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PRIME TIME FOR THE *DROSOPHILA* JAK/STAT PATHWAY

Erika A. Bach and Norbert Perrimon
Department of Pharmacology, New York University School of Medicine, 550 First Avenue, New York, NY 10016 USA; Department of Genetics, Howard Hughes Medical Institute, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115 USA

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1. COMPONENTS OF THE PATHWAY

The JAK/STAT pathway is an evolutionarily conserved phosphotyrosine driven pathway that has been identified in many metazoans. In *Drosophila*, as in mammals, this pathway is used in diverse cellular processes. Studies in *Drosophila* have identified a requirement for this pathway in processes as diverse as sex determination, embryonic segmentation, cell movement, planar polarity, hematopoiesis, cell differentiation and proliferation. Future studies in *Drosophila* have much to contribute to our overall understanding of the JAK/STAT pathway in metazoans. Many *Drosophila* paradigms are now available that will help in identifying the downstream targets regulated by this pathway, and how it interacts with

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others to regulate various biological functions. Furthermore, because of its simplicity, with only one JAK and STAT, studies of this pathway are easier in flies than in vertebrates. In this review we discuss the components, roles and future directions of studies on the *Drosophila* JAK/STAT pathway.

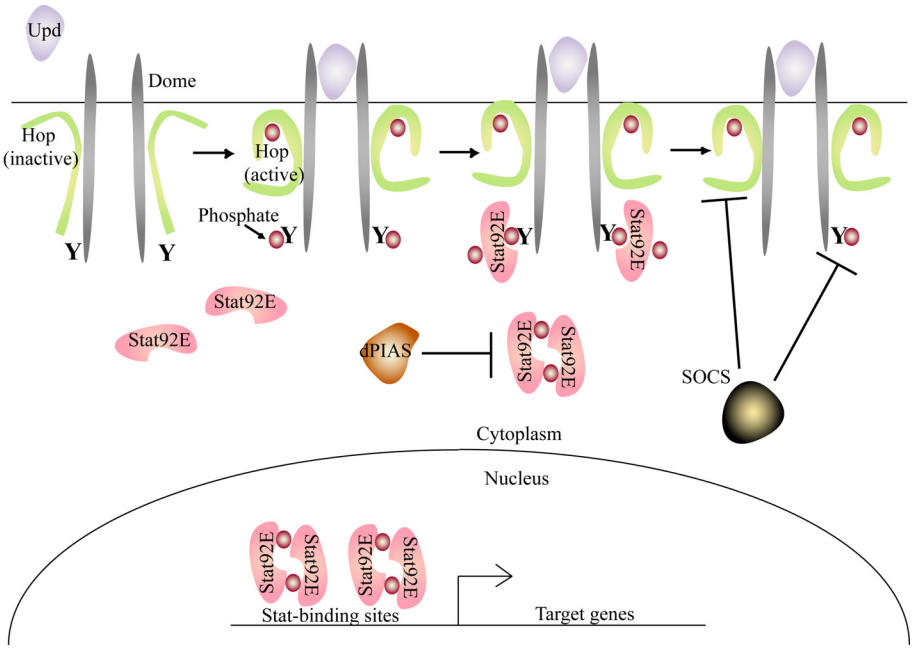

Figure 1. The *Drosophila* JAK/STAT pathway

The canonical *Drosophila* JAK/STAT pathway is identical to the pathway that has been defined by studies in mammalian cells. It is composed of the ligand Unpaired (Upd), the receptor Domeless (Dome), which has also been called Master of Marelle (MOM), the JAK kinase Hopscotch (Hop) and the STAT, Stat92E, also referred to as Marelle (Figure 1). The features of these proteins have been recently reviewed (1, 2).

The ligand of the pathway, Upd, is a novel protein that adheres tightly to the extracellular matrix in tissue culture experiments (Figure 1) (3). Upd can activate cells a few cell diameters away from the Upd source, indicating that Upd protein can diffuse at least a limited distance (4, 5). It is not yet known if Upd acts as a mono- or multimeric protein or if Upd requires post-translational modifications for full activity. Interestingly, there are two additional *upd*-like genes, clustered with the original *upd* gene on the X chromosome (1, 2, 6). A deletion that removes *upd* and the other *upd*-like genes has a stronger phenotype than the individual *upd* mutant, suggesting that the *upd*-like genes encode functional proteins. Future work will reveal the nature of these *upd*-like genes and what processes they regulate.

Dome encodes a type I protein with a single transmembrane pass and five fibronectin binding motifs (FBN) in its extracellular domain, the first two of which bear similar to the cytokine binding modules (CBM) of the interleukin-6 receptor family (Figure 1) (7). The first FBN contains conserved cysteine motifs and the second a partially conserved WSxWS domain, a functionally critical motif found in type I cytokine receptors. The cytoplasmic domain of Dome bears no homology to any known protein (7, 8). Sequence analysis has shown that Dome is distantly related to the gp130 family of cytokine receptors. Biochemical studies indicate that Dome binds to Upd and Stat92E and can activate the pathway *in vitro* (8). Interestingly, a search of the *Drosophila* genome database reveals the presence of a transcription unit CG14225 that is similar to *dome* and is adjacent to *dome* on the X chromosome (1, 2). CG14225 is most similar to gp130 and contains two CBM and a gp130 signature motif. In addition, the function of the *dome*-related gene is unknown as no mutations have yet been isolated in this gene.

