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AAA+ ATPases in the Initiation of DNA Replication

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All cellular organisms and many viruses rely on large, multi-subunit molecular machines, termed replisomes, to ensure that genetic material is accurately duplicated for transmission from one generation to the next. Replisome assembly is facilitated by dedicated initiator proteins, which serve to both recognize replication origins and recruit requisite replisomal components to the DNA in a cell-cycle coordinated manner. Exactly how imitators accomplish this task, and the extent to which initiator mechanisms are conserved among different organisms have remained outstanding issues. Recent structural and biochemical findings have revealed that all cellular initiators, as well as the initiators of certain classes of double-stranded DNA viruses, possess a common adenine nucleotide-binding fold belonging to the ATPases Associated with various cellular Activities (AAA+) family. This review focuses on how the AAA+ domain has been recruited and adapted to control the initiation of DNA replication, and how the use of this ATPase module underlies a common set of initiator assembly states and functions. How biochemical and structural properties correlate with initiator activity, and how species-specific modifications give rise to unique initiator functions, are also discussed.

Keywords DNA replication, initiator, AAA+ ATPase, superfamily III helicase, helicase loader, clamp loader

INTRODUCTION

The timely and faithful transmission of genetic material from one generation to the next is critical for the proliferation of all organisms. The efficient copying of chromosomes is carried out by a large molecular machine, termed the replisome, which couples the highly processive unwinding of parental DNA with the synthesis of new daughter strands. Replisome assembly occurs during a brief window of time known as initiation, during which a variety of requisite catalytic and scaffolding factors are sequentially recruited to DNA. The precise way in which initiation takes place and is temporally controlled remains a major area of inquiry.

The replicon hypothesis posits that initiation begins at a defined region (or set of loci) on the chromosome, termed a replicator or origin (Jacob *et al.*, 1963). At least one trans-acting factor, known as an initiator, is required for origin recognition and to start the replication process. In addition, cells highly regulate initiation to ensure that replication occurs at the correct phase of the cell cycle and that chromosomes are neither mutated nor corrupted between generations. Beyond these general requirements, there exist myriad physical challenges that also must be overcome by the initiation and replication machinery. For example, duplex DNA must be melted to allow access to information encoded by individual bases in single-stranded DNA. Similarly, because DNA is a double-helical polymer, unwinding leads to topological deformations in the chromosome that must be resolved prior to chromosome segregation and cell division.

Despite these common constraints, certain aspects of replication initiation are quite distinct among different types of cells and viruses. This diversity is often a result of physiological requirements specific to a particular species or organism, which can apply selective pressures to alter the initiation process. Some viruses, for example, possess only a single initiation factor that is capable of both melting duplex DNA and functioning as a helicase to mediate processive unwinding (Hickman and Dyda, 2005). For viruses, this parsimony is advantageous for contending with an extremely compact genome that needs to be quickly and efficiently duplicated inside a host cell. In contrast, for bacterial replication initiation, DNA opening and unwinding are performed by two different proteins, DnaA and DnaB, respectively (Kornberg and Baker, 1992; Messer, 2002). This separation of function allows the two proteins to take on additional roles outside of initiation or to be subject to auxilary layers of cell cycle control. For example, DnaA can serve as a transcription factor (Atlung *et al.*, 1985; Braun *et al.*, 1985; Messer and Weigel, 1997), while DnaB participates in replicative/repair pathways

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TABLE 1
A subset of cellular and viral initiation factors

Replication Factor	Bacteria	Eukaryotes	Simian virus 40	Papillomavirus	Archaea
Initiator	DnaA	ORC	L Tag	E1	Orc1/Cdc6
Helicase	DnaB	MCM2-7	L Tag	E1	MCM
Helicase Loader	DnaC	Cdc6	L Tag	E2	Orc1/Cdc6?

that do not require DnaA, such as replication restart (Heller and Kenneth, 2006). Although the number of proteins employed during initiation varies for different types of cells and viruses, they all can be classified and divided into similar categories based on their enzymatic activities (Table 1).

Despite significant differences in their regulation and modes of action, replication initiator proteins possess certain architectural features that are strikingly conserved among different cellular organisms, and even some viruses (Giraldo, 2003; Iyer *et al.*, 2004a; Lee and Bell, 2000; Neuwald *et al.*, 1999). The most notable similarity derives from the consistent reliance on an adenine nucleotide-binding fold belonging to the **A**TPases **A**ssociated with various cellular **A**ctivities (AAA+) superfamily. As their name suggests, AAA+ proteins are known for their participation in a diverse range of cellular functions that, in addition to DNA replication, include vesicle trafficking, proteolytic degradation, and transcriptional regulation (Erzberger and Berger, 2006; Iyer *et al.*, 2004a; Neuwald *et al.*, 1999; Ogura and Wilkinson, 2001). Many initiators also tether dedicated DNA-binding domains to their AAA+ modules, a characteristic which enables their sequence-specific localization to replication origins (Arthur *et al.*, 1988; Chen and Stenlund, 1998; Cunningham and Berger, 2005; Enemark *et al.*, 2002; Fujikawa *et al.*, 2003; Lee *et al.*, 2001; Liu *et al.*, 2000; Meinke *et al.*, 2007; Roth and Messer, 1995; Wilson and Ludes-Meyers, 1991).

One critical property of AAA+ proteins is an ability to form oligomeric, ring-shaped assemblies, which can be stabilized by the binding of nucleotide at the interface between neighboring subunits. A wealth of experimental evidence for AAA+-type initiation proteins indicates that ATP binding plays a crucial role in regulating the onset and execution of the initiation process (Bell and Stillman, 1992; Borowiec and Hurwitz, 1988; Bramhill and Kornberg, 1988; Funnell *et al.*, 1987; Sanders and Stenlund, 1998). While an ATP-bound state often appears to be required to activate initiation factors, conversion to an ADP-state through hydrolysis also serves a critical function. For example, ATP hydrolysis is one mechanism used to deactivate DnaA and prevent re-initiation in bacteria (Kaguni, 2006; Su'etsugu *et al.*, 2004). In eukaryotes, ATP hydrolysis is required to complete helicase loading, a key step in the initiation pathway (Bowers *et al.*, 2004; Randell *et al.*, 2006). ATP turnover likewise drives hexamer formation and the helicase activity of certain viral initiators (Abbate *et al.*, 2004; Borowiec and Hurwitz, 1988; Dean *et al.*, 1987b; Mastrangelo *et al.*, 1989; Yang *et al.*, 1993; Enemark and Joshua-Tor, 2006; Gai *et al.*, 2004b; Hughes and Romanos, 1993; Ray *et al.*, 1992; Sedman and Stenlund, 1998; Stahl *et al.*, 1986).

The observation that cellular and viral initiator subunits can be composed in part of AAA+ domains indicates that the mechanisms of these proteins share particular functional attributes. Some initiator actions are likely dictated by the physical challenges associated with the recognition and melting of origin DNAs, rather than selective pressures specific to any one organism. At the same time, adaptations to the core AAA+ architecture can allow for differential cellular control and the specialization of initiator protein function. This chapter reviews how AAA+ proteins have been recruited and used by different cellular organisms and certain types of viruses to solve the problem of replication initiation. A particular emphasis will be placed on correlating biochemical and structural properties with initiator activity, and on highlighting species-specific modifications that give rise to additional initiator functions. Although a general framework for understanding origin recognition and replisome assembly has been developed for many model organisms, our present understanding of initiator function is highly incomplete at a molecular level.

THE AAA+ SUPERFAMILY

Overview

AAA+ proteins are defined by a structurally conserved ATP-binding module. In its active state, the AAA+ fold can homo- or hetero-oligomerize in a head-to-tail manner to form higher-order (typically ring-shaped), assemblies that undergo conformational changes upon binding and hydrolyzing. Despite notable differences in cellular function, many AAA+ proteins share an ability to couple the enzymatic turnover of ATP to the molecular remodeling of target macromolecules (Neuwald *et al.*, 1999; Iyer *et al.*, 2004a; Hartman and Vale, 1999; Ogura and Wilkinson, 2001).

The AAA+ fold is contained within the broader superfamily of "P-loop"-type nucleoside triphosphate (NTP)-binding proteins (Iyer *et al.*, 2004a). This large group of enzymes is defined in part by the presence of two distinct signature sequences, known as the Walker A (WA) and Walker B (WB) motifs, which are important for nucleotide binding and hydrolysis (Walker *et al.*, 1982) (Figure 1A). Due to the extensive number of proteins that contain these signature sequences, NTPase subfamilies have been constructed based on the organization of secondary structural elements and on the positions of conserved motifs within the core nucleotide-binding fold (Caruthers and McKay, 2002; Iyer *et al.*, 2004a).

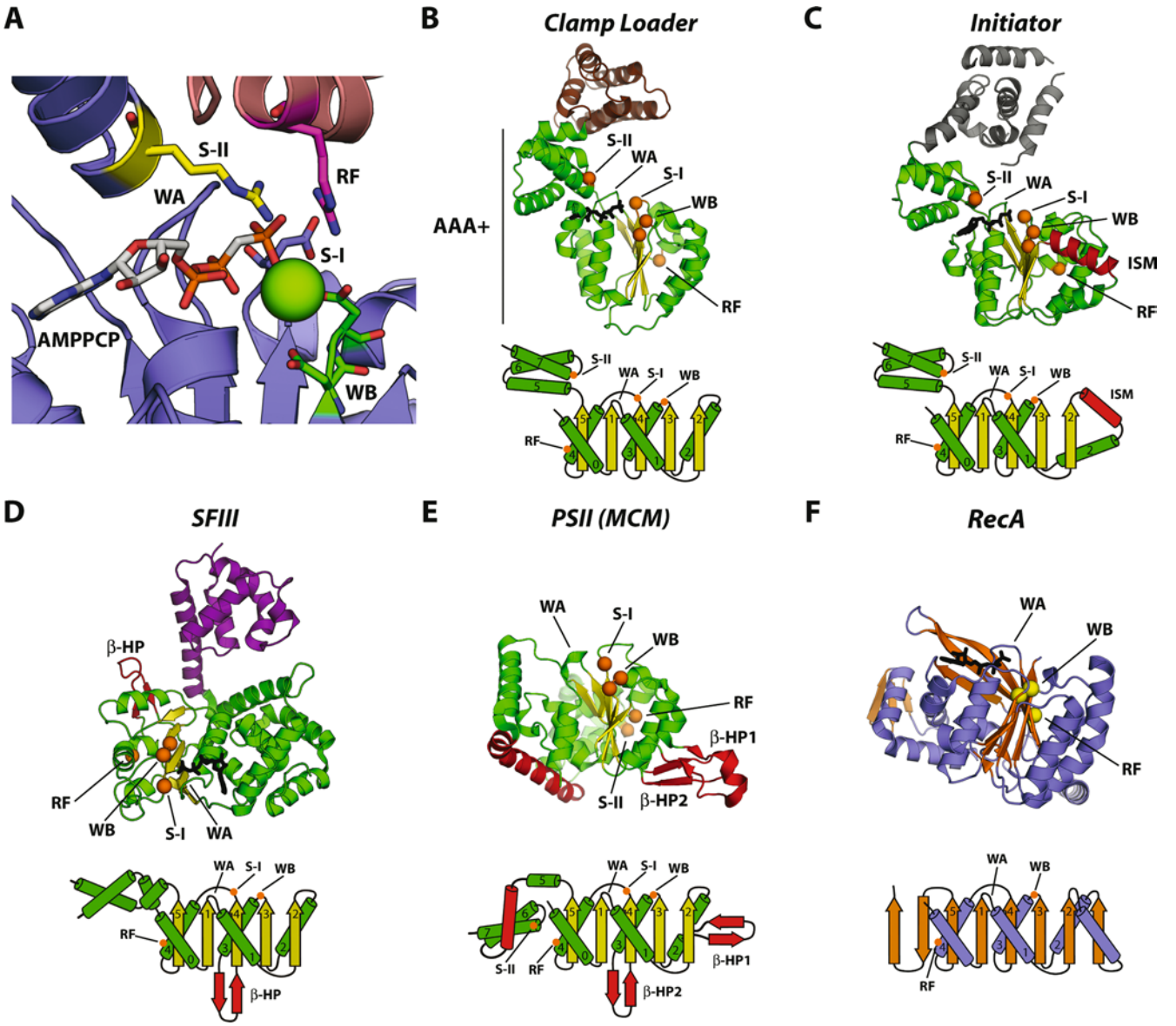

FIG. 1. An abbreviated set of AAA+ clades. For each clade, a representative structure and a topology diagram is displayed. Labeled key motifs include the Walker A (WA), Walker B (WB), sensor-I (S-I), sensor-II (S-II) and arginine finger (RF) elements. Clade-specific insertions, highlighted in red, include the initiator specific motif (ISM) and β-hairpins (β-HP, β-HP1 and β-HP2). Bound nucleotide is shown as CPK or black sticks. The AAA+ domains are displayed in green with additional domains in other colors (B-E). (A) Close-up of a representative AAA+ protein active site (DnaA), with an ATP analog (AMPPCP) bound. The Box VII helix and arginine-finger from an adjacent protomer are shown in pink and magenta. (B) The clamp-loader clade (RFC-B subunit, PDB ID 1SXJ). (C) The initiator clade (DnaA monomer, PDB ID 2HCB). (D) The SuperFamily III helicase clade (SV40 subunit, PDB ID 1SVM). (E) The pre-sensor II (PSII) insertion clade, which includes MCM proteins (BchI magnesium chelatase subunit is shown, PDB ID 1G8P). (F) RecA-type ASCE ATPase fold (RecA monomer, PDB ID 1REA).

One P-loop NTPase subfamily has recently been termed by Aravind and coworkers the "**A**dditional **S**trand **C**onserved **E** family" (ASCE), due to a β-strand insertion between the WA and WB motifs of the classic adenylate kinase fold (Iyer *et al.*, 2004b; Leipe *et al.*, 2003). Within the ASCE family, the nucleotide-binding pocket is positioned between three adjacent, parallel β-strands sandwiched within a globular $\alpha\beta\alpha$ domain. The RecA recombination factor was the first ASCE protein to be characterized structurally (Story and Steitz, 1992); as a consequence, many ASCE ATPases have historically been described

as RecA-like. In the time since the RecA structure, however, many more ASCE proteins have been imaged, revealing a diverse range of ASCE subtypes. Members of the AAA+ superfamily are all predicated on the core ASCE architecture (Erzberger and Berger, 2006; Iyer *et al.*, 2004a).

Different ASCE subfamilies can be distinguished by the organization of secondary structural elements, the presence of certain conserved amino acid sequence motifs, and the relative positioning of those motifs within the P-loop NTPase fold. The organization and position of these structural elements can significantly alter the interface between adjacent subunits, which in turn can change the symmetry and configuration of the resultant higher-order assembly. These structural characteristics are often directly linked to unique functional requirements and catalytic mechanisms that define different protein families (Caruthers and McKay, 2002; Wang, 2004).

The AAA+ superfamily differs from other ASCE members (such as RecA)by the absence of certain β-strand elements at the edges of the core ASCE domain, and by the presence of a small α-helical bundle that is often found fused to the C-terminus of the central $\alpha\beta\alpha$ fold. In addition, a number of distinguishing motifs occupy well-defined positions within the AAA+ domain. For example, a conserved catalytic arginine residue found in many ASCE subfamilies, called an arginine finger (RF) (Wittinghofer *et al.*, 1997), lies within an ASCE core region commonly termed the Box VII or SRC motif (Guenther *et al.*, 1997; Neuwald *et al.*, 1999; Ogura and Wilkinson, 2001). The arginine finger contributes to the formation of a bipartite nucleotide binding pocket upon protomer assembly (Figure 1A), and both assists with ATP hydrolysis and communicates the status of the bound nucleotide (ATP, ADP or apo) from one AAA+ subunit to the next.

The AAA+ fold also contains two nucleotide-interacting motifs termed the sensor-I (S-I) and sensor-II (S-II) elements (Guenther *et al.*, 1997) (Figure 1A). The S-I motif is a polar residue that is thought to help properly orient a water molecule for nucleophilic attack on the γ-phosphate of ATP. The S-II usually provides an arginine to interact with the γ-phosphate of ATP and aid in positioning of the C-terminal α-helical bundle. These highly conserved residues play a key role in ATP hydrolysis in many AAA+ systems (Davey *et al.*, 2002b; Ogura *et al.*, 2004).

The motifs and architectural characteristics described thus far define evolutionarily-conserved features that distinguish the AAA+ superfamily from other ASCE NTPases. Within the AAA+ collection, however, there exist additional adaptations to the fold that define distinct subgroupings, or "clades" (Erzberger and Berger, 2006; Iyer *et al.*, 2004a). An abbreviated collection of AAA+ clades is displayed in Figure 1, with the addition of RecA as an example of a common ASCE NTPase fold that is distinct from the AAA+ branch.

Clamp Loaders

The clamp loader clade constitutes the most architecturally simplified AAA+ subfamily (Figure 1B) (Iyer *et al.*, 2004a; Erzberger and Berger, 2006). Unlike initiators, clamp loaders do not recognize and melt origins; however, they do function as ATP-dependent molecular switches, a property shared with their initiator counterparts. Clamp loaders are responsible for loading ring-shaped, sliding-clamp factors onto primed DNA templates at the replication fork as an aid for increasing DNA polymerase processivity (Davey *et al.*, 2002b; Indiani and O'Donnell, 2006; O'Donnell and Kuriyan, 2006). Clamp loaders are composed of five AAA+ subunits organized in a circular arrangement (Figure 2A). In one of many twists on the AAA+ assembly theme, an α-helical C-terminal domain appended to the central AAA+ region forms a significant portion of the inter-subunit contacts responsible for formation of the pentamer (Bowman *et al.*, 2004; Jeruzalmi *et al.*, 2001a) (Figures 1 and 2A).

The *Escherichia coli* clamp loader (the γ-complex), consists of five AAA+ subunits (a mixture of three γ or τ protomers, along with δ and δ') (Figure 2A) (Maki, 1988; Naktinis *et al.*, 1996; O'Donnell and Kuriyan, 2006). The complete complex also contains two additional, subunits, χ and ψ, which are neither AAA+ proteins nor required for clamp loading (Maki, 1988; Naktinis *et al.*, 1996; O'Donnell and Kuriyan, 2006). Nucleotide binding to the clamp loader occurs at the interface of neighboring subunits, as in other AAA+ assemblies. Although only the γ/τ subunits bind ATP, the δ' subunit provides a functional arginine finger to one ATP active site, while the δ subunit forms inter-subunit contacts important for complex formation and activity (Guenther *et al.*, 1997; Jeruzalmi *et al.*, 2001a; Onrust, 1991; Jeruzalmi *et al.*, 2001a). Between the δ and δ' subunits there is a gap (Figure 2B) (Jeruzalmi *et al.*, 2001a), allowing for the entry and binding of the primer-template junction. This hetero-oligomeric arrangement highlights how adaptations to the underlying AAA+ fold can alter protomer interfaces and influence the assembly of higher-order complexes, tuning a given molecular machine for a specific biological function.

