CMLS, Cell. Mol. Life Sci. 55 (1999) 1578–1584
1420-682X/99/121578-07 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

JAKs and STATs in invertebrate model organisms

C. R. Dearolf

Department of Pediatrics, Jackson 1402, 55 Fruit Street, Massachusetts General Hospital and Harvard Medical School, Boston (Massachusetts 02114, USA), Fax + 1 617 724 3248, e-mail: cdearolf@partners.org

Abstract. Invertebrate organisms provide systems to elucidate the developmental roles of Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signaling pathways, thereby complementing research conducted with mammalian cells and animals. Components of the JAK/STAT protein pathway have been identified and characterized in the fruit fly *Drosophila melanogaster* and the cellular slime mold *Dictyostelium discoideum*. This review summarizes the molecular and genetic data obtained from these model organisms. In particular, a *Drosophila* JAK/STAT pathway regulates normal segmentation, cell proliferation, and differentiation, and hyperactivation of the pathway leads to tumor formation and leukemia-like defects. A *Dictyostelium* STAT regulates the development of stalk cells during the multicellular part of the life cycle. Future research utilizing these organisms should continue to provide insights into the roles and regulation of these proteins and their signaling pathways.

Key words. *Drosophila*; *Dictyostelium*; JAK kinase; STAT; genetics; signaling; transcription.

Introduction

Invertebrate organisms offer advantages as experimental systems for studying Janus kinases (JAKs), signal transducers and activators of transcription (STATs), and the regulatory pathways in which they participate. This review focuses on JAK/STAT signaling in two such model organisms for which the most progress has been made—the fruit fly *Drosophila melanogaster* and the cellular slime mold *Dictyostelium discoideum*. One JAK and one STAT gene have been identified in *Drosophila* [1–3] and three STAT genes have been found in *Dictyostelium* [4; J. G. Williams, personal communication]. A STAT gene has also been identified in the mosquito *Anopheles gambiae* (Ag-Stat) [5], and in the worm *Caenorhabditis elegans* genomic sequence database [EMBL library accession number Z70754], but not in the yeast *Saccharomyces cerevisiae* genomic database [6].

The wealth of genetic and molecular techniques developed in *Drosophila* [7–9] provide the means to clarify the specific developmental roles of these proteins, to identify genetically other components of the pathways, and to conduct extensive in vivo structure-function studies. So far, the characterization of *Drosophila* mutants has demonstrated that JAK and STAT proteins play a critical role in the growth, development and differentiation of cells and tissues [2, 3, 10, 11]. These studies also provided the first evidence that abnormal JAK/STAT function can cause neoplastic cell growth and tumor formation [12, 13]. Several instances have now been identified in which constitutive activation of mammalian Jaks leads to a transformed phenotype [reviewed in ref. 14]. *Dictyostelium* has also been well studied both genetically and biochemically [15–17]. Its life cycle provides a system for studying the functional specificity and regulation of STATs, both at the unicellular and multicellular levels.

Discovery of invertebrate JAKs and STATs

One *D. melanogaster* JAK has been identified, encoded by the hopscotch (hop) gene. Hop was initially studied for belonging to a class of genes in which loss-of-function mutations cause both larval-pupal zygotic lethality as well as specific maternal effects on embryonic development [10]. The name of the locus was derived from

the pattern of missing segmental structures observed in mutant embryos. The gene is located on the X chromosome, at salivary gland position 10B. hop DNA was later cloned in a chromosomal walk of the region and found to be a member of the JAK family [1]. Obvious JAKs have not yet been identified in either the worm *C. elegans* or in the slime mold *D. discoideum*, although a tyrosine kinase that has a JH2-like pseudokinase domain has been reported in *Dictyostelium* [18].

One *Drosophila* STAT has been identified, named Stat92E for its salivary gland chromosome position on the third chromosome (also called D-Stat and Marelle) [2, 3]. Stat92E clones and mutant stocks were independently isolated by two groups using different experimental approaches. In one, a Stat92E cDNA clone was selected by library screening with polymerase-chain-reaction-amplified probes, using degenerate oligonucleotides as primers [2]. A Stat92E mutant strain was then identified from a collection of chemically mutagenized fly stocks, recovered for their ability to suppress the adult lethality caused by a dominant hop mutation [13]. In an alternative approach, a P-element-induced mutation was identified that causes segmentation defects similar to those seen in loss-of-function hop mutants, and the sequences flanking the insertion were cloned [3].

