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Initiation of DNA Replication from Closed Circular DNA

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1. Introduction

The replication of SV40 DNA and that of related viruses (polyoma, papilloma) have been studied for several decades and we know a great deal about the molecules involved in this process. However, there are two major gaps in our understanding of viral DNA replication. First, in spite of a significant amount of structural information on the viral initiator proteins, it's not known exactly how the double stranded DNA is unwound at the origin. Second, although we have known for many years which cellular proteins participate in this reaction and what they do biochemically, we know next to nothing about how they work together as a machine to initiate the replication of the DNA, elongate initiated chains and terminate the process by making two covalently closed daughter circles. The intent of this review is to consolidate the available information into working models of DNA unwinding and replication.

2. The viruses and virus DNAs

The viruses in the polyoma and papilloma groups are unique in their mode of DNA replication. However, these viruses serve as important models of how a circular DS DNA is unwound and copied. The usefulness of these models is brought out by the fact that only one or two viral proteins are involved in the DNA replication process; all other replication factors come from the cell. It is therefore recognized that whatever function these cell factors have in replicating viral DNA, it is likely that the same function is utilized by the host to replicate its own DNA. These cellular proteins associate with each other and with the viral initiator protein to make functional complexes, nanomachines in fact, that most likely also form during the replication of cellular DNA. Importantly, these virus replication systems have the advantages of being relatively simple, are easy to set up in vitro with purified components and can work from a well characterized and easily recognized origin of replication. These features are lacking in cellular replication systems. The viral assays have allowed us to generate complex models of the interactions of a number of proteins with one another and with the DNA to gain insights into mechanisms of replication. Since the structures of many of these cellular proteins have been solved by X-ray crystallography, the models are approaching an atomic understanding of events prior to and during initiation of replication. A historical review of the SV40 DNA replication field and the many advantages of this system are described by Fanning and Zhao (Fanning and Zhao 2009).

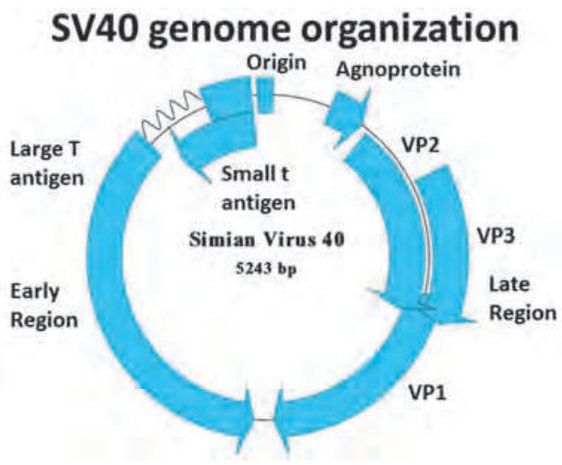


Fig. 1. Genome of SV40. The early genes are shown on the left and the late genes on the right. The origin of DNA replication is at the top.

The organization of genes in a typical polyomavirus (SV40) is shown in Fig. 1. The genome is divided into two parts: an early region and a late region (Simmons 2000). These regions are separated by the regulatory elements that include the origin of replication. Other sequences in the regulatory region are the early and late promoters, an enhancer, and a site (site I) used for repression of the early genes. The minimal origin is 64 bp long and is, by itself, sufficient for T antigen-driven DNA replication in vitro. The early genes are expressed shortly after infection. The early mRNAs are spliced differently to generate two main forms of T antigen, large and small, although numerous other forms can also be made. At about 8 hr PI, sufficient quantities of T antigen accumulate to allow DNA replication to begin. At about the same time, late transcription starts and gives rise to the agnoprotein, probably necessary for virus spread, and the three structural proteins VP1, VP2 and VP3. Early transcription continues late in infection but is down regulated when large T antigen binds site I near the origin.

3. The origin of replication

The origin of replication is tripartite. In the center, also called site II (Fig. 2), are four GAGGC pentanucleotides oriented as shown in Fig. 2 (Deb, DeLucia et al. 1986). Each pentanucleotide is a binding site for one T antigen monomer. Binding to one pentanucleotide is inefficient but the presence of multiple pentanucleotides permits cooperative interactions between T antigen monomers and together, site II constitutes a very sequence-specific strong binding site (Fanning 1992; Fanning and Knippers 1992; Bullock 1997; Simmons 2000; Fradet-Turcotte, Vincent et al. 2007). On the "early" side of site II is a region known as the early palindrome (EP) or inverted palindrome (IP). In fact, this region is not much of a palindrome, but it is the region where melting of the DNA takes place early on (Borowiec and Hurwitz 1988). On the "late" side of site II, there is a 17 bp AT rich stretch called the AT track. Structural changes take place there as well (Dean and Hurwitz 1991). When a monomer T antigen binds the origin, its origin binding domain (OBD) sits on a pentanucleotide and its helicase domain sits on the EP region or on the AT track depending on which pentanucleotide is bound (see Fig. 6) (Kim, Barbaro et al. 1999; Sreekumar, Prack et al. 2000; Reese, Sreekumar et al. 2004) .

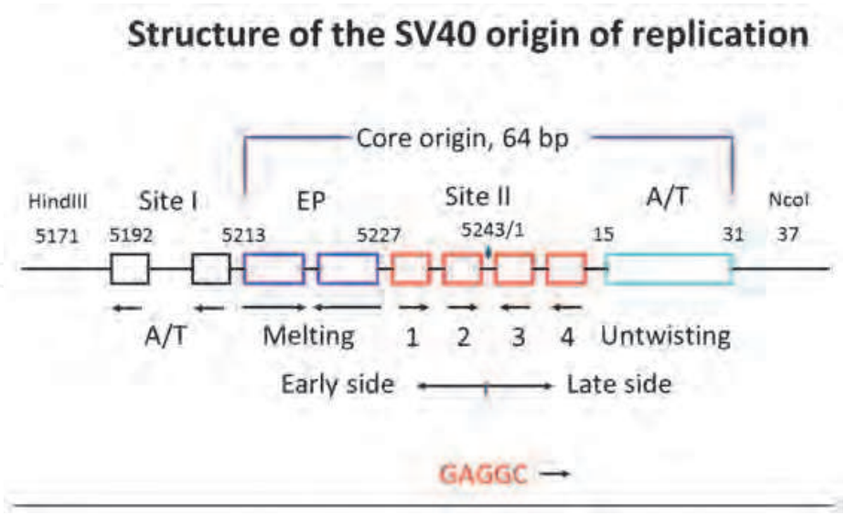


Fig. 2. The SV40 origin of replication. The minimal (core) origin is 64 bp long (Deb, DeLucia et al. 1986) and consists of three regions as shown. Auxiliary sequences that enhance replication, especially in vivo, are on the left and right of the core origin.

4. Initiator proteins

T antigen encoded by the polyomaviruses or the E1/E2 proteins encoded by the papillomaviruses are the only proteins needed from the virus for DNA replication. These proteins are multifunctional and have activities dealing with virus replication and cell transformation/immortalization. In this review, I will concentrate only on the SV40 T antigen system. A domain map of this protein is shown in Fig. 3. Three regions of the protein are important for virus and DNA replication: the J domain and associated linker region (residues 1-131), the origin binding domain (residues 131-246) and the helicase domain (residues 247-627). The C-terminal region (residues 628-708) has host type specific functions but is not directly needed for DNA replication (Simmons 2000).

The structure of the J domain in association with a fragment of the retinoblastoma protein (Rb) has been determined (Kim, Ahn et al. 2001). This region of the protein associates with DNA K-like proteins such as HSP70 and this association is used to functionally alter proteins such as Rb (Srinivasan, McClellan et al. 1997; Stubdal, Zalvide et al. 1997; Zalvide, Stubdal et al. 1998; Sullivan and Pipas 2002). The function of this domain in virus DNA replication is not clearly understood. In SV40, the J domain appears to be needed to support DNA replication in vivo but not in vitro (Sawai, Rasmussen et al. 1994; Weisshart, Bradley et al. 1996; Campbell, Mullane et al. 1997). Fragments of T antigen (such as 110-708) missing the J domain support replication in vitro just fine (Weisshart, Bradley et al. 1996; Simmons, Roy et al. 1998). However, mutants with changes in the J domain are replication defective in monkey cells (Rutila, Imperiale et al. 1986). Binding of this region to cellular proteins, including RPA and topo I (see below), may be more important in vivo.

The origin binding domain of T antigen has a critical role in initiating the replication process. That domain interacts with the GAGGC pentanucleotides within site II in a sequence specific way. The structure of the domain has been determined by NMR and X-ray crystallography (Luo, Sanford et al. 1996; Bochkareva, Martynowski et al. 2006; Meinke, Bullock et al. 2006; Meinke, Phelan et al. 2007). It has a spheroidal structure with two loops

on one side (called A1 and B2) that make sequence specific contacts with at least 4 of the base pairs at each pentanucleotide site. This domain can bind single-stranded DNA in addition to double-stranded origin DNA (Reese, Meinke et al. 2006; Fradet-Turcotte, Vincent et al. 2007; Foster and Simmons 2010; Meinke, Phelan et al. 2010). It also has the ability to hexamerize into a lock-washer structure where there is a gap between the two terminal domains (Fig. 4A) (Meinke, Bullock et al. 2006). It is reasonable to propose that the protein engages the origin as a monomer or dimer attached to each of the four pentanucleotide sites since all four pentanucleotides are needed for DNA replication (Deb, Tsui et al. 1987). Subsequent events (see below) then occur to convert this domain into the lock washer hexamer where it functions during DNA unwinding.

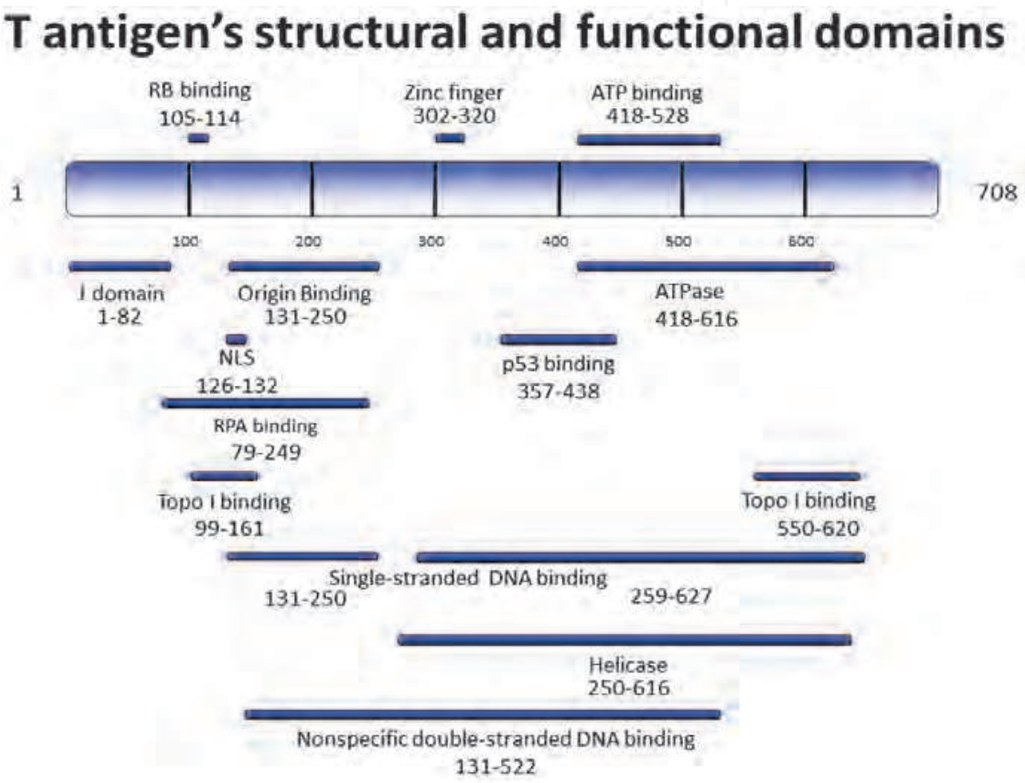


Fig. 3. Structural and functional domains of T antigen. The numbers correspond to the amino acid sequences responsible for each activity.

