A Novel Regulator of Myc Protein Stability

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Myc-family transcription factors are involved in organismal growth and development via their regulation of proliferation, apoptosis, metabolism, and other cellular processes and are also frequently dysregulated in cancer (Eilers and Eisenman, 2008). Due to their importance to gene expression, levels of Myc proteins are tightly regulated in normal cells, and it is of interest to determine how this regulation goes awry in cancer. To identify novel regulators of Myc protein stability, postdoctoral fellow Dr. Ling Li and colleagues in the laboratory of Dr. Robert Eisenman (Basic Sciences Division) undertook a genetic screen in a popular model organism (Li *et al*., 2013). "[Myc] has been highly conserved during evolution. We have taken advantage of this conservation to use the fruitfly Drosophila melanogaster to discover a new pathway involved in the regulation of MYC protein abundance," says Dr. Eisenman. "By screening for mutations that influence MYC-induced cell growth in the Drosophila eye we discovered a gene we call Puffyeye to be a novel controller of MYC abundance. Puffyeye is an enzyme that removes the molecular tags that normally mark MYC for destruction, thereby stabilizing MYC and augmenting its activity."

Overexpression of Drosophila Myc (dMyc) in the developing eye gives a rough eye phenotype. This eye phenotype was previously used by the authors to identify modifiers of Myc-dependent cell growth. The screen identified a gene whose loss suppressed the rough eye phenotype. The authors mapped the location of this gene and named it *Puffyeye* (*puf*) to reflect its role in modulating the dMyc-dependent rough eye phenotype. Analysis of the protein encoded by *puf* showed that it contains a ubiquitin hydrolase domain. Ubiquitin is a small protein tag that often targets proteins for degradation. Thus, Puf is expected to be a ubiquitin-specific protease and, based on this predicted activity, the authors hypothesized that Puf would stabilize Myc.

To test this hypothesis, the authors overexpressed Puf in the developing eye. This resulted in a rough eye phenotype similar to that of dMyc overexpression (see figure, middle column). The eye phenotypes resulting from alterations in Puf levels were found to be modified by alterations in dMyc levels, arguing that changes in dMyc levels contribute to the Puf eye phenotype. The authors also found that overexpression of Puf in the developing wing led to phenotypes similar to those seen with dMyc overexpression.

The authors next examined the relationship between Puf and dMyc on the molecular level. Wing imaginal discs (larval structures that give rise to the adult wings) were harvested from larvae overexpressing either wild-type Puf or Puf carrying a mutation compromising its deubiquitylating activity, as well as control larvae. Importantly, larvae were treated with cycloheximide, an inhibitor of protein synthesis, allowing the authors to assess the effects of Puf on the stability of existing dMyc protein. Control larvae showed a ~95% decrease in dMyc levels 120 minutes after cycloheximide treatment, while larvae overexpressing wild-type Puf displayed only a ~20% decrease at the same time point. In support of this model, larvae overexpressing mutant Puf showed a ~90% decrease in dMyc protein at 60 minutes after cycloheximide treatment, demonstrating the importance of Puf's deubiquitylating activity for stabilizing Myc.

The authors also found that Puf physically interacts with Archipelago (Ago), a ubiquitin ligase that targets Myc for degradation. Interestingly, Puf was found to stabilize Ago, as well as Cyclin E (CycE), another target of Ago. From this, the authors concluded that Puf and Ago antagonistically regulate Myc and, therefore, cell growth. "The *MYC* oncogene is a major driver of the growth of a very wide spectrum of human cancers and its levels of expression are tightly regulated in normal cells," says Dr. Eisenman. "The discovery of Puffyeye raises the possibility of inhibiting its enzymatic activity to promote MYC degradation and lower MYC levels in cancer cells."

Li L, Anderson S, Secombe J, Eisenman RN. 2013. The Drosophila ubiquitin-specific protease Puffyeye regulates dMyc-mediated growth. *Development* 140(23):4776-4787.

See also: Eilers M, Eisenman RN. 2008. Myc's broad reach. *Genes Dev* 22(20):2755-2766.

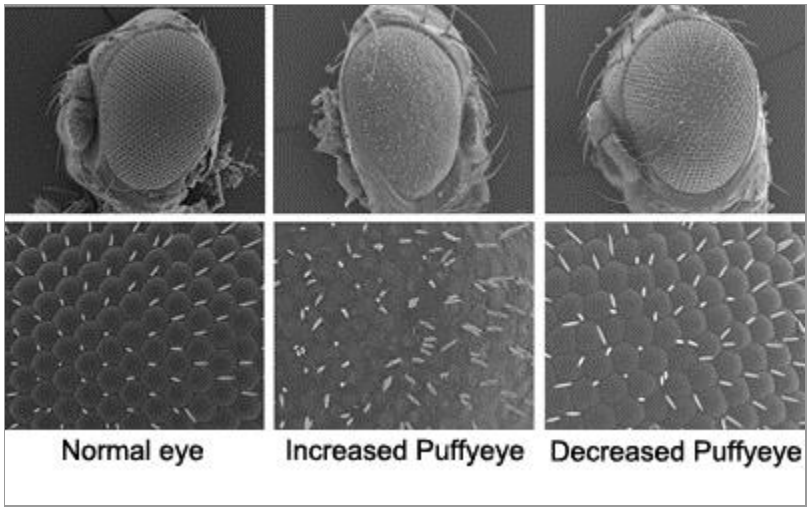

Image provided by Dr. Robert Eisenman

Scanning electron micrographs of Drosophila eyes expressing various levels of Puf. Increased levels of Puf lead to a rough eye phenotype similar to that seen with dMyc overexpression (middle column), while decreased levels of Puf suppress this phenotype (right column).