

## **Mcm10 functions to isomerize CMG-DNA for replisome bypass of DNA blocks.**

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Short title: Mcm10 enables replisome bypass of barriers

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## 1 Abstract

2  
3 Replicative helicases of all cell types are rings that unwind DNA by steric exclusion in which  
4 the helicase ring only encircles the tracking strand, excluding the other strand outside the  
5 ring. Steric exclusion mediated unwinding enables helicase rings to bypass blocks on the  
6 strand that is excluded from the central channel. Unlike other replicative helicases,  
7 eukaryotic CMG encircles duplex DNA at a forked junction and is stopped by a block on the  
8 non-tracking (lagging) strand. This report demonstrates that Mcm10, an essential  
9 replication protein unique to eukaryotes, binds CMG and enables the replisome to bypass  
10 blocks on the non-tracking strand, implying that Mcm10 isomerizes the CMG-DNA complex  
11 to position only one strand through the central channel. A similar CMG-DNA isomerization is  
12 needed at the origin for head-to-head CMGs to bypass one another during formation of  
13 bidirectional replication forks.

## 14 Introduction

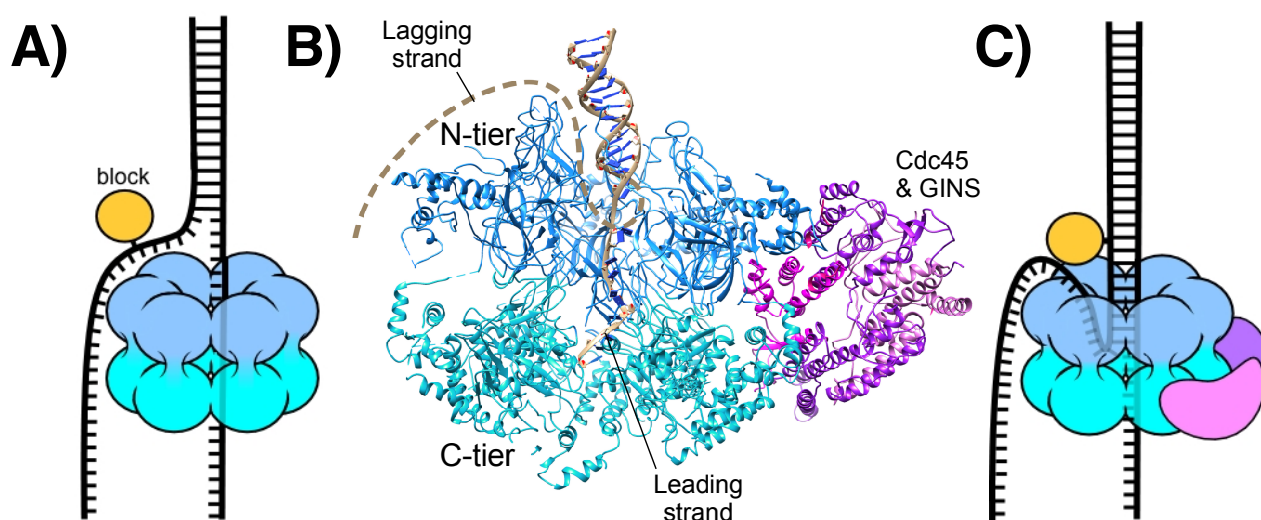
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16 The replication of cellular DNA requires use of a helicase to separate the strands. The  
17 replicative helicase in all domains of life is a circular hexamer. There are four superfamilies  
18 of hexameric helicases, SF3-6, that assort into two main groups, the bacterial helicases  
19 (SF4,5) that have ATP sites derived from the RecA fold and eukaryotic/archaeal helicases  
20 (SF3,6) that have ATP sites derived from the AAA+ fold (Singleton et al., 2007). In all cases,  
21 the subunits of hexameric helicases are composed of two major domains, giving them the  
22 appearance of two stacked rings, an N-tier ring and C-tier ring; the motors are contained in  
23 the C-tier ring. The RecA based bacterial helicases track 5'-3' on DNA with the C-tier motors  
24 leading the N-tier, as determined by crystal structures of bacterial Rho (SF5) and DnaB  
25 (SF4) while eukaryotic helicases track 3'-5' with the N-tier ahead of the C-tier as  
26 demonstrated by structures of bovine papilloma virus E1 (SF3) and *S. cerevisiae* CMG (SF6)  
27 (Enemark and Joshua-Tor, 2006; Georgescu et al., 2017; Itsathitphaisarn et al., 2012;  
28 Thomsen and Berger, 2009).

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30 Hexameric helicases are thought to act by encircling only one strand of DNA upon which  
31 they track and exclude the non-tracking strand to the outside of the ring, thereby acting as a  
32 wedge to split DNA in a process often referred to as steric exclusion and illustrated in  
33 **Figure 1A** (Bell and Labib, 2016; Enemark and Joshua-Tor, 2008; Lyubimov et al., 2011).  
34 Whether a helicase functions by steric exclusion is determined by biochemical experiments  
35 that place a bulky block on one or the other strand of the duplex. A block placed on the non-  
36 tracking strand (i.e. the strand that is excluded from the central channel) does not inhibit  
37 helicase unwinding, while a block placed on the tracking strand stops the helicase because  
38 the bulky block cannot fit through the central channel.

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40 The eukaryotic helicase is the Mcm2-7 heterohexamer which requires five additional  
41 accessory proteins - Cdc45 and the four subunit GINS complex - for full activity. This 11-  
42 subunit assembly is referred to as CMG (Cdc45, Mcm2-7, GINS) (Ilves et al., 2010; Moyer et  
43 al., 2006). The recent cryoEM 3D structure of *S. cerevisiae* CMG helicase at a DNA replication  
44 fork shows a unique DNA binding feature (**Figure 1B**) (Georgescu et al., 2017). Instead of  
45 encircling only ssDNA, the N-tier of CMG encircles dsDNA and the DNA unwinding point is  
46 buried inside the central channel; the unwound leading strand then proceeds through the  
47 central channel into the C-tier motor domain. The lagging strand is not visualized in the  
48 structure, indicating mobility, and is proposed to bend back out of the center of the ring. The  
49 dsDNA is held at a 28° angle to the central channel, surrounded by the zinc fingers at the  
50

51 “top” of CMG. The dsDNA appears to be tightly held because if the CMG-dsDNA contact was  
52 flexible the DNA would have been averaged out during 3D reconstruction.  
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54 The structural evidence that *S. cerevisiae* CMG encircles dsDNA during unwinding is  
55 supported by recent biochemical experiments using strand specific dual streptavidin blocks  
56 that show CMG is halted by a block placed on either the non-tracking (lagging) or the  
57 tracking (leading) strand (**Figure 1C**) (Langston and O'Donnell, 2017). Interestingly, given a  
58 sufficiently long time *S. cerevisiae* CMG can proceed through the lagging strand block  
59 without displacing the streptavidin, suggesting that CMG slowly isomerizes to a steric  
60 exclusion mode that only encircles the tracking (leading) strand. Earlier studies in *Xenopus*  
61 extracts demonstrate that replisome progression is not hindered by a dual streptavidin  
62 block on the lagging strand and conclude that CMG functions in a steric exclusion mode by  
63 only encircling the leading strand (Fu et al., 2011). Considering that isolated *S. cerevisiae*  
64 CMG encircles dsDNA, we proposed that *Xenopus* extracts contain a factor that facilitates  
65 isomerization of CMG-DNA (e.g. such that CMG encircles only ssDNA) enabling it to bypass  
66 blocks on the non-tracking strand (Langston and O'Donnell, 2017).



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69 **Figure 1. CMG-fork structure and isomerization needed for bypass of lagging strand**  
70 **blocks.** (A) Illustration of a hexameric helicase in the steric exclusion mode, encircling the tracking  
71 strand and excluding the non-tracking strand from the central channel. (B) CMG-forked DNA  
72 structure (PDB ID# 5U8S). Duplex DNA penetrates the N-tier and the unwinding point is internal to  
73 the central channel (Georgescu et al., 2017). The Mcm2-7 subunits are shown in two shades of blue  
74 to highlight the N- and C-tiers. The lagging strand tail is present but not visible, indicating mobility,  
75 and is suggested to bend back out the central channel (dashed line). (C) Illustration of CMG  
76 encountering a lagging strand block in the modified steric exclusion mode, partially encircling both  
77 strands of the duplex DNA as shown in (B). To bypass the block, CMG-DNA must isomerize to a full  
78 steric exclusion mode as shown in (A).

79  
80 The present study identifies Mcm10 as the factor that enables CMG to rapidly bypass a block  
81 on the lagging strand. Mcm10 is unique to eukaryotes and is an essential gene product that,  
82 when mutated, causes abortive entry into S-phase (Du et al., 2012; Merchant et al., 1997;  
83 Solomon et al., 1992; Thu and Bielinsky, 2013). Mcm10 is known to bind Mcm2-7 and Cdc45  
84 (Christensen and Tye, 2003; Di Perna et al., 2013; Douglas and Diffley, 2015; Perez-Arnaiz et  
85 al., 2016) and is generally thought to act as an initiation factor (Thu and Bielinsky, 2013).  
86 The licensing of an origin in G1 phase involves several proteins to form a head-to-head  
87 double hexamer of Mcm2-7. Additional initiation factors transform the Mcm2-7 double  
88 hexamer into two head-to-head CMGs during S phase (Bell and Labib, 2016) but Mcm10 is

89 not required for this action (Kanke et al., 2012; van Deursen et al., 2012; Watase et al., 2012;  
90 Yeeles et al., 2015). Mcm10 is only required at the last step of initiation and is proposed to  
91 either activate CMG helicase or help separate the two CMGs for bidirectional replication  
92 forks (Kanke et al., 2012; Lööke et al., 2017; Quan et al., 2015; van Deursen et al., 2012;  
93 Watase et al., 2012; Yeeles et al., 2015). Several recent studies suggest that Mcm10 may also  
94 function at replication forks instead of only at the origin (Chadha et al., 2016; Gambus et al.,  
95 2006; Lööke et al., 2017; Ricke and Bielinsky, 2004).

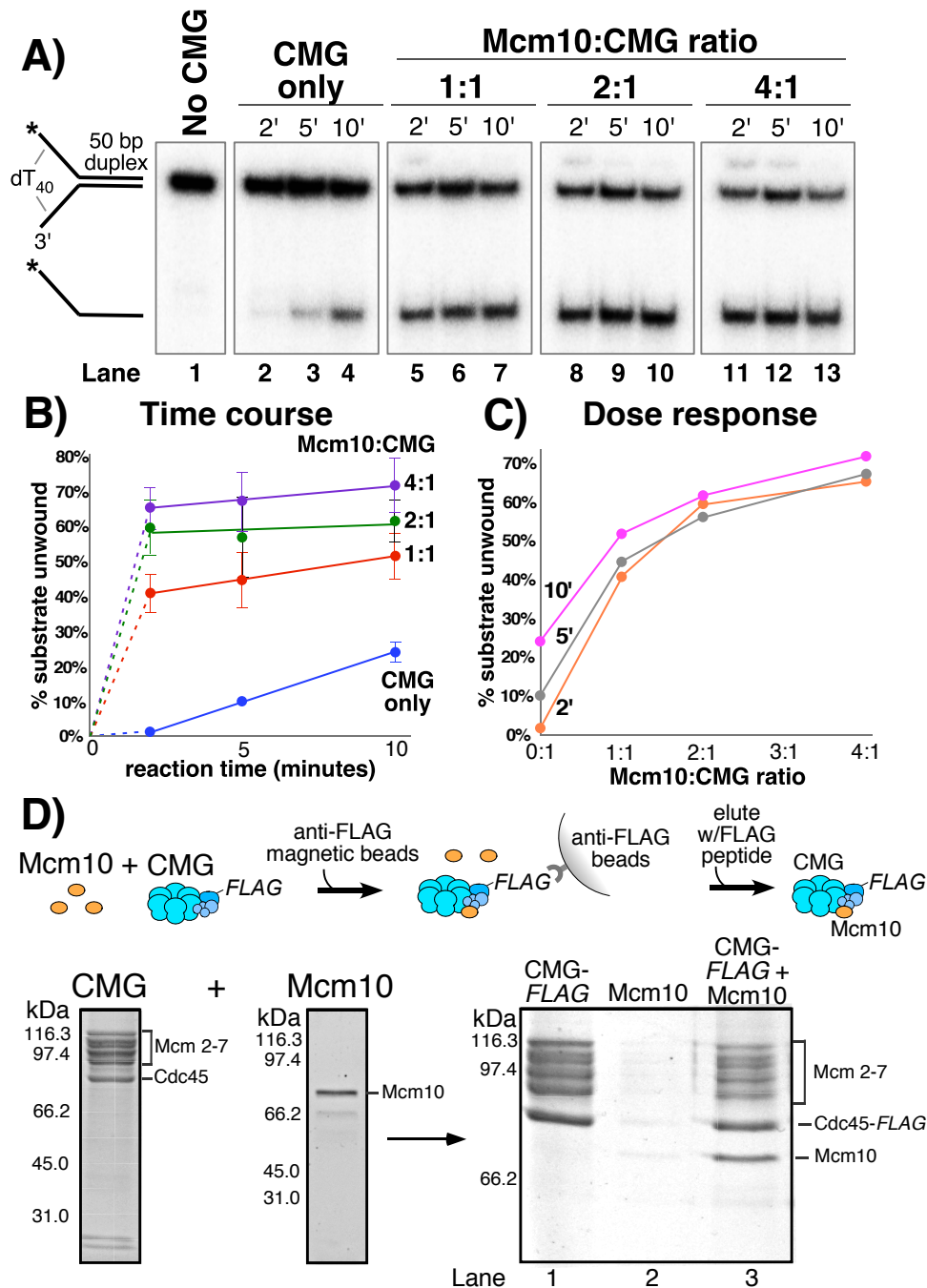
96  
97 The current study demonstrates that Mcm10 forms an isolable stoichiometric complex with  
98 CMG that highly stimulates CMG unwinding (up to 30-fold) and the processivity of CMG in  
99 unwinding. Furthermore, Mcm10 uniquely promotes CMG bypass of a lagging strand block  
100 suggesting that Mcm10 isomerizes the CMG-DNA complex to a steric exclusion mode.  
101 Replisomes also stall at a lagging strand block in the absence of Mcm10 and addition of  
102 Mcm10 is needed to rescue the replisome for continued fork advance past the block.  
103 Interestingly, the rate of DNA synthesis by the replisome is not significantly affected by  
104 Mcm10, yet CMG presumably encircles dsDNA since these replisomes are stalled by a  
105 lagging strand block. Therefore replisome advance is not impeded by CMG encircling dsDNA  
106 until faced with a block on the DNA. Collectively, these studies demonstrate that Mcm10  
107 functions at the level of the helicase to move blocked replisomes past a lagging strand  
108 obstacle. This function might also explain the role of Mcm10 in origin initiation as described  
109 in the Discussion.

