Cell-cycle-regulated interaction of the Bloom syndrome helicase with Mcm6 controls DNA replication speed and is essential for the DNA-damage response.

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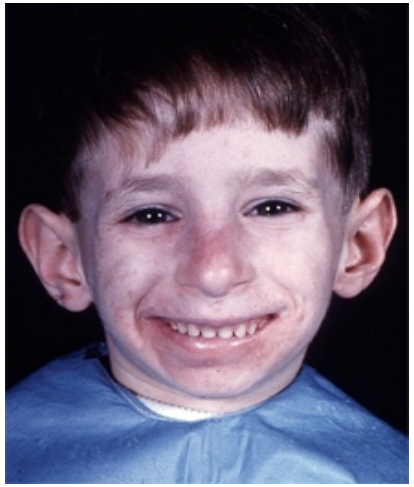
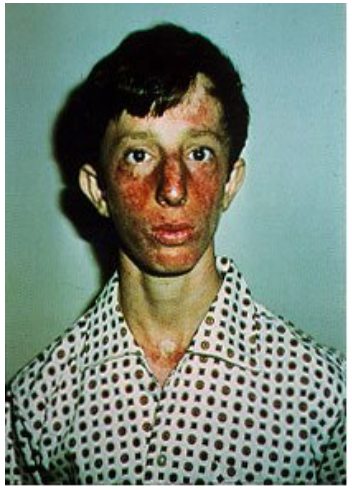
Bloom syndrome

- Rare genetic disorder associated with extreme cancer predisposition
- Affected individuals have two inactive copies of the *BLM* gene which encodes for a DNA helicase belonging to the RecQ helicase family.
- One of the best characterized function of BLM is in the repair of DNA double-strand-breaks by homologous recombination.



German *et al.* 2007



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Potential role of BLM during replication?

A preliminary proteomic screen conducted identified BLM to interact with the Mcm6 subunit of the replicative MCM helicase.

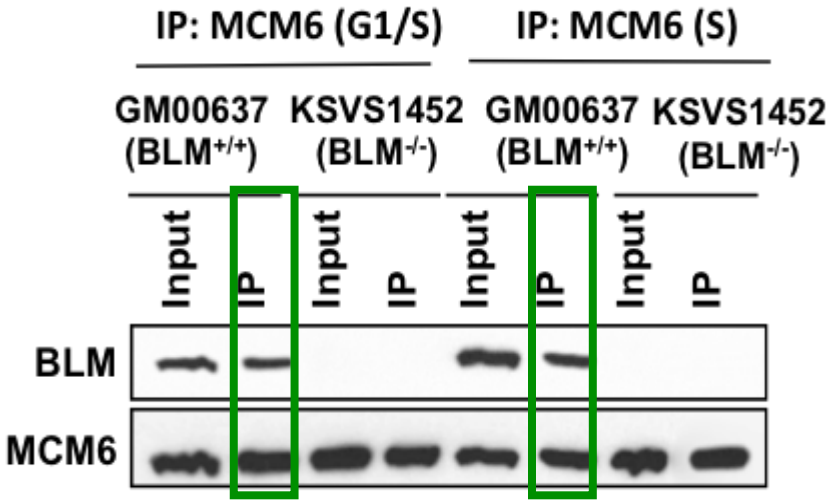

Fig 1: Immunoprecipitation of endogenous MCM6 performed in GM00637 (*BLM*$^{+/+}$) and KSVS1452 (*BLM*^{KO}) cell lines at G1/S and mid S-phase. Cell lines GM00637 and KSVS1452 are an isogenic pair of *BLM*$^{+/+}$ and a CRISPR-mediated BLM knockout cell line respectively.

