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Initial State of the Drosophila Eye before Dorsoventral Specification is Equivalent to Ventral

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Initial state of the *Drosophila* eye before dorsoventral specification is equivalent to ventral

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Summary

Dorsoventral (DV) patterning is crucial for eye development in invertebrates and higher animals. DV lineage restriction is the primary event in undifferentiated early eye primordia of *Drosophila*. In *Drosophila* eye disc, a dorsal-specific GATA family transcription factor *pannier* (*pnr*) controls *Iroquois-Complex* (*Iro-C*) genes to establish the dorsal eye fate whereas *Lobe* (*L*), which is involved in controlling a Notch ligand *Serrate* (*Ser*), is specifically required for ventral growth. However, fate of eye disc cells before the onset of dorsal expression of *pnr* and *Iro-C* is not known. We show that L/Ser are expressed in entire early eye disc before the expression of *pnr* and *Iro-C* is initiated in late first instar dorsal eye margin cells. Our evidence suggests that during embryogenesis *pnr* activity is not essential for eye development. We present evidence that loss of *L* or *Ser* function prior to initiation of *pnr* expression results in elimination of the entire eye, whereas after the onset of *pnr* expression it results only in preferential loss of ventral half of eye. We demonstrate that dorsal eye disc cells also become *L* or *Ser* dependent when they are ventralized by removal of *pnr* or *Iro-C* gene function. Therefore, we propose that early state of the eye prior to DV lineage restriction is equivalent to ventral and requires *L* and *Ser* gene function.

Key words: *Drosophila*, Dorsoventral eye patterning, *Lobe*, *Serrate*, *pannier*, *Iro-C*

Introduction

Development of a field requires generation of lineage restriction boundary, which results in two differently determined cell populations called compartments (Garcia-Bellido et al., 1973). Compartments are the fundamental units of patterning generated by localized expression of transcription factors, which are called selectors as they can confer the compartment-specific properties to the group of cells in which they are expressed (Curtiss et al., 2002; Mann and Carroll, 2002). Activity of these selector genes generate lineage restriction boundary and control signaling at the boundary (Blair, 1995; Wu and Rao, 1999). Signaling between the cells of two compartments contributes to growth and differentiation of an undifferentiated field to its adult counterpart (Blair, 2001).

The development of an imaginal disc into an adult structure requires generation of anteroposterior (AP) and dorsoventral (DV) lineage restrictions. In antenna, wing and leg imaginal discs, early-arising AP boundary is the first lineage restriction event. This is followed by DV boundary generation midway through the growth phase of the disc, which further subdivides these discs into dorsal and ventral compartments (Blair, 1995; Blair, 2001; Diaz-Benjumea and Cohen, 1993; Garcia-Bellido and Santamaria, 1972; Milan and Cohen, 2003; Morata and Lawrence, 1975; Tabata et al., 1995). By contrast, the eye disc does not show a strict anterior versus posterior lineage restriction (Morata and Lawrence, 1978). AP pattern in the eye disc is established dynamically as the morphogenetic furrow (MF), a wave of differentiation, progresses anteriorly, resulting in the distinction of the AP domains. In fact, anterior and posterior domains correspond to undifferentiated (anterior to MF) and differentiated regions (posterior to MF) of eye (Ready et al., 1976; Wolff and Ready, 1993), rather than the compartments of different cell lineages separated by strict lineage restriction boundary. Therefore, the eye disc remains at anterior undifferentiated ground state until the early third larval instar, when MF is initiated to generate the AP pattern (Heberlein and Moses, 1995; Lee and Treisman, 2001). However, unlike the AP axis, DV lineage restriction and domain-specific gene expression of DV patterning genes takes place very early during the eye disc development (Baker, 1978; Cho and Choi, 1998; Dominguez and de Celis, 1998). Consequently, DV lineage restriction, which is secondary event in other imaginal discs becomes the first lineage restriction event in eye disc and is crucial for its growth and differentiation.

Eye disc develops into the adult compound eye, which is a highly precise hexagonal array of 800 ommatidia (Ready et al., 1976; Wolff and Ready, 1993). Two chiral forms of these ommatidial clusters are arranged in mirror image symmetry along the DV midline called equator to form dorsal and ventral eye. Although the mirror image symmetry is generated during third instar of development but the subdivision of eye into dorsal and ventral lineage territories takes place even earlier (Baker, 1978; Cavodeassi et al., 1999; Cho and Choi, 1998; Dominguez and de Celis, 1998; Maurel-Zaffran and Treisman,

2000; McNeill et al., 1997; Papayannopoulos et al., 1998), which is responsible to define the site of differentiation to initiate and promote the growth of eye field.

It has been shown that *pnr* (Maurel-Zaffran and Treisman, 2000) and members of *Iro-C* homeodomain genes viz., *araucan* (*ara*), *caupolican* (*caup*) (Cavodeassi et al., 1999; Gomez-Skarmeta and Modolell, 1996) and *mirror* (*mirr*) (Kehl et al., 1998; McNeill et al., 1997) are expressed in the dorsal region of the prospective eye (Dominguez and de Celis, 1998; McNeill et al., 1997). *pnr* and *Iro-C* genes have been shown to act as dorsal eye fate selectors and can also specify the ommatidial DV planar polarity (Cavodeassi et al., 1999; Maurel-Zaffran and Treisman, 2000). *pnr*, one of the topmost genes known in dorsal eye gene hierarchy, regulates the expression of down stream *Iro-C* genes by Wingless (Wg) signaling (Heberlein et al., 1998; Maurel-Zaffran and Treisman, 2000). *mirr* or *caup* can repress *fringe* (*fng*) and thereby restrict *fng* expression to the ventral eye (Cho and Choi, 1998; Dominguez and de Celis, 1998). These genetic interactions define a signaling pathway that contributes towards the positioning of the equator, which is generated at the boundary of *fng*-expressing and non-expressing cells. Equator is the site for activation of Notch (N) signaling and is crucial for growth and differentiation of the eye (Cho and Choi, 1998; Dominguez and de Celis, 1998; Papayannopoulos et al., 1998).

In the ventral eye, *fng* promotes expression of *Ser* in the cells close to the DV boundary. Notch ligands Ser and Delta (Dl) in turn initiates a *Ser-N-Dl* positive feedback loop that activates N signaling (Huppert, 1997; Irvine, 1999). *Ser* plays dual role in eye development. First, *Ser* contributes to the DV boundary formation and secondly *Ser* is required for ventral eye growth. Expression of *Ser* in the ventral eye is controlled by *L*, which encodes a novel protein containing a poly-glutamine rich region. L protein shares a conserved C-terminal domain with novel insect, mouse and human proteins (Chern and Choi, 2002). *L* has also been proposed to be a component of intracellular pathway that transduces N signaling in the ventral eye probably by interacting with other ventral specific genes such as *Ser* (Chern and Choi, 2002). In contrast to the restricted expression of *pnr* and *Iro-C* in the dorsal domain, *fng* and *Ser* show dynamic expression pattern during eye disc development. Both genes are preferentially enriched in the ventral region of early eye discs but are also expressed dorsally as discs develop further (Cho and Choi, 1998; Dominguez and de Celis, 1998; Papayannopoulos et al., 1998). Conversely, *L* is expressed in the entire eye despite its specific requirement only in the ventral eye development (Chern and Choi, 2002).

DV lineage restriction of eye is associated with onset of expression of dorsal genes (Cavodeassi et al., 2000). Therefore, it would be important to determine the temporal relationship between the expression of dorsal eye selectors and the genes involved in ventral eye development (hereafter *L/Ser*). This will provide new insights into when is the first lineage restriction event of DV boundary formation initiated during eye disc development. Interestingly, we found that expression of *L* and *Ser* is initiated earlier than *pnr* and *Iro-C* in the eye disc. Removal of *L*/*Ser* gene function during early eye development can completely eliminate the eye field, whereas later when dorsal selector *pnr* gene expression is initiated in the dorsal eye, removal of *L*/*Ser* gene function results in selective loss of ventral eye fate. We also present that removal of *pnr* or *Iro-C* gene function from the dorsal eye cells can revert the dorsal eye fate to the ventral, which behaves in a similar fashion to the early eye disc in terms of its sensitivity to L/Ser activity. We also show that early *pnr* expression during embryogenesis has little or no functional contribution to DV patterning of eye. Therefore, we propose that early eye disc has ventral-equivalent state, even before the onset of the dorsal selector genes expression, which results in DV lineage restriction event.

