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Branching Out with DNA Helicases

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Summary

The proper resolution of branched DNA molecules, which arise during processes such as DNA replication, DNA repair, and transcription, is critical for the maintenance of the genome. Disruption of this process can lead to genome instability and cancer progression. In this review, we describe recent progress on several interesting and biologically important enzymes that act upon different types of branched DNA substrates.

Introduction

In eukaryotic cells, DNA is mostly in its double-stranded form (dsDNA). However, single-stranded DNA (ssDNA) and branched DNA molecules are created during normal cellular processes such as transcription, DNA replication, and DNA repair. The unwinding of dsDNA and the generation of branched DNA structures must be carefully regulated as unresolved DNA structures may interfere with other downstream processes and lead to genome instability.

Different types of branched DNA molecules could be present at a replication fork. Forked DNA with ssDNA arms (a.k.a. split-ends and frayed DNA) results from the unwinding of dsDNA and is present at the ends of the unwound region. At the replication fork, forked DNA with two dsDNA arms (a.k.a. three-way junctions) is formed when both leading and lagging strand synthesis has occurred. Forked DNA with one ssDNA arm and one dsDNA arm (a.k.a. flaps) arises when only one of the two template strands has been copied. Finally, four-way junctions refer to branched molecules containing four dsDNA arms and are created at stalled replication forks (a.k.a chicken foot structures) as well as during recombination (e.g. Holliday junctions).

The formation and processing of branched molecules typically involves the action of enzymes that, like traditional helicases, contain an ATPase domain that couples nucleotide hydrolysis with motor activity [1]. Although the enzymes that act upon branched DNA molecules may not necessarily unwind DNA in the same manner as traditional DNA helicases, we will refer to them as helicases because they contain a helicase domain and their action results in a net alteration of the DNA helix. In this review, we highlight recent studies of branched DNA-binding helicases.

Replication Fork Regression

Studies of helicases that bind specifically to branched molecules have led to the identification of a group of factors involved in replication fork regression. Replication fork regression was first reported more than 30 years ago and involves the unwinding of the nascent strands from the template strands at a replication fork. This process allows the template strands to reanneal near the fork while also allowing the unwound nascent strands to anneal to each other [2] and [3]. In essence, replication fork regression results in the conversion of a three-way junction into a four-way junction (Figure 1). Branch migration involves the sliding of a four-way junction and allows the junction to move away from lesions that lead to stalling of the replication fork (Figure 1).

Replication fork regression enables access of specialized repair and replication factors to a lesion in order to repair it or to bypass it for later repair (post-replication repair) (reviewed in [4]). How fork regression allows for lesion bypass is not clear, although one model, referred to as template switching, posits that one of the paired nascent strands of the four-way junction acts as a template for the other nascent strand. An alternative template-switching model suggests that homologous recombination between the nascent strands may occur without the need for fork regression.

Most of our knowledge of fork regression in vitro comes from studies of bacterial RecG, a helicase that binds preferentially to three- and four-way junctions [5], [6] and [7] (Figure 2). Although RecG can unwind short duplex DNA molecules, it preferentially unwinds branched molecules and is able to initiate fork regression from a three-way junction [5], [6], and [8]. Based on its sequence, there does not appear to be a direct homolog of RecG in eukaryotes. However, as discussed below, two eukaryotic helicases, Rad5 and FANCM, exhibit activities that are related to those of RecG.

Rad5

Yeast Rad5 was first predicted to be a helicase in 1992, and it is a member of the SNF2 family of helicases [9]. In addition to its helicase-like domain, Rad5 contains a RING finger domain that confers E3 ubiquitin ligase activity [10].

