NOTES

Replication of Bacteriophage ST-1 in *Escherichia coli* Strains Temperature Sensitive in DNA Synthesis

J. MICHAEL BOWES

Department of Bacteriology, University of California, Davis, California 95616

Received for publication 21 January 1974

Bacteriophage ST-1 replication requires DNA polymerase III (*dnaE*) but not DNA polymerases I or II, DNA ligase, or the products of *dnaA*, *B*, or *C-D*. It was not certain whether *dnaG* was required. These results differ considerably from those reported for ϕ X-174.

Because of their very limited genomic content, small phages such as ϕ X174 (23) and M13 (14) rely heavily upon host-coded gene products. The replication of both phages appears to require all of the products of the *dna* genes (A, B, C-D, E, and G; 27) which are specified and utilized by the host *Escherichia coli* for its own DNA replication (4, 8, 17, 19, 21, 24, 25, 28).

Bacteriophage ST-1 (2) is a single-stranded, DNA-containing phage similar in size, shape, and some physiological properties to ϕ X174. It differs from ϕ X174 in that it infects K-12 rather than C strains of *E. coli* and has a higher temperature range for replication. This paper examines whether the various products involved in the synthesis of host cell DNA are essential for the in vivo replication of ST-1.

If a particular host-coded product is essential for any step in the replication of ST-1, then no phage should be produced when that host product is functionally absent during the entire infection. *E. coli dna-ts* mutants (27) were tested for their ability to support a burst of phage after infection at the restrictive temperature. The basic screening procedure is illustrated in Fig. 1, which shows that the *dnaA* mutation present in E508F'*lac*⁺ is not required for ST-1 phage production. The burst sizes at restrictive (42 or 40 C) and permissive (33 C) temperatures were determined from single-step growth curves as described in Fig. 1. These results are summarized in Table 1.

All representatives of *dnaA*, *dnaB*, *dnaC-D*, and *dnaG* produced bursts of phage at the restrictive temperature, indicating that these particular functions are probably not required for ST-1 DNA replication.

The *dnaE* strains behave differently. The *dnaE-486* mutation (e.g., E613, JG55) shuts off

FIG. 1. Single-step growth curve of ST-1 in E508F'*lac*⁺ at 33 and 42 C after infection at 42 C. A culture of E508F'*lac*⁺ grown overnight in H broth (20) at 33 C without shaking was diluted 1:100 (vol/vol) into N12 (26), supplemented with 4 μ g of thymine per ml, and grown with shaking at 33 C to an OD₄₅₀ of about 0.5 (about 2×10^8 cells/ml). A 5-ml portion was then shifted to 42 C, and 15 min later it was infected with about 2×10^8 PFU/ml of ST-1, prewarmed at 42 C for the same 15-min period. After 5 min, the infected culture was diluted 1,000-fold into prewarmed N12 at either 33 or 42 C. Samples were taken into iced N1 (26) and assayed for PFU/ml as previously described (2).

TABLE 1. Burst size of ST-1 in *dna* strains of *E. coli* K-12

Strain ^a	Pertinent genotype	Reference	Burst size ^b at		Source ^c
			42 (40) C	33 C	
E508	<i>dnaA</i> -508	27	400		1
E508F' ⁺ <i>lac</i> ⁺	-508		440	220	
CRT4634	-46, <i>polA1</i>	15	70		2
D247LT	-47	12	150 ^d	120 ^d	3
CRT4638	-46, <i>polA1</i>	28	110	110	4
CRT46	-46	27	60	50	5
FA21	<i>dnaB</i> -21	5, 27	130	130	6
FA22	-22	5, 27	40 ^d	20 ^d	6
E107	-107	27	240		1
E279	-279	27	450	170	1
HfrH strain 5	-70	1, 9, 19	140		7
CRT266	-266	27	40	180	5
CRT2667	-266, <i>polA1</i>	15	60	30	2
PC-1 ^e	<i>dnaC</i> -D-1	27	(160)	150	1
PC-2	-2	27	(200)	90	5
PC-22	-2, <i>polA1</i>	28	190	120	4
PC-7	-7	27	(130)		1
PC-79	-7, <i>polA1</i>	28	100	120	4
CT28-3b	-28	22	140 ^d	140 ^d	8
CT28-4	-28	1	110	100	8
BW824	-325	29	(40)	30	9
BW833	-325, <i>recA</i> ⁻	29	(50)	40	9
BW828	-325	29	370	130	9
BW832	wild-type trans-ductant	29	420	130	9
E613	<i>dnaE</i> -486	27	<0.1	100	1
E613-R1	-wild-type revertant		100		
JG55	-486	^a	<0.1	100	10
JG55-R1	-wild-type revertant		470		
E511	-511	27	120		1
E293	-293	27	60		1
PC-3	<i>dnaG</i> -3	27	(60)	70	1
PC-3-R1	-wild-type revertant		(190)	210	
NY73	-3, <i>polA1</i>	28	(50)	100	4
N2672	<i>lig</i> -7 (<i>ts</i>) ^h	7	80, 60 ⁱ	90, 70 ⁱ	11
KS253	<i>lig</i> -7 (<i>ts</i>) ^h	11	580	400	12

