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Chromosomal Integration Mechanism of Infecting Mu Virion DNA

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DNA transposition is central to the propagation of temperate phage Mu. A long-standing problem in Mu biology has been the mechanism by which the linear genome of an infecting phage, which is linked at both ends to DNA acquired from a previous host, integrates into the new host chromosome. If Mu were to use its well-established cointegrate mechanism for integration (single-strand nicks at Mu ends, joined to a staggered double-strand break in the target), the flanking host sequences would remain linked to Mu; target-primed replication of the linear integrant would subsequently break the chromosome. The absence of evidence for chromosome breaks has led to speculation that infecting Mu might use a cut-and-paste mechanism, whereby Mu DNA is cut away from the flanking sequences prior to integration. In this study we have followed the fate of the flanking DNA during the time course of Mu infection. We have found that these sequences are still attached to Mu upon integration and that they disappear soon after. The data rule out a cut-and-paste mechanism and suggest that infecting Mu integrates to generate simple insertions by a variation of its established cointegrate mechanism in which, instead of a "nick, join, and replicate" pathway, it follows a "nick, join, and process" pathway. The results show similarities with human immunodeficiency virus integration and provide a unifying mechanism for development of Mu along either the lysogenic or lytic pathway.

Mu DNA in a phage head is linear and covalently attached to host chromosomal DNA packaged during the previous round of infection (29). Fifty to 150 bp of host sequences flank the left end of Mu, and 0.5 to 3 kb flank the right end. Upon infection of an *Escherichia coli* host, noncovalently closed circular forms of Mu have been observed and have been presumed to be integrative precursors (16, 26). Infecting DNA integrates into the host genome without prior replication, a process that has been referred to as "conservative" or "nonreplicative" integration (1, 15, 19). Whether this integration follows the well-established "cointegrate" mechanism described below or occurs by some alternate mechanism is not known. In the ensuing lytic cycle, where Mu DNA is amplified over 100-fold, transposition is known to occur by the cointegrate mechanism (9).

Study of Mu transposition using plasmid substrates in vitro established that single-strand cleavages at Mu ends initially liberate free 3′ OHs which subsequently attack target DNA to generate a θ strand transfer intermediate (Fig. 1A, panel i) (22). The Mu-target fusion joint leaves 3′ OHs on target ends, which can be used as primers for replication through Mu, resolving the θ intermediate to a cointegrate end product, where directly repeated copies of Mu border the target and non-Mu donor sequences (21, 23). The predominant end products of Mu transposition/replication during the lytic cycle are cointegrates (9). If infecting Mu were also to integrate by this mechanism, target-primed replication into the flanking sequences would break the chromosome (Fig. 1A, panel ii). Clear evidence against this possibility exists (10). How, then, does infecting Mu integrate? Does it use a cut-and-paste type of mechanism, where both strands of the transposon are severed from its flanking DNA prior to integration and where the integrated DNA does not generate a branched intermediate and therefore does not need to be resolved by replication (Fig. 1B) (12)? Or does it use an alternate cointegrate mechanism, where the branched strand transfer structure is processed and repaired prior to replicative transposition (Fig. 1C)?

As more transposable elements are studied in vitro, it is apparent that the cointegrate mechanism of transposition is not widely used (14). Mu shares many features of this mode of transposition with retroviral elements, for example, single-strand cleavages at each end followed by a one-step transesterification that joins these ends to 5′ phosphates on the target DNA (11). Most other elements transpose by the cut-and-paste mechanism. In order to distinguish the cut-and-paste mechanism (in which flanking DNA is cut away prior to integration) from the cointegrate mechanism (in which flanking DNA is linked even after integration), we have followed the fate of the flanking DNA upon Mu infection. We show that this DNA remains linked to Mu, ruling out a cut-and-paste mechanism. Our results suggest instead that integration is initiated by the cointegrate pathway, but that the intermediate is processed by repair rather than by replication to generate a simple insertion, similar to how retroviral DNA is thought to integrate.

MATERIALS AND METHODS

Strains. *E. coli* was the host in all experiments. HM8305 is F′ *pro*$^+$ *lac*:Mu *cts*62/Δ*pro lac his met rpsL* Mu^r (5). BU1384 F$^-$ Δ*pro lac sup* (K5070 in reference 8) carries a suppressor of unknown origin. BU1382 (K5461 in reference 8) is isogenic with BU1384 but carries *himA*Δ82. Mu *cts*62 *Aam*1093 was prepared by induction of MH 219 (24). BU1384 and BU40 (Δ*pro lac*) were used as Sup$^+$ and Sup$^-$ hosts for infection with the *Aam* mutant.

