Tramtrack69 Is Positively and Autonomously Required for Drosophila Photoreceptor Development

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ABSTRACT

Cell-fate specification and cellular differentiation are tightly controlled by both positive and negative transcriptional factors during development. The Drosophila BTB/POZ (Bric-a-brac Tramtrack Broad complex/ Fox virus and Zinc finger) domain-containing Tramtrack (Ttk) proteins have been previously shown to be transcriptional repressors and inhibitors of the neuronal fate of cells such as photoreceptors. Here we provide evidence that one of the Ttk proteins, Ttk69, also plays a positive and autonomous role in promoting or maintaining differentiation of photoreceptor neurons at the late stages of Drosophila eye development. Consistent with this notion, the Ttk69 protein, but not Ttk88, is expressed in all photoreceptor cells during pupal stage. Thus, Ttk69 appears to play a dual function by serving negative and positive regulatory roles at different stages of photoreceptor development.

DURING development of multicellular organisms, cells need to be properly specified to become highly specialized cell types such as light-sensitive photoreceptor neurons. Cell lineage and intercellular signaling are two important mechanisms required for initiating processes that lead to cell-fate commitment. Specified cells will then go through progressive steps to eventually become fully differentiated cells. Nuclear factors are required to regulate gene expression essential for cell-fate decisions and cellular differentiation. The Drosophila compound eye provides a model system with which to study molecular genetic mechanisms of cell-fate determination and cellular differentiation. The adult eye consists of ~800 repeating units or ommatidia, each of which contains eight photoreceptor cells, four nonneuronal cone cells, and seven other accessory cells. On the basis of morphology and physiology, eight photoreceptor cells can be further divided into at least three classes: six outer photoreceptors (R1-6), one apical central photoreceptor (R7), and one basal central photoreceptor (R8; reviewed by Dickson and Hafen 1993; Wolff and Ready 1993; Zipursky and Rubin 1994). Retinal differentiation is initiated by hedgehog (hh) signaling, which interacts with decapentaplegic (dpp) and wingless (wg) in the posterior margin during early eye development. hh action, directly or indirectly, leads to a morphological constriction in the apical surface of a dorsoventral strip of cells that is known as the morphogenetic furrow. Further, hh is required to maintain anterior progression of the furrow in the retinal field. In front of the furrow, cells are proliferating; behind the furrow, cells are being specified and recruited for ommatidial assembly (reviewed by Heberlein and Moses 1995; Burke and Basler 1997; Treisman and Heberlein 1998).

Ommatidial development begins during the third larval stage within the eye imaginal disc, a monolayer epithelium. Once the R8 cell is specified, photoreceptors R2/R5 are recruited to begin neural differentiation, followed by sequential differentiation of R3/R4, R1/R6, and finally R7. There are no lineage relationships among cells within an ommatidium, or among cells of different ommatidia. Cell-cell interactions appear to direct cell-fate decisions, with differentiating cells recruiting their uncommitted neighbors. Two receptor tyrosine kinases (RTK), the Drosophila homologue of epidermal growth factor receptor (DER) and Sevenless (Sev), are required for photoreceptor cell specification. Ras1 appears to be a key component of RTK-mediated signaling pathway. A cascade of protein kinases is implied downstream of Ras. They include Draf, Dsor1 (the Drosophila homologue of mitogen-activated protein (MAP) kinase kinase), and DmERK-A (the Drosophila homologue of MAP kinase encoded by the rolled gene). Ras1-mediated signaling needs to be ultimately transmitted into the nucleus to control activities of transcriptional regulators, which include Yan, Tramtrack (Ttk), Pointed (Pnt), D-Jun, Phyllodop (Phyl), Sina, and Prospero (Pros; for recent reviews, see Badenhorst et al. 1996; Cutforth and Gaul 1997; Dickson 1997; Freeman 1997; Klambt 1997; Kumar and Moses 1997; Wasserman and Therrien 1997). Many of the components in this pathway, as well as their mechanisms of action, are remarkably conserved in evolution.
This work focuses on the ttk gene. Through differential splicing, ttk encodes two sequence-specific transcriptional repressors, Ttk69 and Ttk88. These proteins share amino-terminal sequences containing a BTB/POZ domain, but differ in their carboxyl-terminal region where DNA-binding zinc fingers reside (Harrison and Travers 1990; Read and Manley 1992). The role of Ttk88 as an inhibitor of the R7 photoreceptor cell fate is well established. Specific loss of ttk88 function in the ttk3 mutant results in development of ectopic R7 photoreceptors in the eye (Xiong and Montell 1993). This function is autonomously required to prevent neuronal development in cells that have the potential to become photoreceptor neurons (Lai et al. 1996). Furthermore, this autonomous inhibitory function for R7 development is required downstream of Sina (Lai et al. 1996; Yamamoto et al. 1996). For a precursor cell to commit to a neuronal fate, it requires synergistic action of two upstream regulators, Phyllopod (Phyl) and Sina, to target Ttk proteins for degradation (Li et al. 1997; Tang et al. 1997). Consistent with results from loss-of-function studies, gain-of-function analysis indicates that Ttk88 is sufficient to prevent photoreceptor cell differentiation in the eye (Li et al. 1997; Tang et al. 1997).

