DNA Helicases and Human Disease

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Helicases are motor proteins that utilize the energy derived from the hydrolysis of nucleoside triphosphates (NTP/dNTP) to disrupt hydrogen-bond interactions in double- or multi-stranded DNA and RNA. All known helicases are recognized to have at least two intrinsic enzymatic activities: (1) NTP/dNTP-dependent nucleic acid unwinding and (2) DNA/RNA-dependent NTP/dNTP hydrolysis. Unwinding of DNA and RNA, with the generation of single-stranded nucleic acids, is essential for numerous cellular transactions, including DNA replication, repair, and recombination, as well as RNA splicing, transcription, and translation. Helicases therefore are ubiquitous, key players in several aspects of nucleic acid metabolism. The importance of helicases can be gleaned from the fact that, to date, 14 different DNA helicases have been identified in bacteria, 15 in yeast, and 25 in human cells (for a compilation of known helicases, see Tuteja and Tuteja 2004). In addition, mutations in genes encoding DNA helicases have been demonstrated in several inherited human diseases. The fact that these diseases are rare also emphasizes the essentiality of DNA helicases in cellular metabolism. In this chapter we consider the different helicases, the proposed mechanisms for strand separation, their roles in cellular metabolism, and finally, the human diseases associated with mutations in specific DNA helicases.
HELICASE CLASSIFICATION
Helicases are frequently classified by the presence of signature sequence motifs, substrate specificities, or the directionality of unwinding. Four superfamilies have been defined on the basis of the number and sequence of helicase motifs they encode (Gorbalenya and Koonin 1993). Although this number is variable, all helicases contain sequences homologous to the Walker A and B boxes that are characteristic of NTP binding and/or hydrolyzing enzymes. Helicases do not display sequence specificity in unwinding but have preference for the type and structure of the nucleic acid substrate. Unwinding is directional, proceeding either 3′→5′ or 5′→3′ on the translocating strand (Matson and Kaiser-Rogers 1990), although there are exceptions, such as RecBCD, which is a bidirectional helicase (Dillingham et al. 2003; Taylor and Smith 2003).

HELICASE STRUCTURE
Many prokaryotic and viral helicases have been crystallized and/or have been visualized by electron microscopy. Although quaternary structures of monomers, dimers, and hexamers have been proposed, the subunit composition of active complexes of most helicases remains controversial. Structural data are lacking for eukaryotic DNA helicases that, in many cases, are much larger in size than the prokaryotic enzymes. A few eukaryotic DNA helicases, including two human RecQ homologs, the Bloom syndrome protein (BLM) and the Werner syndrome protein (WRN), have been visualized as multimers. BLM forms tetramers and hexamers (Karow et al. 1999), and the amino-terminal domain of WRN exists as a trimer and a hexamer (Xue et al. 2002). For WRN, the subunit composition has important mechanistic implications since the helicase and exonuclease activities (see below, DNA Helicases in Human Diseases) may reside on different subunits.

MECHANISMS OF UNWINDING
Two general mechanisms, termed passive and active, have been postulated to describe unwinding (Lohman and Bjornson 1996). Both processes require ATP hydrolysis; consequently, the term passive is a misnomer.

1. Passive mechanism: In this mode, the helicase facilitates unwinding indirectly. By binding ssDNA that becomes available through
transient breathing of the duplex, the helicase prevents reannealing of the two DNA strands.

2. **Active mechanism:** In this mode, the helicase actively destabilizes duplex DNA in addition to trapping ssDNA. This mechanism requires the helicase to possess at least two DNA-binding sites, one that binds ssDNA and another that simultaneously binds the duplex. Two types of active mechanisms have been envisioned—the “inchworm” and “rolling” modes (Fig. 1) (Lohman and Bjornson 1996).

(a) **Inchworm:** In this model, the helicase has two non-identical DNA-binding sites, a leading site that always interacts with duplex DNA during successive unwinding cycles, and a trailing site that binds only ssDNA. Translocation along ssDNA and DNA unwinding occur through conformational changes coupled with NTP binding and hydrolysis.

**Figure 1.** Active mechanisms of helicase unwinding. Two proposed mechanisms, inchworm (A) and rolling (B), are represented. The oligomeric status of mammalian helicases is unclear; for reasons of simplicity, the enzyme is depicted as a dimer in this scheme. In the inchworm model, one subunit (shown in gray) always contacts duplex DNA and inches forward to disrupt it, while the second (shown in black) always interacts with the unwound ssDNA. In the rolling model, the two subunits are equivalent and alternate between binding to and disrupting dsDNA, and interacting with ssDNA. The rolling of the two subunits is indicated by curved arrows.
(b) **Rolling**: The rolling mechanism requires at least two identical DNA-binding sites that can alternate between binding of ssDNA and dsDNA through allosteric effects of ATP and ADP binding. The helicase rolls along the DNA with translocation coupled to ATP binding and unwinding coupled to ATP hydrolysis.

**FUNCTIONS OF DNA HELICASES**

The role of many helicases in DNA metabolism remains to be established. However, biochemical and genetic studies have implicated the involvement of some DNA helicases in transactions including DNA replication, repair, and recombination. Each of these enzymes may have multiple cellular roles and may substitute for one another.