Rad5 appears to be involved in DNA repair because it is part of the Rad6 epistasis group, and Rad5 deletion mutants are sensitive to UV induced DNA damage [9]. In addition, biochemical studies of purified Rad5 showed that its ATPase activity is stimulated by phage ssDNA but not by dsDNA [11]. A more recent and detailed analysis of Rad5 revealed that its preferred substrates are branched DNA molecules, such as split-end forks as well as three- and four-way junctions [12] (Figure 2). Like bacterial RecG, Rad5 is able to initiate fork regression and to carry out branch migration [12]. The human RecQ-related Bloom Syndrome (BLM) and Werner Syndrome (WRN) helicases may also regress replication forks; however, both the BLM and WRN helicases are able to unwind duplex DNA [13] and [14]. In contrast, Rad5 is unable to unwind duplex DNA [12], and its activity appears to be specific for branched molecules.

Recently, studies involving replication stalling with adozelesin-induced bulky adducts linked Rad5 with lesion bypass and template switching [15]. In wild-type yeast cells, stalled replication forks result in the generation of four-way DNA structures similar to Holliday junctions. In the absence of Rad5, the four-way junctions are not formed. In addition to Rad5, the recombination factors Rad51, Rad52, Rad54 and Rad55 are required to generate the four-way junctions at the stalled replication forks; hence, the four-way junctions appear to be recombination intermediates [15]. These findings suggest that fork regression and DNA recombination are linked, but the mechanisms remain to be clarified.

One possibility is that Rad5 creates an unstable regressed fork that requires subsequent processing by recombination factors.

Two putative Rad5 homologs, SNF2 histone linker PHD RING helicase (SHPRH) and helicase-like transcription factor (HLTF), have been identified in mammals (reviewed in [16•]). Like Rad5, SHPRH and HLTF are SNF2 family proteins that contain a RING finger domain and exhibit E3 ubiquitin ligase activity. HLTF has recently been shown to regress replication forks in vitro and to be required for efficient restart of replication following DNA damage [17•]. Fork regression activity for SHPRH has not yet been reported.

FANCM

FANCM, which is a recently discovered member of the Fanconi Anemia (FA) complementation group, is another helicase that has been implicated in fork regression in humans [[18] and [19]]. FA is a rare disorder that leads to developmental abnormalities, bone marrow failure, and cancer. Mutations in any of the 13 members of the FA complementation group leads to defects in DNA intrastrand crosslink repair and FA (reviewed in [20•]).

FANCM is not a SNF2 family member, but instead contains a helicase domain that is similar to that of RecA [21]. Recent studies on the biochemical activities of FANCM revealed that full-length FANCM displays high affinity binding to three-way junctions, four-way junctions, and D-loops [22•] (Figure 2). FANCM does not bind well to dsDNA, partial duplexes, or splitend DNA molecules, and it does not appear to unwind dsDNA [[19] and [22•]]. Furthermore, like RecG and Rad5, FANCM regresses replication forks in vitro [23•]. The discovery that a helicase unrelated to Rad5 can regress replication forks in vitro suggests that fork regression may be essential for different DNA repair pathways.

Insight into the function of FANCM in cells came from studies using FANCM-null mice and FANCM-null chicken DT40 cells [[24•] and [25•]]. In the absence of FANCM, increased rates of spontaneous sister chromatid exchange (SCE) were observed; hence, FANCM may be involved in recombination. Rescue experiments with FANCM-deficient DT40 cells revealed that the SCE phenotype can be rescued with a wild-type FANCM construct, but not with a FANCM construct containing an inactivating point mutation in the helicase domain [25•]. Thus, it appears that the ATP-dependent helicase activity of FANCM is linked to recombination.

Although recent studies have highlighted several helicases in fork regression there are still unanswered questions about this process. What happens to the replication machinery during fork regression? It is possible, for instance, that the primary role of fork regression factors is not to regress replication forks, but to allow access of recombination and repair factors to the DNA.

Another question concerns how fork regression is reversed. Studies of RecG have suggested that a monomer of RecG binds at the three-way junction and unwinds both nascent strands via a 'wedge' domain [26]. This mechanism implies directionality in fork regression. However, in order for fork regression to be reversed, branch migration must occur in the opposite direction to convert a four-way junction back to a three-way junction. Factors that can mediate reversal at regressed forks have not yet been identified. It is possible that recombination factors known to be involved in branch migration, such as Rad54, might possess such an activity. One general point that needs to be addressed is how a branch migration factor chooses which direction to move the junction.