Phage and DNA procedures. Procedures for prophage Mu induction (42°C for 40 min, followed by a shift down to 37°C until lysis), phage purification (cesium

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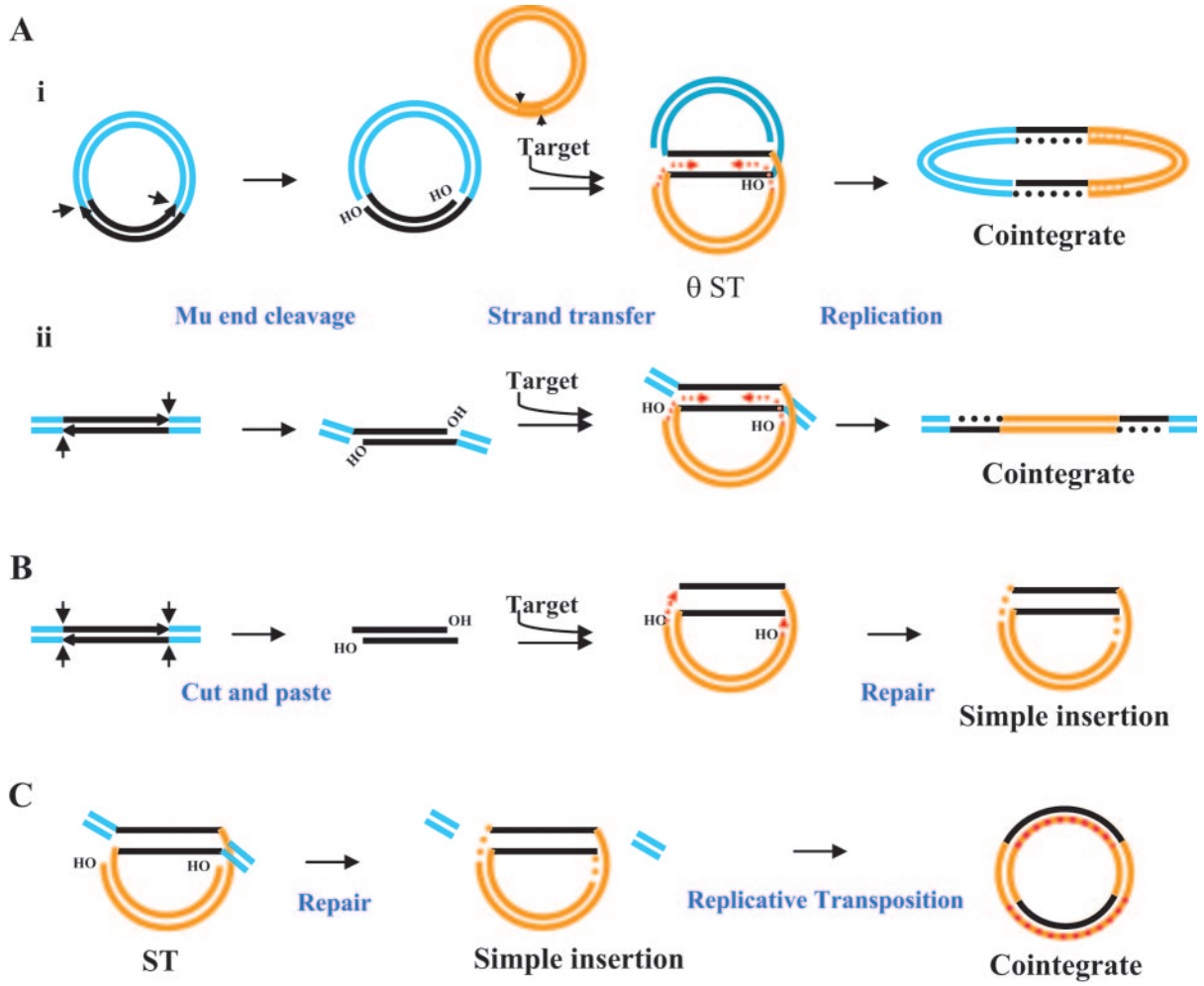

FIG. 1. (A) Transposition from circular (i) or linear (ii) Mu donor DNA substrates by the cointegrate mechanism. Black, Mu DNA; blue, non-Mu DNA; orange, target DNA; OH, 3′ hydroxyl group; dotted lines, replicated DNA; θ ST, strand transfer intermediate. Arrows point to sites of cleavage or strand transfer. (B) Transposition by a cut-and-paste mechanism. Several variations of this mechanism can be envisioned (12). (C) Transposition by an alternate cointegrate mechanism. After integration, flanking sequences in the branched ST intermediate are removed by nucleases and the single-stranded gaps filled by repair. Subsequent amplification of Mu is associated with replication.

chloride gradient centrifugation), and DNA isolation have been described previously (6). We note that preparation of phage by induction is expected to give rise to equal proportions of phage in the positive and negative orientations of the invertible G segment which controls the host range of Mu (18). Even though only the G-positive phage will infect *E. coli*, the fraction of Mu genomes linked to *lacZ* in the infecting population should be the same as that for the whole population.

For infection, strains were grown to an optical density at 600 nm of 0.4 and pelleted, and 2×10^9 cells were resuspended in 1 ml of ice-cold buffer containing 20 mM Tris-HCl, 200 mM NaCl, 2 mM $CaCl_2$, 20 mM $MgCl_2$, pH 7.5. Phage were added at a multiplicity of infection of 5, gently mixed on ice for 2 min, and diluted with 9 ml of prewarmed LB plus 5 mM $MgSO_4$ and 2.5 mM $CaCl_2$ at 37°C. Cells were incubated at 37°C for various times prior to isolation of total DNA as described previously (20). The isolation procedure involved lysis with sodium dodecyl sulfate, followed by treatment with pronase and phenol-chloroform and ethanol precipitation.

