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Biochemistry

DNA or RNA priming of bacteriophage G4 DNA synthesis by *Escherichia coli dnaG* protein

(ADP/DNA binding protein/bacteriophage ST-1 DNA/DNA replication)

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ABSTRACT **_Escherichia coli dnaG_ protein is involved in the initiation of DNA synthesis dependent on G4 or ST-1 single-stranded phage DNAs [Bouche, J.-P., Zechel, K. & Kornberg, A. (1975) *J. Biol. Chem.* 250, 5995–6001]. The reaction occurs by the following mechanism: _dnaG_ protein binds to specific sites on the DNA in a reaction requiring *E. coli* DNA binding protein. An oligonucleotide is synthesized in a reaction involving _dnaG_ protein, DNA binding protein, and DNA. With G4 DNA this reaction requires ADP, dTTP (or UTP), and dGTP (or GTP). Elongation of the oligonucleotide can be catalyzed by DNA polymerase II or III in combination with _dnaZ_ protein and DNA elongation factors I and III, presumably by the mechanism previously reported [Wickner, S. (1976) *Proc. Natl. Acad. Sci. USA* 73, 3511–3515] or by DNA polymerase I.**

Three pathways have been described by which single-stranded circular phage DNAs can be converted to double-stranded DNA in reactions catalyzed by purified *Escherichia coli* proteins (1–3). The three pathways use different mechanisms to prime the template DNA but share a common mechanism of DNA elongation (4). In the elongation reaction, DNA elongation factor III (EF III) and *dnaZ* protein catalyze the transfer of DNA elongation factor I (EF I) to primed DNA in an ATP- (or dATP-) dependent reaction; DNA polymerase III binds to the primed template–DNA EF I complex and catalyzes DNA elongation (4). In the simplest pathway, fd (or M13) DNA synthesis is primed by RNA synthesized by RNA polymerase (1, 3, 5). In the most complex pathway, ϕX174 DNA synthesis requires *dnaB*, *dnaC(D)*, and *dnaG* proteins, DNA binding protein, at least three other *E. coli* proteins [DNA replication factors X, Y, and Z (2)], only ATP of the rNTPs (2), and the same four DNA elongation proteins (1, 2). Most likely, the first seven proteins are involved in primer synthesis. In a third pathway, G4 (ST-1, ϕXtB) DNA synthesis requires *dnaG* protein (6), DNA binding protein, and the DNA elongation proteins (5). In this report, the mechanism of priming of G4 DNA synthesis is examined.

MATERIALS AND METHODS

Materials and Methods. Unless otherwise indicated, these were as described (7). rNTPs were chromatographed on DEAE-Sephadex (8) and dNTPs were treated with 5 μmol of $NaIO_4$ per μmol of nucleotide for 30 min at 25°. [α-^{32}P]dNTPs were from New England Nuclear Corp.

Preparation of DNA. ϕX174 and fd DNA were prepared as described (9, 10). G4 and ST-1 phage were grown (11, 12), banded twice in CsCl, and sedimented in 10–30% sucrose gradients containing 1 M NaCl/1 mM EDTA/30 mM Tris·HCl (pH 8.0). The DNA was extracted with phenol (9).

Preparation of Proteins. DNA polymerases I, II, and III, DNA binding protein, *dnaZ* protein, and *dnaG* protein (both wild-type and thermolabile) were purified by procedures in refs. 13, 14, 15, 16, 5, and 17, respectively. Assay conditions and definitions of units for DNA polymerase III are in ref. 3; those for the other proteins are in the above references. DNA EF I and DNA EF III were purified by procedures to be published elsewhere using the assay described in ref. 4. By native polyacrylamide gel electrophoresis (18), the DNA binding protein was 80% pure and the *dnaG* protein from wild-type cells was 30% pure. The wild-type *dnaG* protein contained 3400 units/mg of protein; the thermolabile *dnaG* protein [purified from *E. coli* strain NY73 (*dnaG3*)] contained 600 units/mg of protein. A unit of *dnaG* protein catalyzes the incorporation of 1 nmol of dTMP in 20 min at 30° in the ϕX174 DNA-dependent complementation assay (17). T4 DNA polymerase was the generous gift of N. Nossal.

