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Deoxyribonucleic Acid Synthesis in a Temperature-Sensitive *Escherichia coli dnaH* Mutant, Strain HF4704S

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The *dnaH* mutant strain HF4704S, isolated by Sakai et al. (1974), was examined for its effect on ϕX174 deoxyribonucleic acid (DNA) synthesis. It was found to carry two mutations affecting DNA synthesis. One mutation had no affect on ϕX174 DNA synthesis, but did affect the ability of the mutant cells to form colonies on agar medium at 41°C, and caused host DNA synthesis to cease after 1 h at 41°C. The mutant marker cotransduced with *ilvD* at a frequency of about 9%. It seems likely that this mutation is in the *dnaA* gene. The second mutation affected the ability of the mutant cells to form colonies on agar medium supplemented with only 2 μg of thymine per ml, and affected both host and ϕX174 DNA synthesis in medium supplemented with only 2 μg of thymine per ml. Both effects could be overcome by adding excess exogenous thymine. We were not able to unambiguously determine the map position of this mutant locus. Our data show that the DNA synthesis phenotype of the mutant strain HF4704S is governed by both of these mutations, neither of which directly affects the replication of ϕX174 DNA.

The study of deoxyribonucleic acid (DNA) replication in *Escherichia coli* has been facilitated by the isolation of mutants that exhibit temperature-sensitive DNA synthesis. These mutations map at several different loci and can be classified as mutations affecting either the initiation of cycles of chromosome replication or the continuation of replication once initiation has occurred. Those reported to affect initiation include *dnaA* (2) and *dnaC* (23). They map at 73 min and 89 min, respectively (28). Those affecting continuation of DNA replication include *dnaB* (30), *dnaE* (20), and *dnaG* (15), which map at 18 min, 4 min, and 60 min, respectively (4, 30).

The small, single-stranded DNA bacteriophage ϕX174 has been a useful probe of the role played by these gene products in DNA replication. Its DNA replication occurs in three distinct stages: the synthesis of the complementary strand on the infecting viral strand template (parental replicative form [RF] synthesis), RF replication, and synthesis of viral strands on RF DNA template (single-strand synthesis) (24). The phage genome codes for eight or nine proteins, only two of which, the gene *A* and gene *H* proteins, appear to be directly involved in the replication of the phage DNA (12, 13, 16). Thus ϕX174 is dependent on the DNA replication apparatus of the host cell. Because the DNA molecule of this phage is small, about 1.7×10^6 daltons, replicating intermediates can easily be isolated intact. Thus, analysis of the DNA synthesized in ϕX174-infected temperature-sensitive *dna* mutants has resulted in valuable information about the roles of the *dna* gene products in DNA replication (8-11, 14, 18).

Sakai and co-workers have recently reported the isolation of a new mutant strain, carrying the *dnaH* mutation, which has a temperature-sensitive effect on both host chromosome replication (21) and replication of infecting ϕX174 DNA (22). They reported that the mutation affects initiation of cycles of chromosome replication and inhibits ϕX174 RF replication at the nonpermissive temperature.

When examining the effects of the *dnaH* mutation on each of the three stages of ϕX174 DNA replication, we discovered that the mutant host strain actually carries two different mutations affecting DNA synthesis. One mutation governs the temperature-sensitive inhibition of cell division, and apparently the initiation of host chromosomal DNA synthesis, but has no detectable effect on ϕX174 DNA synthesis. The second mutation affects the synthesis of ϕX174 DNA in the cell, but this effect can be overcome by high concentrations of thymine in the growth medium.

(These results are drawn from a thesis submitted by P.L.D. to Northwestern University

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in partial fulfillment of the requirements for the Ph.D. degree.)

MATERIALS AND METHODS

Bacteria and phage strains. Table 1 lists the bacterial strains used in the experiments reported here, the conditions under which they were isolated from cultures of strain HF4704S, and the conditions under which they form colonies. Strain HF4704S is a nitrosoguanidine-induced temperature-sensitive (Ts) mutant of HF4704 [*uvrA*$^-$ *thyA*(Ts)] isolated by Sakai et al. (21). We obtained HF4704S from Richard Calendar.

