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JAK/STAT pathway dysregulation in tumors: A *Drosophila* perspective

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Abstract

Sustained activation of the JAK/STAT pathway is causal to human cancers. This pathway is less complex in *Drosophila*, and its dysregulation has been linked to several tumor models in this organism. Here, we discuss models of metastatic epithelial and hematopoietic tumors that are causally linked to dysregulation of JAK/STAT signaling in *Drosophila*. First, we focus on cancer models in imaginal discs where ectopic expression of the JAK/STAT pathway ligand Unpaired downstream of distinct tumor suppressors has emerged as an unexpected mediator of neoplastic transformation. We also discuss the collaboration between STAT and oncogenic Ras in epithelial transformation. Second, we examine hematopoietic tumors, where mutations that cause hyperactive JAK/STAT signaling are necessary and sufficient for “fly leukemia”. We highlight the important contributions that genetic screens in *Drosophila* have made to understanding the JAK/STAT pathway, its developmental roles, and how its function is co-opted during tumorigenesis.

Introduction

Activating mutations in JAK/STAT signaling is a causal event in human leukemia, myeloproliferative neoplasms (MPNs) and solid tumors (Lacronique et al., 1997; Jones et al., 2005; Kralovics et al., 2005; Levine et al., 2005; Moriggl et al., 2005). With respect to the former, the Jak2^{V617F} activating mutation is present in most patients with polycytemia vera and in the majority of patients with essential thrombocytosis and myelofibrosis (reviewed in (Abdel-Wahab, 2011)). Persistent activation of Stat3 is observed all major classes of carcinoma, and cells mis-expressing dominant-active Stat3 cause tumors in immuno-compromised mice (Bromberg et al., 1999; Darnell, 2005). Hyperactivation of the JAK/STAT pathway also causes epithelial and hematopoietic tumors in flies. Interestingly, MPNs and fly hematopoietic tumors both result from over-proliferation of cells in the myeloid lineage. The powerful genetic tools available in *Drosophila*, coupled with the reduced genetic complexity of JAK/STAT and other signaling pathways in this organism,

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has led to *Drosophila* being adopted as a useful model for studying the role of JAK/STAT signaling in tumorigenesis.

The *Drosophila* genome contains a single *JAK* gene called *hopscotch* (*hop*) and a single *STAT* gene called *Stat92E*. Hop is most similar to Jak2 in vertebrates, while Stat92E is most homologous to Stat3 and Stat5 (reviewed in (Arbouzova and Zeidler, 2006)). Three related IL-6-like cytokines, Unpaired (Upd) (also called Outstretched), Upd2 and Upd3, bind to a gp130-like cytokine receptor called Domeless (Dome) (Fig. 1A) that subsequently activates Hop, which stimulates Stat92E. Activated Stat92E dimers induce expression of target genes including *Socs36E*, which encodes a negative regulator of pathway activity. Other inhibitors of the pathway include Eye transformer (ET) (also called Latran (Lat)), a second Upd receptor that antagonizes pathway signaling (Kallio et al., 2010; Makki et al., 2010), and dPIAS (or Su(var)2-10), the sole *Drosophila* PIAS homolog, which inhibits active Stat92E dimers.

The central role of JAK/STAT signaling in epithelial tumors

JAK/STAT signaling and developmental growth control

The JAK/STAT pathway plays important roles during *Drosophila* development, particularly in imaginal discs, which are epithelial tissues set aside during embryogenesis that give rise to the adult structures (Cohen, 1993). In wing and eye imaginal discs, JAK/STAT signaling is an essential regulator of growth and patterning. Pathway activity is detected in all cells in early discs, and JAK/STAT signal transduction is required in a cell autonomous manner for growth (Luo et al., 1999; Mukherjee et al., 2005a; Ekas et al., 2006; Rodrigues et al., 2012). The level of JAK/STAT signaling regulates the size of the adult eye. Animals with reduced JAK/STAT pathway activity have small or ablated eyes, and this phenotype can be rescued by activating Stat92E (Bach et al., 2003; Ekas et al., 2006; Ekas et al., 2010). To maintain proper tissue size, the expression of Upd needs to be tightly regulated. Mis-expression of Upd in the eye or wing disc leads to dramatically overgrown tissue (Fig. 2A,B and (Bach et al., 2003; Tsai and Sun, 2004; Classen et al., 2009; Rodrigues et al., 2012)). Some studies have shown that in wild type eye discs, Notch activation at the midline induces *upd* in cells at the posterior midline, and Upd acts non-cell autonomously to promote growth of the eye field and allow for initiation of neurogenesis (Fig. 2C and (Chao et al., 2004; Reynolds-Kenneally and Mlodzik, 2005; Ekas et al., 2006; Tsai et al., 2007; Djiane et al., 2013)). Up-regulation of Upd can account for much of the non-autonomous growth induced by activated Notch in the eye (Chao et al., 2004; Reynolds-Kenneally and Mlodzik, 2005). However, another report finds that Upd cannot account for the overgrowth induced by sustained Notch signaling. Instead, they find that early in eye development Upd plays a central role upstream of Notch activation (Fig. 2C, green arrow, and (Gutierrez-Avino et al., 2009)). Finally, JAK/STAT signaling can also negatively regulate Notch pathway activity, as Stat92E autonomously represses expression of *Serrate* (*Ser*), which encodes a Notch ligand (Fig. 2C and (Flaherty et al., 2009)). In sum, the relationship between JAK/STAT and Notch pathways in the eye disc is complex and consists of feed-forward as well as inhibitory interactions. Although the relationship of *upd* as a target of Notch signaling is treated in