Materials and methods

Stocks

Stock used were *y w*; *FRT82 pnr^{vx6}/TM6B*, a null allele of *pnr* (Heitzler et al., 1996); *y w hsFLP^{122}; iro^{DFM3} FRT80 /TM6B* (Diez del Corral et al., 1999); *y w; mirr^{B1-12}/TM6B* (Choi et al., 1996); *y w eyFLP* (Newsome et al., 2000); *y w hsFLP^{122}* (Struhl and Basler, 1993); *UAS-Ser^{DN}* (Hukriede et al., 1997); *yw;·FRT42D, L^{rev6-3}/CyO* and *UAS-L* (Chern and Choi, 2002); *ey-GAL4* (Hazelett et al., 1998); *pnr-GAL4* (Calleja et al., 1996); UAS-*Ush* (Fossett et al., 2001) and *P{UAS-GFP.S65T/T10}* (B. J. Dickson, unpublished). These stocks are described in FlyBase (http://flybase.bio.indiana.edu). We have used GAL4/UAS system for targeted misexpression (Brand and Perrimon, 1993). GAL4/UAS crosses were carried out at 18°C, 25°C and 29°C, to sample the effect of different induction level.

Generation of loss-of-function clones

Loss-of-function clones were generated using the FLP/FRT system of mitotic recombination (Xu and Rubin, 1993). To generate loss-of-function clones of *L* in eye, *eyFLP; FRT42 ubi-GFP* females were crossed to *FRT42D L^{rev6-3}* males. For the generation of heat-shock FLP-mediated clones of *L*, *hsFLP^{122}*; *FRT42 ubi-GFP* females were crossed to *FRT42D, L^{rev6-3}* males. Eggs were collected for 2 hours and a single heat shock was administered for 1 hour at 37°C. All larvae were transferred to 25°C for recovery and further development.

To generate the loss-of-function clones of *pnr^{vx6}*, *y w*;+/+ *FRT82 pnr^{vx6}/TM6B*, males were crossed to *eyFLP; FRT82 ubi-GFP* females. *Iro-C* loss-of-function clones were generated by crossing *hsFLP^{122}; FRT80, iro^{DFM3}/TM6Tb* males with the *eyFLP*; *FRT80, ubi-GFP* females.

As *pnr* and *Iro-C* genes play important roles in different developing fields during development, we wanted to generate the flies that have only the eyes homozygous for the *pnr* or *Iro-C* mutation and in the same mutant eye disc overexpress another gene of interest. These flies were generated by using the EGUF (*eyeless*-GAL4 *UAS-FLP*) system (Stowers and Schwarz, 1999). EGUF system has been generated by combining the GAL4/UAS system (Brand and Perrimon, 1993) and the *FLP* recombinase system (Xu and Rubin, 1993) via the UAS-*FLP* transgene (Duffy et al., 1998). The *ey-GAL4* drives UAS-*FLP* recombinase only in the eye and wild-type cells (heterozygous and +/+ twin spot cells) are selectively eliminated by *GMR>hid* later during differentiation (Stowers and Schwarz, 1999). As the clones are generated earlier by *ey-GAL4* and the wild-type cells are killed later by *GMR>hid*, the discs get time to grow.

Temperature shift regimen

Eggs were collected for the genotype, *ey-GAL4; UAS-Ser^{DN} (ey>Ser^{DN})* from a synchronous culture for 2 hours. Each egg collection was divided into several batches. These independent batches were reared at 18°C except for a single shift to 29°C in a 12 hour time window. This single 12 hour heat shock of each sample was performed during different periods of development spanning from *t*=0 hour AEL (after egg laying) to the late third larval instar. These cultures after the 12 hour exposure to 29°C were returned to 18°C for the later part of development until the discs were dissected and stained or till the adult flies emerged (superscript DN indicates dominant negative).

Another temperature shift regimen was carried out for *ey-GAL4; UAS-Ush (ey>Ush*) in a similar way except the time windows of exposure to restrictive temperature were different (see Fig. 2A for details).

Immunohistochemistry

Eye-antenna discs were stained following the standard protocol (Singh et al., 2002). Antibodies used were mouse anti-L (1:100) (Chern and Choi, 2002); rabbit anti-β-galactosidase (1:200) (Cappel); chicken anti-GFP (1:200) (Upstate biotechnology); rabbit anti-Ey (1:500) (a gift from Uwe Walldorf); rat anti-Elav (1:100); mouse 22C10 (1:20); mouse anti-Wg (1:20) (Developmental Studies Hybridoma Bank). Secondary antibodies (Jackson Laboratories) were goat anti-rat IgG conjugated with Cy5 (1:200); donkey anti-rabbit IgG conjugated to Cy3 (1:250); donkey anti-mouse IgG conjugated to FITC (1:200); or donkey anti-chicken IgG conjugated to FITC. *pnr* expression was detected using *pnrGAL4>UAS-GFP*, which has been commonly used to detect *pnr* expression, as seen in wing and eye discs (Calleja et al., 2000; Pichaud and Casares, 2000). Immunofluorescent images were analyzed by using Zeiss LSM laser confocal microscope.

Results

Lobe and *Ser* are expressed earlier than dorsal selectors in eye

To check how and when the DV fates are established in eye, we examined the onset of expression of dorsal eye selector genes and *L/Ser* during larval development. In the first instar eye disc, L is expressed ubiquitously, whereas *pnr* expression is not seen (Fig. 1A; arrows, A′). Expression of *pnr* has been seen in the embryonic eye primordia (Maurel-Zaffran and Treisman, 2000). But in the early first instar eye disc, *pnr* expression was shut off or downregulated to undetectable level. In late first- or early second-instar disc, *pnr* expression is initiated in a small group of cells in the dorsal eye close to its anterior tip (Fig. 1B, arrow; B′), whereas L is expressed in entire disc. This suggests that *pnr* has a very dynamic expression during eye development.

In late second instar, *pnr* expression extends to the dorsal margin of eye (Fig. 1C,C′). In third instar disc, *pnr* is expressed in a wedge of cells on the dorsal margin of the eye, whereas L expression does not change (data not shown). Furthermore, expression of *pnr* is restricted only to the group of cells in the lateral margin of the dorsal eye as previously reported (Maurel-Zaffran and Treisman, 2000). Interestingly, we found that *pnr* is expressed in the dorsal peripodial cells of the eye disc throughout most larval stages, but could not definitively determine whether pnr^+ cells are in the peripodial membrane in the first instar disc.

Expression of *mirr*, an *Iro-C* member, is not initiated in early first instar eye disc (Fig. 1D, arrowhead) whereas Ser is expressed in entire disc (data not shown). In early second instar, *mirr* is restricted to the dorsal eye (Fig. 1E,E′,F,F′), whereas Ser is also preferentially expressed in ventral with a weaker expression in dorsal eye disc (data not shown) (Cho and Choi, 1998). *mirr* expression stays in dorsal region of third instar eye disc (data not shown) (McNeill et al., 1997). *mirr* is expressed in much broader dorsal domain in comparison to *pnr* as it is controlled by secreted Wg, which acts downstream to *pnr* (Maurel-Zaffran and Treisman, 2000). The expression of *ara* using antibody against Ara protein was similar to *mirr* (data not shown).

