

The RecQ DNA helicases: Jacks-of-all-trades or master-tradesmen?

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Cell Research (2008) **18**:328-330. doi: 10.1038/cr.2008.33; published online 3 March 2008

Homologous recombination occurs when a damaged chromosome uses an intact homologous chromosome as a template for its repair. The main steps of recombination are most readily illustrated for the repair of a DNA double-strand-break (DSB). First, DSB-ends are processed to form single-stranded tails, which assemble into nucleoprotein complexes comprising an oligomeric filament of a RecA-family protein (Rad51 in eukaryotes) and associated factors. Rad51 filaments catalyze homologous pairing and strand-exchange between a DSB-end and a double-stranded template to form a joint molecule intermediate. This structure allows *de novo* priming of DNA synthesis to restore sequences that were lost or damaged at the site of the original lesion. Recombination also underpins chromosome replication by facilitating the repair of broken replication forks. In this case, joint molecule formation allows replication to reinitiate. At the final step of recombination, strand-exchange “Holliday” junctions that connect the involved chromosomes are resolved so that segregation can ensue. Joint molecule resolution can occur with one of two outcomes: a crossover, in which chromosome arms are exchanged; or a noncrossover without exchange.

Aberrant recombination causes chromosomal alterations that may activate oncogenes, cause loss-of-heterozygosity

(LOH) for tumor-suppressor genes and ultimately lead to transformation and tumorigenesis. It follows that recombination is regulated at multiple levels. Perhaps most important is to limit the use of recombination to the repair of lesions that cannot be appropriately repaired by other mechanisms. In addition, fidelity is maintained by confining recombination to the S/G2 stages of the cell cycle, when sister-chromatid templates are available. Additional processes function to bias recombination to occur between identical, allelic sequences on the sister-chromatids and to be resolved with a noncrossover outcome. Recombination between parental homologs is less desirable because it can cause local sequence homozygosity, if the outcome is noncrossover, or LOH for all sequences distal to the site of recombination if the outcome is crossover. Moreover, interhomolog crossing-over in S/G2 cells forms mitotic bivalents, which may be prone to segregation errors. Recombination becomes extremely hazardous when it occurs between non-allelic (ectopic) sequences; in such cases, crossing-over will lead to gross chromosomal changes such as translocations, inversions, duplications and deletions. The potential risks of recombination are compounded when non-identical (termed homeologous) templates are utilized.

The importance of regulating homol-

ogous recombination is highlighted by a set of heritable cancer-prone diseases caused by mutations in recombination enzymes. Amongst these are the RecQ helicase disorders Bloom’s syndrome, Werner’s syndrome and Rothmund-Thomson syndrome caused respectively by mutations in the related *BLM*, *WRN* and *RECQL4* genes [1]. The RecQ DNA helicases are an SF-II helicase subfamily with structurally and functionally conserved members in most bacteria and in all eukaryotes. They catalyze the prototypical helicase reaction of ATP-driven separation of complementary DNA strands, but are characterized by a preference for binding and unwinding branched DNA structures that arise during replication and recombination [2].

The role of RecQ enzymes in regulating homologous recombination is exemplified by the archetypal Bloom’s helicase, BLM. Mitotic crossing-over, revealed as cytologically detectable exchanges between both sister and non-sister chromatids, is increased by an order of magnitude in cells lacking BLM. A mechanism for this anticross-over function was revealed by *in vitro* studies showing that BLM and associated factors can resolve joint molecule intermediates to specifically promote the noncrossover outcome of recombination. Most notably, BLM together with the type-I topoisomerase, TOPIII α and the binding/specificity factor, BLAP75,

uniquely catalyze the “dissolution” of double-Holliday junction (dHJ) joint molecule intermediates [3, 4].

Consistent with the distinct pathologies of Werner’s and Rothmund-Thomson syndromes, cells lacking WRN and RECQL4 do not share the hypercrossover phenotype of *BLM* mutants, and both WRN and RECQL4 enzymes have distinct biochemical properties. WRN comprises both RecQ helicase and exonuclease activities and plays an important role in telomere stability, probably by regulating telomere-telomere recombination [1]. RECQL4 is essential for DNA replication in *Xenopus* oocyte extracts, where it helps recruit or stabilize polymerases at replication origins [5]. While RECQL4 is not essential for DNA replication in human cells it is required to arrest cells in S-phase in response to genotoxic stress [6]. Surprisingly, recombinant RECQL4 lacks detectable helicase activity despite being a DNA-dependent ATPase [1]. Perhaps the biological activity of RECQL4 is achieved by translocating along DNA without strand-separation, or by simply binding to a specific DNA structure such as a replication fork. By analogy to the sole budding yeast RecQ helicase, Sgs1, RECQL4 may act to stabilize polymerases at stalled replication forks [7].

Vertebrate genomes contain two additional RecQ proteins, RECQL and RECQL5, which have yet to be associated with heritable human diseases. RECQL is the closest in structure to *E. coli* RecQ, lacking the N- and C-terminal domains characteristic of BLM, WRN and RECQL4. The hyperrecombination and chromosomal instability phenotypes of human and mouse cells lacking RECQL suggest functional overlap with BLM [8]. Also analogous to BLM, RECQL interacts with several components of the DNA mismatch repair system and can disrupt joint molecules [2]. However, RECQL is distinct from BLM in that it cannot catalyze the dHJ dissolution reaction, despite being

able to interact with TOPIII α . Yeast Sgs1 functions with the DNA mismatch-repair machinery to suppress recombination between homeologous sequences [9]. Perhaps the most important function of RECQL is to suppress this risky type of recombination in vertebrates.

