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Pumps, paradoxes and ploughshares: mechanism of the MCM2–7 DNA helicase

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In eukaryotes, numerous lines of evidence have coalesced into a convincing case that the MCM2–7 complex – a heterohexameric ATPase – is the replicative DNA helicase. However, almost nothing is known about how this enzyme functions in a cellular context. Some models for the mechanism of the MCM2–7 helicase envision that it translocates along single-stranded DNA (ssDNA), whereas, more recently, it is has been suggested that it pumps double-stranded DNA (dsDNA) through its central channel. In particular, one model in which a double hexamer of MCM2–7 pumps dsDNA towards the hexamer interface and extrudes ssDNA laterally as a result of torsional strain is gaining popularity. Here, we discuss existing models and propose a new variation in which a single hexamer is the functional unit of the helicase. Duplex DNA is pumped into MCM2–7 and, as it emerges from the complex, a rigid protein that we term the 'ploughshare' splits the duplex.

Introduction

To enable timely duplication of their large genomes, eukaryotic cells initiate DNA replication from multiple sites called origins. Each origin gives rise to two replication forks that move in opposite directions. The replicative DNA helicase is a key component of the replication fork because it is required to unwind DNA ahead of DNA polymerase. Although there is now agreement that MCM2–7 (mini-chromosome maintenance) is likely to be the replicative DNA helicase in eukaryotic organisms, the mechanism by which it unwinds DNA remains a mystery. The assumption that MCM2–7 unwinds DNA by translocating along single-stranded DNA (ssDNA) has been challenged by the more recent idea that this enzyme might unwind DNA by translocating along duplex DNA. A compelling proposal is based on the SV40 large T-antigen DNA helicase, which is thought to function as a double hexamer that pumps double-stranded DNA (dsDNA) towards the hexamer–hexamer interface, causing unwinding (see later). However, this model raises potential complications for the establishment of sister-chromatid cohesion in addition to replication termination. Therefore, we propose a variation of the T-antigen model in which duplex DNA is pumped through a single hexamer of MCM2–7 at each replication fork. A proteinaceous pin or 'ploughshare' that splits the DNA as it emerges from MCM2–7 achieves the unwinding.

MCM2–7: a prime candidate for the eukaryotic replicative DNA helicase

MCM2–7 is a hexameric protein complex found in all eukaryotic organisms that is composed of six different, but highly related, proteins called MCM2, MCM3, MCM4, MCM5, MCM6 and MCM7 [1]. Over the past decade, a large body of indirect evidence has accumulated that strongly argues that MCM2–7 is the eukaryotic replicative DNA helicase (for review, see Ref. [2]). First, as seen for other replicative DNA helicases, each of the six MCM subunits contains an AAA+ ATPase motif [3–5]. Furthermore, electron microscopy (EM) studies indicate that, like other replicative DNA helicases, MCM2–7 adopts a hexameric ring-like structure [6,7]. Indeed, the EM and crystal structures of the N terminus of an archaeal MCM reveal a hexameric complex with a positively charged central channel with an internal diameter of 22 Å, which is large enough to accommodate ssDNA or dsDNA [8]. Second, although the purified MCM2–7 complex is inactive as a DNA helicase, complexes consisting of MCM4, MCM6 and MCM7 exhibit helicase activity in several eukaryotic species, but processivity of this complex is low [9–11]. In archaea, the MCM homohexamer unwinds DNA with moderate processivity [12–14]. The reason why only an MCM4–6–7 sub-complex is found to exhibit helicase activity *in vitro* is presently unclear, but might reflect the ability of this complex to assemble onto DNA in the absence of the many factors that are required *in vivo* to activate the holocomplex. Third, in yeast, MCM4 and MCM7 travel away from origins with the same kinetics as DNA polymerase, indicating that MCM2–7, or at least an MCM4–6–7 sub-complex is present at the replication fork [15]. Fourth, in yeast, at least five out of six MCM2–7 subunits are required for replication-fork progression in S phase [16]. Similarly, in *Xenopus* egg extracts, inactivation of the MCM2–7 complex at any time after initiation arrests DNA replication [17,18]. Fifth,

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inactivation of MCM2–7 in *Xenopus* egg extracts inhibits chromosome unwinding at the replication fork both early and late in S phase [17,18].

At present, the only experiments that might disagree with the MCM2–7 helicase hypothesis are immunofluorescence studies in vertebrate cells because they fail to show co-localization between the MCM2–7 complex and sites of ongoing DNA replication (the 'MCM paradox'; see Box 1). However, as noted, MCM2–7 has recently been directly implicated in chromosome unwinding during DNA replication in *Xenopus* egg extracts [17,18], one of the systems in which the MCM paradox has been observed, suggesting the MCM paradox might be a red herring. Explanations for the MCM paradox that are compatible with MCM2–7 being the replicative DNA helicase are outlined in Box 1.

