Requirements for Epstein-Barr Nuclear Antigen 1 (EBNA1)-induced Permanganate Sensitivity of the Epstein-Barr Virus Latent Origin of DNA Replication*

(Received for publication, June 5, 1997, and in revised form, August 18, 1997)

Heather Summers, Angela Fleming and Lori Frappier‡

From the Cancer Research Group, Institute for Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario, Canada L8N 3S5

Epstein-Barr nuclear antigen 1 (EBNA1) activates DNA replication from the Epstein-Barr virus latent origin of DNA replication, oriP. EBNA1 binds cooperatively to four recognition sites in the dyad symmetry (DS) element of oriP, causing alterations in the origin DNA structure, which can be detected by the increased sensitivity of one Thy residue in two of the binding sites to permanganate oxidation. To better understand the significance of this EBNA1-induced origin distortion, we have investigated the DNA sequence and EBNA1 amino acid requirements for permanganate sensitivity. We have shown that the EBNA1 DNA binding and dimerization domains are sufficient to induce permanganate sensitivity and that amino acids 463–467, which form an extended chain that travels along the minor groove of the EBNA1 recognition site, play an important role in generating the DNA distortion. The EBNA1-induced permanganate sensitivity is independent of cooperative interactions between EBNA1 molecules on the origin and requires a specific sequence within the EBNA1 binding site. Using synthetic EBNA1 binding sites, we found that the inversion of a single AT base pair in the EBNA1 recognition sequence is sufficient to confer EBNA1-induced permanganate sensitivity. These studies indicate that permanganate oxidation can detect very minor alterations in DNA structure.

The first step in the initiation of DNA synthesis in all replication systems involves the melting of the origin DNA. In many systems the disruption of the hydrogen bonds between the bases is a function of the origin binding protein, but in others the interaction of the origin binding protein with the origin is not sufficient to melt the DNA and additional factors are required (for review, see Refs. 1–3). Origin binding proteins that do not melt DNA might, however, contribute to the melting process by destabilizing the structure of the DNA helix. To better understand how localized DNA distortions caused by origin-binding proteins contribute to origin melting, we have examined the structural changes in origin DNA induced by Epstein-Barr virus nuclear antigen 1 (EBNA1). ¹

¹This work was supported by a grant from the National Cancer Institute of Canada (NCIC), which receives funds from the Canadian Cancer Society. 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.

‡Research Scientist of the NCIC. Present address and to whom correspondence should be addressed: Dept. of Medical Genetics and Microbiology, University of Toronto, 1 Kings College Circle, Toronto, Ontario, Canada M5S 1A8. Tel.: 416-946-3501; Fax: 416-978-6885.

¹The abbreviations used are: EBNA1, Epstein-Barr nuclear antigen 1; bp, base pair(s); DS, dyad symmetry; FR, family of repeats; CS, consensus binding site; CSA/T, consensus binding site with an inversion of the AT base pairs at position 6 and 9 (CSA/T) were constructed from the oligonucleotides.

DNA—The construction of pGEMdyad, which contains the DS element of oriP, and pGEMs1, which contains only EBNA1 binding site 1 from the DS element, have been previously described (17, 21). Plasmids containing the palindromic EBNA1 consensus binding site (CS) or the consensus binding site with an inversion of the AT base pairs at position 6 and 9 (CSA/T) were constructed from the oligonucleotides 5′-GGG-TAGCATATGCTAACC-3′ and 5′-GGGAAGCATATGCTTCCC-3′, respectively.

EXPERIMENTAL PROCEDURES

DNA—The construction of pGEMdyad, which contains the DS element of oriP, and pGEMs1, which contains only EBNA1 binding site 1 from the DS element, have been previously described (17, 21). Plasmids containing the palindromic EBNA1 consensus binding site (CS) or the consensus binding site with an inversion of the AT base pairs at position 6 and 9 (CSA/T) were constructed from the oligonucleotides 5′-GGG-TAGCATATGCTAACC-3′ and 5′-GGGAAGCATATGCTTCCC-3′, respectively.