detail below, it is worth bearing in mind that Notch may act downstream of Upd in certain developmental and pathogenic scenarios.

While sustained activation of JAK/STAT signaling accelerates cell cycle progression (Bach et al., 2003; Mukherjee et al., 2005a; Rodrigues et al., 2012), few direct molecular links have been made between this pathway and cell cycle progression or cellular growth. *Cyclin D* transcripts are upregulated in Upd-overexpressing discs (Tsai and Sun, 2004), and the relationship may be conserved as *Cyclin D* is a Stat5 target in vertebrate hematopoiesis (Matsumura et al., 1999). Several screens have been have been carried out in search of JAK/STAT targets in eye disc development (Bach et al., 2003; Mukherjee et al., 2005b; Flaherty et al., 2009). One group has linked Bone Morphogenetic Protein (BMP) signaling with over-growth downstream of JAK/STAT pathway activity in the eye disc (Bach et al., 2003). However, in the wing disc, ectopic JAK/STAT signaling causes increased growth without autonomously increasing BMP signal transduction, and several other developmental regulators involved in tissue growth such as Wingless, Hippo and dMyc are similarly unaffected by increased JAK/STAT activity (Rodrigues et al., 2012). In sum, although it is apparent that the JAK/STAT pathway is a major regulator of developmental growth, it is not yet clear how pathway activity leads to increased cell division and mass accumulation.

Tumors due to epigenetic misregulation of upd

In the last decade, there has been substantial interest in the contribution of epigenetic changes to tumorigenesis. Polycomb Repressor Complexes (PRCs) are essential to this process. PRCs bind specific DNA sequences known as Polycomb Response Elements (PREs), and this interaction leads to gene silencing (reviewed in (Schwartz and Pirrotta, 2007). One complex in particular, PRC1, has been implicated in tumor formation both in flies and mammals (Beuchle et al., 2001; Oktaba et al., 2008; Mills, 2010). Further work has established that loss-of-function mutations in several PRC1 components within *Drosophila* imaginal discs lead to the formation of tumors (Classen et al., 2009; Gonzalez et al., 2009; Feng et al., 2011). These tumors hyper-proliferate and lose normal epithelial organization. Intriguingly, although many PRC1 mutations cause cells to die, they also cause the over-proliferation of neighboring wild type cells (Classen et al., 2009; Feng et al., 2011), suggesting that a secreted signal mediates proliferation downstream of PRC1 loss. Indeed, the regulatory regions of the *upd*, *upd2* and *upd3* loci contain PREs that are bound by PRC1 proteins (Fig. 2C and (Classen et al., 2009; Gonzalez et al., 2009). These PREs lead to the silencing of the *upd* loci in wild type animals, but in PRC1 mutants, *upd* genes are de-repressed. Furthermore, preventing Upd expression or JAK/STAT activation in PRC1 mutants suppresses tumor growth (Classen et al., 2009; Gonzalez et al., 2009; Feng et al., 2011).

