Mcm4,6,7 Uses a “Pump in Ring” Mechanism to Unwind DNA by Steric Exclusion and Actively Translocate along a Duplex*§

Received for publication, July 24, 2003, and in revised form, September 16, 2003
Published, JBC Papers in Press, September 17, 2003, DOI 10.1074/jbc.M308074200

Daniel L. Kaplan‡§, Megan J. Davey‡, and Mike O’Donnell‡¶

From ‡Rockefeller University and ¶Howard Hughes Medical Institute, Laboratory of DNA Replication, New York, New York 10021

Mcm4,6,7 is a ring-shaped heterohexamer and the putative eukaryotic replication fork helicase. In this study, we examine the mechanism of Mcm4,6,7. Mcm4,6,7 binds to only one strand of a duplex during unwinding, corresponding to the leading strand of a replication fork. Mcm4,6,7 unwinding stops at a nick in either strand. The Mcm4,6,7 ring also actively translocates along duplex DNA, enabling the protein to drive branch migration of Holliday junctions. The Mcm4,6,7 mechanism is very similar to DnaB, except the proteins translocate with opposite polarity along DNA. Mcm4,6,7 and DnaB have different structural folds and evolved independently; thus, the similarity in mechanism is surprising. We propose a “pump in ring” mechanism for both Mcm4,6,7 and DnaB, wherein a single-stranded DNA pump is situated within the central channel of the ring-shaped helicase, and unwinding is the result of steric exclusion. In this example of convergent evolution, the “pump in ring” mechanism was probably selected by eukaryotic and bacterial replication fork helicases in order to restrict unwinding to replication fork structures, stop unwinding when the replication fork encounters a nick, and actively translocate along duplex DNA to accomplish additional activities such as DNA branch migration.

Mcm (minichromosome maintenance) proteins 2–7 are required for the initiation and elongation steps of replication in eukaryotes (1–3). These Mcm proteins are components of the prereplicative complex that assembles on replication origins before S phase (4, 5). During replication, all six proteins appear to travel with the replication fork (6). Mcm proteins can form various complexes, but only the Mcm4,6,7 complex has been shown to contain DNA helicase activity (7–9). Interaction of Mcm4,6,7 with Mcm2p or Mcm3,5 modulates the helicase activity of Mcm4,6,7 (10). Mcm4,6,7 may serve as the eukaryotic cellular replication fork helicase, and the other Mcms may have an essential regulatory function.

Mcms share a common ancestor with the Superfamily 3 family of helicases, which includes the SV40 large T antigen, a eukaryotic viral replication fork helicase (11). Mcms, like T antigen, are members of the AAA+ (ATPase associated with a variety of cellular activities) family of proteins, a diverse family of proteins that share a common protein fold (12). The bacterial replication fork helicase, DnaB, is from a different helicase family, called the Family 4 or the DnaB family (13). DnaB shares a common ancestor with RecA and thus contains the RecA fold (14). Thus, the eukaryotic replication fork helicases have a different evolutionary origin and a different protein fold compared with the bacterial replication fork helicases.

Mcm4,6,7 forms a ring-shaped hexamer with a large central channel (10, 15). Other replication fork helicases form ring shapes in systems as diverse as eukaryotic viruses (SV40 T antigen) (16), archaea (mtMcm) (17, 18), bacteria (DnaB) (19), and bacteriophage (T7 gene 4) (20). It has been shown for each of these systems that DNA can pass through the central channel of the helicase ring (10, 16, 21–23).

Two models have been proposed for how Mcm4,6,7 unwinds DNA. In one model, a double-ringed Mcm4,6,7 complex is bound to the replication fork with one ring on the 3′ single strand extension (3′ tail) and one ring on the 5′ single-strand extension (5′ tail; Fig. 1A) (24, 25). In this model, both rings are required for processive unwinding. In a second model, the Mcm ring encircles duplex DNA and twists it, which results in unwinding at a distance (Fig. 1B) (26).

For the bacterial cellular and phage replication fork helicases, a consensus has emerged that these helicases unwind DNA by having one strand pass through the central channel of the ring-shaped protein, whereas the second strand passes outside of the central channel (Fig. 1C) (21, 27, 28). These helicases unwind DNA with 5′ to 3′ polarity (28–30).

The bacterial replication fork helicases require a DNA substrate that resembles a replication fork in order to unwind DNA. In other words, the duplex DNA must have both 5′ and 3′ single-stranded extensions, or “tails,” at one end of the duplex (27, 31). Thermus aquaticus DnaB binds to the 5′ tail but not the 3′ tail during unwinding (28). The 3′ tail is a bulky attachment that stimulates unwinding by forcing steric exclusion of this strand from the central channel of the helicase. As one strand is pulled through the central channel and the other strand is excluded and remains outside the ring, the duplex becomes unwound.

DnaB translocates on the 5′ ssDNA tail, and if the 3′ tail is absent, the DnaB ring continues on the duplex by sliding up over both strands such that they pass through the central channel of the protein (28). However, DnaB only tracks on the one strand during its action on double-stranded DNA. Further, unwinding does not occur when two strands pass through the central channel. While encircling two DNA strands, DnaB actively translocates in the 5′ to 3′ direction with sufficient force to accomplish several functions, including displacing tightly

* This work was supported by National Institutes of Health Grants GM 38839 and GM 62540 and by the Howard Hughes Medical Institute. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ The on-line version of this article (available at http://www.jbc.org) contains a table.

¶ The Leon and Toby Cooperman Fellow of the Damon Runyan Cancer (DRG) Research Foundation (DRG Grant 1663). To whom correspondence should be addressed. Tel.: 212-327-7255; Fax: 212-327-7253; E-mail: dkplan@mod.rockefeller.edu.

1 The abbreviations used are: ssDNA, single-stranded DNA; AMP-PNP, adenylyl imidophosphate; dT, deoxythymidylates.
bound proteins, driving branch migration of a Holliday junction, and unwinding a downstream 3'-tailed DNA strand (32).

In this study, we investigate the unwinding mechanism of Mcm4,6,7 from Saccharomyces cerevisiae. Surprisingly, we find that Mcm4,6,7 helicase modulates DNA structure by a mechanism that is very similar to that of DnaB, although the proteins are not related. A mechanism is proposed, which we refer to as a “pump in ring” model, which explains the diverse actions catalyzed by both Mcm4,6,7 and DnaB. In this model, the helicase ring encircles ssDNA and translocates along it with force in a single direction (5’ to 3’ for DnaB, 3’ to 5’ for Mcm4,6,7). If the second strand contains a bulky group it is sterically excluded from the protein ring, and unwinding is observed. If the second strand does not have a bulky group attached, it passes through the central channel along with the first strand, and no unwinding is observed. Mcm4,6,7 can actively translocate along DNA while encircling two DNA strands, like bacterial DnaB, a function that may be used to accomplish additional functions such as DNA branch migration.

