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Essential roles for *stat92E* in expanding and patterning the proximodistal axis of the *Drosophila* wing imaginal disc

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Abstract

The *Drosophila* wing imaginal disc is subdivided along the proximodistal axis into the distal pouch, the hinge, the surrounding pleura, and the notum. While the genetic pathways that specify the identity of each of these domains have been well studied, the mechanisms that coordinate the relative expansion of these domains are not well understood. Here we investigated the role of the *stat92E* signal transducer and activator of transcription in wing proximodistal development. We find that Stat92E is active ubiquitously in early wing imaginal discs, where it acts to inhibit the induction of ectopic wing fields. Subsequently, Stat92E activity is down regulated in the notum and distal pouch. These dynamics coincide with and contribute to the proportional subdivision and expansion of these primordia. As development proceeds, Stat92E activity becomes restricted to the hinge, where it promotes normal expansion of the hinge, and restricts expansion of the notum. We also find that *stat92E* is required autonomously to specify dorsal pleura identity and inhibit notum identity to properly subdivide the body wall. Our data suggest that Stat92E activity is regulated along the proximodistal axis to pattern this axis and control the relative expansion of the pouch, hinge, and notum.

Keywords

Notum; pleura; hinge; pouch; Upd; Bowl; Eyg; Mirr; Zfh2

Introduction

The subdivision of the proximodistal (PD) axis of developing appendages is critical for their physiological roles in locomotion, sensing, feeding and reproduction. The elaboration of appendage structure depends on signals that emanate from localized sources termed organizers (Lawrence et al., 1996; Mann and Morata, 2000). These signals subdivide nascent appendages into progressively smaller domains along their anteroposterior (AP), dorsoventral (DV) and PD axes. The PD axis of the wing imaginal disc is subdivided into the distal pouch that forms the wing blade, the hinge that connects the blade to the body

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wall, the pleura that surrounds the hinge and forms the lateral plate of the body wall, and the notum that forms part of the dorsal body wall (Fig. 1A-C). While the genetic pathways that specify the identity of these domains have been well studied, the mechanisms that control the expansion of each of these domains remain unclear.

The PD axis of the wing imaginal disc is initially patterned by two opposing signals, Wingless (Wg) and epidermal growth factor (EGF), which specify the wing field and body wall, respectively. *wg* induces the expression of the zinc finger genes *elbow* (*el*) and *no ocelli* (*noc*) (*el/noc*) in the early wing primordium. In turn, *el/noc* represses the expression of the zinc finger gene *teashirt* (*tsh*) in distal cells (Weihe et al., 2004; Wu and Cohen, 2002) to specify wing identity and to inhibit body wall identity (Azpiazu and Morata, 2000; Casares and Mann, 2000). In the notum, the neuregulin-like signal Vein (Vn) activates epidermal growth factor (EGF) receptor signaling to induce expression of the *Iroquois Complex (iro-C)* homeobox genes to specify notum fate and inhibit wing fate (Simcox et al., 1996; Wang et al., 2000; Zecca and Struhl, 2002a; Zecca and Struhl, 2002b). Each of these domains is then progressively subdivided into smaller PD sub domains by the activities of secreted signals and transcription factors.

The elaboration of the wing PD axis depends on signaling centers that are established along both the DV and AP compartment boundaries. Activation of Notch (N) signaling along the DV compartment boundary induces the expression of the wing selector gene *vestigial* (*vg*) in a narrow domain that centers on this boundary (Couso et al., 1995; Kim et al., 1996; Klein and Arias, 1998; Williams et al., 1994). Activation of *vg* by both the Wg signal and the Bone Morphogenetic Protein (BMP)-like signal Decapentaplegic (Dpp) further expands the range of *vg* function (Zecca and Struhl, 2007a; Zecca and Struhl, 2007b). Within the wing field, *vg* activates a set of genes required for the elaboration of the wing PD axis in nested circular domains (Kolzer et al., 2003; Ng et al., 1995; St Pierre et al., 2002; Terriente et al., 2007; Terriente et al., 2008). Different combinations of the wing PD genes progressively subdivide the wing field, from distal to proximal, into the pouch, the distal hinge and the proximal hinge (Cho and Irvine, 2004; Jakobi et al., 1993; Kolzer et al., 2003; Terriente et al., 2008; Dichtel-Danjoy et al., 2009; Perea et al., 2009; Rodriguez del Alamo et al., 2002; Terriente et al., 2007).

The notum is also subdivided into lateral and medial domains, which can be viewed as the most proximal subdivisions of the wing PD axis (Fig. 1A-C). We refer to this axis as the notum mediolateral (ML) axis, and refer to the entire axis spanning both the wing and notum as the wing PD/ML axis. Signaling centers that are established along the notum margins elaborate both the notum ML and AP axes. The Dpp signal is distributed in a medial to lateral gradient at early stages and organizes the notum ML axis. *dpp* promotes expression of the GATA and FoG genes *pannier* (*pnr*) and *u-shaped* (*ush*) in the medial notum, and limits the expression of the *iro-C* genes to the lateral notum (Fromental-Ramain et al., 2008; García-García et al., 1999; Letizia et al., 2007). *Wg* is induced in the lateral notum along the interface with the medial notum (Sato and Saigo, 2000; Tomoyasu et al., 2000) and is required to control cell fate in this region (García-García et al., 1999). As the pathways that specify and subdivide the wing and notum have been well characterized, we explored the mechanisms that control the relative expansion of these primordia.

The JAK/STAT pathway controls numerous developmental processes including the patterning, growth and morphogenesis of epithelial sheets during embryonic, larval and adult life (Arbouzova and Zeidler, 2006; Hombria and Brown, 2002; Hombria and Sotillos, 2008). Canonical JAK/STAT signaling is initiated by the binding of the extracellular ligand Unpaired (Upd) genes (Upd1-3) to the transmembrane Domeless (Dome) receptor. This binding activates the receptor associated Janus kinase (JAK) family member *hopscotch*

(*hop*). Activated JAKs phosphorylate themselves and associated receptors to generate docking sites for the signal transducer Stat92E. Following recruitment and phosphorylation by the receptor-JAK complex, Stat92E dimers assemble and translocate to the nucleus. Nuclear Stat92E dimers bind conserved sequences in downstream target genes to regulate gene expression (Levy and Darnell, 2002). Unphosphorylated Stat92E can translocate to the nucleus and associate with chromatin remodeling factors to stabilize transcriptionally repressed heterochromatin independently of canonical JAK/STAT signaling. Receptor activation of the JAK/STAT pathway reduces the association of Stat92E with chromatin causing global chromatin instability and changes in gene expression (Li, 2008; Shi et al., 2006; Shi et al., 2008).

To test the role of *stat92E* in the elaboration of the wing PD/ML axis, we examined the dynamics of Stat92E activity and the requirements for *stat92E* in wing development from early stages of development using genetic loss- and gain-of-function analyses. We find that *Stat92E* is active ubiquitously at early stages and is then downregulated in a medial to lateral direction in the notum, and in a distal to proximal direction in the pouch. We provide evidence that the dynamics of *stat92E* downregulation controls the relative expansion of gene expression domains along the wing PD/ML axis. We also show that the early ubiquitous activity of Stat92E is required to inhibit ectopic wing induction in ectopic locations, while the later restriction of Stat92E activity to the hinge and pleura is required to promote the expansion of the hinge and the specification of the dorsal pleura. Together, these results suggest novel roles for *stat92E* in patterning and coordinating the expansion of the various subdivisions of the wing PD/ML axis.

