Recombine and Associate to Prevent Genomic Instability and Premature Aging

To overcome obstacles for DNA replication, a plethora of proteins have to perform tasks, such as recognizing the replication-blocking lesion, unwinding local DNA, removing the blockade, filling the gap by de novo DNA synthesis, and finally, DNA ligation. Surprisingly, human somatic cells contain 5 RecQ 3'-5' helicase genes and 11 genes encoding 3'-5' exonucleases, each sufficient to unwind the DNA and to remove blockades to DNA replication [Shevelev and Hübscher, 2002; Hickson, 2003; Keijzers et al., 2014]. This may suggest that evolution generated genetic and molecular redundancy. If so, loss of any one 3'-5' helicase or 3'-5' exonuclease would be without consequence, for a similar enzyme should be able 'to take over'. However, deficiency for a single 3'-5' helicase or a single 3'-5' exonuclease may result in autosomal recessive disorders, which are characterized by distinct phenotypes but also share some features among each other. Both loss of the *BLM*- or the *RECQL4*-encoded helicases in Bloom syndrome (BS) and Rothmund-Thomson syndrome (RTS), respectively, gives rise to short stature, due to reduced growth of the long bones, sun-sensitive skin rash and telangiectasia as well as several other distinct phenotypes. RTS and Werner syndrome (WS) share cataracts, either juvenile in the case of RTS or in adult WS patients. The latter syndrome, which is due to loss of the *WRN* 3'-5' helicase gene, develops from puberty on and presents as a prototypic premature aging disorder, while BS and

RTS are detectable in early childhood and do not include features of accelerated aging. Absence of the *MRE11*-encoded 3'-5' exonuclease produces a syndrome sharing features of both ataxia-telangiectasia and Nijmegen Breakage syndrome, such as chromosomal instability, increased sensitivity to ionizing radiation, defective induction of stress-activated signal transduction pathways, radioresistant DNA synthesis, and cerebellar degeneration without intellectual impairment, yet no signs of premature aging [Stewart et al., 1999]. The cellular features of WS include prolongation of and arrest in the S phase of the cell cycle, hypersensitivity to DNA cross-links and double-strand breaks (DSBs) inducing agents and reduced DNA recombination [Poot et al., 1992, 1999, 2001, 2002, 2004; Ogburn et al., 1997; Kamath-Loeb et al., 2004; Dhillon et al., 2007]. Cultured fibroblasts from WS patients show a typical and diagnostic variegated chromosomal translocation mosaicism and spontaneous deletion formation [Salk et al., 1981; Fukuchi et al., 1989; Rünger et al., 1994; Oshima et al., 2002]. BS cells show characteristically increased rates of chromosomal aberrations, such as chromatid gaps and breaks, telomere associations, quadriradial figures, sister chromatid exchanges, and micronuclei and reticulocytes with M/M and N/N genotypes of the glycophorin A locus, resulting from increased recombination events between homologous chromosomes [German et al., 1965; Chaganti et al., 1974;

Rosin and German, 1985; Langlois et al., 1989; de Renty and Ellis, 2016]. This perplexing multiplicity of organismal and cellular phenotypes resulting from defects in just one of the many 3'-5' helicase or 3'-5' exonuclease genes cannot be explained by merely considering the enzymatic activities of the encoded proteins.

Most 3'-5' helicase or 3'-5' exonuclease genes appear to be involved in either of 2 cellular DSB response pathways: nonhomologous end joining (NHEJ) or homologous recombination (HR). NHEJ does not require homology at the DNA ends of the DSB, while HR uses the undamaged sister chromatid as a template to reconstitute an intact DNA molecule. Thus, HR is the more fitting pathway to faithfully repair replication-dependent DSBs. In contrast, NHEJ may incorrectly rejoin one end of a DSB with another nonhomologous DNA end from a different chromosome, which eventually would produce complex chromosomal rearrangements [Poot and Haaf, 2015]. To elucidate the mechanisms underlying switching between the HR and NHEJ pathways, 2 alternative paradigms have been explored.

