# Fine-tuning of the Replisome: Mcm10 Regulates Fork Progression and Regression

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**Abbreviations:** alt-NHEJ, alternative nonhomologous end-joining; CC, coli-coil motif; CMG, Cdc45/GINS/MCM2-7; CMGM, Cdc45/GINS/Mcm2-7/Mcm10; CPT, camptothecin; CSB, Cockayne Syndrome Group B protein; CTD, C-terminal domain; DSB, double-strand break; DSBR, double-strand break repair; dsDNA, double-stranded DNA; GINS, *go-ichi-ni*-san, Sld5-Psf1-Psf2-Psf3; HJ Dis, Holliday Junction dissolution; HJ Res, Holliday Junction resolution; HR, homologous recombination; ICL, interstrand cross-link; ID, internal domain; MCM, minichromosomal maintenance; ND, not determined; NTD, N-terminal domain; PCNA, proliferating cell nuclear antigen; RPA, Replication Protein A; SA, strand annealing; SE, strand exchange; SEW, steric exclusion and wrapping; ssDNA, single-stranded DNA; TCR, transcription-coupled repair; TOP1, topoisomerase-1

**Abstract:** Several decades of research have identified Mcm10 hanging around the replisome making several critical contacts with a number of proteins but with no real disclosed function. Recently, the O'Donnell laboratory has been better able to map the interactions of Mcm10 with a larger Cdc45/GINS/MCM (CMG) unwinding complex placing it at the front of the replication fork. They have shown biochemically that Mcm10 has the impressive ability to strip off single strand binding protein (RPA) and reanneal complementary DNA strands. This has major implications in controlling DNA unwinding speed as well as responding to various situations where fork reversal is needed. This work opens up a number of additional facets discussed here revolving around accessing the DNA junction for different molecular purposes within a crowded replisome.

**Keywords:** Mcm10, helicase, annealing, unwinding, fork reversal, replisome

## Mcm10 in Replication Fork Establishment and Progression

The role of Mcm10 has been somewhat clandestine since its discovery more than 35 years ago in screens for temperature sensitive mutants involved in S-phase progression.[1, 2] Cumulative evidence over the years suggests that Mcm10 is a scaffold protein at the replication fork coordinating replisome initiation and progression with the DNA damage response.[3, 4] Although it has no enzymatic activity on its own, Mcm10 favors binding single-strand DNA (ssDNA) over double-strand DNA (dsDNA),[5] interacts with the DNA primase (Pol-α),[6] several subunits of the Cdc45-Mcm2-7-GINS helicase (CMG) complex,[7, 8] the processivity clamp (PCNA),[9] and the single-stranded binding protein (Replication Protein A (RPA))[10] making it an integral component of the replisome. Mcm10 is recruited once CMG is formed to further initiate unwinding, transitioning into S-phase to activate the replisome.[11, 12]

Recent work from the O'Donnell laboratory utilized crosslinking mass spectrometry (CX-MS) to map the Mcm10 binding location on CMG to form the CMGM complex.[13] Mcm10 positions itself largely at the leading N-face of the CMG complex, but there are extended binding contacts all the way to the back of the C-face as well (**Figure 1A**). Mcm10 crosslinks significantly with two members (Psf1/Psf2) of the GINS complex, Cdc45, and Mcm2/5/6 on the N-face. Extended contacts were also detected towards the C-face with Cdc45 and Mcm6 on the back side. It is known that Mcm10 stabilizes the entire CMGM complex.[11] There are three domains within the Mcm10 protein: the N-terminal domain (NTD) which facilitates self oligomerization through a coil-coil domain (CC)[14]; the Internal domain (ID) which mediates interactions with DNA, Polα, and PCNA[15]; and the extended C-

