9-2002

Eye Suppression, A Novel Function of Teashirt, Requires Wingless Signaling

Amit Singh
University of Dayton, asingh1@udayton.edu

Madhuri Kango-Singh
University of Dayton, mkangosingh1@udayton.edu

Y. Henry Sun
Academia Sinica

Development 129, 4271-4280 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
DEV5027

Eye suppression, a novel function of *teashirt*, requires Wingless signaling

Amit Singh, Madhuri Kango-Singh and Y. Henry Sun*

Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, 11529 Taiwan, Republic of China
*Author for correspondence (e-mail: mbyhsun@ccvax.sinica.edu.tw)

Accepted 14 May 2002

SUMMARY

***teashirt* (*tsh*) encodes a *Drosophila* zinc-finger protein. Misexpression of *tsh* has been shown to induce ectopic eye formation in the antenna. We report that *tsh* can suppress eye development. This novel function of *tsh* is due to the induction of *homothorax* (*hth*), a known repressor of eye development, and requires Wingless (WG) signaling. Interestingly, *tsh* has different functions in the dorsal and ventral eye, suppressing eye development close to the ventral margin, while promoting eye development near the dorsal margin. It affects both growth of eye disc and retinal cell differentiation.**

Key words: *Drosophila*, Eye, *teashirt*, WG

INTRODUCTION

The compound eye of adult *Drosophila*, which consists of a hexagonal array of about 800 ommatidia, develops from the larval eye disc. The eye disc differentiates progressively in a posterior to anterior direction, with a morphogenetic furrow (MF) marking the front of the differentiation wave. A small number of genes (*eyeless*, *sine oculis*, *eyes absent* and *dachshund*) encoding nuclear factors have been identified to be important for eye formation. Loss-of-function mutations in these genes block eye development, while targeted expression, alone or in combination, can induce ectopic eyes (for reviews, see Desplan, 1997; Treisman, 1999; Heberlein and Treisman, 2000). However, several genes are known to block eye formation. *homothorax* (*hth*), which encodes a homeodomain protein (Rieckhof et al., 1997; Pai et al., 1998; Kurant et al., 1998), is expressed in the anterior margin of the eye disc. Mutant *hth* clones cause ectopic eye formation in the ventral head, whereas ectopic *hth* expression in the eye field blocks MF initiation and progression (Pai et al., 1998; Pichaud and Casares, 2000). Signaling by Wingless (WG), expressed along the anterolateral margins, also blocks MF initiation and progression (Ma and Moses, 1995; Treisman and Rubin, 1995). *extra macrochaetae* (*emc*) and *hairy* (*h*), both of which encode transcription factors, are expressed anterior to the MF and act redundantly to block MF progression (Brown et al., 1995). *teashirt* (*tsh*) encodes a nuclear protein with zinc-finger motifs (Fasano et al., 1991). It is involved in embryonic trunk segmental identity (Fasano et al., 1991; Roder et al., 1992; de Zulueta et al., 1994; Alexandre et al., 1996) and midgut morphogenesis (Mathies et al., 1994), and confers proximal identity in leg development (Erkner et al., 1999; Wu and Cohen, 2000). A role for *tsh* in eye development was initially suggested because the eyes of flies trans-heterozygous for *tsh* and gain-of-function *Antennapedia* mutations were reduced and partially transformed to head cuticle (Bhojwani et al., 1997). Pan and Rubin (Pan and Rubin, 1998) showed that targeted misexpression of *tsh* could induce *eyeless* (*ey*) expression and generate ectopic eyes in the antenna. In this study, we report that *tsh* has a novel function in suppressing eye development. This eye suppression function is achieved through the induction of *hth* and requires WG signaling.

Although the cellular composition of each ommatidium is identical, their spatial arrangements show mirror symmetry over the dorsoventral (DV) midline (equator) in the eye (Wolff and Ready, 1993). Early eye primordia is subdivided into dorsal and ventral compartments (Cho and Choi, 1998; Dominguez and de Celis, 1998; Papayannopoulos et al., 1998; Cavodeassi et al., 1999). Many genes exhibit DV asymmetry in their expression and/or function in the eye. Some genes (e.g. *wg*) have symmetrical DV expression, but are regulated differently or have DV differential functions. For example, the dorsal-specific expression of the *iro-C* genes (McNeill et al., 1997; Dominguez and de Celis, 1998; Cavodeassi et al., 1999; Cavodeassi et al., 2000) requires WG and Hedgehog signaling (Heberlein et al., 1998; Cavodeassi et al., 1999). The dorsal, but not ventral, *wg* expression in turn requires the GATA factor *pannier* (Maurel-Zaffran and Treisman, 2000; Lee and Treisman, 2001). WG induces *mirr* in dorsal and contributes to dorsal eye fate (Heberlein et al., 1998), whereas *wg* on the ventral margin can induce and maintain *hth*, a negative regulator of eye (Pai et al., 1998), and suppress ventral eye fate (Pichaud and Casares, 2000). We found that although *tsh* was expressed in a DV symmetrical pattern in the eye disc, its function in the eye showed DV asymmetry: *tsh* suppresses eye development in the ventral region, but promotes eye development in the dorsal region. The effect of *tsh* is probably on both early eye disc growth and photoreceptor differentiation. We also show that misexpression of *tsh* has DV differential effects in the antennal disc, but not in the wing disc.

These disc- and position-dependent effects are presumably due to the involvement of additional factors.

MATERIALS AND METHODS

Targeted misexpression

We used the *GAL4*/*UAS* system for the targeted misexpression (Brand and Perrimon, 1993). *tsh-GAL4* (Shiga et al., 1996), *dpp-GAL4* (Staehling-Hampton and Hoffmann, 1994), *ey-GAL4* (Hazelett et al., 1998), *bi-GAL4* (Calleja et al., 1996), *UAS-tsh* (Gallet et al., 1998), *UAS-hth* (Pai et al., 1998), *UAS-fluΔarm* (Zecca et al., 1996), *UAS-wg* (Azpiazu and Morata, 1998), *UAS-sgg* (Hazelett et al., 1998) and *UAS-dTCF$^{\Delta N}$* (van de Wetering et al., 1997) were used. The flies were cultured at three different temperatures: 18°C, 25°C and 29°C to sample the effect of different induction level.

Clonal induction of expression

w; P(Act>y^{+}>GAL4)25 P(UAS-GFP^{S65T})/CyO (Ito et al., 1997) and *y w hsFLP^{122}* (Struhl and Basler, 1993) were used for generating expression clones. All other stocks were constructed using these stocks by suitable genetic crosses. Embryos were collected at 12 hours interval at 25°C, and subjected to a single 1 hour heat shock at 37°C at about 24 hours after egg laying (AEL) or as indicated. The larvae were transferred to 25°C for recovery and further development.

wg^{ts} effect on *tsh* function

We used the temperature-sensitive allele *wg^{IL114}* (Nusslein-Volhard et al., 1984; Treisman and Rubin, 1995). The F_1 progeny of the genotypes *w; wg^{IL114}; dpp-GAL4 /SM6-TM6B* and *w; wg^{IL114}*; *UAS-tsh* were grown at 17°C and shifted to 29°C at various developmental stages for a period of 24 hours and returned to 17°C for further development. The eye phenotypes were studied in imaginal discs dissected from Tb^{+} third instar larvae or in pharate adults.

Generation of loss-of-function clones of *tsh*

tsh is located on the second chromosome at 40A, too close to the FRT(40A) for recombination onto the FRT chromosome to generate loss-of-function clones (Xu and Rubin, 1993). A null allele of *tsh*, *tsh^{8}* (Fasano et al., 1991) was used to generate loss-of-function clones by X-ray irradiation following the protocol of Wu and Cohen (Wu and Cohen, 2000). A viable enhancer trap insertion, *tshA8*, with the P[*lacW*] inserted near the *tsh* locus (Sun et al., 1995), served as a marker for the *tsh^{+}* chromosome. *tsh^{8}* clones were generated by irradiating *tsh^{8}/tshA8* larvae (Fig. 4A), from 6 hours egg collections, with 4000 rads of X-ray at 48-72 hours or 72-90 hours AEL. In adult eyes, *tsh^{8}* clones were detected by the loss of eye color, which is dependent on the mini-*white* reporter gene in *tshA8*. As mini-*white* in *tshA8* causes eye color only in the anterior half of the eye, corresponding to the *tsh* expression domain, clones in the *tsh* non-expressing posterior half of the eye cannot be detected. Loss-of-function clones in the posterior region of the third instar eye disc cannot be marked, but only deduced from their effects.