Hop has a functional kinase, a pseudo-kinase, an SH2-like domain and a FERM domain, which has recently been reported to play an important role in transport of the erythropoietin receptor from the endoplasmic reticulum to the plasma membrane (Figure 1) (1, 2, 9, 10). Although no mutations in *hop* have been mapped to the FERM domain, two mutations in *hop* have been shown to result in a hyperactive kinase, the only gain of function mutations in any JAK. The $hop^{Tumorous\text{-}lethal\ (Tum\text{-}l)}$ mutation results in substitution of an amino acid (G341E) that is not conserved among JAK molecules (11, 12). In contrast, hop^{T42} results from substitution of a residue within the kinase-like domain (E695K) that is highly conserved among JAK molecules (11, 12).

Stat92E is similar to both Stat3 and Stat5 and contains most of the functional domains found in other STATs: a DNA-binding domain, an SH2 domain and a functionally critical C-terminal tyrosine residue (Figure 1) (13, 14). Alternative splicing of the *stat92E* mRNA gives rise to an N-terminally truncated protein that acts as a dominant negative Stat92E and inhibits activity of the pathway (15).

Regulators of the JAK/STAT pathway originally identified in mammals have also been found in *Drosophila*, specifically SOCS and PIAS proteins (Figure 1). In mammals PIAS proteins negatively regulate the JAK/STAT pathway by binding to activated STATs and inhibiting their ability to bind to DNA (16). In *Drosophila*, there is at least one PIAS homologue (17-19). Recombinant d-PIAS proteins have been shown to inhibit activated Stat92E. In addition, *d-PIAS* interacts genetically with the JAK/STAT pathway as over-expression of *d-PIAS* alone in the developing eye leads to a small-eye phenotype and exacerbates the small-eye phenotype observed in an adult viable mutation in the *upd* locus called *outstretched* (*os*) (19). Loss-of-function mutations in *d-PIAS* lead to chromosomal aberrations,

indicating either a new role for the JAK/STAT pathway in *Drosophila* or a new role for PIAS proteins independent of their function in the JAK/STAT pathway (18). In mammals, *SOCS* genes frequently are targets of activation of the JAK/STAT pathway. SOCS proteins negatively regulate the JAK/STAT pathway, generally by protein-protein inhibitory interactions with the activated JAK or receptor (20). In *Drosophila*, there are three *SOCS* genes, *SOCS16D*, *SOCS36E* and *SOCS44A*, however there are no reported mutations in any of them (1, 2, 21). Only the *SOCS36E* gene has been characterized. Its expression pattern closely resembles that of *upd*, and mutations in *upd* abolish endogenous expression of *SOCS36E*, indicating that *SOCS36E* expression depends on the activity of the JAK/STAT pathway (22, 23). In addition, the *SOCS36E* gene is induced by ectopic activation of this pathway (23).

2. ROLES OF THE JAK/STAT PATHWAY IN VIVO

2.1 Sex determination

In *Drosophila* gender is determined by the ratio of sex chromosomes to autosomes and is controlled by the *sex-lethal* (*sxl*) gene. *sxl* is initially expressed in females as a result of a balance between positively acting factors encoded on the X chromosome and a negatively acting factor encoded by the *deadpan* gene on an autosome (24). Therefore, in male embryos the reduced genetic dose of positively acting factors due to the presence of only one X chromosome is not sufficient to overcome the negative effects of *deadpan*. In contrast, in female embryos two X chromosomes provide sufficient amounts of positively acting factors that *sxl* expression is induced. One of these positively acting factors is *upd*, and mutations in *upd* (called *sisC*) have reduced *sxl* expression (25). Further experiments have indicated that the JAK/STAT pathway regulates *sxl* expression, however the mechanism of this regulation is not understood (25, 26).

2.2 Gametogenesis

In males and females, both germline and somatic tissues make important contributions to the development of gametes. The germline stem cells generate the spermatozoa or oocytes, which are supported by descendents of the somatic stem cells (27, 28). The germline stem cells are derived from pole cells that differentiate at the posterior end of the blastoderm embryo. During gastrulation the pole cells are invaginated by the posterior

midgut. Afterwards they migrate across the epithelium to join the mesodermal cells, which form the somatic tissue of the gonad. Several signal transduction pathways have been shown to be important for the interaction between the germline and somatic cells in their collaborative efforts to form a functional gonad, including *decapentaplegic* (*dpp*, a *Drosophila* TGFβ homologue) and, most recently, the JAK/STAT pathway (5, 29, 30).

