國立臺灣師範大學生命科學系碩士論文

TBP 蛋白質在聚麩醯胺引起之神經退化性疾病上扮演的角色:與轉錄失調之關聯

Role of TATA-box Binding Protein (TBP) in PolyQ Mediated Neurodegenerative Diseases: Implication of Transcription Dysfunction

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摘要

聚麩醯胺擴增所造成的神經退化性疾病是由於其致病基因的外引子不正常的 CAG 三核苷酸擴增導致。在細胞及分子的層次上，其致病機轉雖尚未完全釐清，但由近幾年的研究發現轉錄失調為這類疾病的主要原因之一，一般認為聚麩醯胺造成轉錄失調有兩種方式：1.干擾轉錄因子間的交互作用； 2. 把轉錄因子捕捉在包涵體中使其無法作用。TATA box binding protein (TBP)為細胞內重要的轉錄因子，當其胺基端聚麩醯胺擴增時會導致第十七型脊髓小腦共濟失調症 (SCA17)。本研究的主要目的即是利用第十七型脊髓小腦共濟失調症果蠅模式株，來探討異常聚麩醯胺擴增的 TBP 蛋白質與相關因子對病理症狀有何影響，並釐清 TBP 在聚麩醯胺擴增造成的神經退化性疾病所伴演的角色。我們研究發現 TBP 功能為果蠅發育所必需，當其功能缺失時果蠅無法孵化，降低 TBP 表現或將其功能在特定組織致默後會造成複眼感光細胞退化，運動行為缺陷及壽命減短等性狀，第十七型小腦萎縮症果蠅疾病模式的性狀也會因其功能的缺失而更加嚴重，相對地增加 TBP 的表現則能有效地改善上述退化及行為等性狀，顯見 TBP 功能的減少為第十七型脊髓小腦共濟失調症致病機轉的一環。此外由於研究指出 High Mobility Group (HMG) 的蛋白質會抑制 TBP 的轉錄作用，我們也籍由 HMG 的同源基因- HmgD 來干擾 TBP，當 HmgD 大量表現時果蠅複眼感光細胞確有退化的現象，SCA17 果蠅模式動物在此背景下也會加劇其病徵。

Abstract

Polyglutamine (polyQ) diseases are a specific group of hereditary neurodegenerative diseases caused by expansion of CAG trinucleotide repeats in the exon of the corresponding gene. The pathological mechanism underlying polyglutamine mediated neurodegenerative diseases has not been fully elucidated in cellular and molecular level. Recently advancement in unraveling the pathogenic mechanism of polyQ mediated neurodegenerative diseases has pointed that transcription dysregulation is one of the major factors that results in death of neurons. It is generally accepted that polyQ causes transcription dysfunction in two ways: 1. polyQ disrupts the normal interaction of transcription factor; 2. many transcription factors were sequestered in polyQ containing inclusions. TATA Box binding protein (TBP) is a general transcription factor that is required for transcription of all three types of RNA polymerases in cell. It has been reported that SCA17 has been attributed to the polyQ expansion at amino terminal of TBP. The major goal of my study is to study how the abnormal TBP leads to SCA17 pathogenesis using Drosophila as model system, and dissect the role of TBP in polyQ mediated neurodegenerative diseases. We have found that TBP is essential for early embryogenesis of Drosophila. Fly embryos can not hatch in the absence of TBP. Reducing the function of TBP in certain tissues by RNA interference causes various phenotypes, including degeneration of photoreceptor cells, defect in mobility and pre-mature death. And pathological phenotype of SCA17 is enhanced in the loss-of-function of TBP. Conversely, increasing the expression of TBP would alleviated abovementioned disorders, suggesting loss-of-function of TBP is involved in the pathogenesis of SCA17, In addition, previous study has show HMG binds to TBP and suppresses TBP mediated transcription. We have found

ectopic HmgD causes retinal degeneration in fly. And co-expression of both mutant TBP and HmgD leads more severe defects.

Introduction

Polyglutamine diseases

The molecular pathology of neurodegenerative trinucleotide repeat diseases is very complex. They can be classified into two different classes. The first class is the polyglutamine disease, and the second class, which trinucleotide repeat is within an untranslated region of a gene (Everett and Wood, 2004).

Polyglutamine (polyQ) diseases are a specific group of hereditary neurodegenerative diseases caused by expansion of CAG trinucleotide repeats in an exon of disease gene which leads to produce a disease protein containing an expanded polyglutamine stretch (Cummings Zoghbi, 2000; Fischbeck, 2001; Gusella and MacDonald, 2000; Orr, 2001). There are nine neurodegenerative disorders including Kennedy's disease, Dentatorubral pallidoluysian atrophy (DRPLA), Huntinton's disease, spinocerebellar ataxias (SCAs) 1, 2, 3, 6, 7, and 17 are known to belong to the polyglutamine diseases (Zoghbi and Orr, 2000 ; Gatchel and Zoghbi, 2005). These disorders are characterized by late-onset progressive neuronal dysfunction, leading to severe neuronal loss.

Accumulated evidences have revealed that multifactorial mechanisms are involved in these polyQ mediated neurodegenerations. Major molecular pathogenesis are listed as followed: aggregation, apoptosis, autophagy, Ca^{2+} homeostasis alterations, excitotoxicity, transcription dysregulation, mitochondrial impairment, oxidative stress, alterations of proteasome degradation, synaptic dysfunction, disruption of axonal transport and vesicle

trafficking, and unfolded protein response (Duenas et al. 2006). PolyQ diseases are clinically distinct, affecting different regions of neurons, although they share common genetic features. In recent years, researchers are still working on the development of therapeutic approaches in polyQ disorders and the relationship of polyQ expansion and neurodegeneration.

CAG trinucleotide repeat

Most polyglutamine diseases except SCA6, when expansion to over 40 repeats leads to onset (Fischbeck, 2001). Because of the length is necessary for polar zipper formation, the threshold number is consistent (Perutz, 1999; Scherzinger et al., 1999). The length of CAG trinucleotide repeats is correlated with the age of onset of these diseases (Brandt et al., 1996; Igarashi et al., 1992; Ikeuchi et al., 1995; Komure et al., 1995; McNeil et al., 1997; Trottier et al., 1994). This phenomenon implicates that the long glutamine tract translated from a sequence containing abnormal repeats of CAG trinucleotide is toxic for neuronal cell. Then transgenic mice expressing mutant polyglutamine show very similar pathology, that means expansion of polyglutamine tract is sufficicient to cause neurodegeneration (Davies et al., 1997 ; Ikeda et al., 1996 ; Magiarini et al., 1996). Besides the affection of expanded polyQ tract on neuronal cell, the specific protein containing expanded polyQ tract itself seems to play an important role in hereditary neurodegenerative diseases.

Nuclear inclusion

Although in all of these diseases, these mutant proteins broadly express in most tissues or cells, only specific neuronal cell would be affected, and the clinical symptoms are different. How do these proteins with polyQ expansion lead neuronal cell to a serial death or loss of function? It is not clear now, but nuclear inclusions may be a clue for this question (Rubinsztein et al., 1999). PolyQ expansions dower mutant proteins with a gain of toxic properties, then these proteins display aberrant interaction with other protein partners and accumulated into neurons to form intranuclear inclusions (NIs), a hallmark of these diseases (Ross, 2002).

Nevertheless, role of protein inclusion in the pathogenesis of polyQ -induced neurodegeneration is paradoxical. Some have suggested that inclusions is harmful because they caused protein misfoding, impact proteintransport (Scherzinger et al. 1999), transcription repression(Steffan et l. 2000), and interference with cytoskeletal and vesicular function(Li et al., 1995; Kalchman et al., 1997; Faber et al., 1998 ; Gusella and McDonald, 1998). But some have suggested that inclusions play a protective role (Saudou et al., 1998). Aggregate formation has been seen in the dentate nucleus of HD cerebellum, a region of the brain unaffected by neurodegeneration in HD (Becher et al., 1998). So aggregate formation doesn't necessarily result in cell death, it even protect cells by enhancing the degradation of toxic polyQ-containing protein (Taylor et al. 2003). Between NIs formation and cell death, cellular models also show the discrepancy. And NIs formation is not a prerequisite for cell death but mutant Htt must be present in the nucleus to cause apoptosis in rat primary striatal neurons (Saudou et al., 1998). Apoptosis in neuroblastoma cell lines was increased in the presence of mutant Htt but the correlation with NIs formation was not discordant (Lunkes and Mandel, 1998; Lunkes et al., 1999). There is also

evidence in transgenic mice for NIs formation without cell death and vice versa (Mangiarini et al., 1996).

However, since one of the primary functions of nuclei is transcription, it is believed that expanded polyQ interferes with transcription machinery and eventually lead to neuronal dysfunction. Besides, most of the polyglutamine disease protein-binding proteins are transcription factor, including TBP, CBP, Sp1 etc.(Okazawa, 2003), which may suggest that transcription dysfunction is a specific cue of polyglutamine disease pathology.

TATA box binding protein and Spinocerebellar ataxia 17

Among the nine polyglutamine diseases, SCA 17 is the dominantly inherited disorder caused by an expansion in the TBP polyglutamine tract.

TATA-binding protein(TBP) is a critical general transcription factor which plays an important role in initiation of transcription of all three RNA polymerases (Gill,1992; Hernandez,1993). These general transcription factors, including TBP, form a preinitiation complex with RNA polymerase II (pol II) and bind to the TATA box (Roeder, 1991). When TBP binds to the TATA box, the DNA is bent and this bending appears to regulate transcription (Tenharmsel and Biggin, 1995). Comparison of TBP sequence from various species reveals that the N-terminal domain (NTD) is species specific and the C-terminal domain (CTD) is highly conserved(Fig. S1). The CTD of TBP is enough for functions of TBP including DNA binding, protein interaction and assembly into TFIID, and directing transcription initiation (Zhou and Berk, 1995; Zhou et al., 1993). The NTD has been supposed to regulate the DNA-binding activity of the CTD (Lescure et al.,

1994). In NTD of TBP of eukaryotes except yeast, there is an uninterrupted glutamine stretch which length varies from 6 residues in chicken TBP to 38 residues in human TBP (Hernandez,1993).

Transcription dysfunction

TBP was found that it is associated with aggregates in several polyglutamine disorders (Perez et al., 1998; Uchihara et al., 2001; van Roon-Mom et al.,2002). It was found subsequently that TBP can cause neurodegeneration itself when the polyglutamine stretch is expanded (Koide et al., 1999).Several studies have demonstrated that reducing the function of TBP due to sequestration of TBP in inclusion leads to neurodegeneration (Huang et al. 1998; Perez et al. 1998; Suhr et al. 2001; Reid et al. 2004). As abovementioned, transcription dysfunction may be a specific cue of polyQ disease pathology, and TBP is the most critical transcription factor. Coincidentally, TBP embraced a tract of polyQ, which expanded would cause pathogenesis of SCA17. Thus, we chose TBP as a theme in this study.

Besides, TfIID transcription factor is a multiprotein complex including TBP and TBP-associated factors (TAFs) (Hernandez, Hanson et al. ,2003). Many TAFs, including SP1, TAFII, CBP/p300, P53, heat shock factor and CREB have been well documented to be involved in neuropathogenesis of polyQ (Sugars and Rubinsztein 2003). In this part of study, we will focus on high mobility group box 1 (HMGB1), whose roles in neurodegeneration have not been extensively studied. It has been reported that HMGB1 physically interacts with the NTD of TBP, the particular domain that polyQ is resided. And the interaction has shown to inhibit TBP-dependent

transcription by disrupting the formation of HMGB1/TBP/TATA preinitiating complex (Sutrias-Grau et al. 1999; Das and Scovell 2001). When a fly homolog of HMGB1, HmgD, was ectopically expressed in photoreceptors, retinal degeneration has evidently observed in this study. This has supported the abovementioned idea that disturbing the function of TBP will result in neurodegeneration. Nevertheless, it is equally possible that the interaction of TBP and HMGB1 is reciprocal, which means that the mutant TBP interferes the normal function of HMGB1. And aberrant activity of HMGB1 will lead to neuronal degeneration.

To answer whether HMGB1 is involved in SCA17 pathogenesis, we plan to put our SCA17 fly model in HmgD background. If HMGB1 does participate in SCA17 pathogenesis, the fly will exhibit stronger phenotype. Phenotypic criteria, including retinal degeneration, mobility and life span, will be used to test if our hypothesis is correct as described in above section. Conversely, it is expected that expression of HmgD may alleviate the disorder. Therefore we will use the UAS/GAL4 expression system to express HmgD to see if our hypothesis is correct.

