Growth of Bacteriophage Mu in *Escherichia coli dnaA* Mutants

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In one-step growth experiments we found that bacteriophage Mu grew less efficiently in nonreplicating *dnaA* mutants than in *dnaA*⁺ strains of *Escherichia coli*. Phage development in *dnaA* hosts was characterized by latent periods that were 15 to 30 min longer and an average burst size that was reduced by 1.5- to 4-fold. The differences in phage Mu development in *dnaA* and *dnaA*⁺ strains were most pronounced in cells infected at a low multiplicity and became less pronounced in cells infected at a high multiplicity. Many of these differences could be eliminated by allowing the arrested *dnaA* cells to restart chromosome replication just before infection. In continuous labeling experiments we found that infected *dnaA* strains incorporated 5 to 40 times more [*methyl*-³H]thymidine than did uninfected cells, depending on the multiplicity of infection. DNA-DNA hybridization assays showed that >90% of this label was contained in phage Mu DNA sequences and that only small amounts of the label appeared in *E. coli* sequences. In contrast, substantial amounts of label were incorporated into both host and viral DNA sequences in infected *dnaA*⁺ cells. Although our results indicated that phage Mu development is not absolutely dependent on concurrent host chromosomal DNA replication, they did strongly suggest that host replication is necessary for optimal growth of this phage.

Temperature-sensitive *dnaA* mutants of *Escherichia coli* are defective in the initiation of chromosomal DNA replication (30). These mutants stop DNA replication at the nonpermissive temperature but only after a delay during which ongoing rounds of chromosome replication are completed (7, 8, 30). Toussaint and Faelen (26) first reported that bacteriophage Mu is able to grow in *dnaA* mutants at the nonpermissive temperature. More recent studies (12, 25) have indicated that some aspects of phage Mu development in *dnaA* strains may differ from the normal course of events seen in wild-type hosts. We report here a detailed study of phage Mu growth in *dnaA* mutant hosts.

Phage Mu integrates at many different sites in the bacterial chromosome, both throughout the lytic cycle (10-12, 19, 20) and in the formation of stable lysogens (1, 24). Recent experiments suggest that both lytic and lysogenic integration events may occur at or near host replication forks (3, 17, 18). Since *dnaA* mutants are deficient in vegetative replication forks at nonpermissive growth temperatures, a comprehensive study of phage Mu development in these mu-

tants should provide a direct test of the extent to which host chromosomal replication forks may be required for normal phage Mu replication and integration. We report here that the growth of phage Mu is less efficient in *dnaA* hosts than in wild-type hosts. The results further suggest that host replication forks, although not absolutely required, are important for efficient phage Mu replication and integration.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. Bacterial strains are described in Table 1. All strains were derived from *E. coli* K-12, except AT3557, which is a *Proteus mirabilis* strain that carries the plasmid RP4::Mucts62. Phage obtained by heat induction of strain AT3557 were the source of phage Mu (*P. mirabilis*) DNA used for hybridization assays (28). Phage Mucts62, Mucts62Aam1093, Mucts62Bam7234, and Mucts62Bam1066 were obtained by heat induction of strains AT3614, MH219, MH2808, and MH213, respectively.

Media and chemicals. Bacteria were grown in L broth (13). Agar plates contained L broth plus 1.8% agar for viable cell counts or 1% agar and 1 mM MgSO₄ for plaque assays. Agar overlays for plaque assays contained L broth plus 0.3% agar and 10 mM MgSO₄. Concentrated phage preparations were stored in Mu buffer as previously described (27). SSC (0.15 M

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TABLE 1. Bacterial strains

Strain	Relevant characteristics	Source ^a (reference)
AT3822	<i>arg met ilv rpsL recA1</i>	P. Kuempel strain 21
AT3706	As AT3822, but <i>ilv</i> ⁺ and <i>dnaA46</i>	Derivative of strain CRT46 (7)
E508	<i>thr-1 leuB6 thyA6 deoC3 thi-1 rpsL67 supE44 dnaA508</i>	P. Kuempel strain 21
E177	As E508, but <i>dnaA177</i>	CGSC strain 5028 (30)
PC5	<i>leuB6 thyA47 deoC3 rpsL153 dnaA5</i>	CGSC strain 5029 (30)
AB259	Hfr H, <i>thi-1</i>	CGSC strain 5944 (30)
AT3557	RP4::Mucts62/ <i>P. mirabilis thy</i>	Laboratory strain
AT3614	Hfr PK191 <i>his-34</i> ::Mucts62	Laboratory strain
MH219	<i>mel-1</i> , SuIII ⁺ , (Mucts62Aam1093)	From CGSC strain 4316 by lysogenization
MH2808	As MH219, but (Mucts62Bam7234)	M. M. Howe (15)
MH213	As MH219, but (Mucts62Bam1066)	M. M. Howe (15)
AT3669	<i>thi-1</i> , SuIII ⁺	M. M. Howe (15)
		Laboratory strain