Results

Downregulation of Stat92E activity coincides with the expansion of the presumptive wing pouch and notum

To understand the role of the JAK/STAT pathway in wing PD axis development, we compared the evolution of Stat92E activity to the gene expression patterns of the PD genes Nubbin (Nub) and Eyegone (Eyg). Nub marks the wing pouch and dorsal hinge (Ng et al., 1995), while Eyg marks a broad anterior part of the notum (Aldaz et al., 2003). To monitor canonical JAK/STAT signaling activity, we utilized a previously characterized reporter composed of 10 tandem Stat92E DNA binding sites inserted upstream of a minimal promoter and a *GFP* reporter (*10× Stat92E-GFP*) (Bach et al., 2007). We found that at second in star, Stat92E was active throughout the disc proper of the wing disc (Fig. 1D). By early third in star, Stat92E activity was repressed in the distal pouch and proximal notum, coincident with the induction of Nub and Eyg in these primordia, respectively (Fig. 1E-F). Analysis of Stat92E protein distribution at third in star revealed nuclear accumulation in the wing hinge, pleura and the anterior border of the notum, and cytoplasmic accumulation in the wing and notum (Sup. Fig. 1). Though slightly more restricted, we detected a similar pattern of expression of the Stat92E activating ligand *upd*, using *Upd-GAL4* driving GFP (Sup. Fig. 2). To confirm these dynamics, we performed a lineage analysis of *upd*-expressing cells, using the G-TRACE system (Evans et al., 2009). We found that most wing disc cells originated from *upd-GAL4* expressing cells except for a small cell population near the disc stalk (Sup. Fig. 3A). This cell population expanded at later stages to occupy most of the medial notum and part of the lateral notum (Sup. Fig. 3B). These dynamics suggested roles for *stat92E* in the elaboration of the wing PD axis.

stat92E suppresses the induction and elaboration of the wing PD axis during early stages of wing development

To test whether Stat92E activity plays a functional role in wing PD development, we analyzed the clonal phenotypes of the strong *stat92E*^{85C9} mutant allele. Because the *stat92E* mutant clones grow poorly in a wild type background (Mukherjee et al., 2005), we generated the clones in a Minute background to provide them with a growth advantage (Garcia-Bellido et al., 1973; Morata and Ripoll, 1975). We found that a subset of the clones induced at 48-72h AEL in a Minute background (equivalent to 34-58h AEL in a wild type background) led to the formation of ectopic wings from the posterior part of the notum in adult flies (Sup. Fig. 6B). Analysis of third in star imaginal discs with molecular markers revealed induction of ectopic wing fields from the posterior region of the notum (Fig. 2). Ectopic wing fields were positively marked by the expression of Nub, Wg and the T-box protein Dorsocross2 (Doc2), (Fig. 2B, D and F, respectively). Clones associated with ectopic wing fields were often small and localized to the disc proper near the disc stalk (Fig. 2B and 2F) and/or the posterior disc margins (Fig. 2D). Wg and Doc2 were expressed in both the pouch and hinge of ectopic wings, indicating that the PD axis was elaborated within ectopic wing fields. Interestingly, the ectopic wings did not substitute for the native notum, as revealed by the expression of both Wg and the Iro-C protein Mirror (Mirr) and Doc2 in the notum (asterisks in Fig. 2D and 2F, respectively). The induction of the notum and wing is dependent on inductive interactions across the AP compartment boundary, which in the notum localizes to the posterior margin (Klein and Arias, 1998; Ng et al., 1996; Williams et al., 1993). Our data suggest that *stat92E* inhibits the competence of this region of the notum to respond to this wing field-inducing signal from the posterior compartment. Ectopic wings were composed of mostly wild type cells suggesting that following wing induction non-wing cells were recruited to the wing field using a non-autonomous mechanism.

To confirm these findings, we analyzed the *stat92E*^{j6C8} and *stat92E*^{397} alleles by clonal analysis. We found that these alleles produce partially overlapping phenotypes with the stronger phenotypes produced by the *stat92E*^{85C9} and *stat92E*^{397} alleles and the weaker phenotypes by the *stat92E*^{j6C8} allele (Sup. Fig. 4, Sup. Tables 1-2 and Materials and Methods). Additionally, we were able to rescue the *stat92E*^{85C9} mutant clone phenotypes using a genomic DNA that spans the *stat92E* locus indicating that the clonal phenotypes resulted from the loss of *stat92E* and not from an associated mutation (Sup. Fig. 5 and Materials and Methods). For the remainder of analyses, we continued to focus on the *stat92E*^{85C9} allele.

Upd is sufficient to inhibit the expansion of the wing and notum

The formation and expansion of the pouch and notum coincided with the downregulation of Stat92E activity in these regions (Fig. 1), and the early loss of *stat92E* function resulted in induction of ectopic wing fields (Fig. 2). These observations suggested that *stat92E* suppresses that formation of the wing and notum at early stages. To determine whether Stat92E activity was sufficient to inhibit the specification of the wing and/or notum, we broadly expressed the JAK/STAT activating ligand *upd* from early stages with the broadly expressed *Esg-GAL4* driver. We found that in severe cases, the *Esg>Upd* discs formed tiny rudiments smaller in size than the leg discs (Fig. 3B-C, E, H). In less severe cases the wing remained larger than the leg, but the morphologies of the wing and notum were impaired (Fig. 3F, I). We examined the expression of Nub and Zfh2 as well as Mirr and Eyg to determine if the wing field and the notum were properly patterned in these discs. Zfh2 is expressed broadly in the early wing field. At later stages Zfh2 becomes restricted to the hinge (Fig. 3A) as expression of pouch-specific genes, such as Nub, are induced in distal cells (Terriente et al., 2008; Wu and Cohen, 2002). We found that Nub was expressed in distal cells in these rudiments as in wild type discs (data not shown). However, Zfh2 was

expressed more broadly across the wing field (Fig. 3B-C compared to wild type in 3A) and failed to be properly restricted to the hinge. Thus, constitutive *upd* expression in the pouch disrupted the elaboration of the wing PD axis.

We detected a similar disruption of ML axis elaboration in the notum. The Pax protein Eyg marks a broad anterior part of the notum, while Mirr marks the lateral part (Fig. 3D and G, respectively) (Aldaz et al., 2003; Kehl et al., 1998). We found that Eyg expression was lost in *Esg>Upd* rudiments in both severely and moderately affected wing discs (Fig. 3E-F compared to wild type in Fig. 3D). *Esg>GFP* expression is excluded from the Eyg domain of late third instar wing discs in the notum (Fig. 3D). However, it was not excluded from the *Esg>Upd* rudiments, and Eyg was not expressed in these rudiments (Fig. 3E-F). Likewise, neither Wg nor Mirr were expressed in these discs suggesting that *upd* expression prevented the elaboration of the intermediate and lateral subdivisions of the notum (Fig. 3H-I compare to wild type in Fig. 3G). Thus, in both the pouch and the notum, suppression of Stat92E activity is required for the elaboration of the PD and ML axes, respectively.

stat92E restricts the scope of the anterior border of the notum

The dynamics of Stat92E activity and the *upd* gain-of-function phenotypes suggested additional roles for *stat92E* in the patterning and expansion of the wing and notum. To test this, we generated *stat92E* mutant clones at later stages of development and examined the clonal phenotypes in adult flies and developing imaginal discs. We found that *stat92E* mutant clones generated at 72-96 hours AEL in a Minute background (equivalent to 51-74 hours AEL in a wild type background) caused severe defects in dorsal closure and cuticle differentiation along the dorsal midline (Sup. Fig. 6C). The anterior and posterior margins of the notum contribute to the closure of the two hemi-not a along the dorsal midline, suggesting that *stat92E* affects the specification, subdivision or expansion of these regions of the wing disc. The Odd-skipped protein Bowl is expressed along the anterior border of the notum, adjacent to cells expressing the Notch signal Delta (Dl) (Fig 4A), where it contributes to dorsal closure (DelSignore et al., 2012). We therefore asked whether *stat92E* mutant clones affect the expression of the Odd-skipped protein Bowl in the notum.

Mutant clones generated at 72-96h caused invaginations between the mutant clones and adjacent wild type cells in developing wing imaginal discs (Figs. 4-6). Clones that were generated in the Bowl domain protruded into the central part of the notum and expanded laterally toward the hinge (Fig. 4B-C). Bowl accumulated in these clones and in wild type cells along clone borders. We speculate that the non-autonomous induction of Bowl was mediated by the induction of the related family member *drumstick (drm)* in the clones, which has been shown to promote Bowl accumulation both autonomously and non-autonomously in the notum (DelSignore et al., 2012). Clones that spanned the posterior notum margin protruded only weakly into the central part of the notum. These results indicate that *stat92E* inhibits the expansion of the anterior border of the notum.

stat92E promotes expansion of the lateral notum and inhibits expansion of the medial notum

The downregulation of Stat92E activity in the notum coincided with the expansion and patterning of the notum mediolateral axis (Fig. 1D-I). We therefore asked whether *stat92E* affects the organization of this axis. *Dpp* induces the expression of *ush* and *pnr* at high and low thresholds in the medial notum, and represses the expression of *mirr* in this region. *wg* is induced at an intermediate position along the border with the medial notum (Fig. 5A). Depletion of *stat92E* in the Ptc domain led to the displacement of the Mirr and Wg domains toward the hinge (Fig. 5B). Likewise, the Mirr and Wg domains were displaced laterally toward the hinge in *stat92E* mutant clones (Fig. 5D-E). The lateral displacement of the Wg

domain coincided with a reduction in the scope of the lateral notum. Reciprocally, ectopic expression of *upd* in the Ptc domain led to the displacement of the Mirr and Wg domains in an opposite direction toward the disc stalk, with a coincidental loss of the medial notum (Fig. 5C). The displacement of the Mirr and Wg domains was also observed at a distance from the Ptc domain. It is possible that this apparent non-autonomy reflects the broader expression of *Ptc-GAL4* at earlier stages of development. Consistent with this idea, lineage tracing revealed that most of the notum originates from the *Ptc-GAL4* expressing cells (Evans et al., 2009). We conclude that *stat92E* restricts the expansion of the medial notum and promotes the expansion of the lateral notum, consistent with the medial to lateral repression of Stat92E activity during notum development (Fig. 1).