The parent strain HF4704 requires thymine for growth at 37°C but not at 30°C (1; P.D., unpublished observation). The mutant strain HF4704S in our hands segregated spontaneously into two phenotypes with respect to thymine requirement: one type (PD102) required high concentrations of thymine (20 μg per ml), and one type (PD103) required low concentrations of thymine (2 μg per ml) for growth at 30°C. The frequencies of these two segregants depended upon the concentration of thymine in the growth medium. Neither type could form colonies at 41°C even in the presence of up to 200 μg of thymine or thymidine per ml. A spontaneous *thyA*$^+$ revertant of strain HF4704S (PD104) was isolated at 30°C on minimal agar plates with no thymine added. These cells could not form colonies at 41°C. Another spontaneous revertant (PD105) was isolated at 41°C by plating strain PD102 on minimal agar plates supplemented with 100 μg of thymine per ml. These cells could not form colonies at 30 or 41°C on minimal agar plates supplemented with only 2 μg of thymine per ml. Strain PD101 was a spontaneous temperature-insensitive revertant isolated at 41°C on plating strain HF4704S on minimal agar plates supplemented with 2 μg of thymine per ml. These cells could form colonies on plates supplemented with 2 μg of thymine per ml at both 30 and 41°C.

Strains AT713 (*thi*$^-$ *argA*$^-$ *cysC*$^-$ *lysA*$^-$ *mtl*$^-$ *xyl*$^-$ *malA*$^-$ *strA*^{r} λ^r λ$^-$ *supE*) and AB2596 (*ilvD*$^-$ *argH*$^-$ *his*$^-$ *trp*$^-$ *strA*^{r}) used in the mapping studies were obtained from the *E. coli* Genetic Stock Center. Hfr strains used were the same as those used by Low (17) and were obtained from the *E. coli* Genetic Stock Center. F-prime donor strains used and the F-prime factors each contained were the following: KLF1/AB2463 (F101), KLF4/AB2463 (F104), ORF4/KL251 (F254), PA200S^{R}F$_1^-$ *gal* (F100), KLF47/KL262 (F147), KLF25/KL181 (F125), KLF48/KL159 (F148), DFF1/JC1553 (F150), KLF29/JC1553 (F129), KLF42/KL253 (F142), KLF43/KL259 (F143), KLF2/JC1553 (F102), MAF1/JC1553 (F140), KLF11/JC1553 (F111), KLF10/JC1553 (F110), KLF12/JC1553 (F112), KLF19/KL132 (F119), and KLF18/KL132 (F118). These were obtained from the *E. coli* Genetic Stock Center

E. coli C is the standard φX174 host used here to prepare stocks of φX174*am3* (gene *E*, lysis defective). HF4714 is an amber suppressor host strain used to measure φX174*am3* plaque-forming units (PFU).

Media and buffers. TPGA is TPG medium (25) with 1.0 g of KH_2PO_4 and 10 g of Casamino Acids per liter. Starvation buffer (SVB) has been described (6). Minimal agar and minimal top agar have been described by Clowes and Hayes (5). LB broth and LB agar have been described (1), as has KC medium (25).

Phage stocks. Nonradioactive phage were prepared as described earlier (7).

Measurement of acid-insoluble radioactivity. Intracellular DNA synthesis in uninfected cells was measured as described by Dumas and Miller (8). The amount of radioactive label incorporated into phage DNA in short pulses was measured as described by Dumas and Miller (9).

Measurement of intracellular PFU. Measurement of intracellular PFU was carried out as previously described (8).

Genetic mapping. The rapid mapping technique (method A) of Low (17) was used to initially assign mutations to small regions of the chromosome. Streptomycin-resistant mutants used as recipients in these crosses were obtained by P1 transduction. Conjugation with F-prime donors was used to assign mutations to still smaller regions of the chromosome. The donor F-prime-carrying strains and recipient temperature-sensitive strains were grown at 30°C in nutrient broth to cell densities of 5×10^8 cells per ml. Using an inoculating loop, portions of each recipient cell culture were transferred in short

TABLE 1. *Bacterial strains derived from HF4704S*

Designation	Conditions for isolation[a]		Colony formation[b]					
			30°C			41°C		
	Temp (°C)	Amt of thymine (μg/ml)	0	2	20	0	2	20
PD101	41	2	−	+	+	−	+	+
PD102	30	100	−	−	+	−	−	−
PD103	30	2	−	+	+	−	−	−
PD104	30	0	+	+	+	−	−	−
PF105	41	100	−	−	+	−	−	+
HF4704			+	+	+	−	+	+

[a] Present on minimal agar plates.

