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A structural framework for replication origin opening by AAA± initiation factors

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Abstract

ATP-dependent initiation factors help process replication origins and coordinate replisome assembly to control the onset of DNA synthesis. Although the specific properties and regulatory mechanisms of initiator proteins can vary greatly between different organisms, certain nucleotide-binding elements and assembly patterns appear preserved not only within the three domains of cellular life (bacteria, archaea, and eukaryotes), but also with certain classes of double-stranded DNA viruses. Structural studies of replication initiation proteins, both as higher-order oligomers and in complex with cognate DNA substrates, are revealing how an evolutionarily-related ATPase fold can support different modes of macromolecular assembly and function. Comparative studies between initiation systems in turn provide clues as to how duplex origin regions may be melted during initiation events.

Introduction

The faithful copying of genetic material depends on the precise spatial and temporal control of chromosome replication. In cells, replication onset is regulated by dedicated ATP-dependent initiation factors, which associate with *cis*-encoded loci (origins) to define start positions for strand synthesis (reviewed in [1]). Once engaged, cellular initiation complexes both unwind origin DNA and recruit requisite catalytic and scaffolding factors needed to construct a functional replisome [2,3]. Although the essential components of many model replication initiation systems largely have been identified, the detailed biophysical mechanisms by which these factors operate are still poorly understood. This deficiency has hindered our knowledge of how initiation programs are executed in an error-free manner to enable cell proliferation and preserve genetic integrity.

Broadly speaking, initiation complexes must carry out at least three key tasks, including origin recognition, origin melting, and the placement of ring-shaped DNA unwinding enzymes (helicases) onto DNA (Table 1). For cellular organisms, these activities often are served by distinct factors, termed initiators and helicase-loaders, respectively. However, in some instances, the replicative helicase can distinguish between origin and non-origin

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regions, or facilitate origin unwinding itself. Once loaded, replicative helicases help recruit strand synthesis machineries such as primases, polymerases, and polymerase clamp-loader complexes to complete replisome formation [2,4].

A sizable number of eukaryotic and prokaryotic replication initiation factors, as well as those of papilloma and polyoma double-stranded DNA viruses, are predicated on the **AAA+** (**A**TPases **A**ssociated with various cellular **A**ctivities) family of nucleotidyl hydrolases (Fig 1) [5]. This structural conservation has suggested that certain mechanistic properties of these proteins may overlap. Consistent with this idea, ATP can modulate the DNA binding and/or melting activities of initiation factors (e.g., see [6–11]), as well as promote the stable formation of higher-order initiator complexes through canonical AAA+-type interactions (Fig 1c, d) [12–18]. However, a paucity of structural models for key nucleotide- and DNA-bound intermediates has limited our understanding of how initiation proteins covert a latent chromosomal origin into an active replication fork. In this review, we detail recent findings that have started to reveal the means by which AAA+ type enzymes required for initiation stretch and untwist DNA to drive replication origin opening, a committing step toward copying the genome.

Initiation pathways in different organisms

Bacteria

In bacteria, replication typically begins with the recognition of a single chromosomal origin, *oriC*, by the initiator protein DnaA (reviewed in [19]). Although *oriC*s of different species vary significantly in size and organization [20], all contain a series of strong and weak DnaA binding sites that lie adjacent to an AT-rich DNA Unwinding Element (DUE) [21,22]. During the first steps of initiation, a subset of DnaA molecules initially engages duplex sites within *oriC* using a **H**elix-**T**urn-**H**elix (**HTH**) type of DNA binding domain that resides C-terminal to the initiator's core AAA+ fold [23–25]. As initiation proceeds (and in the presence of ATP), additional DnaA protomers associate with initiators anchored at the origin to assemble into a large nucleoprotein complex that opens the DUE (Fig 2a) [7,16,18,21,22,26,27]. Following origin opening, the DnaA proteins of many bacterial species collaborate with an auxiliary helicase loader (DnaC in *E. coli*) to recruit and deposit two hexameric DnaB helicases onto the melted origin (reviewed in [3]).

Viruses

The AAA+ ATPase initiators of polyoma and papilloma viruses comprise a different branch of the AAA+ lineage (the superfamily-3 (SF3) clade) [28], and are distinctive in that they act both as an initiator and as the replicative helicase (reviewed in [29]). As with DnaA, initiators such as the SV40 Large T-antigen (LTag) and papillomavirus E1 protein first associate with a viral origin using a dedicated duplex-DNA binding domain [30,31]. These early-binding initiators then recruit additional copies of themselves to the origin, ultimately forming two LTag or E1 hexamers around melted origin DNA strands in an ATP-dependent manner (Fig 2b) [32,33]. Although LTag is principally responsible for origin firing in SV40 [6], chaperoning of the papillomavirus E1 protein onto DNA is aided by a partner protein known as E2 [34,35]. Dissociation of E2 in turn allows for the assembly of a metastable, double-trimer E1 intermediate that facilitates origin melting [32].

