The N-terminal BTB/POZ Domain and C-Terminal Sequences Are Essential for Tramtrack69 to Specify Cell Fate in the Developing Drosophila Eye

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ABSTRACT

The BTB/POZ (broad complex Tramtrack bric-a-brac/Pox virus and zinc finger) domain is an evolutionarily conserved protein-protein interaction motif. Many BTB-containing proteins are transcriptional regulators involved in a wide range of developmental processes. However, the significance of the BTB domain in development has not been evaluated. Here we present evidence that overexpression of the Tramtrack69 (Ttk69) protein not only blocks neuronal photoreceptor differentiation but also promotes nonneuronal cell fate specification in early Drosophila eye development. We show that the BTB domain is essential for Ttk69 function and single amino acid changes in highly conserved residues in this domain abolish Ttk69 activity. Interestingly, the Ttk69 BTB can be substituted by the BTB of the human Bcl-6 protein, suggesting that BTB function has been conserved between Drosophila and humans. We found that the Ttk69 BTB domain is critical for mediating interaction with the Drosophila homolog of C-terminal-binding protein (dCtBP) in vitro, and dCtBP mutations genetically interact with ttk69. Furthermore, the C-terminal region downstream of the DNA-binding zinc fingers is shown to be essential for Ttk69 function. A dCtBP consensus binding motif in the C terminus appears to contribute to Ttk69 activity, but it cannot be fully responsible for the function of the C terminus.

Development of multicellular organisms requires extensive uses of evolutionarily conserved protein motifs. The BTB/POZ (broad complex Tramtrack bric-a-brac/Pox virus and zinc finger) domain is such an evolutionarily conserved protein-protein interaction motif (Bardwell and Treisman 1994; Zollman et al. 1994; reviewed by Albagli et al. 1995). The BTB domain is found in a variety of proteins including actin-binding proteins, pox virus proteins, and many transcriptional regulators that usually contain zinc-finger DNA-binding motifs. The completed yeast and Caenorhabditis elegans genomes reveal 4 and 156 unique BTB-containing sequences, respectively (Cherovitz et al. 1998). It was previously estimated that there are at least 40 BTB sequences in the Drosophila genome based on low-stringency hybridization (Zollman et al. 1994). The Drosophila genome project reveals 64 distinct BTB-containing proteins (Rubin et al. 2000). The human genome contains at least 56 distinct BTB coding sequences (Ahmad et al. 1998).

BTB proteins are involved in a wide variety of regulatory events throughout development. For instance, the abrupt (ab) gene is required for the embryonic formation of a subset of neural-muscular connections and muscle attachments (Hu et al. 1995), and longitudinal lacking (lola) is involved in axonal path finding during Drosophila embryogenesis (Giniger et al. 1994). The fruitless (fru) gene is expressed in a subset of neurons in the central nervous system and is involved in determining male sexual behavior (Ito et al. 1996; Ryner et al. 1996). The Kelch protein is necessary for the construction of ring canals that connect oocyte and nurse cells in the Drosophila ovary (Xue and Cooley 1993). The mammalian calcitonin gene is specifically expressed in testes and is probably involved in a dense nonfilamentous cytoskeletal structure that is tightly associated with the sperm head (von Bulgow et al. 1995). The functions of several BTB genes have been shown to be required for normal Drosophila eye development. In particular, the tramtrack (ttk) gene plays a critical role in the development of photoreceptor (R) cells and cone cells (Xiong and Montell 1993; Lai and Li 1999). In addition, ttk function is required for proper development of embryonic glial cells and sensory organs (Salzberg et al. 1994; Guo et al. 1995; Giesen et al. 1997; Ramaekers et al. 1997). Several human BTB genes are implicated in cancer development. For example, the B cell lymphoma 6 (Bcl-6) gene is implicated in pathogenesis of non-Hodgkin lymphomas (Ye et al. 1993). A fusion protein between the human promyelocytic leukemia zinc-finger protein (PLZF), BTB, and the retinoic acid receptor (RARα) is strongly associated with acute promyelocytic leukemia (Dong et al. 1996).

