Targeting Holliday junctions by origin DNA-binding protein of herpes simplex virus type 1

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In the present paper, the interactions of the origin binding protein (OBP) of herpes simplex virus type 1 (HSV1) with synthetic four-way Holliday junctions (HJs) were studied using electrophoresis mobility shift assay and the FRET method and compared with the interactions of the protein with duplex and single-stranded DNAs. It has been found that OBP exhibits a strong preference for binding to four-way and three-way DNA junctions and possesses much lower affinities to duplex and single-stranded DNAs. The protein forms three types of complexes with HJs. It forms complexes I and II which are reminiscent of the tetramer and octamer complexes with four-way junction of HJ-specific protein RuvA of *Escherichia coli*. The binding approaches saturation level when two OBP dimers are bound per junction. In the presence of Mg2+ ions (≥2 mM) OBP also interacts with HJ in the stacked arm form (complex III). In the presence of 5 mM ATP and 10 mM Mg2+ ions OBP catalyzes processing of the HJ in which one of the annealed oligonucleotides has a 3'-terminal tail containing 20 unpaired thymine residues. The observed preference of OBP for binding to the four-way DNA junctions provides a basis for suggestion that OBP induces large DNA structural changes upon binding to Box I and Box II sites in OriS. These changes involve the bending and partial melting of the DNA at A+T-rich spacer and also include the formation of HJ containing Box I and Box II inverted repeats and flanking DNA sequences.

**Keywords:** herpes simplex virus type 1; viral origin DNA-binding protein; four-way DNA Holliday junction; *E. coli* RuvA protein; ATP

Introduction

The replication origin DNA-binding protein (OBP) of human herpes simplex virus type 1 (HSV1) is encoded by the UL9 gene and plays a key role in the initiation of viral DNA replication. The protein binds to specific sequences within the viral origins of DNA replication OriS and OriL and also functions as an ATP-dependent DNA helicase (Aslani, Macao, Simonsson, & Elias, 2001; Aslani, Olsson, & Elias, 2002; Chattopadhyay & Wellor, 2007; Eom & Lehman, 2002; Fierer & Challberg, 1992; He & Lehman, 2001; Koff, Schwedes, & Tegtmeyer, 1991; Lee & Lehman, 1997; Macao, Olsson, & Elias, 2004; Malik & Wellor, 1996; Olsson et al., 2009; Weir & Stow, 1990). The C-terminal 317 amino acids of the 851 residue protein specify sequence-specific binding to the viral origins of replication and the N-terminal 534 residues contain several motifs characteristic of superfamily SF2 of DNA helicases (Manolaridis et al., 2009; Olsson et al., 2009; Stow, Brown, Cross & Abbotts, 1998). The high-affinity binding sites for OBP in OriS, Box I, and Box II, contain pseudosymmetrical sequences which are separated by A+T-rich spacer. The binding of OBP to the Box I and Box II sites is accompanied by structural changes in the A+T-rich spacer element which become more sensitive to digestion by micrococcal nuclease and to oxidation by potassium permanganate (Gustafsson, Hammersten, Falkenberg, & Ellias, 1994; Koff et al., 1991; Weir & Stow, 1990). In the presence of ATP and viral protein ICP8 (single-strand DNA binding protein) the OBP catalyzes unwinding of the minimal OriS duplex (≈80 bp). The protein also binds to a single-stranded DNA fragment OriS* (63 nt) containing a stable Box I – Box III hairpin and an unstable AT-rich hairpin at the 3'-end (Aslani et al., 2001, 2002; Macao et al., 2004). The viral protein ICP8 forms a 1:1 complex with OBP and stimulates ATP-dependent unwinding of DNA (Boehmer, Craigie, Stow, & Lehman, 1994; He & Lehman, 2001; Lee & Lehman, 1997; Manolaridis et al., 2009). The protein also promotes single-stranded DNA annealing (Tolun, Makhov, Ludtke, & Griffith, 2013).

In the present study, we have used the recombinant HSV1 OBP expressed in *Escherichia coli* cells (Surovaya et al., 2010) to investigate its interaction with various linear oligonucleotide duplexes and branched DNA substrates. The recombinant protein binds selectively to Box I and Box II sequences in OriS and is endowed with helicase and ATPase activities (Bazhulina
et al., 2012; Surovaya et al., 2010). In our previous work, the efficiency of Forster resonance energy transfer (FRET) ( Förster, 1948) between the fluorophore and the quencher covalently linked to the 5'- and 3'-ends of an oligonucleotide (63 nt) containing Box I and Box III sequences and A+T-rich spacer element has been measured (Bazhulina et al., 2014) and used to determine the distance between the fluorophore and the quencher within the complex with OBP. This distance has been estimated to be equal to 53 Å in the absence of ATP and ICP8. This value is consistent with the formation of antiparallel four-way Holliday junction (HJ) (Bazhulina et al., 2014). The kinetics of DNA unwinding by OBP in the presence of ATP has been also studied by the FRET method, using oligonucleotides labeled with fluorophore and quencher at 5'- and 3'- ends. It has been found that synthetic DNA minor groove binding ligands, exemplified by Pt-bis-netropsin and related molecules interact strongly and selectively with AT-clusters containing 8-10 base pairs (Belikov, Grokhovsky, Isaguliants, Surovaya, & Gursky, 2005; Grokhovsky et al., 1998; Gursky et al., 1983; Nikolaev et al., 1996; Surovaya et al., 1997, 2001; Surovaya, Grokhovskii, Bazhulina, & Gurskii, 2008). Some of these polyamides induce virtually complete inhibition of an ATP-dependent unwinding of DNA by OBP at bis-netropsin to the minimal OriS duplex molar ratio of 1:1 (Bazhulina et al., 2014). The observed antiviral activities of Pt-bridged bis-netropsin and related molecules (Andronova, Grokhovskii, Surovaya, & Galegov, 2001; Andronova, Grokhovsky, Surovaya, Gursky, & Galegov, 2007; Andronova et al., 2008; Andronova, Grokhovsky, Galegov, Deriabin, Gursky, & Lvov, 2010; Andronova, Grokhovsky, Surovaya, Gursky, & Galegov, 2013; Bazhulina et al., 2014; Andronova et al., 2010) can be associated with the inhibition of fluctuation opening of AT base pairs at the A+T-rich spacer which is required for initiation of the herpes virus DNA replication.

Two conformations of four-way DNA junctions are observed in solution, a planar open form with a mobile center and four helical arms, and an immobile stacked X form with two continuous strands and two exchangeable (crossover) strands. In the absence of divalent metal ions, four helical arms of junction are believed to be oriented towards the corners of a square, and thymine bases at the center of DNA junction are found to be accessible to modification by osmium tetroxide (Clegg et al., 1992; Duckett et al., 1988; Lilley, 2000). In the presence of magnesium ions (∼15 mM), the junction folds by pairwise stacking of helical arms into the compact stacked X-structure which was confirmed by X-ray crystallography (Eichman, Vargason, Mooers, & Ho, 2001; Ho & Eichman, 2001; Ortiz-Lombardia et al., 1999). In this structure, two of the DNA strands run continuously through a given pair of stacked helices, while the other two serve as crossover strands. NMR studies (Overmars & Altona, 1997) and single-molecule FRET experiments (McKinney, Declais, Lilley, & Ha, 2003) on immobilized four-way DNA junctions indicate that DNA junctions exist in solution as equilibrium mixtures of conformational isomers (Figure 1).

At high salt concentrations there are two alternative conformers of a given junction that differ in the choice of stacking partners. NMR studies, single-molecule FRET experiments, and chemical probing of the junction structure revealed that there is a rapid exchange between alternative stacking conformers in a four-way DNA junction (Grainger, Murchie, & Lilley, 1998; McKinney, Declais, Lilley, & Ha, 2003; Overmars & Altona, 1997). A conformer bias is found to depend upon the nucleotide sequence around the point of strand exchange (Grainger et al., 1998; McKinney, Declais, Lilley, & Ha, 2003). If a junction exists as a mixture of the unfolded (open) form and either of the two possible stacked conformers, a profound effect of magnesium ion concentration on the NMR spectral profiles is observed. This has been demonstrated for a model four-way DNA junction constructed from a 38 residue oligonucleotide containing segments with inverted repeat sequences (Overmars & Altona, 1997; Overmars et al., 1997). In the presence of 5 mM Mg²⁺ ions, the NMR spectrum of the model junction can be represented as a superposition of the NMR spectra characteristic of stacked arm X form of the junction and unfolded (open) form. Increasing the concentration of Mg²⁺ ions from 5 to 10 mM leads to a stabilization of stacked X form with concomitant decrease in the content of open junction form (Overmars et al., 1997). At concentrations of Mg²⁺ ions ≥15 mM the compact stacked arm form dominates, whereas the open form is present at very low concentrations.