Immunohistochemistry

Eye-antennal and wing imaginal discs were dissected from wandering third instar larvae in 1×phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde (in PBS) for 20 minute. They were washed three times with PBST (1×PBS+2% Triton-X-100) for 10 minute each and blocked in 10% normal goat serum for 1 hour. The discs were incubated overnight at 4°C in rat anti-ELAV (1:200) (Developmental Studies Hybridoma Bank) and one of the following primary antibodies: rabbit anti-β-GAL (1:800) (Cappel), mouse anti-WG (1:20) (Steve Cohen), rabbit anti-HTH (1:200) (Pai et al., 1998) or rabbit anti-EY (1:200) (Uwe Walldorf). The discs were washed in PBST twice for 10 minute each and blocked again for 30 minutes in 10% goat serum. Secondary antibodies (Jackson Laboratories) were donkey anti-rat IgG conjugated to Cy5 (1:200), donkey anti-rabbit IgG conjugated to Cy3 (1:400) or goat anti-mouse IgG conjugated to FITC or Alexa Fluor 488 (1:200). The discs were incubated with secondary antibodies for about 2 hour and washed in PBST for 10 minute. The discs were mounted in DABCO (Sigma) mountant in 90% glycerol and photo-documented on a Zeiss LSM510 confocal microscope.

RESULTS

Ectopic *tsh* expression can suppress eye development

Ectopic induction of *tsh* under *dpp-GAL4* (*dpp>tsh*) could occasionally cause the formation of an ectopic eye at the base of the antenna (Fig. 1a, arrowhead), as previously reported (Pan and Rubin, 1998). In addition, about 8% (17/212) of the *dpp>tsh* flies showed a split-eye phenotype. Nearly 42% (89/212) of the *dpp>tsh* individuals arrested and died at the white pupal stage. Their body size was about 50% larger than normal. These larvae have extended larval period (by 2-3 days) and their eye-antennal discs showed overgrowth and distorted morphology. These discs showed both variable ectopic HTH induction and eye suppression phenotypes, from a small group of ELAV-positive photoreceptor cells (Fig. 1b) to near complete absence of ELAV-positive cells (Fig. 1c). Occasionally, HTH induction extended from the posterior margin towards the MF and the endogenous eye field was split into two (Fig. 1b). The frequency of splitting of the eye field was comparable with the split-eye phenotype in the adult flies. These observations suggested that the *dpp>tsh* split-eye phenotype was due to suppression of the eye fate by *tsh*, resulting in the splitting of the endogenous eye field. Consistent with this interpretation, when two copies of *UAS-tsh* were driven by the *dpp-GAL4* (*dpp*>2X*tsh*), the ventral half of the endogenous eye was completely absent in all flies (Fig. 1d), indicating that higher levels of *tsh* expression is capable of completely suppressing ventral eye development. Ectopic induction of *tsh* by *ey-GAL4* (*ey>tsh*), which is expressed in the embryonic eye primordium and in the eye disc (Hazelett et al., 1998), caused a complete suppression of eye development (Fig. 1e,f) with 97% penetrance. Only 2.4% (2/83) of the *ey>tsh* flies have very small eyes.

tsh-mediated eye suppression is *hth*-dependent

In *dpp>tsh*, the frequency of HTH induction correlated with the frequency of eye suppression (see above), suggesting that *hth* may be responsible for the eye suppression. *dpp>tsh* in a heterozygous *hth* mutant background resulted in the reduction of the split-eye frequency to 0.6% (2/164; from 8% in *dpp>tsh*) in pharate adults (Fig. 1g). The pupal lethality was also reduced to 16% (27/164) from 42%. These results suggested that these phenotypes are *hth*-dependent. *dpp>hth* completely suppressed eye development (Pai et al., 1998). Co-expression of *tsh* and *hth* (*dpp>tsh+hth*) also completely suppressed eye development and induced no antennal eye (Fig. 1h), suggesting that *hth* acts downstream of, or in parallel to, *tsh.*

Expression of *tsh* overlaps with *hth* and *ey*

As ectopic *tsh* could regulate *hth* expression, we compared their endogenous expression in imaginal discs. Expression of *tsh* was examined using *tsh-GAL4* (Shiga et al., 1996)-driven *UAS-GFP* (*tsh>GFP*). In the eye disc, *tsh* expression could be detected as

early as first larval instar in the entire disc proper, overlapping with *hth* and the pro-eye gene *eyeless* (*ey*) (Fig. 2a). In the late second instar eye disc (Fig. 2b), *tsh>GFP* expression retracts anteriorly and occupied nearly three quarters of the disc. *hth* expression also retracts anteriorly, as also reported by Pichaud and Casares (Pichaud and Casares, 2000). EY is also expressed in the same region (Fig. 2b) (Halder et al., 1998). In early third instar eye discs (Fig. 2c), *tsh* expression regresses to the anterior two-thirds of the disc. *hth* expression is restricted to the anterior margin in a 10- to 15-cell wide domain. *tsh* and *hth* expression overlaps in a 3- to 4-cell wide stripe. EY expression (Fig. 2c) (Halder et al., 1998) largely overlapped with *tsh*. In late third instar eye disc (Fig. 2d), *tsh>GFP* expression was anterior to the MF and was similar to the expression pattern determined by *tsh-lacZ*, anti-TSH antibody and in situ hybridization (Sun et al., 1995; Bhojwani et al., 1997; Pan and Rubin, 1998). The co-expression of *tsh* and *hth* during the early phase of eye disc development is consistent with the finding that *tsh* induces *hth* expression.

In late third instar wing disc, *tsh* is expressed in a proximal ring around the wing pouch and in most of the notum (Fig. 2e) (Sun et al., 1995; Bhojwani et al., 1995; Casares and Mann, 2000; Azpiazu and Morata, 2000), largely overlapping with, but broader than, *hth* expression (Fig. 2e) (Pai et al., 1998; Azpiazu and Morata, 2000; Casares and Mann, 2000). EY is not expressed in the wing disc (Halder et al., 1995). In the antennal disc, *tsh* is expressed weakly in an anteroproximal region (Bhojwani et al., 1997; Pan and Rubin, 1998), while *hth* is expressed in the proximal region (Rieckhof et al., 1997; Pai et al., 1998; Casares and Mann, 1998).

tsh suppresses eye in ventral margin and promotes eye in dorsal margin

*dpp>*2X*tsh* showed suppression only of the ventral eye (Fig. 1d). We tested this ventral bias using *bi-GAL4*. *bi>GFP* is expressed at the dorsal and ventral margins of the eye disc (Fig. 3a). *bi>tsh* resulted in HTH induction and ELAV suppression only in the ventral region of the eye disc (Fig. 3b, arrow), and the absence of ventral eye in pharate adults (Fig. 3c, arrow). By contrast, *bi>hth* resulted in eye suppression in both dorsal and ventral regions in the eye disc (not shown) and in the adult (Fig. 3d, arrows).

Clonal induction of *tsh* expression [abbreviated *Act>tsh*, as the expression is driven by an *Actin* promoter using the flp-out system of Ito et al. (Ito et al., 1997)] could induce HTH and suppressed photoreceptor development in the eye disc, but only along the ventral margin (Fig. 3e, arrowhead). The induction of *hth* is at the transcription level, because an enhancer trap *(hth$^{1422-4}$) lacZ* reporter is also induced (not shown). *Act>tsh* in the dorsal margin of the eye disc, unlike the ventral clones, could cause overgrowth of the eye cells (Fig. 3e, arrow). Although the *Act>tsh* clones were not marked in the adult eye, adult flies with clonal induction of *tsh* showed ventral eye suppression (Fig. 3f, arrow) and dorsal eye enlargement (Fig. 3g, arrow). Internal *Act>tsh* clones located away from the margin in both the dorsal and ventral eye (not shown), irrespective of their size, did not affect the eye fate.