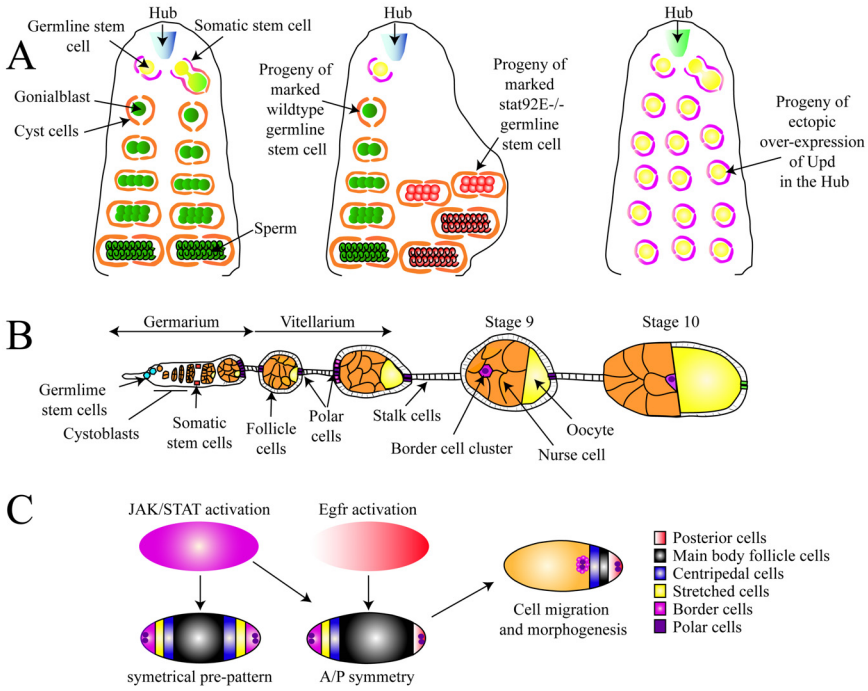

Figure 2. Roles of the JAK/STAT pathway in spermatogenesis (A) and oogenesis (B, C).

2.3 Spermatogenesis

In the testis, germline stem cells and somatic stem cells are found at the anterior tip in contact with a 16-cell structure called the hub (5, 30). Each germline stem cell is surrounded by two somatic stem cells (Figure 2A, left panel). When the germline stem cell divides, the daughter cell proximal to the hub retains stem cell qualities while the cell distal to it become a gonialblast and begins differentiation. The hub has been suggested to provide a niche for the maintenance of the stem cells since cell lineage experiments have shown that the stem cell always remains in contact with the hub while its descendents

are found progressively more distal to the hub. Mutations in *hop* result in male sterility (5, 30). Young testes from *hop* mutants contain normal numbers of primordial germ cells, however, older testes from these animals lack germline and somatic stem cells. Testes that are homozygous mutant for *stat92E* in the germline can make mature sperm, indicating that this pathway is not required for the viability of the male germline. However, stem cells and cells in early stages of spermatogenesis are not found in older testes from *stat92E* mutants, indicating that inactivity of the JAK/STAT pathway in testis results in the loss of self-renewal in stem cells (Figure 2A, center panel). Importantly, it has been recently demonstrated that *upd* is produced exclusively by the cells in the hub. Its highly specific localization suggests that it may be a factor required for the maintenance of stem cells. In fact, ectopic expression of *upd* in the hub cells results in the expansion of both germline and somatic stem cells at the expense of more differentiated cells (Figure 2A, right panel) (5, 30).

2.4 Oogenesis

Germline and somatic stem cells reside in an egg chamber called the germarium localized at the anterior end of the ovary (reviewed in (31)). Unlike the testis, stem cell renewal during oogenesis is mediated by *dpp*, not *upd* (29). In the germarium, a germline stem cell divides asymmetrically and gives rise to a cystoblast (Figure 2B). The cystoblast undergoes four more rounds of incomplete division to generate 16 cells that are connected and form a cyst. Within the cyst, one of the 16 cells becomes the oocyte and moves posteriorly. The remaining 15 become nurse cells and produce proteins and RNAs that are transported to the oocyte. The cysts are surrounded by a layer of follicle cells, which are derived from the somatic stem cells (Figure 2B). The follicle cells migrate in between the cysts and encapsulate them individually by an epithelial cell layer. After encapsulation, the follicle cells then separate from the germline-derived cysts through the formation of a stalk of six to eight cells (called stalk cells). The encapsulated cyst buds off the germarium and forms the first of a series of egg chambers (called the vitellarium) (Figure 2B).

The somatic follicle cells in the germarium can be separated into two populations: one that stops dividing in the germarium and begins to differentiate and the other that keeps dividing and maintains an immature state until later stages (32). The former are precursors of the polar cells and stalk cells. Two polar cells, found at the anterior and at the posterior end of the egg chamber, express specific genes and have inductive properties. Polar cells are required to organize the encapsulation of the cyst and formation of the stalk. Like polar cells, stalk cells are also localized to the anterior and posterior ends

of the egg chambers and function to connect older and younger egg chambers (Figure 2B).

