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Early Events in the Replication of Mu Prophage DNA

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To determine whether the early replication of Mu prophage DNA proceeds beyond the termini of the prophage into host DNA, the amounts of both Mu DNA and the prophage-adjacent host DNA sequences were measured using a DNA-DNA annealing assay after induction of the Mu vegetative cycle. Whereas Mu-specific DNA synthesis began 6 to 8 min after induction, no amplification of the adjacent DNA sequences was observed. These data suggest that early Mu-induced DNA synthesis is constrained within the boundaries of the Mu prophage. Since prophage Mu DNA does not undergo a prophage λ-like excision from its original site after induction (E. Ljungquist and A. I. Bukhari, Proc. Natl. Acad. Sci. U.S.A. 74:3143-3147, 1977), we propose the existence of a control mechanism which excludes prophage-adjacent sequences from the initial Mu prophage replication. The frequencies of the Mu prophage-adjacent DNA sequences, relative to other *Escherichia coli* genes, were not observed to change after the onset of Mu-specific DNA replication. This suggests that these regions remain associated with the host chromosome and continue to be replicated by the chromosomal replication fork. Therefore, we conclude that both the Mu prophage and adjacent host sequences are maintained in the host chromosome, rather than on an extrachromosomal form containing Mu and host DNA.

Several properties of the temperate bacteriophage Mu-1 distinguish it from other temperate bacterial viruses such as λ (3, 6, 11). Among these are the capacities to integrate at random throughout the host chromosome, to generate random mutations (5, 26), to integrate throughout the lytic phase of development (23, 24), and to incorporate random host DNA sequences at both ends of the mature viral genome (2, 4, 7), which are lost when Mu becomes a prophage (1, 12). Further, the circular replicative intermediates characteristic of other temperate bacteriophage such as λ, P2, and P22 (3, 14, 18, 19) have not been observed during Mu development. Intracellular, Mu-containing, circular molecules have been isolated 10 to 12 min after induction of a heat-inducible mutant of phage Mu (28, 30) and after Mu infection of a sensitive host (24). These heterogeneously sized circular DNA forms (Hc-DNA) contain both Mu and host DNA sequences, covalently linked, and their lengths show no consistent correlation to multiples of Mu DNA (24, 29). Thus, they are not analogous to the circular, monomeric replicative DNA forms found in other temperate phage systems, and their role, if any, in Mu DNA replication is unknown.

We have previously reported on the kinetics of Mu-specific DNA synthesis after heat induction of Mu (30). Mu DNA replication begins approximately 6 to 8 min after induction and proceeds at a rate such that by 9 min there is the equivalent of two copies of Mu DNA per cell, by 12 min there are four copies, and by 15 min there are six copies (30). Similar kinetics of Mu DNA replication have been reported following infection of a sensitive host by Mu phage (32).

Ljungquist and Bukhari have shown that, for at least 30 min after induction, the junctions between the Mu prophage and the host DNA from the primary prophage site remain detectable under conditions where the λ prophage-host junctions disappear (20). While it is not known whether the Mu prophage present in the original site at later times is the original prophage or a copy, it is clear from their data that the early replication of Mu DNA occurs while the prophage is attached to the neighboring sequences. In the experiments described in the present communication, we explored whether early Mu DNA replication after heat induction of Mu continues into the adjacent host sequences or, alternatively, is limited by the prophage termini. The first of these alternatives occurs with other prophages such as λ (13); this mechanism also has been invoked for phage Mu in the model of Faelen and Toussaint for the formation of Hc-DNA molecules (9).

The two alternative modes of Mu DNA rep-

lication can be distinguished by monitoring the production of the Mu prophage-adjacent DNA sequences following heat induction of Mu. For this purpose, strains were constructed carrying a Mu prophage with a thermolabile repressor (Mu*cts*) integrated into λ*c*$^+$ prophage. After heat induction of the Mu prophage, the Mu DNA and λ DNA sequences were assayed by DNA-DNA annealing to membrane filters containing purified Mu or λ DNA. Within the limits of the assay, we observed no significant amplification of the λ DNA beyond that passively replicated by the host, while the Mu DNA increased as described above. In addition, our results suggest that the original, prophage-adjacent sequences remain associated with the host chromosome, rather than being excised into an extrachromosomal form containing Mu and host DNA. Therefore, we propose that even though early Mu DNA replication occurs while in association with the host chromosome, this replication does not extend into the adjacent sequences.