A *Dictyostelium* STAT, now named Dd-StatA, was identified as the protein that binds in vitro to a TTGA DNA sequence motif found in the promoters of the ecmA and ecmB genes, which are transcriptionally regulated during prestalk cell differentiation [4]. A more recent genetic analysis indicates that the interaction of Dd-StatA with ecmB, but not ecmA, is biologically meaningful [cited in ref. 19]. Two additional *Dictyostelium* STAT genes, named Dd-StatB and Dd-StatC, have also been identified [J. G. Williams, personal communication].

Molecular biology

Two *Drosophila* hop polyA$^+$ RNAs have been detected by Northern analysis, a 5.4-kb transcript that is expressed throughout development, and a 5.1-kb transcript that is only expressed maternally [1]. Molecular analysis of Hop has focused on clones derived from the larger of the two transcripts. However, the encoded proteins are either identical or at least functionally interchangeable, since in transgenic stocks, a single cDNA construct rescues both the maternal and zygotic defects in hop mutants [20]. The larger transcript is comprised of ten exons. The predicted Hop protein contains 1177 amino acids, and possesses each of the seven JAK homology (JH) domains characteristic of mammalian JAKs [1]. The entire amino acid sequence is 25–27% identical to that of the mammalian JAKs, with the highest degree of identity (39%) found within the kinase domain of the protein.

The Stat92E gene is also expressed throughout development, with the primary polyA$^+$ RNA being 4.0 kb in length [2, 3]. This transcript contains multiple exons, and encodes a predicted protein of 761 amino acids. A full-length Stat92E cDNA clone, encoding a protein with seven fewer amino acids, has also been identified, although possible functional differences between the two proteins are unknown.

Dd-StatA transcripts are expressed at very low levels in growing cells and in cells at stages prior to tip formation, but are highly induced in later stages of differentiation [4]. A single polyA$^+$ RNA has been detected. It is 3.15 kb in length, and encodes a predicted protein of 707 amino acids. Somewhat surprisingly, the Dd-StatA protein is present in growing cells and throughout the development of the multicelluar organism. However, the protein does become tyrosine phosphorylated and localized in the nucleus in a developmentally restricted pattern [19].

Stat92E and Dd-StatA are similar to mammalian STAT proteins in that they contain a DNA-binding domain, an SH2 domain, and a critical tyrosine residue just downstream from the SH2 domain, although both lack a strong SH3 domain [2–4]. Both Stat92E and Dd-StatA become tyrosine phosphorylated and translocated to the nucleus when activated, and likely bind to DNA as multimers of at least dimers. The optimal DNA sequence for *Drosophila* STAT binding in vitro is the TTCnnnGAA motif, similar to the mammalian interferon (IFN)-γ-activating sequence (GAS) element [2]. In contrast, *Dictyostelium* STAT recognizes a binding element that contains a repeat of the TTGA motif, which more closely resembles the mammalian IFN-stimulated response element (ISRE) [4]. The amino acid sequence of Stat92E is 37% identical to that of mammalian STATs 5 and 6, with 25–29% identity when compared to the other mammalian STAT family members. Dd-StatA is more distantly related to other STAT proteins.

Roles of Hop/Stat92E in embyonic segmentation

The *Drosophila* hop and Stat92E genes are required in the developmental regulation of multiple cells and tissues, and throughout the entire life cycle. These roles are discussed in this and the following sections, and the reader is additionally referred to a previous review of the subject [21]. At the molecular level, the best understood of these regulatory pathways is that involved in embryonic segmentation (fig. 1).

