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# JAK/STAT signaling promotes regional specification by negatively regulating *wingless* expression in *Drosophila*

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During development, a small number of conserved signaling molecules regulate regional specification, in which uniform populations of cells acquire differences and ultimately give rise to distinct organs. In the *Drosophila* eye imaginal disc, Wingless (Wg) signaling defines the region that gives rise to head tissue. JAK/STAT signaling was thought to regulate growth of the eye disc but not pattern formation. However, we show that the JAK/STAT pathway plays an important role in patterning the eye disc: it promotes formation of the eye field through repression of the *wg* gene. Overexpression of the JAK/STAT activating ligand Unpaired in the eye leads to loss of *wg* expression and ectopic morphogenetic furrow initiation from the lateral margins. Conversely, tissue lacking *stat92E*, which cannot transduce JAK/STAT signals, is transformed from retinal tissue into head cuticle, a phenotype that is also observed with ectopic Wg signaling. Consistent with this, cells lacking *stat92E* exhibit ectopic *wg* expression. Conversely, *wg* is autonomously repressed in cells with hyperactivated Stat92E. Furthermore, we show that the JAK/STAT pathway regulates a small enhancer in the *wg* 3′ *cis* genomic region. As this enhancer is devoid of Stat92E-binding elements, we conclude that Stat92E represses *wg* through another, as yet unidentified factor that is probably a direct target of Stat92E. Taken together, our study is the first to demonstrate a role for the JAK/STAT pathway in regional specification by acting antagonistically to *wg*.

**KEY WORDS: STAT, JAK, Unpaired, *Drosophila*, *wingless*, Eye imaginal disc, In vivo reporter, Gene expression, Signal transduction**

## INTRODUCTION

In metazoans, homogeneous cell populations acquire regional differences and form specific domains that ultimately give rise to distinct organs. This process of regional specification occurs through a cascade of cell fate decisions, in which broad regional specification is followed by differentiation of increasingly smaller domains. In both *Drosophila* and vertebrates, a limited number of conserved signaling pathways, used reiteratively throughout development, regulate these processes. *Drosophila* imaginal discs represent an excellent model system to dissect the signals that control regional specification. Imaginal discs begin as groups of 20 to 50 cells that are formed as a single layer of epithelium that invaginates from the embryonic ectoderm (Cohen, 1993). Two opposing layers, the peripodial membrane and the disc proper, enclose the disc lumen and give the disc a sac-like appearance. Discs grow and differentiate during the three larval instars and evert during pupal development to become functional in the adult.

The eye imaginal disc is part of the compound eye-antennal disc and differentiates into the adult retina and head capsule (Haynie and Bryant, 1986). Early in development, there is no regional identity in the eye-antennal primordium (Dominguez and Casares, 2005). The eye field emerges during the second larval instar by the action of the selector genes *eyeless* (*ey*) and *twin of eyeless* (Czerny et al., 1999; Quiring et al., 1994). These factors are required for the subsequent expression of the nuclear factor Eyes absent (Eya) in the posterior region of the eye-antennal disc, which is the presumptive eye field (Bonini et al., 1993). Eya is the first of a group of nuclear factors called the 'early retinal genes', which also includes Sine oculis and Dachshund (Dac), that function together in a network to lock in eye fate (Pichaud et al., 2001). Decapentaplegic (Dpp), a secreted factor and BMP family member, is required for Eya expression and is expressed in a similar pattern to Eya (Kenyon et al., 2003). Wingless (Wg), a secreted Wnt factor induced by the GATA transcription factor Pannier (Pnr), acts antagonistically to Dpp and is expressed in the anterior domain of the eye in a pattern complementary to Dpp (Cho et al., 2000; Maurel-Zaffran and Treisman, 2000). By negatively regulating the expression of early retinal genes, Wg controls the specification of retinal territory (Baonza and Freeman, 2002). In early eye development, when most of the cells in the eye disc receive both the Wg and the Dpp signal, retinal determination cannot take place (Cavodeassi et al., 1999; Pichaud and Casares, 2000; Royet and Finkelstein, 1996).

During second instar, the eye disc becomes divided into dorsal and ventral compartments, which promotes growth of the entire disc by localized activation of Notch at the dorsoventral midline. Notch induces expression of Unpaired (Upd), a secreted mitogenic factor that activates the JAK/STAT pathway and promotes growth of the eye disc (Bach et al., 2003; Chao et al., 2004; Reynolds-Kenneally and Mlodzik, 2005; Tsai and Sun, 2004). This growth separates *wg* and *dpp* domains, enabling anterior 'head' and posterior 'eye' regions to form in response to Wg and Dpp signaling, respectively (Kenyon et al., 2003). Following partition, *wg* is localized to the dorsal and ventral anterior margins of the eye disc and is maintained by Pnr and the homeodomain transcription factor Homothorax (Hth), respectively (Maurel-Zaffran and Treisman, 2000; Pichaud and Casares, 2000).

Retinal differentiation begins in third instar at the posterior midline of the eye disc, where the morphogenetic furrow forms and moves in an anterior direction across the eye disc (Ready et al., 1976). The antagonistic relationship between Dpp and Wg restricts the onset of differentiation to a narrow region at the posterior margin in the eye disc (Chanut and Heberlein, 1997; Ma and Moses, 1995; Pignoni and Zipursky, 1997; Treisman and Rubin, 1995).

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Progression of the furrow requires Hedgehog, which is expressed in differentiated photoreceptors posterior to the furrow (Heberlein et al., 1993; Ma et al., 1993).

We have examined the contribution of the JAK/STAT pathway to regional specification. In mammals, this pathway is activated by cytokines and growth factors that bind to specific cell-surface receptors and lead to the activation of JAK tyrosine kinases that phosphorylate and activate latent cytosolic STAT transcription factors (reviewed by Levy and Darnell, Jr, 2002). Activated STATs dimerize and accumulate in the nucleus where they alter transcription of target genes. Studies have elucidated evolutionarily conserved roles for JAK/STAT signaling, including hematopoiesis, immunity and proliferation (Arbouzova and Zeidler, 2006; Levy and Darnell, Jr, 2002).

*Drosophila* serves as an excellent model system to study this pathway, as flies are highly amenable to genetic manipulation and possess only one JAK and one STAT gene, compared with the four JAKs and the seven STATs in mammals (Arbouzova and Zeidler, 2006; Levy and Darnell, Jr, 2002). Genetic studies in *Drosophila* have uncovered several components of this pathway, including Upd and two other Upd-like ligands called Upd2 and Upd3 (Agaisse et al., 2003; Harrison et al., 1995; Hombria et al., 2005); the transmembrane receptor Domeless (Dome), which is distantly related to the gp130 family of cytokine receptors (Brown et al., 2001; Chen et al., 2002); the JAK Hopscotch (Hop) (Binari and Perrimon, 1994); and the STAT protein Stat92E (Hou et al., 1996; Yan et al., 1996). The JAK/STAT pathway is crucial for diverse processes in *Drosophila*, including proliferation and planar polarity in the developing eye (Bach et al., 2003; Luo et al., 1999; Tsai and Sun, 2004; Zeidler et al., 1999).

In this paper we report that the JAK/STAT pathway regulates regional specification of the eye disc by promoting the formation of the eye field via repression of the *wg* gene. Specifically, we demonstrate that JAK/STAT signaling negatively regulates *wg* expression in the eye disc epithelium and in the peripodial membrane and promotes the formation of eye tissue. This is the first demonstration that the repression of *wg* by the JAK/STAT pathway is an important mechanism in regional specification of the eye imaginal disc. Furthermore, this is the first report of a functional interaction between the JAK/STAT and Wnt signaling pathways, and suggests the possibility that a similar interaction exists in higher organisms.

## MATERIALS AND METHODS

### *D. melanogaster* stocks and transgenes

The following stocks are described in FlyBase: *stat92E*$^{85C9}$; *stat92E*$^{397}$; *stat92E*$^{06346}$; *wg*$^{CX3}$ *dpp-lacZ*; *wg-lacZ* (*wg*$^{P}$); *UAS-upd*; *UAS-hop*; *pnr-GAL4*, *UAS-gfp* (*pnr>GFP*); *UAS-gfp; yw*; *P{AyGAL4}25 P{UAS-GFP.S65T}T2; hs-flp MKRS/TM6B*; *upd-GAL4*; *ey-GAL4*; *hop*$^{M13}$; *ey-FLP*; *UAS-flp*; *P{neoFRT}*$^{82B}$ *P{Ubi-GFP(S65T)*$_{nls}$*}3R/TM6B*, *Tb*$^{1}$; *P{neoFRT}*$^{82B}$ *P{arm-lacZ.V}83B/TM6C*, *Sb*$^{1}$ *Tb*$^{1}$; *P{neoFRT}*$^{82B}$ *M(3)96C*, *arm-lacZ*; *P{neoFRT}*$^{82B}$ *RpS3(1)*, *Ubi-GFP*; and *P{neoFRT}*$^{82B}$ *ry*$^{506}$. We used *10XSTAT92E-GFP* (Bach et al., 2006) and *wg2.11Z* (Pereira et al., 2006). All crosses were maintained at 25°C.

