Tsh and CtBP interaction coordinates *Drosophila* eye development

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Abstract

Distinct combinations of conserved transcription factors regulate division of the eye precursor cells, followed by induction of photoreceptor cell specification in the *Drosophila* (the fruit fly) larval eye precursor tissue called the eye disc. During the third instar larval life, a morphogenetic furrow (MF) made of indented cell layers originates at the posterior margin of the eye disc and propagates towards the anterior side of the eye disc. The cells anterior to the MF are in the proliferating stage, and cells posterior to it start to differentiate as photoreceptors. The differentiated retinal cells form the units of the compound adult eyes in *Drosophila*. Previous studies have shown that Teashirt (Tsh), a zinc finger transcription factor, promotes cell division anterior to the MF. The C-terminal Binding Protein (CtBP), a conserved transcriptional co-repressor, was shown to limit cell division in the eye disc. Interestingly, our immunoprecipitation assays revealed that Tsh and CtBP molecules interact in the eye discs. Thus, our research goal is to identify, whether the molecular interaction is associated with the eye development pathways in flies. We have developed fly strains with over-expression of *tsh* & *ctbp* in the dividing cells anterior to the MF. As a result, we found that there is no or tiny adult eyes in the flies with *tsh* over-expression, and subtle larger adult eye developed in the flies with *ctbp* over-expression. Next, we plan to make a double mutant fly by over-expressing both *tsh* & *ctbp* to evaluate the effect of their interaction on eye phenotype. The result will help to identify the processes of eye development regulated by the Tsh and CtBP together.

Introduction

- The developing eye disc of *Drosophila melanogaster* larva is an ideal tissue to study the molecular events that regulate the cell proliferation & differentiation during adult eye development **(figure 1).**

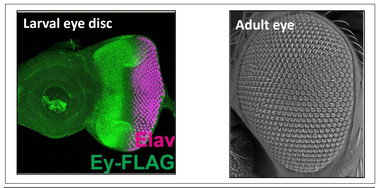


Figure 1. Left: Larval eye-antennal disc; A morphogenetic furrow separates anterior proliferating (green) and posterior differentiating (magenta) cells. **Right:** Adult compound eye.

Figure 2. The retinal determination network regulating *Drosophila* eye development.

- Several molecules participate in retinal determination network to control cell proliferation and differentiation during eye development **(figure 2).**
- Eyeless (Ey), the human ortholog of Pax6, is a master transcription factor in the retinal network. It is expressed in proliferating cells, ahead of the morphogenetic furrow of the eye disc (Bessa et al., 2002; Lopes and Casares, 2010, Curtiss et al., 2007). It controls cell proliferation. Mutation in it causes no or very small adult eye in *Drosophila*.
- The conserved transcription co-regulator C-terminal Binding Protein (CtBP) regulates gene expression by modifying histone assembly (reviewed in Chinnadurai, 2007). It is expressed all over the eye disc (Chinnadurai, 2007). CtBP limits cell proliferation in the eye disc (Hoang et al., 2010).
- Ey & CtBP interact in a molecular complex to control eye development (Hoang et al., 2010).
- Although Ey and CtBP did not show a direct binding in GST-Pulldown assay.
- Teashirt (Tsh) is a conserved Zinc Finger transcription factor. It is expressed in the proliferating cells ahead of the furrow. Ey & Tsh interact in a molecular complex to control cell proliferation (Bessa et al., 2002).
- Tsh has the PxDLS motif. The molecules that bind with CtBP use this motif for CtBP binding.

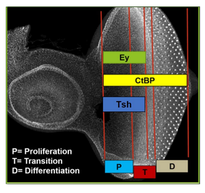


Figure 3. Overlapping expression of Ey, Tsh, and CtBP in the larval antennal eye disc.

- Therefore, due to overlapping expression **(figure 3),** and known interaction among the molecules, we hypothesize that, Ey and CtBP interacts in a complex by the mediator molecule Tsh.

Methods

- Pull-down assays to show evidence for molecular interaction between Tsh & CtBP
- Genotypic – Phenotypic assays to establish genetic interaction
- Immunohistochemistry & Microscopy

Results

Evidence for Molecular Interaction Between Tsh and CtBP

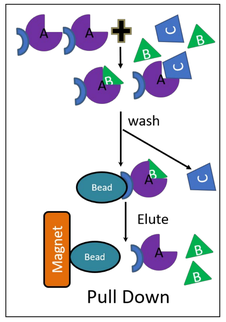


Figure 4. General overview of pulldown assay: The bait protein A specifically binds with protein B in a sample containing other proteins. Next, magnetic beads that bind with the protein A pulls down protein B, and non-specific proteins (such as protein C) are washed away. Presence of protein B in the pulled down sample is identified by Western Blot.

Tsh and CtBP directly bind with each other *in vitro.*

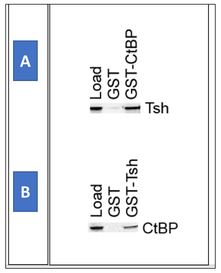
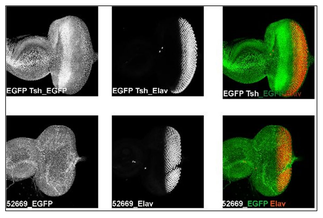
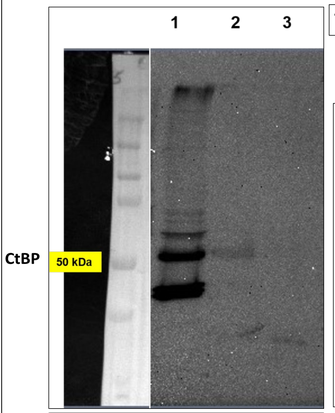


Figure 5. Western Blot of GST pulldown assays.
Panel A: GST-CtBP but not GST pulldowns Tsh.
Panel B: Gst-Tsh but not GST pulldowns CtBP.