^a All strains and derivatives were checked for their auxotrophic markers on appropriate plates. Each strain was also checked for [³H]thymine (or [³H]thymidine incorporation in the presence of AdR if thy⁺) at permissive temperature and after shift to nonpermissive temperature. All strains behaved as expected for the respective mutations based upon previously reported data (1, 5, 12, 15, 22, 27, 29).

^b Burst size was approximated to the nearest 10 by using data from single-step growth curves which were performed as described in Fig. 1, except that in strains which were not low thymine require a supplementation of 20 μ g of thymine per ml was used (instead of 4 μ g/ml).

^c Sources for strains were: (1) J. A. Wechsler, (2) F. Jacob, (3) P. L. Kuempel, (4) J. Hurwitz, (5) Y. Hirota, (6) W. Fangman, (7) C. E. Dowell, (8) M. Dalby, (9) T. Wehr, (10) J. Salstrom, (11) M. Gottesman, and (12) B. Konrad.

^d Cells were grown at 28 rather than 33 C, prior to shift to and infection at restrictive temperature as described in Fig. 1.

^e The single-step growth curve of PC-1 was done in TKMgCa (2) rather than N12, since ST-1 did not adsorb to this strain in N12.

^f CT28-4 is a derivative of CT28-3b, which grows at 37 C but is still temperature-sensitive in initiation at 42 C (M. Dalby, personal communication).

^g JG55 was received from J. Salstrom who received it from J. Gross; presumably it is identical to E613.

^h *ts*, Temperature-sensitive.

ⁱ A single-step growth curve (Fig. 1) was performed after 2 h, rather than 15 min, at 42 C.

host cell DNA synthesis immediately upon shift to the restrictive temperature of 42 C (results not shown; 27). Cells containing this mutation produced less than 0.1 phage per cell at 42 C. However, upon shift down to a permissive temperature (33 C), the 42 C-infected cells gave normal bursts of about 100 phage per cell. Temperature-resistant revertants of these immediate, shut-off *dnaE* strains (e.g. E613-R1 and JG55-R1, which were selected as 42 C-resistant colonies from E613 and JG55, respectively) supported ST-1 phage production at 42 C. These results indicate that polymerase III, the *dnaE*-coded function (6), is required for some step(s) in ST-1 replication, since phage production is blocked at restrictive temperature but restored by reversion of the *dnaE* locus. Furthermore, the *dnaE*-486-imposed block is reversible, since cells infected at nonpermissive temperature are able to produce phage upon shift back to permissive temperature.

The other *dnaE* mutants produced near normal bursts of phage at 42 C. However, these mutants do not shut off host cell DNA synthesis immediately. Instead, they are characterized by an immediate reduction in rate (E511) or a switch to linear rate (E293) of host cell DNA synthesis (27; unpublished data). It is not surprising that *dnaE* strains which are leaky for host cell DNA synthesis would also be leaky for ST-1 DNA synthesis.

Host cell DNA synthesis is not leaky at the restrictive temperature in many of the *dnaA*, *dnaB*, and *dnaC-D* mutants used. Therefore, the ability of ST-1 to replicate in these strains probably really reflects a nonessential role of these functions in ST-1 replication and not a situation analogous to the presumed "leaky" phage production in some *dnaE* mutants.

Only one *dnaG* mutation was tested for ST-1 replication since it is the only one presently available in an ST-1 sensitive host. Lark (13) has recently shown that the *dnaG* function may be involved in the initiation of small, "Okazaki" (16)-sized pieces of DNA. As such, it is involved in the elongation of host cell DNA at the replication fork rather than in the initiation of chromosome replication. The residual amount of DNA synthesis shown by this mutation at the restrictive temperature must indicate that the mutation is at least slightly leaky. Therefore, the reduced ST-1 phage burst observed in the *dnaG* mutant relative to its revertant might be significant and reflect a leaky synthesis of ST-1 DNA analogous to the situation in leaky *dnaE* mutants. Since ST-1 also has an extended latent period in the *dnaG* mutant, any statement regarding the nonessen-

tial nature of the *dnaG* product in ST-1 replication would be premature.

