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Origin DNA Melting and Unwinding in DNA Replication

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Summary

Genomic DNA replication is a necessary step in the life cycles of all organisms. To initiate DNA replication, the double-stranded DNA (dsDNA) at the origin of replication must be separated or melted; this melted region is propagated and a mature replication fork is formed. To accomplish origin recognition, initial DNA melting, and the eventual formation of a replication fork, coordinated activity of initiators, helicases, and other cellular factors are required. In this review, we focus on recent advances in the structural and biochemical studies of the initiators and the replicative helicases in multiple replication systems, with emphasis on the systems in archaeal and eukaryotic cells. These studies have yielded insights into the plausible mechanisms of the early stages of DNA replication.

Introduction

Genomic DNA replication is a common and essential process for all living things. The replication process can be subdivided into three stages: initiation, elongation, and termination. At the initiation stage, multiple replication proteins classified as initiators recognize and bind replication origin DNA and convert it to a replication fork. Subsequently, factors, such as polymerase-primase (pol-prim) and topoisomerase, are recruited to the replication fork to form an elongation-competent “replisome”.

The initiation process in which a replication origin is converted to a mature replication fork involves the following steps. First, initiators assemble around the origin DNA, leading to the eventual melting of the dsDNA origin. Subsequently, the origin melting is propagated to produce a replication fork on each side of the melted origin for bi-directional replication. In this process, topological obstacles must be overcome to fully convert the melted dsDNA origin to a fork structure that can be unwound by the ring-shaped helicases.

The initiators in eukaryotic cells include multiple proteins, including Orc, Cdc6, Cdt1, and MCM (mini-chromosome maintenance) helicase. One of the key factors in the transition from dsDNA origin to the melted and unwound fork DNA is the MCM complex that serves as the replicative helicase in eukaryotic and archaeal cells. MCM forms hexamers that in turn can dimerize into double-hexamers [1–4]. The replicative helicases for simian virus 40 (SV40) and papillomavirus, which replicate the viral genome in eukaryotic cells, also form hexamer/double-hexamer structure. The helicase for SV40 large T antigen (LTag)

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recognizes the origin DNA and assembles into an initiation complex that can melt and unwind origin DNA to form a replication fork without any additional cofactors. In papillomavirus, this process requires the helicase E1 and another protein E2. While in eukaryotes and archaea, melting and unwinding of origin DNA needs multiple initiators in addition to the helicase MCM.

Because of the complexity of the DNA replication system in eukaryotic cells, closely related simpler systems like some viruses (SV40, papillomavirus, etc) and archaea have made significant contributions to the understanding of replication process. In the following sections, we will discuss recent advances in structural and biochemical studies related to the steps from origin recognition and melting to replication fork formation.

Initial melting of replication origin

Biochemical methods can be used to detect the initial melting of origin dsDNA induced by the assembly of initiators at the origin. In prokaryotic cells, origin recognition protein DnaA can initiate origin melting into single-stranded DNA (ssDNA), and the melted ssDNA is the loading site for the hexameric helicase DnaB (reviewed in [5]). For the more complex archaeal and eukaryotic cellular systems, however, the exact timing of the origin melting is unclear. Nonetheless, the origin melting has been shown to be induced by the assembly of LTag alone in SV40, or by E1 and E2 in popillomavirus.

The mechanisms whereby initiators/helicases melt and unwind the origin DNA are not well understood, mainly due to the lack of high-resolution structures that capture the intermediate of melted origin with bound initiators and helicases. Two recent co-crystal structures containing origin DNA and initiators from archaea reveal how these initiators recognize origin dsDNA. The two archaeal Cdc6/Orc-dsDNA complexes [6,7] reveal dsDNA distortion and bending, but no melting (Fig. 1A-B). To induce origin melting, it probably requires additional initiators (such as MCM) to form a higher-order complex at the origin.

