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Replication of Bacteriophage M13: Specificity of the *Escherichia coli dnaB* Function for Replication of Double-Stranded M13 DNA

(*dnaB* mutation/DNA synthesis/RF replication)

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ABSTRACT **Infection of the temperature-sensitive *E. coli* mutant HfrH 165/70 (*dnaB*) with the filamentous single-stranded DNA phage M13 is abortive at the restrictive temperature. Upon infection at 41°, single-stranded phage DNA penetrates the cell and is converted in a rifampicin-sensitive step to the double-stranded replicative form (RF). The parental RF attaches to the cell membrane, but subsequent replication of the RF is blocked. It is concluded that in M13 infection semiconservative RF replication of a double strand to a double strand, in contrast to single-stranded DNA synthesis, depends specifically on the *dnaB* function.**

In strains of *E. coli* containing a temperature-sensitive mutation in the *dnaB* region, infection by the single-stranded DNA phage M13 is inhibited at the restrictive temperature (1). However, synthesis of phage single-stranded (ss) DNA and liberation of mature phage particles continues after cells that are actively producing phage are shifted to the higher temperature (1)†. This result implies that there is an earlier step in the replication process of M13 that depends on the *dnaB* function. Since a precise localization of the block in M13 replication may contribute information both to the replicative process of single-stranded DNA phages and to the biochemical lesion in the *dnaB* mutant, abortive M13 infection at the restrictive temperature was studied in more detail. At the restrictive temperature, M13 ss DNA penetrates the cell and is readily converted to RF. In contrast, neither the parental nor progeny RF could replicate at the high temperature. The data indicate that the block in DNA replication in the *dnaB* mutant involves the inability to perform semiconservative replication of double-stranded RF DNA.

MATERIALS AND METHODS

Bacterial Strain and Media. E. coli HfrH 165/70 (*thy*⁻, B1, *dnaB*), originally isolated by F. Bonhoeffer, was obtained from Dr. D. Beyersmann, Berlin. In this strain DNA synthesis is immediately stopped after a shift from the permissive temperature, 34°, to the restrictive temperature, 41°. Bacteria were grown at 34° in Hanawalt medium (2) supplemented with 20 μg/ml of vitamin B_1 and 2 μg/ml of thymidine.

Infected cells were harvested by centrifugation and resuspended in buffer A [0.05 M Tris·HCl, (pH 7.5)–5 mM EDTA–0.1 M NaCl] and lysed by treatment with 100 μg/ml of lysozyme for 20 min at room temperature, followed by addition of 0.5% Sarkosyl.

Gradient centrifugation of the lysate was performed either on a preformed linear neutral CsCl gradient (3) in an SW 50.1 rotor at 45,000 rpm for 2 hr at 15°, or on 5–20% sucrose in buffer A containing 1 M NaCl in an SW 27 rotor at 4° for 15 hr.

For determination of membrane-bound DNA, cells were lysed by lysozyme treatment in the presence of 15% (w/w) sucrose at 0° for 30 min, followed by 3–4 cycles of freeze–thawing. The lysate was then analyzed by centrifugation in a 20–40% sucrose gradient over a shelf of 60% sucrose (3).

Preparation of ^{32}P-labeled M13 phage and ^{3}H-labeled ss M13 DNA and radioactivity assays have been described (4).

RESULTS

Abortive infection at 41°

To detect abortive infection under the conditions used, *E. coli* HfrH 165/70 (*dnaB*) cells were infected with M13 both at the permissive (34°) and restrictive (41°) temperatures; subsequent phage production was assayed. When cells infected at 34° were shifted to 41° 30 min after infection, phage particles continued to be liberated as in the 34° control culture (Fig. 1). As has been shown in this laboratory, ss DNA synthesis is not inhibited under these conditions. The slightly reduced rate of phage liberation probably reflects a temperature sensitivity of the phage assembly process†. However, when cells were infected at 41°, little or no increase in phage titer was observed (Fig. 1). Since phage adsorption did occur at 41° (5), the block in the M13 replicative cycle might occur either during penetration and conversion of phage ss DNA to RF, or during subsequent RF replication.

Formation of parental RF

To determine if the infecting parental ss DNA penetrates and is converted to RF at the restrictive temperature, HfrH 165/70 cells were infected in the presence of chloramphenicol at 41 and 34° with ^{32}P-labeled M13. Chloramphenicol was added to inhibit the synthesis of phage-specific proteins required for RF replication. It should be noted that chloramphenicol does not prevent conversion of ss DNA to RF (6). 15 min after infection, the cells were harvested and lysed,

Abbreviations: ss, single-stranded phage DNA; RF, double-stranded replicative form of DNA.