## 110 111 **Results:**

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113 **Mcm10 forms a complex with CMG and greatly stimulates helicase activity.** In **Figure**  
114 **2A** we titrated Mcm10 into a helicase assay using a fixed amount of CMG and a synthetic  
115 forked DNA with a 50bp duplex and 40-nt dT ssDNA tails. Stimulation by Mcm10 is  
116 remarkable, with over 30-fold enhancement of CMG unwinding at 2' in the presence of only  
117 one molecule of Mcm10 per CMG (compare lane 5 to lane 2 in **Figure 2A**). Reactions  
118 containing Mcm10 are complete by the 5-minute time point, while the CMG reaction without  
119 Mcm10 continues slow unwinding during the 10-minute time course (**Figure 2B**). The  
120 results at different Mcm10 concentrations indicate that maximal stimulation of CMG is  
121 observed with two subunits of Mcm10 for each CMG complex and further addition of  
122 Mcm10 beyond this amount has little additional effect. (**Figure 2C**).

123  
124 To determine if Mcm10 binds to CMG in a stable fashion, we mixed a 3-fold molar excess of  
125 Mcm10 (as monomer) with FLAG-tagged CMG and then isolated the CMG-Mcm10 complex  
126 using FLAG antibody magnetic beads (**Figure 2D**). The reaction was washed twice with  
127 buffer containing 300 mM NaCl and CMG-Mcm10 complex was eluted using FLAG peptide.  
128 The eluted material was analyzed by SDS/PAGE, and stoichiometric levels of Mcm10 were  
129 clearly visible with CMG (lane 3). Mcm10 was not visible in a control reaction in which  
130 Mcm10 was present with beads in the absence of CMG (lane 2). We also found that the CMG-  
131 Mcm10 complex could be isolated using a MonoQ column and elutes at ~400 mM NaCl  
132 (**Figure 2 - Supplement 1**). The reconstituted complex is functional in unwinding assays  
133 and shows much greater activity on the forked substrate than CMG alone (**Figure 2 -**  
134 **Supplement 2**), comparable to that seen when adding Mcm10 directly to the unwinding  
135 assay in **Figure 2**. The fact that Mcm10 associates with CMG sufficiently tightly to be  
136 isolated by MonoQ chromatography and by immunoaffinity beads using 300 mM salt  
137 washes suggests that CMG-Mcm10 is a stable complex. Densitometric scans from several  
138 preparations of CMG-Mcm10 made using both methods give a stoichiometry of ~1-2 Mcm10  
139 subunits for each CMG complex, consistent with the Mcm10 titration results of **Figure 2**.



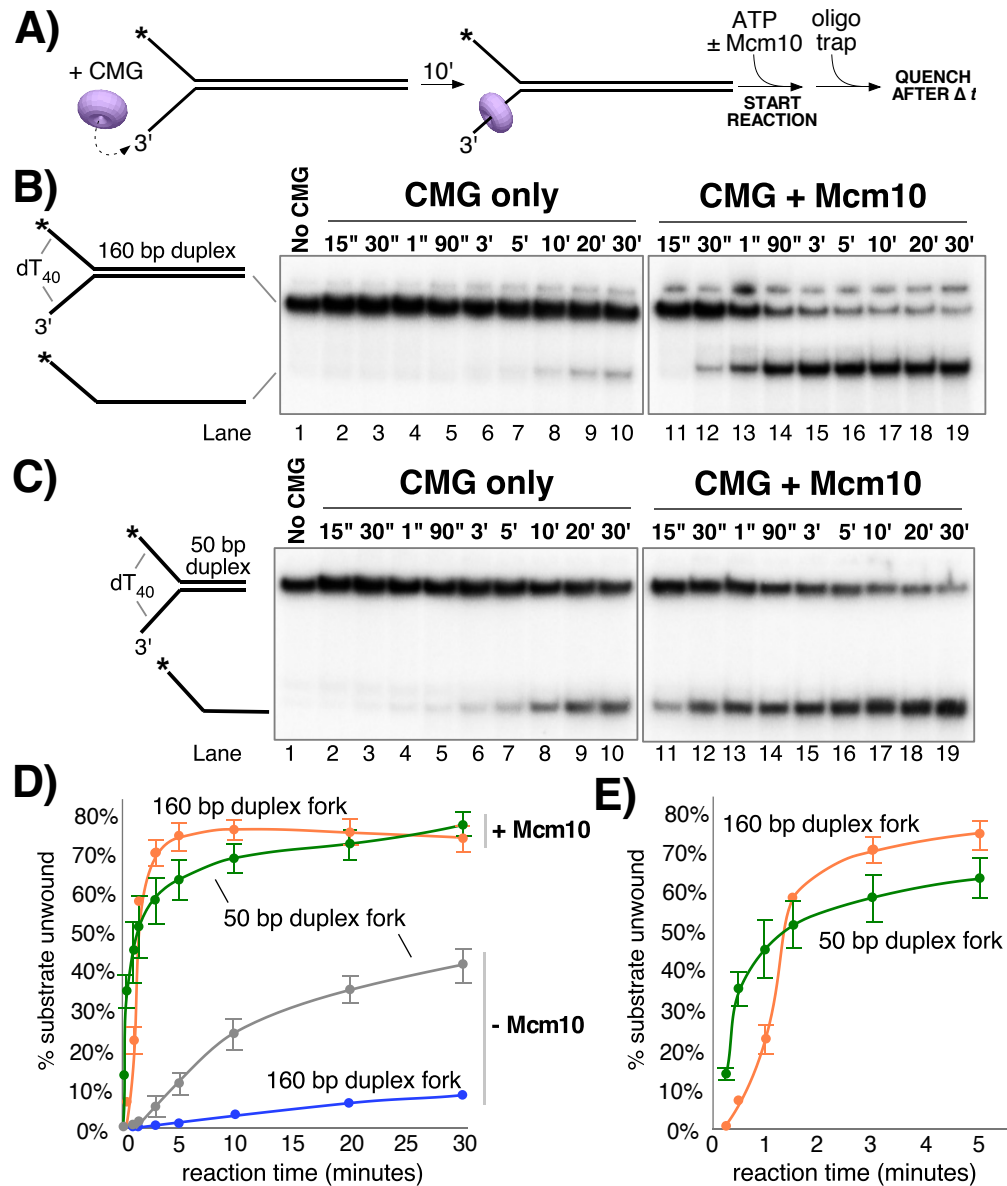


**Figure 2. Mcm10 binds CMG and stimulates its helicase activity.** A) Titration of Mcm10 in a CMG unwinding assay. Reactions contained 25 nM CMG with either: no Mcm10 (lanes 2-4), 25nM Mcm10 (1:1, lanes 5-7), 50 nM Mcm10 (2:1, lanes 8-10) or 100 nM Mcm10 (4:1, lanes 11-13). See Materials and Methods for details. B) Quantification of the data from (A). Values are the average of three independent experiments and the error bars show the standard deviation. C) The same data displayed as a dose-response curve of unwinding as a function of the Mcm10:CMG ratio in the reaction at the three different time points. D) Isolation of CMG-Mcm10 complex using CMG-Flag and anti-Flag beads. CMG and a 3-fold molar excess of Mcm10 were mixed, attached to beads, then washed twice and eluted with Flag peptide. The PAGE gel shows elution from the anti-Flag beads that were loaded with either CMG-flag alone (lane 1), Mcm10 alone (lane 2), or the CMG-flag+Mcm10 mixture (lane 3). The protein bands are identified to the right.

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153 **Mcm10 enhances the processivity of CMG helicase.** The stimulation of unwinding by  
154 Mcm10 observed in **Figure 2** could be attributable to more efficient loading of CMG onto the  
155 substrate, faster unwinding, and/or greater processivity of unwinding. To distinguish  
156 among these possibilities, we compared CMG unwinding of a fork with a longer, 160 bp  
157 duplex region to that of the fork with a 50 bp duplex (as in **Figure 2**) in the presence and  
158 absence of Mcm10 (**Figure 3**). The two substrates have identical 3' and 5' ssDNA tails, so  
159 loading of CMG onto the forks should be the same and any differences in unwinding should  
160 be attributable to differences in CMG unwinding activity over the different lengths of duplex.  
161 For these experiments, CMG was pre-incubated with the DNA substrate for 10' followed by  
162 addition of ATP  $\pm$  Mcm10 to start the reaction (reaction scheme in **Figure 3A**). In the  
163 absence of Mcm10, substantial differences were observed in unwinding of the two  
164 substrates that suggest limited processivity of the helicase, consistent with reports of low  
165 processivity by *Drosophila* and human CMG (Kang et al., 2012; Moyer et al., 2006). CMG  
166 onwound only 3% of the longer 160 bp duplex fork in 10' (**Figure 3B** lanes 1-10 and graph  
167 in **Figure 3D**) compared to 24% for the shorter 50 bp duplex fork at 10' (**Figure 3C**). Even  
168 after 30' only 8% of the 160 bp duplex has been unwound by CMG indicating that the  
169 difference in activity between the two substrates is attributable to low processivity of CMG  
170 rather than simply the additional time it takes to unwind the longer substrate.  
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172 The experiments were repeated in the presence of Mcm10 at a 2:1 ratio to CMG (as  
173 determined in **Figure 2A-C**). Surprisingly, in the presence of Mcm10 the unwinding curves  
174 of the two substrates were very similar, in contrast to experiments in the absence of Mcm10  
175 (**Figure 3B and C**, lanes 11-19 and graph in **Figure 3D**). This result suggests that Mcm10  
176 enhances the processivity of CMG and possibly also stimulates the rate of CMG unwinding.  
177 Examination of the first 5' of unwinding in the presence of Mcm10 shows a short delay in  
178 the appearance of products using the 160 bp duplex compared to the 50 bp duplex (**Figure**  
179 **3E**). We took advantage of this delayed appearance of the longer products to design an  
180 experiment that approximates the average rate of unwinding by CMG-Mcm10 (**Figure 3 -**  
181 **Supplement 1**). CMG was pre-incubated with an equimolar mixture of the two different  
182 length substrates and then the reaction was started by the addition of ATP and Mcm10. The  
183 substrates and products migrate at distinguishable positions in a native PAGE gel allowing  
184 us to observe the appearance of both products in the same reaction. As shown in **Figure 3 -**  
185 **Supplement 1**, appearance of unwound 160 bp duplex products was delayed by ~46  
186 seconds compared to the 50 bp duplex, indicating an average rate of unwinding of 2.4 bp/s  
187 over the additional 110 bp.  
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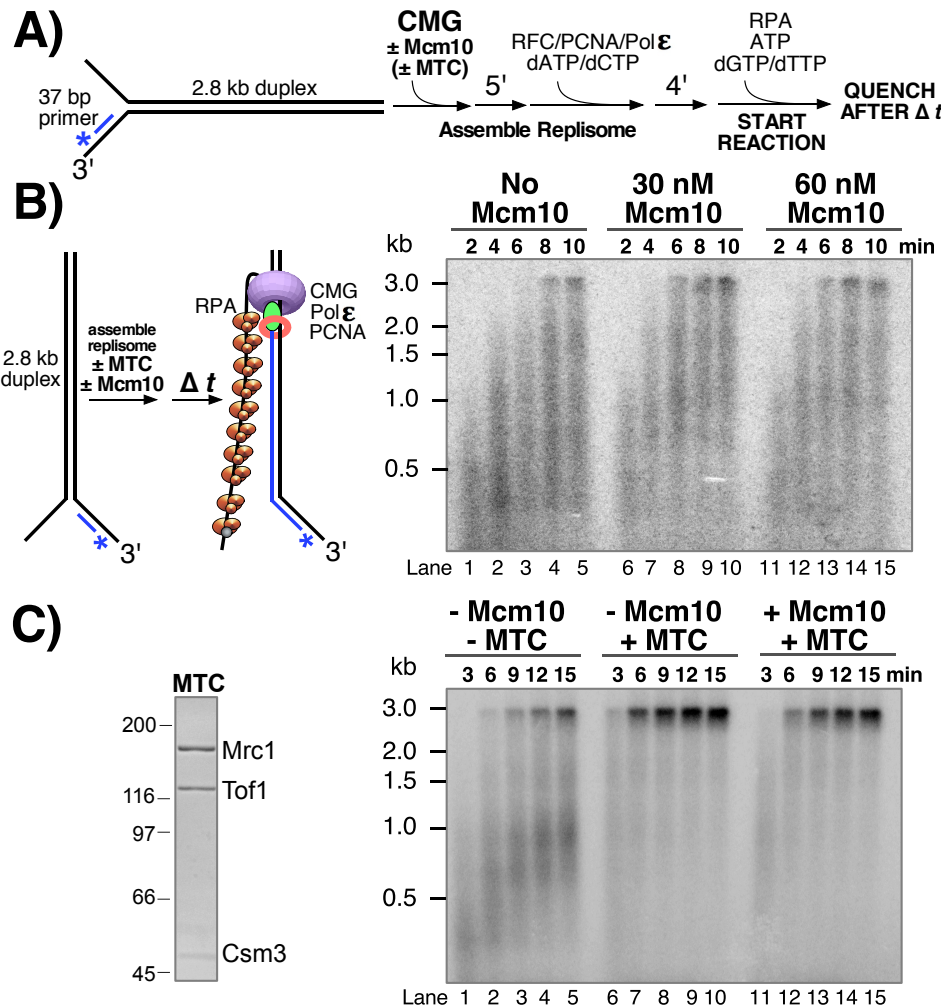
**Figure 3. Mcm10 enhances the processivity of CMG unwinding.** A) Scheme of the reaction. CMG was pre-incubated with the substrates for 10' before addition of ATP  $\pm$  a 2-fold excess of Mcm10 over CMG. B) Native PAGE analysis of CMG unwinding in the absence (lanes 2-10) or presence (lanes 11-19) of Mcm10 using either a 160 bp duplex fork (panel B) or a 50 bp duplex fork (panel C) as indicated by the schematics to the left of the gels. D) Quantification of the data in the gels. E) The first 5' of reactions with Mcm10 are shown to better illustrate the effect of Mcm10 on unwinding by CMG. The values are averages of three independent experiments and the error bars show the standard deviation.