[b] +, Colonies will grow on minimal agar plates supplemented with 0, 2, or 20 μg of thymine per ml; −, colonies will not grow.

patches onto minimal agar plates supplemented with 2 μg of thymine per ml and the patches were allowed to dry. Similar short streaks of donor cells were then made on the same plates so that a different donor streak would intersect and cross each recipient streak. The plates were incubated 1 h at 30°C and then transferred to 41°C. Recipient cells could not grow on these plates because either the temperature of incubation was too high or not enough thymine was present. Donor cells could not grow because medium lacked essential nutrients. Recipient cells receiving an F-prime factor carrying that segment of the chromosomal DNA that included the wild-type allele of the mutation being analyzed could grow under these conditions. These cells were found in the region where the donor and recipient cell streaks intersected. Appropriate controls utilizing K-12 strain nutrient-requiring recipients were included to test the ability of the F-prime donors to transfer the F-prime factors.

Cotransduction was used to further define the location of the mutation on the chromosome. Bacteriophage P1·*kc*·*cc* was grown on the appropriate donor bacterial strains and purified as described by Calendar and Lindahl (3). Transductions were carried out according to procedures described by these same authors with the following modifications. Recipient cells were grown at 30°C and resuspended to a cell density of 10^9 cells per ml in LB medium containing 5 mM $CaCl_2$. Purified P1·*kc*·*cc* was added to a final concentration of 4×10^8 PFU per ml. After 20 min at 30°C, 0.1 volume of 1 M $MgSO_4$ and 1.0 volume of 1 M sodium citrate were added to prevent readsorption of released phage (19). The cells were spun down, and the pellet was resuspended in 1.0 volume of minimal media, 0.1 volume of 1 M $MgSO_4$, and 1.0 volume of 1 M sodium citrate. Portions of this cell suspension were then plated on appropriate media.

Chemicals. [*methyl*-^{3}H]thymidine, 45 Ci/mmol, and [*methyl*-^{3}H]thymine, 21 Ci/mmol, were purchased from Amersham/Searle. Mitomycin C and egg white lysozyme were purchased from Sigma Chemical Co.

RESULTS

In our initial experiments with the mutant strain HF4704S we observed temperature-sensitive host DNA synthesis in uninfected cells and temperature-sensitive phage DNA synthesis in φX174-infected cells, consistent with results previously reported (21, 22). However, we also observed an unusually high rate of incorporation of [^{3}H]thymidine into φX174 at 30°C in this mutant host when compared with the nondefective parent strain. The phage yield was normal at 30°C, suggesting that the specific activity of the labeled phage DNA was higher than normal. Pulse-chase experiments showed that the phage DNA was stable. This led us to check the thymine requirement of the mutant host strain. When this strain was cultured for several generations in 2 μg of thymine per ml, the concentration used in the experiments mentioned above, we found two predominant cell types. Both were temperature sensitive; one strain (PD103) could form colonies at 30°C on agar medium containing 2 μg of thymine per ml; the other strain (PD102) could not, but could when thymine was present at 20 μg/ml. These two strains and three revertant strains are described in further detail in Materials and Methods, and in Table 1. Clones of each of these strains were used in the experiments described below.

Rates of φX174 DNA synthesis. Using these host strains, we were again able to demonstrate a temperature-sensitive effect on φX174 DNA synthesis (Fig. 1 and 2). The rates of φX174 DNA synthesis were measured in 2-min pulses at the indicated times after infection. These data show, however, that the temperature-sensitive effect on φX174 DNA synthesis was only observed in those host strains that require high concentrations of exogenous thymine (strain PD102 and strain PD105, Fig. 1E and 2E, respectively). In all other strains, including the parent host strain, the rate of φX174 DNA synthesis increased upon shifting from 30 to 41°C. Also, the temperature-sensitive effect was observed only when these cells were grown and infected in 2 μg of thymine per ml. When the same strains were infected in medium containing 100 μg of thymine per ml, φX174 DNA synthesis was not temperature sensitive (Fig. 1F and 2F). These data suggest that the apparent temperature sensitivity of φX174 DNA synthesis is an artifact of the low concentration of exogenous thymine in cultures of cells that require high concentrations for normal growth.