We used PCR to generate a full-length *stat92E* cDNA with *Bam*HI (5′) and *Not*I (3′) ends using the following primers (restriction sites underlined): 5′-CGCGGATCCATGAGCTTGTGGAAGCGCATCGCCAGCC-3′ and 5′-TTTCCTTTGCGGCCGCAAAGTTCTCAAAGTTGTAATCGTATCG-3′. After digestion with *Bam*HI and *Not*I, the insert was ligated into the pBluescript KS-based vector C5HA3. The 5′ end of the C5HA3 polylinker includes an ATG immediately upstream of sequence encoding three HA epitopes, followed by an in-frame *Bam*HI site and the *costal2* cDNA (a kind gift of Kent Nybakken, Harvard Medical School, Boston, MA, USA). At the 3′ end, a *Not*I site immediately precedes and is in frame with a stop codon. The C5HA3 plasmid was digested with *Bam*HI and *Not*I to remove the *costal2* sequence. The *3HA-stat92E* insert was excised from C5HA3 by digestion with *Bss*HII. The 3′ recessed termini were filled in with Klenow. This fragment was ligated into *UASp* (Rorth, 1996) that had been cut with *Bam*HI and filled in with Klenow. *UASp-3HA-stat92E* transgenic flies were generated as described (Bach et al., 2003).

### Marked clones of mutant cells

Clones were generated using the FLP/FRT system (Xu and Rubin, 1993). *ey-flp* or *ey-GAL4*, *UAS-flp* (*EGUF*) transgenic animals express FLP in the eye disc (Newsome et al., 2000). Clones were marked using the following chromosomes: *P{neoFRT}*$^{82B}$ *P{Ubi-GFP(S65T)*$_{nls}$*}3R/TM6B, Tb*$^{1}$ or *P{neoFRT}*$^{82B}$ *P{arm-lacZ.V}83B/TM6C, Sb*$^{1}$ *Tb*$^{1}$. *Minute* clones were marked by *P{neoFRT}*$^{82B}$ *M(3)96C*, *arm-lacZ* or *P{neoFRT}*$^{82B}$ *Ubi-GFP*. *P{neoFRT}*$^{82B}$ *ry*$^{506}$ was used as the control. *hop*-expressing or flip-out clones were generated using *UAS-hop* and *P{AyGAL4}25 P{UAS-GFP.S65T}T2; hs-flp MKRS/ TM6B*, in which FLP is under the control of the heat-shock promoter (Ito et al., 1997). Flip-out clones express both Hop and GFP.

### In situ hybridization, antibody staining and microscopy

Sense and antisense *upd* riboprobes were generated as previously described (Bach et al., 2003).

Antibody stainings were performed as previously described (Bach et al., 2003). We used the following primary antibodies: rat anti-Elav (1:50), mouse anti-Wg (1:20), mouse anti-β-galactosidase (1:50), mouse anti-Discs large (Dlg) (1:50), mouse anti-Dac (1:100) (all from Developmental Studies Hybridoma Bank), rabbit anti-Hth (1:300) (a kind gift of H. Sun) (Pai et al., 1998), rabbit anti-β-galactosidase (1:100) (Cappel) and rat anti-HA (1:100) (Roche). We used fluorescent secondary antibodies (Jackson Laboratories) at 1:250. We collected fluorescent images (at 25× magnification) using a Zeiss LSM 510 confocal microscope, scanning electron micrographs (at 100×) using a Leo SEM (Zeiss) (Harvard School of Public Health), and bright field pictures of adults (at 2.5× or 5×) and of discs (at 20×) using a Zeiss Axioplan microscope with a Nikon Digital Sight DL-UL camera.

## RESULTS

### Upd acts in early eye disc development to promote formation of the eye field

To test whether the sole function of JAK/STAT signaling is to promote growth, or whether it also regulates patterning of the eye disc, we examined endogenous Upd expression, assessed the range of Upd activity and misexpressed Upd using the UAS/GAL4 system (Brand and Perrimon, 1993). An *upd-GAL4, UAS-GFP* (*upd>GFP*) reporter revealed that *upd* was expressed in cells residing next to the optic stalk in first and second instar eye discs (Fig. 1A,B). However, *upd* mRNA was not detected after early third instar, suggesting a function in the early eye disc (Fig. 1C,D). Upd is likely to be the only Upd-like molecule involved in eye development. A *upd2*-null mutant is viable and fertile and has no eye defects, whereas *upd3* is not expressed in the eye disc (data not shown).

Upd is a secreted molecule that acts cell non-autonomously (Bach et al., 2003; Tsai and Sun, 2004). To assess the range of Upd activity, we generated a reporter called *10XSTAT92E-GFP* that specifically reflects JAK/STAT pathway activity, as evidenced by the loss of reporter expression in *stat92E* clones (Bach et al., 2006). Although Upd is synthesized only by cells at the posterior midline, it has long-range effects. The *10XSTAT92E-GFP* reporter was activated uniformly throughout the posterior domain of the eye to the lateral margins in first and second instar eye discs (Fig. 1E-H). Using this reporter, we demonstrated that the JAK/STAT pathway is not active in the eye disc after early third instar (Bach et al., 2006). These data indicate that JAK/STAT signaling is active only during the early stages of larval eye disc development. We next assessed the spatial and temporal relationship between JAK/STAT signaling and other pathways, such as Dpp and Wg, that are also active in early eye

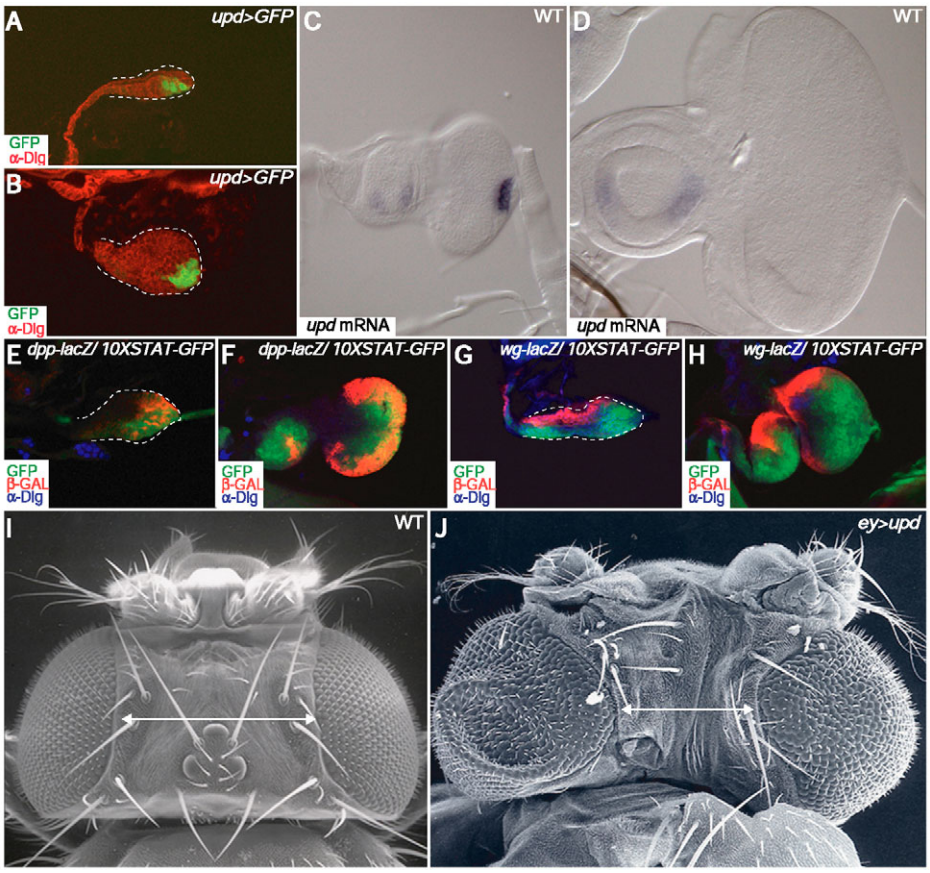



**Fig. 1. Upd acts in the early eye disc to promote regional specification.** (**A**,**B**) *upd>GFP* expression (green) in first (A) and second (B) instar eye discs; Dlg marks the cell outline (red). (**C**,**D**) *upd* mRNA in situ hybridization analysis. *upd* is transcribed at the posterior midline in late second/early third (C), but not in mid-third (D) instar eye discs. (**E-H**) Expression of *10XSTAT92E-GFP* (abbreviated *10XSTAT-GFP*; green), Dlg (blue) and *dpp-lacZ* (anti-β-Gal, red) (E,F) or *wg-lacZ* (anti-β-Gal, red) (G,H). *10XSTAT-GFP* is expressed in the posterior domain of first (E,G) and second (F,H) instar eye discs. This expression partially overlaps with *dpp* (E,F) and abuts *wg* (G,H). (**I**,**J**) Scanning electronic micrographs of wild-type (I) and *ey-GAL4, UAS-upd* (*ey>upd*) (J) adult heads, dorsal view. Overexpression of *upd* results in the expansion of the dorsal eye domain into the lateral head cuticle, reducing the inter-eye distance (arrow). *ey>upd* animals also have patterning defects in both the eye and the head cuticle. In all images, eye discs are oriented with anterior towards the left and dorsal upwards.

development. We used a *dpp-LacZ* reporter and a *wg-lacZ* enhancer trap ($wg^{P}$) that faithfully recapitulate expression of the endogenous genes (Blackman et al., 1991; Kassis et al., 1992). In early larval eye development, the *10XSTAT92E-GFP* reporter partially overlapped with *dpp* but abutted the *wg* expression domain, suggesting functional interactions between these pathways (Fig. 1E-H).