The goal of research

Various molecular processes are implicated in the pathology of polyglutamine diseases caused by the expanded polyglutamine-containing proteins, but the mechanism of pathology is not yet clearly understood. For demonstrating polyQ diseases pathology of utilizing studying SCA 17 is expectable, because by previous studies, TBP may play a critical role in studying polyQ diseases. *Drosophila* is a top priority to be consider as a animal model, which has long been demonstrated to be one of the most valuable animal models for unraveling pathogenic mechanism and finding therapeutic targets of polyQ mediated neurodegeneration.. Highly understanding of *Drosophila* genetics and its versatile technologies can help us elucidate the mechanism of pathology efficiently.

In the present study, I aimed at elucidating the roles of TATA-box binding protein and its polyglutamine tract in neurodegenerative disease by using different mutant and transgenic *Drosophila* lines. As these diseases are genetically dominant, the fly models have been engineered by over-expression of mutant genes which contain expanded glutamine repeats, in central nervous system (*CNS*) or other tissues, such as photoreceptors (Warrick et al. 1998; Marsh et al. 2000; Ross 2002). The photoreceptor and neural cells die with age when elevated level of mutant protein is produced in the corresponding tissues. Fly models develop similar pathological hallmarks, in which they show characteristic late onset, progressive degeneration, abnormal protein aggregation and behavior defects as noted in patients. In a parallel experiment, we have also generated dTBP knock-down fly line by RNA interference (RNAi) techniques (Lee and Carthew 2003). We suspected that reduction of dTBP may enhance and/or sensitize

cytotoxicity of polyQ. We would be able to discern if dTBP play a role in polyQ mediated neurodegeneration

Since TBP itself is a general transcription factor, how the length of polyQ tract in the N terminal domain (NTD) affects its transcriptional activity remains to be elusive. In addition, NTD of TBP interacts with the chromatin association factor, HMGB1 (HmgD). The interaction results in suppressing the formation of transcription initiation complex. Therefore, we would like to investigate whether HMGB1 (HmgD) is participated in SCA17 pathogenesis. We anticipated this study will help to clarify the mystery of pathology of polygluatmine diseases and develop of therapeutic approaches.

Materials and Methods

Drosophila maintenance

All *Drosophila melanogaster* stocks are raised on standard cornmeal-yeast-agar medium supplied by Institute of Molecular Biology Academia Sinica and cultured at 25°C. We anaesthetize flies with CO2 then observe and manipulate them with a stereomicroscope. Most *Drosophila* lines are supplied by Bloomington stock center, and the transgene lines are produced in our laboratory.

UAS-GAL4 system

The UAS-Gal4 system is a method for ectopic gene expression in Drosophila which allows the selective activation of any clone gene in tissue or cell specific patterns (Brand and Perrimon, 1993).

Scanning Electrical Microscope, SEM

To cut 8-10 *Drosophila*'s heads per sample, then immerse them in 2% glutaraldehyde for fixation at 4°C overnight. Rinse in PBS. Immerse in 2% osmium acid at room temperature for 5 hours. Rinse in 30% ethanol, then immerse in 30% ethanol at room temperature for 1.5 hours. Dehydrate through an ethanol series(each twice in 50% and 75% ethanol at room temperature for 30 minutes). Immerse in 100% acetone at 4°C overnight. Critical point dry (CPD) and sputter-coat (Kimmel et al., 1990). After recorded by SEM(20kV, 200X), counting fusion number using Northern Eclipse's Manual count.

Measuring climbing ability and analysis

The analysis for measuring climbing ability in *Drosophila* is modified from a novel assay and analysis (Todd and Brian, 2004). The climbing apparatus (Fig. S2) is a 30 cm long glass tube, with a diameter of 1.5 cm. The tube is held in place by a plastic funnel as a means for transferring flies into the apparatus and acts as both a base for the tube. The glass tube is divided into a series of five sections, starting from the base, each 2 cm in height (scored 1-5), with an buffer zone in the upper portion of the apparatus. Flies are allowed 10 seconds to climb after being tapped down and are given a score based upon the sections reached. The flies are scored ten times (trials) per climbing session, from which a climbing index is calculated as follows:

Climbing index = $\sum(nm) / N$

Where n = number of flies at a given level, m = the score for that level (1-5) and N = total number of flies climbed for that trial. fifty flies from each genotype under investigation are collected within 24 hours of eclosion and separated into groups of ten individuals (each group has 5 males and 5 females). Starting at day one after eclosion, each group is tested for climbing ability and is continually tested every five days throughout their lifespan.

Lifespan analysis

Flies were raised to adulthood at 25°C and newly eclosed flies were placed in vials at low density (10–25 flies per vial) and incubated at 25°C. Males and females were kept in separate vials. Flies were transferred into fresh vials every 5 days, the number of surviving flies was recorded at the same time. Survival curves were generated by calculating the percentage of surviving flies.

ImmunoFluorscence staining

Dissect third-instar larva with fine tweezers to separate the organ we want. Immerse the organs in 3% formaldehyde for 5-10 minutes, then Immerse in PBST. Blocking with 10% BSA at 37°C for 2-4 hours then wash with PBST. Add 1° antibody at 4°C overnight, then wash twice with PBST. Add 2° antibody at room temperature for 1.5 hours, subsequently. Wash with PBST, stored at 4°C ready for confocal microscope.

Results

Drosophila SCA17 model exhibits age-dependent defects

As before mentioned, SCA17 is an autosomal dominant cerebellar ataxia (ADCA) which caused by glutamine repeat expansion within the NTD of TBP. To generate the disease fly model of SCA17, we have overexpressed various human TBP constructs in flies using UAS/GAL4 binary expression system. Targeted expression of the mutant TBP recreated same pathological features of SCA17 (Fig.1). Overexpression of mutant TBP with 109 glutamine residues (TBP-109Q) with GMR-GAL4, causes retinal degeneration while overexpress TBP36Q, TBP54Q, and wild type did not displays defects in photoreceptor. Besides, expressing TBP36Q in neuron, eyes, or whole body in *Drosophila* would not cause neurodegeneration and the phenotype would be the same as wildtype.

Scanning Electrical Microscope (SEM) analysis reveals that only slightly rough can be observed when expression of TBP54Q and TBP109Q (Fig. 2A). And Expression of TBP36Q is similar as wildtype. The degree of neurodegeneration will be quantitated by counting their ommatidia fusion number(Fig. 2B).

Similar age-dependent behavior defect was also observed in disease fly model, while express TBP109Q in neurons of whole body, the climbing ability would decline acutely (Fig. 3A). The lifespan of mutant fly were decreased (Fig. 3B). To our surprise that expression of TBP with 54 glutamines (TBP54Q) did not lead to any morphological phenotypes as seen in TBP109Q. ImmunoFluorescence staining were performed to confirm that TBP-54Q were expressed compatible with those of TBP-109Q, and the inclusion body were clearly observed in the fly tissues (Fig. 4G). Although

our fly model expressing TBP-54Q forms inclusion, degeneration was not observed.

Appropriate glutamine residues within TBP is essential for its function

Since the highly conserved CTD of TBP has been shown to be essential and sufficient for the normal function of TBP, and in TBP with longer polyQ stretch promoted polyQ induced toxicity , we rationed that CTD alone would substitute the function of TBP. Nevertheless, expression of the CTD of TBP or TBP with three glutamine residues (TBP-3Q) and CTD of TBP(DeN-TBP) in photoreceptor cells of flies resulted in retinal degeneration (Fig. S3). In addition, Similar degree of retinal degeneration were observed when TBP109Q was expressed. To better understand that, we analysis their climbing ability and lifespan (Fig. 5), which also reveal similar degeneration were observed when TBP109Q was expressed.

TBP mutant lines also exhibit neurodegenerative symptoms

TBP is a general transcription factor that is required for the transcription of all cells. Using TBP mutant line for decreasing endogenous normal TBP also cause neurodegenerative phenoypes like reducing lifespan and reducing climbing ability(Fig. 6). We then analysis the sequence of mutant TBP for comfirmation(Fig. 7).

TBP-RNAi cause neurodegenerative symptoms are like SCA17 *Drosophila* model's

There are several options to manipulate the expression level of a designate gene. In this study we have mainly chosen RNAi techniques to

silence the function of dTBP because of its effectiveness and versatility.

Using RNAi technique (Lee and Carthew, 2003) to knock down dTBP expression in *Drosophila* will also cause neurodegenerative phenoypes like pigment lost in eyes (Fig. 8), bristles lost or deorganization and ommatidia fusing or deformation in eyes (Fig. 9), reducing lifespan, and reducing climbing ability (Fig. 10).

We also observe some other phenotypes in one of the RNAi lines (Fig. 11), TR0901, which show some kind of blemishes we have never seen before.

Because it's difficult to perform a RT-PCR or western, we co-express UAS-dTBP with TBP-RNAi to test if the RNAi line is work as a replacement (Fig. 12). It reveals that our RNAi line can surely decrease the endogenous dTBP, and increment of dTBP can ameliorate the degeneration. Besides, mildly expression ectopic dTBP can retard the age-dependent reduction of climbing ability (Fig. S4).

Lack of TBP cause neurodegeneration, supply hTBP can ameliorate it but the effect rely on the length of polyQ

Under the TBP-RNAi background, only expression TBP with normal length of polyQ tract can ideally ameliorate the phenotype cause by RNAi, and expression TBP with expansion polyQ tract just can partially ameliorate the neurodegenerative phenotypes(Fig. 13). SEM analysis and quantitated analysis of SEM can also support it (Fig. 14).

TBP with Polyglutamine would affect degeneration caused by HmgD

Overexpession of HmgD would cause neurodegeneration because HmgD can interfere TBP specifically. Co-expression with normal polyQ TBP by GMR-GAL4 will ameliorate the neurodegenerative phenoypes, and coexpression with expansion polyQ TBP will deteriorate the neurodegenerative phenoypes (Fig. 15). Expression by elav-GAL4 then analysis their climbing ability and lifespan found that HmgD would reduce climbing ability of *Drosophila*. TBP36Q can ameliorate the climbing ability, but expanded polyQ would interfere with the amelioration(Fig. 16A). HmgD would't reduce lifespan, But co-expression with TBP109Q would reduce the lifespan a little more than expression of TBP109Q (Fig. 16B).

Discussion

Transcriptional dysfunction has been implicated in pathogenesis of many neurodegenerative diseases. The general transcription factor, TBP has been suggested to be involved in degenerative process. Nevertheless, a direct link between the function of TBP and the neuronal pathogenesis is missing. Our study on the pathogenic mechanisms of SCA17 has given us an possible position to tackle the question. Form our study, we provided evidence that malfunction of TBP may lead to neuronal impairment in SCA17. To make our conclusion more solid, we reduced the expression level of dTBP in animal for more comfirmation.

Overexpression of mutant TBP with 109 glutamine residues (TBP-109Q) with GMR-GAL4, causes retinal degeneration while wild type and GMR-GAL4 did not displays defects in photoreceptor. The lifespan of mutant fly were decreased, Similar age-dependent behavior defect was also observed in disease fly model. To our surprise that expression of TBP with 54 glutamines (TBP54Q) did not lead to any morphological phenotypes as seen in TBP109Q. ImmunoFluorescence staining were performed to confirm that TBP-54Q were expressed compatible with those of TBP-109Q, and the inclusion body were clearly observed in the fly tissues. Previously studies have demonstrated that formation of inclusion body is not necessarily correlated with the pathogenesis of polyQ disorders, because polyQ containing aggregations were not visible while animal developed neurological defects in transgenic mouse models. Our result with TBP-54Q fly model has clearly demonstrated that formation of inclusion is not the primary that causes of neuronal impairment. Other pathogenic factors may involve in the pathogenesis of SCA17 or other polyQ mediated diseases.

Similar to the degree of retinal degeneration of TBP109Q were observed when TBP-3Q and DeN-TBP were expressed. Therefore, we concluded that NTD of TBP with appropriate glutamine residues is essential for its function.

Previous study has shown that TBP with 48 glutamines promotes the CREB dependent transcription up to 4 folds in cultured cells (Reid, et al., 2003). The elevated CREB activity was founded to protect neurons from degeneration and to improve the longevity of fly. Although our fly model expressing TBP-54Q forms inclusion, degeneration was not observed. The fly even exhibits longer life span than expressing TBP36Q. This could be due to the ectopic CREB activity.

