on two separate DNA molecules, none of the 20 revertant clones examined genetically was located at or around 74 min. These clones were crossed with the bacterial strain R410 Δlac (Mu *cts₆₂*) and Lac⁺ clones were selected. The exconjugants were then examined for the amp^R marker carried by the phage, to analyse whether the prophage was still co-transferable with the episome in which it was originally inserted. Crosses, performed with 10 independent Lac⁺ revertants, showed that the amp^R marker was co-transferred with F' and that the exconjugants acquired immunity to Mu superinfection at both 30 °C and 42 °C. Therefore, the Mugem2ts DNA, excised from the *lac* operon carried by F' was always reintegrated in another site of the episome itself. From these results we can conclude that the two sites involved in phage DNA migration must be located on the same DNA molecule, supporting the hypothesis that they are bound by topological constraints.

Effect of the inversion of the DNA region containing the receptor or the donor site on the Mugem2ts DNA reintegration

The strong correlation between the initial prophage location and its subsequent target, and the requirement that the two sites implicated are *in cis* support the hypothesis of the existence of topological constriction in Mugem2ts excision–reintegration process. At this point we may ask what would happen if the receptor site is displaced from its natural location on the *E. coli* map: would the target for Mugem2ts reintegration be anal-

Fig. 3. Mechanism of large chromosomal region inversion. Redrawn after Rebollo *et al.* (1988).

ogously displaced or not? According to the topological-constraint hypothesis, we may expect that the phage DNA migration itself would not be modified, but, genetically, the receptor site would be changed. To answer this question, we studied the reintegration of Mugem2ts excised from the *malT* gene when a large inversion of the DNA region containing the phage receptor site is performed. This experiment was performed using the strategy developed by Rebollo *et al.* (1988), which allows the detection of large region inversions of the *E. coli* chromosome.

In bacterial strain LN1883, the DNA region between *tna* (83 min) and *purE* (12 min) is surrounded by two *tetA* markers, both inactivated, the former with a kan^R and the latter with a sm^R box (Fig. 3). Recombination of the two *tetA* genes gives rise to tet^R clones in which the DNA region between them has been inverted (Rebollo *et al.*, 1988). Strain LN1883 was infected with Mugem2ts and lysogenic clones with the prophage integrated in the *malT* gene were selected. One of Mal[−] clones (R420) was further purified, the localization of the prophage determined as previously described (Ghelardini *et al.*, 1994), and tet^R clones were then selected, purified and controlled for the DNA region inversion. One of the resulting clones, strain R421, was used for excision experiments.

From our previous work (Ghelardini *et al.*, 1994), we know that the prophage excised from *malT* reintegrates at around 94/95 min on the *E. coli* map, which in the case of LN1883 is within the inverted region. Moreover, the inversion modifies the distance between the donor and the receptor site from 18 to 26 min of the conjugative map.

Mal⁺ revertants were selected from R420 and from its tet^R derivative R421. The prophage was localized in two independent Mal⁺ strains derived from R420 and in two from R421 by sequencing the junctions between the

Fig. 4. Location of the Mugem2ts prophage excised from the *malT* gene in strains R420 and R421. (a) The prophage from two independent *Mal*⁺ clones from strain R421 was located at 90.97 min on the *E. coli* map (strain R422 at 4712 bp and strain R423 at 5014 bp). (b) Location of the prophage in two independent *Mal*⁺ clones from strain R421 at 7.78 min on the *E. coli* map (strain R424 at 4379 bp and strain R425 at 4321 bp).

Fig. 5. Effect of donor-site inversion on Mugem2ts migration. The prophage of the *Lac*⁺ clones derived from strains R430 was located at 56.44 min (strain R432) and 58.8 min (strain R433). In *Lac*⁺ clones derived from strain R431 the prophage was located at 41.7 min (strain R435) and 39.21 min (strain R436).

phage and the bacterial DNAs using the RAGE technique. Chromosomal DNA was digested with *Hae*III, ligated to pUC19 plasmid DNA digested with *Hinc*II, amplified by PCR, and the fragments obtained were sequenced and then analysed as described in Methods.

