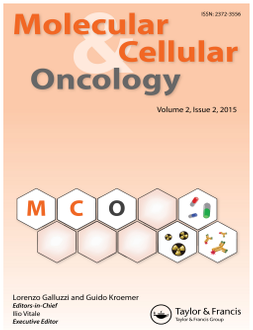

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Roles of DNA helicases in the maintenance of genome integrity

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Keywords: DNA helicase, DnaB, genome integrity, MCM, Mcm2–7, Pif1, RecQ, Rothmund-Thomson syndrome, Twinkle

Abbreviations: BVP, bovine papilloma virus; DSB, double-strand break; dsDNA, double-stranded DNA; HR, homologous recombination; ICL, interstrand crosslink; MCM or Mcm, mini-chromosome maintenance; mtDNA, mitochondrial DNA; PEO, progressive external opthalmoplegia; ssDNA, single-stranded DNA; SF, superfamily; ts, temperature sensitive; TAg, T-antigen

Genome integrity is achieved and maintained by the sum of all of the processes in the cell that ensure the faithful duplication and repair of DNA, as well as its genetic transmission from one cell division to the next. As central players in virtually all of the DNA transactions that occur *in vivo*, DNA helicases (molecular motors that unwind double-stranded DNA to produce single-stranded substrates) represent a crucial enzyme family that is necessary for genomic stability. Indeed, mutations in many human helicase genes are linked to a variety of diseases with symptoms that can be generally described as genomic instability, such as predispositions to cancers. This review focuses on the roles of both DNA replication helicases and recombination/repair helicases in maintaining genome integrity and provides a brief overview of the diseases related to defects in these enzymes.

Introduction

It has been written that "The human body can achieve many things, but perhaps its greatest role is to act as a storage mechanism for the genetic information of the species."[1] However, an organism does not merely store genetic information; the integrity of the genome is also safeguarded through high-fidelity replication, recombination, and repair of genetic information. Indeed, breakdown of genome integrity, a state known as genomic instability, is a characteristic of many diseases such as cancer. Thus, maintaining genomic stability in the face of the approximately 10,000 DNA damaging events that every cell in the human body experiences every day[2] is essential for the faithful propagation of genetic material from one generation to the next.

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Although a multitude of diverse proteins are involved in DNA replication, recombination, and repair, members of only 2 enzymatic families—DNA helicases and DNA polymerases—play roles in virtually all aspects of these processes. This review focuses on the former, but interested readers are directed to several recent excellent reviews on DNA polymerases and their roles in maintaining genome integrity.[3-5]

DNA helicases are molecular motors that in most cases use the power of ATP hydrolysis to unwind double-stranded (ds) DNA and RNA-DNA hybrids into single-stranded (ss) DNA templates. The genomes of all organisms encode a variety of DNA and RNA helicases and helicase-like proteins, from ~30 in model bacteria like *Escherichia coli* and *Bacillus subtilis*, to ~100 in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, to 163 in humans.[6] Based on conserved sequence motifs, helicases have been bioinformatically classified into one of 7 superfamilies (SF-I to SF-VII).[7] They have also been further categorized by their polarity of unwinding (e.g., 3′-5′ [SF-IA] vs. 5′-3′ [SF-IB]) and placed into 18 subfamilies (DnaB/MCM, DEAD-box, DEAH-box, SWI2/SNF2, SKI1, RecD/UPF1, PIF1, MPH1, DinG/RAD3, RECQ, Lhr/HRQ1, UvrD/SRS2, RuvB/RVB, KU, YRF1, HsdR/IRC3/SSL2, PhoH/Rho/SecA/HerA/UvrB/PriA/YgcB, and unclassified) using a variety of bioinformatics techniques (reviewed in[6]). Much has also been written about the various mechanisms used by helicases to unwind dsDNA[8-10] and the biochemical details underpinning this activity.[11-13]

Attempting to address all of these details with respect to the roles of DNA helicases in maintaining genome integrity is beyond the scope of this review. Similarly, each of the multitude of helicases described above cannot be adequately addressed. Instead, this review focuses on well-known members of the replicative and recombination/repair helicases, as well as helicase-linked diseases that result from genomic instability.