The clamp loader in archaea and eukaryotes is known as replication factor C (RFC) (Indiani and O'Donnell, 2006; Prelich *et al.*, 1987). Though AAA+ proteins, archaeal and eukaryotic RFCs differ somewhat from the bacterial γ-complex, as well as from each another. In eukaryotes, RFC consists of four nonidentical small subunits, which are comparable in size and architecture to the bacterial clamp loader proteins, and one large subunit with extensive N- and C- terminal additions to the central AAA+ fold (Figure 2C, *top*) (Bowman *et al.*, 2004; Indiani and O'Donnell, 2006; Prelich *et al.*, 1987; Tsurimoto and Stillman, 1991). Archaeal RFC, by contrast, utilizes a pentamer containing four copies of a single small AAA+ subunit and one large AAA+ subunit (Figure 2C, *bottom*) (Miyata *et al.*, 2005; Seybert *et al.,* 2002; Seybert *et al.,* 2006). The large subunit of both archaeal and eukaryotic RFCs can bind ATP, which results in one additional ATP bound in RFC as compared with the bacterial γ-complex (Indiani and O'Donnell, 2006). Interestingly, while the number of subunits that can productively bind and hydrolyze ATP varies among the three domains of life (Johnson *et al.,* 2006; O'Donnell and

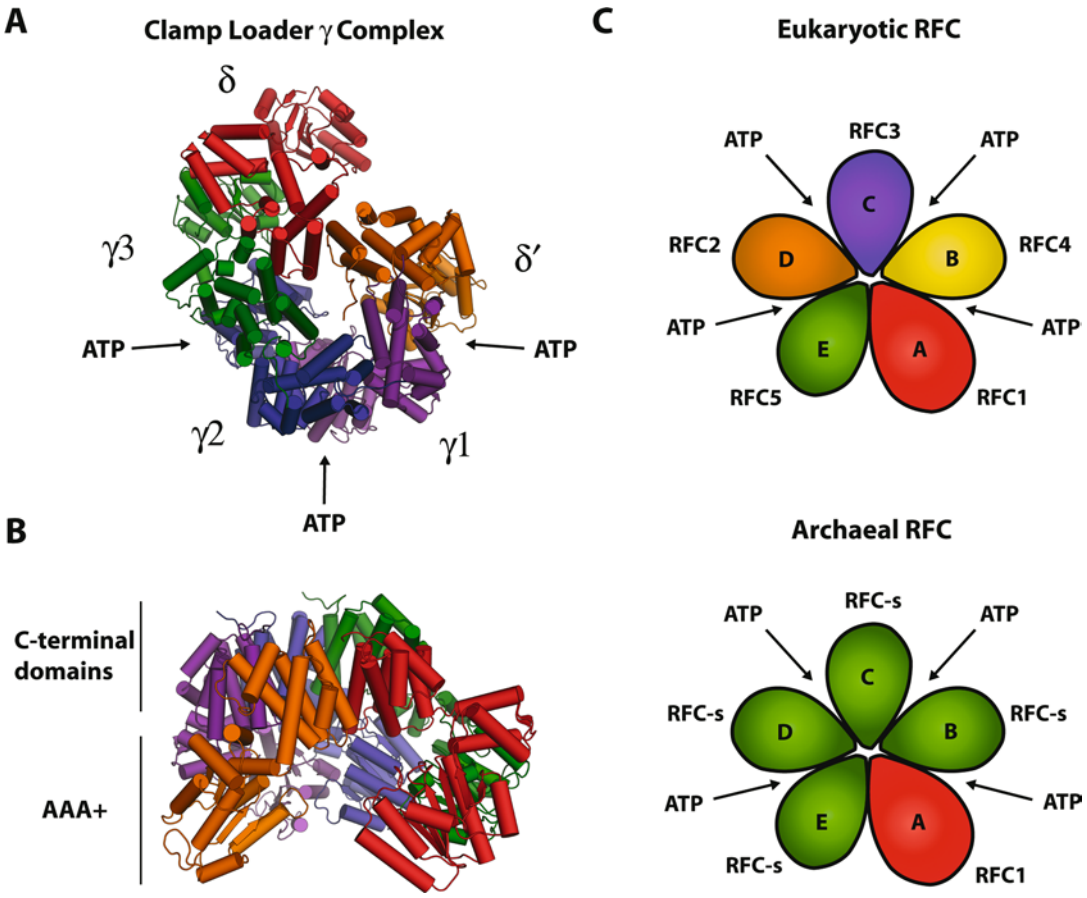

FIG. 2. Architectural features of clamp-loader complexes. (A) Top view of the *E. coli* clamp loader γ complex (PDB ID 1JR3). Each subunit is represented with a different color and ATP binding sites are labeled. (B) Side view of the *E. coli* clamp loader-γ-complex with the same subunit coloring as displayed in panel A. The gap between the δ (red) and δ' (orange) subunits may allow the primer-template junction to enter the pentamer. (C) Global organization of eukaryotic (*upper*) and archaeal (*lower*) replication factor C (RFC) complexes. Subunits are labeled A-E, starting with the largest subunit and moving counterclockwise around the pentamer. The gap is positioned between terminal subunits A and E. The eukaryotic RFC complex is composed of five unique subunits as indicated by the use of different colors. The archaeal RFC complex is composed of four identical small RFC-s subunits (green) and one large RFC1 subunit (red).

Kuriyan, 2006; Seybert *et al.,* 2006), all clamp loaders are equally facile at carrying out the loading function. How each class of clamp loader contends with a different set of active ATPase modules, yet still performs the same basic loading reaction, remains an unresolved issue.

Similar to other AAA+ assemblies, the clamp loader is only active and capable of binding its target (the clamp) when associated with ATP (Bowman *et al.,* 2004; Jeruzalmi *et al.,* 2001b; Johnson *et al.,* 2006; Naktinis *et al.,* 1995; Seybert and Wigley, 2004). Once a clamp loader/clamp complex has formed, ATP hydrolysis is required for release of the clamp and association of the clamp with DNA polymerase. While the central ATPase domain architecture of clamp loaders is quite similar to that of other AAA+ subfamilies, most notably initiators and helicase loaders, the extensive contacts made between the C-terminal helical bundles and the absence of any significant insertions to the core ATPase fold result in a pentameric notched-ring assembly, that defines a unique AAA+ clade.

Replication Initiation Factors

AAA+ ATPases involved in the initation of DNA replication possess architectures derived from the core clamp loader fold (Figure 1). We will first briefly outline how the three AAA+ clades used in this process differ from the clamp loader clade, and then move on to examine how these proteins work together to control replication initiation in more detail.

Initiator/Helicase-Loader Clade

In contrast to clamp loaders, the cellular initiator/helicase-loader clade of the AAA+ superfamily is distinguished by the insertion of an additional α-helix between helix α2 and strand

β2 of the core ASCE fold (Figure 1C) (Erzberger and Berger, 2006; Iyer *et al.*, 2004a). Structural studies thus far suggest that higher-order initiator assemblies favor the formation of open rings or even filaments (Clarey *et al.*, 2006; Erzberger *et al.*, 2006; Speck *et al.*, 2005). For the bacterial initiator DnaA, the extra α-helix serves to rotate adjacent subunits out-of-plane with respect to one another, disrupting the formation of a closed ring, and giving rise to a right-handed protein helix. Structures of archaeal Orc1 proteins bound to DNA have shown that this insert can interact with the duplex directly (Dueber *et al.*, 2007; Gaudier *et al.*, 2007). This so-called initiator-specific motif (or ISM) thus plays a key role in both initiator oligomerization and origin recognition.

SFIII Helicase Clade

Certain classes of viral initiators fall into the AAA+ superfamily, but are distinct from the cellular initiator/helicase-loaders, forming instead a branch comprising the superfamily III (SFIII) helicases (Figure 1D). The SFIII fold is distinguished from other AAA+ subgroups by the insertion of a β-hairpin motif between helix α3 and strand β4 that contains residues required for both DNA melting and processive unwinding (Borowiec and Hurwitz, 1988; Castella *et al.*, 2006a; Kumar *et al.*, 2007; Liu *et al.*, 2007; Reese *et al.*, 2004; Schuck and Stenlund, 2005, 2007). The absence of a helical insertion between helix α2 and strand β2 of the ASCE fold further differentiates the SFIII clade from cellular initiators, allowing for the formation of closed hexameric rings (Enemark and Joshua-Tor, 2006; Li *et al.*, 2003). SFIII helicases also have a highly diverged and partially knotted C-terminal α-helical subdomain that sits off to one side of the core $\alpha\beta\alpha$ ATP-binding fold, replacing the small α-helical bundle that caps the Walker and Sensor motifs in most other AAA+ proteins.

Pre-Sensor II Clade

The replicative helicase found both in archaea and eukaryotes, known as the mini-chromosome maintenance (MCM) complex, belongs to yet another subgroup of the AAA+ superfamily, the pre-sensor II (PSII) clade (Figure 1E). Bioinformatic data have suggested that a defining feature of these proteins may be the insertion of an extra helix in the C-terminal α-helical subdomain before the Sensor II motif of the AAA+ fold. This insertion dramatically rearranges the C-terminal domain, and may reposition the Sensor II such that the motif can engage the active site of a partner subunit in *trans*, rather than acting in *cis* on its own catalytic center (Erzberger and Berger, 2006). The pre-sensor II clade also includes two additional β-hairpin insertions, one within helix α2 and another between helix α3 and strand β4 (Figure 1E). Pre-sensor II AAA+ proteins are not only utilized as replication factors, but also perform a wide variety of other functions (Iyer *et al.*, 2004a). One example is the BchI magnesium chelatase, which adds metal ions to porphyrin rings used in chlorophyll biosynthesis (Fodje *et al.*, 2001).

VIRAL INITIATION

During replication, viruses must overcome challenges that are different from those encountered by cellular systems. For example, to facilitate rapid and abundant proliferation, viruses generally have small, compact genomes, and carefully coordinate their enzymatic activities with their host to ensure survival (Fanning, 1998; Wilson *et al.*, 2002). Many viruses also encode proteins that can hijack the machinery of their host for specific purposes.

Given the enormous diversity of viral genomes and their structures (RNA *vs*. DNA, single- *vs*. double-stranded) it is not surprising that viruses have evolved a multitude of replication strategies. Interestingly, for the papilloma, polyoma and type 2 adeno-associated dsDNA viruses, as well as some small RNA viruses, replication initiation is carried out by members of the SFIII subfamily of AAA+ proteins. In particular, a large number of structural and biochemical studies have been performed on two related SFIII proteins, papillomavirus E1 and simian virus 40 (SV40) large T-antigen. These studies have provided significant insights into the dual initiator and helicase functions of this remarkable protein family (Stenlund, 2003).

Simian Virus 40

The SV40 large T-antigen is composed of three independent functional domains: an N-terminal J domain, a central DNA binding domain, and a C-terminal SFIII-type AAA+ domain. The DNA binding and helicase domains are connected by a flexible linker, which conformationally uncouples the two regions from one another once the protein is localized to the the viral origin (Borowiec *et al.*, 1990; Fanning and Knippers, 1992). Upon assembly, the SV40 T-antigen is thought to form a head-to-head double hexamer that rapidly unwinds the viral genome during DNA replication (Alexandrov *et al.*, 2002; Bullock, 1997; Hickman and Dyda, 2005; Valle *et al.*, 2000).

The SV40 origin consists of a 64-base-pair (bp) region that is essential for the initiation of replication (Figure 3A). Even single base substitutions at many of the positions in the origin can result in a significant reduction of replication (Dean *et al.*, 1987a). The origin is composed of three elements: the early palindrome (EP), the central domain, and an AT-rich tract. The central domain contains a palindrome with four pentanucleotide recognition elements that are bound by the DNA-binding domains of four T-antigen protomers in a precise arrangement (Figure 3B) (Meinke *et al.*, 2006; Meinke *et al.*, 2007). The early palindrome contains an imperfect inverted repeat that unwinds after the initiator binds to the central domain (Borowiec and Hurwitz, 1988; Borowiec *et al.*, 1990). The AT-rich tract comprises a second site that is unwound by large T-antigen, but only after unwinding of the early palindrome has occurred (Borowiec and Hurwitz, 1988; Kumar *et al.*, 2007). Footprinting studies indicate that the AT-rich tract further serves as a landing pad for a second hexamer prior to the onset of replication (Borowiec and Hurwitz, 1988).

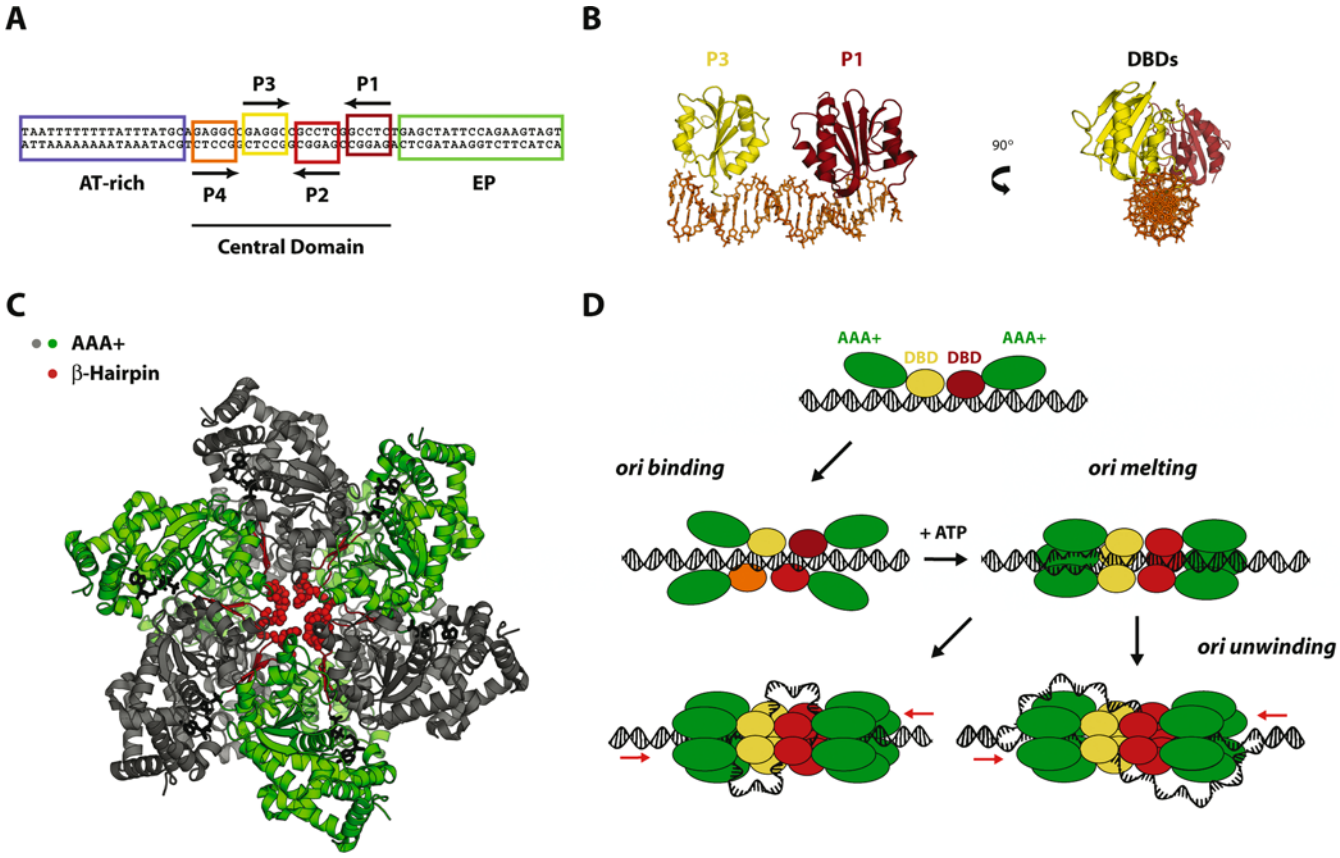

FIG. 3. Simian virus 40 (SV40) initiation. (A) The SV40 origin sequence. Highlighted conserved elements include the site of initial melting (AT-rich), the central domain with each pentanucleotide recognition sequence indicated (P1–P4), and the early palindrome (EP). (B) Front and side views of the SV40 large T-antigen DNA binding domains bound to DNA (PDB ID 2NTC). (C) ATP bound SV40 hexamer with alternating subunits colored green and gray (PDB ID 1SVM). The β-hairpin insertion is highlighted red. (D) Model for SV40 initiation. *(Top, Middle-left)* SV40 large T-antigen monomers bind to the pentanucleotide repeats in the origin. (*Middle-right*) A double-trimer intermediate is responsible for initial melting before hexamer formation. *(Bottom)* Two models for formation of the fully assembled replicative complex. In one model, double-stranded DNA threads through each SV40 AAA+ domain and is unwound either inside the hexamer, or by the action of the two motors pumping against one another *(lower left)*. In the other model, T-antigen melts origin DNA and binds single-stranded DNA in its interior as it assembles *(lower right)*; processive forward motion leads to unwinding by steric separation of the duplex. Note that most models for the SV40 large T-antigen invoke formation of a head-to-head assembly of hexamers by the DNA binding domains. See Figure 4 for an alternative model based on E1.

Assembly of the SV40 initiation complex begins with the sequence-specific association of the DNA binding domains from several SV40 initiators with the viral origin (Figures 3B) (Meinke *et al.*, 2007). Both the DNA binding domains and helicase domains are involved in the ATP-dependent oligomerization of the large T-antigen hexamer at the origin (Figures 3C) (Gai *et al.*, 2004b; Simmons *et al.*, 1993; Weisshart *et al.*, 1999). During assembly, two aromatic residues located in a SFIII-specific β-hairpin that protrudes into the central channel of the assembled hexamer (His513 and Phe459) (Li *et al.*, 2003), and two basic residues located in a neighboring loop off the central AAA+ domain (Lys512 and Lys516), assist in melting duplex DNA (Figure 3C) (Kumar *et al.*, 2007; Reese *et al.*, 2004; Shen *et al.*, 2005). After formation of the hexamer, these residues participate in the 3′–5′, ATP-dependent translocation of DNA through the central channel of the helicase (Gai *et al.*, 2004a).

After two hexamers have assembled around DNA, interactions between the DNA binding domains and the origin are no longer required to maintain the attachment of large T-antigen to the nucleic acid strands. The precise fate of these domains is unclear, but they almost certainly dissociate from their cognate binding sites. There is speculation that this event may free the DNA binding domains of one helicase to bind to a matching set of partner domains on the adjacent large T-antigen assembly, forming a double-hexameric ring (Alexandrov *et al.*, 2002; Meinke *et al.*, 2006; Meinke *et al.*, 2007; Reese *et al.*, 2004).

Such a model may account for the role of residues that appear to be required for double hexamer formation, but that are not seen to contact each other in the structure of two DNA binding domains bound to the SV40 origin (Figure 3B) (Meinke *et al.*, 2007; Weisshart *et al.*, 1999).

Two distinct models have been put forth to explain the processive unwinding mechanism of the SV40 helicase. In one, the AAA+ domains are thought to encircle and translocate along double-stranded DNA (Hickman and Dyda, 2005; Li *et al*., 2003; Valle *et al.*, 2006). During this process, the DNA binding domains would release their binding sites and assemble with each other to form a head-to-head double hexamer (Figure 3D, *bottom left*) (Meinke *et al.*, 2006; Meinke *et al.*, 2007). Melting of the duplex would occur either within the AAA+ domains of the large T-antigen, or from the force of pushing the DNA duplex against itself (Li *et al*., 2003). Single-stranded DNA would then spool out from the center of the dodecamer, through channels formed by contacts between the DNA binding domains, or between the DNA binding and AAA+ domains.

