Involvement of *Escherichia coli* K-12 DNA Polymerase I in the Growth of Bacteriophage Mu

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We examined several aspects of bacteriophage Mu development in *Escherichia coli* strains that carry mutations in the *polA* structural gene for DNA polymerase I (PolI). We found that *polA* mutants were markedly less efficient than PolI wild-type (PolI⁺) strains in their capacity to form stable Mu lysogens and to support normal lytic growth of phage Mu. The frequency of lysogenization was determined for *polA* mutants and their isogenic PolI⁺ derivatives, with the result that mutants were lysogenized 3 to 8 times less frequently than were PolI⁺ cells. In one-step growth experiments, we found that phage Mu grew less efficiently in *polA* cells than in PolI⁺ cells, as evidenced by a 50 to 100% increase in the latent period and a 20 to 40% decrease in mean burst size in mutant cells. A further difference noted in infected *polA* strains was a 10-fold reduction in the frequency of Mu-mediated transposition of chromosomal genes to an F plasmid. Pulse labeling and DNA-DNA hybridization assays to measure the rate of phage Mu DNA synthesis after the induction of thermosensitive prophages indicated that phage Mu replication began at about the same time in both *polA* and PolI⁺ strains, but proceeded at a slower rate in *polA* cells. We conclude that PolI is normally involved in the replication and integration of phage Mu. However, since phage Mu does not exhibit an absolute requirement for normal levels of PolI, it appears that residual PolI activity in the mutant strains, other cellular enzymes, or both can partially compensate for the absence of normal PolI activity.

Replication and integration of bacteriophage Mu DNA are very closely linked processes (17, 35). Random integration into host DNA occurs both in lysogen formation (2, 39) and continually during lytic growth (24, 25, 34). Despite extensive searching, phage mutants which replicate but do not integrate have never been found (31). The mechanism of phage Mu integration is probably similar to that of other transposable elements as evidenced by the lack of site specificity, the ability of phage Mu to generate various DNA rearrangements (for a review, see reference 9), and the fact that Mu prophage are flanked by 5-base-pair direct repeats of host DNA (1, 18; for reviews, see references 3 and 19).

Most models for DNA transposition have a number of features in common. In general, they propose that a staggered double-strand cleavage is made in the target sequence, followed by concomitant ligation and replication of the transposable element into the target site. At some point in the process, a limited amount of repair synthesis must take place to restore host se-

quences interrupted by the staggered double-stranded cuts (12, 13, 16, 37).

E. coli DNA polymerase I (PolI) is known to be involved in many DNA repair processes (for a review, see reference 15). We examined the possible involvement of PolI in Mu development because of the potential need for repair synthesis to complete DNA transposition. Our results show that normal levels of PolI are not absolutely required for Mu growth; however, all aspects of phage development which we examined do occur with reduced efficiency in *polA* mutants compared with PolI⁺ hosts. We conclude that PolI is normally involved in Mu replication and integration.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. Bacterial strains are described in Table 1. All strains were derivatives of *E. coli* K-12, except AT3557, which is a *Proteus mirabilis* strain carrying the plasmid RP4::Mucts62. Phage obtained by heat induction of this strains were the source of Mucts62 (*P. mirabilis*) DNA used in the hybridization assays (42). Strains AT3801, AT3802, and AT3803 are independently isolated methyl methanesulfonate-resistant (MMS^r) revertants of strain AT3760 (*polA1*) which were isolated

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TABLE 1. Bacterial strains

Strain	Relevant characteristics	Source ^a (reference)
AT3760	<i>polA1 endA thyA Pur</i> ⁻	C. C. Richardson, strain D110 (30)
AT3801	MMS ^r revertant of AT3760	This work
AT3802	MMS ^r revertant of AT3760	This work
AT3803	MMS ^r revertant of AT3760	This work
AT3820	As AT3760, but F1-11::Tn10	This work
AT3821	As AT3801, but F1-11::Tn10	This work
AT3836	As AT3760, but (Mucts62)	This work
AT3838	As AT3801, but (Mucts62)	This work
JG110	<i>polA5 thyA36 deoC2 metE70</i>	CGSC, strain 5924 (23, 26)
W3110, Thy ⁻	<i>thyA36 deoC2</i>	CGSC, strain 4916 (8)
RS5064	<i>polA480 trpA33</i>	CGSC, strain 5642 (20)
KS463	<i>trpA33 rha-7</i>	CGSC, strain 5644 (20)
AT3614	Hfr PK191 <i>his-220::Mucts62</i>	CGSC, strain 4316
AT3701	<i>thr-1 leuB6 rpsL8 tsx-461 (λ) F</i> ⁻	Laboratory strain
AT3783	<i>thr-16 proC24 pyrF30 lysA36 purE41 metC65</i> <i>ilv-277 rpsL97 gyrA237 recA1 Mu</i> ^r (Mu) <i>F</i> ⁻	Laboratory strain
AT3557	RP4::Mucts62/ <i>P. mirabilis thy</i>	Laboratory strain
AB259	HfrH, <i>thi-1</i>	Laboratory strain

