The herpes simplex virus, type I origin-binding protein, OBP, is a superfamily II DNA helicase encoded by the UL9 gene. OBP binds in a sequence-specific and cooperative way to the viral origin of replication oriS. OBP may unwind partially and introduce a hairpin into the double-stranded origin of replication. The formation of the novel conformation referred to as oriS* also requires the single-stranded DNA-binding protein, ICP8, and ATP hydrolysis. OBP forms a stable complex with oriS*. The hairpin in oriS* provides a site for sequence-specific attachment, and a single-stranded region triggers ATP hydrolysis. Here we use *Escherichia coli* exonuclease I to map the binding of the C-terminal domain of OBP to the hairpin and the helicase domains to the single-stranded tail. The helicase domains cover a stretch of 23 nucleotides of single-stranded DNA. Using streptavidin-coated magnetic beads, we show that OBP may bind two copies of double-stranded DNA (one biotin-labeled and the other one radioactively labeled) but only one copy of oriS*. It is the length of the single-stranded tail that determines the stoichiometry of OBP-DNA complexes. OBP interacts with the bases of the single-stranded tail, and ATP hydrolysis is triggered by position-specific interactions between OBP and bases in the single-stranded tail of oriS*.

Replication of Herpes simplex virus type I (HSV-1) relies on the activation of the viral origins of replication oriS and oriL by the origin-binding protein (OBP) encoded by the UL9 gene (1, 2). Events catalyzed by OBP assisted by the single-stranded binding protein, ICP8, lead to the assembly of the prototypical herpes virus replisome consisting of the heterodimeric UL30/42 DNA polymerase, the trimeric UL5/8/52 helicase primase complex, and ICP8. The herpes virus replisome is capable of coordinated synthesis of leading and lagging strand products (3). The bulk of replicated DNA appears to be produced by a rolling circle mode of replication forming extensive stretches of genome concatamers (4). However, the failure to demonstrate circular genomes in cells infected with wild type HSV-1 before the onset of DNA synthesis indicates that circular genomes may not be the initial replication template (5). Homologous recombination occurs frequently during virus replication. Events such as genome isomerization, replication of genomic termini, and repair of double-stranded breaks may all involve homologous recombination. Several plausible mechanisms for the recombination events have been suggested and received experimental support (6–10). Nevertheless, the enzymes responsible for recombination remain poorly characterized.

Initiation of HSV-1 DNA replication is probably a rate-limiting step in the production of virus progeny, because DNA synthesis on a linear substrate is much less efficient than it is on a circular template (10). In the latter case, one initiation event will lead to extensive synthesis of daughter molecules. Therefore, it is probable that the initiator protein and the replicator sequence co-evolve to ascertain the necessary specificity and efficiency needed for species-specific amplification of viral genomes. To understand in biochemical terms how this is achieved, we have studied the interaction of HSV-1 OBP and the viral origin of replication oriS as well as the interaction of OBP with a probable intermediate in the activation process referred to as oriS* (11–13). OriS* is an unwound form of double-stranded oriS in which a stable hairpin with a 3′-single-stranded region is formed (Fig. 1, A and B). A role for oriS* during DNA replication in vivo is based on the observations that the sequence arrangement is conserved evolutionarily, and mutations that disrupt the structure of oriS* reduce DNA replication. Second site mutations that restore the ability to form oriS* enhance DNA replication (12). OBP binds tightly and specifically to oriS*; therefore, it is possible to study in detail the positioning of OBP on the DNA molecule and the functional consequences of the protein-DNA interactions.

OriS contains three binding sites for OBP arranged in an evolutionarily conserved manner (12). Boxes I and II are strong binding sites positioned as inverted repeats separated by an AT-rich spacer sequence. Box III is a low affinity site existing as an inverted repeat with box I. Boxes I and III may base pair with each other and form the stable hairpin characteristic of oriS* (Fig. 1, A and B).

