Vaccinia Virus DNA Polymerase

IN VITRO ANALYSIS OF PARAMETERS AFFECTING PROCESSIVITY*

(Received for publication, August 8, 1994, and in revised form, September 21, 1994)

William F. McDonald‡§ and Paula Traktman¶

From the Departments of ‡Microbiology and ¶Cell Biology, Cornell University Medical College, New York, New York 10021

The polymerization and proofreading activities of the vaccinia virus DNA polymerase reside within a 116-kDa catalytic polypeptide. We report here an investigation of the intrinsic processivity of this enzyme on both natural and homopolymeric DNA templates. Inclusion of the Escherichia coli helix destabilizing protein allowed the viral enzyme, which lacks strand displacement activity, to utilize a singly primed M13 DNA template. In the presence of either 10 mM MgCl₂ or 1 mM MgCl₂ + 40 mM NaCl, synthesis was achieved in a highly distributive manner. RFII formation required a significant excess of enzyme, and ~10 nucleotides (nt) were added per primer-template binding event. The apparent rate of primer elongation varied with the enzyme/template ratio and reached a maximum of 8 nts/s. A similar lack of processivity was observed on a poly(dA₉₀;oligo(dT)₁₂₋₁₈) template. In contrast, highly processive synthesis was achieved on both templates in the presence of 1 mM MgCl₂ and the absence of NaCl. A primer extension rate of 30 nts/s was observed, and ~2000 nt were added per binding event. These studies suggest that the catalytic polypeptide of the vaccinia virus DNA polymerase will require accessory protein(s) to form a stable enzyme-template interaction and direct processive DNA synthesis under isotonic conditions in vitro.

DNA polymerases represent the core of the complex enzymatic machinery responsible for the faithful duplication of genomic DNA. These enzymes bind to a primer-template junction and catalyze the addition of the correct nucleotide to the nascent strand. Analysis of viral, procaryotic and, more recently, eucaryotic polymerases has provided a growing insight into the regulation of polymerase-template interactions. The emerging generalization is that there is an inherently weak interaction between polymerases and their templates, which may provide the basis for the identification of polymerase accessory proteins. Preliminary to this goal, we present here an investigation of the mode of synthesis catalyzed by the vaccinia virus DNA polymerase encoded by the E9 gene, which is a 116-kDa protein with both polymerization and 3′→5′ exonuclease activity (6, 9–12). It has strong homology to the α/β family of DNA polymerases (10, 13, 14). We have recently reported the overexpression of the enzyme using the hybrid vaccinia-T7 expression system and its purification to apparent homogeneity (12). The availability of pure enzyme will facilitate the development of an in vitro replication system, which may provide the basis for the identification of polymerase accessory proteins. Preliminary to this goal, we present here an investigation of the mode of synthesis catalyzed by the purified polymerase on homopolymeric and natural templates.

EXPERIMENTAL PROCEDURES

Materials

Poly(dA)₉₀ and oligo(dT)₁₂₋₁₈ were obtained from Pharmacia Biotech Inc. Sequenase kit II was purchased from United States Biochemical Co. (Cleveland, OH). T4 DNA ligase, polynucleotide kinase, and HindIII-digested λ-DNA were purchased from New England Biolabs, Inc. (Beverly, MA) or Boehringer Mannheim and were used according to the instructions provided by the manufacturer. ³²P-Labeled nucleoside triphosphates were acquired from DuPont NEN. Purified E. coli SSB was kindly provided by Dr. M. O'Donnell, Cornell University Medical College. The 24-mer oligonucleotide used to construct primed M13 DNA templates was synthesized on an Applied Biosystems oligonucleotide synthesizer.

Vaccinia DNA Polymerase

We have recently reported the overexpression and purification of the vaccinia virus DNA polymerase (12). The polymerase was overex-

* This work was supported by National Institutes of Health Grant AI 21758 (to P. T.) and by contributions from D. Cofrin and a special group of donors from the Dorothy Rodbell Cohen Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Dept. Biologie du Developpement, Institut Jacques Monod, 2 Place Jussieu, 75231 Paris Cedex 05, France. ¶ Recipient of a Hirsch/Well Caillier Career Scientist Award. To whom correspondence should be addressed: Dept. of Cell Biology, Cornell University Medical College, 1300 York Ave., NY 10021. Tel.: 212-746-6186; Fax: 212-746-1875; E-mail: ptrakt@cumc.cornell.edu.