^a Strains designated CGSC were obtained from B. Bachmann, curator of the Coli Genetic Stock Center at Yale University, New Haven, Conn.

on broth agar plates containing 0.04% methyl methanesulfonate (Eastman Organic Chemicals, Rochester, N.Y.). Revertants such as these are phenotypically Poll⁺ (7, 8, 14). The F::Tn10 plasmid (F1-11) carried by strains AT3820 and AT3821 was constructed as described by Foster et al. (11).

Preparation of phage Mucts62 stocks was by heat induction from strain AT3614. MupAp1, a recombinant of wild-type Mu and the Mucts62pAp1 variant described by Leach and Symonds (22), was constructed in this laboratory. MupAp1 stocks were prepared from plate lysates, with strain AB259 as the host. Bacteriophage lambda lysates were made by inducing strain AT3701 with mitomycin C.

Media and chemicals. Bacterial cultures were grown in L broth (28) supplemented with 10 µg of thymine per ml. The number of viable cells was assayed on agar plates containing L broth plus 1.8% agar. Plaque assay plates for enumerating infective centers and phage particles contained L broth plus 1.0% agar and 1 mM MgSO₄. Soft agar overlays were L broth plus 0.3% agar and also contained 10 mM MgSO₄ for assaying plaque formation. Minimal plates contained Davis minimal medium (Difco Laboratories, Detroit, Mich.), 1.8% agar, 2 mg of glucose per ml, and the required purines, pyrimidines, and amino acids at optimal concentrations between 20 and 170 µg/ml. Media were sometimes supplemented with nalidixic acid, 30 µg/ml; tetracycline, 15 µg/ml; or ampicillin, 25 or 100 µg/ml as indicated.

Frequency of lysogenization measurements. Assays for the frequency of lysogenization took advantage of the β-lactamase encoded by MupAp1. The acquisition of ampicillin resistance among survivors of MupAp1 infection served as an indicator of lysogeny. Bacterial strains were grown to 10⁸ cells per ml, supplemented with CaCl₂ (5 mM), and then infected with a multiplicity of one MupAp1 particle per viable cell (multiplicity of infection [MOI] = 1). At 15 min postinfection, unadsorbed phage were removed by filtering (Nalgene

filter, 0.45-µm pore size) and washing with 0.85% NaCl. After suspension of cells in L broth, the number of infected cells was assayed as infective centers by plating culture dilutions in 2-ml agar overlays with 10⁸ cells of strain AB259 as indicators. Plates were incubated at 37°C. At 30 min postinfection, dilutions of culture in 2-ml agar overlays were poured onto broth agar plates. These plates were overlaid with 2 ml of sterile broth agar and then incubated at 37°C for 2 h to allow for phenotypic expression of ampicillin resistance in all potential lysogenic clones. An additional 3-ml broth agar overlay containing 100 µg of ampicillin per ml was then added, and plates were incubated at 37°C. The number of ampicillin-resistant colonies recovered per infected cell was taken as a measure of the frequency of lysogeny. Uninfected cells did not grow after plating under these conditions.