To test whether the restricted expression of Upd is functionally important, we ectopically misexpressed *upd* throughout the early eye disc using *ey-GAL4*. *ey>upd* adults exhibited overgrowth of the eye field, presumably due to the mitogenic functions of Upd, as well as aberrant patterning of the head cuticle (Fig. 1J). Specifically, lateral head cuticle was replaced by photoreceptors, thereby reducing the distance between the eyes, a phenotype previously reported for loss of *wg* signaling (Fig. 1J) (Ma and Moses, 1995). These data indicate that the early, restricted expression of Upd to the posterior midline is essential for proper formation of the eye field.

## Stat92E is required for eye development

The consequences of loss of Stat92E during eye development have not been reported. To address this issue, we generated mutant *stat92E* clones in the eye using the $stat92E^{85C9}$ allele, which results from an R to P substitution at position 442 in the DNA-binding domain (Silver and Montell, 2001). The $Stat92E^{85C9}$ protein is non-functional, as evidenced by the lack of activation of the *10XSTAT92E-GFP* reporter in $stat92E^{85C9}$ clones (data not shown) (Bach et al., 2006). We induced $stat92E^{85C9}$ mutant clones in the eye disc using *ey-flp*, in which the yeast FLP recombinase is expressed under the control of the eye-specific enhancer of *ey* (Newsome et al., 2000). To generate large patches of *stat92E* mutant tissue we also used a *Minute* mutation, which causes slow growth and recessive lethality in cells possessing the wild-type chromosome (Morata and Ripoll, 1975). When used with *ey-flp* and a mutation on an FRT chromosome, the *Minute* technique produces an eye composed almost entirely of homozygous mutant tissue. Adults carrying a control chromosome in a *Minute* background (hereafter referred to as + $M^{+}$) did not exhibit pupal lethality or eye or head cuticle defects (Fig. 2A,I). By contrast, less than 10% of animals carrying $stat92E^{85C9}$ clones in a *Minute* background (hereafter referred to as *stat92E* $M^{+}$) eclosed from their pupal cases (Fig. 4G). The few adults that eclosed exhibited a small or ablated eye and abnormal head cuticle (Fig. 2B,C). Instead of the smooth head cuticle seen in control animals, $stat92E^{85C9}$ $M^{+}$ adults displayed a ridged cuticle that had a disorganized bristle pattern (Fig. 2A-C). Non-eclosed $stat92E^{85C9}$ $M^{+}$ pupae were frequently headless or exhibited severe eye defects and expansion of the head cuticle (Fig. 2J,K). Similar phenotypes and significant hatching defects were observed with another *stat92E* hypomorphic allele, $stat92E^{397}$, whereas less severe phenotypes were observed with $stat92E^{06346}$ (Fig. 2F,G). We also observed defects in *stat92E* clones in a non-*Minute* background. Frequently, *stat92E* clones within the eye field developed into ectopic head cuticle or formed ambiguous outgrowths (Fig. 2H). The *stat92E* mutant eye and head cuticle phenotypes were due specifically to the loss of Stat92E function. Expression of a wild-type *stat92E* transgene (*UAS-3HA stat92E*) fully rescued all *stat92E* mutant phenotypes (Fig. 2D). However, misexpression of this transgene (*ey>3HA-stat92E*) in a wild-type background did not have a phenotype (Fig. 2E). Taken together, these data suggest that Stat92E, activated by a Upd/Dome/Hop signal, promotes the formation of the eye field. In the absence of Stat92E, cells which are normally part of the eye field form other tissues.

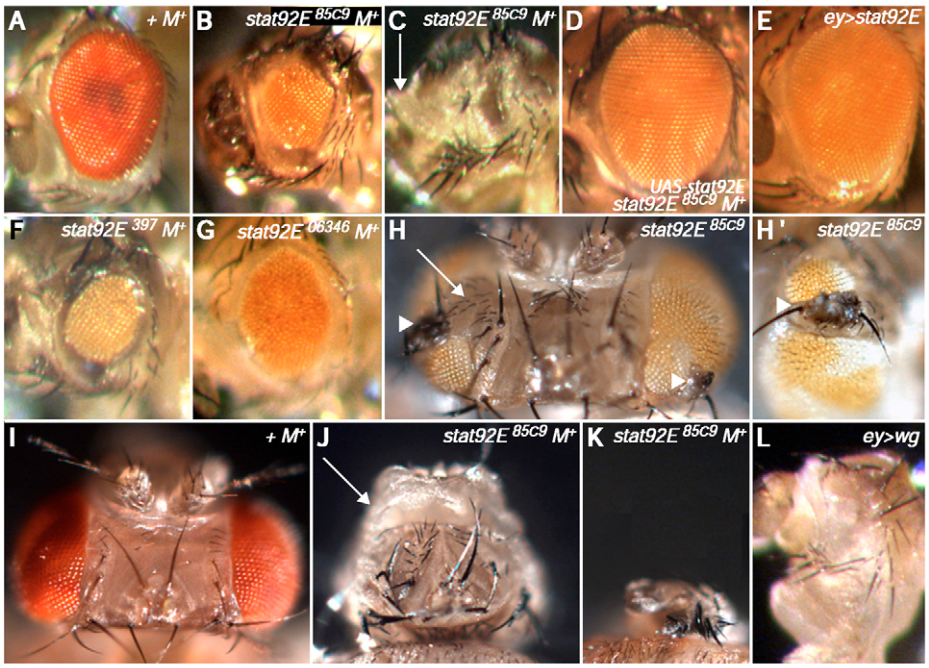



**Fig. 2. Stat92E is required for eye development.** In panels A-C,F,G clones were generated using *ey-flp* in a *Minute* background. (**A**) +$M^+$. (**B**,**C**) $stat92E^{85C9}$ $M^+$. The loss of *stat92E* results in a small-eye (B) or a no-eye (C) phenotype. These adults also exhibit excess head cuticle (C, arrow). (**D**) $stat92E^{85C9}$ $M^+$ adults carrying a *UAS-3HA stat92E* transgene. The $stat92E^{85C9}$ $M^+$ small-eye phenotype can be rescued by the ectopic expression of a full-length copy of *stat92E*. (**E**) Wild-type adults carrying a *UAS-3HA stat92E* transgene. Overexpression of this transgene using the *ey-GAL4* driver (*ey>stat92E*) does not alter the size of the adult eye. (**F**) $stat92E^{397}$ $M^+$. (**G**) $stat92E^{06346}$ $M^+$. Like $stat92E^{85C9}$, these *stat92E* hypomorphic alleles result in a reduction in the size of the adult eye (F,G). (**H**,**H'**) Adults carrying $stat92E^{85C9}$ clones generated using *ey-flp* in a wild-type (i.e. non-*Minute*) background. (H) Dorsal view; (H′) lateral view. Both ectopic cuticle (arrow) and ambiguous, ectopic structures (arrowheads) are observed in the eye field of adults carrying $stat92E^{85C9}$ clones. (**I**) Dorsal view of a + $M^+$ adult that exhibits symmetrically patterned eyes and head cuticle. (**J**,**K**) Dorsal views of a $stat92E^{85C9}$ $M^+$ adult that lacks ommatidia, has an altered pattern of macrochaete and excess head cuticle (arrow) (J) or exhibits only a rudimentary head (K). (**L**) Adult carrying a *UAS-wg* transgene in a wild-type background. Overexpression of a *wg* transgene using the *ey-GAL4* driver (*ey>wg*) results in a no-eye phenotype indistinguishable from the $stat92E^{85C9}$ $M^+$ no-eye phenotype (C).