Replicative helicases

Replicative helicases are the enzymes responsible for the bulk dsDNA unwinding necessary for genome replication during every cell cycle. These enzymes share several features that together distinguish them from all other helicases: (1) they are essential for viability, (2) they are required for both the initiation and elongation steps of DNA replication, (3) they

function at the point of the replication fork, and (4) nearly all of them function as ring-shaped hexamers. Evolution has led to at least 5 distinct families of replicative helicases used by bacteria, archaea, eukaryotes, viruses, and mitochondria (see below).

As enzymes that interact with every base pair of DNA in the genome, replicative helicases are also critical for the maintenance of genome integrity (**Fig. 1**). They are the first portion of the replication fork to encounter DNA lesions and proteins bound to the DNA, both of which can stall DNA replication and lead to genomic instability.[14] DnaB-like helicases, those in the minichromosome maintenance (MCM) family, viral replicative helicases, and mitochondrial replicative helicases are briefly introduced below, and their connections to genome maintenance are described.

Bacterial DnaB-like helicases

All bacteria with sequenced genomes encode a homolog of the well-studied *E. coli* DnaB (R. Ramalho, unpublished), the prototypical bacterial replicative helicase. *In vivo*, the *E. coli* genome is replicated bidirectionally from a single origin of replication, (reviewed in[15]) where homohexameric rings of DnaB are opened and clamped around the ssDNA by the DnaC loader protein.[16] One DnaB hexamer is loaded onto the ssDNA on each side of the origin, and DNA unwinding proceeds in opposite directions around both halves of the circular *E. coli* chromosome.

The importance of DnaB to the integrity of the *E. coli* genome is exemplified by experiments performed with temperature sensitive (ts) alleles of the dnaB gene. At the restrictive temperature, DNA replication elongation is blocked in these cells and newly replicated DNA is extensively degraded.[17] This is in contrast to types of damage that arrest DNA replication without directly targeting DnaB (e.g., UV damage), in which the replication forks are stabilized and protected[18] until the damage can be repaired.[19,20] In any event, DnaB(ts)-mediated replication fork stalling and the associated nascent DNA degradation are disastrous to the cell. Therefore, in *E. coli* (and probably also other bacteria), a properly functioning replicative helicase is essential to maintain genome integrity. As such, small molecules that target and inactivate DnaB-like helicases should function as potent antibiotics.

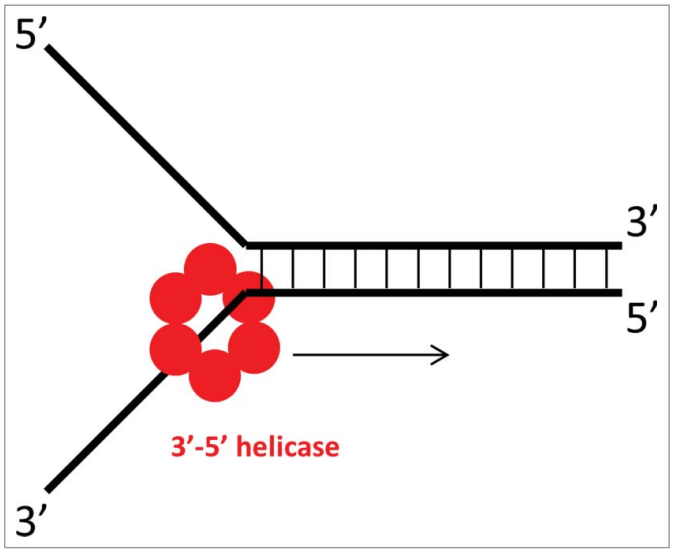

Figure 1. Steric exclusion model of DNA unwinding by a ring-shaped helicase. One strand of ssDNA passes through the central channel of the helicase, while the other is excluded. Unidirectional movement of the helicase (in this case, 3′-5′ as indicated by the arrow) toward the dsDNA and exclusion of the other strand aid in unwinding the DNA duplex.