Pnr activity is not essential for DV patterning during embryogeneis

We could not detect *pnr* in the early first instar eye disc (Fig. 1A), despite its expression in embryo (Maurel-Zaffran and Treisman, 2000). The significance of disappearance of *pnr* expression between embryogenesis to late first instar larva is not yet clear. We performed a functional test to determine whether *pnr* is active in the eye primordium in the embryo. We misexpressed *U-shaped* (*Ush*) using *ey-GAL4* during embryonic development to block *pnr* transcriptional activity. Ush, which is normally not expressed in eye (Maurel-Zaffran and Treisman, 2000; Fossett et al., 2001), encodes a zinc-finger protein that dimerizes with Pnr and acts as a negative regulator of *pnr* transcriptional activity (Haenlin et al., 1997). The aim was to determine if *pnr* has any role in DV patterning of eye during embryogenesis. We used temperature-shift approach in three different conditions as shown in Fig. 2A. First, we maintained the cultures at 29°C all along the development, which served as control and resulted in no eye (Fig. 2B,C) to a small eye phenotype (Fig. 2D) in almost 80% (51/64) of the adult flies scored, also seen by Fossett et al. (Fossett et al.,

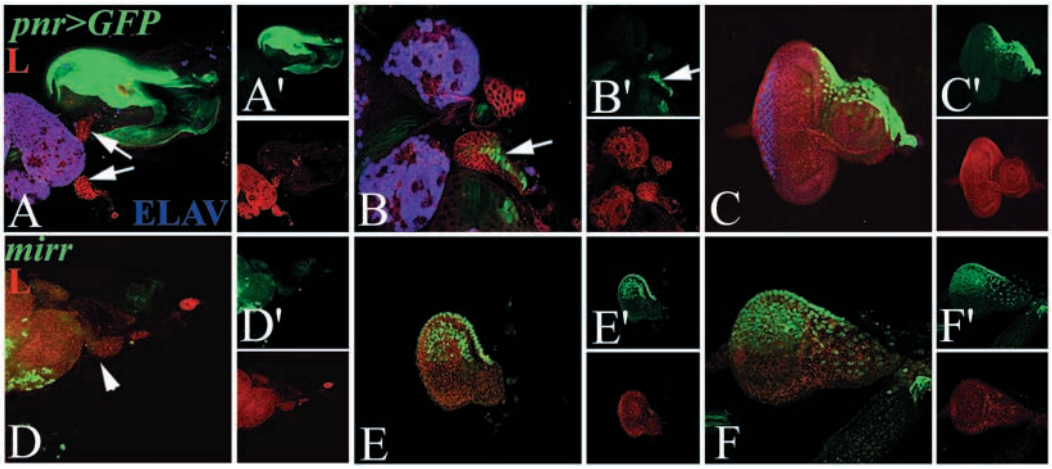

Fig. 1. Expression of L in the larval eye disc is initiated earlier than *pnr* and *Iro-C* genes. All eye discs in this and subsequent figures are oriented anterior towards the right and dorsal towards the top. Eye disc of first- (A,A′, arrows), early second- (B,B′, arrow), and late second- (C,C′) instar larvae stained for *pnr* (green), L (red) and Elav (blue). Expression of *pnr* (green, arrow) begins in late first- to early second-instar disc (B,B′) in a small group of cells at the dorsal margin. Expression of L (red) and *mirr* (green) in first- (D,D′, arrowhead), early second- (E,E′) and mid-second- (F,F′) instar eye disc, respectively. *mirr* is expressed in a broader domain in the dorsal eye as compared with the *pnr*. Individual channels of the images are also shown (′). Magnifications of the images are same.

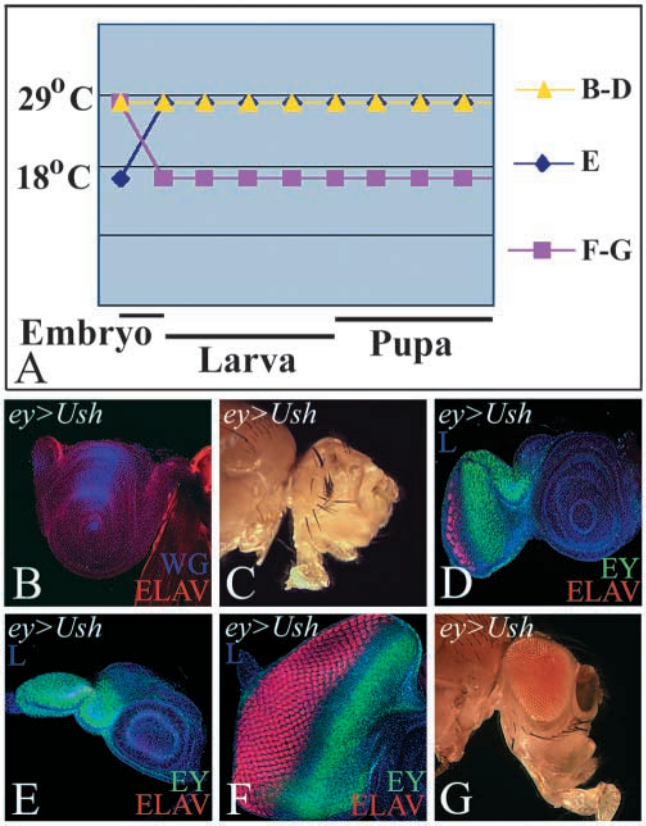

Fig. 2. Pnr is not essential for DV patterning of eye during embryogeneis. *Ush* was misexpressed in eye by *ey-GAL4; UAS-Ush (ey>Ush*) and cultures were shifted to 29°C during different stages of development. (A) Three different restrictive temperature conditions used. (B,D-F) Eye discs were stained for Wg or L (blue), Elav (red) and Ey (green). (B-D) Cultures maintained at 29°C throughout development served as controls and resulted in elimination of entire eye field (B) in eye disc and (C) in adult eye. (D) Some weaker phenotypes of very small eye marked by Elav-positive cells were also observed. (E) Maintenance at 18°C during embryonic development and then shift to 29°C for subsequent development also resulted in complete elimination of eye field. (F,G) When cultures were shifted to 29°C during embryonic development to block *pnr* activity and later allowed to develop at 18°C, they did not show any eye suppression phenotype (F) in the eye disc and (G) in adult eye.

2001). Second, the cultures were maintained at 18°C until embryonic development was over and then shifted to 29°C for the subsequent development to block the *pnr* activity after embryonic development is over. It resulted in elimination of the eye field (Fig. 2E) without affecting the antennal development as seen in the control experiment. Third, we blocked *pnr* activity during embryonic development by maintaining the culture at 29°C and then shifting it back to 18°C for the subsequent part of development and interestingly we found very subtle or no effect on eye development (Fig. 2F,G). We also removed the *pnr* function during early first instar of larval development and then shifted the cultures back to 18°C, which also resulted in a normal eye (data not shown). The results from these experiments further confirm our earlier conclusions that eye disc development during embryogenesis to early first instar of larval development is not sensitive to the loss of *pnr* gene function.

Lobe mutations suppress ventral eye development

As *L* and *Ser* are expressed in eye disc earlier than dorsal eye genes, we checked for the role of *L* and *Ser* during various stages of eye development. *L* mutant shows a selective loss of ventral eye (Fig. 3A) (Chern and Choi, 2002). We generated loss-of-function clones of *L* in the eye during different time windows using $L^{rev6\text{-}3}$ (hereafter L^{-}), a null allele of *L* (Chern and Choi, 2002). These phenotypes can be broadly divided into two groups. First, *ey-FLP* was used (Newsome et al., 2000) to generate loss-of-function clones of *L* exclusively in the eye (Xu and Rubin, 1993). These clones showed asymmetric response in the dorsal and the ventral eye. Loss-of-function clones of *L* in the dorsal eye did not affect the ommatidial development (Fig. 3B, arrow, B′) but the ventral eye disc clones inhibited the ommatidial development (Fig. 3B, arrowhead, B') and corresponding phenotypes were also observed in adult eye (Fig. 3C). In the adult eye, presence of ommatidia in the wild-type twin spot cells (L^{+}/L^{+}) for a ventral (L^{-}/L^{-}) clone suggested that *L* is required for the ventral eye development (Fig. 3C, arrow). We checked the fate of the cells in the loss-of-function clones of *L* by staining the eye discs with antibody against Pax6 homolog protein Eyeless (Ey). Ey marks the undifferentiated cells anterior to the morphogenetic furrow in the third instar disc (Halder et al., 1998). We found that in the ventral clones where the eye fate is blocked, ectopic Ey induction was seen behind the MF (Fig. 3D,D′ arrowhead). This suggested that in the absence of *L* gene function the ventral eye cells remain undifferentiated. As expected, dorsal eye clones where retinal differentiation was not blocked, did not show any ectopic Ey induction (data not shown).