## Loss of JAK/STAT signaling results in abnormal eye disc morphology and ectopic *wg* expression

To determine if the *stat92E* phenotypes observed in the adult arise during larval eye development, we examined the effect of loss of *stat92E* activity in the eye imaginal disc. Indeed, $stat92E^{85C9}$ $M^+$ eye discs display morphological defects consistent with adult *stat92E* mutant phenotypes. The dorsal domain in $stat92E^{85C9}$ $M^+$ eye discs was morphologically abnormal and was marked by protrusions, an elongated appearance and an ectopic eye field (Fig. 3B,C,E and data not shown). In 5% of $stat92E^{85C9}$ $M^+$ discs, the morphogenetic furrow failed to initiate (see Fig. S1 in the supplementary material). In about 85% of these discs, the furrow moved only through the ventral portion of the eye disc (Fig. 3B,C). Ommatidia in $stat92E^{85C9}$ $M^+$ adult eyes are almost entirely of ventral origin. In the eye disc, dorsal cells express the homeodomain protein Mirror (Mirr) (McNeill et al., 1997). The lack of dorsal ommatidia in *stat92E* mutants is not due to altered expression of *mirr*, but due rather to an inability of the furrow to move through the dorsal, *mirr*-positive cells in the $stat92E^{85C9}$ $M^+$ eye disc (see Fig. S1 in the supplementary material).

Many larval and adult phenotypes observed in the absence of *stat92E* are characteristic of either loss of *dpp* or increased *wg* expression. As the expression patterns of *dpp* and *wg* are consistent with regulation by the JAK/STAT pathway (Fig. 1E-H), we next asked if the loss of *stat92E* alters expression of either gene. In wild-type third instar eye discs, *dpp* is expressed in the morphogenetic furrow (Heberlein et al., 1993; Ma et al., 1993). However, in *stat92E* $M^+$ eye discs, *dpp* was lost from the dorsal region of the disc, whereas its ventral expression appeared normal (Fig. 3A-C). *wg* is expressed in dorsal and ventral margin cells, located between the peripodial membrane and the disc proper, and in cells adjacent to them in wild-type eye discs (Fig. 3D) (Cho et al., 2000). By contrast, *wg* was expanded throughout the disc epithelium in *stat92E* $M^+$ eye discs, as well as in eye discs hemizygous for the amorphic mutation $hop^{M13}$ (Fig. 3E,G and data not shown). *wg* was also ectopically expressed in mosaic *stat92E* clones (Fig. 3F). The increased expression domain of the *wg* gene in discs lacking JAK/STAT signaling led to a dramatic increase in Wg protein (Fig. 3C). In *stat92E* $M^+$ eye discs expressing a *stat92E* transgene, ectopic Wg protein was not observed (data not shown). The presence of a ventral furrow in *stat92E* $M^+$ eye discs is consistent with high dorsal levels of Wg, which is observed with ectopic clonal expression of *wg* in the eye disc (data not shown) (Treisman and Rubin, 1995).

In wild-type adults, *wg* is expressed around the periphery of the eye and in the lateral head cuticle (Fig. 3H) (Heslip et al., 1997). However, ectopic *wg* was apparent in *stat92E* clones within the adult eye field, often at the base of ectopic outgrowths (Fig. 3I). Similar outgrowths are observed in *shaggy* clones, which behave as if they have transduced the Wg signal (Heslip et al., 1997). The hypothesis that JAK/STAT signaling functions upstream of Wg is further supported by the observation that the *stat92E* ablated eye phenotype was identical to that observed in *ey>wg* adults, in which *wg* was expressed throughout the developing eye (compare Fig. 2C with 2L). Although these results indicate that JAK/STAT signaling is an important regulator of *wg* expression, *wg* was not ectopically expressed in all cells lacking *stat92E* (Fig. 3E-G,I). This may reflect the time of induction of *stat92E* clones, which are constantly generated in an *ey-flp* background. Alternatively, another factor may be acting redundantly with Stat92E to regulate *wg*.

## Ectopic JAK/STAT signaling autonomously represses *wg* expression

*stat92E* mutants exhibit phenotypes characteristic of expanded *wg* expression. However, the antagonistic relationship between *dpp* and *wg* raises the possibility that Stat92E regulates *dpp*, and *wg*

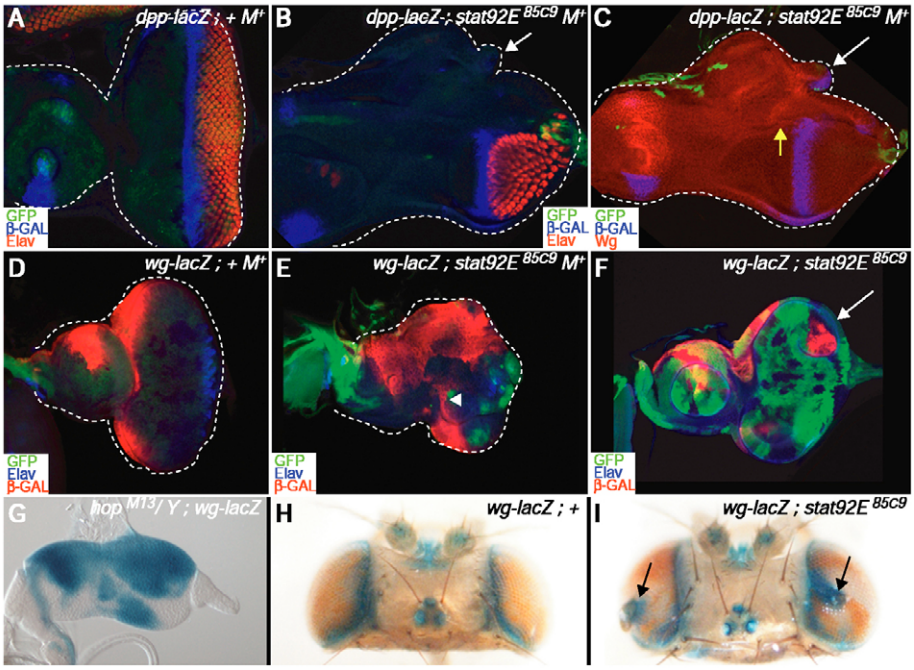



**Fig. 3. Loss of JAK/STAT signaling results in changes in eye disc morphology and ectopic *wg* expression.** Clones were generated using *ey-flp* in a *Minute* (A-E) or non-*Minute* (F,H,I) background. (**A**,**B**) A third instar *dpp-lacZ; + M+* (A) or *dpp-lacZ; stat92E$^{85C9}$ M+* (B) eye disc. Heterozygous tissue (green), Elav (red), *dpp-lacZ* (blue). *dpp* is absent from the dorsal domain of *stat92E$^{85C9}$ M+* eye discs (B). (**C**) A third instar *dpp-lacZ; stat92E$^{85C9}$ M+* eye disc. Heterozygous tissue (green), Wg (red), *dpp-lacZ* (blue). *dpp* is lost from the dorsal domain of the eye disc and Wg expression is expanded into this region (yellow arrow). The morphogenetic furrow [marked by *dpp-lacZ* (anti-β-Gal) expression] moves only through the ventral region of *stat92E$^{85C9}$ M+* eye discs (B,C); these discs also exhibit abnormal dorsal outgrowths (white arrows in B,C). (**D**,**E**) A third instar *wg-lacZ; + M+* (D) or *wg-lacZ; stat92E$^{85C9}$ M+* (E) eye disc. Heterozygous tissue (green), *wg-lacZ* (anti-β-Gal, red), Elav (blue). *wg* is ectopically expressed in *stat92E$^{85C9}$ M+* discs and expansion of both the dorsal *wg* domain and the ventral *wg* domain (arrowhead) is evident (E). (**F**) A third instar *wg-lacZ; stat92E$^{85C9}$* eye disc. Wild-type tissue (green), *wg-lacZ* (red), Dlg (blue). *wg* is expanded in *stat92E$^{85C9}$* clones (arrow). (**G**) A third instar *hop$^{M13}$/Y; wg-lacZ* eye disc. X-gal staining indicates that *wg* is ectopically expressed throughout most of the eye disc in *hop* mutants. (**H**,**I**) Dorsal views of adult heads. X-Gal staining of adults carrying *wg-lacZ* (blue) with an *FRT$^{82B}$* (control) (H) or *stat92E$^{85C9}$* (I) chromosome. Ectopic *wg* is observed in *stat92E$^{85C9}$* clones (marked by white ommatidia) in the eye field, frequently at the base of ectopic outgrowths (arrows) (I). This is never seen in wild-type (*FRT$^{82B}$*) clones (H).

expansion occurs as a secondary effect. We therefore looked at the ability of ectopic JAK/STAT signaling to either induce *dpp* or repress *wg*. Ectopic expression of *hop* using the flip-out technique resulted in ligand-independent, autonomous activation of Stat92E. Ectopic *hop* clones did not induce *dpp* in the eye disc (Fig. 4A). However, they did autonomously repress *wg* in both the dorsal and ventral eye (Fig. 4B). Therefore, JAK/STAT signaling regulates *wg*, and *dpp* is altered as a consequence. This is further supported by the ability of *upd* expressed throughout the eye disc (*ey>upd*) to repress *wg*. In *ey>upd* discs, dorsal *wg* was completely eliminated and ventral *wg* was substantially repressed (Fig. 4C). Furthermore, *ey>upd* discs exhibited precocious furrow initiation from the lateral margins, a phenotype also observed in *wg* mutants (Fig. 4C) (Treisman and Rubin, 1995). This result is consistent with the reduction in the inter-eye distance displayed by *ey>upd* adults (Fig. 1J). To determine if JAK/STAT regulation of *wg* is specific to the eye disc, we examined *hop* flip-out clones in other imaginal discs. Indeed, *hop* expressing clones also repressed *wg* in the notal region of the wing disc, indicating that the JAK/STAT pathway can repress *wg* in other tissues (Fig. 4D).