OBP is a superfamily II DNA helicase (14–16). It is a stable dimer that binds specifically and cooperatively to oriS (17). The C-terminal domain of OBP binds as a monomer to the recognition sequence GTTCGCAC referred to above as box I (18, 19). Cooperative binding to boxes I and II is dependent on the functional consequences of the protein-DNA interactions. Boxes I and II are strong binding sites positioned as inverted repeats separated by an AT-rich spacer sequence. Box III is a low affinity site existing as an inverted repeat with box I. Boxes I and III may base pair with each other and form the stable hairpin characteristic of oriS* (Fig. 1, A and B).
Initially, cooperative binding of OBP to oriS leads to the destabilization of the AT-rich spacer sequence as shown by a hyperchromic effect and an increased sensitivity to nuclease P1 (23). In the second step, OBP assisted by ICP8 and at the expense of ATP hydrolysis is able to separate the two strands completely and produce an OBP-oriS* complex (13). On supercoiled DNA, OBP together with ICP8 catalyzes additional unwinding as seen by the increasing electrophoretic mobility (24). Excessive unwinding of an oriS-containing plasmid also can be carried out by OBP alone but at a much lower efficiency (25).

Here we present results describing the positioning of OBP on oriS*, the stoichiometry of OBP-DNA complexes, and the identification of position-specific base contacts with single-stranded DNA that lead to the activation of ATP hydrolysis. These observations can now be integrated into the model for initiation of Herpes simplex virus, type 1 DNA replication alluded to above.

**Experimental Procedures**

**Proteins and Nucleic Acids**—The UL9 protein, ΔOBP, and ICP8 were purified as described previously (13, 26). Oligonucleotides (Table I) purified by reverse phase-high pressure liquid chromatography were purchased from DNA Technology A/S. The single-stranded oligonucleotide, oriS80, is shown in Fig. 1B.

**Exonuclease I Digestion**—Reactions (40 μl) containing ~10 fmol of 32P-labeled oriS80 (Fig. 1B) and either 0.4 pmol of OBP or 18 pmol of ΔOBP in 10 mM Tris- HCl, pH 7.8, 10% glycerol, 2.5 mM dithiothreitol, 3 mM MgCl2, 2.5 mM ATP·S, 25 mM KC1, and 0.4 μg of bovine serum albumin were incubated 45 min at 37 °C. 10 pmol of ICP8 were included as indicated. Ten units of E. coli exonuclease I (U. S. Biochemical Corp.) was added, and the reaction mixture was incubated for 15 min at 37 °C. The reaction mixtures were extracted by phenol and chloroform. The aqueous phase was precipitated with ethanol according to standard procedure. The samples were analyzed finally on an 8%

**DNA Binding Assays**—The formation of complexes between OBP and the DNA ligands was examined either in gel-retardation experiments using agarose gels or in co-precipitation experiments using biotin-labeled oligonucleotides and streptavidin-coated magnetic beads. For the gel-retardation experiments, the reaction mixtures (10 μl) contained 0.2 nm of radiolabeled oligonucleotide and 20 nm of the UL9 protein in 10 mM Tris- HCl, pH 7.8, 10% glycerol, 2.5 mM dithiothreitol, 3 mM MgCl2, 2.5 mM ATP·S, 25 mM KC1, and 0.4 μg of bovine serum albumin. The samples were mixed on ice and incubated for 15 min at 37 °C. They were centrifuged briefly and transferred to ice before loading onto the agarose gels. The protein-DNA complexes were analyzed on 0.9% agarose gels in standard 1× TE buffer (40 mM Tris acetate, 1 mM EDTA). Gels were run at 7 V/cm for 90 min at ambient temperature. The gels were dried onto DE81 chromatography paper (pH 2.5) and subjected to autoradiography analysis.