Transgenic fly generated for Co-Immunoprecipitation:
Figure 6A. EGFP-tagged *tsh* flies [*EGFP-tsh/+ (2nd)]* generated using CRISPR-Cas9 method. Confocal images of eye-antennal discs showing expression of EGFP in the area where Tsh is expressed (in front of morphogenetic furrow) and expression of Elav in the differentiated neuronal cells behind the furrow in the transgenic flies (top panel). The control flies have no detectable EGFP expression but Elav expression (bottom panel).

Tsh and CtBP directly bind with each other *in vivo.*



Pull-down by Anti-Tsh Antibody and Western Blot with anti-CtBP Antibody

Figure 6B Co-Immunoprecipitation: In the Co-IP, protein lysate is prepared using third instar eye-antennal discs of EGFP-tsh flies. The IP is done using anti-EGFP antibody and the Western Blot is done using anti-CtBP antibody.

Lane 1 shows the presence of the CtBP protein in the total lysate;

Lane 2 shows the presence of the CtBP protein in the precipitated (by EGFP) sample; and

Lane 3 is the negative control to show that the only protein-G beads cannot pulldown CtBP protein.

Results Cont.

Evidence for Genetic Interaction

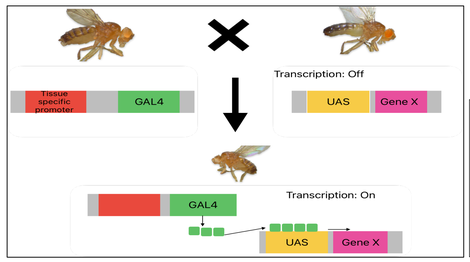


Figure 7. General overview of gene expression regulation using GAL4-UAS system in flies: Elevated transcription from a target GeneX under the UAS enhancer can be achieved due to presence of GAL4 Transcription Factor in a specific tissue.

Transgenic flies are generated to assess genetic interaction between Tsh & CtBP.

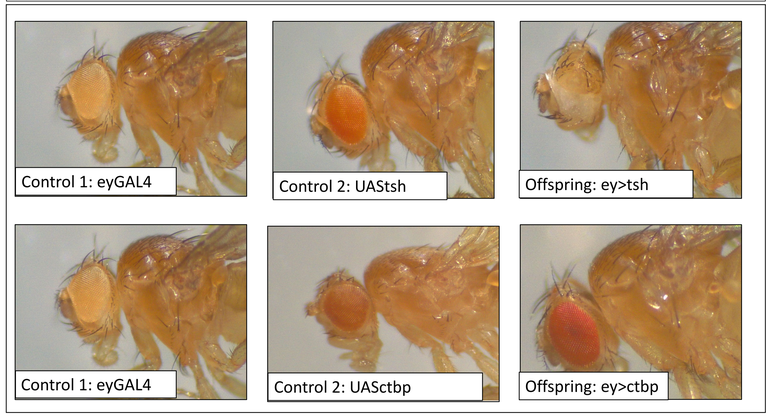


Figure 8.
- The top panel shows the resulting offspring of a cross between a transgenic fly containing eyGAL4 with another transgenic fly, UAStsh. The resulting offspring had a genotype of ey>tsh, and resulted in a new phenotype, No or small eye.
- The bottom panel is a cross between eyGal4 and UASctbp, resulting in an offspring with the genotype of ey>ctbp. The offspring had a larger eye than the controls.

Future Direction

- Genotype-Phenotype analysis in double-mutant flies.
- Immunohistological staining of the eye-antennal disc to show the effect of, Tsh and CtBP interactions on cell proliferation, differentiation, and apoptosis.

References & Acknowledgement

Halder G, Callaerts P, Flister S, Walldorf U, Kloter U, Gehring WJ. 1998. Eyeless initiates the expression of both sine oculis and eyes absent during Drosophila compound eye development. Development 125:2181–2191.
Clements J, Hens K, Merugu S, Dichtl B, Couet G and Callaertsa P. 2009. Mutational analysis of the eyeless gene and phenotypic rescue reveal that an Intact Eyeless Protein is Necessary for normal Eye and Brain Development in Drosophila. Dev Biol. 15; 334(2): 503–512.
Quiring R, Walldorf U, Kloter U. 1994. Homology of the eyeless gene of Drosophila to the Small eye gene in mice and Aniridia in humans. Science 265:785–789.
Silver S and Rebay I. 2005. Signaling circuits in development: Insights from the retinal determination gene network. Development132. 3-13.
Bessa J, Gebelein B, Pichaud F, Casares F, Mann RS. 2002. Combinatorial control of Drosophila eye development by eyeless, homothorax, and teashirt. Genes Dev 16:2415 2427.
Lopes CS, Casares F. 2010. hth maintains the pool of eye progenitors and its downregulation by Dpp and Hh couples retinal fate acquisition with cell cycle exit. Dev Biol 339:78–88
Curtiss J, Burnett M, Mlodzik M. 2007. distal antenna and distal antenna related function in the retinal determination network during eye development in Drosophila. Dev Biol 306:685–702.
Chinnadurai G. 2007. Transcriptional regulation by C-terminal binding proteins. Int J Biochem Cell Biol 39:1593–1607.
Hoang C, Burnett M, and Jennifer Curtiss. 2010. Drosophila CtBP Regulates Proliferation and Differentiation of Eye Precursors and Complexes with Eyeless, Dachshund, Dan, and Danr During Eye and Antennal Development. DEVELOPMENTAL DYNAMICS 239:2367–2385.

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