Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 5768–5772, October 1982
Biochemistry

Escherichia coli host factor required specifically for the φX174 stage III reaction: *In vitro* identification and partial purification

(S_{III} factor/anti-φX174 antiserum)

RUTH WOLFSON AND SHLOMO EISENBERG

Department of Biochemistry, The Weizmann Institute of Science, Rehovot 76100, Israel

Communicated by Arthur Kornberg, May 24, 1982

ABSTRACT **A cell-free extract prepared from φX174-infected *Escherichia coli* cells sustained *in vitro* synthesis of viral DNA (stage III reaction) when supplemented with fraction II from uninfected cells. The reaction was dependent upon deoxyribonucleoside triphosphate, ATP, added φX174 replicative form I DNA template, and the fraction II from uninfected cells. This reaction differed from the stage II reaction (semiconservative replication of duplex replicative form DNA) by the production of stable viral protein–DNA complexes sensitive to anti-φX174 antiserum. Three types of protein–DNA complexes were identified, 50S, 92S, and a 114S complex that cobanded in CsCl and cosedimented in neutral sucrose gradients with a φX174 phage marker. The sensitivity of these complexes to anti-φX174 antiserum and *Staphylococcus aureus* provided a relatively rapid biochemical assay for direct measurement of the amount of DNA synthesized by the stage III reaction. With this assay, an *E. coli* factor (S_{III}) required specifically for the synthesis of viral protein–DNA complexes was identified and purified 200-fold from uninfected *E. coli* cells. The partially purified S_{III} factor was required for the synthesis of DNA and viral protein–DNA complexes in the φX174-infected cell extracts and could not be replaced by rep protein, single-strand binding protein, or DNA polymerase III holoenzyme.**

Three distinct modes characterize the replication of φX174 DNA during its life cycle (1): (*a*) synthesis of a complementary (−) strand on a single-stranded circular DNA template to form a duplex supertwisted replicative form (RF I) DNA (stage I reaction); (*b*) semiconservative replication of the duplex to yield progeny RF DNA (stage II reaction); (*c*) asymmetric displacement and synthesis of viral (+) single-stranded DNA circles coupled to its encapsidation into a phage particle (stage III reaction). Stage I and stage II reactions have been completely resolved and reconstituted *in vitro* (2–6). Because both reactions rely heavily on host enzymes, they served as model systems to investigate the role of these enzymes in the replication of *Escherichia coli* chromosomal DNA.

In addition to some of the stage I and stage II host enzymes, the stage III reaction requires nine proteins (A, A*, B, C, D, F, G, H, and J) coded for by the phage (7, 8). Because synthesis of viral (+) circular DNA is coupled to its encapsidation into a phage particle, at least some of the viral proteins may fulfill multiple functions, acting directly in DNA replication and phage morphogenesis.

Recent *in vitro* studies have established the requirement of purified φX174 prohead and gene C and J proteins for the synthesis of viable phage particles (9, 10). The additional protein requirement for the stage III reaction suggests a novel mechanism for synthesis of viral (+) DNA, different from that operating during duplex RF DNA replication.

In order to elucidate the mechanism of the stage III reaction we have undertaken the task of resolving this process *in vitro*. Here, we describe an enzyme system that sustains the formation of viral protein–DNA complexes starting with an exogenous RF I DNA template and that led to the identification and partial purification of an *E. coli* host activity required for this reaction.

MATERIALS AND METHODS

Bacterial and Phage Strains. Bacterial strains were *E. coli* HF4704repB (11) (from D. T. Denhardt's laboratory), C (12), CR (12), and H560 (13). The phage was φX174am3, a lysis-defective phage with an amber mutation in gene *E.*

Buffers. Buffer A: 50 mM imidazole, pH 6.9/20% (vol/vol) glycerol/0.1 mM EDTA/20 mM 2-mercaptoethanol. Buffer B: 50 mM Tris, pH 7.5/20% glycerol/1 mM EDTA/20 mM 2-mercaptoethanol.

