The JAK–STAT pathway in *Drosophila*

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Recent studies in Drosophila have identified a single JAK and a single STAT protein. Genetic and biochemical analyses reveal that these two proteins operate in the same signal transduction pathway. Phenotypic analyses of JAK and STAT mutants implicate this pathway in a number of developmental decisions, in particular the regulation of pair-rule genes and fly hematopoiesis.

The janus kinase-signal transducer and activator of transcription (JAK–STAT) pathway is an elegant example of how protein signals, outside a cell, trigger a quick response in the nucleus and induce changes in gene expression. This pathway was identified through studies of the transcriptional activation response to a variety of cytokines and growth factors in mammalian cells (reviewed in Refs 1–3). Extensive studies in mammalian cells have led to the model whereby cytoplasmic JAK proteins, which are bound constitutively to the membrane-proximal domain of cytokine receptors, become activated when the receptor homodimerizes in response to cytokine binding. Dimerization of the receptor brings the receptor-associated JAK proteins into apposition, enabling them to *trans*-phosphorylate and, thereby, activate each other. The activated JAK proteins phosphorylate a distal tyrosine residue on the receptor, which is subsequently recognized by the SH2 domain present in the STAT proteins. STAT proteins recruited to the receptor complex then become activated by JAKs through phosphorylation on a tyrosine residue. Activated STAT proteins are competent for homo- or heterodimerization and nuclear translocation, where they subsequently activate gene transcription. This pathway is strikingly different from other signaling mechanisms, such as the RAS–RAF–MEK–MAPK pathway, which uses a long cytoplasmic cascade of protein intermediates to carry a signal to the nucleus. However, it is reminiscent of the fast transcriptional response induced, for example, by steroid hormones and perhaps TGF-β (Ref. 4).

Recent studies in *Drosophila* have provided a paradigm to dissect this pathway genetically. Intracellular signaling pathways employed in this organism are surprisingly similar to those of vertebrates, making it a model system of choice to identify components of signaling pathways genetically. For example, studies on *Drosophila* receptor tyrosine kinases (RTKs) have contributed greatly to our current understanding of the regulation of the RAS–RAF–MEK–MAPK signaling cascade (reviewed in Refs 5, 6). Genetic analysis of RTK signaling has not only helped to order the chain of events triggered by the activated receptor, but has also led to the identification of novel molecules that transduce the signal. In this review, our current understanding of the signaling mechanism and the function of the *Drosophila* JAK–STAT pathway is described.

Characterization of *Drosophila* JAK and STAT

Recently, a *Drosophila* JAK kinase encoded by the gene *hopscotch* (*hop*; Ref. 7), and a STAT protein, encoded by the gene *marelle* also known as *Dstat* or *stat92E* (Refs 8, 9), have been characterized. The agreed name for the *stat* gene is now *stat92E* (M. Ashburner, pers. commun.) and we use this nomenclature in the text below. Mutations in the *stat92E* gene were isolated independently in two studies, as part of a screen for suppressors of the gain-of-function *bcp* mutation *bcp*$^{Tum-l}$ (Refs 9, 10), and in a screen to analyze the maternal effects of zygotic lethal mutations[11].

hop encodes a protein of 1177 amino acids that resembles members of the mammalian JAK family of proteins, which consists of the nonreceptor tyrosine kinases JAK1, JAK2, JAK3 and TYK2 (Ref. 7). These proteins, in addition to a canonical tyrosine kinase domain, contain a second kinase-related domain[12] (Fig. 1). Across the length of the entire protein, HOP shows the highest degree of identity to JAK2 (27%). The tyrosine kinase and kinase-like domains of HOP are 39% and 24% identical to those of JAK2, respectively (Ref. 7).

