Functional characterisation of the Polycomblike protein of *Drosophila melanogaster*

A thesis submitted for the degree of Doctor of Philosophy

by

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Abstract

The Polycomb Group (PcG) of genes are responsible for the epigenetic silencing of genes during *Drosophila* development. To date 14 members have been identified on the basis of a similar mutant phenotype. Epistasis experiments suggest that the encoded proteins are going to act in a multimeric complex to heritably repress gene transcription. Overlapping polytene binding patterns for some PcG proteins, co-immunoprecipitation experiments from embryonic extracts, and direct interactions between some members of the PcG further support the theory of a multimeric complex. However, it is not yet clear what the mechanism of PcG repression is, and an important first step in resolving this is to characterise the role of each PcG member and identify intra-complex interactions.

Polycomblike (*Pcl*) has been shown to be a key member of the PcG. Molecular characterisation of *Pcl* revealed the presence of two PHD fingers, a Cys_4-His-Cys_3 motif in the protein product. The region surrounding and including the PHD fingers is highly conserved with two mammalian homologues, and is termed the conserved domain (cDOM). Yeast two hybrid analysis has demonstrated an interaction between the cDOM of PCL and Enhancer of zeste (E(Z)), another key member of the PcG. Further characterisation of the interaction between the cDOM of PCL and E(Z) using co-immunoprecipitations from embryonic extracts, confirmed their association *in vivo*. *In vitro* mutagenesis was used to demonstrate that the interaction between PCL and E(Z) is mediated through the PHD finger motifs of PCL.

Using an *in vivo* tethering assay, PCL has been shown to be able to initiate heritable silencing of a reporter gene when tethered to DNA via a GAL4 DNA binding domain. This assay was used to identify functionally important regions of PCL. The amino terminus of PCL was sufficient to initiate heritable repression and the repression conferred by both the GAL-PCL fusion protein and the GAL-Amino fusion protein was dependent on the endogenous PcG. The amino terminus was shown by far western analysis to be capable of homotypic interactions. Yeast two hybrid analysis identified a possible interaction between the amino terminus of PCL and a fragment of PC.

The C-terminus of PCL was shown to interact with PHO and SU(Z)2 in a yeast two hybrid assay.

The work described in this thesis has identified key interactions between PCL and other members of the PcG and suggested a model for the role of PCL within the PcG.