



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

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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 (DmcyceE)* is essential and rate limiting for entry into S phase. *DmcyceE* 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 *DmcyceEII* is maternally supplied, while *DmcyceEI* is zygotically expressed. In this study, analysis of the expression of *DmcyceEII* throughout development showed that *DmcyceEII* was present during larval development and oogenesis. This implicates a role for *DmcyceEII* 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 *DmcyceEI* 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 *DmcyceE* proteins as well as N- and C-terminal deletions of *DmcyceEI*, revealed that *DmcyceEII* and N-terminal deletions were able to drive all G1 cells within the MF into S phase, while a C-terminal deletion of *DmcyceEI* could not. Taken together, these results show that *DmcyceEII* is more potent than *DmcyceEI* in driving cells into S phase and that the N-terminal region of *DmcyceEI* contains a negative regulatory domain. A model for this, is that an inhibitor is present in the posterior MF that binds to *DmcyceEI* N-terminus and inhibits *DmcyceEI* 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 *DmcyceEI* inhibition in the posterior MF cells, suggesting that an unknown inhibitory mechanism exists.

To identify the *DmcyceEI* specific inhibitor, genetic interaction and yeast 2-hybrid screens were undertaken. The genetic interaction screen made use of overexpression of *DmcyceEI* 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 *DmcyceEI*. The yeast 2-hybrid screen isolated proteins that bind the N-terminal region of *DmcyceEI* that were then examined for their ability to inhibit *DmcyceE/DmCdk2* function and for an *in vivo* interaction with *DmcyceE*. This analysis revealed that *CG9326*, encoding a MAGUK homologue, may be specific for *DmcyceEI 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.