PARENTAL RF FORMATION OF PHAGES ØX174 AND M13 REQUIRES THE *dnaZ* GENE PRODUCT OF *ESCHERICHIA COLI*

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SUMMARY: A functional *dnaZ* product, known to be essential for host DNA polymerization and for the growth of coliphages ØX174 and M13, is required for the *in vivo* single strand to replicative form conversions of both of these phages.

ØX174 and M13 rely on host cell functions for conversion of infecting single strand DNA (SS) to double strand replicative forms (RF) (1, 2). Several of the *Escherichia coli dna* genes, known to be required for host chromosome replication, have been implicated in these conversions.

The *in vitro* conversion of M13 single strands into double strand molecules requires RNA polymerase and the *dnaE* gene product; a similar reaction on ØX174 templates does not utilize RNA polymerase but has additional requirements which include the *dnaB*, *C*, and *G* gene products (3). DNA polymerization in both cases is catalyzed by the *dnaE* product (DNA polymerase III) (3). The *in vitro* conversion of both M13 and ØX174 primed SS to RF can be catalyzed also by DNA polymerase I (4, 5).

Similar results have been obtained *in vivo*. For M13 parental RF formation, functional *dnaE* product is essential only in host cells which lack DNA polymerase I (6). The *dnaB* and *G* products are dispensable (7, 8) and *dnaC* has not been tested. *In vivo* ØX174 parental RF formation requires *dnaB* and *G* gene products (9, 10); however, in contrast to the *in vitro* data, *dnaE* and *C* products appear to be dispensable (11, 12, 13). The failure to demonstrate a requirement for the *dnaE* product in cells deficient in DNA polymerase I might have resulted from residual *dnaE* activity in the mutant

employed (12), and the dnaC requirement might have been circumvented, in vivo, by a rifampicin sensitive RNA polymerase (14).

The role of the dnaB product in polymerization has not yet been defined, although it has an ATPase activity which is stimulated by single strand DNA (15). The dnaC product appears to participate in the initiation stage of DNA synthesis (3) and is known to interact physically with the dnaB product (16). The dnaG product has rifampicin resistant RNA polymerase activity (17).

In this communication, we report that functional dnaZ product, known to be essential for host DNA polymerization (18) and for the growth of ØX174 and M13 (19), is required for the in vivo SS to RF conversions of both of these phages.

MATERIALS AND METHODS

Strains. The K-12 strain AX727, dnaZts2016, was previously described (18). GN727, a ØX-sensitive dnaZts2016 recombinant of AX737 (K12 F⁻dnaZts2016 leu trpE proC tsx lacY str xyl ara mtl galK azi thi tonA supE44) and HF4726 (ØX-sensitive Hfr) was obtained from G. N. Godson and cured of its prophage, E. coli C from R. McKee, C600 from B. Bachmann, M13 from D. Ray and ØX174h8p (referred to as ØX174) from C. Earhart. AX727 TS⁺ and GN727 TS⁺ were spontaneous temperature-insensitive revertants of AX727 and GN727. F'lac⁺ derivatives were obtained by mating with RV/F'lac⁺ (obtained from M. Malamy).

Analysis of M13 parental RF formation. Cultures of AX727/F'lac⁺ and AX727 TS⁺/F'lac⁺ were grown to $3x10^8$ cells/ml in YET broth (19) at 30°C, followed by incubation at 37°C or 41°C for the desired interval. Chloramphenicol (180 µg/ml) and [^{3}H]-thymidine (10 µCi/0.12 µg/ml) were added to the cultures at 15 min. and 1 min., respectively, prior to infection by M13 at a multiplicity of 100. The pulse was ended 5 minutes after infection by diluting the cells into 2 volumes of cold TES buffer (20) containing 0.1 mg/ml of unlabeled thymidine. The cells were washed, lysed, and the crude lysates analyzed in high salt, 5 to 20%, neutral sucrose gradients by the procedure of Ray and Schekman (21). Centrifugation was performed in a Beckman SW41 rotor at 30,000 rpm for 11.5 hrs at 4°C. Fractions were collected and counted.

Analysis of ØX174 parental RF formation. Cultures of ØX174-sensitive GN727 and GN727 TS⁺ were grown to $3x10^8$ cells/ml in YET broth supplemented with 1.5 mg/ml of $CaCl_2$. The cultures were incubated, inhibited by chloramphenicol, and pulsed as above with these modifications: MOI was 10, pulse duration was 10 minutes, and the pulse was ended by dilution into an equal volume of cold tetrasodium borate (0.05M)-EDTA (0.006M) buffer, pH 8.1. The cells were washed, lysed, and pronase treated by the method of Francke and Ray (22); however, sarkosyl was substituted for SDS in the lysis procedure.