**Helicases in DNA Replication**

Helicases are necessary to melt duplex DNA at replication origins, initiate replication, and unwind DNA ahead of the replication fork. The single strands generated by progressive DNA unwinding serve as templates for processive DNA synthesis by the primase/polymerase complex. Enzymes performing this function have been identified in bacteria and viruses; the situation in yeast is not firmly established, and the involvement of replicative helicase activity in human cells remains to be clarified.

In *Escherichia coli*, DnaB and Rep are the helicases responsible for chromosomal and phage DNA replication, respectively (Matson et al. 1994). T7 gp4, T4 gp41, and SV40 T-Ag are well-studied examples of phage and viral helicases that participate in replication (Patel and Picha 2000). In yeast, the Mcm2–7 complex comprising six origin-binding proteins exhibits helicase activity in vitro (Lee and Hurwitz 2001) and, thus, is the proposed candidate for a replicative helicase. Human homologs of yeast Mcm proteins have also been shown to exhibit helicase activity (Ishimi 1997) and, by analogy, may also function in DNA replication (for review, see Forsburg 2004). Other potential replicative helicases have been identified based on their association and copurification with DNA polymerases or other replisome proteins, such as PCNA and RP-A. In fact, the S-phase defects of Werner and Bloom syndrome cells, the functional or physical interactions of human RecQ helicases with DNA polymerase-δ (pol-δ) (Kamath-Loeb et al. 2000; Szekely et al. 2000), RP-A (Shen et al. 1998a; Brosh et al. 2000), and PCNA (Lebel et al. 1999), and their localization to replication foci (Yan et al. 1998; Yankowski et al. 2000) raise the possibility that these helicases may be involved in DNA replication.
Helicases in DNA Repair

The most studied repair process that involves helicases is the nucleotide excision repair (NER) pathway that removes UV-induced lesions and bulky adducts, such as cyclobutane pyrimidine dimers and 6-4 photoproducts, and cisplatin, respectively. This pathway, which has been reconstituted in vitro with purified components, involves at least four steps: recognition of the lesion, DNA unwinding by helicases, excision of the lesion-containing DNA fragment, and finally, repair synthesis.

In *E. coli*, the UvrABC complex constitutes the major component of NER. The strand-separating activity of the UvrAB complex creates the entry site for UvrC to excise the lesion (Matson et al. 1994). In mammalian cells, recognition is by the XPC-hHR23B complex that creates an open complex and recruits additional factors. Alternatively, the transcription bubble created by the stalled RNA polymerase II (pol II) at the lesion recruits Cockayne syndrome group A and B proteins (CSA and CSB). The DNA around the lesion is unwound by the 5’→3’ and 3’→5’ helicase activities of xeroderma pigmentosum group D and B proteins (XPD and XPB), respectively, to allow access to other repair factors (van Brabant et al. 2000; Lehmann 2001, 2003).

There is also evidence that proteins of the RecF pathway are involved in the repair of UV lesions that stall DNA replication. In particular, the helicase activity of RecQ and the exonuclease activity of RecJ process the nascent lagging strand to allow bypass/repair of the lesion prior to resumption of DNA synthesis (Courcelle and Hanawalt 1999, 2001).

Additional repair helicases include *E. coli* UvrD, implicated in methyl-directed mismatch repair (Matson et al. 1994), and XRCC5, the 80-kD subunit of the Ku protein, involved in the end-joining pathway of double-strand-break repair (Lieber et al. 2003).

Helicases in DNA Recombination

Recombination entails the rearrangement of genes within and between chromosomes. Recombination serves to promote genetic diversity as well as preserve genetic identity on damaged DNA. Double-stranded DNA breaks (DSB) and single-strand gaps, replication fork-blocking lesions that arise from endogenous and exogenous damaging agents, can initiate homologous recombination (HR). By using the undamaged complementary DNA strand for exchange of genetic information, recombination facilitates the repair of damaged DNA and the subsequent reinitiation of DNA replication. This pathway has been extensively studied in *E. coli* and requires the coordinated action of at least 25 proteins, including the helicases, RecBCD, RecQ, RuvAB, RecG, and PriA (for review, see Kowalczykowski 2000).
RecBCD is a nuclease-helicase complex required for the strand-exchange phase of recombination (Smith et al. 1995). It generates ssDNA for strand invasion and the formation of heteroduplex or Holliday junction (HJ) structures. In the absence of RecBCD, the RecQ helicase in conjunction with RecJ exonuclease functions efficiently at DSBs to unwind duplex DNA (Smith 1989). HJ intermediates are processed by RuvAB, another helicase that is specific for branched DNA structures. RuvAB extends the region of DNA heteroduplex by branch migrating the crossover point/HJ. It acts in concert with RuvC, an endonuclease that cleaves the HJ into duplex products (West 1997).

*E. coli* RecG is also a branched DNA-specific recombination helicase that unwinds forked DNA in vitro to form HJ substrates for RuvABC. Current studies suggest that RecG regresses stalled replication forks to create “chicken-foot” structures (Briggs et al. 2004). Fork regression allows access of repair enzymes to damaged sites and subsequent reloading of the replisome by replication restart proteins including the PriA helicase, without the need to cleave DNA and undergo rearrangements.