Second, loss-of-function clones of *L* generated in the eye of early first instar larva using the heat shock FLP source (Struhl and Basler, 1993) could completely eliminate the eye fate, whereas the antennal development in the same disc was not compromised (Fig. 3E,E′). We obtained more consistent results with heat-shock FLP because of controlled induction of FLP during short time windows. Clones with *eyFLP* were probably induced stochastically during any time from embryogenesis onwards, which might cause more variable phenotypes. Earlier it has been shown that loss-of-function of *L* can selectively eliminate the ventral eye fate (Chern and Choi, 2002) but the removal of entire eye within the early loss-of-function clones was a surprise. It suggested that very early during development entire eye may be ventral in fate. Alternatively, it can also be interpreted that loss-of-function clones of *L* results in partial loss of *Ser*, which is also under the control of *fng* (Papayannopoulos et al., 1998). In this case, loss of Ser may prevent the correct establishment of the DV organizing center and hence affecting the growth of the entire disc. But this possibility can be ruled out as it has been shown that *L* mutant clones cause little effect on *Ser* expression near the DV border, although it results in strong reduction of *Ser* in other ventral region (Chern and Choi, 2002). Furthermore, we did not see the similar phenotypes of loss of entire eye or selective reduction of ventral eye when we generated loss-of-function clones of *fng* alone in different time windows. *fng* is preferentially expressed in ventral domain of early eye disc and its loss-of-function clones showed ommatidial polarity defects (Cho and Choi, 1998; Papayannopoulos et al., 1998) rather than complete elimination of ventral eye. On the contrary, loss-of-function clones of *fng* generated ectopic equator (Cho and

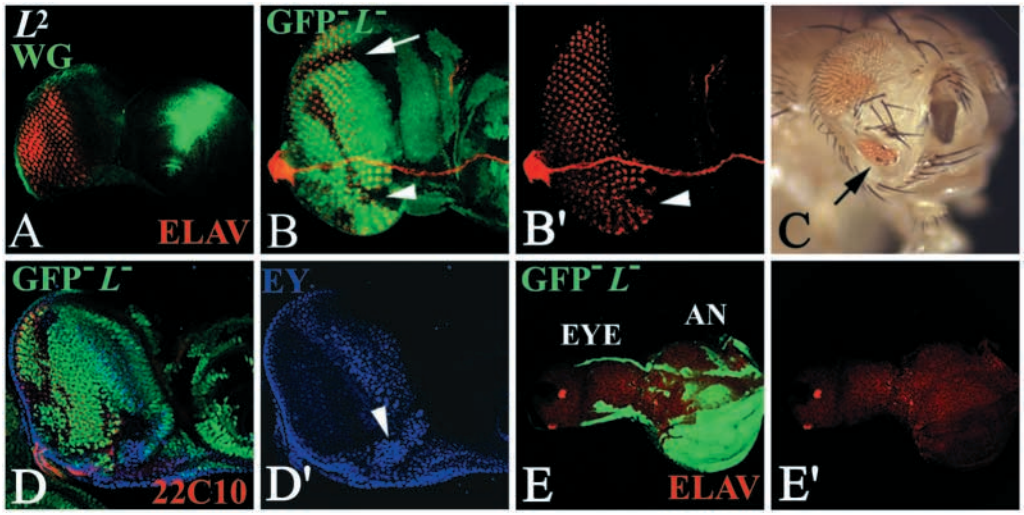

Fig. 3. Loss of function of *L* suppresses ventral eye development. (A) *L* mutant disc showing loss of ventral eye pattern. Loss-of-function clones of *L* were marked by absence of GFP (green) in the eye disc (B) and by absence of *white* gene expression in adult eye (C). Eye discs were stained for 22C10 or Elav. Clone in the dorsal eye shows no effect on eye fate in disc (B,B′, arrow). Ventral clone caused suppression of eye fate as seen by absence of 22C10 in disc (B,B′, arrowhead) and in adult (C, arrow). (D,D′) Ventral loss-of-function clone of *L* also showed ectopic induction of Ey where eye fate is blocked (arrowhead). (E,E′) Early loss-of-function clone of *L* showed complete elimination of eye fate as evident by absence of Elav (red). Note that eye field (EYE) is highly reduced, whereas antennal (AN) development was not affected.

Choi, 1998) and in rare cases (14/159) 5-10% of adult flies scored showed enlargement rather than loss of the ventral eye pattern (A.S. and K.-W.C., unpublished). These results suggest that *Ser* function in the early eye disc is independent of *fng* regulation. Because all the phenotypes of *fng* loss-of-function clones are manifested in terms of effect on polarity suggest that *fng* functions after the early DV lineage restriction is established in the eye.

Ser is required for early eye field development

Ser is known to be the downstream target of genes which affect ventral eye development, such as *fng* (Irvine, 1999; Papayannopoulos et al., 1998) and *L* (Chern and Choi, 2002). Ser^{DN}, a dominant-negative allele encoding a truncated form of Ser, is capable of antagonizing wild-type *Ser* functions (Hukriede et al., 1997). It consists of extracellular domain but lacks the transmembrane domain of Ser. Ser^{DN} was used to generate loss-of-function phenotype of *Ser* (Chern and Choi, 2002; Hukriede et al., 1997; Kumar and Moses, 2001). We used the temperature-dependent expression of the GAL4 enhancer trap (Brand and Perrimon, 1993), to determine the phenocritical period of Ser^{DN} overexpression ($ey>Ser^{DN}$) in the eye (Kumar and Moses, 2001). The rationale was to check the period when the *Ser* function is crucial for DV eye field development. Basically, the phenotypes scored in the eye disc can be grossly classified into three major categories as summarized in Fig. 4A. First category showed complete elimination of eye field to a very small eye. Second category included the eye discs with preferential elimination of the ventral eye pattern. The third category comprised the discs where there were two antennal fields also seen by Kumar and Moses (Kumar and Moses, 2001). These discs were also accompanied by the suppression of eye field. The split of the two antennal fields along with suppression of eye suggests that *Ser* also plays a role in patterning of antennal field.

In the early time window of 12-72 hours of development, misexpression of Ser^{DN} caused complete elimination of the eye field (Fig. 4B,C). These discs had a few Ey-expressing undifferentiated cells of anterior eye, whereas the antennal development was not at all affected in these discs (Fig. 4B). In the same 12- to 72-hour time window, some extremely small eye discs with a few photoreceptors were also seen (Fig. 4D). Misexpression of Ser^{DN} at 72-96 hours of development caused significant reduction in frequency of no-eye phenotype from near 100% to 60% (Fig. 4A) along with an increase in frequency of selective eye suppression in the ventral eye from near 0 to ~40%; (Fig. 4A,E). During 96-168 hours of development, concomitant with the presence of *pnr*-expressing dorsal cells, there is a sharp increase in frequency of eyes showing preferential loss of ventral eye pattern (Fig. 4A,F) when compared with no-eye phenotypes. We found that removal of *L/Ser* gene function during early eye development can completely abolish the entire eye field, whereas later during development these eye inhibition phenotypes become restricted to only the ventral eye. These time-dependent effects of Ser^{DN} further substantiated our view that the fate of early eye disc prior to the emergence of pnr^{+} cells is most probably ventral equivalent.