The co-precipitation experiments were performed in a 10-μl standard reaction mixture containing 50 mM ATP, 10 mM MgCl2, 80 mM KCl, 1 mM dithiothreitol, 2 μg/ml calf thymus DNA, 50 μM ATP·S, 0.02% Nonidet P-40, and 0.2 mg/ml bovine serum albumin. The biotinylated oligonucleotide was added and incubated. The mixture was mixed on ice followed by incubation at 37 °C for 10 min. A 5-μl suspension of 20 μg/ml Dynabeads® M-280 streptavidin (Dynal Biotech ASA) was added, and the mixture was shaken for 10 min at ambient temperature. The beads were set to separate on a magnetic particle concentrator (MPC-S, Dynal Biotech ASA). After the removal of the supernatant, the beads were washed in 100 μl of 50 mM Tris- HCl, pH 7.8, 75 mM NaCl, 1.5 mM MgCl2, and 0.02% Nonidet P-40. The concentrated beads were re-suspended in 20 μl of the same buffer, and the radioactivity was measured by liquid scintillation counting as described above.

**Results**

**Positioning OBP on oriS***—As a starting point for these investigations, we decided to map the interaction between OBP and oriS* using an exonuclease I digestion assay (Fig. 1C). We used a single-stranded oligonucleotide, oriS80, that corresponds to the upper strand of oriS (Fig. 1B and Ref. 13). This oligonucleotide spontaneously adopts the oriS* conformation. We noted that exonuclease I readily digested the 3‘-tail of oriS80. Strong pause sites for exonuclease I were seen at positions 34–36 located on the right at the 3‘-end of the box III-box I hairpin. In the presence of the monomeric C-terminal domain of OBP (ΔOBP), the protected region was extended to position 41, corresponding to eight nucleotides of the single-stranded tail. ΔOBP may bind directly to the single-stranded tail, because it has a capacity to bind single-stranded DNA (27). However, it may also merely shield a part of the single-stranded tail from nuclease digestion. Intact OBP caused a predominant shift of the pause sites to nucleotides 51–53 in the single-stranded tail. Thus, altogether 23 nucleotides of single-stranded DNA are covered by OBP and, as discussed below, directly bind to the helicase domains and trigger ATP hydrolysis. The single-stranded DNA-binding protein, ICP8, is needed to convert an 80-bp double-stranded minimal oriS fragment to oriS*, and it remains bound to the OBP-oriS* complex (13). Therefore, we have examined its effect on the OBP-oriS* interaction using the exonuclease I digestion assay. We could not detect the protection of the single-stranded tail from nuclease digestion by ICP8 alone. However, we noted that there was less full-length material remaining after the incubation period, suggesting that ICP8 melts out the residual secondary structure and facilitates nuclease digestion. ICP8 did not extend the protection of the single-stranded tail caused by ΔOBP. As noted before, an interaction between ICP8 and the single-stranded DNA will lead to a disruption of the ICP8-ΔOBP complex (27). It appears that, under the conditions used in the exonuclease I digestion experiment, the ICP8-DNA interaction is not stable enough to result in protection from enzymatic degradation. Interestingly, ICP8 caused extended protection from nuclease digestion to position 58 in the presence of full-length OBP. In this instance, ICP8 and the helicase domains of OBP will compete for the same stretch of single-stranded DNA and, as a consequence, the ICP8-OBP complex remains stable.

**Stoichiometry of the OBP-oriS* Complex—**Oris* is an efficient activator of ATP hydrolysis catalyzed by OBP (12). A box III-box I hairpin with a single-stranded tail consisting of only 10 nucleotides is enough to cause almost maximal stimulation of ATP hydrolysis. What then is the effect of the additional nucleotides of the single-stranded tail that are protected by OBP from exonuclease I digestion? We first examined the ability of OBP to form complexes with a diverse set of DNA ligands (Fig. 2). In agreement with results published previously (12), we saw that OBP formed one unique complex with wild type oriS* as represented by the oligonucleotide Awt-Bwt (Table I). A DNA ligand consisting of a box III-box I hairpin with a 10-nucleotide single-stranded tail, HP-dT10, gave rise to two complexes. We then replaced the single-stranded oligonucleotide tail of HP-dT10 with an abasic spacer of 10 deoxyribose phosphates terminated by a thymidylate residue (HP-dS10) and found that only a small amount of a single complex was formed. The same result was obtained using only the hairpin as the DNA ligand. Finally, a single-stranded oligonucleotide, T65, did not generate stable complexes with well defined elec-
trophoretic mobilities. These observations are summarized as follows. First, the presence of multiple complexes using HP-dT10 suggests that the stoichiometry of the complexes differs. Second, because the electrophoretic mobilities of the OBP-Awt-Bwt complex and OBP-HP-dT10 differ, it is probable that the charge of the complex determines the electrophoretic mobility. Third, interactions between the helicase domain and the bases of the single-stranded tail strongly affect complex formation as indicated by the small amount of complex formed between OBP and HP-dS10. Fourth, single-stranded DNA alone cannot support complex formation.