Human homologs of *E. coli* RecQ have been identified, and at least two of them, BLM and WRN, are proposed to function in some aspect of recombination (Wu et al. 2001; Saintigny et al. 2002). Likewise, HJ branch migrating and endonuclease activities, comparable to those of *E. coli* RuvABC, have been observed in extracts of human cells (Constantinou et al. 2002).

**DNA HELICASES IN HUMAN DISEASES**

Mutations in DNA helicase genes are associated with rare human diseases, many of which also exhibit a proclivity to develop cancer (Table 1). These diseases provide important insights into the role of helicases in cellular metabolism, development, and carcinogenesis.

**XP Helicases**

XPB and XPD are essential components of the NER pathway. In addition, they are part of the multi-subunit TFIH complex that is involved in basal and activated transcription in eukaryotes (see Fig. 2). Mutations in their encoding genes, particularly in XPD, generate a plethora of human genetic disorders, including xeroderma pigmentosum (XP), XP-Cockayne syndrome (CS), and trichothiodystrophy (TTD) (de Boer and Hoeijmakers 2000; Lehmann 2003).
Table 1. Helicase deficiency human disorders

<table>
<thead>
<tr>
<th>Helicase</th>
<th>Disease</th>
<th>Defective gene</th>
<th>Clinical presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>XP</td>
<td>xeroderma pigmentosum (XP)</td>
<td>XPD</td>
<td>UV sensitivity; skin cancers, neurological abnormalities</td>
</tr>
<tr>
<td></td>
<td>XP-Cockayne syndrome (XP-CS)</td>
<td>XPD</td>
<td>XP phenotypes; dwarfism, mental retardation, retinal and skeletal abnormalities</td>
</tr>
<tr>
<td></td>
<td>trichothiodystrophy (TTD)</td>
<td>XPD</td>
<td>brittle hair, mental retardation, reduced stature, ichthyotic skin, unusual facies</td>
</tr>
<tr>
<td>RecQ</td>
<td>Bloom syndrome (BS)</td>
<td>RecQ2/BLM</td>
<td>predisposition to many cancers: non-Hodgkin's lymphoma, leukemia; dwarfism, sun-induced erythema, type II diabetes, narrow face/prominent ears, male infertility, female sub-fertility</td>
</tr>
<tr>
<td></td>
<td>Werner syndrome (WS)</td>
<td>RecQ3/WRN</td>
<td>premature aging features: cataracts, type II diabetes, osteoporosis, atherosclerosis, graying and loss of hair; mesenchymal tumors: sarcomas, melanoma, thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>Rothmund Thomson syndrome (RTS)</td>
<td>RecQ4/RTS</td>
<td>Juvenile cataracts, skin atrophy and pigmentation changes, skeletal abnormalities, cancer predisposition: chiefly osteosarcoma</td>
</tr>
<tr>
<td></td>
<td>RAPADILLINO syndrome (RS)</td>
<td>RecQ4</td>
<td>developmental and skeletal abnormalities; no cancer risk</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>progressive external ophthalmoplegia (PEO)</td>
<td>Twinkle</td>
<td>external ophthalmoplegia, ptosis, ataxia, peripheral neuropathy, deafness, cataracts</td>
</tr>
</tbody>
</table>
Clinical Presentation

XP: This rare autosomal recessive disorder is characterized by sunlight-induced skin abnormalities ranging from freckles to multiple skin cancers including basal cell carcinoma, squamous cell carcinoma, and malignant melanoma (Cleaver and Crowley 2002). Eight complementation groups (including XPB and XPD helicases), designated XPA–XPG and XPV have been identified. Studies of XP have not only delineated the pathway for NER, but have also provided the first conclusive demonstration that defects in DNA repair are associated with the development of human cancers (Cleaver and Crowley 2002).

XP-CS: CS is also associated with sunlight sensitivity but not with skin cancers. It is characterized by severe dwarfism, mental retardation, microcephaly, and retinal, skeletal, and neurological abnormalities. Two complementation groups, CSA and CSB, have been associated with this disorder; CSB has the seven signature helicase motifs and is also associated with TFIH (Lehmann 2001, 2003). Patients with XP-CS manifest phenotypes of both XP and CS, yet the causative mutations map exclusively to XPD.

TTD: The characteristic feature of TTD is sulfur-deficient brittle hair, caused by the reduced content of cysteine-rich matrix proteins in the hair shaft. TTD is also accompanied by mental retardation, unusual facies, ichthyotic skin, and reduced stature. Many, but not all, TTD patients are sensitive to sunlight, but they do not manifest unusual skin pigmentation nor are they prone to skin cancers.
Molecular Mechanism

XPB and XPD were first identified as components of the NER pathway. The opposing unwinding polarities of XPB and XPD separate both strands of DNA, on either side of the lesion, prior to cleavage by the XPG and ERCC1-XPF nucleases. The involvement of XPB and XPD in transcription, in addition, was unveiled by their identification as subunits of the transcriptional activator protein, TFIIH. Their unwinding role in transcription is to form a promoter open complex for the entry of RNA pol II.