We hypothesized that reducing in the expression of functional TBP gave rise the pathogenesis of SCA17. To test if TBP is involved in survival of neurons, we analyzed the phenotype of dTbp mutants, and reduced the expression of dTbp by RNA interference. We first tested if lost of TBP would lead to neurodegeneration. As shown in our result, dTbp mutants exhibit motor defects and shorter life span as compared to wild-type flies. In addition, knocking down the expression of dTbp by RNAi, the fly showed underdevelopment phenotypes, in which they died at larva or pupa stage. Target silencing the endogenous dTbp activity in photoreceptors of flies leads to retinal degeneration. We, thus, concluded that TBP is required for survival of neurons and normal development of *Drosophila*. In other words, expressing expansion polyglutamine in *Drosophila* would cause neurodegenerative phenotypes like pigment lost in eyes, bristles lost or deorganization and ommatidia fusing or deformation in eyes, reducing lifespan, and reducing climbing ability. These phenotypes can also be observed in TBP muntant lines and RNAi lines.This has further strengthened

our notion that loss-of-function of TBP is implicated in polyQ mediated neurodegeneration. And co-expression human TBP with dBP-RNAi can ameliorate the phenotype mostly cause by RNAi, we can deduce that hTBP may replace dTBP partially, because TBP is very conservative between species.

In this study, we also focus on high mobility group box 1 (HMGB1),which can inhibit TBP-dependent transcription by disrupting the formation of HMGB1/TBP/TATA preinitiating complex.when HmgD, a fly homolog of HMGB1, was ectopically expressed in photoreceptors, retinal degeneration has evidently observed. This has supported our idea that disturbing the function of TBP will result in neurodegeneration. To answer whether HMGB1 is involved in SCA17 pathogenesis, we put our SCA17 fly model in HmgD background. We used these phenotypic criteria, including retinal degeneration, mobility and life span, to test if our hypothesis is correct. Indeed, when we co-express TBP36Q with HmgD, the degeneration would be ameliorated. The TBP with expanded polyQ cannot ameliorate it and even deteriorate it. We deduce that the HmgD could disturb endogenous TBP function and overexpress ectopic hTBP can recover the function of dTBP, but if hTBP embrace expanded polyQ would also disturb TBP function.

The molecular pathology of polyglutamine diseases is very complex. Various molecular processes are implicated in the pathology of polyglutamine diseases caused by the expanded polyglutamine-containing proteins, but the mechanism of pathology is not yet clearly understood. We anticipated this study will help to clarify the enigma of pathology of polygluatmine diseases and develop of therapeutic approaches.

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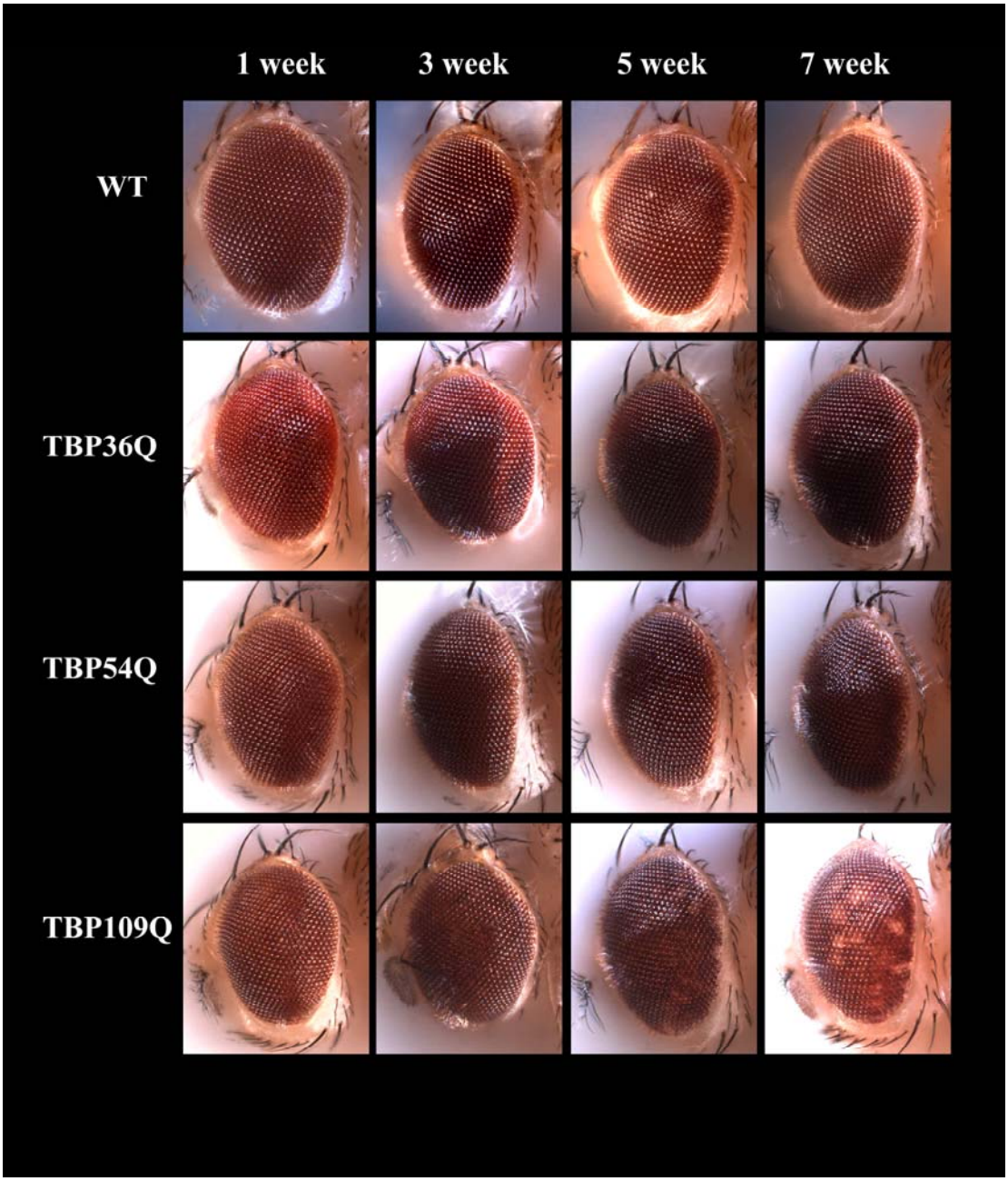
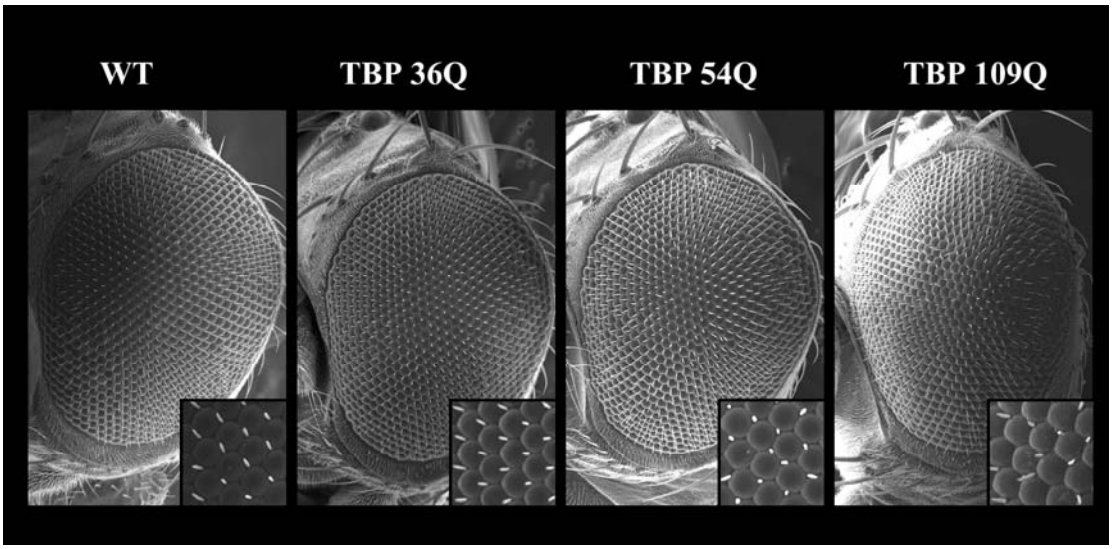


Figure 1. Expression hTBP with polyglutamine expansion in *Drosophila* would induce depigmentation gradually.

Expressing TBP36Q and TBP54Q in eyes of *Drosophila* by GMR-GAL4 would not cause neurodegeneration syndrome and the phenotype would be almost the same as wildtype. Expressing expansion polyglutamine (TBP109Q) in *Drosophila*'s eye would cause depigmentation gradually.

A.



B.

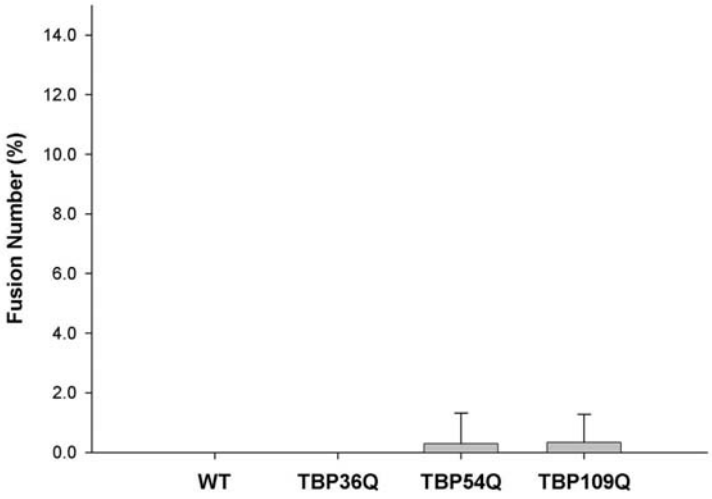
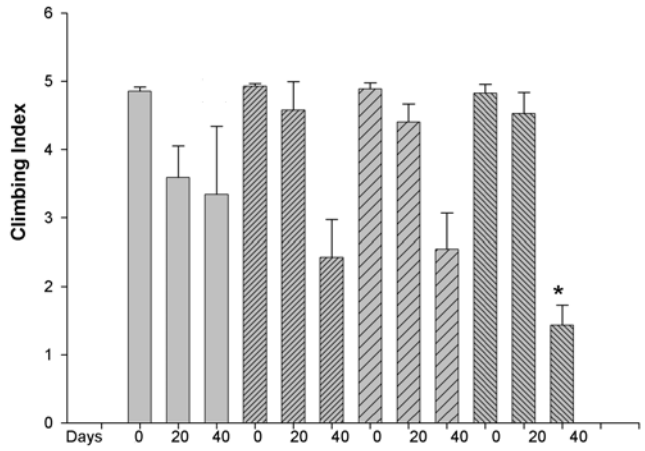


Figure 2. Phenotypes of fly eye models of polyglutamine disease.

(A) SEM images show that expressing TBP36Q in eyes of *Drosophila* by GMR-GAL4 would not cause neurodegeneration syndrome and the phenotype would be almost the same as wildtype which shows regular arrays of ommatia and bristles. Expressing expansion polyglutamine (TBP54Q and TBP109Q) in *Drosophila*'s eye would cause slightly abnormal arrangement of bristles and few ommatia fused. (B) Fusion number analysis of SEM images.

A



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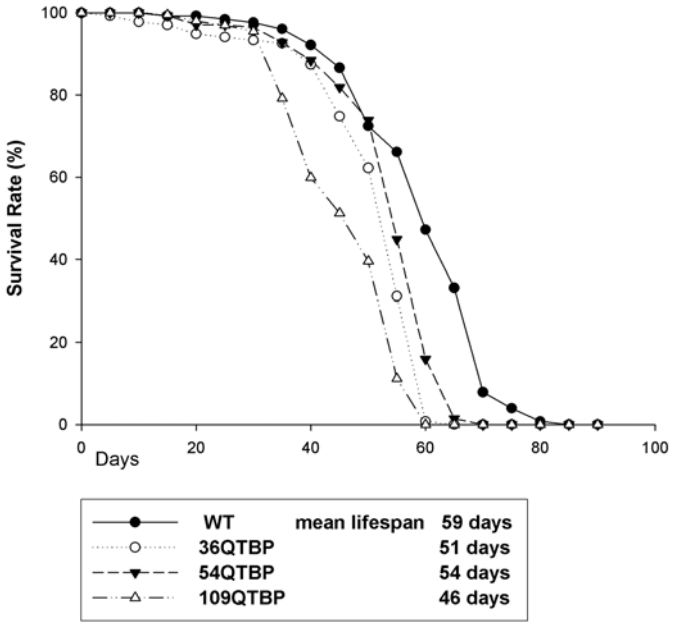

Figure 3. The analysis of climbing ability and lifespan of *Drosophila* model of polyglutamine disease.

