Characterization of Major Recognition Sequences for a Herpes Simplex Virus Type 1 Origin-Binding Protein

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Received 3 June 1988/Accepted 30 July 1988

To investigate early initiation events in the replication of herpes simplex virus type 1, we analyzed interactions of proteins from infected cell extracts with the small origin of herpes simplex virus type 1 (*ori_S1*). Using the mobility shift assay, we detected two origin-specific binding interactions. We characterized the more prominent interaction on both strands of the DNA duplex with DNase I protection and methylation interference assays. Protein binding protects 17 bases of DNA on each strand from DNase I. These sequences are located at the left end of the central palindrome and are shifted four bases relative to one another. On the basis of the DNase I protection pattern, we believe this protein to be related to the origin-binding protein defined by Elias et al. (P. Elias, M. E. O'Donnell, E. S. Mocarski, and I. R. Lehman, *Proc. Natl. Acad. Sci.* 83:6322-6326, 1986). Our DNase I footprint shows both strong and weak areas of protection. The regions strongly protected from DNase I align with the essential contact residues identified by interference footprinting. Methylation interference defines a small binding domain of 8 base pairs: 5'-GTTCGCAC-3'/3'-CAAGCGTG-5'. This recognition sequence contains two inverted 5'-GT(T/G)CG-3' repeats which share a 2-base overlap; thus, the origin-binding protein probably binds to the inverted repeats as a dimer.

Herpes simplex virus type 1 (HSV-1) is an attractive model for DNA replication because the virus encodes most of the proteins required for DNA synthesis. An outline of the events in the replication of HSV-1 suggests mechanisms by which replication may be initiated. A portion of viral DNA is found as circles within the nuclei of infected cells (9, 13, 19). Soon after the onset of viral DNA replication, a number of different forms of viral DNA can be detected. Late in infection, a shift to predominantly large, rapidly sedimenting forms of DNA occurs (1, 13). These large structures suggest that, at least late in infection, the DNA replicates as a rolling circle. The mechanism of the transition from a circle to a rolling circle has not been defined experimentally. In theory, the generation of a rolling circle would require a nick in one strand of the DNA duplex. The nick, in turn, would provide a 3' hydroxyl that could act as a primer for leading strand synthesis. The events leading to rolling circle replication are HSV-1 origin specific (21, 22, 24).

Analysis of defective interfering viral particles (8) identifies two regions of HSV-1 DNA that contain origins. One origin (*ori_S1*) is located in the "c" repeat flanking the short unique segment, and the other (*ori_L1*) is located in an internal region of the long unique segment. Recently, a transient replication assay was used to study HSV-1 origins (21, 22, 24, 26). This procedure allowed the mapping of functional origin sequences. The origins of HSV-1 (22, 24, 28), HSV-2 (14), and varicella-zoster virus (VZV) (23) have been sequenced and compared. All of the origins contain a large palindrome centered on an alternating AT sequence motif. The extent of the palindromic sequences and the length of the AT motif are variable. Alignment of the four origins of HSV-1 and HSV-2 indicates that extensive conservation exists leftward from the center of symmetry. Sequences to the right of the center of symmetry are less homologous. Comparison of the HSV origins with the VZV origin demonstrates conservation in the left end, but not in the right end, of the palindrome. Within the left region, there

is an 11-base-pair (bp) sequence, 5'-CGTTCGCACTT-3', that is completely conserved in all five origins. This conservation implies that the sequence is essential for efficient origin function.

HSV-1 encodes many proteins directly and indirectly involved in viral DNA metabolism. Seven open reading frames encode all of the viral proteins directly involved in viral DNA replication (29). Four of these have been matched with the major DNA-binding protein (ICP8), the viral DNA polymerase, a DNA polymerase accessory protein (65K_{DBP}), and the origin-binding protein (OBP) (18a, 29). In addition, primase activity has also been found in extracts from infected cells (12). Genetic and biochemical studies indicate that many of the replication proteins interact in a multiprotein complex (12, 16, 18, 25). The OBP, purified from a nuclear extract, protects 18 bases of DNA from DNase I (6). These sequences partially overlap the left arm of the palindrome in *ori_S1*. Recently, OBP was also shown to bind to sequences overlapping the right arm of the palindrome in a concentration-dependent manner (5).

