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Genetic analysis of cell cycle regulation during development; the role of a proto-oncogene

During organismal development, control of the cell cycle is complex and is a critical component for determining whether a cell should divide, terminally differentiate, or die. Because it provides a powerful genetic and developmental system in which to dissect cellular and biochemical processes, *Drosophila melanogaster* is an attractive model system for studying the highly conserved biochemical pathways that regulate cellular division and differentiation during development. As a tool for understanding these processes, we are studying the function of *Drosophila myb* (*Dm myb*), a gene related to the proto-oncogene c-*myb*, which is a member of a small gene family in vertebrates. c-*myb* is thought to normally play an important role in controlling cell proliferation, and mutant versions of the gene have been implicated in causing cancer in chickens, mice, and humans. *myb* genes encode sequence-specific DNA-binding proteins that regulate transcription, but a great deal remains unknown about how this activity is governed and which genes are under *myb* control. Elucidating the function of *Drosophila myb* and uncovering the signal transduction pathway(s) in which it participates will help us to understand regulation of the cell cycle during development and provide insights into how mutant versions of the gene cause uncontrolled cell growth.

Using classical genetic screens, we generated two mutant alleles of *Dm myb*. Each contains a single base pair substitution resulting in the change of an amino acid perfectly conserved between *Dm myb* and its vertebrate counterparts. Examination of mutant phenotypes showed that *Dm myb* is important for both embryonic and adult development and that *myb* serves a role in the development of many tissues. Detailed analysis of the wing phenotype revealed that mutant wings contain approximately half the number of cells in wild type, and that the defect occurs during the final cell cycle. Mutant wing cells enter S-phase but are blocked before mitosis, with some of the cells beginning to endoreplicate their DNA. These results indicate that *Dm myb* is required for progression through the G2/M transition and for maintenance of diploidy. Recent studies of the abdominal phenotype have revealed additional roles for *Dm myb* in the cell cycle. The mutant cells proliferate slowly and abnormal mitoses associated with multiple functional centrosomes, unequal chromosome segregation, formation of micronuclei, and/or failure to complete cell division are observed (see Figure 1). These findings demonstrate that in abdominal epidermal cells, *Dm myb* is required to sustain the appropriate rate of proliferation, to suppress formation of supernumerary centrosomes, and to maintain genomic integrity.

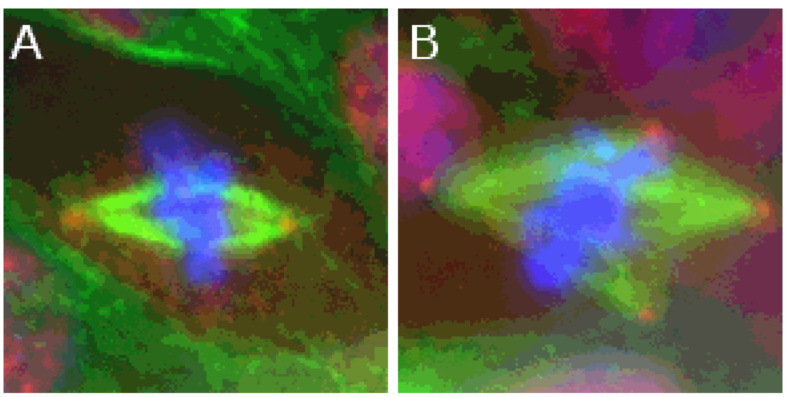

Figure 1. Abnormal mitoses are observed in abdominal epidermal cells that are mutant for *myb*.

Abdominal epidermal samples from wild type (panel A) and *myb2* mutants were triply stained to visualize nuclei (blue), microtubules (green), and red (centrosomes).

We have also generated transgenic animals in which we can induce ectopic expression of wild type and mutant versions of *Dm myb*. We have found that ectopic expression of *Dm myb* within the developing animal can have potent consequences, including inducing pattern disruption and even lethality. Ectopic expression of *Dm myb* can have opposing effects on cell cycle regulation, promoting proliferation in diploid cells (Figure 2), but suppressing endoreduplication in polyploid tissues (Figure 3). Therefore, we conclude that DMyb functions in multiple aspects of the cell division cycle to promote proliferation and maintain the integrity of the genome. A schematic is shown in Figure 4.