Abbreviations: DNA EF I, DNA elongation factor I; DNA EF III, DNA elongation factor III.

RESULTS

Requirements for G4 Phage DNA-Dependent DNA Synthesis. DNA synthesis dependent on G4 and ST-1 DNA required *dnaG* protein, DNA binding protein, DNA polymerase III, *dnaZ* protein, DNA EF 1, and DNA EF III (Table 1*A*). ADP stimulated the reaction; the K_m was about 2 μM. ADP could not be replaced by any other ribo- or deoxyribonucleoside diphosphates or triphosphates, AMP, or α,β-methylene ADP (all at 20 μM). ATP and dADP stimulated DNA synthesis to the same extent but only at higher concentrations; their K_m was about 50 μM. The amount of stimulation by ADP was greater at lower concentrations of *dnaG* protein. With reactions as described in Table 1*A* with G4 DNA and 0.02, 0.4, and 4.0 unit of *dnaG* protein, <0.1, 2.9, and 13.1 pmol of dTMP was incorporated, respectively, in the absence of ADP and 6.2, 32.8, and 33.0 pmol, respectively, in the presence of ADP. Varying the concentration of DNA binding protein or DNA had no effect on the amount of ADP stimulation. Similar results were obtained with ST-1 DNA; however, at all concentrations of *dnaG* protein, DNA synthesis independent of ADP was greater than with G4 DNA.

The requirements for G4 and ST-1 DNA synthesis by DNA polymerase II were similar to those with DNA polymerase III (Table 1*B*). DNA polymerase I (Table 1*C*) and T4 DNA polymerase (results not shown) also catalyzed G4 and ST-1 DNA synthesis in reactions requiring *dnaG* protein, DNA binding protein, and ADP; the reactions did not require the elongation proteins. These results are consistent with the previous observation that DNA elongation of any primed single-stranded DNA by DNA polymerase II or III was stimulated by the elongation components, while elongation by DNA polymerase I or T4 DNA polymerase was not significantly stimulated (4).

In contrast to G4 and ST-1 DNA, fd and ϕX174 DNA were inactive for DNA synthesis by these proteins. With the conditions described in Table 1, <0.1 pmol of dTMP was incorpo-

Table 1. Requirements for G4 and ST-1 DNA-dependent DNA synthesis

Additions	dTMP incorporated (pmol/20 min) G4 DNA	ST-1 DNA
A. DNA polymerase III		
Complete	25.2	25.0
−*dnaG* protein	<0.1	<0.1
−DNA binding protein	0.3	0.3
−DNA EF I	0.5	0.6
−DNA EF III	0.6	0.2
−*dnaZ* protein	0.5	0.2
−DNA polymerase III	0.4	0.4
−ADP	1.9	5.0
−ADP + other nucleotides*	1.7–3.1	—
B. DNA polymerase II		
Complete	29.5	17.3
−*dnaG* protein	0.3	0.2
−DNA binding protein	<0.1	0.4
−DNA EF I	3.2	0.4
−DNA EF III	2.8	3.4
−*dnaZ* protein	2.5	1.5
−DNA polymerase II	0.4	0.4
−ADP	1.9	7.5
C. DNA polymerase I		
Complete	26.4	34.4
−*dnaG* protein	<0.1	<0.1
−DNA binding protein	2.2	3.2
−DNA polymerase I	<0.1	<0.1
−ADP	0.7	9.0

Reaction mixtures (30 μl) contained: reaction buffer (1 mM dithiothreitol/20 μg of rifampicin per ml/50 mM Tris·HCl (pH 7.5)/1 mg of bovine serum albumin per ml/7 mM $MgCl_2$), 50 μM each of dATP, dGTP, dCTP, and [^{3}H]dTTP (800 cpm/pmol), 12 μM ADP, 300 pmol of DNA, 0.8 μg of DNA binding protein, and 0.04 unit of *dnaG* protein. Mixtures for *A* and *B* contained 0.2 unit of *dnaZ* protein, 0.2 unit of DNA EF I, and 0.2 unit of DNA EF III. Mixtures for *A*, *B*, and *C* contained 0.3 unit of DNA polymerase III, 0.3 unit of DNA polymerase II, and 0.2 μg of DNA polymerase I, respectively. A unit of DNA polymerase catalyzes the incorporation of 1 nmol of dTMP in 30 min at 30° with DNase-treated salmon sperm DNA (3). After 15 min at 30°, acid-insoluble radioactivity was measured.