This paper is available on line at http://www.jbc.org

26434
respectively. Each of these oligonucleotides was annealed to itself and then cloned into the Smal site of pBluescript KS to generate pCS and pCSAT. The construct for the expression of EBNAwp was generated by amplifying the EBNA1 gene in two fragments, which encode amino acids 452 to 463 and amino acids 464 to 641, by PCR. The 452–463 fragment was amplified using the primers 5′-CGTGGCATATGTCGGGTGAT-3′ (452 primer) and 5′-CCCTCCTTTTTGCGCCT-3′. The 464–641 fragment was amplified using the primers 5′-GGGCGGCGG-GAAAACGATCTGGTGCAA-3′ and 5′-CCCTCAGGATCCTCTACTCCT-GCCCCTCCTACCC-3′ (641 primer), which converts the Trp and Phe residues at positions 464 and 465 to alanines. The two fragments of the EBNA1 gene were phosphorylated using T4 polynucleotide kinase and ligated together. The ligation products were amplified by PCR using the 452 and 641 primers listed above, which generates an Ndel site at the N terminus and a BamHI site at the C terminus. PCR products were digested with Ndel and BamHI and inserted between the Ndel and BamHI sites of expression vector pET15b (Novagen), downstream of a 6-histidine tag. The EBNA1 fragment in this construct was sequenced and confirmed to be correct except that amino acid 465 was converted to a serine instead of an alanine.

**Protein Expression and Purification—**

beEBNA1 was produced in SF-9 insect cells and purified to homogeneity as described previously (9). EBNA459–619, EBNA463–607, and EBNA468–607 were produced in E. coli and purified to homogeneity as described previously (16, 22). For expression of EBNA1, the pET15b expression construct was used to transform the BL21(DE3) pLysS strain of E. coli, which expresses the β-galactosidase enzyme. The β-galactosidase enzyme was purified and confirmed to be correct except that amino acid 465 was converted to a serine instead of an alanine.

**RESULTS**

**EBNA1 Amino Acids Required for DS DNA Distortion—**

Structural changes in the DS element of oriP, which can be detected by permanganate oxidation, are induced by wild type and internally deleted versions of EBNA1 (15, 18). We wished to determine if the DNA binding and dimerization domains of EBNA1 were sufficient to induce this DNA distortion or if other EBNA1 domains were required. To this end, we compared the permanganate reactivity patterns of the DS element when bound by EBNA459–619, which contains only the DNA binding and dimerization domains, with that induced by a biologically active version of EBNA1 (beEBNA1; Fig. 1). As shown in Fig. 2, beEBNA1 and EBNA459–619 generated identical permanganate reactivity patterns when titrated onto the DS element. Both proteins caused one Thy residue within EBNA1 binding site 1 and one Thy residue within EBNA1 binding site 4, on the opposite DNA strand, to become oxidized by KMnO4. For simplicity, results are shown for one DNA strand only (detecting the oxidized T in site 1); however, in all cases where permanganate sensitivity was detected in site 1, it was also detected in site 4 (data not shown).

We have previously shown that amino acids 461 to 469 from the EBNA1 flanking DNA binding domain forms an extended amino acid chain that travels along the minor groove of the EBNA1 flanking DNA binding domain (20) and the nonessential Gly-Ala repeat (6).