Integrated Mu was separated from free Mu by subjecting 30 to 40 μg of total cellular DNA to pulsed-field gel electrophoresis (PFGE). DNA mixed with sample buffer was heated at 65°C for 2 min before being loading onto a 1% pulsed-field agarose gel (Bio-Rad CHEF-DF II system) in 0.5× Tris-borate-EDTA buffer. Electrophoresis was at 200 V for 17 h at room temperature with a switch time of 1.3 s. After electrophoresis, the gel was stained with ethidium bromide, and the high-molecular-weight DNA was excised and purified using QIAGEN gel purification kit. For more accurate size estimation, purified total DNA was loaded onto a 1% pulsed-field gel as described above, but electrophoresis was at 200 V for 20 h at 4°C with an increasing switch time of 1 to 15 s. Sizes were calibrated using low-range PFG markers and mid-range II PFG markers from New England BioLabs.

Mapping the position of Mu within *lacZ* in HM8305. Initially, primers spanning several regions within *lacZ* were paired with primers pointing outwards from the left and right Mu ends and tested in PCRs for formation of product. MuL-LacZ(Top) and MuR-LacZ(Bot) primer pairs (Table 1) were used for final amplification and identification of insertion joints by sequencing.

PCR. Primers are listed in Table 1. All PCRs used 15 pmol of primer, 10 μmol of deoxynucleoside triphosphate, 5 units of *Taq* polymerase (QIAGEN), 50 to 500 ng of template DNA, and 2.5 mM of magnesium in a 50-μl final volume. The PCR conditions were as follows: initial denaturation at 94°C for 2 min; 40 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 10 min. PCR products were separated on a 1% agarose gel using 1× Tris-acetate-EDTA buffer, stained with ethidium bromide, and photographed.

Chromosomal DNA amounts for PCRs were standardized using the following primer pairs, with the size of the DNA amplified indicated in parentheses: Tsr(F)-Tsr(R) within the *E. coli* serine chemoreceptor gene *tsr* (488 bp), MuA(F)-MuA(R) within Mu*A* (825 bp), LacZ(F)-LacZ(R) within *lacZ* (327 bp), MuR-LacZ(R) across the right Mu end insertion joint (535 bp), Mu-P2 and LacZN-2 across the left Mu end insertion joint (180 bp), and ProB(F)-ProB(R) within *proB* (749 bp).

TABLE 1. Primers used in PCRs

Primer	Sequence
MuL	5′ ACC AAA CTA ATC CCT ACA GAT C 3′
Mu-P2	5′ ATT TTC AAT GAA ACA AAA GC 3′
LacZ(Top)	5′ TAC GGA TTC ACT GGC CGT CG 3′
MuR	5′ CAT TTG AAG CGC GAA AGC TAA AG 3′
LacZ(Bot)	5′ G ACG ACA GTA TCG GCC TCA GGA AGA 3′
Tsr(F)	5′ CAG CGT AAT TGA CGG CAT TGC C 3′
Tsr(R)	5′ ACC ACA GCC GAT GTT TCA CGC TG 3′
MuA(F)	5′ GAA TAA CAT GGA ACT TTG GGT ATC AC 3′
MuA(R)	5′ ATG CAG ATA ACC GTC GCC GTT GAT CCA CTG 3′
LacZ(F)	5′ TCT TCC TGA GGC CGA TAC TGT CGT C 3′
LacZ(R)	5′ CAC CGC GAG GCG GTT TTC TCC GGC 3′
proB(F)	5′ GTT CCG GTA ATC AAT GAG AAC GAT GC 3′
proB(R)	5′ AAC GGC AAC CGG GCA GTA TTC ATA ATC 3′
MuAN	5′ GAGATATACATATGGAACTTTGGGTATCACC 3′
MuAC	5′ CTGGATCCTTAAATGGCTTTTTTACGTCTG 3′
LacZN	5′ TACGGATTCACTGGCCGTCG 3′
LacZN-2	5′ AGC ACA TCC CCC TTT CGC CAG 3′
LacZC	5′ ACCAACTGGTAATGGTAGCG 3′
ECGK1F	5′ TGAGTGACAGCCAGACGCTGGTGG 3′
ECGK2R	5′ AACGGCAACCGGGCCGTATTCATATC 3′
MuA1	5′ GCATTTAATGATGCTGCGGCAGGCCGTGAATAT 3′
LacZ1	5′ GTGCCGGAAAGCTGGCTGGAGTGCG 3′
LacZ2	5′ ACTGCCGTCACTCCAACGCAGCACC 3′
ECGK1R	5′ CGAAAACAGCTGTTCCCACAGTTG 3′

For detection of Mu and *tsr* sequences, 50 ng of DNA was used as the template; 1/10 of the PCR mixture was subjected to electrophoresis. For detection of *lacZ* or *proB* sequences, 500 ng of DNA was used as the template; the entire PCR mixture was used for electrophoresis after concentration.