ESCRT mutants ectopically activate JAK/STAT signaling

Unexpectedly, mutations in endocytic genes revealed a link between endocytosis and tumor formation in epithelia (reviewed in (Vaccari and Bilder, 2009)). During endocytosis, cargo sorting and multi-vesicular body formation require three large protein complexes, the Endosomal Sorting Complexes Required for Transport (ESCRT-I, -II and -III) (reviewed in (Rusten et al., 2012)). Unbiased genetic screens searching for new tumor suppressors in

Drosophila imaginal discs have revealed that many components of the endocytic machinery are essential to suppress overgrowth and to maintain epithelial organization (Lu and Bilder, 2005; Moberg et al., 2005; Thompson et al., 2005; Vaccari and Bilder, 2005; Herz et al., 2006; Menut et al., 2007; Morrison et al., 2008). Mutations in most endosomal and ESCRT components lead to tumor formation in *Drosophila* imaginal discs. In many cases, the mutant cells are eliminated from the tissue and the overgrowths are comprised of neighboring wild type cells. For example, cells mutant for *vps25*, which encodes an ESCRT-II component, are not able to contribute to adult tissue, yet eyes with *vps25* mutant clones are dramatically overgrown (Thompson et al., 2005; Vaccari and Bilder, 2005; Herz et al., 2006). Similar results have been reported for *erupted* (*ept*), the *Drosophila* homolog of *TSG101* that encodes an ESCRT-I and -II component (Moberg et al., 2005). Subsequent work characterized many other ESCRT components and found that mutations in most caused similar overgrown eye phenotypes despite the fact that the mutant clones died (Herz and Bergmann, 2009; Vaccari et al., 2009; Woodfield et al., 2013). In these cases, ESCRT mutants trap the Notch receptor in endosomes, where it signals aberrantly and continues to induce transcription of *upd* (Fig. 2C,D and (Lu and Bilder, 2005; Moberg et al., 2005; Thompson et al., 2005; Vaccari and Bilder, 2005; Herz et al., 2006; Vaccari et al., 2008; Rodahl et al., 2009; Vaccari et al., 2009)). Overgrowth correlates with ectopic Upd expression, and reducing the genetic dose of *Stat92E* suppresses the non-autonomous overgrowth caused by *vps25* and *ept* clones (Moberg et al., 2005; Vaccari and Bilder, 2005; Herz et al., 2006). Interesting recent work has identified endocytic mutants that have increased JAK/STAT activity without a corresponding increase in Notch signaling (Thomas and Strutt, 2014). The outcome of aberrant JAK/STAT activation, however, remains the same: loss of epithelial structure and ectopic growth, which are hallmarks of neoplastic transformation.

One important question is whether JAK/STAT signaling plays an autonomous role within tumor cells themselves. To examine this, two independent groups generated imaginal discs composed almost entirely of ESCRT mutant cells and found autonomous activation of JAK/STAT signaling (Gilbert et al., 2009; Woodfield et al., 2013). Within *ept* tumors, removing one genetic copy of *Stat92E* is sufficient to alter cell size and cell cycle dynamics, and FACS analysis reveals fewer cells entering S-phase following *Stat92E* reduction (Gilbert et al., 2009). In addition, Stat92E phosphorylation and stabilization of Dome within endocytic vesicles is detected within *ept* tumors. While the site of active Dome signaling is debated (Devergne et al., 2007; Vidal et al., 2010), one possibility is that Dome is stabilized in an active state in ESCRT mutants, bypassing the requirement for a secreted ligand (Fig. 2C,D). Moreover, within endocytic tumors, reducing *Stat92E* activity significantly rescues the loss of epithelial polarity (Gilbert et al., 2009; Woodfield et al., 2013; Thomas and Strutt, 2014). These results suggest that the role of JAK/STAT signaling extends beyond simple regulation of proliferation and affects other cell behaviors such as adhesion. One potential effector of this latter function is the apical determinant Crumbs (Crb) (Gilbert et al., 2009). Crb deregulation is itself sufficient to induce neoplastic overgrowth (Lu and Bilder, 2005). While *crb* is a direct target of Stat92E in posterior spiracles (Lovegrove et al., 2006), it is yet to be resolved whether this relationship also exists in endocytic tumors or if *crb* deregulation

is a secondary effect of impaired vesicle recycling (Fig. 2D and (Gilbert et al., 2009; Thomas and Strutt, 2014)).