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

NaCl plus 0.015 M sodium citrate, pH 7.0) was used at varying concentrations for different steps in the hybridization procedure as indicated. The preincubation mixture contained 0.02% each of polyvinylpyrrolidone, Ficoll, and bovine serum albumin in 3× SSC (9).

One-step growth curves. Strain AT3706 (*dnaA46*) was grown to a density of 10⁸ cells per ml at 30°C, and then the cells were incubated at 43°C for 90 min to allow completion of ongoing rounds of DNA replication (7). Pulse-labeling experiments showed that replication ceased after 60 min at 43°C (data not shown). In some experiments, chromosome replication was restarted by incubating the cells at 30°C for 10 min, followed by a return to 43°C before infection. AT3822 (*dnaA*⁺) cells were grown to a density of 1.5 × 10⁸ cells per ml at 43°C with no preincubation at 30°C. Cultures were adjusted to 5 mM CaCl₂ and infected with Mucts62 at the desired multiplicity of infection (MOI). At 15 min postinfection, unadsorbed phage were removed by filtering (Nalgene filter; 0.45 μm pore size) and washing with warm (43°C) 0.85% NaCl. Cells were suspended in warm L broth and then diluted 1:100 into warm L broth to prevent further phage adsorption. At 15-min intervals through 120 min postinfection, dilutions of the culture were plated on plaque assay plates in 2-ml agar overlays, with about 10⁸ AB259 cells used as indicators for infective centers and phage bursts. Plates were incubated overnight at 43°C to develop the plaques. The rate of phage adsorption was measured by assaying the number of chloroform-resistant PFU at various times after infection with an MOI of 0.1.

Single-infection experiments. AT3706 (*dnaA46*) and AT3822 (*dnaA*⁺) cells were grown as described above for one-step growth curves, and CaCl₂ was added to 10 mM. One-tenth milliliter of culture and 0.1 ml of L broth containing about 300 PFU of Mucts62 were mixed and incubated at 43°C for 20 min to allow for phage adsorption. Two milliliters of soft agar plus 10⁸ cells of AB259 were added to the above-described mixtures and poured onto plaque assay plates. Plates were incubated at 43°C overnight, and infective centers were counted. Another set of cultures was grown and infected at 30°C. After plating, the plates were incubated at 30°C for 2 h, followed by overnight incubation at 37°C before counting infective centers. Results were expressed as the efficiency of plating

(EOP) relative to the number of plaques on AT3822 at 30°C, which was arbitrarily designated as EOP = 1.

DNA synthesis measurements. Cultures of *dnaA* cells were grown at 30°C and preincubated at 43°C as described above for one-step growth curves. To prepare cells for efficient labeling with [*meth*yl-³H]thymidine, the cultures were supplemented with 2 μg of thymidine, 200 μg of uridine, and 200 μg of deoxyadenosine per ml (16, 28). Thirty microcuries of [*meth*yl-³H]thymidine (New England Nuclear Corp., Boston, Mass.; specific activity, 80 Ci/mmol) per ml and CaCl₂ to 5 mM were added. One half of each culture was infected, and the other half served as an uninfected control. At various times after infection, 30-μl samples of both infected and uninfected cultures were precipitated on filter paper squares (3MM; Whatman, Inc.) with cold 5% trichloroacetic acid (TCA). Filters were washed twice with cold 5% TCA, once with ethanol, and once with acetone and then dried. The amount of ³H label on filters was counted in liquid scintillation fluid (4.0 g of PPO [2,5-diphenyloxazole] and 0.1 g of bis-MSB [*p*-bis-(*o*-methylstyryl)-benzene] per liter of toluene) with a Beckman LS-7000 counter.