**Mcm10 stimulates CMG-dependent leading strand replication in the absence of Mrc1-Tof1-Csm3.** Having observed stimulation of CMG by Mcm10 in helicase assays, we wished to determine whether Mcm10 also stimulated CMG in the context of the DNA replication fork. To do so, we used a 2.8 kb linear forked DNA primed with a  $^{32}\text{P}$ -5' end-labeled 37mer oligo (Georgescu et al., 2014; Langston et al., 2014) and tested the effect of Mcm10 on extension of the radiolabeled primer by the core leading strand replisome consisting of CMG, Pol  $\epsilon$ , RFC, PCNA, and RPA (**Figure 4**). CMG is pre-incubated with the DNA substrate (in the presence or absence of Mcm10) for 5' at 30 $^{\circ}$  C to allow CMG loading onto the 3' tail of the fork (see reaction scheme in **Figure 4A**). ATP is omitted at this stage to prevent CMG

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from unwinding the DNA before assembly of the replisome. Pol  $\epsilon$ , RFC, PCNA and 2 dNTPs are then added and incubated a further 4' to assemble the core leading strand replisome and the reaction is started upon addition of the remaining dNTPs and ATP along with RPA. Analysis of the autoradiogram in **Figure 4B** shows a modest enhancement of CMG-dependent primer extension by Mcm10. In the presence of Mcm10 the 2.8 kb full length product can be observed at the 6' time point (**Figure 4B**, lanes 8 and 13), yielding a maximum fork rate of 7.8 nucleotides (ntds)/s; 467 ntds/min), while 8' is required to observe the full-length product in the absence of Mcm10 (**Figure 4B**, lane 4; 5.8 ntds/s; 350 ntds/min). This result is consistent with modest stimulation of the rate of DNA synthesis by Mcm10 in an origin-dependent plasmid replisome assembly assay (Lööke et al., 2017).



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**Figure 4 Mcm10 stimulates CMG-dependent leading strand replication in the absence of Mrc1-Tof1-Csm3.** A) Reaction scheme. See Methods for details. B) alkaline agarose gel of leading strand products synthesized by the core leading strand replisome with the indicated amounts of Mcm10. C) Purified MTC complex is shown to the left. Numbers to the left of the SDS Page Gel show the migration of molecular weight markers (kDa). Time courses, to the right, are the core leading strand replisome with or without Mcm10 and MTC as indicated above the gel.

Cell biological and genetic studies have shown that replication forks move about 2-fold slower in cells that lack Mrc1 (Szyjka, Viggiani et al. 2005, Tourrière, Versini et al. 2005, Hodgson, Calzada et al. 2007, Petermann, Helleday et al. 2008) and these observations have



229 been recapitulated in vitro in a origin plasmid replication system (Yeeles, Janska et al.  
230 2016). The in vitro results demonstrated that Mrc1 stimulation of fork speed was most  
231 efficient in the presence of a Tof1-Csm3 heterodimeric complex. The stimulation by Mrc1  
232 +/- Tof1-Csm3 in the origin plasmid based assay did not require Ctf4, FACT, TopI or lagging  
233 strand Pol  $\delta$  extension (Yeeles et al., 2016). Mcm10 is essential for initiation in the plasmid  
234 assay system and this requirement presents difficulty in determining whether Mcm10 also  
235 enhanced the rate of replication fork progression when Mrc1-Tof1-Csm3 was present  
236 (Yeeles et al., 2016). In our experiments, CMG is added as a purified complex enabling a  
237 direct test of whether Mcm10 stimulates fork speed in the presence of these additional  
238 factors. Hence we expressed and purified a Mrc1-Tof1-Csm3 heterotrimer, referred to  
239 herein as MTC complex, and tested its effect on the leading strand replisome with and  
240 without Mcm10 (**Figure 4C**). The autoradiogram shows that in the presence of the MTC  
241 complex, full length 2.8 kb product can be observed after 3 min (**Figure 4C**, lane 6),  
242 indicating a rate of 20 ntds/s (933 ntds/min), close to the 1.5 kb/min rate of replisomes in  
243 vivo (Conti et al., 2007; Hodgson et al., 2007; Sekedat et al., 2010). Furthermore, the MTC  
244 complex clears up the many immature products formed in the absence of MTC, suggesting  
245 that replication forks that stop or pause are brought to full length by the MTC complex.  
246 Addition of Mcm10 to leading strand replisome reactions containing the MTC complex does  
247 not result in a faster replication fork rate under the conditions used and may have even  
248 slightly slowed the replisome (**Figure 4C**, lanes 11-15). Thus, the improved rate effect of  
249 Mcm10 on the core leading strand replisome observed in **Figure 4B** is eclipsed by the rate  
250 enhancement provided by the MTC complex.

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252 **Mcm10 promotes CMG unwinding past a lagging strand block.** Together, the results of  
253 **Figures 2 and 3** show that Mcm10 strongly stimulates CMG unwinding, yet the experiments  
254 of **Figure 4** indicate that Mcm10 is not essential for normal replisome progression in vitro,  
255 particularly in the presence of the MTC complex. To explain these apparently conflicting  
256 results, we hypothesized that Mcm10 is required for replisome progression in particular  
257 circumstances, for example when CMG is hindered by DNA-bound proteins or other  
258 structural impediments on DNA. Indeed, Mcm10 is required for efficient initiation of DNA  
259 unwinding at an origin, a situation in which the two CMG complexes block one another's  
260 progression because they must pass each other in order to establish two bidirectional forks  
261 (see Discussion) (Georgescu et al., 2017).

262  
263 To test the hypothesis that Mcm10 may help CMG overcome blocks, we examined the effect  
264 of Mcm10 on CMG unwinding of forked DNA with strand-specific blocks on the duplex  
265 portion of the DNA (**Figure 5**). We recently showed that CMG unwinding is inhibited by  
266 biotin-streptavidin on either strand and is nearly inactive when two biotin-streptavidin  
267 blocks are present on either strand of the duplex (Langston and O'Donnell, 2017). This is  
268 unique to CMG, as thus far all other hexameric helicases examined are not blocked by an  
269 obstruction on the non-tracking strand and are only blocked by an obstruction on the  
270 translocating strand (Hacker and Johnson, 1997; Kaplan, 2000; Kaplan et al., 2003; Lee et al.,  
271 2014; Nakano et al., 2013). This feature of hexameric helicases is interpreted as a steric  
272 exclusion mode of unwinding in which the non-tracking strand is completely excluded from  
273 the central channel of the helicase ring, thereby explaining why bulky substituents on the  
274 non-tracking strand do not affect helicase unwinding. In contrast, *S. cerevisiae* CMG is  
275 inhibited by a block on the non-tracking strand (Langston and O'Donnell, 2017), consistent  
276 with the CMG-forked DNA structure showing that CMG encircles ds/ssDNA at the fork (see  
277 **Figure 1B-C**) (Georgescu et al., 2017). However, when given sufficient time, the bulk of the  
278 CMGs that progress past the lagging strand block leave streptavidin attached to DNA and

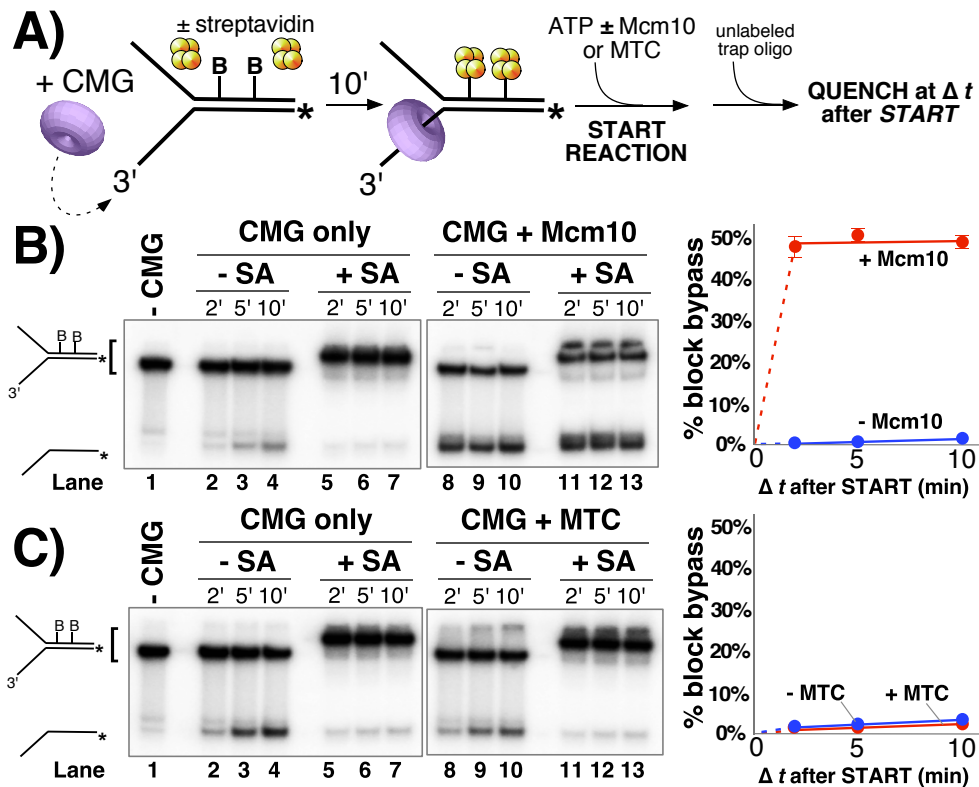


279 thus likely isomerize to encircle only the leading ssDNA to bypass the block without  
280 displacing it (Langston and O'Donnell, 2017).