A second model for unwinding relies on a steric exclusion mechanism thought to be used by many hexameric helicases (Kaplan *et al.*, 2003; Takahashi *et al.*, 2005). In this scheme, the AAA+ domains would distort or melt the origin as they assemble (Kumar *et al*., 2007), eventually forming a single hexamer around each of the two single DNA strands (Figure 3C, *bottom right*). Processive forward movement of the ATPase motors would pull in one strand at the expense of the other, splitting the duplex and unwinding the DNA. Evidence for this model includes the observation that the diameter of the central hole of the large T-antigen hexamer, as seen crystallographically, is not wide enough to accommodate the ∼20 Å diameter of B-form DNA duplex (∼7 Å when bound to ATP, and ∼15 Å in the absence of nucleotide) (Gai *et al.*, 2004a, 2004b; Li *et al.*, 2003). Recent experimental evidence from papillomaviruses, which also use an SFIII helicase for initiation, further supports the idea that the SV40 large T-antigen may translocate along single-stranded DNA during replication (Enemark and Joshua-Tor, 2006).

Papillomavirus

Replication initiation in papillomaviruses occurs by a mechanism similar to that used by SV40, but with a few notable differences. For example, productive origin recognition requires the cooperative binding of two factors, the E1 initiator and the E2 enhancer, rather than a single protein (Mohr *et al.*, 1990; Sedman and Stenlund, 1995). Together, E1 and E2 form a heterotetrameric complex composed of a dimer of both proteins (Abbate *et al.*, 2004; Mendoza *et al.*, 1995). Through the staged loading of additional E1 monomers and dissociation of E2, E1 eventually forms a hexameric helicase (Enemark and Joshua-Tor, 2006; Fouts *et al.,* 1999; Sedman and Stenlund, 1998). This particle is thought to further assemble into a dodecameric structure (Schuck and Stenlund, 2005, 2007), which processively unwinds the papillomavirus genome during the initiation and elongation phases of replication.

E1 is composed of an N-terminal region that contains a nuclear localization signal, followed by a DNA binding domain and a C-terminal, SFIII-type AAA+ domain (Amin *et al.,* 2000; Chen and Stenlund, 1998; Ferran and McBride, 1998). E2 is composed of an N-terminal activation domain that is essential for initiation and other viral functions, followed by a proline-rich hinge region and a C-terminal DNA binding and dimerization domain (Mohr *et al.,* 1990; Sedman and Stenlund, 1995). The DNA binding domains of bovine papillomavirus E1 and SV40 T-antigen possess the same fold, but share <10% amino acid sequence identity (Enemark *et al.,* 2000; Luo *et al.,* 1996).

The papillomavirus origin is a highly conserved 84-bp sequence composed of an AT-tract, a central 18-bp quasi-palindrome region, and a pair of E2 binding sites (Figure 4A) (Seo *et al.,* 1993; Ustav *et al.,* 1991; Wilson and Ludes-Meyers, 1991). The DNA binding domains of multiple E1s bind to the central palindrome on alternating faces of the DNA, an organization similar to that of SV40 large T-antigen (Figure 4B) (Enemark *et al.,* 2002; Meinke *et al.,* 2006; Meinke *et al.,* 2007). The palindrome can be divided into two functional half sites; interestingly, half-integral insertions (5 bp) disrupt replication, whereas full-integral insertions (10 bp) of DNA between the half sites are tolerated (Mendoza *et al.,* 1995). This phasing dependence highlights the importance of the relative orientations of adjacent E1 DNA binding domains for the assembly of an active complex, as half-integral insertions offset all of the binding sites from one another and likely disrupt the formation of E1 dimers (Enemark *et al.,* 2002). The AT-tract closest in proximity to the E1 palindrome serves as the location of initial melting. Recognition and melting of this region is performed by a conserved set of positive (K506) and aromatic (H507) residues in the β-hairpin of the E1 SFIII helicase domain (Liu *et al.,* 2007; Schuck and Stenlund, 2007).

The isolated DNA-binding domain of E1 does not possess sufficient affinity for its binding site to stably engage the viral origin on its own. Instead, the cooperative association of E1 and E2 is used to ensure that target replication origins are appropriately recognized (Mohr *et al.,* 1990; Sedman and Stenlund, 1995). The interface of the E1-E2 complex is formed between the C-terminal helicase domain of E1 and the activation domain of E2, an interaction that occludes the E1 homo-oligomerization surface (Abbate *et al.,* 2004). Through this and other E1-specific contacts, E2 stabilizes a conformation of E1 that is incompatible with ATP binding, an event required for E1 assembly and activity (Castella *et al.,* 2006a; Chen and Stenlund, 2002; Liu *et al.,* 2007; Sanders and Stenlund, 1998; Schuck and Stenlund, 2007). These observations indicate that the E2 homodimer must dissociate from E1 to facilitate the appropriate hexamerization of the helicase and melting of the replication origin.

Once the E1-E2 complex has formed, additional E1 monomers bind the two remaining sites on the central DNA region (Figure 4D, *top*) (Chen and Stenlund, 2002; Enemark *et al.,* 2002). This arrangement is thought to create two pairs of dimers in which the associated helicase domains lie in close proximity

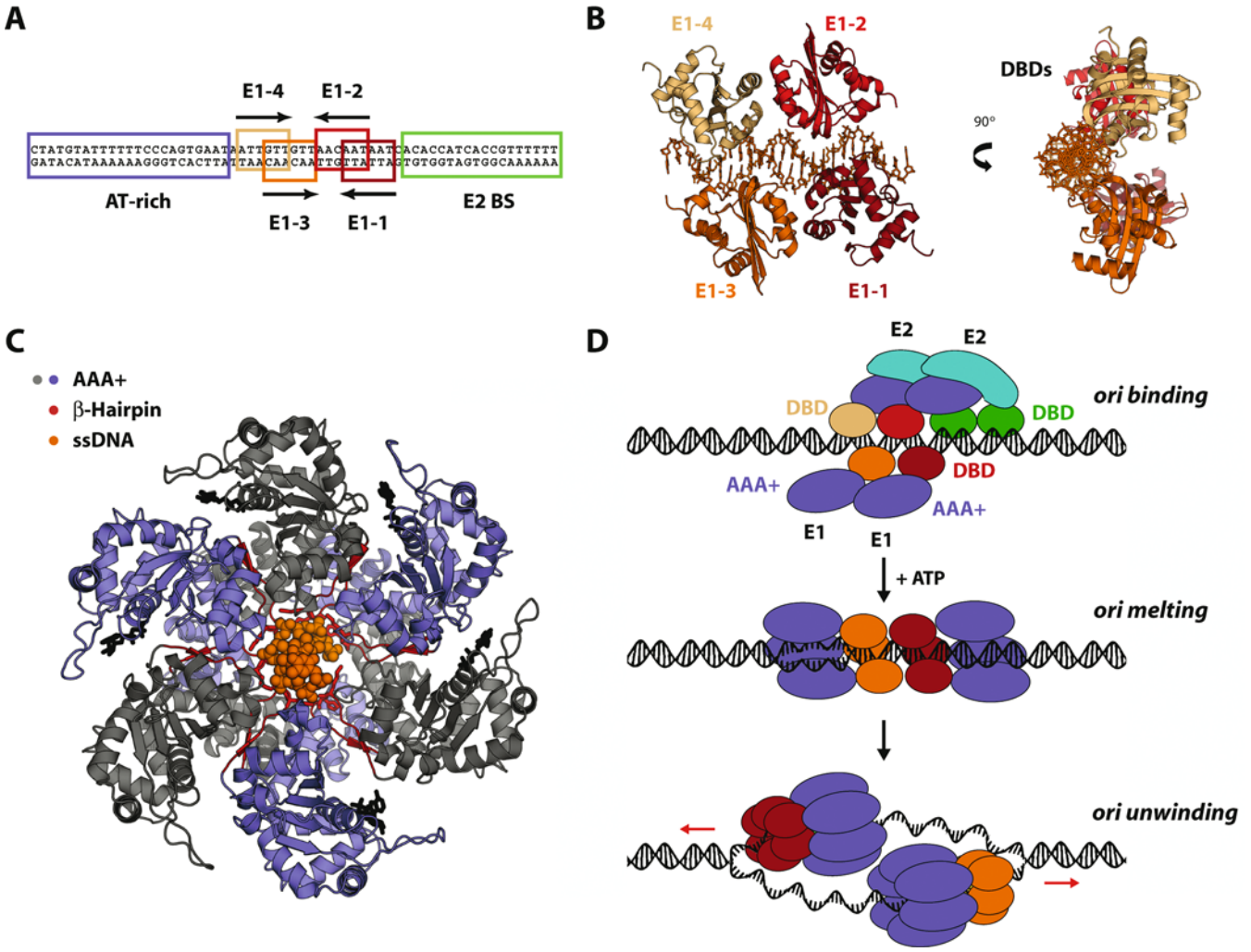

FIG. 4. Papillomavirus initiation. (A) The Papillomavirus origin sequence. Highlighted conserved elements include the site of initial melting (AT-rich), the central region with each E1 recognition sequence indicated (E1-1-E1-4) and the E2 binding sites (E2 BS). (B) Front and side views of the E1 DNA binding domains bound to DNA (PDB ID 1KSX). (C) ADP bound E1 hexamer in complex with single-stranded DNA (PDB ID 2GXA). The β-hairpin insertion is highlighted red. DNA is displayed as orange spheres. (D) Model for E1 initiation. *(Top)* An E1 dimer is recruited to the origin with the help of E2. This event facilitates binding of a second dimer. The color of each DNA binding domain (DBD) matches the corresponding origin binding site in panel A and monomer in panel B. *(Middle)* ATP binding occurs with the loss of E2, and a double-trimer intermediate forms, stabilized by interactions between ATPase domains. This intermediate locally destabilizes the DNA duplex. *(Bottom)* Additional E1 monomers bind, fully melting the duplex and encircling a single DNA strand. The polarity of the DNA strand bound to the E1 hexamer displayed in panel C, is incompatible with the formation of a head-to-head double hexamer, suggesting that the two helicases migrate past one another in the early stages of origin unwinding. The hexamers may then reassociate with each other through ATPase domain interactions. See (Enemark and Joshua-Tor, 2006) for details.

to one another, poised for assembly. The DNA binding domains of the two dimeric E1 complexes associate with the duplex in an offset manner with respect to each other around the DNA helix (Figure 4B; 4D, *top*); each pair primarily contacts only one DNA strand, such that the strand contacted by the upper dimer is different than the strand contacted by the lower dimer. The apparent strand preferences of the DNA binding domains may be functionally important, predisposing each helicase to properly assemble around a different DNA strand (Enemark *et al.,* 2002).

Analysis of the stoichiometry of E1 binding to full length and truncated origin sequences indicates that a metastable, double-trimer intermediate forms prior to double-hexamer formation (Figure 4D, *middle*) (Schuck and Stenlund, 2005; Sedman and

Strenlund, 1996). Trimers of E1 form in the presence of ADP or ATP, but the recognition of AT base pairs in the minor groove is required for template melting, and is dependent on the ATP form (Schuck and Stenlund, 2007). Similarly, E1 hexamer formation only occurs in the ATP bound state, and is stimulated by the presence of single-stranded DNA (Abbate *et al.,* 2004; Castella *et al.,* 2006b; Fouts *et al.,* 1999; Sanders and Stenlund, 1998).

The recently obtained structure of a full hexamer of the E1 helicase domain complexed with a short DNA segment reveals that, once assembled, only a single strand of DNA is threaded though the center of each initiator/helicase ring (Figure 4C) (Enemark and Joshua-Tor, 2006). Positively charged and aromatic residues in the β-hairpin of the E1 SF3 AAA+ domain are used to track phosphates and sugars through the central pore, providing insights into the translocation mechanism of the enzyme. One of many intriguing findings raised by the structure is the orientation of DNA through the helicase ring, which is opposite that predicted for the 3′-5′ tracking polarity of the helicase, and incompatible with head-to-head double hexamer models whereby the DNA binding domains of two E1 hexamers contact each other (cf. Figure 3D for SV40 large T-antigen). This observation suggests that, following melting of the papillomavirus origin, two E1 hexamers may migrate past one another to form a dodecamer with the DNA binding domains on the outer periphery of the complex (Figure 4D, bottom) (Enemark and Joshua-Tor, 2006). Moreover, given their evolutionary similarity, it seems likely that the melting, encirclement, and translocation mechanism used by E1 may also hold for the related SV40 large T-antigen. Further studies will be needed to settle these issues definitively.

BACTERIAL INITIATION

In bacteria, DNA replication begins with the sequence-specific recognition of the chromosomal origin, termed *oriC*, by the initiator protein DnaA (Chakraborty, *et al.* 1982; Fuller *et al.,* 1984; Matsui *et al.,* 1985). Biochemical characterization of *E. coli* DnaA, the archetypal member of this family, has shown that the protein is composed of four primary domains, three of which have well-defined functions (Kaguni, 1997; Messer, 2002). The N-terminal domain associates with the replicative helicase, DnaB, and also may facilitate interactions between DnaA molecules (Abe *et al.,* 2007; Sutton and Kaguni, 1997; Weigel *et al.,* 1999). Following this region is domain II, a poorly conserved and flexible linker segment (Messer *et al.,* 1999; Sutton and Kaguni, 1997). Domain III comprises an AAA+ module, which bears the α-helical insertion to the core $\alpha\beta\alpha$ region (the ISM) that identifies DnaA as a member of the initiator/helicase-loader clade of the AAA+ superfamily (Figure 1C) (Erzberger *et al.,* 2002; Erzberger and Berger, 2006; Kawakami *et al.,* 2006a); the nucleotide state of domain III influences the ability of this region to associate with other DnaA protomers (Erzberger *et al.,* 2006; Sekimizu *et al.,* 1987; Speck *et al.,* 1999). At the extreme C-terminus of DnaA lies domain IV, which consists of a helix-turn-helix-type DNA binding domain that is responsible for recruiting the initiator to the replication origin (Fujikawa *et al.,* 2003; Messer*et al.,* 1999; Roth and Messer, 1995).

Bacterial origin sequences vary widely among different species, but all contain consensus DnaA binding sites traditionally known as DnaA boxes (Mackiewicz *et al.,* 2004). Bacterial origins also contain AT-rich sequences, termed DNA unwinding elements (DUEs) (Kowalski and Eddy, 1989), which melt in response to the cooperative binding of activated DnaA molecules (Bramhill and Kornberg, 1988; Gille and Messer, 1991; Holz *et al.,* 1992). Although bacterial genomes typically contain only one origin, secondary and alternative origin sites have been identified in some instances (Egan and Waldor, 2003; Hassan *et al.,* 1997; Kadoya *et al.,* 2002).

The *E. coli* origin is one of the best characterized to date, and has served as a model for understanding replication initiation in other bacterial species. *E. coli oriC* consists of a 250-bp sequence that contains several 9-bp DnaA boxes known as R1-R5 (Figure 5A). In addition, there are other DnaA binding sites that deviate from the DnaA box consensus sequence, termed I-sites and ATP-DnaA boxes (McGarry *et al.,* 2004; Speck *et al.,* 1999). DnaA has different affinities for each of these different classes of sites. R1, R2 and R4 are occupied throughout most of the cell cycle by either ATP-bound or ADP-bound DnaA (Grimwade *et al.,* 2007; Ryan *et al.,* 2004). R5, R3, I1, I2, and I3 are bound with intermediate affinity, while ATP-DnaA sites are bound with the lowest affinity. Both the I-sites and the ATP-DnaA sites are recognized only in the presence of ATP-bound DnaA (Grimwade *et al.,* 2000; Grimwade *et al.,* 2007; Margulies and Kaguni, 1996; Speck *et al.,* 1999). The relative positioning of each of the DnaA recognition sites with respect to each other and the DUE is critical for proper origin firing and the timing of replication onset (Holz *et al.,* 1992).

oriC also contains sequences recognized by other factors important for the regulation of initiation. The architectural protein integration host factor (IHF), known to cause pronounced distortions in DNA (Craig, 1984; Kuznetsov *et al.,* 2006; Rice *et al.,* 1996; Sugimura and Crothers, 2006), binds between the R1 and R5 sites (Filutowicz, 1990; Polaczek, 1990; Polaczek, 1997). A second architectural protein, known as factor for inversion stimulation (Fis), binds between the R2 and R3 sites (Filutowicz, 1992; Gille, 1991; Roth, 1994). Both IHF and Fis modulate the interaction of DnaA with its moderate and low affinity *oriC* binding sites during initiation. IHF enhances binding to these sites, promoting DnaA complex formation and unwinding of the DUE (Hwang and Kornberg, 1992; McGarry *et al.,* 2004). In contrast, Fis prevents IHF binding and reduces the binding of DnaA to weaker sites, preventing nucleoprotein assembly and melting of the DUE (Hiasa and Marians, 1994; Ryan *et al.,* 2004; Wold *et al.,* 1996). In addition to these proteins, HU, a non-sequence-specific architectural protein, binds around *oriC* and can enhance DNA melting by DnaA (Funnell *et al.,* 1987; Hwang and Kornberg, 1992).