DNA Preparations. φX174 RF I DNA was prepared from φXam3-infected *E. coli* C cells as described (14). pBR322 RF I DNA was prepared by the φX174 procedure from chloramphenicol-treated *E. coli* HB101 cells. Simian virus 40 (SV40) RF I DNA was a gift from E. Winocour.

Growth of Cells and Preparation of Cell-Free Extracts. *E. coli* HF4704repB was grown in AZ medium (15) at 37°C to an OD_{595} of 0.5. To infect the cells, φX174am3 phage was added at a multiplicity of 5–10. After additional 60-min incubation, cells were harvested by centrifugation, resuspended in 50 mM Tris·HCl, pH 8.0/10% (wt/vol) sucrose, frozen in liquid nitrogen, and stored at −80°C. Cell-free extracts [$(NH_4)_2SO_4$ precipitate (fraction II) of gently lysed cells] from either uninfected or φX174-infected *E. coli* cells were prepared essentially as described (16).

Fraction II of the infected cells was further purified through a DEAE-cellulose column equilibrated with buffer B containing 0.2 M NaCl. The $(NH_4)_2SO_4$ precipitate was diluted with buffer B to the conductivity of buffer B/0.2 M NaCl, loaded on the DEAE-cellulose column (25 mg/ml), and eluted with the above buffer. Fractions containing protein were pooled and precipitated with $(NH_4)_2SO_4$ (0.3 g/ml). Precipitates were collected by centrifugation and dissolved in buffer B (40–100 mg/ml).

DNA Synthesis. The standard 25-μl reaction mixture contained: 50 mM Tris·HCl (pH 7.5), 10 mM 2-mercaptoethanol, 12 mM $MgCl_2$, 1.6 mM ATP, 50 μM dATP, 50 μM dGTP, 50 μM dCTP, and 50 μM [α-^{32}P]TTP (400–2,000 cpm/pmol of nucleotides), bovine serum albumin at 120 μg/ml, 2.5–3 μg of φX174 RF I DNA, 50 μg of φX174am3-infected *E. coli* 4704repB (am3/repB) extract and 5 μg of *E. coli* H560 fraction II (for the stage III reaction) or 50 μg of uninfected *E. coli*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: am3/repB, φX174am3-infected *E. coli* 4704repB cells; RF, replicative form; ssB, single-strand binding protein; SV40, simian virus 40.

Table 1. Requirements for the stage III reaction

Medium	DNA synthesized, pmol	DNA immunoprecipitated, pmol
Complete	100	64
Lacking:		
H560, fraction II	20	5
ATP	4	<1
dATP, dCTP, dGTP	1	<1
Mg^{2+}	1	<1
ϕX174 RF I DNA	1	<1
With added:		
SV40 RF I DNA	9	<2
pBR322 RF I DNA	5	<1

After 20-min incubation at 30°C, 10 μl of the reaction mixture was withdrawn for immunoprecipitation. Trichloroacetic acid was added to the remaining 15 μl. Precipitates were collected on GF/C glass fiber filters and the amount of DNA synthesized was determined (Fig. 1). When either SV40 or pBR322 RF I DNA (2.5 μg) was included, ϕX174 RF I DNA was omitted. The values presented here were normalized for a 25-μl reaction mixture.

4704repB fraction II supplemented with purified protein A for the stage II reaction. Reactions were incubated at 30°C for 20 min unless indicated otherwise.

Immunoprecipitation Assay. The 0.1-ml immunoprecipitation reaction mixture contained: 50 mM Tris·HCl (pH 7.5), 0.85 M NaCl, 20 mM EDTA, 5 μl of rabbit anti-ϕX174 antiserum (17), and 10 μl of a DNA synthesis reaction mixture. After 10-min incubation on ice, 50 μl of a *S. aureus* suspension (18) was added and incubation on ice was continued for an additional 10 min. The precipitate was separated by centrifugation in an Eppendorf centrifuge for 1 min, and resuspended in 200 μl of 50 mM Tris·HCl, pH 7.5/1 M NaCl/20 mM EDTA, and centrifuged again. Finally, the pellets were resuspended in 100 μl of the above buffer, transferred onto GF/C glass fiber filters, and washed with trichloroacetic acid and ethanol. Amount of radioactively labeled material retained was measured in a toluene-based scintillation fluid.