Real-time PCR. For generating standard curves for real-time PCR, templates of Mu*A*, *lacZ*, and *proB* were first prepared by PCR amplification of Mu or wild-type *E. coli* genomic DNA using the following primers pairs that amplify the entire gene: MuAN-MuAC (2,010 bp of Mu*A*), LacZN-LacZC (3,048 bp of *lacZ*), and ECGK1F-ECGK2R (1,102 bp of *proB*). The PCR amplicons were gel purified using a QIAGEN gel extraction kit, and the DNA sequences were confirmed by direct sequencing. Purified DNA was UV-spectrophotometrically quantified and used as a calibrator for real-time PCR analysis. Copy numbers of the gene fragments were calculated as follows: concentration of gene fragment/mass of gene fragment $\times 6.02 \times 10^{23}$.

Real-time PCR analysis was done on an Applied Biosystems (AB) 7900HT sequence detection system using their SYBR Green PCR kit, with 2.5 mM $MgCl_2$ and 0.3 μM of each primer in a total volume of 25 μl. The primer pairs selected by Primer Express software (AB) (designed to minimize nonspecific amplification) for Mu*A*, *lacZ*, and *proB* were MuA1-MuAC, LacZ1-LacZ2, and ECGK1F-ECGK1R, respectively; amplified fragments range from 200 to 300 bp. The cycling conditions were 15 min at 95°C followed by 40 cycles of 95°C for 15 s, 60°C for 1 min, and 72°C for 1 min. The threshold cycle number was used to derive a standard curve with the selected primers, using as template serial dilutions of the template DNA containing from 0 to 10^6 copies per 2 μl (in triplicate). The selected primers were used in parallel to amplify DNA from the unknown sample (in triplicate), and their copy number was estimated from the standard curve by using the software 7000 SDS V. 1.0 (AB). The percentage of phage carrying *lacZ* or *pro*B was estimated as follows: copies of *lacZ* or *proB* in unknown sample/copies of Mu*A* in unknown sample × 100%.

RESULTS AND DISCUSSION

Experimental design. The only known pathway for Mu transposition in vivo (during the lytic cycle) and in vitro employs the cointegrate mechanism (Fig. 1A). If infecting Mu initiates integration by the same mechanism, it should remain linked to the flanking host sequences present in the virion. To follow these sequences after Mu integration, we used markers unique to the infecting phage DNA. These markers (*lacZ*/*proB*) were acquired from the host in which the phage had been propagated but were absent in the host being infected. In pilot experiments we observed a stronger PCR signal for *lacZ* sequences in packaged phage when these were obtained after induction of Mu prophage inserted within *lacZ*, compared to those prepared from infection of *lacZ*$^+$ cells. The Mu lysogen HM8305 was therefore used for preparation of phage by induction. The orientation and site of Mu insertion within *lacZ* in HM8305 were determined as described in Materials and Methods and are diagrammed in Fig. 2A. In normal PCRs, *lacZ* sequences linked to the right or left end were detected using pairs of primers described in the Fig. 2 legend. Quanti-