A working hypothesis was formed on the basis of these observations. We suggest that dimeric OBP allows simultaneous and possibly cooperative binding of two double-stranded DNA ligands but only permits the binding of one oriS* ligand with a single-stranded tail exceeding 10 nucleotides. To further examine this idea, we made oligonucleotides containing a hairpin sequence with single-stranded tails of increasing length (Fig. 3). In gel-retardation experiments, we noted that oligonucleotides with tail lengths shorter than 10 nucleotides gave rise to moderate quantities of a complex referred to as complex A (Fig. 3A, lanes 1–3). Oligonucleotides with single-stranded tails longer than 10 nucleotides gave rise to a larger amount of a complex with a slower electrophoretic mobility referred to as

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**Fig. 1. Positioning OBP on oriS*.** A, the Herpes simplex virus, type 1 origin of DNA replication oriS. B, the proposed structure of oriS* represented by oligonucleotide oriS80 (13). C, exonuclease I digestion of oriS80. Arrowheads indicate pause sites at positions 36, 41, 53, and 58 of oriS80. The first lane represents a G + A cleavage of oriS80.
complex B (Fig. 3A, lanes 4–7). The transition from complex A to complex B occurred abruptly when the tail length increased from 8 to 10 nucleotides. As the tail length increased further, so did the electrophoretic mobility of complex B. To see whether complex B contained one or two ligands, a mixing experiment was performed (Fig. 3B). Two oligonucleotides, HP-dT15 and HP-dT29, were mixed and incubated with OBP. We found that only two complexes were formed. The electrophoretic mobilities of these complexes were identical with the electrophoretic mobilities of complexes obtained with a single DNA ligand, and we found no evidence for a complex with intermediate mobility. We conclude that complex B consists of an OBP dimer with a single oriS* ligand bound. By inference, we suggest that complex A corresponds to a complex between an OBP dimer and two DNA ligands. These DNA ligands most probably occupy the binding sites on the C-terminal domains of the OBP dimer.

An alternative way of examining the DNA binding properties of OBP is to look for simultaneous binding of two differently labeled DNA molecules to the same protein molecule. Therefore, we made use of a biotin-labeled duplex oligonucleotide containing the BoxI recognition sequence for OBP, biotin-17/18, [32P]-labeled BoxI duplex oligonucleotide, [32P]P17/18, and a biotin-labeled version of oriS*, biotin-HP-dT29. OBP and its C-terminal domain, ΔOBP, were incubated with the various DNA ligands. Streptavidin-coated magnetic beads then were added to immobilize the biotin-labeled oligonucleotides and their interaction partners. The experiments were made by adding increasing amounts of the biotin-labeled oligonucleotide and keeping the concentration of the radioactively labeled oligonucleotide constant. If complexes containing both biotin-labeled oligonucleotide and [32P]-labeled oligonucleotide are formed, we expect to see an initial increase in the amount of radioactive oligonucleotide bound to the magnetic beads. A maximum will be observed at approximately equimolar concentrations of the oligonucleotides. The amount of radioactive oligonucleotide bound to the magnetic beads will decrease at high concentrations of the biotin-labeled oligonucleotide because of competition between the oligonucleotides for binding to OBP. The ligand binding properties of OBP and ΔOBP were examined in this way (Fig. 4). We found that, in the presence of dimeric OBP, simultaneous binding of biotin-17/18 and [32P]P17/18 to the beads was observed (Fig. 4A). Maximum binding was seen using ~30 nM of a biotin-labeled oligonucleotide. In contrast, monomeric ΔOBP failed to promote the binding of [32P]P17/18 to the streptavidin-coated magnetic beads. In a separate experiment, the effect of protein concentrations was examined. Increasing OBP concentrations resulted in the increased binding of [32P]P17/18 until saturation was achieved. In contrast, increasing concentrations of ΔOBP did not result in an increased binding of [32P]P17/18 to the magnetic beads. Finally, to examine the OBP-oriS* interaction biotin-HP-dT29 was used together with [32P]P17/18 (Fig. 4C). The binding of [32P]P17/18 to the beads now was reduced significantly. This observation suggests that, if one copy of the biotin-HP-dT29 is bound to one OBP dimer, the binding of additional DNA ligands to the dimer is precluded. When the experiment was performed in the presence of ICP8, a further reduction in the amount of [32P]P17/18 bound to the beads was noted (results not shown).