Between initiation events, Fis is associated with *oriC*, along with DnaA molecules at the R1, R2, and R4 sites (Ryan

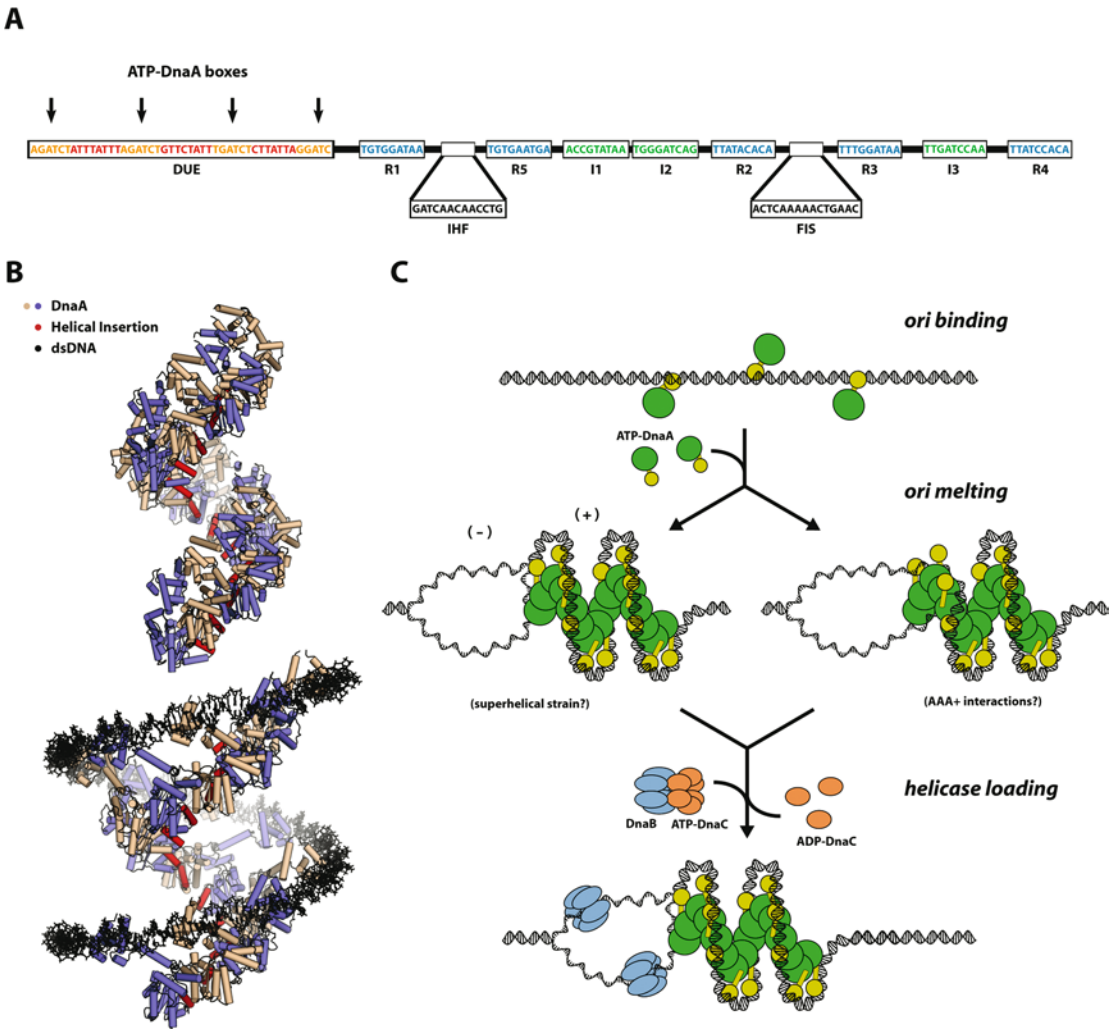

FIG. 5. Bacterial replication initiation. (A) The *Escherchia coli* origin (*oriC*). Highlighted conserved regions include the DNA unwinding element (DUE), DnaA binding sites R1–R5, I1-I3 and ATP-DnaA boxes (arrows), and binding sites for architectural proteins (IHF and Fis). (B) Ribbon diagram of the AMPPCP-bound DnaA filament structure (*top*, PDB ID 2HCB). The helical insertion (ISM) specific to the initiator clade is colored red. (*Bottom*) Hypothetical model in which an outward, rigid-body rotation of domain IV permits the binding and wrapping of DNA. (C) Model for initiation in bacteria. Each DnaA monomer is displayed with a green oval (domains I-III) connected to a yellow oval representing the DNA binding domain. *(Top)* DnaA monomers associate with *oriC*. *(Middle)* In the presence of ATP, DnaA homo-oligomerizes, wrapping DNA and melting the DUE by introducing negative superhelical strain *(left),* actively unwinding DNA through the ATPase domains *(right),* or both. *(Bottom)* Once melted, the DnaB helicase (blue) is loaded onto DNA through the action of DnaA and DnaC (orange).

et al., 2004). The total concentration of DnaA remains constant throughout the cell cycle (Sakakibara and Yuasa, 1982), but the amount of ATP-DnaA is known to increase prior to initiation (Kurokawa *et al.,* 1999). During initiation, Fis becomes displaced from *oriC,* unlocking the remaining DnaA binding sites in the origin (Ryan *et al.,* 2004). This event also promotes the binding of IHF, which further enhances the interaction of DnaA with its lower affinity sites, particularly the I-sites (so named because of their IHF-dependent response) (Grimwade *et al.,* 2000; McGarry *et al.,* 2004). Once the origin has become saturated with ATP-DnaA, strand separation occurs in the DUE, where three unstable 13-mer AT-rich repeats respond to DnaA complex formation by melting (Figure 5C) (Bramhill and Kornberg, 1988). Negative supercoiling is also required for initiation, and likely serves to destabilize the DUE to support efficient melting by DnaA (Funnell *et al.,* 1987; Kowalski and Eddy, 1989).

The membership of DnaA within the AAA+ superfamily initially suggested the initiator might form some type of

ring-shaped complex. Unexpectedly, the structure of the DnaA oligomer responsible for catalyzing DUE melting had remained unknown. Unexpectedly, the structure of the AAA+ and DNA binding domains of *Aquifex aeolicus* DnaA in complex with a non-hydrolyzable ATP analogue (AMPPCP), revealed that the initiator instead assembles into a helical filament (Figure 5B, *top*) (Erzberger *et al.,* 2006). Although the DNA binding domain in this oligomerized structure does not occupy a conformation that directly permits the docking of DNA onto the DnaA oligomer, a helical linker between the DNA binding and AAA+ domains is flexible, and can readily be positioned to accommodate a configuration that would allow DNA to wrap around the filament exterior (Figure 5B, *bottom*). Such a wrap would be predicted to constrain positive DNA supercoils, a prediction verified by topology trapping assays (Erzberger *et al.,* 2006). Since stabilization of positive supercoils within a DnaA-*oriC* complex would lead to the formation of compensatory negative writhe outside of the assembly, it is possible that DNA wrapping around the outside of a DnaA filament may be used to assist in melting of the DUE (Figure 5C, *middle left*). Whether local underwinding actively directs DUE melting or is simply a byproduct of DnaA assembly remains to be resolved.

Structures of distinct DnaA intermediates have provided insights into how nucleotide controls the assembly of the protein (Erzberger *et al.,* 2002; Erzberger *et al.,* 2006). Comparison of ATP- and ADP-bound DnaA states indicates that the presence of a γ-phosphate alters the position of the C-terminal α-helical bundle of the AAA+ domain, primarily through interactions with the sensor II arginine. The resultant conformational change exposes a surface region that allows for the docking of a second ATP-DnaA protomer, which in turn contributes an arginine finger from its BoxVII/SRC motif to the nucleotide binding pocket of the first subunit (Figure 1A). In the ADP state, the α-helical lid appears to sterically block this inter-subunit contact (Erzberger *et al.,* 2006), consistent with biochemical data showing that ADP does not favor oligomerization of the initiator (Speck *et al.,* 1999; Speck and Messer, 2001).

Following melting, ATP-DnaA binds single-stranded DNA in the DUE (Speck and Messer, 2001; Weigel and Seitz, 2002). The ATP dependence of this activity is not understood, but suggests that domain III might be involved. In the ATP-bound DnaA structure, a number of conserved and positively-charged residues line the central axis of the helical filament (Erzberger *et al.,* 2006), consistent with the idea that the AAA+ ATPase domains constitute a secondary DNA binding site. A recent study has shown that mutating residues along the interior of the filament substantially reduces the single-stranded DNA binding activity of DnaA, as well as the ability of the initiator to melt the DUE (Ozaki *et al.,* 2008). Direct association with single-stranded DNA may thus play a role in melting DNA and/or stabilizing the unwound DUE in preparation for downstream stages of replisome assembly (Figure 5C, *middle right*). In this regard, it is worth noting that the interior channels of many ring-shaped DNA binding AAA+ proteins (*e.g.*, E1, SV40 large T-antigen, and clamp loaders) likewise are known to interact with nucleic acid segments.

After unwinding of the DUE, two hexamers of the DnaB helicase are loaded onto *oriC* (Fang *et al.,* 1999). DnaA is known to facilitate loading of DnaB through a direct interaction with its N-terminal domain (Abe *et al.,* 2007; Seitz *et al.,* 2000; Sutton *et al.,* 1998). DnaC, another member of the AAA+ superfamily and a paralog of DnaA, also is required for helicase loading. Interestingly, the ATPase activity of DnaC is not required for deposition of DnaB, but is required for release and helicase activation (Davey *et al.,* 2002a). To date, the exact sequence of events and the types of structural intermediates accessed by the initiator, helicase and loader during the process have not been established. The way in which DnaA and DnaC collaborate to control the precise number of DnaB molecules loaded onto *oriC*, and ensure that each helicase ring is placed on a different DNA strand likewise is not understood.

Bacteria use many different regulatory mechanisms to ensure that replication occurs only once per cell cycle. One mechanism discussed previously involves the nucleotide state of DnaA; however, other processes are also used (Kaguni, 2006; Mott and Berger, 2007; Zakrzewska-Czerwinska *et al.,* 2007). In *E. coli*, these events include the sequestration of the origin between initiation cycles by a protein called SeqA (Boye *et al.,* 1996; Nievera *et al.,* 2006; Slater *et al.,* 1995), and a lowering of the intracellular concentration of free DnaA through the binding of the initiator to genomic regions that contain large numbers of DnaA binding sites (e.g., the *datA* locus) (Kitagawa *et al.,* 1996; Ogawa *et al.,* 2002). In addition, DnaA can regulate its own expression by binding to DnaA boxes flanking the DnaA gene (Atlung *et al.,* 1985; Braun *et al.,* 1985; Messer and Weigel, 1997). Finally, the activity of the initiator is subject to external control processes that include both binding by a protein known as DiaA to ensure initiation synchrony (Ishida *et al.,* 2004; Natrajan *et al.,* 2007), and the Regulatory Inactivation of DnaA (RIDA) pathway (Katayama *et al.,* 1998).

RIDA is particularly noteworthy, as it relies on the Hda protein, a member of the initiator clade of the AAA+ superfamily and a DnaA paralog (Kato and Katayama, 2001). The Hda component of RIDA operates as a homodimer that associates with the *E. coli* β processivity-clamp and then acts to directly catalyze nucleotide hydrolysis by the initiator, converting ATP-DnaA to ADP-DnaA (Kato and Katayama, 2001; Kawakami *et al.,* 2006b; Su'etsugu *et al.,* 2005). The association of Hda with the processivity clamp ensures that nucleotide hydrolysis by DnaA occurs at the proper moment during replisome formation to promote disassembly of the DnaA complex and prevent re-initiation. Consistent with this model, cells that lack Hda display an over-initiation phenotype identical to ATPase-defective mutants of DnaA (Camara *et al.,* 2003; Kato and Katayama, 2001; Nishida *et al.,* 2002). Since Hda is a DnaA paralog, it is likely that the two proteins functionally interact in a canonical head-to-tail arrangement, as seen for other AAA+ proteins. In

support of this model, biochemical studies have shown that the conserved arginine in the BoxVII/SRC motif of Hda is required for its catalytic activity (Su'etsugu *et al.,* 2005). After the active DnaA complex has been disassembled through the action of RIDA, ADP-DnaA is thought to be converted into an ATP-charged state before the next initiation event by acidic phospholipids at the membrane (Aranovich *et al.,* 2006; Kitchen *et al.,* 1999).

Interactions between Hda and DnaA demonstrate how the AAA+ architecture can be used not just as a self-assembly module, but also as a scaffold for promoting association and communication between different replication initiation factors. The proposed mechanism of Hda also is consistent with the observation that ATP-activated DnaA assembles into a filament and not a ring; this architectural organization leaves a pair of interfaces accessible at either end of the oligomerized particle that can potentially interact with other proteins. Given the role of its arginine finger, Hda likely binds directly to an open DnaA active site on one end of the filament. It currently remains unknown which end of the oligomeric DnaA helix faces the DUE, or how the polarity of this assembly is enforced on *oriC*.

EUKARYOTIC INITIATION

The eukaryotic initiator, known as the **O**rigin **R**ecognition **C**omplex (ORC) (Bell and Stillman, 1992), is composed of six subunits (Orc1-Orc6). Three of the six, Orc1, Orc4 and Orc5, were independently identified by multiple groups to contain AAA+ domains (Bell *et al.,* 1995; Schepers and Diffley, 2001; Tugal *et al.,* 1998), and more recently have been shown to fall within the same AAA+ initiator clade as DnaA (Iyer *et al.,* 2004a). The lineage of Orc2 and Orc3 is less certain, however, these proteins also may possess diverged AAA+ folds (Clarey *et al.,* 2006; Speck *et al.,* 2005). Electron microscopy studies have revealed that ORC forms notched- or open-ring particles (Figure 6A) consistent with its phylogenetic relationship to AAA+ proteins (Clarey *et al.,* 2006; Speck *et al.,* 2005). Moreover, a complex of five AAA+ domains taken from an ATP-DnaA filament can be docked comfortably into the open ring region of *Drosphila* ORC (Clarey *et al.,* 2006). These findings suggest that certain architectural features of initiator assemblies may have been preserved throughout the evolution of cellular organisms.

Several ORC subunits, most notably Orc1, Orc4, and Orc5, appear to have winged-helix domains (WHDs) appended to their C-termini. These subunits have been shown to crosslink with DNA (Klemm *et al.,* 1997; Lee and Bell, 1997), but the extent to which the WHDs specifically might be important for DNA recognition and binding is not known. In general, ORCs from different organisms possess a variety of strategies for productively binding to replication origins, including the use of auxiliary DNA binding domains (*e.g.*, the AT-hook domain of *Schizosaccharomyces pombe* Orc4) and interactions with auxiliary partner proteins or nucleosomes (Lee *et al.,* 2001; Lipford and Bell, 2001; Speck and Stillman, 2007).

ORC is capable of productively associating with origins when bound to ATP, and ATP hydrolysis is required for ORC function (Bell and Stillman, 1992; Bowers *et al.,* 2004; Chesnokov *et al.,* 1999; Gillespie *et al.,* 2001; Harvey and Newport, 2003; Klemm *et al.,* 1997; Lee *et al.,* 2000; Remus *et al.,* 2004; Vashee *et al.,* 2001). Consistent with these observations, the nucleotide status of ORC appears to elicit distinct conformational and functional states of the protein (Clarey *et al.,* 2006; Klemm *et al.,* 1997; Lee *et al.,* 2000; Lee *et al.,* 2000; Seki and Diffley, 2000; Speck *et al.,* 2005), some of which may be required for efficient progression beyond the initiation stage of replication. As with other initiators, the strong dependency on ATP binding and hydrolysis for activity indicates that the AAA+ domains play a key role in controlling the activity and properties of ORC.

Eukaryotic replication is initiated by the binding of ORC to multiple origins scattered throughout the genome. Unlike bacterial origins, the sequences of eukaryotic origins vary significantly not only between different organisms, but also within a given cell or even chromosome. In *S. cerevisiae*, **A**utonomously **R**eplicating **S**equences (ARSs) are origins that permit plasmids to be maintained extrachromosomally. Initial work defined *S. cerevisiae* ARS sequences (such as ARS1) as being composed of an 11-bp A-element (or **A**RS **C**onsensus **S**equence—ACS) and three 10- to-15 bp B-elements that are all essential for function (Brewer and Fangman, 1987; Hsiao and Carbon, 1979; Marahrens and Stillman, 1992). ORC binding overlaps with the A- and B1-elements (Bell and Stillman, 1992), whereas the B2 region may serve as a recognition site for another initiation component and B3 is known to bind the transcription factor, Abf1 (Diffley and Stillman, 1988). More recent studies have indicated that there is significant variability in the precise sequences that are recognized as origins in yeast (Poloumienko, 2001; Wyrick, 2001). For example, the ACS sequence of ARS309 differs significantly from the canonical sequence used in ARS1, but is an active chromosomal replicator nonetheless (Theis and Newlon, 1997).

The plasticity of origin sequences observed in yeast is even more extreme in other eukaryotic organisms (Cvetic and Walter, 2005). Indeed, in metazoans, it has been difficult to identify any sequence-specific trends, as even random DNA sequences of sufficient length can confer upon a plasmid the ability to be replicated (Harland and Laskey, 1980; Heinzel *et al.,* 1991; Mechali and Kearsey, 1984). This lack of sequence conservation hints that there must be other mechanisms, such as DNA or chromatin structure (Bell and Dutta, 2002; Lipford and Bell, 2001; Remus *et al.,* 2004), that target initiators to the correct locations in the genome.

Contrary to the action of papilloma and polyoma viral initiators, or DnaA in bacterial replication initiation, ORC has not been observed to induce origin melting (Klemm *et al.,* 1997). Instead, ORC has been suggested to serve as a loading platform for recruiting and assembling dedicated unwinding machinery (Figure 6B). A particularly key event in this process

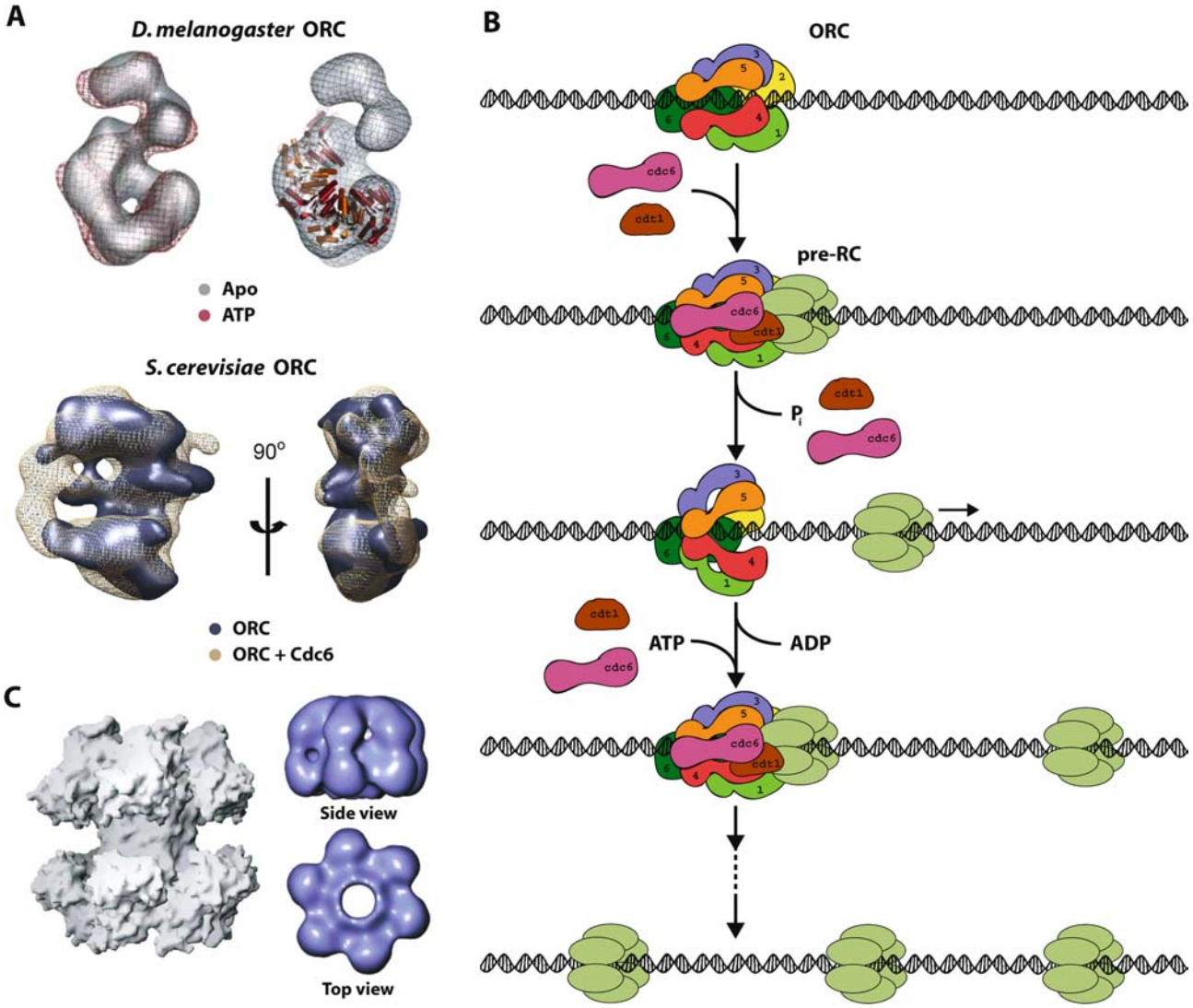

FIG. 6. Archaeal/eukaryotic initiation mechanisms and factors. (A) *(Top)* EM reconstructions of *Drosophila melanogaster* ORC in apo and ATP-bound forms (*left*), and with a pentamer of AAA+ domains from the AMPPCP-bound DnaA filament docked into the apo state (*right*). Reprinted by permission from Macmillan Publishers Ltd: [Nature Structure and Molecular Biology] (Clarey, *et al.*, 2005) *(Bottom)* EM reconstructions of *Saccharomyces cerevisiae* ORC in the absence (*blue*) and presence of Cdc6 (brown). Reprinted by permission from Macmillan Publishers Ltd: [Nature Structure and Molecular Biology]. (Speck *et al.*, 2005) (B) Model of eukaryotic initiation events. Each ORC subunit is numbered and displayed with a unique color (*panel 1*). ORC binds an origin, followed by recruitment of Cdc6 (*pink*), Cdt1 (*brown*), and Mcm2-7 (*light green*) to form the pre-RC *(panels 1-2)*. ORC can deposit multiple MCMs on the origin with ATP turnover occuring during each loading event. C) Archaeal MCM assemblies. *(Left)* The structure of the N-terminus of *Methanobacterium thermoautotrophicum* MCM shows that the domain forms a head-to-head double hexamer (PDB ID 1LTL) Individual chains are represented with alternating colors (green and blue) and zinc ions are represented as spheres (black). *(Right)* Side and top views of an EM reconstruction of the *Methanobacterium thermoautotrophicum* full-length MCM hexamer. Reprinted by permission from Macmillan Publishers Ltd: [EMBO Reports] (Pape *et al.*, 2003).

may be linked to the formation of an intermediate assembly before initiation, the pre-replicative complex (pre-RC), which forms prior to the association of primase, DNA polymerases and the RFC clamp loader assembly (Diffley *et al.,* 1995; Seki and Diffley, 2000). The pre-RC comprises not only ORC, but also several other factors, including the **M**ini-**C**hromosome **M**aintenance (MCM2-7) heterohexameric helicase complex, and the MCM loader/chaperone proteins, Cdc6 and Cdt1.