* Nucleotides substituted for ADP at 20 μM were: all four rNTPs, three other rNDPs, four dNTPs, four dNDPs, AMP, and α,β-methylene-ADP.

rated with fd DNA and 0.4–1.9 pmol was incorporated with φX174 DNA.

Site-Specific Binding of *dnaG* Protein to G4 and ST-1 Phage DNA. The *dnaG* protein bound specifically to G4 and ST-1 DNA in a reaction requiring DNA binding protein. This was shown by incubating DNA binding protein, *dnaG* protein, and G4 DNA and subjecting the mixture to gel filtration (Fig. 1*A*). The excluded volume contained DNA and associated proteins, while the partially included volume contained free proteins. As shown, *dnaG* protein eluted with the G4 DNA. The binding of *dnaG* protein to G4 DNA was reversible: when the isolated DNA–protein complex was dissociated by adjusting it to 1 M NaCl and subjecting it to gel filtration in the presence of 1 M NaCl, *dnaG* protein was no longer associated with the DNA. It eluted as expected for free *dnaG* protein (the recovery of *dnaG* protein from G4 DNA was 40%).

No *dnaG* protein bound to G4 DNA in the absence of DNA

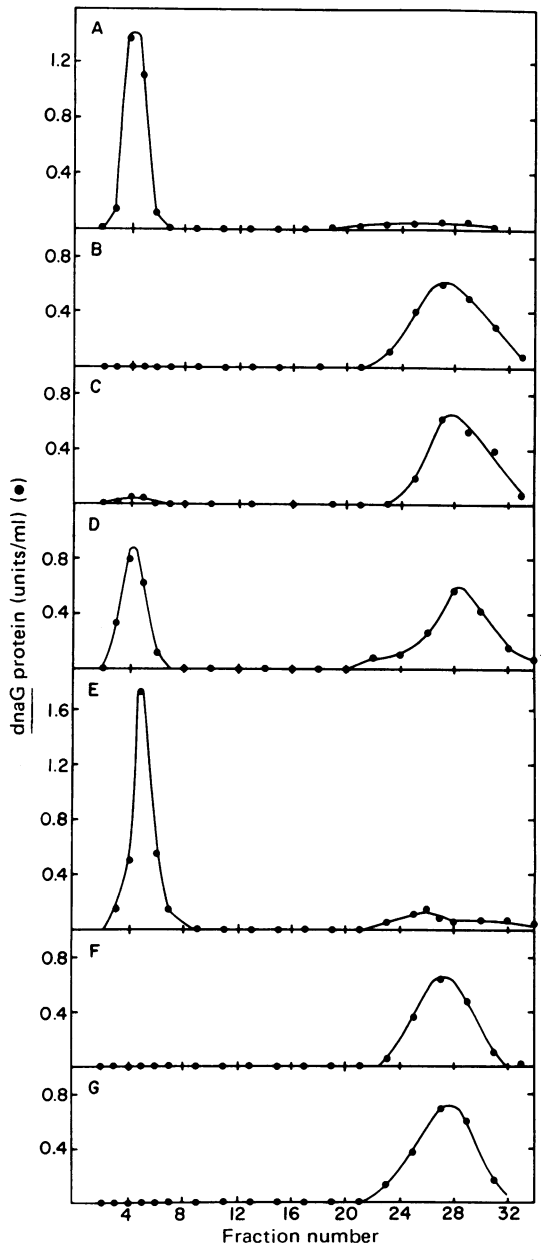

FIG. 1. Site-specific binding of *dnaG* protein to G4 and ST-1 DNA. (*A*) The first reaction mixture (60 μl) contained: reaction buffer as in Table 1, 6 nmol of G4 DNA (as nucleotide), 8 μg of DNA binding protein, and 1.7 units of *dnaG* protein. After 10 min at 30°, the mixture was applied at room temperature to a 22 × 0.5-cm column of Sepharose 6B equilibrated with 50 mM Tris·HCl (pH 7.8)/1 mM dithiothreitol/0.1 mg of bovine serum albumin per ml/5% glycerol/5 mM $MgCl_2$. The column was eluted with the same buffer and 0.11-ml fractions were collected. The excluded volume was fraction 4; the included volume, fraction 36. Fractions were assayed for *dnaG* activity (17). (*B*) The reaction mixture was as in *A* but DNA binding protein was omitted. (*C*) The reaction mixture was as in *A* but contained only 2 μg of DNA binding protein. (*D*) The reaction mixture was as in *A* but contained only 3 nmol of G4 DNA. (*E*, *F*, and *G*) The reaction mixtures were as in *A* but G4 DNA was omitted and 6 nmol of ST-1 (*E*), fd (*F*), and φX174 (*G*) DNA were included.