Segmentation in *Drosophila* has been extensively studied over the past two decades, owing to the identification by genetic means of multiple components of the process

[22; reviewed in 23]. As a simplistic form of the model, the *Drosophila* anteroposterior (segmental) axis is established by the differential expression of genes in response to maternally derived gradients within the embryo. The 'gap' class of genes are transcriptionally regulated by these gradients, and each member is required for the development of a group of multiple, adjacent segmental units. The 'gap' genes in turn regulate the expression of the 'pair-rule' genes, as well as other 'gap' genes. 'Pair-rule' gene expression becomes localized into stripes along the anteroposterior axis, and mutations in the 'pair-rule' genes cause a loss of structures in alternating segmental units throughout the embryo. Finally, the 'pair-rule' genes are required for the expression of the 'segment polarity' genes, which are required for the pattern, and often polarity, of each segmental unit.

Mutations in hop and stat92E cause segmental defects that do not fit neatly into any of the above categories. Instead, even apparent null mutations give rise to a variable phenotype, in which segmental structures are partially or completely deleted from one or more parts of the embryo (fig. 2) [2, 3, 11]. These abnormalities include a loss of characteristic cuticular features, gaps in the ventral nerve cord and in the tracheal trunks, and the loss or fusion of other internal tissues. For both hop and stat92E, these segmentation defects are strongest in embryos lacking both the maternal and zygotic gene component.

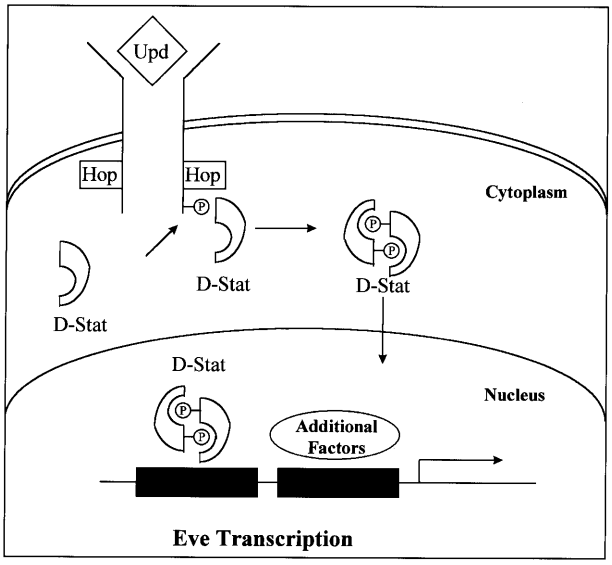

Figure 1. Model for a *Drosophila* JAK-STAT pathway that functions in embryonic development. The extracellular Upd protein activates the Hop JAK-Stat92E (D-Stat) pathway, presumably through a cell surface receptor (whose identity is currently unknown). Stat92E becomes tyrosine phosphorylated, dimerizes through its SH2 domain, and translocates to the nucleus where it activates the transcription of the even-skipped (eve) 'pair-rule' segmentation gene. The activation of eve transcription is strongest in the cells comprising the third of the seven stripes of eve expression.

A possible activating ligand for this pathway has been identified as the Unpaired (Upd) protein [24]. Mutations in the zygotically expressed upd gene cause segmentation defects similar to those observed in hop and stat92E mutants [25], and the overexpression of upd in *Drosophila* S2 tissue culture cells leads to tyrosine phosphorylation of Hop [24]. Upd is a secreted glycoprotein, and has been found to be associated with the extracellular matrix. At present, the sequence of the predicted Upd protein is similar only to the *Drosophila ananassae* Om(1E) protein, about which little is known. Further, the putative receptor for the Upd protein is not yet known.

The transcription of several 'pair-rule' genes, including eve and runt, is significantly reduced by mutations in hop and/or stat92E [1–3]. The clearest example of direct regulation of a segmentation gene by the Hop-Stat92E pathway is the transcriptional activation of the eve gene (fig. 1). eve transcription becomes localized in a seven stripe pattern during early embryogenesis [26]. A 500-bp fragment of the eve promoter is sufficient for the expression of the third stripe and, to a lesser extent, the seventh stripe [27]. This promoter fragment contains two Stat92E-binding elements, which are required for in vivo expression of the third stripe of eve promoter/reporter gene constructs [2]. Further, this stripe of eve expression is strongly reduced or missing in hop and Dstat92E mutants [1–3].