Because SV40 LTag assembly at the origin alone is capable of inducing origin melting and unwinding [8,9], it is considered to be the archetypal initiator/helicase in eukaryotic system, and has served as a model system for studying origin recognition, assembly, and the melting process for many years. Multiple crystal structures of LTag hexamer in the absence of DNA reveal long and narrow central channels (with an openings around ~13–17Å) that appear to be sufficient for binding ssDNA, but not dsDNA that has a diameter of 20Å [10,11] (Fig. 1C-D). Similarly, the central channels observed in bovine papillomavirus (BPV) hexameric E1 structures with or without ssDNA bound [12,13] are also too narrow to bind dsDNA. Indeed, molecular mechanics studies of DNA translocation indicate that a channel with a minimum diameter of 20 Å is required for dsDNA to occupy without loosing its structural integrity [14,15].

The above structural data are the basis for the opinion of “ssDNA-only” in the channel, i.e. only melted ssDNA is encircled in the central channel for hexameric helicases, even during the assembly at the origin (reviewed in [16]). Such thinking was reinforced by the biochemical report suggesting that partial/non-ring-shaped E1 assembly induces initial BPV origin melting before the formation of the ring-shaped hexamer [17]. In this model, two separate trimers of E1 assemble on each end of BPV origin to induce melting. Further supporting evidence would be needed for the formation of a relatively stable trimeric E1 complex that is capable of binding and melting origin dsDNA and that can serve as the obligatory intermediate for a hexamer assembly.

So far, all four published structures of LTag hexamers not only reveal central channels too small for dsDNA, but also show a planar arrangement of the β-hairpins in the central

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channel [10,11,18](Fig. 1C-D). The β-hairpins, together with the neighboring DR/F loops, form two adjacent planar rings to constitute the narrowest part of the channel in the AAA+ domain (Fig. 1D). This planar arrangement of β-hairpins in LTag is in sharp contrast to the staircase arrangement in the two known E1 structures (Fig. 1E-F). In the E1 hexamer, the six β-hairpins (one from each subunit) in the central channel are arranged in a staircase-like structure. The intriguing question is whether this difference of β-hairpin arrangement reflects an intrinsic mechanistic difference of LTag and E1 hexameric helicase.

LTag assembles as a double-hexamer at SV40 origin DNA. Our recent work by cryo-EM reconstruction showed that LTag does bind dsDNA inside the channel by encircling the dsDNA origin sequence as two hexamers, even though the low-resolution (~20Å) structure did not reveal the detailed features for the channel and the DNA inside it [19]. The intriguing question is whether the previously observed narrow channel of LTag can expand to accommodate dsDNA, or if the dsDNA is remodeled by the initiator/helicase to fit the narrow channel. Among the LTag structures in the absence of DNA, the channel does expand slightly (between ~13–17Å) in response to ATP binding and hydrolysis [11]. Whether this would mean even further expansion of the channel in response to dsDNA binding requires data from higher resolution structures of hexameric LTag bound to dsDNA.

However, while all the published LTag and E1 structures in several different liganded states have channel openings narrower than dsDNA dimensions, it is also possible for the LTag hexamer to accommodate the origin dsDNA through its narrower channel. For this scenario to occur, the dsDNA has to be squeezed or crushed by the narrower channel (Fig. 2A), so that the dsDNA is remodeled to fit the narrower channel. A consequence of this squeezing is the disruption of the base-pairs and melting of the dsDNA origin (Fig 2A-B), which is consistent with the literature showing the origin melting through the assembly of LTag initiator/helicase at the AT-rich origin dsDNA [8]. We refer to our origin melting model as the squeeze-to-open model. This model is counter-intuitive, as the expected method of separating the duplex is pull-to-open. This model is in contrast to the model proposed for E1 origin melting where E1 trimers bind dsDNA through insertion of β-hairpins into the DNA to pry open the double-helix [17] (Fig. 2E). Nonetheless, this squeeze-to-open model, albeit awaiting future confirmation, fits the LTag structural data available so far, and is consistent with the biochemical literature on origin DNA melting and unwinding [8,20,21].