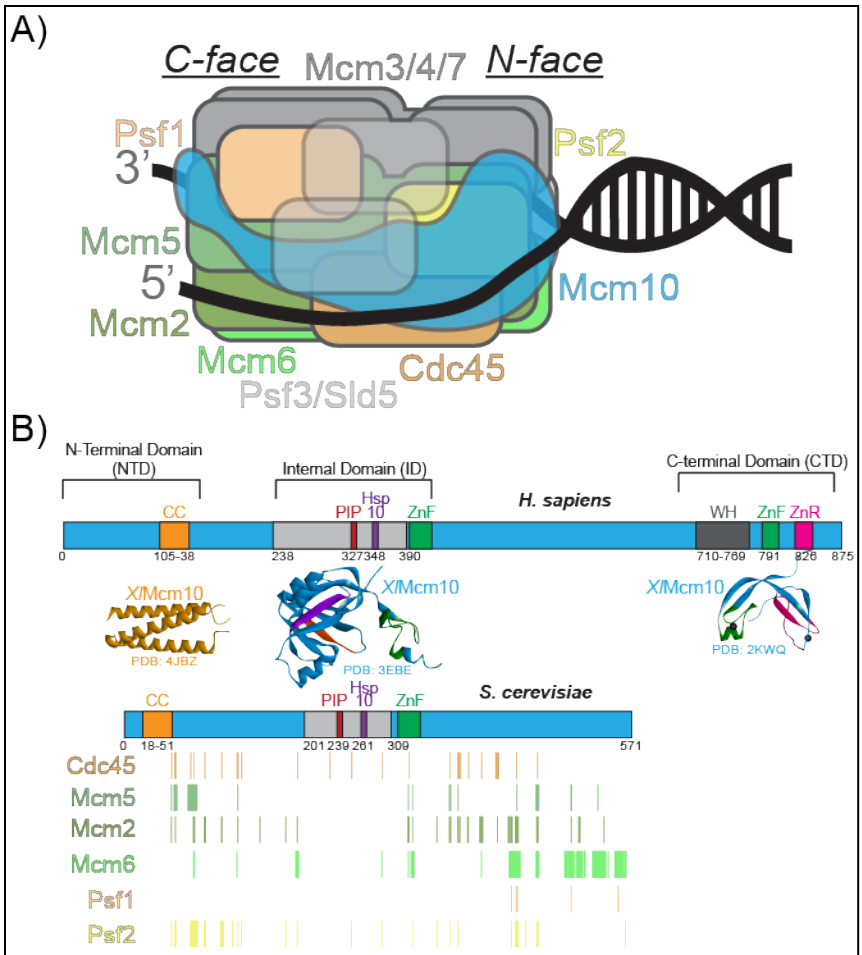


**Figure 1: A)** CMGM complex placing Mcm10 primarily at the N-face but extending laterally to the C-face of CMG. Colored subunits were significantly crosslinked to Mcm10. **B)** Protein schematic for human versus yeast MCM10 showing domains that have been structurally characterized (modelled from Ref 4) and highlighting detected positions on Mcm10 for crosslinks to indicated proteins. The frequency of crosslinks are indicated by the positions and thickness of the lines as taken from the appendix.[13]

terminal domain (CTD)[16] which is only present in metazoans and is largely uncharacterized except for the winged-helix (WH) region that contains zinc binding motifs (**Figure 1B**).[4]

Mapping of yeast Mcm10 binding to CMG has some surprises. First, the shorter C-terminal region in yeast crosslinks in many positions extending from the N-face to the C-face of CMG in a proposed highly extended conformation. Second, the DNA binding ID maps principally to the N-face of the CMG complex, where it may make contacts with both the duplex as well as the unwound excluded lagging strand. There are fewer crosslinks with the ID compared with the NTD or CTD possibly suggesting that it is more mobile and that the NTD and CTD provide the primary stability for binding CMG (**Figure 1B**). Finally, there were a few intermolecular Mcm10 crosslinks within the CC domain in the NTD suggesting that oligomerization may provide for additional allosteric function. This overall binding position for Mcm10 would effectively aid in stabilizing Cdc45 closing of the Mcm2/5 gate important for loading onto DNA[17, 18] as well as be in position to modulate interactions with downstream dsDNA and the recently unwound ssDNA to regulate unwinding.