DNA-DNA hybridization. Hybridization assays were done to determine the proportions of phage Mu and *E. coli* DNA in the total DNA synthesized during infection of strains AT3706 (*dnaA46*) and AT3822 (*dnaA*⁺) by Mucts62. The technique used was a modification of the Denhardt (2) procedure described by Kuempel (9) and used extensively for analysis of phage Mu DNA replication (19, 28, 29).

Nitrocellulose filters were loaded with DNA by a modification of a previously described procedure (23). DNA in 0.1× SSC was denatured with NaOH, neutralized with HCl, diluted in 6× SSC, and then filtered slowly through a nitrocellulose filter (BA85; Schleicher & Schuell Co.). Filters were loaded with either 20 μg of unlabeled AT3822 DNA to analyze the amount of *E. coli* DNA synthesized in infected cells or 5 μg of unlabeled phage Mu (*P. mirabilis*) DNA to analyze phage Mu DNA replication. Phage Mu (*P. mirabilis*) DNA has *P. mirabilis* sequences on its ends rather than *E. coli* sequences; therefore, labeled *E. coli* DNA will not hybridize to these filters (28). After loading, filters were dried overnight, baked for 2 h at 80°C, and then added to Eppendorf tubes containing preincubation mixture. Filters were preincubated at 65°C for 5 h.

Cultures of strains AT3706 (*dnaA46*) and AT3822 (*dnaA*⁺) were grown as described above for one-step growth curves. Labeling conditions were the same as those described for DNA synthesis measurements. The MOI was 10. At various times postinfection, samples of culture were removed, chilled, and adjusted to 0.01 M NaCN and 100 μ g of nalidixic acid per ml. Cells were pelleted, washed with 0.85% NaCl, and resuspended in 0.05 volume of 1 $\times$ SSC. Lysates were prepared by the addition of lysozyme (250 μ g/ml) and incubation of the cells at 0°C for 15 min, followed by the addition of sodium dodecyl sulfate (0.5%) and a further incubation at 65°C for 15 min. One sample of lysate was mixed with a sample of purified ³²P-labeled Mu DNA, and another sample of lysate was mixed with a sample of purified ³²P-labeled *E. coli* DNA. An equal volume of 1 M NaOH was added, and then denaturation and shearing of DNA were completed by boiling for 10 min. Samples were chilled and then neutralized with 0.5 volume of 1 M HCl and 0.5 volume of 1 M Tris (pH 7.5).

Samples of lysate containing about 5×10^3 cpm (2×10^5 to 1×10^6 cpm/ μ g) of [*methyl*-³H]thymidine-labeled DNA from infected cells and 1×10^3 cpm (2×10^6 cpm/ μ g) of purified ³²P-labeled phage Mu DNA were added to preincubated filters loaded with phage Mu (*P. mirabilis*) DNA and to preincubated blank filters. Similar samples containing 5×10^3 cpm of ³H-labeled DNA and 1×10^3 cpm (5×10^5 cpm/ μ g) of purified ³²P-labeled *E. coli* DNA were added to preincubated filters loaded with *E. coli* DNA and to preincubated blank filters. All assays were done in duplicate. Samples of each lysate were also precipitated in duplicate on filter paper squares (GF/A; Whatman, Inc.) with cold 5% TCA to determine the total amount of label added to the hybridization reactions. Hybridization was carried out at 65°C for 48 h, after which time the filters were washed 4 times with 1 $\times$ SSC, dried, and then counted by liquid scintillation as described for DNA synthesis measurements.

The percentage of phage Mu and *E. coli* DNA synthesized during infection was calculated by the method of Waggoner and Pato (28). The amount of ³²P-labeled DNA which hybridized to the filters was used as an internal control to calculate the efficiency of hybridization. Efficiencies of hybridization were typically 75 to 85% for phage Mu DNA hybridization and 45 to 55% for *E. coli* DNA hybridization. Efficiency values were used to correct the observed percentage of ³H-labeled DNA hybridization to a given filter to an efficiency of 100%. Corrected values for the percentage of ³H-labeled DNA representing phage Mu or *E. coli* DNA were used to plot the data as the number of counts per minute representing phage Mu or *E. coli* DNA at each time point.

