Heat Induction of Prophage $\phi 105$ in *Bacillus subtilis*: Bacteriophage-Induced Bidirectional Replication of the Bacterial Chromosome

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A mutant of *Bacillus subtilis*, *dna-1*, which cannot initiate new rounds of DNA replication (obtained from N. Sueoka) was lysogenized with wild-type $\phi 105$ and with the heat-inducible mutant $\phi 105$ cts23. Bacteria were incubated at the permissive temperature in the presence of chloramphenicol and then shifted to the nonpermissive temperature where induction of $\phi 105$ cts23 occurs. DNA made after the shift was labeled with a density label, and the distribution of bacterial and phage markers in replicated and unreplicated DNA was determined. Similar experiments were performed with nonlysogenic *dna-1* infected with phage $\phi 105$ cts23 after the temperature shift. The results show that after induction of $\phi 105$ cts23 prophage, bacterial markers on either side of the prophage replicate at an increased rate compared to more distant markers. No selective stimulation of bacterial DNA synthesis was observed on infection or after shifting bacteria lysogenic for noninducible phage to the higher temperature. Attempts to suppress the initiation mutation *dna-1* by phage $\phi 105$ were unsuccessful.

DNA replication in bacteria is initiated at a specific site on the chromosome, and new protein synthesis is required for each new round of replication initiated (7). Temperature-sensitive initiation mutants have been isolated in several bacteria, e.g., in *Bacillus subtilis* (9). When shifted from a permissive to a nonpermissive temperature, such mutants complete rounds of replication already initiated at the time of the temperature shift, but they cannot initiate new rounds (5, 9).

In lysogenic bacteria prophage replication is controlled by the bacterial replication machinery. Only when entering on a lytic path of growth does the chromosome of a temperate phage replicate autonomously. A shift from bacterial to phage control of phage-DNA synthesis thus occurs after prophage induction. This shift must be associated with the unveiling of a new initiation site on the phage chromosome, either by the unblocking of a previously blocked base sequence on phage DNA after inactivation of the immunity repressor, or by synthesis of initiation protein(s), or by both, with new specificity compared to those involved in replication of the bacterial chromosome.

We recently showed that, after induction of *B. subtilis* lysogenic for the heat-inducible pro-

phage $\phi 105$ cts23, two bacterial markers (phe-1, nia-38) replicate at an increased rate compared to other bacterial markers (2). It was suggested that, after induction, the prophage replicates in situ and that DNA synthesis initiated at the prophage proceeds into adjacent regions of the bacterial chromosome. In these experiments replication of the entire *B. subtilis* chromosome proceeded at its preinduction rate. This made it impossible to establish the extent of the phage-determined replication of the host chromosome. To minimize host-determined DNA synthesis in induced cells I have studied here the effect of prophage induction on bacterial DNA synthesis in *B. subtilis dna-1*, a mutant temperature-sensitive for initiation of DNA synthesis (9).

MATERIALS AND METHODS

In the experiments bacteria were grown at 32°C in the presence of [methyl- 3H]thymidine. Chloramphenicol was then added, and the bacteria were incubated for another 30 min at 32°C. During this time no new rounds of DNA synthesis were initiated. The bacteria were then centrifuged, washed, and suspended in fresh medium at 45°C (nonpermissive and prophage-inducing temperature). To this medium was added 5-bromodeoxyuridine to label DNA made after the temperature shift. At intervals after the shift samples were taken for DNA extraction, and the DNA

was then centrifuged to equilibrium in CsCl. Fractions were collected from the CsCl tubes, and replicated and unreplicated DNA was identified by radioactivity and density. The distribution of various phage and bacterial markers in replicated and unreplicated DNA, respectively, was assayed as previously described (2). The bacterial strains used are listed in Table 1. The temperature-shift experiment was done with the following strains: dna-1 lysogenic for $\phi 105$ wild-type (not heat-inducible), dna-1 lysogenic for $\phi 105$ cts23 (heat-inducible), and dna-1 infected with phage $\phi 105$ cts23 after shifting to 45 C.

RESULTS AND DISCUSSION

The results of the experiments are shown in Fig. 1 and 2 where the percentage of total marker activity found in replicated material is plotted against the position of the particular marker on the *B. subtilis* chromosome (12). The results can be summarized as follows.

Infected bacteria. Thirty minutes after the temperature shift about 70% of all phage-DNA activity, measured by marker rescue, is found in replicated DNA, whereas less than 10% of any

TABLE 1. *Bacillus subtilis* strains employed

Strain	Relevant properties	Source
dna-1	ts ^a DNA initiation thy ⁻ , trp ⁻	N. Sueoka
BD25	purA16, metB5, leu-5, nia-38	A. Adams-Lindahl
BD92	cysB3, hisA1, trpC2	J. Spizizen
BR25	ura-1, purA4, trpC2	J. Spizizen
BR62	purB5, trpC2	J. Spizizen
BR95	phe-1, ilvA1, trpC2	J. Spizizen
BR98	hisB2, ura-2, trpC2	J. Spizizen

^a ts, temperature sensitive.