Oncogenic cooperation: the role of JAK/STAT signaling in polarity-deficient tumors

Many neoplastic tumor suppressor genes identified in genetic screens in *Drosophila* encode regulators of epithelial polarity (Bilder, 2004). In particular, *scribbled* (*scrib*) regulates septate junctions and maintains the separation between apical and basal membranes (Bilder and Perrimon, 2000). Loss of *scrib* in whole tissues leads to epithelial disorganization and tumor formation (Bilder et al., 2000), but clones mutant for *scrib* in proximity to wild type cells are eliminated by a process called cell competition (Brumby and Richardson, 2003; Igaki et al., 2006). However, in cooperation with another oncogene, such as an activated form of Ras called Ras^{V12}, the tumorigenic potential of *scrib* mutant cells is unleashed, and the mutant cells (referred to as Ras^{V12} *scrib*) metastasize (Brumby and Richardson, 2003; Pagliarini and Xu, 2003).

Ras^{V12} *scrib* mutant cells display high Jun N-terminal kinase (JNK) signaling, which can induce expression of all three *upd* genes, leading to systemic JAK/STAT pathway activation (Brumby and Richardson, 2003; Igaki et al., 2006; Pastor-Pareja et al., 2008; Wu et al., 2010). Activated Stat92E and Ras^{V12} then autonomously cooperate to cause massive overgrowth and metastasis (Fig. 2E and (Wu et al., 2010)). In a separate tumor model, Ras^{V12} combined with loss of a JAK/STAT inhibitor leads to metastatic tumor formation, confirming the carcinogenic cooperativity of the two pathways (Herranz et al., 2012). Indeed, preventing JNK activation in *scrib* mutant cells prevents both Stat92E activation and neoplastic transformation, and preventing Stat92E activation in Ras^{V12} *scrib* tumors suppresses both over-proliferation and metastasis (Wu et al., 2010). One model to explain these observations is that expression of Upd ligands in tissues that are damaged, either by direct injury or by cell death, is a mechanism for compensatory proliferation to restore tissue size (Wu et al., 2010). Another interesting aspect of the upregulation of Upd ligands in tumors is that it leads to the proliferation in circulating blood cells called hemocytes (Pastor-Pareja et al., 2008). These cells in turn adhere to tumors and reduce their growth, suggesting that JAK/STAT signaling also plays roles indirectly in altering the tumor microenvironment by affecting hemocyte numbers.

The JAK/STAT pathway as a major driver of hematopoietic tumors

The role of JAK/STAT signaling in melanotic tumors, a “fly leukemia” model

Decades before the discovery of oncogenic mutations in JAK/STAT signaling in myeloproliferative neoplasms, studies in *Drosophila* linked JAK/STAT signaling to lethal blood cell tumors referred to as fly leukemia (Corwin and Hanratty, 1976; Hanratty and Ryerse, 1981). Hemocytes are derived from an embryonic pool as well as from the larval lymph gland (reviewed in (Evans et al., 2003)). The anterior lobe of the lymph gland is subdivided into a niche called the posterior signaling center; a medullary zone where multipotent progenitors called pro-hemocytes reside; and a cortical zone where the pro-hemocytes differentiate into plasmatocytes, crystal cells and lamellocytes (Fig. 3A and (Jung et al., 2005)). Plasmatocytes make up 95% of circulating hemocytes and function as

professional phagocytes to remove bacteria and apoptotic cells. Crystal cells account for 5% of total hemocytes and are required for melanization of foreign tissue. Lamellocytes are absent or at very low levels in wild type animals but are rapidly induced by parasitic wasp infection, and they function to encapsulate objects too large to be phagocytosed (Sorrentino et al., 2002). Although the cell autonomous function of JAK/STAT signaling in maintenance of prohemocytes is debated (Krzemien et al., 2007; Minakhina and Steward, 2010; Mondal et al., 2011), there is agreement that Stat92E is required for plasmatocyte differentiation. This occurs at least in part through JAK/STAT pathway regulation of *pannier*, which encodes a GATA factor, and of *u-shaped*, which encodes a Friend of Gata factor (Fig. 3A,B and (Sorrentino et al., 2007; Gao et al., 2009; Minakhina et al., 2011)).

Tumorous-lethal (*Tum-l*) is a dominant, temperature-sensitive mutation in the *hop* locus that leads to overproliferation of hemocytes and formation of melanotic tumors (Fig. 3C,D). Melanotic tumors are black masses of hemocytes that - in the case of dysregulated JAK/STAT signaling - are invasive and correlated with lethality (Hanratty and Ryerse, 1981; Hanratty and Dearolf, 1993; Lanot et al., 2001; Minakhina and Steward, 2006). $hop^{Tum\text{-}l}$ is caused by a G341E substitution in the JAK homology 4 (JH4) domain (Fig. 1B). A second dominant mutation in *hop*, called *T42*, is functionally identical to *Tum-l* and is caused by a E695K substitution in the JH2 domain (Fig. 1B) (Luo et al., 1997). Both *hop* mutations result in a hyperactive kinase, which hyperphosphorylates Stat92E, leading to increased association of Stat92E with DNA (Fig. 3B and (Harrison et al., 1995; Luo et al., 1995; Luo et al., 1997)). Interestingly, the V617F mutation in Jak2 also results in a hyperactive kinase and this mutation resides in the Jak2 JH2 domain, which normally represses the function of the JH1 kinase domain (Ungureanu et al., 2011; Bandaranayake et al., 2012).

