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Building a double hexamer of DNA helicase at eukaryotic replication origins

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The Mcm2-7 complex, the catalytic core of the eukaryotic replicative DNA helicase, undergoes a complex series of transformations at origins of DNA replication. During G1 phase, it is loaded around double-strand DNA at origins as an inactive double hexamer, which is subsequently remodelled *in situ* to activate the helicase during S phase. Work in this issue of *The EMBO Journal* sheds light on the role of the Cdt1 protein in generating Mcm2-7 double hexamers during the loading reaction.

The chromosome replication machinery in all cells is dependent upon the action of a DNA helicase, which unwinds the parental duplex DNA to form the single-strand DNA template required by DNA polymerases. Replicative DNA helicases are hexameric rings that encircle DNA and use the energy of ATP hydrolysis to track along one strand. Given the apparently simple nature of this task, the replicative DNA helicase in eukaryotic cells is remarkably complex and exquisitely regulated. Unlike most helicases, it is completely inactive when first loaded around its DNA substrate. At each origin of DNA replication, multiple helicase complexes are loaded at the start of the cell cycle, and activated *in situ* only when cells subsequently enter S phase. In this way, each origin fires just once per cell cycle, ensuring that cells make a single copy of each chromosome (Labib, 2010).

The catalytic core of the replicative helicase in eukaryotes is a hetero-hexameric ring of the related Mcm2-7 proteins, originally identified in a yeast screen for defects in *m*inichromosome *m*aintenance. Work in the last few years has indicated that the loading and activation of the Mcm2-7 complex involve a dramatic series of transformations that are still understood very poorly. Away from DNA, the Mcm2-7 complex forms a single hexameric ring, but a head-to-head double hexamer is produced by the loading mechanism at origins, as indicated first by work with budding yeast (Evrin *et al*, 2009; Remus *et al*, 2009). The loaded Mcm2-7 double hexamer stably encircles double-strand DNA but does not yet unwind it. During the subsequent initiation of replication, the double hexamer is activated by an enigmatic mechanism that requires association with two other factors, the Cdc45 protein and the GINS complex, and that is driven by Cdc7 kinase and cyclin-dependent kinase (CDK; Labib, 2010). It seems likely that the double hexamer is split during initiation, so that each Mcm2-7 single hexamer can form the core of one of the two active Cdc45–MCM–GINS helicase complexes (Gambus *et al*, 2006; Moyer *et al*, 2006; Yardimci *et al*, 2010; Figure 1). The head-to-head nature of the inactive double hexamer would ensure that the two active Cdc45–MCM–GINS helicases are properly orientated to support the establishment of two bidirectional DNA replication forks. The Mcm2-7 rings might need to be further remodelled during initiation to encircle single-strand rather than double-strand DNA, as recent work found the active Cdc45–MCM–GINS helicase is stably associated with just one of the two parental DNA strands at each replication fork (Fu *et al*, 2011).

The study by Takara and Bell (2011) provides new insight into the first steps in this elaborate series of transformations, namely the mechanism that loads two Mcm2-7 complexes at origins to form a head-to-head double hexamer around double-strand DNA. Origins of replication represent chromosomal sites bound by the six-subunit origin recognition complex (ORC), which together with the Cdc6 and Cdt1 proteins plays a central role in loading the Mcm2-7 complex. Previous work indicated that Cdt1 recruits Mcm2-7 to ORC, and Takara and Bell provide fresh evidence that association of Cdt1 with Mcm2-7 promotes recruitment of both factors to origins in budding yeast. Following recruitment of Mcm2-7 to the origin, Cdc6 and ORC are thought to mediate loading of the Mcm2-7 ring around DNA, by analogy with other AAA+ ATPases such as the clamp loader complexes that load the PCNA ring around DNA at replication forks. The Mcm2-7 complexes are always loaded as head-to-head double hexamers, and a key question in the last couple of years has been how ORC, Cdc6 and Cdt1 are able to achieve this feat.

Several mechanisms have been envisaged, such as the presence at origins of two neighbouring ORC complexes in opposite orientations, with each ORC recruiting a single Cdt1–MCM complex to form a double hexamer. Alternatively, a single ORC might repeatedly load single Mcm2-7 complexes around DNA, and these might only remain stably loaded if they encounter another complex in the opposite orientation and spontaneously form a double hexamer. The data described by Takara and Bell support a third model that neatly explains how a single ORC could consistently load a head-to-head double hexamer of Mcm2-7 proteins (Figure 1). Earlier work from the same group showed that the budding yeast Orc6 subunit has two separable domains that can bind Cdt1, although it remained unclear whether this really represented the ability of Orc6 to bind two separate Cdt1 molecules, as opposed to binding one Cdt1 molecule in two places (Chen *et al*, 2007). Subsequent work showed that CDK phosphorylation of Orc6 inactivated one of these two sites and reduced

