

## Alexander Brehm - *Biochemical characterization of Drosophila L(3)MBT*

### Background

Mutation of the *lethal (3) malignant brain tumour (l(3)mbt)* gene in *D.melanogaster* causes the transformation of cells of the larval brain and the overgrowth of imaginal discs suggesting that it is involved in cell cycle control (Gateff et al., 1993; Wismar et al., 1995). In addition, *l(3)mbt* is required for germ cell formation (Yohn et al., 2003).

Together with *Sfmbt* and *Scm*, *l(3)mbt* forms a family of three genes coding for proteins containing MBT domains. Some MBT domains have recently been shown to recognize differentially methylated histone tails and have been suggested to participate in chromatin compaction (Francis et al., 2004; Klymenko et al., 2006; Trojer et al., 2007). *Sfmbt* binds to histone H3 tails mono- or dimethylated at K9 (H3K9me1 and H3K9me2) and H4 tails mono- or dimethylated at K20 (H4K20me1/2) (Klymenko et al., 2006). The human MBT protein L3MBTL1 recognizes mono- and dimethylated histones H4 and H1 (H4K20me1/2, H1bK26me1/2) (Trojer et al., 2007).

Several MBT domain containing proteins are subunits of multisubunit protein complexes regulating chromatin structure that control gene expression during development (Klymenko et al., 2006; Saurin et al., 2001). *Sfmbt* is a subunit of PhoRC, a Polycomb group (PcG) complex, *Scm* is a component of distinct PcG complex, PRC1. Both complexes function to maintain tissue specific repression of genes throughout *D.melanogaster* development. L3MBTL1 is part of a complex containing histone H1b, HP1 proteins and pRb and is involved in the regulation of E2F target genes (Trojer et al., 2007).

It is unclear if *D.melanogaster* L(3)MBT also functions within multisubunit chromatin regulating complexes and if it recognizes methylated histone tails. L(3)MBT has been reported to be a substoichiometric member of the Myb-MuvB complex and to be required for the repression of some Myb-MuvB target genes (Lewis et al., 2004). However, independent purification of the same complex did not result in the detection of L(3)MBT (Korenjak et al., 2004).

### Aims

In this project we wish to biochemically characterize the *D.melanogaster* L(3)MBT protein. We will establish *D.melanogaster* cell lines expressing epitope tagged L(3)MBT and affinity purify L(3)MBT associated proteins from extracts of these cells. L(3)MBT interacting proteins will be identified by mass spec analysis and interactions will be confirmed *in vivo* and *in vitro*.

We will characterise antibodies raised against L(3)MBT-derived peptides and use these antibodies to determine L(3)MBT expression in embryos and to perform coimmunoprecipitation and chromatinimmunoprecipitation experiments.

In order to identify genes regulated by L(3)MBT we will RNAi-deplete L(3)MBT from cells and assay changes in gene expression profiles using microarray technology.

Finally, we will employ *in vitro* peptide binding assays to identify histone tail modifications recognized by the MBT domains of L(3)MBT.

### Cited literature illustrating background

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