One-step growth experiments. Cultures of strains AT3760 (*polA1*) and AT3801 (MMS^r) were grown and infected as described for the frequency of lysogenization experiments, except that cells were infected with phage Mucts62 at an MOI of 5. After being filtered and washed, cultures were resuspended in L broth and then diluted 1:100 in L broth to prevent further phage adsorption. Incubation was continued at 37°C, and at various times, dilutions of the culture were plated on plaque assay plates in 2-ml agar overlays containing 10⁸ cells of strain AB259 as indicators for infective centers and free phage. Plates were incubated at 37°C to develop the plaques. Phage Mu development was also assayed in strain RS5064 (*polA480ex*) and an isogenic *polA*⁺ strain, KS463. The *polA480ex* mutation results in a defect in the 5'-to-3' exonuclease activity of Poll at all growth temperatures. The mutation is tolerated at 30°C but is lethal at 43°C (20), presumably owing to a total loss of the coordinated 5'-to-3' polymerization and 5'-to-3' exonucleolytic degradation activities of Poll at the elevated temperature (41). Phage Mu growth in these strains was assayed as described above, except that cells were grown at 30°C

and then infected and assayed for phage growth at 43°C to analyze phage development in the total absence of normal Poll 5'-to-3' exonuclease activity. One-step growth experiments for bacteriophage lambda in strains AT3760 and AT3801 were done as described for phage Mucts62 with the following exceptions: L broth was supplemented with 0.2% maltose, and MgSO₄ (10 mM) rather than CaCl₂ was added before infecting the cells with phage lambda at an MOI of 5.

Frequency of Mu-mediated transposition of chromosomal genes. Cultures of strains AT3820 (*polA1*, F::Tn10) and AT3821 (MMS^r, F::Tn10) were grown and infected as described for the frequency of lysogeny experiments except that the MOI was 5. After a 10-min adsorption, the cultures were filtered, washed, and suspended in L broth. The infected donor cells were mixed with an equal number of recipient cells of strain AT3783, and incubation at 37°C was continued. At various times, the cultures were sampled, mating was interrupted, and dilutions of the culture were plated on agar plates containing nalidixic acid to select against the donor cells and tetracycline to select for those recipient cells receiving the F::Tn10 plasmid. This assay compared the kinetics of F::Tn10 transfer from the infected donors of strains AT3820 (*polA1*) and AT3821 (MMS^r). After 90 min of mating, culture dilutions were plated on minimal plates containing nalidixic acid to select against donor cells, and the plates were supplemented with all the nutritional requirements of AT3783 except either threonine or proline to select for recipient cells which had received a copy of the *thr*⁺ gene or *pro*⁺ gene transposed to the F::Tn10 plasmid. All plates were incubated at 37°C. All *thr*⁺ and *pro*⁺ transconjugants were later tested for ampicillin resistance by replica plating to agar plates containing ampicillin (25 µg/ml). This test insured that all transconjugants contained copies of MupAp1 as expected. Results were expressed as the number of *thr*⁺ or *pro*⁺ transconjugants per input donor cell.

Pulse labeling and DNA-DNA hybridization. The rate of phage Mu DNA replication in thermo-induced Mucts62 lysogens of both *polA* and phenotypically Poll⁺ strains was determined by pulse labeling the DNA at various times after induction and then using DNA-DNA hybridization assays to measure the proportion of labeled DNA which was phage Mu specific. Lysogenic strains AT3836 and AT3838 were grown to 10⁸ cells per ml in L broth at 30°C and then placed at 42°C to induce Mucts62. At various times after induction, samples of cells were removed and added to tubes containing [methyl-³H]thymidine (New England Nuclear Corp., Boston, Mass.; specific activity, 80 Ci/mmol; final concentration, 50 µCi/ml). Culture tubes also contained uridine (final concentration, 200 µg/ml) to assure efficient labeling of the DNA (32, 42). After 5 min at 42°C, the samples were chilled, and an excess of unlabeled thymidine (50 µg/ml), plus NaN₃ (0.02 M) and nalidixic acid (200 µg/ml), was added. Lysates were prepared, and the labeled DNA was hybridized to nitrocellulose filters (BA85; Schleicher & Schuell Co., Keene, N.H.), which were loaded with 5 µg of phage Mu (*P. mirabilis*) DNA per filter. The technique used for sample preparation, DNA-DNA hybridization reactions, and calculations was a modification of the Waggoner and Pato (34, 42) method and is described in detail elsewhere (27).

TABLE 2. Frequency of lysogen formation after infection with MupAp1

Strain	% Lysogens ^a
AT3760 (<i>polA1</i>)	0.68
AT3801 (MMS ^r)	4.48
AT3802 (MMS ^r)	5.40
AT3803 (MMS ^r)	3.09
JG110 (<i>polA5</i>)	0.54
W3110, Thy ⁻ (<i>polA</i> ⁺)	1.63

^a Exponential-phase cultures were infected with one MupAp1 particle per cell. Infective centers and ampicillin-resistant survivors were assayed to obtain the data, which were expressed as the percentage of lysogens formed per infected cell.