It should be noted that the full role of the Hop-Stat92E pathway in embryonic segmentation, and even in the regulation of eve expression, is not yet known. The third and seventh eve stripes are not completely eliminated in mutants lacking Hop or Stat92E, suggesting that additional transcription factors influence eve expression in these segments. In addition, the majority of the eve and runt stripes are largely unaffected in hop and stat92E mutants, leaving unresolved how the Hop and Stat92E proteins influence the development of those segments. Furthermore, the expression of 'downstream' segment polarity genes is also disrupted in hop and stat92E mutants, but it is not clear whether these abnormalities are direct or secondary consequences of the loss of Hop/Stat92E activity.

Roles of Hop/Stat92E in cell proliferation and neoplasia during larval stages

Two lines of genetic evidence indicate that the *Drosophila* Hop and Stat92E proteins play a role in cell proliferation. First, a portion of mutants lacking

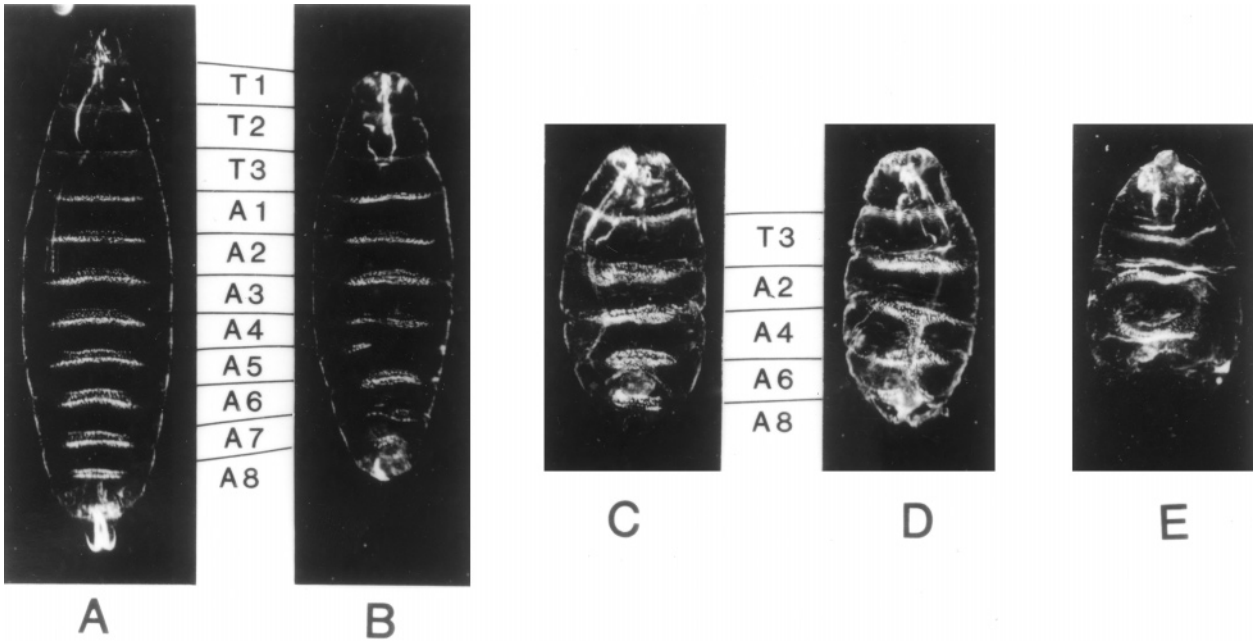

Figure 2. Mutations in the *Drosophila* hop and stat92E genes disrupt embryonic segmentation. Shown are a normal cuticle preparation (*A*) and examples of the range of abnormal cuticles in a stat92E mutant (*B–E*). Most frequently observed is a partial loss of denticle belts (*B*), with 'pair-rule' patterns (*C*, *D*), fusions of segments (*D*), and massive segmental disorganization (*E*) also seen [reprinted from ref. 11].

zygotic but not maternal hop activity survive until the late larval/early pupal stage of development. These mutants lack most of the diploid cells normally found at this stage of development, including imaginal disc cells, ovaries and testes, proliferative cells of the brain, and the hindgut and foregut imaginal rings [10].