The null alleles of *tsh* are embryonic lethal and the available hypomorphic alleles do not show any eye defects (Bhojwani et al., 1997; Pan and Rubin, 1998). We generated loss-of-function clones of *tsh^{8}*, a null allele, by X-ray irradiation (Fig. 4a) (Wu and Cohen, 2000) at different time windows beginning from 48 to 96 hours after egg laying (AEL). Eye phenotypes were observed only in flies irradiated around 52-64 hours AEL. *tsh^{8}* clones located in ventral margin of the adult eye caused ventral eye enlargements (Fig. 4c,d, arrow). Internal ventral clones did not significantly affect eye development (not shown) (Pan and Rubin, 1998). *tsh^{8}* clones in the posterior region of the third

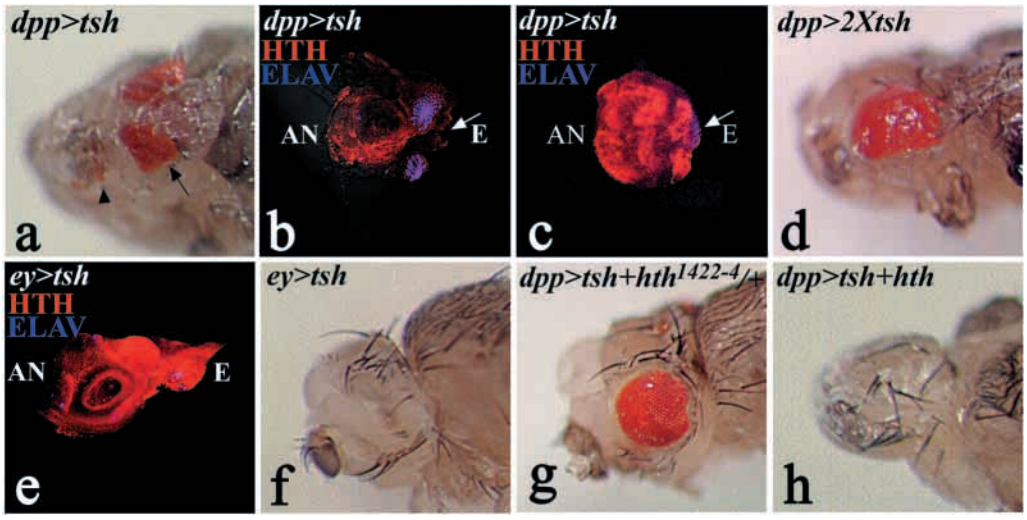

Fig. 1. Ectopic *tsh* can induce HTH and suppress eye development. (a) *dpp>tsh*, which drives expression of *tsh* along the lateral and posterior margin of the eye disc, caused splitting of the endogenous eye (arrow indicates the ventral eye) and induced an ectopic eye at the base of the antenna (arrowhead) in the pharate adult. All eye discs in this and subsequent figures are oriented anterior towards the left and dorsal towards the top. (b,c) *dpp>tsh* eye disc (photoreceptors labeled by ELAV, blue; HTH, red). Eye disc (E) is highly reduced (arrow) relative to the antenna disc (AN). (d) *dpp>2Xtsh* caused suppression of the ventral eye. (e) *ey>tsh* caused complete loss of eye (ELAV, blue) and ectopic induction of HTH (red). The size of the eye disc is extremely reduced. (f) *ey>tsh* adult showed complete loss of eye. (g) *dpp>tsh* in an *hth$^{1422-4}$/+* background resulted in pharate adult with rescue of the *dpp>tsh* split-eye phenotype and (h) *dpp>tsh+hth* caused complete eye loss.

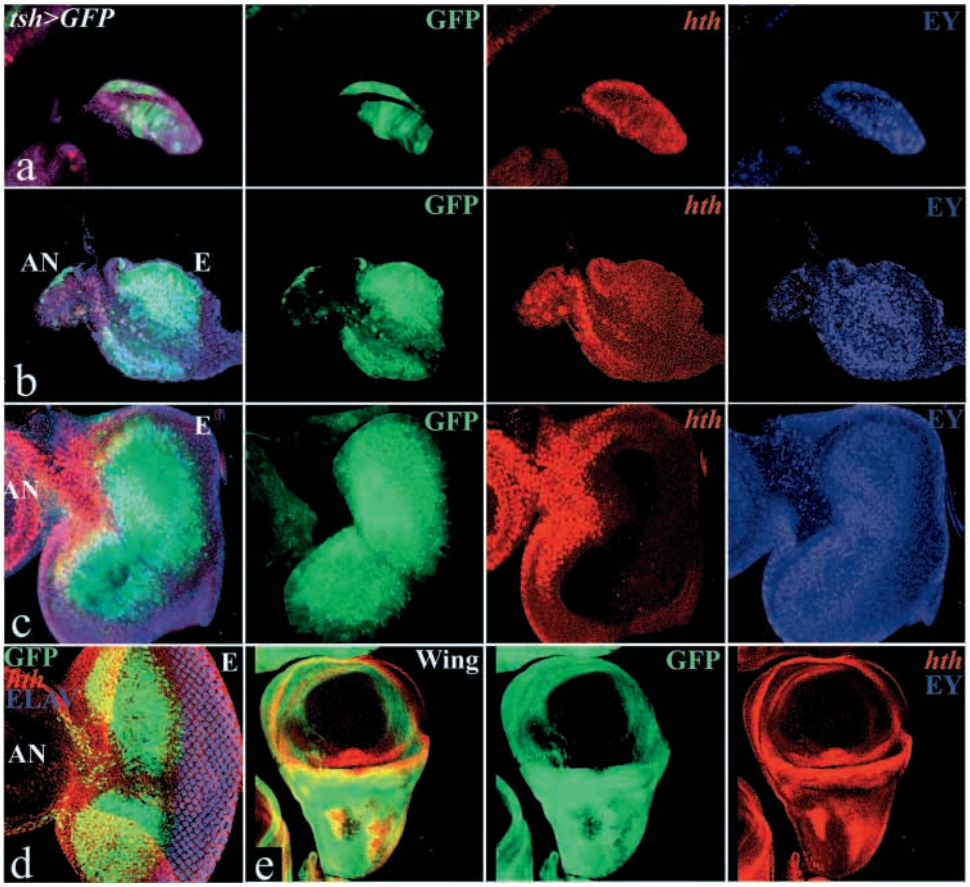

Fig. 2. Expression pattern of *tsh* relative to *hth* and EY in eye and wing discs. *tsh-GAL4* (Shiga et al., 1996) driven *UAS-GFP* and $hth^{1422\text{-}4}$ (an enhancer trap insertion in *hth*) (Kurant et al., 1998) and anti-EY antibody (Halder et al., 1998) were used to examine the expression patterns of *tsh*, *hth* and EY (GFP, green; *hth-lacZ*, red; and EY, blue) in (a) first instar eye-antenna disc, (b) second instar eye disc, (c) early third instar eye disc, (d) late third instar eye disc and (e) late third instar wing disc. Differentiated photoreceptors in d were marked by ELAV (blue). (AN, antenna disc; E, eye disc)

instar eye disc could not be marked, because at this stage *tsh* (and the *tshA8* reporter) expression has already retracted from this region of early expression. However, after clone induction, ventral enlargements of the eye field, corresponding to the adult eye phenotype, were seen in the eye discs (Fig. 4e,f, arrows). In rare cases ectopic ventral eyes were also observed (Fig. 4g, arrow). tsh^{8} clones located in the dorsal eye suppressed eye fate and caused eye-to-cuticle transformation (Fig. 4h,i, arrow). One eye disc had a complete absence of the dorsal eye field (Fig. 4j, arrow). These phenotypes were not found when wild-type larvae were similarly treated by X-ray irradiation. These results suggested that the normal function of *tsh* is to suppress eye fate in the ventral eye and to promote eye fate in the dorsal eye during early second instar.

WG signaling contributes to *tsh* eye-suppression function

Pichaud and Casares (Pichaud and Casares, 2000) reported that *hth* and *wg* are involved in a positive feedback loop only in the ventral eye disc, but not in the dorsal region. Gallet et al. (Gallet et al., 1998; Gallet et al., 1999) showed that TSH binds ARM, a downstream component of WG signaling. We therefore, checked the role of WG signaling in the *tsh*-mediated HTH induction.

Clonal induction of *tsh* together with a constitutively activated ARM (Zecca et al., 1996) caused ectopic induction of HTH and suppressed eye development both in dorsal (Fig. 5a, arrow) and ventral domains, and both in marginal and internal regions of eye disc (Fig. 5a). Some of these *Act>tsh+arm* clones were also associated with tissue overgrowth as seen in adult eye (not shown). These phenotypes were similar to ectopic *hth* expression with the exception of tissue overgrowth (Azpiazu and Morata, 2000; Casares and Mann, 2000; Goto and Hayashi, 1999; Jaw et al., 2000; Pai et al., 1998). *Act>arm* showed variable phenotypes: an internal ventral clone could suppress eye (Fig. 5b, arrow), whereas a clone at the posterior margin could not suppress eye fate (Fig. 5b, arrowhead). *dpp>tsh+arm* resulted in complete suppression of eye (not shown), similar to the *dpp>hth* phenotype (Pai et al., 1998). In *bi>tsh+arm*, eye is reduced in both dorsal and ventral margins in discs and in adults (not shown). *Act>tsh+wg* also resulted in HTH induction and eye suppression in dorsal eye disc (Fig. 5c). In *bi> tsh+wg*, the eye field was extremely reduced at both dorsal and ventral margins in discs (Fig. 5d) and in adults (not shown). These results suggested that WG signaling can collaborate with TSH for HTH-mediated suppression of eye fate.