MATERIALS AND METHODS

Bacteria and bacteriophage. The bacterial strains and their uses in this study are described in Table 1. All are derivatives of *Escherichia coli* K-12 except for AT3557, which is a derivative of *Proteus mirabilis* (PM14) that carries an RP4::Mu*cts*62 plasmid. Normally, Mu is propagated on *E. coli* K-12, and the progeny phage contains covalently attached, random *E. coli* sequences at each terminus of the mature DNA molecule (4, 7). However, Mu can grow on other bacterial strains, including *Proteus mirabilis*, and the terminal host sequences of the Mu genome are most likely homologous to the host in which it is grown (26; R. Fitts and A. L. Taylor, personal communication). To designate Mu phage grown on alternate hosts, the name of the host is placed in parentheses immediately following the Mu genotype, e.g., Mu*cts*62 (*P. mirabilis*). Only for purposes of clarity will the phage derived from *E. coli* be so designated.

Genetic procedures. The methods for growth and labeling of cultures and preparation of samples have been described in detail elsewhere (28). Mu*cts*62 (*P. mirabilis*) was isolated from strain AT3557 in the manner described for Mu. MP300 was isolated after infecting AT4518 (λ*c*$^+$) with Mu*cts*62 and selecting survivors that form Mu plaques at 42°C, produce no λ phage, and still retain λ immunity. The position of the Mu prophage within the λ prophage was determined genetically by A. Toussaint. In strains MP300 and AT4587, the Mu prophage is located at a site somewhere between λ genes F and K, though the exact position is not known. Therefore, one end of the Mu prophage is flanked by 27.5 to 45% of the λ prophage, and the other end is flanked by 55 to 72.5% of the λ DNA. The orientation of Mu DNA with respect to the λ genes is not known for either strain.

DNA-DNA annealing assay. Purified DNA (5 μg of either Mu or λ DNA or 50 μg of *E. coli* DNA) was attached to membrane filters (Schleicher and Schuell Inc., type B5, 24 mm) as described by Kuempel (15). The DNA-DNA annealing assay was performed according to the procedure of Denhardt (8) as modified by Kuempel (15) and has been described in considerable detail elsewhere (28). However, a significant improvement in that procedure has been to use Mu*cts*62 (*P. mirabilis*) DNA on the filters instead of DNA from Mu grown on *E. coli*. This modification eliminates the annealing of labeled *E. coli* DNA sequences to mature Mu DNA on the filters.

If Mu DNA replication is unidirectional and if its replication proceeds through the shorter region of λ DNA, then we would expect the initial round of Mu replication to yield an increase of 27.5 to 45% of the amount of λ DNA present; the DNA-DNA annealing assay was designed to measure increases of this magnitude reproducibly and accurately. Alternatively, should Mu DNA replication proceed through the longer arm of the λ DNA, or bidirectionally through both arms, then larger increases in λ DNA should be detected.

RESULTS

Rationale and validation of DNA-DNA annealing technique. The essence of the experiments described here was to induce a Mu lysogen growing in the presence of [^{3}H]thymidine, and then to analyze, by DNA-DNA annealing, the relative rates of production of Mu DNA and the Mu prophage-adjacent host DNA. For the annealing assay, we constructed *E.*

TABLE 1. *Bacterial strains*

Strain no.	Description	Use in this work	Reference or Source
AT4518	F$^-$, *thyA16 thyR4 thi-1* Sup$^+$ Str^s	Experimental strain	A. L. Taylor
MP300	AT4518 (λ*c*$^+$::Mu*cts*62)	Experimental strain	This work
AT4587	F$^-$, λ*c*$^+$::Mu*cts*61/plasmid[a]	Experimental strain	A. Toussaint
AT4511	AT4518(λcI857)	Source of λ DNA	A. L. Taylor
TP8709	F$^-$, Δ*proA lac trp*::(+Mu*cts*62) Mu^r	Source of Mu (*E. coli*) DNA	A. I. Bukhari via R. Evans
AT3557	*thy met trp nic* Nal^r/RP4::Mu*cts*62 Amp^r Tet^r Kan^r	Source of Mu*cts* (*P. mirabilis*) DNA	A. L. Taylor and R. Fitts
C600	F$^-$, *thr-1 leu-6 thi-1* Sup$^-$	Source of *E. coli* DNA	A. L. Taylor
AT4512	C600 (Muc$^+$)	Titering of λ phage	A. L. Taylor
AT4513	C600 (λ*c*$^+$)	Titering of Mu phage	A. L. Taylor

[a] During the course of this work, strain AT4587 was found to contain an uncharacterized plasmid.