![FIG. 1. The EBNA1 mutants. EBNA1 amino acid numbers are indicated, as are the core and flanking DNA binding domains (20) and the nonessential Gly-Ala repeat (6).](image)
EBNA468–607 on the DNA templates used for the permanganate experiments. The results in Fig. 2B show that all of the EBNA1 proteins, including EBNA468–607, bound to the DS element at the protein concentrations used in the permanganate experiments. For bEBNA1, EBNA459–619, and EBNA463–607, simultaneous filling of the four recognition sites of the DS was observed, at 10 to 30 pmol of protein for both bEBNA1 and EBNA459–619 and at 90 pmol for EBNA463–607. This is the same pattern of filling of the DS that has been reported previously for bEBNA1 (18). In this assay, binding to each of the four binding sites is indicated by the lightening of the bands indicated by the arrows in Fig. 2B. Binding to site 4 is also detected by the appearance of the band indicated by the asterisk, which represents a DMS-hypersensitive adenine. Unlike the larger EBNA1 proteins, EBNA468–607 was observed to bind only to sites 1 and 4 of the DS (at 90–270 pmol of protein); interactions with sites 2 and 3 were not detected at any of the protein concentrations tested.

**DNA Requirements for EBNA1-induced DS Distortion**—The failure of EBNA468–607 to induce permanganate reactivity within sites 1 and 4 of the DS element could be interpreted in either of two ways. First, it might indicate that EBNA1 sequences between amino acid 463 and 468 are required to distort sites 1 and 4. Second, it might indicate that binding to sites 2 and/or 3 is required for the induction of permanganate reactivity within sites 1 and 4 since EBNA468–607 was the only EBNA1 protein tested that did not bind sites 2 and 3. To distinguish between these possibilities, we repeated the permanganate assays on DNA templates that contained only EBNA1 binding site 1 (pGEMs1). The results in Fig. 4 show that the binding of EBNA459–619 and EBNA463–607 to site 1 alone is sufficient to cause permanganate oxidation of the same Thy residue that is reactive in the complete DS sequence. As was the case with the DS DNA templates, induction of perman-
EBNA1-induced Permanganate Sensitivity

FIG. 4. Interactions of EBNA1 mutants with isolated site 1. EBNA1 proteins were titrated onto pGEMs1 containing site 1. A, permanganate assays performed on supercoiled (left panel) and linear (right panel) DNA templates. The position of the EBNA1-induced permanganate-sensitive Thy in site 1 is indicated (arrow). B, methylation protection footprints performed on supercoiled pGEMs1. Arrow indicates the position of the Gua residue that is most obviously and most reproducibly protected by EBNA1.

The role of Trp-464 and Phe-465 in inducing the permanganate reactive T in site 1—The crystal structure of the EBNA1 DNA binding and dimerization domains bound to DNA revealed a peculiar arrangement of the Trp-464 and Phe-465 side chains within the minor groove of the DNA (20) (see Fig. 3). The aromatic rings of these residues are oriented parallel with the minor groove and appear to be pushing on the two sugar phosphate backbones. The minor groove of the DNA is widened by 2–3 Å at this point (20). To determine whether Trp-464 and Phe-465 are responsible for generating the DNA distortion that leads to permanganate reactivity, we repeated the permanganate assays using a version of EBNA1 that contains the complete DNA binding and dimerization domains bound to a single EBNA1 binding site (20). This binding site is a palindromic consensus sequence (Fig. 6) to which EBNA1 binds with high affinity but which does not actually exist in oriP. In the EBNA1-consensus DNA structure, there was no disruption of the hydrogen bonds between the bases of the two DNA strands nor was there any other obvious distortion of the DNA structure that might be expected to cause permanganate sensitivity. However, since little is known about the DNA structural requirements for permanganate oxidation, we tested the EBNA1-induced permanganate sensitivity of the consensus EBNA1 binding site. For these experiments, the consensus 18-bp binding site shown in Fig. 6 was cloned into pBluescriptKS to generate pCS. When bEBNA1 and EBNA468–607 were titrated onto pCS, binding to the consensus site was detected by DMS footprint analysis at as little as 10 pmol of protein (Fig. 7B), but no EBNA1-induced permanganate reactivity was detected at any amount of protein tested (10–90 pmol) (Fig. 7A). Some degree of permanganate reactivity was observed for Thy residues at positions 2 and −1 within the consensus sequence, but this sensitivity was independent of EBNA1.