binding protein (Fig. 1*B*). In addition, when the amount of DNA binding protein was decreased to 25% of the original amount [from about 400 to 100 molecules of protein (molecular weight 20,000) per circle of G4 DNA], no *dnaG* protein bound (Fig. 1*C*). This suggests that the binding of *dnaG* protein to G4 DNA requires that the DNA be covered by DNA binding

Table 2. Requirements for primer synthesis by *dnaG* protein

First reaction components								Isolated DNA complex of proteins and primer			
								Primer synthesis*		DNA elongation†	
DNA	*dnaG* protein	DNA binding protein	ADP	dGTP	dTTP	dATP	dCTP	dGMP	dTMP	−ADP	+ADP
G4	+	+	+	+	+	+	+	1.43	0.32	968	1350
G4	+	+	+	+	+	−	−	1.01	0.44	1620	2160
G4	+	+	+	+	−	−	−	<0.01	N.R.	<10	572
G4	+	+	+	−	+	−	−	N.R.	<0.01	<10	968
G4	+	+	−	+	+	−	−	<0.01	<0.01	<10	464
G4	+	−	+	+	+	−	−	<0.01	N.D.	<10‡	<10‡
G4	−	+	+	+	+	−	−	<0.01	N.D.	108§	1200§
ST-1	+	+	+	+	+	+	+	1.46	N.D.	1280	1920
ST-1	+	+	+	+	−	−	−	1.28	N.R.	1100	1100
ST-1	+	+	−	+	−	+	−	1.46	N.R.	1432	1940
ST-1	+	+	−	+	−	−	−	<0.01	N.R.	42	800
ST-1	+	+	+	−	−	−	−	N.R.	N.R.	64	1080
ST-1	+	+	−	−	−	+	−	N.R.	N.R.	34	864
ϕX174	+	+	+	+	+	+	+	<0.01	<0.01	<10	<10
fd	+	+	+	+	+	+	+	<0.01	<0.01	<10	<10

First reaction mixtures (60 μl) contained: reaction buffer described in Table 1, 6 nmol of DNA (as nucleotide), 2.1 units of *dnaG* protein, 8.0 μg of DNA binding protein, and where indicated, 16 μM ADP, dATP, dCTP, dGTP, or [α-^{32}P]dGTP (50,000 cpm/pmol), and dTTP or [α-^{32}P]dTTP (50,000 cpm/pmol). After 15 min at 30°, mixtures were subjected to gel filtration (see legend to Fig. 1). The excluded volume fractions were measured for ^{32}P. They were also assayed for DNA synthesis in a second reaction (60 μl) containing: 10–50 μl of isolated protein–DNA complex, reaction buffer described in Table 1, 50 μM each of dATP, dCTP, dGTP, and [^{3}H]dTTP (800 cpm/pmol), 12 μM ADP (where indicated), 0.4 unit of *dnaZ* protein, 0.4 unit of DNA EF I, 0.4 unit of DNA EF III, and 0.6 unit of DNA polymerase III. After 10 min at 30°, acid-insoluble radioactivity was measured. The dTMP incorporation measured was the yield. (+) Component included in first reaction; (−) component omitted from first reaction; N.R., not relevant; N.D., not done.