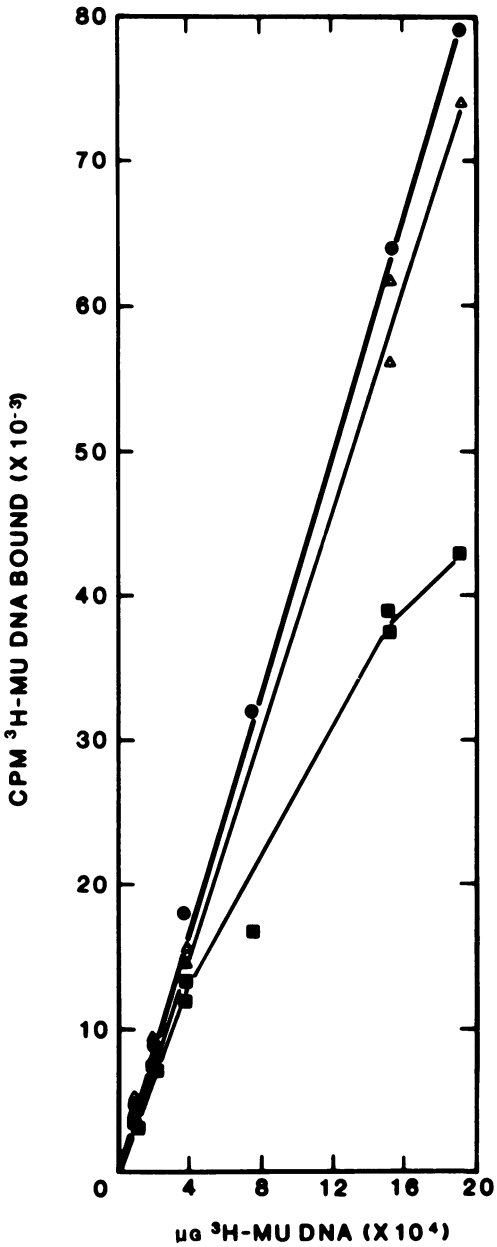

FIG. 1. *DNA-DNA annealing assay for Mu DNA. Each assay contained the indicated amount of ³H-labeled Mu DNA (4.6 × 10⁶ cpm/µg), 3,700 cpm of ³²P-labeled Mu DNA (1.13 × 10⁶ cpm/µg), 20 µg of nonradioactive competitor E. coli DNA, and a filter containing Mu (E. coli) DNA. The assay tubes were incubated for 20 h at 65°C. (●) Trichloroacetic acid-precipitable ³H-labeled Mu DNA input; (■) Counts per minute annealed to filters containing Mu DNA minus background counts per minute bound to filters without DNA; (△) counts per minute adjusted to 100% efficiency of annealing (counts per minute annealed to filters, corrected for background counts, were divided by the fraction of input ³²P-labeled Mu DNA annealed to the same filter).*

coli K-12 lysogens carrying λ*c*⁺ DNA with Mu*cts* DNA inserted within the λ prophage (λ::Mu*cts*62). Thus, the prophage-adjacent DNA may be specifically evaluated by the extent of annealing to filters containing purified λ DNA. Control experiments with induced λ lysogens show that increases in λ DNA sequences indeed can be detected when λ DNA is replicated.

Since mature Mu DNA contains host sequences at both termini (2, 4, 7), precautions must be taken to prevent annealing of *E. coli* DNA in the radioactivity-labeled sample to the host sequences attached to the mature Mu DNA immobilized on membrane filters. In early experiments, nonradioactive *E. coli* DNA was added to each assay as competitor DNA. In later experiments an improved technique was used, whereby Mu DNA loaded onto filters was obtained from phage propagated on *P. mirabilis*. With the use of filters containing Mu*cts* (*P. mirabilis*) DNA, annealing of labeled *E. coli* was eliminated both in the presence and absence of competitor *E. coli* DNA. This demonstrates that there are no *E. coli* DNA sequences associated with the Mu DNA isolated from *P. mirabilis*, and is consistent with phage Mu DNA carrying host sequences only from the strain on which the virus is propagated.