Eukaryotes

In eukaryotes, initiation is coordinated by a six-subunit assembly known as the origin recognition complex (ORC) [36,37]. Phylogenetic and electron microscopy studies have shown that ORC forms a crescent-shaped particle predicated on a central core of as many as five AAA+ subunits (Orc1-Orc5) [28,38,39]. ORC localizes to duplex replication origins

through the use of both specialized DNA-binding domains and interactions with partner proteins or nucleosomes(reviewed in [2]). Once bound to an origin, ORC helps recruit and load the heterohexameric Mini-Chromosome Maintenance (MCM2-7) helicase onto DNA (Fig 2c). ATP is required for MCM2-7 loading by ORC [11,40,41], as are two additional proteins – Cdc6 and Cdt1 [14,42–45]; together with ORC, these factors collectively form a discrete particle known as the pre-replicative complex (Pre-RC) (Table 1) [46]. Like ORC, MCM2-7 and Cdc6 retain a central AAA+ domain. However, only Cdc6 belongs to the same initiator clade as Orc1-Orc5 (and DnaA) [28]; MCM2-7 instead falls within a different "pre-sensor II (PSII)" group, so named for the presence of an extra α-helix that precedes the sensor II motif of the helicase's ATPase fold [28].

Following pre-RC formation, ATP-turnover by ORC and Cdc6 triggers MCM release [43], resetting the initiation complex and allowing for additional MCM2-7 loading events prior to replication [10]. Notably, MCM2-7 complexes are first loaded as inactive double hexamers that encircle double-stranded DNA [47,48]. Activation requires specific phosphorylation events [49,50], as well as the association of the accessory proteins GINS and Cdc45, giving rise to a complex known as the CMG [51–55]. The MCM2-7 double hexamer appears to dissociate either during or after formation of the CMG [56], which itself has recently been shown to encircle only one strand during DNA unwinding [57].

Archaea

Archaeal organisms possess homologs of eukaryotic replication initiation factors (Table 1) (reviewed in [58]). Although archaeal initiators are most closely related to eukaryotic Orc1 and Cdc6 [28], they share a common organization with DnaA, bearing an initiator-type AAA+ domain fused to a C-terminal, duplex-DNA binding domain [59–61]. Co-crystal structures have shown that the Orc1 DNA binding domain, which comprises a winged-helix (WH) fold, can deform cognate origin sites upon engagement; interestingly, however, Orc1 AAA+ domains also can bind to and distort DNA directly [62,63]. At present, archaea appear to use a hexameric MCM-type helicase to unwind template DNA for replication fork progression [58]. How archaeal Orc1 proteins help load MCMs onto origins, and how ATP supports Orc1 function, remains enigmatic [58].

Structural insights into origin firing in bacteria

All replication initiation machineries that act on duplex DNA must overcome certain common physical challenges. Foremost among these is the separation of paired DNA strands to create a replication bubble that exposes template DNA. AAA+ domains are integral for DNA opening by cellular and SF3 initiation systems, although how these elements promote melting is still ill-defined.

A molecular picture of how AAA+ ATPases aid origin opening is starting to emerge, particularly for the bacterial initiator DnaA. ATP binding has been shown to accompany a conformational change in DnaA [64], facilitating the formation of a right-handed, spiral filament both *in vitro* and *in vivo* [65,66]. Helix formation, which is unusual for AAA+ proteins [67], appears to be supported by the presence of an α-helical insertion within the core AAA+ fold of DnaA that nudges adjacent subunits out of plane from each other (Fig. 3a) [64]. This so-termed Initiator Specific Motif (ISM) is present in all cellular initiators and serves to distinguish this group of proteins from other AAA+ ATPases [28,62]. The ISM, together with a pair of neighboring helices (α5–α6), also comprisesa cryptic single-stranded DNA-binding site within DnaAthat is activatedupon self-assembly of the initiator in the presence of ATP and is critical for origin melting [66,68].

A recent crystal structure of DnaA bound to single-stranded DNA shows that upon forming a helical oligomer, the initiator can grip and extend the phosphodiester backbone by ~50% compared to B-form DNA (Fig 3a, b) [9]. An unanticipated feature of this behavior is its striking similarity to the homologous recombination protein, RecA (Fig 3c, d) [69]. Although the cellular function of RecA – catalyzing DNA strand-exchange to support DNA repair [70] – is fundamentally different from that of DnaA, both proteins are based on an evolutionarily-shared ATPase fold belonging to the ASCE superfamily of P-loop NTPases (Fig 1) [28]. This congruence extends to the mechanism of substrate engagement by both proteins, which is manifest by the binding of three nucleotides of DNA to each protomer, concomitant with the introduction of small gaps between tri-nucleotide segments that stretch the nucleic acid substrate (Fig 3c, d). The DNAs bound by RecA and DnaA differ, however, in the relative displacement of their trinucleotide elements: in RecA, successive trinucleotides are oriented in a smoothly-spiraled arrangement to permit the contiguous pairing of a complementary strand [69], whereas in DnaA these segments are offset in a manner that impedes the formation of a continuous duplex (Fig 3e). These similarities and differences suggest that both RecA and DnaA initially extend DNA to promote duplex separation, but that the initiator further reconfigures the substrate to prevent re-pairing and to stabilize an open replication bubble [9].