Recent work has shown that the JAK/STAT pathway is required in the germarium for the development of polar cells and stalk cells (33). Mutations in JAK/STAT genes result in fused egg chambers in the vitellarium due to a lack of or deficit in stalk cells (33-36). *hop* mutant egg chambers also contain an expansion of polar cells. In the germarium, *upd* is expressed in the follicle cells that are the precursors of polar-stalk cells. Ectopic expression of *upd* in the germarium results in an expansion of stalk cells and a decrease in polar cells. Altogether, these data suggest that polar cells and stalk cells arise from a common precursor and that activation of the JAK/STAT pathway is required to promote stalk cell fate.

The JAK/STAT pathway is also involved in the differentiation of the most anterior fate in the follicle epithelium: the border cells (36-38). In the vitellarium, the specific expression of *upd* in the polar cells signals to a group of neighboring cells to differentiate into border cells (Figure 2B and C). A border cell cluster is composed of two centrally located polar cells surrounded by an average of 6-8 follicle cells. At stage 9 of oogenesis, the border cell cluster delaminates from the epithelium and migrates as a unit between the nurse cells towards the posterior side of the egg chamber. By stage 10, a few hours later, the border cells reach the oocyte, after which they migrate dorsally and eventually contribute to the development of the micropyle, through which sperm enter to fertilize the oocyte (Figure 2B and C). Thus, mutations that alter border cell differentiation and migration result in female sterility. In *upd*, *dome*, *hop* and *stat92E* mutants, the number of cells recruited to the border cell cluster is reduced and migration is aberrant or delayed (36-38). Conversely, ectopic expression of *upd* or *hop* leads to the formation at the anterior end of the egg chamber of additional and supernumary border cell clusters, which, interestingly, still have delayed migration (37). In order to identify the mechanism by which the JAK/STAT pathway regulates border cell migration, the expression of a very well characterized marker, *slow border cells* (*slbo*), was examined. *slbo*, a *Drosophila* C/EBP homologue, is specifically expressed in border cells and is required for the migration of the border cell cluster (39). Ectopic activation of the JAK/STAT pathway in border cells upregulated *slbo* expression (37). Conversely, expression of both *slbo* and its target genes were found reduced or absent in *stat92E* mutants (37). However, in a different *stat92E* mutant, *slbo* expression was maintained in border cells (38). Therefore, it is not clear whether the JAK/STAT pathway functions upstream and/or in parallel to *slbo*.

More recently, the JAK/STAT pathway has been shown to provide a graded level of activity that is highest at the termini, the source of Upd, and that different levels of activity influence different cell fates in the follicle epithelium (36). In addition to abnormal border cell fates, other anterior fates

are also altered in JAK/STAT mutants (Figure 2C). Homozygous mutant clones of *hop* in the follicle epithelium leads to expression of *mirror* (*mirr*), which marks the main body follicle cells. In contrast, in clones that ectopically express *hop* or *upd*, *mirr* expression is repressed. These data suggest that the level of activity of the JAK/STAT pathway regulates follicle cell differentiation. In JAK/STAT homozygous mutant clones, stretched and centripetal follicle cells fail to express specific markers and border cells abnormally express a marker of stretched cell identity. In addition, ectopic expression of *upd* or *hop* in cells that should become stretched or centripetal in wild type leads to abnormal morphology and gene expression in these cells.

2.5 Segmentation

The role of the JAK/STAT pathway in segmentation was one of its first described functions (13, 14). Embryos that develop in the absence of *upd*, *dome*, *hop* or *stat92E* gene products harbor a loss of abdominal segment 5 (A5) with partial loss of A4 and frequently loss of A6-8 (3, 7-9, 13, 14). The underlying reason for the specific loss of these segments has been explained at the level of the pair-rule genes. Proper segmentation of the embryo is established during development by several sets of temporally spaced genes: maternal, gap, pair-rule, and segment-polarity (21). In *upd*, *hop* and *stat92E* mutant embryos, the gap genes are expressed normally. However, there are defects in the expression of the pair-rule genes *even-skipped* (*eve*), *fushi tarazu* (*ftz*) and *runt* (*run*). In JAK/STAT mutant embryos, the fifth stripes of *eve*, *ftz*, and *run* are greatly reduced. Consistent with these observations, a 500 bp enhancer of the *eve* gene that controls expression of *eve* stripe 2, 3 and 7 contains two sequences that closely resemble the consensus Stat92E binding site (14). When these sequences are mutated, the expression of *eve* stripes 3 and 7 is abolished (13, 14). Activated Stat92E can bind to the endogenous sites in the *eve* promoter but not to the mutated ones. Taken together, these data indicate that the JAK/STAT pathway is required for expression of *eve* stripe 3.