RESULTS

***polA* mutants have a reduced capacity for phage Mu lysogenization.** Phage Mu derivatives which encode β-lactamase enable one to study various aspects of Mu development by analyzing the inheritance of ampicillin resistance. These derivatives, designated MupAp1, have been used to study the frequency of phage Mu lysogenization (22), cointegrate formation mediated by phage Mu (5, 6), and Mu-mediated transposition of chromosomal genes (A. L. Taylor and C. L. Shellabarger, manuscript in preparation). Here, we utilized phage MupAp1 to compare the frequency of lysogenization of *polA* and Poll⁺ cells. We found that the frequency of lysogenization by MupAp1 was substantially lower in *polA* cells than in Poll⁺ cells (Table 2). Phage MupAp1 lysogenized strains AT3760 (*polA1*) 4 to 8 times less frequently than it lysogenized any of three different MMS^r revertants of strain AT3760. Likewise, the frequency of lysogenization observed after infection of strain JG110 (*polA5*) was threefold lower than in the isogenic *polA*⁺ strain, W3110 Thy⁻. Thus, *polA1* mutants appeared to be less efficient in their ability to support stable Mu lysogen formation than *polA5* mutants when compared with their respective isogenic Poll⁺ derivatives. The *polA5* mutant may support Poll-dependent processes better than the *polA1* mutant because the former retains about 35% and the latter retains only about 1% of normal Poll activity, as measured by in vitro assays (23, 26). We conclude that Poll is normally involved in some part of the process leading to the formation of stable Mu lysogens.

Phage Mu lytic growth is inefficient in *polA* mutants. The course of phage Mu development in *polA* mutants was measured in one-step growth experiments. After infection with Mucts62 (MOI = 5), the growth of phage Mu in strain AT3760 (*polA1*) exhibited latent periods that were 15 to 30 min longer and average bursts

FIG. 1. Mucts62 development of strains AT3760 (*polA1*) and AT3801 (MMS^r). Bacteria were grown to exponential phase at 37°C and then infected with Mucts62 (MOI = 5). Phage development was assayed as infective centers and free phage at 37°C in cultures of strains AT3760 (●) and AT3801 (■).

that were about 20% smaller than those seen in strain AT3801, an MMS^r revertant of strain AT3760 (Fig. 1). One-step growth experiments done after heat induction of Mucts62 lysogenic derivatives of strains AT3760 and AT3801 (strains AT3836 and AT3838, respectively) gave similar results (data not shown). Phage Mu growth in strain RS5064 (*polA480ex*) was assayed at 43°C, the temperature at which normal PolI 5'-to-3' exonuclease activity is undetectable in this mutant (20, 41). The development of phage Mu after infection of RS5064 showed a 15 to 30 min longer latent period and a 40% smaller average burst size than that seen in KS463, an isogenic *polA*⁺ strain grown and infected under identical conditions (Fig. 2). These results suggest that both the polymerase and the 5'-to-3' exonuclease activities of PolI may be important for efficient lytic growth of phage Mu; they are also consistent with the hypothesis that PolI is required for concurrent replication and integration of phage Mu DNA. To examine the effect of *polA* mutations on an unrelated temperate phage that does not depend on integration for the replication of its DNA, we compared the growth

of phage lambda in strains AT3760 and AT3801. In agreement with previous studies on wild-type lambda in *polA* mutants (29, 38), we found that lambda grew just as efficiently in *polA1* cells as in PolI⁺ cells (Fig. 3). Thus, it seems likely that the reduced efficiency of phage Mu development that we have observed in a *polA1* host reflects a specific requirement of this phage for normal levels of PolI activity.