The helicase domain is made up of three subdomains called D1, D2 and D3 (Li, Zhao et al. 2003). D1:D1 and D2:D2 interactions are critical for the helicase to hexamerize into a functional helicase. When assembled as such, a center channel is created which is charged and accommodates DNA (Fig. 4B). The helicase domain also contains an ATPase domain. Various forms of the T antigen helicase domain have been crystallized including a nucleotide free form, one containing ADP and one with ATP (Gai, Zhao et al. 2004). These three forms are somewhat different from each other in overall dimensions, size of the inner channel and relationship between monomers. In the nucleotide free form, the channel is barely large enough to accommodate double stranded DNA but the other two forms are too small and only single stranded DNA can fit. The latter two are similar in this respect to a

structure of the BPV E1 helicase crystallized in association with single stranded DNA (Enemark and Joshua-Tor 2006). The E1 structure shows that the central channel interacts with single-stranded DNA through 6 β-hairpin loops making contacts with 6 successive nucleotides in the DNA (Fig. 5). The proposed mechanism of helicase action from this structure is that the terminal β-hairpin releases its nucleotide and through an allosteric change associated with ATP hydrolysis, the hairpin extends and grabs a nucleotide 6 residues away. All the other β-hairpins move slightly but remain associated with their nucleotide. In this way, the DNA is pulled into the hexameric channel.

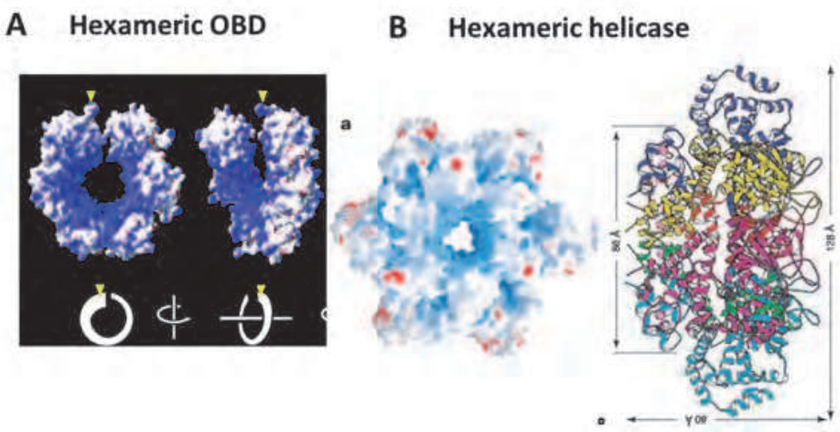


Fig. 4. The origin binding domain and helicase domain assemble into functional hexamers. (A) The crystal structures of hexameric SV40 OBD (Meinke, Bullock et al. 2006). (B) Front and side views of the hexameric helicase domain (Li, Zhao et al. 2003).

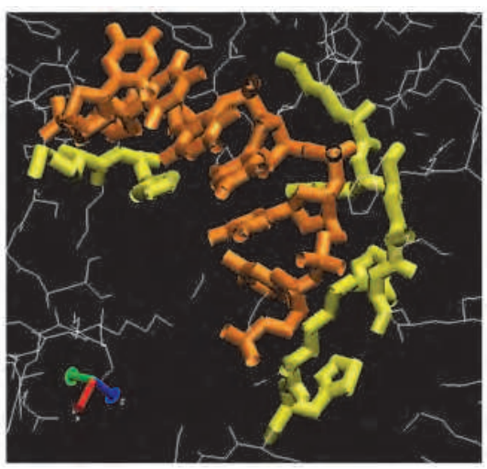


Fig. 5. Structure of the central channel of BPV E1 associated with single-stranded DNA. 12 β-hairpin residues highlighted in yellow (K506 and H507 on each monomer) are attached to the backbone of 6 successive nucleotides of ssDNA (orange) lying within the E1 helicase central channel. [Complete structure is from Enemark and Joshua-Tor (Enemark and Joshua-Tor 2006)]. Most of the other residues have been cut away for clarity. Image generated by VMD program (Humphrey, Dalke et al. 1996).

The mechanism of helicase action is likely to be essentially the same for T antigen. In this case, interactions with the phosphates as well as hydrophobic packing interactions appear to be utilized to attach each nucleotide (Shen, Gai et al. 2005).
The structure of the complete T antigen is not known, but models can be generated from the crystal structures of the three solved domains. These models should be consistent with the imaging and biochemical data. In particular, it's known that in the presence of a nucleotide, T antigen assembles into a double hexamer that covers about 65 to 70 bp of DNA (Mastrangelo, Hough et al. 1989; Gomez-Lorenzo, Valle et al. 2003; Reese, Sreekumar et al. 2004; Cuesta, Nunez-Ramirez et al. 2010). DNAse protection data show that the core origin and flanking sequences are protected by the protein (Tjian 1978; Han and Hurley 1996). EM imaging shows a similar relationship (Gomez-Lorenzo, Valle et al. 2003; Valle, Chen et al. 2006; Cuesta, Nunez-Ramirez et al. 2010). The other consideration is that the helicase domain should be able to access the EP and AT regions, and at least initially, the OBDs should be in contact with the recognition GAGGC sequences within site II (Kim, Barbaro et al. 1999; Sreekumar, Prack et al. 2000; Reese, Sreekumar et al. 2004). Nevertheless, several models can be drawn up and remain in agreement with most of the evidence. The positioning of the J domain has been determined by cryo EM by comparing images with or without this region (Cuesta, Nunez-Ramirez et al. 2010). It seems to lie on the surface of the OBDs where it might be able to interact with cellular proteins. In fact, both RPA and topo I make contacts there or in the neighboring linker region (see below).

5. Basic replication mechanism

Replication is divided into three phases: initiation, elongation and termination. Each one of those steps depends on a particular set of cellular proteins in addition to T antigen. Initiation requires replication protein A (RPA), DNA polymerase alpha/primase (pol/prim) and topoisomerase I (topo I). Elongation requires, in addition, replication factor C (RFC), PCNA and a processive polymerase (most likely pol delta). Termination needs topoisomerase II. Other factors (RNase H and ligase) are needed to remove the RNA primers and seal the resulting nicks.

6. Recognition and unwinding of the origin

In order to replicate the DNA, it must first be unwound. The resulting single strands then act as templates for DNA synthesis. One of the major obstacles in the field is to understand how sequence specific unwinding takes place from circular double stranded DNA. To appreciate the nature of the problem, we first need to consider how T antigen engages the origin. Since there are four pentanucleotide sequences in site II, it makes sense to postulate that all four sites become occupied. Extensive mutagenesis of this region has shown that all four pentanucleotides are not necessary for the formation of a double hexamer over the origin. Two of them (pentas 1 and 3 or 2 and 4) will suffice (Joo, Kim et al. 1998; Sreekumar, Prack et al. 2000), although pentas 1 and 3, which constitute one assembly site, are more effective than pentas 2 and 4 in permitting double hexamers to form. However, unwinding of the DNA (Dean, Borowiec et al. 1987) and its subsequent replication (Deb, Tsui et al. 1987) are both completely dependent on all four pentanucleotides. Therefore, all four pentanucleotides must be engaged with T antigen in order for unwinding and replication to occur. It is unclear if only two pentanucleotides are used initially to allow the double

hexamer to form and the other two (2 and 4) get involved at a later stage in preparation for unwinding. It might be difficult for two T antigen monomers to make sequence specific contacts with the DNA when they are present within a double hexamer since the origin binding domain is known to change orientation when it hexamerizes (Meinke, Bullock et al. 2006). It is somewhat more appealing to assume that all four sites are occupied at the outset (Fig. 6). These four monomers then quickly and effectively recruit additional monomers which become rearranged to form two hexamers. There is some evidence that the hexamer over the EP region (early side of origin) forms first (Parsons, Stenger et al. 1991), followed by the second hexamer on the late side. However, kinetic studies (Junfang Jiao, unpublished results) have demonstrated that the double hexamer forms very quickly (within one minute).

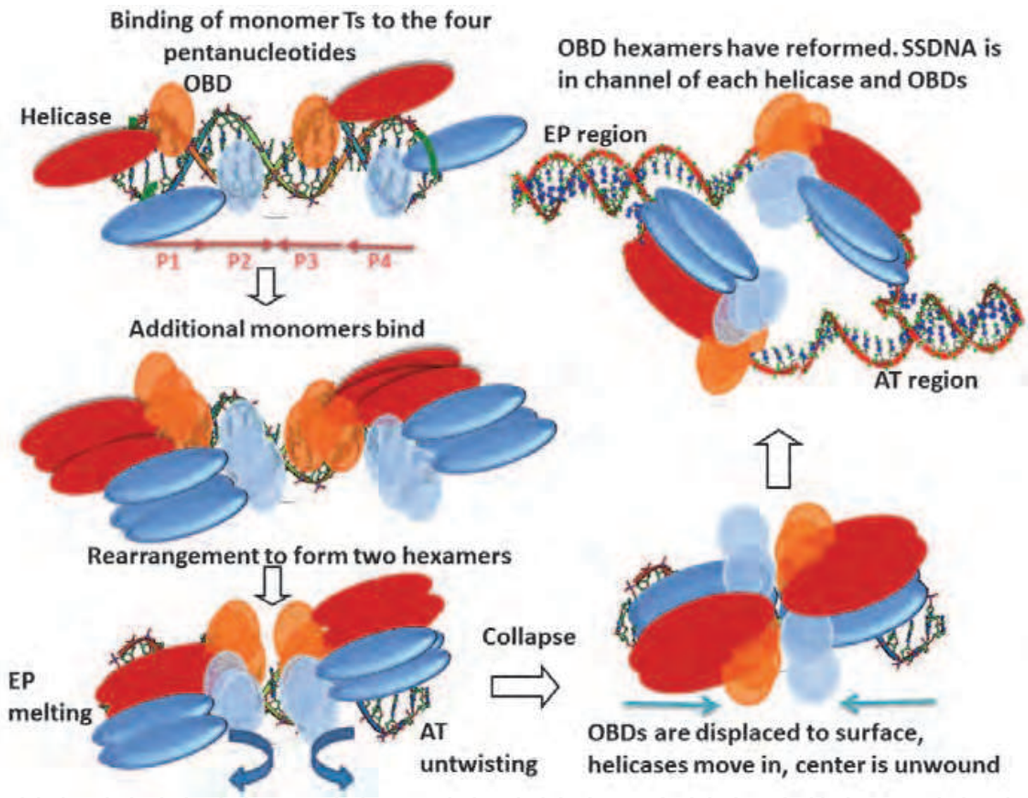


Fig. 6. Proposed model of DNA unwinding from the origin. The model is based on multiple data. Monomers attach first to the pentanucleotides at the origin, additional monomers are recruited to make an intermediate and then two hexamers form. The OBDs in the center undergo displacement to the surface of the helicase domains permitting the helicases to move in closer to the center (collapse reaction). After the center of the origin is unwound, the OBD hexamers form again and the DNA becomes threaded as shown in the top right. The hexamers then become attached to one another as shown in Fig. 8.