Preparation of [^{3}H]-M13. M13 was grown in a low thymine requiring mutant of AX727 TS⁺ in YET broth supplemented with 5 µCi/2 µg/ml of [^{3}H]-thymine. Cells were removed by low speed centrifugation. Phages were pelleted by centrifugation for 5 hours at 40,000 rpm and purified in a 5-20% neutral sucrose gradient similar to that described above. The phages were dialyzed overnight against dilution buffer (23) and had an activity of 10^{-6} counts per minute per plaque forming unit when plated on E. coli C600/F'101.

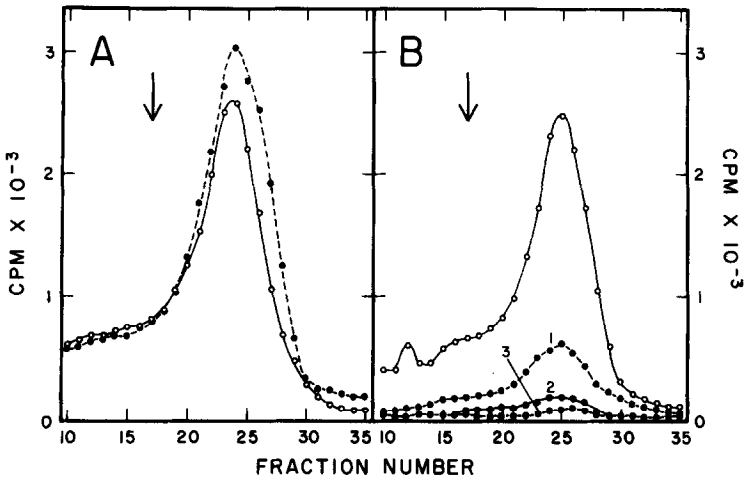

Fig. 1. M13 parental RF formation in dnaZts and TS+ revertant cultures at 37°C and 41°C. (A) A TS+ revertant culture was grown at 30°C and transferred to 37°C (o) or 41°C (•) and infected with M13 at 35 minutes after transfer. (B) A dnaZts culture was grown, transferred to 37°C (o), and infected as in A or transferred to 41°C (•) and infected at 15 (curve 1), 25 (curve 2), or 35 (curve 3) minutes after transfer. Direction of sedimentation was from right to left in this and all other Figures. Vertical arrows indicate the position of ØX174 SS marker DNA.

Biological Assay for ØX174 Marker DNA. Unlabelled ØX174 DNA was prepared by the method of Brown and Dowell (23) and centrifuged with the ØX174 and M13 lysates as a sedimentation marker. Its position on the gradients was assessed by its biological activity in the spheroplast transformation assay (24).

RESULTS

Chloramphenicol (180 µg/ml) prevents the replication, but not formation, of M13 and ØX174 RF DNA (6, 22, 25); therefore, labelled phage DNA formed in the presence of chloramphenicol represents complementary strand synthesis on the infecting viral strands.

M13 infection of F'lac+ derivatives of dnaZts and TS+ cultures at 37°C (selected as the permissive condition) resulted in the formation of similar amounts of parental RF (Fig. 1). However, if the infections were carried out at 41°C, replicative form synthesis was inhibited in the dnaZts mutant. The longer the incubation time at 41°C prior to infection, the less parental RF synthesis occurred (Fig. 1B curves 1, 2, and 3). To ensure that this result was not due to inability of M13 to adsorb to the mutant at 41°C, M13 adsorp-

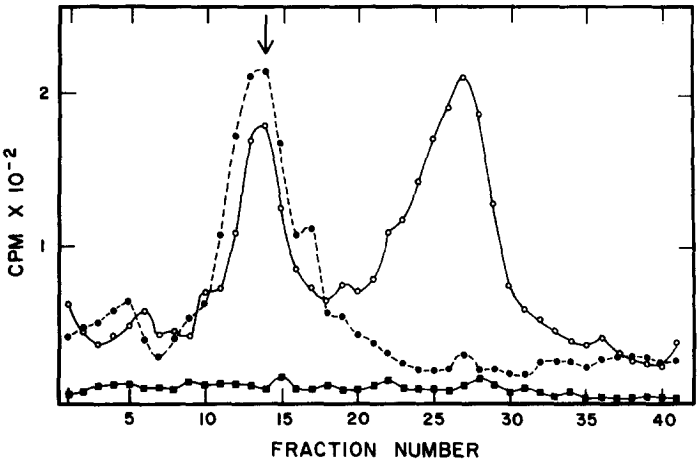

Fig. 2. Adsorption of [^{3}H]-M13 phage at 37°C and 41°C. dnaZts/F'lac$^+$ (o, •) and F$^-$ dnaZts (■) cultures were grown at 30°C to 3x10^8 cells/ml, transferred to 37°C (o) or 41°C (•,■), infected with [^{3}H]-M13 phage at an MOI of 100. Ten minutes after infection, the cells were diluted into cold TES buffer plus 0.01M KCN and washed 3 times to remove unadsorbed phages. Cells were lysed and centrifuged for 10 hr on sucrose gradients as described above. The vertical arrow indicates the position of intact M13 phage particles.

tion to dnaZts/F'lac$^+$ cells was measured at 37 and 41°C (Fig. 2). Cells were infected with [^{3}H]-M13 and unadsorbed phages were washed from the culture. The cells were lysed, which results in stripping adsorbed, whole phage particles from the cell surface, and the lysate centrifuged. Phage particles sedimented to fraction 14. Although conversion of phage DNA to RF (fraction 27) was inhibited at 41°C, adsorption of M13 occurred. The amount of radioactivity at the position of M13 phage infectivity represents 1 to 2 adsorbed phage per cell at 41°C. No adsorption occurred if the host was a female dnaZts strain.