The function of ttk69 is less clearly understood because ttk69-specific mutations had not been previously recognized. However, gain-of-function experiments suggest that ttk69 is a transcriptional repressor and inhibitor of neuronal cell fate (Brown et al. 1991; Read et al. 1992; Brown and Wu 1993; Salzberg et al. 1994; Guo et al. 1995; Giesen et al. 1997; Ramaekers et al. 1997). Like Ttk88 stability, Ttk69 stability is also regulated by Phyl and Sina (Li et al. 1997). In this study, we have analyzed specific loss-of-function mutations in ttk69 and their effect on eye development. Surprisingly, our results demonstrate that ttk69 has a second role in which it is required positively and autonomously for photoreceptor cell differentiation during late stages of Drosophila eye development.

**MATERIALS AND METHODS**

**Fly strains:** Fly culture and crosses were carried out under standard conditions (Ashburner 1989). ttk mutations used in this work were ttk<sup>1870</sup> (Lai et al. 1996; Giesen et al. 1997), ttk<sup>1e11</sup> (Xiong and Montell 1993), and seven EMS-induced ttk alleles (Lai et al. 1997). All of these ttk alleles were used to generate FRT82 ttk<sup>-</sup> recombinant chromosomes by standard procedures. The w<sup>hsFLP1</sup>; FRT82 P[ry<sup>+ w</sup>]90E line was obtained from the Rubin laboratory (University of California at Berkeley), and the y<sup>hsFLP1</sup>; FRT82 P[iaZ<sup>ry</sup>]49E line was obtained from J. Treisman (New York University Medical Center). To stage pupae, white prepupae were collected and maintained at 25°. The white prepupal stage was considered as time zero for pupal development.

**Generation of mitotic ttk<sup>-</sup> clones:** To generate ttk<sup>-</sup> clones in the adult eye, w<sup>hsFLP1</sup>; FRT82 P[ry<sup>+ w</sup>]90E female flies were crossed to w; FRT82 ttk<sup>-</sup> males to obtain progeny flies with a w<sup>hsFLP1</sup>/w; FRT82 P[ry<sup>+ w</sup>]90E/FRT82 ttk<sup>-</sup> genotype. The first-instar larvae were heat treated at 38° for 1 hr and then allowed to develop at 25° until eclosed (Xu and Rubin 1993). The w<sup>+</sup> gene was used as a marker to define mitotic clones in adult eyes. The absence of pigment granules mark cells homozygous mutant for the ttk gene. Because our data with ttk<sup>1e11</sup> external eye phenotypes represent a discrepancy with the data of Xiong and Montell (1993), we used the original ttk<sup>1e11</sup> chromosome to generate adult eye clones through an irradiation method. Of four adult eye ttk<sup>1e11</sup> clones examined by scanning electron microscopy (SEM), all exhibited the same phenotypes (data not shown) as those generated by the FRT<sup>-</sup>/FLP-based method (Figure 1, A–D). Contrary to the results described by Xiong and Montell (1993), no extra mechanosensory bristles were ever observed in ttk<sup>1e11</sup> clones. The y<sup>hsFLP1</sup>; FRT82 P[iaZ<sup>ry</sup>]49E female flies were used to generate ttk mutant clones in both larval and pupal eyes. The iaZ gene at 49E is expressed ubiquitously in all cells behind the morphogenetic furrow in eye discs (Treisman et al. 1997). Areas lacking the β-galactosidase activity (blue/green staining) represent ttk<sup>-</sup> clones.