Recently, MCM8, which is highly homologous to the six subunits of the MCM2–7 complex, has been identified [19]. MCM8 exhibits DNA-helicase activity *in vitro*, and this activity is required for normal fork progression [20]. However, in the absence of MCM8, origin unwinding is unaffected and the rate of DNA replication is reduced only ~50%. Moreover, MCM8 is not conserved among eukaryotes. Therefore, MCM8 is unlikely to be the primary replicative DNA helicase. In summary, although conclusive proof is lacking, there is now extensive evidence that MCM2–7 is the primary engine that unwinds the chromosome during eukaryotic DNA replication. The remainder of this article is based on this view.

Box 1. The MCM paradox: a red herring?

If MCM2–7 is the replicative DNA helicase, it should co-localize with sites of DNA synthesis in living cells. Although this is the case in yeast [15], immunofluorescence studies in vertebrate systems have failed to detect co-localization of MCM2–7 subunits and DNA-replication factories (for review, see Ref. [30]). This discrepancy is known as the 'MCM paradox' [63]. A likely explanation for the paradox comes from the fact that the number of chromatin-bound MCM2–7 complexes far exceeds the number of origins of DNA replication. Thus, in yeast, *Xenopus* egg extracts and human cells, chromatin-bound MCM2–7 complexes exceed the number of active origins by 10–40-fold (see Ref. [52] and references therein). Furthermore, in yeast, there is evidence that a single origin-recognition complex (ORC) normally loads multiple MCM2–7 complexes [48]. In *Xenopus* egg extracts and perhaps in human cells, these MCM2–7 complexes are distributed over a large region of DNA [52,64,65]. Although all the chromatin-bound MCM2–7 complexes are probably functional, only a few seem to be activated as DNA helicases in S phase [52,64]. Given the presence of many dispersed MCM2–7 complexes on chromatin, there are several resolutions to the MCM paradox. First, MCM2–7 complexes might unwind DNA by binding to chromatin at a distance from the site of DNA synthesis [30] (see Figure 1b in the main text). The distance between MCM2–7 and the replication complex would have to be at least several kilo-base pairs to appear separate in light microscopy, the resolution of which is 0.2-μm, especially considering the compaction of DNA into chromatin. A more plausible explanation is that the fluorescence signal from the active MCM2–7 complexes located at the replication fork is too weak compared with the signal emanating from the many latent complexes bound elsewhere. Indeed, the absence of significant MCM fluorescence anywhere near replication sites can be explained if replication initiation leads to removal of all but the active MCM complexes from the replicon [63].

What is the function of the large excess of MCM2–7 complexes? Experiments using *Xenopus* egg extracts show that DNA replication is highly efficient even when the number of MCM2–7 complexes is severely reduced [52,54]. Therefore, excess MCM2–7 is not essential for DNA replication in this system. Instead, it has been proposed that excess MCM2–7 complexes might be activated late in S phase as cells approach mitosis, particularly at loci where long stretches of unreplicated DNA persist [52,66]. In yeast, the situation might be different because a mutation in ORC that prevents the repeated loading of MCM2–7 onto origins is lethal [48].

Activation of MCM2–7: a complex process involving many factors

One of the most extraordinary features of the MCM2–7 helicase is the complex machinery that is required for its assembly onto chromatin and for its activation [21,22] (Box 2). Briefly, the MCM2–7 complex is loaded onto origins of DNA replication in the G1 phase by at least three factors: ORC, Cdc6 and Cdt1. However, the helicase activity of the complex seems to be inactive at this stage. At the G1/S transition, at least eight more factors, including two protein kinases, are needed to activate the helicase activity of MCM2–7 and, thereby, enable origin unwinding. Among these, the initiation factors GINS and Cdc45 are particularly interesting because they are the last known proteins to be recruited before origin unwinding [23–26]. In addition, both GINS and Cdc45 are required for the elongation phase of DNA replication [17,23,27], and both seem to exist in a physical complex with the MCM2–7 complex on chromatin [25,28,29]. As such, Cdc45 and GINS are attractive candidates for factors that might cooperate with MCM2–7 during DNA unwinding. In support of this view, antibodies against Cdc45 block the activity of the replicative DNA helicase when it is uncoupled from the replication fork in *Xenopus* egg extracts [17].

Possible models for DNA unwinding by MCM2–7

The steric-exclusion model

In the 'steric-exclusion model', the MCM2–7 complex translocates along ssDNA in the manner of *Escherichia coli* DnaB, and uses steric exclusion of one strand from the core of the helicase to unwind DNA [10,11]. In this view, the MCM2–7 complex would become engaged with ssDNA upon pre-RC assembly in G1 (Figure 1a).