The disposition of the single-stranded DNA bound to an ATP-charged DnaA oligomer suggests that the complex seen crystallographically likely corresponds to a state after DnaA has unwound DNA [9]. But how does DnaA melt *oriC* in the first place? Clues derive not only from the single-stranded DNA-bound structure of DnaA, but also from biochemical studies showing that DnaA can actively catalyze the disruption of short, model duplexes [9]. This strand separation activity depends on the single-stranded DNA-binding site within DnaA's AAA+ domain, as well as on the active, ATP-dependent assembly of the initiator, indicating that neighboring ATPase regions can directly engage double-stranded DNA and collaborate to pry open the duplex. While there is currently no structural picture of how the DnaA AAA+ domain might engage a double-stranded substrate, consideration of related initiation systems offers some valuable insights. For example, the ISMs of archaeal Orc1 proteins have been observed to bind and distort double-stranded origin DNA directly [62,63]. Viral SF3-class initiators similarly can bind duplexes through their AAA+ fold, and further are able to melt these substrates before enveloping one of the two DNA strands [8,71]. Comparisons of bacterial, archaeal/eukaryl, and papilloma/polyomavirus initiators shows that all of these factors engage the backbone of target DNAs using the same face of their core AAA+ ATPase folds [9] (Fig 4a–d). Moreover, this site and mode of binding further extends to distantly-related polymerase clamp-loaders [72], and appears to be used by MCMs as well (Fig 4e, f). Although the direction of the bound strand can run either 5′→3′ or 3′→5′ through the nucleic-acid binding site of a particular ATPase (Fig 4g), the overall similarities in how these disparate proteins engage client substrates is consistent with a prospective ability of the DnaA AAA+ domain to bind both single and double-stranded DNAs.

Using this logic, a possible sequence of molecular events that underlie origin opening in bacteria can be described as follows. In this scheme, several DnaA monomers first associate with duplex sites adjacent to the DUE using their C-terminal HTH DNA-binding domains (Fig 2a) [23–25]. Following binding, the AAA+ domains of adjacent DnaA molecules co-assemble into a helical oligomer [64,65], forming an organizing complex that wraps duplex *oriC* elements into a solenoidal supercoil [16,64], but that precedes DUE melting [26]. Next, the AAA+ domains of several DnaA protomers collectively engage one strand of a duplex DUE region. A model for this pre-melting state can be generated by aligning individual, ssDNA-bound DnaA protomers onto successive trinucleotide segments of a double-stranded DNA fragment (Fig 5a, b); the resultant arrangement is locally similar to the manner in

which Orc1 AAA+ domains bind to duplex DNA (Fig 5c) [62,63], and further places the nucleotide-binding face of each protomer in close proximity to the arginine finger of a partner subunit (Fig 5b). As additional DnaA protomers bind to the DUE, the additive contributions of their effects on the structure of the duplex destabilize the AT-rich (and labile) region, opening up a bubble that can be sequestered by the stable association of adjacent ATPase domains (Fig 5c). It is at present uncertain how single DnaA protomers are initially recruited to the DUE. Biochemical studies have found evidence for the existence of several types of DnaA *oriC* complexes [16,27,66]; in this vein, the organizing complex bound to the flanking duplex *oriC* sites could act as a nucleation center to support the formation of a dedicated melting subcomplex (Fig. 5c). Once initiation is complete, ATP hydrolysis would promote the disassembly of DnaA oligomers [73,74], freeing DNA strands for copying by replicative polymerases.

Origin melting by other AAA+ ATPases

The nucleic-acid binding strategy employed by DnaA may have parallels with other initiation systems, albeit with some notable distinctions as well. For example, like the bacterial initiator, papillomavirus E1 and SV40 LTag both undergo a monomer/multimer transition, forming higher-order complexes that engage and deform/underwind a duplex AT-rich region [16,18,21,22,26,29,32]. In E1, DNA melting depends on a β-hairpin element [8,29], which emanates from the same face of SF3 AAA+ fold as the ISM of DnaA and Orc1 (Fig. 4). In contrast to DnaA, however, the expansion of two early-stage bubbles appears to require multiple rounds of ATP turnover by two trimeric E1 subcomplexes, which untwists DNA to open the origin (Fig 5d) [8]. After melting, additional E1 protomers assemble with the trimers [8], and eventually fully encircle single-stranded DNA within two topologically-closed rings [71], as opposed to adopting the extended RecA-like spiral.