(A)Expressing TBP109Q in neuron system by elav-GAL4 cause gradually decline in climbing ability as compared with wildtype. (B) Expressing TBP109Q in neuron system by elav-GAL4 reduced the lifespan significantly.

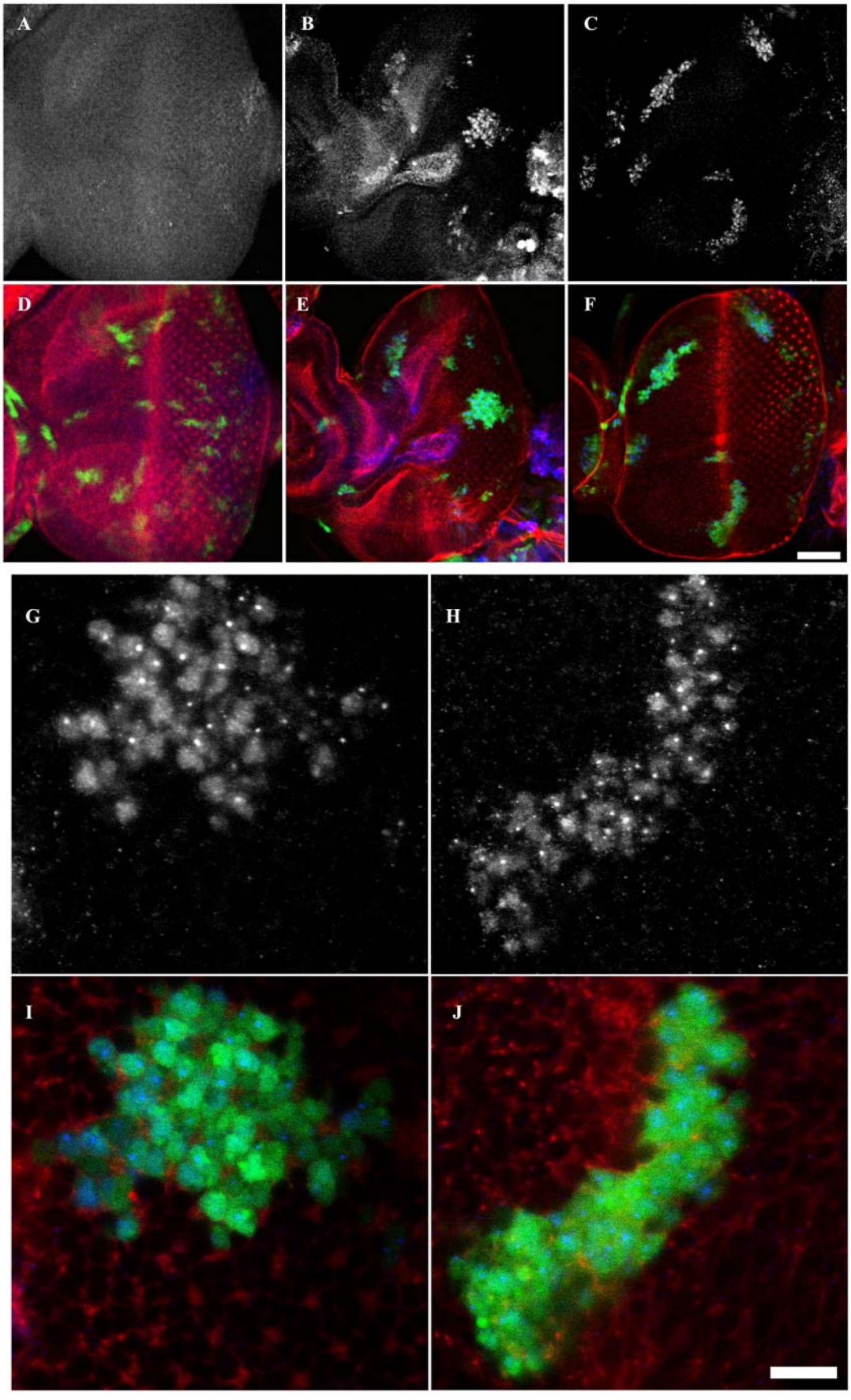
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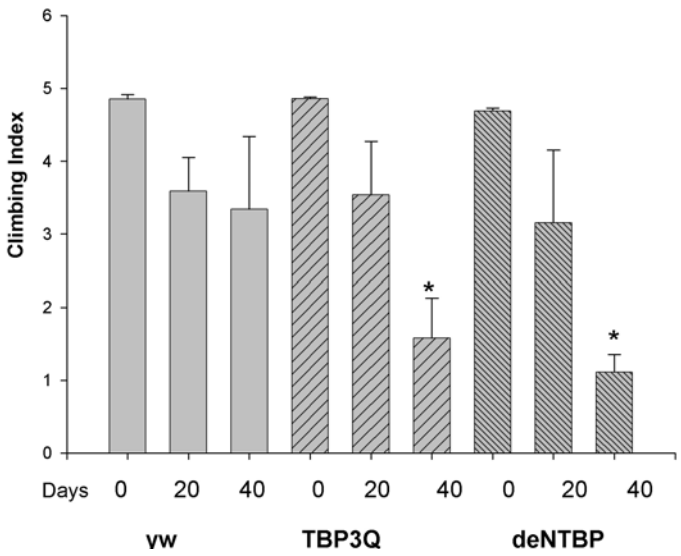
Figure 4. Immunofluorscence staining of *Drosophila* eye disc which express polyQ TBP randomly.

PolyQ TBP would co-express with GFP (green) random spread on eye disc, (D)but random express TBP36Q is hard to detect specific TBP signal(A). Express (E)TBP54Q and (F)TBP109Q can detect specific TBP signal(B & C respectively). (G) and (I) are zoom-in field of (B) and (E), and so forth, (H) and (J) are zoom-in field of (C) and (F), they clearly show that expressing TBP54Q and TBP109Q in eye disc would cause nuclear inclusions formation, the hallmark of polyQ disease.
(A-F scale bar: 40 μm, G-J scale bar: 10 μm)

A.



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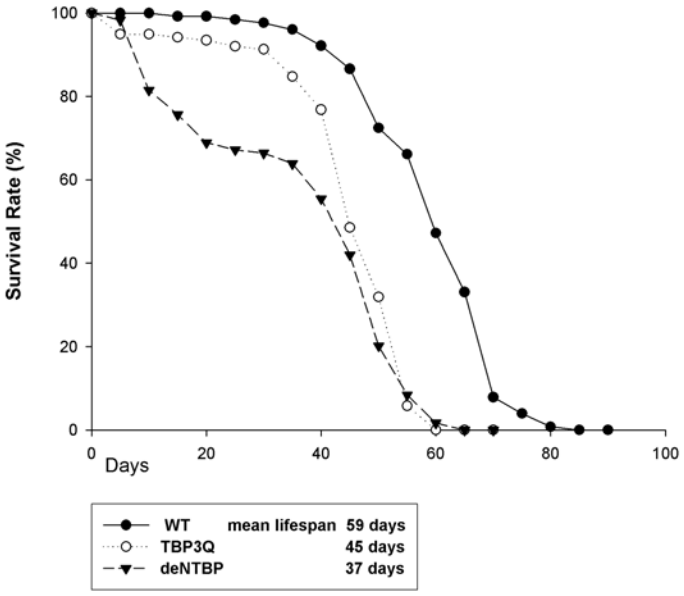
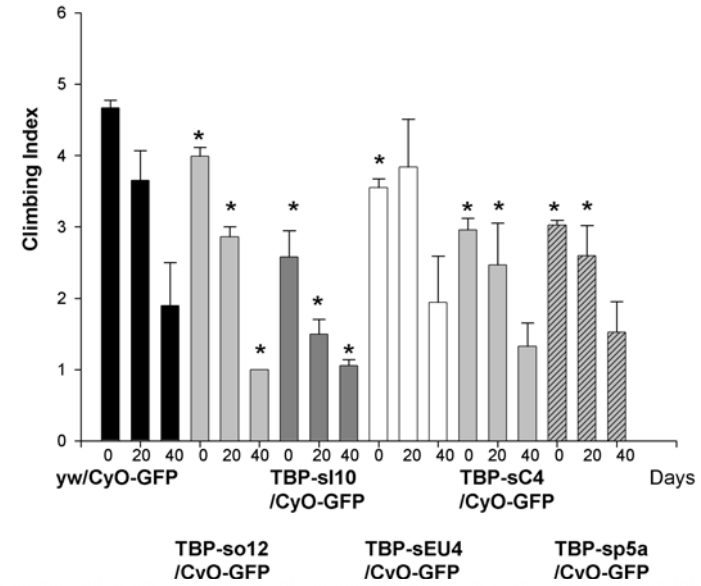


Figure 5. The analysis of climbing ability and lifespan of expressing TBP3Q and DeNTBP.

(A)Expressing TBP3Q and DeNTBP in neuron system by elav-GAL4 cause gradually decline in climbing ability as compared with wildtype. (B) Expressing TBP3Q and DeNTBP in neuron system by elav-GAL4 reduced the lifespan significantly.

A.



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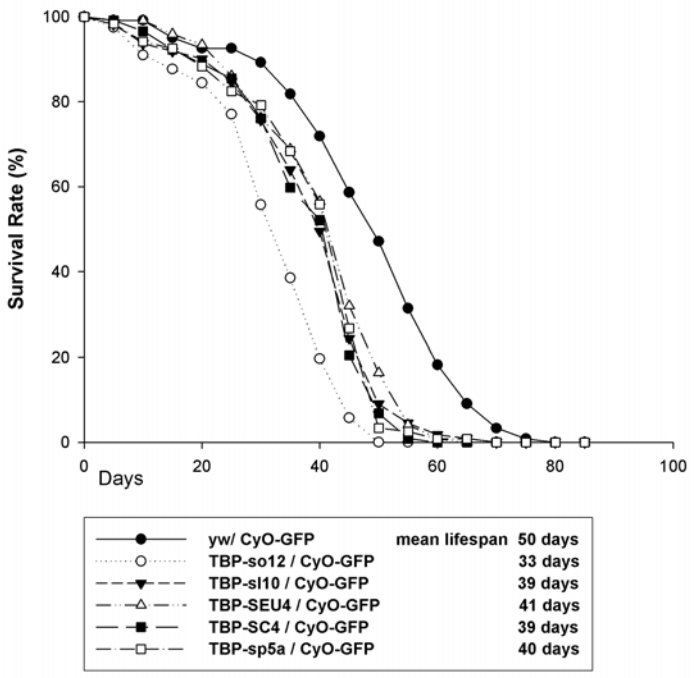


Figure 6. The analysis of climbing ability and lifespan of TBP mutant line.

(A) The *Drosophila* with heterozygotic TBP mutant would gradually decline in climbing ability as compared with wildtype. (B) And their lifespan were reduced significantly.