stat92E encodes a protein of 761 amino acids that resembles members of the STAT family of proteins[1,8,9] (Fig. 1). STAT proteins contain an SRC homology 2 (SH2) domain and a DNA-binding domain. All known members of the STAT family have a single tyrosine residue in the region of residue 700 that becomes phosphorylated during cytoplasmic activation. Accordingly, Tyr711 in STAT92E has been shown to be phosphorylated (Ref. 9). Also in STAT proteins, between amino acid 500 and 600, there is a distinct, probably meaningful, similarity to SRC homology 3 (SH3) domains. The overall identity between STAT92E and STAT5 is 37%. The SH3 domain found in some STAT proteins is less clear in STAT92E, and a putative MAPK phosphorylation site present in some STAT family members (STAT1α, STAT3 and STAT4)[13] is not present in the STAT92E sequence.

Mutations in *hop* and *stat92E* are associated with zygotic lethality, causing death of mutant animals during larval stages (see below). In addition, they exhibit strikingly similar maternal-effect lethal phenotypes that can be identified by examining the phenotypes of embryos derived from females carrying homozygous mutant

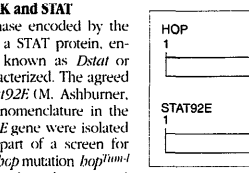

FIGURE 1. Domain structure of HOP and STAT92E. Key: kinase, the canonical kinase domain of HOP; kinase-like, the second kinase-like domain of HOP; DB, DNA-binding domain of STAT92E; SH2, the SRC homology 2 (SH2)-like domain of STAT92E; Y, the tyrosine phosphorylation site at the position of amino acid 711 of STAT92E.

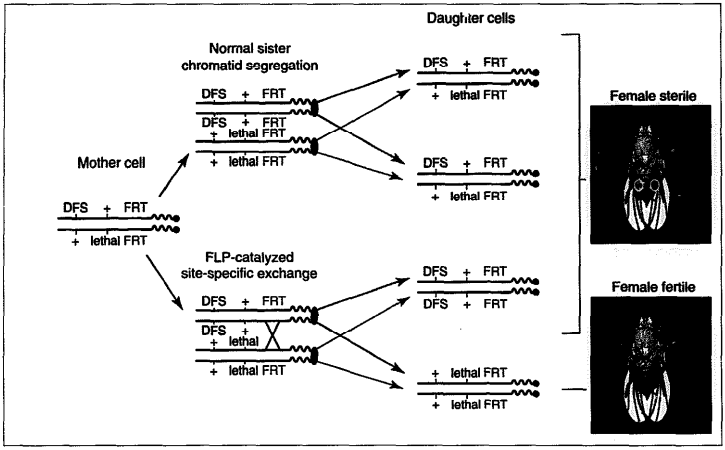

FIGURE 2. The 'FLP–DFS' technique (Refs 33, 34). A chromosomal exchange that occurs in the euchromatin of a fly of genotype *DFS + FRT/+ lethal FRT:FLP/+* is shown. The *FRT* insertion is located proximally to both *DFS* and *lethal*. *hsp70-FLP* can provide recombinase activity following heat shock induction to catalyze site-specific chromosomal exchange at the position of the *FRT* sequences. *FLP*-catalyzed recombination can result in the recovery of almost 100% of females with *lethal/lethal* germline clones. Key: atrophic ovaries are shown as yellow empty ovals, and developed ovaries as red filled ovals; *FLP*-recombinase target sequences (*FRT*) are shown as red boxes; *DFS*, dominant female sterile; *lethal*, recessive zygotic lethal mutation. (Courtesy of Elizabeth Noll.)

