

Examination of Protein Partner Interactions with DNA-DNA Helicase BLM

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Bloom's Syndrome (BS) is an autosomal recessive disorder where afflicted individuals have a predisposition towards genomic instability and hyperinutability generally resulting in the development of cancer. The gene associated with BS was identified and designated as the BLM gene in 1995 (Groden/Ellis). BLM is a member of the RecQ family of 3'-5' DNA-DNA helicases.

Given the BLM protein helicase activity it is hypothesized that the BLM protein functions as part of a protein complex. The aim of this research is to identify the protein partners of the BLM protein to aid in an understanding of its functional role in maintaining DNA stability.

The Yeast Two-Hybrid System was used to search for protein partner interactions. The BLM gene was previously split into three pieces, the N' terminal region, the C' terminal region, and the helicase homologous region and inserted into the *pAS2-1* plasmid. This plasmid contained the DNA binding domain of the GAL-4 transcriptional activator. Brain tissue and B cell cDNA libraries cloned into the *pACT2* plasmid were commercially purchased. The *pACT2* plasmid contained the activation domain of the GAL-4 transcriptional activator. We co-transformed each of the *pAS2*-BLM plasmids and the cDNA libraries into the yeast host strain Y190 and plated on selective media. Surviving yeast colonies were restreaked and assayed for beta-galactosidase (beta-gal) activity. Interaction between the two plasmids was detected via a positive beta-gal result. The beta-gal reactive clones underwent a series of tests to eliminate any false positives, with the remaining potential clones being isolated, sequenced, and identified by comparison using GENBANK.

We were unable to detect any protein partner interactions with the N' terminal region of BLM using the Yeast Two-Hybrid System due to the autoactivation of the Gal-4 transcriptional activator by *pAS2-N'*. Unfortunately contamination of a potential C' terminal protein partner prevented its sequencing and subsequent identification. Two potential protein partners for the helicase domain were isolated. These are currently being screened for potential false positive status and have not been identified to date.

The BLM gene produces a DNA-DNA helicase, it is logical that protein partner interactions occur with this product as seen with other helicases. This research provided a preliminary examination of BLM's potential protein partners.

Reference:

Ellis, N.A.; Groden, J.; Ye, T.-Z.; Straughen, J.; Lennon, D.J.; Ciocci, S.; Proytcheva, M.; German, J.: **The Bloom's syndrome gene product is homologous to RecQ helicases.** *Cell* 83: 655-666, 1995.