The requirement of WG signaling in the *tsh*-mediated eye suppression was examined by co-expressing *tsh* with antagonists of WG signaling. $dTCF^{\Delta N}$, a dominant negative form of dTCF, can block the WG signaling (van de Wetering et al., 1997). Shaggy zeste white-3 (SGG) also acts as an antagonist of WG signaling (Hazelett et al., 1998; Heslip et al., 1997). *Act>*$dTCF^{\Delta N}$*+tsh*, unlike *Act>tsh*, failed to induce HTH and suppress eye development, irrespective of the dorsal or ventral domain, in both discs (Fig. 5e) and adults (not shown). *Act>*$dTCF^{\Delta N}$ did not induce HTH or suppress eye fate (not shown). As expected, *bi>tsh+*$dTCF^{\Delta N}$ did not show suppression of the eye fate both on the dorsal and ventral margins in the eye disc (not shown) and in adult (Fig. 5f). In *ey>tsh+*$dTCF^{\Delta N}$ and *ey>tsh+sgg* eye discs and flies, there was no eye suppression (not shown). Similarly, *Act>tsh+sgg* (Fig. 5g) and *bi>tsh+sgg* (not shown) did not induce HTH or suppress eye development both in the ventral or dorsal margin in the disc and in flies. *Act>sgg* did not suppress eye fate (not shown). These observations suggest

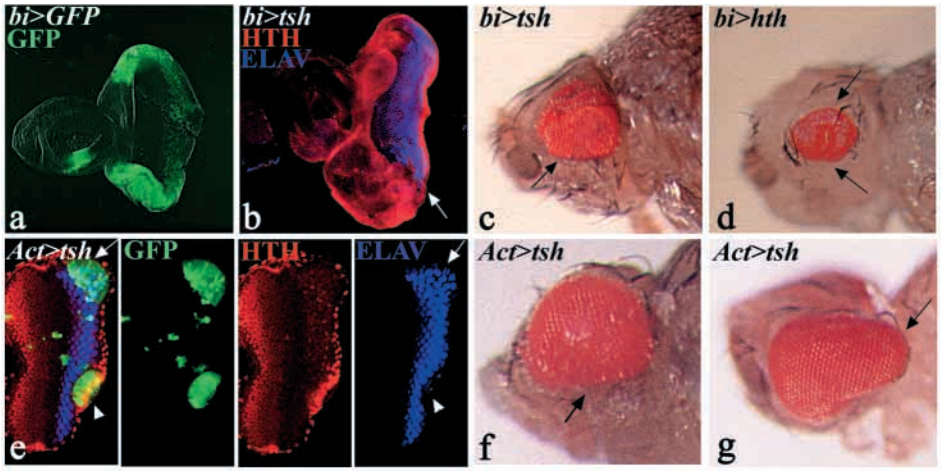

Fig. 3. DV differential effects of *tsh* misexpression. (a) *bi>GFP* marks *bi-GAL4* expression domains along the dorsal and ventral margins of the eye disc, and in a dorsal sector of the antennal disc. The DV axis (as defined by the ventral *wg* and dorsal *dpp* expression) of the antenna disc (Theisen et al., 1996) is reversed from that of the eye disc. (b) *bi>tsh* caused eye suppression and ectopic HTH (red) induction only in the ventral eye margin (arrow) of the eye disc. The eye disc is also enlarged. (c) *bi>tsh* pharate adult showed ventral eye suppression (arrow) and dorsal eye overgrowth. (d) *bi>hth* adult showed eye suppression on both dorsal and ventral eye (arrows). The adult eye phenotype was usually more severe than the disc phenotype. (e) *Act>tsh* clone (marked by GFP: green) induced HTH (red) autonomously and suppressed eye development (ELAV: blue) in the ventral margin of the eye disc (arrowhead). Clone in the dorsal margin did not induce HTH but caused overgrowth (arrow). *Act>tsh* clones were not marked in adults, but flies with clone induction showed ventral suppression (f, arrow) or dorsal enlargement (g, arrow) in the eye.

that WG signaling is required for the ventral eye suppression mediated by *tsh*.

The temporal requirement of WG signaling was examined by misexpressing *tsh* in wg^{ts} mutant flies (see Materials and Methods). When the flies were shifted to the restrictive temperature 48-72 hours AEL, the frequency of split-eye phenotype caused by *dpp>tsh* was reduced to 2% (4/228). The frequency of white pupal lethality was reduced to 19% (43/228). Temperature shifts in other time windows did not affect the frequency and severity of the split-eye phenotype (not shown). This critical period corresponds to the second instar larval stage and is consistent with the above finding that the *tsh* function is required during this period.

TSH affects growth of eye disc

To examine whether *tsh* has a direct effect on eye disc growth, we measured the relative frequency and size of *Act>GFP* and *Act>tsh+GFP* clones induced at the same time (early first instar and second instar). *Act>GFP* clones were equally abundant both in the dorsal and ventral eye (Fig. 6a), but *Act>tsh+GFP* clones were very scarce. The frequency of the dorsal *Act>tsh+GFP* clones (16 clones) was higher than that of the ventral *Act>tsh+GFP* clones (four clones). Twelve of the 16 dorsal *Act>tsh+GFP* clones showed overgrowth (Fig. 6b). By comparison, only one of the four ventral internal *Act>tsh+GFP* clones showed weak overgrowth (Fig. 6c). The other three ventral clones were smaller than the average *Act>GFP* clones (not shown). These results suggested that *tsh* is involved in growth regulation and has opposite effect in the dorsal and ventral region.

TSH also has DV differential effect in antennal disc

tsh also showed DV differential activities in the leg disc (Erkner et al., 1999). We examined whether similar DV differential activities of *tsh*, and the *tsh-hth* relationship, also occurs in the wing and antennal discs. *Act>tsh* in the wing disc induced HTH (Fig. 7a), as previously reported (Azpiazu and Morata, 2000; Casares and Mann, 2000). Induction of *hth* is at the transcriptional level (data not shown**)** **(**Casares and Mann, 2000). Unlike the eye and leg discs, there is no DV differential activity in the wing discs. HTH suppresses *wg* expression in the presumptive wing margin, while enhancing *wg* expression in the hinge region (Azpiazu and Morata, 2000; Casares and Mann, 2000). However, clonal induction of *tsh*, while inducing HTH, has no effect on WG in the wing pouch (Fig. 7b, arrow). These results suggested that TSH, in addition to inducing HTH in the wing, has another function: it prevents *wg* from being suppressed by HTH. *dpp>tsh* induced HTH along the AP compartmental boundary in the wing pouch and resulted in splitting of the wing pouch (Fig. 7c, arrow), as evident from splitting of the DV border-specific WG stripe. *bi>tsh* could induce HTH in the wing pouch (which spans the AP border) and splits the wing pouch (Fig. 7d, arrow), whereas *bi>hth* on its own could not split the wing field (Fig. 7e). These results again suggest that TSH has functions in addition to that of inducing HTH. The induction of HTH in *bi>tsh* wing disc showed no DV difference (Fig. 7d).