germ-line clones[7,8,14]. Loss-of-function mutations in these genes were isolated in large screens designed to analyze the maternal effects of zygotic lethal mutations[11,15], using the technique of germ-line mosaics (Fig. 2). In addition, a *stat92E* mutation was isolated in a screen for suppressor of the *hop*$^{Tum\text{-}l}$ gain-of-function mutation[9]. In the complete absence of maternal and zygotic *hop* or *stat92E* activity, embryos exhibit severe segmentation defects. In the abdominal region, these defects include the deletion of the fifth abdominal segment, as well as variable deletion of the fourth and eighth abdominal segments and the fusion of abdominal segments 6 and 7 (Refs 8, 14). If *hop* or *stat92E* embryos have received a copy of the corresponding wild-type gene from the father, the defects are less severe and are usually restricted to a deletion of the fifth abdominal segment and the posterior mid-ventral portion of the fourth abdominal segment (Fig. 3). Interestingly, most progeny derived from females carrying homozygous germline clones of the weak *hop* allele (*hop*^{msvl}) are associated with subtle defects in A5. These animals can, at a low frequency, give rise to adults that also exhibit segmentation defects in the corresponding region (Ref. 14).

The similarity of phenotypes associated with loss of either *hop* or *stat92E* gene activity suggests that both genes encode components of the same signaling pathway. Additional evidence that *hop* and *stat92E* are functionally related was obtained from genetic interaction studies. A reduction in the amount of *stat92E* gene activity enhances the embryonic phenotypes associated with a weak *hop* mutation, supporting the model that STAT92E is a member of the HOP signal transduction pathway[8]. Also consistent with these results, mutations in *stat92E* suppress the phenotype associated with the dominant *hop*$^{Tum\text{-}l}$ mutation (see below)[8,10].

The JAK–STAT pathway regulates the expression of *even skipped*

Genetic analysis of the mechanisms underlying pattern formation of the *Drosophila* embryo has led to a comprehensive view of the various steps involved in the establishment of the body plan (reviewed in Refs 16, 17). These analyses demonstrate that the egg contains spatial cues that are deposited during oogenesis. Following fertilization, these maternal cues regulate and coordinate the expression of a small number of genes (gap, pair-rule and segment-polarity genes), which are further involved in controlling subsequent steps of body patterning.

Characterization of the expression pattern of segmentation genes in *hop* and *stat92E* mutant embryos reveals that the *hop* and *stat92E* embryonic mutant phenotypes can be explained by defects in the expression of pair-rule genes, such as *runt* (*run*) and *even-skipped* (*eve*) (Refs 7, 8). The expression of pair-rule genes is under the control of the previously expressed zygotic gap genes, whose expression is not perturbed in the absence of maternal *hop* or *stat92E* gene activities.

To characterize further the effect of the HOP–STAT92E pathway on pair-rule gene expression, the regulation of *eve* was analyzed in detail in *hop* and *stat92E* mutant backgrounds. The regulatory elements of the *eve* promoter have been extensively characterized. In particular, a reporter gene containing a 500 bp fragment of the *eve* promoter has been shown to control the expression of *eve* stripe 3 (Ref. 18) as well as, more weakly, stripe 7 expression. The expression of the chimeric *lacZ* reporter gene driven by the 500 bp enhancer is completely abolished when introduced into *hop* or *stat92E* null mutant embryos. This indicates that the activity of the HOP–STAT92E pathway is essential for activation of this *eve* enhancer and that the 500 bp fragment of the *eve* promoter must contain at least one HOP and STAT92E regulatory response element[8,9]. In this fragment, two sequences (TTCCCCGAA and TTCCGCGAA) that closely match the mammalian STAT-binding site[8,9] are present. Yan *et al.*[9] demonstrated that STAT92E, activated by phosphorylation of a single tyrosine residue by HOP in cultured Schneider cells, binds to these two sites in the 500 bp *eve* stripe 3 enhancer. When these sites are mutated, the 500 bp fragment does not drive expression of *eve* stripe 3 any longer[9]. These results strongly suggest that HOP and STAT92E encode components of the same regulatory mechanism.