Synthesis of viable phage. We measured the synthesis of viable phage to see whether it was temperature sensitive in these mutant host strains. We observed normal synthesis of infective phage at 30 and 41°C in all of the host strains when the culture medium contained 100 μg of thymine per ml (Fig. 3). φX174 production was not temperature sensitive, even in strain PD102 (Fig. 3F) and strain PD103 (Fig. 3D), which carry temperature-sensitive mutations. When cultured in 2 μg of thymine per ml, the host strains that require the higher concentrations of thymine for normal cell growth, PD102 and PD105, yielded low levels of viable phage at both 30 and 41°C (Fig. 3E and G, respectively). Thus, viable phage production is not temperature sensitive in these mutant strains, but rather is dependent upon the thymine concentration in the culture medium.

Synthesis of host DNA. To determine the effects of temperature and exogenous thymine concentration on host DNA synthesis, we mea-

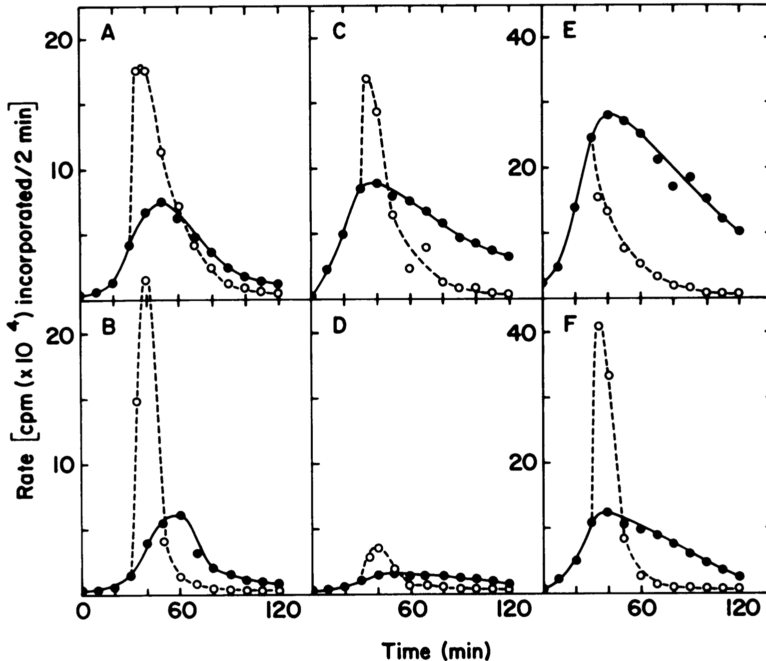

FIG. 1. *Rate of ϕX174am3 DNA synthesis in host bacteria grown in low and high concentrations of thymine. Strains HF4704, PD101, and PD102 were diluted 1:100 from stock cultures grown in KC broth supplemented with 20 μg of thymine per ml and stored at 4°C in TPGA medium supplemented with either 10 or 50 μg of thymine per ml and grown overnight at 30°C. Strain PD102 grown in 10 μg of thymine overnight was diluted 1:10 into TPGA medium supplemented with 2 μg of thymine per ml. The other cultures were diluted 1:50 from TPGA supplemented with 10 μg of thymine per ml into TPGA medium supplemented with 2 μg of thymine per ml, and from 50 μg of thymine per ml into 100 μg of thymine per ml. These cultures were grown at 30°C to cell densities of approximately 4 × 10^8 cells per ml (about three cell doublings). Cells were collected by centrifugation, resuspended in 0.1 volume of starvation buffer, and treated with 100 μg of mitomycin C per ml for 20 min at 30°C. The cells were again collected by centrifugation and resuspended in 1 volume of TPGA medium supplemented with 2 or 100 μg of thymine per ml. ϕX174am3 was added at a multiplicity of 3 (zero time). Thirty minutes after infection a portion of each culture was shifted to 41°C. At the indicated times, 2 ml of each culture at each temperature was transferred into separate vessels containing 5 μCi of [^{3}H]thymidine in 0.5 ml of TPGA medium and incubated for 2 min. Cold acetone (2.5 ml) was added to terminate the pulse. The amount of DNA synthesized during the pulse was then measured as described in Materials and Methods. (A) Strain HF4704 in 2 μg of thymine per ml; (B) strain HF4704 in 100 μg of thymine per ml; (C) strain PD101 in 2 μg of thymine per ml; (D) strain PD101 in 100 μg of thymine per ml; (E) strain PD102 in 2 μg of thymine per ml; (F) strain PD102 in 100 μg of thymine per ml. Symbols: ●, Rate of DNA synthesis at 30°C; ○, rate after shifting to 41°C.*

sured the accumulation of [^{3}H]thymine into DNA in uninfected cells. Host DNA synthesis was temperature sensitive in strains PD103 and PD102, even in medium containing 100 μg of thymine per ml (Fig. 4D and 4F, respectively). DNA synthesis ceased after approximately 1 h at 41°C, whereas it continued at 30°C. Strains PD101 and PD105, which are able to form colonies at 41°C, were able to continue DNA synthesis at 41°C in the same medium (Fig. 4B and H, respectively). Thus, mutant strains carrying the temperature-sensitive mutation governing the ability to form colonies at 41°C are unable to continue host DNA synthesis at 41°C in medium containing high concentrations of thymine, even though ϕX174 DNA synthesis continues normally in these strains under these conditions.