XPB is a core component of TFIIH; consequently, its helicase activity is indispensable for normal transcription processes, and mutations in XPB are commonly lethal. Only three examples have been reported that result either in TTD, or in mild and severe cases of XP-CS (Lehmann 2003). XPD, on the other hand, maintains the stability of the TFIIH complex and, thus, is only required for optimal transcription (de Boer and Hoeijmakers 2000). Many mutations in XPD have been reported; however, no identical substitutions are associated with the three disorders, suggesting that the site of mutation determines the clinical outcome. Most XPD mutations are clustered in the carboxy-terminal region of the protein. The carboxy-terminal domain interacts with the p44 subunit of TFIIH that stimulates the helicase activity of XPD. Mutations that map to this region do not eliminate the intrinsic helicase activity of XPD but abolish its stimulation by p44 (Coin et al. 1998a,b). XP and TTD cell lines also appear to have reduced levels of TFIIH. Thus, the reduced activity (Coin et al. 1998a) and stability of TFIIH (Satoh and Hanawalt 1997) could account for defective NER and, therefore, the hypersensitivity of these cells to UV irradiation.

Mouse Models and Polymorphisms

TFIIH is essential for basal transcription; therefore, deletion of its subunits in mice is lethal. de Boer et al. (1998) have successfully established a mouse model by introducing a human TTD XPD mutation. The mutant mice exhibit features of TTD, including brittle hair and UV sensitivity phenotypes. Unlike human patients, however, TTD mice are more prone to UV-induced skin cancers. This could be due to the fact that rodent cells are less proficient in excising photoproducts from the bulk of their genomic DNA.

Many polymorphisms have been reported in XPD. Two common polymorphisms, D312N and K751Q, are associated with an increased incidence and early age of onset of basal cell carcinomas (Dybdahl et al. 1999) and are present at an elevated frequency in patients with
melanomas (Tomescu et al. 2001). Thus, polymorphisms in the XPD helicase predispose individuals to the development of skin cancer. There are also reports that link these polymorphisms with increased risk of other cancers, including cancers of the breast and lung (Justenhoven et al. 2004; Buch et al. 2005).

The RecQ Helicase Family

The RecQ family of DNA helicases derives its name from the E. coli recQ gene which was identified in a screen for mutations that confer resistance to thymine starvation (Nakayama et al. 1988). Several RecQ homologs have been identified on the basis of sequence homology, genetic, and biochemical evidence. All homologs share a conserved RecQ helicase core, and many of them contain two additional domains carboxy-terminal to the helicase core (Fig. 3). These include the RQC (RecQ family C-terminal) and the HRDC (helicase RNaseD C-terminal) domains believed, respectively, to mediate protein and DNA interactions. In general, prokaryotes and simple eukaryotes have one RecQ member, whereas higher eukaryotes and mammalian cells contain multiple members. For example, human cells possess five RecQ homologs, designated RecQ1 or RecQL, RecQ2 or BLM, RecQ3 or WRN, RecQ4 or RTS, and RecQ5. Mutations in RecQ2, Q3, and Q4 result in the genomic instability disorders, Bloom syndrome (BS), Werner syndrome (WS), and Rothmund-Thomson syndrome (RTS), as well as RAPAPDILINO syndrome (RS), respectively (Ellis 1997; van Brabant et al. 2000; Bachrati and Hickson 2003).

Figure 3. Alignment of RecQ family helicases. The helicase, RecQ carboxy-terminal (RQC) and helicase RNaseD C-terminal (HRDC) motifs are highlighted in RecQ helicases from E. coli (Ec), S. cerevisiae (Sc), S. pombe (Sp), and from humans (Hs). The checker bar, unique to WRN, represents the amino-terminal exonuclease motif. The numbers to the right indicate the number of amino acids in each protein.
BS

Mutations that impair the function of RecQ2/BLM, including missense mutations that result in amino acid substitutions, nonsense codons, and frameshifts, as well as splice-site mutations, result in BS phenotypes.

Clinical Presentation

BS is a rare disorder characterized by proportional dwarfism, sun-induced facial erythema, type II diabetes, narrow face with prominent ears, male infertility, and female sub-fertility. In addition, it is associated with an early onset of cancers, particularly non-Hodgkin’s lymphoma, leukemia, and carcinomas of the breast, colon, and skin. Cancer is the chief cause of death, occurring by age 30.

Molecular Mechanism

BLM is a cell-cycle-regulated DNA helicase with highest levels observed in the S phase (Sanz et al. 2000). BLM localizes to polymorphonuclear leukocyte (PML) nuclear bodies (Zhong et al. 1999), but upon DNA damage, it translocates to distinct nuclear foci. Although BLM is phosphorylated by ATM/ATR kinases, the functional significance of this post-translational modification is not known. It is also sumoylated in vivo; sumoylation controls the intranuclear trafficking of BLM between PML bodies and damage-induced nuclear foci (Eladad et al. 2005).

BS cells are distinguished by an increased frequency of sister chromatid exchanges (SCEs) (Ray et al. 1987), manifested by the persistence of quadriradial chromosomes, a prolonged S phase, and the accumulation of abnormal replication intermediates. BS cells are sensitive to γ-irradiation, to the topoisomerase I inhibitor camptothecin, and hydroxyurea (HU), ethylmethane sulfonate (EMS), mitomycin C (MMC), and 4-nitroquinoline 1-oxide (4-NQO) (Bachrati and Hickson 2003). However, these reports are not without controversy.

