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Role of the Bloom's syndrome helicase in maintenance of genome stability

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Abstract

The RecQ family of DNA helicases has members in all organisms analysed. In humans, defects in three family members are associated with disease conditions: BLM is defective in Bloom's syndrome, WRN in Werner's syndrome and RTS in Rothmund-Thomson syndrome. In each case, cells from affected individuals show inherent genomic instability. The focus of our work is the Bloom's syndrome gene and its product, BLM. Here, we review the latest information concerning the roles of BLM in the maintenance of genome integrity.

Background

The maintenance of chromosome stability is essential for viability in all organisms. In mammals,

a breakdown in genome maintenance is associated with both tumorigenesis and cancer progression (reviewed in [1]). Cells have, therefore, evolved a variety of different mechanisms for ensuring that the integrity of the genome is maintained as far as is possible, given the many and varied 'insults' to which the genome is subjected. One group of proteins that plays a crucial role in the maintenance of genome stability is the RecQ family, named after the *Escherichia coli* RecQ protein, which has multiple members in human cells (reviewed in [2]). One of these human RecQ family proteins, BLM [3], is defective in the rare disorder Bloom's syndrome (BS), which is associated with excessive genomic instability and an enormously elevated incidence of cancers (reviewed in [4]). The biochemical properties and putative cellular roles of BLM will be the subject of this paper.

BLM and other RecQ family proteins

The RecQ family is represented in all organisms that have been analysed. In the commonly used laboratory organisms *E. coli*, *Saccharomyces cere-*

Key words: cancer, genetic recombination, RecQ helicase, topoisomerase.

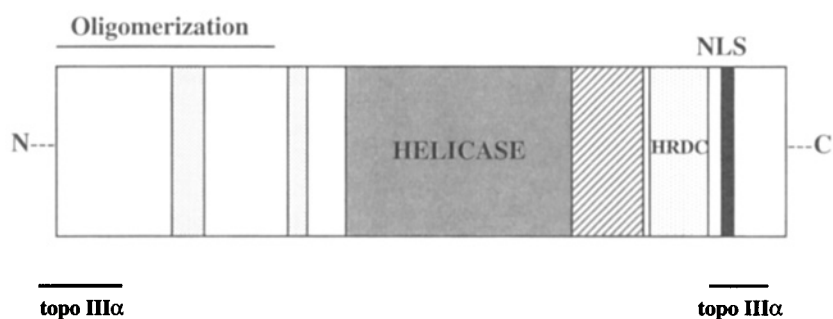
Abbreviations used: BS, Bloom's syndrome; RPA, replication protein A.

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Figure 1

Schematic representation of the domain structure of the BLM protein

The central helicase domain, containing seven conserved motifs, is labelled and shown in grey. The N-terminal domain contains two regions with blocks of acidic residues, shown as stippled boxes. The C-terminal domain contains a region shown with cross-hatching, adjacent to the helicase domain, which shows limited identity with a domain in the other RecQ helicases. Adjacent to this is the HRDC (helicase and RNase D C-terminal) domain, shown with light stippling, which is a putative nucleic acid binding motif. The nuclear localization signal (NLS) sequence in the C-terminal domain is shown by a black vertical bar. The N-terminal 431 amino acids have been shown by Beresten et al. [29] to oligomerize *in vitro*. This domain is indicated by the upper horizontal bar marked 'oligomerization'. The two regions near the N- and C-termini of BLM that mediate interactions with topoisomerase III α are indicated by lower horizontal lines.



