Functional Analysis of Bloom Syndrome Helicase in Development and DNA Repair

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Overview

- Bloom syndrome helicase (*Blm*) facilitates rapid embryonic cell cycles, meiotic chromosome segregation, and homology directed repair of DNA[1,2,3,4]
- *Blm* mutants cause chromosomal abnormalities, loss of heterozygosity (LOH), and genome instability[1,2,3,4]
- I deleted *Blm* regions using CRISPR[5] to test functional significance
- Deletion of amino acids (aa) 576-720 caused significant effects in:
  - Meiotic chromosome segregation (via increased nondisjunction [NDJ])
  - Mitotic crossover (CO) prevalence
  - DNA repair pathway choice
  - Embryonic viability
- This suggests this region is critical to Blm function, possibly via regulation by ATR or ATM kinase (*mei-41/tefu*)

Methods

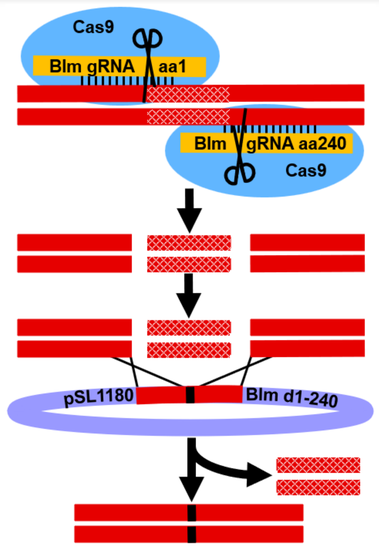

Figure 3. Using CRISPR for *in vivo Blm* deletions. Cas9/gRNA cuts DNA, resulting in deletion by using a supplied template for repair[5]. Deletion of aa 1-240 *Blm* is shown.

♀ *TM3 / TM6B, Hu Tb* ⊗ ♂ *nosCas9 / +* ; *[1-240] / +*

TM3 / TM6B, Hu Tb ⊗ *[d1-240] / TM6B, Hu Tb*

d1-240 / TM6B, Hu Tb ⊗ *d1-240 / TM6B, Hu Tb*

Figure 4. Crosses to recover desired CRISPR deletion (*Blm* d1-240 shown).

Deletions then assessed in four experiments:

1. NDJ Assay: Flies scored using *Bar*⁻ phenotype to scan for X chromosome NDJ.
2. CO Assay: Recessive marker exchange(s) in male germline via mitotic CO are scored in progeny.
3. P{w^a} Assay: Eye color of flies mark SDSA versus non-SDSA repair pathways.
4. Hatch Assay: Hatched embryos counted for viability to determine maternal effect lethality.

Future Directions

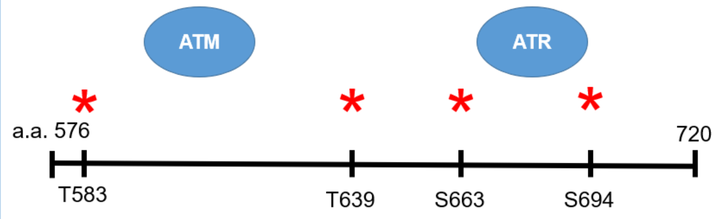

Figure 7. Predicted phosphorylation sites by DNA repair regulatory kinases ATR/ATM (*mei-41/tefu*) within Blm aa 576-720.

- Further examination of aa 576-720 through predicted phosphorylation residue mutations
  - Mutate S/T residues to D (phospho-mimetic) or A (phospho-dead) *in vivo* to evaluate importance of potential phosphorylation
- Characterize C-terminal Blm deletions *in vivo*
- Immunofluorescence to determine Blm regions essential to:
  - Localization to DNA double strand breaks
  - Cell cycle progression roles

Introduction

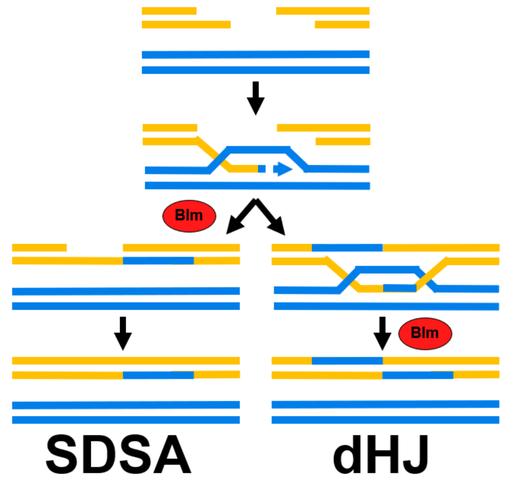

Figure 1. Synthesis Dependent Strand Annealing + double Holliday Junction dissolution DNA repair mechanisms.