The results obtained using the co-precipitation assay agree with the results obtained from the gel electrophoresis experiments. In summary, one OBP dimer can bind two molecules of a double-stranded DNA ligand simultaneously but only one molecule of oriS*. Importantly, the length of the single-stranded tail determines the stoichiometry of the OBP-DNA complex. We also see that contacts between OBP and bases of the single-stranded DNA promote the formation of complexes. It is tempting to speculate that single-stranded DNA may serve as an allosteric effector, although one cannot exclude that competition for the same binding site occurs once the tail length exceeds ten nucleotides.

**Position-specific Base Contacts Trigger ATP Hydrolysis**—Here, we try to identify the contacts between OBP and single-stranded DNA that trigger ATP hydrolysis. To sort out whether interactions between OBP and the DNA backbone or interactions between OBP and the bases of the single-stranded tail are involved in stimulating ATP hydrolysis, we first replaced the oligodeoxythymidylylate tail with a stretch of ten abasic deoxyribose phosphates terminated by a 3’-terminal thymidine residue. This cofactor, HP-dS10, was a poor activator of ATP hydrolysis, indicating that contacts between the bases of the tail and OBP are essential for triggering ATP hydrolysis (Fig. 5A). The oligonucleotide HP-dS5dT6, in which five abasic residues preceded six thymines, behaved as a superactivator of ATP hydrolysis. An oligonucleotide, HP-dT5dT55, with a reverse order in the tail behaved as HP-dS10 (Fig. 5A).

To further localize the residues responsible for activating ATP hydrolysis, a series of oligonucleotides were made in which specific thymines were replaced by abasic spacer residues (Fig. 5B). Again HP-dS5dT6 was a superactivator. The thymines of HP-dS5-dT6 were then replaced one by one by abasic residues in HP-dS6-dT5 and HPdS7-dT4. The effects on ATP hydrolysis were prominent. By replacing thymines at positions 6 and 7 with abasic spacer residues, ATP hydrolysis returned to a level seen with HP-dS10 (Fig. 5B). The rates of ATP hydrolysis were determined from the graphs in Fig. 5B and presented in Table II. We can rationalize these observations by assuming that position-specific base contacts, e.g., positions six and seven in the single-stranded tail, are needed to stimulate ATP hydrolysis. Additional base contacts may stabilize the binding of the DNA activator to OBP, and the absence of these base contacts may facilitate dissociation from and rebinding to single-stranded DNA. In the presence of HPdS5dT6, successive rounds of ATP hydrolysis, therefore, may be facilitated despite the fact that OBP cannot move along the DNA substrate.

We have also performed a competition experiment using HP-dT10 and HP-dS10 as activators of ATP hydrolysis. The result indicates that HP-dS10 and HP-dT10 competes for the same site on OBP, and that the affinity of OBP for the cofactors is very similar (Fig. 5C).