* pmol of dGMP or dTMP incorporated.

† pmol of four dNTPs incorporated.

‡ DNA binding protein (0.8 μg) was added in second reaction.

§ *dnaG* protein (0.2 unit) was added in second reaction.

protein. When the ratio of *dnaG* protein to G4 DNA was doubled, *dnaG* protein was found both free and bound to G4 DNA (Fig. 1*D*). Thus, with excess *dnaG* protein relative to G4 DNA, about one molecule of *dnaG* protein binds per circle of G4 DNA [based on the purity of *dnaG* protein described in *Materials and Methods* and the molecular weight of G4 DNA (11)].

In the presence of DNA binding protein, *dnaG* protein also bound to ST-1 DNA (Fig. 1*E*). In contrast, *dnaG* protein did not bind to fd or ϕX174 DNA (Fig. 1*F* and *G*). Thus, when G4 or St-1 DNA is covered with DNA binding protein, *dnaG* protein binds to specific sites present in those DNAs. DNA synthesis by the isolated complex of G4 DNA, *dnaG* protein, and DNA binding protein required ADP, four dNTPs, and the DNA elongation proteins.

Primer Synthesis Dependent on *dnaG* Protein, DNA Binding Protein, G4 DNA, ADP, dGTP, and dTTP. G4 DNA synthesis was separated into an ADP-dependent priming reaction (first reaction) and an ADP-independent DNA elongation reaction (second reaction). When the first reaction contained DNA binding protein, *dnaG* protein, G4 DNA, ADP, dTTP, dATP, dCTP, and [α-^{32}P]dGTP, label from [α-^{32}P]-dGTP was found with the DNA and associated proteins after gel filtration (Table 2). When the first reaction contained [α-^{32}P]dTTP, [α-^{32}P]dATP, or [α-^{32}P]dCTP instead of [α-^{32}P]-dGTP, label from them was found in the protein–DNA complex (results not shown). Incorporation of ADP into the protein–DNA complex has not been examined. The isolated complex supported DNA synthesis in a second reaction upon addition of four dNTPs, DNA polymerase III, *dnaZ* protein, DNA EF I, and DNA EF III; ADP was no longer required (Table 2). ADP, dTTP, dGTP, *dnaG* protein, and DNA binding protein were required in the first reaction for incorporation of label from [α-^{32}P]dGTP into the protein–DNA complex and for ADP-independent DNA synthesis by the complex in the second reaction (Table 2). In contrast, dATP and dCTP were not required in the first reaction. The results with ST-1 DNA were similar but not identical to those with G4 DNA. When fd or ϕX174 DNA was used, label from [α-^{32}P]dGTP was not associated with the DNA. Thus, only those DNAs that bind *dnaG* protein and whose DNA synthesis is initiated by *dnaG* protein alone incorporate dNTPs into a protein–DNA complex.

As shown in Table 1, the ADP requirement for G4 DNA synthesis was not satisfied by other nucleotides.* In the priming reaction (first reaction) described in Table 2, UTP satisfied the dTTP requirement; dideoxy-TTP and dTDP did not satisfy the requirement. Similarly, GTP substituted for dGTP at a similar concentration (K_m for dGTP was about 5 μM); β,γ-methylene-GTP and dGDP could not be substituted. There was no preference for utilization of GTP over dGTP in the first reaction. To show this, first reactions were prepared as described in Table 2 containing G4 DNA, *dnaG* protein, DNA binding protein, ADP, dTTP, and either (*a*) 15 μM GTP and 15 μM [α-^{32}P]dGTP or (*b*) 15 μM [α-^{32}P]GTP and 15 μM dGTP. After filtration, 0.57 pmol of ^{32}P from dGTP and 0.38 pmol of ^{32}P from GTP were associated with the DNA–protein complex.