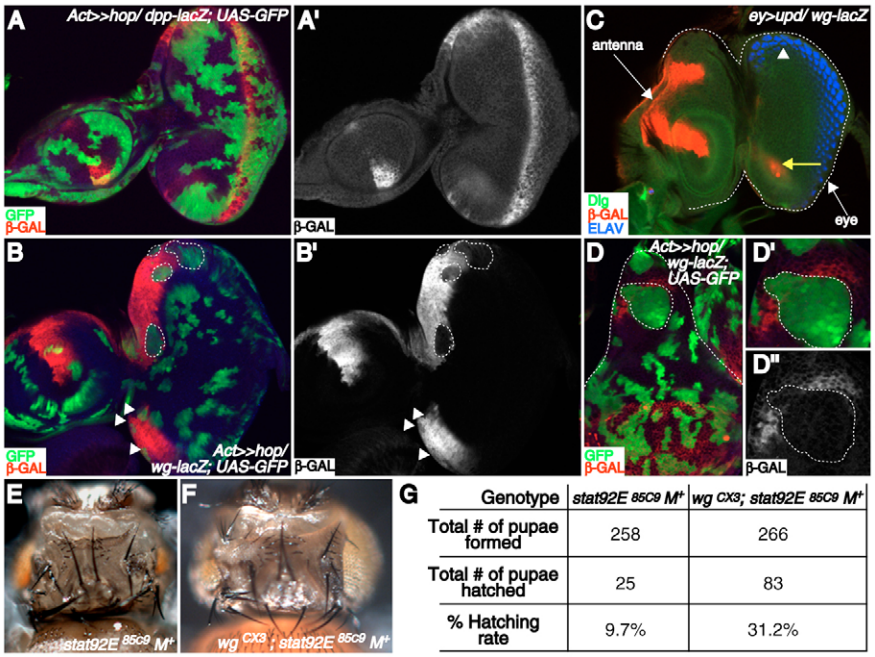
To test whether the effects of *stat92E* loss-of-function are due to ectopic *wg*, we asked whether reducing the dose of *wg*, using the hypomorphic mutation *wg$^{CX3}$*, could rescue *stat92E* mutant phenotypes. As predicted, reducing the dose of *wg* significantly increased the hatching rate of *stat92E$^{85C9}$ M+* flies from 9.7% to 31.2% (Fig. 4G). Furthermore, eclosed *wg$^{CX3}$/+; stat92E$^{85C9}$ M+* flies showed less severe phenotypes when compared with eclosed *+/CyO; stat92E$^{85C9}$ M+* flies, which carry two functional copies of the *wg* gene (Fig. 4E,F). Taken together, these data indicate that Stat92E normally represses *wg* and that ectopic *wg* expression significantly contributes to the *stat92E* mutant phenotype.

## Stat92E negatively regulates a 263 bp region of the *wg* gene

Although the majority of functions attributable to STATs involve transcriptional activation, at least one STAT protein, Dd-STATa, acts as a functional repressor (Mohanty et al., 1999). We therefore assessed the ability of Stat92E to directly repress *wg*. A reporter called *wg2.11Z,* in which β-galactosidase is driven by a 263 base pair enhancer from the 3′ *cis wg* genomic region, was sufficient to recapitulate *wg* expression in the dorsal margin of the disc proper and in the dorsal peripodial membrane (Fig. 5A,B) (Pereira et al., 2006). This reporter was ectopically expressed in mosaic *stat92E* clones as well as in *stat92E M+* clones in a manner similar to that observed for *wg$^{P}$* (Fig. 5C,D). Moreover, *wg2.11Z* was repressed autonomously in *hop*-expressing clones (Fig. 5E). These data indicate that Stat92E can regulate dorsal *wg* expression through the *wg2.11Z* enhancer. *wg2.11Z* does not contain any Stat92E binding sites (TTC(N)$_3$GAA), strongly suggesting that Stat92E does not repress dorsal *wg* directly, but rather regulates another factor which represses *wg* (Hou et al., 1996; Yan et al., 1996).

## Stat92E does not act through known *wg* regulators to repress *wg* in the disc proper

Expression of the *wg2.11Z* enhancer is maintained by *pnr* in dorsal peripodial cells (Pereira et al., 2006). We therefore assessed whether *pnr* expression is altered in the absence of JAK/STAT signaling using a *pnr>gfp* reporter, which mimics expression of the endogenous *pnr* gene (Singh and Choi, 2003). In wild-type eye discs, *pnr* was expressed in dorsal anterior peripodial cells and in dorsal margin cells in the disc proper (Fig. 6E). In *hop$^{M13}$* eye discs, *pnr* expanded throughout the dorsal posterior peripodial membrane, whereas it remained unchanged in the disc proper (Fig. 6F). These




| Genotype | *stat92E* $^{85c9}$ $M^+$ | *wg* $^{CX3}$; *stat92E* $^{85c9}$ $M^+$ |
|---|---|---|
| Total # of pupae formed | 258 | 266 |
| Total # of pupae hatched | 25 | 83 |
| % Hatching rate | 9.7% | 31.2% |

**Fig. 4. Ectopic JAK/STAT signaling represses *wg* expression.** (**A**,**A′**) A third instar eye disc carrying *hop* flip-out clones and *dpp-lacZ* (*Act>>hop/ dpp-lacZ; UAS-GFP*). *hop* flip-out clones (green) do not induce *dpp-lacZ* (anti-β-Gal, red) expression. (A) Merge; (A′) *dpp-lacZ* single channel. (**B**,**B′**) A third instar eye disc carrying *hop* flip-out clones and *wg-lacZ* (*Act>>hop/ wg-lacZ; UAS-GFP*). *hop* flip-out clones (green) repress *wg-lacZ* (anti-β-Gal, red) expression in an autonomous manner. Repression is observed in the dorsal *wg* domain (outlined clones) and in the ventral domain (arrowheads). (B) Merge; (B′) *wg-lacZ* single channel. (**C**) A third instar eye disc carrying *ey>upd* and *wg-lacZ* (*ey>upd; wg-lacZ*). Dlg (green), *wg-lacZ* (red), Elav (blue). Ectopic *upd* expression completely represses dorsal *wg* and strongly represses ventral *wg* (yellow arrow), allowing precocious photoreceptor differentiation from the lateral margin (arrowhead). (**D-D″**) A third instar wing disc carrying *hop* flip-out clones and *wg-lacZ* (*Act>>hop/ wg-lacZ; UAS-GFP*). *hop* flip-out clones (green) autonomously repress *wg* (red) in the notal region of the wing disc. (D) Merge; (D′) magnified view of D; (D″) *wg-lacZ* single channel in D′. (**E**,**F**) Dorsal views of adult heads carrying a *stat92E*$^{85C9}$ chromosome in a *Minute* background alone (E) or with the hypomorphic mutation *wg*$^{CX3}$*/+; stat92E*$^{85C9}$ $M^+$ (F). Reducing the dose of *wg* slightly improves the *stat92E*$^{85C9}$ $M^+$ adult phenotype. (**G**) Reducing the dose of *wg*, using the *wg*$^{CX3}$ allele, significantly increases the number of *stat92E*$^{85C9}$ $M^+$ flies that eclose from the pupal case. Whereas only 9.7% of flies *stat92E*$^{85C9}$ $M^+$ eclose (*n*=258), flies also carrying the *wg*$^{CX3}$ mutation hatch at a rate of 31.2% (*n*=266).

data suggest that Stat92E negatively regulates expression of *pnr* in peripodial cells. To test this hypothesis, we assessed *pnr>gfp* expression when JAK/STAT signaling was ectopically activated. We used the *GMR-upd* transgenic line, in which ectopic Upd activates the *10XSTAT92E-GFP* reporter in anterior cells adjacent to the furrow (Bach et al., 2006; Bach et al., 2003). In mid-third-instar eye discs, *pnr* was expressed in peripodial cells that reside ‘above’ dorsal cells both anterior and posterior to the furrow (Fig. 6G). By contrast, in *GMR-upd* discs, the *pnr* expression domain was significantly repressed and now resided only ‘above’ dorsal cells anterior to the furrow (Fig. 6H). These data suggest that when *stat92E* function is lacking in peripodial cells, *pnr* is ectopically expressed and may induce *wg* expression in these cells via the *wg2.11Z* enhancer.