RESULTS

Characteristics of bacteriophage Mu development in *dnaA*⁺ and *dnaA* hosts. The growth of bacteriophage Mu in strain AT3822 (*dnaA*⁺) exhibited latent periods of less than 45 min and average bursts of about 100 PFU per infected cell (Fig. 1), which is typical of phage Mu development in wild-type *E. coli* cells (27; unpublished data). In contrast, phage Mu growth in

strain AT3706 (*dnaA46*) under nonpermissive conditions was characterized by latent periods that were 15 to 30 min longer and a mean burst size that was 1.5- to 4-fold lower (Fig. 2). The magnitude of these differences was greatest at low MOIs and diminished with increasing MOIs. However, even at an MOI of 10, the latent periods and mean burst size still differed substantially from those seen in wild-type cells.

Based on the Poisson distribution, the percentage of infective centers formed in wild-type cells at different MOIs was consistent with the expectation that a single infecting phage particle per cell would be sufficient to produce an infective center. In *dnaA* mutants, however, these percentages were more consistent with the view that two or more particles per cell were required for infective-center formation. The difference in efficiency of infective-center formation did not appear to be caused by differences in phage adsorption, because the rates of adsorption of phage Mu to the two host strains were found to be nearly identical (Fig. 3). Likewise, the differences could not be attributed to an effect of the

FIG. 1. Mucts62 development in strain AT3822. Bacteria were grown to exponential phase at 43°C, infected at 43°C, and assayed for phage growth at 43°C. The MOI was 1 (●) or 5 (■).

FIG. 2. *Mucts62* development in strain AT3706. Bacteria were grown to exponential phase at 30°C, incubated at 43°C for 90 min to stop vegetative DNA replication, infected at 43°C, and assayed for phage growth at 43°C. The MOI was 1 (●), 5 (■), or 10 (▲).

nonpermissive growth temperature on infection, because both the wild-type and mutant strains were grown at 43°C for equivalent time periods before infection.

The ability of single phage particles to initiate infection in strains AT3706 (*dnaA46*) and AT3822 (*dnaA*⁺) was assayed at both the permissive and nonpermissive temperatures. A small number of phage were mixed with excess host bacteria, and infectivity was measured as the frequency of infective-center formation on a lawn of sensitive indicator cells (AB259) other than AT3706, because the latter will not form a lawn at 43°C. The efficiency of phage Mu plating was comparable in both strains at 30°C and in strain AT3822 at 43°C, whereas the number of infective centers was reduced by more than 80% in strain AT3706 at 43°C (Table 2). The infective centers seen after infection of strain AT3706 at 43°C were smaller than normal, which may be

TABLE 2. Efficiency of plating of *Mucts62* on *dnaA* and *dnaA*⁺ cells at permissive and nonpermissive temperatures

Host strain	Efficiency of plating ^a at following temp (°C):	
	30 ^b	43 ^c
AT3822	1.00	0.92
AT3706	0.87	0.07

^a The number of infective centers formed upon single infection of AT3822 cells at 30°C was set at 1.00 to compute relative efficiencies of plating.

^b Both strains were grown to exponential phase at 30°C and then infected with about 300 PFU per 1.5×10^7 cells before plating for infective centers on a lawn of AB259 indicator cells.

^c AT3822 and AT3706 were grown as described in the legends to Fig. 1 and 2, respectively. A total of 1.5×10^7 cells were then infected with about 300 PFU at 43°C before plating for infective centers on a lawn of AB259 indicator cells.

FIG. 3. Adsorption of *Mucts62* to strains AT3706 (*dnaA46*) and AT3822 (*dnaA*⁺). Bacteria were grown as described in the legends to Fig. 1 and 2 and infected with *Mucts62* at an MOI of 0.1. The percentage of chloroform-resistant PFUs remaining was assayed at 5-min intervals after infection of strain AT3706 (●) or AT3822 (■).

indicative of smaller bursts under these conditions. These results support the view suggested by the one-step growth curves, namely, that two or more infecting phage per cell are required for efficient phage development in strain AT3706 at 43°C.