What is the actual structure of the double hexamers? In the presence of ATP, there is no question that single stranded DNA occupies the entire molecule (Borowiec, Dean et al. 1990; Valle, Gruss et al. 2000; VanLoock, Alexandrov et al. 2002; Gomez-Lorenzo, Valle et al. 2003; Valle, Chen et al. 2006; Cuesta, Nunez-Ramirez et al. 2010). However, in the presence of

ADP or a non-hydrolyzable ATP analogue, double hexamers still form (Borowiec and Hurwitz 1988) and these structures have the ability to melt about 8 bp of DNA at the "early" side of the EP region (Borowiec and Hurwitz 1988). The AT track is either unchanged or perhaps becomes partially untwisted (Borowiec and Hurwitz 1988; Borowiec, Dean et al. 1991) and straightened (Han and Hurley 1996). However, complete untwisting of the AT track depends on ATP (Borowiec and Hurwitz 1988). Therefore, in the absence of a usable energy source, most of the origin DNA remains double stranded within the double hexamer. It is therefore most likely that the double hexamer can accommodate double stranded DNA through most if not all of its length (Cuesta, Nunez-Ramirez et al. 2010). If this is the case, then there are at least two ways in which one strand could be moved to the surface of the helicase. The first possibility is that there is a major reorganization of the hexamer structure such that a DNA strand slips through between adjacent monomers in each hexamer and positions itself on the outside. The other mechanism involves breakage of a DNA strand, rethreading to the outside and religation of the strand. There is no experimental evidence whatsoever for the second case but there is some evidence for the first. Within the large tier of the helicase (Li, Zhao et al. 2003), there are six "hydrophilic channels" that separate individual monomers from one another and the displaced strand would have to go through one of them. Mutagenesis of each hydrophilic residue in this channel, not including the ones involved in monomer to monomer contacts, shows that nearly all of them are needed for origin unwinding (Wang, Manna et al. 2007). Importantly, a number of these mutants are not compromised in helicase activity indicating that these channels are needed specifically for unwinding of the origin.

Mutagenesis of certain residues of the OBD (Foster and Simmons 2010) shows that four amino acids placed close to the gap on one monomer form a trough that can allow single-stranded DNA to go through. One interpretation of the data is that the four residues in question participate in the threading of the single stranded DNA to the outside of the complex. Because single stranded DNA binding proteins relieve the mutants' defect, it's appealing to think that these residues are normally used to prevent back sliding of the single strand during unwinding. This model is shown in Fig. 7.The active form of the helicase is the double hexamer (Alexandrov, Botchan et al. 2002). Threading of single-stranded DNA through the double hexamer complex is of special interest because it allows us to consider how the strand on the surface of the hexamer becomes positioned so that it can be captured by the DNA polymerase for copying. Although we are still some distance from an actual structure, we can make several educated guesses based on the available evidence. First, the two strands of DNA may be separated just before one strand is pulled into the hexamer by the actions of the helicase. The other strand then surrounds the helicase and modeling shows that the OBD hexamer can be placed fairly closely to the small tier of the helicase so that it seems unlikely that the strand reenters the double hexamer between the helicase and OBD. On the other hand, both modeling and EM images of double hexamers of T antigen associated with origin DNA (Gomez-Lorenzo, Valle et al. 2003; Valle, Chen et al. 2006; Cuesta, Nunez-Ramirez et al. 2010) demonstrate that there are two significant holes between the two hexamers. These holes are made when two lock washer OBD hexamers are attached head to head. The placement of monomer OBDs in each hexamer prevents the two hexamers from coming too close to one another and two holes are generated. These holes are certainly large enough to accommodate single-stranded DNA and it makes sense to propose that the DNA reenters the structure there. One of several possible threading models, consistent with most of the data, is shown in Fig. 8.

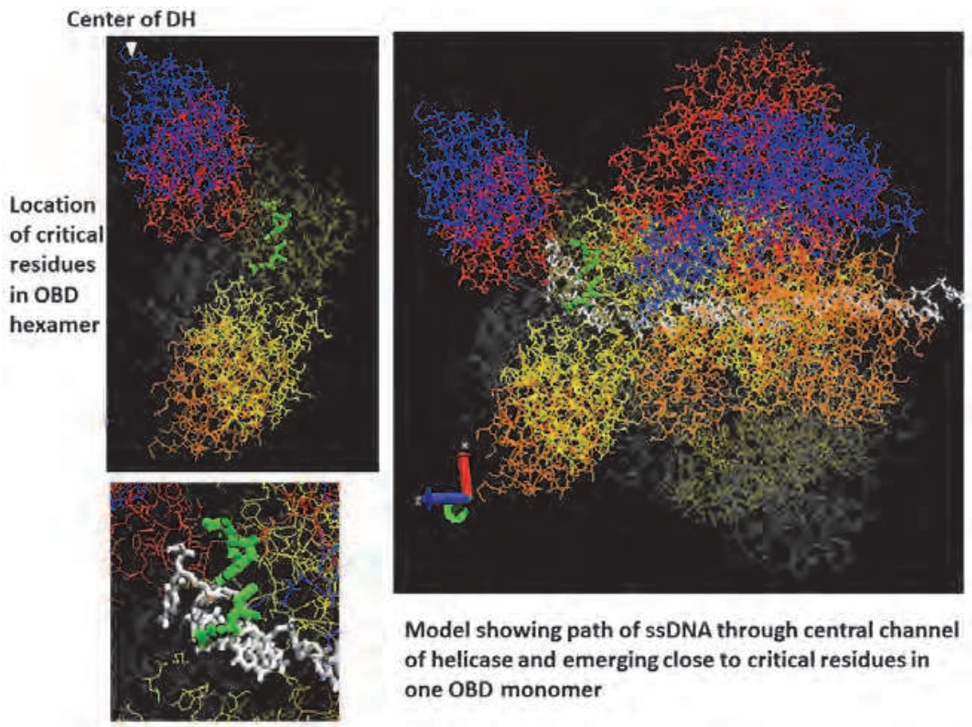


Fig. 7. Model of DNA threading through a hexamer. One strand of DNA (white) is shown to pass within the central channel of the helicase domain and partially through the channel of the OBD lock washer. There, it bends, passes very close to four residues (green) in one OBD monomer and exits the hexamer.

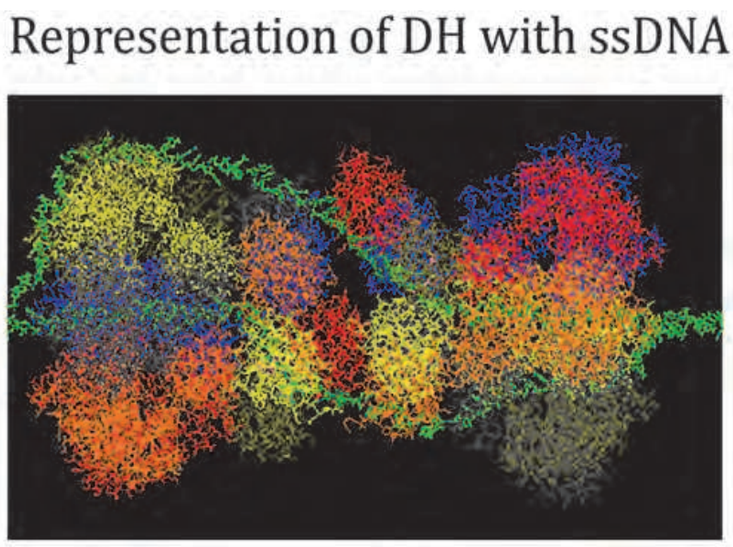


Fig. 8. 2 dimensional projection of an actual 3D model of the threading of single-stranded DNA through a double hexamer of T antigen. The DNA strands are shown in green. Each strand goes through the channel of one hexamer, emerges at the junction between hexamers and surrounds the other hexamer.

A slightly different model of DNA threading was originally proposed by Li et al (Li, Zhao et al. 2003) and Shen et al (Shen, Gai et al. 2005) and more recently by Cuesta (Cuesta, Nunez-Ramirez et al. 2010). They suggest that the β-hairpin structure is the point at which the two strands separate. At that point, one strand would continue to go through the central channel of the hexamer, the other would bend and become threaded through a side channel that exists between the two subdomains of the helicase. This is shown in Fig. 9. There is no direct evidence for either possibility, however. This latter mode of DNA threading appears to be at odds with footprinting data that shows that the DNA in the flanking regions (outside of site II) is accessible to the SSDNA reacting probe phenanthroline copper (Joo, Kim et al. 1998). In either case, a major reorganization of the hexamer structure would be required to transition one strand from the center of the channel to the side channel or to the outside of the helicase. This might be quite tricky for the model shown in Fig. 9. In the absence of strand breaks, one strand would have to move out through a space opened up by two neighboring helicases and then slide into the side channel, which would involve some impressive molecular gymnastics. In the model shown in Fig. 8, each strand would move towards the surface of the helicase altogether. This is the model that is used in the rest of this discussion.

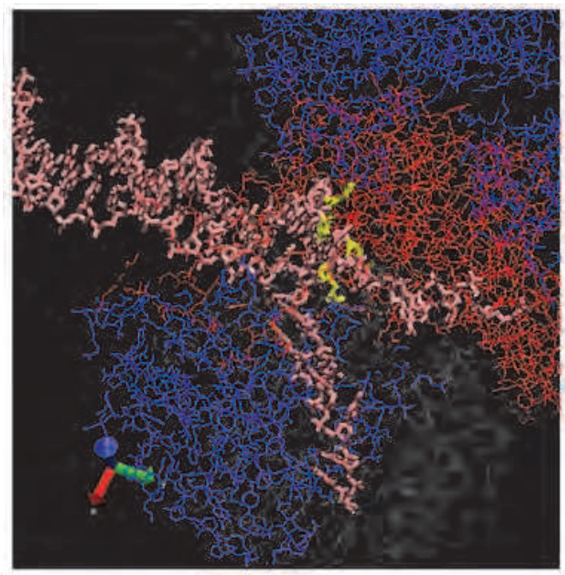


Fig. 9. Model of double stranded DNA (left) entering back end of helicase hexamer (ADP bound form) and separating at β-hairpins with one strand continuing into central channel (right) and the other threading through a side channel (bottom). The DNA is in pink, and four of the 6 His 513 residues in β-hairpin are highlighted in yellow. Some of the surface residues have been cut away for clarity.

