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Functional Interaction between Epstein-Barr Virus DNA Polymerase Catalytic Subunit and Its Accessory Subunit In Vitro

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The Epstein-Barr virus (EBV) DNA polymerase catalytic subunit (BALF5 protein) and its accessory subunit (BMRF1 protein) have been independently overexpressed and purified (T. Tsurumi, A. Kobayashi, K. Tamai, T. Daikoku, R. Kurachi, and Y. Nishiyama, J. Virol. 67:4651–4658, 1993; T. Tsurumi, J. Virol. 67:1681–1687, 1993). In an investigation of the molecular basis of protein-protein interactions between the subunits of the EBV DNA polymerase holoenzyme, we compared the DNA polymerase activity catalyzed by the BALF5 protein in the presence or absence of the BMRF1 polymerase accessory subunit in vitro. The DNA polymerase activity of the BALF5 polymerase catalytic subunit alone was sensitive to high ionic strength on an activated DNA template (80% inhibition at 100 mM ammonium sulfate). Addition of the polymerase accessory subunit to the reaction greatly enhanced DNA polymerase activity in the presence of high concentrations of ammonium sulfate (10-fold stimulation at 100 mM ammonium sulfate). Optimal stimulation was obtained when the molar ratio of BMRF1 protein to BALF5 protein was 2 or more. The DNA polymerase activity of the BALF5 protein along with the BMRF1 protein was neutralized by a monoclonal antibody to the BMRF1 protein, whereas that of the BALF5 protein alone was not, suggesting a specific interaction between the BALF5 protein and the BMRF1 protein in the reaction. The processivity of nucleotide polymerization of the BALF5 polymerase catalytic subunit on singly primed M13 single-stranded DNA circles was low (~50 nucleotides). Addition of the BMRF1 polymerase accessory subunit resulted in a strikingly high processive mode of deoxynucleotide polymerization (>7,200 nucleotides). These findings strongly suggest that the BMRF1 polymerase accessory subunit stabilizes interaction between the EBV DNA polymerase and primer template and functions as a sliding clamp at the growing 3′-OH end of the primer terminus to increase the processivity of polymerization.

Epstein-Barr virus (EBV) is a human herpesvirus with a linear double-stranded DNA 172 kb long (1). EBV has both a latent state and a lytic replicative cycle in the nuclei of EBV-infected lymphoblastoid cells. During the latent phase of the EBV life cycle, the EBV genome is maintained as a circular plasmid molecule synthesized by host DNA polymerases. OriP, one replication origin of EBV, mediates this type of replication (31). However, after induction of the lytic phase of viral replication, EBV replication proteins are induced and the EBV genome is amplified 100- to 1,000-fold. The replication product is a head-to-tail concatemer, which is synthesized by the EBV DNA polymerase (Pol) via a rolling-circle mechanism initiated from the other replication origin, ori-Lyt (8). A number of features of EBV DNA replication make it an attractive model system for the study of eukaryotic DNA replication. Our goal is to understand the enzymatic processes that occur during the lytic phase of EBV DNA replication.

Of the approximately 84 genes that have been identified in the genome, EBV encodes at least 7 viral genes that are essential for ori-Lyt-dependent DNA replication (6). These genes and their functions, which are known or have been predicted on the basis of sequence comparison with herpes simplex virus type 1, are BALF5, the DNA Pol catalytic subunit; BMRF1, the DNA Pol accessory subunit; BALF2, the single-stranded DNA-binding protein; BBLF4 and BSLF1, the helicase and primase; BBLF2/3, a potential homolog of the third component of the helicase-primase complex; and genes encoded by the EBV *Sal*I F fragment, which has an unknown function.

We have focused our efforts on the study of EBV DNA Pol. The 110 kDa of the EBV DNA Pol catalytic polypeptide copurifies with the BMRF1 gene product (11, 15, 24). The neutralization of EBV DNA Pol activity by a monoclonal antibody to the BMRF1 protein (2) and the low level of activity in the DNA Pol fraction lacking the BMRF1 protein (12) strongly suggest that the EBV DNA Pol catalytic subunit forms a tight complex with the BMRF1 protein in EBV-producing cells to function as the Pol holoenzyme. The EBV DNA Pol holoenzyme possesses both DNA Pol and 3′-to-5′ exonuclease activities (24, 26). Furthermore, the EBV DNA Pol holoenzyme exhibits strikingly high processivity, which is a desirable feature in the synthesis of multiple copies of the EBV genome in rolling-circle DNA replication (25).