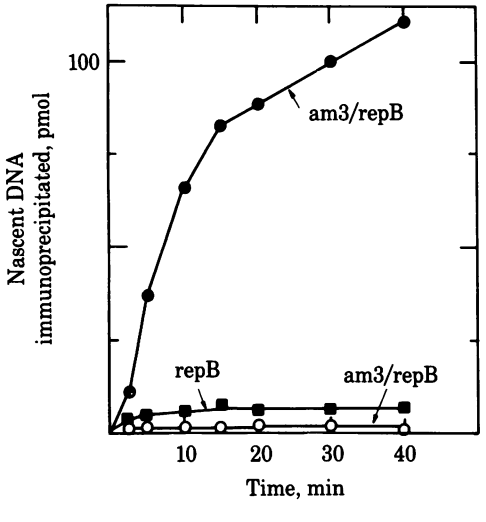

FIG. 1. Time course of the ϕX174 stage III reaction. At the indicated times, 10 μl of the DNA synthesis reaction mixture was withdrawn into an immunoprecipitation mixture, and the amount of nascent DNA immunoprecipitated was determined. ●, DNA synthesized in am3/repB extracts supplemented with fraction II from uninfected *E. coli* H560 cells and precipitated with anti-ϕX174 antiserum; ■, DNA synthesized in extracts of uninfected *E. coli* 4704repB cells supplemented with purified ϕX174 protein A [fraction V (19)], treated with anti-ϕX174 antiserum; ○, DNA synthesized in am3/repB extract supplemented with a fraction II from uninfected *E. coli* H560 cells and treated with normal serum. At 20-min incubation, 220 and 206 pmol of DNA were synthesized in the infected and uninfected extracts, respectively, as measured by precipitating an aliquot with trichloroacetic acid. All values were normalized for a 25-μl reaction mixture.