**DISCUSSION**

Helicases have important and diverse roles in DNA and RNA metabolism (28, 29). Here, we focus our attention on the HSV-1 origin-binding protein, also referred to as the UL9 protein. It is a DNA helicase belonging to superfamily II (14). Enzymes of superfamily I and II share certain structural features. They have two copies of a RecA-like domain, which together form a...
Binds simultaneously to the two monomers of the asymmetric structure of Rep, a single 16-mer of oligodeoxythymidylate case along DNA by an inchworm mechanism (35). In a crystal, interactions appear to contribute to the movement of the helicases, the bases in single-stranded DNA interact extensively with hydrophobic amino acids in the protein. These interactions appear to contribute to the movement of the helicase along DNA by an inchworm mechanism (35). In a crystal structure of Rep, a single 16-mer of oligodeoxythymidylate binds simultaneously to the two monomers of the asymmetric unit (32). The two monomers exist in different conformations, the open and closed forms, yet they bind single-stranded DNA in the same way. The crystal structure of NS3 helicase, a superfamily II RNA helicase, shows that interactions between the protein and the backbone of RNA dominate. Additional domains may target a helicase to well defined DNA structures and/or proteins and make it an integrated partner in a molecular machine. For example, RecG appears to interact with the three arms of a replication fork (36, 37). Surprisingly, the crystal structure indicates that the helicase domains in this

### Table I

| Oligonucleotides used for studies of DNA binding and DNA-dependent ATP hydrolysis |
|-------------------------------------------------|
| Awt-Bwt | 5’-AAAAAGAAGTGAAGACGCAAGGTCGCACTTCGCACTTCCAAATATAATATAATATAATTAGGGCGAA-3’ |
| Hairpin (HP) | 5’-GAGGTAAGACCGAGGTCGCACTTCGCACTTCCAAATATAATATAATATAATTAGGGCGAA-3’ |
| HP-dT3-29 | 5’-AAAAAGAAGTGAAGACGCAAGGTCGCACTTCGCACTTCCAAATATAATATAATATAATTAGGGCGAA-3’ |
| HP-dT4sS5 | 5’-AAAAAGAAGTGAAGACGCAAGGTCGCACTTCGCACTTCCAAATATAATATAATATAATTAGGGCGAA-3’ |
| HP-dS6T5 | 5’-AAAAAGAAGTGAAGACGCAAGGTCGCACTTCGCACTTCCAAATATAATATAATATAATTAGGGCGAA-3’ |
| HP-dS7T4 | 5’-AAAAAGAAGTGAAGACGCAAGGTCGCACTTCGCACTTCCAAATATAATATAATATAATTAGGGCGAA-3’ |
| HP-dS10 | 5’-AAAAAGAAGTGAAGACGCAAGGTCGCACTTCGCACTTCCAAATATAATATAATATAATTAGGGCGAA-3’ |
| PE17 | 5’-GATTCGGAAGGTTCGCACTTCGCACTTCCAAATATAATATAATATAATTAGGGCGAA-3’ |
| PE18 | 5’-GATTCGGAAGGTTCGCACTTCGCACTTCCAAATATAATATAATATAATTAGGGCGAA-3’ |
| Biotin-17 | 5’-XTTTGAGAACGTCGCACTTCGCACTTCCAAATATAATATAATATAATTAGGGCGAA-3’ |
| Biotin-18 | 5’-CCCCCATGGGACGAAATGGGGACGTCGCACTTTCCAAATATAATATAATATAATTAGGGCGAA-3’ |
| Biotin-HP-dT29 | 5’-AAAAAGAAGTGAAGACGCAAGGTCGCACTTCGCACTTCCAAATATAATATAATATAATTAGGGCGAA-3’ |

**Fig. 3.** The length of 3’-single-stranded tail controls the stoichiometry of OBP-oriS* complexes. A, OBP-DNA complexes were formed and analyzed as described in Fig. 2. The length of the single-stranded tail was altered systematically (Table I). Complex A, marked by an arrowhead, corresponds to the faster moving complex also seen in Fig. 2. Complex B is a slower migrating complex whose electrophoretic mobility is affected by the length of the single-stranded tail. **B**, a mixing experiment in which OBP was incubated with two different DNA ligands. Lane 1, HP-dT15 and OBP. Lane 2, HP-dT29 and OBP. Lane 3, Two separate reaction mixtures with OBP and either HP-dT15 or HP-dT29 were prepared. They were mixed immediately before electrophoresis. Lane 4, HP-dT15 and HP-dT29 were mixed prior to incubation with OBP. Note the absence of OBP-DNA complexes of intermediate electrophoretic mobility.