281  
282 In contrast to studies with pure CMG, studies of replication in *Xenopus* egg extracts show  
283 that two adjacent streptavidin blocks on the non-translocating (lagging) strand are quickly  
284 bypassed by the replisome (Fu et al., 2011). The fact that isolated CMG is strongly inhibited  
285 by the same dual streptavidin block, suggests that some other factor in the complete extract  
286 helps CMG bypass lagging strand blocks. To test whether Mcm10 may be the factor that  
287 facilitates CMG bypass of a lagging strand block, we used a forked DNA substrate with two  
288 biotinylated nucleotides on the duplex portion of the lagging strand template (see **Figure**  
289 **5A**), similar to that used in the *Xenopus* study that demonstrated replisome bypass of  
290 lagging strand blocks by steric exclusion (Fu et al., 2011). Control reactions lacking Mcm10  
291 show that CMG helicase is essentially shut down by the streptavidin blocks (**Figure 5B**,  
292 compare lanes 2-4 without streptavidin to lanes 5-7 with streptavidin) as previously  
293 observed (Langston and O'Donnell, 2017). But surprisingly, in the presence of Mcm10,  
294 approximately 50% of the DNA is unwound and the unwinding rate is as fast and as  
295 extensive in the presence of streptavidin as in the absence of streptavidin (**Figure 5B**,  
296 compare lanes 8-10 and 11-13). Hence, Mcm10 enables CMG to rapidly bypass a dual  
297 streptavidin block on the lagging strand. This observation mirrors results observed in the  
298 *Xenopus* system and resolves the discrepancies between the results using purified CMG and  
299 the observations made in *Xenopus* extracts (Fu et al., 2011; Langston and O'Donnell, 2017).

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301 Having shown that the MTC complex greatly stimulates progression of the leading strand  
302 replisome even in the absence of Mcm10 (i.e. in **Figure 4**), we tested the MTC complex in  
303 CMG helicase assays to see if it enhances the ability of CMG to bypass blocks on DNA (**Figure**  
304 **5C**). In contrast to Mcm10, MTC was unable to promote CMG bypass of the block (compare  
305 lanes 11-13 in **Figure 5C** to lanes 11-13 in **Figure 5B**). We also note that MTC did not  
306 stimulate CMG unwinding even in the absence of the block (**Figure 5C** compare lanes 8-10  
307 with MTC to lanes 2-4 without MTC), suggesting that MTC does not function at the level of  
308 CMG unwinding. Together with the results of **Figures 2-4**, these data suggest that Mcm10  
309 and MTC affect fork progression in very different ways. In contrast to Mcm10 mediated  
310 bypass of lagging strand blocks, CMG was strongly repressed by a dual streptavidin block on  
311 the leading strand even with Mcm10 present (**Figure 5 – Supplement 1**).

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**Figure 5. Mcm10, but not MTC complex, enables CMG to bypass a lagging strand block.**

A) Illustration of the reaction scheme. See Methods for details. B) Unwinding assays on dual biotinylated forks with or without streptavidin and with or without Mcm10, as indicated. C) Same as in panel B except reactions contained or omitted MTC complex instead of Mcm10.

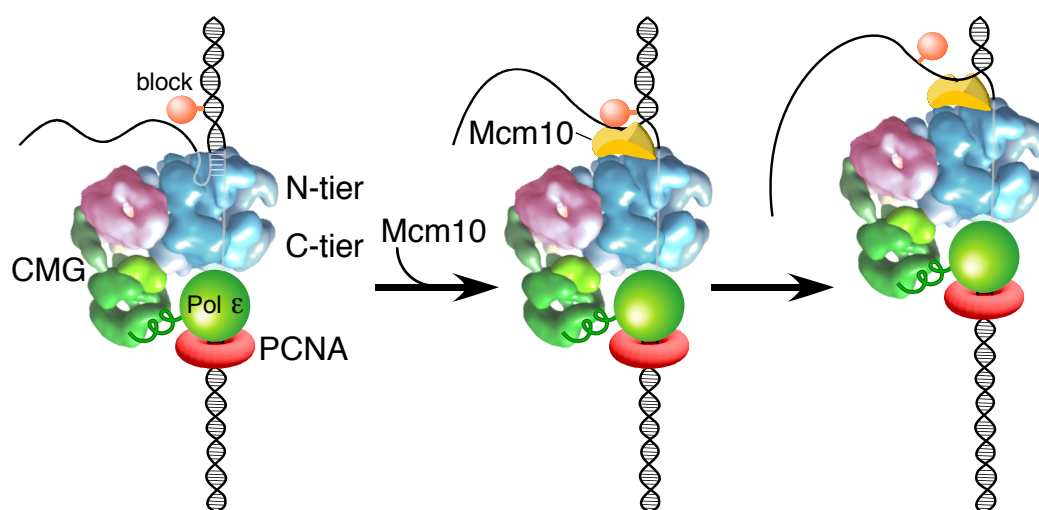
To further investigate the functions of Mcm10 and MTC at a replication fork, we adapted the helicase substrate from **Figure 5** to support DNA replication. The 3' tail of the fork was extended to accommodate a primer for DNA synthesis and the reaction was staged as illustrated in **Figure 5 - Supplement 2 (A)**. First, CMG was added with ATP to allow helicase translocation to the block, followed by Pol  $\epsilon$ /RFC/PCNA/dNTPs to see if the "push" on CMG from a Pol  $\epsilon$ -PCNA motor can overcome the block. The results in **Figure 5 - Supplement 2 (B)** lanes 1-3 show that Pol  $\epsilon$ -PCNA extended the  $^{32}$ P-primer to the stalled CMG (~66bp) but had limited ability to bypass the block to form the 141 bp full length product. The ~91 bp bands result from forks lacking CMG, so Pol  $\epsilon$ -PCNA extends to the fork but cannot proceed further because Pol  $\epsilon$  lacks strand displacement capability and relies on CMG to unwind the DNA for leading strand synthesis (Ganai et al., 2016; Georgescu et al., 2014). MTC has little or no effect on block bypass by the replisome (**Figure 5 - Supplement 2 (B)** lanes 7-9), and is comparable to Pol  $\epsilon$ -PCNA-CMG in lanes 1-3. By contrast, addition of Mcm10 allows the replisome to efficiently and rapidly proceed past these blocks as evidenced by the appearance of full length 141 bp product in lanes 4-6. These results confirm that Mcm10 is needed for replisome bypass of a block and they also indicate that neither MTC nor Pol  $\epsilon$ -PCNA is able to drive the forward movement of a stalled CMG in the absence of Mcm10.

## Discussion

We have shown that a stoichiometric CMG-Mcm10 complex can be reconstituted and isolated (**Figure 2D** and **Figure 2 - Supplement 1**) and that Mcm10 greatly stimulates CMG unwinding and processivity (**Figures 2 and 3**). Notably, we find that Mcm10 enables

341 CMG and the replisome to overcome lagging strand blocks that otherwise bring unwinding  
342 and leading strand synthesis to a halt (**Figure 5 and Figure 5 - Supplement 2**).  
343 Interestingly, the effect of Mcm10 on fork progression *per se* is relatively insignificant in the  
344 absence of blocks, indicating that CMG encircling dsDNA does not slow the rate of fork  
345 progression (**Figure 4**). These results indicate that Mcm10 reorganizes CMG on DNA or  
346 otherwise alters its dynamics such that it rapidly bypasses lagging strand blocks, as  
347 discussed below. This function of Mcm10 is consistent with genetic studies showing that  
348 Mcm10 deficiency leads to genome instability and increased dependence on post-  
349 replication and recombinational repair pathways to maintain viability (Araki et al., 2003;  
350 Lee et al., 2010).

351  
352 Previous reports have solved the structure of the central DNA binding domains of *Xenopus*  
353 Mcm10 and documented that the protein has two DNA binding elements, an OB fold and a  
354 zinc finger, that allow it to bind ssDNA and/or dsDNA, perhaps simultaneously at the forked  
355 junction (Du et al., 2012; Robertson et al., 2008; Warren et al., 2008). Following the internal  
356 DNA binding domains is a C-terminal region that forms the main interaction site to Mcms  
357 (Douglas and Diffley, 2015; Lööke et al., 2017; Quan et al., 2015). Furthermore, Mcm10  
358 appears to bind to the N-tier region of the CMG complex that faces the forked junction  
359 (**Figure 2**), as recent studies show that interaction of Mcm10 with Mcm2-7 is mediated  
360 primarily through an interaction with a conserved domain in the N-terminal region of the  
361 Mcm2 subunit (Lööke et al., 2017). Consistent with these known properties of Mcm10, we  
362 propose in **Figure 6** that, upon encountering a lagging strand block, Mcm10 binds at the N-  
363 tier of CMG and also binds DNA at the fork. This places Mcm10 between CMG and the forked  
364 junction such that duplex DNA no longer enters the central channel of CMG. In this  
365 configuration, Mcm10 converts CMG to a steric exclusion mode where it is capable of  
366 bypassing blocks on the non-tracking strand as observed with other hexameric helicases  
367 and with the complete replisome in the *Xenopus* extract system (Fu et al., 2011). Alternative  
368 isomerization reactions that we have discussed previously for CMG-DNA bypass of lagging  
369 strand blocks could also be promoted by Mcm10 (Langston and O'Donnell, 2017).



370 **Figure 6. Model of Mcm10 function.** Proposed model of Mcm10 mediated isomerization of  
371 CMG-DNA at a replication fork. Left, CMG encounters an impediment on the DNA but cannot  
372 pass it because it surrounds dsDNA in the N-terminal tier of Mcm2-7 (as in **Figure 1C**);  
373 middle, Mcm10 binds to the forked junction and to the N-terminal tier of CMG, releasing  
374 CMG's grip on the duplex DNA and placing CMG in a steric exclusion mode encircling ssDNA  
375 (as in **Figure 1A**); right, with the lagging strand freed from the interior of the Mcm2-7 ring,  
376 CMG can bypass the impediment on the DNA and proceed.

377

378 **Possible role of Mcm10 at the origin.** Ability to rapidly bypass blocks on the non-tracking  
379 strand is inherent in all replicative hexameric helicases examined with the exception of  
380 eukaryotic CMG. One possible reason that CMG evolved a requirement for another protein to  
381 bypass a lagging strand block is for use in regulation of fork initiation and progression. It is  
382 documented from several laboratories that the head-to-head (N-to-N) double hexamer of  
383 Mcm2-7 at an origin matures to form two CMGs on dsDNA without unwinding DNA (Heller  
384 et al., 2011; Kanke et al., 2012; van Deursen et al., 2012; Watase et al., 2012; Yeeles et al.,  
385 2015). Mcm10 is only required at the last step of origin initiation to “activate” CMG to  
386 unwind DNA as detected by RPA binding to the ssDNA products. These observations can be  
387 explained by the findings of this report. Specifically, CMG is known to track with the N-tier  
388 ahead of the C-tier, and therefore head-to-head CMGs at an origin are directed inward  
389 toward one another. Hence, each CMG blocks the other CMG at an origin. However, if Mcm10  
390 activates the CMGs to locally unwind DNA, as observed by head-to-head SV40 T-antigen  
391 hexamers that produce ssDNA “rabbit ears” at the viral origin (Wessel et al., 1992), the two  
392 CMGs could switch to encircle opposite single-strands and pass one another to form  
393 bidirectional forks (see Figure 8 in (Georgescu et al., 2017)). Furthermore, if the CMGs  
394 encircle dsDNA within the N-tier as observed in the CMG-forked DNA structure (Georgescu  
395 et al., 2017), Mcm10 can isomerize the head-to-head CMG-DNA complexes (e.g. as in **Figure**  
396 **6**) so they no longer collide and can bypass one another to produce bidirectional replication  
397 forks.

398

399 **Is Mcm10 a stable component of a moving replisome?** Considering that Mcm10 does not  
400 appear to increase the rate of the replisome, it would not seem to be required at all times.  
401 Mcm10 would be needed to help the replisome bypass blocks on the lagging strand which  
402 conceivably could be performed by a dynamic Mcm10 that interacts only transiently with a  
403 stalled replisome. Alternatively, Mcm10 mediated isomerization of CMG-DNA may remain  
404 stable such that CMG no longer encircles dsDNA at a forked junction, in which case Mcm10  
405 may not be needed for some time after its first use.

406

407 Evidence that Mcm10 could remain attached to the replisome is its tight association with  
408 CMG observed herein. Mcm10 binds remarkably tightly to CMG, eluting as a CMG-Mcm10  
409 complex from beads after high salt washes (**Figure 2D**), and the CMG-Mcm10 complex also  
410 remains associated and elutes from a MonoQ ion-exchange resin at high ionic strength (400-  
411 450 mM NaCl) (**Figure 2 - Supplement 1**). Additionally, several previous studies also  
412 suggest Mcm10 is a component of moving replisomes (Chadha et al., 2016; Gambus et al.,  
413 2006; Lööke et al., 2017; Ricke and Bielinsky, 2004). Future structural and single-molecule  
414 studies of CMG-Mcm10 may be expected to shed light on further details about the dynamics  
415 of Mcm10 and its replisome bypass function at stalled replisomes described in this report.

416

## 417 **AUTHOR CONTRIBUTIONS**

418

419 L.D.L, R.M., G.D.S, N.Y.Y. and R.G. performed the experiments; L.D.L, O.Y., and D.Z. contributed  
420 purified proteins; L.D.L. and M.O.D. wrote the manuscript.