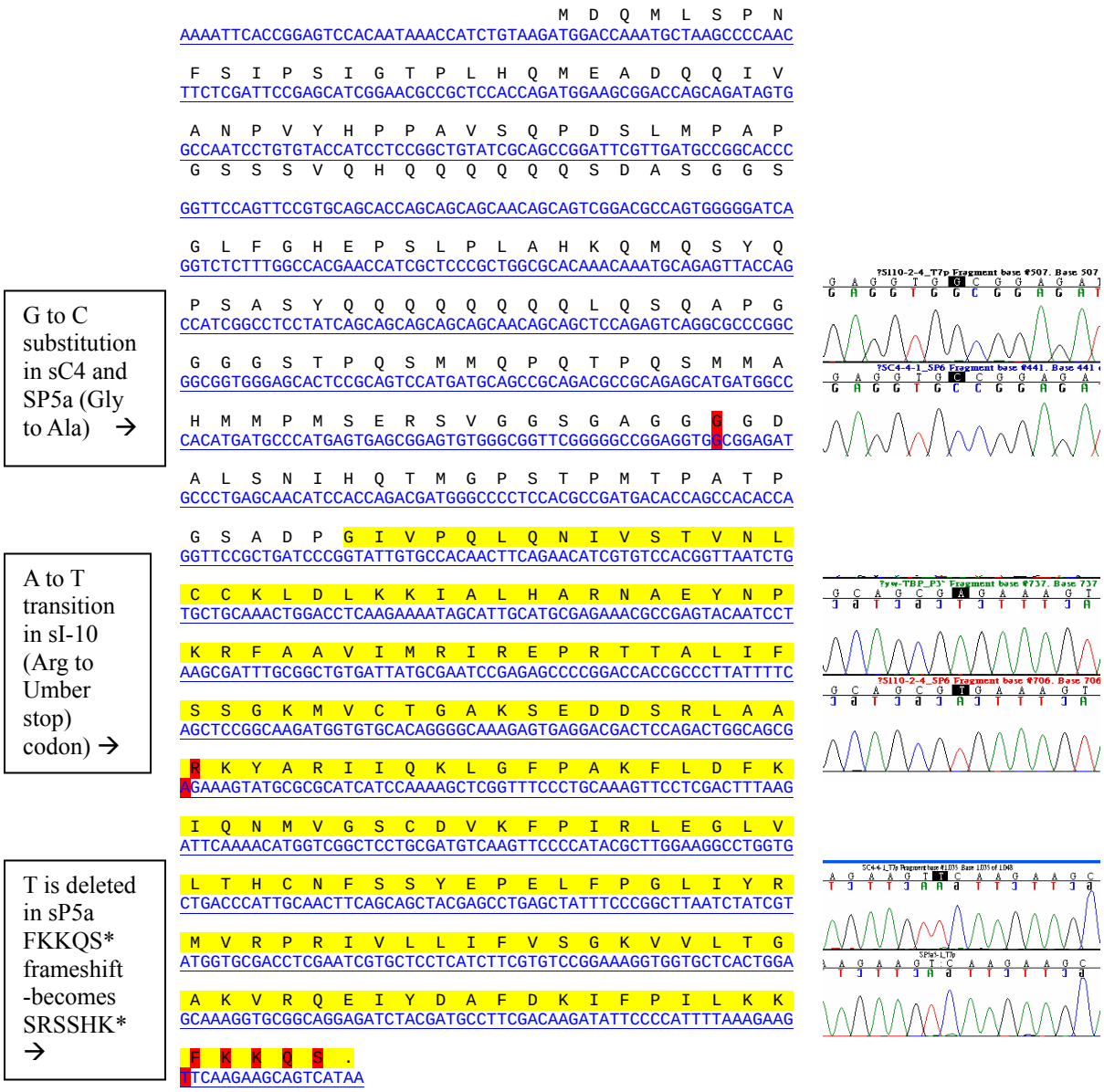


Figure 7. Analysis the molecular genetics background of the TBP mutant line.

Yellow shade shows the conservative C domain, and red shades show the mutants' sites. Chromatograms show the results of sequencing analysis.

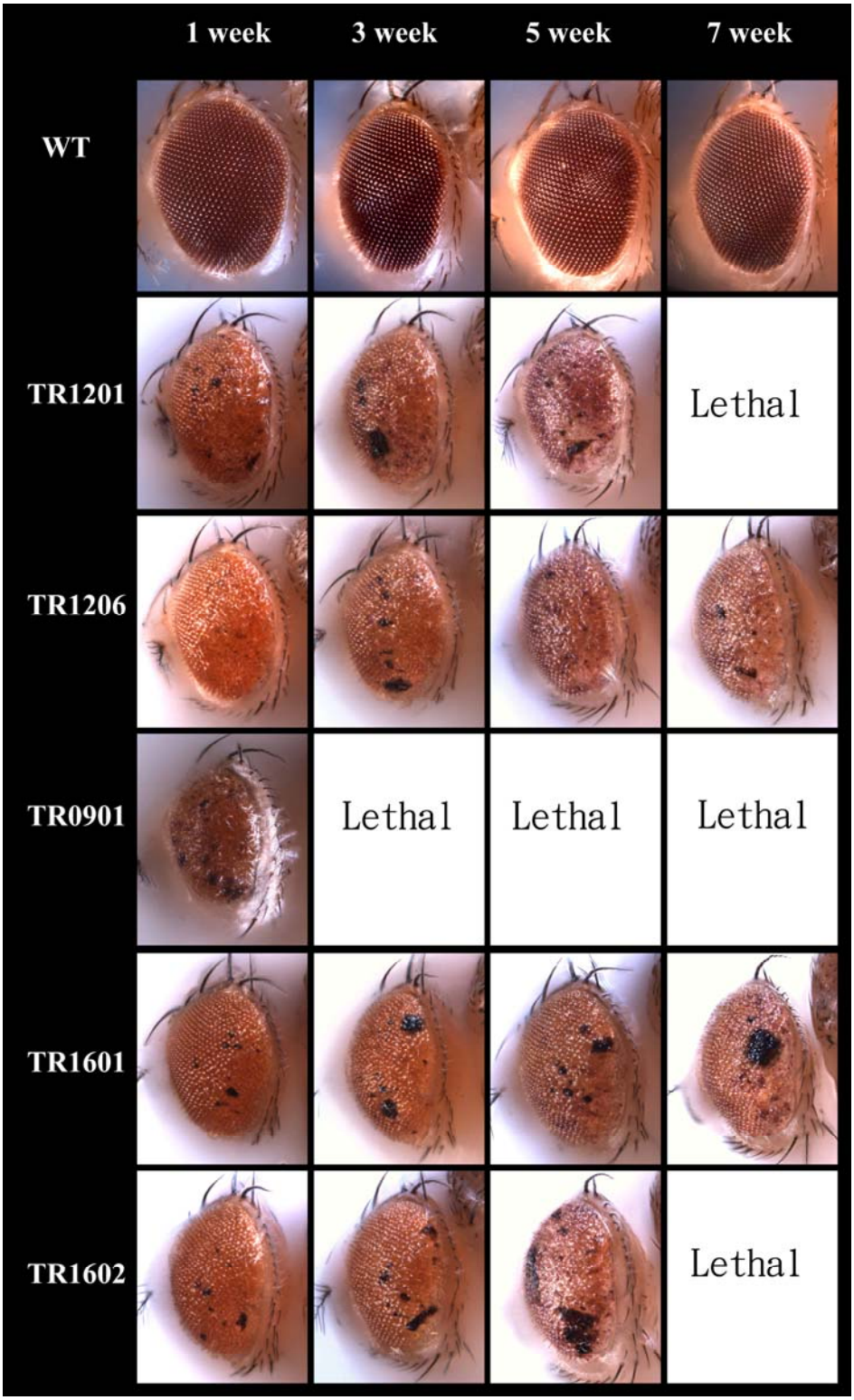
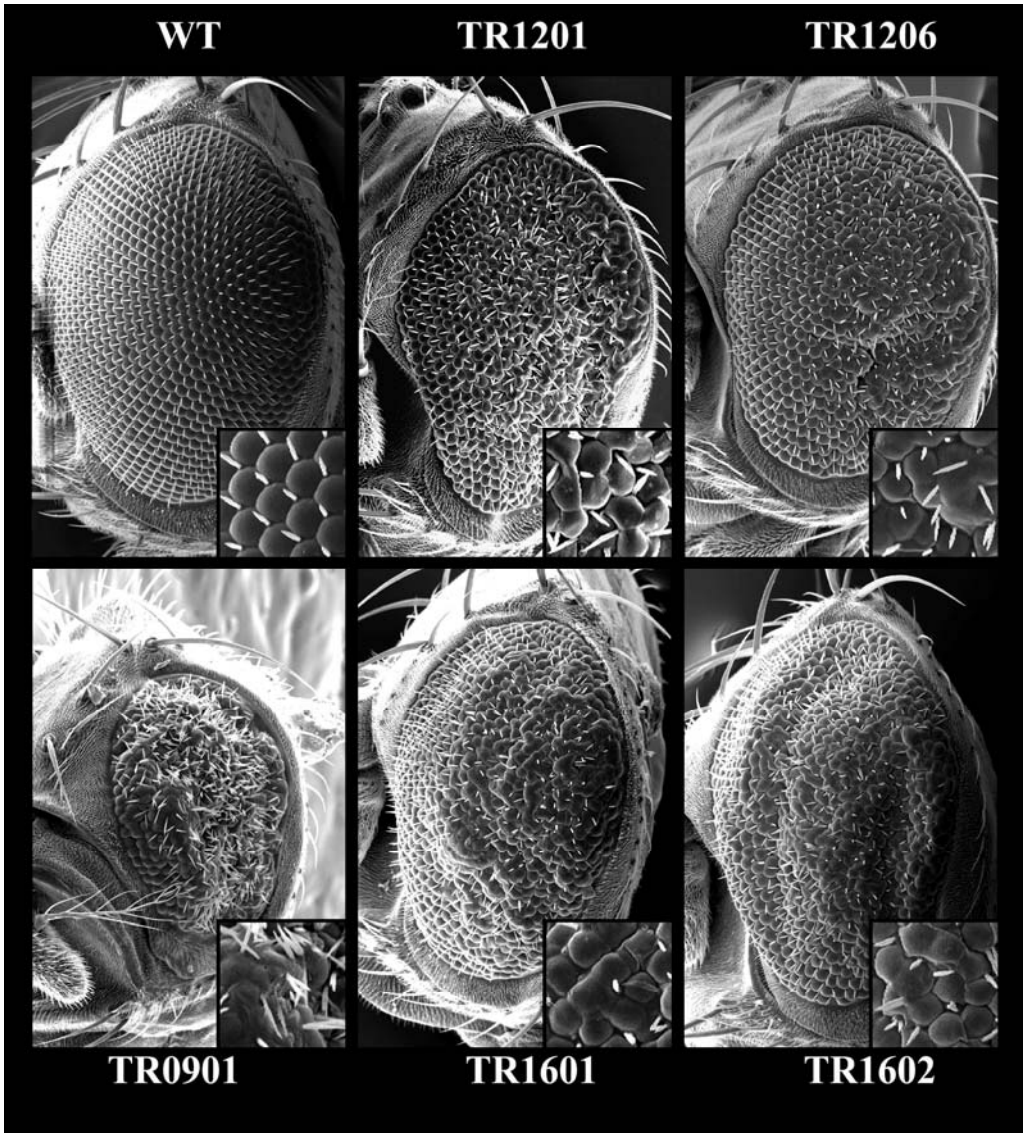


Figure 8. Using RNAi knock down TBP in eyes cause rough eye.

RNAi knock down (dTBP-Ri) cause severe rough eye syndrome, like depigmentation, necrosis, and even shorten the lifespan.

A.



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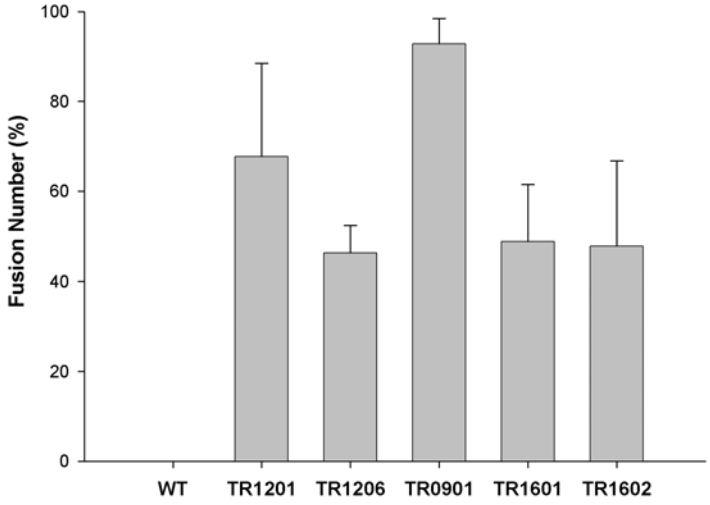


Figure 9. Analysis rough eye caused by dTBP-Ri by SEM.

(A) In flies expressing dTBP-Ri would disrupt the structure and arrangement of ommatidia and bristles. (B) The severe rough syndrome cause a great part of omatidia fused together.

A.

B.