In this study, we demonstrate that the OBP recognition sequence is a small inverted repeat. This inverted repeat is composed of two 5-base recognition elements that have an overlap of 2 bases. DNase I protection, methylation interference, and sequence analysis show that the recognition sequence is asymmetric. These findings suggest that the OBP is involved in an asymmetric event at the origin.

MATERIALS AND METHODS

Cells and virus. Vero cells were maintained as described by Weller et al. (27). Stocks of HSV-1 strain F were grown in Vero cells as described by Roizman and Spear (20). Virus stocks were titered by infecting cells in Dulbecco modified Eagle medium and 1% fetal calf serum. After adsorption of the virus, cells were incubated for 4 h in Dulbecco modified Eagle medium with 5% fetal calf serum. Plaques were counted after a 72-h incubation in medium containing 0.5% pooled human immune serum.

Plasmid constructions. All cloning procedures were per-

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FIG. 1. HSV-1 origin sequences and flanking polylinker sequences of the pHAK clone. Ori_S1 sequences are numbered as described by Stow and McMonagle (24), and restriction sites are shown above the polylinker sequences. The position of the central palindrome is indicated by arrows between the DNA strands. The line above the upper strand represents the OBP DNase I footprint described by Elias et al. (6). Bars under the sequence identify regions of ori_S1 that were used in the analyses described in this paper. pHAK (■) contains a complete origin, and pLAT (▨) has a truncated origin.

formed as described by Maniatis et al. (15), and all enzyme conditions were those recommended by the manufacturer. Origin sequences were cloned into a vector derived from pUC18 by replacement of the *Hinc*II site with a *Bgl*II site by linker insertion. This vector was called pUC18-B. Two origin-containing fragments were derived from the pOR-S series, constructed by Deb and Doelberg (3), after we replaced the *Hind*III site with a *Bgl*II linker. The complete origin was derived from pOR-S. A truncated origin, in which sequences rightward of the AT motif were deleted, was derived from pOR-S-1. The *Bgl*II-to-*Bam*HI origin-containing fragments of pOR-S and pOR-S-1 were inserted into the corresponding sites of pUC18-B to create pHAK and pLAT, respectively (Fig. 1). Plasmids were maintained in *Escherichia coli* HB101, and DNA was purified from CsCl gradients after isolation by the method of Birnboim and Doly (2).

Protein extraction. Vero cells in 30 150-cm² flasks were infected with HSV-1 (strain F) at a multiplicity of infection of 10. Infected cells were collected 16 h after infection by vigorously shaking the flasks. Cells were centrifuged at a low speed and washed with 20 mM HEPES (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid)-sodium hydroxide (pH 7.6)–0.5 mM dithiothreitol (DTT)–150 mM NaCl. Cell pellets were suspended in 2 ml of lysis buffer (20 mM HEPES-sodium hydroxide [pH 7.6], 0.5 mM DTT, 0.5 mM phenyl-methylsulfonyl fluoride [PMSF], 2 µg of leupeptin per ml) and broken with a tight-fitting pestle of a Dounce homogenizer on ice. All subsequent procedures were done at a temperature of 4°C or less. The cell homogenate was adjusted to 10% glycerol (vol/vol) and centrifuged at 100,000 × *g* for 1 h. The supernatant fraction was adjusted to 35% ammonium sulfate and gently mixed for 45 min. The suspension was then centrifuged at 30,000 × *g* for 1 h. The pellet was suspended in 1.5 ml of protein storage buffer (20 mM HEPES-sodium hydroxide [pH 7.6], 0.5 mM DTT, 0.5 mM PMSF, 0.5 mM disodium EDTA [pH 8.0], 10% [vol/vol] glycerol). Supernatant and pellet fractions were dialyzed against protein storage buffer and stored at –70°C. Protein

was quantitated by using a protein assay kit (Bio-Rad Laboratories).