Three-way DNA junctions are observed as intermediates in DNA repair processes. They also play a key role in the genetic recombinations of phages. Comparative gel electrophoresis and NMR studies of three-way junctions, including junctions containing two unpaired pyrimidine residues near the branchpoint, reveal that three-way

![Figure 1](image-url)
junctons can adopt a conformation in which one helix is stacked upon another, forming a quasi-continuous double helix (Assenberg, Weston, Cardy, & Fox, 2002; Leontis et al., 1994). As in four-way junctions, two conformers with different stacking arrangements of the helical arms are possible.

In the present study, we have demonstrated that OBP possesses a capacity to bind selectively to four-way and three-way DNA junctions. It binds less strongly to duplex and single-stranded DNAs. In a close similarity to the HJ-specific protein RuvA of E. coli OBP forms complexes I and II with a HJ in a planar square conformation. In the presence of Mg2+ ions (≥2 mM) OBP also forms a complex with four-way DNA junction in the stacked arm conformation (complex III). In the presence of ATP OBP catalyzes processing of HJ, provided that one of the annealed oligonucleotides has a tail containing 20 unpaired thymine residues at 3′-end.

Materials and methods

DNA substrates

In this study, we have used synthetic oligonucleotides and oligonucleotide constructs corresponding to the four-way Holliday junction and its derivatives (Figure 2). The oligonucleotides S1–S9 and V1–V6 were synthesized by the phosphoramidite method and purified by electrophoresis in polyacrylamide gel (Syntol, Russia). The dyes R6G and BHQ2 were covalently attached to the 3′- and 5′-ends of the oligonucleotides S2–3 and V1–2 via aminohexamethylene linkers. Here, R6G and BHQ2 are the derivatives of 6-carboxyrhodamine G and non-fluorescent dye BHQ2 (Black Hole Quencher 2). The dye R6G was attached to the 5′-ends of the oligonucleotides S2–4 and V1–1. The dye Cy5 was attached to the 3′-end of the oligonucleotide S2–5.

The oligonucleotides were labeled at the 5′-ends with γ-32-P-ATP (Izotop, Moscow) using polynucleotide kinase T4 (Sibenzim), as reported by Sambrook, Fritsch, & Maniatis, 1989. To obtain radiolabeled oligomers appropriate 32P-labeled oligonucleotides and non-labeled oligonucleotides were mixed in a buffer containing 50 mM Tris-HCl pH 7.5, 50 mM NaCl, and 10 mM MgCl2. The mixtures were heated up to 95°C and allowed to cool down slowly to reach temperature. After annealing, the oligonucleotide structures were purified by electrophoresis on a native 12% polyacrylamide gel, electroeluted in 1x TBE, and dialyzed against 10 mM Tris-HCl buffer (pH 8.0) containing 50 mM NaCl and 0.1 mM EDTA.

The formation of branched DNA structures after annealing the oligonucleotides was confirmed by comparing the cleavage profiles generated by bacteriophage T7 endonuclease I (New England Biolabs) in the branched DNA substrates and linear DNA. T7 endonuclease I is known to bind specifically to four-way DNA junctions and promotes their resolution into linear duplexes (Hadden, Déclais, Carr, Lilley, & Phillips, 2007; Parsons & West, 1990). We have found that T7 endonuclease I can resolve branched DNA substrates X2 and X3 and also cleaves unusual DNA substrates X1 and X3*. It does not cleave a linear DNA duplex (supplementary Figure S1).

Expression and purification of recombinant OBP

The full-sized recombinant OBP encoded by the gene UL9 of the herpes simplex virus type 1 (strain L2 from the State Virus Collection of D.I. Ivanovsky Institute of Virology) was synthesized on the basis of a modified plasmid pET14 in E. coli cells BL21(DE3) (Surovaya et al., 2010). This protein contains a histidine “tag,” viz., a cluster of six N-terminal histidine residues enabling protein purification on metal-chelating (Ni-NTA) columns. Subsequent purification of the protein included chromatography on phosphocellulose P11 and dialysis (Surovaya et al., 2010). Protein concentration has been determined spectrophotometrically using a molar extinction coefficient for the OBP monomer equal to 89,000 M−1 cm−1 at 280 nm. The recombinant OBP was stored in 20 mM Tris HCl buffer (pH 7.2) containing 20 mM HEPES-NaOH, 0.54 M NaCl, 0.01% Tween 20, 0.10 mM EDTA, 1 mM dithiothreitol and 20% (v/v) glycerol.

EMSA and helicase assays

In experiments designed to investigate the interaction of OBP with different DNA substrates, the labeled DNA oligomers (100 nM) were preincubated for 1 h at 4°C with the recombinant OBP (300 nM dimers) in 50 mM Tris-HCl buffer (pH 7.8) containing 50 mM NaCl, 10 mM MgCl2, 5 mM DTT, 0.01% Triton X-100, and 4% Ficoll. ATP was added to some samples to a final concentration of 5 mM. The samples were fractionated by 7.5% polyacrylamide gel (29:1) electrophoresis in 0.5x TBE at 100–120 V for 5 h at 4°C. The gels were fixed in 10% acetic acid, dried, and then visualized using a phosphorimager (Perkin Elmer). Interaction of E. coli RuvA protein (Abcam; ab63828) with DNA substrates was studied in the reaction mixtures containing labeled DNA oligomers (100 nM) and indicated amounts of the protein in 50 mM Tris-HCl buffer (pH 7.5) containing 0.5 mM EDTA, 1 mM DTT and 4% Ficoll. Samples were incubated for 1 h at 4°C prior to loading onto 7.5% polyacrylamide gel (29:1) in 0.5x TBE buffer. Electrophoresis was performed in 0.5x TBE buffer at 100–120 V for 5 h at 4°C. The gels were fixed in 10% acetic acid, dried, and visualized using a PhosphorImager (Perkin Elmer).
In order to study the unwinding activity of OBP the labeled DNA oligomers (100 nM) were preincubated for 1.5 h at 37°C with the OBP (300 nM dimers) in 50 mM Tris-HCl buffer (pH 8.4) containing 50 mM NaCl, 10 mM MgCl₂, 5 mM DTT, 0.01% Triton X-100, and 4% Ficoll. ATP was added to some samples to a final concentration of 5 mM. Samples (10 μl) were analyzed as described above for EMSA experiments.

UV melting curves, circular dichroism, and fluorescence measurements

The UV melting curves were recorded on a Jasco V-550 spectrophotometer equipped with a thermostated cuvette holder. The absorbance was registered at λ = 260 nm every 1.0°C across the 15–95°C temperature range. The melting temperatures of DNA oligomers were determined from the first derivative plots of the melting curves using the two-state model for DNA duplex melting process. The circular dichroism (CD) spectra were obtained on a Jasco J-715 spectropolarimeter at 20°C using samples annealed in the same buffer and under the same conditions as those used for the thermal denaturation studies. Fluorescence spectra were measured with a Cary Eclipse fluorescent spectrophotometer (Agilent Technologies) equipped with a Peltier cooled cell holder.

Results and discussion

Interaction of OBP with branched DNA substrates

The results of the electrophoretic mobility shift assay (EMSA) studies of the free oligomers X2, X3, and X4 and their complexes with OBP in the absence and presence of 5 mM ATP are displayed in Figures 3. For
comparison sake, the electrophoretic mobilities of free duplexes $D = S1 + S3$ and $D1 = V1 + V2$, free oligonucleotide $S2–2$, and their mixtures with OBP in the absence and presence of 5 mM ATP are also displayed in Figure 3, (panel a).

As can be seen from Figure 3, the oligomers X2 and X3 form stable complexes with OBP, which manifest lower (compared to the free oligomers) electrophoretic mobilities upon polyacrylamide gel electrophoresis. In the presence of 5–10 mM Mg$^{2+}$ ions oligomers X1, X2, X3, and X4 can exist in solution as a mixture of the open and stacked arm forms of the junction which differed in their electrophoretic mobilities. It is known that at low salt conditions in the absence of magnesium ions a four-way DNA junction exists in a square planar conformation (Clegg et al., 1992; Duckett et al., 1988;
In the presence of magnesium ions (≈5 mM) a part of the DNA oligomers folds into a compact conformation which are stabilized by pairwise coaxial stacking of double-helical arms. The structure of stacked X form has been established by X-ray diffraction method (Eichman et al., 2001; Ho & Eichman, 2001; Ortiz-Lombardia et al., 1999). In the presence of Mg$^{2+}$ ions at concentrations ranging from 5 to 10 mM both the stacked and unfolded (open) forms of the junction coexist in solution.