Several groups have shown that the $hop^{Tum\text{-}l}$ melanotic phenotype is due to hyperactivation of JAK/STAT signaling as heterozygosity of *Stat92E* suppresses the lethality and the tumorigenic phenotype associated with both $hop^{Tum\text{-}l}$ and hop^{T42} (Hou et al., 1996; Yan et al., 1996; Luo et al., 1997). In $hop^{Tum\text{-}l}$ animals, the number of plasmatocytes and lamellocytes in circulation is dramatically increased (Silvers and Hanratty, 1984; Luo et al., 1995; Lanot et al., 2001). In particular, the number of lamellocytes increases 30 fold compared to controls and comprises 40 to 70% of the total hemocyte pool. The tumors caused by the $hop^{Tum\text{-}l}$ lesion represent *bona fide* transplantable neoplasms. When $hop^{Tum\text{-}l}$ larval lymph glands are injected into wild-type adult flies, melanotic masses and invasive hemocytes are observed in various tissues (Hanratty and Ryerse, 1981; Luo et al., 1995). Interestingly, over-expression of wild-type Hop or $Hop^{Tum\text{-}l}$ in the lymph gland is sufficient to generate melanotic tumors (Harrison et al., 1995; Luo et al., 1995; Zettervall et al., 2004). Sustained Stat92E signaling is sufficient to induce melanotic tumors, as these lesions are observed in animals mutant for *dPIAS* as well as those mis-expressing a dominant-active Stat92E (Hari et al., 2001; Ekas et al., 2010). Furthermore, removing one copy of *dPIAS* enhances the $hop^{Tum\text{-}l}$ tumor incidence, while over-expression of *dPIAS* significantly suppresses it (Betz et al., 2001). Taken together, these studies strongly suggest that sustained activation of the JAK/STAT pathway in lymph gland derived-hemocytes is necessary and sufficient to generate melanotic tumors.

The role of pathway modulators and target genes in melanotic tumor formation

Through various screens, several genes have been identified as modulators or effectors of JAK/STAT signaling in tumorigenesis (Fig. 3B). RNAi screens revealed two factors that regulate Stat92E activity (Baeg et al., 2005; Muller et al., 2005). The $hop^{Tum\text{-}l}$ tumor index is suppressed by heterozygosity of *BRWD3*, which encodes a bromodomain and WD40 domain protein, or by mis-expression of Ptp61F, a protein tyrosine phosphatase. Ptp61F has been proposed to de-phosphorylate activated Stat92E dimers and BRWD3 may regulate access of Stat92E dimers to chromatin (Fig. 3B). Loss-of-function mutations in *Nurf301*, a large nucleosome remodeling factor (NURF) subunit, induce melanotic tumors, increase lamellocyte differentiation and enhance the $hop^{Tum\text{-}l}$ phenotype (Badenhorst et al., 2002; Kwon et al., 2008). Microarray analysis of *Nurf301*and $hop^{Tum\text{-}l}$ mutant larvae showed a large overlap of upregulated genes, suggesting that NURF normally represses JAK/STAT targets. The authors propose that this repression occurs by NURF recruitment to STAT binding sites via the transcriptional repressor Ken and Barbie (Ken), which had previously been shown to inhibit STAT targets through competitive binding to STAT responsive DNA elements (Fig. 3B and (Arbouzova et al., 2006; Kwon et al., 2008)). More recent work has revealed that Putzig (Pzg), a component of the TRF2/DREF replication complex, acts in concert with Nurf301 and Ken to repress JAK/STAT target genes (Fig. 3B and (Kugler et al., 2011)). In support of this model, loss of one copy of *Nurf301*, *ken* or *pzg* singly or in combination significantly increases the $hop^{Tum\text{-}l}$ tumor incidence (Kwon et al., 2008; Kugler et al., 2011).