The rotary-pump model

In the rotary-pump model, MCM2–7 translocates along dsDNA during DNA unwinding. Several variations of this idea exist. The first is specifically motivated by the high abundance of MCM2–7 complexes on chromatin [30]. In this case, two cadres of immobilized MCM2–7 complexes located at a distance from the replication fork coordinately pump DNA in opposite directions. The topological underwinding of the DNA that results from pumping leads to strand separation at the fork (Figure 1b). Because the MCM2–7 complex is located at a distance from the site of replication, this model would explain the MCM paradox.

The T-antigen model

In a mechanism related to the rotary-pump model, MCM2–7 might function in a manner analogous to large T antigen (T-ag; Figure 1c), the helicase that unwinds DNA during SV40 virus DNA replication. A seminal

Box 2. Chromatin loading and activation of the MCM2–7 complex requires many factors

The first factor that binds to DNA-replication origins is the origin recognition complex (ORC), a heterohexameric ATPase that binds to chromatin during most or all of the cell cycle. Three of the subunits of ORC – ORC1, ORC4 and ORC5 – contain AAA+ ATPase motifs. ORC recruits two additional polypeptides called Cdt1 and Cdc6. Cdc6 is an AAA+ ATPase, whereas Cdt1 contains a coiled-coil domain but no other known functional motifs. Together, ORC, Cdc6 and Cdt1 recruit MCM2–7 to the origins. MCM2–7 is inactive when it is bound to the pre-replication complex (pre-RC) and its putative helicase activity is only activated at the G1/S transition after an elaborate array of additional factors have acted. Although the precise order of events in pre-RC activation is not clear, a possible model is as follows. This model of pre-RC activation is a composite that is based on experiments in yeast and vertebrates, and on the assumption that the mechanism is fundamentally conserved in all eukaryotes (Figure I). Once cells enter

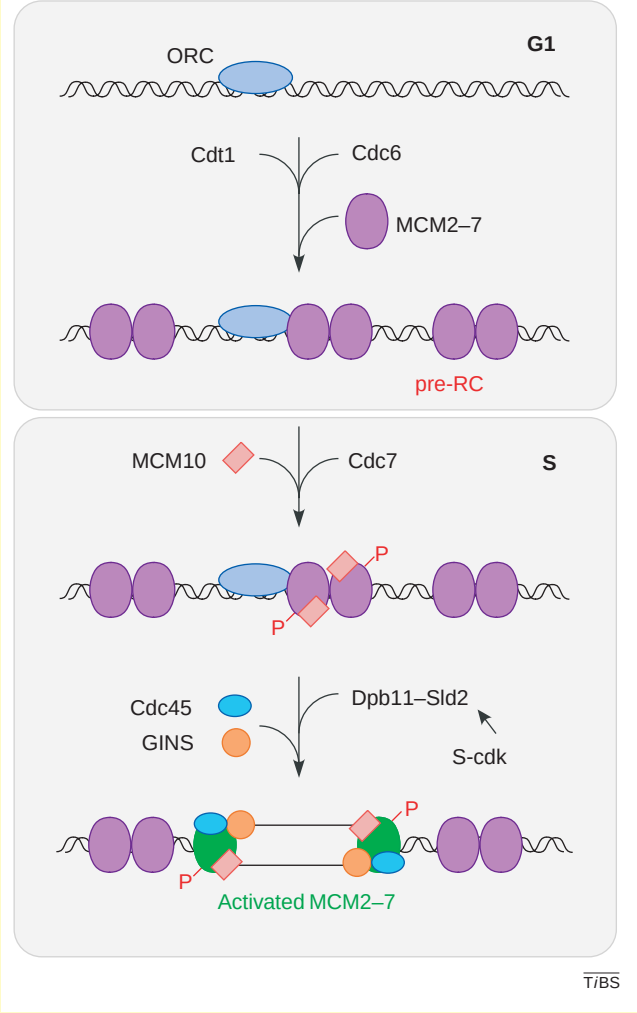

Figure I. Mechanism of helicase activation in eukaryotes.

