The MCM complex: (just) a replicative helicase?

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Abstract
The MCM2–MCM7 (minichromosome maintenance 2–7) complex is involved both in the initiation and the elongation step of eukaryotic DNA replication and is believed to be the replicative helicase. Whereas the mechanism of DNA unwinding at the replication fork has been extensively investigated, the role of the MCM2–MCM7 complex during initiation has not yet been characterized by biochemical studies. Here we summarize the in vivo evidence which supports a role for the MCM complex in origin melting. In addition, we present an overview of the mechanism of action of a number of AAA+ (ATPase associated with various cellular activities) initiators and hexameric helicases, which can be used in turn as models for the steps of recognition, duplex melting, loading and nucleic acid translocation of the MCM helicase.

Introduction
DNA replication in eukaryotes is a highly regulated process that involves a large number of players, and at the core of this process is the loading of the MCM (minichromosome maintenance) proteins on to replication origins. Despite their importance in replication and cancer development, the exact mechanism of action of MCM proteins is not yet well understood. All eukaryotic organisms possess six homologous proteins (MCM2–MCM7) that form hetero-oligomers. They all belong to the family of AAA+ (ATPase associated with various cellular activities) proteins and share similarities to other hexameric helicases [1,2]. Even though additional MCM proteins have been identified in higher eukaryotes, the MCM2–MCM7 complex remains the prime candidate for the replicative helicase [3].

Structure and function of the MCM complex
The complexity of the eukaryotic MCM system has meant that most work addressing the molecular mechanism for unwinding dsDNA (double-stranded DNA) has been carried out on archaeal MCM proteins. Most archaean species possess a single MCM protein that forms homo-oligomeric complexes. The two most commonly studied archaean systems, Methanothermobacter thermautotrophicus MCM (MthMCM) and Sulfolobus solfataricus MCM (SsoMCM), display DNA-stimulated ATPase as well as helicase activities [4,5]. Although the purified MCM2–MCM7 complex does not show any helicase activity on its own, and only a weak activity has been detected for the MCM4–MCM6–MCM7 subcomplex [6], the lack of in vitro unwinding of the eukaryotic complex may be the result of the absence of essential post-translational modifications or protein cofactors needed to perform unwinding in the context of the cell cycle. Indeed, a stable complex comprising Cdc45 (cell division cycle 45), MCM2–MCM7 and GINS (Go, Ichi, Nii, San) can be purified from Drosophila embryo extracts and displays an ATP-dependent helicase activity [7].

The structure of MCM proteins can be divided into three domains (Figure 1): an N-terminal domain, which in MtbMCM can form a head-to-head double hexamer [8], is capable of binding both ssDNA (single-stranded DNA) and dsDNA and modulates the processivity of the helicase [9–11]; an AAA+ domain, which contains two insertions with a critical role in DNA unwinding [5,12]; and a C-terminal domain, which in archaea is predicted to contain a HTH (helix–turn–helix) motif [12].

A high degree of structural polymorphism has been reported for the MCM complexes from a variety of organisms. Initial electron microscopy studies carried out on MtbMCM revealed heptameric [13] as well as hexameric [14] single rings. Subsequent work showed the presence of double ring configurations [15–17] as well as helical arrangements [18]. We have recently characterized a novel interaction between MtbMCM and dsDNA, where the DNA wraps around the N-terminal domain tier of a single hexameric MtbMCM assembly (A. Costa, G. van Duinen, B. Medagli, J. Chong, N. Sakakibara, Z. Kelman, S.K. Nair, A. Patwardhan and S. Onesti, unpublished work; Figure 2A). We suggest that this represents an initial site of interaction between the MCM complex and DNA, distinct from the canonical one in which the protein ring encircles ssDNA (Figure 2B), before the loading and activation of the complex to function as the replicative helicase.

Lessons from other hexameric helicases
Although the atomic structure of the functional helicase domain of an MCM protein has not yet been published, a wealth of high-resolution information has become available on various hexameric helicases, which have provided useful models for the characterization of functional elements involved in DNA unwinding [12,19]. Hexameric helicases...
have been classified into four different SFs (superfamilies), SF3–SF6, with MCM belonging to SF6 [20]. The structures of the catalytic domains of SF3 helicases from eukaryotic viruses, such as bovine papillomavirus E1 and simian virus 40 large T-antigen (Figure 1), have provided a model for the mechanism of ATPase-driven translocation of the molecular motor along ssDNA [21,22]. This is mediated by a characteristic pre-sensor 1 β hairpin, common to SF3 and SF6 helicases, which engages in a nucleotide-state-driven spooling motion.

The bacterial Rho termination factor (an SF5 helicase; Figure 1) supplies a model for the mechanism of recognition and loading of the helicase on to the nucleic acid [23,24]. This involves a primary interaction, where the nucleic acid encircles the N-terminal domain of the hexameric ring (recognition; Figure 2A), and a secondary interaction where the nucleic acid is in turn encircled by the helicase ring (loading; Figure 2B), coming in contact with the functional elements of the catalytic domain which mediate ATPase-dependent translocation.