In investigations of protein-protein interactions between the subunits of the EBV DNA Pol holoenzyme, the overexpression and purification systems of the individual components have been developed by using the baculovirus expression system (27, 29). The BMRF1 gene products expressed in insect cells are made up of two phosphoproteins of 52 and 50 kDa and one unphosphorylated protein of 48 kDa. The BMRF1 DNA Pol accessory subunits exhibit higher binding affinity for double-stranded DNA but have neither DNA Pol nor exonuclease activity (27). The BALF5 gene product is a protein of 110 kDa. The BALF5 DNA Pol catalytic subunit has been demonstrated to catalyze both DNA Pol and 3′-to-5′ exonuclease activities

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(29). However, the catalytic properties of the EBV Pol catalytic subunit are different from those of the EBV DNA Pol holoenzyme from EBV-infected cells in several respects (29), suggesting that the BMRF1 Pol accessory protein does influence the enzymatic properties of the BALF5 Pol catalytic subunit. In this study, we compared the Pol activity catalyzed by the EBV DNA Pol catalytic subunit in the presence or absence of the Pol accessory protein on a short or long single-stranded DNA template to assess the contribution of the BMRF1 Pol accessory protein to the catalytic efficiency of the EBV DNA Pol holoenzyme.

The EBV DNA Pol catalytic subunit was purified from cytosolic extracts of recombinant baculovirus AcBALF5-infected Sf9 cells essentially as previously described (29). The EBV DNA Pol accessory subunit was prepared from nuclear extracts of recombinant baculovirus AcBMRF1-infected Sf9 cells as previously described (27), through the heparin agarose column step. It was purified further as follows. The heparin agarose column fraction of the BMRF1 protein was loaded onto a single-stranded DNA agarose column equilibrated with buffer A containing 20 mM Tris-HCl (pH 7.6), 1 mM EDTA, 25% glycerol, 1 mM dithiothreitol, 4 μg of leupeptin per ml, 4 μg of pepstatin A, and 1 mM phenylmethylsulfonyl fluoride. The single-stranded DNA agarose column was eluted with a 16-ml linear gradient from 0 to 0.6 M NaCl in buffer A. The BMRF1 protein eluted at 0.2 M NaCl was pooled and stored at −80°C. This preparation was nearly homogeneous and had no detectable DNA Pol or nuclease activity.

DNA Pol activity was routinely assayed in a reaction mixture (25 μl) that contained 50 mM Tris-HCl (pH 8.0); 10% glycerol; 6 mM $MgCl_2$; 100 μg of bovine serum albumin per ml; 80 μg of activated calf thymus DNA per ml; 1 mM dithiothreitol; dATP, dCTP, and TTP at 25 μM each, and [^{3}H]dGTP at 10 μM (900 cpm/pmol). The reaction was started by addition of the enzyme fraction, and incubation was for 20 min at 35°C. Reactions were stopped by addition of 30 μl of 2 mg of sonicated salmon sperm DNA per ml containing 0.1 M sodium PPi and quenched with 0.6 ml of cold 10% trichloroacetic acid containing 0.1 M sodium PPi. Incorporation of labeled nucleotides into acid-insoluble material was measured in an Aloka liquid scintillation counter.

