BLM DNA Helicase Protects Against (CAG)•(CTG) Repeat Instability

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Summary

Gene-specific (CAG)•(CTG) repeat tract expansions, which are associated with 16 neuromuscular/neurodegenerative diseases, can give rise to slipped-DNA structures. These slipped-DNAs have previously been observed in expanded (CAG)•(CTG) repeat tracts of the myotonic dystrophy locus in patient tissues[1]. **Ongoing repeat expansions are thought to be driven by aberrant DNA repair of these slipped-DNA structures**.

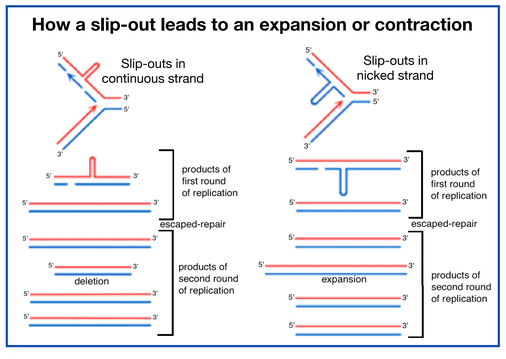
Bloom (BLM) protein is critical to regulation of sister chromatid exchange during DNA replication and repair via its DNA helicase and DNA annealing activities. **BLM's branch migration activity at three- and four-way DNA junctions suggests a possible role for BLM in unwinding slipped-DNAs**.

We assessed the effect of BLM on *in vitro* repair, and observed that **BLM is required for repair of a single (CTG) slip-out, but not for $(CAG)_{20}$**. Further, the helicase domain of BLM, but not annealing domain, is required for slip-out repair.

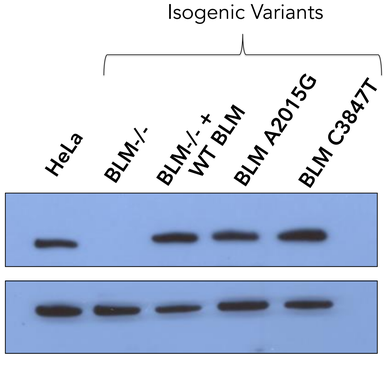
We also examined the effect of BLM on CTG/CAG replication *in vitro*. **The absence of BLM results in reduced DNA replication efficiency when (CAG) is the lagging strand template.** A significant **increase in the frequency of unprocessed slipped-DNAs when (CTG), but not (CAG), is the lagging strand template** was seen. Complementation with **functional BLM extract partially rescued slipped-DNA processing**.

Slipped-DNAs and repeat instability

Slipped-DNAs can involve excess repeats of either the CTG or CAG strand, over various lengths ranging from a single extra repeat to many repeats. Slip-outs in the template strand can be synthesized across by DNA polymerase, leading to contraction products. Slip-outs formed in the nascent strand can lead to expansions.

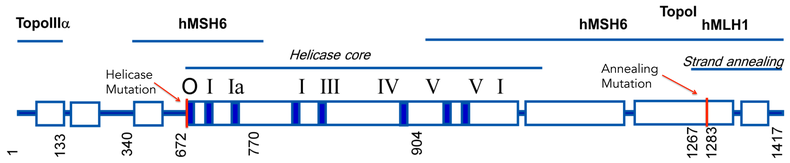


BLM expression in cell extracts



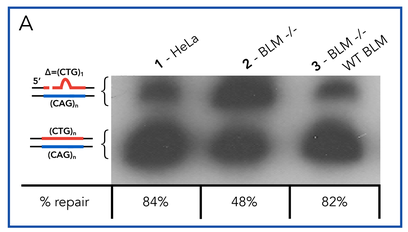
BLM helicase detected by polyclonal anti-BLM ~170kDa (predicted 159kDa). **BLM-/- cell extract derived from patient GM08505, who is homozygous for a BLM truncating mutation, leading to low-to-no functional protein expression.** Point mutations in the helicase and annealing domains (c.2015A>G and c.3847C>T, respectively) do not appear to affect BLM expression.