† The abbreviations used are: kb, kilobase(s); ss, single-stranded; SSB, single-stranded binding protein; nt, nucleotide(s).
by the addition of equal volumes of either 20% trichloroacetic acid, 0.2 M PP, to determine dNTP incorporation. Circular forms of M13 DNA are indicated with arrowheads.

7.5, 40 pg/ml bovine serum albumin, 4% glycerol, 0.1 mM EDTA, 5 mM dithiothreitol, 8 mM MgCl₂, 60 mM NaCl, 20 μM [α-32P]TTP (3500 counts/min/pmol TTP), 25 fmol of singly primed M13 DNA, 10 pmol of tetrameric E. coli SSB where indicated, and the indicated amounts of vaccinia DNA polymerase (VVPol). Reactions were preincubated for 3 min at 30 °C in the presence of two dNTPs (dCTP and dGTP). DNA synthesis was initiated by the addition of dATP and [α-32P]TTP, and reactions were incubated at 30 °C for 30 min. DNA synthesis was stopped by the addition of equal volumes of either 20% trichloroacetic acid, 0.2 mM PP, to determine dNTP incorporation (A), or 40 mM EDTA, 1% SDS to analyze replication products on 0.8% TBE-agarose gels (B). In the graph shown in panel A, assays performed in the absence and presence of SSB are indicated by the filled circles and filled squares, respectively. In panel B, the migration of the single-stranded (SS) and double-stranded (RFII) circular forms of M13 DNA are indicated with arrowheads.

FIG. 1. Stimulation of the vaccinia DNA polymerase by E. coli SSB. Complete replication reactions (25 μl) contained 20 mM Tris-HCl, pH 7.5, 40 μg/ml bovine serum albumin, 4% glycerol, 0.1 mM EDTA, 5 mM dithiothreitol, 8 mM MgCl₂, 60 μM each dCTP, dGTP, and dATP, 20 μM [α-32P]TTP (3500 counts/min/pmol TTP), 25 fmol of singly primed M13 DNA, 10 pmol of tetrameric E. coli SSB where indicated, and the indicated amounts of vaccinia DNA polymerase (VVPol). Reactions were preincubated for 3 min at 30 °C in the presence of two dNTPs (dCTP and dGTP). DNA synthesis was initiated by the addition of dATP and [α-32P]TTP, and reactions were incubated at 30 °C for 30 min. DNA synthesis was stopped by the addition of equal volumes of either 20% trichloroacetic acid, 0.2 mM PP, to determine dNTP incorporation (A), or 40 mM EDTA, 1% SDS to analyze replication products on 0.8% TBE-agarose gels (B). In the graph shown in panel A, assays performed in the absence and presence of SSB are indicated by the filled circles and filled squares, respectively. In panel B, the migration of the single-stranded (SS) and double-stranded (RFII) circular forms of M13 DNA are indicated with arrowheads.

FIG. 2. The formation of true M13 RFII products by the vaccinia DNA polymerase. A standard replication reaction (250 μl) containing 250 fmol of [32P]-primed M13 ssDNA, 100 pmol of E. coli SSB, and 674 fmol of DNA polymerase was incubated at 30 °C. At the indicated times (min), 25-μl aliquots were removed and reactions were quenched with an equal volume of 40 mM EDTA, 1% SDS. At 30 min, an additional 25-μl aliquot was removed and incubated with ATP (1 mM) and T4 DNA ligase (1 unit) for 60 min at 30 °C (30 min + T4 ligase). DNA products were fractionated on 0.8% TBE-agarose and visualized by ethidium bromide staining. Equal amounts of vaccinia DNA polymerase (WPol). Reactions were preincubated for 3 min at 30 °C in the presence of two dNTPs (dCTP and dGTP). DNA synthesis was initiated by the addition of dATP and [α-32P]TTP, and reactions were incubated at 30 °C for 30 min. DNA synthesis was stopped by the addition of equal volumes of either 20% trichloroacetic acid, 0.2 mM PP, to determine dNTP incorporation (A), or 40 mM EDTA, 1% SDS to analyze replication products on 0.8% TBE-agarose gels (B). In the graph shown in panel A, assays performed in the absence and presence of SSB are indicated by the filled circles and filled squares, respectively. In panel B, the migration of the single-stranded (SS) and double-stranded (RFII) circular forms of M13 DNA are indicated with arrowheads.

pressed in BSC40 cells using the hybrid vaccinia virus-T7 overexpression system (15). Clarified cytoplasmic lysates were fractionated sequentially on DEAE-cellulose, phosphocellulose, hydroxypapitate, and heparin-agarose, and DNA polymerase was assayed on an activated salmon sperm DNA template. Fraction V contained a single 116-kDa protein species as visualized on silver-stained SDS-polyacrylamide gels. The purified enzyme had a specific activity of 3023 units/mg (≈285 pmol/unit), where 1 unit is that amount of enzyme that can incorporate 10 nmol of dNTP into acid precipitable material in 30 min at 37 °C.