DNA synthesis in infected and uninfected *dnaA* mutants. A culture of strain AT3706 (*dnaA46*) was incubated at 43°C to arrest chromosome replication and then supplemented with [*methyl*-³H]thymidine as described in Materials and Methods. The incorporation of label into DNA was assayed at various time intervals in an uninfected portion of the culture and in additional portions infected at various MOIs with Mucts62. Little or no incorporation of the label could be detected in uninfected cells, whereas infected cells showed substantial incorporation (Fig. 4). Furthermore, the total amount of label incorporated was markedly dependent on the MOI. At 60 min after infection, DNA synthesis was stimulated 5-, 25-, or 40-fold in cells infected with 1, 5, or 10 phage per viable cell, respectively. Thus, the stimulative effect of increasing the MOI on productive infection and phage yield which was observed in the one-step growth experiments (Fig. 2) is evidently matched by corresponding increases in the amount of DNA synthesis in these cells. Similar results were noted in four different *dnaA* host strains, but the amount of DNA synthesis elicited by phage Mu infection of these mutants varied considerably, with the *dnaA5* mutant showing the least stimulation (Fig. 5).

We considered the possibility that phage Mu infection by itself might cause nonspecific suppression of the *dnaA* phenotype in such a way that infected cells would begin new rounds of chromosome replication. If so, part of the label that was incorporated in the infected cells (Fig. 4) would then reflect host DNA replication. To test this hypothesis, we repeated the labeling experiment in arrested AT3706 cells infected with either Mucts62Aam1093, Mucts62Bam1066, or Mucts62Bam7234. These amber mutants are defective in phage DNA replication and integration in a nonsuppressing host such as AT3706 (15, 31). Little or no label was incorporated after infection of the *dnaA* cells with the phage mutants (Fig. 6). Therefore, infection alone did not cause suppression of chromosomal replication arrest at the nonpermissive temperature. However, these results do not exclude the possibility that subsequent expression of phage Mu replication functions in infected *dnaA* cells might stimulate host DNA replication.

Amount of phage Mu-specific and host-specific DNA synthesized in *dnaA*⁺ and *dnaA* hosts. DNA-DNA hybridization assays were performed to obtain a direct measure of the phage

FIG. 4. DNA synthesis in strain AT3706 infected with Mucts62. Bacteria were grown to exponential phase at 30°C and then incubated at 43°C for 90 min to stop vegetative DNA replication. [*methyl*-³H]thymidine was added at the time of infection. Incorporation of label was assayed as TCA-insoluble counts per minute. Cells were either uninfected (●) or infected at an MOI of 1 (■), 5 (▲), or 10 (◆).

and host-specific DNA sequences produced during phage Mu development and to ascertain whether phage Mu replication in *dnaA* hosts might lead to suppression of the *dnaA* phenotype. DNA was labeled with [*methyl*-³H]thymidine during infection (MOI = 10) of strains AT3706 (*dnaA46*) and AT3822 (*dnaA*⁺) with Mucts62. The cultures were sampled at various times postinfection, and the labeled DNA in each sample was hybridized to nitrocellulose filters loaded with either phage Mu (*P. mirabilis*) DNA or *E. coli* DNA as described in Materials and Methods.

The hybridization assays showed that >90% of the DNA synthesized during infection of *dnaA* cells was of viral origin and that only a

FIG. 5. DNA synthesis in strains AT3706 (*dnaA46*), E508 (*dnaA508*), E177 (*dnaA177*), and PC5 (*dnaA5*) after infection with Mucts62. All strains were grown, labeled, infected, and assayed as described in the legend to Fig. 4. Cells were either uninfected (○) or infected with Mucts62 at an MOI of 10 (●).

small proportion of it was *E. coli* DNA (Fig. 7). Although the amount of *E. coli* DNA synthesized during infection was small, it was clearly greater than the amount produced in uninfected cells (Fig. 4). In contrast, early after infection of *dnaA*⁺ cells, >75% of the DNA synthesized was *E. coli* specific (Fig. 8). At later times, progressively more of the label appeared in viral DNA sequences, so that by the end of the infection cycle, about 65% of the labeled material was phage Mu DNA. After 45 min of infection, DNA synthesis stopped in wild-type cells (Fig. 8), but not in *dnaA* cells (Fig. 7). This is probably a reflection of the fact that phage maturation and cell lysis occur sooner in wild-type hosts than in *dnaA* hosts (Fig. 1 and 2). We concluded that phage Mu replication does not induce extensive host DNA synthesis in arrested *dnaA* cells. Furthermore, these results suggest that the replication of phage Mu is not necessarily dependent on concurrent replication of the host chromosome.