Loss-of-function of dorsal selectors change dorsal eye fate to early ventral-equivalent state

Lack of sensitivity of dorsal cells to *L/Ser* led us to check for the role of dorsal selectors in early DV patterning of eye. We generated loss-of-function clones of *pnr* in the eye using pnr^{VX6}, a null allele generated by a deletion of all but nine amino acids of the coding region (Heitzler et al., 1996). As previously described (Maurel-Zaffran and Treisman, 2000), loss-of-function clones of *pnr* changed the dorsal eye fate to ventral, which resulted in dorsal eye enlargements or ectopic eye caused by generation of new boundary of the *pnr* expressing- and non-expressing cells (data not shown) (Maurel-Zaffran and Treisman, 2000). Loss-of-function clones of *pnr* in the ventral eye had no effect as *pnr* is expressed only in the dorsal eye (Maurel-Zaffran and Treisman, 2000).

We have seen that before the onset of dorsal selector gene function, the entire eye disc is sensitive to *L/Ser* activity. We wanted to check if the eye disc ventralized by eliminating the dorsal selector gene function again becomes sensitive to

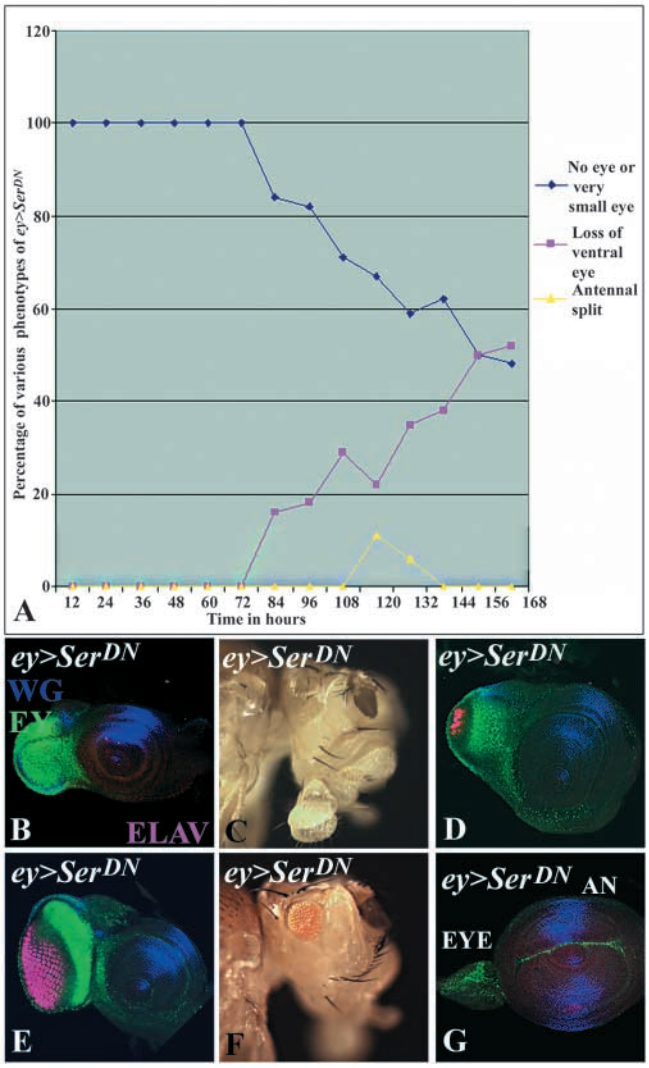

Fig. 4. Loss of *Ser* gene function can abolish the eye fate. (A) Graphical presentation of eye phenotypes generated by targeted misexpression of *ey-GAL4; UAS-Ser^{DN} (ey>Ser^{DN})* along the time course of temperature shifts for the samples collected at every 12 hours of interval until the late third instar. Effect of dominant-negative *Ser* (*Ser^{DN}*) was scored for its effect on eye fate in the discs and phenotypes observed were classified into three main categories: complete loss of eye fate (blue); loss of ventral eye pattern (purple) and generation of two antennal fields (yellow). For each time window, at least 20 discs were scored. (B,D,E,G) Eye discs were stained with Elav (red) and with Ey (green) and Wg (blue). Misexpression of *ey>Ser^{DN}* for 12-72 hours resulted in complete elimination of eye disc (B), adult eye (C) and eye disc with a few photoreceptors (D) (shown in blue in A). For 96-108 hours, *ey>Ser^{DN}* resulted in preferential loss of ventral eye in disc (E) and adult (F) (shown in purple in A). It has been suggested that loss of *Ser* using the same *ey>Ser^{DN}* caused the homeotic transformation of the antenna to the eye fate (Kumar and Moses, 2001). (G) *ey>Ser^{DN}* primarily showed suppression of the eye field and also occasionally (3/35) results in the generation of two antenna fields (shown in yellow in A). This may be due to 'splitting' of the antenna field, as evident from the mirror image duplication of Wg expression in the ventral sector of antenna disc (AN).

L/Ser acitivity as seen in early eye disc. Interestingly, we found that if *L* levels are increased continuously above the wild-type levels by using *ey-GAL4* (*ey>L*), it selectively eliminates the ventral eye pattern (Fig. 5A,B arrows; Table 1) (J. J. Chern, PhD Thesis, Baylor College of Medicine, 2003). This suggests that optimum levels of *L* are required for ventral eye growth and development. We used this property of *L* as an assay system to check if the eye discs when mutated for dorsal selector gene function can revert back to ventral, which is sensitive to levels of *L* gene function. We used the EGUF system (Stowers and Schwarz, 1999) to generate eye disc where all the cells other than those mutant for *pnr* were ablated using *GMR>hid*. The rationale of using this approach is that *GMR>hid* kills the cells later during eye differentiation, therefore these mosaic eye discs could grow. Eye disc mutant for *pnr* gene function showed dorsal overgrowths in disc (Fig. 5C) and in adult eyes (Fig. 5D, Table 1). By contrast, when *L* was overexpressed continuously in eye using *ey-GAL4* driver (*ey>L*), *pnr* mutant discs resulted in very small eye (Fig. 5E,F, arrow and arrowhead; Table 1). The small eye phenotype was different from either of the two controls used; *ey>L* alone causes ventral-specific eye loss (Fig. 5A,B), whereas EGUF clones of *pnr* results in dorsally enlarged eye (Fig. 5C,D). Therefore, these results suggest that small eye phenotype was generated because of suppression of eye by overexpression of L on both dorsal (which has changed to ventral) and ventral eye margins. Furthermore, we also analysed the fate of cells left in the small eyes generated by EGUF clones and overexpression of *L* by sectioning the adult eyes. The polarity of most of the ommatidia left in these eyes was dorsal along with a few ventral or with a polarity defect (data not shown). We also checked the sensitivity of the *pnr* mutant discs to *Ser*

Table 1. Summary of phenotypes shown by EGUF clones of *pnr* and *Iro-C*

Genotypes	Wild type controls (no EGUF)	EGUF *pnr*$^{-}$ clones	EGUF *Iro-C*$^{-}$ clones
Wild type controls (no GAL4/UAS)	Wild-type eye	Dorsal eye enlargements	Dorsal eye enlargements
UAS-L; *ey-GAL4* (*ey>L*)	Twenty percent of flies show ventral eye-specific reduction*	Nearly 20% of the flies show small eye due to reduction of eye on both dorsal and ventral eye margins	Nearly 20% of the flies show small eye due to reduction of eye on both dorsal and ventral eye margins
ey-GAL4; UAS-Ser^{DN} (*ey>Ser^{DN}*) at 25°C	Fifty percent of flies show complete loss of eye to very small eye	Ninety-nine percent of flies show complete loss of eye	Ninety-nine percent of flies show complete loss of eye

Percentages have been calculated based on eye disc and adult eye phenotypes independently and results presented are averages of both. Minimum sample size for each experiment was 20 imaginal discs and 100 adult flies.

*There is low penetrance in *ey-GAL4; UAS-L* phenotype in eye.