As shown in Fig. 4, in strain R420, the prophage excised from *malT* is reintegrated at 91 min of the *E. coli* genetic map. This result is in accordance with our previous results, considering the differences between the two parental strains used and the fact that in the former case, the localization of the prophage insertion was determined genetically whereas in this case it was determined by sequence analysis. It is interesting to note that, after inversion of the genome region the reintegration site remains roughly the same distance from the donor site, i.e. after inversion, the distance between the donor and the receptor site changed from 17 to 14 min of the genetic map.

Instead, if the donor site is relocated the receptor sites will be quite different. The experiment is depicted in Fig. 5. In this case the prophage was inserted in the *lacY* gene

(strain R430), located within the invertible region. From this strain, *tet*^R derivatives were selected. One clone (R431) was purified, controlled for DNA region inversion and used for excision experiments. The prophage from two *Lac*⁺ clones selected from R430 (strains R432 and R433) and two from R431 (strains R435 and R436) was localized as described above. As shown in the Figure, in strains R432 and R433 the prophage is reinserted at about 57 min (56 and 58 min respectively, for the two strains) whereas after inversion of the DNA region containing the donor site, the localization of the prophage excised from *lacY* (strains R435 and R436) changed from about 57 min to about 40 min on the *E. coli* map (41 and 39 for the two strains, respectively).

Even in the case shown in Fig. 4, the reintegration site remains roughly the same distance from the donor site: after inversion, the distance between the donor and the receptor site changed from 49 to 53 min of map (Fig. 5).

From these experiments, we can conclude that the choice of the receptor site is determined only by the

location of the donor site and is independent of the nucleotide sequence of the receptor site itself. In fact, the prophage migration is not substantially affected by the inversion of the region containing the receptor site whereas the receptor site changes dramatically when the donor site is moved.

DISCUSSION

In the classic bacteriophage Mu life cycle, its DNA integrates in the host genome both during the lysogenic cycle (conservative transposition) and the lytic cycle (replicative transposition) (for a review see Pato, 1989). A peculiarity of Mu integration is its inability to excise from the target site, although precise excision of the prophage has been reported with certain defective mutants or particular conditions (Bukhari, 1975; Shapiro, 1984). In all cases, the strains examined lost the prophage.

Reintegration occurs in a discrete region without any sequence recognition

One of the most intriguing peculiarities of the excision process described with the *Mugem2ts* mutant is that the prophage is not lost but is reintegrated at sites which depend on the original location of the prophage (Ghelardini *et al.*, 1994, 1995). This prophage non-random reintegration is somehow reminiscent of the non-random pattern of Tn10-catalysed large inversions in *Salmonella typhimurium*, described by Krug *et al.* (1994).

Two hypotheses have been postulated to explain the relationship between the donor and receptor sites implicated in the Mu excision-reintegration process. The first suggests the existence of specific sequences at the receptor site, which are recognized and preferentially selected for the phage reintegration, for example, a sequence similarity. The second hypothesis presumes, between these two sites, the existence of some constraints that could make the two sites more reactive, for example, increasing the proximity of the two sites, which are otherwise a long way from each other along the host chromosome, or for their ability to recombine. One of these constraints may be nucleoid folding. Alternatively, we can imagine a factory model, based on that elaborated for DNA replication (Lemon & Grossman, 1998). With this model the two sites are brought closer together in a dynamic way at the replication-factory level by the two replication forks, which run across the donor and the receptor site, respectively.

The first hypothesis was tested by comparing the DNA sequences surrounding the Mu DNA at both the donor and receptor sites. Analysis of the receptor sites of phage DNA excised from two independent lysogens with the prophage inserted into the *lacZ* gene showed that they are located at 74 min on the *E. coli* map and that there is neither a unique site of reintegration nor a sequence

similarity between the donor and receptor sites. The receptor site could be defined as a region where there is a cluster of reintegration points and may extend for a number of kilobases.