When comparing the primary structure of the eukaryotic/archaeal replicative helicase (MCM) with the bacterial helicase (the SF4 helicase DnaB), a fundamental difference in domain organization can be detected. In fact, the DNA-interacting N-terminal domain in MCM is replaced by a primase-binding domain [25] (Figure 1). The same difference in domain organization can be observed when comparing replicative helicases from eukaryotic viruses and bacteriophages. Large T-antigen and E1 helicases possess an N-terminal OBD (origin-binding domain; Figure 3C) [26,27]. However, the bacteriophage T7 helicase (also belonging to SF4) possesses a C-terminal helicase domain fused to an N-terminal primase domain. Therefore SF4 helicases appear to be designed to work at the replication fork only, whereas SF3 helicases also act as initiators, being responsible for the recognition and melting of the viral origin of replication.

Intriguingly, a gene was identified in Bacillus cereus, encoded within an integrated phage, which contains an archaeal primase sequence fused to a C-terminal MCM helicase module [19]. This finding provided the rationale behind the observation that the AAA+ domain alone is an active helicase, as reported in a biochemical characterization study of the SsoMCM complex [10]. This scenario supports the notion, suggested by a number of in vivo observations, that the N-terminal DNA-binding domain of MCM might have a role during the initiation, and not the elongation step, of DNA replication, perhaps being involved in origin melting.

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**Figure 1** | Primary structure of various hexameric helicases and AAA+ initiators

Domain organization of Bacillus (now Geobacillus) stearothermophilus DnaB (BstDnaB), T7 GP4 (gene 4 protein), simian virus 40 large T-antigen (SV40 LTAg), E. coli Rho termination factor (EcoRho), MthMCM, Pyrobaculum aerophilum Cdc6 (PaeCdc6) and Aquifex aeolicus DnaA (AaeDnaA). SF4 helicases possess an N-terminal primase-interacting domain (in the case of DnaB) or primase domain (in the case of T7 GP4), and a C-terminal helicase domain (ATPase domain). SF3 helicases possess an N-terminal J domain, followed by an OBD and a C-terminal helicase domain (ATPase domain). SF5 helicases possess an N-terminal nucleic acid-interacting domain (oligonucleotide-binding domain; OB fold) and a C-terminal helicase domain (ATPase domain). MCM helicases, which belong to SF6, contain an N-terminal DNA-interacting domain (DNA-binding domain), a central helicase domain and a C-terminal HTH domain. Among AAA+ initiator factors, Cdc6/Orc1 contains an AAA+ domain, followed by a C-terminal HTH domain; DnaA additionally contains a N-terminal dimerization domain.

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**Figure 2** | Various modalities of protein–DNA interactions

(A) Primary interaction between a helicase and nucleic acid, as observed in the crystal structure of the Rho helicase and in the cryo-electron microscopy structure of MCM bound to long dsDNA molecules (A. Costa, G. van Duenen, B. Medagli, J. Chong, N. Sakakibara, Z. Kelman, S.K. Nair, A. Patwardhan and S. Onesti, unpublished work). (B) Secondary type of interaction, where the nucleic acid is encircled by the active helicase ring, ready for ATP-driven translocation. (C) AAA+ initiator proteins form helical assemblies, with the dsDNA wrapping around the outer surface of the filament. (D) Cartoon representation of a duplex DNA wrapping around a generic initiator protein, modulating the degree of supercoiling of the DNA and facilitating its melting.
AAA+ initiator proteins change the degree of supercoiling of origin DNA and melt the duplex

A number of atomic structures of AAA+ initiator factors have recently become available, which have shed light on the molecular basis of origin melting.

One example is the crystallographic structure of the DnaA protein from bacterium *Aquifex aeolicus* bound to an uncleavable ATP analogue [28]. In this state, DnaA forms a helical fibre, where all intersubunit active-site residues are correctly configured for performing ATP hydrolysis. As with all AAA+ initiator proteins, DnaA contains a characteristic ISM (initiator sequence motif), in between the Walker A and Walker B motifs, which is positively charged, faces a large central cavity within the helical fibre and is found packed against the next neighbouring subunit [29]. The C-terminal domain contains a HTH motif which undergoes a conformational change on oligomerization, resulting in its exposure to the outside of the helical assembly. A DNA duplex can be modelled wrapping around the DnaA filament (Figure 2C), using the crystal structure of the C-terminal HTH domain of *Escherichia coli* DnaA bound to origin DNA [30]. This configuration would stabilize positive supercoils at the origin of replication, resulting in the accumulation of negative supercoils outside the protein complex and providing the required topological stress for the melting of the double helix (Figure 2D) [28].