In some assays, the efficiency of DNA annealing to filters containing bacteriophage DNA was corrected to 100% by including a small amount of ³²P-labeled DNA homologous to that on the filter. Under our experimental conditions, usually 80 to 90% of the input ³²P-labeled Mu (*E. coli*) DNA annealed to filters containing Mu DNA, and 85 to 95% of the input ³²P-labeled λ DNA annealed to filters containing λ DNA. The amount of ³²P-labeled Mu DNA annealing to filters decreased as the amount of input increased (Fig. 1); hence, the need to correct for reduced annealing efficiency is greatest when more Mu sequences are present in the sample. The efficiency of input ³H-labeled *E. coli* DNA annealing to filters containing *E. coli* DNA was approximately 40 to 60% and was not corrected to 100%.

Analysis of Mu and λ DNA synthesis following induction of Mu*cts* in fully labeled cells. Two independently isolated strains carrying the λ::Mu*cts* complex were used in these experiments. Cultures were grown in the presence of [³H]thymidine for several generations to fully label the cellular DNA, and then Mu development was induced by shifting the cultures to 42°C. Samples were subsequently withdrawn,

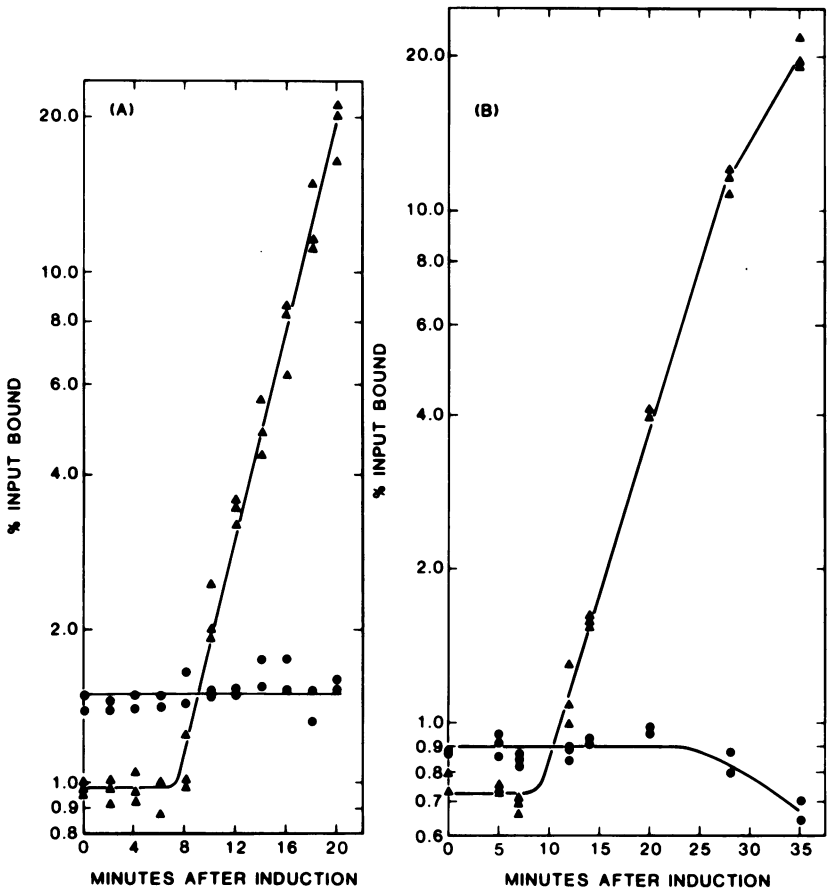

FIG. 2. *Analysis of Mu and λ DNA synthesis following induction of Mucts in fully labeled strains. (A) MP300 was grown in T broth containing 2 μg of thymine and thymidine per ml, 200 μg of uridine and deoxyadenosine per ml, and 50 μCi of [^{3}H]thymidine (specific activity 55 Ci/mmol) per ml at 30°C to 1.5 × 10^8 cells per ml. The generation time under these conditions was about 50 min. Samples for the annealing assay were isolated before and at appropriate intervals after induction at 42°C. Each assay tube contained approximately 50,000 cpm of trichloroacetic acid-precipitable [^{3}H]DNA, 20 μg of unlabeled E. coli DNA, 300 cpm (0.005 μg) of ^{32}P-labeled Mu or λ DNA, and a filter containing Mu (P. mirabilis) or λ DNA. The specific activity of the input [^{3}H]DNA was 1.8 × 10^6 cpm/μg. The annealing was carried out for 20 h at 65°C. The counts per minute annealed to filters containing Mu or λ DNA were corrected as described in legend to Fig. 1. The corrected counts per minute are plotted as percentage bound of the trichloroacetic acid-precipitable [^{3}H]DNA input. The plotted values are averages of duplicate or triplicate determinations from two separate annealing assays. Percentage of input bound to filters containing (▲) Mu DNA or (●) λ DNA. (B) AT4587 was grown in T broth containing (per ml) 2 μg of thymidine, 200 μg of uridine, and 50 μCi of [^{3}H]thymidine at 29 to 30°C for 3 to 4 generations and induced at 42°C at 4 × 10^8 cells per ml. Samples for the annealing assay were isolated at the indicated intervals. Each annealing assay contained approximately 90,000 to 100,000 cpm of trichloroacetic acid-precipitable [^{3}H]DNA, 20 μg of unlabeled E. coli DNA, and a filter containing Mu (E. coli) or λ DNA. The annealing was carried out at 65°C for 40 h. The counts per minute annealed to each filter were corrected by subtracting the background counts bound to a DNA-free filter. No adjustment was made for efficiency of annealing. Percentage of input bound to filters containing (▲) Mu DNA or (●) λ DNA.*

and DNA was isolated as detailed by Kuempel (15) and assayed for the capacity to anneal to filters containing λ or Mu DNA. The results obtained with the two strains are shown in Fig. 2A and B. Since a constant amount of radioactively labeled DNA was added to each annealing assay, the amount bound should remain constant for any DNA species whose relative frequency in the population is unchanged. As observed previously, Mu-specific DNA synthesis was initiated approximately 8 min after induction, and the kinetics of accumulation were sim-