**Histology and immunocytochemistry:** SEM and adult eye sections were carried out as described (Lai et al. 1996). Plastic sections were analyzed using an Axioplan compound light microscope (Zeiss, Thornwood, NY). Immunostaining of eye tissues was as described (Lai and Rubin 1992). Primary antibodies used in this study include rat anti-Ttk69, guinea pig anti-Ttk88, mouse anti-Cut, mouse anti-Elav, and mouse anti-β-galactosidase antibodies (Promega, Madison, WI). Biotinylated secondary antibodies for mouse, rat, and guinea pig immunoglobulin G and Vectastain ABC kit (Vector Laboratories, Burlingame, CA) were used for color reaction. Texas Red-conjugated secondary antibodies for mouse immunoglobulin G (Vector Laboratories) were used for generating fluorescent images that were analyzed using a Zeiss confocal microscope.

**RESULTS**

The ttk69 gene plays a positive and autonomous role in photoreceptor cell development: We were interested in elucidating the role of the ttk69 gene in eye development. Using the FRT/FLP recombination system (Xu and Rubin 1993), we generated clones of ttk- lethal mutations that are known to remove ttk69 function (Xiong and Montell 1993; data not shown). These recessive ttk mutations caused degeneration of the corneal lens (which is secreted by the underlying cone cells and primary pigment cells; Figure 1, A–D) and failure of photoreceptor cell development (Figure 1, E and F). Rhabdomeres of photoreceptors were not observed in the clones, but residual cellular structures in the mutant ommatidia were still recognizable (Figure 1, E and F). Near the boundary of ttk<sup>-</sup> clones, no genetically mosaic ommatidia were ever observed. Although these ommatidia did not contain a full complement of photoreceptor cells, there are no genetically ttk<sup>-</sup> photoreceptors (Figure 1, E and F; Xiong and Montell 1993). This observation demonstrates that the ttk function is autonomously required for photoreceptor cell development. Similar adult eye phenotypes were also observed in seven EMS-induced ttk loss-of-function mutations (Lai et al. 1997; data not shown). The results of these clonal analy-
Role of ttk in Eye Development

Figure 1.—The ttk69 function is positively and autonomously required for photoreceptor differentiation in Drosophila eye development. (A–D) Scanning electron microscopy (SEM) images and (E and F) apical tangential sections of Drosophila adult eyes were from (A, C, and E) ttk\(^{rM730}\)/+ and (B, D, and F) ttk\(^{1e11}\)/+ heterozygous flies. Enlarged views of ttk\(^{-}\) clone regions from A and B are presented in C and D, respectively. (E and F) Lack of orange pigment granules defines the extent of ttk mutant clones. Photoreceptors require at least one copy of wild-type ttk allele for their normal development and some examples are indicated with arrows (E and F). Anterior is to the left.

Figure 2.—The positive function of ttk69 is not required during larval eye development. Third instar larval eye discs of w\(\times\)hsFLP1/; FRT82 ttk\(^{-}\)/FRT82 94F-lacZ flies were dissected for antibody staining after heat treatment for induction of FRT/FLP-mediated recombination. ttk\(^{rM730}\) clones are represented in A, C, E, and G, and ttk\(^{1e11}\) clones in B, D, F, and H. Antibodies used were (A and B) anti-Ttk69, (C and D) anti-Ttk88, (E and F) anti-Elav, and (G and H) anti-Cut. The lacZ gene is ubiquitously expressed in all cells behind the morphogenetic furrow and was used to define ttk mutant clones. Areas lacking β-galactosidase activity, indicated by blue/green staining, represent ttk\(^{-}\) clones. Brown color indicates positive antibody staining in nuclei. Anterior is at the top.