Recombinant BLM exhibits DNA-dependent ATPase and ATP-dependent 3′→5′ unwinding activities (Karow et al. 1997). Like the prototype E. coli RecQ, the processivity of BLM helicase is stimulated by interaction with the human single-strand binding protein, RP-A (Brosh et al. 2000). But unlike RecQ, BLM cannot effectively unwind blunt-ended duplex DNA (Mohaghegh et al. 2001), suggesting the requirement of single-stranded DNA tails for loading and unwinding by BLM. It has become increasingly clear that blunt-ended DNA containing internal single-stranded regions, and noncanonical structures such as quadruplexes
and 4-way junctions, are preferred over standard B-form duplex DNA (Mohaghegh et al. 2001). In fact, BLM can branch migrate HJ over distances as great as 3 kb (Karow et al. 2000), and recently, BLM has been shown to resolve double HJ structures (Wu and Hickson 2003).

BLM interacts functionally and physically with an array of proteins whose primary role is in the maintenance of genome integrity. These include p53, BRCA1 and MLH1, RAD51, Top3α, TRF2, WRN, Fen-1, and Fanconi anemia proteins (Table 2) (Bachrati and Hickson 2003; Hickson 2003). The interaction of BLM with Top3α is evolutionarily conserved and may be the most significant. The concerted action of BLM and Top3α results in the dissolution of a double HJ (Wu and Hickson 2003). This, together with the observations that BS cells have an increased frequency of SCEs and that it colocalizes with RAD51 foci, provides compelling evidence that at least one function of BLM is in HR.

**Mouse Models and Polymorphisms**

Several knockout alleles of murine Blm have been created. However, none of the knockout mice recapitulate the phenotypes of human BS, and all, except one, are embryonic lethal. The embryos are small in size and are developmentally delayed, consistent with the proportional dwarfism seen in BS individuals, and their red blood cells show an increased number of micronuclei, indicative of genetic instability (Chester et al. 1998). Embryonic fibroblasts from some, but not all, Blm−/− mice show the hallmark phenotype of increased SCEs (Goss et al. 2002). The viable Blm−/− mice appear normal but are cancer-prone (Luo et al. 2000). Further analysis has demonstrated that these mice are hypomorphs (rather than Blm−/−) expressing small amounts of Blm that rescue the lethality of the embryos.

No studies associating BLM polymorphisms with disease risk have been reported. However, published data indicate that Ashkenazi Jews with the BLMAsh mutation are about 3 times more likely to develop colorectal cancer than individuals lacking this mutation (Gruber et al. 2002).

**WS**

WRN, the causative gene for WS, is a unique member of the RecQ helicase family, as it encodes both 3′→5′ DNA helicase (Gray et al. 1997) and 3′→5′ DNA exonuclease (Shen et al. 1998b) activities. Mutations that eliminate both activities result in genetic instability, premature aging, and an increased incidence of specific tumors.
<table>
<thead>
<tr>
<th>Associated protein</th>
<th>Interaction determined by</th>
<th>Resultant functional alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication protein A (RP-A)</td>
<td>enzyme activity, ELISA, far western</td>
<td>stimulates activity, enhances processivity</td>
</tr>
<tr>
<td>p53</td>
<td>functional assay</td>
<td>decreases apoptosis; decreases BLM helicase on HJ DNA</td>
</tr>
<tr>
<td>p53 binding protein 1 (53BP1)</td>
<td>GST pull-down colocalization</td>
<td>damage signal transmission*</td>
</tr>
<tr>
<td>Telomere binding protein, TRF2</td>
<td>Co-IP, colocalization</td>
<td>TRF2 stimulates BLM helicase</td>
</tr>
<tr>
<td>RAD51</td>
<td>yeast 2-hybrid, Co-IP, colocalization</td>
<td>N.D.</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Co-IP, colocalization</td>
<td>N.D.</td>
</tr>
<tr>
<td>MLH1 and MSH6</td>
<td>yeast 2-hybrid, Co-IP, colocalization</td>
<td>no effect of BLM on mismatch repair</td>
</tr>
<tr>
<td>Topoisomerase 3α (Top3α)</td>
<td>Co-IP, far western colocalization</td>
<td>BLM stimulates Top3α activity, BLM and Top3α resolve double HJ structures</td>
</tr>
<tr>
<td>Flap endonuclease 1 (Fen-1)</td>
<td>Co-IP</td>
<td>BLM stimulates Fen-1 activity^ BLM inhibits WRN exonuclease</td>
</tr>
<tr>
<td>WRN</td>
<td>Co-IP, ELISA</td>
<td>WRN exonuclease</td>
</tr>
<tr>
<td>Fanconi anemia proteins (FA)</td>
<td>Co-IP, colocalization</td>
<td>S-phase checkpoint activation</td>
</tr>
<tr>
<td>Mus81 endonuclease</td>
<td>Co-IP, colocalization</td>
<td>BLM stimulates Mus81 activity^</td>
</tr>
<tr>
<td>ATM/ATR kinase</td>
<td>yeast 2-hybrid, Co-IP, genetic</td>
<td>phosphorylates BLM</td>
</tr>
<tr>
<td>Chromatin assembly factor 1 (CAF-1)</td>
<td>yeast 2-hybrid, Co-IP, colocalization</td>
<td>BLM inhibits CAF-1-mediated chromatin assembly during DNA repair^</td>
</tr>
</tbody>
</table>

*(ELISA) Enzyme-linked immunosorbent assay; (Co-IP) coimmunoprecipitation; (N.D.) not determined.