ilar to those previously reported (30). However, and most importantly, no significant increase in the amount of Mu prophage-adjacent λ DNA was observed; the frequency of the λ DNA relative to other *E. coli* genes did not change substantially after onset of Mu DNA synthesis. The relatively constant fraction of input which bound to filters containing λ DNA suggests also that the host chromosomal replication of the λ DNA continued at an unaltered rate during Mu development (see Discussion). That overall host DNA replication is not substantially altered following Mu induction was shown by continued [^{3}H]thymidine incorporation in the nonlysogen as compared to the lysogen at 42°C (29).

The results presented here would be difficult to interpret should Mu replication not occur in a large portion of the induced cells. We routinely observed that the induced cultures that initiate lysis by 35 to 45 min rapidly complete lysis by 50 to 60 min and that fewer than 1% of these cells survive induction. This suggests that, under our conditions of induction, the lytic cycle of Mu is a relatively synchronous and complete process.

The absolute values shown in Fig. 2A for the amount of Mu and λ DNA present in the uninduced culture (0 min) are approximately as expected for an exponentially growing culture with a single replication fork; these theoretical values are approximately 1.0% for Mu prophage and 1.3% for λ prophage (2, 10).

Analysis of Mu and λ DNA synthesis in pulse-labeled cells. As an additional approach for determining whether Mu-specific DNA replication proceeds beyond the termini of the prophage, we analyzed the DNA produced during short pulse-labeling intervals throughout the replication period. This procedure will greatly amplify the detectability of any change in rate of replication of a DNA species. For example, during the 2-min pulse-labeling period used in the experiment of Table 2, the Mu and λ prophage DNA was replicated by the chromosomal replication fork in only about 5% of the cells, whereas virtually all the cells were replicating Mu DNA about 8 min after induction. Therefore, if all Mu DNA replication forks proceed past one or both termini of the Mu prophage and replicate the adjacent λ DNA, large increases in the amount of labeled λ DNA will be observed. Cultures were grown in nonradioactive medium, as described in the footnotes to Tables 2 and 3. At intervals after induction of the Mu prophage, samples were labeled with [^{3}H]thymidine for either 2 or 5 min, and then DNA was purified for the annealing assay; the results of these experiments are shown in Tables 2 and 3. The fractions of the purified DNA samples which annealed to filters containing Mu DNA increased substantially 8 to 10 min after induction, while annealing to filters containing λ DNA remained essentially constant throughout the course of both experiments. We estimate that "read-through" into λ DNA by approximately 5 to 10% of the Mu DNA replication forks would have yielded an amount of labeled λ DNA equal to that generated by the chromosomal replication fork. The values obtained in samples drawn prior to the onset of Mu DNA synthesis represent the amount of prophage DNA replicated by the host chromosomal replication fork during the labeling interval. The constancy of this value for λ suggests again that chromosomal replication is not significantly impaired during the early portion of the lytic cycle of Mu (see Discussion).