Several lines of evidence suggest that label from [α-^{32}P]dGTP

* Reactions were not inhibited by 20 mM potassium phosphate (pH 7.5). Less than 10 pmol of AMP was incorporated from [α-^{32}P]ADP by DNA binding protein plus *dnaG* protein with conditions as in Table 2 minus template.

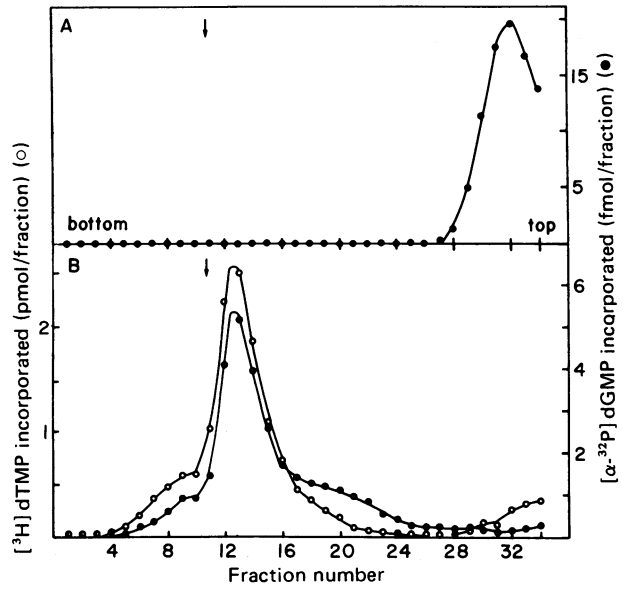

FIG. 2. Alkaline sucrose sedimentation of primer and DNA elongated primer. The first reaction contained 2.1 units of *dnaG* protein, 8 μg of DNA binding protein, 6 nmol of G4 DNA, 15 μM each of ADP, dTTP, and [α-^{32}P]dGTP (95,000 cpm/pmol), and reaction buffer as described in Table 2. After 15 min at 30°, the reaction mixture was subjected to gel filtration (see legend to Fig. 1). (*A*) One portion of the isolated primed DNA complex was adjusted to 20 mM EDTA/0.8 M NaCl/0.2 M NaOH in 100 μl. The sample was sedimented in a 10–25% sucrose gradient containing 2 mM EDTA/0.8 M NaCl/0.2 M NaOH for 6 hr in an SW50.1 Spinco rotor at 50,000 rpm at 10°. Thirty-four fractions were collected and radioactivity from [α-^{32}P]dGTP was measured directly (●). (*B*) Another portion of the isolated DNA complex was incubated in a second reaction (60 μl) in the absence of ADP with dATP, dCTP, dGTP, and [^{3}H]dTTP as described in Table 2 but with 0.6 μg of T4 DNA polymerase. After 10 min at 30°, the reaction mixture was adjusted to 20 mM EDTA/0.8 M NaCl/0.2 M NaOH in 100 μl and sedimented as in *A*. Thirty-four fractions were collected and acid-insoluble radioactivity from [α-^{32}P]dGTP (●) and from [^{3}H]dTTP (○) was measured. The arrow refers to the position of φX174 [^{14}C]DNA. The primer was also found to be elongated by DNA polymerase I or by the combination of DNA polymerase III, *dnaZ* protein, DNA EF I, and DNA EF III (results not shown).