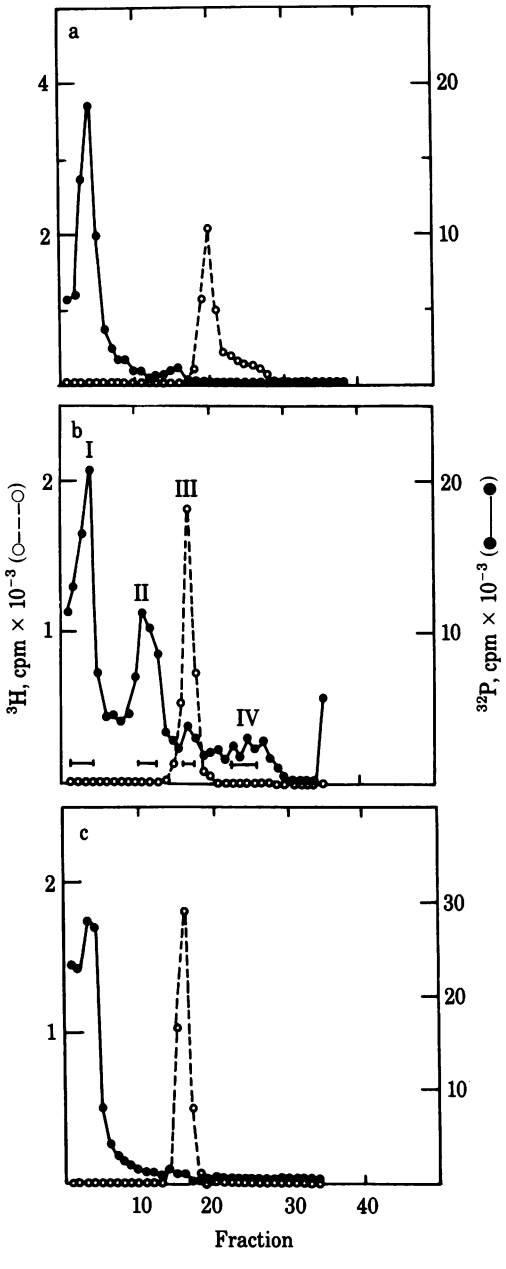

FIG. 2. Analysis of stage II and stage III reaction products by banding in CsCl equilibrium density gradients. After 20-min incubation at 30°C the DNA synthesis reaction mixtures were diluted to 2.5 ml with 50 mM Tris·HCl, pH 7.5/10 mM EDTA and CsCl was added (0.625 g/ml of solution). To each mixture, ^{3}H-labeled ϕX174 marker (12) was added, and centrifugation was carried out for 48 hr at 20°C and 40,000 rpm in the SW 50.1 rotor. Fractions (0.1 ml) were collected from the bottom of the tube on GF/C glass fiber filters. The filters were washed with trichloroacetic acid and ethanol and their radioactivity was measured in a toluene-based scintillation fluid. (*a*) DNA products synthesized in uninfected *E. coli* 4704repB cell extracts supplemented with purified ϕX174 protein A [fraction V (19)]. (*b*) DNA products synthesized in am3/repB extracts supplemented with a fraction II from uninfected *E. coli* H560 cells. (*c*) DNA products as in *b* treated for 1 hr at 37°C with proteinase K (prior to centrifugation) in a reaction mixture containing 50 mM Tris (pH 8.0), 50 mM EDTA, 0.5% $NaDodSO_4$, and proteinase K at 0.25 mg/ml. ○—○, ϕX174 marker.

RESULTS

The Stage III Reaction: Synthesis of Viral Protein–DNA Complexes in Extracts Prepared from am3/repB. *In vivo*, the repB mutation in *E. coli* cells leads to decreased levels of RF DNA replication (stage II) and a decreased production of phage particles of which most are noninfectious (11). The limited *in vivo* utilization of phage proteins in this mutant makes am3/repB a good source of phage proteins for the *in vitro* synthesis of viral DNA coupled to phage assembly (stage III).

Extracts prepared from am3/repB and supplemented with a fraction II from uninfected *E. coli* sustained *in vitro* DNA synthesis. The reaction was dependent upon the addition of a protein fraction from uninfected cells, ϕX174 RF I DNA, ATP, and deoxyribonucleoside triphosphates (Table 1). SV40 and pBR322 RF I DNA could not substitute for the ϕX174 DNA as template in the reaction. More than 50% of the newly synthesized DNA was precipitated by anti-ϕX174 antiserum and *S. aureus*, indicating that nascent DNA was associated with phage capsid proteins.