421

## 422 **MATERIALS AND METHODS**

423

424 **Reagents:** Radioactive nucleotides were from Perkin Elmer and unlabeled nucleotides were  
425 from GE Healthcare. DNA modification enzymes were from New England Biolabs. DNA  
426 oligonucleotides were from Integrated DNA Technologies. Streptavidin was from  
427 Pierce/Thermo Scientific. 5 mg of streptavidin powder was resuspended in 0.5 ml distilled



428 water to make a 10 mg/ml stock. Protein concentrations were determined using the Bio-  
429 Rad Bradford Protein stain using BSA as a standard. *S. cerevisiae* CMG, Pol  $\epsilon$ , RFC, PCNA and  
430 RPA were overexpressed and purified as previously described (Georgescu et al., 2014;  
431 Langston et al., 2014).

432  
433 MBP-Mcm10. Mcm10, with a Maltose Binding Protein (MBP) tag at the N-terminus, was  
434 purified by applying clarified extract from 12L *E. coli* overexpression cells at 1 ml/min to a  
435 12 ml amylose column (New England Biolabs) in MBP buffer (20 mM Tris-Cl pH 7.5, 0.5 M  
436 NaCl, 1 mM DTT and 1 mM EDTA). The column was washed with 100 ml MBP buffer and  
437 eluted with 60 ml MBP buffer supplemented with 10 mM D-(+)-maltose (Sigma Aldrich)  
438 collecting 1.5 ml fractions. Peak fractions containing MBP-Mcm10 were pooled (~20 ml/85  
439 mg) and dialyzed 2 hours vs. 2 L Buffer S (25 mM Hepes-KOH pH 7.5, 10% glycerol, 2 mM  
440 DTT, 2 mM MgCl<sub>2</sub>, and 1 mM EDTA). The dialysate was spun at 5,000 rpm in a swinging  
441 bucket rotor in an RC3B centrifuge to remove any precipitated material. The supernatant  
442 was applied to a 10 ml SP-Sepharose column that had been previously equilibrated in Buffer  
443 S supplemented with 100 mM NaCl and washed with 50 ml of the same buffer. The column  
444 was eluted with a 100 ml linear gradient from 100 to 600 mM NaCl in Buffer S. Peak  
445 fractions containing full-length MBP-Mcm10 were dialyzed overnight vs. 2L buffer S/200  
446 mM NaCl and re-applied to a second 10 ml SP-Sepharose column and eluted with a step  
447 gradient from 20% to 50% of the buffer in pump B in 5% increments (pump A contained  
448 S/100 and pump B contained S/600). The peak of full-length MBP-Mcm10 eluted at 50%  
449 pump B and was pooled and dialyzed against S/200 in the presence/absence of PreScission  
450 Protease to remove the MBP tag. Dialyzed pools were aliquoted, flash frozen in liquid  
451 nitrogen and stored at -80°C. Unless otherwise indicated, the cleaved form of the protein  
452 (with the MBP tag removed) was used in all assays.

453  
454 His-FLAG Mcm10. Mcm10 with a hexahistidine tag at the N-terminus and a 3X FLAG tag at  
455 the C-terminus, was purified by first applying clarified extract from 72L *E. coli*  
456 overexpression cells to a column containing 10 ml Chelating Sepharose Fast Flow (GE  
457 Healthcare) charged with 50 mM NiSO<sub>4</sub>. The column had been previously washed with  
458 binding buffer (20 mM Tris-Cl pH 7.9, 5 mM imidazole, 500 mM NaCl, 0.01% NP-40) and the  
459 *E. coli* extract was adjusted to approximate the ionic strength of the binding buffer and  
460 applied to the column at 1 ml/min. The column was washed with binding buffer and then  
461 eluted with the same buffer containing 375 mM imidazole. The eluted material was then  
462 applied to a column containing 6 ml ANTI-FLAG M2 Affinity Gel (Sigma) at 0.1 ml/min. The  
463 FLAG column had been previously equilibrated in FLAG buffer (20 mM Tris-Cl pH 7.5, 10%  
464 glycerol, 500 mM NaCl, 1 mM DTT, 1 mM MgCl<sub>2</sub>, 0.01% NP-40) and after loading the column  
465 was washed with 50 ml of FLAG buffer. The bound material was eluted with 20 ml FLAG  
466 buffer containing 0.2 mg/ml 3X FLAG peptide (EZ Biolab, Carmel, Indiana USA) and  
467 collecting 1.5 ml fractions. The elution buffer was applied to the column in two 6 ml  
468 increments pausing 30' after each increment followed by 3 ml increments with 30' pauses  
469 until the elution was complete. Eluted material was aliquoted, flash frozen in liquid  
470 nitrogen, and stored at -80°C.

471  
472 Mcm10-Tof1-Csm3 complex. The 3 subunits of the MTC complex (Mrc1-Tof1-Csm3) were  
473 co-expressed in yeast. Genes were integrated into the chromosome of strain OY01 (ade2-1  
474 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1 $\Delta$  MATa pep4::KANMX6) with  
475 Mrc1<sup>Flag</sup> integrated at the Ade2 locus, untagged Tof1 at the His3 locus, and HisCsm1 at the  
476 Leu2 locus, each under control of the Gal1/10 promoter. Cells were grown under selection  
477 at 30 °C in SC glucose, then split into 18L YP-glycerol and grown to OD600 of 0.7 at 30 °C  
478 before induction for 6 h upon addition of 20 g of galactose/L. After 6 h, cells were harvested



479 by centrifugation, resuspended in a minimal volume of 20 mM HEPES, pH 7.6, 1.2%  
480 polyvinylpyrrolidone, and protease inhibitors and frozen by dripping into liquid nitrogen.  
481 Purification of MTC was performed by lysis of 18 L equivalent of frozen cells with a SPEX  
482 cryogenic grinding mill. Ground cell powder was thawed in the cold room and resuspended  
483 to 25 ml final volume with 5X FLAG binding buffer (1x is 250 mM K glutamate, 50 mM  
484 HEPES pH 7.5, 1 mM EDTA pH 8.0) plus protease inhibitors and stirred slowly for 30'. Cell  
485 debris was removed by centrifugation (19,000 r.p.m. in a SS-34 rotor for 1 h at 4 °C) and the  
486 supernatant was collected and mixed with 1.5 ml anti-Flag M2 affinity resin (Sigma)  
487 equilibrated in 1X FLAG binding buffer with 10% glycerol. The mixture was rotated on an  
488 orbital platform in the cold room at 30 rpm for 1 h. To collect the bound proteins, anti-FLAG  
489 resin was pelleted at 1000 X g in 50 ml conical tubes and washed 5 times with 5 ml of FLAG  
490 binding buffer followed by centrifugation. After the final wash step, the anti-Flag affinity  
491 resin was resuspended in 2 ml of FLAG binding buffer with 10% glycerol, loaded onto a  
492 gravity column and washed twice with 7.5 ml of FLAG binding buffer containing 10%  
493 glycerol. Bound protein was eluted with the same buffer containing 0.2 mg/ml 3X FLAG  
494 peptide (EZ Biolab, Carmel, Indiana USA). Eluted protein was concentrated to 0.75 ml of 1.5  
495 mg/ml protein and injected onto a 24 ml Superose 12 gel filtration column equilibrated in  
496 2X PBS with 10% glycerol in two separate runs of 0.5 ml and 0.25 ml. Fractions were  
497 analyzed on a 7.5% SDS-PAGE gel and MTC-containing fractions were pooled, aliquoted,  
498 flash frozen in liquid nitrogen, and stored at -80°C.  
499

500 **Helicase substrates.** For all radiolabeled oligonucleotides, 10 pmol of oligonucleotide was  
501 labeled at the 5' terminus with 0.05 mCi [ $\gamma$ -<sup>32</sup>P]-ATP using T4 Polynucleotide Kinase (New  
502 England Biolabs) in a 25  $\mu$ l reaction for 30' at 37°C according to the manufacturer's  
503 instructions. The kinase was heat inactivated for 20' at 80°C. For annealing, 4 pmol of the  
504 radiolabeled strand was mixed with 6 pmol of the unlabeled complementary strand, NaCl  
505 was added to a final concentration of 200 mM, and the mixture was heated to 90°C and then  
506 cooled to room temperature over a time frame of >1 h. DNA oligonucleotides used in this  
507 study are listed in Table I.  
508

509 **Helicase assays with forked DNA substrates.** For the assays in **Figures 2 and 3C**, the  
510 forked DNA was formed using the following two oligos (Table 1): 50duplex LEAD and 5'-<sup>32</sup>P-  
511 50duplex LAG. For the assays in **Figure 3B**, the forked DNA was formed using unlabeled  
512 160mer duplex LEAD and 5'-<sup>32</sup>P-160mer duplex LAG. Oligos were annealed as described  
513 above.  
514

515 Reactions in **Figure 2A** contained 25 nM CMG and either 0 nM, 25 nM, 50 nM or 100 nM of  
516 Mcm10 (as indicated) with 0.5 nM DNA substrate and 1 mM ATP in 40  $\mu$ l final volume of  
517 buffer A (20 mM Tris Acetate pH 7.6, 5 mM DTT, 0.1 mM EDTA, 10 mM MgSO<sub>4</sub>, 30 mM KCl,  
518 40  $\mu$ g/ml BSA). Reactions were mixed on ice and started by placing in a water bath at 30° C.  
519 1' after starting the reaction, 25 nM unlabeled 50duplex LAG oligo was added as a trap to  
520 prevent re-annealing of unwound radiolabeled DNA. At the indicated times, 12  $\mu$ l aliquots  
521 were removed, stopped with buffer containing 20 mM EDTA and 0.1% SDS (final  
522 concentrations), and flash frozen in liquid nitrogen. Frozen reaction products were thawed  
523 quickly in water at room temperature and separated on 10% native PAGE minigels in TBE  
524 buffer. Gels were washed in distilled water, mounted on Whatman 3MM paper, wrapped in  
525 plastic and exposed to a phosphor screen that was scanned on a Typhoon 9400 laser imager  
526 (GE Healthcare). Scanned gels were analyzed using ImageQuant TL v2005 software (e.g. for  
527 **Figure 2B and 2C**). For all quantitations of helicase assays, the small % background of  
528 unannealed radiolabeled primer in the "No CMG" lane was subtracted from the % unwound  
529 at each time point.

530

531 Reaction conditions in **Figure 3** were similar to those in **Figure 2A** but using 20 nM CMG  
532 and 40 nM Mcm10 (where indicated). CMG was mixed with the substrate on ice in the  
533 absence of ATP and placed at 30° C for 10' to allow CMG to load onto the substrates without  
534 unwinding. To start the reaction, ATP was added with or without Mcm10 (as indicated). 1'  
535 after starting the reaction, 50 nM unlabeled lagging strand oligo was added as a trap to  
536 prevent re-annealing of unwound radiolabeled DNA. Total reaction volumes were 126 µl,  
537 and 11 µl aliquots were stopped at the indicated times after addition of ATP and processed  
538 as described for the assays of **Figure 2A**.

539

540 **Helicase assays using a dual biotin fork DNA:** For the assays in **Figure 5**, the forked DNA  
541 was formed by annealing 50 duplex LAG dual biotin and 5'-<sup>32</sup>P-50duplex LEAD (see Table I).  
542 The biotinylated dT nucleotides are 13 and 20 bases from the forked junction. Oligos were  
543 annealed as described above. Reaction conditions were similar to those in **Figure 3** except  
544 that the final reaction volume was 45 µl and 4 µg/ml streptavidin was added (where  
545 indicated) during the 10' CMG pre-incubation. CMG was at 25 nM and Mcm10 or MTC was at  
546 50 nM (final concentrations) when present. In these assays, the trap oligo was 50 nM  
547 unlabeled 50duplex LEAD oligo. 12 µl aliquots were removed at the indicated times after  
548 addition of ATP, terminated with EDTA/SDS stop buffer, flash frozen and processed as  
549 above.

550

551 **Mcm10 binding to CMG.** To determine if Mcm10 binds to CMG in a stable fashion (**Figure**  
552 **2D**), we mixed 40 pmol of FLAG-CMG with 120 pmol Mcm10. The mixture was incubated for  
553 15 minutes on ice and then spun in a microcentrifuge at 15,000 rpm for 10' at 4°C. The  
554 volume of the protein solution was adjusted to 150 µl with binding buffer (25 mM Hepes, pH  
555 7.5; 10% glycerol; 0.01% Nonidet P-40; 300 mM NaCl) and mixed with 25 µl anti-FLAG M2  
556 magnetic beads (50% suspension; Sigma-Aldrich). The protein-bead mixture was incubated  
557 on ice for 1 h and then the beads were collected with a magnetic separator and the  
558 supernatant (containing unbound proteins) was removed. The beads were washed three  
559 times with 250 µl binding buffer and bound proteins were eluted by incubating in 62.5 µl of  
560 the same buffer supplemented with 0.2 mg/ml 3X FLAG peptide on ice for 30'. The beads  
561 were collected with a magnetic separator and eluted proteins were collected and analyzed  
562 in an 8% SDS-polyacrylamide gel stained with Denville Blue.