The receptor site is determined by the location of the donor site

Since Mu reintegration appears to be restricted to a discrete DNA region without any sequence recognition, we postulated that the receptor and donor sites are bound to each other by nucleoid folding or the replication factory models. Both these two models postulate that in the excision-reintegration process of Mu DNA, the receptor site should always be located on the same DNA molecule as the donor site (*cis*-dependence of the process). As a matter of fact, Mu DNA excised from the *lacZ* gene carried by the episome F' does not reintegrate *in trans*, at or around 74 min on the *E. coli* chromosome, but *in cis* in another region of the episome. Moreover, since F' does not contain the '74 min region', this experiment confirms that the choice of the target of the excised DNA is not determined by any nucleotide sequence, as informational content, but rather by some constraint imposed on the region itself.

In support of the sequence-independent model, we observed that the inversion of a large chromosomal region containing the receptor site does not affect the migration of the excised Mu DNA. In fact, it integrates at about the same distance from the donor site, independently of the nature of the DNA sequence. In other words, the phage DNA excised from *malT* integrates at 91 min in LN1883 derivative strains (i.e. at a distance of 17 min from the donor site) and at 7 min (i.e. at a distance of 14 min from the donor site) after inversion of the genomic region. Hence, the recognition of the zone for the reintegration of the phage DNA excised from *malT* is not affected by the inversion of a chromosomal region of about 26 min (between *tnaA* and *purE*) where it is located. In fact, after inversion, the prophage recognizes this region as though it were not inverted, since its reintegration occurs at a site which is only 3 min away from the integration site in the non-inverted chromosome.

The result changes dramatically if the donor site is in the invertible region. In this case, the excised prophage migrates to a new target, after inversion. In fact, the prophage migrates to about 57 min, before inversion, while, after inversion it goes to about 40 min. It is interesting to note that this last site is symmetrical with the preceding one, as was the localization of the *lacY* gene (where the prophage was originally inserted) before and after chromosome inversion.

Previous results, obtained with strain RS54, showed that the prophage excised from *lacZ* was reintegrated at a region around 74 min of the *E. coli* map. There is a difference with the results obtained with R430. In this case, the prophage was reintegrated around 57 min. We ascribe this difference to the great differences between

the genetic background of RS54 and R340 rather than to the difference in the integration site of the prophage (*lacZ* in RS54 and *lacY* in R340). In R430 the donor site is within a genetic region which underwent significant rearrangements, due to the presence of two transposons and a transfer origin near the *lac* operon.

The replication-factory model predicts a symmetry between the two interacting sites as regards *oriC*. Most of the acceptor sites (4 out of 6) are symmetrical about *oriC*, and in the cases where donor and acceptor sites break down (the inversion strains) the replication symmetry might be altered because of *ter* sites. On the other hand, symmetry (or reciprocal results) may not be necessary in the case of the nucleoid-folding model and we have already observed that the Mu DNA excised from the *gal* operon (17 min) migrates to a chromosome region between 2 and 5 min, without any symmetry as regards *oriC* (Ghelardini *et al.*, 1995). For these reasons, we believe that this last model could be more plausible. In any event, from these results, we can conclude that, during the excision–reintegration process, the receptor site is determined with regard to the location of the donor site.

Is the excision–reintegration process a general property of the Mu bacteriophage?

At this point, we may ask whether the excision–reintegration process is a peculiarity of the *Mugem2ts* mutant or a general property of the Mu bacteriophage. In fact, there is an important discrepancy between the behaviour of Mu wild-type and the *Mugem2ts* bacteriophages as far as the ability to give rise to precise excision is concerned.