Active dnaZ product also is required for the conversion of ØX174 SS to RF (Fig. 3). Infection of chloramphenicol-treated, ØX174-sensitive dnaZts mutant and revertant cultures at 37°C resulted in substantial amounts of RF DNA synthesis in both cultures (Fig. 3A). If the infections were carried out at 41°C, RF synthesis in the revertant culture decreased approximately 50%, and, in the mutant, was almost totally eliminated (Fig. 3B).

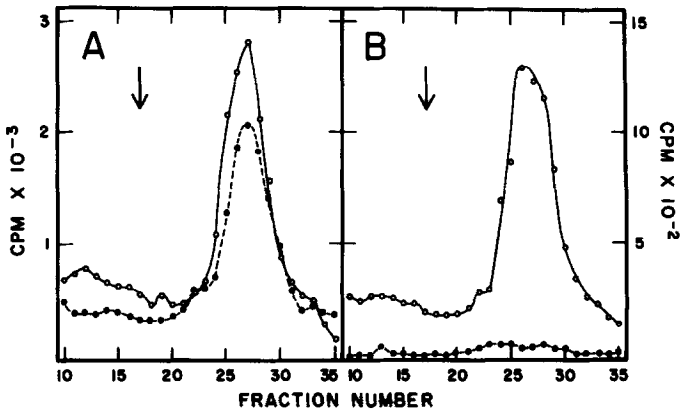

Fig. 3. ØX174 parental RF formation in dnaZts and TS+ revertant cultures at 37°C and 41°C. dnaZts (•) and TS+ (o) cultures were grown at 30°C, shifted to 37°C (A) or 41°C (B), and infected with ØX174 at 35 minutes after the shift. Arrows indicate the position of ØX174 SS marker DNA.

DISCUSSION

The product of the E. coli polymerization gene dnaZ is required for the conversion of ØX174 and M13 single strand DNA molecules into parental RF. In addition, it is essential for replication of M13 RF (unpublished results) and for ØX174 growth after infection with ØX174 RF DNA (26). Experiments are in progress to determine the requirement for dnaZ gene product for synthesis of M13 single strands. M13 parental RF can be polymerized in vivo by DNA polymerase I or polymerase III; this conclusion is based on the finding that M13 parental RF synthesis is inhibited at the nonpermissive temperature in a dnaEts polA double mutant, but not in the double mutant at the permissive temperature or in the dnaEts single mutant at the nonpermissive temperature (6). The dnaZts mutant is polA+ and dnaE+. Therefore, the dnaZ product is involved either in a process which precedes the stage in RF synthesis at which DNA polymerase is employed (i.e., RNA priming), or in a DNA polymerizing function which is common to both DNA polymerases I and III. The first possibility is less attractive because different RNA priming mechanisms are employed by M13 and ØX174 (3) and dnaZ activity is required for parental RF formation for both phages. Moreover, M13 infection of dnaZts cells at 41°C

renders parental RF synthesis resistant to rifampicin when the temperature is subsequently reduced, a result which suggests that priming occurs at 41°C (unpublished data). The more likely possibility is that the dnaZ product is a factor required for DNA polymerization per se.

Two models for a polymerization function seem plausible. First, dnaZ product might be required for activity of DNA polymerases I and III in parental RF formation. It has been reported that the in vitro conversion of M13 and ØX174 SS → RF, on RNA primed templates, requires only DNA polymerase III* and co-polymerase III* as the protein components of a reaction mixture in which spermidine is substituted for the DNA unwinding protein (27). DNA polymerase III can perform a similar function if it is supplemented by two protein factors (Factor I and II) (28). It has been suggested that DNA polymerase III* is equivalent to factor II plus DNA polymerase III and that co-polymerase III* is equivalent to factor I (28, 29). Perhaps dnaZ codes for Factor I or II, proteins for which the genes have not been identified, or for a new factor not yet detected by in vitro experiments. dnaZ does not appear to code for the unwinding protein (K. Geider, personal communication). In as much as dnaZ mutants cannot synthesize RF in vivo by either DNA polymerase I or III, the dnaZ product might also be required for DNA polymerase I activity in this reaction in vitro. Factor I has been shown to stimulate DNA polymerase I activity in vitro (30) (although there is contradictory evidence on this point (27)) and might, therefore, be the dnaZ gene product.

Second, the dnaZ product might play an exclusively in vivo function in DNA synthesis (e.g., by stabilizing the replication complex within the cell). If this is the case, its role might be difficult to assess by in vitro assay.

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