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† Staudenbauer, W. & Hofschneider, P. H. (1972) *Eur. J. Biochem.*, submitted.

and the products were analyzed by sedimentation through a CsCl gradient. As is shown in Fig. 2, nearly all the parental ss DNA was converted to RF at both the permissive and restrictive temperatures. We conclude that M13 ss DNA can enter the cell at 41°. Moreover, the presence of the *dna*B mutation does not inhibit the replicative conversion of the ss viral DNA into the double-stranded RF.

Although single strands of DNA are effectively converted into RF at 41°, it is possible that this does not represent normal replication. Recently, it was shown that *in vivo* (7) and in soluble extracts of *E. coli* (8), the conversion of M13 ss DNA into RF is inhibited by rifampicin. When cells of HfrH 165/70 were treated with 200 μg/ml of rifampicin for 10 min and then infected with ^{32}P-labeled M13, the antibiotic quantitatively inhibited the conversion of ss DNA to RF at both 34 and 41°.

In the wild-type host, the parental RF of M13 is bound to the cell membrane early after infection (3, 9). The attachment is a necessary condition for further replication of the parental RF. When the RF formed at 41 and 34° were centrifuged through a 5–20% sucrose gradient containing a cushion of 60% sucrose, it was found that at both temperatures about 77% of the infecting M13 DNA was associated with the fast-sedimenting cell membrane component. This material was further characterized as RF.

Inhibition of RF replication

Because it appeared that the parental RF could be formed at the restrictive temperature, we therefore asked if the next step, RF to RF replication, was also blocked at the restrictive temperature. To investigate if the parental RF, once formed, could undergo subsequent replication at 41°, cells were infected in the presence of chloramphenicol. After an absorption period, the chloramphenicol was washed away and the cells were shifted to 34 and 41°. Samples taken after 10 and 20 min of further incubation were pulse-labeled with [^{3}H]-thymidine. Analysis of the cell lysates by sucrose gradient sedimentation (Fig. 3) indicated that replication of the parental RF did not occur at 41°.

The possibility has been suggested that the progeny RF molecules could replicate semiconservatively by a different mechanism than the parental RF (10). Therefore, it was of interest to determine if the block caused by the *dna*B mutation was only in the first replication of the parental RF, or whether it also affected later RF replication. Cells infected at 34° were shifted to 41° at various times after infection, followed by pulse-labeling with [^{3}H]thymidine. As seen in Fig. 4, little label is found at the RF position in gradients containing lysates of cells shifted to 41°, even after RF replication had been initiated at 34° before the shift.

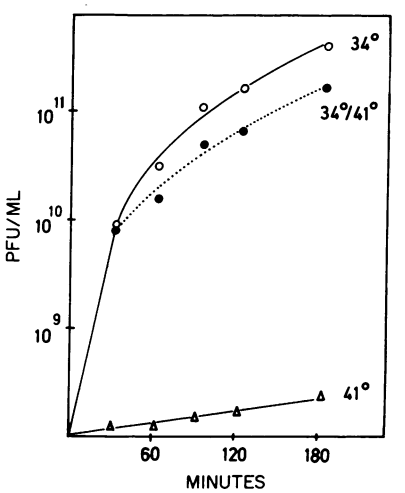

FIG. 1. Phage production in *E. coli* HfrH 165/70. *E. coli* HfrH 165/70 was grown at 34° in H medium to a concentration of 2 × 10^8 cells per ml. One-third of the culture was then shifted to 41°, and, after 10 min of further incubation, both cultures were infected with M13 at multiplicity of infection of one. 30 min after infection half of the 34° culture was shifted to 41° and incubation of the three cultures was continued for 150 min. Samples were taken from the culture at the indicated times and assayed for plaque forming units (PFU)/ml.