A key issue regarding the role of these BTB-containing proteins is to reveal the function of the BTB domain.
A general property of the BTB domain is to mediate homomeric dimerization (e.g., Bardwell and Treisman 1994). The crystal structure of the PLZF BTB domain revealed that BTB monomers are tightly intertwined as dimers (Ahmad et al. 1998). However, the BTB domain has also been shown to be involved in heteromeric interactions with a number of proteins. In the case of Bcl6 and PLZF, the BTB domain directly interacts with the silencing mediator of retinoid and thyroid receptor (SMRT) corepressor to form a transcriptional repressor complex that includes another corepressor, mSin3a, and the HDAC1 histone deacetylase (Dhordain et al. 1997, 1998; Hong et al. 1997; Lin et al. 1998; Wong and Privalsky 1998). When fused with a heterologous DNA-binding domain, the BTB domain is effective in mediating transcriptional repression (e.g., Deweindt et al. 1995; Chang et al. 1996) that suggests an autonomous role of BTB. It is believed that histone deacetylase in the BTB protein complex modifies chromatin structure necessary for transcriptional repression. Interestingly, the BTB domain has been reported to be capable of mediating transcriptional activation as well (Kaplan and Calame 1997; Kobayashi et al. 2000).

The Drosophila eye provides a useful system to investigate protein function in cell specification and differentiation (reviewed by Wolff and Ready 1993; Zipursky and Rubin 1994; Treisman and Herberlein 1998). Using this system, we have investigated structural requirements for Ttk69 to function as a neural inhibitor during early eye development. We show that the BTB domain is essential for Ttk69 function and that the BTB function appears to be conserved during evolution. Moreover, the C-terminal region downstream of the zinc fingers is also essential. Our results suggest that the transcriptional corepressor Drosophila homolog of human C-terminal-binding protein (dCtBP) might be an interacting partner of Ttk69 for the control of cell fate decision and cellular differentiation.

MATATELRS AND METHODS

Fly strains: Two Gal4 lines, severeless (sev)-Gal4 and eyeless (ey)-Gal4, were used to drive eye-specific gene expression. The following mutants were used to test if they genetically interact with the ttk69 gene: dCtBP<sup>PR160</sup>, dCtBP<sup>PR20-10</sup>, Rpd3<sup>S1556</sup>, Sin3A<sup>2029</sup>, and Sin3A<sup>2026</sup>. All the strains were obtained from the Bloomington Drosophila Stock Center. Fly culture and crosses were carried out under standard conditions (Ashburner 1989).

Molecular analysis and germline transformation: The ttk69 deletion and point mutations were generated by polymerase chain reaction (PCR) and the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). Some Ttk69 constructs were tagged with the hemagglutinin (HA) epitope. To achieve this, we generated a pUAST-HA vector in which three copies of the HA epitope sequences flankned by EcoRI and NotI sites were cloned into the pUAST vector. A Drosophila translational start site consensus sequence (CACG) followed by a translation initiation codon (ATG) was inserted upstream of the HA sequence in frame by PCR. The full-length and mutant ttk69 cDNA molecules were cloned into the pUAST (Brand and Perrimon 1993) or pUAST-HA vectors for germline transformation. 35S-labeled full-length Ttk69 and the Ttk69 derivatives were synthesized in vitro, using the TNT coupled reticulocyte lysate system (Promega, Madison, WI), and tested for their ability to associate with glutathione S-transferase (GST)-dCtBP or GST proteins in affinity pulldown assays.