A comparison of the sequences of the EBNA1 binding sites in the origin and the consensus EBNA1 binding site is shown in Fig. 6. For both of the EBNA1 binding sites that have been previously protected by EBNA1.

2 A. Fleming, and L. Frappier, unpublished data.
EBNA1-induced Permanganate Sensitivity

Permanganate oxidation is commonly used to detect alterations in the structure of DNA helices, but the structural requirements for permanganate sensitivity are not well understood. While double-strand DNA is largely insensitive to permanganate oxidation, pyrimidines (particularly Ts) in single-strand DNA are oxidized at the 5,6-double bond by this reagent to form pyrimidine glycols (24, 25). Therefore permanganate is useful for the detection of melted regions of double-strand DNA (26). In some cases, however, permanganate-sensitive residues have been detected in regions of double-strand DNA that, by other criteria, are not melted. For example, the lac repressor (27), SV40 large T antigen (28), repressor activator protein 1 (RAP1) (29), and EBNA1 (18, 19) have all been reported to induce localized structural changes in the DNA to which they bind, resulting in the increased sensitivity of one or more T residues to permanganate oxidation. The nature of the structural changes that cause these permanganate sensitivities are not clear.

Although EBNA1 does not appear to melt oriP DNA, several observations suggest that the interaction of this protein with its recognition sites in the DS element of oriP structurally alters the DNA helix. First, electron microscopic studies of EBNA1 on the DS element show pronounced bending of the DS DNA at the position where the four EBNA1 dimers are assembled (17). Second, modeling studies using the EBNA1-DNA co-crystal structure suggest that the cooperative assembly of EBNA1 dimers on adjacent binding sites in the DS must be accompanied by a change in structure of the DNA, most likely unwinding and/or unbending of the DNA (20). Third, EBNA1 binding to the DS element, both in vivo and in vitro, has been shown to induce permanganate sensitivity in one Thy residue in each of the two outer EBNA1 binding sites (15, 18, 19).

The studies presented here were conducted to better understand the nature of and the requirements for the EBNA1-induced distortion of the DS element that results in permanganate sensitivity. We have shown that this permanganate sensitivity occurs on single EBNA1 binding sites, and therefore the DNA distortion detected by permanganate is distinct from that predicted by the modeling studies to accompany cooperative assembly of EBNA1 on two adjacent sites of the DS. This conclusion is consistent with the results of Harrison et al. (14), who showed that point mutations in sites 2 or 3 in the DS element did not abrogate the EBNA1-induced permanganate sensitivity of sites 1 and 4 and that this sensitivity was independent of the spacing between the EBNA1 binding sites. In their experiments, Harrison et al. (14) used the full-length EBNA1 protein, which contains a domain that mediates interactions at a distance between DNA-bound EBNA1 molecules (21, 30–32). Therefore, although the cooperative filling of adjacent sites was disrupted by virtue of their DNA templates, it is likely that interactions occurred between EBNA1 molecules on distant sites (i.e. between sites 1 and 4, 2 and 4, or 1 and 3, and between different DNA molecules). Since EBNA1 interac-

**DISCUSSION**

Permanganate oxidation is commonly used to detect alterations in the structure of DNA helices, but the structural requirements for permanganate sensitivity are not well understood. While double-strand DNA is largely insensitive to permanganate oxidation, pyrimidines (particularly Ts) in single-strand DNA are oxidized at the 5,6-double bond by this reagent to form pyrimidine glycols (24, 25). Therefore permanganate is useful for the detection of melted regions of double-strand DNA (26). In some cases, however, permanganate-sensitive residues have been detected in regions of double-strand DNA that, by other criteria, are not melted. For example, the lac repressor (27), SV40 large T antigen (28), repressor activator protein 1 (RAP1) (29), and EBNA1 (18, 19) have all been reported to induce localized structural changes in the DNA to which they bind, resulting in the increased sensitivity of one or more T residues to permanganate oxidation. The nature of the structural changes that cause these permanganate sensitivities are not clear.