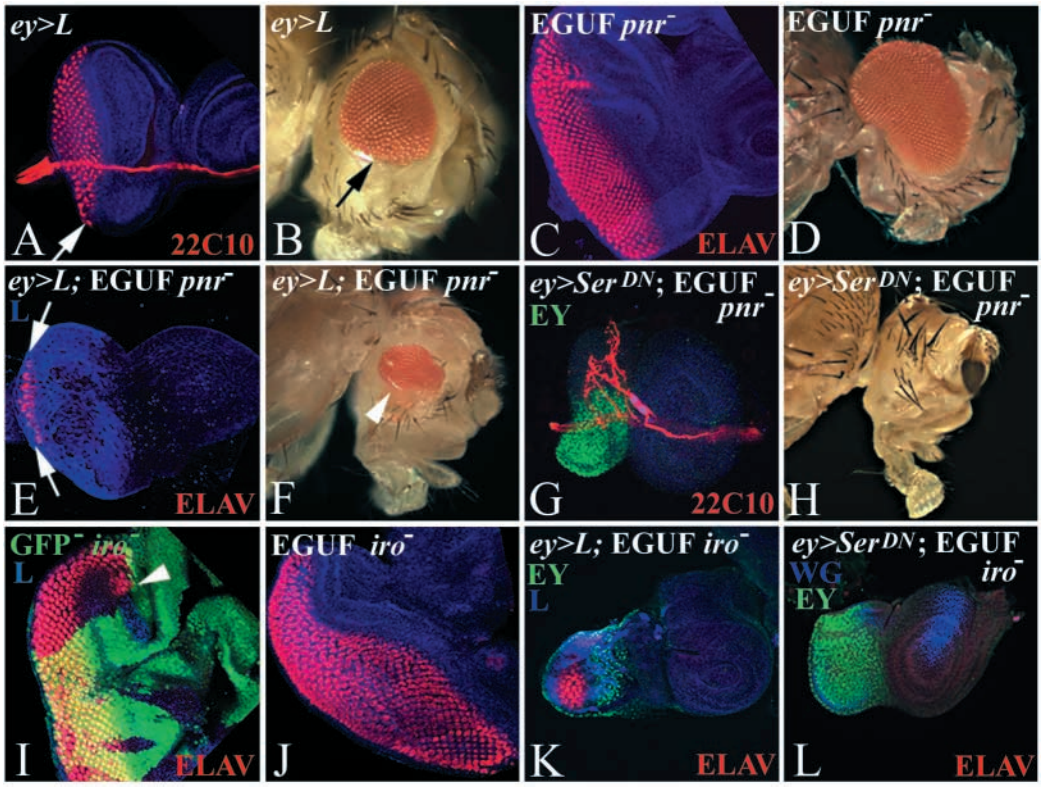

Fig. 5. Loss-of-function of *pnr* and *Iro-C* changes dorsal eye sensitivity to ventral. (A,B) Overexpression of *L* by *ey>L* causes selective ventral eye suppression in disc (A, arrow) and adult (B, arrow). Loss-of-function clones of *pnr* were generated in eye using EGUF approach, which resulted in dorsal eye enlargement (C) in disc and (D) in adult. In the ventralized disc with EGUF *pnr* clones in dorsal when *L* (*ey>L)* was overexpressed resulted in small eye because of suppression of eye fate on both dorsal and ventral margin of (E) disc (arrows) and in (F) adult eye (arrowhead). Misexpression of Ser^{DN} (*ey>* Ser^{DN}) in the ventralized disc with EGUF *pnr* clones completely abolished the entire eye fate (G) in disc and in (H) adult eye. Loss-of-function clones of *Iro-C* show dorsal eye enlargement in (I) disc. (J) EGUF *Iro-C* clones in eye disc also result in dorsal eye enlargements. (K,L) In the ventralized eye disc mutant for *Iro-C*, overexpression of *ey>L* (K) or *ey>*Ser^{DN} (L) results in suppression of eye on both DV margins and complete removal of the eye fate, respectively.

activity. Misexpression of Ser^{DN} continuously during development in the same *pnr* mutant discs at 25°C completely abolished the eye fate in nearly 99% of discs (Fig. 5H, Table 1), and corresponding phenotypes were also seen in the unhatched pupae that were dissected out to check their phenotypes (data not shown). These results suggest that removal of *pnr* gene function in the eye disc changes the dorsal eye fate to ventral, which makes the entire disc sensitive to *ey>L* or Ser^{DN} as observed in early eye disc.

Loss-of-function clones of *Iro-C* mutation were generated in the eye using iro^{DFM3}, a deficiency for all three members, i.e. *ara*, *caup* and *mirr* (Diez del Corral et al., 1999). These clones also showed enlargement in the dorsal eye (Fig. 5I) (Cavodeassi et al., 1999), a phenotype similar to that seen in the *pnr* loss-of-function clones, whereas in the ventral eye there was no effect of these clones (data not shown) (Cavodeassi et al., 1999). Eye discs mutant for *Iro-C* gene function generated by EGUF approach resulted in enlarged disc (Fig. 5J), as seen in loss-of-function clones in Fig. 5I. Overexpression of *L* (*ey>L*) in eye disc with *Iro-C* EGUF clones resulted in small eyes with suppression of the eye on both dorsal and ventral margins (Fig. 5K, Table 1), but the phenotypes were not as severe as seen in *pnr*. Misexpression of Ser^{DN} in *Iro-C* mutant eye discs completely abolished the eye fate (Fig. 5L). These results suggest that when the eye fate changes from dorsal to ventral in response to removing dorsal selector gene function, the eye fate reverts back from the dorsal to its default ventral state. Therefore, the entire eye disc responds to *L/Ser* activity in a similar fashion to that seen in the early eye disc before the onset of expression of dorsal eye selector genes.

Discussion

We have addressed a basic question of how patterning and growth of early eye primordium are regulated. Our results provide an important insight into the role of genes controlling ventral eye growth. Previously, *L/Ser* were thought to be required for ventral eye growth after the DV lineage restriction boundary was established, which corresponds to the onset of expression of dorsal eye selectors. Our results clearly suggest that *L/Ser* are required much earlier for the growth of the entire early eye disc, even before the DV patterning is established. In contrast to the function of dorsal selector genes in eye patterning, *L* and *Ser* have been shown to play a distinct role in controlling ventral-specific growth of eye disc.

Temporal requirement of genes controlling ventral eye development

It has been shown that loss-of-function phenotypes of *L* or *Ser* are restricted to the ventral eye (Chern and Choi, 2002). We checked the spatial as well as temporal requirement of these genes in the ventral eye pattern formation. We found that extent of loss of ventral eye pattern in loss-of-function clones of *L/Ser* varied along the temporal scale. During early eye disc development, prior to onset of *pnr* expression in dorsal eye, removal of *L* or *Ser* function resulted in complete elimination of the eye field, whereas later when dorsal eye selector genes starts expressing the eye suppression phenotype becomes restricted only to the ventral eye (Figs 2-5). Therefore, DV lineage border in the eye can also be interpreted as the border between the cells sensitive and insensitive to the *L/Ser* gene function.

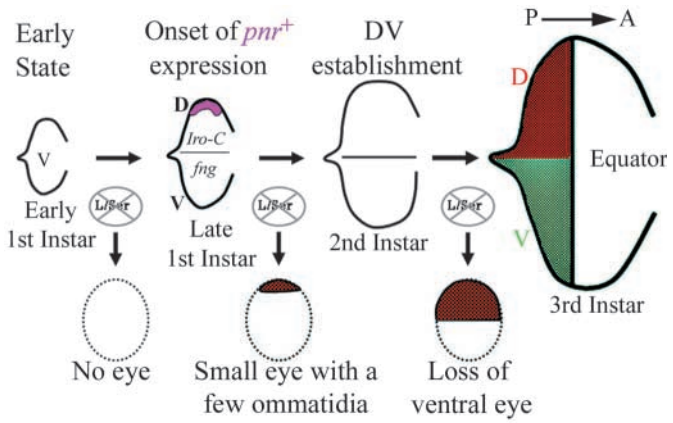

Fig. 6. Larval eye primordia arise from an initial state comprising a group of cells that require *L/Ser* function for growth and maintenance. Removal of *L*/Ser function in these initial cells can completely eliminate eye. During late first instar of development, the *pnr*$^{+}$ cells emerge and initiate the expression of downstream *Iro-C* genes, which results in DV specification of eye. Establishment of DV lineage in eye restricts the *L/Ser* requirement to the ventral cells only. *pnr*$^{+}$ and *Iro-C*$^{+}$ cells become independent of *L/Ser* requirement. Therefore, the initial state prior to DV specification is probably equivalent to ventral eye in nature.