In the antennal disc, clonal induction of *tsh* in the ventral domain (Fig. 7f, arrow) caused a duplication of the antennal field as shown by duplication of the ventral WG expression domain (Fig. 7f). This phenotype could also be seen in an adult where the antennal segments distal to AN2 were duplicated (Fig. 7g, arrow). HTH is repressed within the *Act>tsh* clones (Fig. 7f). In the dorsal domain, the effect depends on the spatial location. *bi-GAL4* reflects the expression pattern of *optomotor-blind* (*omb*), which is expressed in a dorsal sector (spanning the AP compartmental border), in the antennal disc (Fig. 3a). *bi-tsh* caused no obvious antennal phenotype (not shown), suggesting that *tsh* has no effect in this dorsal domain of the antennal disc. *Act>tsh* clone in the dorsoproximal region (Fig.7h, arrow) and near the border between the eye and the antenna discs (Fig. 7h, arrowhead) did not affect HTH level but could cause overgrowth in the posterior dorsal region (Fig. 7i). Induction of *tsh* by the *dpp-GAL4*, which drives expression in a dorsal sector at the AP border in the antennal disc could induce eye formation in the anteroproximodorsal region of the antenna (Fig. 1a) (Pan and Rubin, 1998). A *tsh*-expressing clone in the same anteroproximodorsal region also caused ectopic eye formation (Fig. 7j, arrow). *dpp>tsh* did not cause

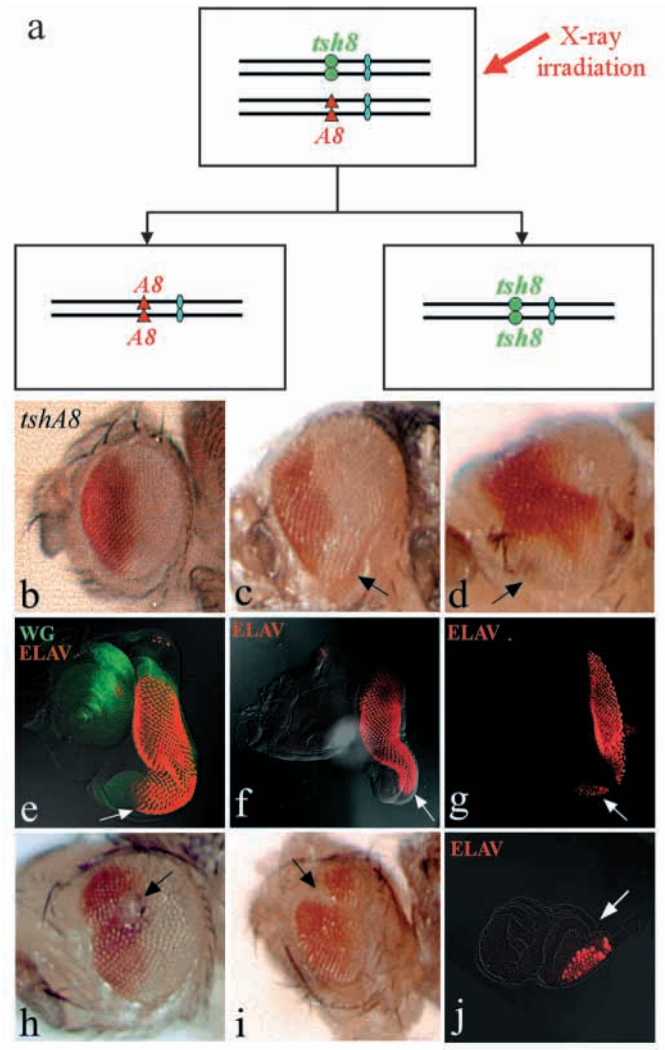

Fig. 4. *tsh* mutant clones can cause ventral enlargement and dorsal suppression in eye. (a) tsh^8 clones were generated by X-ray irradiation of *w; tsh^8/tshA8* larvae. The tsh^8 clones were marked by the loss of the *tshA8* mini-*white* reporter in the adult eye. (b) Wild-type expression of the *tshA8* reporter, a P[*lacW*] insertion at *tsh* locus and expresses in the anterior half of the adult eye. (c,d) tsh^8 clones located at the ventral margin caused overgrowth (arrow). (e-g) tsh^8 clones were not marked in the disc, but eye discs treated for clone induction could have ventral enlargement (e,f, arrow) and ectopic eye field (g, arrow). (h,i) tsh^8 clones located in the anterior dorsal eye can suppress eye (arrow). (j) tsh^8 clone induction can cause nearly complete elimination of the dorsal eye field (arrow).

antennal duplication, unlike *dpp>hth* (Yao et al., 1999), consistent with the non-induction of HTH by *tsh* (Fig. 7h,i). These results indicate that TSH has differential functions along the DV axis in the antennal disc, similar to the eye disc.

DISCUSSION

A novel function of TSH in eye suppression

We showed that ectopic expression of *tsh* could suppress photoreceptor development, while loss-of-function *tsh* clones induced ectopic eye formation. This novel function of *tsh* occurs only at the ventral margin of the eye disc. Pan and Rubin (Pan and Rubin, 1998) noted that targeted expression of *tsh* could induce ectopic eye formation in the antennal disc, but did not observe the eye-suppression phenotype. The discrepancy may be in that they induced *tsh* expression by insertional activation using a P element carrying a *dpp* disc-enhancer coupled with a *hsp70* basal promoter (Pan and Rubin, 1998), while we drove *UAS-tsh* expression using a *dpp-GAL4*.

Interestingly, although *tsh* is expressed symmetrically in the dorsal and ventral halves of the eye disc, overexpressing *tsh* in these regions suppressed eye development in the ventral region, while promoted eye development in the dorsal region. Why would overexpressing *tsh* in a region where it is normally expressed caused phenotype reciprocal to the loss-of-function *tsh* mutant phenotype? It is possibly a dose effect, as the ectopic expression of two copies of *tsh* transgene caused stronger effect (Fig. 1d). The normal level of TSH may be balanced with some opposing forces for proper development, thus too little and too much of TSH will cause reciprocal effects. A similar case is WG, which is normally expressed in both dorsal and ventral margins. Reducing WG level caused ectopic MF formation (Ma and Moses, 1995; Treisman and Rubin, 1995), while raising WG level blocks MF initiation (Treisman and Rubin, 1995).

TSH collaborates with WG signaling to induce *hth* transcription and suppress eye development

The eye-suppression function of *tsh* is accompanied by the induction of *hth* at the transcriptional level. Eye suppression is reduced when the *hth* dose is reduced, suggesting that HTH is the major mediator of *tsh*-induced eye suppression. This is consistent with the known role of *hth* as a repressor of eye development (Pai et al., 1998; Jaw et al., 2000; Pichaud and Casares, 2000). In the wing disc, *tsh* also induces HTH, but our results show that *tsh* has additional effects (e.g. protecting *wg* from suppression by HTH and splitting the wing pouch). Whether *tsh* has additional effects in the eye disc awaits further study.

The eye-suppression function of *tsh* requires WG signaling, as blocking WG signaling by co-expressing $dTCF^{\Delta N}$ or *sgg* with *tsh*, or overexpressing *tsh* in a wg^{ts} mutant at the non-permissive temperature blocked the suppression effect. The critical time for *wg* involvement is 48-72 hours AEL, corresponding to the second instar larval stage. At this stage, the expression patterns of *tsh*, *hth* and *wg* in the eye disc overlap considerably (Fig. 2b) (Pichaud and Casares, 2000; Royet and Finkelstein, 1997), consistent with their functional interaction.

TSH could induce HTH and suppress eye development only in the ventral margin of the eye disc. Internal *Act>tsh* clones had no eye-suppression effects. The restriction of eye suppression to the eye disc margin, where *wg* is expressed, suggests that *tsh* does not induce *wg* but requires high level WG signaling. Indeed, clonal expression of *tsh* internal in the eye disc does not induce WG expression (not shown). When TSH is co-expressed with WG or an activated ARM, eye suppression could occur away from the margin, possibly because higher level of WG signaling is provided by the ectopic expression. TSH also requires high level of WG to repress *Ubx* transcription in the embryonic midgut (Waltzer et al., 2001).