**Fig. 4.** Co-precipitation of DNA ligands in complex with OBP or OBP using streptavidin-coated magnetic beads. Biotin-17/18, [32P]17/18 and biotin HP-dT29 were the DNA ligands used. The concentration of [32P]17/18 was 10 nM in all of the experiments. The concentrations of biotin-17/18 and biotin-HP-dT29 are indicated in the graphs. OBP and the DNA ligands were preincubated prior to the addition of streptavidin-coated magnetic beads. A, co-precipitation of biotin-17/18 and [32P]17/18 in the presence of OBP (open squares) and OBP (closed squares). B, co-precipitation of biotin-17/18 and [32P]17/18 in the presence of increasing concentrations OBP (open squares) and ΔOBP (closed squares). C, co-precipitation of biotin-17/18 and [32P]17/18 (open circles) or biotin-HP-dT29 and [32P]17/18 (closed circles) with OBP.
instance might interact with duplex DNA (37).

In this discussion, we examine how the structural and functional properties of the helicases described in the previous paragraph may relate to HSV-1 OBP and its ability to activate the viral origin of replication oriS. OBP binds in a cooperative and sequence-specific manner to the viral origin of replication oriS. Together with the single-stranded DNA-binding protein ICP8, it converts double-stranded oriS to a novel conformation oriS* in a reaction that requires ATP hydrolysis (13). The function of OBP is controlled by DNA structure. The distance between the binding sites must be appropriate to allow cooperative binding and initiation of DNA synthesis (38). We have suggested that the exposure of single-stranded DNA will cause a conformational change in ICP8 and release it from OBP (27). Unwound oriS must be able to adopt a novel conformation, oriS*, with stable hairpin structures presenting binding sites for OBP and a single-stranded tail interacting with the helicase domains of OBP to support DNA replication (11–13).

We have found that only a short piece, 10 nucleotides, of single-stranded DNA attached to a hairpin with a unique sequence, HP-dT10, is needed to stimulate ATP hydrolysis and that contacts not only with the DNA backbone but also with the bases of single-stranded DNA are needed for maximum binding to DNA as well as ATP hydrolysis. The bases at positions 6 and 7 of the tail appear to be especially important. A series of the mutations have been made in the helicase motifs of OBP, and it has been found that mutations in motif Ia result in proteins that no longer support replication of HSV-1 (22). In at least some cases, it appears that these residues directly contact single-stranded DNA as judged by comparison with Rep and NS3. Perhaps such studies could be extended to identify the amino acid residues in OBP, which contact the bases in single-stranded DNA and trigger ATP hydrolysis.

The exonuclease I digestion experiments indicate that as much as 23 nucleotides of single-stranded DNA may be covered

### Table II

| Oligonucleotide co-factor | ATP hydrolysis (pmol/min) |
|--------------------------|--------------------------|
| No DNA                   | 3.4                      |
| T65                      | 8.5                      |
| HP-dT10                  | 16.7                     |
| HP-dS5dT6                | 26.8                     |
| HP-dS6dT5                | 15.6                     |
| HP-dS7dT4                | 11.5                     |
| HP-dS10                  | 8.2                      |

#### FIG. 5

**Base contacts are required for efficient activation of ATP hydrolysis by single-stranded DNA.** The ATPase assay is described under “Experimental Procedures.” The oligonucleotides are presented in Table I. A, the oligonucleotides were HP-dT10 (open squares), HP-dS10 (open circles), HP-dS5dT6 (closed squares), and HP-dT5dS5 (closed circles). B, the concentration of oligonucleotide cofactors was 25 nM. The symbols represent no DNA (closed diamonds), T65 (closed squares), HP-dT10 (closed triangles), HP-dS10 (open circles), HP-dS5dT6 (open triangles), HP-dS6dT5 (open squares), and HP-dS7dT4 (open diamonds). C, competition experiment with the DNA cofactors HP-dT10 and HP-dS10. The closed squares represent an experiment in which the concentration of HP-dS10 was increased gradually as indicated. The closed diamonds represent an experiment in which both DNA cofactors HP-dT10 and HP-dS10 were included in the same reaction mixture. The concentration of HP-dT10 was kept constant at 25 nM, and the concentration of HP-dS10 was increased as indicated.