To determine whether other known regulators of *wg* are altered in *stat92E* mutant discs, we examined the expression patterns of Dac, Hth and the Paired-domain protein Eyegone (Eyg). Although Eyg

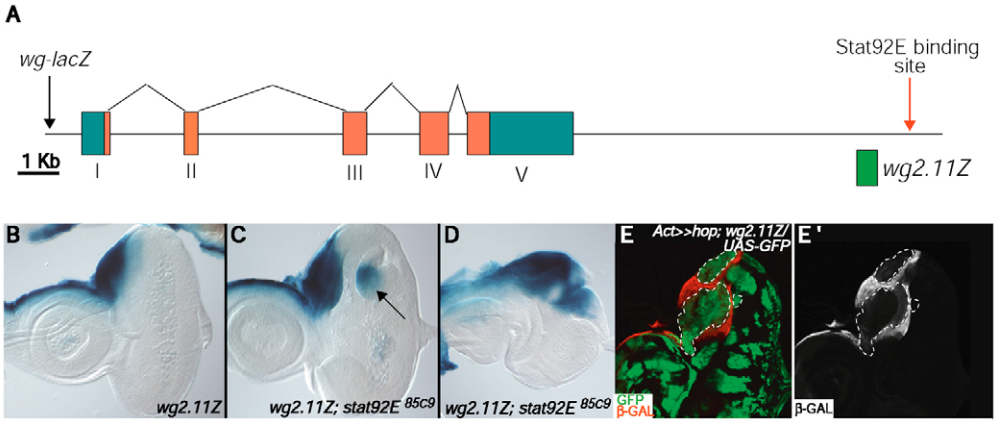



**Fig. 5. Stat92E regulates *wg* through a small enhancer in the *wg* 3′ *cis* genomic region.** (**A**) Schematic representation of ~20 kb of the *wg* gene, from just 5′ of the start site, through untranslated and coding exons (turquoise and red boxes, respectively), and including ~9 kb of 3′ *cis* genomic region. *wg2.11Z* is a 263 bp enhancer from the *wg* 3′ *cis* genomic region that is sufficient to recapitulate *wg* expression in the dorsal eye (B). This ~20 kb region contains only one putative Stat92E binding site (TTC(N)$_3$GAA; red arrow), which resides downstream of the *wg2.11Z* enhancer. (**B-D**) X-gal staining of *wg2.11Z* (blue). Third instar eye discs carrying the *wg2.11Z* reporter (B) and a *stat92E*$^{85C9}$ chromosome in a non-*Minute* (C) or *Minute* (D) background. Clones were generated using *ey-flp*. *wg* is expanded in *stat92E*$^{85C9}$ non-*Minute* (C) and *Minute* (D) clones in a manner similar to *wg-lacZ* (see Fig. 3E,F). Outgrowths of tissue containing ectopic *wg2.11Z* are evident during larval stages (C, arrow). (**E**,**E′**) A third instar eye disc carrying the *wg2.11Z* reporter (anti-β-Gal, red) and *hop* flip-out clones (*Actin>>GAL4; UAS-hop/ wg2.11Z*; green). *hop* flip-out clones autonomously repress *wg2.11Z* expression. (E) Merge; (E′) *wg2.11Z* single-channel.

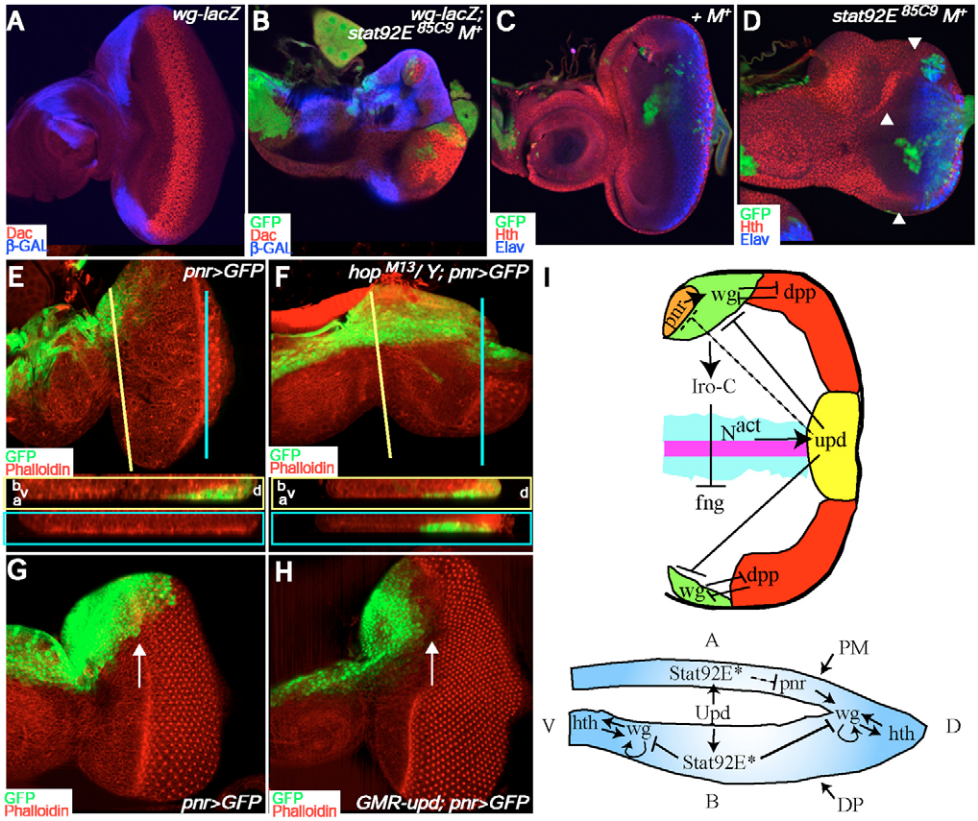



**Fig. 6. The JAK/STAT repression of *wg* in the disc epithelium is not mediated by known *wg* regulators.** (**A**,**B**) A third instar eye disc carrying *wg-lacZ* in a wild-type background (A) or in a *stat92E*$^{85C9}$ *Minute* background (B). Heterozygous tissue (green), Dac (red), *wg-lacZ* (anti-β-Gal, blue). Dac expression is lost in tissue that ectopically expresses *wg* (B). (**C**,**D**) A third instar eye disc carrying a wild-type (C) or a *stat92E*$^{85C9}$ *M*$^{+}$ (D) chromosome. Heterozygous tissue (green), Hth (red), Elav (blue). Hth is expanded in both dorsal and ventral domains of *stat92E*$^{85C9}$ *M*$^{+}$ eye discs (D, arrowheads). (**E**,**F**) A third instar eye disc carrying *pnr>GFP* in a wild-type (E) or a *hop*$^{M13}$*/Y* background (F). *pnr>GFP* (green) and Phalloidin (red). *pnr* is expanded posteriorly in the peripodial membrane of *hop*$^{M13}$ eye discs (F); increased *pnr* expression also evident in *xz* sections from the anterior (yellow box) and posterior (blue box) regions of the disc. (**G**,**H**) A late-third instar eye disc carrying *pnr>GFP* in a wild-type (G) or *GMR-upd* (H) background. *pnr>GFP* (green) and Phalloidin (red). Ectopic *upd* expression represses *pnr* to the region anterior to the furrow (H, arrow). (**I**) Model of JAK/STAT activity in *Drosophila* eye imaginal discs. fng, fringe; N$^{act}$, activated Notch receptors; Iro-C, Iroquois-C; A, apical; B, basal; D, dorsal; V, ventral; DP, disc proper; PM, peripodial membrane.

negatively regulates *wg* expression, we found that Eyg expression was normal in mosaic *stat92E* clones (data not shown) (Hazelett et al., 1998). Dac and Wg mutually repress each other's expression (Baonza and Freeman, 2002; Treisman and Rubin, 1995). Dac expression was reduced in *stat92E M*$^{+}$ discs; however, its loss did not coincide with the boundary of *stat92E* clones but rather with regions of the eye disc that contained high levels of ectopic *wg* (Fig. 6A,B). This suggests that the loss of Dac expression is an indirect consequence of ectopic *wg*, as has been reported previously (Baonza and Freeman, 2002). The observation that Dac was not induced by clones expressing ectopic *hop*, strongly supports the conclusion that Dac expression is lost as a result of the ectopic Wg expressed in *stat92E M*$^{+}$ discs (data not shown). Wg positively regulates expression of the *hth* gene. In addition, ectopic Hth can increase expression of existing *wg* in both the dorsal and ventral margins of the eye disc (Pichaud and Casares, 2000). Hth was expressed at the anterior edge of the third instar eye disc (Fig. 6C). By contrast, in *stat92E M*$^{+}$ discs, Hth expanded into the posterior dorsal and ventral domains (Fig. 6D). Clones expressing ectopic *hop* cannot induce Hth expression, suggesting that the effects on *hth* are an indirect consequence of the ectopic Wg observed when JAK/STAT signaling is impaired (data not shown).