Figure 3 shows that addition of OBP the intensities of bands corresponding to the free oligomers X2 and X3 diminish, and new bands corresponding to complexes between OBP and branched DNA substrates emerge (Figure 3). The DNA junction X4 contains longer double-helical arms than junction X3. It was used as a DNA substrate to study the activities of the HJ-specific helicase RuvAB and endonuclease RuvC of E. coli (Lloyd, & Sharples, 1993; Whitby, Bolt, Chan, & Lloyd, 1996).

Distinct bands are observed for complexes of OBP with all four-way DNA junctions used in our experiments. We have observed the formation of complexes exhibited low electrophoretic mobilities (complexes I and II) and fast moving complex III which, presumably, reflects the binding of OBP dimers to a DNA junction at high Mg$^{2+}$ ions concentration. Distinct bands which may reflect the interaction of OBP multimers with an immobile X4 junction have been also observed (Figure 3, panel d). OBP is known to exist as a dimer in solution. It tends to form associates of the higher order in the presence of Mg$^{2+}$ ions concentration (Ariyoshi et al., 1998; Ho & Eichman, 1999). In the complex, the DNA junction is sandwiched between the RuvA tetramer and X3 junction has been also observed (Figure 3). The observed preference of OBP for binding to the replication origin OriS and promotes the interaction of OBP multimers (presumably, tetramers) with DNA. These complexes exhibit a lower electrophoretic mobility and are observed in some cases, even in the absence of h-Tid-l protein (see, for instance, complex IV in Figure 3, panels b and d). It is important to note that under the experimental conditions used in our work nonspecific linear duplex D and single-stranded oligonucleotide S2–2 interact rather weakly with OBP (Figure 3). The observed preference of OBP for binding to the branched DNA substrates can be attributed to the structure recognition, although sequence-specific binding of OBP to the replication origins OriS and OriL plays an important role in the initiation of the viral DNA replication.

A similarity in the binding behavior between OBP and HJ-specific protein RuvA of E. coli

The RuvA protein of E. coli is known to form two types of complexes with HJ which correspond to the binding of one and two RuvA tetramers to the junction (Figure 4, panel a). The three-dimensional structure of a complex between the RuvA tetramer and X3 junction has been established by X-ray diffraction method (Ariyoshi, Nishino, Iwasaki, Shinagawa, & Morikawa, 2000; Nishino, Ariyoshi, Iwasaki, Shinagawa, & Morikawa, 1998; Rafferty et al., 1996). In the complex, two AT base pairs near the branchpoint of the junction are unpaired (Ariyoshi et al., 2000). Each RuvA monomer has two consecutive alpha-helix-hairpin-alpha-helix domains bridged by a connector alpha-helix and related by an approximate twofold symmetry (Ariyoshi et al., 2000; Doherty, Serpell, & Ponting, 1996; Nishino et al., 1998; Rafferty et al., 1996; Shao & Grishin, 2000). These motifs are known to be implicated in the recognition of the open form of HJ by the RuvA tetramer. We have found that there is a similarity of the amino acid sequence of E. coli RuvA protein and sequences of HSV1 OBP and its homologs in other alpha- and beta-herpes viruses which suggests that HSV1 OBP belongs to a family of HJ-binding helicases (Bazhulina et al., 2014). The structure of octameric RuvA–HJ complex is also determined. In the complex, the DNA junction is sandwiched between two RuvA tetramers (Roe et al., 1998). Gel shift studies of the complexes formed by E. coli RuvA protein and HSV1 OBP with the same DNA junction show that there is a remarkable similarity in the binding properties of these two proteins (Figure 4).

From data presented in Figure 4 one can conclude that complexes I and II of OBP with the X3 junction are reminiscent of the corresponding complexes formed by the RuvA protein with the same DNA junction. However, in contrast to the binding of E. coli RuvA protein, the HSV1 OBP in the presence of Mg$^{2+}$ ions forms an additional complex with X3 junction that will be further referred to as the complex III. It moves faster in the gel than complexes I and II do under the same conditions. We suggest that complex III reflects the interaction of OBP dimers with X3 junction in a stacked arm conformation, whereas complexes I and II correspond to the binding of one and two OBP dimers, respectively, to the junction in an unfolded planar square conformation.

In a close similarity to the binding of HJ-specific proteins RuvA, UvrD, and RecG of E. coli (Carter, Tahmasch, Compton, & Matson, 2012; Mahdi, McGlynn, Levett, & Lloyd, 1997; Parsons, Tsaneva, Lloyd, & West, 1992; Whitby & Lloyd, 1998) HSV1 OBP exhibits a high preference for binding to three-way and four-way branched DNA substrates. OBP has nearly the same affinity for binding to DNA oligomers X2 and Y1 (Figure 5). The protein exhibits a lower tendency to form a type II complex with 3-way junction Y2, as compared to the binding to Y1 junction. Despite the fully complementary sequence the Y2 junction contains unpaired nucleotides near the branchpoint, as revealed from single-molecule FRET and time-resolved fluorescence.
measurements which were carried out for another fully complementary three-way junction (Sabir et al., 2012).

The observed difference in the electrophoretic mobilities of complexes formed by OBP with junctions X2, Y1 and Y2 can be attributed to characteristic structural features of these complexes.

**OBP–HJ interaction monitored by FRET method**

In order to determine the stoichiometry of OBP binding to the four-way DNA junctions, we have studied the interaction of OBP with junctions X5 and X6 containing the fluorescently labeled oligonucleotides S2–3, S2–4 and S2–5 (Figure 2). The oligonucleotide S2–3 contains a fluorophore R6G (6-carboxyrhodamine G) and a quencher BHQ2 (Black Hole Quencher 2) linked via hexamethylene linkers to the 5'- and 3'-ends of the oligonucleotide S2–3. The junction X6 contains two fluorescently labeled oligonucleotides, viz., the S2–4 oligonucleotide carrying the fluorophore R6G at the 5'-end and S2–5 oligonucleotide carrying the fluorophore Cy5 covalently attached to the 3'-end. After annealing the oligonucleotides, the fluorophores R6G and Cy5 become spatially approximated in the X6 junction, enabling transfer of the electron excitation energy from R6G to Cy5 by the FRET mechanism. In Figure 6 normalized fluorescence intensities \( I_\lambda \) are plotted as functions of the molar ratio of OBP dimers to the junction (panel a). Here \( I_0 \) is the fluorescence intensity measured at 559 or 670 nm for junctions X5 and X6, respectively, in the absence of OBP. \( I_\lambda \) is the fluorescence intensity measured at wavelength \( \lambda \) in the presence of OBP. \( C_{OBP} \) is the concentration of OBP dimers. X is the concentration of the junction. We found that fluorescence intensity at 670 nm \( (I_{670}) \) increases after the addition of OBP. It approaches the saturation level at the molar ratio of added OBP dimers to the junction of 2:1 (Figure 6, panel a). The experimentally determined dependence of the fluorescence intensity on time can be described by the equation: \( I_{559}(t) = A_0 + A_1 \exp (-t/T_1) \), where \( I_{559}(t) \) is the fluorescence intensity of the oligonucleotide complex with OBP at time t measured from the moment of ATP addition, \( T_1 \) is the characteristic time of the process, and \( A_0 \) is the limiting value of \( I_{559}(t) \) when t approaches infinity. \( A_1 \) is the constant. The solid curve (panel b) represents the theoretically calculated dependence \( I_{559}(t) \).
wherein the sum of the squares of the deviations of experimental data points from the calculated values of $I_{559}(t)$ approaches the minimum. The best fit values of $A_0$, $A_1$ and $T_1$ are indicated in Figure 6.

In the presence of OBP, the fluorescence intensity at 559 nm ($I_{559}$) diminishes due to structural changes induced upon binding of OBP to the junction. Presumably, the average distance between the fluorophore R6G and the quencher BHQ2 is diminished upon binding of OBP to the junction X5. This leads to the quenching of the R6G fluorescence by BHQ2. The binding approaches the saturation level when two OBP dimers are bound per junction. After addition of ATP the fluorescence intensity of the complex containing two OBP dimers per X5 junction increases, and then a slow time-dependent process is observed (Figure 6, panel b). The characteristic time of the ATP-dependent process lies within the interval of 20–30 min.