Growth of phage Mu in *dnaA* hosts after reinitiation of host chromosome replication. Chromosome replication in arrested cells of strain AT3706 was restarted by shifting the cells from 43 to 30°C for 10 min and then returning them to 43°C (Fig. 9). To study the effect of reinitiation of host chromosome replication on phage Mu

development, we incubated one half of a culture of strain AT3706 (previously grown at 43°C) at 30°C for 10 min and kept the other half at 43°C during this time. Both cultures were infected with Mucts62 at an MOI of 2 and then incubated at 43°C for the remainder of the experiment. Development of phage Mu was assayed as previously described for the one-step growth curves (Fig. 10). Phage Mu growth in the nonreplicating *dnaA* cells followed a pattern similar to that described before (Fig. 2), whereas growth in the replicating cells more closely approximated that seen in a wild-type host (Fig. 1). Restarting chromosome replication before infection resulted in a slight decrease in the latent period and a marked increase in the average burst size from 40 to 100 PFU per infected cell.

DISCUSSION

Toussaint and Faelen (26) first described the productive growth of phage Mu in *dnaA* cells that were placed at the nonpermissive temperature at either the time of infection or 30 min before infection. These observations clearly showed that phage Mu replication could proceed in the absence of a functional *dnaA* gene product, but they did not indicate to what extent phage Mu replication might have been dependent on residual vegetative DNA synthesis in

FIG. 6. DNA synthesis in strain AT3706 infected with various Mu phages. Growth, labeling, infection, and assay were performed as described in the legend to Fig. 4. The MOI was 10. The phages used were Mucts62 (●), Mucts62Bam7234 (■), Mucts62Aam1093 (▲), and Mucts62Bam1066 (◆).

these bacteria. The data of Hirota et al. (7), as well as pulse-labeling studies done under our conditions (data not shown), indicate that *dnaA* mutants must be grown at the nonpermissive temperature of 43°C for at least 50 to 60 min to arrest DNA replication completely. In this study, we analyzed the growth of phage Mu under conditions in which no residual DNA synthesis could be detected in *dnaA* hosts at the time of infection (Fig. 4). We found that fully arrested *dnaA* cells were permissive for phage Mu DNA synthesis (Fig. 4) and for the subsequent release of progeny phage (Fig. 2). It is therefore evident that phage Mu can grow in host cells that are no longer actively engaged in normal chromosome replication at the time of infection.

Teifel and Schmieger (25) reported that the amount of phage Mu produced in *dnaA* cells decreased progressively with increasing time of incubation at the nonpermissive temperature before infection at an MOI of 5. They concluded that phage Mu can grow in *dnaA* mutants as long as there are residual vegetative replication forks present, but that once host replication stops,

phage Mu can no longer grow. Our results do not agree with this interpretation, since we were consistently able to demonstrate phage Mu replication in various *dnaA* mutants that had been fully arrested by growing them at 43°C for 90 min before infection. We did observe, however, that phage Mu development in arrested *dnaA* cells infected at a low MOI was less efficient than in comparably infected wild-type cells. The lytic growth cycle in *dnaA* cells was marked by delays in the onset of lysis and a smaller-than-normal burst size that could be enlarged to some extent by increasing the MOI (Fig. 2).

We considered the possibility that arrested *dnaA* cells might not be able to support efficient growth of phage Mu because they are deficient in replication functions required by phage Mu. This seems unlikely for two reasons. First, the growth of bacteriophage lambda is unaffected by the *dnaA* mutation, whether growth is measured

FIG. 7. Mu-specific and *E. coli*-specific DNA synthesis in strain AT3706 infected with Mucts62 at an MOI of 10. Growth, labeling, and infection were performed as described in the legend to Fig. 4. Percentages of total DNA which were phage Mu specific or *E. coli* specific were assayed by DNA-DNA hybridization as described in the text. Data were plotted as total DNA synthesis (---●---), phage Mu DNA synthesis (—●—), and *E. coli* DNA synthesis (—○—).

