

**Structural and functional studies on the G3842S  
mutation in the Mixed Lineage Leukemia protein  
(MLL)**

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ARISE 2006

## Abstract

The Mixed Lineage Leukemia (MLL) protein is a histone H3 methyltransferase that methylates lysine 4 on histone H3, which is an epigenetic signal for transcriptional activation (Strahl *et al.* 2000). The genes MLL positively regulates are Hox genes, which are homeotic regulators of development (Wiederschain *et al.* 2003). The Mixed Lineage Leukemia (MLL) protein is mutated in 5 to 10% of children and adults that have acute lymphoblastic leukemia (Canaani *et al.*, 2004). The prognosis of those cancer patients is poor (Pui *et al.*, 2002). The purpose of this study is to provide an understanding of how MLL functions in order to help facilitate the development of new cancer treatments, which could improve the prognosis of individuals with MLL related leukemia. This will be done by studying the Trithorax Z11 (TRX Z11) mutation in MLL. Doing this will show how a critical amino acid affects the function and structure of MLL.

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The trithorax protein is the *Drosophila* homolog of the human MLL protein. Genetic screens in *Drosophila* identified a single amino acid within the trithorax SET domain that when mutated results in lethal developmental defects (Krajewski *et al.* 2004, Katsani *et al.* 2001). The mutation results from the replacement of glycine to serine (Stassen *et al.* 1995, Krajewski *et al.* 2004), which has been shown experimentally to impede histone binding (Katsani *et al.* 2001). Therefore, we hypothesize that the same mutation in the MLL SET domain will also impede histone binding, by an, as yet, unknown mechanism. The main goals of this project are to determine if the TRX Z11 mutation alters the function of MLL, and if so, determine the molecular mechanisms involved. We expect that this research will lead to an improved understanding of the

structure and function of the MLL SET domain, which will facilitate the development of new therapeutic strategies for the treatment of cancer.

## Methods

A BLAST sequence alignment was done in order to determine the locations of the conserved domains on the two proteins and the location for the site directed mutagenesis.

Figure 1

TRX Mutation:(G3601 -> S)

TRX Mutation: ( 10801 -> )

MLL Mutation:(G3842 -> S)

Ref<<http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&val=184394>>

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LOCUS      AAA58669                3969 aa          linear   PRI 31-DEC-1994
DEFINITION HRX.
ACCESSION  AAA58669
VERSION    AAA58669.1  GI:184394
DBSOURCE   locus HUMHRX accession L04284.1
KEYWORDS   .
SOURCE     Homo sapiens (human)
   ORGANISM Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE  1 (residues 1 to 3969)
   AUTHORS  Tkachuk,D.C., Kohler,S. and Cleary,M.L.
   TITLE    Involvement of a homolog of Drosophila trithorax by 11q23
            chromosomal translocations in acute leukemias
   JOURNAL  Cell 71 (4), 691-700 (1992)
   PUBMED   1423624
COMMENT    Method: conceptual translation.
FEATURES   Location/Qualifiers
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                                   /note="CXXC zinc finger; pfam02008"
                                   /db_xref="CDD:42016"
   Region                1433..1482

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/region\_name="PHD"

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Seq alignment

Steps for purification are adapted steps for purification of the wild type enzyme, which include amylose affinity chromatography, Tev-protease cleavage of the affinity tag, and further chromatographic steps (hydroxyapatite and gel filtration chromatography) to separate the mutant SET domain from the liberated MBP affinity tag.

The purified mutant MLL protein will be used to answer the following questions:

- 1) does the G3842S mutation impede histone binding in MLL as it does in the trithorax *Drosophila* homologue? This will be tested by enzyme kinetic assays where the  $K_m$  and  $k_{cat}$  kinetic constants will be determined and compared with that of the wild-type enzyme.
- 2) Does the mutation affect the overall folding of the protein? This will be tested using UV CD spectroscopy, which gives easily obtainable low resolution information about the overall fold of the protein. We will compare spectra from the mutant and wild type forms of MLL. If the CD spectra show relatively little global disruption of the protein, we will determine the three-dimensional structure of the mutant MLL SET domain by x-ray crystallography and compare it to that of the wild-type enzyme.

## Results

Current progress on this project includes the generation of the G3842S mutation in MLL, which is the equivalent site that is mutated in the TRX Z11 protein. This was accomplished by site directed mutagenesis, where a single base pair was changed in order to change the respective amino acid glycine to a serine. We then transformed the mutated plasmid into an expression strain of *Escherichia Coli*, and worked out optimal expression conditions. Currently, conditions for purification are being optimized.

## **Conclusion**

## References

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