<u>Hypothesis</u>: BLM regulates DNA replication through its interaction with Mcm6, and loss of this regulatory arrangement leads to replication defects, increased DNA damage and genome instability

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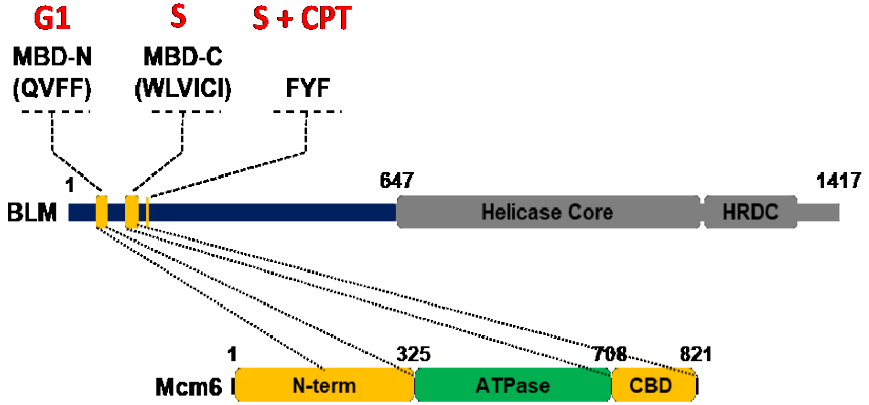
BLM uses different binding sites to associate with Mcm6 in unperturbed G1, S-phase and after DNA damage

Using a combination of mammalian two-hybrid assay, co-immunoprecipitation, fluorescence microscopy we determined that BLM uses distinct binding sites to associate with Mcm6 in a cell-cycle specific manner



BLM mutant	Mcm6 binding in		
	G1	S	S + CPT
Blm	✓	✓	✓
Blm KO	✗	✗	✗
Blm-QVFF (MBD-N)	✗	✓	✓
Blm-WLVICI (MBD-C)	✓	✗	✗
Blm-FYF (MBD-D)	✓	✓	✗
Blm-QVFF-WLVICI	✗	✗	✗
Blm-QVFF-FYF	✗	✗	✗

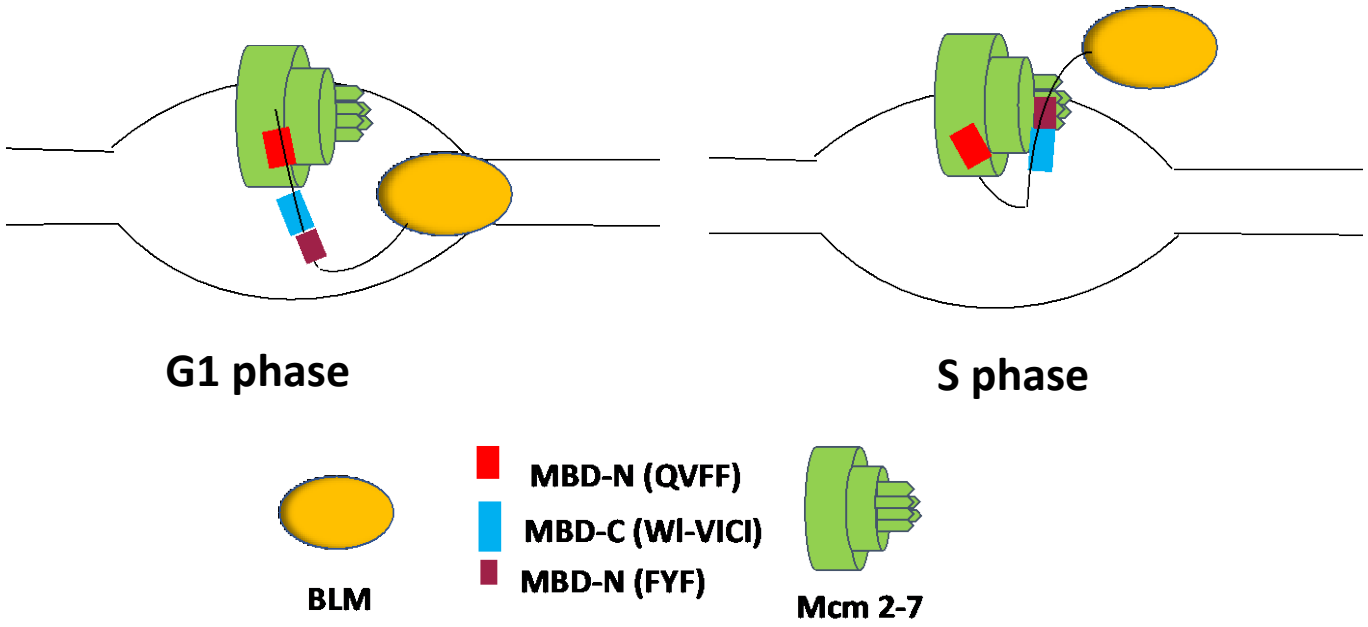


Fig 2:Schematic showing binding sites in BLM that facilitate binding to Mcm6 in G1, in unperturbed S-phase and after DNA damage