Mcm10 binds both single-strand (ss) and double-strand (ds) DNA but has a greater preference for ssDNA.[19] Based on the binding position, Mcm10 may also engage the duplex fork junction and stabilize the newly exposed excluded ssDNA strand on the N-face of CMGM. The excluded strand would be guided along the mobile ID of Mcm10 before being bound by RPA and handed off to Pol$\alpha$ for priming. This function for Mcm10 is analogous to the steric exclusion and wrapping unwinding (SEW) model proposed previously for hexameric helicases[20] with an added benefit of being able to reverse directions for annealing when needed to control the unwinding rate. This overall binding position allows Mcm10 to remain part of the replisome and provide an integral role in regulating elongation.

### Mcm10: Unwinding ←→ Annealing

Interestingly, addition of Mcm10 to the CMG complex increases DNA unwinding dramatically but only with inclusion of a trapping oligo in the reaction. In the absence of a trap, CMGM appears to reanneal the unwound product in a somewhat futile cycle. Stimulation of DNA unwinding can be effectively explained by Mcm10 forming a more stable toroidal structure[11] (aiding Cdc45 in closing the Mcm2-5 gate) (**Figure 1**) with a slower off-rate that promotes forward translocation,[21] but the DNA annealing activity is more interesting. It appears that Mcm10 on its own is able to stimulate reannealing of DNA forks in the absence of ATP. It is not clear whether that is facilitated through oligomerization of the NTD of Mcm10 to bring bound DNA strands in proximity, but this seems likely. Interestingly, inclusion of CMG did not inhibit Mcm10 DNA reannealing; and so, whether CMG plays a role or whether this activity come solely from Mcm10 remains to be determined. Increasing concentrations of Mcm10 appear to stimulate DNA annealing consistent with an oligomer. Future experiments investigating the oligomeric state of Mcm10 (either directly or through mutations) and its impact on annealing should be performed.

Some of the most striking experiments come from preincubated RPA bound complementary ssDNA strands being efficiently reannealed by Mcm10. There was only a slight decrease (~25%) in the overall annealed product when RPA was present compared with naked DNA substrates. RPA has 40-fold tighter binding affinity for ssDNA than Mcm10,[6] and so the question remains of how Mcm10 is able to strip RPA from a ssDNA template. Mcm10 has no ATPase activity on its own that can be utilized to actively displace RPA. It will be interesting to determine

if binding energy is gained from Mcm10 oligomerization that facilitates annealing and direct displacement of RPA. Separation of function mutants that disrupt oligomerization[14] or DNA binding[22] should be utilized to get at this mechanism.

At the end of the article, the authors propose a unique mechanism for Mcm10 that may also have a role in establishing bidirectional replication forks. The CMGM complex (along with Pol$\varepsilon$) proceeding N-first pass over each other to establish two unwound forks, reannealing the complementary origin sequences behind the replisomes. The authors suggest that Mcm10 facilitates reannealing of the unwound origin; however, it is not clear how this would happen given Mcm10's position at the N-face of the CMGM complex, nor whether this is actually needed to establish bidirectional replication. Pol$\alpha$ priming would occur on the lagging strands and be extended by Pol$\delta$. At this point, the original lagging strand is synthesized up to the other replisome and becomes the leading strand. Then, there would be an exchange between Pol$\delta$ and Pol$\varepsilon$ to establish the leading strand replisome, and lagging strand synthesis would reinitiate a second time for Okazaki fragment synthesis. Therefore, DNA priming would never *de facto* occur directly on the leading strand, and lagging strand DNA is synthesized first only to ultimately become the leading strand. Deciphering the individual steps involved in converting and activating CMGM into active bidirectional replication forks by following specific DNA syntheses as well as required replisome components will be a worthwhile but challenging task.