As Stat92E activity remains high in the hinge throughout development (Fig.1), we asked whether *stat92E* was required to limit the scope of the lateral notum along the notum/hinge interface. In the wild type disc, the lateral notum and dorsal pleura marked by Eyg and Zfh2 are adjacent along the anterior disc margin (arrow in Fig. 5F). Further posterior, a small region separates the Eyg and Zfh2 domains (bracket in Fig. 5F inset). In large *stat92E* mutant clones that spanned the Eyg and Zfh2 domains this intervening domain between the Eyg and Zfh2 domains was missing. As a consequence, the Eyg and Zfh2 domains were adjacent in the clones (arrow in Fig. 5G inset). In addition, the Eyg domain extended along the anterior margin from the notum into the dorsal pleura (arrow in Fig. 5G). Similarly, the broad expression of *UAS-Stat92ERNAi* with *C311-GAL4* resulted in the expansion of the Eyg domain toward the dorsal hinge and pleura and coincided with the downregulation of Zfh2 expression in this region (Sup.Fig. 7). Mirr and Eyg were also expressed cell-autonomously in smaller *stat92E* mutant clones that were generated in the dorsalpleura (arrows in Fig. 5H and I, respectively). These phenotypes coincided with the patterning defects in the pleura surrounding the hinge in adult flies baring *stat92E* mutant clones (Sup. Fig. 6E-F). Thus, *stat92E* is required to limit the lateral expansion of the Eyg domain and to promote the expansion of the intervening domain between the Eyg domain and the dorsal hinge. *stat92E* also acts cell-autonomously to promote dorsal pleura identity and inhibit notum identity in this region.

stat92E promotes the expansion of the hinge

While Stat92E activity is downregulated in the pouch and notum, high levels of Stat92E activity persist in the hinge during all stages of development, suggesting additional roles for *stat92E* in hinge development. The mature hinge is subdivided morphologically along its PD axis by a series of epithelial folds (Fig. 6A, zoom of the dorsal hinge in Fig. 6B), and is subdivided molecularly by the restricted expression of different combinations of gene products (Fig. 6A). Zfh2 is expressed broadly in the hinge (Terriente et al., 2008; Whitworth and Russell, 2003), while Nub is restricted to the distal part of the dorsal hinge (Ng et al., 1995), and Wg to two concentric rings within the distal and the proximal parts of the hinge (Baker, 1988; Couso et al., 1993). Tsh is expressed proximal to the Zfh2 domain in the presumptive body wall (Azpiazu and Morata, 2000; Casares and Mann, 2000). Early in development Nub and Tsh are expressed in nearly adjacent domains. As development proceeds, the intervening domain (bracketed in Z1 Fig. 6D) expands and displaces the Nub and Tsh domains from one another (Zirin and Mann, 2007). *stat92E* mutant clones that were generated in the dorsal hinge caused a reduction in the scope of the Zfh2 domain (Fig. 6C), as well as a reduction or loss of the intervening domain between the Nub and Tsh domains (Fig. 6D arrow in Z2). Likewise, clones that were generated in the ventral hinge caused a reduction in the scope of the Zfh2 domain (opposing arrows, Fig. 6C).

To determine if Stat92E activation is sufficient to promote hinge expansion, we broadly expressed *upd* with *Bx-GAL4* in a region that spans the dorsal hinge. Ectopic expression of *upd* in this region promoted the expansion of the distal subdivision of the dorsal hinge

(arrow, Fig. 6E). Likewise, expression of *upd* in FLP-out clones, using a combination of the FLP-FRT and UAS-GAL4 systems, led to the expansion of both the dorsal and ventral hinge (Fig. 6F).

Wg is expressed in two concentric rings within the distal and proximal hinge (Baker, 1988; Couso et al., 1993; Couso et al., 1994; Dichtel-Danjoy et al., 2009; Perea et al., 2009; Rodriguez del Alamo et al, 2002; Terriente et al., 2008) where it promotes hinge cell proliferation (Neumann and Cohen, 1996). To determine if *stat92E* affects hinge growth by promoting the expression of the hinge mitogenic signal Wg (Neumann and Cohen, 1996), we examined Wg protein expression in *stat92E* mutant clones. We found that Wg expression was generally maintained in the *stat92E* mutant clones ruling out the possibility that *stat92E* promotes hinge growth by regulating Wg production (Fig. 6G also see Fig. 5D). We note that the contour of Wg expression was displaced proximally in patches of wild type cells relative to the contour of Wg expression in surrounding mutant cells (arrow in Fig. 6G), further supporting a role for *stat92E* in hinge expansion.

Requirements for canonical JAK/STAT signaling in wing PD axis development

Stat92E has been shown to translocate to the nucleus independent of receptor activation via a non-canonical mechanism (Li, 2008; Shi et al., 2006; Shi et al., 2008). To determine the role of canonical JAK/STAT signaling in wing development we examined the role of *upd* and the *upd* receptor *dome* in wing PD patterning. Broad depletion of *upd* in the wing by RNAi resulted in a mild outstretched wing phenotype (data not shown), suggesting a mild requirement for *upd* in hinge development. Similarly, expressing a dominant negative *dome* receptor (*UAS-dome*$^{\Delta CYT}$) (Brown et al., 2001) with *Tsh-GAL4* resulted in a mild to moderate reduction of notum size (Sup. Fig. 8G-H). By contrast, depleting *stat92E* function by RNAi caused a range of phenotypes which were generally more severe than the Tsh>*dome*$^{\Delta CYT}$ phenotypes (Sup. Fig. 8D-F). In severe cases, the entire wing disc formed a tiny rudiment and the pouch failed to expand (Sup. Fig. 8F). Further analysis of *dome* function by clonal analysis revealed only mild requirements for *dome* in wing PD patterning (Fig. 7). Mutant clones for the strong *dome*^{468} allele generated in a wild type background were recovered readily in the pouch and notum. However, only small clones were recovered in the hinge, while mostly wild type twin spot clones were recovered in the dorsal hinge (Fig. 7A). *dome* mutant clones that were generated in a Minute background caused only mild patterning defects. Clones that spanned the hinge disrupted the normal pattern of Wg expression (arrow, Fig. 7C), while a subset of large clones caused a reduction in the growth of the ventral hinge (arrow, Fig. 7D). Furthermore, *dome* mutant clones in both the dorsal and ventral hinge lost expression of 10× Stat92E-GFP reporter activity (Fig. 7A). However, in general we did not recover phenotypes as severe as those generated by the *stat92E* mutant clones such as wing duplication or severe reduction in the growth of the dorsal hinge.

Our data is largely consistent with a canonical role for *stat92E* in wing development with the exception of the *dome*^{468} loss-of-function phenotypes. However, there are several possible explanations for the difference between the *dome*^{468} and *stat92E* clonal phenotypes that are consistent with a canonical role for *stat92E* in wing development. It is conceivable that the *dome*^{468} clones produce a more severe loss of growth and proliferation due to stronger loss of *stat92E* function that precludes formation of more dramatic clonal phenotypes. In addition, the lack of the receptor could cause an increase of the pool of the non-phosphorylated cytoplasmic Stat92E leading to an increase in the level of unphosphorylated heterochromatin bound protein. This effect could mask the requirements of *stat92E* in the canonical pathway. Additional experiments will be necessary to define the role of *stat92E* in the canonical and non-canonical pathways during wing development.