DISCUSSION

M13 cannot produce progeny phage after infection of *E. coli* HfrH 165/70 (*dna*B) when the infection occurs at the restrictive temperature (Fig. 1). However, when the cells are infected at the permissive temperature and, later in the infection, shifted to the restrictive temperature, single-strand synthesis and phage production continue†. Therefore, the *dna*B mutation of the host affects an early event in M13

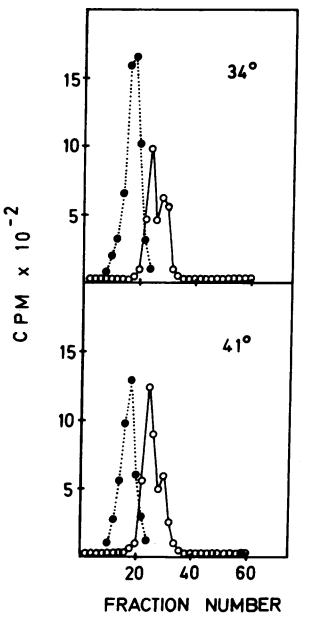

FIG. 2. Formation of parental RF. HfrH 165/70 was grown to 3 × 10^8 cells per ml in 20 ml of H medium at 34° and chloramphenicol (100 μg/ml) was added. The culture was then divided into two 10-ml subcultures, one of which was shifted to 41° and one left at 34°. The cultures were then incubated for 10 min, followed by infection with ^{32}P-labeled M13 phage (2.2 × 10^{-6} cpm/PFU) at an MOI of 100. After 15 min of further incubation, the cultures were quickly cooled by placing them in a dry ice–ethanol bath, and KCN was added to 0.02 M. The cells were harvested by centrifugation, washed twice with a Waring Blendor to remove F-pili and absorbed phage (3), and lysed as described in *Methods*. Samples of the lysates were mixed with ^{3}H-labeled M13 ss DNA and analyzed by CsCl sedimentation gradient centrifugation. Fractions were collected from the bottom of the tube and assayed for Cl_3CCOOH-precipitable radioactivity. Sedimentation in this and all subsequent gradients is from *right* to *left*. O——O, ^{32}P-labeled parental DNA, ●···●, ss [^{3}H]DNA.

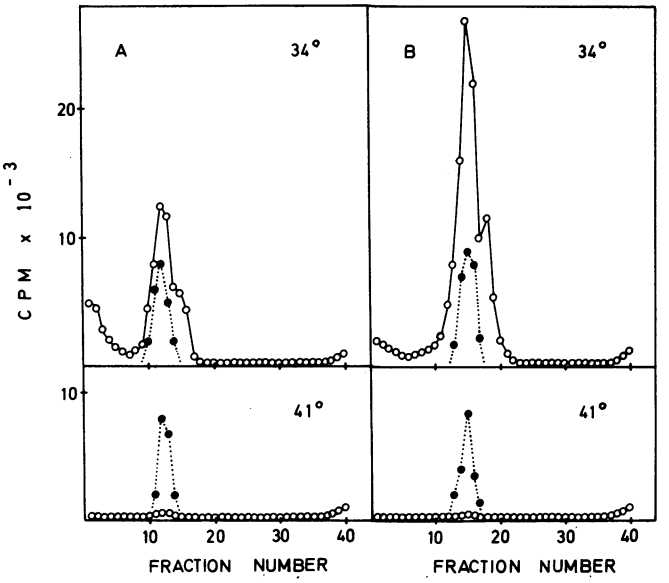

FIG. 3. Replication of parental RF. Exponentially growing HfrH 165/70 (2×10^8 cells per ml) incubated at 34° were treated with chloramphenicol (100 μg/ml) for 5 min, then infected with M13 (MOI = 100). After 10 min the culture was divided in half; the infected cells were harvested by centrifugation at room temperature and washed twice with H medium without glucose or thymidine. After washing, the cells were resuspended in warmed H medium containing glucose and thymidine and immediately placed in water baths at 34 and 41°. After 10 and 20 min of incubation 2-ml samples of each culture were pulse-labeled (at 34 and 41°) with [^{3}H]thymidine (10 μCi/ml) for 1 min. After the pulse, incorporation was stopped by addition of KCN (0.02 M), and the sample was immediately placed in a dry ice–ethanol bath. The cells were lysed by incubation with 5 mM EDTA and (100 μg/ml) of lysozyme for 20 min, followed by addition of 0.5% Sarkosyl. ^{32}P-Labeled M13 RF I DNA was added as a marker, and the entire viscous lysate was chilled and layered on a 5–20% sucrose gradient (1 M NaCl) and centrifuged 15 hr at 25,000 rpm in an SW 27 rotor at 4°. Fractions (1 ml) were collected from the top of the tube by pumping 60% sucrose into the bottom of the gradient, and were assayed for Cl_3CCOOH-precipitable radioactivity. (*A*) Pulse-labeled 10 min after removal of chloramphenicol. (*B*) Pulse-labeled 20 min after removal of chloramphenicol. O——O, pulse-labeled [^{3}H]DNA; ●···●, ^{32}P-labeled M13 RF I.