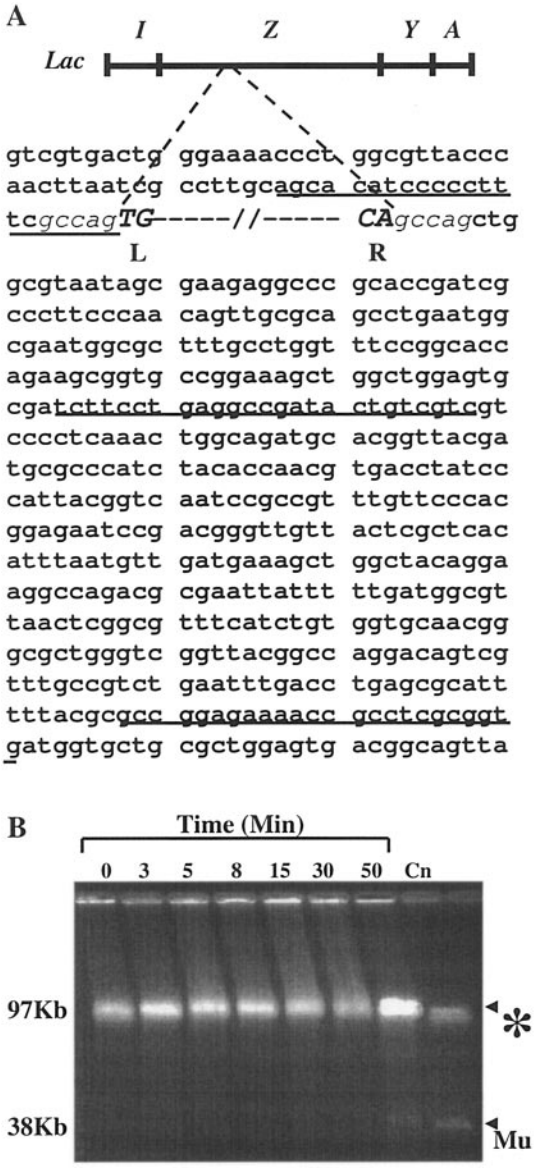

FIG. 2. (A) Orientation and site of Mu insertion (top strand) in the *lacZ* gene of the Lac operon (*lacIZYA*) in strain HM8305. Only the terminal dinucleotides TG/CA at the left (L) and right (R) ends of Mu are shown; the 5-bp duplication of host sequences flanking the Mu insertion is indicated in lowercase italic. Underlined sequences indicate primers [LacZ(F), LacZ(R), and LacZN2] used for detection of this region in integrated Mu in later experiments (see Table 1). (B) PFGE of total DNA from BU1384, isolated at indicated times after infection with Mu derived from HM8305. Cn, control lane with a mixture of genomic DNA from uninfected cells and purified phage DNA. Positions of free Mu and chromosomal DNA (*) are indicated. Known DNA markers were used to estimate sizes. See Materials and Methods for details.

tative PCRs (see Materials and Methods) showed that the fraction of Mu DNA carrying *lacZ* was 3.6% in the induced population (data not shown). *proB* was less well represented, with only 0.1% of the input phage carrying this marker. The 36-fold enrichment for the specific marker *lacZ* (adjacent to the integrated prophage) over *proB* (the nonadjacent marker) in the induced population is likely because of a high probability of packaging the preexisting insertion.

Time course of Mu integration. BU1384 (Δ*pro lac*) was infected with Mu obtained from induction of HM8305 (Fig. 2A). Samples were removed at various times up to the onset of lysis at 50 min for extraction of total DNA, which was subjected to PFGE to separate integrated Mu in the larger chromosomal fragments from the smaller free Mu DNA (Fig. 2B). Mature Mu-length genomes first appeared at the last time point assayed, suggesting that packaging of replicated Mu has begun by this point. In the control lane, uninfected genomic DNA was mixed with Mu virion DNA in order to gauge the extent of contamination of the chromosomal DNA band with free Mu in subsequent PCRs. We note that the chromosomal DNA, which runs at approximately 97 kbp, is fairly sheared by the extraction procedure; when electrophoresed for longer times, it runs as a smear between 70 and 150 kbp (not shown). It is unlikely, therefore, that any Mu molecules would remain somehow topologically trapped in these fragmented chromosome pieces that have been subjected to protease treatment, phenol extraction, and ethanol precipitation (see Materials and Methods).

Flanking host sequences remain linked to Mu DNA upon integration. Chromosomal DNA bands throughout the sampled time course (Fig. 2B) were isolated and subjected to PCRs with different primer sets (Fig. 3). Amounts of chromosomal DNA used as templates were standardized with primers to the chromosomal serine chemoreceptor gene *tsr* (Fig. 3F). Controls included DNA from the control lane in Fig. 2B (Cn), Mu virion DNA used for infection, and a negative no-template control to monitor for spurious contamination.

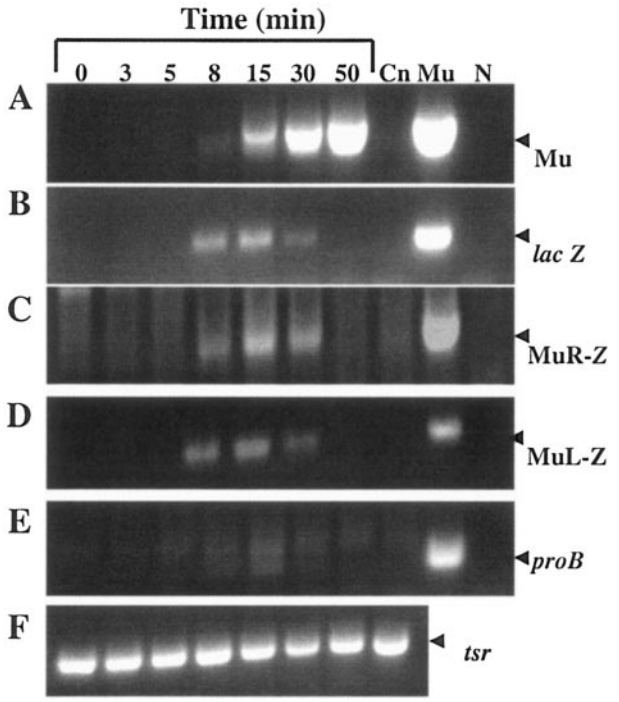

FIG. 3. Time course of Mu integration and fate of flanking DNA. Genomic DNA separated from free Mu DNA (see Fig. 2B) was amplified in PCRs to detect the sequences indicated on the right. DNA loading was standardized to a chromosomal marker, *tsr*. PCRs shown in panels A and F contained similar amounts of DNA, while those in panels B to E contained larger amounts because *lac* and *pro* sequences constituted only a fraction of the input Mu DNA (see Materials and Methods). The band above the amplified *proB* fragment in panel E is a nonspecific product present in all the lanes. Controls included DNA from the control lane in Fig. 2B (Cn), Mu virion DNA used for infection (Mu), and a negative no-template control (N) to monitor for spurious contamination.