BMRF1 Pol accessory protein drastically changes the salt sensitivity of the DNA Pol activity of the BALF5 protein on an activated DNA template. The DNA Pol activity of the EBV DNA Pol holoenzyme purified from EBV-producing lymphoblastoid cells was stimulated by ammonium sulfate when activated DNA was used as the template primer (11, 25). This feature is common to DNA Pols in the herpesvirus family (11, 13, 25). In sharp contrast, the Pol activity of the EBV Pol catalytic subunit purified from AcBALF5-infected Sf9 cells was inhibited by the salt in a dose-dependent manner (29). To determine whether complex formation between the BALF5 and BMRF1 proteins results in aquisition of the salt-stimulatory property, the salt concentration dependency of DNA Pol activity was determined after preincubation with the purified BALF5 and BMRF1 proteins at 0°C for 20 min (Fig. 1). The BMRF1 Pol accessory protein alone did not exhibit DNA Pol activity at any concentration of ammonium sulfate. As reported previously (29), the activity of the Pol catalytic subunit alone was sensitive to the salt (50% inhibition at 50 mM ammonium sulfate). However, addition of the BMRF1 Pol accessory protein to the reaction resulted in a drastic change in salt sensitivity. In the absence of ammonium sulfate, the BMRF1 Pol accessory protein inhibited Pol activity 13-fold. However, with increasing concentrations of ammonium sulfate, the Pol activity catalyzed by the BALF5 Pol catalytic subunit along with the BMRF1 protein was stimulated gradually and maximum stimulation was observed at around 100 to 125 mM ammonium sulfate. At 100 mM ammonium sulfate, the BMRF1 protein enhanced Pol activity more than 10-fold compared with that of the Pol catalytic subunit alone. Thus, the salt-stimulatory property of EBV DNA Pol was recovered by preincubation of both subunits in vitro.

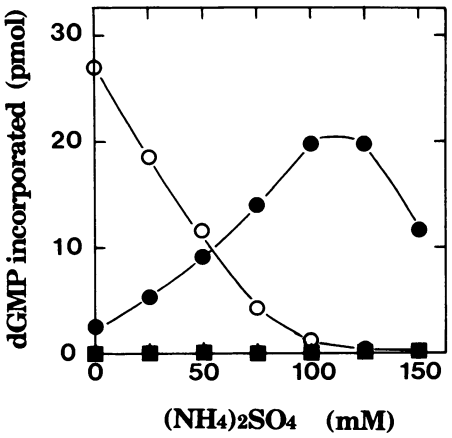

FIG. 1. Effect of ammonium sulfate on the Pol activity catalyzed by the BALF5 Pol catalytic subunit in the absence (○) and presence (●) of the BMRF1 Pol accessory subunit. DNA Pol activity was assayed by using an activated DNA template as described in Materials and Methods, except that ammonium sulfate concentrations were varied as indicated. Prior to the start of the reaction, the BALF5 Pol catalytic subunit (50 ng) and the BMRF1 protein (50 ng) were mixed and preincubated at 0°C for 20 min. The same amount of the BALF5 protein alone (○) or the BMRF1 protein alone (■) was also preincubated as described above. Datum points represent averages of duplicate assays.

Stoichiometric levels of BMRF1 protein with respect to BALF5 protein can stimulate DNA Pol activity. To determine the molar ratio of BMRF1 protein to BALF5 protein which can enhance EBV DNA Pol activity maximally, Pol assays were performed with an activated calf thymus DNA template in the presence of 100 mM ammonium sulfate (Fig. 2). Rate determination was based on 20 min of incubation under reaction conditions in which Pol activity was linear with time for at least 40 min (data not shown). A constant amount of BALF5 protein and the amounts of BMRF1 Pol accessory protein indicated in Fig. 2 were preincubated for 20 min at 0°C, and then the Pol assays were performed. Substoichiometric levels of BMRF1 protein with respect to BALF5 protein caused a dose-dependent increase in DNA synthesis. Maximal stimulation occurred at molar BMRF1/BALF5 protein ratios of 2 or more. Further addition of the BMRF1 protein did not change the maximum activity. Thus, the interaction of the Pol accessory protein with the Pol catalytic subunit was apparently stoichiometric.

Anti-BMRF1 protein MAb neutralizes the Pol activity catalyzed by the EBV DNA Pol holoenzyme reconstituted in vitro. Monoclonal antibody (MAb) 9240 (Dupont, NEN) is an anti-BMRF1 protein-specific MAb (19, 21) and is known to neutralize the DNA Pol activity of EBV DNA Pol purified from EBV-producing lymphoblastoid cells (2). The EBV DNA Pol catalytic subunit and the same amount of the BMRF1 Pol accessory subunit were mixed and preincubated at 0°C for 10 min. The amounts of MAb 9240 indicated in Fig. 3 were added to the mixture and further incubated at 0°C for 20 min. The