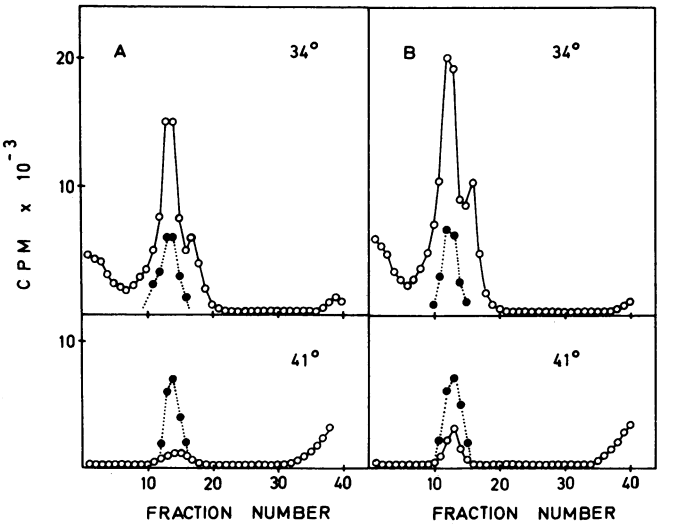

FIG. 4. Replication of progeny RF. Exponentially growing HfrH 165/70 (2×10^8 cells per ml) were infected with M13 at a MOI of 100 at 34°. Incubation was continued at 34° and, after 15 min and 30 min, two 2-ml samples were removed from the culture and shifted to 41 and 34°. After 10 min of incubation, these samples were pusle-labeled with [^{3}H]thymidine (10 μCi/ml) for 1 min. Incorporation was stopped, the cells were lysed, and the labeled products were analyzed as described in Fig. 3. (*A*) Temperature shift after 15 min. (*B*) Temperature shift after 30 min. O——O, pulse-labeled [^{3}H]DNA; ●···●, ^{32}P-labeled M13 RF I.

infection. In this paper, we have shown that at 41° the phage ss DNA enters the cell and is converted into the double-stranded RF (Fig. 2). This process is sensitive to rifampicin, as it is in the wild-type host (7). However, subsequent semiconservative replication of the parental RF is blocked at the restrictive temperature (Fig. 3), i.e., it apparently depends on the *dna*B function.

There is evidence that suggests that the defect in the *dna*B mutant involves an alteration in the cell membrane (11–13). Furthermore, it is known that replication of M13 involves membrane-bound intermediates (3, 9). We have observed that parental RF can attach to the membrane at 41°. Therefore, either the *dna*B mutation is not involved with the binding ability of the membrane for M13 RF, or we have observed a nonfunctional attachment. The situation might be similar to that seen with phage lambda, where the phage DNA binds to the cell membrane in a *dna*B host at the restrictive temperature, but further replication is blocked (14). M13 RF replication is also blocked at the restrictive temperature after progeny RF have been formed. This result implies that progeny RF replication, if it takes place (10), also depends on the *dna*B function.

Thus, M13 RF replication may well serve as a model system for further investigation of the *dna*B function in semiconservative DNA replication in *E. coli*. On the other hand, M13 ss DNA synthesis and the parental ss DNA to RF conversion might resemble transfer replication of the *E. coli* chromosome, which is not affected by the *dna*B mutation (15). Certainly, in the RF replication described here, no progeny ss DNA to RF conversion is involved. As we have recently demonstrated, this type of RF formation can occur at 41°, provided no M13 gene 5 product is available†.

It appears from these data that the process of semiconservative replication of double strand to double strand in M13, but not the synthesis of ss DNA, depends specifically on the *dna*B function. It is not known whether this observation will hold for other ss DNA phages. In this regard, conflicting data has been published on the replication of φX174 in *dna*B mutants (16, 17). Furthermore, it should be mentioned that some small plasmids (col E_I and minicircular DNA of *E. coli* 15) can replicate in *dna*B mutants at the restrictive temperature (18). These plasmids may use an alternate replicative mechanism, as indicated by their requirement for DNA polymerase I (19). This activity is essential neither for the replication of the *E. coli* chromosome nor for all other DNA phages tested, including M13, φX174,

and lambda (20). That phages T4 and P1 can replicate in HfrH 165/70 at 41° (21) is due to their ability to specify a function replacing the *dna*B product (22).

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