Gel mobility shift binding assay. The target fragment containing ori_S1 was made by digesting pHAK with *Hind*III and *Eco*RI, and the 5' overhangs were filled with [α-³²P]dATP by the Klenow fragment of DNA polymerase. The labeled DNA fragment was purified by electrophoresis through a 50 mM HEPES-sodium hydroxide (pH 7.6)–5% polyacrylamide gel at 10 V/cm. Probe DNA was eluted by soaking the gel slice in 0.5 M ammonium acetate and 1 mM disodium EDTA (pH 8.0) overnight at 42°C and was subsequently precipitated with ethanol. DNA was suspended at 0.1 µg/ml of 10 mM Tris hydrochloride–1 mM disodium EDTA (pH 8.0). End-labeled DNA (0.1 ng) was incubated with various amounts of protein and poly(dI-dC) · poly(dI-dC) (indicated in figure legends) in binding buffer (50 mM HEPES-sodium hydroxide [pH 7.6], 0.1 mM disodium EDTA [pH 8.0], 5 mM MgCl₂, 0.5 mM DTT, 100 mM NaCl). Protein-DNA complexes were allowed to form for 30 min on ice. Just prior to electrophoresis, sample buffer (50 mM HEPES-sodium hydroxide [pH 7.6], 0.5 mM DTT, 80% glycerol, 0.1% bromophenol blue, 0.1% xylene cyanol) was added to one-fourth of the final volume. The sample was applied to a 50 mM MOPS (morpholinopropanesulfonic acid)–sodium hydroxide (pH 7.6)–5% polyacrylamide gel and electrophoresed at a constant voltage of 10 V/cm for 2.5 h. Gels were dried on Whatman 3MM paper, and autoradiography was done by using intensifier screens at –70°C.

DNase I footprinting. pHAK (1 µg) was 3' labeled at either the *Hind*III or *Bam*HI end as previously described (4). The origin-containing fragment was ethanol precipitated and suspended in 10 mM Tris-hydrochloride–1 mM disodium EDTA (pH 8.0) at a concentration of 200,000 cpm/µl for use in a binding reaction. Approximately 55 µg of protein in the 35% ammonium sulfate extract was incubated with the DNA in the presence of 8 µg of poly(dI-dC) · poly(dI-dC) in a standard binding reaction. DNase I (0.4 U) was added, and the tube was incubated on ice for 1 min. The DNase I was stopped by adjusting the binding reaction to 50 mM disodium

EDTA (pH 8.0) and by the addition of 10 μ g of salmon sperm DNA. Sample buffer was added, and free and protein-bound DNAs were separated by using the gel mobility shift assay described above except that 10 mM disodium EDTA was added to the gel and running buffer. The gel was exposed at 4°C for 4 h, and appropriate fragments were excised and eluted. DNA was concentrated by ethanol precipitation and suspended in 10 μ l of 95% formamide sample buffer. Samples in equivalent counts per minute, of the bound and unbound fractions were loaded onto a 7 M urea–10% polyacrylamide gel and electrophoresed at 55°C. Gels were dried and exposed to X-ray film by using intensifier screens at –70°C. Autoradiographs were then analyzed by densitometry on an UltroScan XL system (LKB Instruments, Inc.).

Methylation interference. Origin fragments were end labeled as described for DNase I footprinting. Methylation of the DNA was performed as described by Wright et al. (30), except that the sodium cacodylate and $MgCl_2$ buffer was replaced with binding buffer. The dimethyl sulfate (DMS) was quenched by the addition of 50 μ l of 1.0 M β -mercaptoethanol and 1.5 M sodium acetate and by ethanol precipitation. The DNA was ethanol precipitated three more times and was suspended in 10 mM Tris-hydrochloride–1 mM disodium EDTA (pH 8.0) at 200,000 cpm/ μ l. The binding reaction was performed under the same conditions used for DNase I footprinting. Free and bound fractions of DNA were resolved by using the gel mobility shift assay. DNA was isolated from the gel fragment, precipitated with ethanol, and broken at the modified G residues as described by Maxam and Gilbert (17) for the G > A reaction. After the final lyophilization, the DNA was suspended and treated as described in DNase I footprinting.