In order to estimate the distance between the donor and acceptor chromophores within the junction X5, fluorescence intensities of the donor should be measured in two systems: one containing the junction conjugated with a fluorophore and a quencher ($I_{DA}$) and another one in which the junction contains the oligonucleotide conjugated with the fluorophore only ($I_D$). In the latter case, the oligonucleotide S2–3 in the X5 junction has been replaced by oligonucleotide S2–4 (Figure 2). Assuming random orientation of the transition moments of the donor and the acceptor, the distance between them can be calculated as:

$$R = R_0 ((I_{DA}/I_D)/(1 - I_{DA}/I_D))^{1/6}$$  \(1\)

where $(1 - I_{DA}/I_D)$ is the efficiency of the energy transfer between the donor and the acceptor; $I_{DA}$ and $I_D$ are the intensities of the donor fluorescence in the presence and absence of the acceptor, respectively. $R_0$ is the critical
distance between the excited molecule of the donor and the non-excited molecule of the acceptor wherein the probability of the energy transfer from the excited donor molecule to the acceptor molecule is equal to the probability of the energy transfer from the excited donor molecule of the acceptor wherein the fluorophore-quencher distance can be attributed to a partial dissociation of the complex between OBP and X5 junction in the presence of 5 mM ATP, as revealed from EMSA studies of the protein complexes with X3 junction (Figure 3, panel b).

Dissociation of the X7 DNA junction induced upon binding of OBP in the absence and presence of ATP

We have found that OBP binds strongly to four-way DNA junctions, including X7 junction, in which the S1–2T oligonucleotide contains a stretch of 20 unpaired thymine residues at 3′-end. Figure 7 shows that OBP forms two types of complexes with X7 junction, complexes I and II. Complex III is not observed in the reaction mixture in the presence of 10 mM MgCl2. This is presumably attributed to a dissociation of the X7 junction into two partial duplexes which is catalyzed by OBP in the presence of 10 mM Mg2+ ions. Fast moving constituents of the X7 junction are observed in the mixture containing X7 junction (100 nM) and OBP (300 nM dimers) in 50 mM Tris-HCl buffer (pH 8.4) in the presence of 50 mM NaCl, 10 mM MgCl2, 5 mM DTT, 0.2% Triton X-100, 0.5 mg/ml BSA, and 4% Ficoll (see Figure 7, lane 9). Addition of ATP (5 mM) leads to an increase in the amount of dissociated component parts of the X7 junction (Figure 7, lane 10).

In order to identify the final products of the dissociation reaction the samples (10 μl) containing OBP (200 nM) and X7 junction (100 nM) in 50 mM Tris-HCl buffer (pH 8.4) in the presence of 50 mM NaCl, 10 mM MgCl2, 5 mM DTT, 0.2% Triton X-100, 0.5 mg/ml BSA, and 4% Ficoll were preincubated for 90 min at 37°C in the absence and presence of ATP (5 mM). The reactions were stopped by the addition of SDS (0.1%), EDTA (20 mM), CaCl2 (1 mM), and proteinase K (Amresco) at a concentration of 4 μg/ml. The solutions were then kept for additional 30 min at 37°C and loaded onto 7.5% PAGE (29:1) and electrophoresed at 4°C for 5 h (Figure 7, lanes 6 and 7). The electrophoretic mobilities of the reaction products were compared with the mobilities of unbound oligonucleotide markers (Figure 7, lanes 2–4) and mobilities of the products generated by OBP in the absence and presence of ATP (5 mM). EMSA analysis shows that partial duplexes D3 and D4 serve as the final products of the dissociation reaction catalyzed by OBP in the presence of 10 mM Mg2+ ions and 5 mM ATP.

The effects of Mg2+ ions and synthetic DNA-binding polyamide cis-diammine Pt(II)-bridged bis-distamycin (Pt-bis-Dst) (supplementary Figure S2) on dissociation of the X7 junction have been also studied (Figure 8). Addition of a synthetic polyamide Pt-bis-Dst to the X7 junction at polyamide/junction molar ratio equal to approximately 1.0 is accompanied by a decrease in the band intensities corresponding to the fast moving junction.
constituents generated by OBP in the presence of 10 mM Mg²⁺ ions. Detailed analysis shows that the amount of dissociated oligonucleotide species is diminished in the presence of Pt-bis-Dst (Figure 8, lane 7). These results deserve some comment. Previous DNase I footprinting and CD spectroscopy studies have shown that 5′-TTTTAAAA-3′ is the strongest affinity binding site for cis-diammine Pt(II)-bridged bis-netropsin and bis-distamycin (for chemical structures see supplementary Figure S2). These polyamides bind less strongly to a DNA region containing the sequence 5′-AAAATTTT-3′. Pt-bis-Dst exhibits much lower affinities for binding to
short AT clusters containing only 2 to 4 successive AT-base pairs (Grokhovsky et al., 1998). From CD spectroscopy studies we have found that Pt-bis-Dst binds to the X7 junction in a monodentate manner (using only one distamycin-like fragment) with an affinity constant of $4 \times 10^5$ M$^{-1}$ (data are not shown).

Figure 8. Effect of Mg$^{2+}$ ions and synthetic DNA-binding polyamide ligand Pt-bis-Dst on the dissociation of the junction X7 by OBP.

Notes: Lanes 1–3, S1–2T oligonucleotide and partial duplexes D3 and D4, respectively. Each marker substrate was used at concentration 50 nM. Lane 4, unbound X7 junction (100 nM) alone. Lanes 5 and 6, mixtures of the X7 junction (100 nM) and OBP (200 nM) preincubated for 90 min at 37°C in 50 mM Tris-HCl buffer (pH 8.4) containing 50 mM NaCl, 10 mM MgCl$_2$, 5 mM DTT, 0.2% Triton X-100, 0.5 mg/ml BSA and 4% Ficoll in the absence and presence of ATP (5 mM), respectively. Lane 7, the same mixture as in lane 6 which was preincubated in the presence of Pt-bis-Dst at concentration 100 nM. Lane 8, X7 junction (100 nM) alone. Lanes 9 and 10, reaction mixtures containing X7 junction (100 nM) and OBP (200 nM) which were preincubated for 90 min at 37°C in 50 mM Tris HCl (pH 7.5), 30 mM NaCl, 1 mM MgCl$_2$, 0.5 mM EDTA, 1 mM DTT, 0.2% Triton X-100, 0.5 mg/ml BSA, and 4% Ficoll in the absence and presence of ATP (5 mM), respectively. Lane 11, the same mixture as in lane 10 which was preincubated in the presence of Pt-bis-Dst at concentration 100 nM.
Decreasing the concentration of Mg$^{2+}$ ions from 10 mM to approximately 1 mM is accompanied by inhibition of the dissociation process. At 1 mM Mg$^{2+}$ ions the dissociated components of the junction (which are moving faster than the native junction) were not observed, even in the presence of 5 mM ATP (Figure 8, lanes 9 and 10). Under these conditions, the protein forms predominantly complexes I and III with the junction. The interaction of Pt-bis-Dst with X7 junction is accompanied by a decrease in the intensity of a band that reflects the formation of complex III with concomitant increase in the band intensity characteristic of complex I between the OBP and X7 junction.

DNase I footprinting studies have shown that Pt-bis-Dst and related molecules bind selectively to the A+T-rich spacer in the linear duplex form of OriS (Andronova et al., 2008; Bazhulina et al., 2014). The binding increases the melting temperature of the A+T-rich spacer element and suppresses the local opening of AT base pairs at the A+T-rich spacer which is a prerequisite to the unwinding of DNA by OBP (Bazhulina et al., 2014; He & Lehman, 2000; Surovaya et al., 2010). Since Pt-bis-Dst has a little influence on the binding of OBP to the X7 junction, it seems likely that the observed antiviral activity of Pt-bis-Dst is associated with inhibition of local opening of AT-base pairs at the A+T-rich spacer in the linear duplex form of OriS.

**Interaction of OBP with duplex and single-stranded oligonucleotides containing Box I, Box II, and Box III sequences**

Previous studies have demonstrated that a single OBP dimer can bind simultaneously two DNA duplexes containing specific binding sites which are represented by Box I and Box II sequences (Macao et al., 2004; Surovaya et al., 2010). Since these duplexes contain inverted repeat sequences they may assemble into four-way DNA junctions. It seems likely that the V3 oligonucleotide alone, after annealing at 95°C for 20 min and subsequent cooling to room temperature, may form intramolecular hairpin, self-associated dimer, and tetramer species. Gel retardation experiments have shown that complex of OBP with the V3 oligonucleotide is characterized by a low electrophoretic mobility which is comparable to the mobility of the complex I formed by OBP with X2 junction (Figure 9).

We suggest that OBP promotes assembly of V3 oligonucleotides into a four-way DNA junction which is stabilized by complementary base pairing of Box I and Box III sequences (supplementary Figure S3). OBP exhibits a high affinity to a four-way junction (V3)$_4$. It binds less strongly to a fully complementary duplex D2 = V3 + V4 which has a specific Box I site for the interaction with OBP.