Cdc6 was originally identified in a screen for mutants that caused changes in the cell division cycle (Hartwell, 1973). Interestingly, Cdc6 is a paralog of Orc1 (Bell *et al.,* 1995; Iyer *et al.,* 2004a), and is predicted to contain both AAA+ and winged helix domains (Liu *et al.,* 2000). In many organisms, Cdc6 is required for Mcm2-7 association with replication origins (Aparicio *et al.,* 1997; Liang and Stillman, 1997; Mendez and Stillman, 2003; Perkins and Diffley, 1998; Weinreich *et al.,* 1999). Mutations in the conserved sensor-I region of the *S. cerevisiae* Cdc6 AAA+ fold result in an inactive but stable protein, and further lead to constitutive MCM loading and over-replication (Liang and Stillman, 1997; Liu *et al.,* 2000; Schepers and Diffley, 2001). The ability of Cdc6 to hydrolyze nucleotide is necessary for Mcm2-7 loading, and occurs at a distinct moment in the loading process before ATP hydrolysis by ORC (Perkins and Diffley, 1998; Randell *et al.,* 2006). In *S. cerevisiae*, the ATPase activity of Cdc6 also ensures that pre-RCs are only formed at origins (Speck and Stillman, 2007). In the presence of random DNA, ORC stimulates ATP hydrolysis by Cdc6 to prevent pre-RC formation, while the presence of origin DNA inhibits ATP hydrolysis by Cdc6, thereby allowing pre-RC assembly (Speck and Stillman, 2007). Taken together, these findings have established Cdc6 as a crucial component for pre-RC assembly and Mcm2-7 loading, and highlight the importance of its ATPase activity for proper function. In this regard, the activity of Cdc6 parallels that of bacterial DnaC to some extent (Lee and Bell, 2000; Robinson and Bell, 2005), although a direct Cdc6/MCM interaction has not yet been mapped.

Cdt1's role as a key component of the pre-RC was first demonstrated by mutations that inhibited DNA replication and caused defects in the S-phase checkpoint (Hofmann and Beach, 1994; Nishitani *et al.,* 2000). *S. pombe* Cdt1 has been shown to associate with the C-terminus of Cdc18 (the fission yeast ortholog of Cdc6) to promote the association of Mcms with origin DNA (Nishitani *et al.,* 2000). Only in the presence of both Cdc6 and Cdt1 can ORC participate in the loading of Mcm2-7, after which Mcm2-7 remains associated with other pre-RC components until nucleotide hydrolysis by ORC induces release (Randell *et al.,* 2006). In budding yeast, origin DNA is known to inhibit ATP hydrolysis by ORC, consistent with a requirement for ORC to remain in an ATP-bound state until Mcm2-7 loading is complete (Klemm *et al.,* 1997; Lee *et al.,* 2000). ATP hydrolysis by ORC is still essential, however, as mutation of the arginine finger of Orc4 disrupts the hydrolysis of ATP by Orc1 and is lethal (Bowers *et al.,* 2004).

Unlike the action of DnaA and DnaC on DnaB, the ORC-Cdc6-Cdt1 complex is capable of loading more than two Mcm2-7 complexes onto origin DNA. This multiple loading behavior requires ATP hydrolysis (Bowers *et al.,* 2004; Randell *et al.,* 2006), and may be partly responsible for the so-called "MCM paradox," in which anywhere from 4 to 20 copies of the helicase are loaded and present at replication origins prior to initiation (Figure 6B) (Lei *et al.,* 1996; Walter and Newport, 1997). This processive action further suggests that pre-RC assembly may enhance ATP hydrolysis by ORC and Cdc6 as part of an event to elicit a conformational change that both releases Mcm2-7 and resets ORC for further loading events.

Interestingly, the six distinct subunits of the Mcm2-7 complex are themselves members of the AAA+ superfamily. MCMs do not fall within either the initiator or SFIII helicase clades, but instead belong to the pre-sensor II (PSII) subfamily (Iyer *et al.,* 2004a) (Figure 1E). Eukaryotic Mcm2-7 also appears to rely on a set of partner proteins, the GINS complex (Kanemaki *et al.,* 2003; Kubota *et al.,* 2003; Takayama *et al.,* 2003) and Cdc45 (Gambus, 2006; Moir *et al.,* 1982; Pacek and Walter, 2004), for stimulating DNA unwinding (Moyer *et al.,* 2006; Pacek *et al.,* 2006). To date, structural studies on the eukaryotic Mcm2-7 complex have not been straightforward. Based on biochemical and electron microscopy studies of a three-subunit subassembly that displays limited helicase activity (comprising Mcm4, Mcm6, and Mcm7) (Ishimi, 1997; Kaplan *et al.,* 2003; Sato, 2000; Yabuta, 2003; You, 1999), and by analogy with archaeal MCM homologs (see below) and other hexameric helicases, Mcm2-7 is predicted to be ring shaped (Figure 6C) (Costa *et al.,* 2006a; Fletcher *et al.,* 2003; Takahashi *et al.,* 2005). Given this finding, it has been proposed that the pre-RC proteins may load Mcm2-7 by changing the conformation of the helicase and opening the ring to allow passage of DNA into its central channel (Bowers *et al.,* 2004; Mendez and Stillman, 2003; Randell *et al.,* 2006; Takahashi *et al.,* 2005). Such an activity would again parallel to some extent the mechanism by which DnaC is thought to help load DnaB onto DNA (Davey and O'Donnell, 2003).

ARCHAEA

Upon sequencing of the first archaeal genome (Bult, 1996), it became clear that archaea possess replication proteins more closely related to those of eukaryotes than bacteria (Edgell and Doolittle, 1997; Grabowski and Kelman, 2003). Indeed, it now appears that most factors responsible for catalyzing a variety of essential nucleic acid transactions (replication, transcription, and repair) in archaea are akin to eukaryotic machineries, albeit typically in a more simplified form (Barry and Bell, 2006). These similarities have encouraged the study of replication initiation in archaea in the hope of gaining general insights into this process in more complex eukaryotic organisms (Barry and Bell, 2006; Kelman and Kelman, 2003).

Archaeal initiation is thought to rely on a two-domain protein known as Cdc6/Orc1, so named due to its roughly equal homology with regions of eukaryotic Orc1 and Cdc6. The N-terminal domain consists of an initiator-type AAA+ ATPase module that is highly similar structurally to the AAA+ domain of DnaA (Erzberger *et al.,* 2002; Iyer *et al.,* 2004a; Liu *et al.,* 2000). Following the AAA+ region is a winged-helix domain (Liu *et al.,* 2000), which enables the sequence-specific recognition of replication origins (Capaldi and Berger, 2004; Robinson *et al.,* 2004; Singleton *et al.,* 2004). The number of Cdc6/Orc1 homologs varies between different archaeal species, with some organisms possessing only a single initiator, and others encoding multiple

paralogs (Grabowski and Kelman, 2003; Ng *et al.,* 2000; Norais *et al.,* 2007; She *et al.,* 2001). Some DNA elements recognized by Cdc6/Orc1 proteins contain discrete nucleotide repeats, while others present more divergent sequences. In those organisms that possess multiple Cdc6/Orc1 variants, the specific sequence recognized by one paralog can be completely unrelated to the sequence recognized by a different paralog (Robinson *et al.,* 2004).

The first archaeal origin to be identified experimentally and through sequence analysis techniques derived from *Pyrococcus abyssi* (Lopez *et al.,* 1999; Myllykallio *et al.,* 2000). Since this initial classification, more archaeal origins have been determined, revealing some basic properties of their structure (Berquist and DasSarma, 2003; Kelman and Kelman, 2004; Robinson *et al.,* 2004). A surprising finding from these studies has been that replication origins in archaea often bear a hybrid resemblance to the organization and structure of origins found in both bacteria and eukaryotes (Forterre *et al.,* 2002; Kelman and Kelman, 2003; Myllykallio *et al.,* 2000). For example, like bacteria, many archaeal origins possess well-defined patterns of sequence repeats, termed Origin Recognition Boxes (ORBs), that serve as binding sites for particular Cdc6/Orc1 proteins (Capaldi and Berger, 2004; Gaudier *et al.,* 2007; Grainge *et al.,* 2006; Matsunaga *et al.,* 2007; Robinson *et al.,* 2004). AT-rich regions that serve as DUEs during initiation also are often evident near these repeats. Unlike many bacteria, however, archaeal chromosomes often contain multiple origins, similar to the situation observed in eukaryotes (Kelman and Kelman, 2004; Robinson *et al.,* 2004). Moreover, some archaeal origins do not contain obvious recognition sequences for Cdc6/Orc1 proteins, but nonetheless serve as *bone fide* start points for DNA synthesis (Robinson *et al.,* 2007).

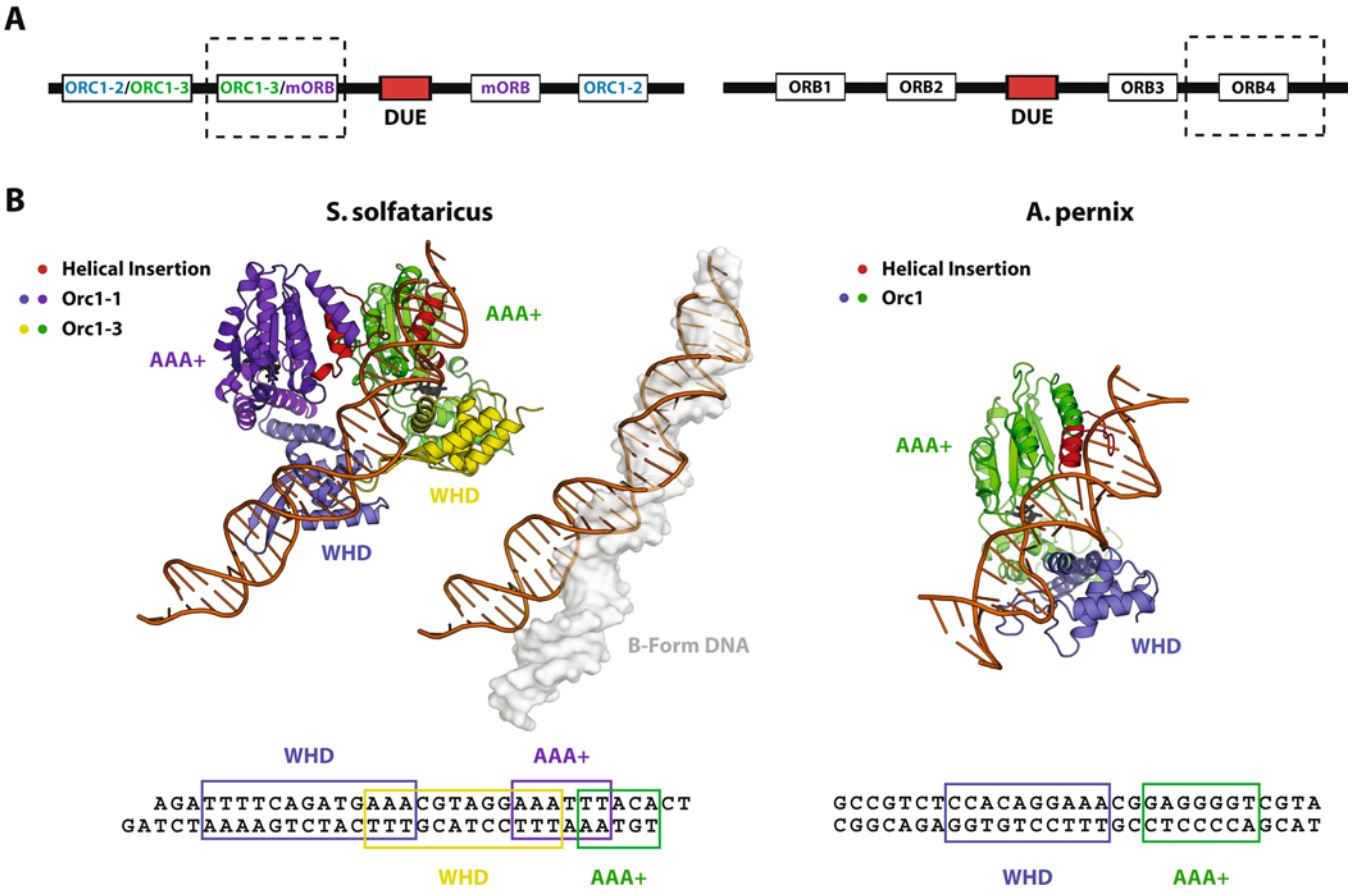

FIG. 7. Achaeal origins and intiators. (A) Two different archaeal origins. The DNA unwinding element (DUE) in each origin is indicated by a red box. *(Left)* The *oriC2* origin from *S. solfataricus*. Binding sites for all three of its ORC proteins are indicated. ORBs and mORBs are bound by class I Orc1 proteins (Grainge *et al.*, 2006). *(Right)* Origin from *A. pernix.* Only one of this organism's two ORC proteins has binding sites in this region. Dotted boxes refer to binding sites used in structural studies. (B) Initiator/DNA complexes. The helical insertion specific to the initiator clade is colored red. *(Left) S. solfataricus* Orc1-1/Orc1-3 heterodimer bound to the dual site indicated for *oriC2* (PDB ID 2V1U). (*Middle*) DNA bound by the *S. solfataricus* initiators (*orange*) is deformed compared to B-form DNA (*white*). *(Right) A. pernix* Orc1 from bound to ORB4 (PDB ID 2V1U). *(Bottom)* DNA sequences and interaction regions for the two initiator complexes.

Structural studies have begun to reveal how archaeal initiators engage their cognate DNA recognition sequences in replication origins (Dueber *et al.,* 2007; Gaudier *et al.,* 2007) (Figure 7). One recent effort focused on a complex between two Cdc6/Orc1 paralogs (Orc1-1 and Orc1-3) from *S. solfataricus* and a fragment of the *S. solfataricus oriC2* origin that contains a pair of overlapping binding sites for each protein (Dueber *et al.,* 2007). A second, parallel paper revealed how *A. pernix* Orc1 binds to one of four ORBs (ORB4) present in the organism's *oriC1* region (Gaudier *et al.,* 2007). Unexpectedly, in both structures DNA binding was observed not only by the WHD, but also by direct associations with the ATPase domain through the ISM α-helix that typifies the AAA+ initiator clade. Very few sequence-specific contacts to DNA were observed in either instance, a finding that may help explain the rather substantial affinity of Orc1-type proteins for nonorigin DNA. The joint actions of the AAA+/WHD associations also were observed to induce a marked bending and underwinding of the DNA duplex (Figure 7B, *middle*). The importance of these deformations is not yet clear, but it is possible that they may be tied to the melting of duplex DNA prior to the onset of replication.

Recruitment of the archaeal helicase is thought to occur after the Cdc6/Orc1 initiators have assembled at the origin (Matsunaga *et al.,* 2001). Echoing a theme seen for other archaeal initiation factors, the replicative helicase in these organisms is most homologous not to bacterial DnaB (a RecA-family helicase), but to the Mcm2-7 proteins found in eukaryotes. One notable difference is that most archaeal genomes appear to encode only a single Mcm protein, rather than six paralogous subunits as seen for eukaryotic Mcm2-7. Another distinction is that archaeal MCM proteins show relatively robust helicase activity *in vitro* (Chong *et al.,* 2000; Grainge, 2003; Kelman *et al.,* 1999; Shechter *et al.,* 2000), a property not yet observed for their eukaryotic counterparts. Based on structural studies, the functional assembly state of archaeal MCM is believed to be a hexamer or double hexamer (Figure 6C) (Chen *et al.,* 2005; Costa *et al.,* 2006a; Costa *et al.,* 2006c; Fletcher *et al.,* 2003; Pape *et al.,* 2003), although heptamers (Yu *et al.,* 2002), double-hexamers/heptamers (Costa *et al.,* 2006a), and even filaments have also been observed (Chen *et al.,* 2005; Costa *et al.,* 2006b).

It is currently unknown how the archaeal MCM helicase is loaded onto DNA. Archaea do not appear to possess an obvious homolog of Cdt1, which plays a crucial role during MCM loading in eukaryotes. However, Cdc6/Orc1 can associate with MCM on forked DNA substrates (DeFelice, 2004), suggesting that archaea may use initiator ATPases for MCM loading. In *Methanothermobacter thermautotrophicus*, the activity of the MCM helicase also is inhibited by the presence of a corresponding Orc1/Cdc6 paralog from the same species (De Felice *et al.,* 2004; Kasiviswanathan *et al.,* 2006; Shin *et al.,* 2003). Nonetheless, as with eukaryotes, MCM loading has not been reconstituted outside the cell using purified components. Whether this is because some other factor has yet to be identified, or because the target DNA has to be in a state that reflects the organization and/or status of chromatin in the cell, remains to be determined.