Recently, it has been reported that mutations in *cyclin dependent kinase 4* (*cdk4*) have an embryonic segmentation phenotype that overlaps with those seen in JAK/STAT mutants (40). *cdk4* mutants also have a the loss of pair-rule gene expression observed JAK/STAT mutants. *cdk4* may act upstream of *stat92E* to activate it because ectopic expression of *cdk4* or *cyclinE* (*cycE*) in *hop* null embryos partially rescues the loss of denticle bands in abdominal segments. However, *cdk4* does not rescue *stat92E* null embryos (40). Cdk4 has been shown in mammalian cells to form a complex with Cyclin D (CycD) to promote phosphorylation of Retinoblastoma (41). Interestingly, in protein extracts from *Drosophila* cells and embryos, Stat92E

co-immunoprecipitates with both CycD-Cdk4 and CycE-Cdk2 complexes (40). This interaction apparently serves to stabilize Stat92E, as Stat92E protein expression is dramatically reduced in *cdk4* mutants. Conversely, ectopic expression of CycD-Cdk4 increased Stat92E protein expression. Using a reporter construct where canonical Stat92E binding sites were fused to a luciferase gene, it was demonstrated that co-expression of Hop, CycD-Cdk4 or CycE-Cdk2 with Stat92E increased Stat92E-dependent transcriptional output. Thus, Cdk4 helps to stabilize Stat92E, which in turn increases the transcriptional activity of Stat92E. It is important to note that the stabilization of Stat92E protein (i.e., increased and sustained protein expression) may positively regulate its activity in *Drosophila* tissues and cells. However, this has not been proven experimentally. Nonetheless, these experiments raise the interesting possibility that in the context of pattern formation Cdk4 acts through the regulation of Stat92E.

2.6 Tracheal development

The essential role of the JAK/STAT pathway in development of the tracheal system in *Drosophila* was initially revealed from studies of the *dome* gene (7, 8). In *Drosophila*, the trachea is a system of tubes that carry oxygen to tissues, which is connected to the external environment by posterior spiracles. *dome* was identified in a screen for mutations that affected the development of the posterior spiracle (7). An early marker of tracheal pits, *trachealess* (*trh*) is required for the expression of tracheal genes and is essential for tracheal development. In *dome* and *stat92E* mutants, *trh* is not expressed (7, 8). These results indicate that Stat92E is the earliest transcription factor required for trachea specification and may regulate *trh*. The specific role of the JAK/STAT pathway in formation of the tracheal system remains uncharacterized. However, the information available so far suggests that this pathway is involved in the specification of the tracheal placodes (7, 8).

2.7 Posterior hindgut formation

upd is expressed in a highly localized position at the anterior of the embryonic hindgut, suggesting that the JAK/STAT pathway plays a role in the development of this tissue (42). The elongation of this organ has been shown to occur largely through cell rearrangement and is independent of proliferation or apoptosis (reviewed in 43). Recent studies have shown that the JAK/STAT pathway is required for full elongation of the embryonic hindgut (42). In null *upd* mutants, the hindgut is patterned normally, but it is

only 40-50% as long as wild type and with a greater number of cells in the circumference. This same phenotype was observed for *hop* and *stat92E* mutants and animals expressing a dominant negative form of *dome* in the hindgut. Neither the decreased length nor increased cell number in circumference is due to a change in the overall number of cells in the hindgut when compared with wild type. This indicates that the JAK/STAT pathway is required for elongation of the hindgut. This is the first example of a required role for JAK/STAT signaling in orienting cell rearrangement that drives elongation of an epithelium. Since STAT activation has been demonstrated to alter transcriptional responses, it is intriguing to think about how a graded level of JAK/STAT activation and STAT protein could facilitate cell rearrangements. This question is particularly pertinent because STAT is required for other types of cell movement/rearrangements, such as border cell migration in *Drosophila* and convergent extention in zebrafish (44). This also raises the question of whether STAT regulates the expression of cytoskeletal proteins that control adhesion.