563

564 **Replication Assays with 2.8 kb duplex substrate.** Leading strand replication experiments  
565 in **Figure 4** used a singly primed 2.8 kb forked linear DNA substrate that was previously  
566 described (Georgescu et al., 2014). The duplex portion of the DNA substrate is linearized  
567 pUC19 DNA to which a synthetic fork junction has been ligated to one end of the duplex. The  
568 fork is primed for leading strand DNA replication with 5'-<sup>32</sup>P-C2 oligo (Table 1). Reactions  
569 were 25 µL and contained 30 nM CMG, 10 nM Pol ε, 5 nM RFC, 25 nM PCNA, 600 nM RPA  
570 and 1.25 nM linear forked template (final concentrations) in a buffer consisting of 25mM  
571 Tris Acetate pH 7.5, 5% glycerol, 40 µg/ml BSA, 3 mM DTT, 2mM TCEP, 10 mM magnesium  
572 acetate, 50 mM K glutamate, 0.1 mM EDTA, 5 mM ATP, and 120 µM of each dNTP.  
573 Replication assays were performed by first incubating CMG (and the indicated amount of  
574 Mcm10 and/or MTC, where indicated) with linear forked template for 5' at 30° C, followed  
575 by addition of RFC, PCNA, and Pol ε for 4' in the presence of dATP and dCTP to support  
576 clamp loading and polymerase binding while preventing 3'-5' exonuclease activity on the  
577 primer. Reactions were started by addition of ATP, RPA, and the withheld nucleotides (dGTP  
578 and dTTP). The reactions proceeded for the indicated amount of time at 30°C and were  
579 stopped with an equal volume of 2X stop solution (40 mM EDTA and 1% SDS). Reaction

580 products were analyzed on 1.3% alkaline agarose gels at 35 V for 17 h, backed with DE81  
581 paper, and dried by compression. Gels were exposed to a phosphorimager screen and  
582 imaged with a Typhoon FLA 9500 (GE Healthcare).  
583

584 **Stalled Replisome Assays:** For the experiments in **Figure 5 – Supplement 2**, the substrate  
585 was made by annealing Blocked Fork LEAD, Blocked Fork LAG and 5'-<sup>32</sup>P-Blocked Fork  
586 Primer (Table 1). The lagging strand oligo has two biotinylated dT nucleotides that are 13  
587 and 20 bases from the forked junction. To form the lagging strand block, streptavidin (75  
588 nM) was added to the substrate (1.25 nM) and incubated at 30°C for 5' in buffer consisting  
589 of 20 mM Tris Acetate, 4% glycerol, 0.1 mM EDTA, 5 mM DTT, 40 µg/mL BSA and 10 mM  
590 MgSO<sub>4</sub> (all amounts are the final concentration in the complete reaction). Next, 30 nM CMG  
591 was added along with 0.5 mM ATP for 3' to allow CMG to translocate to the block and then  
592 replication was initiated by adding 5 nM RFC, 20 nM PCNA, 20 nM Pol ε, 5 mM ATP, and 30  
593 nM MTC (when present) along with adding 115 µM of each dNTP. After a further 2'  
594 incubation, Mcm10 was either added at 60 nM or omitted. Aliquots of each reaction were  
595 collected 1, 3, and 10 min later and quenched with an equal volume of Stop Buffer  
596 containing 78% formamide, 8 mM EDTA, and 1% SDS. Samples were boiled and then  
597 analyzed by PAGE in a 10% Urea gel. Gels were washed in distilled water, mounted on  
598 Whatman 3MM paper, wrapped in plastic and exposed to a storage phosphor screen that  
599 was scanned on a Typhoon 9400 laser imager (GE Healthcare).  
600

**Table I. Oligonucleotides Used in this Study.** All oligonucleotides used in this study were ordered from IDT with the indicated modifications.

Oligo Name	Sequence (5' to 3')	Modification(s)
50dupex LAG	TT TTTTTTTTTACGCTGCCGAATCTGGCTTGCT AGGACATTACAGGATCGTTCGGTCTC	None
50dupex LAG dual biotin	TT TTTTTTTTTACGCTGCCGAAT <b>TCTGGCTTGCT</b> AGGACATTACAGGATCGTTCGGTCTC	Two biotin-modified thymidine residues in <b>BOLD</b>
50dupex LEAD	GAGACCGAACGATCCTGTAATGTCCTAGCAAG CCAGAA <b>TTCGGCAGCGTCTTTTTTTTTTTTTTT</b> TTTTTTTTTTTTTTTTTTTT* <b>T</b> * <b>T</b> * <b>T</b> * <b>T</b> * <b>T</b>	The six dT residues at the 3' end are connected by phosphorothioate bonds (*)
50dupex LEAD dual biotin	GAGACCGAACGATCCTGTAATGTCCTAGCAA GCCAGAA <b>TTCGGCAGCGTCTTTTTTTTTTTTTTT</b> TTTTTTTTTTTTTTTTTTTT* <b>T</b> * <b>T</b> * <b>T</b> * <b>T</b> * <b>T</b>	Two biotin-modified thymidine residues in <b>BOLD</b> ; the six dT residues at the 3' end are connected by phosphorothioate bonds (*)
160mer duplex LEAD	AGAGAGTAGAGTTGAGTTGTGATGTGTAGAG TTGTTGTAGAGAAGAGTTGTGAAGTGTGAG TAGAGAAGAGAAGAGAAGTGTGTGATGTGT TGAGTAGTGTAGAGTTGAGAAGTAGAGATGT GTTGAGATGAGAAGAGTTGTAGTTGAGTTGA AGTGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTT* <b>T</b> * <b>T</b> * <b>T</b> * <b>T</b> * <b>T</b>	The five dT residues at the 3' end are connected by phosphorothioate bonds (*)
160mer duplex LAG	TT TTTTTTTTTCCACTTCAACTCAACTACAACCTCT TCTCATCTCAACACATCTCTACTTCTCAACTC TACACTACTCAACACATCACAACACTTCTCTT CTCTTCTCTACTCAACACTTCACAACCTTCTCT CTACAACAACCTACACATCACAACCTCAACTC TACTCTCT	None
Blocked Fork LEAD	ACCGGAGACCGAACGATCCTGTAATGTCCTAG CAAGCCAGAA <b>TTCGGCAGCGTCTTTTTTTTTTT</b> TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGA GGAAAGAATGTTGGTGAGGGTTGGGAAGTGG AAGGATGGGCTCGAGAGTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTT* <b>T</b> * <b>T</b> * <b>T</b> * <b>T</b> * <b>T</b>	The five dT residues at the 3' end are connected by phosphorothioate bonds (*)
Blocked Fork LAG	TT TTTTTTTTTTTTTTTTTTTTT <b>TGACGC</b> TGCCGAAT <b>TCTGGCTTGCTAGGACATTACAG</b> GATCGTTCG* <b>G</b> * <b>T</b> * <b>C</b> * <b>T</b> * <b>C</b>	Two biotin-modified thymidine residues in <b>BOLD</b> ; the five dT residues at the 3' end are connected by phosphorothioate bonds (*)
Blocked Fork Primer	CCTCTCGAGCCATCCTTCCACTTCCAACCCT CACC	None
C2	CCTCTCGAGCCATCCTTCCACTTCCAACCCT CACC	None

## References

- 602  
603  
604 Araki, Y., Kawasaki, Y., Sasanuma, H., Tye, B.K., and Sugino, A. (2003). Budding yeast  
605 *mcm10/dna43* mutant requires a novel repair pathway for viability. *Genes to Cells* *8*, 465-  
606 480.
- 607 Bell, S.P., and Labib, K. (2016). Chromosome duplication in *Saccharomyces cerevisiae*.  
608 *Genetics* *203*, 1027-1067.
- 609 Chadha, G.S., Gambus, A., Gillespie, P.J., and Blow, J.J. (2016). *Xenopus* Mcm10 is a CDK-  
610 substrate required for replication fork stability. *Cell Cycle* *15*, 2183-2195.
- 611 Christensen, T.W., and Tye, B.K. (2003). *Drosophila* MCM10 interacts with members of the  
612 prereplication complex and is required for proper chromosome condensation. *Molecular*  
613 *biology of the cell* *14*, 2206-2215.
- 614 Conti, C., Saccà, B., Herrick, J., Lalou, C., Pommier, Y., and Bensimon, A. (2007). Replication  
615 fork velocities at adjacent replication origins are coordinately modified during DNA  
616 replication in human cells. *Molecular biology of the cell* *18*, 3059-3067.
- 617 Di Perna, R., Aria, V., De Falco, M., Sannino, V., Okorokov, A.L., Pisani, F.M., and De Felice, M.  
618 (2013). The physical interaction of Mcm10 with Cdc45 modulates their DNA-binding  
619 properties. *Biochemical Journal* *454*, 333-343.
- 620 Douglas, M.E., and Diffley, J.F.X. (2015). Recruitment of Mcm10 to Sites of Replication  
621 Initiation Requires Direct Binding to the MCM Complex. *Journal of Biological Chemistry* *291*,  
622 5879-5888.
- 623 Du, W., Stauffer, M.E., and Eichman, B.F. (2012). Structural biology of replication initiation  
624 factor Mcm10. In *The Eukaryotic Replisome: a Guide to Protein Structure and Function*  
625 (Springer), pp. 197-216.
- 626 Enemark, E.J., and Joshua-Tor, L. (2006). Mechanism of DNA translocation in a replicative  
627 hexameric helicase. *Nature* *442*, 270-275.
- 628 Enemark, E.J., and Joshua-Tor, L. (2008). On helicases and other motor proteins. *Current*  
629 *Opinion in Structural Biology* *18*, 243-257.
- 630 Fu, Y.V., Yardimci, H., Long, D.T., Ho, T.V., Guainazzi, A., Bermudez, V.P., Hurwitz, J., van Oijen,  
631 A., Scharer, O.D., and Walter, J.C. (2011). Selective bypass of a lagging strand roadblock by  
632 the eukaryotic replicative DNA helicase. *Cell* *146*, 931-941.
- 633 Gambus, A., Jones, R.C., Sanchez-Diaz, A., Kanemaki, M., van Deursen, F., Edmondson, R.D.,  
634 and Labib, K. (2006). GINS maintains association of Cdc45 with MCM in replisome  
635 progression complexes at eukaryotic DNA replication forks. *Nat Cell Biol* *8*, 358-366.
- 636 Ganai, R.A., Zhang, X.-P., Heyer, W.-D., and Johansson, E. (2016). Strand displacement  
637 synthesis by yeast DNA polymerase  $\epsilon$ . *Nucleic Acids Research* *44*, 8229-8240.
- 638 Georgescu, R., Yuan, Z., Bai, L., de Luna Almeida Santos, R., Sun, J., Zhang, D., Yurieva, O., Li,  
639 H., and O'Donnell, M.E. (2017). Structure of eukaryotic CMG helicase at a replication fork  
640 and implications to replisome architecture and origin initiation. *Proceedings of the National*  
641 *Academy of Sciences* *114*, E697-E706.