How origins are opened by eukaryotic initiation factors is less well-resolved. Nevertheless, there are hints that this process may exhibit an intriguing blend of similarities and differences compared to bacterial and viral SF3-type initiation systems. For example, based on phylogenetic signatures, the AAA+ subunits of ORC and Cdc6 likely all contain an ISM [62,63]. ORC, however, has not yet been observed to melt duplexes, either prior to or concomitant with helicase loading [11,47,48]. In this regard, it is notable that the local architecture of ORC-type ISMs differs from that of DnaA (at least in archaeal Orc1 orthologs), forming not a “V”-shaped helical wedge, but rather a parallel, helix-loop-helix motif (Fig 4a, b). Because the DnaA ISM appears at least partly responsible for pushing oligomerized initiator protomers into a spiral and extending single-stranded DNA [9,64], this structural difference raises the possibility that assembled ORC subunits may adopt a more planar configuration that allows adjoining ISMs to engage duplex DNA, but not to stretch and melt it.

If ORC does not melt origin DNA directly, the most likely candidate for such a function would seem to be MCM2-7. However, while MCM double hexamers loaded by ORC do encircle double-stranded DNA, they are not immediately competent to unwind the duplex [47,48]. This finding, along with three other observations a need for phosphorylation, the stable binding of Cdc45•GINs to activate MCM2-7 [47,75], and the apparent encirclement of single DNA strands by the resultant CMG [57] – suggests that origin melting could occur as a consequence of MCM2-7 isomerization between double and single-hexamer states [57,76,77]. Were MCM2-7 to prove capable of catalyzing the initial origin melting event (in addition to its demonstrated role as a replicative helicase), the heterohexamer’s AAA+ domains would likely drive the process, potentially emulating viral SF3 enzymes by engaging one strand of duplex DNA, restructuring the nucleic acid substrate through ATP-dependent conformational changes, and eventually sequestering a single DNA strand within

a heterohexameric ring (Fig 5e) [57,76,77]. How isomerization might take place alongside DNA opening is not clear, but could rely on an ATP-dependent untwisting approach analogous to that proposed for E1. Alternatively, a wide variety of structural forms have been observed for eukaryotic and archaeal MCMs, including an intriguing helical state [78], which we note displays right-handed chirality and pitch similar to that used by DnaA to promote DNA unpairing. Additional studies will be needed to resolve these issues.

Conclusions

Although many structural and mechanistic aspects of replication initiation are still poorly understood, trends pertaining to how duplex origin segments are melted are starting to emerge. Cellular and SF3-type initiation factors are predicated on an evolutionarily-related AAA+ ATPase fold that assembles into higher-order oligomers through the formation of a canonical, bipartite catalytic center. Despite bearing numerous distinguishing structural augmentations, initiation proteins display a striking similarity in how they engage nucleic acid targets, in particular the use of a common face of the AAA+ region to bind to the backbone of a single DNA strand (even when that substrate is paired within a duplex). However, architectural differences between these substrate-binding surfaces also correlate with distinct structural states formed upon oligomerization (e.g., spirals vs. closed rings), and in turn appear to underpin unique functional traits. Hence, the AAA+ domains of eukaryotic and archaeal initiators assist with origin recognition and the loading of a hexameric helicase (which itself may melt DNA); SF3 viral initiators by contrast both bind and open origin regions, and then further interconvert into hexameric helicases that processively unwind DNA. Bacterial DnaA exhibits a blend of these properties, with an ability to engage and melt *oriC* and to aid helicase loading. Future work will undoubtedly provide more surprises regarding how cellular and viral systems have adapted and exploited a common ancestral ATPase fold to accommodate specialized roles for supporting the initiation of DNA replication.

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Highlights

AAA+ ATPase proteins in the initiation of replication engage DNA in a similar manner.

ATP-dependent assembly stretches and untwists DNA to drive replication origin opening.

Distinct spiral and ring-shaped assemblies conduct initiation in different systems.