**EXPERIMENTAL PROCEDURES**

**Proteins and DNA**—Proteins were expressed in *E. coli* and purified as described: Mcm2p, Mcm4p, Mcm6p, and Mcm7p (33), RuvA and RuvB (34), DnaB (35), and UvrD (36), except an additional purification step was added to Mcm2p and Mcm7p.

Mcm2p was dialyzed against 20 mM Hepes, pH 7.5, 0.1 mM EDTA, 2 mM dithiothreitol, and 10% glycerol, and then applied to an ssDNA-agarose column equilibrated in the same buffer. The solution that flowed directly through the column contained pure Mcm2p.

Mcm7p was diluted in Buffer A (20 mM Tris, pH 7.5, 0.1 mM EDTA, 2 mM dithiothreitol, and 10% glycerol) until its conductivity was equal to that of Buffer A containing 50 mM NaCl. Mcm7p was then loaded onto a MonoS column (Amersham Biosciences) pre-equilibrated in Buffer A containing 50 mM NaCl. Mcm7p was eluted with Buffer A containing 100 mM NaCl.

Mcm4,6,7 was reconstituted, and the complex was purified from free subunits as follows. 44 nmol (monomer) each of Mcm4p, Mcm6p, and Mcm7p were incubated in 1 ml of buffer A containing 50 mM NaCl at 15°C for 30 min. The proteins were then applied to a 1-ml MonoQ column (Amersham Biosciences) equilibrated in Buffer A containing 50 mM NaCl. Proteins were eluted from the column with a 20–50 mM NaCl gradient in buffer A. Mcm4,6,7 complex elutes later than any of the free subunits (~350–400 mM NaCl).

DNA oligonucleotides used to construct the substrates in this report are listed in Supplemental Table I and were synthesized as described (28). Oligonucleotide strands were labeled with 32P at the 5’-end as described (28).

Enzyme reactions were incubated at 37°C for the time indicated, and they contained (unless otherwise stated) 1 nM DNA substrate (concentration of labeled strand), 586 nM Mcm4,6,7 complex, 5 mM ATP, 5 mM creatine phosphate, 20 μg/ml creatine kinase, 20 mM Tris–HCl, pH 7.5, in a final volume of 6 μl. Reactions were quenched upon adding 1 μl of Proteinase K (10 mg/ml) and incubated at 37°C for an additional 1 min, followed by the addition of 5 μl of 2X SDS and 80 mM EDTA. For gel analysis, 5 μl of 15% Ficoll (Type 400; Amersham Biosciences) and 0.25% xylene cyanol FF was added. Samples were snap frozen in a dry ice/ethanol bath and stored at −20°C until electrophoretic analysis was performed. Prior to gel electrophoresis, each reaction tube was incubated in a room temperature water bath for 5 min to reduce intrastrand base pairing.

Assays containing DnaB, UvrD, or RuvAB were identical to those using Mcm4,6,7, except that Mcm4,6,7 was replaced with 500 nM DnaB (hexamer), 50 nM UvrD (monomer), or 300 nM RuvA (monomer) and 1 μM RuvB (monomer). RuvA was preincubated with substrate DNA for 1 min at 37°C prior to the addition of RuvB for the indicated time.

For assays containing streptavidin, streptavidin was pre-equilibrated with substrate DNA before adding to the assay mixture. The concentration of the streptavidin tetramer in the final reaction mixture was 100 nM.

DNA products were separated from DNA substrate in an 8% polyacrylamide gel (29:1 acrylamide/bisacrylamide) using 1× TBE (90 mM Tris-HCl-borate, 2 mM EDTA), 175 V, and room temperature until the unannealed labeled strand was near the bottom of the gel.

**FIG. 1. Models for replication fork unwinding.** A, an Mcm4,6,7 ring encircled each of the 3’ and 5’ single-stranded extensions of a replication fork (24, 25). In this model, both rings are required for processive unwinding. B, the Mcm ring translocated along duplex DNA and twisted it, causing unwinding at a distant replication fork (26). C, a DnaB ring encircled the 5’ single-stranded extension of a replication fork (28). DnaB translocated with 5’ to 3’ polarity to unwind the DNA.
Mcm4,6,7 Uses a “Pump in Ring” Mechanism

Gels were dried at 80 °C for 1 h and then exposed to a phosphorimaging screen (Amersham Biosciences). Band intensities were quantified, and background counts were subtracted using the IQMac software package, version 1.2 (Amersham Biosciences). For all reactions, unwinding is defined as the fraction of radiolabeled DNA species that is single-stranded. The percentage product in an unreacted sample varied from 0 to 5%. To normalize for the slight variability in these values, the percentage of product was calculated using the equation,

\[ \%\text{Unwound} = (\%U - \%U_0)/(1 - \%U_0) \]  

(Eq. 1)

where \( \%U \) represents the percentage unwound in the sample lane of interest, and \( \%U_0 \) is the percentage unwound in the unreacted substrate.

Representative experiments are shown in each figure, each performed under standard conditions. Each experiment was performed at least twice. The data were not averaged, because the conditions in some of the repeat experiments differed from the standard conditions.

RESULTS

Mcm4,6,7 from S. cerevisiae Unwinds Duplex DNA—In human, mouse, and Schizosaccharomyces pombe, Mcm4,6,7 exhibits helicase activity in vitro (7–9). In the current study, we have reconstituted the S. cerevisiae Mcm4,6,7 complex from pure subunits expressed in E. coli and studied the resulting Mcm4,6,7 complex for helicase activity. To test the ability of Mcm4,6,7 from S. cerevisiae to unwind DNA, the protein complex was incubated with radiolabeled duplex DNA and ATP (Fig. 2A). The DNA substrate contains a duplex region of 31 base pairs and two single-stranded extensions to form a “forked duplex” (see Fig. 2A). Each single strand extension or “tail” was composed of 60 deoxythymidylates (60 dT). The products of Mcm4,6,7 on this substrate were analyzed on a native polyacrylamide gel, which separates single-stranded product from double-stranded substrate. Mcm4,6,7 converted almost all of the substrate to product (Fig 2A, top left gel). In a previous study, S. cerevisiae Mcm4,6,7 did not show unwinding activity (37). The Mcm proteins were overexpressed using baculovirus in the previous study, which may result in post-translational modifications that inhibit helicase activity.

In many experiments of this report, we perform side-by-side comparisons of Mcm4,6,7 with E. coli DnaB under identical conditions to compare Mcm4,6,7 to DnaB action. DnaB also unwinds the forked DNA duplex in Fig. 2A (top right gel). The total extent of DnaB unwinding is not as great as with Mcm4,6,7, in part because DnaB is optimally active in unwinding at higher pH (31).