## DISCUSSION

In this paper, we report a new role for the *Drosophila* JAK/STAT pathway. Our study demonstrates for the first time that JAK/STAT signaling promotes formation of the eye field through repression of *wg* gene transcription in both the dorsal and ventral halves of the eye disc epithelium (Fig. 6I). By monitoring Upd expression and activity, we have shown that the JAK/STAT pathway is normally activated early in eye development, during first and second instar. Ectopic activation of this pathway leads to abnormal patterning of the head capsule and a reduction in the inter-eye distance through an increase in dorsal ommatidia. By contrast, loss of activity of this pathway, using strong hypomorphic *stat92E* mutations, frequently resulted in the development of a rudimentary head. When the head capsule did form, *stat92E* mutants often had small or ablated adult eyes and excessive head cuticle. *wg* was ectopically expressed in *stat92E* clones and *hop* mutant eye discs, and was repressed by ectopic activation of the JAK/STAT pathway. Reduction in the dose of *wg* partially rescued *stat92E* mutants by increasing the rate of eclosion and by mitigating the phenotypes of *stat92E* mutant animals. Lastly, we showed that *wg* regulation by the JAK/STAT pathway is independent of the known *wg* regulators Eyg, Dac, Hth and Pnr.

Our results conflict with those of a previous study, which reported that JAK/STAT signaling does not repress *wg* in the eye disc. This conclusion was reached on the basis of wild-type Wg protein expression in eye discs that contained ectopic *upd*-expressing clones (Zeidler et al., 1999). However, we found that in the absence of *stat92E*, *wg* was ectopically expressed in both dorsal and ventral halves of the eye disc. It is likely that our examination of the *wg* gene using the $wg^{P}$ enhancer trap is a more sensitive measure of *wg* expression than monitoring Wg protein. Zeidler and colleagues also reported that the JAK/STAT pathway negatively regulates *mirr* expression. This conclusion was drawn after finding a preponderance of dorsal, *mirr*-positive ommatidia in adult eyes containing unmarked *upd* loss-of-function clones (Zeidler et al., 1999). However, using marked clones, we showed that Mirr is expressed normally in eye tissue that is largely homozygous mutant for *stat92E*. Moreover, we demonstrated that *stat92E* $M^{+}$ adult eyes are largely composed of Mirr-negative ommatidia, which indicates their ventral origin. Thus, our data indicate that *mirr* is not regulated by JAK/STAT pathway activity.

## Stat92E repression of the *wg* gene

Previous work has shown that the 3′ *cis* region of the *wg* gene regulates its expression in imaginal discs. Several *wg* mutations that specifically affect imaginal disc development, as well as disc-specific enhancers, map to this region (Baker, 1988; Couso et al., 1993; Neumann and Cohen, 1996; Pereira et al., 2006; van den Heuvel et al., 1993). In this study, we showed that Stat92E negatively regulates dorsal *wg* through a small enhancer (*wg2.11Z*) in the 3′ *cis* genomic region of the *wg* gene. This enhancer is ectopically expressed in *stat92E* and *hop* mutants and is autonomously repressed by ectopic activation of Stat92E. The DNA binding preferences of Stat92E and other STAT proteins have been well characterized (Hou et al., 1996; Seidel et al., 1995; Yan et al., 1996). Because there are no Stat92E binding sites in the *wg2.11Z* enhancer, we favor the interpretation that Stat92E does not directly repress dorsal *wg* but rather acts through another factor. This repressor may be encoded by a direct Stat92E target gene, because *wg* is autonomously repressed by the JAK/STAT pathway. However, we cannot rule out the possibility that Stat92E regulates *wg* through other transcription factors, such as Dorsal or vHNF-4, which have putative sites in *wg2.11Z* (Pereira et al., 2006). It is also possible that there are cryptic Stat92E binding sites in this *wg* enhancer, through which Stat92E may directly repress *wg*. Additional experiments will be needed to test these possibilities.

We also demonstrated that Stat92E represses ventral *wg* in the eye disc epithelium. This is presumably independent of the *wg2.11Z* enhancer, which recapitulates *wg* expression in the dorsal but not the ventral eye disc (Pereira et al., 2006). Moreover, we showed that Stat92E negatively regulates *pnr* in peripodial cells. In the absence of JAK/STAT signaling, *pnr* is dramatically expanded into the posterior peripodial membrane. However, we stress that because *pnr* is an intracellular protein, the ectopic *pnr* in the peripodial membrane cannot account for the ectopic *wg* observed in the disc proper of *stat92E* mutants. Currently, we do not know whether Stat92E regulates *wg* in the ventral eye disc epithelium and the peripodial membrane in the same manner as in the dorsal eye. All three *wg* expression domains may be regulated by the same as yet unidentified factor. Alternatively, Stat92E may regulate *wg* expression domains through different mechanisms. For example, dorsal *wg* may be regulated indirectly, whereas ventral and peripodial *wg* may be regulated directly by Stat92E. The *wg* gene 3′ *cis* genomic region contains one putative Stat92E binding site, which resides downstream of the *wg2.11Z* enhancer. Therefore, it is possible that Stat92E regulates ventral and peripodial *wg* through this site. Future work will be needed to address these issues.

## Do mammalian STATs repress Wnt genes?

Our study raises the possibility that the JAK/STAT pathway negatively regulates expression of vertebrate Wnts. Interestingly, both pathways are active in the developing vertebrate eye. One recent study indicates that activation of canonical Wnt signaling in the developing chick eye inhibits retinal progenitor gene expression and the differentiation of retinal neurons (Cho and Cepko, 2006). This led to the proposal that canonical Wnt signaling is required for peripheral eye development, which is similar to the role of *wg* in peripheral eye tissue in *Drosophila*. In contrast to Wnt signaling, the roles of the JAK/STAT pathway in the development of the vertebrate eye are poorly understood. However, numerous cytokines that activate this pathway, such as ciliary neurotrophic factor, are expressed in the developing retina and induce proliferation of lens cells and the expression of glial intermediate filament protein (Potts et al., 1998; Wang et al., 2002). It is therefore possible that, in mammals, activation of the JAK/STAT pathway in the developing retina inhibits Wnt expression, thus promoting retinal progenitor gene expression and the differentiation of retinal neurons.

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### Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/133/23/4721/DC1

### References

**Agaisse, H., Petersen, U. M., Boutros, M., Mathey-Prevot, B. and Perrimon, N.** (2003). Signaling role of hemocytes in *Drosophila* JAK/STAT-dependent response to septic injury. *Dev. Cell* **5**, 441-450.

**Arbouzova, N. I. and Zeidler, M. P.** (2006). JAK/STAT signalling in *Drosophila*: insights into conserved regulatory and cellular functions. *Development* **133**, 2605-2616.

**Bach, E. A., Vincent, S., Zeidler, M. P. and Perrimon, N.** (2003). A sensitized genetic screen to identify novel regulators and components of the *Drosophila* janus kinase/signal transducer and activator of transcription pathway. *Genetics* **165**, 1149-1166.

**Bach, E. A., Ekas, L. A., Ayala-Camargo, A., Flaherty, M. S., Lee, H., Perrimon, N. and Baeg, G. H.** (2006). GFP reporters detect the activation of the *Drosophila* JAK/STAT pathway in vivo. *Gene Expr. Patterns* doi: 10.1016/j.modgep.2006.08.003.

**Baker, N. E.** (1988). Transcription of the segment-polarity gene wingless in the imaginal discs of *Drosophila*, and the phenotype of a pupal-lethal wg mutation. *Development* **102**, 489-497.

**Baonza, A. and Freeman, M.** (2002). Control of *Drosophila* eye specification by Wingless signalling. *Development* **129**, 5313-5322.

**Binari, R. and Perrimon, N.** (1994). Stripe-specific regulation of pair-rule genes by hopscotch, a putative Jak family tyrosine kinase in *Drosophila*. *Genes Dev.* **8**, 300-312.

**Blackman, R. K., Sanicola, M., Raftery, L. A., Gillevet, T. and Gelbart, W. M.** (1991). An extensive 3′ cis-regulatory region directs the imaginal disk expression of decapentaplegic, a member of the TGF-beta family in *Drosophila*. *Development* **111**, 657-666.

**Bonini, N. M., Leiserson, W. M. and Benzer, S.** (1993). The eyes absent gene: genetic control of cell survival and differentiation in the developing *Drosophila* eye. *Cell* **72**, 379-395.

