Functional Analysis of Bloom Syndrome Helicase in Development and DNA Repair
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Bloom Syndrome is a rare autosomal recessive disorder in humans caused by mutation of the *BLM* gene that leads to increased genome instability and cancer. The *BLM* gene codes for a helicase (BLM) that works together with Topoisomerase 3-alpha (Top3α) in homology-directed repair of DNA. Top3α directly binds to BLM and helps release the torsional stress on DNA as BLM unwinds recombination intermediates. These proteins preserve genome stability and have been shown in many organisms to operate together in the prevention of detrimental mitotic (non-meiotic) crossovers via two main DNA repair pathways, synthesis-dependent strand annealing and double Holliday junction dissolution. In *Drosophila*, *BLM* (known as *Blm*) also has roles in proper meiotic chromosome segregation and rapid cell cycle progression of the developing embryo. Each of these BLM functions are not well characterized and limit development of treatments for *BLM*-related disorders. To investigate the Blm-Top3α interaction in DNA repair, I performed a yeast 2-hybrid (Y2H) assay using the *Drosophila* genes. I found the interaction was specific to certain regions of Blm, with the strongest interaction observed at a C-terminal region conserved among several *Drosophila* species, amino acids (aa) 1381-1487. Based on this data, I created specific deletions of the *Blm* gene via CRISPR/Cas9 editing to characterize the various roles of Blm *in vivo*. First, I assessed the effects of *Blm* deletions on known *Blm* roles in meiotic chromosome segregation via a nondisjunction assay. Both aa 576-720 and N1 produced measurable defects compared to the *wild type* suggesting functional value of aa 576-720. Significance of this Blm region in preventing mitotic crossovers and DNA repair was evaluated by a crossover assay showcasing a lack of significant effect by aa 576-720 relative to the true null allele, N1, but still increased relative to *wild type*. These studies showcase the importance of aa 576-720 and other Blm regions in the roles of meiotic segregation and DNA repair. Blm aa 576-720 will be further assessed by examining the relevance of predicted ATR/ATM phosphorylation sites within the region required for proper Blm function. Additional Blm roles in embryonic development will also be explored via an embryo hatching assay. By characterizing the functions of Blm in *Drosophila*, we will better understand and improve BLM function within humans and the detrimental health effects associated with *BLM* mutations.

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