DNA Polymerase Assays

Singly Primed M13 DNA—To construct a primed template, a 24-mer oligonucleotide primer (5'-CGCAGGTTTTCCCATCGAACS-3') was annealed to M13mp10 ssDNA at a molar ratio of 20:1. To construct a 5'-32P-primed, the primer was radiolabeled with T4 polynucleotide kinase and [γ-32P]ATP prior to annealing to M13mp10 ssDNA. Radiolabeled primer/template was purified on a Bio-Gel A1.5 m sizing column to remove free label and unannealed primer. Unless indicated otherwise, DNA polymerase assay was performed in reactions (25 μl) that contained 10 mM Tris-Cl, pH 7.5, 40 μg/ml bovine serum albumin, 4% glycerol, 0.1 mM EDTA, 5 mM dithiothreitol, 8 mM MgCl₂, 25 fmol of singly primed M13mp10 ssDNA, 750 ng of E. coli SSB (10 pmol of tetramer), 60 μM each of dCTP, dGTP, and dATP, and 20 μM [32P]TTP (2400 counts/min/pmol/μl). Reactions were preincubated with enzyme and two of the four dNTPs (dCTP and dGTP) at 30 °C for 3 min. Primer extension was initiated by the addition of dATP and [32P]TTP, and incubation was continued at 30 °C. To measure the incorporation of radiolabeled nucleotide into acid precipitable material, DNA synthesis was stopped by the addition of an equal volume of 20% trichloroacetic acid, 0.2 mM NP-40, Trichloroacetic acid precipitates were collected and washed on glass fiber filters, and radioactivity was quantitated by Cerenkov counting. To visualize primer extension products, reactions were quenched with an equal volume of 1% SDS, 40 mM EDTA and fractionated on 0.8% TBE-agarose gels containing 0.125 mg/ml EtBr. Gels were cast and run in 1 x TBE (50 mM Tris, 50 mM boric acid, and 1 mM EDTA), dried, and subjected to autoradiography.

Poly(dA)-Oligo(dT)—Radiolabeled poly(dA)-oligo(dT) was made by annealing 5'-32P-oligo(dT)₂₅₋₅₀ to poly(dA)ₙ using a molar ratio of 1:1. DNA polymerase assay was performed in reactions (25 μl) that contained 10 mM Tris-Cl, pH 7.5, 40 μg/ml bovine serum albumin, 4% glycerol, 0.1 mM EDTA, 5 mM dithiothreitol, 8 mM MgCl₂, 336 fmol of poly(dA)ₙ, 5'-32P-oligo(dT)₂₅₋₅₀, and 20 μM dTTP. DNA synthesis was initiated by the addition of enzyme, and reactions were incubated at 30 °C. Primer extension was terminated by organic extraction and ethanol precipitation. DNA pellets were resuspended in loading buffer (1 x TBE, 90% formamide, bromphenol blue, and xylene cyanol) and fractionated on 0.4 mm urea, 12% polyacrylamide gels cast and run in 1 x TBE. To quantitate the incorporation of [32P]TTP into acid-precipitable material, samples were quenched and subjected to trichloroacetic acid precipitation as described above.

RESULTS

Single-stranded M13 phage DNA annealed to an oligonucleotide primer has served as a useful template for the biochemical characterization of diverse DNA polymerases. An advantage to using this molecule over activated (nicked and gapped) DNA is that enzyme processivity, or the ability of polymerases to syn-
the inability of the vaccinia DNA polymerase to overcome these barriers, and hence to direct significant primer elongation, was described some years ago in the classic biochemical study of this enzyme (16). In the intervening years, it has become clear that utilization of such a template can be facilitated by inclusion of a helix destabilizing SSB in experimental assays. Because no vaccinia-encoded SSB has yet been identified, and because the E. coli SSB has been shown to support DNA synthesis by a variety of heterologous polymerases (17–20), we explored the use of the E. coli protein in our analysis of the processivity of the purified vaccinia DNA polymerase (12).