**Strand Annealing Proteins and Their Roles in Fork Remodeling**

When replication forks stall due to a blocking DNA lesion, depleted nucleotide pool, or pharmacological inhibition of DNA polymerase activity, the forks undergo remodeling to preserve their stability and enable them to eventually restart.[23] Replication fork remodeling may involve the reannealing of parental strands (fork regression) to form a Holliday Junction-like structure or branch-migration of the regressed (reversed) fork in the opposite direction to reform the replication fork structure. The ability of Mcm10 to inhibit SMARCAL1-catalyzed fork regression *in vitro* raises the questions if the activity occurs *in vivo*, how it is regulated by other fork stability factors, and whether other proteins are capable of performing strand annealing or exchange in certain cellular contexts. Shown in **Table 1** is a list of potential candidates that catalyze strand annealing *in vitro* and are implicated in the maintenance of genomic stability. Inspection of **Table 1** reveals several overarching principles that can be related to Mcm10:

1) The human RecQ helicases all share the ability to perform strand annealing and strand exchange; however, unlike Mcm10, their strand annealing activities are all inhibited by RPA pre-coated onto the complementary single-strands. Strand annealing by human Pif1 is also inhibited by RPA bound to the ssDNA.[24, 25] In the Mcm10 studies, RPA is not included in the SMARCAL1 fork regression reaction mixtures,[13] raising the question on whether Mcm10 is capable of inhibiting SMARCAL1-mediated fork regression on substrates that have RPA pre-coated on the single-stranded gaps in the leading or lagging strand.

2) The homologous recombination (HR) protein Rad52[26] and FANCA, a key component of a protein complex implicated in interstrand cross-link (ICL) repair and a factor that was also recently implicated in double-strand break repair (DSBR),[27] both catalyze annealing of RPA-coated single-strands in a manner like Mcm10; however, these proteins were not

tested for their effect on translocase-mediated fork regression. It remains to be seen if Rad52 or FANCA play a role in fork remodeling to maintain fork stability and integrity.

3) Strand annealing by each of the RecQ helicases is inhibited by the presence of ATPγS in the reaction mixture (with the exception of RECQL4, which to our knowledge was not tested). ATPγS in the reaction mixture was shown to cause a conformational change in RECQL1, as evidenced by partial proteolysis experiments.[28] Mutation of the nucleotide binding domain of RECQL5 eliminated its strand annealing function.[29] Both these findings indicate that nucleotide binding diminishes strand annealing by the respective RecQ helicases. Unlike the RecQ helicases, Mcm10 does not contain a nucleotide binding motif and has not been reported to hydrolyze ATP; therefore, nucleotide binding is not likely to regulate strand annealing by Mcm10. It remains to be seen if other proteins regulate strand annealing by Mcm10. As hypothesized by Mayle et al., local concentration of Mcm10 at actively replicating forks may be enhanced by its interaction with the CMG helicase complex;[13] however, it is unclear if CMG modulates Mcm10-mediated strand annealing and/or the precise mechanism of Mcm10's inhibition of SMARCAL1-catalyzed fork regression. A valuable Mcm10 site-directed mutant to test in the *in vitro* reactions would be one that is specifically defective in strand annealing but retains its interaction with the CMG complex. If such a Mcm10 mutant defective in strand annealing is unable to inhibit SMARCAL1-mediated fork regression, then one could conclude that Mcm10-catalyzed strand annealing is vital in limiting the translocase-mediated reversal of the fork.