Although there is still a large gap in our understanding of how T antigen becomes a double hexamer with single-stranded DNA going through it, the general features of this structure are fairly well accepted in the field. As shown in Fig. 8, all DNA is single-stranded. Therefore, if this were origin DNA, the center of the origin would also be completely denatured. The difficulty is that there is no obvious way for T antigen to unwind the center of the origin. When the double hexamer first forms, the helicase domains are most likely positioned over the EP and AT regions (Kim, Barbaro et al. 1999; Reese, Sreekumar et al.

2004) where they induce structural distortion. Since T antigen is a 3′ to 5′ helicase, the 3′ end of the strand that lies within the channel faces the center of the origin. Helicase activity would have to take place from the EP and AT regions OUTWARDS. The helicase has no apparent way to access the center of the origin in order to unwind it.

Evidence that the helicase does in fact structurally distort and unwind the center of the origin (mostly site II) exists. While WT T antigen can specifically unwind short substrate DNAs containing primarily site II, mutants with single substitutions in the helicase domain cannot (Wang and Simmons 2009). These mutants are structurally normal, have normal helicase activity, form double hexamers with origin DNA and bind to a variety of DNA substrates normally. Their defect appears to be primarily an inability to unwind origin DNA and, as a consequence, they are totally defective at DNA replication. It makes complete sense to presume, therefore, that helicase domain actively unwinds these short origin DNAs. However, modeling shows that in the context of a double hexamer, the ends of the DNA are far removed from the β-hairpins of the helicase domains. As shown in Fig. 10A, there is no way for the helicase domain to gain access to the ends of the DNA. Other data support the idea that a major structural change takes place in T antigen to unwind the center of the origin. First, Borowiec (Borowiec and Hurwitz 1988) showed that in the presence of ATP, the center of the origin becomes structurally distorted. Second, analysis of the unwinding of various mutant DNA substrates (Wang and Simmons 2009) demonstrated that pentanucleotides 1 and 4 are the most important for the unwinding of small origin DNAs. One way to solve this problem is to assume that the helicase domains are permitted to move into the center of the origin. The only way in which this could be done is by displacing the OBDs and relocating them to the surface of the helicase. The link between the OBD and helicase domain is a long flexible region that could easily be used to move the OBD some distance. Modeling shows that once the OBDs are displaced, the helicase domains could move close enough together so that the ends of these small DNAs can overlap with the β-hairpins (Fig. 10B). This proposed reaction is called "collapse". During collapse, the helicase would have to move 5′ to 3′ relative to the single stranded DNA within the channel. Although T antigen is primarily a 3′ to 5′ helicase, it does have a small amount of 5′ to 3′ activity (Goetz, Dean et al. 1988; Wiekowski, Schwarz et al. 1988).

The idea is also supported by what happens during unwinding of papilloma virus DNA. There, two initiator proteins are used: E1 the helicase and E2 which serves as the origin recognition protein. It has been pretty well documented that E2 binds the origin first, recruits E1 and when E1 assembles into a double hexamer, E2 is displaced and is no longer used (Sedman and Stenlund 1995; Sanders and Stenlund 2000). The situation is analogous to the collapse reaction for T antigen, but because the OBD and helicase are part of the same protein, the OBD can't leave altogether. Our model (Fig. 6) is that the collapse reaction occurs after the EP and AT regions are structurally distorted and this permits the helicase domains to move into the center of the origin where they melt all but about 15 bp. After collapse, the DNA would start moving the other (normal) direction relative to the helicases and the OBDs might very well reform their hexameric structures. The very center of the origin could become melted as the OBDs rehexamerize allowing only one strand to become threaded through the center of the hexamer (last image in Fig. 6). Simultaneously, the two hexamers would orient towards each other head to head, perhaps as illustrated in Fig. 8.

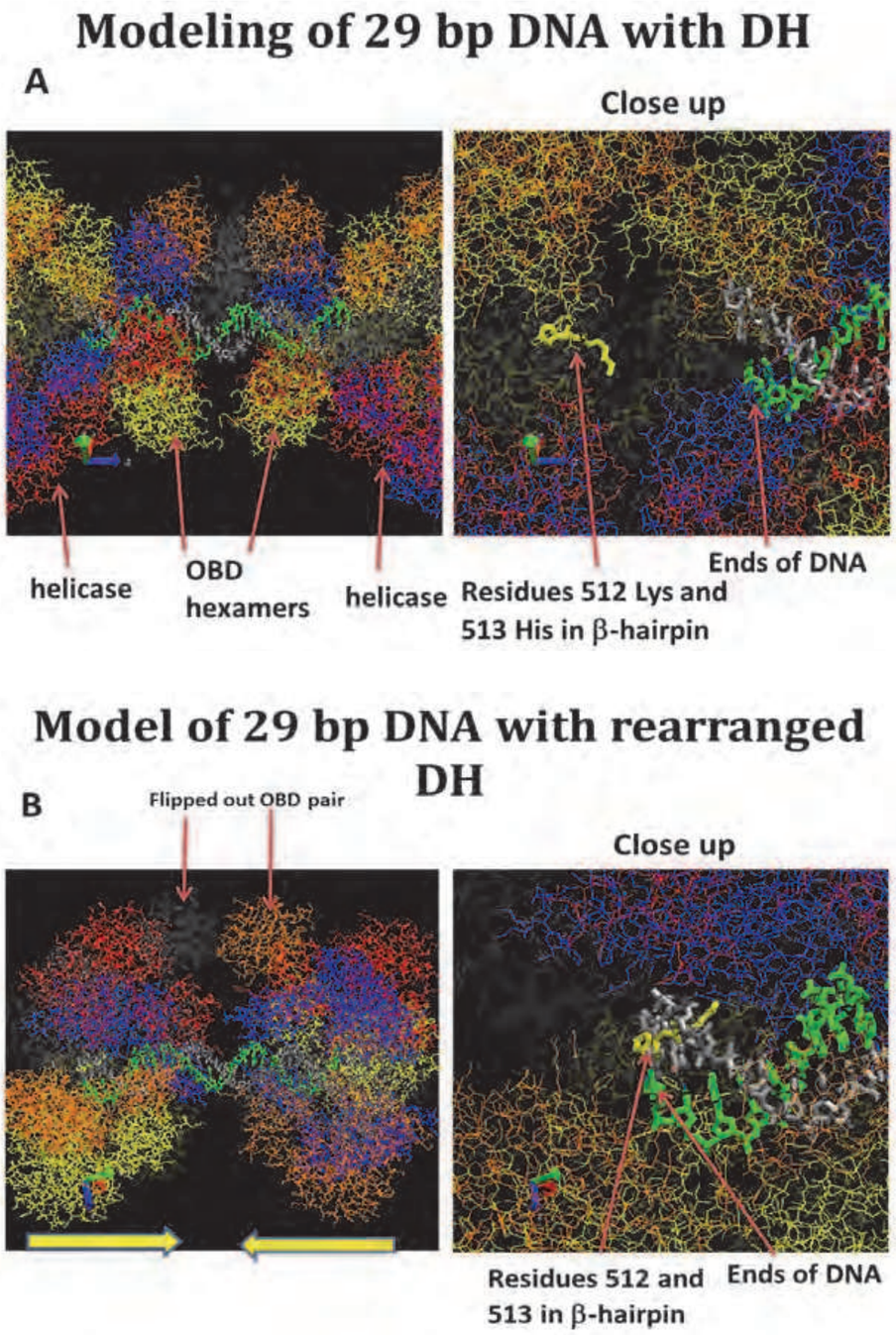


Fig. 10. (A) Modeling of a 29 bp length of DNA in the center of a double hexamer with the OBD lock washers facing each other head to head. In this model, the ends of the DNA are some distance away from the β-hairpins of the helicase domains (close up on right). (B) The OBD lock washers have been removed and the helicase domains have been relocated closer to one another. A pair of OBDs (Bochkareva, Martynowski et al. 2006; Meinke, Phelan et al. 2007) is shown on the surface of the helicases. In this structure, the β-hairpins are close to the ends of the DNA (right).

7. Initiation of DNA synthesis

Three cellular proteins are needed for efficient initiation of new chains. Although RPA and pol/prim are absolutely essential for DNA synthesis (Simmons 2000), some DNA synthesis can take place in vitro in the absence of topo I (Simmons, Trowbridge et al. 1998). However, topo I stimulates replication by up to 10-folds (Trowbridge, Roy et al. 1999). This observation holds for assays performed with crude cell extracts depleted of topo I as well as for in vitro assays with purified proteins. Topo I is needed for the efficient formation of completed form I DNA molecules as well as for the formation of replicative intermediates (Trowbridge, Roy et al. 1999). The cellular enzyme is needed from the beginning of DNA synthesis and is most likely a component of the replication initiation machine (Halmer, Vestner et al. 1998; Trowbridge, Roy et al. 1999). This was demonstrated in a number of ways, but the most convincing was that reactions started with catalytically inactive mutants of topo I could not be rescued by the later addition of WT topo I suggesting that once topo I becomes incorporated into the initiation complex, it cannot be substituted by soluble enzyme. The enzyme is therefore not merely one that is needed for the relaxation of torsionally strained DNA, but is an integral component of the replication complex, and this applies to the physiologically relevant chromatin state as well (Halmer, Vestner et al. 1998).

The order in which the three cellular proteins are recruited to the initiation complex has been studied (Simmons, Gai et al. 2004). In the presence of pol/prim, topo I binds first followed by RPA. Pol/prim was not detected in the complex probably because it has a fleeting association. Of the three cellular proteins, we know the most about how topo I is recruited to the replication machine.

8. Association of topo I with the initiation complex

There are two independent binding sites on T antigen for topo I (Simmons, Melendy et al. 1996; Roy, Trowbridge et al. 2003), one located near the C-terminal end between residues 550 and 627 and another near the N-terminal end between residues 99 to 161. The way in which topo I is recruited to T antigen at the C-terminal binding site is reasonably well understood. The binding site consists of a patch of 6 residues on the "back" side of the helicase (Khopde and Simmons 2008) (Fig. 11). Only double hexamers associated with DNA can recruit topo I (Gai, Roy et al. 2000). Single hexamers and intermediate structures are inactive. The stoichiometry of topo I to T antigen in the double hexamer is very close to 1:6 (Gai, Roy et al. 2000; Simmons, Gai et al. 2004), that is, one molecule of topo I per hexamer. It has been demonstrated that DNA flanking the core origin on both sides is necessary for efficient recruitment of topo I (Gai, Roy et al. 2000). The most obvious mechanism of topo I recruitment is therefore that one molecule first binds to the DNA on each side of the origin. Each enzyme then slides over and when it encounters a binding site on one of the helicase subunits, it "snaps" into place. This model (Fig. 12) explains all of the binding data as well as the known stoichiometry. Interestingly, given the predicted structure of the helicase-topo I complex (Khopde and Simmons 2008), DNA going through the center of topo I would have to bend around by about 165°. The idea then is that double-stranded DNA would thread through the topo I molecule, severely bend, and then separate just before entering the "back" of the helicase with one strand funneled through the central channel and the other delivered to the outside of the helicase (Fig. 12).