In growth medium containing only 2 μg of thymine per ml, the rate of accumulation of [^{3}H]thymine into DNA declined upon shifting cultures of strains PD102 and PD105 to 41°C (Fig. 4E and G, respectively). These are the two strains that can neither grow normally nor support normal levels of ϕX174 phage production in medium containing only 2 μg of thymine per ml. In addition, the rate of accumulation of radioactive label into DNA was slower in these two strains at 30°C than in the strains requiring less exogenous thymine for normal growth

(compare Fig. 4E with C, for example). The decline in the rate of [^{3}H]thymine uptake at 41°C and the slow synthesis of DNA at 30°C were unique to these two strains. Both defects were overcome in medium containing 100 μg of thymine per ml (Fig. 4F and H, respectively). These effects are apparently artifacts of low concentrations of exogenous thymine in cultures of cells that require high concentrations for normal growth.

Genetic analyses. The results presented thus far suggest that two different mutations affect DNA synthesis in these strains: one, carried by strain PD103, affects the ability to form colonies at 41°C, stops *E. coli* DNA synthesis after 1 h at 41°C, and has no effect on φX174 DNA synthesis; the other, carried by strain PD105, affects the ability to form colonies in medium containing 2 μg of thymine per ml, and affects both host and φX174 DNA synthesis in 2 but not 100 μg of thymine per ml. Strain PD102 carries both of these mutations, whereas strain PD101 carries neither. We sought evidence that would allow us to determine the position of each genetic marker on the *E. coli* chromosome.

Using the rapid mapping technique of Low (17), we were able to place the marker in strain PD103 governing the ability to form colonies at 41°C between *ilvE* at 74.5 min and *strA* at 64 min. F-prime factor F111 complemented the mutant gene, whereas F-prime factor F140 did not. F111 carries a region of chromosomal DNA bounded by *malB* (81 min) and *pyrE* (72 min); F140 carries a region of chromosomal DNA bounded by *mtl* (71 min) and *argG* (61 min). Therefore, the marker must be located between *ilvE* (74.5 min) and *pyrE* (72 min).

Strain PD103 was then infected with P1 grown on strain AB2596 (*ilvD*$^-$) and plated on minimal agar medium supplemented with thymine (2 μg per ml), isoleucine, and valine. Of 232 temperature-insensitive transductants tested, 22 required isoleucine and valine. Therefore, the cotransduction frequency of the temperature-sensitive marker with *ilvD* is about 9%.