A genetic screen for $hop^{Tum\text{-}l}$ modifiers revealed a link between JAK/STAT tumorigenesis and chromatin modifiers. Mutations in *Su(var)205* and *Su(var)3-9*, which encode Heterochromatin Protein 1 (HP1) and a histone methyltransferase, respectively, were identified as enhancers of $hop^{Tum\text{-}l}$ tumorigenicity (Shi et al., 2006). HP1 and Su(var)3-9 show reduced association with heterochromatin in $hop^{Tum\text{-}l}$ flies and increased association in animals heterozygous for a *hop* loss-of-function allele (Shi et al., 2006). Despite the established linear relationship from Hop to Stat92E, they were reported to regulate HP1 in opposite manners (Shi et al., 2008). These results have led to a model of “non-canonical” pathway activity in which unphosphorylated Stat92E is bound to heterochromatin with HP1. This association is disrupted with Stat92E phosphorylation by Hop leading to heterochromatin instability (Shi et al., 2008).

Finally, expression profiling has revealed potential JAK/STAT targets that promote tumorigenesis. *eukaryotic initiation factor 1A (eIF-1A)* mRNA, which encodes a component of the translation machinery, is significantly upregulated in $hop^{Tum\text{-}l}$ lymph glands, providing a potential link between increased JAK/STAT activity and growth, but its functional relevance is unclear (Myrick and Dearolf, 2000). The Stat92E target gene *chinmo* causes melanotic tumors when mis-expressed, but it is not yet known how Chinmo affects blood cells (Flaherty et al., 2010). Transcripts encoding a G protein subunit Gα73B (also called Gαf) are increased in cultured cells treated with Upd (Bina et al., 2010). Decreasing Gα73B suppresses $hop^{Tum\text{-}l}$ tumorigenesis, while Gα73B over-expression increases the tumor burden (Bausek and Zeidler, 2014). Gα73B likely mediates hemocyte motility and tumor invasion downstream of Stat92E.

Conclusions

Sustained JAK/STAT signaling accounts for the overgrowth and neoplastic appearance caused by loss of several distinct tumor suppressors, including PRC1, ESCRT and *scrib*. In most cases, mutation of the tumor suppressor results in ectopic expression of Upd. However, overgrowth of the eye disc is also observed with loss of *C-terminal Src kinase* (*Csk*) (Read et al., 2004), in which Stat92E protein is activated autonomously without upregulating the ligand Upd (Fig. 3C). Thus, sustained Stat92E activation is sufficient for tissue overgrowth. One emerging theme is that tumorigenesis involves co-operation between oncogenes, such Ras and STAT, in mammals and in flies (Wu et al., 2010; Corcoran et al., 2011; Herranz et al., 2012). Melanotic tumor phenotypes have also been observed with hyperactivation of Ras and Toll pathways (Minakhina and Steward, 2006). One outstanding question is whether the JAK/STAT, Ras, and Toll pathways act in parallel in this process or if there are cooperative interactions between them in melanotic tumor formation in *Drosophila*. Although the causal link between JAK/STAT activity and oncogenesis is clear both in flies and mammals, the relevant targets that mediate transformation still need to be identified. The lower complexity of the JAK/STAT pathway in *Drosophila* and the conservation of some genetic relationships (e.g. STAT and Ras) in tumorigenesis in flies and mammals, suggest that research in *Drosophila* will continue to yield fruitful avenues for understanding tumor formation.

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Highlights

!! JAK/STAT signaling is central to epithelial and hematopoietic tumors in *Drosophila*.

!! Unpaired is ectopically induced by loss of distinct tumor suppressors.

!! Ras and JAK/STAT pathways cooperate to induce metastatic tumors.

!! Dominant-active JAK mutations cause melanotic tumors, a fly leukemia.

!! Genetic screens have identified new pathway effectors in blood cell tumors.