Initial state of eye is ventral equivalent

The eye antennal disc has the most complex origin in the embryo. The eye disc is initiated from a small group of ~70 precursor cells on each side contributed by six different head segments of the embryo (Jurgens and Hartenstein, 1993). These embryonic precursors do not physically separate from the surrounding larval primordia and are therefore difficult to discern morphologically.

Once the cells for the eye-antennal disc are committed, these discs proliferate and undergo differentiation into an adult eye, which requires generation of DV lineage restriction in eye. There are possibly three different ways by which genesis of DV lineage in the eye can be explained. Early first instar larval eye disc may initiate either from only dorsal, only ventral or from both DV lineages. Based on our results from studies of expression patterns (Fig. 1) and analysis of mutant phenotypes (Figs 2-5), we propose that larval eye primordium initially comprises only the ventral-equivalent state (Fig. 6) rather than well-defined DV or dorsal states alone. We have referred the initial state of eye as ventral equivalent state because, at this stage, dorsal and ventral identity is not yet generated. DV lineage restriction is established later after the onset of *pnr* expression. The cells of the initial ventral-equivalent state are similar to the ventral eye cells that are generated after DV specification. The similarity is in terms of their requirement of *L/Ser* for growth and maintenance, and the absence of the dorsal selector expression. How dorsal lineage is initiated in the early eye disc is not yet clear. Once the DV lineage restriction is established, N signaling is initiated at the equator, a border between dorsal and ventral compartments. Activation of N signaling promotes proliferation, which is followed by differentiation of eye disc into adult compound eye.

Our ventral-equivalent state model is supported by two observations. First, presence of *Ser* and *L* expression in the dorsal and ventral eye disc of the early first instar larva. Second, change of dorsal eye fate to ventral upon removal of dorsal selectors. It has been observed that the mutants, which affect ventral eye development, show two major phenotypes in eye: either there is no or very small eye, or there is a preferential loss of ventral eye based on the time they affect their function but none of the mutants for dorsal eye selectors show phenotypes of loss of only dorsal eye. Conversely, loss-of-function clones of *pnr* or *Iro-C* causes dorsal eye enlargement or ectopic eye formation rather than loss of only dorsal eye clonal tissue (Fig. 4) (Maurel-Zaffran and Treisman, 2000; Cavodeassi et al., 1999). This phenotype is probably due to generation of ectopic boundary of *pnr*-expressing and non-expressing cells (rather than absence of *pnr*), which could be important for promoting eye growth (Maurel-Zaffran and Treisman, 2000). Overexpression of Ush or Fog proteins in eye discs results in loss of *pnr* activity, causing complete elimination of eye development (Fossett et al., 2001). By removing *pnr* activity at different time points we found that *pnr* activity in embryo and early first instar is not essential for eye disc development (Fig. 2). Later, *pnr* becomes essential for DV patterning consistent with its strong expression in dorsal margin of eye disc after early first instar stage.

Dorsal selectors and *Lobe/Ser* affect the eye development at two different tiers

In contrast to enlargements or ectopic eyes induced by loss-of-function clones of dorsal selectors (Cavodeassi et al., 1999; Maurel-Zaffran and Treisman, 2000), the loss-of-function clones of *L* or *Ser* always resulted in the elimination of the eye fate. *L/Ser* are primarily required for the maintenance and development of ventral or ventral-equivalent state of the eye, whereas dorsal genes establish the DV border. This suggests that dorsal genes and *L/Ser*, although involved in a common goal of generation of DV lineage in eye, probably affect eye development at two different tiers.

Fng, another essential component of DV patterning in eye, is expressed preferentially in the ventral domain of early eye disc and is required for restriction of N signaling to the DV border (Cho and Choi, 1998; Dominguez and de Celis, 1998; Papayannopoulos et al., 1998). Although *fng* is known to act upstream of *Ser* in the wing and eye discs (Irvine, 1999), there is also an apparent difference between the two genes. Unlike *L/Ser*, the main function of *fng* seems to affect DV ommatidial polarity but not the growth (Cho and Choi, 1998; Dominguez and de Celis, 1998; Papayannopoulos et al., 1998). This suggests that *fng* may be selectively required for DV patterning after dorsal selectors initiate domain specification. This may be the reason why phenotypes of loss-of-function clones of *fng* are different from those of *L* and *Ser* in the eye. It has been observed that the pattern of *fng* expression is not altered in *L* mutants, and vice versa, supporting the independent functions of these two genes in controlling DV border formation and growth of ventral domain (data not shown).

Functional conservation of dorsal selector Pnr

The function of Pnr in organizing the DV pattern from an initial ventral-equivalent state raises an interesting question of whether similar patterning processes occur in other developing tissues and organs. Interestingly, Pnr is expressed in a broad dorsal domain in early embryos, but later refined

in a longitudinal dorsal domain extending along the thoracic and abdominal segments. During this stage, Pnr has an instructive and selector-like function, determining the identity of the medial dorsal structures (Calleja et al., 2000). It has been shown that loss of *pnr* eliminates the dorsomedial pattern in the larval cuticle whereas the dorsolateral pattern extends dorsally without cell loss (Herranz and Morata, 2001). This suggests that DV pattern in the larval cuticle is established with the onset of Pnr expression in the dorsomedial domain, and ventral may be the initial fate of epidermal cells.

The compound eye of *Drosophila* shares some similarities with the vertebrate eye (Hartenstein and Reh, 2002). Like *Drosophila*, in higher vertebrates dorsal eye genes (e.g. *Bmp4* and *Tbx5*) also act as 'dorsal selectors' and restrict the expression of genes involved in ventral eye development (e.g. *Vax* and *Pax2*) to the ventral eye (Koshiba-Takeuchi et al., 2000; Peters and Cepko, 2002). These DV expression domains correspond to developmental compartments (Peters, 2002) and thereby generate DV lineage restrictions in a way similar to *Drosophila* eye. Furthermore, conservation is also seen at the level of genes and probably their functions. For example, *Ser* has a vertebrate homolog *Jag1*, the loss of function of which shows a strong eye reduction phenotype (Xue et al., 1999). Other dorsal eye genes, such as *pnr* and *Iro-C*, are also highly conserved. *Iro-C* genes are involved in neural development in vertebrates (Gomez-Skarmeta and Modolell, 2002). There is conservation even in the eye patterning mechanism because the wave of neurogenesis in the vertebrate eye is analogous to the morphogenetic furrow in the fly eye (Holt and Harris, 1993; Neumann and Nuesslein-Volhard, 2000; Peters, 2002). Therefore, it would be interesting to see whether the DV lineage in the vertebrate eye also develops from a ventral-equivalent initial state. It has been observed that DV patterning regulates the connectivity of retinal ganglion cells to their targets in brain (Peters, 2002). Therefore, the study of DV patterning in vertebrate eye holds immense potential.

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References

Baker, W. K. (1978). A clonal analysis reveals early developmental restrictions in the *Drosophila* head. *Dev. Biol.* **62**, 447-463.

Blair, S. S. (1995). Compartments and appendage development in *Drosophila*. *BioEssays* **17**, 299-309.

Blair, S. S. (2001). Cell lineage: compartments and Capricious. *Curr. Biol.* **11**, R1017-R1021.

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.

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

Calleja, M., Herranz, H., Estella, C., Casal, J., Lawrence, P., Simpson, P. and Morata, G. (2000). Generation of medial and lateral dorsal body domains by the *pannier* gene of *Drosophila*. *Development* **127**, 3971-3980.

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.

Chern, J. J. and Choi, K. W. (2002). *Lobe* mediates Notch signaling to control domain-specific growth in the *Drosophila* eye disc. *Development* **129**, 4005-4013.