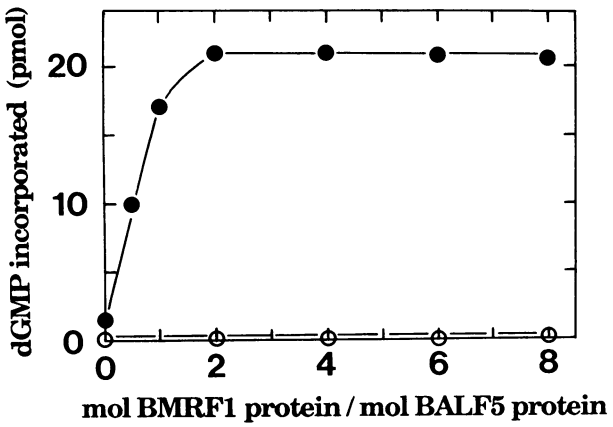

FIG. 2. Stimulation of the DNA Pol activity of the BALF5 Pol catalytic subunit by the BMRF1 Pol accessory subunit. Pol assays were performed on an activated DNA template in the presence of 100 mM ammonium sulfate. The BALF5 protein (50 ng) and the indicated amounts of the BMRF1 protein were mixed and preincubated at 0°C for 20 min, and then the reactions were carried out for 20 min at 35°C as described in Materials and Methods. Acid-insoluble dGMP incorporation was determined. Symbols: ●, reactions performed in the presence of the BALF5 protein; ○, reactions performed in the absence of the BALF5 protein. Datum points represent averages of duplicate assays.

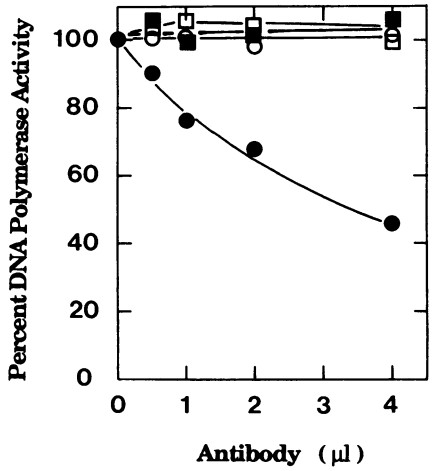

FIG. 3. Effect of anti-BMRF1 protein-specific MAb on the DNA Pol activity catalyzed by the BALF5 protein in the presence or absence of the BMRF1 protein. We mixed the BALF5 protein (50 ng) and the BMRF1 protein (50 ng), preincubated them at 0°C for 10 min, and then added MAb 9240 (R3 mouse immunoglobulin G1 MAb to EBV EA-D [19, 21]; Dupont, NEN) at 100 μg/ml (circles) or anti-BBLF4 protein-specific rabbit immunoglobulin at 1 mg/ml (squares) and further incubated the mixture for 20 min at 0°C. Pol assays were performed on an activated DNA template in the presence of 100 mM ammonium sulfate for 20 min at 35°C. The 100% value of the EBV DNA Pol activity in the presence of the BMRF1 protein was 20 pmol of dGMP incorporated per 20 min. Pol assays done in the absence of the BMRF1 protein were done without ammonium sulfate. The 100% value of EBV DNA Pol activity in the absence of the BMRF1 protein was 27 pmol of dGMP incorporated per 20 min. Datum points represent averages of duplicate assays. Closed symbols represent reactions performed in the presence of the BMRF1 protein. Open symbols are reactions performed in the absence of the BMRF1 protein.

DNA Pol reactions were carried out in the presence of 100 mM ammonium sulfate at 35°C for 20 min. The DNA Pol assays for the EBV DNA Pol catalytic subunit alone were performed in the absence of salt. The results are depicted in Fig. 3. MAb 9240 neutralized the DNA synthesis catalyzed by the BALF5 Pol catalytic subunit in the presence of the BMRF1 protein but had no effect on that of the EBV Pol catalytic subunit alone. The effect of an anti-BBLF4 protein-specific antibody (29) on EBV DNA Pol activity was also examined as a control. The anti-BBLF4 protein antibody did not affect the Pol activity catalyzed by the BALF5 protein in the presence or absence of the BMRF1 Pol accessory protein.