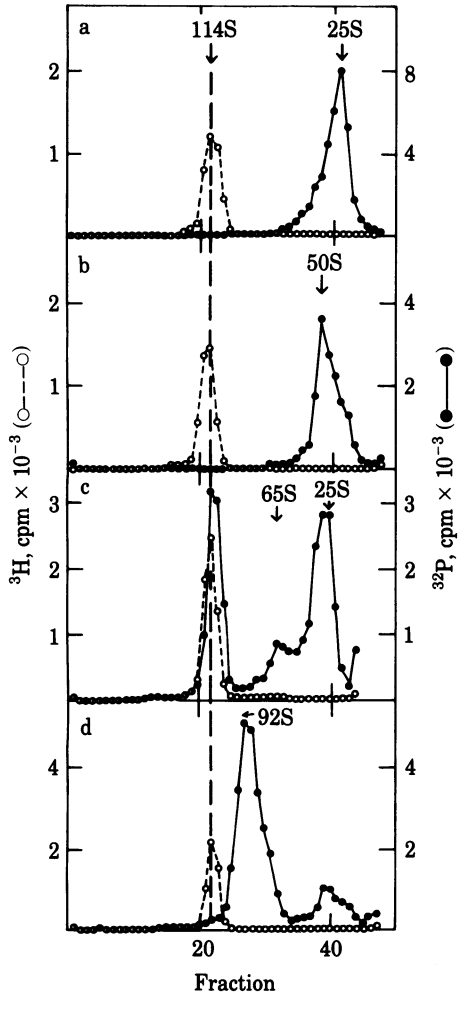

FIG. 3. Sedimentation analysis (neutral sucrose gradients) of DNA products synthesized in am3/repB extracts. DNA was synthesized in infected extracts and banded in CsCl as in Fig. 2*b*. The DNA peaks (I, II, III, and IV) were pooled separately and dialyzed against 50 mM sodium tetraborate. Each DNA peak was mixed with ^{3}H-labeled ϕX174 phage marker and sedimented through a 5–20% neutral sucrose gradient containing 50 mM Tris (pH 8.0), 0.2 M NaCl, 1 mM EDTA, and bovine serum albumin at 0.2 mg/ml. Sedimentation was for 45 min at 0°C and 50,000 rpm in the SW 50.1 rotor. Fractions (0.1 ml) were collected onto GF/C glass fiber filters and the amount of radioactively labeled material was determined as before (Fig. 1). (*a*) DNA peak I; (*b*) DNA peak II; (*c*) DNA peak III; (*d*) DNA peak IV.

The immunoprecipitation of nascent DNA was specific for DNA synthesized in infected extracts. Normal serum did not precipitate this DNA. DNA synthesized by the stage II reaction [uninfected extracts supplemented with ϕX174 protein A (16)] were not sensitive to anti-ϕX174 antiserum (Fig. 1).

DNA Products Synthesized in am3/repB Extracts. The synthesis of stable viral protein–DNA complexes was verified by banding the reaction products in CsCl (Fig. 2*b*). DNA peaks II, III, and IV (Fig. 2*b*) were sensitive to proteinase K treatment (Fig. 2*c*) and anti-ϕX174 antiserum (see Fig. 4) and were not formed in the stage II reaction (Fig. 2*a*).

The DNA of peaks I, II, and IV (Fig. 2*b*) sedimented in neutral sucrose with sedimentation coefficients of 25, 50, and 92 S, respectively (Fig. 3). About 40% of the DNA in peak III cosedimented with 114S ^{3}H-labeled ϕX174 marker, indicating that intact phage particles were formed.

The DNA of the 50S complex [believed to be an intermediate and precursor for the synthesis of intact phage (20–22)] was seen as a rolling circle in a formamide spread in the electron microscope; upon denaturation, the DNA sedimented on alkaline sucrose gradients as longer-than-unit-length ϕX174 DNA (unpublished data). The DNA of peak III was a mixture of single-stranded linear and circular ϕX174 unit-length DNA, whereas the 92S complex (peak IV) contained DNA fragments of heterogeneous size but all shorter than ϕX174 unit length, presumably generated by a premature DNA cleavage of the 50S protein–DNA complex (unpublished data).

Identification and Partial Purification of an *E. coli* (S_{III}) Factor Required for the ϕX174 Stage III Reaction. DNA synthesis and formation of viral protein–DNA complexes was dependent upon a protein fraction from uninfected *E. coli* cells (Table 1), suggesting a requirement for an *E. coli* host factor. The immunoprecipitation assay, which clearly distinguishes

Table 2. Partial purification of the *E. coli* H560 S_{III} factor

Step	Fraction	S_{III} factor specific activity, units × 10^3/mg of protein	Recovery, %
Extracts	I	(0.45)	(100)
$(NH_4)_2SO_4$	II	1.4	100
DEAE-Sephacel	III	29	63
Hydroxylapatite	IV	94	45