Second, two dominant, gain-of-function mutations, $hop^{Tum\text{-}l}$ and hop^{T42}, cause tumors in larvae, as well as hematopoietic defects that include overproliferation (fig. 3) [12, 28]. In *Drosophila* larvae, approximately 90% of the circulating blood cells are rounded cells termed plasmatocytes, which serve both in innate defense (phagocytosis of microorganisms and synthesis of anti-microbial peptides) and in normal development (phagocytosis of cell debris and secretion of extracellular matrix components). Plasmatocytes are thought to terminally differentiate into larger, flat cells (lamellocytes) at the end of the larval stage of development, or in response to parasitization [reviewed in ref. 29].

The $hop^{Tum\text{-}l}$ and hop^{T42} mutations cause hypertrophy of the lymph gland, the reported source of larval blood cells. In addition, the circulating blood cells overaccumulate (fig. 3), with the concentration in the hemolymph reaching or exceeding 30 times the normal level [30]. Both mutations are temperature sensitive. At low culture temperatures, mutant larvae have an excess of plasmatocytes, although the larvae and adults are healthy. At high temperatures, the majority of mutants have an overabundance of both plasmatocytes and lamellocytes, and hemizygotes/homozygotes die prior to the adult stage. The circulating plasmatocytes and lamellocytes frequently aggregate into masses containing hundreds of cells, and may become melanized. The hematopoietic defects are clonal in origin, as the transplantation of mutant lymph glands into normal adult hosts leads to tumor formation in the host [12]. Melanized tumors of the lymph gland and gut are also common, and similar tumors can be induced by the overexpression of wild-type hop constructs [31]. Interestingly, neither the hyperactive hop mutations nor the overexpression of wild-type hop leads to tumor formation or overproliferation of imaginal disc cells.

The lesion in both $Hop^{Tum\text{-}l}$ and Hop^{T42} is an amino acid substitution [20, 28, 31]. The $Hop^{Tum\text{-}l}$ protein contains a G341E substitution, located in or near the JH4 domain. The Hop^{T42} protein contains an E695K substitution. The affected glutamic acid residue is located in the JH2 domain, and is conserved among the mammalian JAK family members. The dominant $hop^{Tum\text{-}l}$ and hop^{T42} phenotypes are significantly suppressed by mutations in stat92E, and both the $Hop^{Tum\text{-}l}$ and Hop^{T42} proteins hyperactivate Stat92E when over-

expressed in tissue culture cells. Therefore, the developmental defects in these mutants result from a misregulation of both the JAK and STAT proteins. The hemotopoietic phenotype in $hop^{Tum\text{-}l}$ and hop^{T42} mutants can also be mildly suppressed by a mutation in the awd gene, a *Drosophila* member of the mammalian Nm23 gene family [32]. However, the specificity of this latter interaction is not certain.

Roles of Hop/Stat92E in adult flies

Defects in adult tissues and cuticle, resulting from the loss of Hop and Stat92E function, have been studied by several methods. First, some hop and stat92E alleles retain partial activity, and a percentage of mutants can survive until the adult stage [10, 11]. Second, homozygous somatic clones of hop- or Stat92E-cells can be made in an otherwise heterozygous (and phenotypically normal) animal [H. Luo, H. Asha, T. Parke, C. R. Dearolf and M. Mlodzik, unpublished observations]. In addition, the effects of wild-type hop overexpression have been examined in adults [31].

These studies have identified several abnormalities in adult structures, which seem to involve changes in individual cell fates or more global developmental patterning. A frequently observed abnormality is the appearance of ectopic veins in the wings of hypomorphic (partial-loss-of function) hop and stat92E mutants [11]. Ommatidia in the adult eye are often missing, or misoriented [H. Luo, H. Asha, T. Parke, C. R. Dearolf and M. Mlodzik, unpublished observations]. In addition, hop and Stat92E males and females are sterile [10; K. Baska and C. R. Dearolf, unpublished observations].

Dd-StatA in *Dictyostelium* prestalk cell development

The cellular slime mold, *D. discoideum*, exists as a single-cell amoeba which, upon starvation, will ultimately form a multicellular organism with differentiated cell types, the stalk and spore cells [reviewed in ref. 15]. Starved amoebae secrete and respond to cAMP, leading the individual cells to aggregate into a mound. During this time, cells are specified into either a prestalk or prespore lineage. The prestalk cells migrate to the top of the mound, forming a tip, leading to the formation of a standing slug that will fall on its side and become a migrating slug. Finally, the slug develops into a mature fruiting body, containing terminally differentiated stalk cells and spores.