Fork remodeling activity has been reported for the human RecQ helicases WRN, BLM, RECQL1, and RECQL5 (**Table 2**). *In vitro*, WRN,[30] BLM,[30, 31] and RECQL5[32] have all been shown to catalyze fork regression using model oligonucleotide-based DNA substrates, whereas RECQL1 has a strong preference to catalyze fork restoration on model DNA substrates.[33] WRN and BLM are also capable of restoring the fork from a regressed version *in vitro;*[34] however, to date there is not strong experimental evidence that fork regression or fork restoration by WRN or BLM has relevance *in vivo*. A good RecQ candidate for fork restoration in a biological setting is the human RECQL1 helicase which has been implicated in fork restart. In 2013, Berti *et al.* presented biochemical and cell biological data supporting a model that RECQ1 plays a unique role among the RecQ helicases to restart regressed replications forks that arise due to pharmacological inhibition of DNA topoisomerase I,[33] an enzyme that relieves torsional stress during transcription and DNA replication.[35] They demonstrated that RECQL1 could reverse branch-migrate a synthetic oligonucleotide-based DNA structure resembling a model regressed replication fork, the so-called "chicken-foot" intermediate, thereby restoring the model replication fork.[33] However, given that precoating RPA on complementary single-strands was demonstrated to strongly inhibit RECQ1-mediated strand annealing,[28] potential difference in activities catalyzed by Mcm10 and RECQL1 may hinge on their relative abilities to inhibit fork regression when RPA is bound to the single-strand gaps. Alternatively, RECQL1 may act upon the fully regressed fork in an ATP-dependent branch-migration fashion, whereas CMGM may operate during initial steps of fork regression or continuously during DNA synthesis elongation to control speed, potentially relying on its strand annealing activity.

In the latest work from the O'Donnell lab, the fork regression assays with SMARCAL1 and/or Mcm10 did not contain RPA in the reaction mixtures.[13] This may be important to address because the Cortez lab published results indicating that RPA binding to the single-stranded

region of model fork structures plays an important role in dictating SMARCAL1 substrate specificity in remodeling the forks.[36] Given estimates that RPA is a highly abundant nuclear protein that binds ssDNA with very high affinity,[37] it would be valuable to know how RPA bound to ssDNA gaps at a stalled fork would affect fork dynamics in the context of SMARCAL1 fork regression and Mcm10 fork restoration.

Returning our attention to RECQL1, Berti *et al*. showed by electron microscopic analysis of genomic DNA from cells treated with the Topoisomerase I (TOP1) inhibitor camptothecin (CPT) that RECQL1 depletion led to the accumulation of regressed forks, consistent with the idea that RECQL1 plays an instrumental role in fork restart upon drug-targeted TOP1 inhibition.[33] A key regulator in RECQL1's fork restart activity was determined to be Poly(ADP-Ribose) Polymerase 1 (PARP-1), which keeps RECQL1's fork restoration activity in check, presumably to prevent untimely fork progression.[33] Based on the recent paper from the O'Donnell lab suggesting that Mcm10 inhibits fork regression catalyzed by the ATP-dependent DNA translocase SMARCAL1, [13] it would be of interest to determine if RECQL1 behaves similarly in its outcome and if its mechanism of operation is unique or related to that of Mcm10. Perhaps more importantly, the acquisition of cell- and genome-based experimental data supporting Mcm10's proposed role in fork restart will greatly strengthen the latest biochemical results. If evidence is supportive of a role of Mcm10 to restore forks *in vivo*, it would be valuable to determine if its activity is regulated by PARylated PARP1, as observed for RECQL1,[33] or if other factors modulate Mcm10's activity. Moreover, the cellular context in which Mcm10 or RECQL1 restores stalled or regressed forks to resume replication may be very important. While experimental evidence suggests that RECQL1 is a key player in fork restoration when TOP1 is inhibited by cellular exposure to CPT, it remains to be seen under what conditions Mcm10's fork restoration activity is relevant in cells.