CONCLUSION

The variety of initiation mechanisms discussed in this chapter illustrate that the AAA+ ATPase fold can be readily augmented to carry out many different specialized functions. One modification arises from the tethering of additional functional domains to the conserved AAA+ core. For example, most initiators contain one or more DNA binding domains appended to either the N-terminus (SV40, E1) or C-terminus (cellular initiators) of the central AAA+ fold (Figure 1) that help guide initiators to replication origins. Flexible linkers tethering the AAA+ and DNA binding domains enable the two regions to move independent of each other. Still other variations may assist with helicase loading or other regulatory functions.

Another important set of adaptations have occurred within the AAA+ module itself. These include α-helix and β-strand additions to the core ATP-binding fold, and rearrangements of the C-terminal α-helical subdomain. In some cases, these additions appear to alter the architectural organization of higher-order complexes. For example, the extra α-helix in the AAA+ fold of DnaA favors the formation of helical filaments (Figure 5B), and may be responsible for the open-ring architectures observed for ORC (Figure 6A). Such open-ring structures may be useful for allowing initiator AAA+ domains to bind other AAA+ proteins and/or to engage DNA directly (Figures 5 and 7). In other instances, small structural additions to the core AAA+ module can modulate enzymatic functions. For example, the addition of a β-hairpin to the AAA+ fold of SFIII-type viral initiators is necessary not only the melting of duplex DNA, but also processive function as a helicase once the initiator has been properly assembled on a replication origin (Figures 3 and 4).

Despite these alterations, initiators across all cellular domains of life have retained the ability to bind adenine nucleotides. Changes in nucleotide state alter the conformation of the AAA+ assembly, creating in some instances a regulatory switch for oligomerization and, in others, a means for remodeling or translocating along DNA. The activation of cellular initiators by ATP binding, and their inactivation by ATP hydrolysis, further provides a control mechanism to prevent over-initiation, an event that can lead to changes in gene ploidy, genomic instabilities, and loss of proliferative control. Future studies are needed to help elucidate the precise molecular effects of nucleotide on initiator function, and to understand how the AAA+ scaffold has been modified during evolution to carry out such a wide variety of essential cellular tasks.

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REFERENCES

Abbate, E.A., Berger, J.M., and Botchan, M.R. 2004. The X-ray structure of the papillomavirus helicase in complex with its molecular matchmaker E2. *Genes Dev* 18:1981–1996.

Abe, Y., Jo, T., Matsuda, Y., Matsunaga, C., Katayama, T., and Ueda, T. 2007. Structure and function of DNAA N-terminal domains: Specific sites and mechanisms in inter-DNAA interaction and in DNAB helicase loading on oriC. *J Biol Chem* 282:17816–27.

Alexandrov, A.I., Botchan, M.R., and Cozzarelli, N.R. 2002. Characterization of simian virus 40 T-antigen double hexamers bound to a replication fork. The active form of the helicase. *J Biol Chem* 277:44886–44897.

Amin, A.A., Titolo, S., Pelletier, A., Fink, D., Cordingley, M.G., and Archambault, J. 2000. Identification of domains of the HPV11 E1 protein required for DNA replication in vitro. *Virology* 272:137–150.

Aparicio, O.M., Weinstein, D.M., and Bell, S.P. 1997. Components and dynamics of DNA replication complexes in S. cerevisiae: redistribution of MCM proteins and Cdc45p during S phase. *Cell* 91:59–69.

Aranovich, A., Gdalevsky, G.Y., Cohen-Luria, R., Fishov, I., and Parola, A.H. 2006. Membrane-catalyzed nucleotide exchange on DnaA. Effect of surface molecular crowding. *J Biol Chem* 281:12526–12534.

Arthur, A.K., Hoss, A., and Fanning, E. 1988. Expression of simian virus 40 T antigen in Escherichia coli: localization of T-antigen origin DNA-binding domain to within 129 amino acids. *J Virol* 62:1999–2006.

Atlung, T., Clausen, E.S., and Hansen, F.G. 1985. Autoregulation of the dnaA gene of Escherichia coli K12. *Mol Gen Genet* 200:442–450.

Barry, E.R., and Bell, S.D. 2006. DNA replication in the archaea. *Microbiol Mol Biol Rev* 70:876–887.

Bell, S.P., and Dutta, A. 2002. DNA replication in eukaryotic cells. *Annu Rev Biochem* 71:333–374.

Bell, S.P., Mitchell, J., Leber, J., Kobayashi, R., and Stillman, B. 1995. The multidomain structure of Orc1p reveals similarity to regulators of DNA replication and transcriptional silencing. *Cell* 83:563–568.

Bell, S.P., and Stillman, B. 1992. ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. *Nature* 357:128–134.

Berquist, B.R., and DasSarma, S. 2003. An archaeal chromosomal autonomously replicating sequence element from an extreme halophile, Halobacterium sp. strain NRC-1. *J Bacteriol* 185:5959–5966.

Borowiec, J.A., Dean, F.B., Bullock, P.A., and Hurwitz, J. 1990. Binding and unwinding—how T antigen engages the SV40 origin of DNA replication. *Cell* 60:181–184.

Borowiec, J.A., and Hurwitz, J. 1988. Localized melting and structural changes in the SV40 origin of replication induced by T-antigen. *EMBO J* 7:3149–3158.

Bowers, J.L., Randell, J.C., Chen, S., and Bell, S.P. 2004. ATP hydrolysis by ORC catalyzes reiterative Mcm2-7 assembly at a defined origin of replication. *Mol Cell* 16:967–978.

Bowman, G.D., O'Donnell, M., and Kuriyan, J. 2004. Structural analysis of a eukaryotic sliding DNA clamp-clamp loader complex. *Nature* 429:724–730.

Boye, E., Stokke, T., Kleckner, N., and Skarstad, K. 1996. Coordinating DNA replication initiation with cell growth: differential roles for DnaA and SeqA proteins. *Proc Natl Acad Sci USA* 93:12206–12211.

Bramhill, D., and Kornberg, A. 1988. Duplex opening by dnaA protein at novel sequences in initiation of replication at the origin of the E. coli chromosome. *Cell* 52:743–755.

Braun, R.E., O'Day, K., and Wright, A. 1985. Autoregulation of the DNA replication gene dnaA in E. coli K-12. *Cell* 40:159–169.

Brewer, B.J., and Fangman, W.L. 1987. The localization of replication origins on ARS plasmids in S. cerevisiae. *Cell* 51:463–471.

Bullock, P.A. 1997. The initiation of simian virus 40 DNA replication in vitro. *Crit Rev Biochem Mol Biol* 32:503–568.

Bult, C. 1996. Complete genome sequence of the methanogenic archaeon, Methanococcus jannaschii. *Science* 273:1058–1073.

Camara, J.E., Skarstad, K., and Crooke, E. 2003. Controlled initiation of chromosomal replication in Escherichia coli requires functional Hda protein. *J Bacteriol* 185:3244–3248.

Capaldi, S.A., and Berger, J.M. 2004. Biochemical characterization of Cdc6/Orc1 binding to the replication origin of the euryarchaeon Methanothermobacter thermoautotrophicus. *Nucleic Acids Res* 32:4821–4832.

Caruthers, J.M., and McKay, D.B. 2002. Helicase structure and mechanism. *Curr Opin Struct Biol* 12:123–133.

Castella, S., Bingham, G., and Sanders, C.M. 2006a. Common determinants in DNA melting and helicase-catalysed DNA unwinding by papillomavirus replication protein E1. *Nucleic Acids Res* 34:3008–3019.

Castella, S., Burgin, D., and Sanders, C.M. 2006b. Role of ATP hydrolysis in the DNA translocase activity of the bovine papillomavirus (BPV-1) E1 helicase. *Nucleic Acids Res* 34:3731–3741.

Chakraborty, T., Yoshinaga, K., Lother, H., and Messer, W. 1982. Purification of the E. coli dnaA gene product. *EMBO J* 1:1545–1549.

Chen, G., and Stenlund, A. 2002. Sequential and ordered assembly of E1 initiator complexes on the papillomavirus origin of DNA replication generates progressive structural changes related to melting. *Mol Cell Biol* 22:7712–7720.

Chen G., and Stenlund, A. 1998. Characterization of the DNA-binding domain of the bovine papillomavirus replication initiator E1. *J. Virol* 72:2567–2576.

Chen, Y.J., Yu, X., Kasiviswanathan, R., Shin, J.H., Kelman, Z., and Egelman, E.H. 2005. Structural polymorphism of Methanothermobacter thermautotrophicus MCM. *J Mol Biol* 346:389–394.

Chesnokov, I., Gossen, M., Remus, D., and Botchan, M. 1999. Assembly of functionally active Drosophila origin recognition complex from recombinant proteins. *Genes Dev* 13:1289–1296.

Chong, J.P., Hayashi, M.K., Simon, M.N., Xu, R.M., and Stillman, B. 2000. A double-hexamer archaeal minichromosome maintenance protein is an ATP-dependent DNA helicase. *Proc Natl Acad Sci USA* 97:1530–1535.

Clarey, M.G., Erzberger, J.P., Grob, P., Leschziner, A.E., Berger, J.M., Nogales, E., and Botchan, M. 2006. Nucleotide-dependent conformational changes in the DnaA-like core of the origin recognition complex. *Nat Struct Mol Biol* 13:684–690.

Costa, A., Pape, T., van Heel, M., Brick, P., Patwardhan, A., and Onesti, S. 2006a. Structural basis of the Methanothermobacter thermautotrophicus MCM helicase activity. *Nucleic Acids Res* 34:5829–5838.

Costa, A., Pape, T., van Heel, M., Brick, P., Patwardhan, A., and Onesti, S. 2006b. Structural studies of the archaeal MCM complex in different functional states. *J Struct Biol* 156:210–219.

Craig, N. 1984. E. coli integration host factor binds to specific sites in DNA. *Cell* 39:707–716.

Cunningham, E.L., and Berger, J.M. 2005. Unraveling the early steps of prokaryotic replication. *Curr Opin Struct Biol* 15:68–76.

Cvetic, C., and Walter, J.C. 2005. Eukaryotic origins of DNA replication: could you please be more specific? *Semin Cell Dev Biol* 16:343–353.

Davey, M.J., Fang, L., McInerney, P., Georgescu, R.E., and O'Donnell, M. 2002a. The DnaC helicase loader is a dual ATP/ADP switch protein. *EMBO J* 21:3148–3159.

Davey, M.J., Jeruzalmi, D., Kuriyan, J., and O'Donnell, M. 2002b. Motors and switches: AAA+ machines within the replisome. *Nat Rev Mol Cell Biol* 3:826–835.

Davey, M.J., and O'Donnell, M. 2003. Replicative helicase loaders: ring breakers and ring makers. *Curr Biol* 13:R594–R596.

De Felice, M., Esposito, L., Pucci, B., De Falco, M., Rossi, M., and Pisani, F.M. 2004. A CDC6-like factor from the archaea Sulfolobus solfataricus promotes binding of the mini-chromosome maintenance complex to DNA. *J Biol Chem* 279:43008–43012.

Dean, F.B., Borowiec, J.A., Ishimi, Y., Deb, S., Tegtmeyer, P., and Hurwitz, J. 1987a. Simian virus 40 large tumor antigen requires three core replication origin domains for DNA unwinding and replication in vitro. *Proc Natl Acad Sci USA* 84:8267–8271.

Dean, F.B., Bullock, P., Murakami, Y., Wobbe, C.R., Weissbach, L., and Hurwitz, J. 1987b. Simian virus 40 (SV40) DNA replication: SV40 large T antigen unwinds DNA containing the SV40 origin of replication. *Proc Natl Acad Sci USA* 84:16–20.

DeFelice, M. 2004. A CDC6-like factor from the archaea Sulfolobus solfataricus promotes binding of the mini-chromosome maintenance complex to DNA. *J Biol Chem* 279:43008–43012.

Diffley, J.F., Cocker, J.H., Dowell, S.J., Harwood, J., and Rowley, A. 1995. Stepwise assembly of initiation complexes at budding yeast replication origins during the cell cycle. *J Cell Sci Suppl* 19:67–72.

Diffley, J.F., and Stillman, B. 1988. Purification of a yeast protein that binds to origins of DNA replication and a transcriptional silencer. *Proc Natl Acad Sci USA* 85:2120–2124.

Dueber, E.L., Corn, J.E., Bell, S.D., and Berger, J.M. 2007. Replication origin recognition and deformation by a heterodimeric archaeal Orc1 complex. *Science* 317:1210–1213.

Edgell, D.R., and Doolittle, W.F. 1997. Archaea and the origin(s) of DNA replication proteins. *Cell* 89:995–998.

Egan, E.S., and Waldor, M.K. 2003. Distinct replication requirements for the two Vibrio cholerae chromosomes. *Cell* 114:521–530.

Enemark, E.J., Chen, G., Vaughn, D.E., Stenlund, A., and Joshua-Tor, L. 2000. Crystal structure of the DNA binding domain of the replication initiation protein E1 from papillomavirus. *Mol Cell* 6:149–158.

Enemark, E.J., and Joshua-Tor, L. 2006. Mechanism of DNA translocation in a replicative hexameric helicase. *Nature* 442:270–275.

Enemark, E.J., Stenlund, A., and Joshua-Tor, L. 2002. Crystal structures of two intermediates in the assembly of the papillomavirus replication initiation complex. *EMBO J* 21:1487–1496.

Erzberger, J.P., and Berger, J.M. 2006. Evolutionary relationships and structural mechanisms of AAA+ proteins. *Annu Rev Biophys Biomol Struct* 35:93–114.

Erzberger, J.P., Mott, M.L., and Berger, J.M. 2006. Structural basis for ATP-dependent DnaA assembly and replication-origin remodeling. *Nat Struct Mol Biol* 13:676–683.

Erzberger, J.P., Pirruccello, M.M., and Berger, J.M. 2002. The structure of bacterial DnaA: implications for general mechanisms underlying DNA replication initiation. *EMBO J* 21:4763–4773.

Fang, L., Davey, M.J., and O'Donnell, M. 1999. Replisome assembly at oriC, the replication origin of E. coli, reveals an explanation for initiation sites outside an origin. *Mol Cell* 4:541–553.

Fanning, E. 1998. Introduction to simian virus 40: getting by with more than a little help from its host cell. *Devel Biol Stand* 94:3–8.

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

Ferran, M.C., and McBride, A.A. 1998. Transient viral DNA replication and repression of viral transcription are supported by the C-terminal domain of the bovine papillomavirus type 1 E1 protein. *J Virol* 72:796-801.

Filutowicz, M. 1992. Involvement of Fis protein in replication of the Escherichia coli chromosome. *J Bacteriol* 174:398–407.

Filutowicz, M. 1990. The requirement of IHF protein for extrachromosomal replication of the Escherichia coli oriC in a mutant deficient in DNA polymerase I activity. *New Biologist* 2:818–827.

Fletcher, R.J., Bishop, B.E., Leon, R.P., Sclafani, R.A., Ogata, C.M., and Chen, X.S. 2003. The structure and function of MCM from archaeal M. Thermoautotrophicum. *Nat Struct Biol* 10:160–167.

Fodje, M.N., Hansson, A., Hansson, M., Olsen, J.G., Gough, S., Willows, R.D., and Al-Karadaghi, S. 2001. Interplay between an AAA module and an integrin I domain may regulate the function of magnesium chelatase. *J Mol Biol* 311:111–122.

Forterre, P., Brochier, C., and Philippe, H. 2002. Evolution of the Archaea. *Theor Popul Biol* 61:409–422.

Fouts, E.T., Yu, X., Egelman, E.H., and Botchan, M.R. 1999. Biochemical and electron microscopic image analysis of the hexameric E1 helicase. *J Biol Chem* 274:4447–4458.

Fujikawa, N., Kurumizaka, H., Nureki, O., Terada, T., Shirouzu, M., Katayama, T., and Yokoyama, S. 2003. Structural basis of replication origin recognition by the DnaA protein. *Nucleic Acids Res* 31:2077–2086.

Fuller, R.S., Funnell, B.E., and Kornberg, A. 1984. The dnaA protein complex with the E. coli chromosomal replication origin (oriC) and other DNA sites. *Cell* 38:889–900.

Funnell, B.E., Baker, T.A., and Kornberg, A. 1987. In vitro assembly of a prepriming complex at the origin of the Escherichia coli chromosome. *J Biol Chem* 262:10327–10334.

Gai, D., Li, D., Finkielstein, C.V., Ott, R.D., Taneja, P., Fanning, E., and Chen, X.S. 2004a. Insights into the oligomeric states, conformational changes, and helicase activities of SV40 large tumor antigen. *J Biol Chem* 279:38952–38959.

Gai, D., Zhao, R., Li, D., Finkielstein, C.V., and Chen, X.S. 2004b. Mechanisms of conformational change for a replicative hexameric helicase of SV40 large tumor antigen. *Cell* 119:47–60.

Gambus, A. 2006. GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. *Nature Cell Biol* 8:358–366.

Gaudier, M., Schuwirth, B.S., Westcott, S.L., and Wigley, D.B. 2007. Structural basis of DNA replication origin recognition by an ORC protein. *Science* 317:1213–1216.

Gille, H. 1991. The FIS protein binds and bends the origin of chromosomal DNA replication, oriC, of Escherichia coli. *Nucleic Acids Res* 19:4167–4172.

Gille, H., and Messer, W. 1991. Localized DNA melting and structural pertubations in the origin of replication, oriC, of Escherichia coli in vitro and in vivo. *EMBO J* 10:1579–1584.

Gillespie, P.J., Li, A., and Blow, J.J. 2001. Reconstitution of licensed replication origins on Xenopus sperm nuclei using purified proteins. *BMC Biochem* 2:15.

Giraldo, R. 2003. Common domains in the initiators of DNA replication in Bacteria, Archaea and Eukarya: combined structural, functional

and phylogenetic perspectives. *FEMS Microbiol Rev* 26:533–554.

Grabowski, B., and Kelman, Z. 2003. Archeal DNA replication: eukaryal proteins in a bacterial context. *Annu Rev Microbiol* 57:487–516.

Grainge, I., Gaudier, M., Schuwirth, B.S., Westcott, S.L., Sandall, J., Atanassova, N., and Wigley, D.B. 2006. Biochemical analysis of a DNA replication origin in the archaeon Aeropyrum pernix. *J Mol Biol* 363:355–369.

Grainge, I. 2003. Biochemical analysis of components of the pre-replication complex of Archaeoglobus fulgidus. *Nucleic Acids Res* 31:4888–4898.

Grimwade, J.E., Ryan, V.T., and Leonard, A.C. 2000. IHF redistributes bound initiator protein, DnaA, on supercoiled oriC of Escherichia coli. *Mol Microbiol* 35:835–844.

Grimwade, J.E., Torgue, J.J., McGarry, K.C., Rozgaja, T., Enloe, S.T., and Leonard, A.C. 2007. Mutational analysis reveals Escherichia coli oriC interacts with both DnaA-ATP and DnaA-ADP during pre-RC assembly. *Mol Microbio* 66:428–439.