Distinct types of prestalk cells can be identified in the anterior part of the slug, including the prestalk (pst) A, O, and AB cells [33]. The ecmB gene, which encodes an extracellular matrix protein, serves as a molecular marker for a subset of these cells. It is expressed at high levels in the migrating slug in the pstAB cells, but not in the pstA and pstO cells [34, 35]. Dd-StatA has been implicated as the transcription factor that represses ecmB expression in these pstA and pstO cells (fig. 4) [4, 19]. Dd-StatA binds to two repressor elements located in the ecmB promoter region. One element consists of inverted TTGA repeats, the other of inverted TTGA and TTGT motifs [36].

The activation of Dd-StatA in these cells is dependent upon both extracellular cAMP and the cAR1 receptor [19], one of a family of serpentine, G-protein-coupled cell surface receptors. The requirement for this receptor has been shown genetically, as Dd-StatA is neither tyrosine phosphorylated nor translocated to the nucleus in mutants lacking cAR1 activity. Other components of

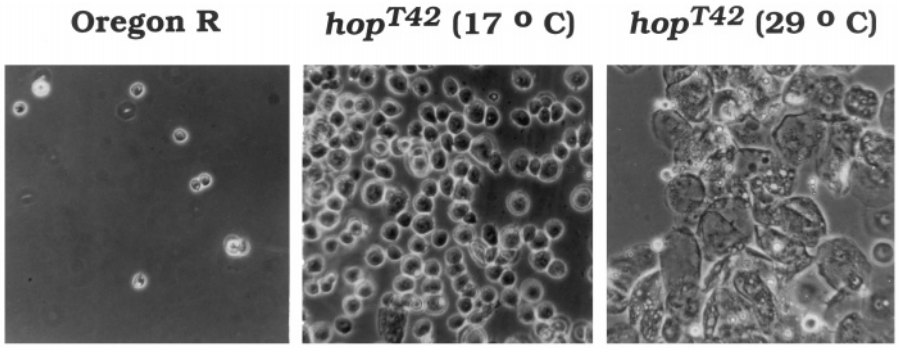

Figure 3. Hyperactivation of the *Drosophila* JAK-STAT pathway causes overproliferation and premature differentiation of larval blood cells. Shown are hemolymph preparations of circulating blood cells from normal Oregon R larvae (left panel), and hyperactive hop mutant larvae (middle and right panels). The mild mutant phenotype includes an overaccumulation of primarily rounded plasmatocytes (middle), whereas the more extreme phenotype includes an overaccumulation and aggregation of both plasmatocytes and differentiated lamellocytes (right) [reprinted from ref. [28]].

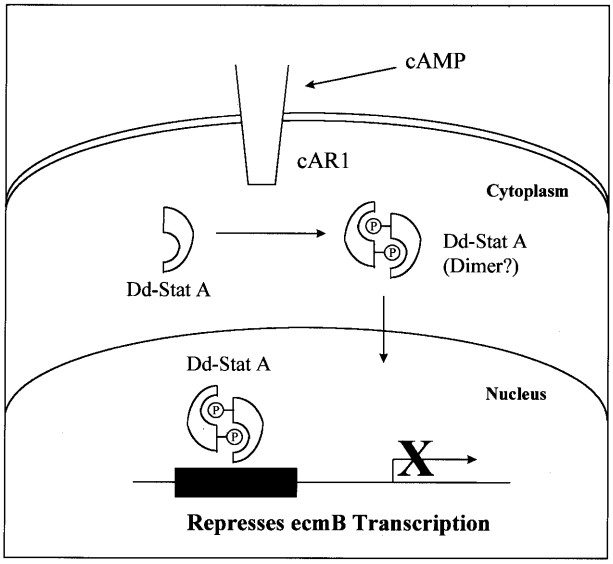

Figure 4. Model for the function of a *Dictyostelium* Stat during stalk cell development. Dd-StatA represses the transcription of the ecmB gene in a subset of prestalk cells. Dd-StatA becomes activated in response to cAMP, by a mechanism that requires the cAR1 receptor. The identities of additional intermediate proteins, including the source of tyrosine kinase activity, are not yet known.

the activation pathway are not yet known. The involvement of the cAR1 receptor suggests that Dd-StatA activation might be dependent upon heterotrimeric G proteins. However, activation occurs in the absence of the only known G_β subunit, suggesting that the process is either independent of G protein signaling or utilizes a currently unknown subunit [19].