RESULTS

Overexpression of Ttk69 transforms photoreceptor neurons to nonneuronal cells: During early Drosophila eye development, Ttk proteins accumulate only in cone cells but not in R cells (Li et al. 1997). One of the Ttk proteins, Ttk88, has been shown to be an inhibitor of R cell development through loss- and gain-of-function analysis (Xiong and Montell 1993; Li et al. 1997; Tang et al. 1997). To investigate the role of the other Ttk protein, Ttk69, in early eye development, the full-length Ttk69 was overexpressed in cells of the R<sub>7</sub> equivalent group that include the R<sub>3</sub>/R<sub>4</sub>, R<sub>7</sub>, and cone cell precursors by using the Gal4/UAS expression system (Brand and Perrimon 1993; Figure 1). In sev-Gal4/UAS-ttk69 eye discs, all R<sub>3</sub>/R<sub>4</sub> and many R<sub>7</sub> precursor cells fail to express the Elav protein, which has been commonly used as a neural-specific marker (Robinson and White 1991; Figure 2, A, C, and I). Consequently, these cells do not become R cells. With one copy of the ttk69 transgene, all ommatidia contain four or fewer outer R cells (on average 3.3 outer R cells per ommatidium), and 45% of the ommatidia have no R<sub>7</sub> cell (Figure 3E). When driven by ey-Gal4 for expression in the anterior region of the developing eye, Ttk69 effectively blocked eye formation (Figure 6B). Thus, Ttk69 is sufficient to block neural differentiation in eye discs, which is consistent with the idea that Ttk69 is a negative regulator of R cell fate during larval stages of the eye development. At late pupal stages, Ttk69 is positively and autonomously required for facilitating R cell differentiation (Lai and Li 1999).

To determine if Ttk69 also plays a role in cone cell specification, we stained sev-Gal4/UAS-ttk69 eye discs...
with an antibody made against the Cut protein, which is normally expressed in all cone cells (Blochlinger et al. 1993) and has been commonly used as a cone cell marker. We found that all R3/R4 and many R7 precursor cells are positive for Cut expression (Figure 2, E and K). The anterior and posterior cone cell precursors are not recruited into the ommatidial clusters in the mutant eye disc. However, it appears that the polar and equatorial cone cells can be occasionally recruited. Consequently, each ommatidium contains two to five cone cells (Figure 2G). Mystery cells often become Cut positive as well, but they appear only for several hours before being excluded from the clusters (Figure 2L). Thus, ttk69 overexpression appears to transform photoreceptor neurons to nonneuronal cone cells. Consistent with this observation, ttk69 has been shown to be sufficient to transform neurons into support cells in sensory organs (Guo et al. 1995; Ramaekers et al. 1997). Loss-of-function analysis indicates that Ttk69 is necessary for the development of cone cells (Lai and Li 1999).

The BTB domain is essential for Ttk69 function: To evaluate the significance of the BTB domain for Ttk69 function, deletion analyses and site-directed mutagenesis were carried out to examine the sequence requirements within the BTB domain necessary for Ttk69 function. Mutant protein activity is monitored in transgenic eyes as assayed by the overexpression phenotype using the sev-Gal4 driver described above (Figure 1). Deletion of the BTB domain completely abolished the activity of Ttk69 in repressing R cell development (Figure 2, B
lies exhibit wild-type phenotype (Fig. 1). It is remarkable that a single amino acid change at these positions is as effective as the BTB deletions in inactivating Ttk69. Thus, the conserved residues (D32, H45, and L49) are essential for BTB activity. On the basis of the crystal structure of the BTB domain of PLZF, His-45 and Leu-49 are located within the core of the BTB domain, whereas Asp-32 is positioned in the putative ligand-binding groove (Ahmad et al. 1998). These residues are likely to play a critical role in maintaining proper structure and function of the BTB domain. An essential role of the BTB domain for Ttk69 function was also demonstrated using the ey-Gal4 driver (Figure 6, C, D, and G).