**Is Fork Restoration by Mcm10 Unique to SMARCAL1-Mediated Fork Regression?**

In addition to SMARCAL1, the DNA translocases zinc finger RANBP2 type-containing 3 (ZRANB3) and helicase-like transcription factor (HLTF) remodel forks leading to fork reversal.[38] Cellular deficiencies of SMARCAL1, ZRANB3 or HLTF all lead to elevated sensitivity to agents that induce DNA damage or impose replication stress, suggesting that they each have a unique role to maintain genomic stability. While all three are ATP-dependent dsDNA translocases capable of catalyzing fork regression, it is still unclear how their duties are delegated *in vivo*. Biochemical studies with model DNA substrates suggest that there are differences in their substrate specificities.[39, 40] SMARCAL1 shows some preference to regress forks that contain a lagging strand gap, whereas fork regression by HLTF or ZRANB3 is not affected by leading or lagging strand gaps. As alluded to above, RPA is a key player in recruiting SMARCAL1 to forks and directs SMARCAL1 action on forks with a leading strand gap *in vitro.*[39] The RPA-regulated fork regression activities of SMARCAL1 is distinct from that of ZRANB3. RPA binding to the leading strand inhibits fork regression by ZRANB3, suggesting a different mode of fork remodeling regulation compared to SMARCAL1.[39] For HLTF, RPA binding to the lagging strand gap inhibits fork regression. Importantly, the stimulatory effect of RPA on SMARCAL1 regression of forks with a leading strand gap may translate into a unique dependence on Mcm10 to restore the fork. Further studies are required to determine how the fork remodeling activities of ZRANB3 or HLTF are affected by Mcm10, and if this modulation is distinct from that of SMARCAL1 and influenced by RPA binding to gaps on the leading or lagging strands of the fork. SMARCAL1, ZRANB3, and HLTF can all also catalyze fork restoration, leaving it open that

they may collaborate with Mcm10 in this process. In the future, it will be important to build upon our understanding of the apparent differences among the fork remodelers SMARCAL1, ZRANB3, and HLTF as they relate to their molecular functions in coordinating with fork protection factors such as Mcm10. This may provide insight to how their properties relate to cellular phenotypes and human disease in which mutations in SMARCAL1, ZRANB3, and HLTF uniquely manifest in Schimke immunooseous dysplasia, endometrial cancer, and colorectal cancer, respectively.[38]

**Perspective of Mcm10 Strand Annealing**

Understanding the significance of Mcm10's newly discovered strand annealing function and its relevance to fork dynamics during normal DNA replication or under conditions of replication stress is an important next step in the field. While it is tempting to speculate that the RPA-tolerant strand annealing and strand exchange activities as well as inhibition of SMARCAL1-mediated fork regression catalyzed by Mcm10 *in vitro* mirrors the *in vivo* situation, it remains to be seen if or how Mcm10 coordinates its activities with other fork remodeling DNA translocases and helicases. Interestingly, Mcm10-deficient cells activate the ATR checkpoint kinases, Mec1 and Rad53, recognizing a buildup of ssDNA to activate downstream effectors.[41, 42] In fact, Mcm10 deficient cells are synergistic and synthetically lethal with several members of the DSBR pathway. Therefore, it fits that loss of Mcm10 results in a build-up of ssDNA that was dependent on Mcm10 annealing activity for prevention and that this excess ssDNA becomes susceptible to DSBs. Moreover, it is plausible that Mcm10 utilizes its strand pairing activities for fork reversal, directly in DSBR, or other aspects of DNA metabolism. Building on the biochemical results to extrapolate the findings *in vivo* is now paramount. While it seems likely that Mcm10's newly identified strand annealing and exchange activities are relevant for fork progression/regression, it remains to be seen how other nucleic acid transactions such as DNA repair or transcription are affected.

Finally, the replisome has some crowded real estate. The CMGM complex is really just the beginning of the story. Trimeric Ctf4 (or AND-1 in humans) interacts with the Sld5 subunit of GINS to coordinate multiple CMGs or Pol$\alpha$ primases for lagging strand synthesis.[43, 44] Could Mcm10 have a supportive role here as well creating tripartite contacts between Pol$\alpha$ and Ctf4 to better organizing lagging strand priming or will there be competition between Mcm10 and Ctf4 for binding to CMG? It is also not clear how the oligomeric state of Mcm10 affects its binding to CMG or regulates its activities. Is a higher order Mcm10 complex (through the CC motif in the NTD) required for stimulated unwinding or reannealing or to control DNA priming by Pol$\alpha$? Finally, the human form of Mcm10 contains an extended and largely uncharacterized CTD (**Figure 1B**). Does this additional domain effect the functions described for yeast Mcm10, or is this extended CTD only utilized to mediate (either directly or indirectly through posttranslational modifications) further interactions within a more complex metazoan replisome? We are excited to witness these forthcoming answers during this current structural and biochemical revolution.