Although the stripe 3 expression of the chimeric *lacZ* reporter gene driven by the 500 bp *eve* enhancer is completely abolished when introduced into *hop* or *stat92E* null mutant embryos, endogenous *eve* stripe 3 expression is not completely eliminated in *hop* or *stat92E* null embryos. This observation suggests that at least one additional activator (refer to product X in Fig. 4) operates outside of the 500 bp *eve* enhancer. This additional factor(s) cooperates with STAT92E to activate stripe 3 expression[8,9]. Further, mutating both STAT92E-binding sites in the 500 bp *eve* enhancer abolishes stripes 3 and 7 expression, but stripe 7 expression is not affected in *hop* and *stat92E* null embryos, suggesting the presence of at least one other activator acting in the 500 bp enhancer for stripe 7 expression[8,9]. Cooperativity in a number of positive regulator mechanisms might be required to provide an appropriate level of expression of *eve* in certain stripes. Thus, the function of the HOP–STAT92E pathway during embryonic segmentation might simply be to upregulate the expression of *eve* (and other segmentation genes) in specific stripes. According to this model, the level of activation provided by the HOP–STAT92E system will depend on the number of STAT-binding sites present in the stripe-specific enhancer regions.

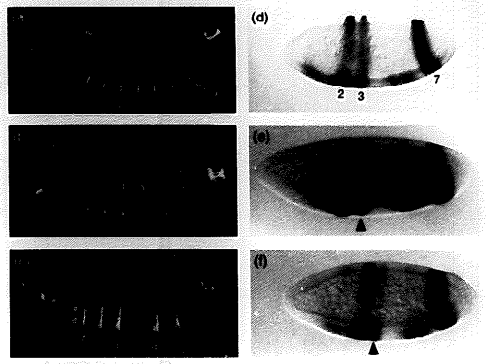

FIGURE 3. Embryonic phenotypes of *hop* and *stat92E* mutant embryos. Dark-field photographs of the embryonic cuticular elements in a wild-type (a), *hop* (b), and *stat92E* (c) are shown. A3 and A6 indicate the position of the abdominal segments 3 and 6, respectively. Note that abdominal segment 5, and parts of segment 4, are missing in *hop* and *stat92E* embryos. *lacZ* expression, driven by the reporter gene construct *eve–lacZ*, is shown in a wild-type (d), a *hop* (e), and a *stat92E* (f) embryo. In wild type, *eve–lacZ* drives *lacZ* expression in second, third and seventh *eve* stripes. In *hop* and *stat92E* embryos, *lacZ* expression corresponding to *eve* stripe 3 is almost completely missing (arrowhead). Embryos are oriented with anterior to the left and dorsal at the top.

Mechanisms of activation of the HOP–STAT92E pathway during embryogenesis

In *Drosophila*, after fertilization, the zygotic nuclei divide 13 times before cellularization to form the precellular blastoderm. Because segmentation genes, such as *eve*, are expressed in the precellular blastoderm, one of the issues raised by the mutant phenotypes of *hop* and *stat92E* embryos is how this JAK–STAT pathway becomes activated in a precellular context.

The results discussed above suggest that the HOP–STAT92E pathway is stored maternally and is required zygotically for proper activation of the pair-rule genes. Although *hop* and *stat92E* transcripts are produced maternally and uniformally distributed, it is not clear whether HOP and STAT92E proteins are translated during oogenesis, or whether translation of these proteins only begins following fertilization. Regardless, it is clear that the HOP and STAT92E only need to be present during zygotic development because the *hop* and *stat92E* maternal effects are partially rescuable when embryos have received a copy of the corresponding wild-type gene from the father. In addition, injection of wild-type *stat92E* mRNA at preblastoderm stages can rescue the absence of maternal *stat92E* product[8], indicating that zygotic translation of STAT92E protein is sufficient for proper regulation of pair-rule gene expression.