Although EBNA1 does not appear to melt oriP DNA, several observations suggest that the interaction of this protein with its recognition sites in the DS element of oriP structurally alters the DNA helix. First, electron microscopic studies of EBNA1 on the DS element show pronounced bending of the DS DNA at the position where the four EBNA1 dimers are assembled (17). Second, modeling studies using the EBNA1-DNA co-crystal structure suggest that the cooperative assembly of EBNA1 dimers on adjacent binding sites in the DS must be accompanied by a change in structure of the DNA, most likely unwinding and/or unbending of the DNA (20). Third, EBNA1 binding to the DS element, both in vivo and in vitro, has been shown to induce permanganate sensitivity in one Thy residue in each of the two outer EBNA1 binding sites (15, 18, 19).

The studies presented here were conducted to better understand the nature of and the requirements for the EBNA1-induced distortion of the DS element that results in permanganate sensitivity. We have shown that this permanganate sensitivity occurs on single EBNA1 binding sites, and therefore the DNA distortion detected by permanganate is distinct from that predicted by the modeling studies to accompany cooperative assembly of EBNA1 on two adjacent sites of the DS. This conclusion is consistent with the results of Harrison et al. (14), who showed that point mutations in sites 2 or 3 in the DS element did not abrogate the EBNA1-induced permanganate sensitivity of sites 1 and 4 and that this sensitivity was independent of the spacing between the EBNA1 binding sites. In their experiments, Harrison et al. (14) used the full-length EBNA1 protein, which contains a domain that mediates interactions at a distance between DNA-bound EBNA1 molecules (21, 30–32). Therefore, although the cooperative filling of adjacent sites was disrupted by virtue of their DNA templates, it is likely that interactions occurred between EBNA1 molecules on distant sites (i.e. between sites 1 and 4, 2 and 4, or 1 and 3, and between different DNA molecules). Since EBNA1 interac-

![Fig. 5. The role of W464 and F465 in site 1 distortion. EBNAWF was titrated onto pGEMs1, and permanganate assays (KMnO4) or methylation footprints (DMS) were performed. Arrows mark the position of the permanganate-sensitive T in the permanganate assay and the protected G in the methylation protection footprints.](Image 95x414 to 261x729)

**FIG. 5.** The role of W464 and F465 in site 1 distortion. EBNAWF was titrated onto pGEMs1, and permanganate assays (KMnO4) or methylation footprints (DMS) were performed. Arrows mark the position of the permanganate-sensitive T in the permanganate assay and the protected G in the methylation protection footprints.

![Fig. 6. EBNA1 binding sites. The four EBNA1 binding sites from the DS element and the consensus binding site with and without the AT inversion at position 6/6 are shown. EBNA1-induced permanganate-sensitive Thy residues are in bold type.](Image 326x584 to 545x729)

**FIG. 6.** EBNA1 binding sites. The four EBNA1 binding sites from the DS element and the consensus binding site with and without the AT inversion at position 6/6 are shown. EBNA1-induced permanganate-sensitive Thy residues are in bold type.
tions at a distance might have substituted for the interaction between EBNA1 molecules on adjacent sites in inducing permanganate sensitivity, we felt that it was important to test permanganate sensitivity under conditions in which neither type of EBNA1 interaction was possible (i.e. on individual binding sites using EBNA1 proteins that lack the domain that mediates interactions at a distance).