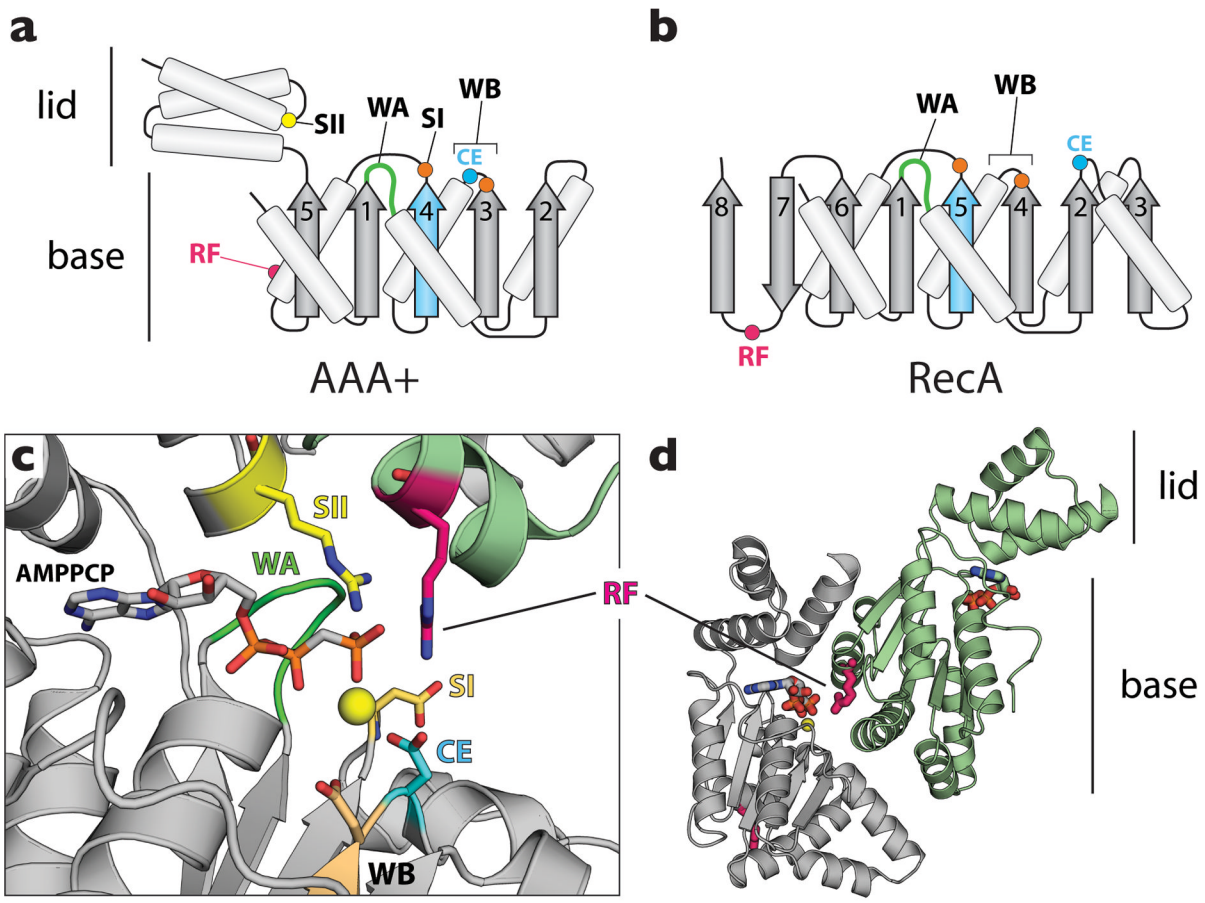

Figure 1.

ASCE ATPase architecture. AAA+ enzymes share a common structural core with RecA-type ATPases, together forming the Additional Strand Catalytic glutamate (E) (ASCE) supergroup of P-loop NTPases [79]. **(a)** Topology diagram for a AAA+ ATPase. AAA+ proteins frequently possess a nucleotide-binding pocket formed by two subdomains, a base αβ α-nucleotide-binding fold and a C-terminal α-helical lid domain. **(b)** Topology diagram for a RecA ATPase. In panels **a** and **b**, the additional strand and catalytic glutamate are highlighted in cyan. **(c)** Signature sequence motifs of AAA+ proteins. WA – Walker A (dark green), a glycine-rich element that forms a distinctive loop for cradling the phosphates of ATP. WB – Walker B (cyan and orange), a motif that contains acidic residues that interact with an associated magnesium ion (yellow sphere). SI/SII – sensor I (orange) and sensor II (yellow) elements, which reside near and can interact with the γ-phosphate of ATP. **(d)** Assembly patterns in AAA+ ATPase oligomers. A typical AAA+ dimer is shown (DnaA in this instance; PDB ID: 3R8F [9]). A conserved arginine residue – the arginine finger (RF, magenta) – extends from one AAA+ subunit (light green) to contact the γ-phosphate of ATP bound to a neighboring protomer (gray).

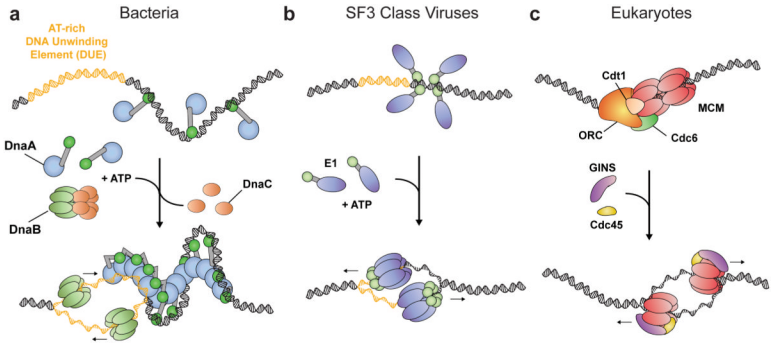

Figure 2.

Replication initiation mechanisms in cells and papilloma/polyomaviridae. **(a)** Bacteria. DnaA monomers are displayed as a blue oval (AAA+ domain) connected to a green oval (DNA binding domain). (*Top*)DnaA monomers associate with *oriC*. (*Bottom*) DnaA homo-oligomerizes in the presence of ATP, melting the DUE (yellow) and aiding the loading of the DnaB helicase (light green) with DnaC (orange). **(b)** SF3 viruses. Initiators are displayed as a purple oval (AAA+ domain) connected to a light green oval (DNA binding domain). (*Top*) monomers associate with the origin. (*Bottom*) Viral initiators melt the origin in the presence of ATP (yellow – DUE), eventually assembling into two hexameric helicases. **(c)** Eukaryotes. (*Top*) Activation of ORC by ATP stimulates formation of the pre-replicative complex (pre-RC), composed of ORC (orange), an MCM2-7 double-hexamer (red ovals), and the loader/chaperone proteins Cdc6 (green) and Cdt1 (tan). (*Bottom*) After loading, Cdc45 (yellow) and the GINS (purple) complex stimulate duplex melting by MCM2-7, giving rise to two hexameric helicases.