Similar mapping studies resulted in data in-

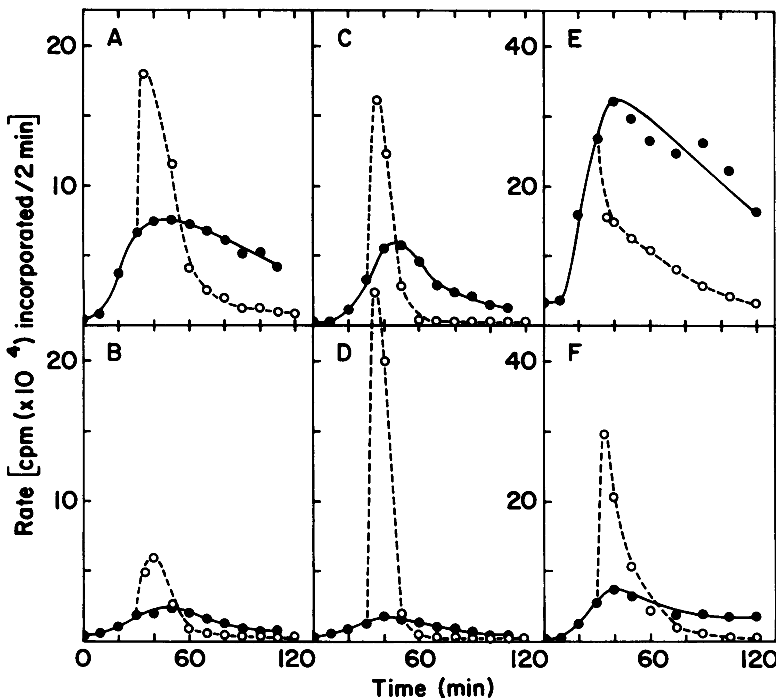

FIG. 2. *Rate of φX174am3 DNA synthesis in host bacteria grown in low and high concentrations of thymine. Strains PD103, PD104, and PD105 were grown, treated with mitomycin C, and resuspended in medium as described in Fig. 1. (Strain PD105 grown overnight in TPGA medium supplemented with 10 μg of thymine per ml was diluted 1:10 into TPGA medium supplemented with 2 μg of thymine per ml.) The experiment was completed as described in Fig. 1. (A) Strain PD103 in 2 μg of thymine per ml; (B) strain PD103 in 100 μg of thymine per ml; (C) strain PD104 in 2 μg of thymine per ml; (D) strain PD104 in 100 μg of thymine per ml; (E) strain PD105 in 2 μg of thymine per ml; (F) strain PD105 in 100 μg of thymine per ml. Symbols: ●, Rate at 30°C; ○, rate at 41°C.*

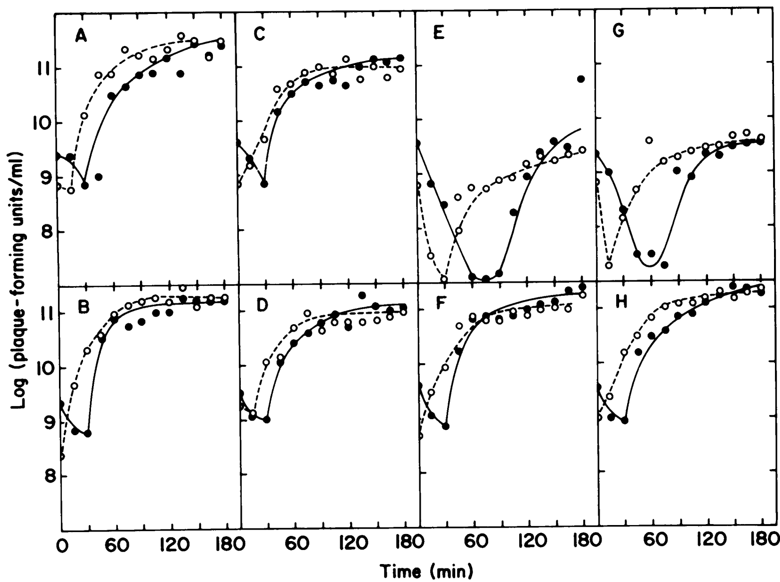

FIG. 3. *Synthesis of infectious φX174am3 in host bacteria grown in low and high concentrations of thymine. Strains PD101, PD102, PD103, and PD105 were grown at 30°C in TPGA medium as described in Fig. 1 and 2. φX174am3 was added at a multiplicity of 3. Five minutes after infection, half of each culture was shifted to 41°C (zero time) and aeration was continued for 3 h. At the indicated times, 0.1-ml samples of each culture were transferred into 1.2 ml of lysis buffer. Total PFU were then determined as described in Materials and Methods. (A) Strain PD101 in 2 μg of thymine per ml; (B) strain PD101 in 100 μg of thymine per ml; (C) strain PD103 in 2 μg of thymine per ml; (D) strain PD103 in 100 μg of thymine per ml; (E) strain PD102 in 2 μg of thymine per ml; (F) strain PD102 in 100 μg of thymine per ml; (G) strain PD105 in 2 μg of thymine per ml; (H) strain PD105 in 100 μg of thymine per ml. Symbols: ●, 30°C; ○, 41°C.*