Mcm4,6,7 unwinding is inhibited by pre-incubation with Mcm2p (Fig. 2A, bottom left gel), consistent with previous studies (8, 9). Unwinding is not inhibited by the buffer solution that Mcm2p is stored in, showing that the inhibition is due to the Mcm2p protein, and not by salt or other solution components.

Incubation of Mcm4,6,7 with ATP or dATP yields product, but no product is observed when Mcm4,6,7 is incubated with AMP-PNP, ADP, or no nucleotide (Fig. 2A, bottom middle graph). Thus, the protein complex requires a hydrolyzable NTP or dNTP to unwind DNA, consistent with the energy requirement of an active helicase and with other studies (7, 8).

To determine whether AMP-PNP is bound to the active site in the experiment described above, we incubated the forked substrate with Mcm4,6,7, ATP (fixed concentration), and increasing amounts of AMP-PNP (Fig. 2A, bottom right gel). AMP-PNP inhibits unwinding in a concentration-dependent manner (lanes 2 and 3). AMP-PNP may be inhibiting unwinding by competing for Mg\(^{2+}\). Thus, the experiment was also conducted with additional Mg\(^{2+}\) to compensate for the increased AMP-PNP (Mg\(^{2+}\) added in a 1:1 molar ratio with additional AMP-PNP). Again, AMP-PNP inhibited unwinding in a concentration-dependent manner (lanes 4 and 5), suggesting that AMP-PNP is competing directly with ATP.

Taken together, the data suggest that Mcm4,6,7 requires NTP hydrolysis for unwinding.

Mcm4,6,7 Requires Two Single-stranded Extensions (“Tails”) for Unwinding—Processive unwinding catalyzed by Mcm4,6,7 from S. pombe requires a DNA substrate that bears two single-stranded tail extensions, called a “forked” DNA substrate (25). Next we examined Mcm4,6,7 from S. cerevisiae for activity on DNA substrates bearing either a 5’ and 3’ tail (fork) or only a 3’ tail or 5’ tail (Fig. 2B). Mcm4,6,7 requires a forked structure for activity, consistent with the previous study (25). DnaB also shares this same dependence for a fork, as illustrated in Fig. 2B, and described previously (29, 31).

Mcm4,6,7 Binds to the 3’ Tail, not the 5’ Tail during Unwinding—The observation that a forked DNA structure is required for processive Mcm4,6,7 unwinding, combined with data indicating that as many as two Mcm4,6,7 complexes may bind to forked DNA, led to the previous model shown in Fig. 1A (24, 25). In this model, two Mcm4,6,7 rings bind to the two DNA tails for processive unwinding. To test this model, we replaced each single-stranded DNA tail extension with a biotin-streptavidin group (Fig. 3A). Replacement of the 5’ tail with biotin/streptavidin stimulated unwinding, suggesting that Mcm4,6,7 does not need to bind to the 5’ tail during unwinding. Replacement of the 3’ tail with biotin/streptavidin completely abolished activity, suggesting that Mcm4,6,7 binds to the 3’ tail during unwinding. Thus, Mcm4,6,7 binds to the 3’ tail and does not need to bind a 5’ tail for unwinding. Since Mcm4,6,7 typically requires a forked DNA for activity, it would appear that the biotin/streptavidin moiety can substitute for the 5’ tail. It will be demonstrated below that the 5’ tail acts as a steric factor to stimulate Mcm4,6,7 unwinding.

For comparison, DnaB unwinding was abolished by replacement of the 5’ tail with biotin/streptavidin, whereas activity was stimulated by replacement of the 3’ tail with biotin/streptavidin. These data suggest that DnaB binds to the 5’ tail, but not the 3’ tail, during unwinding, as previously proposed (28). DnaB, like Mcm4,6,7, was more active on the substrate containing a biotin/streptavidin in place of an ssDNA tail (except on the 3’ instead of the 5’ end).

Mcm4,6,7 Unwinds DNA with 3’ to 5’ Polarity—The polarity of Mcm4,6,7 from human and S. pombe has been examined previously using a long ssDNA substrate with a short duplex region on either end and determining which duplex end was unwound. These studies indicated that Mcm4,6,7 unwinds DNA 3’ to 5’ (7, 8). However, later studies demonstrated that processive unwinding requires a forked structure, in which case unwinding may occur with either 3’ to 5’ or 5’ to 3’ polarity (25).

In Fig. 3B, we examined the polarity of Mcm4,6,7 unwinding using a different system. Forked DNA substrates were constructed that have “reverse polarity” linkages on one or the other ssDNA tail. To reverse the polarity of the 3’ tail, a 3’-3’ connection was incorporated at the junction of single- and double-stranded DNA. This generates a DNA substrate with two 5’ tails. To reverse the polarity of the 5’ tail, a 5’ to 5’ connection was incorporated at the single-stranded/double-stranded DNA junction, thus making a DNA substrate with two 3’ tails. We also reversed the polarity of both tails to produce a DNA substrate with a 5’ tail and a 3’ tail, but the polarity of both strands reverses at the duplex junction.

Mcm4,6,7 is active on the substrate with two 3’ tails, but not on the substrate with two 5’ tails. This confirms that Mcm4,6,7 unwinds DNA with 3’ to 5’ polarity. It also confirms that Mcm4,6,7 is insensitive to the chemistry of the 5’ tail, since it can be flipped around backward with no substantial loss in activity. As one might expect, Mcm4,6,7 cannot unwind the
DNA with two reverse tails. This shows that the protein recognizes the polarity of the DNA strand to which it is bound during binding and unwinding. It also demonstrates that the protein cannot switch strands during the course of the reaction.

DnaB unwinds the substrate with two 5' tails but not two 3' tails or two reverse tails. This is consistent with the known 5' to 3' polarity of DnaB and further supports the conclusion that Mcm4,6,7 has opposite polarity to that of DnaB.

The 3' Tail Passes through the Central Channel of Mcm4,6,7 during Unwinding, whereas the 5' Tail Is Sterically Excluded—For DnaB, the 5' tail passes through the central channel during unwinding, whereas the 3' tail passes on the outside of the protein because it is sterically excluded from the central channel. The polarity of unwinding for Mcm4,6,7 is opposite to that of DnaB. Thus, if the mechanism of unwinding is similar for these two ring-shaped helicases except for the reversal of po-
larity, one would expect the 3' tail to pass through the central channel of Mcm4,6,7, whereas the 5' tail is sterically excluded from entering the central channel.

To test this prediction, we used biotin/streptavidin as a steric block. The diameter of streptavidin is ~45 Å, which is likely to be larger than the central channel of Mcm4,6,7. Thus, streptavidin bound to a DNA strand should prevent it from passing through the central channel of Mcm4,6,7, and movement should be blocked. In contrast, when streptavidin is bound to the DNA strand that passes on the outside of the Mcm4,6,7 ring, activity should not be inhibited.