- Blm helicase unwinds DNA during DNA repair[1,2,3]
- This prevents mitotic COs[1,4], helps avoid meiotic chromosome NDJ[4], promotes SDSA[4], and aids rapidly replicating embryos by fixing stalled/broken replication forks[1,3,4]
- Improper Blm function can lead to Bloom syndrome and cancer[1]

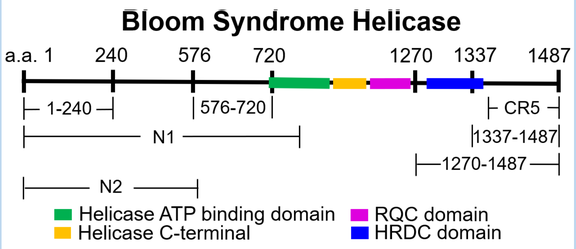

Figure 2. Studied *Blm* fragments, alleles, and functional domains (Flybase). Fragments are conserved Blm regions across *Drosophila* species closely related to *D. melanogaster*.

Results

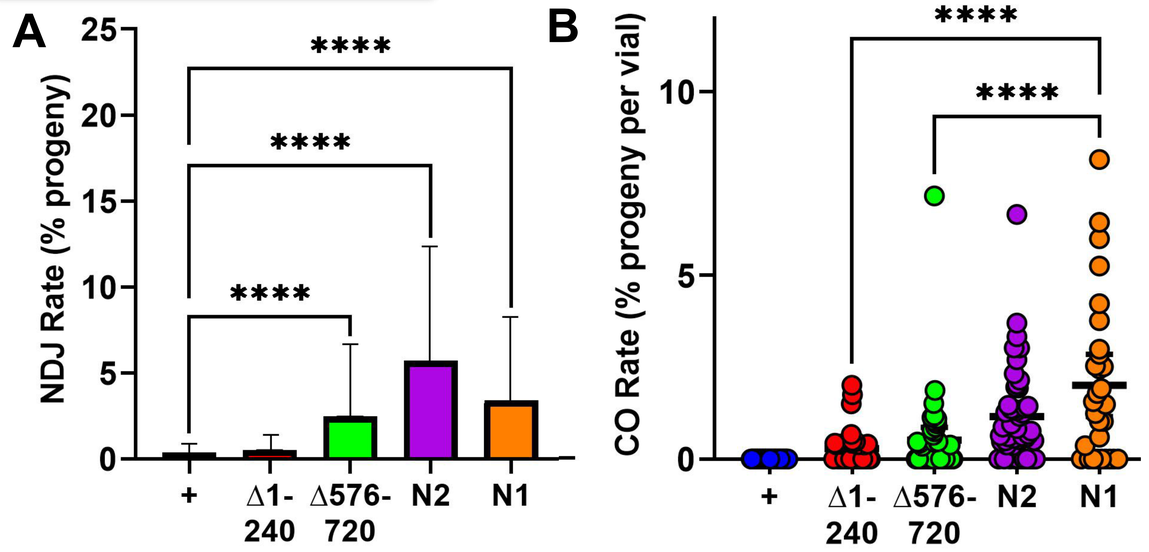

Figure 5. (A) NDJ rate in *wt* and *Blm* deletions over the classic *Blm* N1 null allele[4]. While d1-240 has a NDJ rate close to *wt*, classic *Blm* alleles N1 and N2 and *Blm* d576-720 show increased NDJ rate versus *wt* (*p<0.05, unpaired t-test, each). (B) Male germline mitotic CO rates for *wt* and *Blm* deletions over *Blm* N1 null allele[4]. *Blm* d1-240 and d576-720 have significantly lower CO rates versus Blm alleles N1 and N2 though greater than *wt* (*p<0.05, ANOVA with Tukey's post-hoc).