MCM helicases

The replicative helicases of all prokaryotes studied to date are homohexamers, including those found in archaea. However, unlike the bacterial DnaB helicases, archaeal genomes encode MCM replicative helicases. Although they are functional homologs (i.e., they are both localized at replication forks to unwind genomic DNA during replication), DnaB and MCM helicases are not orthologous. Further, archaeal MCMs translocate along DNA with an opposite polarity to DnaB (3′-5′ vs. 5′-3′; reviewed in[21,22]). The eukaryotic replicative helicase is also a 3′-5′ MCM family enzyme. However, in contrast to the archaeal MCM, the eukaryotic Mcm2–7 complex is a heterohexamer comprised of 6 distinct subunits (individually numbered Mcm2 through Mcm7).[23] As with DnaB in bacteria, though, the MCM/Mcm2–7 helicases are the vanguards of the replication forks in archaea and eukaryotes.

Biochemical studies of the simpler and more stable archaeal MCM complexes, especially those from thermophilic archaea, have yielded a tremendous wealth of structural (e.g.,[24-27]) and mechanistic (e.g.,[28-31]) information about this enzyme family. However, most work connecting MCM helicases to genomic stability has been performed with Mcm2–7 and eukaryotic model organisms.

Because of its essential role in DNA replication, which must occur once and only once per cell cycle in eukaryotes, loading and activation of the Mcm2–7 complex at origins of replication are tightly and redundantly controlled processes.[32] As stated above, genomic instability is a hallmark of cancers, thus perturbing Mcm2–7 regulation or activity can lead to carcinogenesis. For example, work in *S. cerevisiae* and mammals indicates that a hypomorphic allele of Mcm4 ($Mcm4^{Chaos3}$) is linked to increased rates of loss[33] and mutation[34] of genetic information in yeast and a variety of defects in mice, including mammary adenocarcinomas (**Table 1**).[33] Similarly, deregulating Mcm7 expression actively increases tumor formation in a mouse chemical carcinogenesis model.[35] Indeed, altering the expression levels of any of the 6 Mcm2–7 subunits renders cells susceptible to chromosome loss, increased recombination rates, altered viability, and/or early-onset cancer.[36,37]

The loss of genome integrity in tumor cells allows them to rapidly accumulate mutations that lead to their uncontrolled replication and can also lead to the development of resistance to chemotherapeutic agents. However, as a vital player in DNA replication, targeting the Mcm2–7 complex with drugs is