Hu *et al.* [11] recently provided new insights into the biological functions of RECQL5. Previous work by the Luo group showed that *Recql5*^{-/-} mutant mouse cells, like *Blm*^{-/-} cells, have elevated frequencies of crossing-over [10]. They now show that *Recql5*^{-/-} knockout mice are also highly cancer prone [11]. The functions of RECQL5 and BLM are clearly not redundant, however, as epistasis analysis demonstrates that *Recql5* and *Blm* function nonredundantly to suppress crossing-over [10]. Striking differences between RECQL5- and BLM-deficient cells provide further evidence for distinct cellular functions of these two helicases.

First, LOH is not elevated in *Recql5*^{-/-} ES cells, whereas LOH increases by at least 10-fold in *Blm*^{-/-} cells. This result is perplexing given the increased crossing-over detected in *Recql5*^{-/-} cells and raises the question of why *Recql5*^{-/-} cells are cancer prone? A possible explanation is suggested by the response of *Recql5*^{-/-} cells to camptothecin (CPT), an inhibitor of topoisomerase I that induces fork stalling and breakage; a ~10-fold induction of chromosomal aberrations is detected, including chromosome breaks and fusions, multiradial structures (indicative of interchromosomal crossing-over) and complex rearrangements. A similar effect of CPT on chromosomal stability was not detected in *Blm*^{-/-} cells. Thus, the cancer susceptibility of *Recql5*^{-/-} mice may be primarily due to oncogene activation caused by chromosomal rearrangements. This idea raises the testable prediction that the tumors that develop in *Recql5*^{-/-} and *Blm*^{-/-} mice will have distinct underlying etiologies. Furthermore, *Recql5*^{-/-} and *Blm*^{-/-} mice might be expected to develop distinct types of tumor. The prominence of lung

adenocarcinomas in *Recql5*^{-/-} mice hints that this could be the case.

Consistent with the inference that RECQL5 regulates recombination, the frequency and lifespan of Rad51 immunostaining foci is increased in *Recql5*^{-/-} cells, indicating an abnormal accumulation and/or persistence of recombination intermediates in these cells. Revealingly, the fraction of DSBs that are repaired via homologous recombination is increased in *Recql5*^{-/-} cells. Taken together, these data suggest that RECQL5 acts to prevent “unscheduled” recombination. Moreover, the additional lesions that are channeled into the recombination pathway in *Recql5*^{-/-} cells appear to be repaired abnormally.

The relationship between murine *Recql5* and *Blm* parallels that of the Srs2 and Sgs1 helicases, two key regulators of recombination in budding yeast. Sgs1 appears to be structurally and functionally analogous to BLM. The unrelated Srs2, an SF-I superfamily helicase, prevents unscheduled recombination at replication forks by displacing Rad51 from single-stranded DNA [12]. This antirecombination effect is mediated by an interaction between Srs2 and the replicative clamp, PCNA. Extending the analogy with Srs2, Hu *et al.* [11] showed that human RECQL5 similarly inhibits Rad51 from catalyzing joint molecule formation by displacing it from single-stranded DNA. Moreover, RECQL5 also interacts with PCNA and localizes to replication forks. Is RECQL5 the vertebrate ortholog of yeast Srs2? This seems unlikely as vertebrate genomes encode an additional helicase, FBH1, which may be structurally and functionally more closely equivalent to Srs2 than is RECQL5 [13].

Accompanying the study of Hu *et al.* [11], an investigation by Bugreev *et al.* [15] demonstrated that human BLM is also capable of disrupting Rad51 filaments *in vitro*. Thus, at least three DNA helicases, BLM, RECQL5 and Srs2 (and presumably vertebrate FBH1) may negatively regulate recom-

ination by disrupting Rad51 filament formation (note that RECQL and WRN cannot catalyze this reaction). Differences between the activities of these three helicases argue against simple redundancy. Srs2 can disrupt filaments comprised of ssDNA and either yeast Rad51, human Rad51, or even *E. coli* RecA indicating a general displacement activity. However, Srs2 does not dissociate Rad51-coated strand-exchange products (joint molecules), in which the Rad51 is now bound to the double-stranded (ds) product of strand-exchange. Consistently, Srs2 cannot disrupt Rad51 filaments assembled onto linear dsDNA [14]. In contrast, human BLM can disrupt Rad51-coated joint molecules although it is unclear whether this activity involves direct dissociation of dsDNA-Rad51 complexes [15]. Also, BLM can only disrupt cognate filaments of human Rad51, suggesting the need for a specific BLM-Rad51 protein-protein interaction; filaments comprised of yeast Rad51 or human Dmc1, a Rad51 homolog, are not disrupted. Moreover, disruption by BLM only occurs when Rad51 filaments are in the inactive, ADP-bound state. Finally, experiments using ATPase defective Rad51 protein suggest that RECQL5 can, distinctively, displace active ATP-bound Rad51-filaments [11].

As the complexity of the RecQ helicases unfolds, what can we surmise about the relationships between the five vertebrate RecQ proteins? First, differences between their structures and biochemical activities, and the distinct phenotypes of human and mouse mutants argue against substantial redundancy. It

is notable, however, that BLM appears to have some redundancy with most or all of the other RecQ homologs. We can suggest that the primordial eukaryotic RecQ helicase evolved the plethora of activities that can be demonstrated for human BLM and yeast Sgs1 proteins. From this “Jack-of-all-trades”, via gene amplification, mutation and selection, specific activities may have been parceled-out to produce a set of “master-tradesmen”, dedicated to one or a few activities. Ultimately, this division of RecQ labor may have enabled metazoans to exert finer spatial and temporal regulation over specific RecQ activities.

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