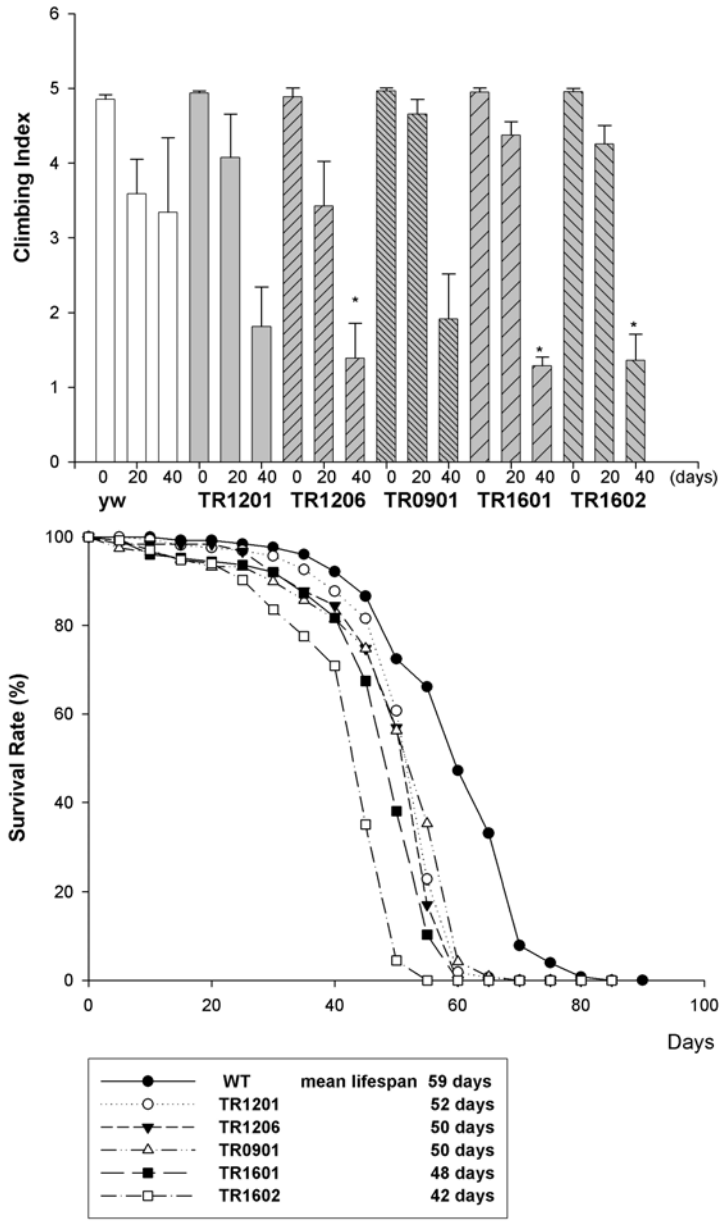


Figure 10. The analysis of climbing ability and lifespan of expressing dTBP-RNAi .

(A) Using RNAi knock down TBP in neuron system by elav-GAL4 cause gradually decline in climbing ability as compared with wildtype, (B) Also reduced their lifespan significantly.

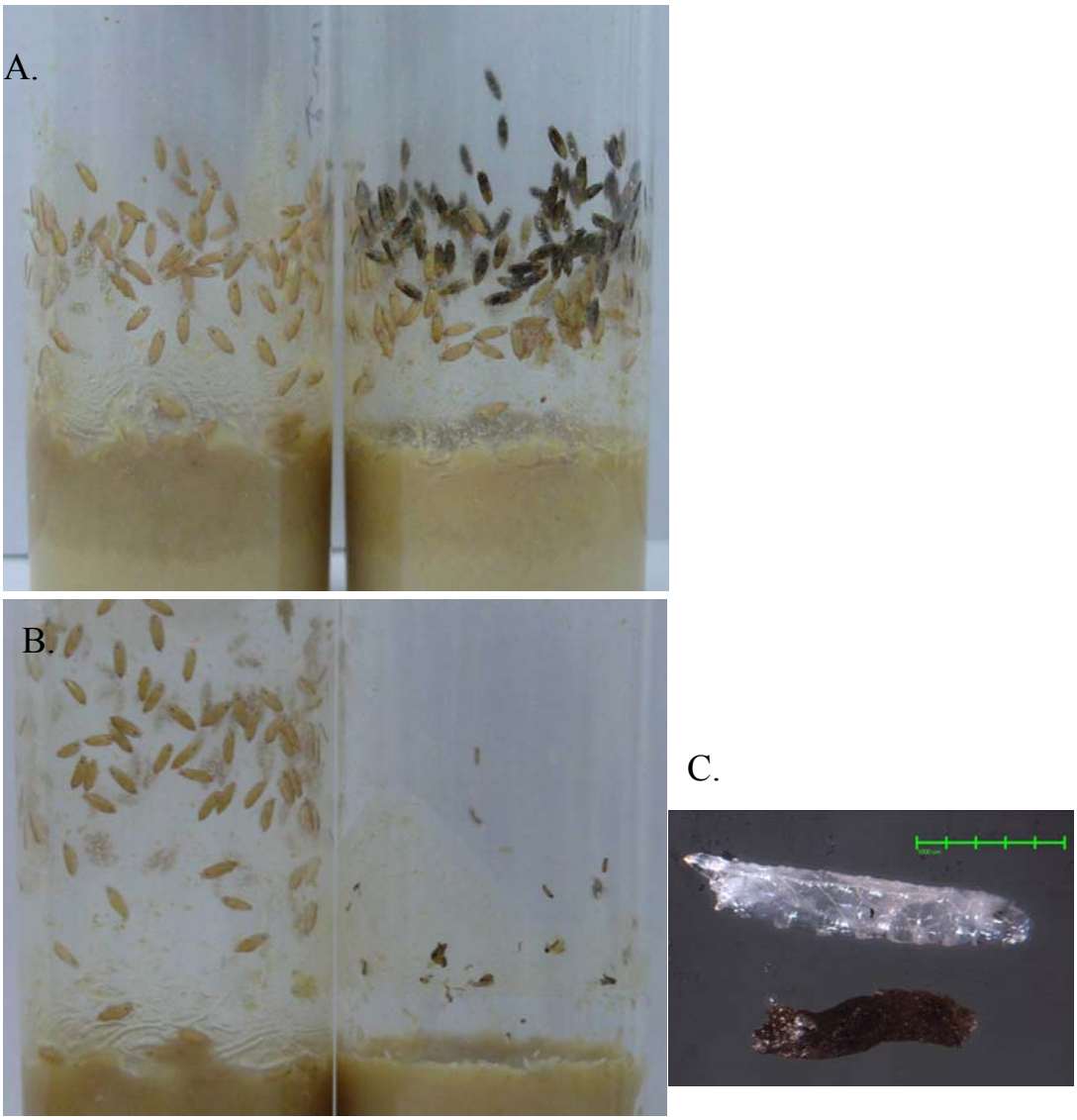
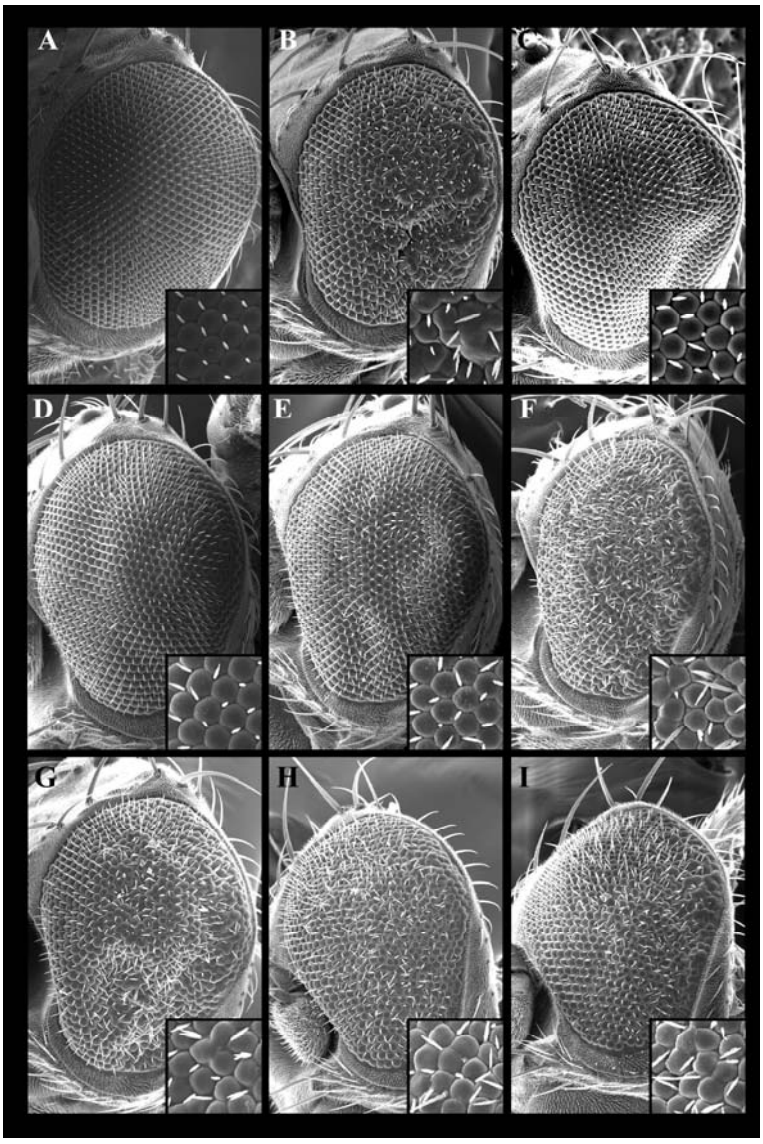


Figure 11. Special phenotype cause by RNAi.

(A) One of the TBP-RNAi lines TR0901 driven by GMR-GAL4 would reduce the eclosion rate severely. Most pupa get dark and die, only one adult, two most, will eclose alive per vial (left vial is wildtype). (B) TR0901 driven by arm-GAL4, which expresses in whole body, would cause second-instar larva climb upward and get dark and die(C, upper is the wildtype second-instar larva).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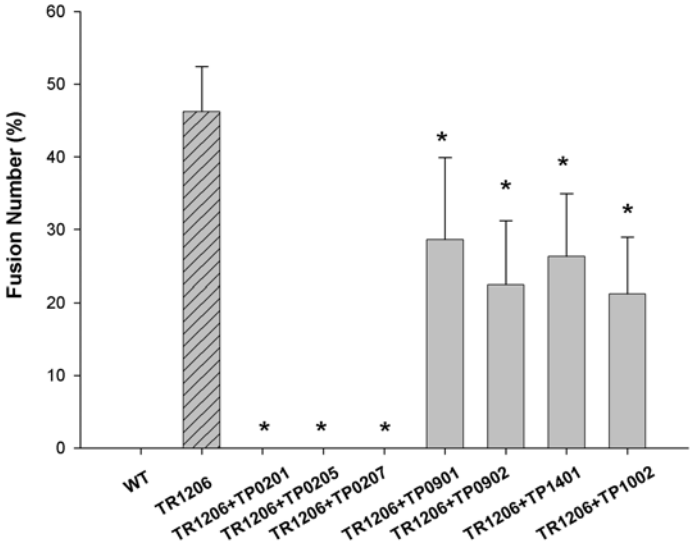

Fusion Number (%)
60
50
40
30
20
10
0
*
*
*
*
*
*
*
WT
TR1206
TR1206+TP0201
TR1206+TP0205
TR1206+TP0207
TR1206+TP0901
TR1206+TP0902
TR1206+TP1401
TR1206+TP1002

Figure 12. Co-express UAS-dTBP would ameliorate the RNAi syndrome.

(A) Wildtype, (B) TR1206, (C-I) co-express TR1206 with different UAS-dTBP lines, which are TP0201, TP0205, TP0207, TP0901, TP0902, TP1401, and TP1002, respectively. (J) Analysis the percentage of fusion number of ommatidia reveals that the co-expression of UAS-TBP can ameliorate the degeneration syndrome caused by RNAi.