Table 1. Helicase-linked diseases

Helicase	Disease(s)	Symptoms	Types of genomic instability	References
Mcm2–7 (Mcm4^{Chaos3})	Cancer	Predisposition to cancers (e.g., mammary adenocarcinoma) and increased tumor growth	Chromosome breaks	33,35-37
T-antigen	Cancer	Malignant transformation (uncontrolled cellular proliferation)	Inactivation of the Rb and p53 tumor suppressors	40,47
E1	Carcinomas	Malignant transformation (uncontrolled cellular proliferation)	Inactivation of tumor suppressors and activation of telomerase	41,48
Twinkle	Progressive external opthalmoplegia	Weak/paralyzed eye muscles, drooping eyelids, and general skeletal muscle weakness	DNA damage from reactive oxygen species, replication fork stalling, and mtDNA loss	54,55,100
BLM	Bloom syndrome	Increased cancer risk, sun sensitivity, and short stature	Increased levels of sister chromatid exchange	85,99,100
WRN	Werner syndrome	Premature aging and increased cancer risk	Defects in DNA repair, reduced p53-dependent apoptosis, and accelerated telomere loss	85,99,100
RECQ4	Rothmund-Thomson syndrome, Baller-Gerold syndrome, & Rapadilino syndrome	Increased cancer risk, slow growth, skeletal defects, poikiloderma, sparse hair, cataracts	Chromosome copy number alterations and sensitivity to DNA damaging agents	85,99,100,106
PIF1	Cancer	Predisposition to inherited breast cancer	Increased direct repeat recombination	98
FANCJ	Fanconi anemia	Bone marrow failure, increased rates of blood and skin cancers, congenital defects	Increased sensitivity to DNA interstrand crosslinking agents, sensitivity to G-quadruplex stabilizing ligands	101,107
FANCM	Fanconi anemia	Bone marrow failure, increased rates of blood and skin cancers, congenital defects	Increased sensitivity to DNA interstrand crosslinking agents and increased levels of sister chromatid exchange	101,108
CHLR1/DDX11	Warsaw Breakage syndrome	Growth retardation, intellectual disabilities, microcephaly, congenital defects	Increased sensitivity to DNA interstrand crosslinking agents and sister chromatid cohesion defects	102
RTEL1	Dyskeratosis congenital & Hoyeraal-Hreidarsson syndrome	Nail dystrophy, hyperpigmentation, growth retardation, aplastic anemia	Dysfunctional telomere maintenance, increased levels of spontaneous DNA damage and anaphase bridges	103,104
XPB	Xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy	Sensitivity to UV light and increased levels of skin cancers	Defects in DNA repair, sensitivity to oxidative stress	70,105
XPD	Xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy	Sensitivity to UV light and increased levels of skin cancers	Defects in DNA repair and reduced p53-dependent apoptosis	70,105

*Mcm, mini-chromosome maintenance; mtDNA, mitochondrial DNA.

hypothesized to be a viable method to fight cancer.[38] If a drug is able to inhibit all 6 Mcm2–7 subunits, 6 mutational events would be needed to develop resistance if drug resistance is even possible (as these are essential proteins, many mutations will simply be lethal). Schwacha and colleagues are screening small molecules to uncover Mcm2–7-specific inhibitors and their effects on yeast and human cells.[38]

Viral replicative helicases

The replicative helicases from DNA viruses are members of SF-I to SF-III,[39] with well-studied examples that include simian virus 40 T-antigen (SV40 TAg)[40] and the bovine papilloma virus (BVP) E1 protein.[41] Both of these helicases bind to origins of replication in their respective viral genomes in a sequence-dependent manner. However, TAg is unique among replicative helicases in that it associates with origin DNA on its own;[40] all of the other helicases require additional DNA replication initiation proteins to help target them to origins of replication (e.g., DnaA and DnaC for *E. coli* DnaB[15,16] and the papillomavirus E2 protein for E1).[41] In most other respects, however, TAg and E1are quite similar. They both load at origins, where they undergo an ATP-dependent multistep oligomerization process to form 2 ring-shaped head-to-head hexamers (i.e., double hexamers) with the DNA topologically constrained within the central channels of the hexameric rings. Based on a crystal structures of BVP E1 in

the presence and absence of DNA and nucleotides,[42,43] it is believed that these ring-shaped helicases contain ssDNA within their central channels and thus move along only one strand of the DNA, melting the double helix by steric exclusion of the unbound strand (**Fig. 1**). Similar unwinding models have also been proposed for DnaB and the MCM/Mcm2–7 helicases (see[29,44] and references therein).

Initially, the replicative helicases from eukaryotic viruses served as models to begin delineating the similarities and differences between bacterial and eukaryotic DNA replication *in vitro*. This is because the Mcm2–7 helicase has only recently been found to be amenable to biochemical investigations through the use of buffer conditions that more closely resemble the nuclear milieu[45] and the discovery of associated factors that stimulate its activity.[46] However, like Mcm2–7, both SV40 TAg and the E1 proteins of papillomaviruses are linked to genome stability. For example, SV40 (a non-human primate virus that was widely introduced into the human population through polio vaccines contaminated with the virus),[47] and related polyomaviruses induce malignant transformation of cells.[40] This process occurs when TAg binds to and suppresses the activity of the tumor suppressor proteins p53 and Rb, inducing uncontrolled cellular proliferation and rendering the genome susceptible to damage. Papillomaviruses are similarly linked to tumorigenesis.[48] As the most conserved protein encoded by papillomavirus genomes and the only one with enzymatic activity, E1 is vital for the virus to commandeer the normal DNA replication machinery of the cell.[41] Thus, although the TAg and E1 helicases may help to ensure the integrity of their viral genomes, they also lead to genetic instability in host cells.