The function of the BTB domain has been conserved during evolution: To investigate if the function of the BTB domain is conserved during evolution, Ttk69 BTB domain was replaced with the BTB of the human Bcl-6 protein. Surprisingly, the Bcl-6 BTB domain can functionally substitute for the Ttk69 BTB domain, although these two sequences are only 25% identical. Like Ttk69, the Bcl6-Ttk69 chimeric protein is effective in inhibiting R cell differentiation, which suggests that the mechanism of BTB action might be conserved between Drosophila and humans. Interestingly, the R7 cells are more sensitive than the outer R cells to the inhibitory effect of the Bcl6-Ttk69 protein when compared to the full-length Ttk69 protein. In eyes of sev-Gal4/UAS-Bcl6ΔN153 flies, there are on average 5.2 outer R cells per ommatidium and up to 70% of the ommatidia are missing the R7 cell (Figure 3, G and J). Consistent with these observations, most R3/R4 cells developed properly but R7 cells failed to become neurons in the larval eye discs (data not shown). Curiously, the Bcl6-Ttk69 fusion protein is much less effective in disrupting eye formation than the full-length Ttk69 protein when misexpressed in the anterior precursor cells through the ey-Gal4 driver (Figure 6F). Consistent with the idea that BTB function has been conserved during evolution, a BTB domain of the Drosophila GAGA protein was shown to be able to functionally replace the Ttk69 BTB domain (Figure 6E). The GAGA BTB domain is 40% identical to Ttk69 BTB and is distantly related compared to other Drosophila BTB proteins (data not shown).

The BTB domain of the Bcl-6 protein has been shown to be necessary and sufficient for interaction with HDAC-1 histone deacetylase, SMRT, and mSIN3A corepressors to mediate transcriptional repression (Dhorain et al. 1997, 1998; Wong and Privalsky 1998). Ttk69 might use similar mechanisms to specify cell fate in the developing eye. To investigate this possibility, loss-of-function mutations in Drosophila Sin3A and Rpd3 histone deacetylase were tested for their ability to dominantly modify the mutant eye phenotypes caused by ttk69 overexpression. Our results showed that a reduction in either Sin3A or Rpd3 function does not dramati-
cally modulate the mutant eye phenotypes of *sev-Gal4 UAS-ttk69/+* flies (Figure 4, B, E, and F).

**The C-terminal region is essential for Ttk69 function:** To examine the significance of the C-terminal region, the sequence [amino acids (aa) 568–641] downstream of the DNA-binding zinc-finger domains in Ttk69 was deleted (Figure 1). The eyes of the *sev-Gal4/UAS-ttk69ΔC68* flies are normal (Figure 3, H and K), indicating that the C-terminal sequence is essential for Ttk69 function. Further deletion of the C-terminal region that includes the zinc-finger motifs (aa 500–641) also inactivates Ttk69 in the transgenic eye discs (Figure 1). Similarly, these C-terminally truncated Ttk69 proteins are also inactive when tested using the *ey-Gal4* driver (Figure 6H and data not shown). Thus, the C-terminal region is indispensable for Ttk69 function.

**A P-DLS motif appears to contribute to Ttk69 activity:** We have identified a P-DLS consensus sequence (aa 591–595) located within the C-terminal region, which might mediate direct interaction with the dCtBP corepressor (Nibu et al. 1998; Poortinga et al. 1998). To investigate the significance of this motif, the PPDLS sequence was converted into AAAAS through site-directed mutagenesis. In the eyes of *sev-Gal4/UAS-ttk69PPDLS/AAAAS* flies, the mutant Ttk69 protein is less effective in blocking R cell development, since there are fewer ommatidia that are missing the R7 cell as compared to the eyes that are expressing the wild-type Ttk69 protein (13 vs. 43%, respectively). Moreover, there are on average 4 outer R cells in each ommatidium (Figure 3L), whereas the number is 3.2 in the eyes expressing the wild-type Ttk69 protein (Figure 3E). When driven by the *ey-Gal4* regulator, Ttk69PPDLS/AAAAS is not as effective as the wild-type Ttk69 protein in blocking eye formation (Figure 6I). Thus, the P-DLS motif appears to contribute to Ttk69 function.