^Sengupta et al. (2004).
^Zhang et al. (2005).
^Jiao et al. (2004).
Clinical Presentation

WS patients present with premature onset of age-related conditions including cataracts, atherosclerosis, osteoporosis, and type II diabetes (Furuichi 2001). A limited spectrum of cancers is observed in WS; mesenchymal tumors such as sarcomas, melanomas, meningiomas, and thyroid cancers predominate. Death (median age 47) occurs due to cancer or cardiovascular disease.

Molecular Mechanism

WRN is a constitutively expressed helicase that localizes predominately in the nucleolus (Opresko et al. 2003). Like BLM, it translocates into the nucleus and localizes to distinct nuclear foci upon stress and exposure to DNA-damaging agents (Bachrati and Hickson 2003; Opresko et al. 2004). Frameshift, splice-site, and nonsense mutations causal to WS truncate the encoded protein. WS was believed to result from a failure of the truncated proteins to localize to the nucleus. However, neither the transcript nor the truncated protein is detectable in WS cells (Yamabe et al. 1997; Moser et al. 2000), suggesting that the absence of WRN, rather than its impaired function, is associated with WS phenotypes. Unlike BS and many inherited genetic diseases, no point mutations that result in amino acid substitutions have been reported in WS. Phosphorylation by ATM/ATR kinases (Kim et al. 1999), DNA-PK (Karmakar et al. 2002), and c-Abl tyrosine kinase (Cheng et al. 2003), as well as sumoylation (Kawabe et al. 2000), has been reported. However, the functional consequences of these modifications have not been elucidated.

Primary cells from WS patients have a reduced replicative life span in culture, consistent with the premature-aging phenotypes of WS (Martin et al. 1970). Genomic instability is manifested at the chromosomal level by rearrangements termed variegated translocation mosaicism, and at the molecular level by large DNA deletions (Fry 2002). WS cells show increased sensitivity to 4-NQO, to cross-linking agents such as MMC and cisplatin, camptothecin, and HU (Bachrati and Hickson 2003; Opresko et al. 2004). Recently, it has been reported that WS cells are also sensitive to methylation agents but only when the repair systems that remove these lesions are compromised (Blank et al. 2004).

WRN is a poorly processive DNA helicase requiring RP-A to unwind long stretches of DNA sequence (Shen et al. 1998a; Brosh et al. 1999). Unwinding of B-form duplex DNA by WRN requires a 3’ ssDNA tail (Kamath-Loeb et al. 1998). However, WRN, like BLM, preferentially unwinds in vitro alternate DNA structures, such as replication bubbles, tetraplex DNA, forked DNA, and D-loops (Mohaghegh et al. 2001; Orren et al.
2002), and can branch-migrate HJ (Constantinou et al. 2000). Although the DNA substrate specificities of WRN and BLM overlap, WRN helicase exhibits a preference for unwinding bubble DNA over HJ DNA.

WRN is a unique member of the RecQ family, in that it also possesses 3´→5´ exonuclease activity encoded within the amino-terminal domain (Shen et al. 1998b). The exonuclease of WRN resembles the proofreading exonuclease activity of DNA polymerases in its propensity to remove a single mismatched nucleotide from 3´ recessed duplex DNA (Kamath-Loeb et al. 1998). However, unlike the proofreading exonuclease of DNA polymerases, WRN exonuclease does not degrade ssDNA or blunt-ended duplexes. Similar to the helicase, WRN exonuclease prefers DNA with noncanonical structures, acting effectively on DNA containing replication bubbles (Shen and Loeb 2000). The similar substrate preferences of WRN helicase and exonuclease suggest that the two activities cooperate in vivo. In fact, this has been observed in vitro with forked DNA substrates (Opresko et al. 2001).

WRN, like BLM, interacts with RP-A, p53, RAD51, and TRF2 (Table 3) (Fry 2002; Bachrati and Hickson 2003). In addition, it associates physically and functionally with proteins of the DNA replication machinery, including pol-δ, PCNA, and Fen-1. WRN markedly stimulates the activities of Fen-1 and the polymerase activity of pol-δ in vitro and alleviates pausing of pol-δ at replication roadblocks such as tetraplex DNA structures formed by G-rich sequences (Kamath-Loeb et al. 2001). Consistent with the in vitro observations, WS cells are defective in lagging-strand DNA synthesis of G-rich telomere sequences (Crabbe et al. 2004). It has been proposed that replication of G-rich telomeric DNA requires the helicase activity of WRN to prevent telomere dysfunction and consequent genomic instability.