Guenther, B., Onrust, R., Sali, A., O'Donnell, M., and Kuriyan, J. 1997. Crystal structure of the delta' subunit of the clamp-loader complex of E. coli DNA polymerase III. *Cell* 91:335–345.

Gulbis, J.M., Kazmirski, S.L., Finkelstein, J., Kelman, Z., O'Donnell, M., and Kuriyan, J. 2004. Crystal structure of the chi:psi subassembly of the Escherichia coli DNA polymerase clamp-loader complex. *Eur J Biochem* 271:439–449.

Harland, R.M., and Laskey, R.A. 1980. Regulated replication of DNA microinjected into eggs of Xenopus laevis. *Cell* 21:761–771.

Hartman, J.J., and Vale, R.D. 1999. Microtubule disassembly by ATP-dependent oligomerization of the AAA enzyme katanin. *Science* 286:782–785.

Hartwell, L.H. 1973. Three additional genes required for deoxyribonucleic acid synthesis in Saccharomyces cerevisiae. *J Bacteriol* 115:966–974.

Harvey, K.J., and Newport, J. 2003. Metazoan origin selection: origin recognition complex chromatin binding is regulated by CDC6 recruitment and ATP hydrolysis. *J Biol Chem* 278:48524–48528.

Hassan, A.K., Moriya, S., Ogura, M., Tanaka, T., Kawamura, F., and Ogasawara, N. 1997. Suppression of initiation defects of chromosome replication in Bacillus subtilis dnaA and oriC-deleted mutants by integration of a plasmid replicon into the chromosomes. *J Bacteriol* 179:2494–2502.

Heinzel, S.S., Krysan, P.J., Tran, C.T., and Calos, M.P. 1991. Autonomous DNA replication in human cells is affected by the size and the source of the DNA. *Mol Cell Biol* 11:2263–2272.

Heller, Ryan C Marians,Kenneth J. 2006. Replisome assembly and the direct restart of stalled replication forks. *Nat Rev Mol Cell Biol* 7:932.

Hiasa, H., and Marians, K.J. 1994. Fis cannot support oriC DNA replication in vitro. *J Biol Chem* 269:24999–25003.

Hickman, A.B., and Dyda, F. 2005. Binding and unwinding: SF3 viral helicases. *Curr Opin Struct Biol* 15:77–85.

Hofmann, J.F., and Beach, D. 1994. cdt1 is an essential target of the Cdc10/Sct1 transcription factor: requirement for DNA replication and inhibition of mitosis. *EMBO J* 13:425–434.

Holz, A., Schaefer, C., Gille, H., Jueterbock, W.R., and Messer, W. 1992. Mutations in the DnaA binding sites of the replication origin of Escherichia coli. *Mol. Gen. Genet.* 233:81–88.

Hsiao, C.L., and Carbon, J. 1979. High-frequency transformation of yeast by plasmids containing the cloned yeast ARG4 gene. *Proc Natl Acad Sci USA* 76:3829–3833.

Hughes, F.J., and Romanos, M.A. 1993. E1 protein of human papillomavirus is a DNA helicase/ATPase. *Nucleic Acids Res* 21:5817–5823.

Hwang, D.S., and Kornberg, A. 1992. Opening of the replication origin of Escherichia coli by DnaA protein with protein HU or IHF. *J Biol Chem* 267:23083–23086.

Indiani, C., and O'Donnell, M. 2006. The replication clamp-loading machine at work in the three domains of life. *Nat Rev Mol Cell Biol* 7:751–761.

Ishida, T., Akimitsu, N., Kashioka, T., Hatano, M., Kubota, T., Ogata, Y., Sekimizu, K., and Katayama, T. 2004. DiaA, a novel DnaA-binding protein, ensures the timely initiation of Escherichia coli chromosome replication. *J Biol Chem* 279:45546–45555.

Ishimi, Y. 1997. A DNA helicase activity is associated with an MCM4, -6, and -7 protein complex. *J Biol Chem* 272:24508–24513.

Iyer, L.M., Leipe, D.D., Koonin, E.V., and Aravind, L. 2004a. Evolutionary history and higher order classification of AAA+ ATPases. *J Struct Biol* 146:11–31.

Iyer, L.M., Makarova, K.S., Koonin, E.V., and Aravind, L. 2004b. Comparative genomics of the FtsK-HerA superfamily of pumping ATPases: implications for the origins of chromosome segregation, cell division and viral capsid packaging. *Nucleic Acids Res* 32:5260–5279.

Jacob, F., Brenner, S., and Cuzin, F. 1963. On the regulation of DNA replication in bacteria. *Cold Spring Harbor Symp Quant Biol* 28:329–348.

Jeruzalmi, D., O'Donnell, M., and Kuriyan, J. 2001a. Crystal structure of the processivity clamp loader gamma (gamma) complex of E. coli DNA polymerase III. *Cell* 106:429–441.

Jeruzalmi, D., Yurieva, O., Zhao, Y., Young, M., Stewart, J., Hingorani, M., O'Donnell, M., and Kuriyan, J. 2001b. Mechanism of processivity clamp opening by the delta subunit wrench of the clamp loader complex of E. coli DNA polymerase III. *Cell* 106:417–428.

Johnson, A., Yao, N.Y., Bowman, G.D., Kuriyan, J., and O'Donnell, M. 2006. The replication factor C clamp loader requires arginine finger sensors to drive DNA binding and proliferating cell nuclear antigen loading. *J Biol Chem* 281:35531–35543.

Kadoya, R., Hassan, A.K., Kasahara, Y., Ogasawara, N., and Moriya, S. 2002. Two separate DNA sequences within oriC participate in accurate chromosome segregation in Bacillus subtilis. *Mol Microbiol* 45:73–87.

Kaguni, J.M. 2006. DnaA: controlling the initiation of bacterial DNA replication and more. *Annu Rev Microbiol* 60:351–375.

Kaguni, J.M. 1997. Escherichia coli DnaA protein: the replication initiator. *Mol Cells* 7:145–157.

Kanemaki, M., Sanchez-Diaz, A., Gambus, A., and Labib, K. 2003. Functional proteomic identification of DNA replication proteins by induced proteolysis in vivo. *Nature* 423:720–724.

Kaplan, D.L., Davey, M.J., and O'Donnell, M. 2003. Mcm4,6,7 uses a "pump in ring" mechanism to unwind DNA by steric exclusion and actively translocate along a duplex. *J Biol Chem* 278:49171–49182.

Kasiviswanathan, R., Shin, J.H., and Kelman, Z. 2006. DNA binding by the Methanothermobacter thermautotrophicus Cdc6 protein is inhibited by the minichromosome maintenance helicase. *J. Bacteriol* 188:4577–4580.

Katayama, T., Kubota, T., Kurokawa, K., Crooke, E., and Sekimizu, K. 1998. The initiator function of DnaA protein is negatively regulated by the sliding clamp of the E. coli chromosomal replicase. *Cell* 94:61–71.

Kato, J., and Katayama, T. 2001. Hda, a novel DnaA-related protein, regulates the replication cycle in Escherichia coli. *EMBO J* 20:4253–4262.

Kawakami, H., Ozaki, S., Suzuki, S., Nakamura, K., Senriuchi, T., Su'etsugu, M., Fujimitsu, K., and Katayama, T. 2006a. The exceptionally tight affinity of DnaA for ATP/ADP requires a unique aspartic acid residue in the AAA+ sensor 1 motif. *Mol Microbiol* 62:1310–1324.

Kawakami, H., Su'etsugu, M., and Katayama, T. 2006b. An isolated Hda-clamp complex is functional in the regulatory inactivation of DnaA and DNA replication. *J Struct Biol* 156:220–229.

Kelman, L.M., and Kelman, Z. 2004. Multiple origins of replication in archaea. *Trends Microbiol* 12:399–401.

Kelman, L.M., and Kelman, Z. 2003. Archaea: an archetype for replication initiation studies? *Mol. Microbiol.* 48:605–615.

Kelman, Z., Lee, J.K., and Hurwitz, J. 1999. The single minichromosome maintenance protein of Methanobacterium thermoautotrophicum DeltaH contains DNA helicase activity. *Proc Natl Acad Sci USA* 96:14783–14788.

Kitagawa, R., Mitsuki, H., Okazaki, T., and Ogawa, T. 1996. A novel DnaA protein-binding site at 94.7 min on the Escherichia coli chromosome. *Mol Microbiol* 19:1137–1147.

Kitchen, J.L., Li, Z., and Crooke, E. 1999. Electrostatic interactions during acidic phospholipid reactivation of DnaA protein, the Escherichia coli initiator of chromosomal replication. *Biochemistry* 38:6213–6221.

Klemm, R.D., Austin, R.J., and Bell, S.P. 1997. Coordinate binding of ATP and origin DNA regulates the ATPase activity of the origin recognition complex. *Cell* 88:493–502.

Kornberg , A., and Baker, T.A. 1992. *DNA Replication* New York, W.H. Freeman and Company.

Kowalski, D., and Eddy, M.J. 1989. The DNA unwinding element: a novel, cis-acting component that facilitates opening of the Escherichia coli replication origin. *EMBO J* 8:4335–4344.

Kubota, Y., Takase, Y., Komori, Y., Hashimoto, Y., Arata, T., Kamimura, Y., Araki, H., and Takisawa, H. 2003. A novel ring-like complex of Xenopus proteins essential for the initiation of DNA replication. *Genes Dev* 17:1141–1152.

Kumar, A., Meinke, G., Reese, D.K., Moine, S., Phelan, P.J., Fradet-Turcotte, A., Archambault, J., Bohm, A., and Bullock, P.A. 2007. Model for T-antigen-dependent melting of the simian virus 40 core origin based on studies of the interaction of the beta-hairpin with DNA. *J Virol* 81:4808–4818.

Kurokawa, K., Nishida, S., Emoto, A., Sekimizu, K., and Katayama, T. 1999. Replication cycle-coordinated change of the adenine nucleotide-bound forms of DnaA protein in Escherichia coli. *EMBO J* 18:6642–6652.

Kuznetsov, S.V., Sugimura, S., Vivas, P., Crothers, D.M., and Ansari, A. 2006. Direct observation of DNA bending/unbending kinetics in complex with DNA-bending protein IHF. *Proc Natl Acad Sci USA* 103:18515–18520.

Lee, D.G., and Bell, S.P. 2000. ATPase switches controlling DNA replication initiation. *Curr Opin Cell Biol* 12:280–285.

Lee, D.G., and Bell, S.P. 1997. Architecture of the yeast origin recognition complex bound to origins of DNA replication. *Mol Cell Biol* 17:7159–7168.

Lee, D.G., Makhov, A.M., Klemm, R.D., Griffith, J.D., and Bell, S.P. 2000. Regulation of origin recognition complex conformation and ATPase activity: differential effects of single-stranded and double-stranded DNA binding. *EMBO J* 19:4774–4782.

Lee, J.K., Moon, K.Y., Jiang, Y., and Hurwitz, J. 2001. The Schizosaccharomyces pombe origin recognition complex interacts with multiple AT-rich regions of the replication origin DNA by means of the AT-hook domains of the spOrc4 protein. *Proc Natl Acad Sci USA* 98:13589–13594.

Lei, M., Kawasaki, Y., and Tye, B.K. 1996. Physical interactions among Mcm proteins and effects of Mcm dosage on DNA replication in Saccharomyces cerevisiae. *Mol Cell Biol* 16:5081–5090.

Leipe, D.D., Koonin, E.V., and Aravind, L. 2003. Evolution and classification of P-loop kinases and related proteins. *J Mol Biol* 333:781–815.

Li, D., Zhao, R., Lilyestrom, W., Gai, D., Zhang, R., DeCaprio, J.A., Fanning, E., Jochimiak, A., Szakonyi, G., and Chen, X.S. 2003. Structure of the replicative helicase of the oncoprotein SV40 large tumour antigen. *Nature* 423:512–518.

Liang, C., and Stillman, B. 1997. Persistent initiation of DNA replication and chromatin-bound MCM proteins during the cell cycle in cdc6 mutants. *Genes Dev* 11:3375–3386.

Lipford, J.R., and Bell, S.P. 2001. Nucleosomes positioned by ORC facilitate the initiation of DNA replication. *Mol Cell* 7:21–30.

Liu, J., Smith, C.L., DeRyckere, D., DeAngelis, K., Martin, G.S., and Berger, J.M. 2000. Structure and function of Cdc6/Cdc18: implications for origin recognition and checkpoint control. *Mol Cell* 6:637–648.

Liu, X., Schuck, S., and Stenlund, A. 2007. Adjacent residues in the E1 initiator beta-hairpin define different roles of the beta-hairpin in Ori melting, helicase loading, and helicase activity. *Mol Cell* 25:825–837.

Lopez, P., Philippe, H., Myllykallio, H., and Forterre, P. 1999. Identification of putative chromosomal origins of replication in Archaea. *Mol Microbiol* 32:883–886.

Luo, X., Sanford, D.G., Bullock, P.A., and Bachovchin, W.W. 1996. Solution structure of the origin DNA-binding domain of SV40 T-antigen. *Nat Struct Biol* 3:1034–1039.

Mackiewicz, P., Zakrzewska-Czerwinska, J., Zawilak, A., Dudek, M.R., and Cebrat, S. 2004. Where does bacterial replication start? Rules for predicting the oriC region. *Nucleic Acids Res* 32:3781–3791.

Maki, S. 1988. DNA polymerase III holoenzyme of Escherichia coli. II. A novel complex including the gamma subunit essential for processive synthesis. *J Biol Chem* 263:6555–6560.

Marahrens, Y., and Stillman, B. 1992. A yeast chromosomal origin of DNA replication defined by multiple functional elements. *Science* 255:817–823.

Margulies, C., and Kaguni, J.M. 1996. Ordered and sequential binding of DnaA protein to oriC, the chromosomal origin of Escherichia coli. *J Biol Chem* 271:17035–17040.

Mastrangelo, I.A., Hough, P.V., Wall, J.S., Dodson, M., Dean, F.B., and Hurwitz, J. 1989. ATP-dependent assembly of double hexamers of SV40 T antigen at the viral origin of DNA replication. *Nature* 338:658–662.

Matsui, M., Oka, A., Takanami, M., Yasuda, S., and Hirota, Y. 1985. Sites of dnaA protein-binding in the replication origin of the Escherichia coli K-12 chromosome. *J Mol Biol* 184: 529–533.

Matsunaga, F., Forterre, P., Ishino, Y., and Myllykallio, H. 2001. In vivo interactions of archaeal Cdc6/Orc1 and minichromosome maintenance proteins with the replication origin. *Proc Natl Acad Sci USA* 98:11152–11157.

Matsunaga, F., Glatigny, A., Mucchielli-Giorgi, M.H., Agier, N., Delacroix, H., Marisa, L., Durosay, P., Ishino, Y., Aggerbeck, L., and Forterre, P. 2007. Genomewide and biochemical analyses of DNA-binding activity of Cdc6/Orc1 and Mcm proteins in Pyrococcus sp. *Nucleic Acids Res* 35:3214–3222.

McGarry, K.C., Ryan, V.T., Grimwade, J.E., and Leonard, A.C. 2004. Two discriminatory binding sites in the Escherichia coli replication origin are required for DNA strand opening by initiator DnaA-ATP. *Proc Natl Acad Sci USA* 101:2811–2816.

Mechali, M., and Kearsey, S. 1984. Lack of specific sequence requirement for DNA replication in Xenopus eggs compared with high sequence specificity in yeast. *Cell* 38:55–64.

Meinke, G., Bullock, P.A., and Bohm, A. 2006. Crystal structure of the simian virus 40 large T-antigen origin-binding domain. *J Virol* 80:4304–4312.

Meinke, G., Phelan, P., Moine, S., Bochkareva, E., Bochkarev, A., Bullock, P.A., and Bohm, A. 2007. The crystal structure of the SV40 T-antigen origin binding domain in complex with DNA. *PLoS Biol* 5:e23.

Mendez, J., and Stillman, B. 2003. Perpetuating the double helix: molecular machines at eukaryotic DNA replication origins. *Bioessays* 25:1158–1167.

Mendoza, R., Gandhi, L., and Botchan, M.R. 1995. E1 recognition sequences in the bovine papillomavirus type 1 origin of DNA replication: interaction between half sites of the inverted repeats. *J Virol* 69:3789–3798.

Messer, W. 2002. The bacterial replication initiator DnaA. DnaA and oriC, the bacterial mode to initiate DNA replication. *FEMS Microbiol Rev* 26:355–374.

Messer, W., Blaesing, F., Majka, J., Nardmann, J., Schaper, S., Schmidt, A., Seitz, H., Speck, C., Tungler, D., Wegrzyn, G. et al. 1999. Functional domains of DnaA proteins *Biochimie* 81:819–825.

Messer, W., and Weigel, C. 1997. DnaA initiator–also a transcription factor. *Mol Microbiol* 24:1–6.

Miyata, T., Suzuki, H., Oyama, T., Mayanagi, K., Ishino, Y., and Morikawa, K. 2005. Open clamp structure in the clamp-loading complex visualized by electron microscopic image analysis. *Proc Natl Acad Sci USA* 102:13795–13800.

Mohr, I.J., Clark, R., Sun, S., Androphy, E.J., MacPherson, P., and Botchan, M.R. 1990. Targeting the E1 replication protein to the papillomavirus origin of replication by complex formation with the E2 transactivator. *Science* 250:1694–1699.

Moir, D., Stewart, S.E., Osmond, B.C., and Botstein, D. 1982. Cold-sensitive cell-division-cycle mutants of yeast: isolation, properties, and pseudoreversion studies. *Genetics* 100:547–563.

Mott, M.L., and Berger, J.M. 2007. DNA replication initiation: mechanisms and regulation in bacteria. *Nat Rev Microbiol* 5:343–354.

Moyer, S.E., Lewis, P.W., and Botchan, M.R. 2006. Isolation of the Cdc45/Mcm2-7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. *Proc Natl Acad Sci USA* 103:10236–10241.

Myllykallio, H., Lopez, P., Lopez-Garcia, P., Heilig, R., Saurin, W., Zivanovic, Y., Philippe, H., and Forterre, P. 2000. Bacterial mode of replication with eukaryotic-like machinery in a hyperthermophilic archaeon. *Science* 288:2212–2215.

Naktinis, V., Onrust, R., Fang, L., and O'Donnell, M. 1995. Assembly of a chromosomal replication machine: two DNA polymerases, a clamp loader, and sliding clamps in one holoenzyme particle. II. Intermediate complex between the clamp loader and its clamp. *J Biol Chem* 270:13358–13365.

Naktinis, V., Turner, J., and O'Donnell, M. 1996. A molecular switch in a replication machine defined by an internal competition for protein rings. *Cell* 84:137–145.

Natrajan, G., Hall, D.R., Thompson, A.C., Gutsche, I., and Terradot, L. 2007. Structural similarity between the DnaA-binding proteins HobA (HP1230) from Helicobacter pylori and DiaA from Escherichia coli. *Mol Microbiol* 65:995–1005.

Neuwald, A.F., Aravind, L., Spouge, J.L., and Koonin, E.V. 1999. AAA+: A class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes. *Genome Res* 9:27–43.

Ng, W.V., Kennedy, S.P., Mahairas, G.G., Berquist, B., Pan, M., Shukla, H.D., Lasky, S.R., Baliga, N.S., Thorsson, V., Sbrogna, J. et al. 2000. Genome sequence of Halobacterium species NRC-1. *Proc Natl Acad Sci* USA 97:12176–12181.