In the experiment in Fig. 3C, a dT was replaced with a biotin-dT within the duplex region of the test strand, and then excess streptavidin was added to the reaction to create a steric block. Biotin-dT pairs with dA on the complementary strand and has a spacer arm connecting biotin to dT. When biotin-dT/streptavidin is present on the strand bearing the 3' tail, Mcm4,6,7 activity is abolished. However, when biotin-dT/streptavidin is present on the strand bearing the 5' tail, activity is slightly stimulated. Thus, the tail bearing the 3' tail probably passes through the central channel of Mcm4,6,7, whereas the strand bearing the 5' tail passes on the outside of the protein.
Mcm4,6,7 Uses a “Pump in Ring” Mechanism

As a control, biotin/streptavidin blocks DnaB when it is positioned on the strand bearing the 5′ tail but not the 3′ tail (Fig. 3C). This is consistent with previous studies of *Thermus aquaticus* DnaB showing that the strand bearing the 5′ tail, but not the 3′ tail, passes through the central channel of the protein during unwinding (28). There is some weak unwinding activity when biotin/streptavidin is positioned on the strand bearing the 5′ tail, probably because the protein has some ability to displace proteins during unwinding (32).

Summarizing our conclusions thus far, Mcm4,6,7 binds to the 3′ tail, encircles the 3′ tail, and translocates with 3′ to 5′ polarity to unwind DNA. If Mcm4,6,7 does not contact the 5′ tail during unwinding, why is it required for unwinding? An analogous situation occurs for DnaB. In this case, the 3′ tail is required for unwinding, but it passes on the outside of the protein and does not contact the protein ring. For DnaB, the 3′ tail acts as a steric factor to promote unwinding. In other words, the 3′ tail is a bulky group that blocks entry of double-stranded DNA into the central channel of DnaB, thereby stimulating unwinding. To determine whether the 5′ tail acts like a steric factor for Mcm4,6,7, tandem duplex substrates were tested as described below.

**Mcm4,6,7 Rapidly Translocates along Duplex DNA while Encircling Two DNA Strands**—As shown above, Mcm4,6,7 unwinds a duplex if it contains a 5′ ssDNA tail or other bulky group at this position. If there is no 5′ tail or bulky group, there is no unwinding. Perhaps when no 5′ tail is present, Mcm4,6,7 encircles both DNA strands with no resultant unwinding, as occurs for DnaB. Alternatively, the Mcm4,6,7 may simply stop translocating when it encounters a duplex with no 5′ tail.

To distinguish between these two possibilities, Mcm4,6,7 was incubated with substrates that contain two duplexes positioned in tandem (Fig. 4). In these substrates, the bottom strand is continuous, but the top strand contains a nick in the middle of the duplex region. This nick separates the substrate into two duplexes, one on the left (labeled duplex 1) and one on the right (labeled duplex 2). There is only one 3′ tail for loading of Mcm4,6,7, located at the left side of the substrate. Thus, Mcm4,6,7 should bind to the 3′ tail and then move in the 3′ to 5′ direction to sequentially unwind duplex 1, followed by unwinding duplex 2 (Fig. 4A).

As shown in Fig. 4A (lanes 3–6), the first product to appear is one of intermediate electrophoretic mobility. Unwinding of duplex 1, but not duplex 2, produced this product. Later in the reaction, the single-stranded product with fast electrophoretic mobility accumulates. Unwinding of duplex 2 produced this product. Thus, Mcm4,6,7 first unwinds duplex 1, and then it unwinds duplex 2. This suggests that Mcm4,6,7 binds to the 3′ tail on the left side of the substrate and then translocates in the 3′ to 5′ direction to sequentially unwind duplex 1, followed by duplex 2. The sequential unwinding is confirmed by the unwinding quantitation (filled circles in Fig. 4, F and G), showing that duplex 1 is unwound faster than duplex 2.

Next, the 5′ tail of duplex 1 was removed from the DNA to yield the substrate shown in Fig. 4, B and C. The substrates of Fig. 4, B and C, are identical to each other, except duplex 1 is radiolabeled in Fig. 4B, and duplex 2 is radiolabeled in Fig. 4C. Mcm4,6,7 should bind to the 3′ tail of this substrate and then move in the 3′ to 5′ direction until it encounters duplex 1 with no 5′ tail. If translocation stops at this junction, then no unwinding of duplex 1 or 2 should occur. If translocation continues, then duplex 2 may be subsequently unwind. Fig. 4B (lanes 3–6) shows that when duplex 1 is labeled, only an intermediate product is formed, which results from unwinding only duplex 2. Fig. 4C (lanes 3–6) shows that when duplex 2 is labeled, only the free single strand is produced with no intermediate, confirming that only duplex 2 is unwound.

Overall the results may be explained as follows. Mcm4,6,7 continues translocation after encountering the flush duplex end with no 5′ tail, moving in the 3′ to 5′ direction by pulling both strands through the central channel. No unwinding of duplex 1 is observed while Mcm4,6,7 translocates along it due to the fact that it encircles both strands (Fig. 4, B, lanes 3–6, and F, open squares). When the helicase encounters duplex 2, which has a bulky 5′ tail, it continues to translocate in the 3′ to 5′ direction, but the 5′ tail of duplex 2 cannot fit into the inside of the protein ring, and therefore duplex 2 is unwound (Fig. 4, C, lanes 3–6, and G, open squares). If duplex 2 bears no 5′ tail, then no unwinding of duplex 1 or 2 is observed (no gel is shown; Fig. 4, F and G, show the quantitation as filled diamonds).

An alternative explanation to that described above is that rather than first binding the 3′ single-stranded tail and then translocating onto duplex 1, Mcm4,6,7 binds directly to the duplex 1 region. To distinguish between these two possibilities, the Mcm4,6,7 was incubated with substrates identical to those in Fig. 4, B and C, except the 3′ tail was removed (Fig. 4, D and E). Mcm4,6,7 produces no products from these substrates (Fig. 4, D and E, lanes 3–6, and Fig. 4, F and G, open triangles), and therefore Mcm4,6,7 loads onto the 3′ single-stranded tail first and then translocates onto the duplex region.

The results of Fig. 4 support the idea that Mcm4,6,7 translocates while encircling two DNA strands. Unwinding of duplex 2 is faster if duplex 1 bears no 5′ tail (compare open squares with filled circles in Fig. 4G). This suggests that Mcm4,6,7 translocates faster when encircling two DNA strands with no unwinding compared with encircling one strand with unwinding. The results in Fig. 4 are analogous to that of *T. aquaticus* DnaB, except the strand polarity is reversed (28). Thus, the two replication fork helicases appear to share a common mechanism.