From origin DNA melting to replication fork formation

The conversion from a melted origin induced by the assembly of initiators to an active replication fork requires expansion of the melted region and positioning of the hexameric/double-hexameric helicase on the fork correctly, a process with many topological obstacles to be resolved. One possible model for replication fork formation from the melted origin, named “squeeze-pumping” model, is shown in Fig. 2A-D. This model is based on available LTag structural data, including the narrow channel, the pumping motion of the AAA+ motor domain, the side channels for a possible ssDNA exit, and the weak inter-Zn domain interactions within a hexamer. In this model, the DNA melted by the squeezing/crushing force of the narrow channel of the AAA+ motor domain is pumped toward the Zn-domain, where there is a local larger “chamber” between the junction of AAA+ and Zn-domains to allow the expansion of the melted region (Fig. 2B). Continued DNA pumping would result in building up the ssDNA loop [11], which could exit the channel by extruding the ssDNA between two neighboring Zn-domains where inter-subunit interactions are weak [10,11,22] (Fig. 2C-D). The Zn-domain may then return to the closed conformation, leaving one or both strands of ssDNA in the side channels. This loop exit process proposed here still needs future testing.

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Another model previously proposed for E1 suggests that after two adjacent E1 trimers assemble at both sides of the origin to induce melting, a ring-shaped E1 hexamer is then assembled from the trimer intermediate around the already separated ssDNA to form a fork (Fig. 2E-F) [17]. In this model, the fork can then be unwound by E1 translocating along one ssDNA strand (reviewed in [16]). This model circumvents the topological obstacle of the transition from the dsDNA origin to the replication fork formation. However, it does not explain how initial melting, which is proposed to be generated by an E1 trimer intermediate, is expanded.

Archaeal MCM helicases reportedly exist in multiple conformations, including filament and open rings [23–26]. This observed structural plasticity led to the proposal of the ring-filament transition model. In this model, the helicase converts between a fully closed ring and an open filament to release unpaired DNA strands out of the central channel. Prompt closure of the ring ensures processive helicase activity during elongation. Several truncated helicases form helical filaments [27,28]. However, none of them have been observed to form filament on dsDNA. Thus, the ring-filament transition model awaits experimental support.

Fork progression–translocation of ssDNA or partially denatured dsDNA

Currently, the most widely accepted model of fork unwinding is the steric exclusion model that is mostly suggested from several lines of evidence from studying the prokaryotic DnaB helicases. This model states that a ring-shaped helicase encircles and migrates down one DNA strand toward the dsDNA fork, displacing the other strand. The E1 structure with ssDNA bound in the central channel can be explained by this steric exclusion model [12]. The structure of ssRNA bound rho hexamer, which is not a replicative helicase per se, but a RNA helicase for transcription termination, is also proposed to operate by a steric exclusion mechanism [29]. Biochemical data with artificial fork DNA substrates also suggest that the steric exclusion model may be the working mechanism for *in vitro* unwinding for several hexameric helicases (reviewed in [16]).

However, LTag and MCM form double-hexamers over origin DNA [2–4,9]. Though active as single-hexamers *in vitro*, the double-hexameric LTag and MCM reportedly have higher unwinding activity[30,31]. Furthermore, LTag is unique in having the ability to unwind long blunt-ended dsDNA that contains an internal origin sequence *in vitro*, likely as a double-hexamer [19,32]. The LTag double-hexamer is shown to be the active helicase for viral replication. Residues needed for head-to-head interactions between two LTag hexamers are critical for *in vivo* DNA replication [33]. These data are consistent with our previously proposed double-pump looping model for the bi-directional fork progression in eukaryotic and archaeal systems [10,11,34]. With the recent advances, we are able to refine the double-pump looping model as shown in Fig. 3, in which both hexamers pump dsDNA into the double-hexamer to generate ssDNA loops that can exit from the side channels. The double-pump looping model is compatible with the finding that the pol-prim and ssDNA binding protein RPA dock on the side of LTag double-hexamer [35,36], positioning the pol-prim and RPA to capture the emerging ssDNA from the side-channels (Fig. 3A-B). Additionally, this model is also compatible with the observed double-hexameric architecture of MCM helicases [1,3,4,26,31]. Furthermore, this model also makes it easy for the two forks to communicate with each other in case of DNA damage and fork stall.

