PERSPECTIVES: MOLECULAR BIOLOGY

DNA Ends RecQ-uire Attention

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Bloom's syndrome is an autosomal recessive disorder of humans characterized by short stature, sensitivity to sunlight, reduced fertility, and a greatly elevated incidence of cancer. Among cancer-susceptibility syndromes, this disease is of particular interest because affected individuals succumb to the full range of cancers seen in the general population. The *BLM* gene, which is mutated in Bloom's syndrome, encodes a member of the highly conserved RecQ family of DNA helicases (*1*). Remarkably, defects in two other human RecQ family members give rise to disorders characterized by cancer predisposition and premature aging: The WRN protein is defective in Werner's syndrome, and RECQ4 in Rothmund-Thomson syndrome (*1*). Although it is not clear what these helicases do in the cell, cells with a defective form of RecQ helicase have an unstable genome, which may be the underlying cause of the elevated cancer risk seen in these three syndromes. In a recent issue of *Science*, Kusano *et al.* (*2*) reported the isolation of fly mutants with defects in *Dmblm*, the fly homolog of *BLM*. These fly mutants recapitulate certain features of human Bloom's syndrome. More important, the investigators found that Ku70—a protein that binds to the ends of breaks in double-stranded DNA and facilitates their repair—blocks genetic inactivation of *Dmblm*, thereby implicating BLM in the repair of damaged DNA.

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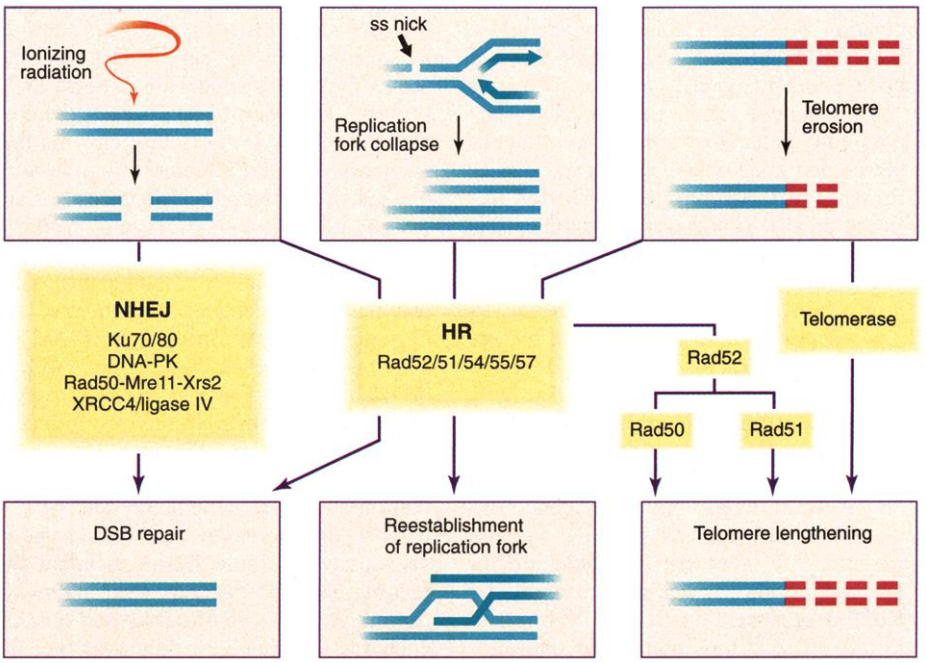
Like human patients with Bloom's syndrome, *Dmblm* mutant flies have impaired fertility that is probably due to a defect in meiosis—the process by which germ cells are produced for sexual reproduction. The defect in male *Dmblm* mutants appears to result in homologous chromosomes becoming interlinked, such that the chromosomes do not segregate correctly into daughter cells during cell division. Mutation of the *SGS1* gene, encoding the sole RecQ homolog in budding yeast, also gives rise to similar chromosome segregation defects (*3*). Kusano *et al.* (*2*) show, as expected, that insertion of a wild-type copy of the *Dmblm* gene into the fly genome partially rescued the sterility of male *Dmblm* fly mutants. However, more surprisingly, they found that introduction of a third copy of *Drosophila* Ku70 or a human Ku70 transgene that could be switched on and off also resulted in partial rescue of the sterile phenotype (*2*). This mild overexpression of Ku70 also partially relieved the hypersensitivity of *Dmblm* mutants to alkylating agents that damage DNA.