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.Disruption of Mcm6-binding to BLM in S-phase causes high DNA replication speed in unperturbed cells

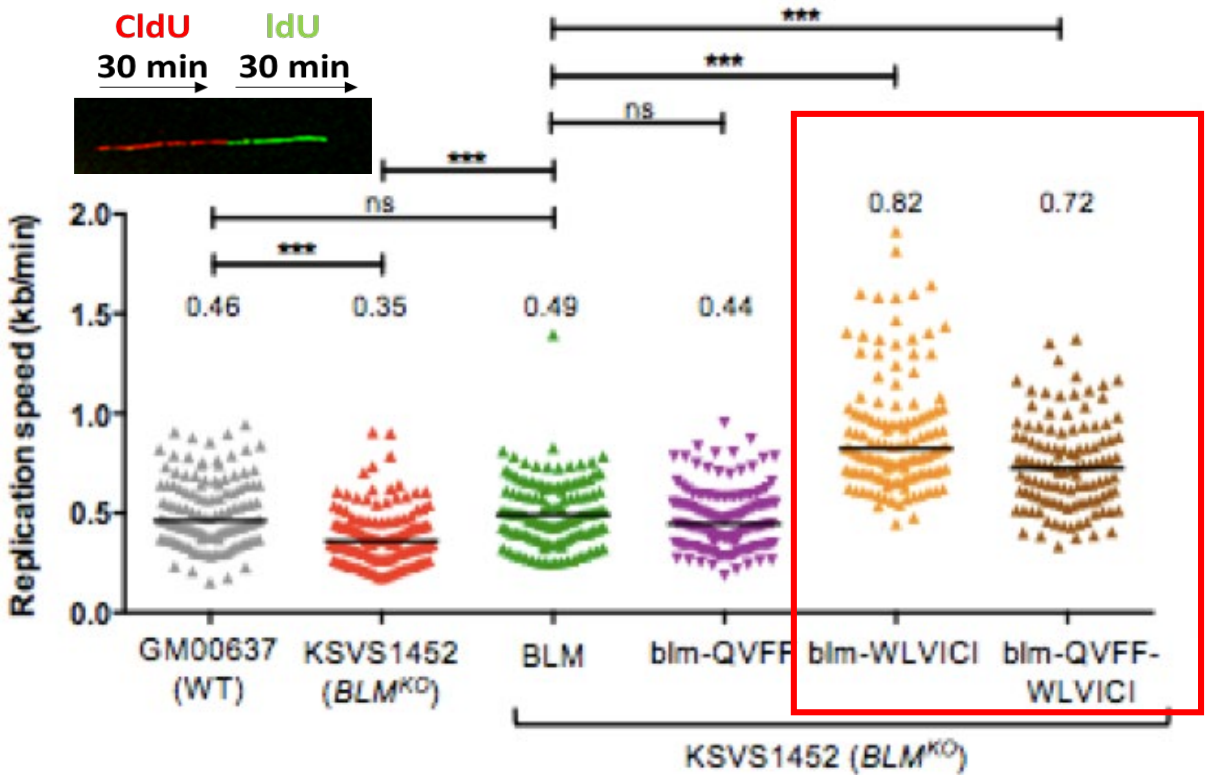

Fig 3: DNA fiber analysis demonstrating replication speed in cells lacking BLM/Mcm6 interaction

BLM not only plays a role in the response to DNA damage and replication stress, but its physical interaction with Mcm6 is needed in unperturbed cells, most notably in S-phase as a negative regulator of replication speed.

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