visiae and *Schizosaccharomyces pombe*, there is a single representative: RecQ, Sgs1p and Rqh1p respectively. In higher eukaryotes there are multiple members, with at least five in humans, designated BLM, WRN, RECQL, RECQ4 and RECQ5 (reviewed in [2,5,6]). In recent years there has been a tremendous upsurge in interest in this family following the realization that defects in RecQ proteins give rise to at least three clinically distinct disorders. As mentioned above, BLM is defective in BS, while WRN is defective in Werner's syndrome [7] and RECQ4 is defective in Rothmund-Thomson syndrome [8]. The main feature of Werner's syndrome is the development at an abnormally early age of many of the manifestations of the normal aging process, including loss and greying of the hair, formation of cataracts, loss of subcutaneous fat and osteoporosis (reviewed in [9]). Rothmund-Thomson syndrome is associated with skin and skeletal abnormalities, but the cellular phenotype has not been characterized extensively [10]. All of these disorders are associated with a predisposition to cancer, although this is a particularly striking feature of BS. Indeed, BS is unique among cancer predisposition syndromes in that individuals with this disorder show an increased incidence of all (or nearly all) types of cancers, including leukaemias, lymphomas and the common solid epithelial cancers. Other features of this disorder include pro-

portional dwarfism, sunlight sensitivity, immunodeficiency and reduced fertility [4].

All RecQ family proteins include a domain of approx. 450 amino acids that contains seven highly conserved motifs, including a 'Walker A-box' ATP-binding motif and a DExH box which is a characteristic of this family (Figure 1). These motifs are found in several DNA and RNA helicases. As discussed below, RecQ family proteins are 3'-5' DNA helicases. In some cases, such as with RecQ and RECQL, the protein comprises little more than this 'helicase' domain. For other family members, including Sgs1p, Rqh1p, BLM, WRN and RECQ4, long N- and C-terminal domains are also present [2]. The possible functional roles of these domains are discussed below.

Phenotype of BS cells

BS cells show several manifestations of genetic instability. The hallmark feature, which is used in the diagnosis of the disorder, is an approx. 10-fold elevation in the frequency of sister chromatid exchanges [4]. These apparently reciprocal exchanges between sister chromatids have been shown to represent homologous recombination events [11]. BS cells also show a mutator phenotype and an increased accumulation of quadriradial chromosomes and other chromosomal aberrations, which appear to signify a failure to

suppress recombination events occurring between homologous chromosomes. BS cells show abnormalities in DNA replication, but these have not been characterized in detail. There are reports of the accumulation of aberrant replication intermediates in BS cells, and of a retarded rate of replication fork progression [12].

Biochemical properties of BLM

BLM has been purified in recombinant form from budding yeast and shown to be a DNA helicase that translocates along DNA in the 3' → 5' direction [13]. The active form of BLM is oligomeric, with a molecular mass of approx. 850 kDa. Consistent with this, BLM appears to form oligomeric rings, most of which are hexameric [14]. The BLM helicase can unwind a wide variety of structurally diverse DNA substrates. However, the substrate specificity of BLM is rather atypical for a DNA helicase, which may provide clues as to the cellular function of the enzyme. BLM does not unwind or even bind to blunt-ended, fully duplex DNA molecules [15]. It can unwind partial duplex molecules if these either contain a single-stranded 3' tail or form a forked structure. Nevertheless, BLM can unwind certain blunt-ended duplexes. In particular, it has been shown that the insertion of a 12 bp 'bubble' in the centre of an otherwise duplex molecule creates a good substrate for unwinding by BLM (P. Mohaghegh and I. D. Hickson, unpublished work). Moreover, BLM can unwind a synthetic X-structure, comprising four blunt-ended arms which are a model for the Holliday junction recombination intermediate (see below). Given that these X-structures are not thought to contain any single-stranded DNA under the conditions used for unwinding (in the presence of Mg²⁺), it may be that BLM effects DNA substrate recognition through the recognition of particular DNA structures and not the mere presence of single-stranded DNA. Consistent with an ability to disrupt X-structures, recent data indicate that BLM can promote the ATP-dependent branch migration of Holliday junctions *in vitro* [15]. It has been proposed that this activity, which is conserved in WRN [16], is involved in the suppression of hyper-recombination at sites of blocked replication forks. For models of BLM function during replication, see [2,6].