TABLE 2. *Analysis of Mu and λ DNA in pulse-labeled samples from strain MP300 after the induction of Mu*[a]

Labeling interval (min)	Percentage of input DNA annealed to filters containing:	
	Mu (*P. mirabilis*) DNA (5 μg)	λ DNA (5 μg)
0–2	0.63	1.17
2–4	0.87	1.36
4–6	0.78	1.15
6–8	1.05	1.13
8–10	6.84	1.14
10–12	16.40	1.15
12–14	24.58	1.17
14–16	26.22	1.25
16–18	32.52	1.14
18–20	39.38	1.04

[a] MP300 was grown in T broth containing 2 μg of thymine and thymidine per ml and 200 μg of uridine and deoxyadenosine per ml at 29°C until the culture reached 2×10^8 bacteria per ml. The cells were filtered, washed, and suspended in T broth supplemented with 2 μg of thymine per ml, then grown for 10 min at 30°C before induction at 42°C. Samples, withdrawn at intervals, were pulse-labeled for 2 min with 40 μCi of [^{3}H]thymidine per ml. The specific activity of the DNA in the first sample was approximately 3×10^5 cpm/μg. The onset of lysis occurred between 42 and 43 min after induction. Each annealing assay contained about 40,000 cpm of [^{3}H]DNA sample, 300 cpm of ^{32}P-labeled Mu or λ DNA, and a filter containing Mu (*P. mirabilis*) or λ DNA. The annealing was carried out at 65°C for 22 h. The values listed are averages of duplicate determinations and are plotted as percentage of input bound.

DISCUSSION

These experiments show that, within the limits of our assay, the induction of Mu-specific DNA synthesis in a Mu lysogen does not simultaneously induce the replication of the DNA

TABLE 3. *Analysis of Mu and λ DNA in pulse-labeled samples from strain AT4587 after the induction of Mu*[a]

Labeling interval (min)	Total TCA-precipitable input [^{3}H]-DNA (cpm)	Percentage of input DNA annealed to filters containing:			
		Mu (*P. mirabilis*) DNA (5 μg)	λ DNA (5 μg)	*E. coli* DNA (50 μg)	No DNA
1–6	57,418	0.88	1.29	47.2	0.06
3–8	58,310	1.91	1.43	46.8	0.08
7–12	53,600	14.51	1.30	36.4	0.09
9–14	52,128	20.05	1.27	35.9	0.09

[a] Cultures of AT4587 were grown at 29°C in T broth containing 200 μg of uridine per ml and induced at 42°C at a cell density of 1.5×10^8 cells per ml. Samples were pulse-labeled with 20 μCi of [^{3}H]thymidine per ml for 5-min intervals. The specific activity of the DNA in the first sample was 2.1×10^5 cpm/μg. Each annealing assay contained [^{3}H]DNA sample, 300 cpm of ^{32}P-labeled Mu or λ DNA, and a filter containing Mu (*E. coli*) or λ DNA. The values listed represent an average of triplicate determinations within the same experiment, and are plotted as percentage of input bound to filters. TCA, Trichloroacetic acid.

sequences which flank the primary Mu prophage. Since early Mu replication occurs while the prophage is attached to the prophage-adjacent host sequences (20), we propose that a control mechanism exists which allows the replication of the Mu prophage, but prevents amplification of these flanking sequences.

Replication of an integrated viral genome without replication of adjacent host DNA can be accomplished by utilizing specific replication termination and initiation sites to contain replication within the Mu prophage. For example, Mu replication could be initiated at one (or the other) of its termini and proceed unidirectionally, as proposed by Wijffelman and van de Putte (33), to a termination signal present at the opposite end of the viral genome. Alternatively, Mu replication could be initiated within the Mu prophage and proceed bidirectionally to be terminated at specific sites present at both ends of the prophage. Unique termination regions for the replication of *E. coli* and plasmid R6K have been proposed (16, 21, 22). A third model would invoke initiation sites at both ends of the viral prophage, with replication proceeding medially, away from the Mu-host junctions; termination could occur at a specific site within the prophage or upon collision of the two replication forks, in much the same manner as reported for simian virus 40 (17). An alternative model, which cannot be excluded by these data, invokes replication of a small segment of the adjacent host DNA followed by specific cleavage at the termini of the Mu DNA in the replicas.