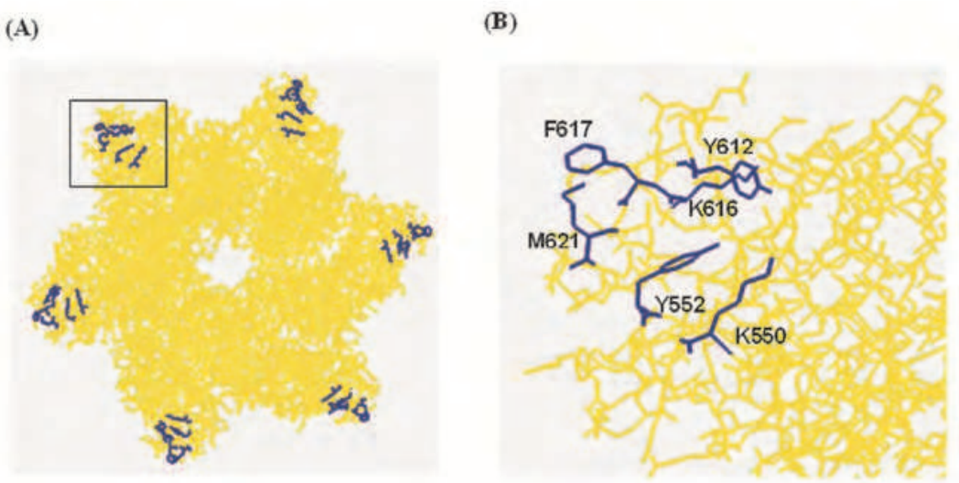


Fig. 11. (A) Structure of the "back" end of the hexameric helicase (Khopde and Simmons 2008) showing a patch of 6 residues that most likely contact topo I. (B) A close up of one of the monomers.

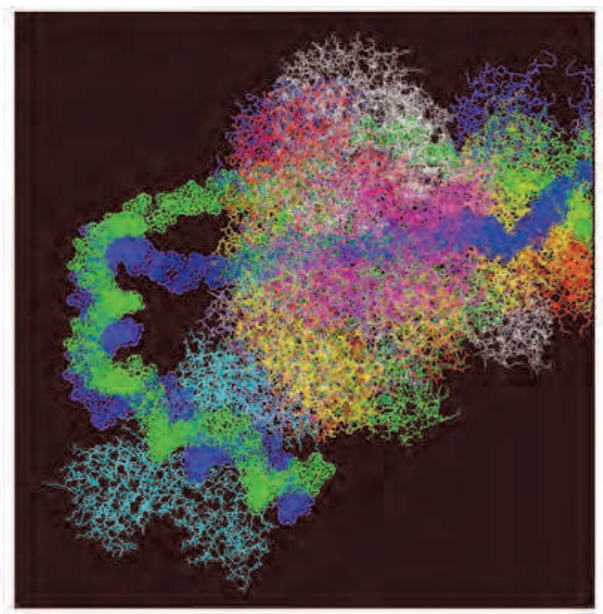


Fig. 12. Model of a T antigen hexamer with DNA threaded as shown in Fig. 8. Topo I (cyan) is shown associated with one of the T antigen helicase monomers through the six residues shown in Fig. 11. The predicted structure shows that double stranded DNA (in green and blue) going through topo I would have to bend before entering the back of the helicase with one strand going through the center channel and one strand going around the helicase domains.

Associated in this way, topo I would be in a perfect position to relax the DNA just before the DNA strands are separated by the helicase. Surprisingly, this is not its function. If this were the case, topo I binding defective mutants would be expected to be compromised in their

ability to unwind a circular DNA molecule in the presence of topo I. Khopde et al (Khopde, Roy et al. 2008) showed that all of these mutants are perfectly normal in DNA unwinding. What could the function of the bound topo I be? Since topo I stimulates DNA replication and is needed from the point of initiation, it makes sense to hypothesize that it is needed for activating or recruiting the polymerase. This was confirmed when it was shown that topo I stimulates pol/prim to synthesize about 6 to 7 times as much RNA-DNA primer (Khopde, Roy et al. 2008). These primers are made by the primase and polymerase activities of pol/prim and are about 36 nucleotides long (Bullock, Seo et al. 1991; Bullock, Tevosian et al. 1994). The most obvious way topo I could stimulate pol/prim is by directly communicating with the polymerase during initiation. At this time, however, there is no evidence that these two proteins can interact with one another.

The function of the topo I binding site near the N-terminal end of T antigen is not well understood. Mutagenesis of this region (unpublished results) has shown that it shares many of the same features as the C-terminal binding site. Mutants that are topo I binding defective cannot support DNA replication normally, although the effects are not quite as dramatic as they are for mutants with substitutions at the C-terminal region. Nevertheless, the data are convincing that both sites are needed for efficient DNA replication. If true, the stoichiometry of topo I to T antigen in the initiation complex should be 2 per hexamer, which is double the observed ratio (Gai, Roy et al. 2000; Simmons, Gai et al. 2004). There are several possible solutions to this dilemma. One is that the same topo I flips back and forth between its two binding sites (the same molecule cannot bind to both sites at once). In principle, this may be possible but it seems unlikely. Two, it's possible that binding to one site or the other is only transiently needed and no stable complex forms. For example, it's possible that the binding to the N-terminal site is only needed in the "collapsed" state described above. Since this structure is most likely very fleeting, a stable association of topo I at this site in the context of a preinitiation complex would not occur. Three, it's possible that the stoichiometry measured previously reflects that of a preinitiation complex, that is one that hasn't actually fired the origin and that initiation depends on another molecule of topo I binding each hexamer. At this time, it's not possible to clearly differentiate between these latter two choices.

9. Association of RPA with the initiation complex

RPA is a single-stranded DNA binding protein that is essential for SV40 DNA replication (Simmons 2000). The binding site for RPA on T antigen has been mapped to part of the origin binding domain, including residues 170-190, (Weisshart, Taneja et al. 1998; Arunkumar, Klimovich et al. 2005) and to the J domain (residues 79 to 97) (unpublished results). An NMR structure of the RPA 32 subunit bound to the OBD has been obtained and the contact sites on T antigen for this RPA subunit are near the C-terminal end of the domain (residues 240 to 260) (Arunkumar, Klimovich et al. 2005). The structure of part of RPA70 associated with single-stranded DNA has been determined (Bochkarev, Pfuetzner et al. 1997) but it is not known where exactly it binds to T antigen. There are probably a number of sites of interactions between the two proteins. RPA stimulates the DNA unwinding activity of T antigen (Iftode and Borowiec 1997) and an interaction with T antigen appears to be necessary for DNA replication (Weisshart, Taneja et al. 1998); (Melendy and Stillman 1993). The binding of RPA70 to the T antigen DNA binding domain stimulates the association of RPA with the emerging single stranded DNA during DNA

replication (Jiang, Klimovich et al. 2006). Given the known sites of interaction between T antigen and RPA, it is possible to construct several models that fit the data. Fig. 13 shows one such model. Only RPA70 associated with single-stranded DNA is included in the model and this protein is shown to interact with the OBD as well as with the J domain. How RPA32 and RPA 13 would fit in this structure is nearly impossible to predict based on available structures.

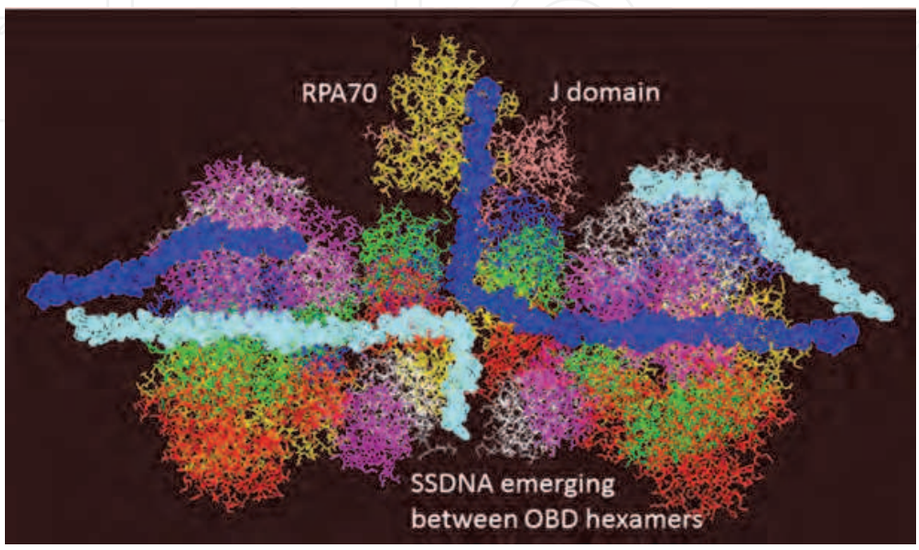


Fig. 13. One possible way in which RPA could associate with a double hexamer. RPA is shown to make contacts with the origin binding domain and with the J domain (mauve). Importantly, the single-strand shown to be emerging from the right hexamer becomes threaded through RPA (yellow). Another RPA molecule (not shown) would be associated with the light blue DNA strand at the bottom.

RPA is known to interact with pol/prim as well (Dornreiter, Erdile et al. 1992; Nasheuer, von Winkler et al. 1992; Braun, Lao et al. 1997). Its interaction with the polymerase complex most likely explains its ability to activate the polymerase during DNA synthesis (Wold 1997). The site on RPA 70K responsible for activating polymerase activity corresponds to the region involved in binding (Braun, Lao et al. 1997; Jacobs, Lipton et al. 1999). Given the critical nature of RPA in DNA synthesis, it makes perfect sense to assume that, in the initiation complex, the polymerase associates with the bound RPA and with T antigen at the same time (see below). The polymerase complex is a huge enzyme, more than two thirds the size of a T antigen hexamer. Its structure is not known, although the structure of the heterodimeric yeast pol alpha has been determined (Klinge, Nunez-Ramirez et al. 2009). The human enzyme has been shown to bind T antigen (Dornreiter, Hoss et al. 1990; Collins and Kelly 1991; Collins, Russo et al. 1993; Dornreiter, Copeland et al. 1993) and this interaction is needed for DNA replication (Taneja, Boche et al. 2007); (Huang, Zhao et al. 2010). Given its size, it might very well cover a substantial part of a hexamer. Nevertheless, these results, in summation, indicate that the "business" end of the T antigen hexamer is near the N-terminal end. This idea actually fits in quite nicely with where the single-stranded DNA is likely to emerge from the double hexamer (Fig.7). We could postulate that this same strand might be captured by an RPA molecule [stoichiometry in double hexamer preinitiation complex is the

same as topo I (Simmons, Gai et al. 2004)] and therefore thread into RPA as it emerges from the holes in the middle of the double hexamer. The polymerase might sit "behind" RPA and the DNA could thread into it as well. The threaded single stranded DNA would then be copied to make the RNA-DNA primer. This primer consists of about 7 nucleotides of RNA and 25-30 nucleotides of DNA (Bullock, Seo et al. 1991; Bullock, Tevosian et al. 1994).
An association between RPA and topo I has also been detected (Khopde, Roy et al. 2008). Since RPA binds to the N-terminal end of T antigen and topo I does as well, it is possible that all three proteins interact with one another there. A possible model incorporating these data is shown in Fig. 14.