**Primer Extension by the Vaccinia Enzyme on M13 ssDNA Is Stimulated by E. coli SSB—**A fixed amount of primed M13 DNA (25 fmol), uncoated or coated with E. coli SSB, was incubated with various amounts of the purified vaccinia DNA polymerase (Fraction V) (12). After a 3-min preincubation designed to permit the polymerase to bind to the primer terminus, DNA synthesis was initiated and allowed to proceed for 30 min at 30 °C. Reactions were terminated and products were analyzed by quantitation of the incorporation of radiolabeled dNTP into acid-precipitable material and by fractionation of the products on neutral agarose gels under conditions that maximize separation of template from product. As seen in Fig. 1A, dNTP incorporation by the vaccinia enzyme was greatly increased in the presence of E. coli SSB. When the vaccinia polymerase was present in a 7-fold excess over template, a maximum stimulation of 40-fold was observed. Electrophoretic analysis of the radiolabeled products (Fig. 1B) elucidated the nature of this stimulation. Only in the presence of SSB could the vaccinia enzyme synthesize the full complement of the template strand to form products whose migration was indistinguishable from RFII (double-stranded nicked circle). Although a significant amount of dNTP incorporation was detected in the absence of SSB, the radiolabeled material co-migrated with (or migrated slightly above) the single-stranded DNA template. These products, in which the primer was only extended a short distance, reflect the inability of the enzyme to displace barriers of secondary structure in the naked template.

To determine whether the presumed RFII product obtained in the presence of enzyme excess and SSB was completely
plex in nature, a modified experiment was performed. Reactions contained unlabeled dNTPs and a population of primer-templates in which some of the primers contained a 32P-radiolabeled 5' terminus. A molar excess of the vaccinia DNA polymerase was incubated with 25 fmol of this template, and DNA synthesis was initiated and terminated as described previously. The DNA products were fractionated electrophoretically on neutral agarose gels containing ethidium bromide. Examination of the total DNA population by ultraviolet illumination revealed that all of the single-stranded template was converted to RFII DNA in 15' at 30 °C (Fig. 2A). Under these conditions, primer elongation by the vaccinia enzyme occurred at an average rate of 8 nt/s. To confirm that the completed DNA products contained nicks and not gaps, bacteriophage T4 DNA ligase and ATP were added subsequent to the production of RFII, and ligation was allowed to proceed for 1 h at 30 °C. Visualization of the products by autoradiography revealed that the radiolabeled RFII molecules (i.e. those products extended from a primer containing a phosphorylated 5' terminus) were completely converted to the rapidly migrating RFII form (Fig. 2B, lane 8). The covalently closed RFII molecules become positively supercoiled during migration through EtBr-containing gels and hence migrate more rapidly than nicked RFII molecules. Because the majority of the annealed primers contained unphosphorylated 5' termini, and the vaccinia enzyme has no detectable 5'-3' exonuclease activity (12), most of the RFII products (those which are not radiolabeled) were not converted to RFII (compare the products seen by ultraviolet illumination (Fig. 2A, lane 8)) with those seen by autoradiography (Fig. 2B, lane 8)). These results demonstrate that vaccinia polymerase-catalyzed DNA synthesis on SSB-coated M13 templates is indeed complete and results in the formation of true RFII molecules.

Characterization of Polymerization Efficiency on Singly Primed M13 DNA and Poly(dA)-Oligo(dT) — In the analysis described above, we failed to observe completely extended M13 products when enzyme levels below 185 fmol were incubated with 25 fmol of an SSB-coated M13 template for 30 min at 30 °C (Fig. 1). At lower enzyme concentrations (<7-fold excess), all of the available primed template, as visualized by EtBr staining of fractionated replication products (data not shown), was acted upon by the enzyme, yet no complete RFII product was detected. These findings suggested that DNA synthesis was catalyzed by the vaccinia polymerase in a distributive, or nonprocessive, fashion. Further support for this conclusion came from our observation that the time required to complete RFII formation depended upon the amount of enzyme added; whereas RFII formation was evident by 15 min when 674 fmol were assayed, more than 10 min were required when 185 fmol were assayed (data not shown).