Discussion

stat92E promotes cell proliferation in the second instar larval wing disc and inhibits cell proliferation in the pouch at later stages (Mukherjee et al., 2005). *stat92E* activity levels also regulate cell competition, such that *stat92E* mutant cells are eliminated by cell competition, while cells with hyper-activated Stat92E eliminate surrounding wild type cells (Rodrigues et al, 2012). While the above studies assigned general roles for *stat92E* in control of cell proliferation and cell competition, the current study relates the temporal pattern of Stat92E activity to the patterning and expansion of the wing PD axis. We show that Stat92E is active ubiquitously at early stages and acts to inhibit the induction of ectopic wing fields. Subsequently, Stat92E activity is repressed in the notum and pouch to allow for the proper expansion of these primordia. At later stages, Stat92E is active in and surrounding the hinge and is required for the expansion of the hinge and the specification of the dorsal pleura. Thus, Stat92E is regulated along the wing PD/ML axis to coordinate the expansion of this axis and control its proper subdivision.

The integration of Stat92E activity with other patterning signals

Secreted signals that emanate from localized organizers operate in concert to pattern fields of cells (Lawrence et al., 1996; Mann and Morata, 2000). In some contexts, organizer signals emanate from field boundaries and act reciprocally and antagonistically to organize pattern across the intervening field. In relatively simple systems, such as the patterning of the fly embryonic epidermis and vertebrate neural tubes, two organizers are established at field boundaries to pattern the intervening field of cells (Hatini & DiNardo, 2001; Wilson and Maden, 2005; Gomez-Skarmeta et al., 2003). In more complex systems, such as the patterning of the vertebrate limb bud, the coordinated activities of more than two organizers are required to elaborate the pattern (Duboc and Logan, 2009). Our findings suggest that Dpp, the Odd-skipped proteins and Stat92E interact functionally to organize both the AP and ML axes of the notum (Fig. 8B). Dpp and the Odd-skipped proteins act antagonistically and reciprocally to organize the notum AP axis (DelSignore et al., 2012). We now find that *stat92E* restricts the scope of the Odd-skipped domain and thereby the organization of the notum AP axis (Fig. 4). In addition, we find that *stat92E* acts antagonistically to Dpp to pattern the notum ML axis (Fig. 5).

Unpaired signals, which activate the JAK/STAT pathway, are produced in several localized sources surrounding the pouch and the lateral border of the notum and Stat92E is activated in and surrounding these sources (Fig. 1 and Sup. Fig. 2). Moreover, the dynamics of canonical Stat92E activation coincides with the requirement of *stat92E* in wing development. Thus, our data is largely consistent with a canonical role for *stat92E* in wing development. According to this model the coordinated activities of the three pattern organizing signals that emanate from the three borders of the notum are coordinated to control notum growth and patterning (Fig. 8B). *stat92E* regulates *wg* expression to pattern the eye, and *wg* and *dpp* expression to pattern the leg and antenna (Ayala-Camargo et al., 2007; Ekas et al., 2006). Thus, *stat92E* appears to regulate the expression or modulate the activity of other organizer signals to pattern developing imaginal discs. Although unlikely, it remains possible that *stat92E* acts primarily through a non-canonical mechanism, a model that rules out the idea that a third organizer expressing *upd* signals is required for the elaboration of the wing PD/ML axis. Instead, this model predicts that a balance of non-canonical Stat92E activity and a temporally regulated pattern of Stat92E repression are superimposed on existing patterning systems to elaborate this axis (Fig. 8).

stat92E controls the relative expansion of gene expression domains along the notum ML axis

The pouch is organized by several signals that are produced along the AP and DV compartment boundaries (Lawrence et al., 1996; Mann and Morata, 2000), while the notum is organized by signals that emanate from the anterior and posterior borders of the notum (DelSignore et al., 2012; Fromental-Ramain et al., 2008; García-García et al., 1999; Letizia et al., 2007). However, it remains unclear how the programs of gene expression controlled by these organizers are coordinated to control relative expansion of these primordia. We provide evidence that Stat92E activity is regulated across the wing disc PD/ML axis to coordinate the relative expansion of the pouch, hinge, and notum. Our data reveals that Stat92E activity is downregulated in the presumptive notum and pouch with dynamics that coincide with the expansion of these primordia (Fig. 8A). The broad constitutive activation of canonical Stat92E signaling blocked the induction of Eyg, Mirr and Wg expression in the notum as well as the general expansion of the notum (Fig. 3E-F and H-I, respectively). In the wing field, constitutive *upd* expression impaired the expansion of the wing field and the restriction of the Zfh2 domain to an intermediate PD position (Fig. 3B-C). These results suggest that the downregulation of Stat92E activity in the notum and pouch are required for the proper expansion and patterning of these domains from early stages.

Analysis of *stat92E* mutant clones that were generated at later stages revealed that *stat92E* affects the relative scope of various domains along the notum ML axis and wing PD axis. *stat92E* restricted the scope of the medial notum and promoted the expansion of the lateral notum. In addition, *stat92E* was required to promote the expansion of the lateral part of the notum between the Eyg domain and the dorsal hinge (Fig. 5F-G) indicating a more general role for *stat92E* in controlling the scope of various gene expression domains along the entire notum ML axis (Fig. 8). How might *stat92E* restrict the expansion of medial notum identities while promoting expansion of lateral identities? It is conceivable that *stat92E* inhibits cell proliferation in early wing discs and downregulation of Stat92E activity stimulates cell proliferation by relief-of-repression at later stages. According to this model, the subdivision and expansion of the notum are coupled with the downregulation of Stat92E activity in a medial to lateral direction. This model predicts that the untimely removal of Stat92E activity in early wing disc would lead to the untimely proliferation and expansion of medial notum identities at the expanse of lateral identities as a result of precocious relief-of-repression in lateral cells.

While *stat92E* restricted the expansion of the notum, it was required to promote the expansion of the hinge. In particular, *stat92E* was required for the generation of the intervening domain between the Tsh and Nub domains in the dorsal hinge as well as the expansion of the ventral hinge. The failure to generate the intervening domain could result from the lateral expansion of the Tsh domain at the expense of the intervening domain. Alternatively, it could result from a role for *stat92E* in controlling cell proliferation in this region of the wing hinge. Consistent with the latter, both *dome* and *stat92E* mutant clones survived and grew poorly in this region of the wing disc (Fig. 7A) (Mukherjee et al., 2005). This observation suggests differential roles for *stat92E* along the wing PD/ML axis including a negative role in notum and pouch expansion and a positive role in hinge expansion.

stat92E suppresses ectopic wing PD axis specification

In addition to specific roles in the elaboration of the notum and wing ML/PD axis, we also find that *stat92E* represses inappropriate induction of ectopic wing fields (Fig. 2). Stat92E is active ubiquitously in early wing discs and inhibits the induction of ectopic wing fields in part of the wing disc that normally adopts body wall identity (Figs. 1-2, Sup. Fig. 6B).

Conversely, ectopic activation of Stat92E activity suppressed the elaboration of the wing PD axis (Fig. 3). Vn-dependent EGF receptor signaling promotes notum identity and antagonizes wing identity (Wang et al., 2000; Zecca and Struhl, 2002a, 2002b). *stat92E* mutant clones in the presumptive notum caused induction of ectopic wing fields despite the specification of the notum in these wing discs (Fig. 2). This observation indicates that the Vn-dependent mechanisms that specify the notum are insufficient to inhibit wing induction, and implies that additional mechanisms are required to inhibit wing induction in this region. The induction of the wing field depends on signals that are delivered by the posterior compartment to the adjacent anterior compartment in early wing discs (Klein and Arias, 1998; Ng et al., 1996; Williams et al., 1993). Our data indicate that wing-inducing signals are also delivered in the notum, but the competence of the notum to respond to these signals is blocked by *stat92E*. It has been previously reported that wing inducing signals are also delivered in the peripodial epithelium (PE) of the wing imaginal disc, but the competence of this tissue to respond to these signals is blocked by the *odd-skipped* genes (Nusinow et al., 2008). We therefore propose that the broad competence of the early wing disc to respond to wing inducing signals is antagonized in the presumptive notum by *stat92E* and in the PE by the *odd-skipped* genes. This may be significant at early stages of development when the wing disc is relatively small and the organizing-centers that specify the notum and wing reside in close proximity to one another. During this stage *stat92E* may act as a buffer to prevent inappropriate pathway activation in ectopic locations.