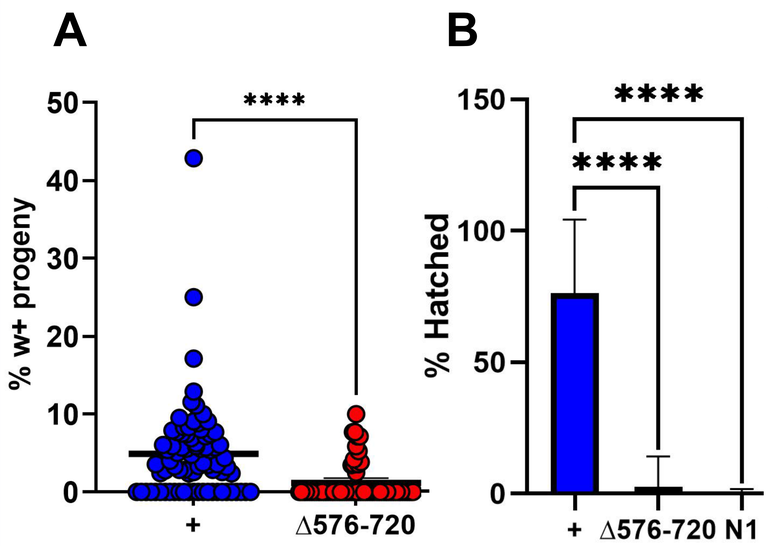

Figure 6. (A) P{w^a} assay data for *wt* and *Blm* d576-720 over *Blm* D2 null allele. *Blm* d576-720 saw significant decrease in SDSA indicating use of end-joining repair pathways (*p<0.05, unpaired t-test). (B) Embryonic hatch assay results. To examine maternal effect lethality, embryo hatching is evaluated. *Blm* N1 null allele[4] and d576-720 cause significantly compromised embryonic development (*p<0.05 vs. *wt*, ANOVA with Tukey's Post Hoc).

Conclusions

- Deletion of Blm aa 576-720 causes:
  - Increased NDJ rates
  - Increased mitotic COs, though not at levels of N1 and N2 null alleles
  - Increased end-joining repair
  - Increased embryonic maternal effect lethality
- Blm aa 576-720 is important to Blm DNA repair and embryonic development functions
- Pinpointing regulatory sites of functional significance will allow us to better examine effects of *Blm* mutations in *Drosophila* and potentially in disease

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Citations

1. Heyer, W. D., Ehmsen, K. T., & Liu, J. (2010). Regulation of homologous recombination in eukaryotes. *Annual review of genetics, 44*, 113–139. https://doi.org/10.1146/annurev-genet-051710-150955
2. Wu, L., Davies, S. L., North, P. S., Goulaouic, H., Riou, J. F., Turley, H., Gatter, K. C., & Hickson, I. D. (2000). The Bloom's syndrome gene product interacts with topoisomerase III. *The Journal of biological chemistry*, 275(13), 9636–9644. https://doi.org/10.1074/jbc.275.13.9636
3. Davies, S., North, P. & Hickson, I. (2007). Role for BLM in replication-fork restart and suppression of origin firing after replicative stress. *Nat Struct Mol Biol*, 14, 677–679. https://doi.org/10.1038/nsmb1267
4. McVey, M., Andersen, S. L., Broze, Y., & Sekelsky, J. (2007). Multiple functions of Drosophila BLM helicase in maintenance of genome stability. Genetics, 176(4), 1979–1992. https://doi.org/10.1534/genetics.106.070052Fields, S., & Song, O. (1989). A novel genetic system to detect protein-protein interactions. *Nature, 340*(6230), 245–246. https://doi.org/10.1038/340245a0
5. Gratz, S. J., Ukken, F. P., Rubinstein, C. D., Thiede, G., Donohue, L. K., Cummings, A. M., & O'Connor-Giles, K. M. (2014). Highly specific and efficient CRISPR/Cas9-catalyzed homology-directed repair in Drosophila. Genetics, 196(4), 961–971. https://doi.org/10.1534/genetics.113.160713