To address the issue of distributivity versus processivity more directly, we performed a template challenge experiment. Enzyme was preincubated with an equimolar amount of SSB-coated, 32P-primed-M13 template in the presence of 2 dNTPs. Upon initiation of DNA synthesis by the addition of the other two dNTPs, a 5-fold molar excess of an unlabeled challenge template was added to half of the reaction. Aliquots were removed at various times, DNA synthesis was terminated, and reaction products were analyzed on denaturing polyacrylamide gels. As shown in Fig. 3, an examination of primer elongation on M13 DNA revealed distinct termination sites. Such polymerase pause sites are typically observed with natural DNA templates and may reflect residual M13 secondary structure. Under the conditions used in this experiment we observed an average elongation rate of one nt every 2 s. However, in the presence of challenge DNA, a 15-fold decrease in the rate of primer elongation was seen: approximately one nt was incorporated every 30 s. The majority of the extended products were one nt longer than the original primer. As G is the first nucleotide to be added with the primer used in these studies, it is probably incorporated during the preincubation (idling) step of the reaction when both dCTP and dGTP are available. These results indicated that the enzyme readily dissociated from the first template shortly after the addition of the second. The enzyme displayed a low processivity number, with only one to two nt incorporated/polymerase binding event.

To eliminate any effect E. coli SSB might have on the stabil-
The Effect of MgCl₂ on Enzyme Processivity on a Primed M13 Template. Reactions (250 µl) containing 250 fmol of primed M13 ssDNA, 10 pmol of *E. coli* SSB, 370 fmol of DNA polymerase, 60 µM each dCTP, dGTP, dATP, 20 µM [α-³²P]dTP and 8 mM or 1 mM MgCl₂, as indicated, were incubated at 30 °C. 25 µl aliquots removed at the indicated times (min) and DNA synthesis was stopped by the addition of equal volumes of either 20% trichloroacetic acid/0.2 M PPI to quantitate dNTP incorporation or 40 mM EDTA/1% SDS to analyze replication products on 0.8% TBE-agarose gels. (A) Incorporation of dNTP into acid-precipitable material. Assays performed in the presence of 1 and 8 mM MgCl₂ are indicated by squares and circles, respectively. (B) Autoradiographic visualization of products. The positions to which the RFII and SS forms of M13 DNA migrated are shown at the right.
template was used. The relative abundance of primer-template junctions (1/390 base pairs of template) in the homopolymeric template must reduce the contribution of primer-template reassociation to the net rate of DNA synthesis.

To further demonstrate that the dramatic increase in primer elongation rate at lower MgCl₂ concentrations was due to an increase in the processivity of the enzyme, extension products from single polymerase binding events were analyzed (Fig. 7). Replication reactions containing M13 template, various amounts of enzyme, and 1 mM MgCl₂ were incubated for 10 min at 30°C. Products were fractionated on a 0.5% alkaline agarose gel. Lane M contains ³²P-labeled HindIII-digested λ-DNA; the sizes of the fragments (in kb) are shown to the left.

**DISCUSSION**

Based on sequence homology with other eucaryotic DNA polymerases, the vaccinia virus DNA polymerase has been classified as a member of the α/β family. Seven domains of the vaccinia protein are shared with DNA polymerase α from human and yeast, and an additional five domains, which include three putative exonuclease motifs, are shared with δ enzymes (10, 13, 14, 25). A structure-function analysis designed to explore the relationship between these conserved regions and enzyme function has begun to yield structural information regarding active sites of the protein. To date, seven drug-resistant and two temperature-sensitive alleles have been isolated and their lesions mapped within the gene (10, 14, 26–28). Several drug-resistant mutations that display altered mutation rates in vivo (14, 26) are predicted to contain lesions that affect nucleotide binding and exonuclease activity.