RESULTS

Gel mobility shift analysis of protein-DNA interactions at *ori_S1*. To define protein-DNA interactions at *ori_S1*, we used the gel mobility shift assay (11). In initial experiments, we incubated an unfractionated infected cell extract with the *Hind*III-to-*Eco*RI origin containing restriction fragment from pHAK. We expected numerous protein-DNA interactions to retard the migration of the target fragment to a variety of positions in the gel during electrophoresis. To reduce non-specific protein-DNA interactions, we added 1 μ g of competitor poly(dI-dC) · poly(dI-dC). When 5 μ g of crude extract from HSV-1-infected Vero cells (S100) were incubated with the probe, we detected four distinct protein-DNA interactions (Fig. 2). These interactions are numbered 1 to 4 on the basis of their proximity to the free DNA. None of these four interactions was evident when extracts of uninfected cells were used in this assay (data not shown). Increasing the amount of S100 from infected cells in the binding reaction caused the DNA at all positions to accumulate at the top of the gel. We interpreted this finding to mean that multiple proteins bound to the DNA because of the high protein concentration used in binding.

To simplify these interactions, we separated the S100 extract into two fractions by precipitation with 35% ammonium sulfate. When 5 μ g of protein in the 35% ammonium sulfate supernatant fraction was used in the mobility shift assay, the probe was retarded to positions 2, 3, and 4 but not to position 1. Increased amounts of the supernatant fraction caused all of the DNA to remain at the top of the gel. Protein in the precipitate fraction of the 35% ammonium sulfate reaction retarded DNA migration primarily to position 1 and to the top of the gel. Less DNA was shifted to positions 2, 3,

FIG. 2. Gel mobility shift assay for OBPs. Proteins, in crude extracts (S100) or 35% ammonium sulfate fractions of the crude extracts from infected cells, were bound to the *ori_S1* probe in the presence of 1 μ g of poly(dI-dC) · poly(dI-dC). Bound and free DNAs were separated by polyacrylamide gel electrophoresis. The positions of the bound and free DNAs are indicated on the left. Lines connect similar DNA positions induced by the different extracts.

and 4. Thus, the fractionated proteins increased the accumulation of DNA in positions 1 to 4, and we had sufficient material for analysis of these protein-DNA interactions.

Specificity of protein-DNA interactions. We used competition analysis to determine which of the mobility shift positions were induced by HSV-1 origin recognition proteins. A variety of competitor DNAs were added to the labeled origin DNA fragment, described previously for the binding reaction, and were incubated with the 35% ammonium sulfate precipitate fraction. Control, nonorigin DNAs included linear copolymers of poly(dI-dC) · poly(dI-dC) and form I pUC18-B DNA. As specific competitors for origin DNA sequences, we used form I pHAK and pLAT DNAs. The wild-type plasmid, pHAK, contains all of the origin sequences in the radiolabeled probe. The truncated plasmid, pLAT, has origin DNA but lacks the sequences to the right of the AT segment (Fig. 1). To reduce nonspecific interactions, twice the usual amount of poly(dI-dC) · poly(dI-dC) was added to all of the binding reactions. The competitors were added in a 20,000-fold excess of total DNA. The specific competitors also had a 100-fold excess of origin sequences. Fractionation of DNA-binding proteins and an increase in the amount of total competitor DNA in the binding reaction allowed the resolution of two components at position 2. Figure 3 shows the effects of competitor DNAs on DNA binding by proteins in the 35% ammonium sulfate precipitate fraction.