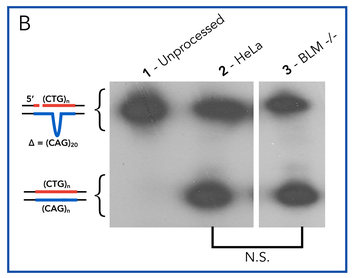
BLM gene functional domains



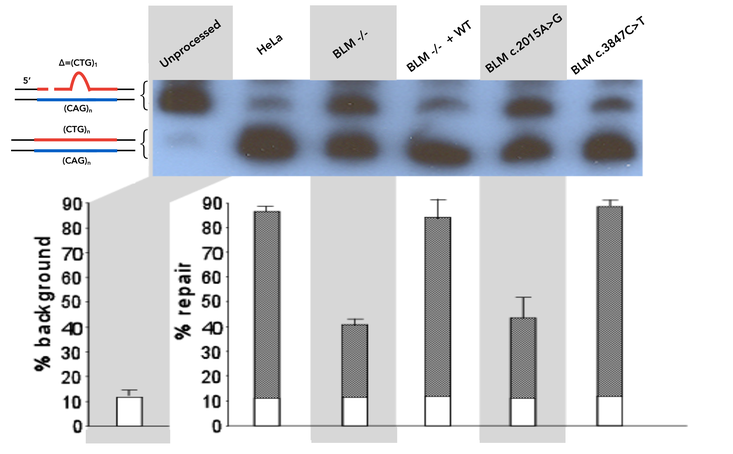
The helicase and annealing domain mutations are both associated with BLM syndrome. Helicase domain mutation c.2015A>G (Q672R) is a naturally-occurring missense mutation allowing for normal DNA binding but impaired ATPase activity. Annealing domain mutation c.3847C>T (Q1283X) introduces a pre-mature stop codon, impairing ability for BLM to anneal DNA strands.

BLM is required for *in vitro* DNA repair of a single (CTG) slip-out





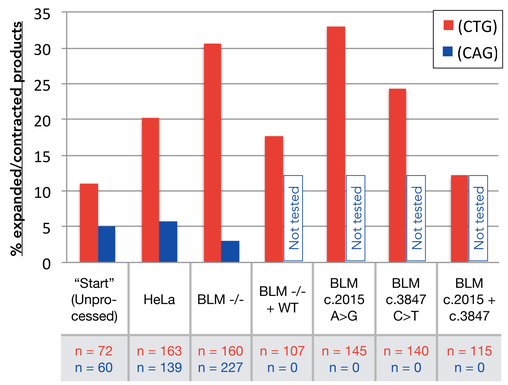
Nick-directed *in vitro* repair of plasmids containing pre-formed DNA slip-outs with an excess of $(CTG)_1$ or $(CAG)_{20}$. A single (CTG) repeat mismatch is processed to a linear substrate in the presence of functional BLM (panel A, lanes 1 and 3, 84% and 82% repair, respectively). In the absence of functional BLM, repair processing is markedly less efficient (48%). DNA containing a $(CAG)_{20}$ repeat mismatch is similarly repaired in the presence and absence of functional BLM (panel B, lanes 2 and 3).

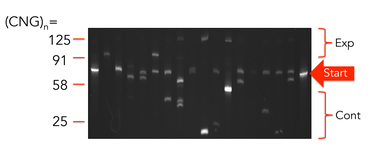


Point mutations known to render the helicase or annealing domains of BLM non-functional were induced[2,3]. Cell extracts made from these mutated BLM cell lines were tested for *in vitro* repair efficiency with single (CTG) repeat slip-out substrates. **Mutating BLM's helicase core notably impairs repair of single-(CTG) slip-outs, while the annealing domain does not decrease repair efficiency, suggesting functional redundancy for the annealing role of BLM.**

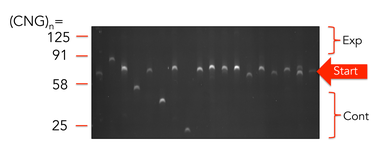
$(CAG)_n$•$(CTG)_n$ length changes increase following *in vitro* replication in the absence of BLM

DNA plasmids containing 79 paired (CAG)•(CTG) repeats underwent *in vitro* replication and repeat-length analysis. **In the absence of BLM, high levels of repeat expansions and contractions are observed when (CTG) is the lagging strand template.** This can be rescued by the addition of functional BLM cell extract. **The helicase-null mutant extract also results in a significant increase in repeat length changes following *in vitro* replication**, while the annealing mutant does not.



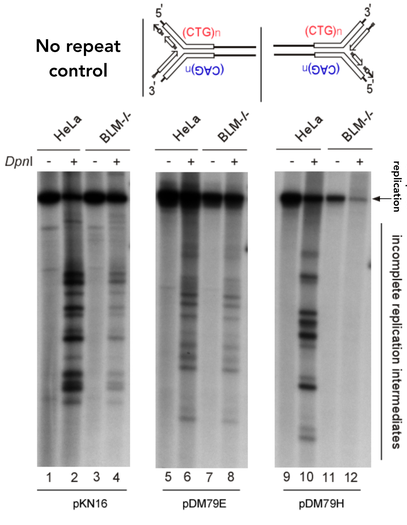


(CAG)79•(CTG)79 DNA replicated in BLM-/- cell extract.



(CAG)79•(CTG)79 DNA replicated in BLM-/- + WT cell extract.

How does lagging strand template sequence affect replication efficiency?



Via radioactive incorporation during *in vitro* replication, we observed that **in the presence or absence of functional BLM**, DNA plasmids containing $(CAG)_{79}$•$(CTG)_{79}$ were **efficiently replicated when (CTG) was the lagging strand template, however replication was notably impaired when (CAG) was the lagging strand template.** These results support the low frequency of repeat length changes observed when (CAG) is the lagging strand template for replication. Replication fork stalling/collapse in the absence of BLM may be involved, however further study is needed to understand this observation.

References & Acknowledgements

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