The eyes of *sev-Gal4/UAS-ttk69ΔN153* flies exhibit several subtle mutant phenotypes that include R4 to R3 transformation in ~10% of the ommatidia, occasional transformation of R4 into R7-like cell (1%), missing R7 cell (0.5%), and missing retinal bristles (Figures 2O and 3D). Further deletion of the N terminus (Figure 3, M and P) or C terminus (Figure 3, N and Q) results in complete inactivation of the Ttk69 protein. Remarkably, the PPDSLs/AAAAS mutation also completely abolishes the Ttk69ΔN153 activity (Figure 3, O and R), which supports the idea that the P-DLS motif plays a contributing role in Ttk69 activity.

**Figure 3.** The BTB domain and C-terminal sequences are essential for Ttk69 function. SEM images (A–C, G–I, and M–O) and apical tangential sections (D–F, J–L, and P–R) of adult eyes are presented. (A and D) Wild-type eyes. Ommatidia are regularly arrayed to form a smooth surface (A). In an apical section, rhabdomeres of six outer cells (R1–6) and one central R7 cell are arranged in a trapezoidal configuration. The central R8 cell is located at a basal level and is not visible in this section. Each R cell is identified as shown in the inset in D. (B and E) *sev-Gal4/UAS-ttk69.* The eye appears rough and retinal bristles are abnormally patterned (B). The ommatidia never contain more than four outer R cells, and 43% (*n* = 341) of the ommatidia are missing the R7 cell (E). (C and F) *sev-Gal4/UAS-ttk69ΔN153.* (C) Eyes are normal in size and appearance except many retinal bristles are missing. (F) R4 to R3 cell transformation is seen in 10% (*n* = 225) of the ommatidia (circled), and occasionally R4 is transformed as an R7-like cell (1%; indicated by an arrowhead) and R7 is absent (0.5%; indicated by an arrow). (G and J) *sev-Gal4/UAS-Bcl6ΔN153.* The eye defects are almost as severe as that caused by the full-length ttk69; the eyes are apparently rough (G) and the R7 cell is missing in >70% (*n* = 392) of the ommatidia (J). (H and K) *The sev-Gal4/UAS-ttk69ΔC68* eyes are wild type both externally (H) and internally (K). (I and L) The *sev-Gal4/UAS-ttk69ΔN153ΔC68* eyes are still rough in appearance (I). However, the mutant Ttk69 protein is less active as R7 cell development is blocked only in ~13% (*n* = 344) of the ommatidia, and many ommatidia (circled) contain more than four outer R cells (L). Eye development is basically normal in *sev-Gal4/UAS-ttk69ΔN153 (M and P), sev-Gal4/UAS-ttk69ΔN153ΔC68 (N and Q),* and *sev-Gal4/UAS-ttk69ΔN153PPDSLs/AAAAS (O and R).*
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**Figure 5.** The dCtBP protein interacts with Ttk69 in vitro. 

Figure 4.—Tests on genetic interactions between ttk69 and mutations in the dCtBP, Rpd3, and Sin3A genes. Apical tangential sections of adult eyes are presented. The genotypes of the flies are as follows: (A) wild type, (B) sev-Gal4 UAS-ttk69/+, (C) sev-Gal4 UAS ttk69/UAS ttk69, (D) sev-Gal4 UAS ttk69/+, dCtBP<sup>W79A</sup>/+, (E) sev-Gal4 UAS ttk69/+, Rpd3<sup>30156/+</sup>, and (F) sev-Gal4 UAS ttk69/Sin3A<sup>09269</sup>.