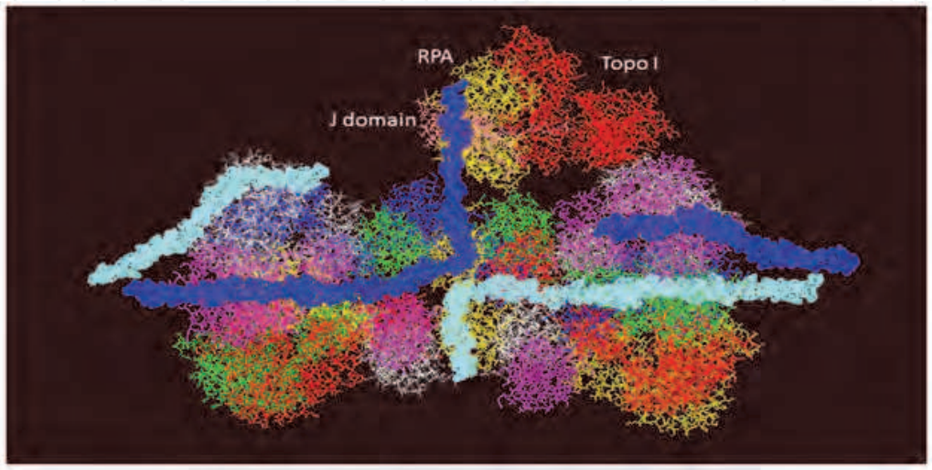


Fig. 14. Model of double hexamer with bound RPA and topo I associated with the N-terminal binding site on T antigen. Topo I binds primarily to the hinge region between the J domain and origin binding domain. In this orientation, topo I is likely to make contacts with RPA as well as with T antigen.

10. Overall mechanism of initiation

Taking all the data into accounting, we have come up with a model (Fig. 15) for how the replication machine forms and works to replicate the DNA. First, a double hexamer of T antigen forms over the origin as described above and shown in Fig. 6. The DNA is initially embedded in the channels of the OBD and helicase hexamers, mostly in double stranded form (structure 1). The only exception is probably at the EP region where about 8 nucleotides are single-stranded. How the two DNA strands of that region are threaded through the helicase is of immense interest because this information should tell us the detailed mechanism of unwinding from the origin. One possibility is that one strand is associated with the 6 β-hairpins in the center of the hexameric helicase and the other strand is threaded in a different place of the helicase. This place could be one of the side channels, but modeling suggests instead that the second strand is in one of the hydrophilic channels opened up after two monomers become separated from each other. The AT track is probably initially double stranded but untwisted (Borowiec, Dean et al. 1991; Dean and Hurwitz 1991), so all of that region may fit into the channel of the other hexamer. After collapse and strand separation, one strand gets displaced to the surface of the helicase (structure 2). Topo I then binds, first to the DNA on both sides of the origin then snaps into

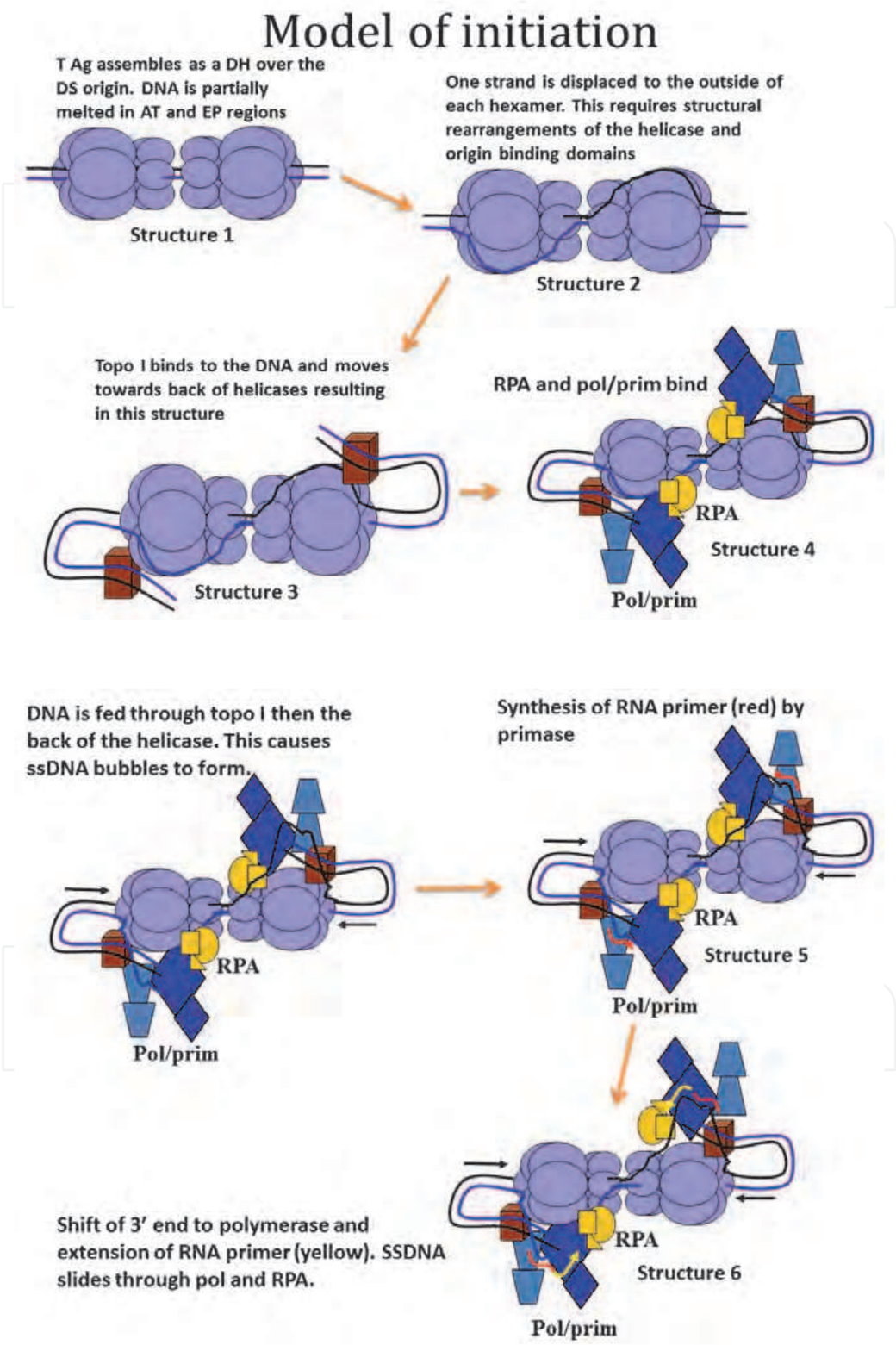


Fig. 15. Model of initiation of DNA synthesis from SV40 DNA. (see text)

place when it meets one of the six binding sites on the "back" end of each hexameric helicase (structure 3). The DNA bends severely before it enters the helicase. RPA is recruited next although it's not known at what point pol/prim becomes associated with the complex. In this model, we show RPA and pol/prim binding together (structure 4). The RPA molecule is shown to bind to its site near the N-terminal end of T antigen. In order for topo I to stimulate the polymerase to synthesize the RNA/DNA primer as described above, we can assume that pol/prim will attach to the complex via interactions with topo I as well as with T antigen and with RPA. All of these interactions appear to be essential for efficient synthesis of DNA. In this way, pol/prim is now activated and can begin the synthesis of the RNA primer (red, structure 5) followed by DNA (yellow, structure 6). During this whole time, from the time structure 2 is formed, DNA is being pulled into the complex from both sides, and consequently, the single-stranded DNA loops get larger and larger. However, the polymerase probably quickly copies each strand at a particular site leaving single-stranded DNA only on the "back" side of the reaction (Fig. 16, structure 7).

11. Elongation reaction and synthesis of Okazaki fragments

Pol/prim is replaced with the processive polymerase after it has finished making the initial primer. This elongation reaction has been well characterized (Simmons 2000). Presumably, pol/prim is displaced when RFC, the clamp loader, or an RFC-PCNA (clamp) complex attaches to the DNA at the primer/template boundary (Ellison and Stillman 1998). Pol delta (Melendy and Stillman 1991; Tsurimoto and Stillman 1991) binds and processively copies the template to make the leading strands on both sides (structures 7-9) (Fig. 16). Behind the first primers, single-stranded DNA accumulates as the helicase separates the parental strands. This DNA is copied discontinuously into 200 nucleotide Okazaki sized segments. Each one is made when another complex of topo I, RPA and pol/prim associate with the template (structure 9). Pol/prim synthesizes only a short RNA/DNA primer as it did at first, and the elongation machinery takes over to make the remainder of the Okazaki fragment. In this way, about half of each strand is copied continuously from the point of initiation and the other half of each strand is copied discontinuously behind the point of initiation as described above (Fig. 17). Termination of DNA synthesis and separation of the two daughter strands require the action of topoisomerase II. Completion of each strand requires, in addition, repair enzymes such as RNase H and DNA ligase.

12. Remaining questions

The model described above does not consider the participation of topo I at the N-terminal side of T antigen. This is still a remaining question. The various possible explanations for this were described above. It's not understood how topo I stimulates pol/prim to make an RNA/DNA primer. Does it just recruit the enzyme? The other major question is the exact way in which the double-stranded DNA becomes denatured and threaded through the double hexamer complex. Insights into this process probably will require the determination of additional T antigen-DNA structures. Another major gap in our knowledge is the interaction between the initiation and elongation machinery. Does the elongation machinery attach to T antigen? If so, where? It is thought that RPA is required for elongation as well as initiation. What is the connection between the RPA molecule that is used in initiation, those involved in coating the single-stranded DNA molecules and the molecules needed for elongation? Finally, how do these protein complexes dissociate from the DNA at