Two strains (N2672 and KS253) harboring a mutation (*lig ts7*; 18) in DNA ligase were also examined for the ability to support ST-1 replication. In both strains burst size was normal and about equal at both 33 and 42 C. Strain N2672 also gave a similar burst of ST-1 at both temperatures after a 2-h incubation at 42 C prior to infection (Table 1). Therefore, unlike the situation in ϕ X174 (10, 20a), the host cell ligase does not seem to be essential for ST-1 replication.

ST-1 does not require DNA polymerase II, since it plates with full efficiency on HMS83 (3), a strain deficient in both DNA polymerase I and II (results not shown). It also plates with full efficiency on a series of *rec*⁻ strains obtained from A. J. Clark: *recA-13* (JC2926), *recB-21* (JC5743), *recC-22* (JC5489), *recF-143* (JC9259), *recL-152* (JC8471), *recD(?)*-34 (KMBL-239), and *recG-162* (PCO-297), indicating that these host cell functions are also dispensable for ST-1 replication.

From the results presented in this paper, it should be apparent that ST-1 differs considerably from ϕ X174 and M13 at least as regards reliance on host cell functions. Why ST-1 should be so much more autonomous, at least relatively, in its host cell replicative requirements is not understood. However, assuming that other host cell functions are not able to replace defective ones (quite possibly a bad assumption), these results imply that ST-1 utilizes either (i) a much simpler replicative process with fewer functions, (ii) viral-coded functions instead of the analogous host-coded products required for ϕ X174 and M13 replication, or (iii) a different set of host-coded functions. Clearly, more extensive experiments are necessary to distinguish between these alternatives.

I would like to extend my deepest appreciation to David Pratt for allowing this work to be completed in his laboratory and for critically reading the manuscript. The very capable technical assistance of Ellen Martin, Jackie Hoffmann, and Tim Zundel is also greatly appreciated.

This work was supported by Public Health Service grant AI-10201 from the National Institute of Allergy and Infectious Diseases and in part by Public Health Service postdoctoral research fellowship GM-52104 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Bonhoeffer, F. 1966. DNA transfer and DNA synthesis during bacterial conjugation. *Z. Vererbungslehre* **98**:141-149.
2. Bowes, J. M., and C. E. Dowell. 1974. Purification and properties of bacteriophage ST-1. *J. Virol.* **13**:53-61.