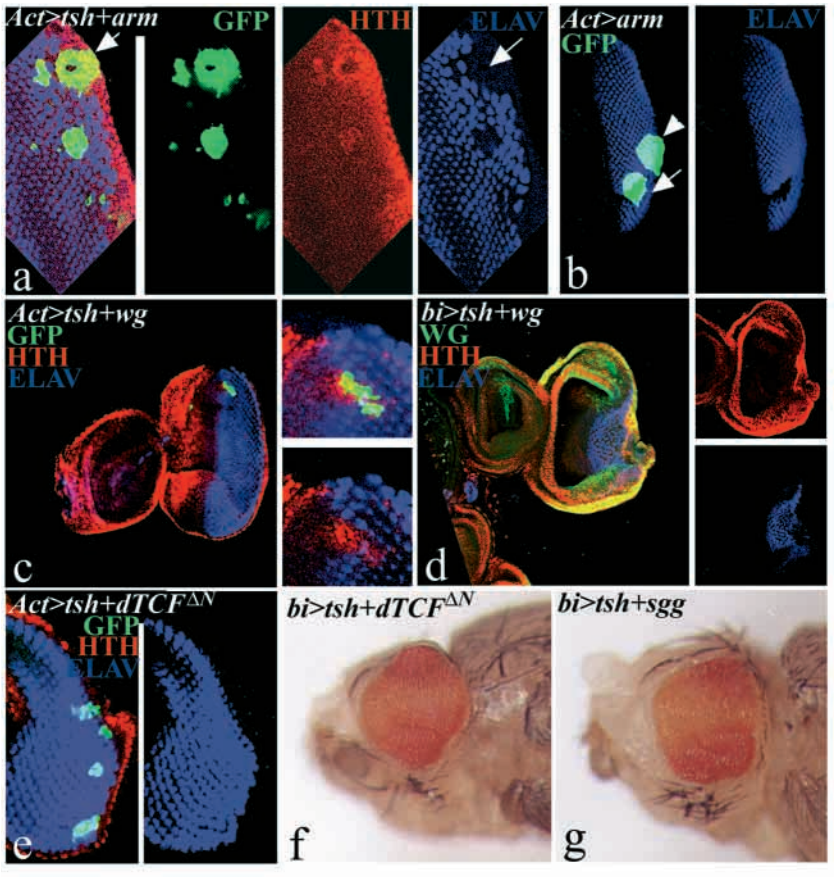

Fig. 5. TSH collaborates with WG signaling for ventral eye suppression. (a) *Act>tsh+arm* clone (marked by GFP, green) suppressed eye fate (ELAV, blue) by ectopic induction of HTH (red) in both dorsal (arrow) and ventral (not shown) domains. (b) *Act>arm* clone did not always suppress eye development. Two *Act>arm* clones (GFP: green) in the eye disc; one near the margin could suppress (arrow), but the other on the margin could not (arrowhead). (c) *Act>tsh+wg* clone (GFP, green) also suppressed eye by ectopic HTH (red) induction near the dorsal margin. (d) *bi>tsh+wg* resulted in ectopic induction of HTH (red) and eye suppression on the dorsal and ventral margins in the in adult eye. (e) *Act>tsh+dTCF*$^{\Delta N}$ clones (GFP, green) did not suppress eye fate. (f) *bi >tsh+dTCF*$^{\Delta N}$ did not induce HTH (red) or suppress eye fate on the dorsal or ventral margin in adult. (g) *Act>tsh+sgg* clone failed to suppress eye development in the ventral margin (arrow).

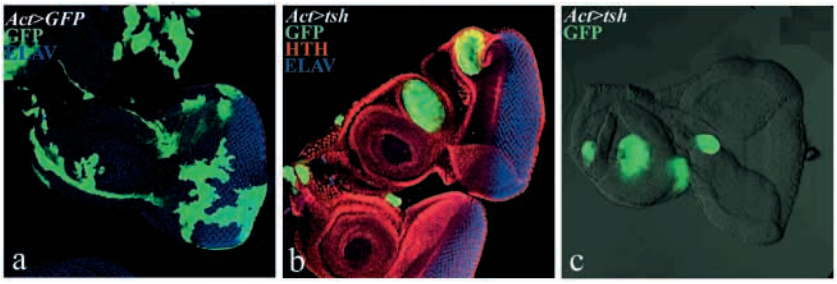

Fig. 6. Effect of *tsh* on growth in eye disc. (a) *Act>GFP* clones (GFP, green) were equally distributed in both dorsal and ventral region in eye disc. Distribution was also equivalent before and after MF. (b) *Act>tsh+GFP* clone (GFP: green) in the dorsal region in the eye disc caused overgrowth. (c) The ventral *Act>tsh+GFP* clone (GFP, green) did not cause overgrowth.

Ectopic expression of WG in the region ahead of MF induces HTH, while blocking WG signaling (by clonal expression of *dTCF*$^{\Delta N}$) reduced HTH in the presumptive head region of the eye disc (Pichaud and Casares, 2000). These locations correspond to *tsh* expression domain, consistent with the TSH-WG collaboration. *Act>hth* clones could block MF initiation without inducing ectopic *wg* expression (Pichaud and Casares, 2000), also suggesting that *hth* acts downstream of WG. Thus, these results suggest that TSH collaborates with WG signaling to induce HTH to suppress eye development.

TSH and WG signaling also collaborate during embryonic development. TSH acts in the late phase of WG signaling to promote the naked cuticle cell fate of larvae (Gallet et al., 1998). TSH phosphorylation and nuclear accumulation is partially promoted by WG signaling (Gallet et al., 1998; Gallet et al., 1999). Hypophosphorylated TSH can bind directly to the intracellular ARM (Gallet et al., 1999). The effect of TSH overexpression on embryo development is dependent on the interaction with ARM (Gallet et al., 1999). TSH can also associate with SGG, an inhibitory component of WG signaling that promotes ARM degradation and acts downstream of SGG (Gallet et al., 1999). Whether the same molecular interaction operates in the eye disc awaits further study.

DV asymmetry in *tsh* function in eye

Based on the loss-of-function phenotype and overexpression phenotype, *tsh* suppresses eye development only in the ventral eye, while promoting eye development in the dorsal eye. The DV difference in TSH function is not likely to be due to *wg*, as *wg* is expressed in both dorsal and ventral margins, with even higher levels in dorsal parts (Ma and Moses, 1995; Treisman and Rubin, 1995). In a *wg* temperature-sensitive mutant, an ectopic MF initiates more on the dorsal side (Ma and Moses, 1995). WG signaling upregulates *hth* in both dorsal and ventral regions of the eye disc (Pichaud and Casares, 2000). Thus, *wg* can induce *hth* and suppress eye development in both ventral and dorsal margins, but through different mechanisms. TSH collaborates with WG signaling for eye

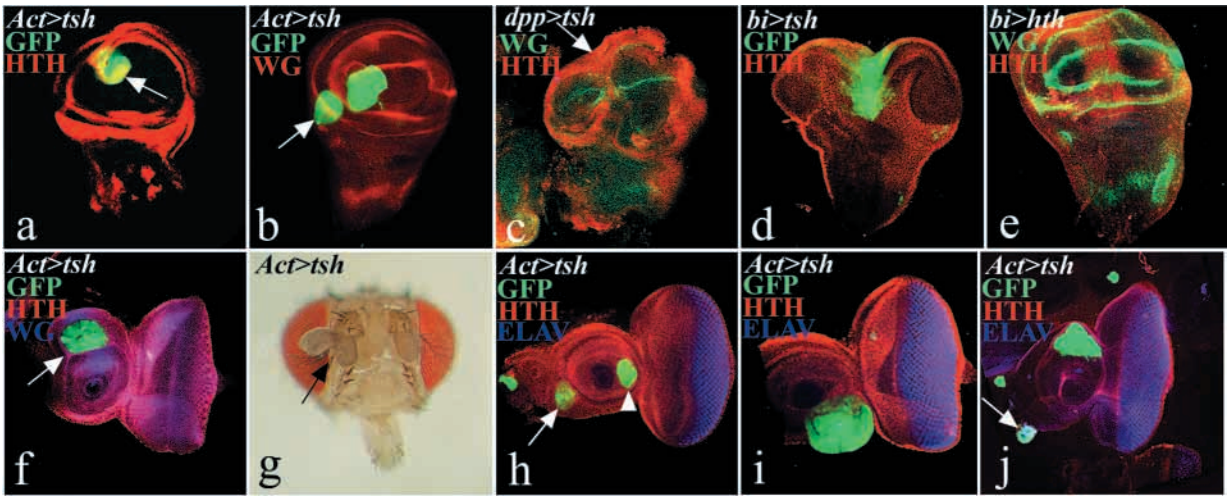

Fig. 7. DV differential functions of *tsh* in the antennal disc but not in the wing disc. (a-d) Effects of *tsh* misexpression in wing disc (anterior is towards the left and ventral is towards the top). (a) *Act>tsh* clone (GFP, green) could induce HTH (red) in a cell-autonomous manner. (b) *Act>tsh* located in the wing pouch region did not suppress WG (red). (c) *dpp>tsh* caused a splitting of the wing pouch by ectopic induction of HTH (red) along the AP compartmental boundary (arrow). (d) *bi>tsh* could split the wing pouch by ectopic induction of HTH (red). (e) *bi>hth* did not suppress WG (green) and did not split the wing pouch. (f-j) Effects of *tsh* misexpression in the antennal disc. Note that the DV axis in the antennal disc (dorsal is upwards and ventral is downwards in these figures) is inverted compared to that of the eye disc. (f,g) *Act>tsh* clone (GFP, green) located in the ventral *wg* expression domain (arrow) resulted in duplication of the antenna field in the disc (f) and in an adult (g). (h) *Act>tsh* clone (GFP: green) located in the anterior (arrow) or posterior (arrowhead) proximal regions did not affect HTH (red) and caused no ectopic eye induction (ELAV, blue). (i) *Act>tsh* clone in the DP region can cause overgrowth. (j) An *Act>tsh* clone in the anteroventroproximal region caused ectopic eye formation (ELAV, blue, arrow).

suppression only in the ventral margin, but not in the dorsal margin. Whether WG requires other co-factors in the dorsal margin is not known.

tsh promoted eye development in the dorsal margin (Fig. 3b,e,g). When TSH is co-expressed with WG or an activated ARM, the dorsal enlargement is blocked (Fig. 5a,c,d). When WG signaling is blocked in *bi>tsh+dTCF$^{\Delta N}$* (Fig. 5f) and *bi>tsh+sgg* (not shown), eye enlargement occurred in both dorsal and ventral sides. These results suggested that in the dorsal eye, WG signaling blocks eye development at a step downstream of *tsh* function.