S phase, MCM10 – a ssDNA-binding protein (which was isolated in the same genetic screen as MCM2–7 but is otherwise unrelated) – and the protein kinase Cdc7 cooperate to cause phosphorylation of one or more of the MCM2–7 subunits [59,60,67–70]. Subsequently, S-phase-specific cyclin-dependent kinase (S-cdk) phosphorylates Sld2, which enables it to bind to Dpb11, a protein with multiple BRCT motifs [71,72]. Together, Dpb11 and Sld2 enable the chromatin binding of Cdc45 and a heterotetramer called the GINS complex [24,73–75]. Cdc45 and GINS contain no known functional motifs, but EM studies suggest that GINS forms a ring structure [73]. Finally, after GINS and Cdc45 have loaded, the origin is unwound and polymerases are recruited.

feature of this model is that a T-ag double hexamer is the functional helicase unit. Duplex DNA is pumped into each T-ag hexamer and ssDNA is extruded from the hexamer interface. In support of this model, EM images of T-ag engaged in DNA unwinding reveal a proteinaceous structure bound to duplex DNA from which two loops of ssDNA emanate [31,32]. In addition, T-ag double-hexamer formation strongly stimulates DNA unwinding [33–35]. Recent crystal structures of the helicase domain of T-ag suggest the following mechanism for DNA unwinding [36,37]. Nucleotide-dependent movements of a β-hairpin extending into the central channel of T-ag pulls DNA into the channel, and rotation of one section of the channel relative to another causes DNA unwinding. A large, positively charged chamber inside the hexamer would enable strand separation to occur. Finally, positively charged channels located at the interfaces of each of the six subunits would enable egress of ssDNA and, thereby, prevent re-annealing. Interestingly, potential ssDNA channels are present in the archaeal MCM complex [8]. It has been suggested that, like T-ag, MCM complexes might function as a double hexamer [22,38].

The proposed ploughshare model

Finally, we propose a new model in which MCM2–7 might unwind DNA by translocating along duplex DNA as a single hexamer (Figure 1d). Because DNA tracking by a single hexamer cannot induce sufficient torsional strain to unwind DNA, strand separation is achieved by a protein that sterically separates the two strands as they emerge from the helicase. We refer to this as the 'ploughshare model' (ploughshare: a sharp steel wedge on a plough that cuts the slice of earth at the bottom of the furrow). The 'ploughshare' protein must be located where DNA exits from the helicase because, if it was located where the DNA enters, subsequent re-annealing within the pump would be possible. For several prokaryotic DNA helicases, including RecG [39], PcrA [40] and UvrB [41], it is thought that DNA unwinding occurs by passing the duplex DNA over a 'pin'. A particularly striking example is seen in the RecBCD-repair helicase, whereby DNA is denatured when it is pulled by the RecB and RecD motor domains over a β hairpin that protrudes from the surface of the RecC subunit at the entrance of the RecBCD complex [42]. Unlike these examples, we envision that the pin is dragged behind MCM2–7, much as a plough is pulled by a tractor. We note that T-ag could be seen to fit the ploughshare model because it seems to contain a pump and a ploughshare (the positively charged channels). Thus, strand separation would result from the pump forcing ssDNA through the channel and not necessarily from unwinding within the channel. If the ploughshare idea applies to T-ag, the only real difference between the T-antigen model (Figure 1c) and the ploughshare model (Figure 1d) is whether the helicase dimerizes or not.

Which model is correct?

As noted recently [43], present biochemical experiments do little to distinguish between the models described here because the MCM2–7 holocomplex is largely inert, whereas the MCM4–6–7 sub-complex interacts with