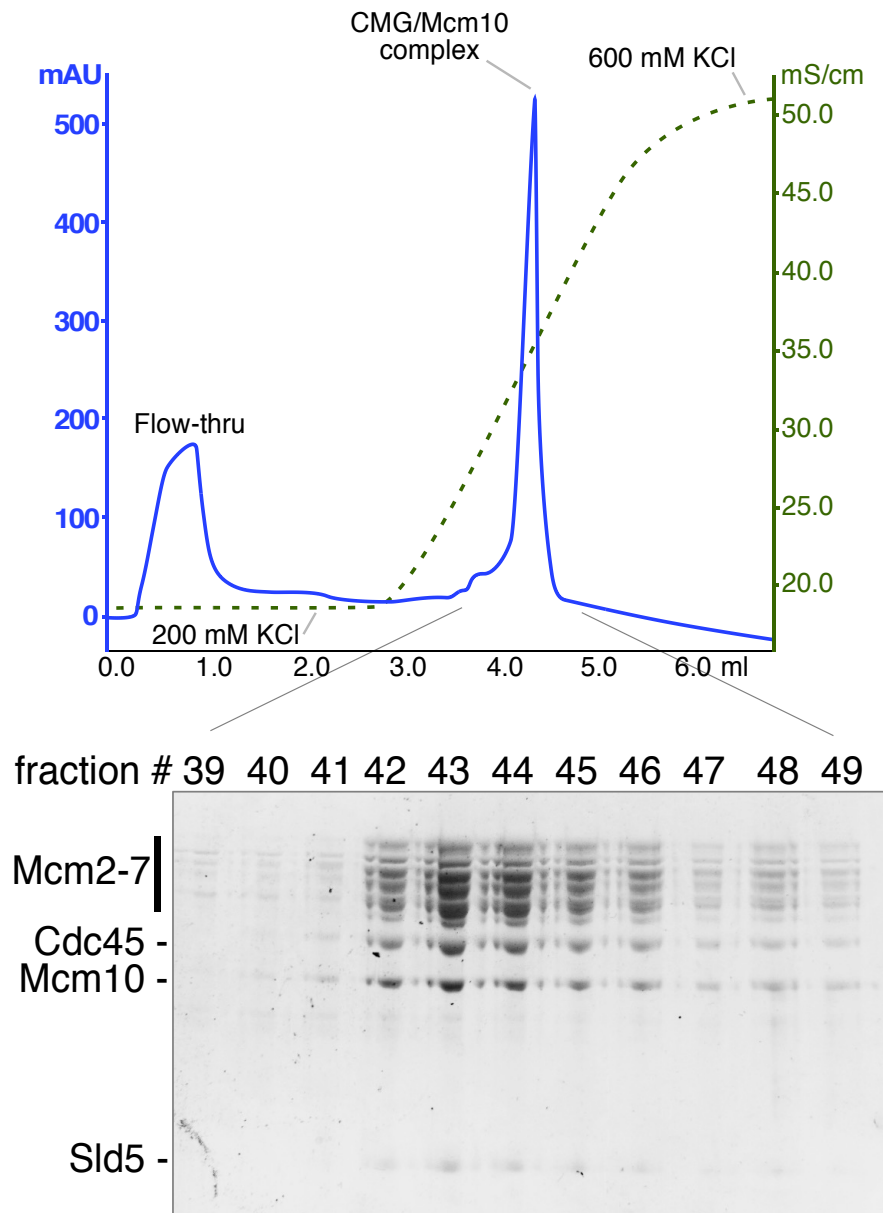


- 642 Georgescu, R.E., Langston, L., Yao, N.Y., Yurieva, O., Zhang, D., Finkelstein, J., Agarwal, T., and  
643 O'Donnell, M.E. (2014). Mechanism of asymmetric polymerase assembly at the eukaryotic  
644 replication fork. *Nat Struct Mol Biol* *21*, 664-670.
- 645 Hacker, K.J., and Johnson, K.A. (1997). A Hexameric Helicase Encircles One DNA Strand and  
646 Excludes the Other during DNA Unwinding. *Biochemistry* *36*, 14080-14087.
- 647 Heller, R.C., Kang, S., Lam, W.M., Chen, S., Chan, C.S., and Bell, S.P. (2011). Eukaryotic Origin-  
648 Dependent DNA Replication In Vitro Reveals Sequential Action of DDK and S-CDK Kinases.  
649 *Cell* *146*, 80-91.
- 650 Hodgson, B., Calzada, A., and Labib, K. (2007). Mrc1 and Tof1 regulate DNA replication forks  
651 in different ways during normal S phase. *Molecular biology of the cell* *18*, 3894-3902.
- 652 Ilves, I., Petojevic, T., Pesavento, J.J., and Botchan, M.R. (2010). Activation of the MCM2-7  
653 helicase by association with Cdc45 and GINS proteins. *Mol Cell* *37*, 247-258.
- 654 Itsathitphaisarn, O., Wing, Richard A., Eliason, William K., Wang, J., and Steitz, Thomas A.  
655 (2012). The Hexameric Helicase DnaB Adopts a Nonplanar Conformation during  
656 Translocation. *Cell* *151*, 267-277.
- 657 Kang, Y.H., Galal, W.C., Farina, A., Tappin, I., and Hurwitz, J. (2012). Properties of the human  
658 Cdc45/Mcm2-7/GINS helicase complex and its action with DNA polymerase epsilon in  
659 rolling circle DNA synthesis. *Proc Natl Acad Sci U S A* *109*, 6042-6047.
- 660 Kanke, M., Kodama, Y., Takahashi, T.S., Nakagawa, T., and Masukata, H. (2012). Mcm10 plays  
661 an essential role in origin DNA unwinding after loading of the CMG components. *Embo J* *31*,  
662 2182-2194.
- 663 Kaplan, D.L. (2000). The 3'-tail of a forked-duplex sterically determines whether one or two  
664 DNA strands pass through the central channel of a replication-fork helicase. *J Mol Biol* *301*,  
665 285-299.
- 666 Kaplan, D.L., Davey, M.J., and O'Donnell, M. (2003). Mcm4,6,7 uses a "pump in ring"  
667 mechanism to unwind DNA by steric exclusion and actively translocate along a duplex. *J Biol*  
668 *Chem* *278*, 49171-49182.
- 669 Langston, L.D., and O'Donnell, M.E. (2017). Action of CMG with strand-specific DNA blocks  
670 supports an internal unwinding mode for the eukaryotic replicative helicase. *eLife* *6*,  
671 e23449.
- 672 Langston, L.D., Zhang, D., Yurieva, O., Georgescu, R.E., Finkelstein, J., Yao, N.Y., Indiani, C., and  
673 O'Donnell, M.E. (2014). CMG helicase and DNA polymerase epsilon form a functional 15-  
674 subunit holoenzyme for eukaryotic leading-strand DNA replication. *Proc Natl Acad Sci U S A*  
675 *111*, 15390-15395.
- 676 Lee, C., Liachko, I., Bouten, R., Kelman, Z., and Tye, B.K. (2010). Alternative mechanisms for  
677 coordinating polymerase alpha and MCM helicase. *Molecular and cellular biology* *30*, 423-  
678 435.
- 679 Lee, S.J., Syed, S., Enemark, E.J., Schuck, S., Stenlund, A., Ha, T., and Joshua-Tor, L. (2014).  
680 Dynamic look at DNA unwinding by a replicative helicase. *Proc Natl Acad Sci U S A* *111*,  
681 E827-E835.

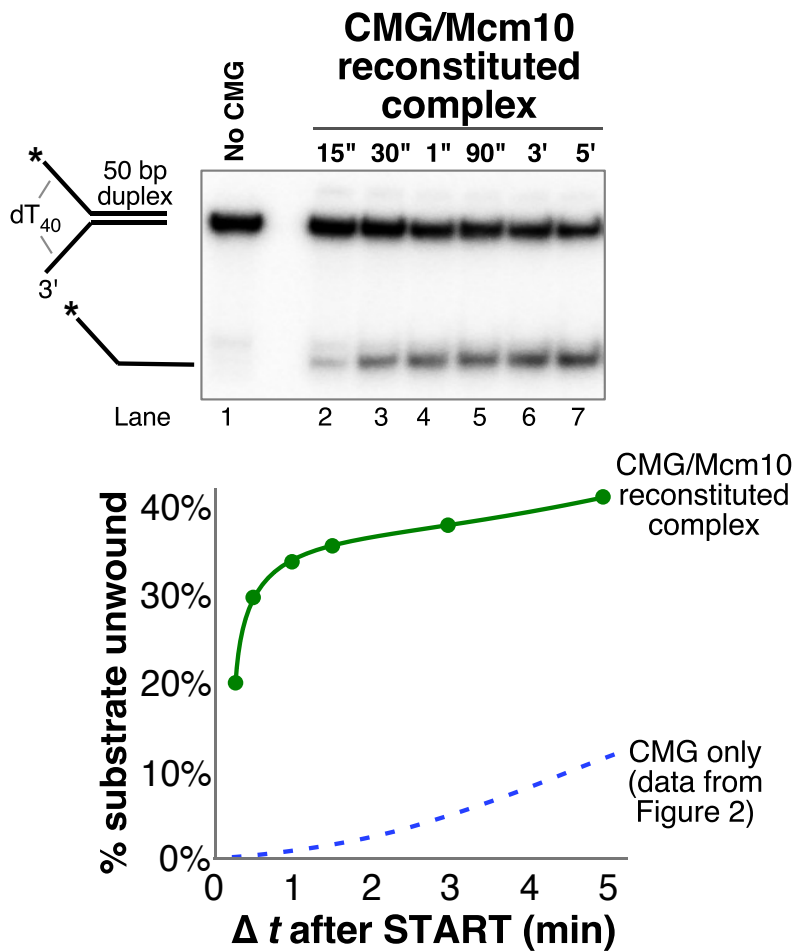
- 682 Lõoke, M., Maloney, M.F., and Bell, S.P. (2017). Mcm10 regulates DNA replication elongation  
683 by stimulating the CMG replicative helicase. *Genes & Development* *31*, 291-305.
- 684 Lyubimov, A.Y., Strycharska, M., and Berger, J.M. (2011). The nuts and bolts of ring-  
685 translocase structure and mechanism. *Current Opinion in Structural Biology* *21*, 240-248.
- 686 Merchant, A.M., Kawasaki, Y., Chen, Y., Lei, M., and Tye, B.K. (1997). A lesion in the DNA  
687 replication initiation factor Mcm10 induces pausing of elongation forks through  
688 chromosomal replication origins in *Saccharomyces cerevisiae*. *Molecular and cellular*  
689 *biology* *17*, 3261-3271.
- 690 Moyer, S.E., Lewis, P.W., and Botchan, M.R. (2006). Isolation of the Cdc45/Mcm2-7/GINS  
691 (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. *Proc Natl Acad*  
692 *Sci U S A* *103*, 10236-10241.
- 693 Nakano, T., Miyamoto-Matsubara, M., Shoulkamy, M.I., Salem, A.M., Pack, S.P., Ishimi, Y., and  
694 Ide, H. (2013). Translocation and stability of replicative DNA helicases upon encountering  
695 DNA-protein cross-links. *J Biol Chem* *288*, 4649-4658.
- 696 Perez-Arnaiz, P., Bruck, I., and Kaplan, D.L. (2016). Mcm10 coordinates the timely assembly  
697 and activation of the replication fork helicase. *Nucleic acids research* *44*, 315-329.
- 698 Quan, Y., Xia, Y., Liu, L., Cui, J., Li, Z., Cao, Q., Chen, Xiaojiang S., Campbell, Judith L., and Lou, H.  
699 (2015). Cell-Cycle-Regulated Interaction between Mcm10 and Double Hexameric Mcm2-7 Is  
700 Required for Helicase Splitting and Activation during S Phase. *Cell Reports* *13*, 2576-2586.
- 701 Ricke, R.M., and Bielinsky, A.K. (2004). Mcm10 regulates the stability and chromatin  
702 association of DNA polymerase-alpha. *Mol Cell* *16*, 173-185.
- 703 Robertson, P.D., Warren, E.M., Zhang, H., Friedman, D.B., Lary, J.W., Cole, J.L., Tutter, A.V.,  
704 Walter, J.C., Fanning, E., and Eichman, B.F. (2008). Domain architecture and biochemical  
705 characterization of vertebrate Mcm10. *Journal of Biological Chemistry* *283*, 3338-3348.
- 706 Sekedat, M.D., Fenyo, D., Rogers, R.S., Tackett, A.J., Aitchison, J.D., and Chait, B.T. (2010). GINS  
707 motion reveals replication fork progression is remarkably uniform throughout the yeast  
708 genome. *Mol Syst Biol* *6*, 353.
- 709 Singleton, M.R., Dillingham, M.S., and Wigley, D.B. (2007). Structure and Mechanism of  
710 Helicases and Nucleic Acid Translocases. *Annu Rev Biochem* *76*, 23-50.
- 711 Solomon, N.A., Wright, M.B., Chang, S., Buckley, A.M., Dumas, L.B., and Gaber, R.F. (1992).  
712 Genetic and molecular analysis of DNA43 and DNA52: Two new cell-cycle genes in  
713 *Saccharomyces cerevisiae*. *Yeast* *8*, 273-289.
- 714 Thomsen, N.D., and Berger, J.M. (2009). Running in reverse: the structural basis for  
715 translocation polarity in hexameric helicases. *Cell* *139*, 523-534.
- 716 Thu, Y.M., and Bielinsky, A.K. (2013). Enigmatic roles of Mcm10 in DNA replication. *Trends*  
717 *Biochem Sci* *38*, 184-194.
- 718 van Deursen, F., Sengupta, S., De Piccoli, G., Sanchez-Diaz, A., and Labib, K. (2012). Mcm10  
719 associates with the loaded DNA helicase at replication origins and defines a novel step in its  
720 activation. *Embo J* *31*, 2195-2206.

- 721 Warren, E.M., Vaithiyalingam, S., Haworth, J., Greer, B., Bielinsky, A.-K., Chazin, W.J., and  
722 Eichman, B.F. (2008). Structural Basis for DNA Binding by Replication Initiator Mcm10.  
723 *Structure* 16, 1892-1901.
- 724 Watase, G., Takisawa, H., and Kanemaki, M.T. (2012). Mcm10 plays a role in functioning of  
725 the eukaryotic replicative DNA helicase, Cdc45-Mcm-GINS. *Curr Biol* 22, 343-349.
- 726 Wessel, R., Schweizer, J., and Stahl, H. (1992). Simian virus 40 T-antigen DNA helicase is a  
727 hexamer which forms a binary complex during bidirectional unwinding from the viral origin  
728 of DNA replication. *Journal of Virology* 66, 804-815.
- 729 Yeeles, J.T., Deegan, T.D., Janska, A., Early, A., and Diffley, J.F. (2015). Regulated eukaryotic  
730 DNA replication origin firing with purified proteins. *Nature* 519, 431-435.
- 731 Yeeles, J.T.P., Janska, A., Early, A., and Diffley, J.F.X. (2016). How the Eukaryotic Replisome  
732 Achieves Rapid and Efficient DNA Replication. *Molecular Cell* 65, 105-116.