Some dorsal- or ventral-specific factor(s) may determine the outcome of TSH function. One possible mechanism is by affecting the collaboration between TSH and WG signaling (ARM or SGG). Our preliminary results indicated that the dorsal-expressing *auracuan* (*ara*) and *Delta* (*Dll*) can confer the dorsal specificity, and the ventral-expressing *Serrate* (*Ser*) can confer the ventral specificity to TSH function (A. S. and Y. H. Sun, unpublished).

The DV differential effect of *tsh* also occurs in the leg and antennal discs, but not in the wing disc. In the leg discs, when away from the border between the proximal *tsh*-expressing and distal *Dll*-expressing cells, clonal *tsh* induction caused no effect in the dorsal domain, but affected cell adhesion property and patterning when in the ventral domain. In the distal region of the leg disc, where *tsh* is not expressed, clonal *tsh* induction can lead to TSH protein accumulation only in the ventral domain, because of WG signaling (Erkner et al., 1999). In the antennal disc, *Act>tsh* clones in the ventral domain could cause HTH repression and antenna duplication (Fig. 7g). In the dorsal domain, the effect of *tsh* misexpression depends on the location. In the *omb* expression region, which spans the dorsal AP border, there is no effect on HTH and on antenna development. But further away from the AP border, *tsh* dorsal misexpression could cause overgrowth (in the posterior-proximal region) and ectopic eye formation (in the anterior-proximal region). These disc- and position-dependent differences in *tsh* function suggest the involvement of additional factors in determining the functional outcome of TSH.

The effect of *tsh* is on both growth and differentiation

The critical period for eye suppression by *tsh* is in the second instar larval stage, based on *tsh* mutant clones and on misexpression of *tsh* in *wg^{ts}* background. At this time, morphogenetic furrow has not initiated and photoreceptor differentiation has not begun. *tsh* mutant clones induced in second instar caused enlargement in the ventral eye field and reduction of eye cells in the dorsal eye field. In the ventral overgrowth, not all cells have differentiated into photoreceptors. These results suggest that the primary effect of *tsh* function is on growth in the early eye disc. When the relative frequency and size of *Act>GFP* and *Act>tsh+GFP* clones were compared (Fig. 6), the results showed that *tsh* promoted growth in the dorsal and suppressed growth in the ventral region. A dorsal clone anterior to the MF showed overgrowth (Fig. 6b), suggesting that the effect can be a general growth promotion and not limited to differentiating retinal cells.

However, *tsh^{8}* mutant clones in the dorsal eye caused a transformation of eye cells into cuticle fate, suggesting that *tsh* also plays a role in promoting eye fate (in dorsal). This role is consistent with the finding that *tsh* could induce ectopic eye

formation in antenna (Pan and Rubin, 1998). In the ventral eye disc, a role in directly suppressing photoreceptor fate is also supported by the finding of an isolated ventral eye field in the eye disc with tsh^{8} clone induction (Fig. 4g). This direct role is consistent with the ventral activation of *hth*, which can directly suppress photoreceptor differentiation (Pai et al., 1998). Thus, *tsh* can affect both the growth of the eye disc and the differentiation of photoreceptors.

We thank Shigeo Hayashi, Stephen Kerridge, Steve Cohen, Jessica Treisman, Mark Muskavitch, Sonsoles Campuzano, Kwang-Wook Choi and the Bloomington Stock Center for *Drosophila* strains; Steve Kerridge, Steve Cohen, Gert Pflugfelder, Uwe Walldorf, Isabel Guererro and the Developmental Studies Hybridoma Bank (DSHB) for antibodies; Steve Kerridge, Steve Cohen, Kwang-Wook Choi and Cheng-ting Chien for critical comments on the manuscript; Chiou-Yang Tang for fly stocks; and Chun-lan Hsu and Lili Chen for preparing the fly media. This study and M. K.-S. were supported by the National Science Council of the Republic of China (NSC-88-2312-B-001-016). A. S. was supported by a postdoctoral fellowship from Academia Sinica, Republic of China.

REFERENCES

Alexandre, E., Graba, Y., Fasano, L., Gallet, A., Perrin, L., de Zulueta, P., Pradel, J., Kerridge, S. and Jacq, B. (1996). The *Drosophila teashirt* homeotic protein is a DNA-binding protein and modulo, a HOM-C regulated modifier of variegation, is a likely candidate for being a direct target gene. *Mech. Dev.* **59**, 191-204.

Azpiazu, N. and Morata, G. (1998). Functional and regulatory interactions between Hox and *extradenticle* genes. *Genes Dev.* **12**, 261-273.

Azpiazu, N. and Morata, G. (2000). Function and regulation of *homothorax* in the wing imaginal disc of *Drosophila*. *Development* **127**, 2685-2693.

Bhojwani, J., Singh, A., Misquitta, L., Mishra, A. and Sinha, P. (1995). Search for *Drosophila* genes based on patterned expression of mini-*white* reporter gene of P *lacW* vector in adult eyes. *Roux's Arch. Dev. Biol.* **205**, 114-121.

Bhojwani, J., Shashidhara, L. S. and Sinha, P. (1997). Requirement of *teashirt (tsh)* during cell fate specification in developing head structures in *Drosophila*. *Dev. Genes Evol.* **207**, 137-146.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Brown, N. L., Sattler, C. A., Paddock, S. W. and Carroll, S. B. (1995). Hairy and Emc negatively regulate morphogenetic furrow progression in the *Drosophila* eye. *Cell* **80**, 879-887.

Calleja, M., Moreno, E., Pelaz, S. and Morata, G. (1996). Visualization of gene expression in living adult *Drosophila*. *Science* **274**, 252-255.

Casares, F. and Mann, R. S. (1998). Control of antennal versus leg development in *Drosophila*. *Nature* **392**, 723-726.

Casares, F. and Mann, R. S. (2000). A dual role for *homothorax* in inhibiting wing blade development and specifying proximal wing identities in *Drosophila*. *Development* **127**, 1499-1508.

Cavodeassi, F., Diez del Corral, R., Campuzano, S. and Dominguez, M. (1999). Compartments and organising boundaries in the *Drosophila* eye: the role of the homeodomain Iroquois proteins. *Development* **126**, 4933-4942.

Cavodeassi, F., Modolell, J. and Campuzano, S. (2000). The Iroquois homeobox genes function as dorsal selectors in the *Drosophila* head. *Development* **127**, 1921-1929.

Cho, K. O. and Choi, K. W. (1998). Fringe is essential for mirror symmetry and morphogenesis in the *Drosophila* eye. *Nature* **396**, 272-276.

de Zulueta, P., Alexandre, E., Jacq, B. and Kerridge, S. (1994). Homeotic complex and *teashirt* genes co-operate to establish trunk segmental identities in *Drosophila*. *Development* **120**, 2287-2296.

Desplan, C. (1997). Eye development: governed by a dictator or a junta? *Cell* **91**, 861-864.

Dominguez, M. and de Celis, J. F. (1998). A dorsal/ventral boundary established by Notch controls growth and polarity in the *Drosophila* eye. *Nature* **396**, 276-278.

Erkner, A., Gallet, A., Angelats, C., Fasano, L. and Kerridge, S. (1999). The role of Teashirt in proximal leg development in *Drosophila*: ectopic Teashirt expression reveals different cell behaviours in ventral and dorsal domains. *Dev. Biol.* **215**, 221-232.

Fasano, L., Roder, L., Core, N., Alexandre, E., Vola, C., Jacq, B. and Kerridge, S. (1991). The gene *teashirt* is required for the development of *Drosophila* embryonic trunk segments and encodes a protein with widely spaced zinc finger motifs. *Cell* **64**, 63-79.