WRN also interacts functionally with repair proteins, pol-β and apurinic/apyrimidinic endonuclease (APE1), involved in short patch base excision repair, the DNA-PK-Ku complex involved in nonhomologous DNA end-joining, and poly(ADP-ribose)polymerase I (PARP-1) (Table 3). The Ku70/86 heterodimer, in fact, dramatically increases the exonuclease activity of WRN (Cooper et al. 2000). There is also evidence to suggest a role for WRN in homologous recombination. First, WRN interacts with the HR proteins, MRN (Mre11-Rad50-Nbs1) and BLM (Table 3). Second, WS cells lack the ability to faithfully complete recombination, and expression of bacterial HJ resolvase, RusA, or a dominant-negative form of RAD51 reduces the elevated spontaneous and damage-induced recombination rate in WS cells (Saintigny et al. 2002). Although the principal role of WRN remains to be established, current data, including the prolonged S phase of WS cells, their sensitivity to replication-
Table 3. Physical and functional interactions of WRN with associated proteins

<table>
<thead>
<tr>
<th>Associated protein</th>
<th>Interactions determined by</th>
<th>Resultant functional alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication protein A (RP-A)</td>
<td>enzyme activity, Co-IP</td>
<td>increases helicase activity; enhances processivity</td>
</tr>
<tr>
<td>p53</td>
<td>functional assay, Co-IP, enzyme activity</td>
<td>decreases apoptosis; increases p53-dependent transcription; inhibits WRN helicase and exonuclease</td>
</tr>
<tr>
<td>Telomere binding protein, TRF2</td>
<td>GST pull-down, Co-IP, ELISA</td>
<td>TRF2 stimulates WRN helicase</td>
</tr>
<tr>
<td>DNA polymerase-δ (pol-δ)</td>
<td>enzyme activity</td>
<td>WRN increases pol-δ activity, WRN allows synthesis past tetraplex DNA,</td>
</tr>
<tr>
<td>PCNA</td>
<td>yeast 2-hybrid copurification, Co-IP</td>
<td>WRN recruits pol-δ to the nucleolus</td>
</tr>
<tr>
<td>Flap endonuclease-1 (Fen-1)</td>
<td>GST pull-down, Co-IP</td>
<td>WRN stimulates cleavage of 5’ flap substrates</td>
</tr>
<tr>
<td>DNA polymerase-β (pol-β)</td>
<td>GST pull-down, Co-IP</td>
<td>WRN stimulates strand displacement synthesis by pol-β</td>
</tr>
<tr>
<td>AP endonuclease-1 (APE1)</td>
<td>GST pull-down, ELISA</td>
<td>APE1 inhibits WRN helicase^a</td>
</tr>
<tr>
<td>Ku70/80</td>
<td>affinity chromatography</td>
<td>increases WRN exonuclease on undamaged and damaged DNA; alters WRN exonuclease substrate specificity</td>
</tr>
<tr>
<td>DNA-dependent protein kinase (DNA-PK)</td>
<td>IP-nuclear extracts enzyme activity</td>
<td>phosphorylates WRN in vitro and in vivo; inhibits WRN activity by phosphorylation</td>
</tr>
<tr>
<td>c-Abl tyrosine kinase Poly(A/DP-ribose) polymerase-1 (PARP-1)</td>
<td>Co-IP, ELISA GST pull-down, ELISA, Co-IP, mouse crosses</td>
<td>phosphorylates WRN in vivo? PARP-1 inhibits WRN helicase and exonuclease^b, early onset cancers; increased chromatid breaks/rearrangements in PARP-1, null/Wrn^Δhel/Δhel MEFs</td>
</tr>
<tr>
<td>RAD51</td>
<td>genetic, cellular</td>
<td>suppresses aberrant recombination of WS cells</td>
</tr>
<tr>
<td>RAD52</td>
<td>yeast 2-hybrid, far western, Co-IP</td>
<td>inhibits WRN helicase</td>
</tr>
<tr>
<td>Mre11-Rad50-Nbs1 (MRN)</td>
<td>GST pull-down, Co-IP</td>
<td>MRN complex stimulates WRN helicase^c</td>
</tr>
<tr>
<td>Bloom syndrome protein (BLM)</td>
<td>Co-IP, ELISA</td>
<td>BLM inhibits WRN exonuclease</td>
</tr>
</tbody>
</table>

blocking lesions, the preference of WRN to unwind and degrade replication and recombination intermediates, as well as its interaction with proteins of the replication and recombination machinery, hint at its involvement in resolving stalled replication forks by a recombinational repair pathway.

**Mouse Models and Polymorphisms**

Two different knockout alleles of *Wrn* have been established. Whereas one lacks the helicase domain, the second simulates a human mutation and, thus, lacks detectable Wrn protein (Lebel and Leder 1998; Lombard et al. 2000). Although neither model shows overt WS phenotypes, both reveal defects when crossed with other mice. *WrnΔhel/Δhel* p53−/− and *WrnΔhel/Δhel* PARP-1−/− mice develop tumors more rapidly than either p53−/− or PARP-1−/− mice (Lebel et al. 2001; Lebel et al. 2003) and *WrnΔhel/Δhel* PARP-1−/− mice display accelerated mortality (Lombard et al. 2000). *WrnΔhel/Δhel* mice also show an elevated frequency of somatic mutations, revealed by crossing them with a pink-eyed unstable indicator mouse strain (Lebel 2002). Furthermore, *WrnΔhel/Δhel* mice crossed with *terc* (encoding the telomerase RNA component) null mice (Chang et al. 2004; Du et al. 2004) exhibit a classic WS-like premature-aging phenotype and show an increased incidence of nonepithelial cancers that is characteristic of WS (Chang et al. 2004; Du et al. 2004). These phenotypes are not exhibited by *terc* null mice that express Wrn.