Mu sequences were first detected in the 8-minute sample (Fig. 3A), consistent with detection times reported previously (8, 15). Concomitant with detection of Mu, *lacZ* sequences were also detected (Fig. 3B) (note that larger amounts of template DNA were used in Fig. 3B to E). Primers within Mu (R and L ends) and *lacZ* were used to ensure that the *lacZ* sequences were indeed linked to Mu (Fig. 3C and D). The signal for *proB* sequences was weaker than that for *lacZ*, as expected from its lower representation in the input DNA; nonetheless, *proB* was also detected concomitant with the earliest detection of Mu (Fig. 3E). From the Cn control lane it is evident that the chromosomal DNA bands are not contaminated with free Mu DNA, nor is Mu DNA amplified from the chromosomal DNA bands up to the 5-min time point; thus, any Mu DNA amplified from the chromosomal fragments must represent bona fide integration.

While the flanking sequences were found in the chromosome at the same time point that Mu sequences were, their subsequent detection patterns were different. Mu DNA continued to increase up to 50 min (Fig. 3A), consistent with its replication during lytic development, which begins soon after integration (15). *lacZ* and *proB* sequences, however, were both maximally detected at 15 min, diminished by 30 min, and were undetectable by 50 min (Fig. 3B to E). This suggests the operation of a mechanism that specifically removes flanking sequences after integration. Since phage adsorption and injection are not synchronized, the maximal detection of flanking sequences at 15 min likely represents the peak of integration of the infecting phage population.

To determine what fraction of integrated Mu genomes bring along the flanking sequences, quantitative PCR was employed (see Materials and Methods). Genomic DNA isolated 8 min after infection was used as the template, because this was the earliest integration time point. Primers used in these experiments are described in Materials and Methods and listed in Table 1. We estimated that 1.4% of the integrated Mu genomes carry *lacZ* at the 8-min time point (data not shown). Since 3.6% of the input Mu had associated *lacZ*, this means that flanking *lacZ* sequences can be detected in approximately 40% of integrated Mu. This value is likely to be an underestimate, since degradation of flanking DNA has probably begun even at this earliest time. Given that the values for input and integrated genomes agree within a factor of 2 to 3, we conclude that the fractions of Mu genomes linked to *lacZ* are similar in the input and integrated samples. Therefore, the majority of infecting Mu integrates by this pathway. The input *proB* signal

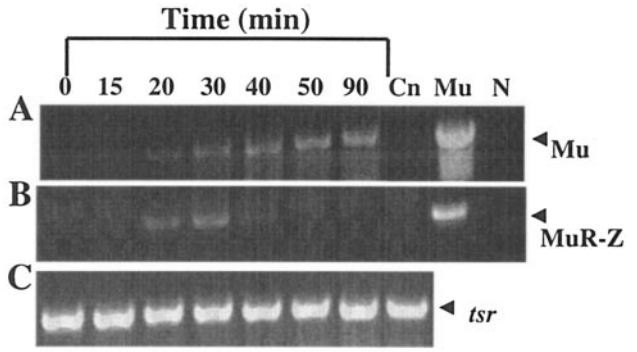

FIG. 4. Mu integration into a *himA* host. All procedures were similar to those used for Fig. 3. Panels A, B, and C correspond to panels A, C, and E in Fig. 3. A nonspecific product is seen in panel A in the 0-min, 15-min, and Cn lanes and in panel B above the MuR-Z band in all lanes.

was determined to be too low to obtain meaningful data from integrated Mu.

Additional controls included monitoring integration of an *Aam* mutant of Mu (MuA is the transposase), which is known to be defective in integration (24), in Sup^+ and Sup^- hosts (see Materials and Methods). At the 30-min time point, integration of the *Aam* mutant was detected only in the Sup^+ host (data not shown). Thus, the Mu integration we detect is the result of the action of the transposase and not some illegitimate recombination event.

We conclude that soon after infection, the flanking DNA present on both the left and right ends of the input genomes is still attached to the majority of integrated Mu genomes. While our data do not address whether flanking sequences are attached to both ends of the same genome, this is a reasonable assumption. Thus, it appears that infecting Mu integrates by nicking and strand transfer, similar to the initial steps of the cointegrate pathway (Fig. 1B). However, the flanking sequences are processed and removed, unlike in the cointegrate pathway (Fig. 1C).

Similar kinetics of flanking sequence removal in a host permissive for integration but not for Mu replication. In lysogens, Mu integrations are recovered as simple insertions. It has been speculated that these insertions might be formed by a cut-and-paste mechanism (29). We were curious, therefore, to follow the fate of the flanking sequences in a strain which showed efficient lysogeny. We chose a *himA* mutant of *E. coli*, which can be lysogenized by Mu at high levels but does not support Mu replication (3, 4). The *him* locus encodes integration host factor, which is involved in the regulation of expression of the early region of Mu (17) and is one of several host functions required for normal Mu development (25). While transposition during the lytic pathway is reduced by more than a factor of 100 in *himA* mutants, the integration of ^{32}P-labeled Mu DNA was reported to be depressed by a factor of 2 to 3 and showed a slower integration kinetics (8).