The formation of four-way junctions by single-stranded oligonucleotides bearing inverted repeat sequences might be a widespread phenomenon. The oligonucleotide bearing the inverted repeat sequence 5′-CCGGTACCGG-3′ is known to crystallize in the stacked arm form whose structure was determined at the atomic level (Eichman et al., 2001). The oligonucleotide 5′-CCGGACCGG-3′ designed initially to study the geometry of G. A mismatched base pairs in the B DNA duplex is also crystallized as a four-way junction (Ho & Eichman, 2001). There is evidence that the ACC trinucleotide adjacent to the position of a branchpoint favors the formation of HJ in solution and in crystals (Hays, Schirf, Ho, & Demeler, 2006; Ho & Eichman, 2001). Clearly, this may reflect the fact that oligonucleotides with inverted repeat sequences tend to form four-way DNA junctions in solution which are in the concentration-dependent equilibria with dimers and monomers (Hays et al., 2006). UV melting curves obtained for self-associated species of the V3 oligonucleotide exhibit a typical biphasic character that reflects the melting of a G+C-rich duplex region (which is stabilized by complementary base pairing of Box I and Box III inverted repeats) and the melting of a A+T-rich hairpin which is formed at the A+T-rich spacer and flanking sequences. The melting temperatures are equal approximately to (81.4 ± 0.7)°C and (47.8 ± 0.5)°C, respectively (supplementary Figure S4). The melting temperature of fully complementary (V3 + V4) duplex is equal to (80.2 ± 0.5)°C, whereas the melting temperature of the X3 junction is equal to (59.7 ± 0.5)°C. In the presence of ATP OBP catalyzes dissociation of the junction into two partial duplexes which are in concentration-dependent equilibrium with the monomers. Each monomer can be folded into a hairpin-shaped structure stabilized by complementary base pairing of Box I and Box III sequences.

OBP is known to bind strongly to the D1 duplex containing specific sites, Box I and Box II, for interaction with the protein. Our gel shift experiments demonstrate that OBP exhibits approximately five times higher affinity to the X2 junction (complex I) as compared to its affinity to the Box I site in duplex D1 (Figure 3, panel c). The protein forms two types of complexes with tetramer (V1)$_4$ which are reminiscent of complexes I and II formed by OBP with junctions X2 and X3 (Figure 10). The HJ-specific protein RuvA of *E. coli* binds to the oligonucleotides V2 and V3 and, presumably, promotes assembly of these oligonucleotides into four-way DNA junctions (Figure 11).

The protein forms two types of complexes with oligonucleotides V2 and V3 which manifest low mobilities during electrophoresis in the polyacrylamide gel (Figure 11). The results of our previous experiments as well as data of other investigators show that the V3 hairpin containing stretches of unpaired nucleotides at 5′-
3’- ends represents a minimal DNA substrate to which OBP binds with a high affinity (Macao et al., 2004; Surovaya et al., 2010). However, this complex moves faster during gel electrophoresis than complexes of OBP with four-way junctions. From data presented in Figures 10 and 11 it seems likely that *E. coli* RuvA and HSV1 OBP both promote the assembly of V2 and V3 oligonucleotides into four-way DNA junctions. Experiments are now in progress to test the validity of these suggestions.

**Structural rearrangement induced upon binding of OBP dimers to the OriS duplex containing Box I and Box II sites separated by A+T-rich spacer**

We propose that two OBP dimers bind to the linear duplex form of OriS in a sequence-specific manner and promote structural rearrangement of the OriS duplex and formation of a HJ (Figure 12). The presumed HJ involves Box I and Box II sequences, a pseudosymmetric sequence in the A+T-rich spacer element and flanked DNA regions. The junction has two horizontal arms extruded in the upper and lower strands of OriS duplex (63 base pairs) upon binding of OBP. Each horizontal arm contains 18 unpaired adenine and thymine residues which serve as “nucleus” facilitating the helix-coil transition at the A+T-rich region of the arm. In addition, three consecutive GC pairs in each arm are flanked by unpaired nucleotides.

An important property of the HJ is its ability to undergo branch migration through regions of sequence homology. This process involves the melting and annealing of base pairs and results in the movement of branch point along the arms within the region of sequence homology (George et al., 2002; Panyutin, Biswas, &
The viral protein ICP8 is known to interact with single-stranded DNA segments. It may also facilitate the melting of five consecutive AT base pairs at each horizontal arm. Each bound ICP8 molecule occupies a region extending over 10 ± 1 nucleotides (Boehmer & Lehman, 1993; Gourves, Le Gael, Villanit, Boehmer, & Johnson, 2000). Since horizontal arms are related by a two-fold symmetry branch migration is possible along the arms at the expense of ATP hydrolysis. The DNA junction seems to serve as a platform for interaction between bound OBP and ICP8 molecules. Due to the unwinding activity of OBP and strong preference of ICP8 for binding to single-stranded DNA segments, one can suggest that both Box I and Box II sequences and A+T-rich spacer can be turned to unwound states.

The model shown in Figure 12 is supported by electron microscopy studies showing that stable preunwinding complexes were formed when OBP and ICP8 were incubated with negatively supercoiled DNA containing OriS site (Makhov, Lee, Lehman, & Griffith, 2003). Extrusion of a cruciform at OriS favors the assembly of OBP dimers and ICP8 and facilitates melting of the A+T-rich region which connects Box I and Box II sites. The presumed structure of the four-way DNA junction (Figure 12) is also supported by the results of our FRET measurements. The efficiency of energy transfer was measured in two systems containing duplexes (V1–1+V2) and (V1–2+V2) (Figure 2) which were mixed with OBP and contained the...

Figure 10. EMSA analysis of complexes formed by OBP with the junction X2, duplex D1, and self-associated duplex (V1)2.

Notes: Lanes 1, 4, 7 – X2 junction (100 nM), D1 duplex (100 nM), and V1 oligonucleotide (100 nM), respectively; lanes 2, 5, 8 – reaction mixture containing OBP (300 nM) and one of the following DNA substrates: X2 junction (100 nM), D1 duplex (100 nM), and V1 oligonucleotide (100 nM), respectively; lanes 3, 6 and 9 – reaction mixture containing OBP (300 nM), ATP (5 mM) and one of the following DNA substrates: X2 junction (100 nM), D1 duplex (100 nM), and V1 oligonucleotide (100 nM), respectively.
same molar ratio of added OBP dimers to DNA oligomer. We have found that the fluorescence intensity of the duplex (V1\(^{-2}+V2\)) carrying both the fluorophore and the quencher diminishes nearly threefold upon addition of OBP, while that of the system containing (V1\(^{-1}+V2\)) duplex (which carries no quencher), diminishes only by about 20% in the presence of OBP. Evidently, in the latter case, the quenching effect is not associated with Forster mechanism of resonance energy transfer. Presumably, it is mediated by a direct contact between the OBP and R6G fluorophore conjugated with (V1\(^{-1}+V2\)) duplex (Marras, Kramer, & Tyagi, 2002). The distance between the fluorophore and the quencher can be measured by estimating the efficiency of the resonance energy transfer from the fluorophore to the quencher in the complex between OBP and duplex (V1\(^{-2}+V2\)):

\[
R = R_0\left(\frac{I_{DA,OBP}/I_{D,OBP}}{(1 - I_{DA,OBP}/I_{D,OBP})^{1/6}}\right)
\]  

Here \(I_{D,OBP}\) and \(I_{DA,OBP}\) are the fluorescence intensities of the donor in complexes of OBP with the duplexes (V1\(^{-1}+V2\)) and (V1\(^{-2}+V2\)), respectively. \(R_0\) is the critical distance between the fluorophore and the quencher wherein the probability of the resonance energy transfer from the fluorophore to the quencher is equal to the probability of spontaneous emission of the light by the fluorophore in the absence of the quencher. The DNA oligomer concentrations and the molar ratio of OBP to the DNA oligomer for these complexes practically coincide. The estimated values of distances between the fluorophore and quencher are based on \(R_0 = 63\) Å for a pair R6G–BHQ2 (Bazhulina et al., 2014) and numerical factor \(K^2 \approx 2/3\) where \(K^2\) depends on the mutual orientation.

Figure 11. EMSA analysis of complexes formed by _E. coli_ RuvA protein with X3 junction and self-associated duplexes (V3)\(_2\) and (V2)\(_2\).