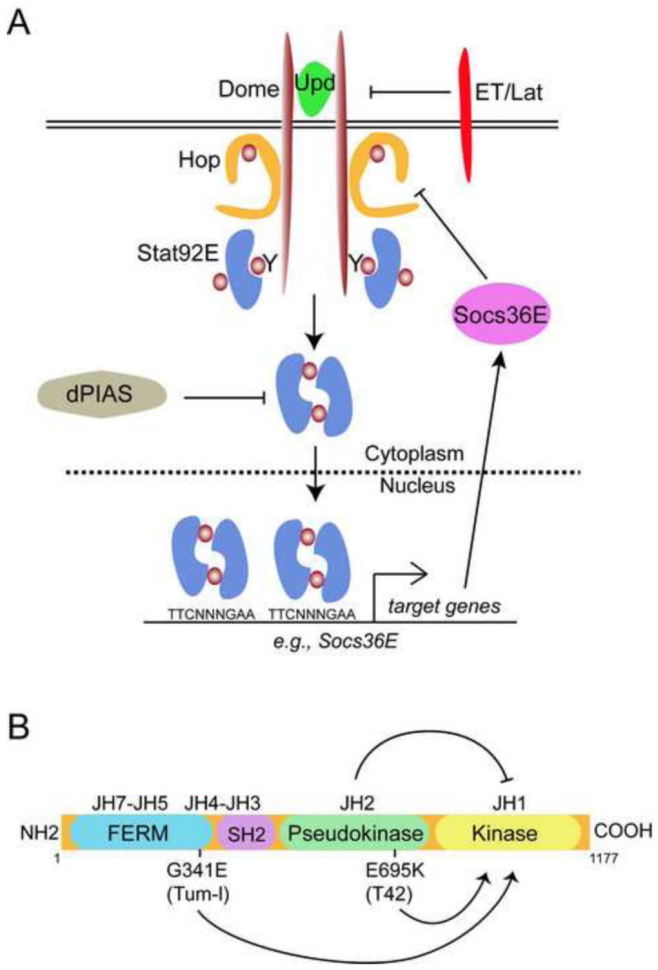

Fig. 1. The *Drosophila* JAK/STAT pathway

(A) The *Drosophila* JAK-STAT pathway consists of three Upd ligands collectively referred to as Upd (green). Upd activates the receptor Dome (brown), which results in activation of Hop (orange), leading to tyrosine phosphorylation (brown circles) on Dome. Stat92E dimers (blue) bind to the phosphorylated receptor. Once bound, Stat92E is phosphorylated on tyrosine 711, generating an active Stat92E dimer that undergoes nuclear translocation, where it binds to a consensus TTCNNNGAA site and alters gene expression. *Socs36E* is a Stat92E target gene that encodes a negative regulator (magenta) of Dome/JAK activity. A second receptor ET/Lat (red) inhibits JAK/STAT signaling. dPIAS (gray) inhibits activated Stat92E dimers.

(B) Domain structure of Hop. Hop contains a *bona fide* tyrosine kinase domain (JH1, yellow), a pseudokinase domain that lacks kinase activity (JH2, green), an atypical SH2 domain (magenta), and a FERM domain (blue) that mediates attachment to cytokine receptors. In the wild type Hop protein, the JH2 domain prevents the activation of the kinase domain, JH1 (top arrow). The E695K (T42) or G341E (Tum-l) mutations lead to activation (bottom arrows) of JH1.

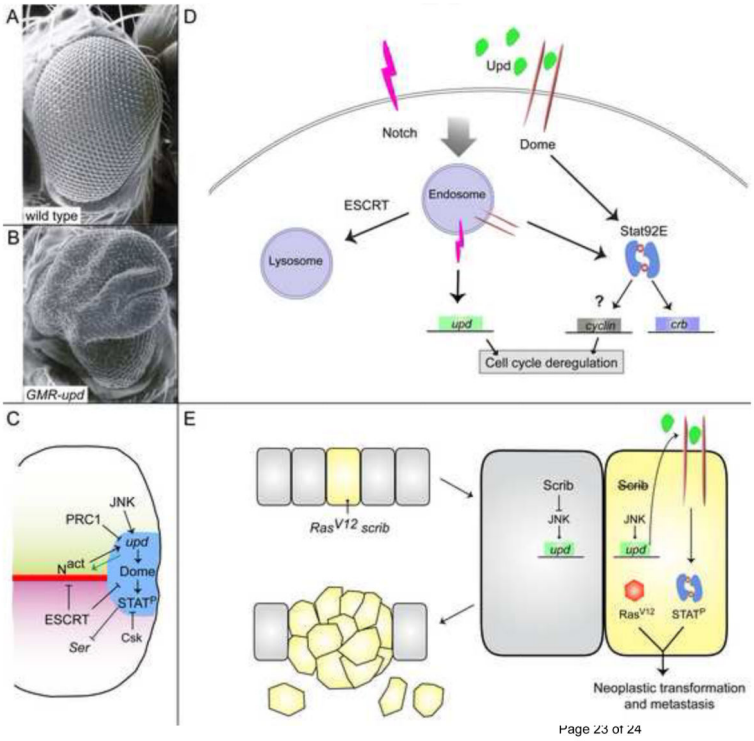

Fig. 2. JAK/STAT signaling in eye development and imaginal disc-derived tumors

(A,B) Scanning electron micrograph of a wild type (A) or *GMR-upd* (B) adult eye. Note the dramatic overgrowth in B.