We have explored the EBNA1 amino acids required to induce permanganate reactivity and found that amino acids 463–607, containing the DNA binding and dimerization domains of EBNA1, are sufficient to elicit this response. Deletion of part of the flanking DNA binding domain that forms an extended chain positioned along the base of the minor groove of the EBNA1 binding site (amino acids 463–467) abrogated the ability of EBNA1 to induce permanganate reactivity. We have previously shown, using electrophoretic mobility shift assays, that this deletion had little effect on the ability of EBNA1 to bind to site 1 from the DS element (Kd for EBNA463–607 and EBNA468–607 was 110 and 125 nM, respectively) (16). The methylation protection footprints presented here also confirm that EBNA468–607 binds site 1, both in the context of the DS element and in isolation. However, the weaker footprint generated by this protein suggests that EBNA468–607 binds less tightly to site 1 than the larger versions of EBNA1 tested. Therefore, the requirement for amino acids 463–467 for permanganate reactivity could either reflect a specific requirement for the extended chain in the minor groove of the DNA or a requirement for tight binding. We favor the former possibility because other mutations in the EBNA1-flanking DNA binding domain that reduce binding to site 1 still induce permanganate sensitivity. An example of this is the EBNA467–607 mutant. This mutant gives a weaker footprint on site 1 (Fig. 5) and binds site 1 with an affinity that is approximately 15-fold lower than the same fragment of EBNA1 with the wild-type sequence (as determined by electrophoretic mobility shift assays), yet EBNA467–607 still induces permanganate reactivity in site 1.

Investigation of the DNA sequence requirements for EBNA1-induced permanganate reactivity revealed that the differences in permanganate sensitivity of different EBNA1 binding sites is solely due to the inversion of a single AT base pair at position 6/–6 relative to the axis of dyad symmetry of the palindromic binding site. Permanganate sensitivity is only seen when the Thy residue is at position 6, generating the sequence CCCTTCGTA. This suggests that EBNA1 binding to this sequence causes a distortion in the DNA structure that does not occur when EBNA1 binds to recognition sites containing the sequence CCCATCGTA. This distortion might be facilitated by the 6-bp polypurine/polypyrimidine tract that is generated by the AT inversion since polypurine/polypyrimidine tracts have a tendency to adopt alternative DNA structures (33). Alternatively, the structures of the EBNA1-bound CCCTTCGTA and CCCATCGTA binding sites might be the same, and the Thy residue at position –6 might simply be inaccessible to the permanganate reagent due to positioning of EBNA1 amino acids. These two alternative interpretations can only be resolved by solving the high resolution structure of the EBNA1 DNA binding and dimerization domains on binding sites that are and are not permanganate-sensitive.

We have already solved the structure of the EBNA1 DNA binding and dimerization domains on the consensus binding site that is not permanganate sensitive (20). In keeping with the results of the permanganate assays on the consensus site, distortion of the DNA helix at position 6/–6 was not apparent from this structure. We have also solved the structure of the EBNA1 DNA binding domain on the permanganate-sensitive

![Figure 7](image-url)
site 1 at 2.8 Å resolution. The structure of the site 1 DNA was indistinguishable from that of the consensus site, however the particular packing arrangement of EBNA1 in the crystal form used to solve the structure would permit the EBNA1-DNA complex to crystallize in either of two orientations. As a result, the final structure of the site 1 DNA might represent an average of the two halves, and hence even moderate structural perturbations on one side of the pseudopalindromic DNA may not be revealed. To conclusively determine the structure of EBNA1-bound permanganate-sensitive DNA, we require a palindromic permanganate-sensitive EBNA1 binding site in which the structural distortion would be manifested on both halves of the palindrome. We have shown that we can generate such a binding site by inverting the AT base pair at position 6/6 in both halves of the consensus palindrome. This finding should facilitate the structural determination of permanganate-sensitive DNA bound by EBNA1, which in turn will help us to understand the structural requirements for permanganate oxidation.

Acknowledgments—We gratefully acknowledge Dr. Alexey Bochkarev for Fig. 3, Elena Bochkareva for the pCS and pCSA/T constructs, and Kathy Shire for technical assistance. We also thank Dr. Aled Edwards for helpful comments throughout the course of this work and critical reading of the manuscript.