Notes: Experiments were performed in 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM MgCl\(_2\), 0.5 mM EDTA, 1 mM DTT, 5 mg/ml BSA and 4% Ficoll. Lanes 1, 5 and 9, X3 junction (100 nM), V3 oligonucleotide (100 nM) and V2 oligonucleotide (100 nM) in the absence of RuvA protein, respectively. Lanes 2–4, mixtures containing the X3 junction (100 nM) and RuvA tetramers at concentrations 75, 250 and 600 nM, respectively. Lanes 6–8, mixtures containing the V3 oligonucleotide (100 nM) and RuvA tetramers at concentrations 75, 250 and 600 nM, respectively. Lanes 10–12, mixtures containing the V2 oligonucleotide (100 nM) and RuvA tetramers at concentrations 75, 250 and 600 nM, respectively.
of the transition moments of the fluorophore emission and quencher absorbance. The value of $K^2 \approx 2/3$ does not lead to a substantial error because the fluorophore-quencher distance depends on inverse six power of $K^2$. The experimental value of $I_{DA,OBP}/I_{D,OBP}$ for complexes of OBP with the duplexes ($V1\!–\!2+V2$) and ($V1\!–\!1+V2$) is $\approx 0.35$ and the distance between the fluorophore and the quencher, $R \approx 57$ Å. This value is close to that expected for a separation distance between the fluorophore and quencher linked covalently to $5'$- and $3'$-ends of the $V1\!–\!2$ oligonucleotide, which is incorporated in the HJ as shown in Figure 12. Since the linear DNA duplex contains 63 base pairs, our FRET measurements showed that the DNA underwent large-scale structural changes upon binding of OBP for which interactions between bound OBP dimers are responsible. It seems likely that the viral DNA-binding protein, ICP8, interacts with single-stranded regions in the horizontal arms of the presumed HJ and, in combination with OBP, induces activation of the replication origin OriS. The single-stranded regions produced by OBP and ICP8 can be used as loading sites for the helicase–primase complex.

Interesting that OBP binds to the X9 DNA junction obtained by annealing of the oligonucleotides $V1$, $V5$, and $V6$ (see Figures 2 and 13). The X9 junction is a derivative of the branched DNA structure which is assumed to be formed in the viral replication origin upon binding of OBP and ICP8 (Figure 12).

The X9 junction contains oligonucleotides bearing Box I and Box II sequences and also has an AT-rich hairpin. Gel mobility shift experiments show that OBP forms a stable complex with the X9 junction in the absence of ATP and ICP8 (Figure 13). In the presence of 5 mM ATP the complex of OBP with X9 junction dissociates into the two partial duplexes ($V1 + V5$) and ($V1 + V6$). Two fast moving products were observed in the reaction mixture: that with the electrophoretic mobility characteristic of the duplex ($V1 + V6$) in the absence of OBP and that whose mobility is close to the mobility of the single-stranded oligonucleotide $V1$. A partial duplex ($V1 + V5$) seems to dissociate into single-stranded oligonucleotides $V1$ and $V5$. It should be noted that OBP exhibits a high affinity for binding to the X9 junction. It exhibits a weaker affinity to the ($V1 + V6$) duplex and single-stranded oligonucleotides $V1$ and $V5$ (data are not shown). Interesting that ATP-dependent processing of the X9 junction by OBP is observed at $37^\circ$C in the absence of ICP8 (Figure 13), whereas two proteins, OBP and ICP8, are needed for the unwinding of minimal OriS duplex containing Box I and Box II.
inverted repeats and A+T-rich spacer (Manolaridis et al., 2009; He & Lehman, 2000; He & Lehman, 2001).

**Conclusion**

The data presented in this work expand the known functions of the initiator protein OBP of HSV1. This multifunctional protein binds in a sequence-specific manner to Box I and Box II sites in the replication origin OriS and possesses helicase and ATPase activities. It also forms a specific complex with the single-stranded DNA-binding protein ICP8. The data obtained in the present work show that the OBP binds strongly to synthetic four-way HJs and exhibits much lower affinities to a linear duplex and single-stranded DNAs. The protein forms three types of complexes with synthetic HJs. In a close similarity to the HJ-specific protein RuvA of *E. coli* OBP forms complexes I and II with X3 junction in a planar square conformation. OBP also forms a complex with X3 junction in a stacked arm conformation under high salt conditions (complex III). It should be noted that a histone-like

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Figure 13. EMSA analysis of complexes formed by OBP with four-way DNA junction X9.
Notes: Lane 1, unbound X9 junction (20 nM) alone; Lanes 2 and 3, reaction mixtures containing X9 junction (20 nM) and OBP (40 nM) in the absence and presence of ATP (5 mM), respectively. The mixtures were incubated for 1 h at 37°C in 50 mM Tris-HCl buffer (pH 8.4) containing 50 mM NaCl, 10 mM MgCl2, 5 mM DTT, 0.2% Triton X-100, 0.5 mg/ml BSA and 4% Ficoll and then loaded onto 7.5% PAGE (29:1) and electrophoresed at 4°C for 5 h. Lane 4, unbound V1 oligonucleotide (5 nM) which serves as a marker.
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protein HU binds to HJs predominantly in the stacked X form (Vitoc & Mukerji, 2011). Interesting there are a wide group of proteins which bind to HJs and stabilize them exclusively in the open conformation (Pohler, Norman, Bramham, Bianchi, & Lilley, 1998). The DNA recombinase Cre (Gopaul, Guo, & Van Duyne, 1998) and some endonucleases (Hadden et al., 2001; Kvaratskhelia, George, Cooper, & White, 1999) also interact predominantly with HJs in open conformations. Evidently, HSV1 OBP belongs to a group of HJ-specific DNA binding proteins which can bind synthetic HJs both in an unfolded (open) conformation and stacked X form. We have found that in the presence of ATP the OBP catalyzes processing of a HJ in which one out of four annealed oligonucleotides carries a 3′-terminal segment containing 20 unpaired thymine residues. In the presence of OBP and ATP the DNA junction dissociates into two partial DNA duplexes. A conclusion can be drawn that OBP plays a key role in the DNA structural rearrangement during the initiation of the viral DNA replication. Further studies of the origin DNA unwinding catalyzed by OBP in the presence of ICP8 and ATP may provide a deeper insight into molecular mechanisms underlying the functional roles of OBP and ICP8 in the initiation of viral DNA replication.

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No potential conflict of interest was reported by the authors.

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Supplementary material
The supplementary material for this paper is available online at http://dx.doi.org/10.1080/07391102.2016.1161561. Supplementary material dealing with electrophoretic analysis of the cleavage products generated by T7 endonuclease I in the branched DNA substrates (Figure S1), chemical structures of cis –di-ammine Pt(II) bridged bis-diaminocytanin and Pt-bridged bis netropsin (Figure S2), the presumed structure of four-way junction formed by four V3 oligonucleotides (Figure S3) and the melting curves for the duplex (V3+V4), single-stranded oligonucleotides V3 and X3junction (Figure S4) are available from the authors at: http://dnaprotolab.eimb.ru/jbsd2016.html.