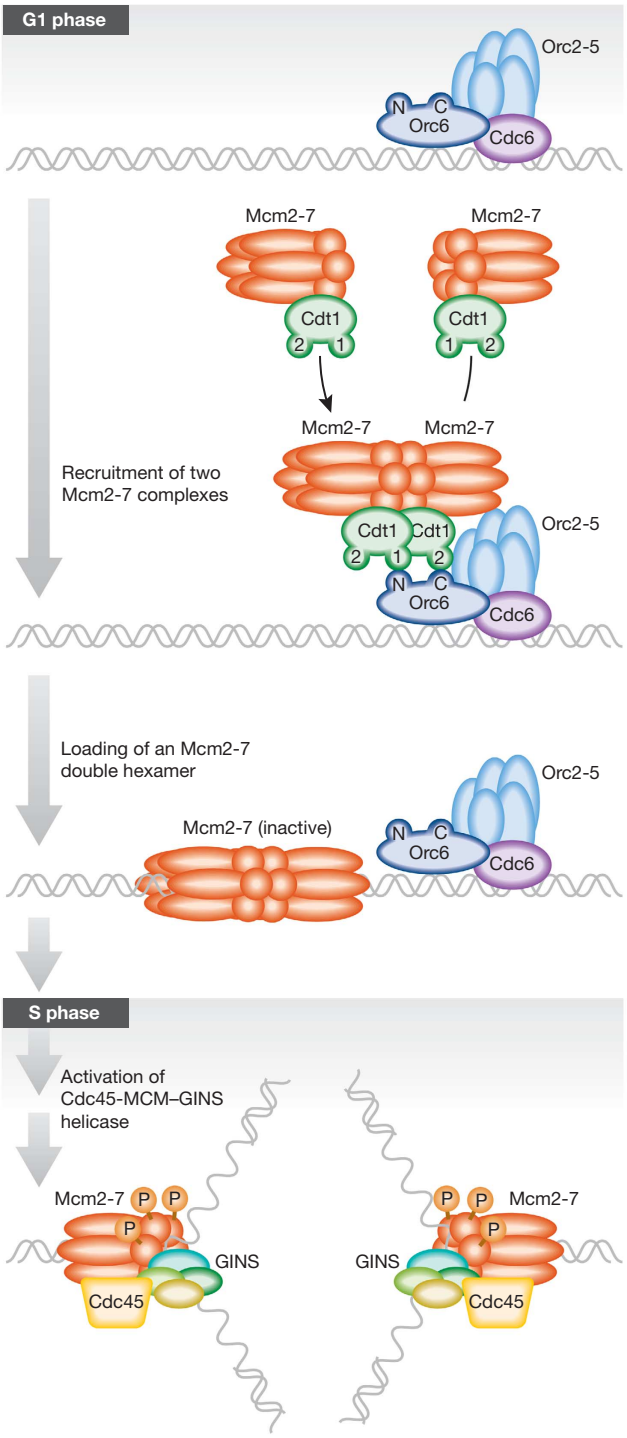

Figure 1 A model to explain how single hexamers of Mcm2-7 proteins are assembled into double hexamers at DNA replication origins. Activation of the Mcm2-7 double hexamer to form the Cdc45-MCM-GINS helicase is still understood poorly.

recruitment of Mcm2-7 by about 50%, which was however sufficient to block loading almost completely (Chen and Bell, 2011). The new evidence by Takara and Bell (2011) now indicates that Cdt1 has two sites that mediate its recruitment to origins, and the authors argue that these can associate with the two Cdt1-binding sites in Orc6 (Figure 1). By reconstituting *in vitro* the recruitment of Cdt1–MCM to ORC onto origin DNA, the authors show that more than one Cdt1 molecule can be recruited to the origin, even under conditions where the origin DNA used should only support binding of a single ORC complex. These data support the notion that two Cdt1–MCM complexes are recruited to a single ORC, and the relative orientation of the two binding sites for Cdt1 on Orc6 might serve to ensure that the two Mcm2-7 complexes are properly orientated to promote formation of a double hexamer. Consistent with this view, a truncated version of Cdt1 lacking one of the presumed binding sites for ORC is less efficient at recruiting Mcm2-7 to the origin, and cannot

support stable loading of a double hexamer (Takara and Bell, 2011).

In addition, the authors identify a novel role for the N-terminal domain of Cdt1 in helicase activation. Removal of this region still allows Cdt1 to support the loading of an apparently stable double hexamer of Mcm2-7, which can also be efficiently phosphorylated by Cdc7 kinase; however, subsequent recruitment of Cdc45 and GINS is very inefficient and replication is largely blocked. Overall, these data suggest that budding yeast Cdt1 plays multiple roles during the loading reaction, helping to ensure that a stable double hexamer of Mcm2-7 is loaded around origin DNA, in a conformation that is compatible with the subsequent steps of the initiation process.

It remains to be seen whether the loading reaction in other species also involves recruitment of two Cdt1–MCM complexes to a single ORC via interaction with Orc6. Recent work indicated that the *Xenopus* Mcm2-7 complex is loaded as a double hexamer like its yeast counterpart (Gambus *et al*, 2011), and it seems likely that the loading reaction in most if not all eukaryotes will share the same basic features. The last couple of years have brought a series of exciting new insights into the initiation of chromosome replication in eukaryotes, and it should be just as fascinating in the future to determine the complex mechanisms by which the inactive double hexamer of Mcm2-7 around double-strand DNA at origins is transformed into an active Cdc45–MCM–GINS helicase at replication forks.

Conflict of interest

The authors declare that they have no conflict of interest.

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