**Mcm4,6,7 Stops Unwinding upon Encountering a Leading Strand Nick**—The experiments above suggest that if Mcm4,6,7 encounters a nick on the strand that it is not translocating along, it will go up on the duplex and encircle both DNA strands and thereby stop unwinding. What happens if Mcm4,6,7 encounters a nick on the strand that it is translocating along? To evaluate this possibility, the effect of Mcm4,6,7 on a substrate containing a long duplex was examined (Fig. 5, left panel). Mcm4,6,7 unwinds the long duplex (left gel). Mcm4,6,7 was then tested with a similar substrate that has a nick in the strand Mcm4,6,7 is translocating along (Fig. 5, middle and right panels). Mcm4,6,7 stopped unwinding when it encountered this nick (middle and right gels). Thus, Mcm4,6,7 stops unwinding DNA when it encounters a nick on the strand it translocates along.

To summarize, the experiments of Figs. 4 and 5 show Mcm4,6,7 stops unwinding upon encountering a nick on either DNA strand. When Mcm4,6,7 encounters a nick on the leading strand, unwinding and translocation will both stop. When encountering a nick on the lagging strand, unwinding will stop, but the helicase will continue to translocate along duplex DNA.

**Mcm4,6,7 Drives Branch Migration of Holliday Junctions**—Thus far, Mcm4,6,7 behaves like DnaB on all of these various DNA substrates, except the polarity is reversed. Another activity of DnaB is its ability to drive branch migration of Holliday junctions (32). To determine whether Mcm4,6,7 can drive branch migration, we constructed a synthetic Holliday junction (X-junction) that contains a 3′ single-strand extension for Mcm4,6,7 loading (Fig. 6). The junction contains heterologous duplex arms to prevent spontaneous branch migration.

If Mcm4,6,7 functions like DnaB, it should translocate onto the duplex region of the Holliday junction and then pump the strand bearing the 3′ tail through the ring to melt both duplex...
Mcm4,6,7 actively translocates along duplex DNA while encircling two DNA strands. A–E, Mcm4,6,7 or UvrD was incubated with the substrate shown above the gel for the times indicated. Products were resolved on native gel as shown. The diagrams and arrows to the right of each gel indicate the position of each product in the gel as determined by analysis of standards. F and G, bands from gels (A–E) (A, filled circles; B and C, open squares; D and E, open triangles) and from other gels not shown (filled diamonds) were quantified, and the percentage unwound is plotted as a function of time for Mcm4,6,7. The percentage unwound is defined as the fraction of radiolabeled DNA species that is single-stranded.

FIG. 4. Mcm4,6,7 actively translocates along duplex DNA while encircling two DNA strands. A–E, Mcm4,6,7 or UvrD was incubated with the substrate shown above the gel for the times indicated. Products were resolved on native gel as shown. The diagrams and arrows to the right of each gel indicate the position of each product in the gel as determined by analysis of standards. F and G, bands from gels (A–E) (A, filled circles; B and C, open squares; D and E, open triangles) and from other gels not shown (filled diamonds) were quantified, and the percentage unwound is plotted as a function of time for Mcm4,6,7. The percentage unwound is defined as the fraction of radiolabeled DNA species that is single-stranded.
arms of the Holliday junction (see scheme in Fig. 6A). This action requires that the helicase actively translocate while it is on the duplex, not simply diffuse along it. (During active translocation, the helicase consumes ATP to move with force along the DNA. Passive translocation would require no ATP hydrolysis, being powered only by Brownian motion, and thus movement should lack force.) The result of Mcm4,6,7 action on the synthetic Holliday junction is shown in Fig. 6B. Consistent with Mcm4,6,7 acting according to the scheme shown in Fig. 6A, the major product formed during the reaction corresponds to that of branch migration (Fig. 6B, lanes 5–8). Therefore, Mcm4,6,7, like DnaB, actively translocates along duplex DNA with force sufficient to melt the two arms of the synthetic X junction at the same time. RuvAB can bind to either the top and bottom or right and left duplex arms, and therefore it can drive branch migration in either direction to produce the two bands in Fig. 6B, lane 3. DnaB requires a 5′ single-strand extension for branch migration (32), and thus it cannot drive branch migration of this particular Holliday junction (Fig. 6B, lane 4).

To determine the nucleotide dependence for branch migration, Mcm4,6,7 was incubated with the Holliday junction substrate shown in Fig. 6B and various nucleotide analogs (Fig. 6C). Mcm4,6,7 is only active in the presence of a hydrolyzable nucleotide triphosphate, suggesting that NTP hydrolysis is required for branch migration.
Removal of the 3’ tail of the Holliday junction abolishes Mcm4,6,7 activity (Fig. 6D, lanes 5–8). These data further support that Mcm4,6,7 acts according to the model shown at the top of Fig. 6. This result also illustrates that Mcm4,6,7, like DnaB, does not bind to Holliday junctions directly.

The ability of Mcm4,6,7 to unwind Holliday junctions has important mechanistic implications. It shows that Mcm4,6,7 actively translocates along duplex DNA while encircling two DNA strands, since branch migration is an active process. Furthermore, Mcm4,6,7 is unwinding two duplexes simultaneously during branch migration, confirming that the protein complex unwinds DNA by steric exclusion.

**DISCUSSION**

In this study, we examine the mechanism of DNA unwinding catalyzed by Mcm4,6,7 from *S. cerevisiae*. The protein binds to and encircles the 3’ single stranded tail extension. If the DNA substrate contains a bulky group attached to the other strand, then the protein migrates with 3’ to 5’ polarity to unwind the duplex. During unwinding, the strand with the 3’ tail passes through the central channel, and the other strand is positioned outside the protein ring. If the DNA substrate lacks a bulky group on the nonbinding strand, then Mcm4,6,7 will not unwind the duplex. Instead, the protein ring will slip up over the duplex to encircle two DNA strands. While encircling two DNA strands, Mcm4,6,7 continues to actively translocate along one DNA strand with 3’ to 5’ polarity. This property enables the protein to unwind a downstream DNA strand provided it contains a bulky attachment, as shown in Fig. 4C. The protein can also drive branch migration of a Holliday junction while encircling two DNA strands, which involves active unwinding of two DNA duplexes simultaneously. Thus, the mechanism of Mcm4,6,7 is remarkably similar to DnaB, except the polarity is reversed.

A “Pump in Ring” Model for Bacterial and Eukaryotic Cellular Replication Fork Helicases—The results of this study demonstrate that Mcm4,6,7 operates in an analogous manner to DnaB, except with opposite strand polarity. Previous models for DnaB-type helicases include the torsional, helix-destabilizing, and wedge models (38). In the torsion model, the helicase binds to both DNA tails, ripping the duplex apart. Mcm4,6,7 and DnaB bind to only one tail, and therefore this model can be excluded. In the helix-destabilizing model, the helicase binds to and directly melts duplex DNA ahead of the helicase. The helix-destabilizing model applies to the Superfamily 1 helicases like Rep and UvrD but not to the replication fork helicases, as discussed in depth below (see “Comparison of Replication Fork Helicases with UvrD Type Helicases”). In the wedge model, the helicase binds to only one DNA strand and drives its protein ring as a wedge to separate the two strands. DnaB and Mcm4,6,7 interact with only one DNA strand, consistent with the wedge model. However, the wedge model does not explain how these helicases can actively translocate along DNA while encircling two DNA strands.