References
Andronova, V. L., Grokhovsky, S. L., Galegov, G. A., Deriabin, P. G., Gorsky, G. V., & Lvov, D. K. (2010). Antiviral properties of the derivatives of netropsin and distamycin against herpes simplex virus type 1 and variolovaccine. Voprosy Virusologii (Russian), 35, 24–27.
Andronova, V. L., Grokhovsky, S. L., Surovaya, A. N., Arkhipova, V. S., Gorsky, G. V., & Galegov, G. A. (2008). Antiviral and cytotoxic activity of netropsin derivatives in vero cells infected with vaccinia virus and herpes simplex virus type I. Doklady Biochemistry and Biophysics, 422, 688–693.
Andronova, V. L., Grokhovskii, S. L., Surovaya, A. N., Gorski, G. V., & Galegov, G. A. (2001). Antitumor and antiviral activity of dimeric derivatives of netropsin. Doklady Biochemistry and Biophysics, 380, 345–348.
Andronova, V. L., Grokhovsky, S. L., Surovaya, A. N., Gorsky, G. V., & Galegov, G. A. (2007). Effect of dimeric derivatives of netropsin and their combinations with acyclovir on herpes simplex virus type 1 infection in mice. Doklady Biochemistry and Biophysics, 413, 830–834.
Andronova, V. L., Grokhovsky, S. L., Surovaya, A. N., Gorsky, G. V., & Galegov, G. A. (2013). Estimation of the activities of bis-netropsin derivatives on a model of an experimental cutaneous herpes simplex disease of Guinea Pigs. Voprosi Virusologii (Russian), 38, 32–35.
Ariyoshi, M., Nishino, T., Iwasaki, H., Shinagawa, H., & Morikawa, K. (2000). Crystal structure of the Holliday junction DNA in complex with a single RuvA tetramer. Proceedings of the National Academy of Sciences of the USA, 97, 8257–8262.
Aslani, A., Macao, B., Simonsson, S., & Elias, P. (2001). Complementary intrastrand base pairing during initiation of Herpes simplex virus type 1 DNA replication. Proceedings of the National Academy of Sciences of the USA, 98, 7194–7199.
Aslani, A., Olsson, M., & Elias, P. (2002). ATP-dependent unwinding of a minimal origin of DNA replication by the origin-binding protein and the single-strand DNA-binding protein ICP8 from herpes simplex virus type I. Journal of Biological Chemistry, 277, 41204–41212.
Assenberg, R., Weston, A., Cardy, D. N. L., & Fox, K. R. (2002). Sequence-dependent folding of DNA three-way junctions. Nucleic Acids Research, 30, 5142–5150.
Bazhulina, N. P., Surovaya, A. N., Gursky, Y. G., Andronova, V. L., Arkhipova, V. S., Golovkin, M. V., … Gorsky, G. V. (2012). Inhibition of herpes simplex virus helicase UL9 by netropsin derivatives and antiviral activities of bis-netropsins. Biophysics, 57, 153–162.
Bazhulina, N. P., Surovaya, A. N., Gursky, Y. G., Andronova, V. L., Moiseeva, E. D., Nikitin, A. M., … Gorsky, G. V. (2014). Complex of the herpes simplex virus type 1 origin binding protein UL9 with DNA as a platform for the design of a new type of antiviral drugs. Journal of Biomolecular Structure & Dynamics, 32, 1456–1473.
Belikov, S. V., Grokhovsky, S. L., Isagulians, M. G., Surovaya, A. N., & Gorsky, G. V. (2005). Sequence-specific minor groove binding ligands as potential regulators of gene expression in xenopus laevis oocytes. Journal of Biomolecular Structure & Dynamics, 23, 193–202.
Boehmer, P. E., Craigie, M. C., Stow, N. D., & Lehman, I. R. (1994). Association of origin binding protein and single strand DNA-binding protein, ICP8, during herpes simplex virus type 1 DNA replication in vivo. Journal of Biological Chemistry, 269, 29329–29334.
Boehmer, P. E., & Lehman, I. R. (1993). Herpes simplex virus type 1 ICP8: Helix-destabilizing properties. *Journal of Virology, 67*, 711–715.

Carter, A. S., Tahmaseb, K., Compton, S. A., & Matson, S. W. (2012). Resolving Holliday junctions with Escherichia coli UvrD helicase. *Journal of Biological Chemistry, 287*, 8126–8134.

Chattopadhyay, S., & Weller, S. K. (2007). Direct interaction between the N- and C-terminal portions of the herpes simplex virus type 1 origin binding protein UL9 implies the formation of a head-to-tail dimer. *Journal of Virology, 81*, 13659–13667.

Clegg, R. G., Murchie, A. I. H., Zechel, A., Carlborg, C., Diekmann, S., & Lilley, D. M. J. (1992). Fluorescence resonance energy transfer analysis of the structure of the four-way DNA junction. *Biochemistry, 31*, 4846–4856.

Doherty, A. J., Serpell, L. C., & Ponting, Ch. P. (1996). The helix–hairpin–helix DNA-binding motif: A structural basis for non-sequence-specific recognition of DNA. *Nucleic Acids Research, 24*, 2488–2497.

Duckett, D. R., Murchie, A. I. H., Diekmann, S., von Kitzing, E., Kemper, B., & Lilley, D. M. J. (1988). The structure of the Holliday junction, and its resolution. *Cell, 55*, 79–89.

Eichman, B. F., Vargason, J. M., Mooers, B. H. M., & Ho, P. S. (2001). The Holliday junction in an inverted repeat DNA sequence: Sequence effects on the structure of four-way junctions. *Proceedings of the National Academy of Sciences of the USA, 97*, 3971–3976.

Eom, C. Y., & Lehman, I. R. (2002). The human DnaJ protein, hDnJ-I, enhances binding of a multimer of the herpes simplex virus type 1 UL9 protein to oriS, an origin of viral DNA replication. *Proceedings of the National Academy of Sciences of the USA, 99*, 1894–1898.

Fierer, D. S., & Challberg, M. D. (1992). Purification and characterization of UL9, the herpes simplex virus type 1 origin-binding protein. *Journal of Virology, 66*, 3986–3995.

Förster, T. (1948). Intermolecular energy migration and fluorescence. *Annalen der Physik, 2*, 55–75.

George, H., Kuraoka, I., Nauman, D. A., Kobertz, W., Wood, R. D., & West, S. C. (2002). RuvAB-mediated branch migration does not involve extensive DNA opening within the RuvB hexamer. *Current Biology, 10*, 103–106.

Gopal, D. N., Guo, F., & Van Duyne, G. D. (1998). Structure of the Holliday junction intermediate in Cre-loxP site-specific recombination. *The EMBO Journal, 17*, 4175–4187.

Gourves, A.-S., Le Guel, N. T., Villaniit, G., Boehmer, P. E., & Johnson, N. P. (2000). Equilibrium binding of single-stranded DNA with herpes simplex virus type I-coded single-stranded DNA-binding protein, ICP8. *Journal of Biological Chemistry, 275*, 10864–10869.

Grainger, R. J., Murchie, A. I. H., & Lilley, D. M. J. (1998). Exchange between stacking conformers in a four-way DNA junction. *Biochemistry, 37*, 23–32.

Grokhosvsky, S. L., Surovaya, A. N., Burkhartd, G., Pismensky, V. F., Chernov, B. K., Zimmer, C., & Gursky, G. V. (1998). DNA sequence recognition by bis-linked netropsin and distamycin derivatives. *FEBS Letters, 439*, 346–350.

Gursky, G. V., Zasosatelev, A. S., Zhure, A. L., Khorlin, A. A., Grokhovsky, S. L., Streltsov, S. A., ... Gottikh, B. P. (1983). Synthetic Sequence-specific ligands. *Cold Spring Harbor Symposium on Quantitative Biology, 47*, 367–378.

Gustafsson, C. M., Hammarsten, O., Falkenberg, M., & Elias, P. (1994). Herpes simplex virus DNA replication: A spacer sequence directs the ATP dependent formation of a nucleo-protein complex at OriS. *Proceedings of the National Academy of Sciences, 91*, 4629–4633.

Hadden, J. M., Déclais, A. C., Carr, S. B., Lilley, D. M. J., & Phillips, S. E. (2007). The structural basis of Holliday junction resolution by T7 endonuclease I. *Nature, 449*, 621–624.

Hays, F. A., Schirv, V., Ho, P Sh, & Demeler, B. (2006). Solution formation of Holliday junctions in inverted repeat DNA sequences. *Biochemistry, 45*, 2467–2471.

He, X., & Lehman, I. R. (2000). Unwinding of a herpes simplex virus type 1 origin of replication (OriS) by a complex of the viral origin binding protein and the single-stranded DNA binding protein. *Journal of Virology, 74*, 5726–5728.

He, X., & Lehman, I. R. (2001). An initial ATP-independent step in the unwinding of a herpes simplex virus type I origin of replication by a complex of the viral origin-binding protein and single-stranded DNA-binding protein. *Proceedings of the National Academy of Sciences, 98*, 3024–3028.

Ho, P. S., & Eichman, B. F. (2001). The crystal structures of DNA Holliday junctions. *Current Opinion in Structural Biology, 11*, 302–308.

Koff, A., Schwedes, J. F., & Tegtmeyer, P. J. (1991). Herpes simplex virus origin-binding protein (UL9) loops and distort the viral replication origin. *Journal of Virology, 65*, 3284–3292.

Kvaratskhelia, M., George, S. J., Cooper, A., & White, M. F. (1999). Quantitation of metal ion and DNA junction binding to the Holliday junction endonuclease Cce I. *Biochemistry, 38*, 16613–16619.

Lee, S. S., & Lehman, I. R. (1997). Unwinding of the box 1 element of a herpes simplex virus type 1 origin by a complex of the viral origin binding protein and single-stranded DNA binding protein, and single-stranded DNA. *Proceedings of the National Academy of Sciences, 94*, 2838–2842.

Leontis, N. B., Hills, M. T., Piotto, M., Ouporov, I. P., Malhotra, A., & Gorenstein, D. G. (1994). Helical stacking in DNA three-way junctions containing two unpaired pyrimidines: proton NMR studies. *Biophysical Journal, 68*, 251–265.