Structural studies of the archael and eukaryotic ORC (origin recognition complex) suggest that the mechanism of origin melting by AAA+ initiator factors might be conserved throughout the three domains of life [31–34]. Striking structural similarities can be detected by comparing the MCM proteins and AAA+ initiators: first, MCM is the only protein among hexameric helicases for which DnaA-like helical fibres have been described [18]; secondly, as with all AAA+ initiators, archaeal MCM proteins possess a predicted HTH C-terminal domain (although whether this domain is involved in DNA interaction is a matter of controversy [10–12]); thirdly, MCM is unique among hexameric helicases as it possesses an insertion (helix 2 insertion) within the AAA+ domain, located in a position similar to the ISM element, which is essential for helicase activity and is believed to have a role in DNA binding [12].
Large T-antigen origin melting

No clear mechanistic model has been proposed for the origin-melting role of viral helicases, such as the large T-antigen. When the crystal structure of a monomer of the large T-antigen OBD bound to dsDNA [26] is modelled on to the hexameric notched-ring configuration determined in the absence of dsDNA [35], a circular arrangement of dsDNA interacting with the C-terminal face of the OBD ring is obtained (Figure 3C), reminiscent of the model suggested for the AAA+ initiators (Figure 2D), with origin DNA wrapping around the OBD, potentially creating a topological tension instrumental in the melting of the duplex.

This type of interaction is supported by NMR studies, which have identified the C-terminal face of the OBD as an essential site of DNA interaction [36], and by electron microscopy observations of the E1 helicase in complex with origin DNA [37]. The model we suggest is in good agreement with a single-particle reconstruction study carried out on the large T-antigen bound to origin DNA [38], although we propose a different interpretation of the electron-density map (as shown in Figure 3B).

In the original publication, the electron-density protrusions emerging from the OBD were interpreted as unwound ssDNA [38]. However, since the protein was incubated with ADP and origin dsDNA, the simplest explanation is that the structure represents a loading intermediate.

Functional evidence for the dual role of the MCM complex

A wealth of functional and structural information suggests that the MCM2–MCM7 complex does not just work as a DnaB-like helicase unwinding the replication fork, but also has an active role during the initiation step of DNA replication [39].

Up to 40 MCM protein complexes per origin of replication have been found bound to chromatin, at sites all around the ORC [40]. This abundance is hard to reconcile with a simple model of a canonical helicase, acting at the replication fork [41]. If the complex is involved in origin melting initiation and in the unwinding of the fork during elongation, the former step could require multiple copies of the MCM complex, whereas the latter step would only require one active helicase assembly per fork.

The first indication that the MCM2–MCM7 complex might possess a dual role comes from genetic experiments, which showed that many MCM thermosensitive mutants had defects in DNA replication initiation, but were capable of completing initiated replication events [42,43], whereas later experiments using thermosensitive degron mutants showed that all six proteins were essential both for the initiation and the elongation phase, suggesting an immediate arrest at the replication fork [44]. These results indicate that the role of the complex in DNA initiation is more critically sensitive to the presence of partially inactive MCM proteins, and that the two different activities, during DNA replication initiation and fork progression, can be decoupled.

Genetic studies have provided a wealth of information on the role of the MCM complex during the initiation step of DNA replication. In particular, DDK (Dbf4-dependent kinase)-mediated phosphorylation of various subunits of the MCM2–MCM7 complex is believed to trigger the switch which starts DNA replication [39]. A point mutation in the S. cerevisiae MCM5 protein is able to bypass the requirement for DDK phosphorylation, resulting in premature firing of the origin of replication [45]. A MthMCM mutant bearing the equivalent residue change was characterized in terms of biochemical and structural behaviour: the mutant appeared to retain wild-type levels of ATP hydrolysis and DNA binding, but displayed a reduced helicase activity [46], and the crystal structure showed a conformational change involving the N-terminal domain [8]. These findings explain the reduced efficiency of origin firing, but not the premature entry in S-phase reported from S. cerevisiae in vivo experiments [39]. One possible explanation is that this mutation interferes with both the postulated initiator as well as the helicase functions of the MCM protein complex, activating the former and attenuating the latter. This would represent further evidence of the fact that the two activities of the MCM complex may be decoupled.

Conclusions

It is intriguing to speculate that the similarities in domain organization of the MCM proteins, the viral replicative helicases and the AAA+ initiators are reflected in an active role for this protein complex in assisting origin melting during the initiation step of DNA replication. However, it should be noted that, unlike AAA+ initiator proteins and SF3 helicases, the MCM proteins do not possess any sequence specificity, but rather associate with origin DNA on recruitment as part of the pre-replication complex. We envisage that the cross-talk between MCMs and the AAA+ initiator proteins ORC and Cdc6 might result in the complete unwinding of the replication bubble and the concomitant loading of the MCM helicase at the fork.

References

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