The purification and biochemical characterization of the vaccinia DNA polymerase were originally reported by Challberg and Englund (9, 16). In addition to defining the 5'-3' polymerization and intrinsic 3'-5' exonuclease activities of the enzyme, their studies documented a lack of strand displacement capability. This inability of the viral polymerase to pass through regions of DNA secondary structure precluded an in depth analysis of enzyme processivity on a natural DNA template. In the analysis presented here, removal of structural barriers by the inclusion of the E. coli SSB protein enabled processivity determinations to be performed on natural as well as homopolymeric DNA templates. The vaccinia polymerase was pu-
As mentioned above, low enzyme processivity was observed when reaction conditions were optimal for DNA synthesis on gapped DNA (9). Conversion of singly primed M13 DNA to the duplex RFII product required the presence of a helix destabilizing protein (E. coli SSB). RFII formation was completed at a synthetic rate that varied with enzyme concentration; higher polymerase concentrations resulted in faster apparent rates of primer elongation. A maximal rate of dNTP incorporation of 8 nt/s at 30 °C was detected when enzyme concentrations were in vast excess over the primed DNA template. A clue to the relative slowness of this elongation rate was provided by template challenge experiments, which indicated that perhaps only one or two nucleotides were incorporated during a single cycle of enzyme-template binding.

A distributive mode of DNA synthesis was also observed using a poly(dA)-oligo(dT) template, whose full extension did not require a helix destabilizing protein. To determine the number of consecutive nucleotides incorporated during each interaction of the polymerase with this template, assays were performed under conditions of template excess. At a template to enzyme ratio of 35:1, incorporation of fewer than 10 dTMPs/ primer was observed. As the homopolymeric template studies were performed in the absence of E. coli SSB, we conclude that the distributive behavior of the vaccinia DNA polymerase (processivity number ~10) is an intrinsic property of the catalytic enzyme and not due to the presence of the E. coli protein.

The effect of individual reaction components on polymerase activity was examined to determine their influence, if any, on enzyme processivity. A significant increase in processivity was detected when MgCl₂ concentrations were reduced in assays where primed M13 DNA or poly(dA)-oligo(dT) served as replication templates. Analysis of extension products from single interactions of the enzyme with the M13 template revealed a >200-fold increase in enzyme processivity when the concentration of MgCl₂ was reduced from 8 to 1 mM. A corresponding increase in the apparent rate of primer elongation from 8 nt/s (with excess polymerase) to an average rate of 30 nt/s at 30 °C was also observed. That the reduction in MgCl₂ was augmenting enzyme processivity, and not catalysis per se, was confirmed in studies using poly(dA)-oligo(dT) as a template. TTP incorporation was maximal at 4 mM MgCl₂; approximately 2-fold lower levels of incorporation were obtained in the presence of either 1 or 8 mM MgCl₂. Moreover, varying the MgCl₂ concentration within this same range had no effect on 5'-3' exonuclease activity (data not shown). Finally, the increase in processivity observed at low concentrations MgCl₂ could be reversed by the addition of 40 mM NaCl without bringing about a severe reduction in net DNA synthesis.

This dramatic fluctuation in processivity with varying MgCl₂ and NaCl concentrations suggest that polymerase-DNA interactions are highly sensitive to subtle changes in ionic strength. As mentioned above, low enzyme processivity was observed when reaction conditions were optimal for synthesis on templates that contain short single-stranded regions. Indeed, salt concentrations that favor cycling of the DNA polymerase are likely to increase net DNA synthesis in reactions where primer-template concentrations are high and single-stranded template regions are relatively short. A similar sensitivity of enzyme processivity to Mg²⁺ and salt concentrations has been described for DNA polymerases from other systems (18, 21-24). The processivity of calf thymus DNA polymerase α can be reduced 20-fold by raising the Mg²⁺ concentration from 1 to 10 mM (from 50–100 nt incorporated/binding event to 2–5, pH 8.0) (23, 24).

In a recent study of the Drosophila mitochondrial DNA polymerase (22), an increase in processivity with decreasing MgCl₂ and NaCl concentrations, and an inverse relationship between polymerase processivity and enzyme activity, were demonstrated. However, no reaction conditions were described that allowed for maximal processivity and polymerase activity simultaneously. We did observe an increase in DNA synthesis with increasing processivity in assays where MgCl₂ and NaCl concentrations were reduced and singly primed M13 DNA served as a template. Because this substrate presents a low concentration of primer-template junctions (1/7200 nt), the contribution of enzyme recycling to the apparent rate of DNA synthesis is likely to be significant. It is therefore not surprising that ionic conditions which reduce enzyme dissociation (low ionic strength) stimulate the rate of dNTP incorporation.