**Loss-of-function mutations in dCtBP dominantly modulate certain mutant eye phenotypes caused by ttk69 overexpression:** The Drosophila CtBP protein has been shown to be a corepressor of several transcriptional repressors (Nibu et al. 1998; Poortinga et al. 1998). To begin investigating if dCtBP could be involved in coordinating Ttk69 function, the sev-Gal4 UAS-ttk69 eye was used as a sensitized system to assay for any genetic interaction, as it is clear that the mutant eye phenotypes are dosage dependent (Figure 4, B and C). In the eyes of sev-Gal4 UAS ttk69 flies, a subset of R cells failed to develop due to the inhibitory action of Ttk69 protein. However, other defects were also observed that include increased spacing between ommatidia and the absence of all R cells in some ommatidia (Figure 4, B and C). Interestingly, the latter phenotypes can be dominantly enhanced by loss-of-function mutations of the dCtBP gene (Figure 4D), which suggests that dCtBP genetically interacts with ttk69. Since ttk69 was overexpressed in only a subset of R cells with the sev-Gal4 driver, the absence of all R cells in some ommatidia must be due to both autonomous and nonautonomous effects of Ttk69.

**Ttk69 BTB interacts with the dCtBP protein in vitro:** To test if Ttk69 directly interacts with dCtBP, pull-down assays were performed using GST-dCtBP and labeled Ttk69 proteins. The full-length Ttk69 protein binds to dCtBP specifically (Figure 5A). However, mutant proteins Ttk69<sup>ΔC568</sup> and Ttk69<sup>PPDLS/AAAAS</sup> still associate with dCtBP (Figure 5, B and C). These results suggest that dCtBP interacts with Ttk69 but the PPDLS motif is not essential. Some other sequences in Ttk69 might mediate direct interaction with dCtBP. Indeed, the BTB domain appears to be a good candidate since its deletion greatly reduced the ability of Ttk69 to bind to dCtBP (Figure 5D). It is known that sequences other than P-DLS can interact with dCtBP. For instance, a PVNLA motif in CtBP<sup>87De-10</sup>, (E) sev-Gal4 UAS ttk69/+, Rpd3<sup>30156/+</sup>, and (F) sev-Gal4 UAS ttk69/Sin3A<sup>09269</sup>.

**DISCUSSION**

The Drosophila ttk gene is one of the founding members of the BTB/POZ gene family. Studies over the last 10 years have revealed that ttk69 plays a critical role in cell fate specification in a number of developmental systems (reviewed by Badenhorst et al. 1996). In the eye, ttk69 plays a dual function as a positive and negative regulator during R cell development. The ttk69 gene is autonomously required for facilitating or maintaining R cell differentiation during late stages of eye development (Lai and Li 1999). During the third instar larval stage, however, ttk69 appears to be an inhibitory regula-
tor of R cell fate and this function is best illustrated by the genetic relationship between ttk69 and yan. The yan gene product is a general inhibitor that prevents specification of a number of cell types (Rebay and Rubin 1995). Loss of yan function results in the formation of ectopic R cells in the eye, and this mutant phenotype can be dominantly enhanced by the reduction of ttk69 function (Lai et al. 1997 and data not shown). If ttk69 is solely a positive regulator of R cells, one would predict that loss of the ttk69 function should suppress the ectopic R cell phenotype of yan mutants. Moreover, overexpression of ttk69 in third instar eye discs blocks R cell specification, which supports the inhibitory role of Ttk69 in early R cell development (Li et al. 1997 and this study). As Ttk69 is known to be required for the formation of at least a subset of cone cells (Lai and Li 1999), it is not surprising that ttk69 appears to be sufficient to transform photoreceptor neurons to nonneuronal cone cells in the developing sensory organs, overexpression of ttk69 transforms neurons to support cells (Guo et al. 1995; Ramaekers et al. 1997).