Similarities and differences among helicase systems

For the various DNA replication schemes in prokaryotes, archaea, eukaryotes, and viruses, closely parallel designs and underlying mechanisms are frequently observed. For example, the ring-shaped structure is common to replicative helicases. However, obvious differences exist in the diverse systems. For instance, the prokaryotic DnaB helicases only exists as

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single-hexamers, whereas LTag and some MCMs are shown to form double-hexamers. The architectural differences of the helicases may well mirror their mechanistic diversity. For example, the single-hexamer ring in DnaB has its N-terminal end free for docking with primases. Functionally, the N-terminal docking makes it convenient for the primase to capture the ssDNA emerging from the N-terminal domain during the fork unwinding, as proposed in the steric exclusion model (Fig. 2F) [28,37,38]. In contrast, LTag and MCM form double-hexamers through N-N interactions [19], making the N-termini unavailable for primase docking. This may explain why the pol-prim and RPA dock on the side of LTag double-hexamer (Fig 3).

Even for the closely related SV40 and BPV viral systems, differences in the observed features are just as apparent as the similarities. On the structural side, while LTag and E1 hexamers both possess essential βhairpins in the narrow central channels [10–13,20,21,39], surprising differences are observed in the β-hairpin arrangements. The β-hairpins in the E1 adopt a staircase-like arrangement, independent of binding to nucleotide and ssDNA [12,13] (Fig. 1E-F). Whereas the LTag six β-hairpins are arranged in planar manner, yet again independent of various nucleotide ligand binding [10,11,17] (Fig. 1C-D). The most convenient explanation for these differences is that the staircase arrangement in E1 is essential for ssDNA translocation, whereas the planar arrangement in LTag is critical for melting dsDNA origin or fork. This raises intriguing questions of whether E1 binds and melts its dsDNA origin with helically arranged β-hairpins, and how LTag translocates over ssDNA with its planar β-hairpins.

Another apparent difference observed between different hexameric helicases is the mode of ATP binding/hydrolysis. The T7gp4, a DnaB family helicase, has a maximum of four nucleotide sites occupied in one hexamer, with two of the four sites having lower occupancy [40]. For BPV-E1, only one out of the six binding sites exhibit low occupancy [12]. Biochemistry data indicates that T7gp4 may bind and hydrolyze ATP possibly in a sequential manner. Though no nucleotide binding structure for MCM exists, biochemical studies of an archaeal MCM suggest a so-called “semi-sequential” ATP binding and hydrolysis [41]. In LTag, all six nucleotide sites in the apo, ATP, ADP structures uniquely display an all-or-none nucleotide occupancy [11], i.e. all six sites are either occupied fully with ATP, or ADP, or empty. The all-or-none mode is consistent with biochemical analysis that shows a 1:1 binding of ATP or ADP by LTag in solution [42], which suggests a concerted or highly coordinated mode for ATP binding/hydrolysis, as well as for the associated conformational switches between the different nucleotide states [11]. Therefore, the available data appear to suggest that each hexameric helicase may operate differently, even for evolutionarily related helicases. If so, this class of hexameric helicases is another showcase for how nature has found different solutions to the same problem of genomic DNA duplication.

Conclusions

Recent progress has significantly advanced our understanding of molecular events in early stages of DNA replication. However, details of these processes are far from clear. Critical questions, such as how origin DNA is melted, how the initial melted origin expands to form a replication fork, and how the origin melting and fork formation by the initiators/helicases is coupled to other replication proteins, and how a double-hexamer unwinds the replication forks during replication, require future investigation, which likely involves a major undertaking with cross-disciplinary approaches, including, but not limited to, structural, computational, single-molecule analysis, biochemistry and kinetics.

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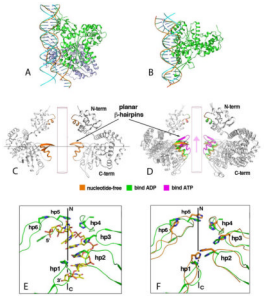

Figure 1.