Material and Methods

Generation and analysis of *stat92E* mutant alleles by mosaic analysis

The $Rps3^{Plac190}$ Minute mutation prolongs larval development by 51 hours (Saeboe-Larssen et al., 1998). Mitotic *stat92E* clones were induced using the Minute FLP/FRT technique at 48-72, 72-96 hours AEL, which correspond to approximately first and second larval in star in the $Rps3^{Plac190}$ Minute background. Flies of the genotype *y w hs-FLP; FRT82B Ubi-GFP* $Rps3^{Plac190}$ */TM6C, Sb* were used to induce marked Minute FLP/FRT clones.

We examined the clonal phenotypes of the $stat92E^{85C9}$, $stat92E^{397}$, $stat92E^{j6C8}$, and $stat92E^{06346}$ mutant alleles to rule out the possibility that phenotypes arose from independent genetically linked mutations. We found that $stat92E^{85C9}$, $stat92E^{397}$ and $stat92E^{j6C8}$ mutant clones generated at second and early third in star caused morphological defects in the appendages and body wall of adult flies (Sup. Fig. 6, Sup. Tables 1-2). Analysis of mutant clones in developing imaginal discs revealed strong morphological defects and changes in expression of patterning markers in $stat92E^{85C9}$ and $stat92E^{397}$ mutant clones and only weaker morphological anomalies in $stat92E^{j6C8}$ clones (Sup. Fig. 4, Sup. Tables 1-2). In comparison, neither $stat92E^{06346}$ mutant clones nor control clones produced any obvious defects (data not shown). To rule out the possibility that the *stat92E* clonal phenotypes resulted from an associated mutation we rescued the strongest $stat92E^{85C9}$ clonal phenotypes with genomic DNA that spans the *stat92E* locus (P[acman] Bac clone CH321-73F24). A super folder *EGFP* (*sf-EGFP*) was fused in frame with *stat92E* coding region in this genomic DNA by recombineering (gift from R. Spokony), and the modified Bac was inserted by site-specific recombination at 22A3. The distribution of the Stat92E:sf-EGFP protein coded by the Bac coincided with the distribution of the endogenous protein and the expression of the 10× Stat92E-GPF reporter in developing wing imaginal discs (Sup. Fig. 5, and data not shown). Expression of the Stat92E:sf-EGFP protein in the $stat92E^{85C9}$ mutant clones rescued to wild type the patterning defects and the abnormal morphology of the clones (Sup. Fig. 5; 27 wing discs bearing clones stained with Dapi and Wg were analyzed). We thus conclude that the $stat92E^{85C9}$ clonal phenotypes resulted from the loss of *stat92E* function and not from an associated mutation. We note that

the rescue does not exclude the possibility that the *stat92E*^{85C9} chromosome carry a second mutation close to the stat92E locus though this possibility is unlikely.

The nature of the *stat92E* alleles is consistent with why some produce strong phenotypes and others do not. Both *stat92E*^{06346} and *stat92E*^{j6C8} have P-element insertions 5′ to the start site of the *stat92E* gene, which presumably lower *stat92E* gene transcription (Hou et al., 1996; Spradling et al., 1999). The 761 amino acid Stat92E protein is composed on an N-terminal region, a coiled-coil domain, a DNA-binding domain, a linker region, an SH2 domain, and a C-terminal transactivation domain. The *stat92*^{85C9} allele is caused by a substitution of an Arg at position 442 to a Pro in the DNA binding domain and this substitution abolishes the transcriptional activity of the protein *in vitro* (Ekas et al., 2010). The *stat92E*^{397} allele is caused by substitution of a Trp to a stop codon at position 594 3′ of the DNA binding domain resulting in truncation of part of the SH2 domain and the C-terminal transactivation domain (Silver and Montell, 2001). The in vitro transcriptional activity of this variant has not been examined. Analysis of the zygotic lethal phase of these *stat92E* alleles placed over a chromosomal deficiency that removes the *stat92E* locus suggested the following stat92E allelic series: *85C9=397>j6C8>06346*. In support of this conclusion it has been reported that the *stat92E*^{85C9} and *stat92E*^{397} clones generated stronger phenotypes than *stat92E*^{06346} or *stat92E*^{j6C8} clones in the eye-antennal, wing or leg disc (Ayala-Camargo et al., 2007; Ekas et al., 2006). Our analysis in the wing imaginal disc is largely consistent with these reports (Sup. Tables 1-2).

Analysis of *dome* function by clonal analysis

Flies of the genotype *Ubi-mRFPhsFLP FRT19A* were used to generate *dome*^{A68} mutant clones in a wild type background. Females of the genotype *Ubi-GFP M(1)o*^{sp}*FRT19A*/FM7; hsFLP/TM6b (G. Struhl) were crossed to *dome*^{A68}*FRT19A*/FM7 mutant males that were rescued to viability with a genomic fragment spanning the *dome* locus inserted at 65B2 (Dp DC365) in trans to a TM6b balancer chromosome. Marked Minute FLP/FRT clones were induced in female larvae of the genotype *Ubi-GFP M(1)o*^{sp}*FRT19A/dome*^{A68}*FRT19A; hsFLP*/+.45 wing imaginal discs stained with Wg and Dapi were examined for phenotypes.

UAS transgenes

Flies of the genotype *UAS-RedStinger, UAS-FLP, Ubi>STOP>Stinger* were used to trace the lineage of *Upd-GAL4* expressing cells (green) and simultaneously detect the source of *upd* expression (red) (Evans et al., 2009). *UAS-updRNAi* (VDRC ID-3282), *UAS-Stat92ERNAi* (VDRC ID-43866, ID-43867, strong and weak insertion, respectively), *UAS-dome*$^{\Delta CYT3.2}$ (J. Castelli-Gair) where used to inhibit canonical JAK/STAT signaling. *UAS-upd* was used to activate canonical signaling (Harrison et al., 1998). *Esg>Upd* and *Esg>dome*$^{\Delta CYT3.2}$ larvae were raised for 5-6 days at 30°C prior to dissection.

GAL4 lines

The following drivers were used: *UAS-GFP; Tsh-GAL4*^{md621}, *Esg-GAL4*^{NP5130}, *UAS-GFP, C311-GAL4* (G. Schubiger); *UAS-GFP, Ptc-GAL4* (Speicher et al., 1994), *UAS-GFP; Ubx-GAL4* (Pallavi and Shashidhara, 2003) and *Bx-GAL4*^{ms1096}. Tsh-GAL4^{md621} is broadly expressed and excluded from the pouch and distal hinge, *Esg-GAL4*^{NP5130} is broadly expressed and excluded from a broad anterior domain in the notum, *C311-GAL4* is broadly expressed and is upregulated along the anterior notum margin, *Ptc-GAL4* is expressed in a narrow domain along the AP compartment boundary, *Ubx-GAL4* is expressed in the peripodial epithelium and *Bx-GAL4*^{ms1096} is expressed at high levels in the dorsal hinge and pouch and at lower levels in the ventral wing field and notum. *upd-GAL4* (E132) was used to highlight the sources of *upd* expression.

Immunofluorescence, reporter lines and microscopy

10× Stat92E reporter driving expression of GFP (*10× Stat92E-GFP*) or destabilized GFP (*10× Stat92E-DCFP*) were used to monitor the pattern of Stat92E activity (Bach et al., 2007). Both reporters were expressed in a similar pattern. Antibody staining protocols have been described elsewhere (Hatini et al., 2005). Primary antibodies used were: mouse anti-Nub (Ng et al., 1995), rabbit anti-Tsh (Wu and Cohen, 2002), rat anti-Zfh2 (M. Lundell), mouse anti-Wg (4D4, DSHB), rabbit anti-Bowl (DelSignore et al., 2012), guinea pig anti-Eyg (Aldaz et al., 2003), rabbit anti-Doc2 (M. Frasch), mouse anti-Ubx (R. White), mouse anti-Tup (DSHB), rat anti-Mirr (H. McNeill) and rabbit anti-Stat92E (Cell Signaling).

Typically 50-100 wing imaginal discs were stained, mounted and inspected per experiment. Of those, 5-10 representative imaginal disc were imaged by confocal microscopy for detailed analysis. Imaginal discs were scanned using a Zeiss LSM510 confocal microscope in multitracking mode and images were assembled and adjusted using Adobe Photoshop CS3.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

► *stat92E* suppresses wing induction in early wing discs. ► Stat92E repression coincides with the expansion of the pouch and notum. ► *stat92E* controls the relative expansion of the lateral and medial notum. ► *stat92E* promotes hinge expansion.