Mitochondrial replicative helicases

It is widely believed that mitochondria arose as the result of an ancient endosymbiosis between a eukaryotic cell and an α-proteobacterium.[49] As such, these organelles contain a separate genome (mtDNA) from the nuclear DNA that encodes genes with homology to bacteria and bacteriophages. It has also become clear in recent years that the replication of mtDNA involves a different repertoire of enzymes than replication of the nuclear genome, including a mitochondrial replicative helicase known as Twinkle in metazoans.[50]

Twinkle is a 5′-3′ helicase[51,52] that is more similar to bacteriophage and DnaB-like helicases than to MCM proteins, supporting a bacterial origin for mitochondria. Like all of the replicative helicases described above, though, Twinkle forms a hexameric complex to unwind DNA, and its proper function is linked to maintaining genome integrity. For example, in tissues under high oxidative stress, high Twinkle levels are necessary to overcome replication fork stalling and reduce mtDNA mutations caused by damage from reactive oxygen species.[53] Mutations in the gene encoding human TWINKLE are causative of autosomal dominant progressive external opthalmoplegia (PEO) as a result of associated deletions in the mtDNA (**Table 1**).[54] PEO is a disease characterized by weak or paralyzed eye muscles, drooping eyelids, and general skeletal muscle weakness that can be exacerbated by exercise and results from depletion of mitochondria,[55] i.e., from loss of mtDNA as a result of genomic instability.

Although all multicellular and most unicellular eukaryotes have mitochondria (very simple parasitic eukaryotes lack them[56]), not all of these organisms encode a Twinkle homolog. Such organisms include the well-studied budding yeast *S. cerevisiae* and kinetoplastid parasites such as *Trypanosoma brucei*. However, these organisms encode one or more members of the Pif1 family of helicases (reviewed in[57]), which in *S. cerevisiae*[58-62] and *T. brucei*[63-65] are necessary for mtDNA maintenance. It is tempting to speculate that Pif1 helicases may act as replicative helicases in these cases, although unlike the enzymes discussed above, Pif1 proteins are not known to form hexamers[66] nor do they display the levels of processivity (the number of base pairs unwound per helicase-DNA binding event) that one would expect to be necessary to unwind the mtDNA genome.[67] Speculation aside, the roles of Pif1 family helicases in maintaining the integrity of the nuclear genome are discussed in greater detail below.

Recombination and repair helicases

In addition to replicative helicases, cells encode a cadre of additional helicases that have a variety of functions. For example, accessory helicases such as *E. coli* Rep, UvrD, and DinG and *S. cerevisiae* Rrm3 can act in conjunction with their replicative helicases to drive replication fork progression past impediments (e.g., protein-bound DNA) *in vivo*.[68,69] Additionally, helicases can serve more than one role, such as the human XPB and XPD enzymes (*S. cerevisiae* Ssl2 and Rad3, respectively) that function in both transcription and nucleotide excision repair.[70] Many more have niche roles in DNA recombination and repair, both of which are essential for maintaining genome integrity. Examples of such helicases from 2 evolutionarily conserved families and their roles in genome maintenance are discussed below.