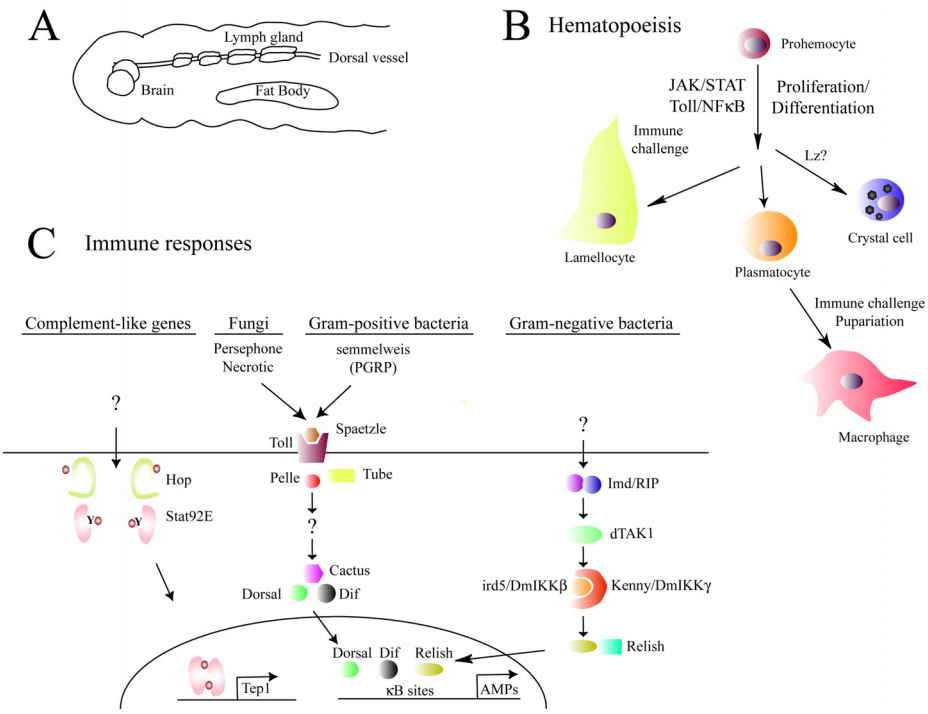

Figure 3. Roles of the JAK/STAT pathway during hematopoiesis and innate immunity

2.8 Blood cell development

In embryogenesis, prohemocytes, the precursor of hematopoietic cells termed hemocytes, migrate throughout the embryo and differentiate into two major cell types: the plasmatocyte and the crystal cell (45, 46). Plasmatocytes differentiate into macrophages and remodel tissue and phagocytose dying cells (47). The function of embryonic crystal cells, named for their cytoplasmic crystalline inclusions, is not known. Mice and humans deficient in certain JAK or STAT genes exhibit hematopoietic defects. This suggests a role for the JAK/STAT pathway in the proliferation, differentiation or functional activity of embryonic hemocytes in *Drosophila* (48). However, additional studies are needed to assess this.

In larval, pupal and adult stages, the blood cells not only perform phagocytic functions but are also involved in immune responses (Figure 3A). During larval stages, the primary organ of hematopoiesis is the lymph gland, a multi-lobed organ that is associated with the dorsal vessel. Little is known about the ontogeny of larval hemocytes. However, a tentative cell lineage has been proposed based on analyses of phenotypic characteristics of hemocytes and markers (49). The prohemocytes self-renew in the lymph gland and differentiate into specific types of hemocytes, which are then released into the hemolymph, the *Drosophila* circulatory system that is open and surrounds all internal organs (Figure 3A and B). Though rudimentary, the fly circulatory system is similar to the mammalian one as it carries materials throughout the insect. Larval plasmatocytes respond to pupariation, injury or immune challenge by becoming activated and increasing in number. Lamellocytes, the largest cells in the hemolymph, encapsulate objects too large to be phagocytosed by forming multi-cell layers around the object or pathogen. The encapsulated object is then melanized by the third major type of larval hemocyte, the crystal cell.

$hop^{Tum\text{-}l}$ and hop^{T42}, both temperature-sensitive, dominant, gain-of-function mutations, exhibit over-proliferation and precocious differentiation of hemocytes (11, 12). The increased numbers of plasmatocytes and abundant lamellocytes aggregate into masses that often become melanized, called "melanotic tumors". A "melanotic tumor" appears as a black mass within the body cavity of the fly. $hop^{Tum\text{-}l}$ lymph glands give rise to increased numbers of hemocytes and melanotic tumors when transplanted into wild type hosts (11, 12). In addition, mis-expression of the hop^{+} cDNA in the larval lymph gland also causes tumors. These data support the hypothesis that the *hop* gain-of-function mutations disrupt the signal transduction pathways that control proliferation and differentiation in hematopoietic cells. However, reducing the dose of the *stat92E* gene product can suppress the incidence of precocious differentiation but not the over-proliferation of hemocytes in $hop^{Tum\text{-}l}$ flies (11,

12). Suppression of the hyperplastic phenotype may require a greater reduction in the amount of *stat92E*.

Although the *hop* gain-of-function mutations have been well characterized, our knowledge of the function of the JAK/STAT pathway in larval hematopoiesis is limited. We do not know whether the hemolymph of *hop* or *stat92E* homozygous mutant larvae contains fewer hemocytes. We also do not know if Upd or an Upd-like molecule activates the JAK/STAT pathway during hematopoiesis and through what receptor (48). Lastly, only two target genes of this pathway have been identified in blood cells, *D-eIF1A*, the *Drosophila* homologue of a eukaryotic initiation factor and, as described below, *thiolester-containing protein 1* (*tep1*) (50, 51).

2.9 Immunity

The immune system in *Drosophila* is highly homologous to the innate immune system in mammals (reviewed in 52). Immune responses in *Drosophila* generally trigger one or more of three major lines of defense: 1) proteolytic cascades; 2) humoral responses; and 3) cellular responses (Figure 3C). The rapid induction of proteolytic cascades results in melanization, coagulation, and production of signaling molecules. In the complement cascade foreign organisms trigger the proteolysis of thiolester-containing C3 protein, thus mediating inflammatory reactions, opsonization of microorganisms, and direct killing of some pathogens. In *Drosophila*, there appears to be a primitive complement-like cascade that is involved in host defense. Four genes called *tep1–4* exhibit significant homology to the complement C3- α2-macroglobulin superfamily (51). *tep1* is expressed in the fat body (the *Drosophila* analog of the mammalian liver) and in other cells, including hemocytes. Expression of *tep1* is constitutive in $hop^{Tum\text{-}l}$ gain-of-function mutants and is substantially reduced in hypomorphic hop^{M38} mutants. The increased expression of *tep1* is concomitant with the overproliferation, premature differentiation and development of melanotic tumors seen in $hop^{Tum\text{-}l}$ animals. However, it is currently not known whether *tep1* expression is causally related to the $hop^{Tum\text{-}l}$ blood cell phenotype.