Fraction I (lysate supernatant, 145 ml) was prepared from 40 g of *E. coli* H560 cells as described (13). Fraction II was prepared by the addition of $(NH_4)_2SO_4$ (0.29 g/ml of solution). The precipitate was collected by centrifugation and dissolved in buffer A (16 ml). This solution was dialyzed against buffer A/50 mM NaCl and passed through a DEAE-Sephacel column (25 mg of protein per ml of bed volume) equilibrated with buffer A/50 mM NaCl. The column was washed with 1 column volume of buffer A/50 mM NaCl followed by 5 column volumes of buffer A/0.25 M NaCl. The S_{III} factor activity was eluted with buffer A/0.5 M NaCl (fraction III, 80 ml). Fraction III was diluted by the addition of an equal volume of buffer A/0.2 mM KP_i and passed through a hydroxylapatite column (5 mg of protein per ml of bed volume) equilibrated with buffer A/0.25 M NaCl/0.1 mM KP_i. The column was washed successively with 5 column volumes of buffer A/0.25 M NaCl/1 mM KP_i, 5 mM KP_i, and 25 mM KP_i. The S_{III} factor activity was eluted with buffer A/0.25 M NaCl/100 mM KP_i (fraction IV, 20 ml). Fraction IV was frozen in liquid nitrogen and stored at −80°C. All operations were carried out at 0–4°C. Assays of fraction I were not reliable. Calculation of yield was based on the activity recovered from fraction I in an $(NH_4)_2SO_4$ pellet: 1 unit of S_{III} factor activity is defined as the amount of protein catalyzing synthesis of 1 pmol of immunoprecipitable DNA in 1 min.

between stage II and stage III reactions, was used for partial purification of this *E. coli* stimulating activity (Table 2).

The synthesis of viral protein–DNA complexes in extracts of ϕX174-infected cells was absolutely dependent upon the addition of the purified S_{III} factor (Fig. 4). The S_{III} factor appeared to stimulate the formation of the 50S and 114S complexes (Fig. 4*b*), which were sensitive to treatment with anti-ϕX174 antiserum (Fig. 4*c*).

The purified S_{III} factor had no effect on the *in vitro* stage II (+) (23) reaction (Table 3), indicating that it is free of any rep protein, DNA polymerase III holoenzyme, or single-strand binding protein (ssB) activity. Moreover, the stage II (+) enzymes could not substitute for the S_{III} factor in our *in vitro* stage III reaction (Table 4).

Hence, we infer that the S_{III} factor is required specifically for the stage III reaction.