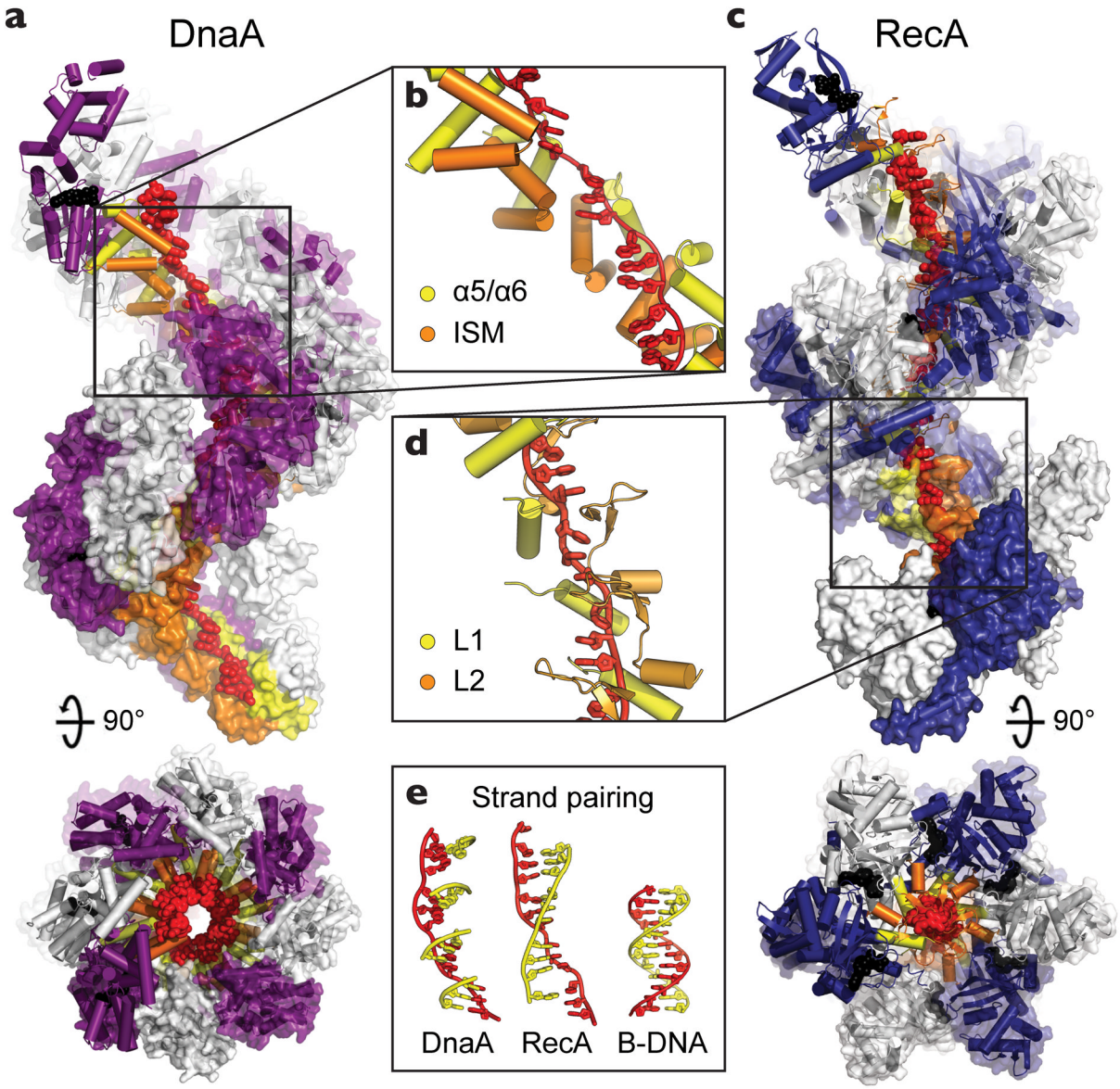

Figure 3.