## SUPPLEMENTAL FIGURES

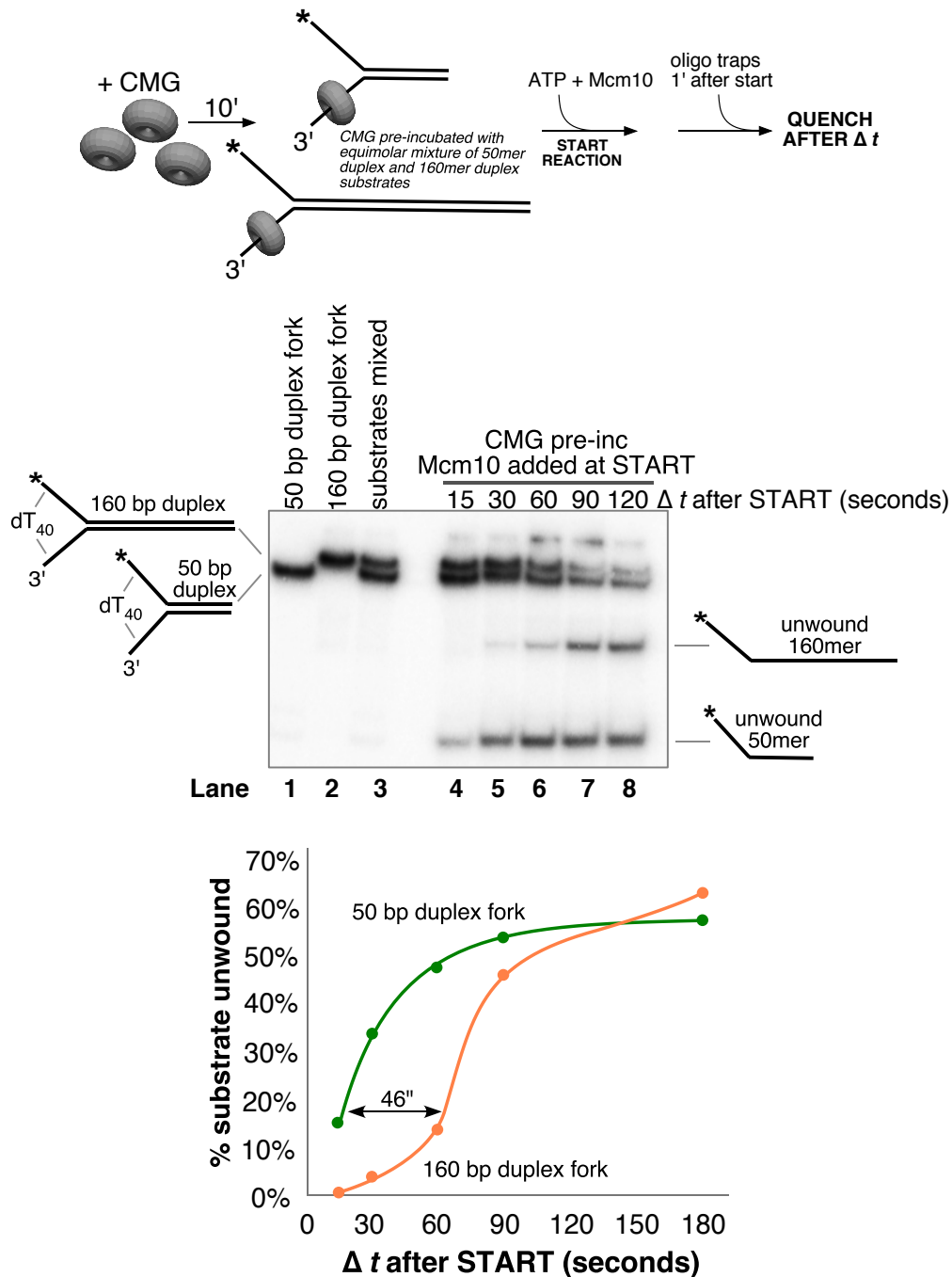


733 **Figure 2 – Supplement 1. MonoQ reconstitution of CMG/Mcm10 complex.** To form a  
734 CMG-Mcm10 complex, 600  $\mu$ g purified CMG (765 pmol) was mixed with 250  $\mu$ g purified  
735 MBP-Mcm10 (3.1 nmol) from which the tag had been previously removed using PreScission  
736 protease (GE Healthcare). The mixture was incubated on ice for 30' and spun in a  
737 microcentrifuge at 15,000 rpm for 3' at 4°C to remove any precipitated proteins. The  
738 CMG/Mcm10 mixture was injected at 0.1 ml/min onto a 100  $\mu$ l monoQ column that had  
739 previously been equilibrated in a buffer consisting of 20 mM Tris-Cl pH7.5, 10% glycerol,  
740 200 mM KCl, 2 mM DTT and 2 mM MgCl<sub>2</sub>. The column was washed and then eluted in the  
741 same buffer using a 2.5 ml gradient from 200 mM to 600 mM KCl, collecting 0.1 ml fractions.  
742 Peak fractions were analyzed in an 8% SDS-polyacrylamide gel stained with Denville Blue.

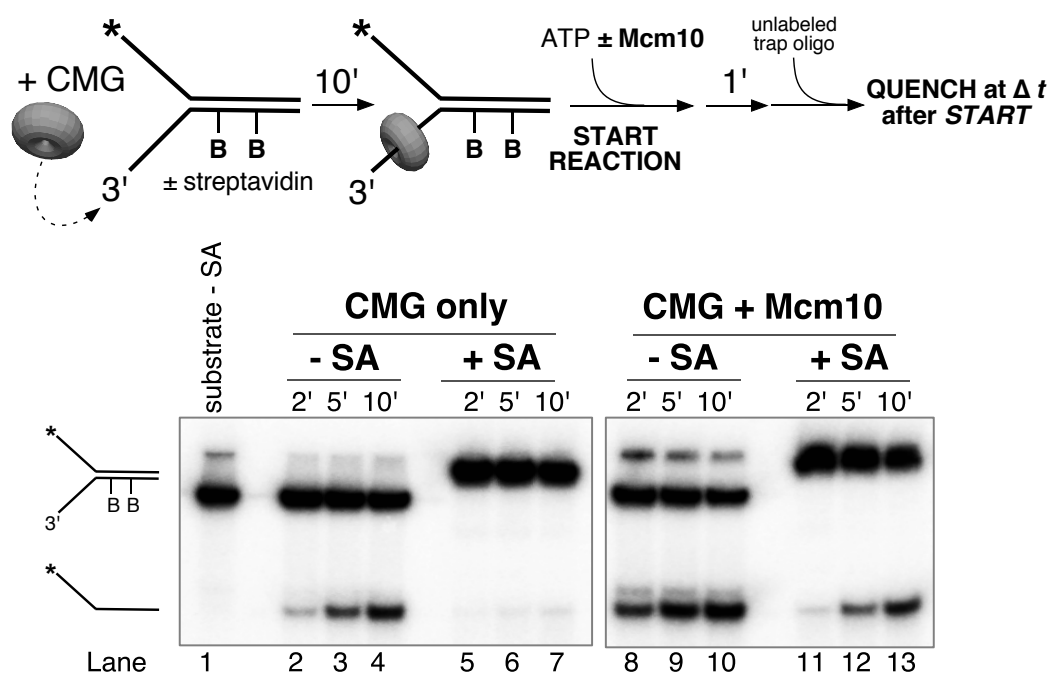


743 **Figure 2 - Supplement 2. The CMG-Mcm10 complex reconstituted on monoQ is**  
744 **functional.** 30 nM (final concentration) of reconstituted CMG-Mcm10 complex (**Figure 2 -**  
745 **Supplement 1**) was preincubated with the 50 bp duplex fork substrate (0.5 nM) for 10' in  
746 the absence of ATP in buffer containing 20 mM Tris Acetate pH 7.6, 5 mM DTT, 0.1 mM  
747 EDTA, 10 mM MgSO<sub>4</sub>, 30 mM KCl, 40 μg/ml BSA. The reaction was started by addition of 1  
748 mM ATP and 40" later unlabeled 50duplex LAG oligo was added (to 50 nM) as a trap for  
749 unwound DNA. The total reaction volume was 78 μl and at the indicated times, 11 μl  
750 aliquots were removed, stopped with buffer containing 20 mM EDTA and 0.1% SDS (final  
751 concentrations), and flash frozen in liquid nitrogen. Frozen reaction products were thawed  
752 quickly in room temperature water and separated on 10% native PAGE minigels. Gels were  
753 washed in distilled water, mounted on Whatman 3MM paper, wrapped in plastic and  
754 exposed to a storage phosphor screen that was scanned on a Typhoon 9400 laser imager  
755 (GE Healthcare). The scanned gel was analyzed using ImageQuant TL v2005 software to  
756 obtain the quantitations shown in the graph below the gel.



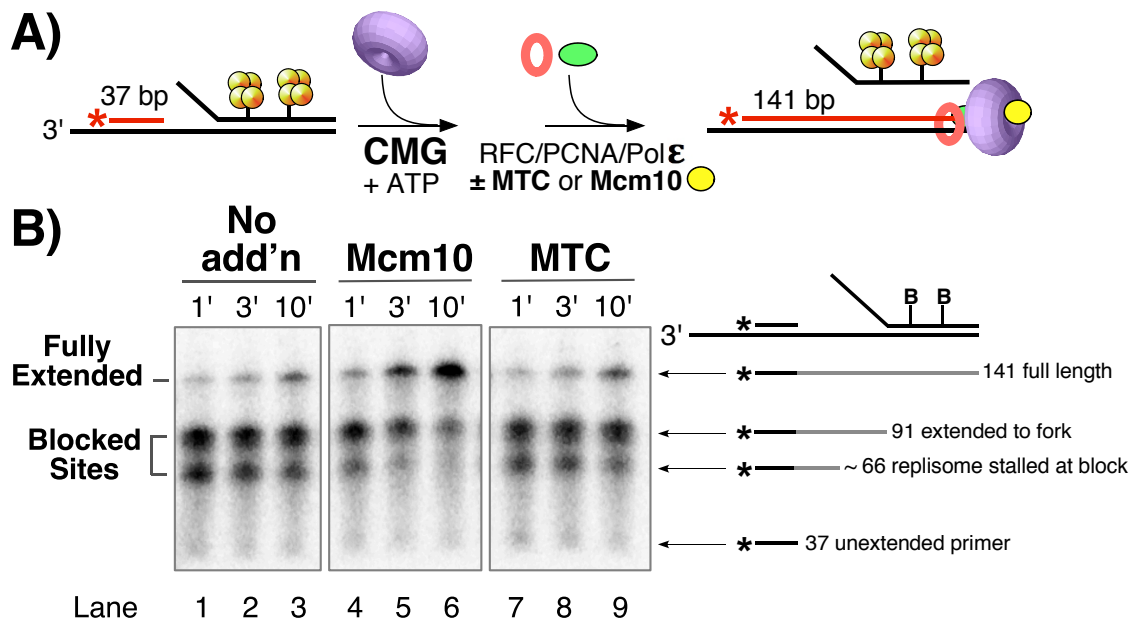


757 **Figure 3 – Supplement 1. Delayed replication of longer duplex allows estimation of**  
 758 **unwinding rate.** A helicase assay identical to those in **Figure 3** was performed except that  
 759 both the 50 bp duplex and 160 bp duplex for substrates were added to the reaction at 0.5  
 760 nM each substrate and pre-incubated together with 20 nM CMG in the absence of ATP as  
 761 shown in the scheme above the gel. The reaction was started by the addition of ATP (1 nM)  
 762 and Mcm10 (40 nM) and unlabeled traps for each substrate (25 nM) were added 1' later to  
 763 prevent re-annealing of unwound DNA. The total reaction volume was 91  $\mu$ l and at the  
 764 indicated times, 11  $\mu$ l aliquots were removed, stopped with buffer containing 20 mM EDTA  
 765 and 0.1% SDS (final concentrations), and flash frozen in liquid nitrogen. The positions of the  
 766 two substrates are indicated in lanes 1-3 of the gel showing that they migrate at  
 767 distinguishable positions. Unwinding of each substrate was calculated as  
 768 [unwound/(unwound + unreacted substrate)] for each substrate separately and the values  
 769 are shown in the graph below the gel.



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**Figure 5 - Supplement 1. A leading strand block inhibits CMG unwinding even in the presence of Mcm10.** Helicase reactions were performed as in **Figure 5B** except that the substrate contained a dual biotin-streptavidin block on the leading strand (50duplex LEAD dual biotin); the lagging strand (50duplex LAG) was radiolabeled. Oligo sequences are in Table I.



775 **Figure 5 – Supplement 2. Mcm10 enables the replisome to bypass a lagging strand**  
 776 **block.** A) Reactions contained a synthetic minifork with a dual biotin-streptavidin (SA)  
 777 block on the lagging strand template. CMG and ATP were added for 3 min to allow CMG to  
 778 bind DNA and translocate to the SA blocks, then Pol ε, RFC and PCNA were added (± MTC)  
 779 along with all four dNTPs to assemble and extend the primer up to the blocked CMG,  
 780 followed by addition of Mcm10 (or no protein) and timed aliquots were quenched at the  
 781 indicated times after ± Mcm10 addition. See Methods for details. B) Timed aliquots were  
 782 analyzed in a 10% urea PAGE gel. The primer, two stalled products and the full-length  
 783 product are indicated to the right of the gel.