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News and Views, Cell Cycle: Boskovic et al., Molecular architecture of the recombinant human MCM207 helicase in complex with nucleotides and DNA

Title: MCM2-7 Conformational Changes in Presence of DNA

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Main text:

Eukaryotic replication requires a high degree of spatial and temporal coordination. The mini-chromosome maintenance helicase MCM2-7 is a key factor during replication initiation and elongation[1]. During the G1 phase, MCM2-7 is recruited to the origin of replication by the origin recognition complex and two additional factors, cell division cycle 6 (Cdc6) and Cdt1 (Figure). Together these proteins form the pre-replication complex (preRC). Two MCM complexes are recruited sequentially into the preRC to form an inactive double hetero-hexamer and a ring around the double-stranded DNA [2]. MCM proteins belong to the AAA+ ATPase superfamily using ATP for DNA unwinding. In addition to the C-terminal ATPase domain, they comprise an N-terminal domain with an oligonucleotide/oligosaccharide-binding fold responsible for interaction with dsDNA and ssDNA. At the end of the G1 phase the pre-RCs bound to the origins are licensed. In the subsequent S phase formation of new preRCs is not possible in order to avoid re-replication. The MCM helicases in existing preRCs are then activated by Cdc45, the GINS complex, cyclin-dependent kinase 2 and other factors, leading to recruitment of the DNA polymerase machinery (Pol). Subsequently, MCM2-7 helicase migrates together with Cdc45 and the GINS hetero-tetramer in front of the replication fork on the leading strand to unwind the dsDNA, and Pol synthesizes the complementary strand.

During replication initiation and activation MCM2-7 helicase undergoes a series of conformational rearrangements regulated by binding of nucleotides, DNA, other replication factors as well as phosphorylation. To bind DNA, MCM2-7 must open to capture dsDNA and reclose, a process that requires ATP binding. DsDNA is suggested to enter MCM2-7 via the gate formed by Mcm2 and Mcm5 subunits [3]. Several EM structures of MCM hexamers from different species indicate that two major arrangements are possible: (1) a closed ring was observed in yeast [3] and in *Drosophila* [4] and (2) a spiral 'lock-washer' conformation. The latter was predominant in *Drosophila* [4] and in *Encephalitozoon cuniculi* MCM complexes in presence of the non-hydrolysable nucleotide analogue ATPγS [5]. A study in this issue by Boskovic, Montoya *et al* now uses fully recombinant active human MCM2-7 hexamer which was produced in insect cells [6]. The authors show the stabilization of this highly flexible complex by addition of ATPγS. Significant conformational changes in MCM2-7 are observed upon binding of single-stranded DNA [6]. The 3D reconstruction of MCM2-7 with ATPγS shows a planar left-handed ring where the N-terminal domains form a closed circle while the AAA+ domains have a gap, presumably between subunits Mcm2 and Mcm5. In the presence of ssDNA and ATPγS, the N- and C-terminal domains rotate with respect to each other resulting in a rather elongated cylindrical conformation of the helicase [6]. At

the same time, the Mcm2-5 gate in the C-terminal domains remained only slightly opened. Binding of GINS and Cdc45 to MCM2-7 is expected to close this gap in the Mcm2-5 gate such that the leading strand remains in the centre and the lagging strand excluded from the complex. In the Cdc45-MCM-GINS complex a spiral right-handed arrangement of the then fully active MCM2-7 helicase has been reported[7].

ATP hydrolysis and concomitant conformational changes in MCM2-7 are essential for double hexamer formation. In the cryo-EM structure of the yeast MCM double hexamer the two MCM complexes were tilted and twisted [2]. This could introduce a kink of the dsDNA in the constricted central channel, at the hexamer interface formed by the N-terminal domains of MCM. Melting of dsDNA at the origin could be achieved by this. In the double hexamer, several AAA+ ATPase domains adopt an unproductive arrangement with low nucleotide occupancy [2], consistent with low activity observed for the double hexamer.

The molecular mechanism of MCM2-7 activation including DNA melting, exclusion of the lagging strand from the MCM rings, binding of Cdc45 and GINS to both rings and separation of the double hexamer remains poorly understood and will have to await future studies. Clearly, MCM helicase activation is cooperative, leading to simultaneous activation and bi-directional origin firing events. The availability of recombinant MCM helicase, achieved in this study [6], and of its interaction partners, will allow probing and challenging the molecular mechanisms by mutational studies, thus greatly advancing our understanding of origin licencing and activation.

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Figure Legend:

Model for MCM conformational changes during replication initiation. ORC·Cdc6 recruit Cdt1 and MCM2-7·ATP to the origin. MCM2-7 with bound nucleotide adopts a left-handed ring conformation with an open Mcm2-5 gate. ATP hydrolysis is required for double hexamer formation. MCM2-7 bound to single-stranded DNA adopts a more planar ring. GINS- and Cdc45-binding activates the helicase, inducing a right-handed spiral MCM2-7 conformation.