DnaA binds single-stranded DNA in a manner similar to RecA. **(a)** Side and top views of oligomerized DnaA (PDB ID: 3R8F), showing twelve DnaA subunits, each colored white or purple, complexed with DNA (red). AMPPCP and Mg^{2+} are shown as black spheres and the DNA engagement elements, helices α5/α6 and the ISM, are colored yellow and orange, respectively. **(b)** Close-up of the DnaA oligomer bound to the backbone of DNA. **(c)** Side and top views of a RecA filament, constructed by aligning four copies of a RecA pentamer model (PDB ID: 3CMW [69]). Twelve RecA subunits, each colored white or blue, are shown complexed with DNA (red). ADP•AlF_4 and Mg^{2+} are shown as black spheres and the DNA engagement elements, loops L1 and L2, are colored yellow and orange, respectively. **(d)** Close-up of the RecA oligomer bound to the backbone of DNA. Both DnaA and RecA bind DNA in an extended conformation with large gaps between trinucleotide base stacks. **(e)** (*left*) Cartoon model showing how complementary base triplets (yellow) would pair (in a B-DNA manner) with single-stranded-DNA bound to DnaA (red). Formation of a continuous base-paired strand is prevented by the orientation of successive DnaA-bound

triplets. (*middle*) Same DNA view, but as seen in RecA, which orients triplets to allow pairing of an extended complementary strand for promoting duplex formation and strand exchange (PDB ID: 3CMX [69]). (*right*) B-DNA.

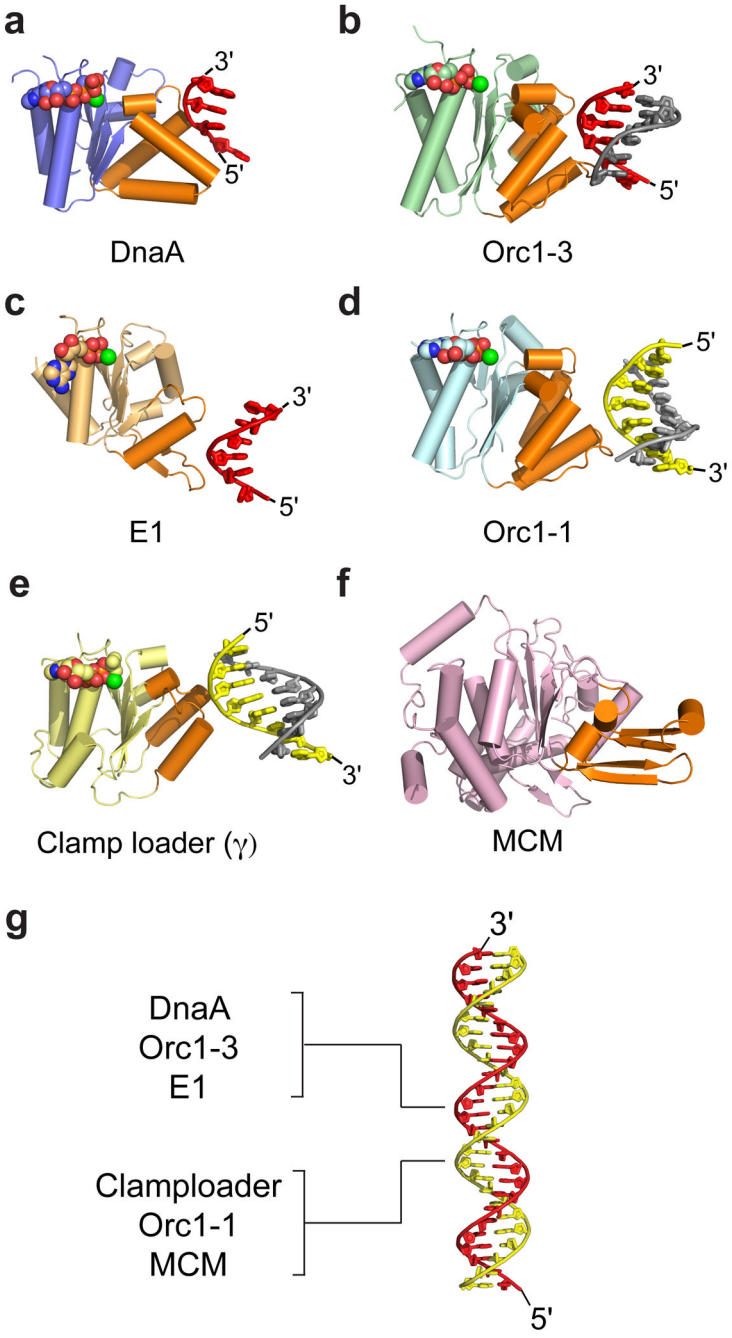

Figure 4.

DNA recognition by replicative AAA+ proteins. Nucleic acid substrates are all engaged using structural elements that reside on the same face of the AAA+ domain (orange). **(a)** Bacterial initiator DnaA (AAA+ domain, blue) bound to single-stranded DNA [9]. **(b)** Archaeal initiator Orc1-3 (AAA+ domain, green) bound to duplex DNA (PDB ID: 2QBY [62]). **(c)** Viral initiator E1 (AAA+ domain, light orange) bound to single-stranded DNA (PDB ID: 2GXA [71]). **(d)** Archaeal initiator Orc1-1 (AAA+ domain, cyan) bound to duplex DNA (PDB ID: 2QBY). **(e)** Bacterial clamp-loader subunit γ (AAA+ domain, yellow) bound to primer-template DNA (PDB ID: 3GLF [72]). **(f)** Archaeal Mcm (AAA+ domain, pink) (PDB ID: 3F9V [80]); although a DNA-complex has yet to emerge for this enzyme,

mutagenesis studies have implicated the highlighted regions in binding substrate and helicase activity [81,82]. **(g)** Duplex DNA (red/yellow) with the strand bound by various AAA+ proteins indicated. For panels **a–f**, DNA is shown as red/grey or yellow/gray cartoons. Nucleotide co-factors are represented as spheres colored by atom.