Gallet, A., Erkner, A., Charroux, B., Fasano, L. and Kerridge, S. (1998). Trunk-specific modulation of Wingless signalling in *Drosophila* by Teashirt binding to Armadillo. *Curr. Biol.* **8**, 893-902.

Gallet, A., Angelats, C., Erkner, A., Charroux, B., Fasano, L. and Kerridge, S. (1999). The C-terminal domain of Armadillo binds to hypophosphorylated Teashirt to modulate Wingless signalling in *Drosophila*. *EMBO J.* **18**, 2208-2217.

Goto, S. and Hayashi, S. (1999). Proximal to distal cell communication in the *Drosophila* leg provides a basis for an intercalary mechanism of limb patterning. *Development* **126**, 3407-3413.

Halder, G., Callaerts, P. and Gehring, W. J. (1995). Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*. *Science* **267**, 1788-1792.

Halder, G., Callaerts, P., Flister, S., Walldorf, U., Kloter, U. and Gehring, W. J. (1998). Eyeless initiates the expression of both *sine oculis* and *eyes absent* during *Drosophila* compound eye development. *Development* **125**, 2181-2191.

Hazelett, D. J., Bourouis, M., Walldorf, U. and Treisman, J. E. (1998). *decapentaplegic* and *wingless* are regulated by *eyes absent* and *eyegone* and interact to direct the pattern of retinal differentiation in the eye disc. *Development* **125**, 3741-3751.

Heberlein, U., Borod, E. R. and Chanut, F. A. (1998). Dorsoventral patterning in the *Drosophila* retina by *wingless*. *Development* **125**, 567-577.

Heberlein, U. and Treisman, J. E. (2000). Early retinal development in *Drosophila*. *Results Probl. Cell Differ.* **31**, 37-50.

Heslip, T. R. T., Walker, H. and Marsh, J. L. (1997). SHAGGY and DISHEVELLED exert opposite effects on *wingless* and *decapentaplegic* expression and on positional identity in imaginal discs. *Development* **124**, 1069-1078.

Ito, K., Awano, W., Suzuki, K., Hiromi, Y. and Yamamoto, D. (1997). The *Drosophila* mushroom body is a quadruple structure of clonal units each of which contains a virtually identical set of neurones and glial cells. *Development* **124**, 761-771.

Jaw, T. J., You, L. R., Knoepfler, P. S., Yao, L. C., Pai, C. Y., Tang, C. Y., Chang, L. P., Berthelsen, J., Blasi, F., Kamps, M. P. et al. (2000). Direct interaction of two homeoproteins, homothorax and extradenticle, is essential for EXD nuclear localization and function. *Mech. Dev.* **91**, 279-291.

Kurant, E., Pai, C. Y., Sharf, R., Halachmi, N., Sun, Y. H. and Salzberg, A. (1998*). dorsotonals/homothorax*, the *Drosophila* homologue of *meis1*, interacts with *extradenticle* in patterning of the embryonic PNS. *Development* **125**, 1037-1048.

Lee, J. D. and Treisman, J. E. (2001). The role of Wingless signaling in establishing the anteroposterior and dorsoventral axes of the eye disc. *Development* **128**, 1519-1529.

Ma, C. and Moses, K. (1995). *wingless* and *patched* are negative regulators of the morphogenetic furrow and can affect tissue polarity in the developing *Drosophila* compound eye. *Development* **121**, 2279-2289.

Mathies, L. D., Kerridge, S. and Scott, M. P. (1994). Role of the *teashirt* gene in *Drosophila* midgut morphogenesis: secreted proteins mediate the action of homeotic genes. *Development* **120**, 2799-2809.

Maurel-Zaffran, C. and Treisman, J. E. (2000). *pannier* acts upstream of *wingless* to direct dorsal eye disc development in *Drosophila*. *Development* **127**, 1007-1016.

McNeill, H., Yang, C. H., Brodsky, M., Ungos, J. and Simon, M. A. (1997). mirror encodes a novel PBX-class homeoprotein that functions in the definition of the dorsal-ventral border in the *Drosophila* eye. *Genes Dev.* **11**, 1073-1082.

Nusslein-Volhard, C., Wieschaus, E. and Kluding, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster* I. Zygotic loci on the second chromosome. *Wilhelm Roux's Arch. Dev. Biol.* **193**, 267-282.

Pai, C. Y., Kuo, T. S., Jaw, T. J., Kurant, E., Chen, C. T., Bessarab, D. A., Salzberg, A. and Sun, Y. H. (1998). The Homothorax homeoprotein activates the nuclear localization of another homeoprotein, extradenticle, and suppresses eye development in *Drosophila*. *Genes Dev.* **12**, 435-446.

Pan, D. and Rubin, G. M. (1998). Targeted expression of *teashirt*

induces ectopic eyes in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **95**, 15508-15512.

Papayannopoulos, V., Tomlinson, A., Panin, V. M., Rauskolb, C. and Irvine, K. D. (1998). Dorsal-ventral signaling in the *Drosophila* eye. *Science* **281**, 2031-2034.

Pichaud, F. and Casares, F. (2000). *homothorax* and *iroquois-C* genes are required for the establishment of territories within the developing eye disc. *Mech. Dev.* **96**, 15-25.

Rieckhof, G. E., Casares, F., Ryoo, H. D., Abu-Shaar, M. and Mann, R. S. (1997). Nuclear translocation of extradenticle requires homothorax, which encodes an extradenticle-related homeodomain protein. *Cell* **91**, 171-183.

Roder, L., Vola, C. and Kerridge, S. (1992). The role of the *teashirt* gene in trunk segmental identity in *Drosophila*. *Development* **115**, 1017-1033.

Royet, J. and Finkelstein, R. (1997). Establishing primordia in the *Drosophila* eye-antennal imaginal disc: the roles of *decapentaplegic*, *wingless* and *hedgehog*. *Development* **124**, 4793-4800.

Shiga, Y., Tanaka-Matakatsu, M. and Hayashi, S. (1996). A nuclear GFP/beta-galactosidase fusion protein as a marker for morphogenesis in living *Drosophila*. *Dev. Growth Differ.* **38**, 99-106.

Staehling-Hampton, K., Jackson, P. D., Clark, M. J., Brand, A. H. and Hoffmann, F. M. (1994). Specificity of bone morphogenetic protein-related factors: cell fate and gene expression changes in *Drosophila* embryos induced by *decapentaplegic* but not *60A*. *Cell Growth Differ.* **5**, 585-593.

Struhl, G. and Basler, K. (1993). Organizing activity of wingless protein in *Drosophila*. *Cell* **72**, 527-540.

Sun, Y. H., Tsai, C. J., Green, M. M., Chao, J. L., Yu, C. T., Jaw, T. J., Yeh, J. Y. and Bolshakov, V. N. (1995). *white* as a reporter gene to detect transcriptional silencers specifying position-specific gene expression during *Drosophila melanogaster* eye development. *Genetics* **141**, 1075-1086.

Theisen, H., Haerry, T. E., O'Connor, M. B. and Marsh, J. L. (1996). Developmental territories created by mutual antagonism between Wingless and Decapentaplegic. *Development* **122**, 3939-3948.

Treisman, J. E. (1999). A conserved blueprint for the eye? *BioEssays* **21**, 843-850.

Treisman, J. E. and Rubin, G. M. (1995). *wingless* inhibits morphogenetic furrow movement in the *Drosophila* eye disc. *Development* **121**, 3519-3527.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A. et al. (1997). Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. *Cell* **88**, 789-799.

Waltzer, L., Vandel, L. and Bienz, M. (2001). Teashirt is required for transcriptional repression mediated by high Wingless levels. *EMBO J.* **20**, 137-145.

Wolff, T. and Ready, D. F. (1993). Pattern formation in the *Drosophila* retina. *Cold Spring Harbor Laboratory Press* **II**, 1277-1325.

Wu, J. and Cohen, S. M. (2000). Proximal distal axis formation in the *Drosophila* leg: distinct functions of *teashirt* and *homothorax* in the proximal leg. *Mech. Dev.* **94**, 47-56.

Xu, T. and Rubin, G. M. (1993). Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* **117**, 1223-1237.

Yao, L. C., Liaw, G. J., Pai, C. Y. and Sun, Y. H. (1999). A common mechanism for antenna-to-Leg transformation in *Drosophila*: suppression of homothorax transcription by four HOM-C genes. *Dev. Biol.* **211**, 268-276.

Zecca, M., Basler, K. and Struhl, G. (1996). Direct and long-range action of a *wingless* morphogen gradient. *Cell* **87**, 833-844.