In the humoral response, the fat body and the hemocytes rapidly produce substantial quantities of antimicrobial peptides that are released directly into the circulatory system (Figure 3C). The *Drosophila* NFκB/Rel proteins are activated in response to infection and presumably induce transcription of antimicrobial genes, as the promoter region of all of the antimicrobial genes examined to date contain κB sites. Currently, it is not known whether the JAK/STAT pathway is involved in the humoral response (48). However, in another insect species, *Anopheles gambiae*, a STAT protein, Ag-STAT, is activated quickly (30-45 minutes) in response to

bacterial infection (53). What activates Ag-STAT or whether its activation depends upon prior gene induction by the NFκB/Rel pathway is not yet clear. In mammalian immune responses, the JAK/STAT pathway is generally activated sequentially after the NFκB/Rel pathway. Therefore, it is likely that a similar paradigm will exist in immune responses in insects.

In the cellular response, hemocytes expand, differentiate and participate in phagocytosis and encapsulation. Although it is not known whether the JAK/STAT pathway plays a role in the cellular response, this is probable because many interleukins and cytokines that activate the JAK/STAT pathway in mammals play critical roles in the mammalian counterpart of the insect cellular immune response.

2.10 Eye development

The adult compound eye consists of approximately 800 repeating units called ommatidia that contain eight photoreceptors and their support cells (reviewed in 54). The photoreceptors form stereotypic opened-ended trapezoids. Two mirror image forms of this trapezoid exist in an adult eye, a dorsal form that is present in ommatidial clusters dorsal to the dorsoventral (DV) midline called the equator, and a ventral form that is present ventrally. During third instar larval development, differentiation of ommatidial clusters occurs when the morphogenetic furrow, a morphologically distinct indentation that runs from the dorsal to ventral pole of the eye disc, sweeps across the eye from posterior to anterior. After the eye precursors are released from the morphogenetic furrow, the ommatidia begin to develop as preclusters and additional cells are recruited to the growing clusters. Thus, the movement of the furrow from posterior to anterior leads to increasingly more mature ommatidia in successive rows. The clusters become polarized on the DV axis, as the developing ommatidia rotate 90º towards the equator (21). In wild type the rotation of ommatidial clusters occurs with amazing regularity, and thus mutants that effect ommatidial rotation can be easily scored. The direction of this rotation is hypothesized to be due to a gradient of positional information that emanates from the midline and/or the poles. Interestingly, *hop* loss-of-function mutant clones cause inversion of the polarity of ommatidia, indicating that the JAK/STAT pathway is involved in ommatidial rotation (4, 55).

The JAK/STAT pathway is also involved in growth of the eye disc. The most compelling data come from analyses of loss-of-function mutations in genes in the pathway. The eye primordium begins as a sac of 20-50 epithelial cells at the end of embryogenesis. By the beginning of third instar, the eye disc contains several thousand cells and thus several rounds of mitosis have occurred (reviewed in 56). Transheterozygous combinations of *hop* loss-

of-function mutations result frequently in a small-eye phenotype with fewer ommatidia, irregular arrays and duplicated bristles (55). Occasionally, the eye in the transheterozygotes is completely abolished with a concomitant duplication of the antenna. Similarly, *os* flies have small eyes with fewer ommatidia but, unlike *hop* mutants, do not have irregular ommatidial arrays or duplicated bristles (57). The molecular lesion that causes this phenotype is not definitively known, but it is likely to be in the regulatory region of the *upd* locus because *os* is a viable mutation and a null allele of *upd* (*yc43*) is embryonic lethal (3, 4, 25). The small-eye phenotype in *os* flies can be fully rescued by ectopic misexpression of *upd* (57).

