Analysis of the function of *Drosophila* Cyclin E isoforms and identification of interactors

A thesis submitted for the degree of Doctor of Philosophy

Donna Crack, B.Sc. (Hons.)

Department of Molecular Biosciences

The University of Adelaide

August 2002

Table of Contents

Abstract

Regulation of proliferation acts primarily during G1 phase of the cell cycle. The G1 to S phase transition is regulated by the activity of Cdk2(cyclin dependent kinase)/Cyclin E. As in mammals, *Drosophila cyclin E* (*DmcycE*) is essential and rate limiting for entry into S phase. *DmcycE* gives rise to alternative transcripts encoding two proteins that differ at their N termini. Both proteins are nuclear localised during interphase, and become distributed throughout the cytoplasm during mitosis. During embryogenesis DmcycEII is maternally supplied, while DmcycEI is zygotically expressed. In this study, analysis of the expression of DmcycEII throughout development showed that DmcycEII was present during larval development and oogenesis. This implicates a role for DmcycEII outside of early embryogenesis.

Drosophila eye development requires the synchronisation of cells in G1 phase within the morphogenetic furrow (MF) of the eye imaginal disc. Ectopic expression of DmcycEI in the eye imaginal disc has been previously shown to drive the anterior, but not the posterior, G1 phase cells within the MF into S phase. In this study, ectopic expression analyses using full-length DmcycE proteins as well as N- and C-terminal deletions of DmcycEI, revealed that DmcycEII and N-terminal deletions were able to drive all G1 cells within the MF into S phase, while a C-terminal deletion of DmcycEI could not. Taken together, these results show that DmcycEII is more potent than DmcycEI in driving cells into S phase and that the N-terminal region of DmcycEI contains a negative regulatory domain. A model for this, is that an inhibitor is present in the posterior MF that binds to DmcycEI N-terminus and inhibits DmcycEI function.

Two possible candidates for this inhibition, the Cdk inhibitors Dacapo and Roughex, were examined. Both Dacapo and Roughex were shown by yeast 2-hybrid, co-immunolocalization and *in vivo* functional studies not to be the mediator of the DmcycEI inhibition in the posterior MF cells, suggesting that an unknown inhibitory mechanism exists.

To identify the DmcycEI specific inhibitor, genetic interaction and yeast 2-hybrid screens were undertaken. The genetic interaction screen made use of overexpression of DmcycEI in the eye imaginal disc to produce a dose-sensitive rough eye phenotype. This phenotype was then used to screen a collection of *P*-element alleles, and identified dominant enhancers. One of the enhancers, *CG7394*, encoding a DnaJ homologue, appears to be specific for DmcycEI. The yeast 2-hybrid screen isolated proteins that bind the N-terminal region of DmcycEI that were then examined for their ability to inhibit DmcycE/DmCdk2 function and for an *in vivo* interaction with DmcycE. This analysis revealed that *CG9326,* encoding a MAGUK homologue, may be specific for DmcycEI *in vivo*. Further analysis of these and other interactors identified in both the genetic interaction and yeast 2-hybrid screens, will reveal novel mechanisms for the control of cell proliferation during development.