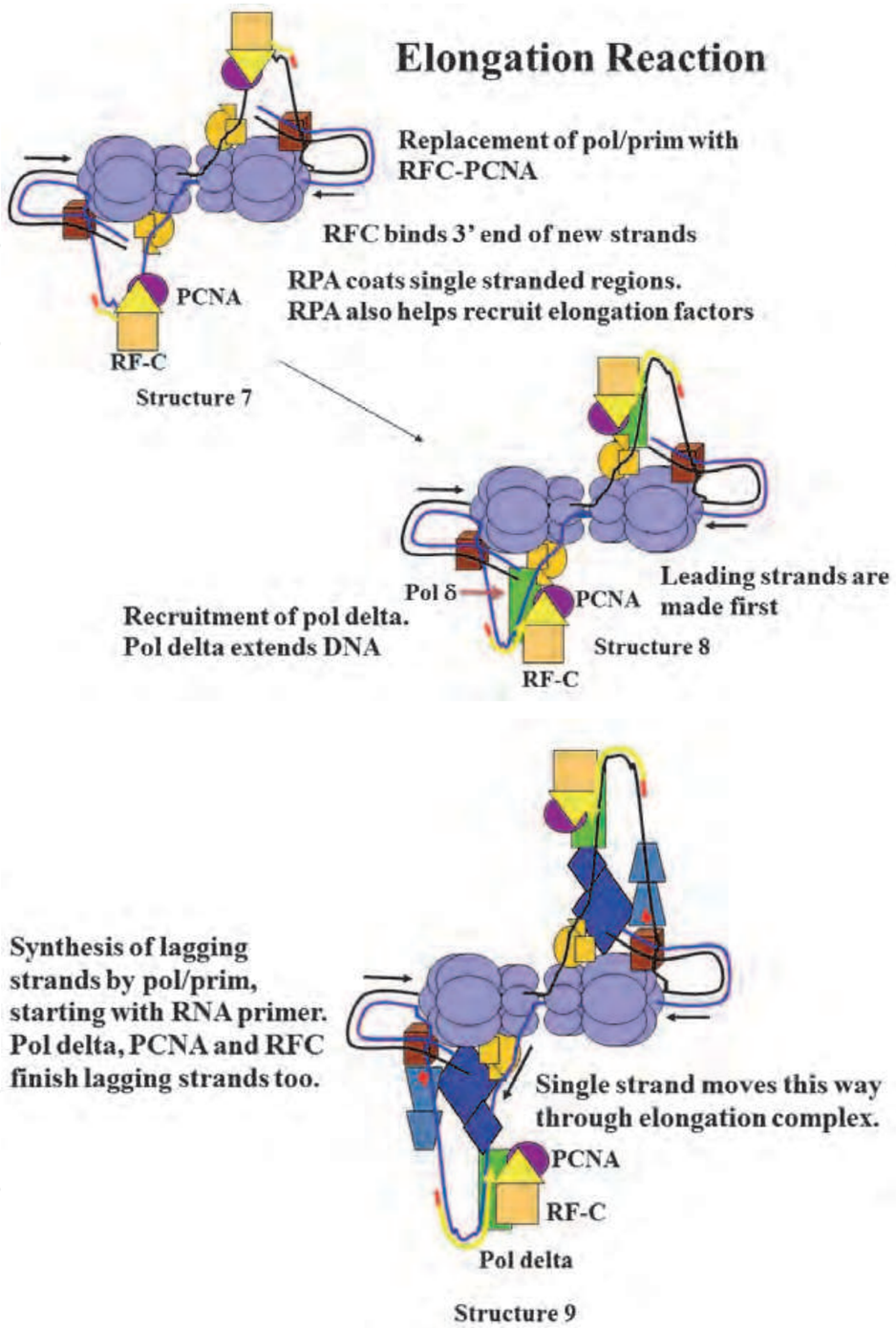


Fig. 16. Model of elongation reaction. After pol/prim has finished making an RNA-DNA primer from each strand, the enzyme is displaced and replaced by a complex of RFC-PCNA-pol δ. This complex extends the primer (DNA is shown in yellow). As single-stranded DNA accumulates "behind" the first primer, it becomes associated with a new initiation complex which synthesizes another primer. This machinery is again replaced by an elongation complex to complete the synthesis of each Okazaki fragment.

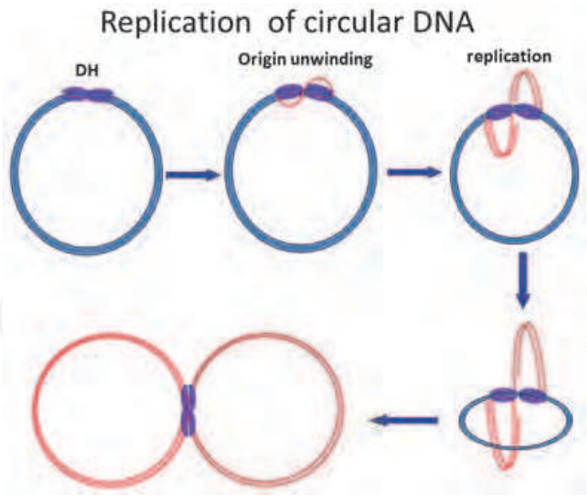


Fig. 17. Duplication of circular SV40 DNA. A double hexamer complex assembles over the origin, unwinds it and cellular proteins copy each strand as described in the text. Each strand is copied continuously and discontinuously.

termination? If either of the threading models in Fig. 9 and 10 is correct, the hexamers would have be disrupted in order for the DNA to be unraveled. A related question is how the last bit of DNA gets replicated. Is it through a repair process?

Although we have made significant progress in our understanding of the mechanism of virus DNA replication, we are still a long way from understanding these steps at the atomic level. This must be our eventual goal. Additional structural and biochemical information will have to be used to complete this difficult task.

13. Acknowledgements

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14. References

Alexandrov, A. I., M. R. Botchan, et al. (2002). "Characterization of simian virus 40 T-antigen double hexamers bound to a replication fork. The active form of the helicase." J Biol Chem 277(47): 44886-97.

Arunkumar, A. I., V. Klimovich, et al. (2005). "Insights into hRPA32 C-terminal domain--mediated assembly of the simian virus 40 replisome." Nat Struct Mol Biol 12(4): 332-9.

Bochkarev, A., R. A. Pfuetzner, et al. (1997). "Structure of the single-stranded-DNA-binding domain of replication protein A bound to DNA." Nature 385(6612): 176-81.

Bochkareva, E., D. Martynowski, et al. (2006). "Structure of the origin-binding domain of simian virus 40 large T antigen bound to DNA." Embo J 25(24): 5961-9.

Borowiec, J. A., F. B. Dean, et al. (1990). "Binding and unwinding--how T antigen engages the SV40 origin of DNA replication." Cell 60(2): 181-4.

Borowiec, J. A., F. B. Dean, et al. (1991). "Differential induction of structural changes in the simian virus 40 origin of replication by T antigen." J Virol 65(3): 1228-35.

Borowiec, J. A. and J. Hurwitz (1988). "Localized melting and structural changes in the SV40 origin of replication induced by T-antigen." Embo J 7(10): 3149-58.

Braun, K. A., Y. Lao, et al. (1997). "Role of protein-protein interactions in the function of replication protein A (RPA): RPA modulates the activity of DNA polymerase alpha by multiple mechanisms." Biochemistry 36(28): 8443-54 issn: 0006-2960.

Bullock, P. A. (1997). "The initiation of simian virus 40 DNA replication in vitro." Crit Rev Biochem Mol Biol 32(6): 503-68.

Bullock, P. A., Y. S. Seo, et al. (1991). "Initiation of simian virus 40 DNA synthesis in vitro." Mol Cell Biol 11(5): 2350-61.

Bullock, P. A., S. Tevosian, et al. (1994). "Mapping initiation sites for simian virus 40 DNA synthesis events in vitro." Mol Cell Biol 14(8): 5043-55.

Campbell, K. S., K. P. Mullane, et al. (1997). "DnaJ/hsp40 chaperone domain of SV40 large T antigen promotes efficient viral DNA replication." Genes-Dev 11(9): 1098-110 issn: 0890-9369.

Collins, K. L. and T. J. Kelly (1991). "Effects of T antigen and replication protein A on the initiation of DNA synthesis by DNA polymerase alpha-primase." Mol Cell Biol 11(4): 2108-15.

Collins, K. L., A. A. Russo, et al. (1993). "The role of the 70 kDa subunit of human DNA polymerase alpha in DNA replication." Embo J 12(12): 4555-66.

Cuesta, I., R. Nunez-Ramirez, et al. (2010). "Conformational rearrangements of SV40 large T antigen during early replication events." J Mol Biol 397(5): 1276-86.

Dean, F. B., J. A. Borowiec, et al. (1987). "Simian virus 40 large tumor antigen requires three core replication origin domains for DNA unwinding and replication in vitro." Proc Natl Acad Sci U S A 84(23): 8267-71.

Dean, F. B. and J. Hurwitz (1991). "Simian virus 40 large T antigen untwists DNA at the origin of DNA replication." J Biol Chem 266(8): 5062-71.

Deb, S., A. L. DeLucia, et al. (1986). "Domain structure of the simian virus 40 core origin of replication." Mol Cell Biol 6(5): 1663-70.

Deb, S., S. Tsui, et al. (1987). "The T-antigen-binding domain of the simian virus 40 core origin of replication." J Virol 61(7): 2143-9.

Dornreiter, I., W. C. Copeland, et al. (1993). "Initiation of simian virus 40 DNA replication requires the interaction of a specific domain of human DNA polymerase alpha with large T antigen." Mol Cell Biol 13(2): 809-20.

Dornreiter, I., L. F. Erdile, et al. (1992). "Interaction of DNA polymerase alpha-primase with cellular replication protein A and SV40 T antigen." EMBO-J 11(2): 769-76 issn: 0261-4189.

Dornreiter, I., A. Hoss, et al. (1990). "SV40 T antigen binds directly to the large subunit of purified DNA polymerase alpha." EMBO-J 9(10): 3329-36 issn: 0261-4189.

Ellison, V. and B. Stillman (1998). "Reconstitution of recombinant human replication factor C (RFC) and identification of an RFC subcomplex possessing DNA-dependent ATPase activity." J Biol Chem 273(10): 5979-87.

Enemark, E. J. and L. Joshua-Tor (2006). "Mechanism of DNA translocation in a replicative hexameric helicase." Nature 442(7100): 270-5.

Fanning, E. (1992). "Simian virus 40 large T antigen: the puzzle, the pieces, and the emerging picture." J. Virol. 66: 1289-1293.

Fanning, E. and R. Knippers (1992). "Structure and function of simian virus 40 large tumor antigen." Annu. Rev. Biochem. 61: 55-85.

Fanning, E. and K. Zhao (2009). "SV40 DNA replication: from the A gene to a nanomachine." Virology 384(2): 352-9.

Foster, E. C. and D. T. Simmons (2010). "The SV40 large T-antigen origin binding domain directly participates in DNA unwinding." Biochemistry 49(10): 2087-96.

Fradet-Turcotte, A., C. Vincent, et al. (2007). "Quantitative analysis of the binding of simian virus 40 large T antigen to DNA." J Virol 81(17): 9162-74.

Gai, D., R. Roy, et al. (2000). "Topoisomerase I associates specifically with simian virus 40 large-T-antigen double hexamer-origin complexes." J Virol 74(11): 5224-32.

Gai, D., R. Zhao, et al. (2004). "Mechanisms of conformational change for a replicative hexameric helicase of SV40 large tumor antigen." Cell 119(1): 47-60.

Goetz, G. S., F. B. Dean, et al. (1988). "The unwinding of duplex regions in DNA by the simian virus 40 large tumor antigen-associated DNA helicase activity." J Biol Chem 263(1): 383-92.

Gomez-Lorenzo, M. G., M. Valle, et al. (2003). "Large T antigen on the simian virus 40 origin of replication: a 3D snapshot prior to DNA replication." Embo J 22(23): 6205-13.

Halmer, L., B. Vestner, et al. (1998). "Involvement of Topoisomerases in the Initiation of Simian Virus 40 Minichromosome Replication." J Biol Chem 273(52): 34792-34798.

Han, F. X. and L. H. Hurley (1996). "A model for the T-antigen-induced structural alteration of the SV40 replication origin based upon experiments with specific probes for bent, straight, and unwound DNA." Biochemistry 35(24): 7993-8001.

Huang, H., K. Zhao, et al. (2010). "A specific docking site for DNA polymerase {alpha}-primase on the SV40 helicase is required for viral primosome activity, but helicase activity is dispensable." J Biol Chem 285(43): 33475-84.