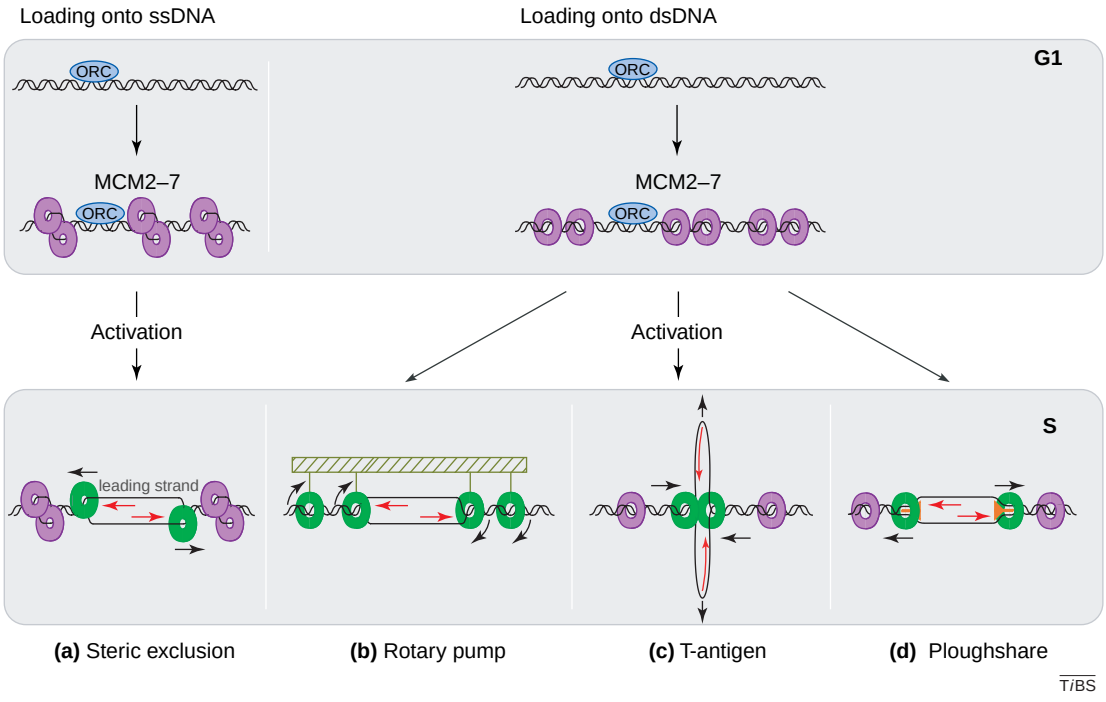

Figure 1. Four models for helicase activity of MCM2–7. **(a)** The steric-exclusion model, whereby a helicase translocates on a ssDNA while displacing the complementary DNA strand by steric exclusion. **(b)** The rotary-pump model, whereby two groups of distributed helicases encircling dsDNA rotate the DNA in opposite directions, causing torsional stress that unwinds duplex DNA. The hatched box denotes a nuclear matrix to which the MCM2–7 complexes are attached. **(c)** The T-antigen model, whereby two physically connected helicases that encircle dsDNA pump DNA towards the helicase interface. The resulting single strands of DNA are extruded laterally from positively charged channels. **(d)** The ploughshare model, whereby a helicase encircles and translocates on dsDNA. Unwinding of duplex DNA is achieved by a rigid 'ploughshare' located at the trailing edge of the helicase that is inserted between DNA strands and, thus, cleaves the duplex. The orange object is the ploughshare. It is assumed that multiple pairs of MCM2–7 hexamers (purple) are loaded onto each replicon in the G1 phase, and that only one of these pairs (green) is activated as a DNA helicase. The exception is the rotary-pump model (b), in which all the chromatin-bound MCMs participate in unwinding. Red arrows indicate where leading-strand DNA synthesis takes place in relation to the active helicase. Black arrows indicate the directional or rotational movement of the helicase.

DNA by multiple mechanisms that are compatible with most of the models presented in Figure 1. Thus, MCM4–6–7 can load onto DNA via a 3′-ssDNA tail along which it then translocates. When it encounters a complementary strand, two outcomes are possible [10,11]: (i) if the complementary strand contains a 5′ flap or a bulky moiety such as streptavidin, MCM4–6–7 begins unwinding the duplex DNA, which is consistent with the steric-exclusion model (Figure 2a); (ii) if no 5′ flap is present, MCM4–6–7 can slide onto, and translocate, along the duplex DNA (Figure 2b) – this translocation is an active, ATP-driven process that is consistent with models in which MCM2–7 pumps duplex DNA. Finally, it was also shown that if two MCM4–6–7 assemblies are loaded onto different arms of the same heterologous four-way junction, they coordinately unwind DNA by translocation along dsDNA [43] (Figure 2b). If one imagines that origins of replication contain symmetrical hairpins that load the MCM2–7 complex, the biochemical activity described for dsDNA translocation (Figure 2b) could result in chromosome unwinding by a 'heterologous junction model' [43] (Figure 2c). Considering how many MCM2–7 complexes load onto the chromatin in the G1 phase (Box 1), this model would require formation of numerous hairpins on the chromosome, a process that would be energetically unfavorable. Given the ambiguities in biochemical experiments, we consider largely cell biological criteria in evaluating the merits of each proposal here.

The idea that MCM2–7 unwinds DNA by steric exclusion in the manner of DnaB was initially attractive (Figure 1a). Indeed, ORC emerged as the obvious ortholog to DnaA, the bacterial initiator protein that first melts the origin (OriC), whereas Cdc6 was an attractive candidate for the eukaryotic counterpart to DnaC, the bacterial factor that deposits DnaB onto ssDNA. However, the following considerations suggest that the DnaB model might not be strictly applicable. First, attempts to observe ORC- or MCM2–7-dependent DNA-strand separation in *Saccharomyces cerevisiae* [44] or in *Xenopus* egg extracts (C. Cvetic and J.C. Walter, unpublished results) have been unsuccessful, although a recent report suggests that *Schizosaccharomyces pombe* ORC can destabilize DNA [45]. Second, in eukaryotic cells, where the delay between pre-RC assembly and replication initiation can be many hours, assembling the helicase around ssDNA upon loading would be undesirable because it could lead to premature origin firing or DNA damage resulting from prolonged exposure of ssDNA. The problem of ssDNA exposure is particularly acute because so many MCM2–7 complexes are loaded onto chromatin at each origin of DNA replication (Box 1). Third, in this model, it is unclear why so many factors are needed to activate MCM2–7 after it has been loaded (Box 2).

