Seeing is believing
The MCM2–7 helicase trapped in complex with its DNA loader

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Complete DNA replication prior to cell division is essential, so that each daughter cell receives a full genetic complement from the mother cell. DNA duplication is a carefully choreographed process, where numerous proteins synergize to assemble a large DNA replication machinery at replication origins. This assembly reaction is a 2-step process: Helicase loading at a DNA replication origin occurs in G1-phase of the cell cycle, and helicase activation and replisome assembly occurs in early S-phase. The helicase loading reaction is frequently misregulated in cancer, and this misregulation results in genomic instability. On the other hand, it was found that cancer cells are hypersensitive to helicase loading inhibitors,1 indicating a potential route for treatment. Studying helicase loading will provide insights into a biological process of major importance, and equally, this work could spur the development of new anticancer therapeutics.

The eukaryotic replicative helicase MCM2–7 is a hetero-hexamer, the 6 distinct subunits of which assemble into a ring shaped complex. The central channel of the ring is wide enough to accommodate double-stranded DNA. Loading of the helicase onto DNA, also termed pre-replicative complex (pre-RC) formation, is facilitated by the 6-subunit origin recognition complex (ORC), as well as Cdc6 and Cdt1. ORC recognizes the DNA replication origin and, once these 2 complexes meet, we expect that helicase loading starts. Although we have only a limited understanding about pre-RC formation, we know that 2 multi-step reactions must occur: first, the MCM2–7 ring needs to be opened; double-stranded DNA must be inserted; and then the ring must be closed again. Second, during helicase loading, 2 MCM2–7 hexamers need to be assembled into a double-hexamer, with the N-terminal domains of each hexamer interacting with each other.2,3 Importantly, the final product is an MCM2–7 double-hexamer that can slide along on DNA in an ATP hydrolysis-independent manner.

Figuring out how the helicase loader interacts with the large helicase is instrumental to understanding the mechanism of helicase loading and could also inform us about the MCM2–7 double-hexamer formation process. Structural information is key to analyzing complex reactions. Unfortunately, crystallographic approaches for the analysis of dynamic and flexible complexes are usually very challenging. However, cryo-electron microscopy (cryo-EM) has the potential to visualize the stable envelope of a protein complex and—in combination with specific labeling approaches—this technique can even pinpoint the location of individual subunits within the EM structure.

We have recently employed cryo-EM to study helicase loading.4 By using ATPγS, an ATP analog that can be only very slowly hydrolyzed, we successfully captured a helicase loading intermediate. This complex, which contains all 14 pre-RC polypeptides, reveals for the first time how the eukaryotic helicase interacts with its loader (Fig. 1A). Importantly, we found that the C-terminal section of MCM2–7 latches onto the ATPase domains of the helicase loader, ORC-Cdc6, thereby defining the main surfaces of this interaction. In this configuration the N termini of MCM2–7 remain free to interact. This finding is particularly satisfying, since we know that following ATP-hydrolysis a second MCM2–7 hexamer is recruited to the MCM2–7 N terminus5—therefore, the structure also suggests a mechanism for double-hexamer formation.

How the initial recruitment of Cdt1-MCM2–7 by ORC-Cdc6 is realized has been unclear for a long time. Now, we and others found that Mcm3 is essential for this process.6,4 We have shown that Mcm3 interacts with purified Cdc6, suggesting that the 2 proteins facilitate recruitment of the helicase by the helicase loader. Similarly, John Diffley’s group found that Mcm3 activates the ORC/Cdc6 ATPase.6 However, within the cryo-EM structure, Mcm3 is only partially engaged with ORC-Cdc6 (Fig. 1A). One possibility could be that this interaction is flexible, and therefore not visible in the structure. Another interesting hypothesis is that an initial contact is made between Mcm3 and Cdc6, but that a subsequent reorganization of the complex separates Mcm3 and Cdc6 again.

On the other hand, 3 out of the 6 Mcm subunits appear strongly engaged with ORC-Cdc6—namely Mcm6, Mcm2, and Mcm5. Interestingly, Mcm2 and Mcm5 have been suggested to form a gate for DNA entry into the ring.2 Thus,
the observed interactions of ORC-Cdc6 with Mcm6, Mcm2, and Mcm5 could have a role in DNA loading (Fig. 1B). Importantly, upon inspection of the cryo-EM structure, we also noticed DNA entering into the ORC-Cdc6 complex and traversing partially into the MCM2–7 ring (Fig. 1B). The DNA exit site near the MCM2–7 N termini was not defined, suggesting that only partial DNA loading occurs prior to ATP hydrolysis. Clearly, the process of DNA loading and the role of the putative Mcm2-Mcm5 gate needs to be addressed in more detail.

The general architecture of the pre-RC complex, with DNA entering through a central channel of ORC-Cdc6, displays structural and functional similarity to the RFC-PCNA-DNA complex. RFC is a pentameric protein complex that loads the trimeric PCNA ring onto DNA in an ATP hydrolysis-dependent manner. Once PCNA has been loaded onto DNA it serves as a polymerase processivity factor during DNA synthesis. Interestingly, RFC encircles DNA in a similar manner to ORC-Cdc6, and both RFC and ORC-Cdc6 load ring-shaped complexes onto DNA. So this is another fascinating case of convergent evolution where two ATPase complexes have evolved in a similar manner to load toroidal protein complexes onto DNA.

Future work will be needed to define the mechanism of MCM2–7 ring opening, which is still mysterious. Cryo-electron microscopy will be of importance to solve this next puzzle.

References

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