If the mechanism of activation of the JAK–STAT pathway is conserved between mammals and *Drosophila*, then HOP should be activated by its interaction with a membrane-bound receptor lacking a kinase domain. In addition, if a ligand–receptor system activates the HOP–STAT92E pathway, which, in turn, regulates

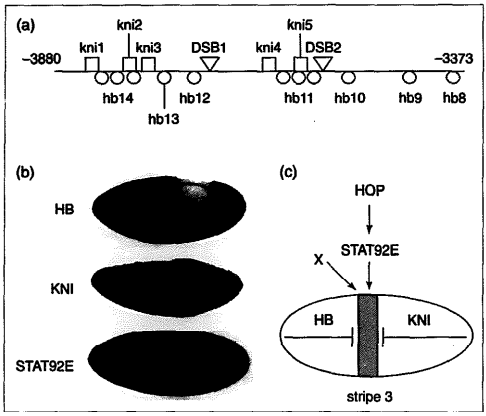

FIGURE 4. A model for HOP–STAT activation in the precellular embryo. (a) Shows the 500 bp enhancer that directs expression of *eve* at the position of stripe 3 (for details see Small *et al.*[14]). The 16 putative HB (circles) and KNI (squares) repressor sites are shown, along with the two STAT92E activator sites (triangles, indicated by DSB–DSTAT binding). (b) HB, KNI and STAT92E protein expression in a late cycle 14 embryo. The antibody against STAT92E was provided by R. Yan and J. Darnell. (c) A cellularizing embryo is oriented with anterior to the left and dorsal to the top. Genetic studies, promoter fusion assays, and DNA-binding experiments suggest that the stripe 3 enhancer is regulated by the ubiquitously distributed activator STAT92E, and at least one other activator depicted as X. The anterior and posterior borders of stripe 3 are established by the HB and KNI repressors, respectively. (Figure 4a is redrawn from Ref. 8.)

pair-rule gene expression, one question that arises is whether this system is spatially regulated along the anteroposterior axis of the embryo. The suggestion that the ligand–receptor system does not need to be localized comes from examining the mechanism of *eve* stripe 3 regulation.

Genetic analysis has shown that the anterior and posterior borders of *eve* stripe 3 are set through transcriptional repression by the HUNCHBACK (HB) and KNIRPS (KNI) proteins, respectively[18] (Fig. 4). Removal of HB activity causes an anterior shift and expansion of *eve* stripe 3. Mutations in *kni* result in a posterior expansion of stripe 3, and removing *bb* and *kni* gene activities allows this enhancer to be active throughout the embryo. Thus, *eve* stripe 3 is expressed in the region that falls between the domains of expression of HB and KNI, and HOP–STAT92E are required for activation of the 500 bp *eve* stripe 3 fragment (Fig. 4). Consistent with this model, the 500 bp modular enhancer contains 11 HB, five KNI- and two STAT92E-binding sites. Because the HB- and KNI-binding sites are involved in defining the sharp on–off borders of gene expression, and the STAT92E-binding sites are involved in activating transcription, the HOP–STAT92E signaling pathway does not need to be spatially activated.

In principle, the HOP–STAT92E pathway could be activated in a manner similar to the terminal system, which is required for the proper establishment of the most anterior and posterior embryonic cell fates. The *Torso* (*Tor*) mRNAs are deposited maternally and translated zygotically. When *Tor* receptors reach the cell surface of the precellular embryo they become activated as they interact with their ligands. Because the ligand activity of *Tor* is localized during oogenesis (by cues that originate from specialized groups of follicle cells located at both ends of the oocyte) it triggers the localized activation of *Tor* at the embryonic termini (reviewed in Ref. 19). In the case of the HOP–STAT92E system, however, it is possible that the ligand activity is not localized because the specificity of the effects observed on pair-rule gene expression can be explained by the repressor activities of the gap gene proteins. Thus, we propose that a uniformly distributed ligand located in the perivitelline space leads to uniform activation of the JAK–STAT pathway throughout the embryo.

Altogether the analysis of the HOP–STAT92E pathway in *eve* regulation points towards a permissive, rather than instructive, role for this JAK–STAT pathway in gene activation. The specific effects on pair-rule gene expression observed in the absence of *hop* or *stat92E* gene activities might simply reflect the expression of the localized transcriptional repressors, such as HB and KNI.