Frequency of Mu-mediated transposition of chromosomal genes is reduced in *polA* mutants. During lytic growth, phage Mu is capable of translocating bacterial genes from the chromosome to a transmissible plasmid (10). The transposed bacterial DNA segment is flanked by two copies of phage Mu in direct repeat. These transposition events can be detected by mating the infected donor cells with an auxotrophic, *recA*, Mu-immune, Mu-resistant recipient and then selecting for the bacterial gene of interest (9, 10; Taylor and Shellabarger, manuscript in preparation). We constructed derivatives of strains AT3760 (*polA1*) and AT3801 (MMS^r) which carry an F::Tn10 plasmid, strains AT3820 and AT3821, respectively. Using these strains, we measured the frequency of Mu-mediated

FIG. 2. Mucts62 development in strains RS5064 (*polA480ex*) and KS463 (*polA*⁺). Bacteria were grown to exponential phase at 30°C and then shifted to 42°C for 5 min before infection with Mucts62 (MOI = 5). Phage development was assayed at 42°C in cultures of strains RS5064 (●) and KS463 (■).

transposition of chromosomal genes to the F::Tn10 plasmids to test the effect of the *polA1* mutation on the ability of phage Mu to promote rearrangements normally associated with the lytic growth cycle. Cultures of AT3820 and AT3821 were infected with MupAp1 and then mated with AT3783, a polyauxotrophic recipient strain. The transfer of F::Tn10 was assayed at various times by selecting for the inheritance of tetracycline resistance, and then after 90 min of conjugation, *pro*⁺ and *thr*⁺ transconjugants were selected. F::Tn10 transfer from both AT3820 and AT3821 after MupAp1 infection occurred with similar kinetics (Fig. 4), which indicated that the frequency of plasmid transfer was not itself adversely affected by the *polA1* mutation. Thus, any differences in the frequency of Mu-mediated transposition of chromosomal genes detected should reflect an absolute difference in the number of these events occurring in the two donor strains. The frequency of recovery of *pro*⁺ and *thr*⁺ transconjugants of recipient strain AT3783 was 8- to 10-fold lower in matings with strain AT3820 (*polA1*, F::Tn10) than in comparable matings with strain AT3821 (MMS^r, F::Tn10) (Table 3). These results demonstrate a further inefficiency of phage Mu growth in *polA*

FIG. 3. Lambda development in strains AT3760 (*polA1*) and AT3801 (MMS^r). Bacteria were grown to exponential phase at 37°C and then infected with lambda (MOI = 5). Phage infective centers and free phage were assayed at 37°C in cultures of strains AT3760 (●) and AT3801 (■).

FIG. 4. Transfer of F::Tn10 to strain AT3783 by MupAp1-infected strains AT3820 (*polA1*, F::Tn10) and AT3821 (MMS^r, F::Tn10). Exponential-phase donor cultures were infected with five phage per cell. After 10 min for phage adsorption, an equal number of infected donor cells and recipient cells were mixed. At various times, mating was interrupted and tetracycline- and nalidixic acid-resistant transconjugants were selected to assay the kinetics of F::Tn10 transfer from infected strains AT3820 (●) and AT3821 (■).

hosts and suggest that the ability of phage Mu to generate the various DNA rearrangements normally seen during lytic growth is significantly affected by the *polA* mutation.

Phage Mu DNA replication proceeds at a slow rate in *polA* mutants. To determine the rate of phage Mu DNA replication in *polA* compared with that in *PolI*⁺ cells, cultures of lysogenic derivatives, strains AT3836 (*polA1*, Mucts62) and AT3838 (MMS^r, Mucts62), were induced at 42°C. At various times after induction, samples of culture were pulse-labeled for 5 min with [methyl-³H]thymidine (50 μCi/ml), and then DNA was prepared and annealed to nitrocellulose filters loaded with phage Mu (*P. mirabilis*) DNA as described above. Phage Mu DNA replication began at about the same time in both AT3836 and AT3838, but it proceeded at a slower rate and comprised a smaller percentage of the total DNA synthesized in the *polA1* host, strain AT3836 (Fig. 5). These results are consistent with the longer latent period and smaller average burst size seen after phage infection of nonlysogenic *polA1* cells (Fig. 1). Thus, the inefficiency of phage Mu growth in *polA* mutants appears to be at the level of phage DNA replication.

TABLE 3. Frequency of Mu-mediated transposition of chromosomal genes after infection with MupAp1

Donor strain	Transposition frequency ^a	
	<i>pro</i> ⁺	<i>thr</i> ⁺
AT3820 (<i>polA1</i> , F::Tn10)	6.2×10^{-7}	3.1×10^{-7}
AT3821 (MMS ^r , F::Tn10)	4.8×10^{-6}	3.3×10^{-6}

^a Exponential-phase donor cultures were infected with five MupAp1 particles per cell and then mixed with an equal number of recipient cells of strain AT3783. *pro*⁺ and *thr*⁺ transconjugants were selected. The transposition frequency is the number of transconjugants per input donor cell.