Additional evidence comes from ectopic mis-expression of *upd* in the developing eye (8, 40, 57). *GMR-upd* transgenic animals that over-express *upd* in the eye have dramatically enlarged eyes with prominent dorsal outgrowths (57). The enlarged eye phenotype occurs as a result of more mitoses in the precursor eye cells within the developing eye (57). How is the enlarged-eye phenotype generated? As a soluble protein that associates with the extra-cellular matrix, Upd presumably can diffuse away from the Upd-producing cells. Interestingly, *dome* mRNA is dramatically upregulated in the undifferentiated cells anterior to the furrow in *GMR-upd* discs (57). In contrast, its expression is nearly undetectable in wild type third instar eye discs. Taken together with other data, *dome* appears to be a target of activation of the JAK/STAT pathway (8, 35, 57). Therefore, the Dome-expressing cells anterior to the furrow respond to the diffusing Upd produced posterior to the furrow and may undergo additional rounds of mitosis (57). Consistent with this, many more precursor eye cells in the anterior part of the eye disc in *GMR-upd* animals are in mitosis relative to wild type. In addition, cell cycle analysis by flow cytometry indicates that more cells anterior to the furrow in *GMR-upd* cells discs are in the G2/M phase of the cell cycle relative to the same population in wild type (57). As discussed above, a recent report indicates that *cdk4* functions downstream of *hop* in the several tissues in *Drosophila*, including the eye (40). Co-expression of *cdk-4* and *cycD* with *upd* in the eye using the *GMR* promoter promotes synergistic outgrowths of the eye (40). However, it has been previously reported that ectopic mis-expression of *cdk4* and *cycD* in post-mitotic photoreceptors in the eye using the GMR promoter produces an overgrowth phenotype that is due to a dramatic increase in cell volume and not to an increase in cell number (58, Figure 3). Therefore, the synergistic effects seen by overexpressing *upd*, *cdk4* and *cycD* together may reflect the ability of *upd* to control the number of mitoses in cells anterior to the furrow and of *cycD-cdk4* to give rise to increased cell volumes behind the furrow. Future experiments will be necessary to confirm whether the interaction documented between Stat92E and CycD-Cdk4 in the embryo also holds true for the eye.

2.11 Chromatin remodeling and transcription

The importance of mammalian STAT proteins in promoting transcription has been recently reviewed (59). In contrast to the larger number of STAT-induced genes identified in mammalian cells, there are relatively few genes in *Drosophila* that have been demonstrated to be regulated by the JAK/STAT pathway. However, the number is steadily growing. In addition to *dome*, *SOCS36E*, *D-eIF1A*, *tep-1*, *eve* stripe 3 enhancer, one other Stat92E target gene is *raf* (60). Other potential target genes include *trh*, *slbo* and *RNrS* (7, 37, 40). It will be important to establish *in vivo* Stat92E reporters for all stages of *Drosophila* development in order to accurately assess activation of the pathway.

In mammalian systems, the C-terminal transactivation domain of all STATs has been shown to interact with a histone acetyl transferase (HAT), in particular p300/CBP (CREB-binding protein) (59). HATs acetylate lysines on the tails of histones, and acetylation has been shown to be required for the opening of chromatin from the nucleosome and hence a positive regulator of transcription. Although we do not know if Stat92E associates with HATs, recently, a protein involved in nucleosome remodeling, NURF301, has been shown to interact genetically with the JAK/STAT pathway (61). Interestingly, almost 100% of homozygous *nurf301* mutants have melanotic tumors and dramatically increased numbers of circulating hemocytes, a phenotype reminiscent of $hop^{Tum\text{-}l}$ flies. Reduction of *nurf301* levels in $hop^{Tum\text{-}l}$ flies dramatically increased the incidence of tumors in $hop^{Tum\text{-}l}$ flies but not in an activated *Toll* mutant background (61). In addition, *tep1*, which is constitutively expressed in $hop^{Tum\text{-}l}$ mutants, is also induced in *nurf301* mutants. Lastly, the held-out wing phenotype in *os* is restored to wild type when the dose of *nurf301* is reduced in *os* flies (61). These data very strongly indicate that NURF301 acts as a negative regulator of JAK/STAT pathway.

In a genetic screen to identify mutations that dominantly modified the enlarged-eye phenotype in *GMR-upd* animals, we identified several mutations in genes known to be involved in chromatin structure and in transcription: *pineapple eye* (*pie)*, *Dichaete* (*D*), *Histone 2A variant* (*His2Av)*, *kohtalo (kto)*, and *plexus (px)* (57). Pie is a nuclear protein that is thought to facilitate chromatin-mediated transcriptional regulation. D is a high mobility group protein and a member of a family of relatively low molecular weight non-histone components in chromatin. *His2Av* belongs to the H2AZ variant subclass, which is involved in chromatin stability, chromatin remodeling and transcriptional control. Since STATs have been shown to mediate transcriptional changes within seconds or minutes of activation, histone modification may need to be coordinated with transcriptional co-activation. Kto is the homologue of thyroid-hormone receptor associated protein

(TRAP230), which was originally identified as part of the *trithorax* group, a large transcriptional co-activation complex. Px is a nuclear protein that is associated with the nuclear lamin. Future work will elucidate any biochemical interaction between Stat92E and these transcription/nuclear factors and also whether they regulate the transcription of a common set of genes.

3. CONCLUSIONS

In this review we have summarized the various roles of the JAK/STAT pathway that have emerged from studies in *Drosophila*. First, although this has been observed for other signaling pathways, the evolutionary conservation of this pathway is remarkable. Thus, further analyses of this pathway in flies using genetic methods such as enhancer/suppressor screens will without a doubt lead to the characterization of new components of this pathway. Second, the picture that emerges from the *Drosophila* studies is that this pathway is involved in a multitude of developmental events. This is in contrast to studies in mammals where this pathway has been mostly studied for its function during hematopoiesis and immunity. Thus, studies of the JAK/STAT pathway in *Drosophila* nicely complement the vertebrate studies and future findings promise to be quite exciting.

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