The role of HOP–STAT92E pathway in cell proliferation

In addition to its embryonic functions, *hop* has been implicated in the control of cell proliferation. Loss of zygotic *hop* activity results in larval to pupal lethality. Larval diploid structures are reduced in size suggesting an important role for this pathway in the control of cell proliferation[14]. Interestingly, a dominant gain-of-function point mutation in *hop*, $hop^{Tum\text{-}l}$, is associated with the formation of melanotic tumors and hypertrophy of the larval lymph glands, the hematopoietic organs[20–22] (Fig. 5). These melanotic tumors, when transplanted into wild-type animals, become metastatic and lead to death of the host. The $hop^{Tum\text{-}l}$ tumor phenotype is caused by a single amino acid substitution of glycine to glutamic acid at residue 341, which results in a hyperactive kinase. Furthermore, overexpression of wild-type *hop* throughout the larvae also results in melanotic tumors and lethality, identical to the $hop^{Tum\text{-}l}$ mutant phenotype. The $hop^{Tum\text{-}l}$ mutation and overexpression of wild-type *hop* are both presumed to potentiate mitogenic signals in hematopoietic organs and cause melanotic tumors. A reduction in the amount of *stat92E* gene activity suppresses the phenotype associated with $hop^{Tum\text{-}l}$, which is consistent with a model whereby

STAT92E operates downstream of HOP in transducing a signal that regulates blood cell development[8].

The consequence of *hop* overexpression is not restricted to larval blood cells. Overexpression of *hop* or *hop*$^{Tum\text{-}l}$ (directed by the *GAL4* upstream-activation-sequence system) in imaginal tissues causes defects in the adult fly. For example, expression of *hop* in the wing disc can cause defects of the wing blade itself, such as ectopic veins in the proximal wing, loss of veins in the distal wing, ectopic sensory organs and duplications of wing structures[22]. The mechanisms by which these defects occur have yet to be determined, but *hop* over-activity in imaginal discs is clearly not associated with neoplasia or melanotic masses. These defects primarily involve gain of ectopic structures or apparent changes in cell fate, suggesting that *hop* overexpression can interfere with proper determination and/or differentiation of imaginal tissues. Further analyses are required to determine the function of HOP in patterning the imaginal discs. Interestingly, some of the phenotypes caused by overexpressing *hop* are reminiscent of phenotypes exhibited by activated forms of *Drosophila* MAP kinase, suggesting a possible function of HOP in receptor tyrosine kinase signaling (see discussion in Ref. 22).

In mammals, the JAK–STAT pathways appear to be instructive, in that they elicit different effects that are regulated by combinations of ligands, receptors, JAKs and/or STATs. Knockout experiments in mice indicate that each STAT is very 'coupled' to specific cytokines, in spite of the fact that the cytokines activate multiple signaling pathways. For example, most, if not all, functions of the (interferons) are lost in *stat1*$^{-/-}$ mice[23,24], most functions of IL-4 (interleukin 4) are lost in *stat6*$^{-/-}$ mice[25], and most functions of IL-12 are lost in *stat4*$^{-/-}$ mice[26,27]. Similarly, a knockout of *jak3* has indicated a very precise role for this kinase in lymphoid lineage function[28,29]. Another remarkable similarity is that the functions are related to innate or acquired immunity. Indeed, it is striking that STAT4 is as critical to IL-12-enhanced TH1 (T helper cell type 1) differentiation[26,27] as STAT6 is to IL-4-enhanced TH2 differentiation[25]. We have argued that in the *Drosophila* embryo, the HOP–STAT92E pathway is permissive, and that its apparent specificity is dictated by transcriptional repressors that define the domain where the system can act. It will be important to characterize the regulation of the HOP–STAT92E pathway in the hematopoietic organs, as well as during imaginal disc development, to determine whether this pathway plays roles that are inductive rather than permissive in these tissues.