Together with the structurally related protein Ku80, Ku70 is crucial for the repair of double-stranded breaks in the DNA (*4*). There are two principal pathways to repair this type of DNA damage: the error-free homologous recombination (HR) pathway, and the error-prone nonhomologous end-joining pathway (see the figure). In yeast, HR requires genes of the *RAD52* epistasis group, including *RAD51* (*5*). Rad51p catalyzes the central step in HR, namely, the pairing and exchange of DNA strands between homologous sequences. The Ku70-Ku80 heterodimer acts at a very early step in the nonhomologous end-joining pathway (see the figure). Ku binds to the ends of broken DNA and then recruits and activates the catalytic subunit of a DNA-dependent protein kinase (*4*). End-joining components are required not only for repair of x-ray induced double-strand breaks in DNA, but also for V(D)J recombination of immunoglobulin genes, which is necessary for generating a large antibody repertoire (*4*).

At first sight, the discovery that Ku70 suppresses *Dmblm* mutations appears to link BLM to the end-joining pathway. Several lines of evidence, however, suggest that the RecQ helicases are more likely to be involved in the HR pathway for DNA double-strand break repair. This is apparently not an essential catalytic role, because RecQ helicase mutants appear proficient in performing "generalized" HR such as double-strand break repair. Indeed, the most visible abnormality in RecQ helicase mutants is hyperrecombination (an elevated frequency of recombination events between homologous DNA sequences). The hallmark of Bloom's syndrome (used in diagnosis of the disorder) is hyperrecombination, particularly between sister chromatids but also between homologous chromosomes. Consistent with this hyperrecombination phenotype, the RAD51 homologous re-



Maintaining genomic stability. Depicted are the major pathways and key proteins that repair breaks in double-stranded DNA (DSB) and maintain the ends of chromosomes (telomeres). RecQ helicases are thought to be important in homologous recombination (HR) and in the RAD52-dependent pathway for maintaining the lengths of telomeres. These enzymes may also be involved in nonhomologous end joining (NHEJ). Single strand, ss.

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combination pathway is apparently constitutively activated in Bloom's syndrome cells (*6*). Moreover, BLM exists in a complex with the RAD51 protein in human cells, and BLM can process certain recombination intermediates such as D-loops and Holliday junctions in vitro (*6–8*). The proposed involvement of BLM in homologous recombination is apparently conserved, because budding yeast *sgs1* mutants also show hyper-recombination, and Sgs1p and Rad51p operate in the same recombination repair pathway that protects yeast DNA from the damaging effects of alkylating agents (*1*, *6*).

It may appear contradictory that mutants lacking a protein proposed to be involved in homologous recombination display elevated rates of homologous recombination. The way in which RecQ helicases achieve the apparently conflicting tasks of both promoting HR and suppressing "promiscuous" HR is not yet clear. One possibility is that they are able to influence the choice of template DNA used during recombination repair of DNA double-strand breaks. That this may be the case is suggested by analyses of diploid *sgs1* yeast mutants, which are unable to use the homologous chromosome to repair γ-ray–induced double-strand breaks, but are still competent for sister-chromatid recombination repair (*9*). Moreover, Sgs1p is known to prevent recombination between homologous (similar but nonidentical) sequences (*10*).