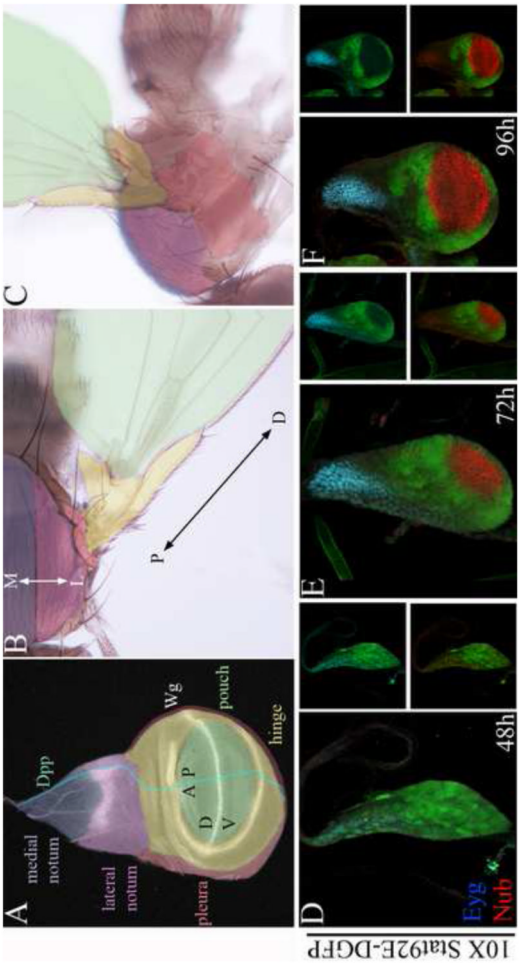

Figure 1. Downregulation of Stat92E activity coincides with the expansion of the pouch and notum

(A-C) Schematics of third in star wing disc (A) and its derivatives in adult flies shown in dorsal (B) and lateral (C) orientations. The third in star wing disc (A) is subdivided along the PD axis into the distal pouch (green), the intermediate hinge (yellow),the pleura (red), and the proximal notum (lateral notum in mauve, and medial notum in purple). *wg* expression (white) defines the border between the medial and lateral notum, the hinge, and the DV compartment boundary in the wing field. *dpp* (cyan) is expressed in the anterior compartment along the AP compartment boundary. (B-C) The notum and presumptive pleura give rise to dorsal and lateral parts of the adult mesothorax, respectively, while the wing field gives rise to the wing hinge and blade. The notum mediolateral (ML) and wing proximodistal (PD) axes are indicated by double arrows in B. (D-F) *10× Stat92E-DGFP* (green), Nub (red), Eyg (cyan). Downregulation of Stat92E activity in the presumptive notum and pouch coincided with the expansion of these primordial marked with Eyg and Nub, respectively.

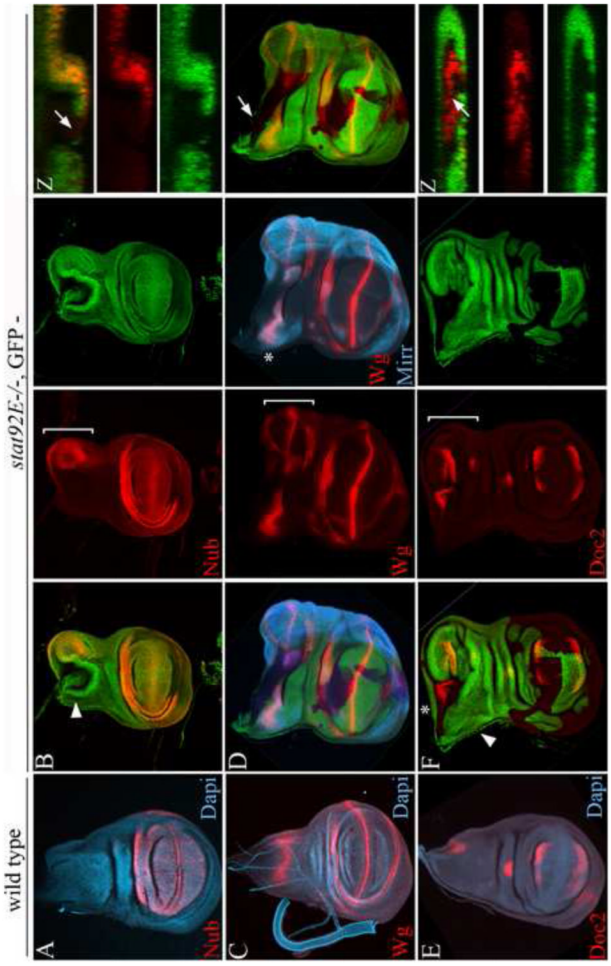

Figure 2. *stat92E* inhibits the induction of ectopic wing fields

(A, C, E) Wild type wing discs, and (B, D, F) wing discs bearing negatively marked *stat92E* mutant clones generated at first to second in star, and stained for Nub (A-B),Wg (C), both Wg and Mirr (D), and Doc2 (E-F). Arrowheads in B and F point to planes of Z sections shown in corresponding insets. (A) Nub is expressed in the pouch and distal hinge. (C) Wg is expressed along the DV compartment boundary in the pouch, in two concentric rings in the hinge and at an intermediate position in the notum. Lateral notum is marked with Mirr in D. (E) Doc2 is expressed in dorsal and ventral rims in the pouch, in a spot in the central region of the dorsal hinge, and in a narrow domain along the posterior notum margin. (B, D, F) Early *stat92E* mutant clones that were generated in the disc proper near the disc stalk led to the induction of ectopic wing fields in the posterior part of the notum (demarcated by brackets in red channel). Ectopic wings were marked by the stereotypic expression of Nub, Wg and Doc2. Note that the notum specific expression of Mirr and Wg (asterisk in D), as well as Doc2 (asterisk in F) persisted in discs baring ectopic wings fields.

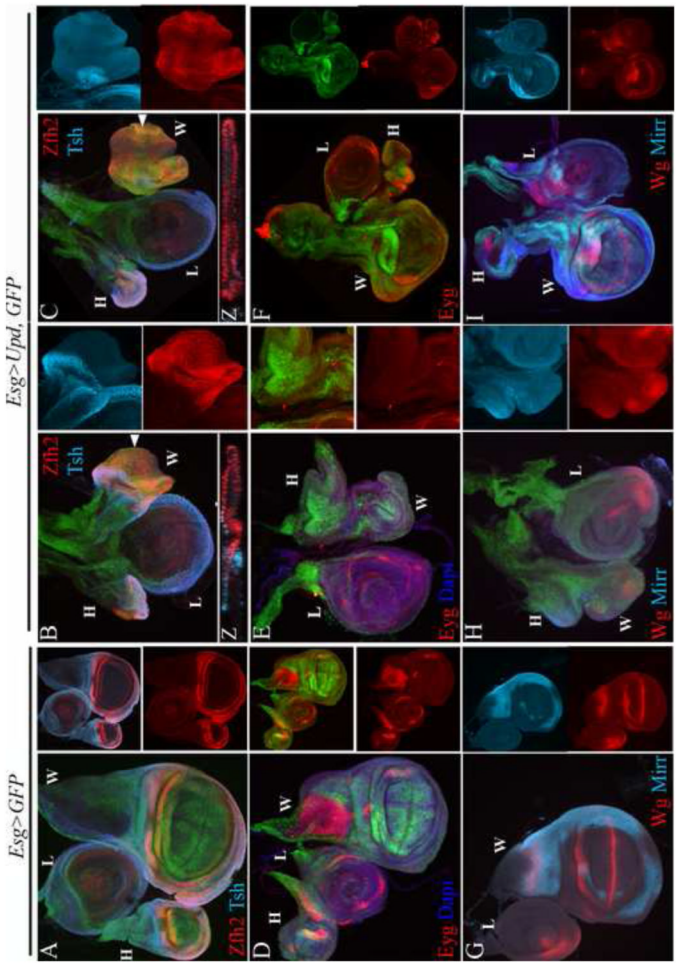

Figure 3. The Stat92E activating signal *upd* is sufficient to suppress the elaboration of the wing PD axis and notum ML axis

(A, D, G) Wild type; (A) Zfh2 highlights the hinge and Tsh the surrounding body wall; (D) Eyg highlights a broad part of the anterior notum; (G) Mirr highlights the lateral notum and Wg an intermediate position along the notum ML axis, as well as the hinge and DV compartment boundary in the wing field. (B-C, E-F, H-I) *Esg-GAL4/UAS-Upd; UAS-GFP* (*Esg>Upd, GFP*). Broad and constitutive expression of *upd* in the wing field led to severe (middle column: B, E, H, right column: C) to moderate (right column: F, I) reduction in wing size and defects in wing and notum patterning. Broad *upd* expression prevented the proper restriction of the Zfh2 domain to the hinge (B-C, and corresponding Z sections). In addition, broad *upd* expression led to loss or a severe reduction of Eyg (E-F) as well as (H-I) Mirr and Wg expression in the presumptive notum. H-haltere, L-leg, W-Wing.