We propose a simple “pump in ring” mechanism to explain all activities of DnaB and Mcm4,6,7 on DNA (Fig. 7A). In this model, a single-stranded DNA pump is positioned within the central channel of the ring-shaped helicase, as illustrated in Fig. 7A. (The ssDNA pump is depicted as two hands pulling ssDNA). The single-stranded pump pulls DNA in the 5’ to 3’ direction for DnaB and in the 3’ to 5’ direction for Mcm4,6,7. While the pump pulls on one DNA strand, it does not contact the second strand. If this second strand contains a bulky attachment such as streptavidin, it cannot fit inside the ring, and thus the second strand is stERICALLY EXCLUDED from the ring (Fig. 7A, upper left diagram). The closest base pair to the ring is the weakest chemical bond in this system, and it is therefore broken as the pump pulls on the first DNA strand. Thus, the helicase does not need to interact with the second strand, or the duplex, to accomplish unwinding. Unwinding is the result of steric exclusion.

If the second strand contains a long ssDNA tail instead of streptavidin, it also acts as a bulky attachment, and thus the second strand is usually excluded from the protein ring, and unwinding is observed (Fig. 7A, upper right diagram). However, if the second strand does not have a bulky group attached, it is pulled into the protein ring along with the first strand (Fig. 7A, lower left diagram). Mcm4,6,7 and DnaB helicases can accommodate a second strand within the central channel. In fact, the pump continues to pull on the first strand while the second strand is in the channel. This enables the helicase to unwind a downstream strand of DNA if it contains a bulky attachment (Fig. 4C). (It is possible that transient unwinding occurs while the two strands are inside the central channel, but once they exit the central channel they rapidly anneal. Alternatively, the helicase may simply translocate along the duplex without unwinding.)

If Mcm4,6,7 or DnaB encounters a Holliday junction while encircling two DNA strands, the helicase will continue to pump single-stranded DNA through the central channel. Since the four-way junction cannot fit into the ring, the two duplexes are ripped apart by the helicase by steric exclusion, resulting in DNA branch migration (Fig. 7A, lower right diagram). A single-stranded pump situated inside a protein ring is a simple model that explains how Mcm4,6,7 and DnaB accomplish a variety of DNA modulating activities. We have previously shown that T7 gp4 ring-shaped helicase also drives branch migration of Holliday junctions by this mechanism (32). Thus, the “pump in ring” mechanism would appear to apply to wide variety of replication fork helicases.

**Comparison of Replication Fork Helicases with UvrD Type Helicases**—Mcm4,6,7 and DnaB act differently from UvrD, a member of the Superfamily 1 family of helicases (13). UvrD does not form a ring shape or a hexamer (39, 40). UvrD is not stimulated by a tail extension attached to the nonbinding strand (41). Thus, UvrD does not use steric exclusion to unwind DNA. Superfamily 1 helicases like UvrD appear to catalyze unwinding by making intimate and direct chemical contacts with the forked junction (42). Thus, they directly melt the DNA by binding to the duplex and each single-stranded extension. DnaB and Mcm4,6,7 do not melt the duplex directly in an analogous manner. DnaB and Mcm4,6,7 unwinding require the nonbinding tail to have a bulky attachment to exclude it from the central channel. A direct melting mechanism would not require a bulky group on the nonbinding strand. Thus, UvrD and related helicases probably separate DNA by directly melting the DNA, whereas replication fork helicases unwind DNA by steric exclusion.

**Revision of Previous Models for Mcm4,6,7 Mechanism**—In one previous model for Mcm4,6,7 activity, one protein ring is positioned on the 5’ tail, and one protein ring is positioned on the 3’ tail (Fig. 1A) (24). This model derives from the fact that two tails are required for processive unwinding, and protein cross-linking indicates that two Mcm4,6,7 complexes bind a forked DNA (25). This is a very interesting observation, and we suggest a slightly different interpretation. The current study shows that the Mcm4,6,7 ring on the 3’ tail catalyzes unwinding, but the one on the 5’ tail is not required. The 5’ tail is a bulky attachment that forces steric exclusion of the DNA strand. Furthermore, this report demonstrates that an Mcm4,6,7 ring positioned on the 5’ tail will translocate away from the duplex region, because it only translocates with 3’ to 5’ polarity. However, in *vivo*, replication mainly occurs at in-
**Fig. 7.** Mcm4,6,7 uses a “pump in ring” mechanism to unwind DNA by steric exclusion and actively translocate along a duplex. 

**A.** Unwinding of Streptavidin Tail

The ring-shaped helicase, which can be Mcm4,6,7 or DnaB, is shown as a cylinder. The ssDNA pump inside the central channel is shown as two hands tugging on a DNA strand. **Upper left diagram**, streptavidin is shown as a large filled circle. The helicase pumps one DNA strand through its central channel, and the bulky streptavidin group forces steric exclusion of the second strand. Unwinding is the result. **Upper right diagram**, the second strand bears a long single-stranded extension (“tail”) that is usually sterically excluded from the ring, resulting in unwinding. **Lower left diagram**, with no bulky group on the second strand, the second strand is pulled through the central channel with the first strand, with no resultant unwinding. The helicase now translocates along duplex DNA. **Lower right diagram**, while actively translocating along duplex DNA, the helicase can drive branch migration of a Holliday junction.

**B.** Mcm4,6,7 As Double Ring Unwinds Two Replication Forks

**C.** Communication in Replisome (Lagging polymerase excluded)

**Eukarya**

Eukaryotic systems have a different mechanism for unwinding the second strand. 

**Bacteria**

In bacterial systems, the second strand is unwound by a different mechanism.

**D.** Unwinding Stops at Nicks

When a nick is encountered, the leading strand polymerase is unable to continue, causing unwinding to stop.

---

*Note: The diagram is a visual representation of the unwinding process and the mechanisms described in the text.*
ternal regions of DNA (i.e., a bubble) rather than at the end of a forked linear molecule. Thus, if Mcm4,6,7 forms a ring on each ssDNA strand of the bubble, then the two rings would translocate in opposite directions to form two forks for bidirectional replication (Fig. 7B). Mcms acting together as in Fig. 7B is also consistent with Mcms forming distinct replication foci in vivo (43).