Several observations suggest that MCM2–7 encircles duplex DNA, as seen on certain DNA templates for MCM4–6–7 *in vitro* (Figure 2b). There are interesting

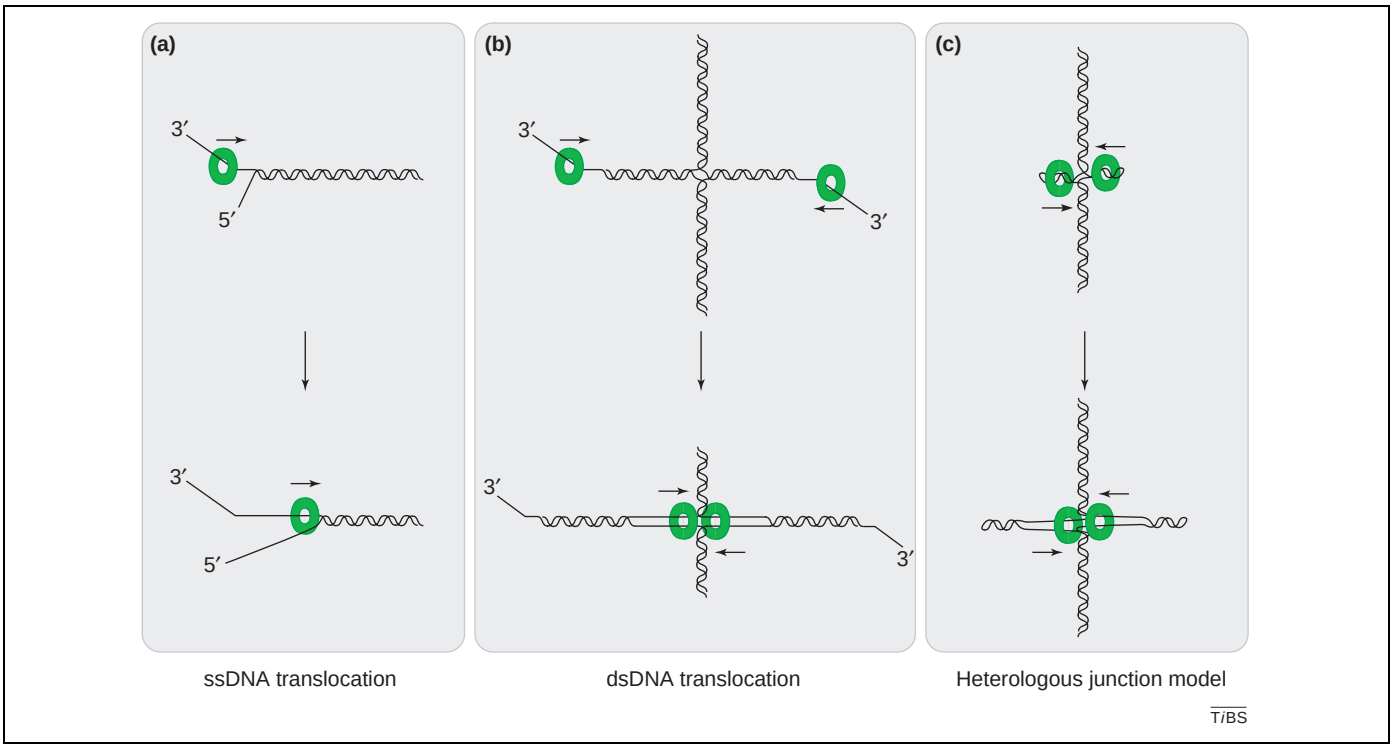

Figure 2. Biochemical activities of the MCM4–6–7 complex. MCM4–6–7 can load onto and translocate along a 3′-ssDNA tail. If it then encounters a 5′ flap **(a)**, it continues translocation and unwinds DNA by exclusion of the 5′-flap-containing strand from the central channel of the helicase. If the translocating MCM4–6–7 encounters a complementary strand without a 5′ flap **(b)**, it slides over the 5′ end and continues translocation along duplex DNA. On DNA substrates that contain a four-way junction with two 3′-ssDNA-loading sites on opposite arms, the two MCM4–6–7 complexes translocating towards each other can unwind the arms. Note that, in this case, MCM4–6–7 unwinds DNA by encircling two non-complementary DNA strands. **(c)** In the heterologous junction model, the origins are proposed to consist of short duplex-DNA hairpins, onto which MCM2–7 is loaded by ORC, Cdc6 and Cdt1. The dsDNA-translocation activity (b) then leads to DNA unwinding.