Specific interaction between the Pol catalytic subunit and its accessory subunit. To determine whether the effect of the EBV BMRF1 protein on DNA Pol activity is specific for the EBV BALF5 Pol catalytic subunit or not, the ability of the BMRF1 protein to influence the activities of other DNA Pols was examined. With activated calf thymus DNA as the template, we tested the effect of the BMRF1 protein on two other DNA Pols: the *Escherichia coli* PolI Klenow fragment and T4 DNA Pol (data not shown). The Pol assays were performed in the presence or absence of 100 mM ammonium sulfate under the reaction condition in which Pol activity was linear with time and dose. Neither stimulation nor inhibition of the Pol activity of the *E. coli* PolI Klenow fragment and T4 DNA Pol by the BMRF1 protein was observed in the presence or absence of ammonium sulfate. These findings indicate that the BMRF1 protein specifically interacts with the EBV DNA Pol catalytic subunit and rule out the possibility of a nonspecific stimulatory or inhibitory effect on the template or other components of the reaction mixture.

EBV DNA Pol accessory protein increases the polymerization rate and processivity of the Pol catalytic subunit. An oligodeoxynucleotide with the sequence CACAATTCCACA CAAC, complementary to nucleotides 6170 to 6185 of M13mp18 single-stranded DNA (30) was purchased from New England BioLabs, Inc. To form singly primed M13 single-stranded DNA, the synthetic 16-mer DNA was annealed at a molar ratio of 20:1 to M13mp18 single-stranded DNA in a buffer (20 mM Tris-HCl [pH 8.0], 5 mM $MgCl_2$, 0.3 M NaCl). The hybridization mixture was incubated at 90°C for 5 min, allowed to cool to room temperature for 1 h, and incubated for a further 1 h at 30°C. The primed M13 single-stranded DNA was separated from excess primer by centrifugation through a spun column of Chroma Spin-100 (Clontech Laboratory, Inc.).

To determine the function of the BMRF1 Pol accessory protein in determining the catalytic efficiency of the EBV DNA Pol holoenzyme on the long single-stranded DNA template, the holoenzyme was reconstituted onto singly primed M13mp18 single-stranded DNA circles by using stoichiometric levels of BALF5 protein along with BMRF1 protein. DNA synthetic processivity was measured by using the singly primed M13 template in an excess molar ratio of primer-template to Pol, so that each Pol molecule was bound to a primer terminus. In this assay, each Pol molecule catalyzes multiple rounds of synthesis, but the probability that a particular primer will be extended more than once is very low because of the low Pol-to-primer ratio that is maintained throughout the experiment. dATP and dGTP are needed to prevent removal of the DNA primer by the 3′-to-5′ exonuclease activity of the BALF5 protein. Synchronous DNA synthesis by the reconstituted forms of the holoenzyme was initiated upon addition of the remaining deoxynucleoside triphosphates, the reaction was sampled at various times, DNA synthesis was quantitated (Fig. 4A), and DNA products were analyzed in a native agarose gel