Lilley, D. M. J. (2000). Structures of helical junctions in nucleic acids. *Quarterly Reviews of Biophysics, 33*, 109–159.

Lloyd, R. G., & Sharples, G. J. (1993). Processing of recombinant intermediates by the RecG and RuvAB proteins of *Escherichia coli*. *Nucleic Acids Research, 21*, 1719–1725.

Macao, B., Olsson, M., & Elias, P. (2004). Functional Properties of the herpes simplex virus type I origin-binding protein are controlled by precise interactions with the activated form of the origin of DNA replication. *Journal of Biological Chemistry, 279*, 29211–29217.

Mahdi, A. A., McGlynn, P., Levett, S. D., & Lloyd, R. G. (1997). DNA binding and helicase domains of the *Escherichia coli* recombination protein RecG. *Nucleic Acids Research, 25*, 3875–3880.

Makhov, A. M., Lee, S. S. K., Lehman, I. R., & Griffith, J. D. (2003). Origin-specific unwinding of herpes simplex virus 1 DNA by the viral UL9 and ICP8 proteins: Visualization of a specific preunwinding complex. *Proceedings of the National Academy of Sciences, 100*, 898–903.

Malik, A. K., & Weller, S. K. (1996). Use of transdominant mutants of the origin-binding protein (UL9) of herpes simplex virus type 1 to define functional domains. *Journal of Virology, 70*, 7859–7866.
Manolaridis, I., Mumtsidu, E., Konarev, P., Makhov, A. M., Fullerton, S. W., Sinz, A., … Tucker, P. A. (2009). Structural and biophysical characterization of the proteins interacting with the herpes simplex virus 1 origin of replication. *Journal of Biological Chemistry*, 284, 16343–16353.

Marras, S. A. E., Kramer, F. R., & Tyagi, S. (2002). Efficiencies of fluorescence resonance energy transfer and contact-mediated quenching in oligonucleotide probes. *Nucleic Acids Research*, 30, 122e.

McKinney, S. A., Déclais, A.-C., Lilley, D. M. J., & Ha, T. (2003). Structural dynamics of individual Holliday junctions. *Nature Structural Biology*, 10, 93–97.

Nikolaev, V. A., Grokhovsky, S. L., Surovaya, A. N., Leinsoo, T. A., Sidorova, N Yu, Zasedatelev, A. S., … Gursky, G. V. (1996). Design of sequence-specific DNA binding ligands that use a two-stranded peptide motif for DNA sequence recognition. *Journal of Biomolecular Structure & Dynamics*, 14, 31–47.

Nishino, T., Ariyoshi, M., Iwasaki, H., Shinagawa, H., & Morikawa, K. (1998). Functional analyses of the domain structure in the Holliday junction binding protein RuvA. *Structure*, 6, 11–21.

Olsson, M., Tang, K.-W, Persson, C., Wilhelmsson, L. M., Billetter, M., & Elias, P. (2009). Stepwise evolution of the herpes simplex virus origin binding protein and origin of replication. *Journal of Biological Chemistry*, 284, 16246–16255.

Ortiz-Lombardia, M., Gonzalez, A., Eritja, R., Ayamini, J., Azorin, F., & Coll, M. (1999). Crystal structure of a DNA Holliday junction. *Nature Structural Biology*, 6, 913–917.

Overmars, F. J. J., & Altona, C. (1997). NMR study of the exchange rate between two stacked conformers of a model Holliday junction. *Journal of Molecular Biology*, 273, 519–524.

Overmars, F. J. J., Lanzotti, V., Galeone, A., Pepe, A., Mayol, L., Pikkemaat, J. A., & Altona, C. (1997). Design and NMR study of an immobile DNA four-way junction containing 38 nucleotides. *European Journal of Biochemistry*, 249, 576–583.

Panyutin, I. G., Biswas, I., & Hsieh, P. (1995). A pivotal role for the structure of the Holliday junction in DNA branch migration. *EMBO Journal*, 14, 1819–1826.

Panyutin, I. G., & Hsieh, P. (1993). Formation of a single base mismatch impedes spontaneous DNA branch migration. *Journal of Molecular Biology*, 230, 413–424.

Parsons, C. A., Tsaneva, I., Lloyd, R. G., & West, S. C. (1992). Interaction of Escherichia coli RuvA and RuvB proteins with synthetic Holliday junctions. *Proceedings of the National Academy of Sciences*, 89, 5452–5456.

Parsons, C. A., & West, S. C. (1990). Specificity of binding to four-way junctions in DNA by bacteriophage T7 endonuclease I. *Nucleic Acids Research*, 18, 4377–4384.

Pohler, J. R. G., Norman, D. G., Bramham, J., Bianchi, M. E., & Lilley, D. M. J. (1998). HMG box proteins bind to four-way DNA junctions in their open conformation. *The EMBO Journal*, 17, 817–826.

Rafferty, J. B., Sedelnikova, S. E., Hargreaves, D., Artyumiak, P. J., Baker, P. J., Sharples, G. J., … Rice, D. W. (1996). Crystal structure of DNA recombination protein RuvA and a model for its binding to the Holliday junction. *Science*, 274, 415–421.

Roe, S. M., Barlow, T., Brown, T., Oram, M., Keeley, A., Tsaneva, I. R., & Pearl, L. H. (1998). Crystal structure of an octameric RuvA–Holliday junction complex. *Molecular Cell*, 2, 361–372.

Sabir, T., Toulmin, A., Ma, L., Jones, A. C., McGlynn, P., Schröder, G., & Magennis, S. W. (2012). Branchpoint expansion in a fully complementary three-way DNA junction. *Journal of the American Chemical Society*, 134, 6280–6285.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular cloning: A laboratory manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Shao, X., & Grishin, N. V. (2000). Common fold in helix-hairpin-helix proteins. *Nucleic Acids Research*, 28, 2643–2650.

Stow, N. D., Brown, G., Cross, A. M., & Abbotts, A. P. (1998). Identification of residues within the herpes simplex virus type 1 origin-binding protein that contribute to sequence-specific DNA binding. *Virology*, 240, 183–192.

Sun, W., Mao, C., Liu, F., & Seeman, N. C. (1998). Sequence dependence of branch migratory minima. *Journal of Molecular Biology*, 282, 59–70.

Surovaya, A. N., Burckhardt, G., Grokhovsky, S. L., Birch-Hirschfeld, E., Gursky, G. V., & Zimmer, Ch (1997). Hairpin polyamides that use parallel and antiparallel side-by-side peptide motifs in binding to DNA. *Journal of Biomolecular Structure and Dynamics*, 14, 595–606.

Surovaya, A. N., Burckhardt, G., Grokhovsky, S. L., Birch-Hirschfeld, E., Nikitin, A. M., Fritzsche, H., … Gursky, G. V. (2001). Binding of bis-linked netropsin derivatives in the parallel-stranded hairpin form to DNA. *Journal of Biomolecular Structure and Dynamics*, 18, 689–701.

Surovaya, A. N., Grokhovskii, S. L., Bazhulina, N. P., & Gurskii, G. V. (2008). DNA-Binding activity of bis-netropsin containing a cis-diaminoplatinum group between two netropsin fragments. *Biophysics*, 53, 344–351.

Surovaya, A. N., Grokhovsky, S. L., Gurskii, Ya. G., Andronova, V. L., Arkhipova, V. S., Bazhulina, N. P., … Gursky, G. V. (2010). Complex of the herpes simplex virus initiator protein UL9 with DNA as a platform for the design of a new type of antiviral drugs. *Biophysics*, 55, 206–216.

Tolun, G., Makhov, A. M., Ludtke, S. J., & Griffith, J. D. (2013). Details of ssDNA annealing revealed by an HSV-1 ICP8–ssDNA binary complex. *Nucleic Acids Research*, 41, 5927–5937.

Vitoc, C. L., & Mukerji, I. (2011). HU binding to a DNA four-way junction probed by Förster resonance energy transfer. *Biochemistry*, 50, 1432–1441.

Weir, H. M., & Stow, N. D. (1990). Two binding sites for the herpes simplex virus type 1 UL9 protein are required for efficient activity of the OriS replication origin. *Journal of General Virology*, 71, 1379–1385.

Whitby, M. C., Bolt, E. L., Chan, S. N., & Lloyd, R. G. (1996). Interactions between RuvA and RuvC at Holliday junctions: Inhibition of junction cleavage and formation of a RuvA-RuvC-DNA complex. *Journal of Molecular Biology*, 264, 878–890.

Whitby, M. C., & Lloyd, R. C. (1998). Targeting Holliday junctions by the RecG Branch migration protein of *Escherichia coli*. *Journal of Biological Chemistry*, 273, 19729–19739.