DISCUSSION

We found that both lysogenic and lytic aspects of bacteriophage Mu development occur inefficiently in *polA* mutants. These results lead us to suggest that PolI normally participates in the replication and integration of phage Mu. Our observation that *polA* mutants are partially permissive for Mu lysogenization and productive phage Mu growth more than likely reflects both residual PolI activity and compensation for reduced PolI activity by other enzymes in the mutant cells. Since phage Mu shares many of the properties exhibited by other transposable elements (3, 19), it is noteworthy that previous reports have implicated PolI in the integration of some of these elements (4, 36).

Phage Mu lysogenized *polA* hosts 3 to 8 times less frequently than PolI⁺ cells (Table 2). Mu prophage in lysogenic bacteria are flanked by 5-base-pair direct repeats of host DNA (1, 18) which have been postulated to arise from staggered double-strand cleavages in host DNA at the sites of phage Mu insertion (12, 13, 16, 37). PolI could be responsible for completing the integration process by repairing the gaps at Mu-host junctions left by the staggered cuts. The reduced lysogenization of *polA* hosts would be consistent with PolI involvement in this process, if one postulates that repair of Mu-host junctions is an important determinant for stable integration of Mu prophage. Delays in completion of the repair process in *polA* mutants might decrease the frequency of lysogenization either by favoring a lytic response or by aborting the infection before a stable prophage can be established.

The lytic growth of phage Mu in *polA* hosts was characterized by longer latent periods, smaller average bursts (Fig. 1 and 2), and decreases in the frequency of Mu-mediated transposition of chromosomal genes (Table 3), all of which correlated with a reduced rate of phage Mu DNA synthesis (Fig. 5). The precise mechanism of phage Mu DNA replication remains

unknown, although it appears that repeated integration into host DNA is an integral part of the process (24, 25, 34). In spite of some differences, it is generally thought that the mechanisms of both lysogenic and lytic integration are similar (16, 17, 35, 37). Since it is thought that Mu DNA replication and integration occur concomitantly (17, 35), our observation that the rate of phage DNA synthesis in *polA* cells is reduced suggests that PolI could be involved in either the replication or the integration of Mu DNA or both. Furthermore, the results observed with phage lambda (Fig. 3) suggest that PolI-deficient cells are rate limiting for events that are particular to phage Mu and not to lambda which, unlike Mu, does not depend on integration for replicating its DNA. We propose that the essential role of PolI in Mu development may be to complete the

FIG. 5. The rate of phage Mu DNA replication in heat-induced lysogens AT3836 (*polA1*, Mucts62) and AT3838 (MMS^r, Mucts62). Bacteria were grown to exponential phase at 30°C and then induced at 42°C. At various times, DNA was pulse-labeled with [methyl-³H]thymidine, lysates were prepared, and DNA was hybridized to filters containing phage Mu (*P. mirabilis*) DNA as described in the text. Data were expressed as the percentage of labeled DNA which was phage Mu specific after induction of strains AT3836 (●) and AT3838 (■).

replication of newly integrated copies of Mu DNA, which then serve as templates for further rounds of replication and integration. If the efficiency of completion of a round of phage Mu replication is rate limiting for the initiation of subsequent replication events, there are at least two possible ways in which PolI could be involved in this process. First, PolI could repair the gaps which are produced at Mu-host junctions during lytic integration, in the same manner as postulated above for lysogen formation. In *polA* mutants, delays in the completion of this repair process could slow down the rate of Mu DNA synthesis if one supposes that the presence of unrepaired gaps prevents initiation of the next replication event. Second, the slow rate of joining of Okazaki fragments which is characteristic of *polA* mutants (21, 33) could also limit the rate of formation of complete, double-stranded phage DNA templates which are required for the initiation of subsequent cycles of replication and integration. Either or both of these postulated roles for PolI could satisfactorily account for the reduced rate of phage Mu DNA synthesis that we have observed in PolI-deficient host cells.

Phage Mu relies heavily on host functions for its propagation. In addition to the involvement of PolI reported here, Mu also requires the *E. coli* *dnaB*, *dnaC*, probably *dnaE* (40), and the *dnaZ* (unpublished data) gene products during lytic growth. The involvement of PolI in phage Mu lysogenization is the first description of a defined host function which is utilized for the formation of stable Mu lysogens.

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