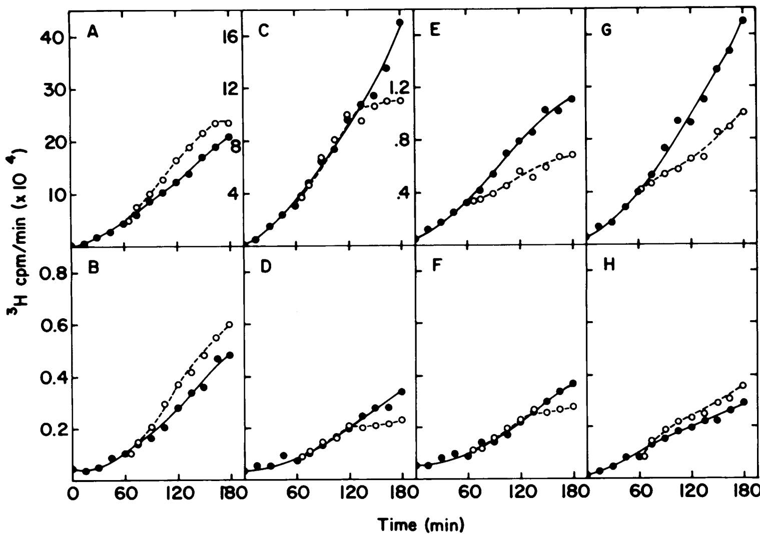

FIG. 4. *DNA synthesis in uninfected host bacteria grown in low and high concentrations of thymine. Strains PD101, PD102, PD103, and PD105 were grown at 30°C in TPGA medium as described in Fig. 1 and 2. [³H]thymine was added to a final concentration of 10 μCi per ml (zero time). Sixty minutes later half of each culture was shifted to 41°C. At 15-min intervals, 0.1-ml portions were removed into 1 ml of 5% trichloroacetic acid. Two-tenths milliliter of calf thymus DNA (1 mg per ml) was added as carrier. The amount of acid-insoluble radioactivity was then determined as described in Materials and Methods. (A) Strain PD101 in 2 μg of thymine per ml; (B) strain PD101 in 100 μg of thymine per ml; (C) strain PD103 in 2 μg of thymine per ml; (D) strain PD103 in 100 μg of thymine per ml; (E) strain PD102 in 2 μg of thymine per ml; (F) strain PD102 in 100 μg of thymine per ml; (G) strain PD105 in 2 μg of thymine per ml; (H) strain PD105 in 100 μg of thymine per ml. Symbols: ●, 30°C; ○, shifted to 41°C at 60 min.*

dicating that the marker in strain PD105 that governs the requirement for high concentrations of exogenous thymine was closely linked to *thyA*, *lysA*, and *argA*. In transduction experiments, strain AT713 (*lysA*$^-$ *thyA*$^+$ *argA*$^-$) served as the donor. Transductants of strain PD105 were first allowed to form colonies at 41°C on agar medium supplemented with only 2 μg of thymine per ml plus the appropriate amino acids. These were then scored for the presence of the three donor markers. We found apparent cotransduction frequencies of 14 to 22%, 6%, and 5% between the recipient marker and the *thyA*, *lysA*, and *argA* markers, respectively. However, all of the 99 apparent cotransductants tested were Thy$^+$ (required no exogenous thymine). None of the *lysA*$^-$ or *argA*$^-$ apparent cotransductants was still *thyA*$^-$. It seems certain that the Thy$^+$ phenotype would mask the presence of the marker necessitating high concentrations of exogenous thymine. Thus, the apparent cotransductants may still have carried the defective recipient marker. We cannot, therefore, interpret these data unambiguously. The marker may be closely linked to *thyA*, as previously reported for the *dnaH* marker (21). Alternatively, and perhaps more likely, the apparent cotransduction may be an artifact of the Thy$^+$ phenotype of these cells, in which case the mutant marker would not be closely linked to *thyA*, *lysA*, or *argA*.

DISCUSSION

We conclude from our analyses of the segregants and spontaneous revertants we isolated from the HF4704S stock culture that strain PD102 carries two mutations: one affects the ability to form colonies at 41°C, stops *E. coli* DNA synthesis after 1 h at 41°C, and has no affect on φX174 DNA synthesis; the other affects the ability to form colonies on agar medium supplemented with only 2 μg of thymine per ml, and affects both host cell and φX174 DNA synthesis. We argue that strain HF4704S is equivalent to strain PD102, and that cultures of strain HF4704S grown in medium containing only 2 μg of thymine per ml consist of mixtures of primarily strains PD102 and PD103.