To resolve this issue, expression of Ttk69 and Ttk88 isoforms was examined in ttk\(^{-}\) clones in third instar larval eye discs using isoform-specific antibodies. ttk\(^{-}\) clones were marked with a cell-autonomous lacZ reporter that exhibits ubiquitous expression in all cells behind the morphogenetic furrow. Among the ttk mutations used in this clonal analysis, the ttk\(^{rM730}\) hypomorphic mutation was caused by a P-element insertion about 1.2 kb upstream of the first exon (Giesen et al. 1997), resulting in the loss of both ttk69 and ttk88 expression (Figure 2, A and C). Normally, both Ttk69 and Ttk88 proteins are found in all four cone cells in the larval eye disc (Li et al. 1997). Another ttk mutation, ttk\(^{1e11}\),
carries a deletion in the translated region of ttk69 and was considered a null allele for both ttk69 and ttk88 (Xiong and Montell 1993). Indeed, no Ttk69 protein can be detected in ttk111 cells in third instar larval eye discs (Figure 2B). However, the expression of ttk88 was not dramatically affected (Figure 2D). Complementation tests were then carried out to confirm the finding that only ttk69 function is required in ttk111 mutation. If indeed ttk111 has a specific loss of ttk69, one might expect that it would complement ttk1, because only ttk88 function has been removed in the ttk1 mutation. It appeared that ttk111 did effectively complement ttk1 mutant eye phenotype, because up to 94% (n = 329) of the ommatidia in ttk1/ ttk111 flies were wild type. In contrast, there was only 65% (n = 353) of normal ommatidia in ttk1/ ttkW730 flies and most mutant ommatidia contain ectopic photoreceptors. ttk1 homozygotes contain ~50–60% normal ommatidia (Xiong and Montell 1993; Lai et al. 1996). These data further confirm that there is a relatively specific loss of ttk69 in ttk111 mutation, and the mutant eye phenotype observed in the adult eye clones must be mainly caused by the loss of ttk69 function. Contrary to the gain-of-function data whereby ttk69 is an inhibitor of photoreceptor cell fate, evidence presented here reveals a positive function of ttk69 in photoreceptor cell development. Thus, ttk69 might play a dual function as both positive and negative regulator in this process.

**Clonal analysis of ttk mutations suggests that the positive role of ttk69 is not required during larval eye development:** To determine the time at which the positive function of ttk69 for photoreceptor development is required, expression of a neuronal-specific protein, Elav, was examined in ttk69- clones in third instar larval eye discs. Elav expression appeared normal in both ttkW730 and ttk111 mutant cells (Figure 2, E and F). These Elav-positive ttk- cells were more basally located. No ectopic neurons could be found between or underneath the developing ommatidia in ttk- clones (Figure 2, E and F). However, ommatidial clusters were somewhat disorganized. Therefore, photoreceptors developed properly in the absence of ttk69 function, although loss of ttk69 might interfere with ommatidial assembly in larval eye discs. Our previous work demonstrated that Ttk proteins do not accumulate in larval photoreceptors (Li et al. 1997), which is consistent with the notion that the positive and autonomous function of ttk69 in photoreceptor cells is not required during larval development but rather at a later stage.

Because ttk69 and ttk88 are expressed in cone cells at the larval stage, we wanted to determine if there is a requirement for ttk function for cone-cell development. To address this issue, cone-cell-specific cut expression was examined in third instar eye discs. The cut gene is normally expressed in cone cells at this stage. cut expression appeared normal in ttk- ommatidia (Figure 2, G and H), suggesting that cone cells are properly specified in the absence of ttk69 function.

**Ttk69 but not Ttk88 is expressed at high levels in all photoreceptor cells in the developing pupal eye:** Following larval development, it takes about 4 days to complete metamorphosis. To investigate how ttk might function during pupal eye development, it is essential to characterize its expression at this stage. Antibodies specific to Ttk69 and Ttk88 proteins were used to examine Ttk distribution in wild-type pupal eye tissues. Forty hours into the pupal stage, both ttk69 and ttk88 continue to be expressed in cone cells (Figure 3, A and E). Primary pigment cells (Figure 3, B and F) and secondary and tertiary pigment cells (Figure 3, D and H) are also positive for both ttk69 and ttk88 expression. Of the four cells composing the bristle group, we detected high levels of Ttk69 and Ttk88 proteins in one, while the other two expressed lower levels of Ttk proteins (Figure 3, D and H). One striking difference between ttk69 and ttk88 expression in the pupal eye was that ttk69, but not ttk88, was expressed at high levels in all eight photoreceptor cells (Figure 3, C and G). Expression of ttk69 protein in photoreceptors of pupal eyes was detected as early as 36 hr, and photoreceptors in 48-hr-old pupal eyes continued to express Ttk69 protein (data not shown). The expression of ttk69 in photoreceptor cells during pupal eye development provides corroborative evidence for an autonomous role of ttk69 in late differentiation events.