3. Campbell, J. L., L. Soll, and C. C. Richardson. 1972. Isolation and characterization of a mutant of *Escherichia coli* deficient in DNA polymerase II. Proc. Nat. Acad. Sci. U.S.A. **69**:2090-2094.
4. Dumas, L. B., and C. A. Miller. 1973. Replication of bacteriophage ϕ X174 DNA in a temperature-sensitive mutant of *Escherichia coli*. J. Virol. **11**:848-855.
5. Fangman, W. L., and A. Novick. 1968. Characterization of two bacterial mutants with temperature-sensitive synthesis of DNA. Genetics **60**:1-17.
6. Gefter, M. L., Y. Hirota, T. Kornberg, J. A. Wechsler, and C. Barnoux. 1971. Analysis of DNA polymerases II and III in mutants of *Escherichia coli* thermosensitive for DNA synthesis. Proc. Nat. Acad. Sci. U.S.A. **68**:3150-3153.
7. Gottesman, M. M., M. L. Hicks, and M. Gellert. 1973. Genetics and function of DNA ligase in *Escherichia coli*. J. Mol. Biol. **77**:531-547.
8. Greenlee, L. L. 1973. Replication of bacteriophage ϕ X174 in a mutant of *Escherichia coli* defective in the dnaE gene. Proc. Nat. Acad. Sci. U.S.A. **70**:1757-1760.
9. Gross, J. D. 1971. DNA replication in bacteria. Curr. Top. Microbiol. Immunol. **57**:39-74.
10. Iwaya, M., S. Eisenberg, K. Bartok, and D. T. Denhardt. 1973. Mechanism of replication of ϕ X174 DNA. VII. Circularization of the progeny viral strand. J. Virol. **12**:808-846.
11. Konrad, E. B., P. Modrich, and I. R. Lehman. 1973. Genetic and enzymatic characterization of a conditional lethal mutant of *Escherichia coli* K12 with a temperature-sensitive DNA ligase. J. Mol. Biol. **77**:519-529.
12. Kuempel, P. L. 1969. Temperature-sensitive initiation of chromosome replication in a mutant of *Escherichia coli*. J. Bacteriol. **100**:1302-1310.
13. Lark, K. G. 1972. Genetic control over the initiation of the synthesis of the short deoxynucleotide chains in *E. coli*. Nature N. Biol. **240**:237-240.
14. Marvin, D. A., and B. Hohn. 1969. Filamentous bacterial viruses. Bacteriol. Rev. **33**:172-209.
15. Mordoh, J., Y. Hirota, and F. Jacob. 1970. On the process of cellular division in *Escherichia coli*. V. Incorporation of deoxynucleotide triphosphates by DNA thermosensitive mutants of *Escherichia coli* also lacking DNA polymerase activity. Proc. Nat. Acad. Sci. U.S.A. **67**:773-778.
16. Okazaki, R., T. Okazaki, K. Sakabe, K. Sugimoto, and A. Sugino. 1968. Mechanism of DNA chain growth. I. Possible discontinuity and unusual secondary structure of newly synthesized chains. Proc. Nat. Acad. Sci. U.S.A. **59**:598-605.
17. Olsen, W. L., W. L. Staudenbauer, and P. H. Hofschneider. 1972. Replication of bacteriophage M13: specificity of the *Escherichia coli* dnaB function for replication of double-stranded M13 DNA. Proc. Nat. Acad. Sci. U.S.A. **69**:2570-2573.
18. Pauling, C., and L. Hamm. 1969. Properties of a temperature-sensitive, radiation-sensitive mutant of *Escherichia coli*. II. DNA replication. Proc. Nat. Acad. Sci. U.S.A. **64**:1195-1202.
19. Primrose, S. B., L. R. Brown, and C. E. Dowell. 1968. Host cell participation in small virus replication. I. Replication of M13 in a strain of *Escherichia coli* with a temperature-sensitive lesion in deoxyribonucleic acid synthesis. J. Virol. **2**:1308-1314.
20. Salivar, W. O., H. Tzagoloff, and D. Pratt. 1964. Some physical-chemical and biological properties of the rod shaped coliphages M13. Virology **24**:359-371.
- 20a. Schekman, R. W., and D. S. Ray. 1971. Polynucleotide ligase and ϕ X-174 single strand synthesis. Nature N. Biol. **231**:170-173.
21. Schekman, R., W. Wickner, O. Westergaard, D. Brutlag, K. Geider, L. Bertsch, and A. Kornberg. 1972. Initiation of DNA synthesis: synthesis of ϕ X174 replicative form requires RNA synthesis resistant to rifampicin. Proc. Nat. Acad. Sci. U.S.A. **69**:2691-2695.
22. Schuback, W. H., J. D. Whitmer, and C. I. Davern. 1973. Genetic control of DNA initiation in *Escherichia coli*. J. Mol. Biol. **74**:205-221.
23. Sinsheimer, R. L. 1968. Bacteriophage ϕ X174 and related viruses, p. 115-169. In J. N. Davidson and W. E. Cohn (ed.), Progress in nucleic acid research and molecular biology, vol. 8. Academic Press Inc., New York.
24. Staudenbauer, W. L., W. L. Olsen, and P. H. Hofschneider. 1973. Analysis of bacteriophage M13—DNA replication in an *Escherichia coli* mutant thermosensitive in DNA polymerase III. Eur. J. Biochem. **32**:247-253.
25. Steinberg, R. A., and D. T. Denhardt. 1968. Inhibition of synthesis of ϕ X174 DNA in a mutant host thermosensitive for DNA synthesis. J. Mol. Biol. **37**:525-528.
26. Tseng, B. Y., and D. A. Marvin. 1972. Filamentous bacterial viruses. V. Assymetric replication of fd duplex deoxyribonucleic acid. J. Virol. **10**:371-383.
27. Wechsler, J. A., and J. D. Gross. 1971. *Escherichia coli* mutants temperature-sensitive for DNA synthesis. Mol. Gen. Genet. **113**:273-284.
28. Wickner, R. B., M. Wright, S. Wickner, and J. Hurwitz. 1972. Conversion of ϕ X174 and fd single-stranded DNA to replicative forms in extracts of *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **69**:3233-3237.
29. Wolf, B. 1972. The characteristics and genetic map location of a temperature-sensitive DNA mutant of *E. coli* K12. Genetics **72**:569-593.