**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, S., Hu, N. and Hombria, J. C.** (2001). Identification of the first invertebrate interleukin JAK/STAT receptor, the *Drosophila* gene domeless. *Curr. Biol.* **11**, 1700-1705.
**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.
**Chanut, F. and Heberlein, U.** (1997). Role of decapentaplegic in initiation and progression of the morphogenetic furrow in the developing *Drosophila* retina. *Development* **124**, 559-567.
**Chao, J. L., Tsai, Y. C., Chiu, S. J. and Sun, Y. H.** (2004). Localized Notch signal acts through eyg and upd to promote global growth in *Drosophila* eye. *Development* **131**, 3839-3847.
**Chen, H. W., Chen, X., Oh, S. W., Marinissen, M. J., Gutkind, J. S. and Hou, S. X.** (2002). mom identifies a receptor for the *Drosophila* JAK/STAT signal transduction pathway and encodes a protein distantly related to the mammalian cytokine receptor family. *Genes Dev.* **16**, 388-398.
**Cho, K. O., Chern, J., Izaddoost, S. and Choi, K. W.** (2000). Novel signaling from the peripodial membrane is essential for eye disc patterning in *Drosophila*. *Cell* **103**, 331-342.
**Cho, S. H. and Cepko, C. L.** (2006). Wnt2b/{beta}-catenin-mediated canonical Wnt signaling determines the peripheral fates of the chick eye. *Development* **133**, 3167-3177.
**Cohen, S. M.** (1993). Imaginal disc development. In *The Development of* Drosophila melanogaster (ed. M. Bate and A. Martinez-Arias). Cold Spring Harbor, NY: Cold Spring Harbor Press.
**Couso, J. P., Bate, M. and Martinez-Arias, A.** (1993). A wingless-dependent polar coordinate system in *Drosophila* imaginal discs. *Science* **259**, 484-489.
**Czerny, T., Halder, G., Kloter, U., Souabni, A., Gehring, W. J. and Busslinger, M.** (1999). twin of eyeless, a second Pax-6 gene of *Drosophila*, acts upstream of eyeless in the control of eye development. *Mol. Cell* **3**, 297-307.
**Dominguez, M. and Casares, F.** (2005). Organ specification-growth control connection: new in-sights from the *Drosophila* eye-antennal disc. *Dev. Dyn.* **232**, 673-684.
**Harrison, D. A., Binari, R., Nahreini, T. S., Gilman, M. and Perrimon, N.** (1995). Activation of a *Drosophila* Janus kinase (JAK) causes hematopoietic neoplasia and developmental defects. *EMBO J.* **14**, 2857-2865.
**Haynie, J. L. and Bryant, P. J.** (1986). Development of the eye-antenna imaginal disc and morphogenesis of the adult head in *Drosophila* melanogaster. *J. Exp. Zool.* **237**, 293-308.
**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., Wolff, T. and Rubin, G. M.** (1993). The TGF beta homolog dpp and the segment polarity gene hedgehog are required for propagation of a morphogenetic wave in the *Drosophila* retina. *Cell* **75**, 913-926.
**Heslip, T. R., Theisen, H., 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.
**Hombria, J. C., Brown, S., Hader, S. and Zeidler, M. P.** (2005). Characterisation of Upd2, a *Drosophila* JAK/STAT pathway ligand. *Dev. Biol.* **288**, 420-433.
**Hou, X. S., Melnick, M. B. and Perrimon, N.** (1996). Marelle acts downstream of the *Drosophila* HOP/JAK kinase and encodes a protein similar to the mammalian STATs. *Cell* **84**, 411-419.
**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.
**Kassis, J. A., Noll, E., VanSickle, E. P., Odenwald, W. F. and Perrimon, N.** (1992). Altering the insertional specificity of a *Drosophila* transposable element. *Proc. Natl. Acad. Sci. USA* **89**, 1919-1923.
**Kenyon, K. L., Ranade, S. S., Curtiss, J., Mlodzik, M. and Pignoni, F.** (2003). Coordinating proliferation and tissue specification to promote regional identity in the *Drosophila* head. *Dev. Cell* **5**, 403-414.
**Levy, D. E. and Darnell, J. E., Jr** (2002). Stats: transcriptional control and biological impact. *Nat. Rev. Mol. Cell Biol.* **3**, 651-662.
**Luo, H., Asha, H., Kockel, L., Parke, T., Mlodzik, M. and Dearolf, C. R.** (1999). The *Drosophila* Jak kinase hopscotch is required for multiple developmental processes in the eye. *Dev. Biol.* **213**, 432-441.
**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.
**Ma, C., Zhou, Y., Beachy, P. A. and Moses, K.** (1993). The segment polarity gene hedgehog is required for progression of the morphogenetic furrow in the developing *Drosophila* eye. *Cell* **75**, 927-938.
**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.
**Mohanty, S., Jermyn, K. A., Early, A., Kawata, T., Aubry, L., Ceccarelli, A., Schaap, P., Williams, J. G. and Firtel, R. A.** (1999). Evidence that the Dictyostelium Dd-STATa protein is a repressor that regulates commitment to stalk cell differentiation and is also required for efficient chemotaxis. *Development* **126**, 3391-3405.
**Morata, G. and Ripoll, P.** (1975). Minutes: mutants of *Drosophila* autonomously affecting cell division rate. *Dev. Biol.* **42**, 211-221.
**Neumann, C. J. and Cohen, S. M.** (1996). Sternopleural is a regulatory mutation of wingless with both dominant and recessive effects on larval development of *Drosophila* melanogaster. *Genetics* **142**, 1147-1155.
**Newsome, T. P., Asling, B. and Dickson, B. J.** (2000). Analysis of *Drosophila* photoreceptor axon guidance in eye-specific mosaics. *Development* **127**, 851-860.
**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.
**Pereira, P. S., Pinho, S., Johnson, K., Couso, J. P. and Casares, F.** (2006). A 3′ cis-regulatory region controls wingless expression in the *Drosophila* eye and leg primordia. *Dev. Dyn.* **235**, 225-234.
**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.
**Pichaud, F., Treisman, J. and Desplan, C.** (2001). Reinventing a common strategy for patterning the eye. *Cell* **105**, 9-12.
**Pignoni, F. and Zipursky, S. L.** (1997). Induction of *Drosophila* eye development by decapentaplegic. *Development* **124**, 271-278.
**Potts, J. D., Kornacker, S. and Beebe, D. C.** (1998). Activation of the Jak-STAT-signaling pathway in embryonic lens cells. *Dev. Biol.* **204**, 277-292.
**Quiring, R., Walldorf, U., Kloter, U. and Gehring, W. J.** (1994). Homology of the eyeless gene of *Drosophila* to the Small eye gene in mice and Aniridia in humans. *Science* **265**, 785-789.
**Ready, D. F., Hanson, T. E. and Benzer, S.** (1976). Development of the *Drosophila* retina, a neurocrystalline lattice. *Dev. Biol.* **53**, 217-240.
**Reynolds-Kenneally, J. and Mlodzik, M.** (2005). Notch signaling controls proliferation through cell-autonomous and non-autonomous mechanisms in the *Drosophila* eye. *Dev. Biol.* **285**, 38-48.
**Rorth, P.** (1996). A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. *Proc. Natl. Acad. Sci. USA* **93**, 12418-12422.
**Royet, J. and Finkelstein, R.** (1996). hedgehog, wingless and orthodenticle specify adult head development in *Drosophila*. *Development* **122**, 1849-1858.
**Seidel, H. M., Milocco, L. H., Lamb, P., Darnell, J. E., Jr, Stein, R. B. and Rosen, J.** (1995). Spacing of palindromic half sites as a determinant of selective STAT (signal transducers and activators of transcription) DNA binding and transcriptional activity. *Proc. Natl. Acad. Sci. USA* **92**, 3041-3045.
**Silver, D. L. and Montell, D. J.** (2001). Paracrine signaling through the JAK/STAT pathway activates invasive behavior of ovarian epithelial cells in *Drosophila*. *Cell* **107**, 831-841.
**Singh, A. and Choi, K. W.** (2003). Initial state of the *Drosophila* eye before dorsoventral specification is equivalent to ventral. *Development* **130**, 6351-6360.
**Treisman, J. E. and Rubin, G. M.** (1995). wingless inhibits morphogenetic furrow movement in the *Drosophila* eye disc. *Development* **121**, 3519-3527.
**Tsai, Y. C. and Sun, Y. H.** (2004). Long-range effect of upd, a ligand for Jak/STAT pathway, on cell cycle in *Drosophila* eye development. *Genesis* **39**, 141-153.
**van den Heuvel, M., Harryman-Samos, C., Klingensmith, J., Perrimon, N. and Nusse, R.** (1993). Mutations in the segment polarity genes wingless and porcupine impair secretion of the wingless protein. *EMBO J.* **12**, 5293-5302.
**Wang, Y., Smith, S. B., Ogilvie, J. M., McCool, D. J. and Sarthy, V.** (2002). Ciliary neurotrophic factor induces glial fibrillary acidic protein in retinal Muller cells through the JAK/STAT signal transduction pathway. *Curr. Eye Res.* **24**, 305-312.
**Xu, T. and Rubin, G. M.** (1993). Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* **117**, 1223-1237.
**Yan, R., Small, S., Desplan, C., Dearolf, C. R. and Darnell, J. E., Jr** (1996). Identification of a Stat gene that functions in *Drosophila* development. *Cell* **84**, 421-430.
**Zeidler, M. P., Perrimon, N. and Strutt, D. I.** (1999). Polarity determination in the *Drosophila* eye: a novel role for unpaired and JAK/STAT signaling. *Genes Dev.* **13**, 1342-1353.