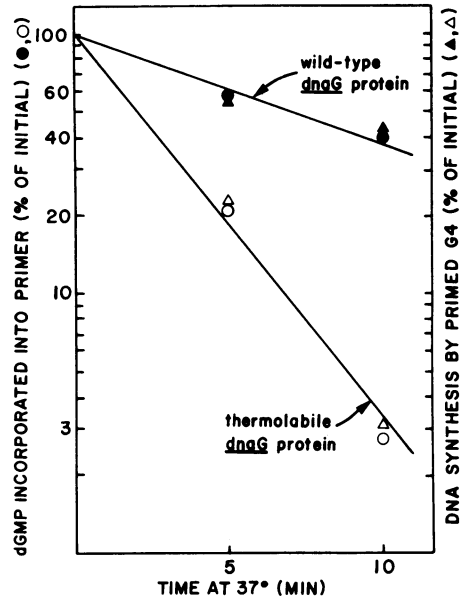

FIG. 3. Thermolability of primer synthesis catalyzed by thermolabile *dnaG* protein. *dnaG* proteins (wild-type and thermolabile) were heated [diluted to 300 units/ml in 10% glycerol/50 mM Tris·HCl (pH 8.0)/1 mM dithiothreitol/1 mg of bovine serum albumin per ml] for 0, 5, or 10 min at 37°. The *dnaG* proteins (10 μl) were then incubated in first reactions with reaction buffer, G4 DNA, DNA binding protein, ADP, dTTP, and [α-^{32}P]dGTP (as described in Table 2) and subjected to gel filtration (as described in legend to Fig. 1). ^{32}P was measured in the DNA complexes isolated from reactions containing wild-type *dnaG* protein (●) and from reactions containing thermolabile *dnaG* protein (○). One hundred percent represents 1.02 and 0.80 pmol of dGMP incorporated with wild-type and thermolabile *dnaG* protein–DNA complexes, respectively. ADP-independent dTMP incorporation in a second reaction was measured as described in Table 2 with DNA complexes formed using wild-type *dnaG* protein (▲) and thermolabile *dnaG* protein (△). One hundred percent represents 1040 and 712 pmol of total nucleotide incorporated with wild-type and thermolabile *dnaG* protein–DNA complexes, respectively. When the first reaction contained a mixture of wild-type and thermolabile *dnaG* proteins heated 10 min at 37°, both dGMP incorporation into primer and ADP-independent DNA elongation were the expected sums.

associated with the protein–DNA complex was in a primer molecule: (*a*) The same components were required to incorporate label from [α-^{32}P]dGTP into the complex and to form a complex active for DNA synthesis. (*b*) After DNA elongation of the isolated complex, the label from [α-^{32}P]dGTP sedimented in an alkaline sucrose gradient with nearly full-length linear DNA (Fig. 2). (*c*) The label from [α-^{32}P]dGTP was recovered from the complex as a phenol-extractable oligonucleotide; label could be recovered as dGMP after treatment with snake venom phoshodiesterase.

***dnaG* Protein Is Involved in G4 Primer Synthesis.** The priming reaction was thermolabile when thermolabile *dnaG* protein [previously shown to be thermolabile *in vitro* (17)] was used. In the experiments presented in Fig. 3, wild-type and thermolabile *dnaG* proteins were heated and then incubated in first reactions with DNA binding protein, G4 DNA, ADP, dTTP, and [α-^{32}P]dGTP. The products of the first reactions were isolated by gel filtration. Both [α-^{32}P]dGMP incorporation into primer in the first reaction and ability of the isolated template to support DNA elongation in the second reaction were more thermolabile when thermolabile *dnaG* protein was used than when wild-type *dnaG* protein was used. As further proof that *dnaG* protein was involved in the priming reaction, *dnaG* complementing activity (17) comigrated on native polyacrylamide gel electrophoresis (18) with both [α-^{32}P]dGMP incorporation into G4 primer and formation of a complex that supported ADP-independent DNA elongation (measured as described in Table 2; results not shown).

The *dnaG* protein was directly involved in primer synthesis and not merely involved in binding an oligonucleotide to G4 DNA. To demonstrate this, reactions as described in Fig. 3 were repeated, and the products formed were isolated by Sephadex G-25 chromatography. With this procedure, small oligonucleotides should be excluded from the column. The DNA–protein complex (excluded from Sephadex G-25) contained 0.05 pmol of label from [α-^{32}P]dGTP when heated thermolabile *dnaG* protein (10 min at 37°) was used and 0.35 pmol of label when heated wild-type *dnaG* protein was used. The isolated complex formed with heated thermolabile *dnaG* protein catalyzed incorporation of 17 pmol of total nucleotide; that formed with heated wild-type *dnaG* protein, 556 pmol. Thus, *dnaG* protein is involved in G4 DNA-dependent primer synthesis.