It is clear from the analyses presented here that the vaccinia DNA polymerase must associate with additional factors in order to replicate a template such as the 192 kb viral genome efficiently. The need for a helix destabilizing activity and the lack of duplex invasion capabilities indicate that an SSB and perhaps a helicase are essential. In addition, the weak enzyme-template interactions seen in the presence of moderate levels of salt suggest that other accessory factors are needed for ensuring processive DNA synthesis under isotonic conditions. Because the purified enzyme was overexpressed with vaccinia-infected cells, viral factors that stimulate polymerase activity or processivity might be expected to copurify with the catalytic subunit. In our purification protocol, however, assays containing an activated DNA template and moderate salt levels were used to follow polymerase activity. In retrospect, this approach would have selectively enriched for a distributive catalytic activity. To investigate further the potential role of accessory proteins in processive viral DNA synthesis, a careful fractionation of the vaccinia enzyme from wild-type infected cells is underway. Using a primed M13 ssDNA template to monitor polymerase activity over the course of purification, we have partially purified a processive form of the vaccinia enzyme.² This activity catalyzes DNA synthesis rapidly and processively under reaction conditions that permit only a distributive mode of synthesis with the purified enzyme. Although significant progress has been made in the purification of this processive activity, subunit composition has not been determined and awaits further biochemical analysis.

Acknowledgments—We thank the members of the O'Donnell laboratory for their generous gift of E. coli SSB and many helpful conversations.

REFERENCES
1. Nossal, G. N. (1992) FASEB J. 6, 871–878
2. Challberg, M. D. (1991) Semin. Virol. 2, 247–256
3. O'Donnell, M., Ovresak, R., Dean, P. B., Chen, M., and Hurwitz, J. (1993) Nucleic Acids Res. 21, 1–3
4. Campbell, J. L. (1993) J. Biol. Chem. 268, 25261–25264
5. Waga, S., and Stillman, B. (1994) Nature 370, 207–212
6. Traktman, P. (1990) Curr. Top. Microbiol. Immunol. 183, 93–123
7. Traktman, P. (1990) Cell 62, 621–626
8. Traktman, P. (1991) Semin. Virol. 2, 291–304
9. Challberg, M. D., and Englund, P. T. (1979) J. Biol. Chem. 254, 7812–7819
10. Earl, P. L., Jones, E. V., and Moss, B. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3659–3660
11. Traktman, P., Sridhar, P., Condit, R. C., and Roberts, B. E. (1984) J. Virol. 49, 125–131
12. McDonald, W. F., and Traktman, P. (1994) Protein Expression Purif. 5, 409–421

² N. Klenerman, W. F. McDonald, and P. Traktman, unpublished results.
Processivity of the Vaccinia Virus DNA Polymerase

13. Wang, T. S.-F. (1991) Annu. Rev. Biochem. 60, 513-552
14. Taddie, J. A., and Traktman, P. (1993) J. Virol. 67, 4323-4336
15. Moss, B., Rivoir-Stein, O., Mizukami, T., Alexander, W. A., and Fuerst, T. R. (1990) Nature 345, 91-92
16. Challberg, M. D., and Englund, P. T. (1979) J. Biol. Chem. 254, 7820-7826
17. Gottlieb, J., Marcy, A. J., Coen, D. M., and Challberg, M. D. (1990) J. Virol. 64, 5976-5987
18. Lee, S.-H, Pan, Z.-Q., Kwong, A. D., Burgers, P. M. J., and Hurwitz, J. (1991) J. Biol. Chem. 266, 22707-22717
19. Burgers, P. M. J. (1991) J. Biol. Chem. 266, 22698-22706
20. O'Donnell, M. E., Elias, P., and Lehman, I. R. (1987) J. Biol. Chem. 262, 4252-4259
21. Burgers, P. M. J. (1988) Nucleic Acids Res. 16, 6297-6307
22. Williams, A. J., Wernette, C. M., and Kaguni, L. S. (1993) J. Biol. Chem. 268, 34855-34862
23. Burgers, P. M. J. (1989) Prog. Nucleic Acid Res. 37, 235-280
24. Sabatino, R. D., Myers, T. W., Bambara, R. A., Shin, O. K., Morraccino, R. L., and Fricke, P. H. (1988) Biochemistry 27, 2999-3004
25. Blanco, L., Bernad, A., and Salas, M. (1992) Gene (Amst.) 112, 139-144
26. Taddie, J. A., and Traktman, P. (1991) J. Virol. 65, 869-879
27. Traktman, P., Kelvin, M., and Pacheco, S. (1988) J. Virol. 63, 841-846
28. Defilippes, P. M. (1989) J. Virol. 63, 4060-4063