All of the competitors reduced the DNA-binding reactions that result in the retention of DNA at the top of the gel (compare Fig. 2 and 3). Neither the addition of pUC18-B nor the addition of poly(dI-dC) · poly(dI-dC) DNAs reduced the binding interactions at positions 1 and 2b. In contrast, pHAK and pLAT DNAs strongly interfered with these interactions (Fig. 3). We did not identify any origin-specific interactions in the 35% ammonium sulfate supernatant fraction under these binding conditions (data not shown). We conclude that only the interactions at position 1 and 2b were origin specific, because no other protein-DNA interactions were dramatically affected by the addition of the origin-containing competitors. pLAT competed slightly less effectively than pHAK for position 1-inducing protein. Perhaps, the region deleted in pLAT has a minor role in the binding of the protein that causes the retardation of DNA to position 1. We did not analyze position 2b further for two reasons. First, under these conditions, positions 2a and 2b were not well resolved. Second, when these positions were resolved,

FIG. 3. Identification of origin-specific interactions by competition analysis. The ammonium sulfate-precipitated fraction of protein (10 μ g) was added to the labeled *HindIII-EcoRI* origin probe from pHAK in the presence of 2 μ g of poly(dI-dC) · poly(dI-dC). Additional competitor DNA (2 μ g) was added to the samples: lane 1, form I pUC18-B (pUC); lane 2, form I pLAT; lane 3, form I pHAK; and lane 4, linear poly(dI-dC) · poly(dI-dC). Lane 5 contained only the origin probe. Free and bound DNAs were separated by gel electrophoresis. Bound species are numbered on the right.

the amount of DNA at position 2b was insufficient to allow adequate analysis. The remainder of this study focuses on the more prominent binding interaction at position 1.

DNase I footprinting analysis. We used DNase I footprinting analysis (10) to define the sequences covered by protein at position 1. We incubated the 35% ammonium sulfate precipitate fraction with a singly end-labeled, origin-containing restriction fragment from pHAK (Fig. 1) and allowed protein-DNA complexes to form. DNA was subsequently cut with DNase I under conditions which generated less than one nick per molecule of DNA. We separated the bound from the free DNA by using gel mobility shift electrophoresis. Free DNA and DNA at position 1 were eluted from the mobility shift gel and run on a denaturing polyacrylamide gel.

Protein protected nucleotides 571 through 587 on the upper strand from DNase I (top of Fig. 4). The DNase I footprint of the upper strand was very similar to the footprint of the partially purified OBP described by Elias et al. (6), except that our footprint was one nucleotide shorter on the 5' end. We interpret this to mean that the position 1-inducing protein is probably the OBP. In the present study (Fig. 4), OBP protected regions in this domain to different extents. DNase protection from nucleotides 571 through 577 was strong, while protection from nucleotides 578 through 587 was weaker. On the lower strand, the OBP protected nucleotides 567 through 583 from DNase I (bottom of Fig. 4). This region was the same length as the footprint on the upper strand but shifted 4 bases to the left. Protection of nucleotides 577 through 580 was stronger than protection at either end of the footprint. We suggest that the areas of stronger DNase protection on both strands are likely to represent the more important recognition sequences for OBP.

Identification of OBP contact sites. We used methylation interference to define the DNA recognition sequence of the OBP. We treated the end-labeled origin probe with DMS to methylate the N7 position of a single guanine per DNA molecule. After incubation of the modified probe with the 35% ammonium sulfate precipitate fraction, the free and bound DNAs were separated by using the mobility shift assay. We eluted free DNA and DNA at position 1 from the gel and treated them with piperidine to break the DNA at the

modified positions. The cleavage products were identified on a denaturing polyacrylamide gel. This DMS assay depends on the ability of modifications at certain positions to interfere with protein binding. Modifications of these positions would be underrepresented in the bound DNA fraction and overrepresented in the free DNA fraction.

Figure 5 shows DMS interference footprints for both strands of the origin DNA. Most guanine modifications appeared with equal frequency in the bound and free fractions. These positions are unlikely to be important in protein recognition and binding. On the upper strand, nucleotides at positions 573 and 577 were virtually absent in the bound DNA and were overrepresented in the free DNA (top of Fig. 5). On the lower strand, guanines at positions 576, 578, and 580 interfered with binding (bottom of Fig. 5). Position 576 played a minor role in the formation of the OBP-DNA complex because it was only slightly underrepresented in the bound DNA. Significantly, all interfering guanines occur in an 8-bp segment of DNA from nucleotides 573 through 580. Furthermore, the nucleotide contacts identified by methylation interference align closely with the areas of strong DNase I protection on both strands. Consequently, we think that the OBP recognizes the sequence 5'-GTTCGCAC-3'/3'-CAAGCGTG-5'.