We have used the ttk69 transgenic eye as an in vivo assay to investigate the structural requirement of Ttk69 function. One prominent feature of the Ttk69 protein is the presence of an evolutionarily conserved BTB domain in its N terminus. Our data demonstrate that the BTB domain is essential for Ttk69 function. Single amino acid changes in three residues (D32, H45, and L49), which are conserved in all BTB sequences, completely abolished Ttk69 activity. On the basis of the structural data of another BTB domain (Ahmad et al. 1998), we expected these residues to be critical for proper structure and function of the BTB domain. Interestingly, the Ttk69 BTB domain can be functionally replaced by the BTB domain of a human protein Bcl-6 in certain developmental contexts. These results suggest that not only the structure but also the function of the BTB domain is conserved during evolution. Furthermore, our deletion analyses also demonstrate the significance of the C-terminal region downstream of the zinc-finger DNA-binding domain. As Ttk69 dimerizes through its BTB domain and specifically binds to DNA through the zinc-finger motifs, one would expect that truncated forms of Ttk69 should interfere with the function of the wild-type Ttk69 protein. Surprisingly, neither the N-terminally nor the C-terminally truncated Ttk69 derivatives behaved as dominant negative forms. One possibility is that the truncations cause drastic overall structural defects such that the Ttk69 derivatives are completely inactivated. However, point mutations in Ttk69 also do not exhibit any dominant negative effect on the wild-type Ttk69 protein.

To begin to investigate mechanisms by which Ttk69 acts to specify cell fate, we first tested the possibility that the Sin3a corepressor and Rpd3 histone deacetylase could interact with Ttk69 to specify cell fate in the developing eye. This is based on the facts that Ttk69 has been shown to function as a transcriptional repressor (Read et al. 1992; Brown and Wu 1993) and that the Bcl-6 BTB domain interacts with mSin3A and HDAC-1 histone deacetylase to repress transcription (Dhordain et al. 1997, 1998; Wong and Privalsky 1998). The Drosophila homolog of Sin3A is essential for embryonic viability (Pennetta and Pauli 1998). Clonal analysis reveals that the Sin3A gene is required for cell survival and proliferation in developing tissues such as the eye (Neufeld et al. 1998). The Drosophila Rpd3 histone deacetylase is essential for embryonic segmentation and viability (Mannervik and Levine 1999). In our approach, eyes expressing the ttk69 transgene were used as a sensitized assay to monitor potential genetic interactions between ttk69 and mutations in the Sin3A and Rpd3 genes. Interestingly, the reduction of either the Sin3A or Rpd3 histone deacetylase activity does not dominantly modify the mutant eye phenotype caused by the ttk69 transgene, indicating that there is a lack of genetic interaction between these genes. Functional redundancy might provide part of the explanation. Indeed, two additional Drosophila histone deacetylase homologs have been identified (Bornemann et al. 1999). Alternatively, the ttk69 transgenic eye might simply not be sensitized enough to detect changes in the level of Sin3A and Rpd3 proteins.

Ttk69 might use other mechanisms to specify cell fate. In particular, the dCtBP corepressor may interact with Ttk69 to form a repressive complex for transcriptional repression. A putative dCtBP-binding motif that appears to contribute to Ttk69 activity is found in the C-terminal
region of Ttk69. Although this motif is not essential for dCtBP binding, Ttk69 does interact with dCtBP through its BTB domain and the possibility of BTB and P-DLS mediating multivalent Ttk69-dCtBP interaction cannot be excluded at the moment. In a genetic assay, reduction in the level of the dCtBP protein might free some Ttk69 proteins that might be recruited in other kinds of complexes. Ttk69 might form oligomers to cause the nonautonomous effect in disrupting R cell development. Supporting this hypothesis, Ttk69 proteins in the sev-Gal4 UAS-ttk69/+; dCtBP/+ genotype appear to be as effective as those in the sev-Gal4/2xUAS-ttk69 genotype (Figure 4). Further supporting evidence comes from studies on the GAGA protein. A recent study demonstrates that the GAGA protein can form oligomers in a BTB-dependent manner (Katsani et al. 1999). We showed that the Ttk69 BTB can be replaced by the BTB domain of the GAGA protein (Figure 6E and data not shown). However, protein crosslinking experiments using eye disc protein extracts failed to detect Ttk69 in the form of oligomers (data not shown). As BTB proteins are involved in a wide variety of biological events, further studies on mechanisms of BTB action would help to understand how complex developmental processes can be controlled by BTB-containing regulators.

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