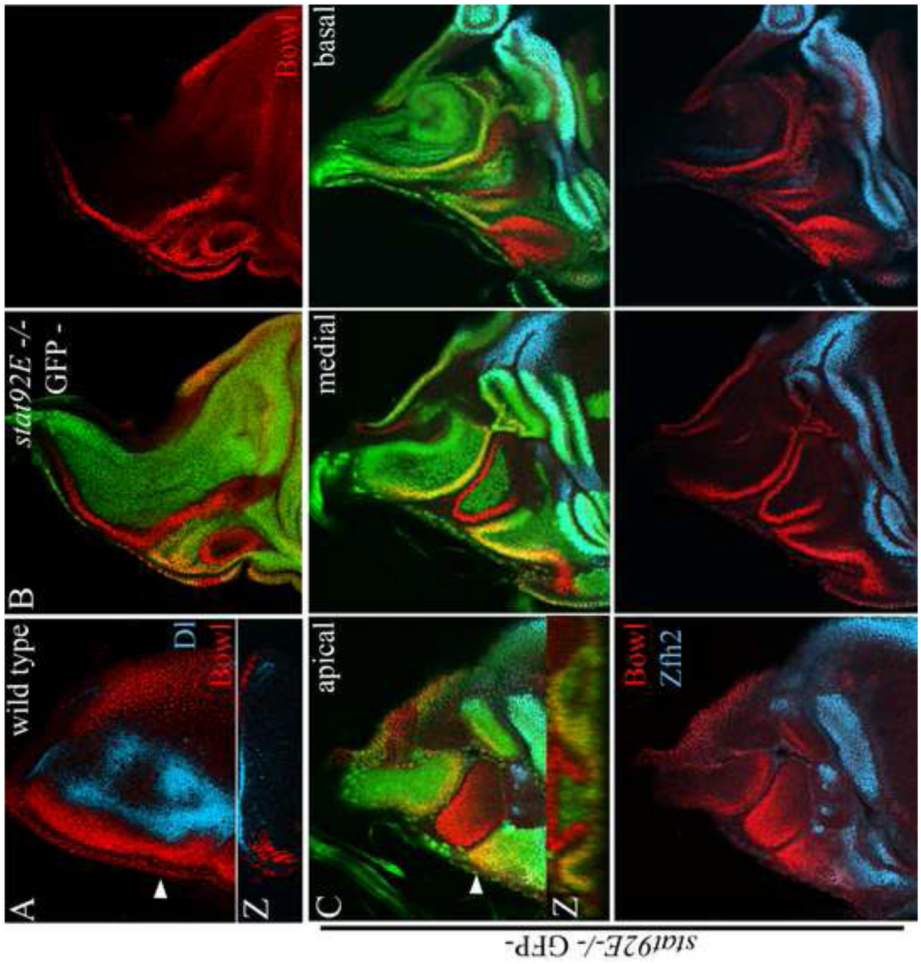

Figure 4. *stat92E* inhibits the expansion of the anterior border of the notum

(A) Wild type; Bowl (red) accumulates along the anterior border of the notum adjacent to Dl expressing cells (cyan). (B-C) Negatively marked *stat92E* mutant clones; Bowl (red), Zfh2 (cyan). In *stat92E* mutant clones that spanned the anterior border of the notum, the Bowl domain expanded posteriorly toward the central part of the notum and laterally toward the hinge. Bowl was also expressed in wild type cells along clone borders (yellow). Images in C are optical sections at the apical, medial and basal planes of a single wing disc. Arrowheads in A, C point to plane of Z-sections shown in corresponding insets.

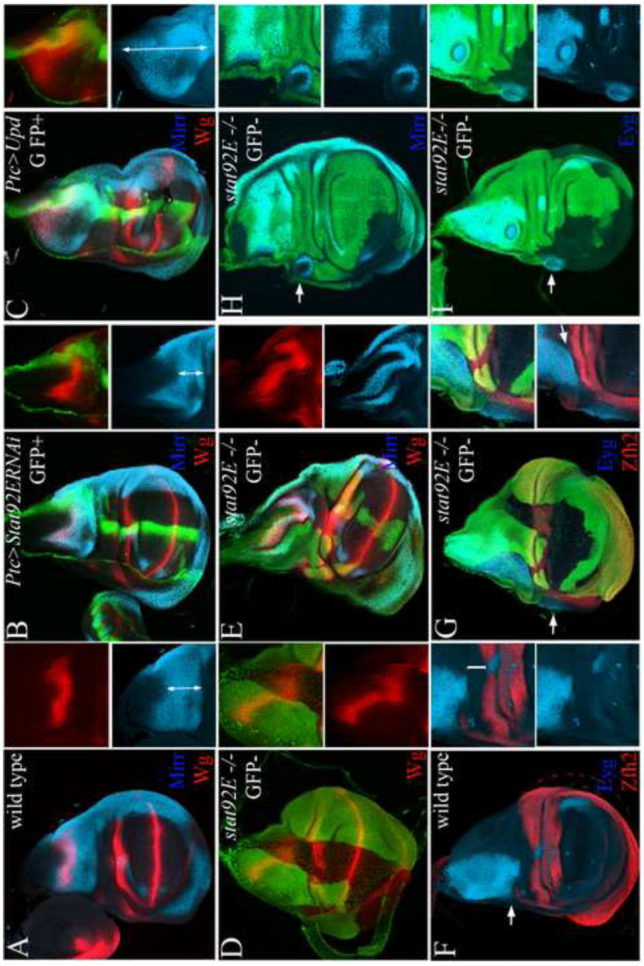

Figure 5. *stat92E* restricts the expansion of the medial notum and specifies the dorsal pleura cell-autonomously

(A) Mirr (cyan) is expressed in the lateral notum and Wg (red) in the lateral notum along the border with the medial notum. (B) Depletion of Stat92E activity in the Ptc domain (green) displaced the Mirr and Wg domains toward the hinge. (C) Expression of *upd* in the Ptc domain resulted in the opposite displacement of the Mirr and Wg domains toward the disc stalk. Double arrows demarcate the scope of the lateral notum marked with Mirr in A-C. To quantify the effects, we measured the scope of the lateral notum relative to scope of the entire notum ML axis and found that a reduction in *Ptc>StatRNAi* (0.275 +/− 0.05 SE, N=7) and expansion in *Ptc>Upd* (0.86 +/− 0.1 SE, N=6) compared to wild type (0.395 +/− 0.5 SE, N=8). (D-E) The Wg and Mirr expression domains were also displaced laterally in *stat92E* mutant clones. (F) Eyg (cyan) is expressed in the notum in a broad anterior domain, and Zfh2 (red) is expressed in the hinge. An intervening domain separates the Eyg and Zfh2 domains (bracket in inset in F). Only along the anterior margin the Eyg and Zfh2 domains are adjacent (arrow in F). (G-I) In large *stat92E* clones that spanned the Eyg and Zfh2 domains, the intervening domain between the Eyg and Zfh2 domains was missing (arrow in inset in G). In addition, the Eyg domain was displaced laterally into the pleura (arrow in G). (H) Mirr was expressed ectopically in *stat92E* mutant clones laterally to the Mirr domain along the wing margin (arrow). (I) Eyg was expressed ectopically in *stat92E* mutant clones in the dorsal pleura (arrows).