DISCUSSION

Elias et al. (6) originally showed that a protein partially purified from extracts of HSV-1-infected cells binds to the left arm of the large palindrome in the *ori_S1*. More highly purified OBP also binds to a site on the right arm of the palindrome with a lower efficiency (5). In the present study, we have identified two *ori_S1*-specific protein-DNA interactions by a mobility shift assay and have characterized the more prominent interaction with high-resolution footprinting techniques. Because our DNase I footprint closely resembles that identified by Elias et al. on the left arm of the palindrome (6), we assume that we are describing a related interaction. In the present study, we have defined the nucleotide recognition sequences for OBP within the DNase I footprint domain by using methylation interference analysis.

DNase I footprints of origin DNA show that the OBP protects 17 bases of both DNA strands. The two footprints overlap but are four nucleotides out of register. In our studies, the extent of DNase protection within the footprint area is not uniform; small regions of the DNA are strongly protected from DNase, while other regions are only partly protected. Perhaps, the strong areas of protection correspond to close contacts between DNA and protein, and the weaker protection represents steric hindrance of DNase over adjacent areas. These variations in the extent of DNase protection are not evident in the footprints of Elias et al. (6). We do not know whether the origin-binding activity of our preparation represents a single protein or a complex of proteins. Complexed proteins could influence the affinity and the pattern of DNA binding by OBP. This possibility may explain the small differences in the DNase protection patterns reported in this and in previous studies. Other subtle variations in the preparation of extracts or in the footprinting conditions could also explain the differences.

The DNase I footprint domain and flanking areas contain many repeat motifs (Fig. 6A). To determine which, if any, of these motifs are involved in protein binding, we used a methylation interference analysis on both strands of the DNA. Methylation events cause strong interference of OBP binding within the areas strongly protected from DNase I.

FIG. 4. DNase I footprints of OBP bound to both DNA strands of the HSV-1 origin. The *Hind*III-*Bam*HI fragment from pHAK was labeled at either end and used as the probe. Protein (55 μ g) from the 35% ammonium sulfate precipitate was added to the origin probe in the presence of 8 μ g of poly(dI-dC) · poly(dI-dC). After protein-DNA complexes had formed, the binding reactions were treated with DNase I. Free and bound DNAs were resolved by gel electrophoresis. Eluted DNA fractions were analyzed by electrophoresis through a denaturing gel. Position markers (not shown) were generated by the G reaction-sequencing technique described by Maxam and Gilbert (17). A partial sequence of *ori*_{S1} is shown in the center of the figure. The central palindrome is indicated by arrows. Autoradiograms and densitometer tracings of the footprinted DNAs are adjacent to the appropriate DNA strands. Shading between the densitometer tracings of bound and unbound DNAs highlights areas protected by OBP.

The area of interference is confined to an 8-bp 5'-GTTCCG CAC-3'/3'-CAAGCGTG-5' sequence overlapping the left arm of the palindrome. All of the guanines in this region appear to bind the OBP. This 8-bp segment corresponds

exactly to two inverted and overlapping repeated sequences but not to any other group of repeated sequences (Fig. 6A). The DNA repeat sequence is 5'-GT(T/G)CG-3'. Figure 6B shows that this inverted repeat motif occurs in the same

FIG. 5. Methylation interference footprints of OBP on both DNA strands. The *Hind*III-*Bam*HI fragment from pHAK was labeled at either end and modified by DMS for use as the origin probe. Protein (55 μ g) from the 35% ammonium sulfate precipitate was added to the origin probe in the presence of 8 μ g of poly(dI-dC) · poly(dI-dC). Free and bound DNAs were separated by gel electrophoresis. Eluted DNAs were broken at the modified residues with piperidine and analyzed by electrophoresis through a denaturing gel. The figure is arranged as described in Fig. 4.