Conclusion

It is likely that invertebrate model systems will continue to provide knowledge that will complement the study of mammalian JAK and STAT proteins. In *Drosophila*, for example, a number of signal transduction pathways have been (and continue to be) elucidated from studies of eye, wing, and oocyte development. Both Hop and Stat92E activity are required for the development of these tissues, and one can envision the discovery of a regulatory interaction between the JAK-STAT pathway and one (or more) of these additional pathways. Further, *Dictyostelium* is emerging as a system to study the effects of STAT specificity on cell lineage determination and differentiation. This organism, along with the worm *C. elegans*, will likely provide insights into the JAK-independent activation of STATs.

Acknowledgements. I thank Jeffrey Williams for sharing unpublished data concerning the *Dictyostelium* STATs, and members of my laboratory for helpful comments and discussions. This work was funded by grants from the National Institutues of Health.

1 Binari R. and Perrimon N. (1994) Stripe-specific regulation of pair-rule genes by hopscotch, a putative Jak family tyrosine kinase in *Drosophila*. Genes Dev. **8:** 300–312
2 Yan R., Small S., Desplan C., Dearolf C. R. and Darnell J. E. Jr (1996) Identification of a Stat gene that functions in *Drosophila* development. Cell **84:** 421–430
3 Hou X. S., Melnick M. B. and Perrimon N. (1996) marelle acts downstream of the *Drosophila* HOP/JAK kinase and encodes a protein similar to the mammalian STATs. Cell **84:** 411–419
4 Kawata T., Shevchenko A., Fukuzawa M., Jermyn K. A., Totty N. F., Zhukovskaya N. V. et al. (1997) SH2 signaling in a lower eukaryote: a STAT protein that regulates stalk cell differentiation in *Dictyostelium*. Cell **89:** 9090–9916
5 Barillas-Mury C., Han Y.-S., Seeley D. and Kafatos F. C. (1999) Anopheles gambiae Ag-STAT, a new insect member of the STAT family, is activated in response to bacterial infection. EMBO J. **18:** 959–967
6 Hunter T. and Plowman G. D. (1997) The protein kinases of budding yeast: six score and more. Trends Biochem. Sci. **22:** 18–22
7 Ashburner M. (1989) *Drosophila*: A Laboratory Handbook, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
8 Goldstein L. S. B. and Fyrberg E. (1994) *Drosophila melanogaster*: Practical Uses in Cell and Molecular Biology, Academic Press, San Diego
9 Greenspan R. J. (1997) Fly Pushing—The Theory and Practice of *Drosophila* Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
10 Perrimon N. and Mahowald A. P. (1986) l(1)hopscotch, a larval-pupal zygotic lethal with a specific maternal effect on segmentation in *Drosophila*. Dev. Biol. **118:** 28–41
11 Yan R., Luo H., Darnell J. E. Jr and Dearolf C. R. (1996) A JAK-STAT pathway regulates wing vein formation in *Drosophila*. Proc. Natl. Acad. Sci. USA **93:** 5842–5847
12 Hanratty W. P. and Ryerse J. S. (1981) A genetic melanotic neoplasm of *Drosophila melanogaster*. Dev. Biol. **83:** 238–249
13 Hanratty W. P. and Dearolf C. R. (1993) The *Drosophila* Tumorous-lethal hematopoietic oncogene is a dominant mutation in the hopscotch locus. Mol. Gen. Genet. **238:** 33–37
14 Leonard W. J. and O'Shea J. J. (1998) JAKS and STATS: biological implications. Annu. Rev. Immunol. **16:** 293–322
15 Mutzel R. (ed.) (1995) Molecular biology, growth and development of the cellular slime mold *Dictyostelium discoideum*. Experientia **51:** 1103-1196
16 Fisher P. R. (1997) Genetics of phototaxis in a model eukaryote, *Dictyostelium discoideum*. BioEssays **19:** 397–407
17 Thomason P., Traynor D. and Kay R. (1999) Taking the plunge: terminal differentiation in *Dictyostelium*. Trends Genet. **15:** 15–19
18 Adler K., Gerisch G., Hugo U. von, Lupas A. and Schweiger A. (1996) Classification of tyrosine kinases from *Dictyostelium discoideum* with two distinct, complete or incomplete catalytic domains. FEBS Lett. **395:** 286–292
19 Araki T., Gamper M., Early A., Fukuzawa M., Abe T., Kawata T. et al. (1998) Developmentally and spatially regulated activation of a *Dictyostelium* STAT protein by a serpentine receptor. EMBO J. **17:** 4018–4028
20 Luo H., Hanratty W. P. and Dearolf C. R. (1995) An amino acid substitution in the *Drosophila* hopTum1 Jak kinase causes leukemia-like hematopoietic defects. EMBO J. **14:** 1412–1420
21 Hou X. S. and Perrimon N. (1997) The JAK-STAT pathway in *Drosophila*. Trends Genet. **13:** 105–110