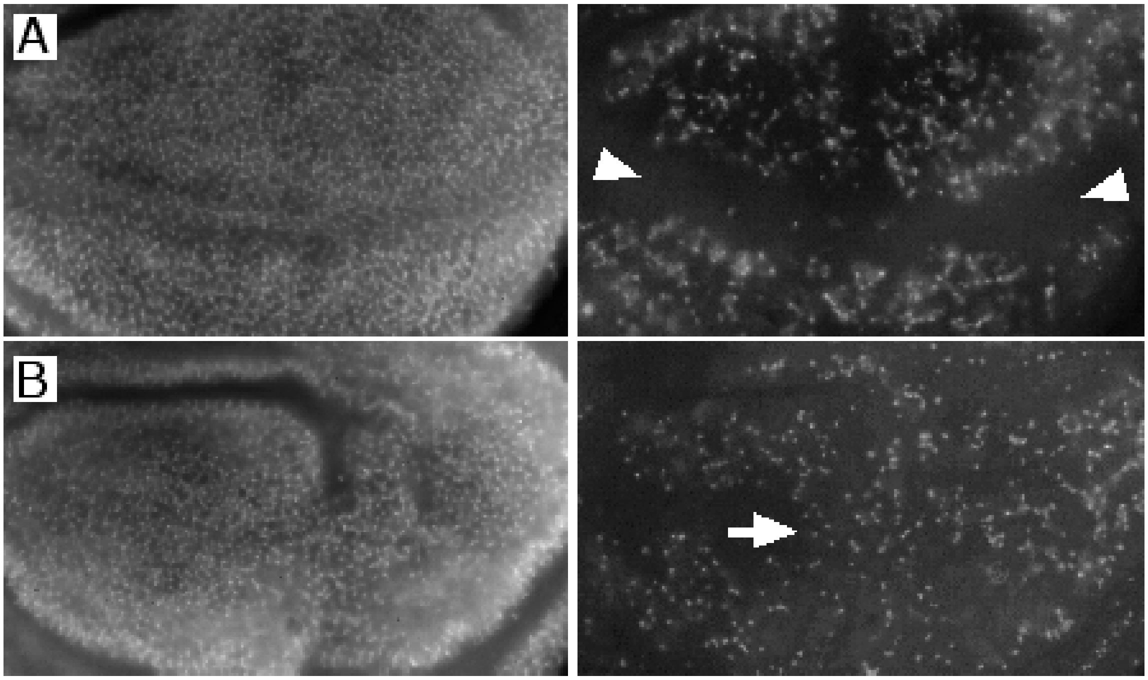

Figure 2. Ectopic expression of DMyb promotes S-phase in the ZNC of larval wing discs.

Wing discs that were doubly stained to visualize nuclei (left panels) and for DNA synthesis by BrdU incorporation (right panels). (A) In wild type control discs, BrdU incorporation was not detected in the zone of non-proliferating cells (ZNC), which is composed of cells at the dorsoventral boundary (see arrowheads). (B) However, when DMyb is ectopically expressed in the posterior compartment of the disc (right side of the disc), BrdU incorporation could be detected in the posterior ZNC (indicated by arrow).