Expression of a ttk-lacZ reporter gene (from the ttkO219 line, Lai et al. 1996) was characterized to verify the pattern of ttk expression in pupal eye tissues. Like Ttk proteins, β-galactosidase protein was detected in cone cells (Figure 3I), primary pigment cells (Figure 3J), secondary and tertiary pigment cells (Figure 3L), and three bristle cells (Figure 3K, with one of them expressing a higher level of β-galactosidase). No β-galactosidase protein was detected in photoreceptor cells. One possibility is that the lacZ reporter gene does not detect a photoreceptor-specific enhancer that drives ttk expression in pupal photoreceptors. Regulation of ttk expression at the post-transcriptional level or translational/post-translational levels might determine that only Ttk69 protein is produced at a high level in pupal photoreceptors.

**The positive function of ttk69 for photoreceptor cell development is likely required at late-pupal stage:** To directly examine whether ttk69 is required for photoreceptor cell development at pupal stage, ttk111 clones in the eye were analyzed at midpupal stage by examining the expression of the neuronal-specific Elav protein. Within the clone, where ttk69 function was abolished, photoreceptor cells continued to express Elav proteins (Figure 4, A–C). Similarly, cone cells lacking ttk69 activity were still positive for cut expression. However, it is clear that some cone cells failed to develop in the absence of ttk69 function (Figure 4, D and E), suggesting...
that there is a partial requirement for ttk69 function for proper cone-cell differentiation. Because ttk69− photoreceptors appear normal even up to midpupal stage, the corneal structure defects and failure of photoreceptor cell development observed in the adult eye are likely to be initiated sometime during late-pupal stage, when expression of many photoreceptor cell-specific genes such as Rhodopsin (Rh) is activated.

Figure 3.—Ttk69 but not Ttk88 is expressed at a high level in photoreceptors in the pupal eye. Eye tissues from 40-hr-old pupae (at 25°C) of (A–H) wild-type and (I–L) ttk69+/− flies were stained with (A–D, and G) anti-Ttk69, (E, F, and H) anti-Ttk88, and (I–L) anti-β-galactosidase antibodies. All of these antibodies gave nuclear staining in (A, E, and I) cone cells (c), (B, F, and J) primary pigment cells (ppc), (D, H, and L) secondary and tertiary pigment cells (spc and tpc), and (D, H, and K) bristle cells (bc). (C) Only Ttk69 protein was detected at high levels in the nuclei of all photoreceptor cells (G) an enlarged view of one ommatidium with all eight photoreceptors labeled as 1–8 is shown. Anterior is to the left.

Figure 4.—Phenotypic analysis of loss-of-ttk69-function mutations in pupal eye development. Eye tissues from 40-hr-old ttk69+/+ pupae (at 25°C) were dissected and doubly labeled with (A, B, and C) anti-Elav and anti-Ttk69 antibodies and (D and E) with anti-Cut and anti-Ttk69 antibodies to illustrate ttk mutant phenotypes in ttk− clones. Lack of Ttk69 staining (in red as detected with a Texas Red-conjugated secondary antibody) defined a ttk69 clone that is outlined with broken white lines in A–C. (B and C) In the same tissue, Elav protein (in green as detected with an FITC-conjugated secondary antibody) was detected in all photoreceptors, including those genotypically mutant for ttk69. Images from A and B are superimposed to demonstrate that Ttk69 and Elav are coexpressed in photoreceptors (yellow in C) and that Ttk69 but not Elav is expressed in primary pigment cells (red in C). (C) ttk69 mutant cells in the clone continued to express neuronal-specific Elav protein. (D) Cut protein in cone cells was detected by black staining first, which was followed by a (E) brown staining to detect Ttk69 expression in cone cells (in D, previous black staining masked the brown staining) and photoreceptor cells. D is an apical plane and F is a basal plane of the same tissue. The absence of Ttk69 protein in photoreceptor cells defines a ttk69 mutant clone as outlined in D and E.
DISCUSSION

Photoreceptor cell-fate decisions in the developing Drosophila eye are mainly mediated by an RTK signaling pathway that eventually leads to changes of activities of nuclear regulatory molecules. Inside the nucleus, transcriptional activation and repression provide important mechanisms for tight regulation of target gene expression. For instance, transcriptional activator Pnt is required to promote neuronal fate, whereas transcriptional repressor Yan acts to inhibit inappropriate commitment to a neuronal cell fate. Like Yan, Ttk88 and Ttk69 proteins are two inhibitors of neuronal cell fate. Through clonal analysis of loss-of-function mutations in ttk69, we demonstrated that ttk69 plays a critical role in promoting photoreceptor cell differentiation at the late stages of eye development. Therefore, it appears that Ttk69 plays a dual function by serving negative and positive regulatory roles at different stages of photoreceptor development.