A *himA* mutant host (BU1382) was infected with Mu phage as before. Samples were collected for up to 90 min after infection, because of the reported slower Mu integration in this strain. Chromosomal DNA was separated from free Mu by PFGE and subjected to PCR as before (Fig. 2 and 3). Mu integration was first detected at 20 min postinfection and appeared to level off between 40 to 50 min (Fig. 4A). Mu sequences were not amplified, consistent with lack of Mu replication in this strain (compare Fig. 4A to Fig. 3A). *lacZ* sequences, however, were detected concomitant with Mu integration at 20 min (Fig. 4B; compare with Fig. 3C). These sequences were processed with kinetics similar to that seen during the infection of a wild-type host: they were maximally detected at 30 min, diminished at 40 min, and were undetectable by 50 min. Thus, Mu integration in the *himA* mutant strain, which supports efficient lysogeny but not Mu replication, is similar to that in the wild-type strain in that flanking Mu sequences are attached to the integrated genomes under both conditions and appear to be similarly processed.

Summary and conclusions. The results in this study show that the majority of infecting Mu DNA integrates by a nick-join-process mechanism to give simple insertions, in a variation of the cointegrate mechanism (Fig. 1C). Given that all known transposases cleave to generate 3′ OH ends and strand transfer these to 5′ P groups (2, 14), the polarity of the strand cleavage/joining reactions during integration is likely to be the same as that established for the cointegrate pathway. Indeed, the Mu lysogen originally obtained by integration of infecting DNA has a tell-tale 5-bp duplication at the insertion site (Fig. 2A); this must have arisen as a result of gap repair, a process that involves extension of 3′ OHs left on the target.

Our studies do not address how the flanking sequences are processed in vivo. Parallel loss of these sequences within minutes following Mu integration, irrespective of whether or not Mu DNA replication follows (Fig. 3 and 4), eliminates models involving replication followed by recombination. A replication-recombination mechanism is also not favored by earlier experiments which showed that both strands of Mu throughout the Mu genome are recovered in the integrated population (10). A more plausible mechanism involves degradation of the integrated flanking sequences followed by gap repair, irrespective of whether Mu develops along the lysogenic or the lytic pathway (Fig. 1C). Gap repair has been invoked to explain the apparent replication of a fraction of integrated Mu DNA in a *dam himA* host (15). A related mechanism was suggested earlier to explain the generation of both cointegrates and simple inserts upon incubation of the θ intermediate with cell extracts in vitro; in this scheme, simple inserts would be the product of gap repair of the θ intermediate, while cointegrates would result from replication (21). Resolution of the θ structure by replication would work only with circular donors (Fig. 1A). For linear donors, if a second round of transposition were attempted immediately after integration and prior to replication, the resulting cleavages at the 3′ ends of Mu would lead to Mu excision (consider the consequence of nicking the 3′ ends of Mu in the strand transfer intermediate shown in Fig. 1C) unless the 5′ ends had been first repaired and joined to the target to give a simple insertion. This would mean that the first integration event is truly nonreplicative; i.e., replication of the first insertion must occur only after repair is completed and during the second round of transposition as shown in Fig. 1C. (We note that the term "nonreplicative transposition" used to describe this integration refers to the replication status of Mu prior to integration [1, 10, 15, 19].) It appears, therefore, that integration of linear Mu DNA has similarities to integration of

linear human immunodeficiency virus DNA, which is thought to be processed by gap repair (11).

We are left with several questions, the most important of which is the mechanism of removal and repair of flanking host sequences. We also do not know why the initial integrant is processed differently from subsequent integrations, although the structure of the infecting Mu genome, i.e., a noncovalently closed circle joined at the tips by the injected phage protein N, has long been evoked as the decisive factor (13, 16, 26, 28, 29). Other differential requirements for phage and host proteins during these two types of events are also known (7, 27, 29). Clearly, there is much to be learned.