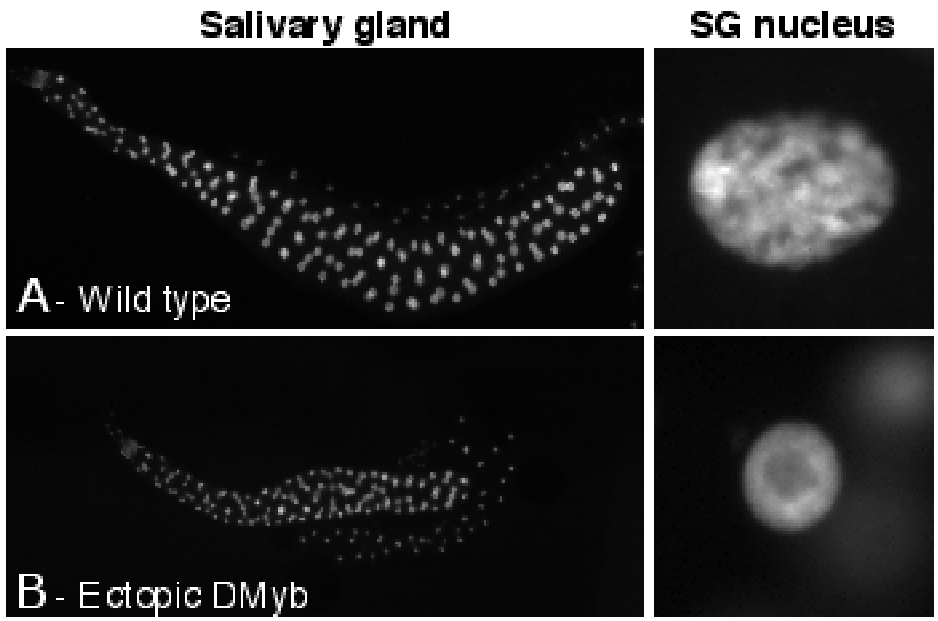

Figure 3. Ectopic DMyb activity inhibits endoreduplication and growth in salivary glands.

Salivary glands were stained with DAPI to visualize nuclei. Salivary glands dissected from wild type larvae (A) were considerably larger than those dissected from larvae in which an activated form of DMyb had been ectopically expressed (B). In the right panels, a representative nucleus from each salivary gland is shown at higher magnification.

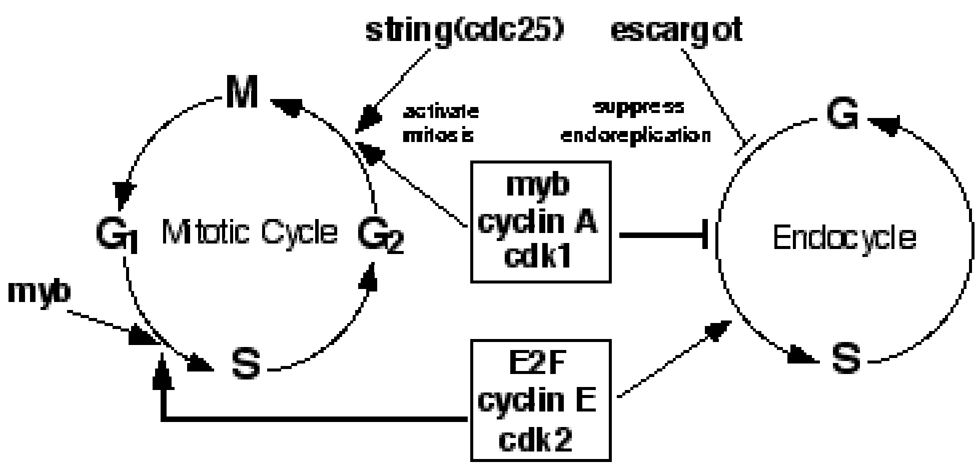

Figure 4. Schematic representation of the multiple roles DMyb plays in regulating the mitotic and endoreplicative cell cycles.

DMyb is a positive regulator of both progression from G_1 into S-phase and G_2 into mitosis, but is a negative regulator of endoreplication, indicating that it plays an important role maintaining diploidy and preserving genomic integrity.

Our laboratory continues to pursue research that will help us to: 1) gain further understanding of *myb* function in regulating the cell cycle and differentiation during *Drosophila* development; 2) identify genes that participate in the same signal transduction pathway(s) as *myb* through genetic approaches; and 3) address the functional relationship between *Drosophila myb* and its vertebrate counterparts.

Selected Publications

Fitzpatrick, C.A., N.V. Sharkov, G. Ramsay, and **A.L. Katzen**. *Drosophila myb* exerts opposing effects on S-phase, promoting proliferation and suppressing endoreduplication. Manuscript submitted.

Fung, S-M., G. Ramsay, and **A.L. Katzen**. (2002) Mutations in *Drosophila myb* lead to centrosome amplification and genomic instability. *Development* **129**, 347-359.

Jackson, J., N.V. Sharkov, E. Lium, G. Ramsay, and **A.L. Katzen**. (2001). The role of transcriptional activation in the function of the *Drosophila myb* gene. *Blood Cells, Molecules, and Diseases* **27**:446-455.

Katzen, A.L., J. Jackson, B.P. Harmon, and J.M. Bishop. (1998). *Drosophila myb* is required for the G_2/M transition and maintenance of diploidy. *Genes Dev*. 12:831-843.

Katzen, A.L. and J.M. Bishop. (1996). *myb* provides an essential function during *Drosophila* development. *Proc. Natl. Acad. Sci. USA* 93,13955-13960.