**Table 1. Properties of Human Nuclear Strand Annealing Proteins**

| Protein[a] | Pathway[b] | SA[c] | SA Tolerant of RPA[d] | SA Modulated by ATP/ATPγS[e] | SE[f] | Helicase[g] | Reference |
|---|---|---|---|---|---|---|---|
| Mcm10 | Replication | + | + | ND | + | - | 13 |
| RECQL1 | Fork Restart, HJ Res | + | - | + | + | + | 28, 45 |
| WRN | Fork Restart, HJ Res | + | - | + | + | + | 46 |
| BLM | Fork Restart, HJ Dis | + | - | + | + | + | 46, 47 |
| RECQL4 | Replication | + | - | + | + | + | 48-50 |
| RECQL5 | DSB Repair | + | - | + | + | + | 29, 51, 52 |
| Rad52 | DSB Repair | + | + | - | + | - | 26, 53-55 |
| FANCA | ICL Repair, DSB Repair | + | + | - | + | - | 27 |
| Mcm2-7[h] | Replication | + | ND | + | ND | +[i] | 56 |
| Pif1 | Replication, Telomere | + | - | ND | ND | + | 24, 25 |
| CSB | TCR | + | - | + | - | - | 57 |
| Polθ helicase[j] | alt NHEJ | + | + | +[k] | ND | + | 58, 59 |

[a]Purified recombinant protein tested.
[b]Protein implicated in indicated pathway of DNA metabolism.
[c]SA, Strand Annealing of two complementary ssDNA molecules.
[d]Annealing of two complementary ssDNA molecules pre-coated with the ssDNA binding protein RPA.
[e]ATPγS present in reaction mixture inhibited strand annealing by RECQL1, WRN, BLM, and RECQL5 whereas DNA unwinding is ATP-dependent. Presence of ATP in reaction mixture stimulated RECQL4 helicase activity, thereby diminishing strand annealing activity; effect of ATPγS on RECQL4 strand annealing was not reported. Presence of ATP in reaction mixture inhibited Mcm2~7 strand annealing but did not affect strand annealing by Rad52 or FANCA.
[f]SE, Strand Exchange of ssDNA from a DNA duplex to another complementary ssDNA molecule.
[g]Unwinds dsDNA molecule in an ATP-dependent manner.
[h]Mouse Mcm2~7 tested.
[i]In complex with Cdc45 and GINS.
[j]Polθ helicase domain fragment tested.
[k]ATP required for annealing of RPA-coated complementary single-stranded DNA but does not affect annealing of naked ssDNA molecules.
+, biochemical activity of recombinant protein detected; -, marginal or no biochemical activity detected;

**Table 2. Fork Remodeling by Mcm10 and RecQ Helicases**

| Protein[1] | Fork Regression Catalysis[2] | Fork Restoration Catalysis[3] | Translocase-Mediated Fork Regression[4] | Reference |
|---|---|---|---|---|
| Mcm10 | - | ND | Inhibition | [13] |
| WRN | + | + | ND | [30, 33, 34] |
| BLM | + | + | ND | [30, 31, 34] |
| RECQL1 | - | + | ND | [33] |
| RECQL5 | + | ND | ND | [32] |

[1]Purified recombinant protein tested
[2]ATP-dependent fork regression with synthetic oligonucleotide-based DNA substrate
[3]ATP-dependent fork restoration with synthetic oligonucleotide-based DNA substrate
[4]Inhibition of SMARCAL1-catalyzed fork regression with synthetic oligonucleotide-based DNA substrate

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