Given the large body of evidence implicating RecQ helicases in HR, we suggest that the most likely explanation for the suppression of *Dmblm* mutations by Ku70 overexpression (which by implication leads to stimulation of the end-joining repair pathway) is that this suppression activates an alternate repair pathway that deals with the failure to efficiently process double-strand breaks in the absence of a RecQ helicase. Nevertheless, the intriguing possibility exists that the suppression of *Dmblm* mutations by a component of the end-joining pathway reflects some as yet unidentified molecular link between end-joining and HR, which until now have been viewed as functionally separate processes. Indeed, it has been known for some time that (in certain genetic backgrounds) *recQ* mutants of *Escherichia coli* are defective in both promotion of HR and suppression of illegitimate recombination events that may have arisen through a process akin to end joining.

The Kusano *et al.* (*2*) report is not the first to connect Ku70 with a RecQ helicase. The Ku70-Ku80 heterodimer has been shown to physically interact with and stimulate one of the catalytic activities of WRN (*11*, *12*), possibly reflecting a common function for both proteins in end joining. However, in addition to acting at the ends of damaged DNA, Ku70 binds to the natural ends of chromosomes within telomeres, the specialized structures that "cap" the ends of linear chromosomes in eukaryotes. Ku70 appears to be required for gene silencing in telomeres, correct localization of telomeres to the nuclear periphery, and regulation of telomere length (*4*). The interaction between WRN and Ku70 may, therefore, be particularly important for telomere maintenance. Primary fibroblasts from Werner's syndrome individuals, like Ku-deficient murine cells, display excessive telomere shortening and premature replicative senescence, which is thought to contribute to the early onset of aging seen in Werner's syndrome patients (*13*). Indeed, expression of telomerase, the specialized reverse transcriptase enzyme that normally regulates telomere length, suppresses the accelerated telomere shortening and premature senescence of Werner's syndrome cells (*14*). Yeast Sgs1p is also required for a telomere maintenance pathway that is independent of telomerase and dependent on recombination (*15–17*). This telomere defect in *sgs1* mutants can be partially overcome by overexpression of WRN, indicating a conserved mechanism of action for RecQ helicases in telomere maintenance (*17*). In yeast, there are two mechanistically and genetically distinct telomere maintenance pathways that are telomerase-independent and recombination-dependent. Both pathways require *RAD52*, but *RAD51* and *RAD50* each operate in only one pathway (see the figure). RecQ helicases and Rad51p act together during homologous recombination under some circumstances. However, telomere maintenance mediated by Sgs1p is Rad51-independent, but does require the Rad50p component of the Rad50-Mre11-Xrs2 nuclease complex that probably operates in both HR and end joining (*15*, *16*). It is possible, therefore, that RecQ helicases and Ku70 act together in at least some aspect of telomere length control.

Maintenance of genomic stability requires not only the repair of DNA damage, but also the coordination of DNA repair with processes such as DNA replication and chromosome segregation. The relative insensitivity of RecQ helicase mutants to agents that induce DNA double-strand breaks suggests that RecQ helicases are likely to act in a "specialized" pathway for repair of double-strand breaks, perhaps during a specific phase of the cell cycle. Most evidence points to their involvement during DNA replication, because RecQ helicase mutants are defective in exiting from replication arrest, and RecQ helicases interact with replication proteins and localize to sites of ongoing replication (*18–21*). DNA ends (whether they are damage-induced or naturally occurring at telomeres) pose a particular problem for the cell's replication machinery. It is now apparent that there is a great deal of cross talk between the replication machinery and processes required for minimizing the loss of genetic material at DNA ends. We have suggested previously (*7*, *22*) that RecQ helicases process lesions that arise at replication forks (or are generated by the translocating fork itself) and in doing so prevent unrepaired DNA strand breaks from initiating "promiscuous" recombination. Indeed, the interlinking of homologous chromosomes through aberrant replication-dependent recombination may account for the meiotic defects in *Dmblm* mutants (*2*). It is clear that further analysis of the interplay between RecQ helicases, DNA replication, and the DNA repair machinery will be required to fully delineate the parts played by RecQ helicases in the maintenance of genome integrity. A broader ambition for those of us working in this field is to characterize the molecular basis for genomic instability in cancer-prone disorders with the hope that this will help us to understand more fully the pathogenesis of sporadic cancers in the human population.

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