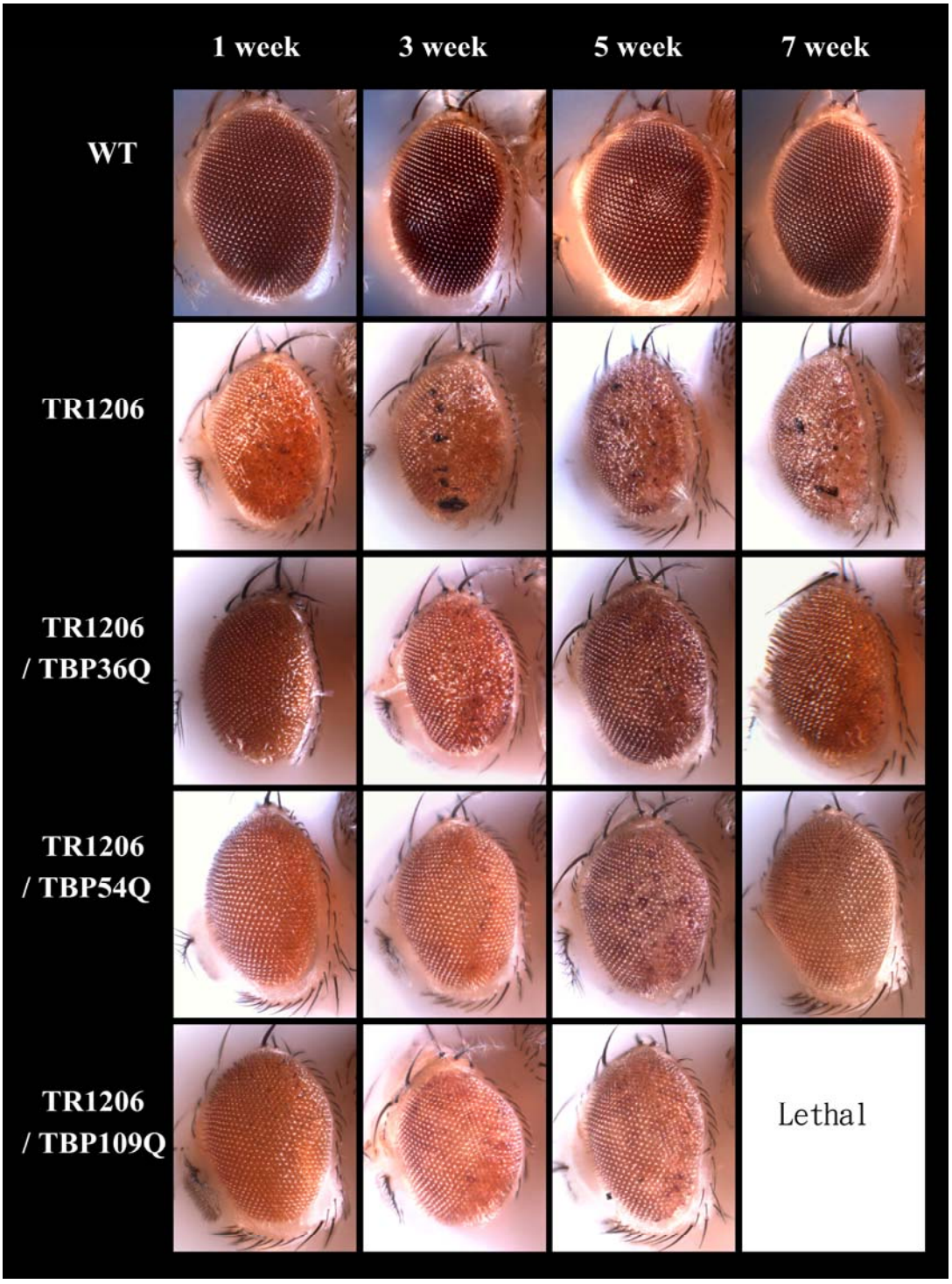
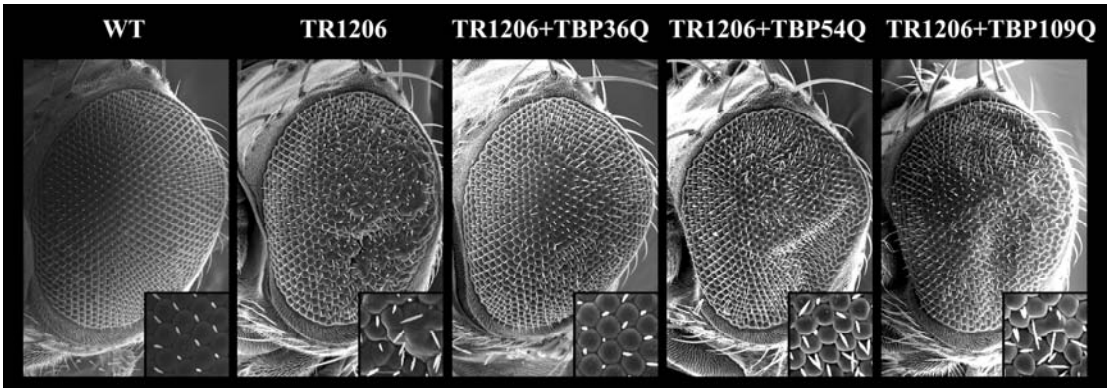


Figure 13. Co-express human polyglutamine TBP with TBP-RNAi.

Human TBP36Q can ameliorate the syndrome cause by RNAi, like deformation of ommaditia and depigmentaion. The effect of co-expression TBP54Q or TBP109Q is less then TBP 36Q.

A.



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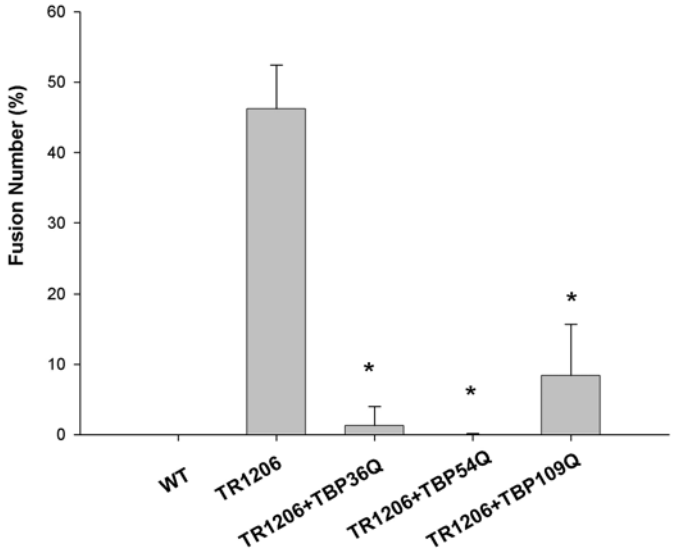


Figure 14. Analysis SEM images of co-express human polyglutamine TBP with TBP-RNAi.

(A) co-express TBP36Q can ameliorate the deformation caused by RNAi greatly. TBP54Q and TBP109Q can also ameliorate the syndrome, but we can observe a part of deformation on ommatidia and abnormal bristles arrangement, and the effect cause by co-epression TBP109Q is less then TBP54Q. (B) Counting the fusion number reveal that co-expression of human polyglutamine TBP can reduce the number of ommatidia fusion.

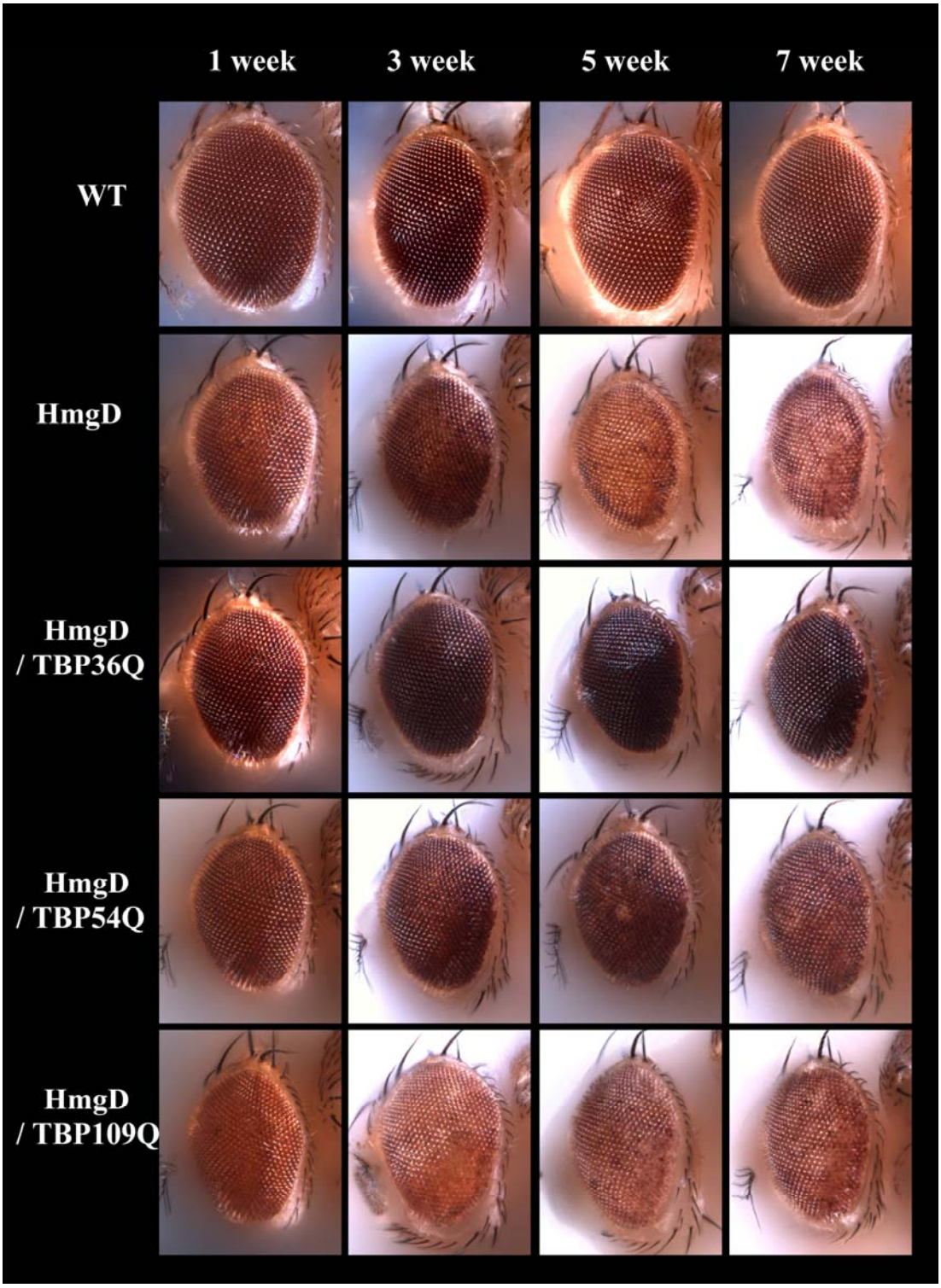
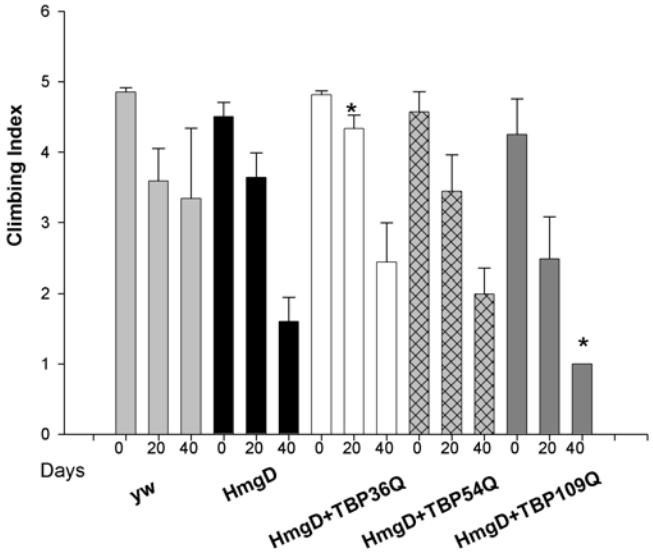


Figure 15. HmgD can cause depigmentation on *Drosophila* eye. TBP36Q can ameliorate pigment lost, but expanded polyQ would interfere with the amelioration.

Transcription repressor HmgD can inactivate TBP function, overexpress HmgD in Drosophila eye would cause depigmentation. Co-expression TBP36Q with HmgD can ameliorate it. Co-expess TBP54Q can also ameliorate it, but pigment still lost gradually. Co-express TBP109Q with HmgD cause more severe depigmentation than expression of HmgD only.

A.



B.