parallels between the factors that load the MCM2–7 complex onto origins and 'clamp loaders', molecular machines that deposit ring-shaped processivity factors onto dsDNA (for review, see Ref. [22]). For example, the eukaryotic clamp loader, replication factor C (RFC), contains five subunits, all of which are in the AAA+ family of ATPases. Importantly, the MCM2–7-loading machinery contains at least four AAA+ ATPases (ORC1, ORC4, ORC5 and Cdc6). Among these, Cdc6 exhibits sequence and structural similarity to subunits of clamp loaders [46,47]. Another parallel is seen in the activities of MCM2–7 and clamp-loading factors: recent experiments from Bell and colleagues in *S. cerevisiae* indicate that a mutation in the arginine finger of ORC4 that enables ATP binding but not hydrolysis by ORC1 is deficient in recycling the ORC complex for multiple rounds of MCM2–7 loading [48]. Similarly, in the absence of ATP hydrolysis, the RFC complex is able to load PCNA onto dsDNA but is unable to dissociate [49,50]. Another piece of circumstantial evidence is that, when MCM2–7 is bound to pre-RCs, it is extremely resistant to high ionic strength [51–53]. Because there is no evidence for melted DNA in the pre-RC, it is plausible that the MCM2–7 complex achieves high affinity for DNA by encircling the duplex. If MCM2–7 does indeed encircle dsDNA in G1, we note that it is difficult to envision that it would become associated with ssDNA in S phase because this would involve an awkward mechanism whereby it engages and then disengages from dsDNA.

Among the models that envision MCM2–7 binding to duplex DNA, the rotary-pump model (Figure 1b) represents a creative proposal to account for the large number of MCM2–7 complexes present on the chromatin and their apparent absence from replication foci [30], but it has several drawbacks. First, experiments in *Xenopus* egg extracts have shown that the number of MCM2–7 complexes bound to chromatin can be drastically reduced without compromising the efficiency of DNA replication [52,54], and that only a few of the chromatin-bound MCM2–7 complexes are normally activated in the extract [52]. These experiments indicate that large numbers of chromatin-bound MCM2–7 complexes are not essential to execute DNA unwinding, in contrast to the rotary-pump model. Second, because the two cadres of pumping MCM2–7 complexes are located at a distance from each other, there is no obvious way to prevent topoisomerases from short-circuiting the unwinding mechanism.

The T-ag pumping model addresses the major difficulties of the rotary-pump model by using only two functional helicase units that are closely apposed to one another. Thus, DNA is unwound within the double hexamer such that a topoisomerase cannot intervene. Interestingly, the single MCM protein from *Methanobacterium thermoautotrophicum* seems to be a double hexamer in crystallographic, size exclusion and EM studies, suggesting that this prototypical helicase might function using a T-ag-like mechanism [8,12,14,55]. At present, too little evidence is available to determine whether eukaryotic MCM complexes form single or double hexamers. Taken literally, the T-ag model has several important implications for eukaryotic DNA replication. First, if two MCM2–7 complexes must be physically coupled for each to unwind

DNA efficiently, then termination by one helicase should not lead to its dissociation until the other helicase within the double hexamer has also terminated. Second, there is a problem concerning the mechanism by which sister-chromatid cohesion is established. Upon their replication, sister chromatids are connected to one another by a ring-shaped molecule called cohesin, probably via topological linkage. An attractive mechanism for cohesion establishment is that the replication fork passes directly through the cohesin ring during S phase [56,57]. However, due to the helicase-mediated connection between the two replication forks envisioned in the T-ag model, neither fork could pass through the cohesin ring (Figure 3a). These potential complications do not affect SV40 DNA replication because it uses a single DNA-replication origin and cohesion of sister chromatids is unlikely to occur.

We find the ploughshare model for a single MCM2–7 hexamer (Figure 1d) appealing because (i) it is consistent with data suggesting that the MCM2–7 complex encircles dsDNA, (ii) it does not complicate cohesion establishment, and (iii) it does not run afoul of topoisomerases. In this model, ORC, Cdc6 and Cdt1 deposit the MCM2–7 complex on duplex DNA in an inactive form during the G1 phase. The factors that function at the G1/S transition would have three functions. First, they would melt the DNA on the side of the MCM2–7 complex that lies opposite the eventual direction of movement. Because there is evidence of DNA melting in the G1 phase in yeast cells carrying a mutation in MCM5 that bypasses the need for Cdc7 function [58], we speculate that Cdc7 stimulates origin melting. The ssDNA-binding activity of MCM10 might cooperate with Cdc7 and MCM2–7 in performing this function [59,60]. Second, the 'ploughshare' is inserted into the melted region. To melt DNA, the ploughshare need not encircle one strand of DNA (eliminating the need for another clamp loader); it need only bisect the channel where DNA exits from the MCM2–7 complex, with one strand passing on either side. The ploughshare could be part of the MCM2–7 complex itself, or it might correspond to another protein. Third, the MCM2–7 ATPase motor must be jump-started. Because Cdc45 and GINS load onto origins at the same time and are required for replication elongation [17,27], each is a reasonable candidate for the ploughshare or the helicase activator.