#### FIG. 6

**A model of the activation of HSV-1 oriS and oriL by OBP.** We make use of the structural information of *E. coli* Rep and its interaction with DNA to suggest a model for the OBP-oriS and OBP-oriS* complexes (32). The model proposes conformational changes of OBP upon binding the different DNA ligands. The model also takes into account that the DNA binding sites in oriS and oriL occur as inverted repeats in duplex oriS and that single-stranded DNA binds in the same direction to both subunits of the OBP dimer. It highlights the importance of interactions between the helicase domains for cooperative binding to DNA, but it does not predict the surfaces involved in the formation of a single dimer and a complex between dimers. The open/closed (OC) conformation OBP has been drawn using the crystal structure of *E. coli* Rep in complex with single-stranded DNA and ADP as a starting point (32). An OBP protomer consists of a C-terminal sequence-specific DNA binding domain (∆OBP) and two RecA-like helicase domains. One protomer has been highlighted with a broad contour. We propose that an open/open (OO) conformation of OBP can be formed by rotation of the ∆OBP domain. We also propose in accordance with our experimental results that the OC conformation may bind two double-stranded DNA ligands but that the OC conformation may bind only one oriS* ligand. In our model, two dimers of OBP in the OO conformation bind cooperatively to double-stranded oriS and oriL (Fig. 1 and Ref. 42). The OC conformation of OBP binds to oriS*. Note that one hairpin has been left out from each strand, because it cannot support the stable binding of OBP (10) and may be eliminated by ICP8. Note also that oriS is only able to form one OBP-oriS* complex but that oriL may form two OBP-oriL* complexes.
by the helicase domains of an OBP dimer. One then would want to speculate that approximately 8–10 nucleotides of single-stranded DNA interact with each of the two subunits of OBP, an observation that is in good agreement with the binding site of single-stranded DNA on Rep (32). We have also found that an OBP dimer may bind two molecules of a double-stranded DNA ligand but only one molecule of oriS*. The ability of a dimer of OBP to the simultaneously bind two sites on double-stranded DNA may be essential for the correct cooperative positioning of OBP on oriS. OBP and oriS then must undergo mutual conformational changes, possibly in a concerted reaction, and establish novel interactions in the OBP-oriS* complex. A single-stranded region of defined length must be exposed at this stage to assist in the conversion of the 2:2 (OBP:ligand) complex to a 2:1 (OBP:ligand) complex. The structural studies and functional studies of E. coli Rep may help to understand this process. It has been shown, as mentioned above, that Rep in complex with single-stranded DNA may form an asymmetric dimer consisting of protomers in open and closed conformations (32). Although the functional implications of the putative Rep dimer are poorly understood, there is accumulating evidence for dimers as active forms of the Rep and UvrD helicases (39, 40). In the case of Rep, the two subunits bind to the same piece of single-stranded DNA. These observations lead us to suggest that OBP may be able to form either a dimer with the protomers in open/open conformation capable of binding two double-stranded DNA ligands or an asymmetric dimer with the protomers in open/closed conformation capable of binding only one molecule of oriS* (Fig. 6). These conformational changes may be facilitated by chaperones (41). We do not know with certainty the number of OBP molecules needed for the activation of HSV-1 oriS and oriL, but it is not an unlikely assumption that the two dimers may be required (25). Therefore, we suggest that the initial complex between OBP and oriS/oriL would be a symmetric arrangement of two dimers in the open/open conformation (Fig. 6). Upon exposure of single-stranded DNA accompanied by hydrolysis of ATP, the two dimers are converted to two dimers in the open/closed conformation positioned on opposite strands. The role of ICP8 is unclear. Perhaps its role is to facilitate strand separation and to prevent reannealing of the complementary strands in a reaction that is tightly coupled to the structural transitions of the OBP-oriS complex. A better understanding of the structure OBP and its interaction partners would be of great value to resolve these issues.

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