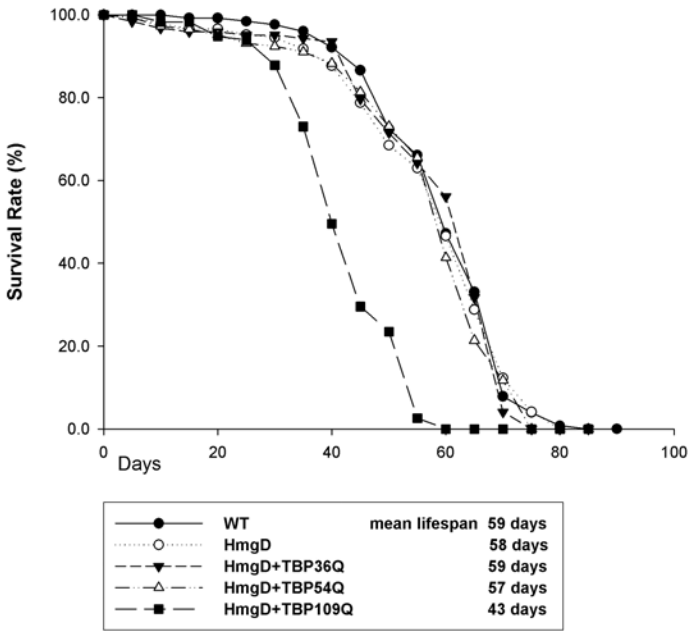


Figure 16. The analysis of climbing ability and lifespan of expression of HmgD and co-expression with human polyQ TBP.

(A) HmgD would reduce climbing ability of *Drosophila* . TBP36Q can ameliorate the climbing ability, but expanded polyQ would interfere with the amelioration. (B) HmgD would't reduce lifespan, But co-expression with TBP109Q would reduce the lifespan a little more than expression of TBP109Q.

Supplemental figure

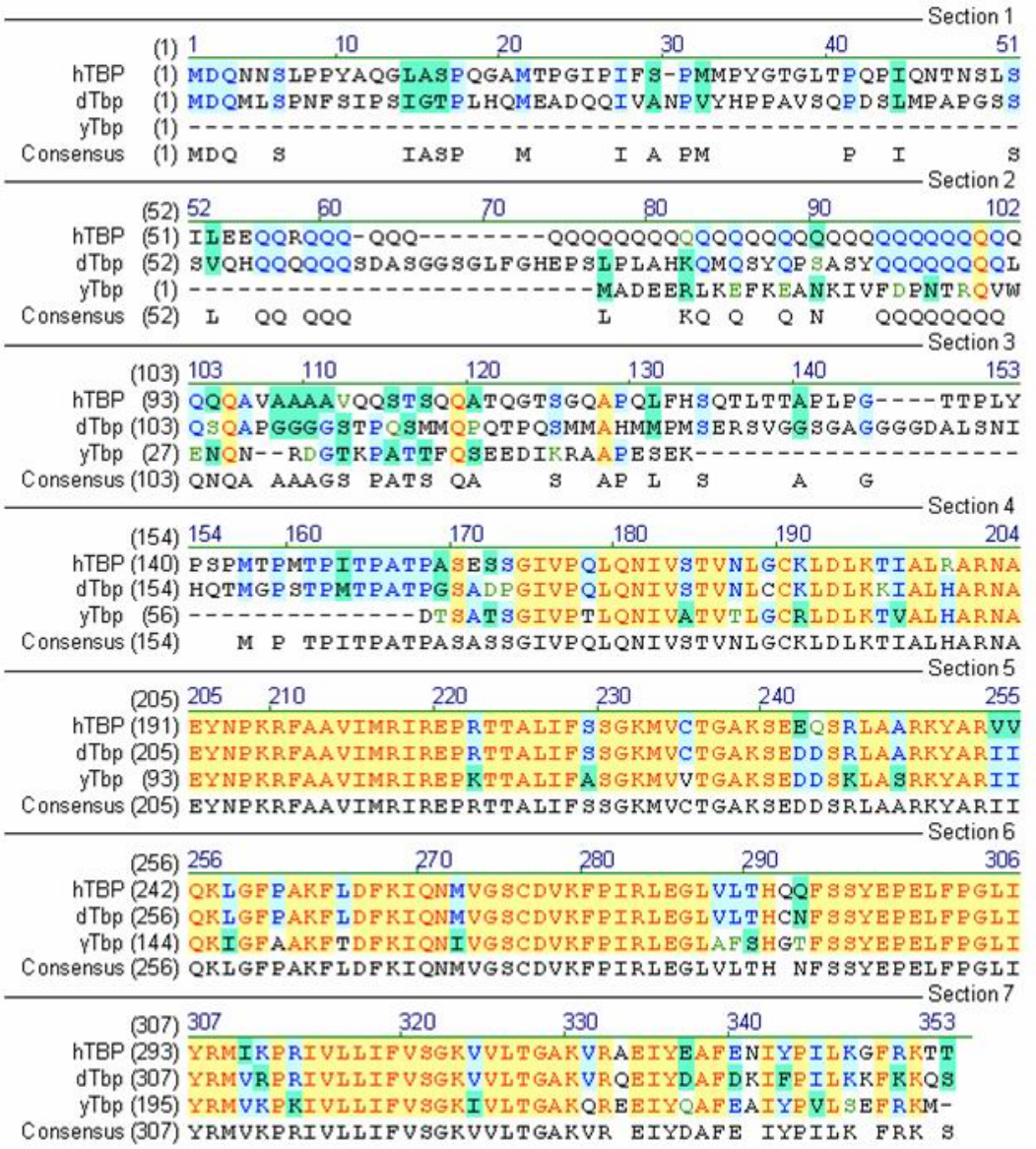


Figure S1. Comparison of different species amino acid sequence of TBP.

N-terminal domain (NTD) is species specific and the C-terminal domain (CTD) is highly conserved. The CTD of TBP is enough for functions of TBP including DNA binding, protein interaction and assembly into TFIID, and directing transcription initiation. The NTD has been supposed to regulate the DNA-binding activity of the CTD. In NTD of TBP of eukaryotes except yeast, there is an glutamine stretch in TBP.

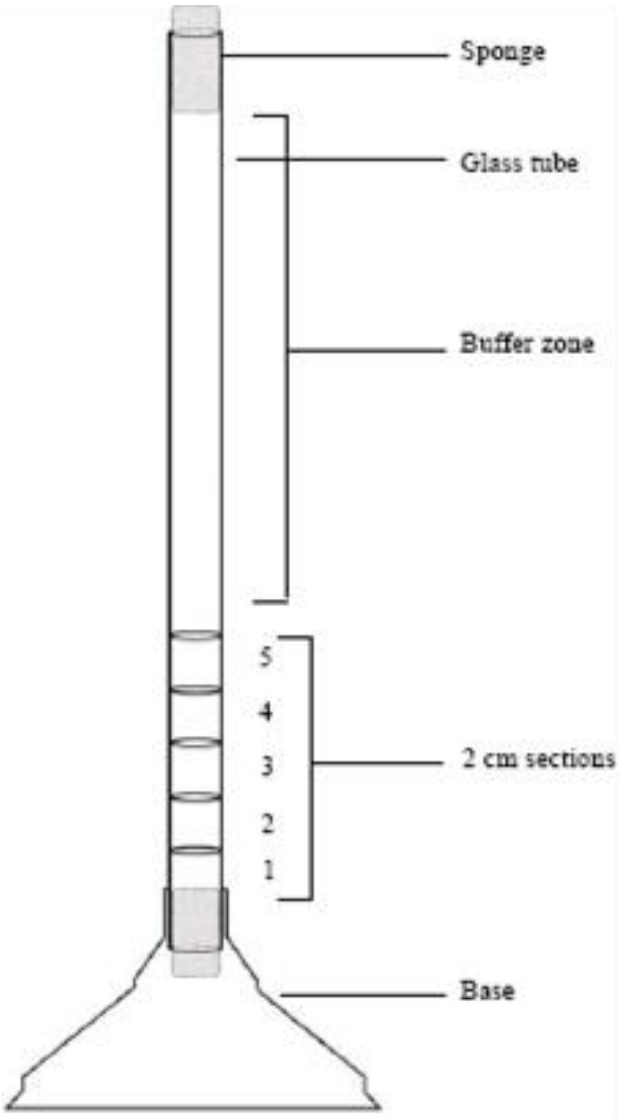


Figure S2. Schematic representation of the climbing apparatus.

The apparatus consists of a 30 cm long glass tube, with a diameter of 1.5 cm. The tube is held in place by a stale base. The glass tube is divided into five, 2 cm sections (1-5) with a buffer zone at the top of the apparatus. The ends of the tube are plugged with sponges to prevent escape of flies (Todd and Brian, 2004).

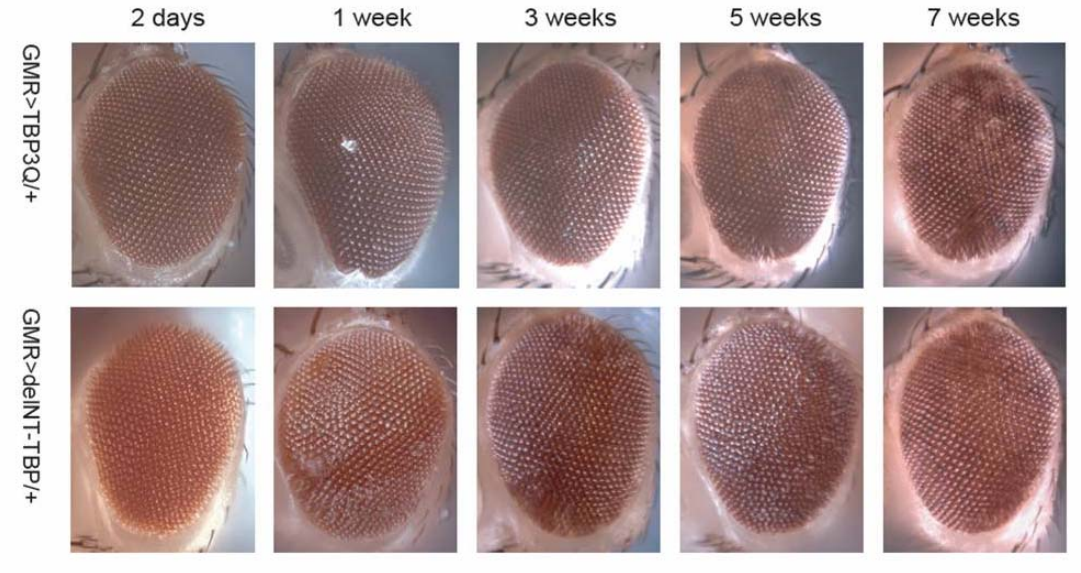


Figure S3. Expression TBP3Q and DelNT-TBP in *Drosophila* would induce depigmentation gradually. (by Dr. Wang, Cheng-Kuang)

The truncated form of human TBP, TBP3Q and C terminal domain(DelN-TBP) would cause depigmentation gradually, the phenotype are similar to TBP109Q.

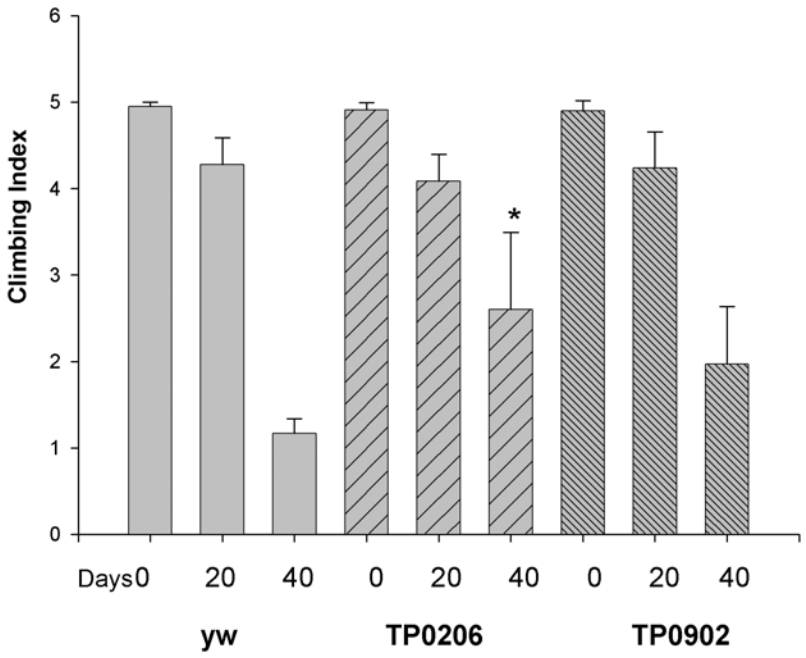


Figure S4. Express UAS-dTBP can retard the age-dependent reduction of climbing ability.

Slight express UAS-dTBP in whole body by hs-GAL4 can retard the age-dependent reduction of climbing ability.