Understanding the roles of RecQ helicases in the maintenance of genome integrity and suppression of tumorigenesis

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Abstract
RecQ helicases are evolutionarily conserved enzymes required for the maintenance of genome stability. Mutations in three of the five known human RecQ helicase genes cause distinct clinical disorders that are characterized by genome instability and cancer predisposition. Recent studies have begun to reveal the cellular roles of RecQ helicases and how these enzymes may prevent tumorigenesis at the molecular level.

The RecQ family of DNA helicases has received considerable interest due to their putative roles in suppression of cancer and premature ageing in humans [1]. Mutations in three different human RecQ helicase genes cause BS (Bloom’s syndrome; BLM), Werner’s syndrome (WRN) and Rothmund–Thomson syndrome (RECQ4). All of these disorders show genomic instability associated with cancer predisposition. Additionally, individuals with Werner’s syndrome or Rothmund–Thomson syndrome show features resembling premature ageing. We primarily focus on BLM, the gene mutated in BS. Since BS patients are susceptible to the full range of cancers typically observed in the normal population, a clearer understanding of the role of BLM may provide an insight into cellular processes that exist to prevent cells becoming neoplastic.

Role of the BLM protein
Previous studies have suggested that BLM is involved in HR (homologous recombination), a process that is conserved in all species from bacteria to man. By copying the DNA sequence from a homologous non-damaged template, HR is required for the repair of double-strand breaks and single-stranded gaps that may arise from damaged replication forks and/or discontinuities in DNA replication (Figure 1).

We have reported previously that BLM interacts with, and stimulates the activity of, hTOPOIIIα, a type IA topoisomerase [2]. Type IA topoisomerases are evolutionarily conserved enzymes that catalyse strand passage of DNA molecules and have also been implicated in HR. We have recently demonstrated that recombinant BLM-hTOPOIIIα act together to resolve a synthetic DNA substrate containing a DHJ (double-Holliday junction) structure [3]. This type of structure is considered to be a key intermediate formed during HR repair (Figure 1). One way that DHJs can be resolved is by symmetrical endonucleolytic cleavage of the individual junctions followed by rejoining of the DNA molecules (Figure 1B). When the two junctions of a DHJ are resolved in opposite orientations, ‘crossing over’ (reciprocal exchange of genetic information) occurs.

However, we reported that BLM-hTOPOIIIα catalyses a novel reaction, termed ‘DHJ dissolution’, which resolves DHJs without any crossing over of genetic material [3]. More specifically, we propose that BLM catalyses branch migration of DHJs, producing a hemicatenane intermediate which hTOPOIIIα resolves (Figure 1C). This finding may also explain the high SCE (sister chromatid exchange) frequency that is the diagnostic feature of BS, since recombination intermediates may be processed by an alternative pathway(s) in BS cells resulting in elevated levels of crossing over.

Studies on RecQ helicases in yeast
In Saccharomyces cerevisiae (budding yeast), Sgs1, the sole RecQ helicase in this organism, is closely associated with Top3, the sole type IA topoisomerase [4]. Furthermore, Sgs1 and Top3 have been recently demonstrated to act in the same pathway to suppress crossing over during HR repair of a DNA double-strand break [5]. This probably occurs by a mechanism similar to that discussed above for BLM-hTOPOIIIα, with Sgs1-Top3 catalysing DHJ dissolution. Further evidence for Sgs1 in preventing crossovers also comes from the observations that Sgs1 localizes to chromosomal sites of crossing over in meiosis and that sgs1 cells display an increase in meiotic crossovers [6].

Interestingly, mutation of another (unrelated) DNA helicase, SSR2, also causes an increase in crossing over [5]. However, genetic and kinetics analyses revealed that Srs2 and Sgs1-Top3 act in two different pathways to prevent crossovers. Srs2 has been demonstrated to displace Rad51 (which mediates strand invasion into a homologous sequence).
from DNA [7,8]. Whereas Sgs1-Top3 probably resolves DHJs formed by HR, Srs2 probably prevents HR at an early stage and promotes an alternative repair pathway (Figure 1A). Since simultaneous inactivation of both SGS1 and SRS2 is lethal, these helicases probably process recombination intermediates that spontaneously arise during DNA replication [9]. Together, they probably maintain genome integrity by co-ordinating HR with DNA replication and cell-cycle progression.

No obvious human homologue of SRS2 has yet been identified, but a homologue exists in S. pombe (fission yeast) [10]. If a situation analogous to that in yeast also exists in human cells, mutations in RecQ helicases (or a human SRS2 homologue) could have more severe effects under conditions where the parallel pathway is limiting. Interestingly, we find that overexpression of SGS1 can suppress some phenotypes of the srs2 mutant [11]. Our ongoing studies are attempting to investigate further the mechanistic basis for this genetic interaction and elucidate how cells ‘choose’ which particular repair pathway to utilize. Additionally, we are using two-dimensional gel electrophoresis to identify DNA structural intermediates formed during DNA replication in sgs1, srs2 and top3 cells.

**Physiological relevance of suppressing crossing over**

Recent studies have implicated RecQ helicases in suppression of crossing over and provided an explanation for the high SCE frequency that is the diagnostic feature of BS cells. Although (equal) SCEs by themselves are non-mutagenic and therefore unlikely to be harmful, this may not hold true for aberrant recombination events that occur between homologous chromosomes or repetitive sequences at ectopic sites. The resultant reciprocal translocations or loss of heterozygosity may contribute to the genomic instability seen in many cancer cells. Indeed, such genomic alterations have been observed in BLM-deficient mice [12].

Despite these recent advances, much still remains to be discovered about the physiological roles of RecQ helicases. Further studies in this exciting field will undoubtedly improve our understanding of the molecular basis of tumorigenesis and, possibly, extend the range of potential therapeutic targets.

**References**


Received 2 July 2004