Although Ljungquist and Bukhari demonstrated that the original Mu-host junctions persist after Mu induction (20), it cannot be determined from their data whether this region remains associated with the host chromosome or exists as an extrachromosomal form of Mu, generated by the excision of both the Mu prophage and the adjacent host sequences. If the primary Mu prophage DNA is excised with adjacent host sequences, a likely candidate for this extrachromosomal form would be the heterogeneously sized, covalently closed, circular Hc-DNA, which contains a complete Mu genome covalently linked to *E. coli* DNA (24, 29). However, the Hc-DNA is first detected 4 min after the onset of Mu DNA synthesis (30), in quantities considerably less than 1 Hc-DNA molecule per cell. While we cannot rule out the possibility that Mu is "imprecisely" excised to yield a DNA form that is not a covalently closed circle, we feel this is unlikely for the following reason: the relative content of the λ DNA was essentially unchanged after onset of Mu-specific DNA synthesis. This level would be maintained if the chromosomal replication fork continued to replicate the λ DNA. An alternative explanation would involve a decrease in the replication of λ DNA by the chromosomal replication fork, fortuitously matched by an increase in λ DNA replication resulting from "read-through" from the Mu-specific replication in a small number of cells. We consider as more likely the former explanation, which suggests that the replication of the λ DNA remains under the control of the host during early Mu development, and therefore that λ sequences remain physically associated with the chromosome. Since the Mu-λ junction is preserved after Mu induction (20), it follows that both Mu prophage and prophage-adjacent λ DNA are in the host chromosome. Hence, we conclude that the early replication of Mu DNA occurs while it is still associated with the host chromosome. Indeed, as Mu integrates repeatedly into the chromosome throughout its life cycle (20, 23, 24; unpublished data), these secondary prophage sites may also function as sites of Mu replication.

Since we propose that the Mu replication fork

does not proceed into adjacent host sequences, while the chromosomal replication fork can continue past the Mu DNA termini, a product coded by Mu DNA may be required to effect the proposed specific termination events. The product of the A gene of Mu has been implicated in both integration and replication (27, 31, 32); it may be capable of recognizing the ends of Mu DNA and could be responsible for terminating Mu-specific DNA replication. However, replication of λ DNA by Mu-specific replication forks conceivably could occur in a small fraction of cells. This might explain why, in some experiments, the amount of λ DNA does not fall as rapidly as expected at later times after induction, when the increased amount of Mu DNA should displace nonamplified DNA in the samples used in the annealing assay.

Our proposal, that Mu replication is initiated and terminated within the boundaries of Mu-specific DNA sequences to generate progeny lacking prophage-adjacent host sequences, may relate to several phenomena associated with Mu integration and replication (also, see reference 20 for discussion). For example, it is known that the host DNA sequences located at both termini of the infecting mature Mu phage genome are not integrated into a stable lysogen (1, 12). This could be explained if the Mu DNA replication is initiated within the incoming mature Mu genome and if it is the replica of the Mu DNA, devoid of non-Mu sequences, that is inserted into the host chromosome. A similar suggestion has been made by Bukhari (3, 20).

Another interesting implication of our proposal that Mu replication is confined to the Mu prophage concerns the role of Hc-DNA molecules in Mu replication (6, 11). It has been suggested that these circular forms represent late replicative forms of Mu and are intermediates in a rolling circle type of replication (11, 24). However, replication of Hc-DNA in toto would not occur unless the mechanism of Mu DNA replication which excludes the replication of the adjacent host sequences were abated; a rolling circle type of mechanism a priori requires replication past the termini of Mu DNA into the prophage-adjacent sequences. The actual role of Hc-DNA in Mu development remains unknown.

As a simple scenario for Mu development, we envision that, after the induction of a Mu lysogen, Mu DNA replicas without attached host sequences at their termini are formed at the primary Mu prophage site. These replicas are rapidly integrated into the host DNA, generating secondary prophage sites which, in turn, can serve as sites of replication of Mu DNA. At the end of the lytic cycle, Mu DNA is packaged directly from these prophage sites by a "head full mechanism" (see reference 3). We are currently testing various aspects of this scenario. Our preliminary results suggest that most of the Mu progeny present in the cell are associated with the folded host nucleoid until late in the developmental cycle.

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