(C) Model of regulation of JAK/STAT pathway signaling in the developing eye imaginal disc. Notch signaling (N^{act}) induces *upd* in cells at the posterior midline (blue area). Upd activates Dome and Stat92E (labelled $STAT^{P}$) in adjacent cells. PRC1 normally repress *upd* expression, while JNK signaling can induce it. ESCRT components normally restrict Notch and Dome activity, while Csk represses Stat92E activity. In addition, early in eye development, Upd can act upstream of Notch and induce Notch activity at the midline (green arrow). Activated Stat92E ($STAT^{P}$) also restricts Notch activity by repressing expression of *Ser*, which encodes a Notch ligand.

(D) Model of regulation of JAK/STAT pathway signaling by ESCRT components. Active Notch (magenta) and Dome (brown) receptors are trafficked into endosomes, where they can induce target genes like *upd* (green) and *crb* (blue), respectively. ESCRT factors promote the trafficking of Notch and Dome into the lysosome for degradation. In ESCRT mutants, Notch and Dome are trapped in an activated state in endosomes, where their unbridled activity causes cell cycle deregulation and transformation.

(E) Model of metastatic tumors caused by gain of Ras^{V12} and loss of *scrib* in the eye disc. In wild type epithelial cells (gray), Scrib repressed JNK activity. In a cell that has Ras^{V12} and lacks *scrib* (yellow), JNK signaling is now activated and *upd* genes are ectopically expressed (green). The ectopic Upd protein (green) leads to autocrine and paracrine (not depicted) activation of Dome (brown) and Stat92E (blue). The autonomous collaboration of Ras^{V12} and activated Stat92E causes neoplastic transformation and metastasis.

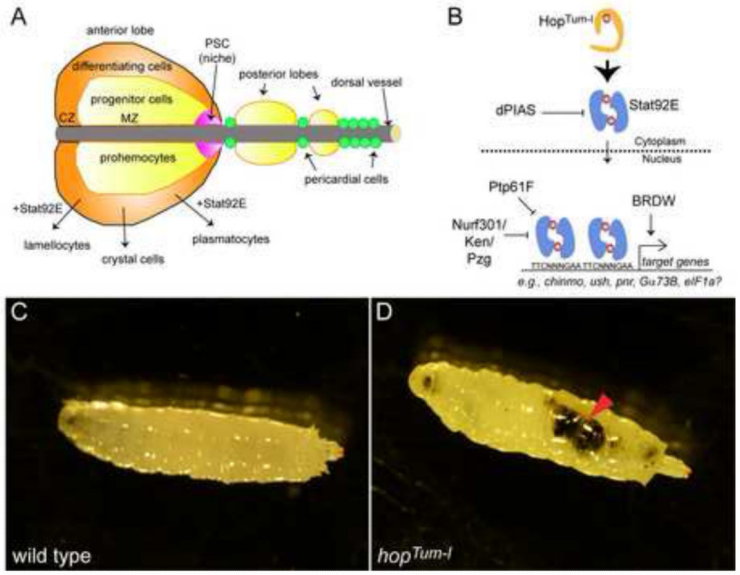

Fig. 3. JAK/STAT signaling in hematopoiesis and melanotic tumors

(A) The lymph gland is the larval hematopoietic organ. In the anterior lobe, there are three zones. Cells in the posterior signaling center (PSC, magenta) form the niche for multipotent progenitors called prohemocytes that reside in the medullary zone (MZ, yellow). Prohemocytes give rise to all *Drosophila* blood lineages, plasmatocytes, crystal cells and lamellocytes. Differentiation of hemocytes occurs in the cortical zone (CZ, orange).

(B) Model of Stat92E dimer activity (blue) and gene regulation downstream of $Hop^{Tum\text{-}l}$. See text for details.

(C,D) Micrograph of a wild type larva (C) or a $hop^{Tum\text{-}l}$ larva (D) reared at the restrictive temperature of 29°C. There is a large melanotic tumor in the abdomen of the $hop^{Tum\text{-}l}$ larva (D, arrowhead) but none in the wild type control (C).