In a second model for eukaryotic Mcm action, the protein ring encircles two DNA strands and actively translocates along the duplex (Fig. 1B) (26). This may alter the twisting of the DNA, resulting in unwinding at a distance from the Mcm protein complex. The present study shows that Mcm4,6,7 can actively translocate along a duplex, as predicted by this theoretical paper. However, the results also show that Mcm4,6,7 directly catalyzes DNA unwinding if one strand passes through the central channel and one strand passes outside the central channel. With one strand inside and one strand outside the protein ring, the strands are physically separated from each other, which can allow single-stranded DNA to be directly delivered to the replication fork polymerase (Fig. 7C). Thus, Mcm unwinding at a replication fork with one strand inside the protein ring and one strand outside the ring is a more likely model for replication fork unwinding in vivo. It is possible that while they are on duplex DNA the Mcm proteins may serve other functions away from the replication fork or perhaps assist in unwinding the replication fork from a distance. This possibility is supported by the observation that Mcm proteins are found at sites away from replication forks (44).

Consequences of a Leading Strand Compared with a Lagging Strand Replication Fork Helicase—Mcm4,6,7 is proposed to be the replication fork helicase in eukaryotes. If not, it may still serve as a suitable mechanistic model for the replication fork helicase, especially if it is ring-shaped as all known replicative helicases are. For example, the Mcm2–7 is also thought to possibly be the replication fork helicase (contains Mcm4,6,7 as well as Mcm2,3,5), except it has not been demonstrated to contain helicase activity.

Mcm4,6,7 unwinds DNA with 3′ to 5′ polarity, whereas DnaB unwinds DNA with 5′ to 3′ polarity. This positions Mcm4,6,7 on the leading strand and DnaB on the lagging strand during replication (Fig. 7C). Since these proteins only contact one strand during unwinding, Mcm4,6,7 does not contact the lagging strand. The primary function of a replication fork helicase is to provide the replication polymerases with single-stranded templates for replication. In this function, the eukaryotic system seems ideally suited, since the replication fork helicase is positioned on the same strand as the leading strand polymerase, making it easy to couple the movement of the two proteins. In this arrangement, the strand exiting the helicase central channel can conceivably enter the leading strand polymerase directly. The helicase is on the opposite strand in the bacterial system, which may explain why bacteria contain the ι subunit that physically bridges the leading strand polymerase to the replicative helicase (45).

There is a second critical protein–protein interaction for the replication helicase, the recruitment of primase on the lagging strand for each cycle of Okazaki fragment synthesis (46). In this respect, the bacterial system seems ideally suited, since the helicase and primase are positioned on the same strand. The T7 system emphasizes this point, since the gp4 polypeptide has dual helicase and primase functions. It is not yet known how the eukaryotic replication fork recruits primase to act on the lagging strand. Perhaps eukaryotes use a protein bridge to link helicase on one strand to primase on the other, analogous to bacterial ι. Cdc45p may serve this function, since it binds to both Mcms and a DNA polymerase/primase (Fig. 7C) (47).

In Vivo Advantages of the “Pump in Ring” Mechanism—The “pump in ring” mechanism for Mcm4,6,7 and DnaB ensures that these cellular replication fork helicases only unwind DNA at forked structures. For example, this mechanism ensures that helicase unwinding and polymerase synthesis are coordinately aborted upon replication fork encounter with a nick on either strand. If Mcm4,6,7 were to encounter a nick in the lagging strand during replication, it would slip over the duplex and encircle both DNA strands (Fig. 7D). DNA unwinding would cease, as the helicase proceeds forward on the parental duplex. Moreover, the helicase would be cleared from the site of damage and may displace downstream proteins as it actively translocates with force along the duplex (32). This protein clearing function may enable repair proteins to bind and repair the broken ends.

DNA unwinding would also cease if Mcm4,6,7 were to encounter a nick on the leading strand. Mcm4,6,7 or DnaB cannot unwind past a nick along the strand it is translocating along. Unwinding requires the continued pulling of ssDNA by these helicases. The ssDNA pump in Mcm4,6,7 and DnaB is probably inside the helicase ring, and therefore the pump cannot gain access to DNA beyond a nick. Thus, if Mcm4,6,7 or DnaB encounters a nick on either strand, unwinding ceases.

The “pump in ring” mechanism may have been selected by eukaryotic and bacterial cellular replication fork helicases to coordinate abortion of leading and lagging strand synthesis if the replisome encounters a nick. In addition, if Mcm4,6,7 or DnaB were to mistakenly bind to a single-stranded gap region of DNA, it may be disastrous for the cell if the genomic DNA was unwound in a highly processive fashion. However, if Mcm4,6,7 or DnaB bound to a gap, the proteins would harmlessly pass onto the duplex region, with no resultant DNA unwinding. Thus, one role of active translocation along double-stranded DNA may be to restrict unwinding to fork structures. A Holliday junction is a likely DNA structure to be formed near a replication fork during the repair process following replisome encounter with a DNA lesion. The Holliday junction may be formed either by regression of the replication fork or by DNA recombination involving the daughter strands at the replication fork (48, 49). Mcm4,6,7 and DnaB can drive branch migration of Holliday junctions and are therefore candidates for branch migration catalysis in vivo.

The “Pump in Ring” Mechanism Was Selected by Eukaryotic and Bacterial Replication Fork Helicases—Mcm proteins are related to the Superfamily 3 helicases (11), and these have the structural fold of the ancient AAA+ family (12). DnaB and T7 gp4 are members of the Family 4 helicase proteins (13) and thus contain the RecA structural fold (14). DnaB is related to RecA and probably originated from a duplication of a RecA-like ancestor after the divergence of the bacteria from the archaea and eukarya (50). Thus, the replication fork helicases in bacteria and eukarya have evolved independently and have different structural folds.

Despite having a distinct protein fold and a different evolutionary origin, Mcm4,6,7 and DnaB modulate DNA structure by a similar mechanism. However, the strand polarity is opp-
Mcm4,6,7 Uses a “Pump in Ring” Mechanism

site for these two proteins. Thus, in this example of convergent evolution, eukaryotes and bacteria selected the “pump in ring” mechanism for cellular replication fork helicases, but they did not select the same polarity. This implies that the “pump in ring” mechanism is critical for a cellular replication fork helicase, but strand polarity is not. Two obvious advantages that a ring shape confers to a replication fork helicase are processive DNA unwinding and physical separation of DNA strands. The ability to actively translocate along double-stranded DNA may have been selected by eukaryotic and bacterial cellular replication fork helicases to restrict unwinding to replication fork structures, stop unwinding when the replication fork encounters a nick, and perform additional functions such as branch migration of a Holliday junction.

Acknowledgments—We thank Dr. Zvi Kelman and Dr. Jerard Hurwitz for communicating results on a similar subject in different species. We thank Dr. Kenneth Marnris for providing the DNA plasmids for overexpression of RuvA and RuvB. We thank everyone in the O’Donnell laboratory.