Genetic dissection of the JAK–STAT pathway: perspective

In this review, our current understanding of the HOP–STAT92E pathway in *Drosophila* is summarized. One of the important issues that needs to be addressed is to determine whether, like in mammals, the fly pathway is regulated by the binding of a ligand to a receptor. A genetic approach might result in the identification of new loci with mutant phenotypes similar to *hop* and *stat92E* and might identify such receptors and ligands. Interestingly, mutations in the gene *unpaired* (Ref. 30) are associated with an embryonic lethal phenotype similar to the maternal effect of *hop* and *stat92E*. Current analysis of this locus is consistent with it playing a role in the HOP–STAT92E pathway (D. Harrison, R. Binari and N. Perrimon, unpublished).

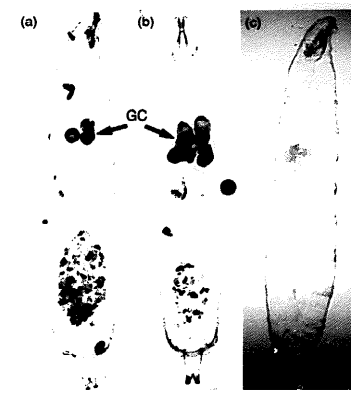

FIGURE 5. Melanotic tumor formation in *hop*$^{Tum\text{-}l}$ larvae. Melanotic masses of various sizes can be seen in several tissues of *hop*$^{Tum\text{-}l}$ larvae reared at the restrictive temperature of 29°C. (a, b) Show that the gastric cecae (GC), part of the larval anterior midgut, are melanized in most *hop*$^{Tum\text{-}l}$ animals. (c) Wild-type larva. (Courtesy of D. Harrison.)

Two types of genetic screens are likely to be fruitful for further study of this pathway. The first one is to pursue screens that led to the characterization of *hop* and *stat92E* functions during embryonic development. *hop* and *stat92E* are used in more than one developmental stage and the discovery of their roles was dependent on screens designed to determine the maternal effect of zygotic lethal mutations[11,15]. Such screens have not been completed to saturation and might, in principle, lead to the identification of additional genes with phenotypes similar to *hop* and *stat92E*. The second genetic approach to identify members of this pathway is to conduct screens in sensitized genetic backgrounds[31]. Indeed, this approach has already proved successful for studying the *Drosophila* HOP pathway, because Yan *et al.*[9,10] identified an allele of *stat92E* in a screen for dominant suppressors of *hop*$^{Tum\text{-}l}$. In addition, a number of mutations that generate melanotic tumors have been isolated in *Drosophila*[32] and these should be examined for their possible roles in the HOP–STAT92E pathway.

Another question whose answer will further our understanding of the HOP–STAT92E pathway is whether other genes encoding JAKs and STATs in *Drosophila* exist. In mammals, four JAK family members and six STAT family members have been isolated to date. Thus, it is possible that, in addition to *hop* and *stat92E*, other JAK and STAT genes are present in the *Drosophila* genome.

The HOP–STAT92E pathway and the study of cancer

During the 1980s, the first cellular oncogenes, which when mis-expressed are responsible for forcing a cell to

become cancerous, were characterized. Many of these cellular oncogenes are normal components of signal transduction pathways, and the dissection of the intracellular signal pathways that control cell growth and proliferation has become a primary focus of cellular biology and cancer research. The *Drosophila* homologs of a number of proto-oncogenes have been cloned and characterized, with the hope that their normal biological functions might be studied in a genetically tractable organism. However, mutations in these genes do not lead to the formation of neoplasms in the fly but, instead, interfere with establishment of cell fates. The *Tum-l* allele of *hop* is the first known dominant *Drosophila* mutation encoding an activated cytoplasmic tyrosine kinase that results in transplantable neoplastic tumors. Furthermore, the reduction of cell growth in diploid larval tissues in *hop* and *stat92E* recessive alleles also clearly implicates the fly JAK–STAT pathway in cell proliferation. Studies on the HOP–STAT92E pathway in *Drosophila*, therefore, hold the promise of a new genetic paradigm with which to characterize the mechanisms involved in tumorigenesis.

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