Structures of initiators and helicases in eukaryotic and archaeal systems. **(A)** Structure of *S. solfataricus* Cdc6-ORC1 binding the origin DNA (in orange), bending the DNA (for comparison, B-form dsDNA is in cyan) [6]. **(B)** Structure of *A. pernix* ORC1 binding the origin DNA (in orange) through two separate domains, bending the origin DNA (B-form dsDNA is drawn in cyan) [7]. The initiator binding does not induce origin melting. **(C)** Side view of the apo LTag hexamer, showing the β-hairpins are on the same plane (planar arrangement, indicated by the horizontal line). To show the planar β-hairpins clearly, the subunits in the front and back of the hexamer are removed. **(D)** Superposition of LTag structures in ATP-bound, ADP-bound, and nucleotide-free states. Major structural shifts of the β-hairpins (β-hp, colored as indicated in the figure) in the C-to-N direction occur upon ATP binding, hydrolysis, and release. The channel diameter of these hexamers vary between ~13–17Å, too small for dsDNA to pass through. (**E)** The staircase-arranged six β-hairpins (hp1-6) in a E1 hexamer, showing the right-handed helical path along the central channel from C-to-N direction (indicated by a vertical bar), along which the six-nucleotide ssDNA (drawn as sticks in yellow/orange) is anchored [12]. The structure shows that the 5’ end of the ssDNA is on the N-terminal side (upper), and the 3’ on the C-terminal side (lower), which is in the opposite orientation from the model drawn in Fig 2F. **(F)** Alignments of E1 β-hairpins (hp1-6) in ssDNA-bound state [12] (in green) and DNA-free state [13] (in orange), showing the same staircase arrangement for the β-hairpins in the presence and absence of ssDNA and nucleotide. This same staircase arrangement was seen in the DNA-free and nucleotide-free E1 structure, suggesting that the ssDNA adopts a conformation to fit the staircase-arranged β-hairpins.

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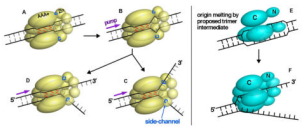

Figure 2.

Models for the conversion from a melted origin to a replication fork proposed for LTag, the" squeeze-pumping" model shown in **(A-D),** and for E1, the "trimer assembly" model in **(E-F). (A)** The first step in the squeeze-pumping model, the initial melting of the origin in the AAA+ domain of a LTag hexamer, which probably is generated by the squeeze/crush of the narrow channel on the dsDNA (or the squeeze-to-open mechanism). The squeeze or crush on the dsDNA can lead to base-pair disruption and thus origin melting (shown in red bars). **(B)** Another round of ATP binding/hydrolysis in the AAA+ motor domain pumps the melted origin DNA toward the Zn-domain, expanding and building up the melted DNA region. **(C)** Further ATP binding/hydrolysis pump the DNA and push the separated ssDNA loop against the channel wall, which may eventually force the ssDNA loops through the gaps between two subunits at the Zn-domain, allowing the growing ssDNA strands to exit from the side channels. **(D)** A slightly different topology of the unwinding fork from that in panel C. Here, only one ssDNA exits the central channel via a side channel while the other strand passes through the Zn-domain channel. **(E, F)** A model previously proposed for E1 origin melting and fork formation [17,39]. The model proposes that two adjacent E1 trimers (one trimer shown) assemble at the origin to melt the origin (E). Then, a ring-shaped E1 hexamer is formed around the melted ssDNA to form a fork (F). This model allows the E1 helicase to unwind the fork by the steric exclusion unwinding model, in which E1 will translocate on ssDNA in a 3'-5' direction to unwind the fork on the C-terminal domain end.

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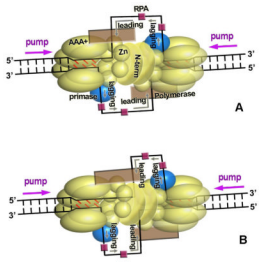

Figure 3.

Our refined double-pump looping model [10,11] for bi-directional DNA unwinding by a double hexameric helicase (such as LTag or MCM). **(A)** Two hexamers stay together through N-N interactions. The C-terminal AAA+ motor domain pumping the dsDNA ahead of the fork into the double hexamer, extruding the separated ssDNA as loops. The dsDNA traversing the narrow channel in the AAA+ domain may be squeezed and melted and is poised to be separated into two strands by the next cycle of pumping toward the N-terminus. The separated ssDNA may force its way between two subunits in the Zn-domain area, extruding through two side channels on each hexamer as loops [11]. During replication, primase, polymerase, RPA, and other replication proteins dock on the side of the double hexamer near the side-channel, capturing the emerging ssDNA loops as the template for synthesizing the leading and lagging strands. **(B)** A slight variation of the looping topology from panel-A, showing an asymmetric looping. Here only one ssDNA exits from the helicase domain side channel, and the other ssDNA exits around the junction between two double hexamers.

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