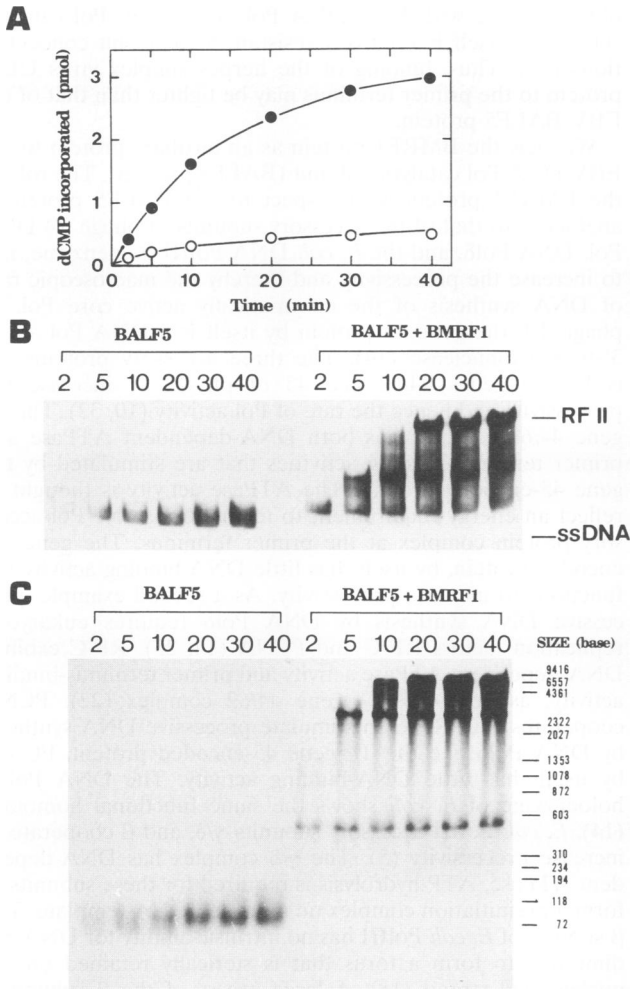

FIG. 4. Time course of DNA replication on singly primed M13 single-stranded DNA by the BALF5 Pol catalytic subunit in the presence (●) or absence (○) of the BMRF1 Pol accessory subunit. The BALF5 and BMRF1 proteins (115 ng of each) were preincubated at 0°C for 20 min and then added to a reaction mixture (175 μl) containing 50 mM Tris-HCl (pH 8.0), 3 mM $MgCl_2$, 1 mM dithiothreitol, 100 μg of bovine serum albumin per ml, 10% glycerol, 20 μg of singly primed M13 single-stranded DNA (8.3 pmol as a circle), 80 mM NaCl, 0.5 mM ATP, and 50 μM each dATP and dGTP and further incubated at 0°C for 5 min. To start the reaction, 50 μM dTTP and 4 μM [α-^{32}P]dCTP (3,000 Ci/mmol) were added, and the replication reaction was incubated at 35°C. Samples (25 μl) were removed at the times indicated and quenched by addition to an equal volume of 1% sodium dodecyl sulfate–50 mM EDTA. After quenching, the samples were divided into three parts. One was analyzed for DNA synthesis by measurement of the radioactivity incorporated into acid-insoluble material (A). The second was used for analysis of the replication products by electrophoresis in neutral 0.7% agarose gels (B). The third was used for analysis of the replication products by electrophoresis in an alkaline 1.2% agarose gel as previously described (20) (C). The arrows mark the positions of replicative form II DNA (RF II; circular duplex DNA with a small gap or nick in one strand) and singly primed single-stranded DNA (ssDNA) standards. Heat-denatured 5′-terminally labeled *Hin*dIII DNA fragments of λ DNA and *Hae*III DNA fragments of φX174 DNA were run as size markers. The positions of the nucleotide length standards are indicated at the right of panel C. Removal of ATP from the reactions had no effect on DNA synthesis. The BMRF1 protein alone exhibited no DNA Pol activity.

(Fig. 4B) or in an alkaline agarose gel (Fig. 4C). The reaction without added Pol verified the lack of Pol contamination in the BMRF1 Pol accessory protein (data not shown).

With substoichiometric levels of the EBV Pol catalytic subunit alone, low levels of DNA synthesis were detected (Fig. 4A). The EBV Pol catalytic subunit extended the primer slightly (~50 nucleotides), and no full-length product was observed (Fig. 4B and C). Analyses of the replication products in an alkaline agarose gel showed no increase in product size throughout the time course, indicating the distributive action of the Pol catalytic subunit in the absence of the BMRF1 Pol accessory protein. Addition of the BMRF1 protein, however, resulted in accumulation of full-length replicative form II. Some part of the products consisted of specific bands of pause sites on the template. We presume that these pause sites represent sites of a substantial helical region on the single-stranded DNA template. In the presence of the BMRF1 protein, the BALF5 Pol catalytic protein moved through these barriers and completed synthesis of some of the 7.2-kb M13mp18 template within 20 min. Most of the primed single-stranded DNA remained unchanged, as detected by UV-induced ethidium bromide fluorescence during the time in which full-length products (replicative form II) were formed (data not shown). These findings support the highly processive mode of nucleotide polymerization (>7,200 nucleotides) by the EBV DNA Pol holoenzyme reconstituted in vitro, consistent with previous findings on the EBV DNA Pol holoenzyme purified from EBV-producing lymphoblastoid cells (25). The 20 min required for complete replication of an M13 single-stranded DNA circle (7.2 kb) yields an average nucleotide turnover of six nucleotides per s per Pol molecule. In the absence of the accessory protein, the template was replicated at a rate of about 1.5 nucleotides per s (quantified by measuring the kinetics of deoxyribonucleotide incorporation). Thus, addition of the BMRF1 protein resulted in a highly processive mode of polymerization and thereby at least fourfold stimulation of the rate of incorporation to six nucleotides per s.