First, the tissue expression patterns of *WRN*, *BLM*, and *RecQL4* were found to differ widely [de Renty and Ellis, 2016]. While *WRN* is expressed in quiescent cells, expression of *BLM* and *RecQL4* was close to the detection limit [Kawabe et al., 2000]. Expression of *WRN*, *BLM*, and *RecQL4* increased upon transformation with Epstein-Barr virus and simian virus 40 and decreased when cell cultures reached quiescence [Kawabe et al., 2000]. Expression of *BLM* is limited to lymphoid cells and actively proliferating cells in normal tissues; it is upregulated in tumor cells of lymphoid and epithelial origin [Turley et al., 2001]. These findings are consistent with the tissues affected by malignant disease in BS patients [de Renty and Ellis, 2016]. Loss of the *WRN* gene, in contrast, affects slowly dividing mesenchymal cells in the vasculature, fat, bone, and dermis. Likewise, WS patients show loss of proliferative homeostasis in their mesenchymal tissues and soft-tissue sarcomas, osteosarcomas, thyroid cancer, and atypical melanomas as well as a diagnostic variegated translocation mosaicism in cultured mesenchymal cells [Epstein et al., 1966; Norwood et al., 1979; Salk et al., 1981; Goto et al., 1996]. Loss of *BLM* produces elevated rates of sister chromatid exchanges. Conversely, *WRN* is required to prevent translocations and complex chromosomal rearrangements, whereas *BLM* helicase activity is needed to preclude sister chromatid exchange formation.

Second, studies of protein complex formation indicated that the *WRN*, *BLM* and *RTS* helicases and the *WRN* and *MRE11* exonucleases participate in distinct, but pos-

sibly mutually complementing, biochemical pathways. *WRN*-deficient cells show hypersensitivity towards the DNA topoisomerase I-trapping drug camptothecin [Poot et al., 1999]. In response to topoisomerase-I-DNA covalent complexes, the *WRN* protein activates the ATR-CHK1-induced S-phase checkpoint, recruits and stabilizes Rad51, and limits the activity of the *MRE11*-encoded exonuclease [Patro et al., 2011; Su et al., 2014]. Thus, in the absence of *WRN*, the *MRE11*-encoded 3'-5' exonuclease degrades newly synthesized DNA at collapsed replication forks. This may account for the deletions found at ligated DSBs in *WRN*-deficient cells [Rünger et al., 1994; Oshima et al., 2002]. The *BLM* protein, on the other hand, forms a complex with topoisomerase III α , RMI1, and RMI2 and cooperates with the DNA2 helicase-exonuclease and RPA to promote DNA-end resection [Wu et al., 2000; Cejka et al., 2010; Sturzenegger et al., 2014]. In addition, both *BLM* and *WRN* interact physically with DNA2 and coordinate their enzymatic activities to mediate 5'-3' DNA-end resection in an RPA-dependent pathway [Sturzenegger et al., 2014]. Thus, *WRN* and *BLM* act epistatically with DNA2 in the long-range resection of DSBs at stalled DNA replication forks [Sturzenegger et al., 2014].

A recent study sheds light on the mechanisms by which the DNA resection and the switch between HR and NHEJ is regulated [Palermo et al., 2016]. With extensive immunoprecipitation and nuclear localization experiments as well as DNA fiber analysis, the authors demonstrate that phosphorylation of serine residue 1133 of the *WRN* protein by CDK1 controls DNA2-dependent DNA-end resection at DSBs during DNA replication. Serine 1133 phosphorylation promotes HR-mediated replication recovery and assures chromosomal stability. In addition, serine 1133 phosphorylation is dispensable for *WRN* relocalization to nuclear foci, but it governs the interaction of *WRN* with the *MRE11* complex. Loss of *WRN* phosphorylation reduces *MRE11* foci formation and acts in a dominant-negative manner to prevent long-range DNA resection. Therefore, collapsed forks have to be 'repaired' by an alternative mechanism, e.g., NHEJ. Taken together, these findings demonstrate that CDK1-dependent phosphorylation regulates *WRN*-DNA2-mediated DNA resection and serves as a molecular switch between the HR and NHEJ pathways for DSB repair during DNA replication. Thus, intact *WRN* function is required to prevent complex chromosomal rearrangements.

Individuals carrying heterozygous *WRN* mutations, e.g., the parents of *WRN* patients, occur at a rate of 1:200 in the general population. Their cells also show elevated

sensitivities to DNA damage induced by DNA cross-linking agents [Ogburn et al., 1997; Poot et al., 1999]. Since these individuals are not affected by WS, they are not thoroughly studied, so no information regarding possible susceptibility to malignant disease has as yet been reported. In conclusion, the WRN 3'-5' helicase or 3'-5' exonuclease protein is by no means 'redundant'. To the contrary, it appears to be pivotal to the recovery from DSBs during DNA replication by switching between HR and NHEJ. Defects of this molecular switch will require other

3'-5' helicases and 3'-5' exonucleases to take over, which will result in genomic instability, in particular somatic variegated translocation mosaicism and eventually premature aging as well as an elevated risk for malignant disease of mesenchymal tissues. The non-enzymatic functions of WRN may hold the key to WS and possibly to the disorders associated with the other 3'-5' helicases and 3'-5' exonuclease genes, which merit further study.

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