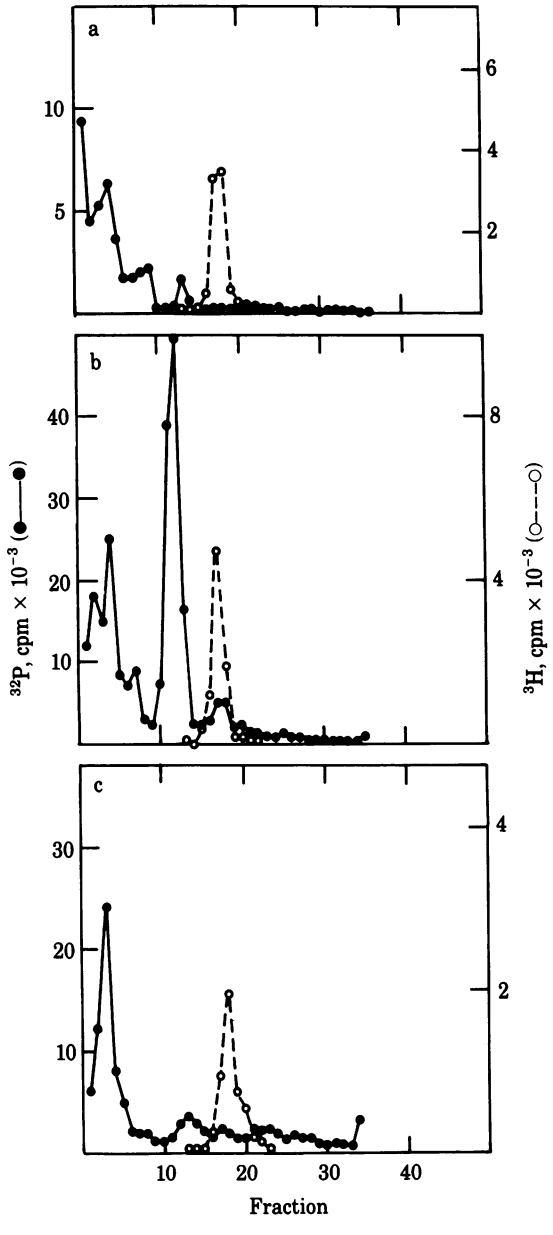

FIG. 4. Synthesis of viral protein–DNA complexes in am3/repB extracts is dependent on the presence of the partially purified S_{III} factor. The DNA products were analyzed by banding in CsCl as described in Fig. 2. (*a*) Banding in CsCl of DNA products that were synthesized in a reaction mixture including only am3/repB extracts. (*b*) Banding in CsCl of DNA products that were synthesized in a reaction mixture including am3/repB extracts and partially purified S_{III} factor (0.03 μg of fraction IV, Table 2). (*c*) Banding in CsCl of DNA products remaining in the supernatant after treatment of the reaction mixture of *b* with anti-ϕX174 antiserum and *S. aureus*. ○----○, ϕX174 marker.

Table 3. The S_{III} factor is free of DNA polymerase III holoenzyme, rep protein, and ssB activities

Medium	DNA synthesis, pmol
Complete	158
Lacking:	
S_{III} factor	165
Holoenzyme	1
rep protein	7
ssB	4

The complete mixture for the stage II (+) (24) reaction contained deoxyribonucleoside triphosphates, ATP, ϕX174 RF I DNA, $MgCl_2$, ϕX174 protein A [0.05 μg, fraction V (19)], DNA polymerase III holoenzyme, rep protein, ssB (as described in Table 4), and S_{III} factor (0.6 μg, fraction IV, Table 2). The incubation was for 10 min at 30°C. The amount of DNA synthesized was determined as before (23).

DISCUSSION

We have developed an enzyme system and an assay for the ϕX174 stage III reaction. The enzyme system takes advantage of the inefficient *in vivo* consumption of phage proteins in am3/repB (11) which then become available for the *in vitro* synthesis of viral protein–DNA complexes in the cell-free extracts.

Three major types of viral protein–DNA complexes were identified in the *in vitro* reaction. The 92S structure is presumably an artifact of the *in vitro* system because it contains DNA fragments shorter than ϕX174 unit-length DNA. The 50S DNA–protein complex, which contains DNA in a rolling circle form associated with phage proteins, is presumed to be an intermediate and precursor for the formation of a phage particle, as suggested by others (20–22). The formation of the 114S complex suggests that intact phage particles were synthesized.

The ability to immunoprecipitate the DNA products synthesized in the extracts of am3/repB by anti-ϕX174 antiserum provided a relatively easy, rapid, and specific assay for the *in vitro* stage III reaction.

Table 4. *E. coli* DNA polymerase III holoenzyme, rep protein, and ssB cannot substitute for the S_{III} factor in the stage III reaction

Addition to medium	DNA synthesized, pmol	DNA immunoprecipitated, pmol
am3/repB extract	9	3
Plus:		
S_{III} factor	145	67
Holoenzyme	18	5
rep protein	30	6
ssB	20	3
Holoenzyme rep protein, & ssB	37	8

After 20-min incubation at 30°C, 10 μl of each reaction mixture was withdrawn for treatment with anti-ϕX174 antiserum and *S. aureus*. The remaining newly synthesized DNA was precipitated by trichloroacetic acid. This acid-precipitable material represents the total DNA synthesized. The values presented were normalized for a 25-μl reaction mixture. The am3/repB extracts were supplemented, in separate reactions, with: S_{III} factor (0.03 μg, fraction IV, Table 2); DNA polymerase III holoenzyme [0.4 μg, fraction IV (25)]; rep protein [0.7 μg, fraction V (24)]; ssB [0.9 μg, fraction IV (26)].

The S_{III} factor activity was precipitated from uninfected *E. coli* cell lysates (fraction I) by $(NH_4)_2SO_4$ at 0.29 g/ml. It bound tightly to DEAE-cellulose and was eluted at a NaCl concentration higher than 0.3 M. The profound effect of the S_{III} factor on the synthesis of viral protein–DNA complexes became apparent when the extracts from infected *E. coli* cells were prepared by chromatography through DEAE-cellulose, which presumably generated a deficiency of this activity in the extracts.