FIG. 1. The bacteria were grown at 32 C in NY broth with 1 μ Ci of [3 H]thymidine per ml (specific activity 6.7 Ci/mmol, NEN chemicals GmbH, Dreieichenhain, Germany) to about 5×10^7 cells per ml. To the culture was then added 100 μ g of chloramphenicol per ml. After 30 min in the presence of this drug the cells were washed twice with minimal-CH medium (1) and suspended in 0.5 volume of this medium at 45 C. After 5 min the culture was diluted into an equal volume of minimal-CH medium at 42 C supplemented with 40 μ g of L-tryptophan, 20 μ g of uracil, and 20 μ g of 5-bromodeoxyuridine per ml, respectively. At intervals, samples were withdrawn, and DNA was extracted with phenol as described (2). The DNA was centrifuged to equilibrium in CsCl, and fractions were collected. Each fraction was assayed for radioactivity and for biological activity of various phage and bacterial markers. The techniques employed have all been described recently (2). The results of the experiment are given as percentage of total marker activity for any marker tested that was recovered at hybrid density. In this figure are given results obtained with DNA extracted 15 min after shifting the cells to 45 C. Symbols: ($\square$ — $\square$) dna-1 infected with $\phi 105$ cts23, ($\circ$ — $\circ$) dna-1 ($\phi 105$), and ($\bullet$ — $\bullet$) dna-1 ($\phi 105$ cts 23).

FIG. 2. Distribution of bacterial and phage markers in DNA extracted 30 min after shifting the cells to 45°C. Symbols are as described for Fig. 1.

bacterial marker tested (except the terminal marker metB) replicated at the same time. For metB about 20% of the marker activity is found in replicated DNA. This probably reflects rounds of replication initiated at 32°C but not finished during the 30 min of incubation in the presence of chloramphenicol at 32°C. Thus, a nonintegrated autonomously replicating phage will not induce replication of the host chromosome.

Bacteria lysogenic for wild-type $\phi 105$. No preferential replication of phage markers over that of any bacterial marker tested is seen. Otherwise the results are similar to those obtained with infected bacteria.

Bacteria lysogenic for $\phi 105$ cts23. After 15 min at 45°C more than 70% of the phage DNA activity is found in replicated material. Concomitant with phage-DNA synthesis there is replication of bacterial markers adjacent to the prophage attachment site. The following percentages of total marker activities were found in replicated DNA after 5 min (not shown in Fig. 2): phe, 8%; leu, 8%; and ura-1, 3%; after 15 min: phe, 27%; leu, 14%; and ura-1, 13%; after 30 min: phe, 31%; leu, 26%; and ura-1, 17%. This is compared with infected bacteria where less

than 10% of either phe, leu, or ura-1 activity replicated after 30 min. Significant replication of the early marker purA16 is also seen in the induced bacteria. This may be due to simultaneous induction of prophage PBSH which is known to be associated with selective replication of this marker (4). PBSH particles are often found in electron micrographs of lysates from induced $\phi 105$ lysogenic bacteria (3).

At 30 min after the temperature shift about twice the amount of prelabeled [3 H]DNA replicates in the cts23 lysogen compared to the other strains, yet close to 90% of the host chromosome remains unreplicated at this time. The rate of replication of host DNA decreases between 15 and 30 min (Fig. 3). In previous experiments we showed that host DNA synthesis continues at constant rate for at least 30 min after induction of a non-ts DNA strain lysogenic for $\phi 105$ cts23 (2). This difference between the present results and previous ones may be trivial and reflects the chloramphenicol treatment or differences between the bacterial strains employed.

The above results thus confirm our previous finding that heat induction of prophage $\phi 105$ is followed by an increased rate of synthesis of bacterial markers adjacent to the prophage. It

FIG. 3. Rate of replication of prelabeled [^3H]DNA after shifting the cells to 45°C. Symbols: (Δ — Δ) *dna-1* infected with $\phi 105$ cts23, (●—●) *dna-1* ($\phi 105$), and (○—○) *dna-1* ($\phi 105$ cts23).

also extends our previous results by showing that phage-induced bacterial DNA replication is bidirectional. Whether this also reflects bidirectional replication of the phage chromosome remains to be determined. In lambda-lysogenic *Escherichia coli*, Imae and Fukasawa (6) showed that after prophage induction, there is an overrun of DNA synthesis into the host *gal* genes. The sensitivity of their technique does not allow a decision as to whether overrun synthesis also occurs in direction of the biotin genes, e.g., whether it is bidirectional or not. Replication of lambda DNA is bidirectional, however (11). DNA initiation mutations in *E. coli* can be suppressed by an integrated episome such as the F factor (10) or phage P2 (8). I have isolated revertants of *dna-1* lysogenic for $\phi 105$ wild-type, $\phi 105$ cts23, or $\phi 105$ cts23 tsA15. Gene A determines some post-replicative function (1). The reversion frequency was lower in the lysogenic strains compared to nonlysogenic bacteria. Five to ten revertants of each strain were examined by back-crosses and were found to have reverted because of mutations at or close to the original *dna-1* mutation. Phage isolated from the revertants did not suppress the *dna-1* mutation. The *E. coli* DNA initiation mutant suppressed by P2 was found by Lindahl et al. (10) to have the prophage integrated close to

met E, whereas in normal lysogenization, integration occurs equally frequent close to his. It may be that the F factor is a more complete suppressor of initiation mutations, whereas structural requirements, such as direction of integration (8), are important for suppression by temperate phage. Phage $\phi 105$ with only one known attachment site is not well suited for studying this problem.

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