Another good substrate for BLM, and indeed for other RecQ helicases, is G-quadruplex DNA [17]. It may be significant that other classes of helicase cannot unwind this structure. G-quadruplex DNA is an unusual form of DNA that can

form (at least *in vitro*) within G-rich stretches of DNA, and comprises a square planer structure stabilized by Hoogsteen base pairing. G-DNA is significantly more thermostable than a corresponding B-DNA structure of the same sequence. Sequences that can form G-DNA *in vitro* are present in the G-rich strand of human telomeric DNA, within ribosomal DNA, and at certain other genetic loci (reviewed in [18]). One potential role for BLM and/or other RecQ family helicases is, therefore, to disrupt G-DNA structures as and when they form in cellular DNA, to permit DNA metabolism to occur. For example, during DNA replication, G-DNA might form in the single-stranded DNA at the fork, and need to be disrupted to prevent fork blockade or chromosomal breakage/deletion.

Interactions between BLM and other cellular proteins

BLM has extended N- and C-terminal domains that are not obviously conserved in other RecQ family helicases [2,3]. It is likely, therefore, that these domains impart properties that distinguish the functions of BLM from those of other family members. One probable role of these extended domains is to direct interactions with other nuclear proteins. We have studied in detail two protein partners for BLM, both of which are likely to be relevant to the functions of the enzyme *in vivo*. One partner, replication protein A (RPA), is the major binding protein for single-stranded DNA in human cells, with roles in DNA replication, repair and recombination. The interaction between BLM and RPA is a direct one, as indicated by the fact that purified BLM and RPA form a complex *in vitro* [19]. The interaction with BLM is mediated by the 70 kDa subunit of the heterotrimeric RPA protein. The interaction has been shown to be a functional one, with RPA increasing the processivity of the BLM helicase reaction, permitting BLM to unwind longer substrates (≥ 259 bp) than it is capable of doing alone. This stimulation of BLM helicase activity is not a consequence solely of binding of single-stranded DNA by RPA, since heterologous single-stranded-DNA-binding proteins do not stimulate BLM.

A second binding partner for BLM is topoisomerase III α , a so-called type IA topoisomerase. This interaction is conserved in evolution; for example, Sgs1p and Top3p form a complex in budding yeast [20]. Moreover, the genes encoding Sgs1p and Top3p in budding yeast, and Rqh1p

and Top3p in fission yeast, show a similar genetic interaction, in that the defects seen in *top3Δ* mutants are in each case suppressed by mutation of the corresponding RecQ helicase gene [20–22]. Yet another connection between RecQ helicases and topoisomerase III comes from the finding that RECQ5 interacts with both topoisomerase III α and III β , the two known isoforms of topoisomerase III in human cells [23]. The interaction between BLM and topoisomerase III α is direct and is mediated by residues present in both the N- and C-terminal domains of BLM [24]. In the absence of BLM, in BS cells, topoisomerase III α is mis-localized in the nucleus, opening up the possibility that defects in topoisomerase III α function might underlie at least some of the phenotypes of BS cells [24]. The precise role of the BLM–topoisomerase III α complex is not clear at this stage, but Harmon et al. [25] have shown that, together, *E. coli* RecQ and topoisomerase III α can catalyse the catenation of plasmid DNA. It has been suggested that such an activity may be involved in resolving aberrant recombination intermediates. For a more detailed discussion of this and other roles of this helicase–topoisomerase complex, see Wu et al. [26].

Subcellular localization of BLM

Using antibodies that are specific for BLM, several groups have analysed the subcellular distribution of BLM in human cells. There is general agreement that the bulk of BLM protein is found in specific nucleoplasmic foci, where it co-localizes with topoisomerase III α [24]. These foci correspond to one class of previously characterized nuclear structure, termed PML nuclear bodies, the functional role of which is currently uncertain [27]. BLM is also found at sites of ongoing DNA replication, at least under certain circumstances, and in nucleoli [6,28]. Whether BLM translocates from site to site to effect its functions is not yet clear.

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