Elongation of G4 DNA primed by thermolabile *dnaG* protein at 37° was not thermolabile even though *dnaG* protein was present (results not shown) in the isolated DNA complex. For these experiments, DNA complexes prepared with unheated wild-type and thermolabile *dnaG* proteins were isolated and

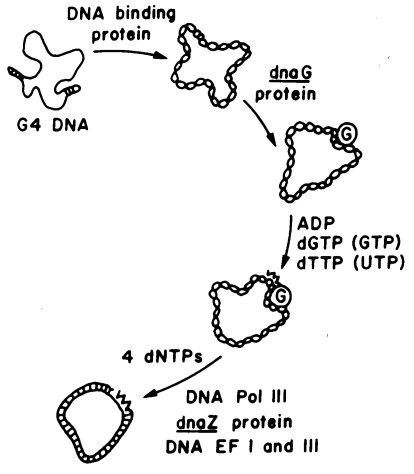

FIG. 4. Mechanism of priming of G4 DNA-dependent DNA synthesis.

the DNA elongation reactions (second reactions) were carried out at either 37° or 27° in the absence of ADP (as described in Fig. 3). With the DNA complex primed by wild-type *dnaG* protein, the ratio of dTMP incorporation at 37° to the incorporation at 27° in the second reaction was 2.1; with the DNA complex primed by thermolabile *dnaG* protein, that ratio was 1.4. In contrast, when reaction mixtures (as described in Table 1) containing thermolabile *dnaG* protein were incubated directly at 37° or 27°, the ratio of dTMP incorporation at 37° to the incorporation at 27° was 0.12; when reactions contained wild-type *dnaG* protein, that ratio was 1.04.

DISCUSSION

These results suggest the following mechanism of initiation of G4 DNA synthesis (Fig. 4). DNA binding protein covers G4-DNA and facilitates binding of *dnaG* protein to a specific site on the DNA. An oligonucleotide is synthesized by this protein–DNA complex in a reaction requiring ADP, dTTP (or UTP), and dGTP (or GTP). The oligonucleotide is elongated by DNA polymerase II or III in combination with *dnaZ* protein, DNA EF I, and DNA EF III, or by DNA polymerase I.

The data presented above, showing the increased thermolability of primer synthesis by thermolabile *dnaG* protein when compared to wild-type *dnaG* protein, conclusively prove that *dnaG* protein is involved in G4 DNA-dependent primer synthesis. It had previously been claimed that *dnaG* protein was an RNA polymerase (1, 6, 19, 20) capable of synthesizing a unique transcript of 28 nucleotides (20) with G4 DNA as template in a reaction requiring two (19) or four (6) rNTPs. It is shown here that *dnaG* protein-dependent G4 DNA synthesis does not require any rNTPs and that *dnaG* protein can synthesize deoxyoligonucleotides in an ADP-stimulated reaction. This discrepancy is partially resolved by the demonstration here that *dnaG* protein can synthesize ribo-, deoxyribo-, and mixed ribo- and deoxyribooligonucleotides.

If the function of *dnaG* protein in φX174 phage DNA synthesis is similar to its function in G4 DNA synthesis, then perhaps the *dnaB* and *dnaC(D)* proteins, replication factors X, Y, and Z, and DNA binding protein are needed to form a site on the φX174 DNA that can be recognized by *dnaG* protein. DNA binding protein alone is able to create such a site on G4 DNA. The site on φX174 DNA where the structure recognized by *dnaG* protein is built must itself be a unique site, since these proteins cannot use fd DNA (2). Perhaps, once bound to φX174 DNA, *dnaG* protein synthesizes an oligonucleotide primer for DNA synthesis. S. Wickner and Hurwitz (2) have consistently found that only ATP of the rNTPs is required for φX174 DNA synthesis, while Schekman *et al.* (1) have claimed that all four rNTPs were required for this reaction.

Since *E. coli* chromosome elongation uses many of the same proteins as φX174 DNA synthesis, it is hoped that an understanding of how these proteins function in phage DNA replication will give insight into their function in *E. coli*. The ability of *dnaG* protein to use either ribo- or deoxyribonucleotides in primer formation raises the possibility that Okazaki fragments may be primed with either DNA or RNA or with both.

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