Humphrey, W., A. Dalke, et al. (1996). "VMD: visual molecular dynamics." J Mol Graph 14(1): 33-8, 27-8.

Iftode, C. and J. A. Borowiec (1997). "Denaturation of the simian virus 40 origin of replication mediated by human replication protein A." Mol-Cell-Biol 17(7): 3876-83 issn: 0270-7306.

Jacobs, D. M., A. S. Lipton, et al. (1999). "Human replication protein A: global fold of the N-terminal RPA-70 domain reveals a basic cleft and flexible C-terminal linker." J Biomol NMR 14(4): 321-31.

Jiang, X., V. Klimovich, et al. (2006). "Structural mechanism of RPA loading on DNA during activation of a simple pre-replication complex." Embo J 25(23): 5516-26.

Joo, W. S., H. Y. Kim, et al. (1998). "Assembly of T-antigen double hexamers on the simian virus 40 core origin requires only a subset of the available binding sites." Mol Cell Biol 18(5): 2677-87.

Khopde, S., R. Roy, et al. (2008). "The Binding of Topoisomerase I to T Antigen Enhances the Synthesis of RNA-DNA Primers during Simian Virus 40 DNA Replication." Biochemistry 47(36): 9653-60.

Khopde, S. and D. T. Simmons (2008). "Simian virus 40 DNA replication is dependent on an interaction between topoisomerase I and the C-terminal end of T antigen." J Virol 82(3): 1136-45.

Kim, H. Y., B. Y. Ahn, et al. (2001). "Structural basis for the inactivation of retinoblastoma tumor suppressor by SV40 large T antigen." Embo J 20(1-2): 295-304.

Kim, H. Y., B. A. Barbaro, et al. (1999). "Sequence requirements for the assembly of simian virus 40 T antigen and the T-antigen origin binding domain on the viral core origin of replication." J Virol 73(9): 7543-55.

Klinge, S., R. Nunez-Ramirez, et al. (2009). "3D architecture of DNA Pol alpha reveals the functional core of multi-subunit replicative polymerases." Embo J 28(13): 1978-87.

Li, D., R. Zhao, et al. (2003). "Structure of the replicative helicase of the oncoprotein SV40 large tumour antigen." Nature 423(6939): 512-8.

Luo, X., D. G. Sanford, et al. (1996). "Solution structure of the origin DNA-binding domain of SV40 T-antigen." Nat-Struct-Biol 3(12): 1034-9 issn: 1072-8368.

Mastrangelo, I. A., P. V. C. Hough, et al. (1989). "ATP-dependent assembly of double hexamers of SV40 T antigen at the viral origin of DNA replication." Nature 338: 658-662.

Meinke, G., P. A. Bullock, et al. (2006). "Crystal structure of the simian virus 40 large T-antigen origin-binding domain." J Virol 80(9): 4304-12.

Meinke, G., P. Phelan, et al. (2007). "The crystal structure of the SV40 T-antigen origin binding domain in complex with DNA." PLoS Biol 5(2): e23.

Meinke, G., P. J. Phelan, et al. (2010). "Structure-based analysis of the interaction between the simian virus 40 T-antigen origin binding domain and single-stranded DNA." J Virol 85(2): 818-27.

Melendy, T. and B. Stillman (1991). "Purification of DNA polymerase delta as an essential simian virus 40 DNA replication factor." J Biol Chem 266(3): 1942-9.

Melendy, T. and B. Stillman (1993). "An interaction between replication protein A and SV40 T antigen appears essential for primosome assembly during SV40 DNA replication." J Biol Chem 268(5): 3389-95.

Nasheuer, H. P., D. von Winkler, et al. (1992). "Purification and functional characterization of bovine RP-A in an in vitro SV40 DNA replication system." Chromosoma 102(1): S52-9.

Parsons, R. E., J. E. Stenger, et al. (1991). "Cooperative assembly of simian virus 40 T-antigen hexamers on functional halves of the replication origin." J Virol 65(6): 2798-806.

Reese, D. K., G. Meinke, et al. (2006). "Analyses of the interaction between the origin binding domain from simian virus 40 T antigen and single-stranded DNA provide insights into DNA unwinding and initiation of DNA replication." J Virol 80(24): 12248-59.

Reese, D. K., K. R. Sreekumar, et al. (2004). "Interactions required for binding of simian virus 40 T antigen to the viral origin and molecular modeling of initial assembly events." J Virol 78(6): 2921-34.

Roy, R., P. Trowbridge, et al. (2003). "The Cap Region of Topoisomerase I Binds to Sites near Both Ends of Simian Virus 40 T Antigen." J Virol 77(18): 9809-16.

Rutila, J. E., M. J. Imperiale, et al. (1986). "Replication and transformation functions of in vitro-generated simian virus 40 large T antigen mutants." J Virol 58(2): 526-35.

Sanders, C. M. and A. Stenlund (2000). "Transcription factor-dependent loading of the E1 initiator reveals modular assembly of the papillomavirus origin melting complex." J Biol Chem 275(5): 3522-34.

Sawai, E. T., G. Rasmussen, et al. (1994). "Construction of SV40 deletion mutants and delimitation of the binding domain for heat shock protein to the amino terminus of large T-antigen." Virus Res 31(3): 367-378.

Sedman, J. and A. Stenlund (1995). "Co-operative interaction between the initiator E1 and the transcriptional activator E2 is required for replicator specific DNA replication of bovine papillomavirus in vivo and in vitro." Embo J 14(24): 6218-28.

Shen, J., D. Gai, et al. (2005). "The roles of the residues on the channel beta-hairpin and loop structures of simian virus 40 hexameric helicase." Proc Natl Acad Sci U S A 102(32): 11248-53.

Simmons, D. T. (2000). "SV40 large T antigen: Functions in DNA replication and transformation [In Process Citation]." Adv Virus Res 55: 75-134.

Simmons, D. T., D. Gai, et al. (2004). "Assembly of the replication initiation complex on SV40 origin DNA." Nucleic Acids Res 32(3): 1103-12.

Simmons, D. T., T. Melendy, et al. (1996). "Simian virus 40 large T antigen binds to topoisomerase I." Virology 222(2): 365-74.

Simmons, D. T., R. Roy, et al. (1998). "The Activity of Topoisomerase I Is Modulated by Large T Antigen during Unwinding of the SV40 Origin." J Biol Chem 273(32): 20390-6.

Simmons, D. T., P. W. Trowbridge, et al. (1998). "Topoisomerase I stimulates SV40 T antigen-mediated DNA replication and inhibits T antigen's ability to unwind DNA at nonorigin sites." Virology 242(2): 435-43.

Sreekumar, K. R., A. E. Prack, et al. (2000). "The simian virus 40 core origin contains two separate sequence modules that support T-antigen double-hexamer assembly." J Virol 74(18): 8589-600.

Srinivasan, A., A. J. McClellan, et al. (1997). "The amino-terminal transforming region of simian virus 40 large T and small t antigens functions as a J domain." Mol-Cell-Biol 17(8): 4761-73 issn: 0270-7306.

Stubdal, H., J. Zalvide, et al. (1997). "Inactivation of pRB-related proteins p130 and p107 mediated by the J domain of simian virus 40 large T antigen." Mol Cell Biol 17(9): 4979-90.

Sullivan, C. S. and J. M. Pipas (2002). "T antigens of simian virus 40: molecular chaperones for viral replication and tumorigenesis." Microbiol Mol Biol Rev 66(2): 179-202.

Taneja, P., I. Boche, et al. (2007). "Different activities of the largest subunit of replication protein A cooperate during SV40 DNA replication." FEBS Lett 581(21): 3973-8.

Tjian, R. (1978). "Protein-DNA interactions at the origin of simian virus 40 DNA replication." Cold Spring Harbor Symp. Quant. Biol. 43: 655-662.

Trowbridge, P. W., R. Roy, et al. (1999). "Human Topoisomerase I Promotes Initiation of Simian Virus 40 DNA Replication In Vitro." Mol Cell Biol 19(3): 1686-1694.

Tsurimoto, T. and B. Stillman (1991). "Replication factors required for SV40 DNA replication in vitro. II. Switching of DNA polymerase alpha and delta during initiation of leading and lagging strand synthesis." J Biol Chem 266(3): 1961-8.

Valle, M., X. S. Chen, et al. (2006). "Structural basis for the cooperative assembly of large T antigen on the origin of replication." J Mol Biol 357(4): 1295-305.

Valle, M., C. Gruss, et al. (2000). "Large T-antigen double hexamers imaged at the simian virus 40 origin of replication." Mol Cell Biol 20(1): 34-41.

VanLoock, M. S., A. Alexandrov, et al. (2002). "SV40 large T antigen hexamer structure: domain organization and DNA-induced conformational changes." Curr Biol 12(6): 472-6.

Wang, W., D. Manna, et al. (2007). "Role of the hydrophilic channels of simian virus 40 T-antigen helicase in DNA replication." J Virol 81(9): 4510-9.

Wang, W. and D. T. Simmons (2009). "Simian virus 40 large T antigen can specifically unwind the central palindrome at the origin of DNA replication." J Virol 83(7): 3312-22.

Weisshart, K., M. K. Bradley, et al. (1996). "An N-terminal deletion mutant of simian virus 40 (SV40) large T antigen oligomerizes incorrectly on SV40 DNA but retains the ability to bind to DNA polymerase alpha and replicate SV40 DNA in vitro." J-Virol 70(6): 3509-16 issn: 0022-538x.

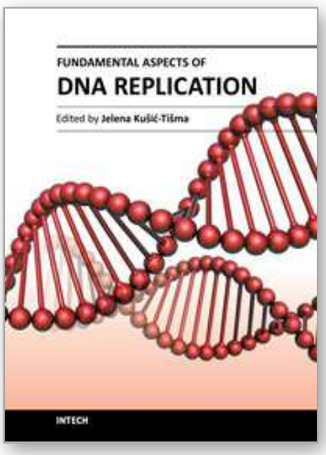
Weisshart, K., P. Taneja, et al. (1998). "The replication protein A binding site in simian virus 40 (SV40) T antigen and its role in the initial steps of SV40 DNA replication [In Process Citation]." J Virol 72(12): 9771-81.

Wiekowski, M., M. W. Schwarz, et al. (1988). "Simian virus 40 large T antigen DNA helicase. Characterization of the ATPase-dependent DNA unwinding activity and its substrate requirements." J Biol Chem 263(1): 436-42.

Wold, M. S. (1997). "Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism." Annu Rev Biochem 66: 61-92.

Zalvide, J., H. Stubdal, et al. (1998). "The J domain of simian virus 40 large T antigen is required to functionally inactivate RB family proteins." Mol Cell Biol 18(3): 1408-15.

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DNA replication, the process of copying one double stranded DNA molecule to produce two identical copies, is at the heart of cell proliferation. This book highlights new insights into the replication process in eukaryotes, from the assembly of pre-replication complex and features of DNA replication origins, through polymerization mechanisms, to propagation of epigenetic states. It also covers cell cycle control of replication initiation and includes the latest on mechanisms of replication in prokaryotes. The association between genome replication and transcription is also addressed. We hope that readers will find this book interesting, helpful and inspiring.

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