REFERENCES

1. Brush, G. S., and Kelly, T. J. (1996) in DNA Replication in Eukaryotic Cells (DePamphilis, M. L., ed) p. 1–44, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2. DePamphilis, M. L. (1996) in DNA Replication in Eukaryotic Cells (DePamphilis, M. L., ed) p. 45–86, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

3. Kelman, Z., and O’Donnell, M. (1994) Curr. Opin. Genet. Dev. 4, 185–195
4. Adams, A. (1987) J. Virol. 61, 1743–1746
5. Yates, J. L., and Guan, N. (1991) J. Virol. 65, 483–488
6. Yates, J. L., Warren, N., and Sugden, B. (1985) Nature 313, 812–815
7. Rawlins, D., Milman, G., Hayward, S. D., and Hayward, G. S. (1985) Cell 42, 859–868
8. Reisman, D., Yates, J., and Sugden, B. (1985) Mol. Cell. Biol. 5, 1822–1832
9. Frappier, L., and O’Donnell, M. (1991) J. Biol. Chem. 266, 7819–7826
10. Ambinder, R. F., Mullen, M., Chang, Y., Hayward, G. S., and Hayward, S. D. (1991) J. Virol. 65, 1466–1478
11. Gahn, T. A., and Schildkraut, C. L. (1989) Cell 58, 527–535
12. Niller, H. H., Glaser, G., Knauch, R., and Wolf, H. (1995) J. Biol. Chem. 270, 12864–12868
13. Wysockenski, D. A., and Yates, J. L. (1989) J. Virol. 63, 2657–2666
14. Harrison, S., Fisenne, K., and Hearing, J. (1994) J. Virol. 68, 1915–1925
15. Hsieh, D.-J., Camilo, M. S., and Yates, J. L. (1993) EMBO J. 12, 4933–4944
16. Summers, H., Barwell, J. A., Pfitzner, R. A., Edwards, A. M., and Frappier, L. (1996) J. Virol. 70, 1228–1231
17. Frappier, L., and O’Donnell, M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10875–10879
18. Frappier, L., and O’Donnell, M. (1992) J. Virol. 66, 1786–1790
19. Hearing, J., Mulhaupt, Y., and Harper, S. (1992) J. Virol. 66, 941–970
20. Bochkarev, A. Barwell, J., Pfitzner, R., Bochkareva, E., Frappier, L., and Edwards, A. M. (1996) Cell 84, 791–800
21. Goldsmith, K., Bendell, L., and Frappier, L. (1993) J. Virol. 67, 3418–3426
22. Barwell, J., Bochkarev, A., Pfitzner, R., Tong, H., Yang, D., Frappier, L., and Edwards, A. (1995) J. Biol. Chem. 270, 20556–20559
23. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Methods Enzymol. 185, 60–89
24. Hayatsu, H., and Ukita, T. (1967) Biochem. Biophys. Res. Commun. 29, 556–561
25. Iida, S., and Hayatsu, H. (1970) Biochim. Biophys. Acta 213, 1–13
26. Sasse-Dwight, S., and Gralla, J. D. (1989) J. Biol. Chem. 264, 8074–8081
27. Boroweic, J. A., Zhang, L., Sasse-Dwight, S., and Gralla, J. D. (1987) J. Mol. Biol. 196, 101–111
28. Boroweic, J. A., Dean, F. B., and Hurwitz, J. (1991) J. Virol. 65, 1228–1235
29. Gilson, E., Roberge, M., Giraldo, R., Rhodes, D., and Gasser, S. M. (1993) J. Mol. Biol. 231, 283–310
30. Frappier, L., Goldsmith, K., and Bendell, L. (1994) J. Biol. Chem. 269, 1057–1062
31. Laine, A., and Frappier, L. (1995) J. Biol. Chem. 270, 30914–30918
32. Mackey, D., Middleton, T., and Sugden, B. (1995) J. Virol. 69, 6199–6208
33. Wells, R. D. (1988) J. Biol. Chem. 263, 1095–1098

3 A. Bochkarev, A. Edwards, and L. Frappier, unpublished data.