Nievera, C., Torgue, J.J., Grimwade, J.E., and Leonard, A.C. 2006. SeqA blocking of DnaA-oriC interactions ensures staged assembly of the E. coli pre-RC. *Mol Cell* 24:581–592.

Nishida, S., Fujimitsu, K., Sekimizu, K., Ohmura, T., Ueda, T., and Katayama, T. 2002. A nucleotide switch in the Escherichia coli DnaA protein initiates chromosomal replication: evidnece from a mutant DnaA protein defective in regulatory ATP hydrolysis in vitro and in vivo. *J Biol Chem* 277:14986–14995.

Nishitani, H., Lygerou, Z., Nishimoto, T., and Nurse, P. 2000. The Cdt1 protein is required to license DNA for replication in fission yeast. *Nature* 404:625–628.

Norais, C., Hawkins, M., Hartman, A.L., Eisen, J.A., Myllykallio, H., and Allers, T. 2007. Genetic and physical mapping of DNA replication origins in Haloferax volcanii. *PLoS Genet* 3:e77.

O'Donnell, M., and Kuriyan, J. 2006. Clamp loaders and replication initiation. *Curr Opin Struct Biol* 16:35–41.

Ogawa, T., Yamada, Y., Kuroda, T., Kishi, T., and Moriya, S. 2002. The datA locus predominantly contributes to the initiator titration mechanism in the control of replication initiation in Escherichia coli. *Mol Microbiol* 44:1367–1375.

Ogura, T., Whiteheart, S.W., and Wilkinson, A.J. 2004. Conserved arginine residues implicated in ATP hydrolysis, nucleotide-sensing, and inter-subunit interactions in AAA and AAA+ ATPases. *J Struct Biol* 146:106–112.

Ogura, T., and Wilkinson, A.J. 2001. AAA+ superfamily ATPases: common structure–diverse function. *Genes Cells* 6:575–597.

Onrust, R. 1995. Assembly of a chromosomal replication machine: two DNA polymerases, a clamp loader, and sliding clamps in one holoenzyme particle. I. Organization of the clamp loader. *J Biol Chem* 270:13348–13357.

Onrust, R. 1991. Analysis of the ATPase subassembly which initiates processive DNA synthesis by DNA polymerase III holoenzyme. *J Biol Chem* 266:21681–21686.

Ozaki, S., Kawakami, H., Nakamura, K., Fujikawa, N., Kagawa, W., Park, S.Y., Yokoyama, S., Kurumizaka, H., and Katayama, T. 2008.

A common mechanism for the ATP-DnaA-dependent formation of open complexes at the replication origin. *J Biol Chem* 283:8351–8362.

Pacek, M., Tutter, A.V., Kubota, Y., Takisawa, H., and Walter, J.C. 2006. Localization of MCM2-7, Cdc45, and GINS to the site of DNA unwinding during eukaryotic DNA replication. *Mol Cell* 21:581–587.

Pacek, M., and Walter, J.C. 2004. A requirement for MCM7 and Cdc45 in chromosome unwinding during eukaryotic DNA replication. *EMBO J* 23:3667–3676.

Pape, T. 2003. Hexameric ring structure of the full-length archaeal MCM protein complex. *EMBO Reports* 4:1079–1083.

Perkins, G., and Diffley, J.F. 1998. Nucleotide-dependent prereplicative complex assembly by Cdc6p, a homolog of eukaryotic and prokaryotic clamp-loaders. *Mol Cell* 2:23–32.

Polaczek, P. 1997. Role of architectural elements in combinatorial regulation of initiation of DNA replication in Escherichia coli. *Mol Microbiol* 26:261–275.

Polaczek, P. 1990. Bending of the origin of replication of E. coli by binding of IHF at a specific site. *New Biologist.* 2:265–271.

Poloumienko, A. 2001. Completion of replication map of Saccharomyces cerevisiae chromosome III. *Molecular Biol Cell.* 12:3317–3327.

Prelich, G., Kostura, M., Marshak, D.R., Mathews, M.B., and Stillman, B. 1987. The cell-cycle regulated proliferating cell nuclear antigen is required for SV40 DNA replication in vitro. *Nature* 326:471–475.

Pritchard, A. 2000. A novel assembly mechanism for the DNA polymerase III holoenzyme DnaX complex: association of deltadelta' with DnaX(4) forms DnaX(3)deltadelta'. *The EMBO J* 19:6536–6545.

Randell, J.C., Bowers, J.L., Rodriguez, H.K., and Bell, S.P. 2006. Sequential ATP hydrolysis by Cdc6 and ORC directs loading of the Mcm2-7 helicase. *Mol Cell* 21:29–39.

Ray, S., Anderson, M.E., Loeber, G., McVey, D., and Tegtmeyer, P. 1992. Functional characterization of temperature-sensitive mutants of simian virus 40 large T antigen. *J Virol* 66:6509–6516.

Reese, D.K., Sreekumar, K.R., and Bullock, P.A. 2004. Interactions required for binding of simian virus 40 T antigen to the viral origin and molecular modeling of initial assembly events. *J Virol* 78:2921–2934.

Remus, D., Beall, E.L., and Botchan, M.R. 2004. DNA topology, not DNA sequence, is a critical determinant for Drosophila ORC-DNA binding. *EMBO J* 23:897–907.

Rice, P.A., Yang, S., Mizuuchi, K., and Nash, H.A. 1996. Crystal structure of an IHF-DNA complex: a protein-induced DNA U-turn. *Cell* 87:1295–1306.

Robinson, N.P., and Bell, S.D. 2005. Origins of DNA replication in the three domains of life. *FEBS J* 272:3757–3766.

Robinson, N.P., Blood, K.A., McCallum, S.A., Edwards, P.A., and Bell, S.D. 2007. Sister chromatid junctions in the hyperthermophilic archaeon Sulfolobus solfataricus. *EMBO J* 26:816–824.

Robinson, N.P., Dionne, I., Lundgren, M., Marsh, V.L., Bernander, R., and Bell, S.D. 2004. Identification of two origins of replication in the single chromosome of the archaeon Sulfolobus solfataricus. *Cell* 116: 25–38.

Roth, A. 1994. Functions of histone-like proteins in the initiation of DNA replication at oriC of Escherichia coli. *Biochimie* 76:917–923.

Roth, A., and Messer, W. 1995. The DNA binding domain of the initiator protein DnaA. *EMBO J* 14:2106–2111.

Ryan, V.T., Grimwade, J.E., Camara, J.E., Crooke, E., and Leonard, A.C. 2004. Escherichia coli prereplication complex assembly is regulated by dynamic interplay among Fis, IHF and DnaA. *Mol Microbiol* 51:1347–1359.

Sakakibara, Y., and Yuasa, S. 1982. Continuous synthesis of the dnaA gene product of Escherichia coli in the cell cycle. *Mol Gen Genet* 186:87–94.

Sanders, C.M., and Stenlund, A. 1998. Recruitment and loading of the E1 initiator protein: an ATP-dependent process catalysed by a transcription factor. *EMBO J* 17:7044–7055.

Sato, M. 2000. Electron microscopic observation and single-stranded DNA binding activity of the Mcm4,6,7 complex. *J Mol Biol.* 300:421–431.

Schepers, A., and Diffley, J.F. 2001. Mutational analysis of conserved sequence motifs in the budding yeast Cdc6 protein. *J Mol Biol* 308:597–608.

Schuck, S., and Stenlund, A. 2007. ATP-dependent minor groove recognition of TA base pairs is required for template melting by the E1 initiator protein. *J Virol* 81:3293–3302.

Schuck, S., and Stenlund, A. 2005. Assembly of a double hexameric helicase. *Mol Cell* 20:377–389.

Sedman, J., and Stenlund, A. 1998. The papillomavirus E1 protein forms a DNA-dependent hexameric complex with ATPase and DNA helicase activities. *J Virol* 72:6893–6897.

Sedman, J., and Stenlund, A. 1996. The initiator protein E1 binds to the bovine papillomavirus origin of replication as a trimeric ring-like structure. *EMBO J* 15:5085–5092.

Sedman, J., and Stenlund, A. 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* 14:6218–6228.

Seitz, H., Weigel, C., and Messer, W. 2000. The interaction domains of the DnaA and DnaB replication proteins of Escherichia coli. *Mol Microbiol* 37:1270–1279.

Seki, T., and Diffley, J.F. 2000. Stepwise assembly of initiation proteins at budding yeast replication origins in vitro. *Proc Natl Acad Sci USA* 97:14115–14120.

Sekimizu, K., Bramhill, D., and Kornberg, A. 1987. ATP activates dnaA protein in initiating replication of plasmids bearing the origin of the E. coli chromosome. *Cell* 50:259–265.

Seo, Y.S., Muller, F., Lusky, M., and Hurwitz, J. 1993. Bovine papilloma virus (BPV)-encoded E1 protein contains multiple activities required for BPV DNA replication. *Proc Natl Acad Sci USA* 90:702–706.

Seybert, A., Scott, D.J., Scaife, S., Singleton, M.R., and Wigley, D.B. 2002. Biochemical characterisation of the clamp/clamp loader proteins from the euryarchaeon Archaeoglobus fulgidus. *Nucleic Acids Res* 30:4329–4338.

Seybert, A., Singleton, M.R., Cook, N., Hall, D.R., and Wigley, D.B. 2006. Communication between subunits within an archaeal clamp-loader complex. *EMBO J* 25:2209–2218.

Seybert, A., and Wigley, D.B. 2004. Distinct roles for ATP binding and hydrolysis at individual subunits of an archaeal clamp loader. *EMBO J* 23:1360–1371.

She, Q., Singh, R.K., Confalonieri, F., Zivanovic, Y., Allard, G., Awayez, M.J., Chan-Weiher, C.C., Clausen, I.G., Curtis, B.A., De Moors, A. et al. 2001. The complete genome of the crenarchaeon Sulfolobus solfataricus P2 *Proc Natl Acad Sci* USA 98:7835–7840.

Shechter, D.F., Ying, C.Y., and Gautier, J. 2000. The intrinsic DNA helicase activity of Methanobacterium thermoautotrophicum delta H minichromosome maintenance protein. *J Biol Chem.* 275:15049–15059.

Shen, J., Gai, D., Patrick, A., Greenleaf, W.B., and Chen, X.S. 2005. The roles of the residues on the channel beta-hairpin and loop structures of simian virus 40 hexameric helicase. *Proc Natl Acad Sci USA* 102:11248–11253.

Shin, J.H., Grabowski, B., Kasiviswanathan, R., Bell, S.D., and Kelman, Z. 2003. Regulation of minichromosome maintenance helicase activity by Cdc6. *J Biol Chem* 278:38059–38067.

Simmons, D.T., Upson, R., Wun-Kim, K., and Young, W. 1993. Biochemical analysis of mutants with changes in the origin-binding domain of simian virus 40 tumor antigen. *J Virol* 67:4227–4236.

Singleton, M.R., Morales, R., Grainge, I., Cook, N., Isupov, M.N., and Wigley, D.B. 2004. Conformational changes induced by nucleotide binding in Cdc6/ORC from Aeropyrum pernix. *J Mol Biol* 343:547–557.

Slater, S., Wold, S., Lu, M., Boye, E., Skarstad, K., and Kleckner, N. 1995. E. coli SeqA protein binds oriC in two different methyl-modulated reactions appropriate to its roles in DNA replication initiation and origin sequestration. *Cell* 82:927–936.

Speck, C., Chen, Z., Li, H., and Stillman, B. 2005. ATPase-dependent cooperative binding of ORC and Cdc6 to origin DNA. *Nat Struct Mol Biol* 12:965–971.

Speck, C., and Messer, W. 2001. Mechanism of origin unwinding: sequential binding of DnaA to double- and single-stranded DNA. *EMBO J* 20:1469–1476.

Speck, C., and Stillman, B. 2007. Cdc6 ATPase activity regulates ORC x Cdc6 stability and the selection of specific DNA sequences as origins of DNA replication. *J Biol Chem* 282:11705–11714.

Speck, C., Weigel, C., and Messer, W. 1999. ATP- and ADP-dnaA protein, a molecular switch in gene regulation. *EMBO J* 18:6169–6176.

Stahl, H., Droge, P., and Knippers, R. 1986. DNA helicase activity of SV40 large tumor antigen. *EMBO J.* 5:1939–1944.

Stenlund, A. 2003. Initiation of DNA replication: lessons from viral initiator proteins. *Nat Rev Mol Cell Biol* 4:777–785.

Story, R.M., and Steitz, T.A. 1992. Structure of the recA protein-ADP complex. *Nature* 355:374–376.

Su'etsugu, M., Shimuta, T.R., Ishida, T., Kawakami, H., and Katayama, T. 2005. Protein associations in DnaA-ATP hydrolysis mediated by the Hda-replicase clamp complex. *J Biol Chem* 280:6528–6536.

Su'etsugu, M., Takata, M., Kubota, T., Matsuda, Y., and Katayama, T. 2004. Molecular mechanism of DNA replication-coupled inactivation of the initiator protein in Escherichia coli: interaction of DnaA with the sliding clamp-loaded DNA and the sliding clamp-Hda complex. *Genes Cells* 9:509–522.

Sugimura, S., and Crothers, D.M. 2006. Stepwise binding and bending of DNA by Escherichia coli integration host factor. *Proc Natl Acad Sci USA* 103:18510–18514.

Sutton, M.D., Carr, K.M., Vicente, M., and Kaguni, J.M. 1998. Escherichia coli DnaA protein. The N-terminal domain and loading of DnaB helicase at the E. coli chromosomal origin. *J Biol Chem* 273:34255–34262.

Sutton, M.D., and Kaguni, J.M. 1997. The Escherichia coli dnaA gene: four functional domains. *J Mol Biol* 274:546–561.

Takahashi, T.S., Wigley, D.B., and Walter, J.C. 2005. Pumps, paradoxes and ploughshares: mechanism of the MCM2-7 DNA helicase. *Trends Biochem Sci* 30:437–444.

Takayama, Y., Kamimura, Y., Okawa, M., Muramatsu, S., Sugino, A., and Araki, H. 2003. GINS, a novel multiprotein complex required for chromosomal DNA replication in budding yeast. *Genes Dev* 17:1153–1165.

Theis, J.F., and Newlon, C.S. 1997. The ARS309 chromosomal replicator of Saccharomyces cerevisiae depends on an exceptional ARS consensus sequence. *Proc Natl Acad Sci USA* 94:10786–10791.

Tsurimoto, T., and Stillman, B. 1991. Replication factors required for SV40 DNA replication in vitro. I. DNA structure-specific recognition of a primer-template junction by eukaryotic DNA polymerases and their accessory proteins. *J Biol Chem* 266:1950–1960.

Tugal, T., Zou-Yang, X.H., Gavin, K., Pappin, D., Canas, B., Kobayashi, R., Hunt, T., and Stillman, B. 1998. The Orc4p and Orc5p subunits of the Xenopus and human origin recognition complex are related to Orc1p and Cdc6p. *J Biol Chem* 273:32421–32429.

Ustav, M., Ustav, E., Szymanski, P., and Stenlund, A. 1991. Identification of the origin of replication of bovine papillomavirus and characterization of the viral origin recognition factor E1. *EMBO J* 10:4321–4329.

Valle, M., Chen, X.S., Donate, L.E., Fanning, E., and Carazo, J.M. 2006. Structural basis for the cooperative assembly of large T antigen on the origin of replication. *J Mol Biol* 357:1295–1305.

Valle, M., Gruss, C., Halmer, L., Carazo, J.M., and Donate, L.E. 2000. Large T-antigen double hexamers imaged at the simian virus 40 origin of replication. *Mol Cell Biol* 20:34–41.

Vashee, S., Simancek, P., Challberg, M.D., and Kelly, T.J. 2001. Assembly of the human origin recognition complex. *J Biol Chem* 276:26666–26673.

Walker, J.E., Saraste, M., Runswick, M.J., and Gay, N.J. 1982. Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J* 1:945–951.

Walter, J., and Newport, J.W. 1997. Regulation of replicon size in Xenopus egg extracts. *Science* 275:993–995.

Wang, J. 2004. Nucleotide-dependent domain motions within rings of the RecA/AAA(+) superfamily. *J Struct Biol* 148:259–267.

Weigel, C., Schmidt, A., Seitz, H., Tungler, D., Welzeck, M., and Messer, W. 1999. The N-terminus promotes oligomerization of the Escherichia coli initiator protein DnaA. *Mol Microbiol* 34: 53–66.

Weigel, C., and Seitz, H. 2002. Strand-specific loading of DnaB helicase by DnaA to a substrate mimicking unwound oriC. Mol Microbiol 46: 1149–1156.

Weinreich, M., Liang, C., and Stillman, B. 1999. The Cdc6p nucleotide-binding motif is required for loading mcm proteins onto chromatin. *Proc Natl Acad Sci USA* 96:441–446.

Weisshart, K., Taneja, P., Jenne, A., Herbig, U., Simmons, D.T., and Fanning, E. 1999. Two regions of simian virus 40 T antigen determine cooperativity of double-hexamer assembly on the viral origin of DNA replication and promote hexamer interactions during bidirectional origin DNA unwinding. *J Virol* 73:2201–2211.

Wilson, V.G., and Ludes-Meyers, J. 1991. A bovine papillomavirus E1-related protein binds specifically to bovine papillomavirus DNA. *J Virol* 65:5314–5322.

Wilson, V.G., West, M., Woytek, K., and Rangasamy, D. 2002. Papillomavirus E1 proteins: form, function, and features. *Virus Genes* 24:275–290.

Wittinghofer, A., Scheffzek, K., and Ahmadian, M.R. 1997. The interaction of Ras with GTPase-activating proteins. *FEBS Lett* 410: 63–67.

Wold, S., Crooke, E., and Skarstad, K. 1996. The Escherichia coli Fis protein prevents initiation of DNA replication from oriC in vitro. *Nucleic Acids Res* 24:3527–3532.

Wyrick, J. 2001. Genome-wide distribution of ORC and MCM proteins in S. cerevisiae: high-resolution mapping of replication origins. *Science* 294:2357–2360.

Yabuta, N. 2003. Mammalian Mcm2/4/6/7 complex forms a toroidal structure. *Genes Cells* 8:413–421.

Yang, L., Mohr, I., Fouts, E., Lim, D.A., Nohaile, M., and Botchan, M. 1993. The E1 protein of bovine papilloma virus 1 is an ATP-dependent DNA helicase. *Proc Natl Acad Sci USA* 90:5086– 5090.

You, Z. 1999. Biochemical analysis of the intrinsic Mcm4-Mcm6-mcm7 DNA helicase activity. *Mole Cell Biol* 19:8003–8015.

Yu, X., VanLoock, M.S., Poplawski, A., Kelman, Z., Xiang, T., Tye, B.K., and Egelman, E.H. 2002. The Methanobacterium thermoautotrophicum MCM protein can form heptameric rings. *EMBO Rep* 3:792–797.

Zakrzewska-Czerwinska, J., Jakimowicz, D., Zawilak-Pawlik, A., and Messer, W. 2007. Regulation of the initiation of chromosomal replication in bacteria. *FEMS Microbiol Rev* 31:378–387.

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