As the early function of ttk69 in the developing eye is to inhibit photoreceptor cell fate, loss of ttk69 function would allow formation of extra photoreceptors in third instar larval eye discs. However, no ectopic photoreceptor cells can be found when ttk69 or both ttk69 and ttk88 functions are abolished. Similarly, extra photoreceptor cells are not observed in larval eye discs when ttk88 function is specifically removed, even though there are extra photoreceptor cells in adult retinal (Lai et al. 1996). This argues that depletion of these inhibitory factors might not be sufficient to cause an immediate switch toward photoreceptor cell fate that is measured by neuronal-specific Elav expression, although both Ttk69 and Ttk88 are normally required to prevent inappropriate neuronal fate at this stage. Additional factors are required to promote neuronal fate and subsequent differentiation in cells that are normally prevented from realizing their potential for neural development. Possible redundant function might also be responsible for the failure to observe extra photoreceptors at this stage.

Expression of Ttk69, but not Ttk88, in pupal photoreceptors provides strong supporting evidence for an autonomous role of Ttk69 in promoting photoreceptor differentiation at late stages of eye development. Although ttk69 and ttk88 are coexpressed in most tissues during development, differential expression of ttk isoforms has also been observed in the developing embryonic CNS where ttk69 but not ttk88 is expressed (Giesen et al. 1997). A photoreceptor-specific enhancer might be used to drive ttk transcription in pupal photoreceptors. However, a regulatory mechanism at the post-transcriptional level or translational/post-translational levels must be responsible for producing only ttk69 transcripts or Ttk69 proteins. In larval eye discs, Ttk proteins can be accumulated only in cone cells but not in photoreceptors because of action of two upstream regulators Phyllopod and Sina (Li et al. 1997; Tang et al. 1997).

It is unclear at this moment how ttk69 might act to promote photoreceptor differentiation at the late stages of eye development. One possible scenario is that ttk69 could be involved in activating expression of genes required for terminal differentiation of photoreceptors. Rh genes might be one of the targets of ttk69. Supporting this hypothesis, Ttk69 protein was found to bind specifically to the Rhodopsin upstream sequence 4A (RUS4A) element (Z.-C. Lai, M. E. Fortini and G. M. Rubin, unpublished data), which is essential for Rh4 gene expression in a subset of R7 photoreceptors (Fortini and Rubin 1990). Interestingly, the absence of Rh gene expression leads to a similar neural degeneration phenotype as seen in the ttk69- adult eye (Kumar and Ready 1995). This would suggest a potential role for ttk69 in initiating and maintaining late differentiation events in eye development. However, ectopic expression of ttk69 in adult retinal reduces expression of the Rh4-lacZ gene (data not shown). It suggests that Ttk69 is intrinsically a transcriptional repressor required for its neural inhibitory activity. Other mechanisms convert Ttk69 into a positive factor required for terminal differentiation of photoreceptors.

The conversion of ttk69 from a neural inhibitor into a factor positively required for development of photoreceptor neurons poses an intriguing developmental mechanism. It appears that Ttk69 acts intrinsically as a transcriptional repressor, mediating its neural inhibitory function. Many transcriptional repressors act by competing with activators for DNA sequences. They may also interact directly with activators or the transcriptional machinery to turn off transcription. A number of transcriptional repression systems utilize corepressors to prevent transcription. As a transcriptional repressor, Ttk69 may utilize such mechanisms to inhibit inappropriate photoreceptor cell fate in early eye development. Interestingly, Ttk69 is expressed in photoreceptor cells at later stages, where Ttk69 activity might be changed through protein modification, availability of cofactors, or changes in the context of the target gene promoter. Consequently, Ttk69 becomes a positive regulator critical for photoreceptor development.

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LITERATURE CITED


Brown, J. L., and C. Wu, 1993 Repression of Drosophila pair-rule


