



**Functional characterisation of POLYCOMBLIKE and a
novel, chromosomal protein interactor from
*Drosophila melanogaster***

A thesis submitted for the degree of Doctor of Philosophy

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Abstract

The Polycomb-Group of genes is responsible for the maintenance of repression of many genes during *Drosophila* development. Sequence analyses of those members of the Polycomb-Group that have been cloned provide few clues about the mechanism of this repression. However, some genetic and molecular data suggest that the mechanism is similar to that operating during the repression of genes in Position Effect Variegation.

In order to determine the mechanism of Polycomb-Group repression, the identification of proteins that interact with a key member of the Polycomb-Group, POLYCOMBLIKE, was undertaken. Two proteins were found to interact with POLYCOMBLIKE. One of these, ENHANCER of ZESTE, is a previously characterised Polycomb-Group member that is known to be essential for the attachment of Polycomb-Group proteins to chromosomes. The other, temporarily named 2.1, is a novel protein. A region of POLYCOMBLIKE containing two PHD fingers, a recently identified, putative protein-interaction motif, appears to be responsible for binding ENHANCER of ZESTE but not 2.1.

Antibodies were raised against 2.1 for use as a molecular probe to characterise its nature and distribution. It was found that 2.1 is present ubiquitously during embryogenesis, as has been found for members of the Polycomb-Group, however, it was distributed differently on polytene chromosomes. Its interbanded deposition and close physical abutment to sites of deposition of POLYCOMBLIKE suggest a role for 2.1 in boundary element structures that abate the spread of Polycomb-Group repression, as a general chromosomal factor, or as a general transcriptional activator.

A previously identified mutant, *l(3)SG23*, was defined as being a novel Polycomb-Group gene in the course of attempting to identify a mutation within the 2.1 gene.

The mechanism by which the Polycomb-Group protein complex attaches to DNA is presently unknown. During the course of this study, significant sequence similarity was detected between POLYCOMBLIKE and a murine protein, M96, that was reported to have DNA binding activity, thus potentially providing a mechanism for the DNA attachment of the Polycomb-Group protein complex. However, it appears that POLYCOMBLIKE is not able to bind to DNA *in vitro*.