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REFERENCES

1. **Akroyd, J. E., and N. Symonds.** 1983. Evidence for a conservative pathway of transposition of bacteriophage Mu. Nature **303:**84–86.
2. **Baker, T. A., and K. Mizuuchi.** 2002. Chemical mechanisms for mobilizing DNA, p. 12–23. *In* N. L. Craig, R. Craigie, M Gellert, and A. M. Lambowitz (ed.), Mobile DNA II. ASM Press, Washington, D.C.
3. **Bourret, R. B., and M. S. Fox.** 1988. Intermediates in bacteriophage Mu lysogenization of *Escherichia coli him* hosts. J. Bacteriol. **170:**1683–1690.
4. **Bourret, R. B., and M. S. Fox.** 1988. Lysogenization of *Escherichia coli him*$^+$, *himA*, and *himD* hosts by bacteriophage Mu. J. Bacteriol. **170:**1672–1682.
5. **Bukhari, A. I.** 1975. Reversal of mutator phage Mu integration. J. Mol. Biol. **96:**87–99.
6. **Bukhari, A. I., and E. Ljungquist.** 1977. Bacteriophage Mu: methods for cultivation and use, p. 749–756. *In* A. I. Bukhari, J. A. Shapiro, and S. L Adhya (ed.), DNA insertion elements, plasmids and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Chaconas, G., E. B. Giddens, J. L. Miller, and G. Gloor.** 1985. A truncated form of the bacteriophage Mu B protein promotes conservative integration, but not replicative transposition, of Mu DNA. Cell **41:**857–865.
8. **Chaconas, G., G. Gloor, J. L. Miller, D. L. Kennedy, E. B. Giddens, and C. R. Nagainis.** 1984. Transposition of bacteriophage Mu DNA: expression of the A and B proteins from lambda pL and analysis of infecting Mu DNA. Cold Spring Harbor Symp. Quant. Biol. **49:**279–284.
9. **Chaconas, G., R. M. Harshey, N. Sarvetnick, and A. I. Bukhari.** 1981. Predominant end-products of prophage Mu DNA transposition during the lytic cycle are replicon fusions. J. Mol. Biol. **150:**341–359.
10. **Chaconas, G., D. L. Kennedy, and D. Evans.** 1983. Predominant integration end products of infecting bacteriophage Mu DNA are simple insertions with no preference for integration of either Mu DNA strand. Virology **128:**48–59.
11. **Craigie, R.** 2002. Retroviral DNA integration, p. 613–630. *In* N. L. Craig, R. Craigie, M. Gellert, and A. M. Lambowitz (ed.), Mobile DNA II. ASM Press, Washington, D.C.
12. **Curcio, M. J., and K. M. Derbyshire.** 2003. The outs and ins of transposition: from mu to kangaroo. Nat. Rev. Mol. Cell Biol. **4:**865–877.
13. **Gloor, G., and G. Chaconas.** 1988. Sequence of bacteriophage Mu N and P genes. Nucleic Acids Res. **16:**5211–5212.
14. **Haren, L., B. Ton-Hoang, and M. Chandler.** 1999. Integrating DNA: transposases and retroviral integrases. Annu. Rev. Microbiol. **53:**245–281.
15. **Harshey, R. M.** 1984. Transposition without duplication of infecting bacteriophage Mu DNA. Nature **311:**580–581.
16. **Harshey, R. M., and A. I. Bukhari.** 1983. Infecting bacteriophage mu DNA forms a circular DNA-protein complex. J. Mol. Biol. **167:**427–441.
17. **Higgins, N. P., D. A. Collier, M. W. Kilpatrick, and H. M. Krause.** 1989. Supercoiling and integration host factor change the DNA conformation and alter the flow of convergent transcription in phage Mu. J. Biol. Chem. **264:**3035–3042.
18. **Koch, C. M., G. Ruct, F. Kahmann, R. Kanaar, R. Plasterk, R. Van De Putte, P. Sandulache, and R. Kamp.** 1987. The invertible G segment, p. 75–91. *In* N. T. Symonds, A. Toussaint, P. Van De Putte, and M. M. Howe (ed.), Phage Mu. Cold Spring Laboratory, Cold Spring Harbor, N.Y.
19. **Liebart, J. C., P. Ghelardini, and L. Paolozzi.** 1982. Conservative integration of bacteriophage Mu DNA into pBR322 plasmid. Proc. Natl. Acad. Sci. USA **79:**4362–4366.
20. **Ljungquist, E., and A. I. Bukhari.** 1977. State of prophage Mu DNA upon induction. Proc. Natl. Acad. Sci. USA **74:**3143–3147.
21. **Mizuuchi, K.** 1984. Mechanism of transposition of bacteriophage Mu: polarity of the strand transfer reaction at the initiation of transposition. Cell **39:**395–404.
22. **Mizuuchi, K.** 1992. Transpositional recombination: mechanistic insights from studies of mu and other elements. Annu. Rev. Biochem. **61:**1011–1051.
23. **Nakai, H., V. Doseeva, and J. M. Jones.** 2001. Handoff from recombinase to replisome: insights from transposition. Proc. Natl. Acad. Sci. USA **98:**8247–8254.
24. **O'Day, K., D. Schultz, W. Ericsen, L. Rawluk, and M. Howe.** 1979. Correction and refinement of the genetic map of bacteriophage Mu. Virology **93:**320–328.
25. **Pato, M. L.** 1989. Bacteriophage Mu, p. 23–52. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. ASM Press, Washington, D.C.
26. **Puspurs, A. H., N. J. Trun, and J. N. Reeve.** 1983. Bacteriophage Mu DNA circularizes following infection of Escherichia coli. EMBO J. **2:**345–352.
27. **Sokolsky, T. D., and T. A. Baker.** 2003. DNA gyrase requirements distinguish the alternate pathways of Mu transposition. Mol. Microbiol. **47:**397–409.
28. **Sonti, R. V., D. H. Keating, and J. R. Roth.** 1993. Lethal transposition of Mud phages in Rec- strains of Salmonella typhimurium. Genetics **133:**17–28.
29. **Symonds, N. T., A. Toussaint, P. Van De Putte, and M. M. Howe (ed.).** 1987. Phage Mu. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.