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

Choi, K. W., Mozer, B. and Benzer, S. (1996). Independent determination of symmetry and polarity in the *Drosophila* eye. *Proc. Natl. Acad. Sci. USA* **93**, 5737-5741.

Curtiss, J., Halder, G. and Mlodzik, M. (2002). Selector and signalling molecules cooperate in organ patterning. *Nat. Cell Biol.* **4**, E48-E51.

Diaz-Benjumea, F. J. and Cohen, S. M. (1993). Interaction between dorsal and ventral cells in the imaginal disc directs wing development in *Drosophila*. *Cell* **75**, 741-752.

Diez del Corral, R., Aroca, P., Gomez-Skarmeta, J.L., Cavodeassi, F. and Modolell, J. (1999). The Iroquois homeodomain proteins are required to specify body wall identity in *Drosophila*. *Genes Dev.* **13**, 1754-1761.

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.

Duffy, J. B., Harrison, D. A. and Perrimon, N. (1998). Identifying loci required for follicular patterning using directed mosaics. *Development* **125**, 2263-2271.

Fossett, N., Tevosian, S. G., Gajewski, K., Zhang, Q., Orkin, S. H. and Schulz, R. A. (2001). The Friend of GATA proteins U-shaped, FOG-1, and FOG-2 function as negative regulators of blood, heart, and eye development in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **98**, 7342-7347.

Garcia-Bellido, A., Ripoll, P. and Morata, G. (1973). Developmental compartmentalisation of the wing disk of *Drosophila*. *Nat. New Biol.* **245**, 251-253.

Garcia-Bellido, A. and Santamaria, P. (1972). Developmental analysis of the wing disc in the mutant *engrailed* of *Drosophila melanogaster*. *Genetics* **72**, 87-104.

Gomez-Skarmeta, J. L. and Modolell, J. (1996). *araucan* and *caupolican* provide a link between compartment subdivisions and patterning of sensory organs and veins in the *Drosophila* wing. *Genes Dev.* **10**, 2935-2945.

Gomez-Skarmeta, J. L. and Modolell, J. (2002). *Iroquois* genes: genomic organization and function in vertebrate neural development. *Curr. Opin. Genet. Dev.* **12**, 403-408.

Haenlin, M., Cubadda, Y., Blondeau, F., Heitzler, P., Lutz, Y., Simpson, P. and Ramain, P. (1997). Transcriptional activity of pannier is regulated negatively by heterodimerization of the GATA DNA-binding domain with a cofactor encoded by the *u-shaped* gene of *Drosophila*. *Genes Dev.* **11**, 3096-3108.

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.

Hartenstein, V. and Reh, T. A. (2002). Homologies between vertebrate and invertebrate eyes. In *Drosophila Eye Development*, Vol. 37 (ed. K. Moses), pp. 219-51. Berlin: Springer-Verlag.

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. and Moses, K. (1995). Mechanisms of *Drosophila* retinal morphogenesis: the virtues of being progressive. *Cell* **81**, 987-990.

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

Heitzler, P., Haenlin, M., Ramain, P., Calleja, M. and Simpson, P. (1996). A genetic analysis of *pannier*, a gene necessary for viability of dorsal tissues and bristle positioning in *Drosophila*. *Genetics* **143**, 1271-1286.

Herranz, H. and Morata, G. (2001). The functions of *pannier* during *Drosophila* embryogenesis. *Development* **128**, 4837-4846.

Holt, C. E. and Harris, W. A. (1993). Position, guidance, and mapping in the developing visual system. *J. Neurobiol.* **24**, 1400-1422.

Hukriede, N. A., Gu, Y. and Fleming, R. J. (1997). A dominant-negative form of Serrate acts as a general antagonist of Notch activation. *Development* **124**, 3427-3437.

Huppert, S. S., Jacobsen, T. L. and Muskavitch, M. A. (1997). Feedback regulation is central to Delta-Notch signalling required for *Drosophila* wing vein morphogenesis. *Development* **124**, 3283-3291.

Irvine, K. D. (1999). Fringe, Notch, and making developmental boundaries. *Curr. Opin. Genet. Dev.* **9**, 434-441.

Jurgens, G. and Hartenstein, V. (1993). The terminal regions of the body pattern. In *The Development of* Drosophila melanogaster, Vol. I (ed. M. Bate and A. Martinez-Arias), pp. 687-746. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Kehl, B. T., Cho, K. O. and Choi, K. W. (1998). *mirror,* a *Drosophila* homeobox gene in the Iroquois complex, is required for sensory organ and alula formation. *Development* **125**, 1217-1227.

Koshiba-Takeuchi, K., Takeuchi, J. K., Matsumoto, K., Momose, T., Uno, K., Hoepker, V., Ogura, K., Takahashi, N., Nakamura, H., Yasuda, K. et al. (2000). Tbx5 and the retinotectum projection. *Science* **287**, 134-137.

Kumar, J. P. and Moses, K. (2001). EGF receptor and Notch signaling act upstream of Eyeless/Pax6 to control eye specification. *Cell* **104**, 687-697.

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.

Mann, R. S. and Carroll, S. B. (2002). Molecular mechanisms of selector gene function and evolution. *Curr. Opin. Genet. Dev.* **12**, 592-600.

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.

Milan, M. and Cohen, S. M. (2003). A re-evaluation of the contributions of Apterous and Notch to the dorsoventral lineage restriction boundary in the *Drosophila* wing. *Development* **130**, 553-562.

Morata, G. and Lawrence, P. A. (1975). Control of compartment development by the *engrailed* gene in *Drosophila*. *Nature* **255**, 614-617.

Morata, G. and Lawrence, P. A. (1978). Anterior and posterior compartments in the head of *Drosophila*. *Nature* **274**, 473-474.

Neumann, C. J. and Nusslein-Volhard, C. (2000). Patterning of the zebrafish retina by a wave of sonic hedgehog activity. *Science* **289**, 2137-2139.

Newsome, T. P., Asling, B. and Dickson, B. J. (2000). Analysis of *Drosophila* photoreceptor axon guidance in eye-specific mosaics. *Development* **127**, 851-860.

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.

Peters, M. A. (2002). Patterning the neural retina. *Curr. Opin. Neurobiol.* **12**, 43-48.

Peters, M. A. and Cepko, C. L. (2002). The dorsal-ventral axis of the neural retina is divided into multiple domains of restricted gene expression which exhibit features of lineage compartments. *Dev. Biol.* **251**, 59-73.

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.

Ready, D. F., Hanson, T. E. and Benzer, S. (1976). Development of the *Drosophila* retina, a neurocrystalline lattice. *Dev. Biol.* **53**, 217-240.

Singh, A., Kango-Singh, M. and Sun, Y. H. (2002). Eye suppression, a novel function of *teashirt*, requires Wingless signaling. *Development* **129**, 4271-4280.

Stowers, R. S. and Schwarz, T. L. (1999). A genetic method for generating *Drosophila* eyes composed exclusively of mitotic clones of a single genotype. *Genetics* **152**, 1631-1639.

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

Tabata, T., Schwartz, C., Gustavson, E., Ali, Z. and Kornberg, T. B. (1995). Creating a *Drosophila* wing de novo, the role of *engrailed*, and the compartment border hypothesis. *Development* **121**, 3359-3369.

Wolff, T. and Ready, D. F. (1993). Pattern formation in the *Drosophila* retina. In *The Development of* Drosophila melanogaster, Vol. 2 (ed. M. Bate and A. Martinez-Arias), pp. 127-132. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Wu, J. Y. and Rao, Y. (1999). Fringe: defining borders by regulating the *Notch* pathway. *Curr. Opin. Neurobiol.* **9**, 537-543.

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

Xue, Y., Gao, X., Lindsell, C. E., Norton, C. R., Chang, B., Hicks, C., Gendron-Maguire, M., Rand, E. B., Weinmaster, G. and Gridley, T. (1999). Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1. *Hum. Mol. Genet.* **8**, 723-730.