The 50S and 114S viral protein–DNA complexes were synthesized only when the S_{III} factor was present in the reaction mixture (Fig. 4). Thus, the S_{III} factor may have a role in the initial phase of the stage III reaction, perhaps in the assembly of a virus–host protein complex, at the origin of replication, which might actively participate in the coupled reaction of DNA synthesis and phage assembly. However, more work is needed to elucidate the precise function and mechanism of action of the S_{III} factor in this reaction. To shed light on the *in vivo* function of the S_{III} factor, a mutant of *E. coli* deficient in this activity is needed.

We believe that the immunoprecipitation assay we have used here will enable biochemical resolution of the stage III reaction, purification of the anticipated phage proteins, identification and isolation of other host cell factors that might be needed, and a detailed analysis of the mechanism of viral DNA synthesis coupled to phage assembly.

This work was supported in part by a grant from a United States–Israel Binational Research Fund and in part by funds granted by the Charles H. Revson Foundation.

1. Denhardt, D. T. (1975) *CRC Crit. Rev. Microbiol.* **4**, 161–223.
2. Meyer, R. R., Shlomai, J., Kobori, J., Bates, D. L., Rowen, L., McMacken, R., Ueda, K. & Kornberg, A. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 289–294.
3. Eisenberg, S., Scott, J. F. & Kornberg, A. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 295–302.
4. Sumida-Yasumoto, C., Ikeda, J.-E., Benz, E., Marians, K. J., Vicuna, R., Sugrue, S., Zipursky, L. S. & Hurwitz, J. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 311–330.
5. Shlomai, J., Polder, L., Arai, K. & Kornberg, A. (1981) *J. Biol. Chem.* **256**, 5233–5238.
6. Arai, N., Polder, L., Arai, K. & Kornberg, A. (1981) *J. Biol. Chem.* **256**, 5239–5246.
7. Tessman, E. S. & Tessman, I. (1978) in *The Single-Stranded DNA Phages*, eds. Denhardt, D. T., Dressler, D. & Ray, D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 9–30.
8. Hayashi, M. (1978) in *The Single-Stranded DNA Phages*, eds. Denhardt, D. T., Dressler, D. & Ray, D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 530–548.
9. Mukai, R., Hamatake, R. K. & Hayashi, M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4877–4881.
10. Aoyama, A., Hamatake, R. K. & Hayashi, M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7285–7289.
11. Iwaya, M. (1971) Dissertation (Harvard Univ., Cambridge, MA).
12. Iwaya, M., Eisenberg, S., Bartok, K. & Denhardt, D. T. (1973) *J. Virol.* **12**, 808–818.
13. Wickner, W., Brutlag, D., Schekman, R. & Kornberg, A. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 965–969.
14. Eisenberg, S., Harbers, B., Hours, C. & Denhardt, D. T. (1975) *J. Mol. Biol.* **99**, 107–123.
15. Ueda, K., McMacken, R. & Kornberg, A. (1978) *J. Biol. Chem.* **253**, 261–270.
16. Eisenberg, S., Scott, J. F. & Kornberg, A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1594–1597.
17. Herbert, W. J. (1973) in *Handbook of Experimental Immunology*, ed. Weir, D. M. (Blackwell, Oxford), pp. A2.1–A2.14.
18. Kessler, S. W. (1975) *J. Immunol.* **6**, 1617–1624.
19. Eisenberg, S. & Kornberg, A. (1979) *J. Biol. Chem.* **254**, 5328–5332.
20. Fujisawa, H. & Hayashi, M. (1977) *J. Virol.* **24**, 301–313.
21. Koths, K. & Dressler, D. (1980) *J. Biol. Chem.* **255**, 4328–4338.
22. Sumida-Yasumoto, C. & Hurwitz, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4195–4199.
23. Eisenberg, S., Scott, J. F. & Kornberg, A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3151–3155.
24. Scott, F. J. & Kornberg, A. (1978) *J. Biol. Chem.* **253**, 3292–3297.
25. McHenry, C. & Kornberg, A. (1977) *J. Biol. Chem.* **252**, 6478–6484.
26. Weiner, H. J., Bertsch, L. L. & Kornberg, A. (1975) *J. Biol. Chem.* **250**, 1972–1980.