In addition, when replication stalls, the replicative helicase can uncouple from the polymerase and continue to unwind DNA [27]. In this case, a split-end fork would be formed downstream of the lesion. In order for fork regression to occur, the resulting ssDNA strands would have to be rewound until a three-way junction is reestablished with the nascent DNA strands. The recent discovery of another branch-specific helicase has shed light on the resolution of split-end forked DNA and how ssDNA strands formed due to helicase uncoupling could be resolved at the replication fork.

HARP

HARP (also known as SMARCAL1 and DNA-dependent ATPase A) is a SNF2 family protein that preferentially binds to branched DNA molecules. Based on the sequence of its helicase-like domain, HARP has been classified as a distant member of the SNF2 family [[28] and [29]]. Mutations in HARP in humans lead to a rare pleiotropic disorder known as Schimke immuno-osseous dysplasia (SIOD), which is characterized by T-cell immunodeficiency, kidney failure, and bone growth abnormalities [30]. Early biochemical studies of bovine HARP showed that the ATPase activity of HARP is stimulated by phage ssDNA and DNA molecules containing both ssDNA and dsDNA [[31] and [32]].

Recently, it was found that the specific preferred substrate for HARP is a split-end fork DNA [33•] (Figure 2). Unlike fork regression and branch migration factors, HARP does not appear to bind well to four-way junctions (Yusufzai and Kadonaga, unpublished data). However, similar to those factors, HARP fails to unwind partial duplex DNA, even when the duplex contains split ends [33•]. Instead, HARP was found to possess an ATP-dependent DNA-rewinding activity that acts to anneal complementary ssDNA stabilized by the ssDNA-binding protein, replication protein A (RPA) [33•] (Figure 1). In the absence of DNA rewinding, HARP is unable to remove RPA that is bound to ssDNA or a non-complementary loop. Thus, HARP appears to catalyze DNA rewinding by binding to the split-end fork and translocating along the DNA in a manner that resembles the closing of a zipper. This ATP-dependent activity, termed annealing helicase, can act in opposition to unwinding helicases and is, thus far, unique to HARP.

Studies of purified HARP identified RPA as the primary interacting partner for HARP [[34•], [35•], [36•], [37•] and [38•]]. Although the interaction between HARP and RPA is dispensable for annealing helicase activity, it serves to recruit HARP to sites of DNA repair [[34•], [35•], [36•], [37•] and [38•]]. In cells with reduced levels of HARP, there is an increase in spontaneous DNA damage as well as replication fork stalling following DNA damage [[35•], [36•] and [37•]]. Thus, it appears as if HARP acts to limit the amount of ssDNA generated following replication fork stalling, possibly by rewinding ssDNA generated from an uncoupled replicative helicase.

Whether the annealing helicase activity of HARP is required for the process of DNA repair remains to be determined. Future studies of HARP should shed light on which repair pathways depend on its activity. In addition, the identification of other annealing helicases would increase our knowledge of their roles in the cell. Indeed, we have recently identified a HARP-related protein with ATP-dependent rewinding activity [39•].

In conclusion, studies of helicases that reorganize branched DNA molecules have provided new avenues of investigation into DNA replication and repair. Moreover, it is likely that we have yet to uncover the full range of cellular processes in which these enzymes are involved. In the future, it will be interesting and exciting to see the discoveries and insights that will emerge from the analysis of these intriguing factors.

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Figure 1.
Current models of helicase activity on branched molecules discussed in this review.

RecG	—	—	+	+
Rad5	—	+	+	+
FANCM	—	—	+	+
HARP	—	+	n.d.	—

Figure 2. DNA binding properties of helicases discussed in this review. The “+” and “—” signs denote whether or not the indicated DNA substrates stimulate DNA binding and/or ATPase activity. n.d., not determined.