22 Nusslein-Volhard C. and Weischaus E. (1980) Mutations affecting segment number and polarity in *Drosophila*. Nature **287:** 795–801
23 Lawrence P. A. (1992) The Making of a Fly: The Genetics of Animal Design, Blackwell, Oxford
24 Harrison D. A., McCoon P. E., Binari R., Gilman M. and Perrimon N. (1998) *Drosophila* unpaired encodes a secreted protein that activates the JAK signaling pathway. Genes Dev. **12:** 3252–3263
25 Wieschaus E., Nusslein-Volhard C. and Jurgens G. (1984) Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. Part III. Zygotic loci on the X-chromosome and fourth chromosome. Wilhelm Roux Arch. Dev. Biol. **193:** 296–307
26 Macdonald P., Ingham P. and Struhl G. (1986) Isolation, structure, and expression of even-skipped: a second pair-rule gene of *Drosophila* containing a homeobox. Cell **47:** 721–734
27 Small S., Blair A. and Levine M. (1996) Regulation of two pair-rule stripes by a single enhancer in the *Drosophila* embryo. Dev. Biol. **175:** 314–324
28 Luo H., Rose P., Barber D., Hanratty W. P., Lee S., Roberts T. M. et al. (1997) Mutation in the Jak kinase JH2 domain hyperactivates *Drosophila* and mammalian Jak-Stat pathways. Mol. Cell. Biol. **17:** 1562–1571
29 Dearolf C. R. (1998) Fruit fly ''leukemia.''. Biochem. Biophys. Acta **1377:** M13–M23
30 Silvers M. and Hanratty W. P. (1984) Alterations in the production of hemocytes due to a neoplastic mutation of *Drosophila melanogaster*. J. Invert. Pathol. **44:** 324–328
31 Harrison D. A., Binari R., Nahreni T. S., Gilman M. and Perrimon N. (1995) Activation of a *Drosophila* Janus kinase (JAK) causes hematopoietic neoplasia and developmental defects. EMBO J. **14:** 2857–2865
32 Zinyk D. L., McGonnigal B. G. and Dearolf C. R. (1993) *Drosophila* awdK-pn, a homologue of the metastasis suppressor gene nm23, suppresses the Tum-1 haematopoietic oncogene. Nat. Genet. **4:** 195–201
33 Jermyn K. A. and Williams J. G. (1989) A new anatomy of the prestalk zone in *Dictyostelium*. Nature **340:** 144–146
34 Early A. E., Gaskell M. J., Traynor D. and Williams J. G. (1993) Two distinct populations of prestalk cells within the tip of the migratory *Dictyostelium* slug with differing fates at culmination. Development **118:** 353–362
35 Ceccarelli A., Mahbubani H. and Williams J. G. (1991) Positively and negatively acting signals regulating stalk cell and anterior-like cell differentiation in *Dictyostelium*. Cell **65:** 983–989
36 Harwood A. J., Early A. and Williams J. G. (1993) A repressor controls the timing and spatial localisation of stalk cell-specific gene expression in *Dictyostelium*. Development **118:** 1041–1048