REFERENCES
1. Lahib, K., Terreno, J. A., and Diffley, J. F. X. (2000) Science 288, 1643–1647
2. Yan, H., Merchant, A. M., and Tye, B. K. (1993) Genes Dev. 7, 2149–2160
3. Gómez, E. B., Catlett, M. G., and Forb싱g, S. L. (2002) Genetics 160, 1305–1318
4. Lahib, K., Kearssey, S. E., and Diffley, J. F. X. (2001) Mol. Biol. Cell 22, 3658–3667
5. Lindner, K., Gregun, J., Montgomery, S., and Kearssey, S. E. (2002) Mol. Biol. Cell 13, 435–444
6. Aparicio, O. M., Weinstein, D. M., and Bell, S. P. (1997) Cell 91, 59–69
7. Ishimi, Y. (1997) J. Biol. Chem. 272, 24508–24513
8. Lee, J.-K., and Hurwitz, J. (2000) J. Biol. Chem. 275, 18871–18878
9. You, Z., Komamura, Y., and Ishimi, Y. (1999) Mol. Cell. Biol. 19, 8003–8015
10. Sato, M., Gotow, T., You, Z., Komamura-Kohno, Y., Uchiyama, Y., Yabuta, N., Nojima, H., and Ishimi, Y. (2000) J. Mol. Biol. 300, 421–431
11. Koonin, E. V. (1993) Nucleic Acids Res. 21, 2541–2547
12. Li, D., Zhao, R., Liljestrom, W., Gal, D., Zhang, R., DeCaprio, J. A., Fanning, E., Jochimski, A., Staskewyl, G., and Chen, X. S. (2003) Nature 425, 512–518
13. Gorbalenya, A. E., and Koonin, E. V. (1993)Curr. Opin. Struct. Biol. 3, 419–429
14. Sawaya, M. R., Gao, S., Taber, S., Richardson, C. C., and Ellenberger, T. (1999) Cell 99, 167–177
15. Yabuta, N., Kajimura, N., Mayanagi, K., Sato, M., Gotow, T., Uchiyama, Y., Ishimi, Y., and Nojima, H. (2003) Genes Cells 8, 413–421
16. VanLoock, M., Alexander, A., Yu, X., Cozzarelli, N., and Egelman, E. (2002) Curr. Biol. 12, 472–476
17. Chong, J. P. J., Hayashi, M. K., Simon, M. N., Xu, R.-M., and Stillman, B. (1999) Proc. Natl. Acad. Sci. U. S. A. 97, 1530–1535
18. Yu, X., VanLoock, M. S., Poplawski, A., Kelman, Z., Xiang, T., Tye, B. K., and Egelman, E. H. (2002) EMBO Rep. 3, 792–797
19. Yang, S., Yu, X., VanLoock, M., Jezewska, M., Bujalowski, W., and Egelman, E. (2002) J. Mol. Biol. 321, 839–849
20. VanLoock, M. S., Chen, Y. J., Yu, X., Patel, S. S., and Egelman, E. H. (2001) J. Mol. Biol. 311, 951–956
21. Hacker, K. J., and Johnson, K. A. (1997) Biochemistry 36, 14980–14987
22. Egelman, E. H., Yu, X., Wild, R., Hingorani, M. M., and Patel, S. S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3869–3873
23. Fisher, R. J., Bishop, B. E., Leon, R. P., Schafini, R. A., Ogata, C. M., and Chen, X. S. (2000) Nat. Struct. Biol. 10, 160–167
24. Kelman, Z., and Hurwitz, J. (2003) Nat. Struct. Biol. 10, 148–150
25. Lee, J.-K., and Hurwitz, J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 54–59
26. Laskey, R. A., and Madine, M. A. (2003) EMBO Rep. 22, 704–712
27. Ahnert, P., and Patel, S. S. (1997) J. Biol. Chem. 272, 32267–32273
28. Kaplan, D. L. (2000) J. Mol. Biol. 301, 285–299
29. LeBowitz, J. H., and McMacken, R. (1986) J. Biol. Chem. 261, 4738–4748
30. Richardson, R. W., and Nussal, N. G. (1989) J. Biol. Chem. 264, 4725–4731
31. Kaplan, D. L., and Steitz, T. A. (1999) J. Biol. Chem. 274, 6889–6897
32. Kaplan, D. L., and O’Donnell, M. (2002) Mol. Cell 10, 647–657
33. Davey, M. J., Indiani, C., and O’Donnell, M. (2003) J. Biol. Chem. 278, 4491–4499
34. Tsaneva, I., IJling, G., Lloyd, R., and West, S. (1992) Mol. Gen. Genet. 235, 1–10
35. Yuzhakov, A., Turner, J., and O’Donnell, M. (1996) Cell 86, 877–886
36. Runyan, G. T., Wong, I., and Lohman, T. M. (1993) Biochemistry 32, 606–612
37. Schwacha, A., and Bell, S. P. (2001) Mol. Cell 8, 1093–1104
38. Patel, S. S., and Picha, K. M. (2000) Annu. Rev. Biochem. 69, 651–697
39. Malof, K. K., Fiecher, C. J., and Lohman, T. M. (2003) J. Mol. Biol. 325, 913–935
40. Mechanic, L. E., Hall, M. C., and Matson, S. W. (1999) J. Biol. Chem. 274, 17502–17512
41. Runyan, G. T., and Lohman, T. M. (1989) J. Biol. Chem. 264, 17502–17512
42. Velazquez, S. S., Solbana, P., Dilinger, M. S., Subramanya, H. S., and Wigley, D. B. (1999) Cell 97, 75–84
43. Hozak, P., Hassam, A. B., Jackson, D. A., and Cook, P. R. (1993) Cell 73, 361–373
44. Dimitrova, D., Todorov, I. T., Melendy, T., and Gilbert, D. M. (1999) J. Cell Biol. 146, 709–722
45. Kim, S., Dallmann, H. G., McHenry, C. S., and Marnris, K. J. (1996) Cell 84, 643–650
46. Kerber, A., and Baker, T. A. (1999) DNA Replication, 2nd Ed., pp. 275–303, W. H. Freeman and Co., New York
47. Bell, S. P., and Dutta, A. (2002) Annu. Rev. Biochem. 71, 333–374
48. Lusetti, S. L., and Cox, M. M. (2002) Annu. Rev. Biochem. 71, 71–100
49. Papes, F., and Haber, J. E. (1999) Microbiol. Mol. Biol. Rev. 63, 349–404
50. Leipe, D. D., Aravind, L., Grishin, N. V., and Koonin, E. V. (2000) Genome Res. 10, 5–16
Mcm4,6,7 Uses a "Pump in Ring" Mechanism to Unwind DNA by Steric Exclusion and Actively Translocate along a Duplex
Daniel L. Kaplan, Megan J. Davey and Mike O'Donnell

J. Biol. Chem. 2003, 278:49171-49182.
doi: 10.1074/jbc.M308074200 originally published online September 17, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M308074200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2003/10/15/M308074200.DC1

This article cites 47 references, 20 of which can be accessed free at
http://www.jbc.org/content/278/49/49171.full.html#ref-list-1