The mutation resulting in temperature-sensitive colony formation maps between 72 and 74.5 min on the *E. coli* genetic map, as judged by conjugation experiments, and cotransduces with *ilvD* at a frequency of 9%. Cells carrying this mutation exhibit temperature-sensitive DNA synthesis. The observation that DNA synthesis continues for about 1 h at the nonpermissive temperature (Fig. 4) is consistent with that expected of a mutant defective in the initiation of DNA synthesis. In addition, the gene product is not required for φX174 DNA synthesis (Fig. 2). Markers in the *dnaA* gene cotransduce with *ilvD* at frequencies of 4 to 13% (30). Cells mutated in the *dnaA* gene are defective in the initiation of host chromosome synthesis (2), but are able to support normal φX174 infection (26). It seems likely, therefore, that the mutation resulting in temperature-sensitive colony formation is in the *dnaA* gene. Although mutants carrying a defect in the *dnaP* gene have also been reported to be defective in the initiation of host chromosome synthesis (29), but not in φX174 infection (27), the gene maps between *cya* (75 min) and *metE* (75.5 min). It is not likely, therefore, that the temperature-sensitive mutation carried in strains PD102 and PD103 is in the *dnaP* gene.

The second mutation is more difficult to analyze, and our genetic analysis of strains carrying it is less easily interpreted. We observed that more [^{3}H]thymidine is incorporated into φX174 DNA at 30°C in cultures of mutant cells grown in 2 μg of thymine per ml than in similar cultures of nondefective cells (Fig. 1 and 2), whereas phage yields are lower in the mutant cells (Fig. 3). Thus, the specific activity of [^{3}H]thymidine-labeled DNA is unusually high under these conditions. In addition, phage DNA synthesis appears to be temperature sensitive when measured by rate of [^{3}H]thymidine incorporation (Fig. 1 and 2), although the synthesis of infective phage particles is not (Fig. 3). Thus, the specific activity of [^{3}H]thymidine-labeled DNA is reduced at 41°C compared with 30°C.

When exogenous thymine is present at 100 μg per ml, the phage yield is normal (Fig. 3), and DNA synthesis no longer appears temperature sensitive (Fig. 1 and 2). When the concentration of exogenous thymine is no longer limiting, the specific activity of the [^{3}H]thymidine-labeled DNA no longer changes with temperature. Thus, the actual amount of DNA synthesis is dependent upon the thymine concentration in the medium, not the temperature. The apparent temperature sensitivity of φX174 DNA synthesis measured by the rate of incorporation of [^{3}H]thymidine is thus an artifact of the limiting thymine concentration in the medium.

Similarly, uninfected cells carrying this mutation (strain PD105) exhibit slow DNA synthesis in 2 μg of thymine per ml, and this DNA synthesis is temperature sensitive. But in 100 μg of thymine per ml, DNA synthesis is normal (Fig. 4). Again, the amount of DNA synthesis in uninfected cells carrying this mutation is

primarily dependent upon the exogenous thymine concentration, not the temperature.

Perhaps the simplest interpretation of these data is that the second mutation causes a defect in the utilization of exogenous thymine which can be overcome by the addition of high concentrations of thymine to the growth medium. At limiting thymine concentrations the specific activity of [^{3}H]thymidine in DNA would be high, assuming its utilization is not defective, and the amount of DNA made would be low. The addition of excess exogenous thymine would result in a lower specific activity and a higher rate of DNA synthesis.

We were able to generate data from genetic analyses that suggest that the mutant thymine utilization locus is closely linked to the *thyA* gene. Sakai et al. reported that the *dnaH* marker is closely linked to *thyA* (21). Our genetic evidence also might suggest cotransduction with *lysA* and *argA*. However, all of the apparent cotransductants we observed were Thy$^+$ (required no exogenous thymine), and the Thy$^+$ phenotype could mask the presence of a mutant thymine utilization marker. Thus all of our apparent cotransductants could still have carried the mutant allele. We suggest, therefore, that the mutant marker may not in fact be closely linked to *thyA*, *lysA*, or *argA*. Further genetic analyses are necessary to allow its position to be accurately determined.

Others have reported (21, 22) that *E. coli* strain HF4704S carries a temperature-sensitive mutation in the *dnaH* gene which causes temperature-sensitive ϕX174 DNA synthesis in medium containing 2 μg of thymine per ml, and causes temperature-sensitive initiation of host DNA synthesis. They also reported that the mutant locus is closely linked to *thyA*. From our observations, we conclude that strain HF4704S carries the two different mutations reported here, both of which affect DNA synthesis. We suggest, however, that only one of these mutations directly affects DNA synthesis, and that this mutation is probably in the *dnaA* gene. The other mutation apparently affects the cell's requirement for exogenous thymine. We cannot unambiguously assign its position on the genetic map.

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