RecQ helicase family

RecQ proteins are 3′-5′ helicases that have DNA structure-specific roles *in vivo*, often functioning at recombination intermediates (reviewed in[71]). *E. coli* expresses the founding member of this family, known simply as RecQ, but eukaryotes tend to express several RecQ helicases. Indeed, the human genome encodes 5 RecQs (RECQ1, BLM, WRN, RECQ4, and RECQ5), and even single-celled eukaryotes like yeasts express 2 or 3 RecQs.[72-75] Mutations in 3 of the human RecQ helicases (BLM, WRN, and RECQ4) cause diseases characterized by a predisposition to cancers and/or premature aging (**Table 1**), pathologies that are linked to loss of genome integrity.[71]

Perturbation of the expression levels and biochemical activities of the RecQ helicases have such negative consequences on genome integrity because these enzymes interact with a host of important protein cofactors. Indeed, RecQs affect DNA replication (RECQ1[76] and RECQ4[77]), recombination (all 5 human RecQs[71]), repair (all 5[71]), and telomere maintenance (BLM,[78] WRN,[79] and RECQ4[80]), as well as transcription (BLM,[81] WRN,[82] and RECQ5[83]) and mtDNA maintenance (RECQ4[84]). In other words, one or more of the RecQ helicases function in virtually all aspects of DNA metabolism.

RecQs are perhaps best known for their roles in homologous recombination (HR; reviewed in[85]). Indeed, they are involved in multiple steps of the HR repair pathway, from beginning to end (**Fig. 2**). In human cells, when a DNA double-strand break (DSB) occurs, the DNA ends are initially resected in the 5′-3′ direction. The resulting 3′ ssDNA is the perfect substrate for the 3′-5′ BLM helicase, which partners with the nuclease DNA2 to processively unwind dsDNA and degrade the resulting 5′ ssDNA strand, leading to further resection. The remaining 3′ ssDNA is eventually coated by the RAD51 recombinase, which aids in the homology search, strand invasion, and D-loop formation necessary to carry out HR. One pathway used to resolve the D-loop involves the formation of double Holliday junctions, which themselves are resolved by BLM in a complex with TOP3 (a topoisomerase) and RMI1/RMI2 (factors that stimulate TOP3 activity). Furthermore, biochemical experiments suggest that RECQ1,[86] BLM,[87] and RECQ5[88] can inhibit or correct the formation of unproductive recombination intermediates. All of these steps are vital to proper HR, a DSB repair pathway that does not result in loss of genetic information and hence aids in maintaining genomic integrity.