Discussion and Conclusions. Several lines of evidence support the view that EBV DNA Pol is a holoenzyme consisting of the BALF5 and BMRF1 proteins (11, 15, 24). Despite extensive efforts, it has been difficult to isolate the BALF5 Pol catalytic subunit free from the BMRF1 Pol accessory subunit (28). Only by cloning the genes for BALF5 and BMRF1 and overproducing these products did it become possible to specify their functions. Therefore, we separately overexpressed these proteins in a baculovirus expression system and purified them to homogeneity. In this study, we compared the enzymatic properties of the DNA Pol activity catalyzed by the BALF5 Pol catalytic subunit in the presence or absence of the BMRF1 Pol accessory protein by using a short or long single-stranded DNA template to facilitate the study of the role of each of these two components in the EBV DNA Pol reaction.

Under conditions of an excess of a long single-stranded DNA template over BALF5 protein molecules, the BALF5 protein initiates DNA synthesis from the same number of primers in the absence as in the presence of the BMRF1 protein. In the absence of the BMRF1 protein, the extensions are short, around 50 nucleotides long. Even when given sufficient time for DNA synthesis, the product size was unchanged although nucleotide incorporation increased with time. The BALF5 protein dissociates from the primer after incorporation of around 50 nucleotides. Thus, the BALF5 Pol catalytic subunit by itself is a quasiprocessive enzyme and acts distributively. Furthermore, in the gap-filling reaction on an activated calf thymus DNA template, increasing concentrations of ammonium sulfate progressively reduced the DNA Pol

activity catalyzed by the EBV DNA Pol catalytic subunit. On the basis of these findings, we speculate that the binding affinity of the Pol catalytic subunit for the primer terminus is weak and that high ionic strength destabilizes the catalytic protein-primer terminus interaction. With the BMRF1 Pol accessory protein present in the reaction, the salt dependency of EBV DNA Pol activity changed drastically. Although there is no direct evidence that the BALF5 and BMRF1 proteins form a complex in vitro, the BMRF1 Pol accessory protein appears to interact with the BALF5 Pol catalytic subunit and stabilize the binding of the BALF5 Pol catalytic subunit to the primer terminus. The complex formation appears to be specific and tight, because maximal stimulation of EBV DNA Pol activity was obtained with stoichiometric levels of BMRF1 protein with respect to the BALF5 protein present. In the presence of the BMRF1 protein, the BALF5 protein polymerizes thousands of nucleotides on the same primer template without dissociation, while the Pol catalytic subunit alone normally falls off the growing 3′ end of the DNA chain. The double-stranded DNA-binding activity of the BMRF1 protein may play a significant role in the affinity between the holoenzyme and the growing 3′-OH end of the primer template. To clarify these speculations, an in vitro study of the interaction between the BALF5 and BMRF1 proteins with regard to binding affinity for the primer terminus is in progress.

Unexpectedly, in the absence of ammonium sulfate, the BMRF1 protein greatly inhibited the DNA Pol activity of the BALF5 protein on an activated DNA template. However, the BMRF1 protein did not inhibit the DNA Pol activity catalyzed by the *E. coli* PolI large fragment or bacteriophage T4 DNA Pol on an activated DNA template (data not shown). Although the mechanism for Pol inhibition by the BMRF1 protein at low ionic strength is not clear, the above-described results indicate that the inhibition is also due to a specific interaction between the BALF5 and BMRF1 proteins.

Kiehl and Dorsky (12) have reported that the EBV Pol catalytic subunit alone exhibited no DNA Pol activity in an in vitro translation system. However, expression of both the EBV BALF5 and BMRF1 proteins generated DNA Pol activity in the reticulocyte lysate. On the other hand, Lin et al. (16) have found that the BALF5 protein exhibited DNA Pol activity in an in vitro translation system. However, addition of the BMRF1 protein resulted in a decrease in Pol activity. Our study has solved the discrepancy between them; the salt concentration assayed affects EBV DNA Pol activity strongly. That is to say, at low salt concentrations the BALF5 protein exhibits DNA Pol activity but addition of the BMRF1 protein decreases Pol activity. At high salt concentrations the BALF5 protein exhibits little Pol activity but addition of the BMRF1 protein increases Pol activity greatly.