Mugem2ts prophages are known to affect DNA supercoiling in host lysogens and to change the expression of many genes and a possible role of chromosome supercoiling in the ability of *Mugem2ts* to excise has been suggested (Ghelardini *et al.*, 1994). The mutation was mapped within the *gem* promoter at position –44 (8153 bp from the left end of the phage genome) (La Valle *et al.*, 1996). Since the only genetic defect carried by *Mugem2ts* and involved in the aforesaid process can not be ascribed to a modified (thermosensitive) protein, but to modified expression of the wild-type *gem* operon, it should be possible to induce the excision–reintegration process of wild-type Mu in the presence of *gem* overexpression and, in particular, of GemA, which is responsible for the effects on DNA supercoiling. Preliminary results obtained with a recombinant plasmid carrying the *gemA* gene under the control of an inducible promoter indicate that in the presence of extra copies of the *gemA* gene, the wild-type Mu prophage is able to excise from its original localization and to reintegrate elsewhere on the host genome. Therefore, as far as the excision–reintegration process is concerned, it behaves as the *gem2ts* mutant (P. Ghelardini & J. C. Lièbart,

unpublished). Hence, it is obvious that this process could be included in general Mu biology and could open up a new field of investigation on Mu illegitimate recombination, besides raising interesting questions on the nature of GemA activity.

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REFERENCES

- Bukhari, A. I. (1975). Reversal of mutator phage Mu integration. *J Mol Biol* **96**, 87–99.
- Casadeus, J. & Roth, J. R. (1989). Transcriptional occlusion of transposon targets. *Mol Gen Genet* **216**, 204–209.
- Craig, N. L. (1997). Target site selection in transposition. *Annu Rev Biochem* **66**, 437–474.
- Fuchs, R., Stoehr, P., Rice, P., Omond, R. & Cameron, G. (1990). New services of the EMBL data library. *Nucleic Acids Res* **18**, 4319–4323.
- Ghelardini, P., Lièbart, J. C., Di Zenzo, G., Micheli, G., D'Ari, R. & Paolozzi, L. (1994). A novel illegitimate recombination event: precise excision and reintegration with *Mugem* mutant prophage. *Mol Microbiol* **13**, 709–718.
- Ghelardini, P., Lièbart, J. C., Fabozzi, G., Tomassini, B., D'Ari, R. & Paolozzi, L. (1995). Reversal of *Mugem2ts*-induced mutations. *FEMS Microbiol Rev* **17**, 171–176.
- Grindley, N. D. & Reed, R. R. (1985). Transpositional recombination in prokaryotes. *Annu Rev Biochem* **54**, 863–896.
- Kleckner, N. (1981). Transposable elements in prokaryotes. *Annu Rev Genet* **15**, 341–404.
- Krug, P. J., Gileski, A. Z., Code, R. J., Torjussen, A. & Schmid, M. B. (1994). Endpoint bias in large Tn10-catalyzed inversions in *Salmonella typhimurium*. *Genetics* **136**, 747–756.
- La Valle, R., Fabozzi, G., Ghelardini, P. & Paolozzi, L. (1996). Bacteriophage *Mugem2ts*, which induces synchronous cell division in the infected host, is mutated in the *gem* operon promoter. *Res Microbiol* **147**, 615–618.
- Lemon, K. & Grossman, A. D. (1998). Localization of bacterial DNA polymerase: evidence for a factory model of replication. *Science* **282**, 1216–1219.
- Mahillon, J. & Chandler, M. (1998). Insertion sequences. *Microbiol Mol Biol Rev* **62**, 725–774.
- Miller, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- Mizobuchi, M. & Frohman, L. A. (1993). Rapid amplification of genomic DNA ends. *Biotechniques* **15**, 213–216.

Pato, M. (1989). Bacteriophage Mu. In *Mobile DNA*, pp. 23–52. Edited by D. E. Berg & M. M. Howe. Washington, DC: American Society for Microbiology.

Rebollo, J. E., Francois, V. & Louarn, J. M. (1988). Detection and possible role of two large nondivisible zones on the *Escherichia coli* chromosome. *Proc Natl Acad Sci USA* **24**, 9391–9395.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning; a Laboratory Manual*, 2nd edn. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

Shapiro, J. (1984). Observations on the formation of clones containing *araB*–*lacZ* cistron fusions. *Mol Gen Genet* **194**, 79–90.

Wang, X. & Higgins, P. (1994). ‘Muprints’ of the *lac* operon demonstrate physiological control over the randomness of in vivo transposition. *Mol Microbiol* **12**, 665–677.

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