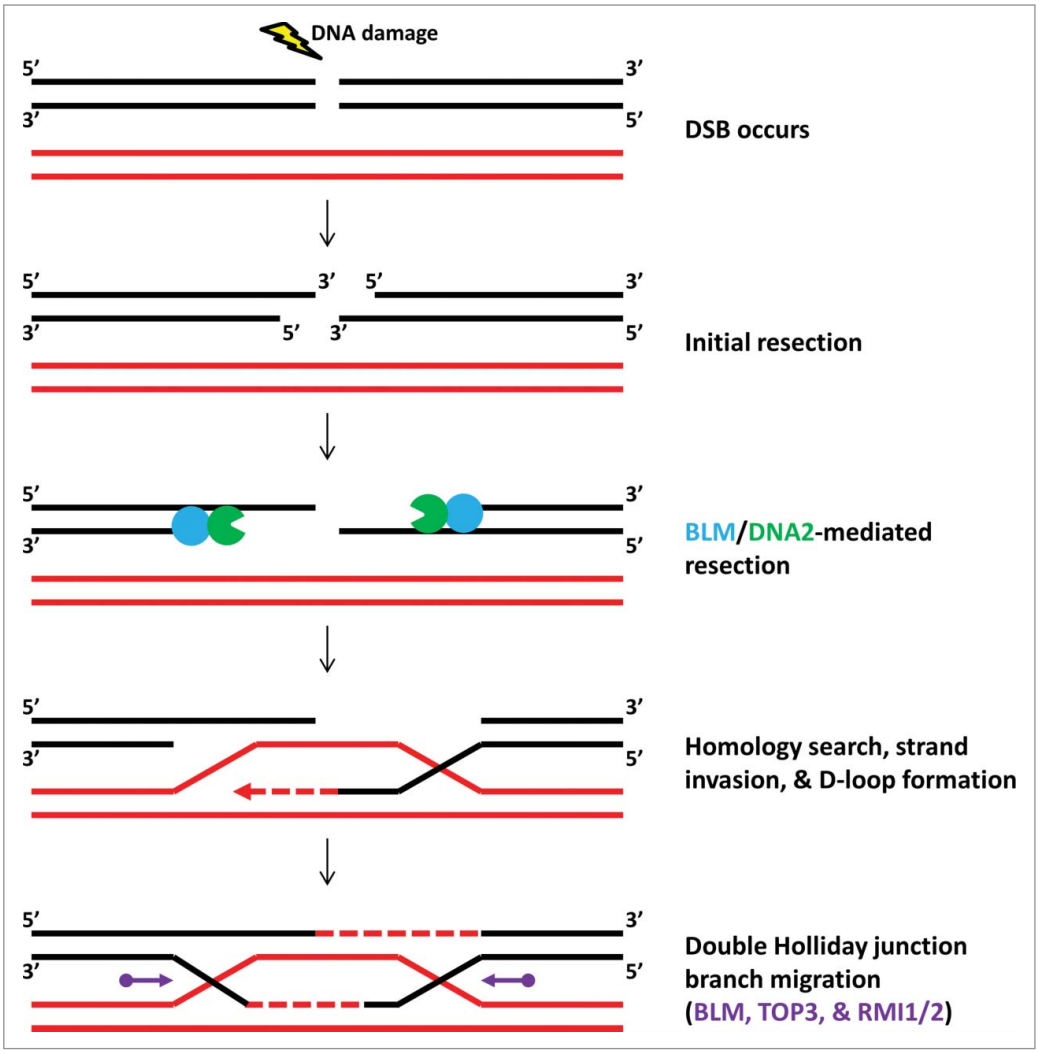

Figure 2. Simplified model of double-strand break (DSB) repair. When a DSB occurs, the DNA surrounding the break is initially resected in the 5′-3′ direction to produce 3′ ssDNA overhangs. The BLM helicase can load onto this 3′ ssDNA and translocate in the 3′-5′ direction, unwinding the double helix to create additional 5′ ssDNA that the DNA2 nuclease degrades to further resect the DNA away from the lesion. The RAD51 recombinase (not pictured) coats the ssDNA to initiate a homology search, strand invasion, and D-loop formation on the undamaged chromosome (red). DNA synthesis (red dashed arrow) serves to copy the missing genetic information. One of the pathways used to resolve these recombination intermediates involves the formation of double Holliday junctions and their branch migration and resolution by a complex composed of BLM, TOP3, RMI1, and RMI2 (purple).

Pif1 helicase family

Pif1 helicases function with a 5′-3′ polarity, and like the RecQs, perform a diverse set of known and hypothesized functions *in vivo*.[57] Although Pif1s were originally thought to be present only in eukaryotes, genes encoding these enzymes have recently been identified in numerous bacteria, bacteriophages, and eukaryotic viruses.[89] To date, little is known about the functions of Pif1s in bacteria and viruses, but the roles of Pif1 helicases in genome maintenance in *S. cerevisiae*, *S. pombe*, and other eukaryotes have been investigated by several groups (reviewed in[57]).

Unlike most model eukaryotes (e.g., mice and humans), *S. cerevisiae* encodes 2 Pif1 family helicases: the founding member Pif1 and its paralog Rrm3.[57] Neither protein is essential, nor are cells lacking both the PIF1 and RRM3 genes inviable (N. Ahmad and M. Bochman, unpublished). However, Pif1 and Rrm3 perform multiple (and often opposing) functions to help maintain both nuclear and mitochondrial genome integrity.[57] As the best-studied family member, the *S. cerevisiae* Pif1 is focused on here.