overlapping form at the left end of all sequenced alpha-herpesvirus origins. With the exception of VZV, the repeated sequences are also present at the right end of herpesvirus origins. The two bases that flank the repeated sequence are also well conserved at both ends of herpesvirus origins. These flanking nucleotides are either both cytosines or both adenines. The presence of cytosine or adenine residues appears to be related to the nucleotide at position 3 in the

core sequence. Flanking bases and the base at position 3 are either all purines or all pyrimidines. The binding sites on the right end of both *ori*_{S1} and *ori*_{S2} have variations from the consensus sequence at position 2, and *ori*_{S1} also has a mismatch at the 5'-flanking nucleotide (underlined in Figure 6B). The mismatched bases in *ori*_{S1} may explain the binding of OBP to both ends of the HSV-1 origin with different affinities (5).

FIG. 6. The mechanism of OBP binding to the HSV-1 origin. (A) Identification of the repeat motif responsible for OBP binding. The arrows between the ori_S strands locate repeated sequences in the protein-binding region. The highlighted arrows indicate the core pentanucleotides for OBP binding. The solid bar below the upper strand indicates the DNase protection domain of OBP reported by Elias et al. (6). The bars on the outside of the DNA strands show areas of DNase protection determined in the present study. Solid segments indicate strong protection, and open segments correspond to weak protection. Circles identify methylated guanines that interfere with protein binding in our studies. Solid and open circles indicate strong and weak interference, respectively. Dotted lines align the interfering bases with the inverted repeats. (B) Comparison of this sequence with presumptive binding sites in other alpha-herpesviruses. The table arranges the binding motifs identified in the left arm of the ori_S 1 palindrome with homologous motifs in the left and right arms of the origins from HSV-1, HSV-2, and VZV. The core pentanucleotide is numbered 1 to 5, and flanking nucleotides are shown. A consensus sequence includes the seven nucleotides shown. Two nonequivalent copies of the consensus sequence appear at each binding site. Base changes from the consensus sequence are indicated by underlining.

The use of overlapping inverted repetitions as binding motifs in origins of replication is not unique. The T antigens of simian virus 40 and polyomavirus both bind to the pentanucleotide 5'-GAGGC-3' in a palindrome at the center of their origins of replication (7). Each arm of the palindrome contains two pentanucleotides oriented as direct repeats with 1-bp spacing between them. The arms of the palindromes are arranged differently in these two viruses. Simian virus 40 has a 1-bp separation between the inverted repeats, while the inverted repeats of polyoma virus overlap by two base pairs. This alternative arrangement of polyoma sequences indicates that recognition sequences for protein subunits can overlap in replication origins.

Our findings suggest that the herpesvirus OBP binds to the inverted repeat motif as a dimer. In every putative binding site, there is an asymmetry of the repeats at position 3 and within the flanking bases. This asymmetry could be important for subsequent protein function. OBPs have two potential roles in the initiation of replication. One possible role is to assemble a preinitiation complex at the origin. In this case, the asymmetrical binding site could impart directionality to a bound complex of proteins. The other possibility is that the OBP could participate in an origin initiation event; the asymmetry of the OBP-binding site could focus origin events to one strand of origin DNA. For example, the initiation of a rolling circle may require the nicking of one and only one strand of duplex DNA. Our findings add to the framework of information that can be used for the design of genetic and biochemical approaches to these functional problems.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grants CA-18808, CA-38146, and 5-T32 CA-09176 awarded by the National Cancer Institute, Department of Health and Human Services.

ADDENDUM

Pure protein encoded by the UL9 gene of HSV-1 (18a) has the same methylation interference pattern as the OBP described in this study (Koff and Tegtmeyer, unpublished data; P. D. Olivo, N. J. Nelson, and M. D. Challberg, unpublished data). Thus, OBP is the product of the UL9 gene.

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