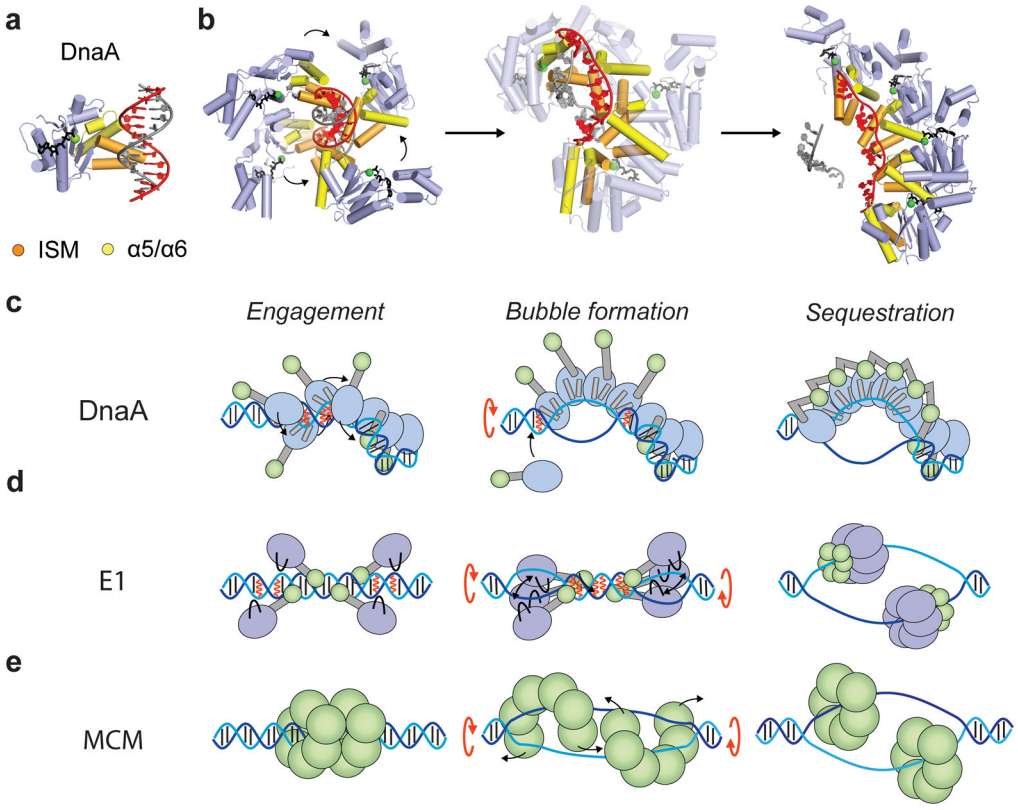

Figure 5.

Models for origin melting. **(a)** Alignment of a single-stranded, DNA-bound DnaA monomer onto one strand of duplex DNA (*red*) results in an arrangement similar to that seen for Orc1 when bound to a double-stranded substrate (cf. Fig 4c). **(b)** Possible mechanism for DNA opening by DnaA. Four DnaA molecules are shown bound to consecutive trinucleotide segments along one strand of the duplex DUE. DnaA binding destabilizes this metastable region, promoting bubble formation and sequestration of a single DUE strand by a DnaA oligomer. The intermediate state (*middle*) corresponds to a linear interpolation between the initial and final states (Yale Morph Server) [83]. Bound nucleotide and Mg^{2+} are represented as black sticks and green spheres, respectively. **(c)** Stages of origin opening by DnaA. Each DnaA monomer is displayed as a blue oval (AAA+ domain) connected to a green oval (DBD). DNA binding elements (ISM and α5/α6) are displayed as gray cylinders. **(d)** Stages of origin opening by E1. Each E1 monomer is displayed as a purple oval (AAA+ domain) connected to a green oval (DBD). DNA binding elements are displayed as black loops. **(e)** Stages of duplex DNA melting by MCM. MCM monomers are represented as green ovals. In panels **c–e** the two strands of duplex DNA are displayed in blue and cyan with black lines for bases. Red lines represent regions where duplex DNA is destabilized. Black arrows refer to protein movements and red arrows to DNA untwisting as a bubble is formed.

Table 1

A subset of cellular and viral initiation factors.

Replication Factor	Bacteria	Simian virus 40	Papillomavirus	Eukaryotes	Archaea
Initiator	DnaA	L Tag	E1	ORC	Orc1/Cdc6
Helicase	DnaB	L Tag	E1	MCM2-7	MCM
Helicase Loader	DnaC	L Tag	E2	Cdc6/Cdt1	Orc1/Cdc6?