As hypothesized above, Pif1 may be the *S. cerevisiae* mitochondrial replicative helicase.[58-62] In the nucleus, however, Pif1 is a veritable jack-of-all-trades. It acts as a catalytic inhibitor of

telomerase by using its helicase activity to physically evict telomerase from chromosome ends and DSBs.[90] Additionally, Pif1 is involved in Okazaki fragment maturation, where it probably creates long ssDNA flaps that are degraded by the Dna2 nuclease during Okazaki fragment processing.[91,92] Pif1 also helps to oppose DNA replication at rDNA repeats, where it aids in establishing replication fork barriers to prevent the head-on collision of replication and transcription.[93] More recently, *S. cerevisiae* Pif1 was shown to suppress genomic instability at DNA motifs with the potential to form very stable secondary structures that can impede replication fork progression (G-quadruplex motifs)[94,95] and to promote break-induced repair by helping to migrate a bubble-like replication fork.[96,97]

It is unclear how many of these activities are conserved in the human PIF1 helicase, but defects in any of them could explain why mutation of a conserved residue in the PIF1 ATPase/helicase domain is linked to inherited breast cancer (**Table 1**).[98] It is also unclear what bacterial Pif1 helicases do *in vivo*, especially in organisms encoding more than one family member.[89] However, if the bacterial Pif1s are as vital to genome integrity as *S. cerevisiae* Pif1, they may also prove to be useful drug targets in human pathogens.

Helicase-linked diseases

An obvious theme that arises from the above examples of helicases and their roles in maintaining genome integrity is that when the activity of these enzymes is altered (either by mutation or changes in expression level), disease ensues. Many of these pathologies are predispositions to cancer, suggesting that a large number of helicases are tumor suppressors (see **Table 1**). Additional helicases that are known to be linked to disease have been covered in several excellent reviews.[99,100] Some of the best studied include those linked to Fanconi anemia (FANCJ and FANCM in humans; Chl1 and Mph1 in *S. cerevisiae*), a genetic disease leading to cancer and bone marrow failure in most patients as a result of defects in repairing DNA interstrand crosslinks (ICLs).[101]

ICLs are covalent linkages between the two strands of the double helix and are particularly dangerous DNA lesions because they block both replication and transcription. Indeed, mutations in many other human helicases are linked to ICL sensitivity, including BLM, CHLR1/DDX11, HELQ, the Mcm8/9 complex, RECQ4, RECQ5, RTEL1, and WRN (**Table 1**) (Rogers, van Kessel, and Bochman, in press). Unsurprisingly, there are known and suspected disease links with these enzymes, such as the *CHLR1/DDX11* mutations that cause Warsaw breakage syndrome, which is characterized by defects in sister chromatid cohesion and Fanconi anemia-like symptoms.[102] Similarly, RTEL1 mutations are associated with dyskeratosis congenita[103] and Hoyeraal-Hreidarsson syndrome,[104] related diseases that are characterized by bone marrow and telomere maintenance defects.

Adjacent nucleotides in DNA can also be crosslinked (i.e., form intrastrand crosslinks), such as the thymine-thymine dimers caused by UV irradiation. Although not as deleterious as ICLs to cells, intrastrand lesions must still be repaired to maintain genomic integrity, and helicases are involved in this repair. Indeed, mutations in the XPB and XPD helicases mentioned above are linked to xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy – diseases that share the symptom of light sensitivity due to deficiencies in repairing UV damage.[70,105]

Conclusions

Based on their evolutionary conservation, known and hypothesized *in vivo* roles, and links to diseases when mutated, it is clear that DNA helicases are essential for maintaining genomic integrity. What is unclear, however, is how defects in these enzymes lead to disease. Indeed, many of the helicases described above are multifunctional, and deficiencies in any one (or more) of the *in vivo* processes that they take part in could result in a predisposition to cancer. For example, do mutations in RECQ4 alter its activities in DNA replication, recombination, repair, telomere maintenance, or mtDNA maintenance? Furthermore, do different mutations differentially affect RECQ4, accounting for the spectrum of diseases that it is linked to? In the future questions such as these must be addressed, both biochemically using purified protein and *in vivo* using mutant cell lines and simple model systems (e.g.,[72]). Such investigations will delineate exactly which of the pathways these helicases are involved in safeguard genome integrity and suggest targets for clinical interventions in helicase-linked diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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