The BMRF1 Pol accessory protein increased the processivity and thereby the polymerization rate of the BALF5 protein to six nucleotides per s. The value obtained with the EBV DNA Pol holoenzyme reconstituted in vitro is close to 12 nucleotides per s, the value obtained with the EBV DNA Pol holoenzyme purified from EBV-producing lymphoblastoid cells (25). Addition of the EBV single-stranded DNA-binding protein (BALF2 protein) to the reaction mixture may increase the polymerization rate more. Further study is needed.

The herpes simplex virus type 1 UL42 protein is a herpes simplex virus DNA Pol accessory protein that increases the processivity of polymerization (4, 7, 9). The UL42 protein also has double-stranded DNA-binding activity and specifically interacts with the UL30 Pol catalytic protein. Gottlieb et al. (7) have reported that the UL42 protein had no effect on the DNA Pol activity of the UL30 Pol catalytic subunit when activated DNA was used as a primer template, in contrast to the results obtained here with EBV DNA Pol. The UL30 Pol catalytic subunit by itself is relatively resistant to high salt concentrations (17). Thus, binding of the herpes simplex virus UL30 protein to the primer terminus may be tighter than that of the EBV BALF5 protein.

We view the BMRF1 protein as an auxiliary protein to the EBV DNA Pol catalytic subunit (BALF5 protein). The role of the BMRF1 protein with respect to the BALF5 protein is analogous to that of the accessory subunits of phage T4 DNA Pol, DNA Polδ, and the *E. coli* DNA PolIII holoenzyme, i.e., to increase the processivity and thereby the macroscopic rate of DNA synthesis of the enzymatically active core Pol. In phage T4, the gene 43 protein by itself is a DNA Pol and a 3′-to-5′ exonuclease (14). The three accessory proteins encoded by genes 44/62 and 45 of phage T4 increase the processivity and hence the rate of Pol activity (10, 32). The T4 gene 44/62 complex has both DNA-dependent ATPase and primer terminus-binding activities that are stimulated by the gene 45-encoded protein. The ATPase activity is thought to reflect an energy requirement to maintain a stable Pol-accessory protein complex at the primer terminus. The gene 45-encoded protein, by itself, has little DNA-binding activity but functions to increase processivity. As a second example, processive DNA synthesis by DNA Polδ requires eukaryotic replication factors RF-C and PCNA (14, 23). RF-C exhibits DNA-dependent ATPase activity and primer terminus-binding activity, as does the T4 gene 44/62 complex (22). PCNA cooperates with RF-C to stimulate processive DNA synthesis by DNA Polδ like the T4 gene 45-encoded protein. PCNA, by itself, has little DNA-binding activity. The DNA PolIII holoenzyme of *E. coli* shows the same functional homology (14). *E. coli* PolIII accessory subunits γ/δ, and β cooperate to increase processivity (5). The γ/δ complex has DNA-dependent ATPase. ATP hydrolysis is required for these subunits to form a preinitiation complex on a primed DNA template. The β subunit of *E. coli* PolIII has no intrinsic affinity for DNA but dimerizes to form a torus that is sterically retained on the nucleic acid strand (18). A large excess of the β subunit is required, probably because of the weak interaction of the β subunit with DNA PolIII* (3). In all three systems, the action of the accessory proteins can be visualized as clamping of the core Pol to the primer template, enabling processive DNA synthesis to occur. In contrast to these complex cases, the BMRF1 protein appears to be simple and bifunctional. Although the BMRF1 protein does not exhibit DNA-dependent ATPase activity (27), it possesses double-stranded DNA-binding activity for primer recognition and acts as a sliding clamp to increase processivity. The DNA-binding property of the EBV BMRF1 protein is different from that of the accessory proteins of the three systems. For example, the BMRF1 protein binds double-stranded DNA without ATP hydrolysis and does not necessarily require a primer terminus, whereas RF-C binds specifically to the primer terminus junction but not to single- or double-stranded DNA (22). Thus, the BMRF1 protein efficiently stabilizes the BALF5 protein at the primer terminus without an energy requirement and slides on the single-stranded DNA template. However, it remains to be clarified how the BMRF1 Pol accessory protein can slide to follow the elongating primer without acting as a brake.

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