FIG. 8. Phage Mu-specific and *E. coli*-specific DNA synthesis in strain AT3822 infected with Muc62 at an MOI of 10. Bacteria were grown at 43°C. Labeling and infection were as described in the legend to Fig. 4. Assays were performed as described in the legend to Fig. 7 and in the text. Data were expressed as total DNA synthesis (---●---), phage Mu DNA synthesis (—●—), and *E. coli* DNA synthesis (—○—).

after infection of cells under conditions similar to ours (7) or after UV induction of a prophage (14). Since some of the host replication proteins required by phage Mu are also utilized by phage lambda (22, 26), at least these proteins should be present in sufficient amounts for Mu replication in *dnaA* cells. Second, we found that a brief incubation of arrested *dnaA* cells at 30°C just before infection at 43°C was sufficient to restore the phage yield to nearly normal levels (Fig. 10). The results of this experiment suggest that, except for the absence of vegetative replication forks, *dnaA* cells grown at the nonpermissive temperature are physiologically competent to support normal phage Mu development.

Repeated integration (transposition) of phage Mu DNA into the host chromosome seems to be an integral part of normal phage Mu replication

(10–12, 15, 19). Furthermore, there is genetic evidence which suggests that at least the earliest of these integration events occurs preferentially in the vicinity of host chromosomal replication forks (3). In the absence of these preferred sites in arrested *dnaA* cells, we postulate that phage Mu may be forced to search for alternative integration sites that might be less abundant or less accessible than those normally present in replicating bacteria. Nonreplicating host cells would thus be limited in their capacity to produce optimal phage yields. This view has the merit of being consistent with the experiments which show nearly complete reversal of the abnormalities in phage Mu growth when *dnaA* cells are allowed to begin chromosome replication at the time of infection (Fig. 10). In this regard, we find it noteworthy that the amount of parental phage Mu DNA which becomes covalently bound to chromosomal DNA after infection has been shown to be considerably lower in nonreplicating host cells than in replicating cells (12). Limited availability of integration sites may also help to explain our observation that singly infected *dnaA* cells seldom produce infective

FIG. 9. Restart of chromosome replication in strain AT3706. Bacteria were grown to exponential phase at 30°C and then incubated at 43°C for 80 min before adding [methyl-³H]thymidine to the culture. Ten minutes later, the culture was divided in half. One half was left at 43°C (●), and the other half was incubated at 30°C for 10 min and then returned to 43°C (○). Label incorporation was assayed as TCA-insoluble counts per minute as described in the text.

FIG. 10. Growth of phage Mu in replicating and nonreplicating cells of strain AT3706. Bacteria were grown to exponential phase at 30°C and then incubated at 43°C for 90 min to stop vegetative DNA replication. Chromosome replication was restarted in one half of the culture as described in the legend to Fig. 9, and the other half was left at 43°C. Both cultures were infected with Mucts62 at an MOI of 2. Phage development was assayed in replicating cells (■) and nonreplicating cells (●).

centers, whereas multiply infected cells are generally productive (Table 2 and Fig. 2). For example, if it is assumed that an infecting DNA molecule must initiate the integration-replication process within a fixed period of time before it is degraded or otherwise inactivated by the host cell, then multiple infection of a cell that contains fewer than normal integration sites might increase the probability that a productive infection will ensue. Other possible consequences of multiple infection, such as synthesis of increased amounts of the phage Mu transposition and replication proteins encoded by genes *A* and *B*, may also contribute to the successful infec-

tion of *dnaA* cells, but there is no direct evidence for such an effect at the present time.

The small amount of *E. coli* DNA synthesis which can be detected in infected *dnaA* cells but not in uninfected cells (Fig. 4 and 7) is probably more indicative of repair synthesis than true chromosome replication. If phage Mu were able to relieve the block imposed by the *dnaA* mutation and permit the host to begin normal rounds of chromosome replication, a much higher level of label incorporation into *E. coli* DNA would be expected at early times after infection (see Fig. 8 and 9). A small amount of repair synthesis, however, might be expected on the basis of current DNA transposition models which require some host DNA synthesis to complete the process of transposition and replication (4-6, 21).

The work reported here has shown that phage Mu grows less efficiently in nonreplicating *dnaA* hosts than in wild-type hosts and that efficient phage Mu growth can be restored by allowing *dnaA* cells to begin chromosome replication. On the basis of these and earlier observations, we propose that it is the presence of chromosomal replication forks which is primarily responsible for optimal phage propagation in replicating host cells. Clearly, more studies will be required to confirm this hypothesis and, in particular, it would be desirable to define precisely the sites of phage Mu integration in arrested *dnaA* cells.

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