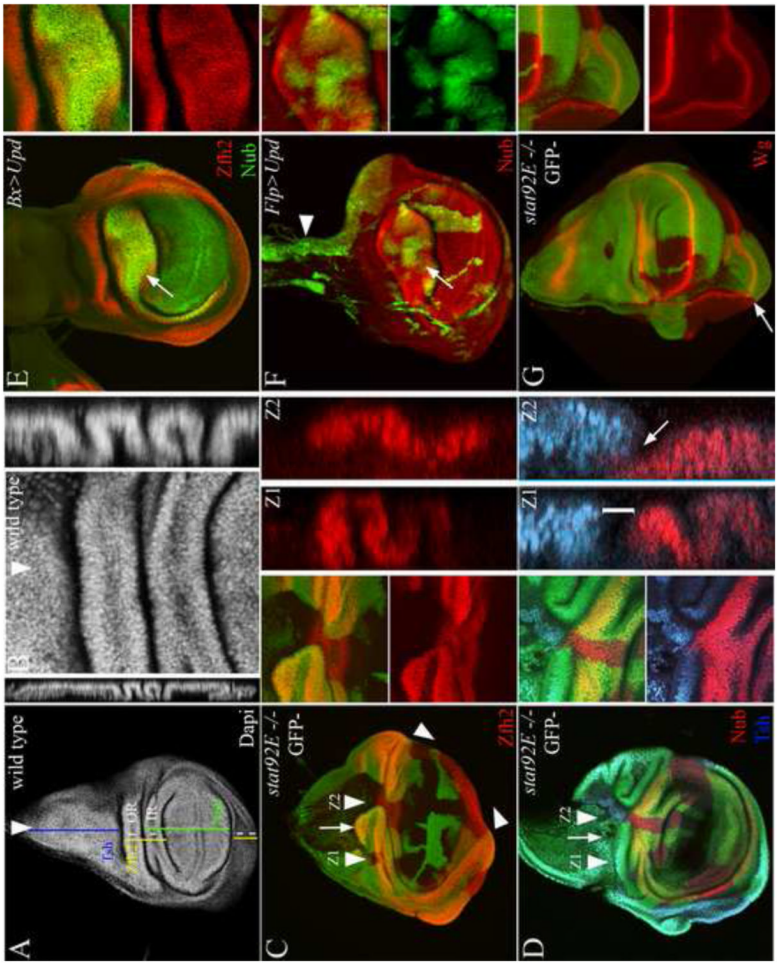

Figure 6. *stat92E* promotes the expansion and fine subdivision of the hinge

(A-B) Wild type wing disc stained with Dapi to highlight the folded appearance of the mature dorsal hinge. Arrowheads indicate planes of Z sections shown in corresponding insets. The Nub, Zfh2, Wg and Tsh expression domains are indicated schematically in A. (C) The scope of the dorsal hinge was reduced in *stat92E* mutant clones. Corresponding Z-sections reveal a reduced folding of the Zfh2 domain in mutant clones (Z2) compared to wild type tissue (Z1). Also note the thinning of the ventral hinge in a *stat92E* mutant clone (demarcated with opposing arrowheads). (D) An intervening domain intercalates between the Nub and Tsh domains in wild type tissue (demarcated with a bracket in Z1). This intervening domain was missing in *stat92E* mutant clones (arrow in Z2 shows absence of the intervening domain in *stat92E* mutant clone). (E) Expression of *upd* with *Bx-GAL4* caused expansion of the distal part of the dorsal hinge (arrow). (F) Likewise, ectopic expression of *upd* in FLP-out clones caused expansion of the dorsal hinge (arrow). However, *upd* clones that were generated in the notum severely perturbed notum size and morphology (arrowhead). (G) Wg expression in the hinge was maintained in the *stat92E* mutant clones. The contour of Wg expression in patches of wild type cells was displaced proximally (arrow) relative to surrounding patches of *stat92E* mutant cells.

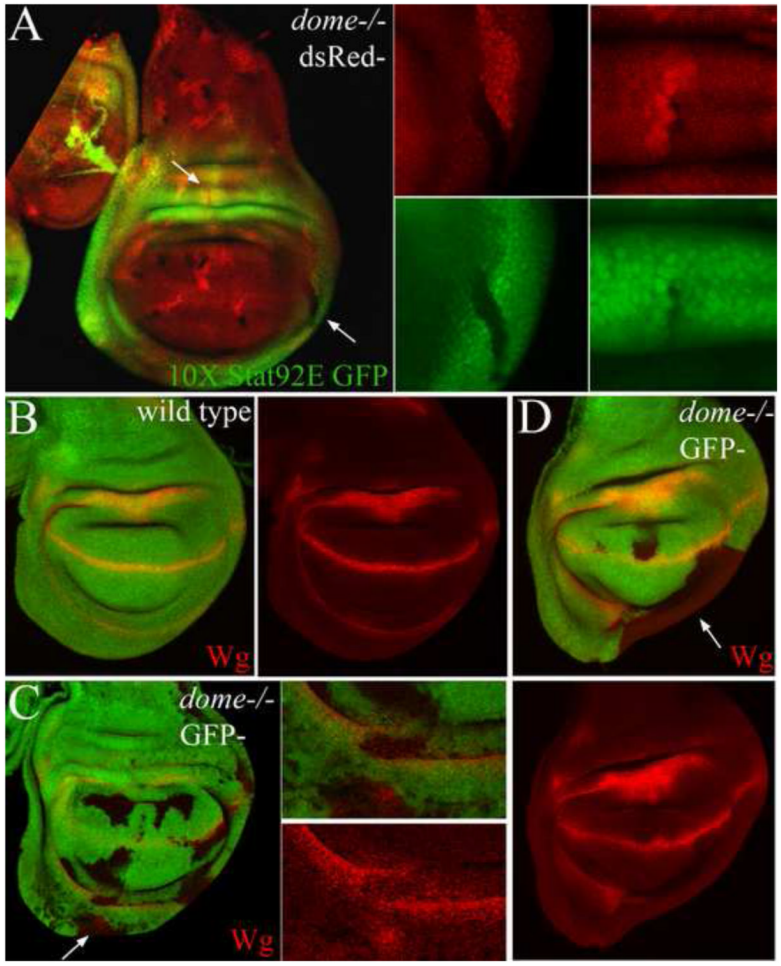

Figure 7. The role of canonical JAK/STAT Signaling in the elaboration of the wing PD/ML axis

(A) Negatively marked *dome*^{468} mutant cell clones generated in a wild-type background survived and grew poorly in the dorsal hinge. Expression of the 10× Stat92E-GFP reporter was missing in *dome* clones in either the dorsal or ventral hinge (arrows). (B) Wg expression in a wild type wing field. (C-D) Negatively marked *dome* mutant clones generated in a Minute background caused mild defects in Wg expression in clones that spanned the hinge region (C). Similar defects in Wg expression were observed in the ventral hinge in *stat92E* mutant clones (see examples in Fig. 2D and Fig. 5D). (D) In rare cases, the hinge was more severely affected in the clones (in 2 large clones in 45 discs examined).

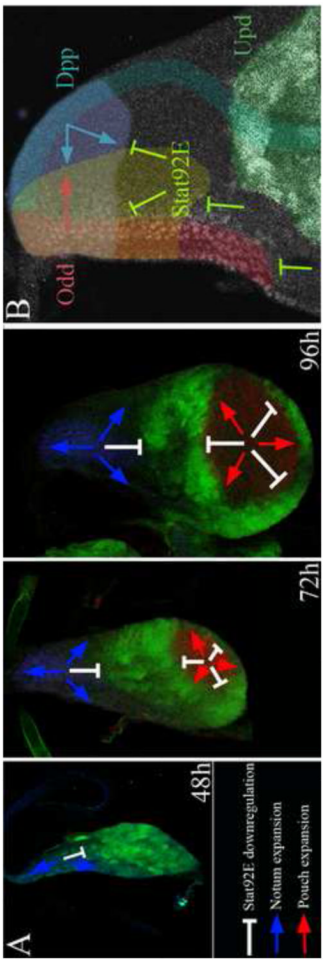

Figure 8. Model illustrating *stat92E* regulation and role in elaboration of the wing PD/ML axis

(A) A cartoon depicting the relationship between the progressive downregulation of Stat92E activity (white inhibitory arrows) and the expansion of the notum (blue arrows) and pouch (red arrows). The dynamics of Stat92E donwregulation coincided and contributed to the expansion of the pouch and notum, while the persistent activity of Stat92E in the hinge promoted the expansion of this domain. (B) A cartoon depicting the activity of Stat92E in notum patterning in relationship to the role of *dpp* and the *odd-skipped* genes. *dpp* plays two distinct roles in notum patterning. *dpp* promotes medial notum identity and represses lateral identity. In addition, *dpp* promotes anterior notum identity and represses posterior identity. The *odd-skipped* genes have been proposed to regulate the production of an unknown signal at the anterior border of the notum, which acts reciprocally and antagonistically to *dpp* to promote anterior identity and repress posterior identity. We provide evidence that *stat92E* restricts the lateral expansion of medial notum and thus acts antagonistically to *dpp* to pattern the notum ML axis. *stat92E* also restricts the scope of the Bowl domain to the anterior border of the notum and thereby the patterning of the notum AP axis.