There are no published studies that examine the disease/cancer risk of carriers of WS mutations. However, although inconclusive, there are reports which suggest that the common Cys1367Arg WRN polymorphism may be associated with coronary artery disease risk (Ye et al. 1997; Bohr et al. 2004). Parallel studies have examined the effect of known WRN polymorphisms on the helicase and exonuclease activities. The R834C polymorphism significantly compromises the helicase and helicase-dependent exonuclease activities of WRN (Kamath-Loeb et al. 2004). Whether a decrease in helicase and/or exonuclease activity increases the probability of developing disease/tumors remains to be determined.

**RTS and RS**

Mutations in *RecQ4* give rise to two distinct disorders: RTS, a premature-aging disease with genomic instability and a preponderance of osteosarcomas, and RS, a developmental disorder without elevated cancer risk (Kitao et al. 1999; Siitonen et al. 2003). There are no reports which demonstrate that RecQ4 has helicase activity, nor have mouse models
been established. However, the phenotypes of both disorders implicate RecQ4 function in bone development.

**Mitochondrial DNA Helicase and Progressive External Ophthalmoplegia**

Like chromosomal DNA replication, mitochondrial DNA (mtDNA) replication and maintenance also require the action of DNA helicases. In *Saccharomyces cerevisiae*, Pif1p is involved in repair and recombination of mtDNA, and Hmip is required for maintenance of wild-type (rho+) mtDNA but is dispensable for that of rho– (partially deleted) genomes (Lahaye et al. 1991; Sedman et al. 2000). Not much was known about DNA helicases that function in mtDNA replication of mammalian cells until the identification of Twinkle—mtDNA helicase that is one of the genes mutated in the neuromuscular disorder progressive external ophthalmoplegia (PEO) (Spelbrink et al. 2001).

**Clinical Presentation**

PEO is a rare, autosomal neuromuscular disorder characterized by the accumulation of large-scale mtDNA deletions. Both recessive and dominant forms of this disorder exist, although the dominant form is more frequent. Autosomal dominant PEO (adPEO) is characterized by external ophthalmoplegia (lack of eye movement), ptosis, progressive skeletal muscle weakness, peripheral neuropathy, deafness, ataxia, cataracts, and hypogonadism.

**Molecular Mechanism**

AdPEO has been linked to mutations in three genes located at 4q34-35, 10q24, and 15q25. The gene on chromosome 4q encodes adenine nucleotide translocator 1 (ANT1), whereas 15q25 encodes the gene for the large subunit of the mtDNA polymerase, pol-γ. ANT1 controls ATP and ADP shuttling at the mitochondrial inner membrane and may regulate the level of deoxynucleotides. Imbalance in the nucleotide pools or defects in pol-γ could stall mtDNA replication forks and initiate recombination that results in large DNA deletions (Wanrooij et al. 2004).

The gene at 10q24 is homologous at the sequence level to the phage T7 gene 4 hexameric DNA helicase. The gene product colocalizes with mtDNA and exhibits a punctate, star-like staining; the gene product is
therefore called “Twinkle” (Moraes 2001; Spelbrink et al. 2001). Twinkle exhibits 5’→3’ DNA helicase activity that is stimulated by mtSSB. Unlike most helicases, Twinkle prefers UTP as the cofactor for hydrolysis during unwinding (Korhonen et al. 2003). A minimal mtDNA replisome has been reconstituted in vitro using purified pol-γ and Twinkle (Korhonen et al. 2004). Although pol-γ cannot use dsDNA templates for synthesis, and Twinkle cannot unwind long stretches of DNA on its own, the combination of the two enzymes allows processive replication to occur. The addition of mtSSB yields products as large as the mtDNA genome at a synthesis rate equivalent to the in vivo rate, suggesting that Twinkle participates in mtDNA replication.

**Mouse Models and Polymorphisms**

Two transgenic mouse lines have been created, one overexpressing Twinkle and the other expressing a Twinkle-specific small interfering RNA. Overexpression of Twinkle results in a threefold higher mtDNA copy number, whereas a decrease in its amount causes a rapid decline in copy number (Tyynismaa et al. 2004). No polymorphisms in the gene encoding Twinkle helicase have yet been characterized.

The demonstration of helicase activity, functional interaction with pol-γ, and regulation of mtDNA copy number all provide evidence that Twinkle acts at the mtDNA replication fork. It also explains why mutations in both pol-γ and Twinkle manifest identical disease phenotypes.

**CONCLUSION**

The multiplicity of DNA helicases in eukaryotic cells, their association with human diseases and pathological processes, and the fascinating mechanisms required for unwinding DNA guarantee that studies on DNA helicases will be central to our understanding of DNA synthetic processes. Considering that even a simple eukaryote like *S. cerevisiae* contains more than 100 open reading frames which encode potential DNA helicases (van Brabant et al. 2000), we must conclude that we are only at the tip of the helicase iceberg.

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