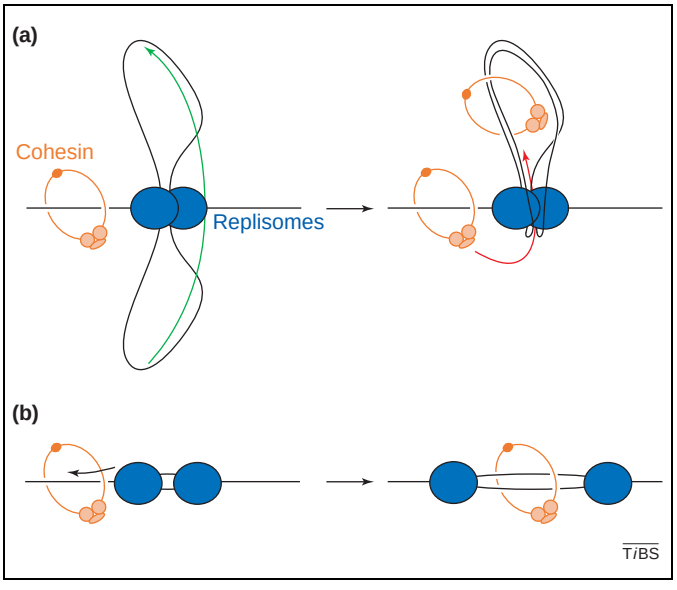

Figure 3. Establishment of chromosome cohesion. Two mechanisms are presented, each of which assumes that cohesin forms a ring around parental DNA and that the replisome must pass through the ring to establish cohesion. **(a)** In models where DNA is extruded from opposite sides of a helicase complex, sister chromatids must first be juxtaposed to enable cohesion establishment (green arrow). In addition, assuming that the helicases are physically linked, the linkage would have to be transiently severed to enable passage of cohesin (red arrow). **(b)** In models where DNA is extruded behind a helicase that is not physically linked to another helicase, the replisome must only pass through the cohesin ring. Black lines represent duplex DNA.

A potential complication with all the models in which MCM2–7 encircles duplex DNA concerns the mechanism of termination. Biochemical studies of MCM2–7 indicate that the functional unit of the helicase interacts with ~80 base pairs of DNA [52], and structural studies of the archaeal MCM complex suggest that much of this DNA would be sequestered within a central channel as duplex DNA [8]. In this view, when two MCM2–7 complexes emanating from adjacent origins meet during termination, it is unclear how the DNA sequestered within each helicase is unwound. One helicase might disassemble to enable passage of the other, but the last helicase remaining on the chromatin will still have a 'dead volume' of DNA that cannot be actively unwound by helicase translocation. One solution is that, after the final helicase has disassembled, another helicase such as MCM8 unwinds the remaining short stretch of duplex DNA. Another solution is that helicase disassembly causes denaturation of the helicase-associated DNA.

Concluding remarks

A diverse set of models has been proposed for the mechanism of MCM2–7 action, but many of these have important drawbacks. Thus, models in which MCM2–7 associates with ssDNA upon loading are unlikely given the large amount of ssDNA that would be exposed for prolonged periods. The torsional unwinding mechanism of the rotary-pump model is in conflict with the fact that cells contain topoisomerases. This leaves the T-ag and the ploughshare models, which differ with respect to the question of whether the active helicase is a monomer or a dimer. A monomeric helicase offers advantages because it can better accommodate currently favored mechanisms of cohesion establishment. We note that both models are compatible with the idea that DNA replication takes place in fixed factories because either a monomeric or dimeric helicase could be attached to the nuclear matrix.

How will researchers distinguish between the models discussed here? The development of cell-free systems for pre-RC assembly in yeast is a promising development [48,61] because it could enable more sophisticated footprinting and structural studies to discern the structure of pre-RCs. For example, a major indication that T-ag encircles duplex DNA came from its uniform protection of both strands of the double helix [62]. In *Xenopus*, EM studies of simple DNA substrates undergoing DNA replication might help determine whether replisomes are physically coupled or not. High-resolution structural information on the MCM2–7 complex and associated proteins such as GINS and Cdc45 will hopefully yield

unanticipated insights. However, researchers might have to hold their breaths for the answer until eukaryotic DNA replication is reconstituted with purified components.

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