Developmental Cell, Vol. 3, 765–778, December, 2002, Copyright ©2002 by Cell Press

The JAK/STAT Pathway in Model Organisms: Emerging Roles in Cell Movement

Review

Steven X. Hou,[1,3] Zhiyu Zheng,[1] Xiu Chen,[1] and Norbert Perrimon[2]
[1]The Laboratory of Immunobiology
National Cancer Institute at Frederick
Building 560, Room 12-70
Frederick, Maryland 21702
[2]Howard Hughes Medical Institute
Department of Genetics
Harvard Medical School
Boston, Massachusetts 02115

The JAK/STAT pathway was originally identified in mammals. Studies of this pathway in the mouse have revealed that JAK/STAT signaling plays a central role during hematopoeisis and other developmental processes. The role of JAK/STAT signaling in blood appears to be conserved throughout evolution, as it is also required during fly hematopoeisis. Studies in *Dictyostelium*, *Drosophila*, and zebrafish have shown that the JAK/STAT pathway is also required in an unusually broad set of developmental decisions, including cell proliferation, cell fate determination, cell migration, planar polarity, convergent extension, and immunity. There is increasing evidence that the versatility of this pathway relies on its cooperation with other signal transduction pathways. In this review, we discuss the components of the JAK/STAT pathway in model organisms and what is known about its requirement in cellular and developmental processes. In particular, we emphasize recent insights into the role that this pathway plays in the control of cell movement.

The Janus kinase (JAK)/signal transducers and activators of transcription (STATs) cascade is a ubiquitous intracellular signaling pathway required for response to many extracellular ligands (Decker, 1999; Levy, 1999; Mui, 1999; Yeh and Pellegrini, 1999; Imada and Leonard, 2000; O'Shea et al., 2002). In vertebrates, this pathway is activated by a large number of cytokines and growth factors. These signals induce proliferation or cell fate determination and are crucial to the proper growth and development of mammalian tissues. Both decreases and increases in activity of this signaling pathway have severe consequences. In particular, constitutive activation of JAKs and/or STATs correlates with several oncogenic transformations (Lacronique et al., 1997; Bromberg et al., 1999; Bromberg, 2002).

A canonical JAK/STAT pathway has been characterized from studies in cytokine signaling in mammalian cells (Figure 1). Cytokine receptors have no intrinsic tyrosine kinase activity but they constitutively associate with JAKs, and this allows activation of the signaling cascade. When a cytokine binds to its receptor, it induces conformational changes that lead to activation of the associated JAKs. Activated JAKs autophosphorylate and/or transphosphorylate, and then phosphorylate the cytokine receptors. The phosphorylated receptor tyrosine motifs generated act as docking sites for the SH2 domains of STATs. After binding, the STATs are activated by tyrosine phosphorylation and are released from the receptor, and then dimerize, translocate to the nucleus, bind to specific DNA elements in the promoters of genes, and activate transcription (Schindler and Darnell, 1995).

There are four known members of the JAK family in mammals: JAK1–3 and Tyk2, and seven STAT factors (STAT1–6; two isoforms of *stat-5* called *stat-5a* and *stat-5b* exist as closely linked genes; Table 1). In addition to the JAKs and STATs, three other regulators of the pathway have been identified (Figure 1; Table 1): protein inhibitor of activated STAT (PIAS), suppressor of cytokine signaling (SOCS), and signal transducing adaptor molecule (STAM). PIAS proteins negatively regulate the JAK/STAT pathway by binding to, and inhibiting, STAT activity (Shuai, 2000). This family has grown to five members: PIAS1, PIAS3, PIASxα, PIASxβ, and PIASy, each containing a putative Zn binding finger. SOCS proteins also act as negative regulators of the pathway and are thought to bind to and inhibit the activity of the receptor or JAK, and thus play key roles in negative regulation of the pathway (Starr and Hilton, 1999). The SOCS family comprises at least eight members, each with a central SH2 domain and an $\sim$40 amino acid C-terminal region referred to as the SOCS box. The STAM proteins associated with JAKs are phosphorylated in response to cytokines, and serve to increase signaling (Lohi and Lehto, 2001). So far, four members of the STAM family have been identified in human (STAM1, STAM2A, STAM2B, and EAST). All STAMs have a 140 amino acid VHS (present in *V*ps-27, *H*rs, and *S*TAM) domain in their N terminus, a central SH3 domain, and an ITAM motif (except STAM2B) in their C terminus.

Several animal model systems, including the mouse, *Drosophila melanogaster*, *Dictyostelium discoideum*, and *Zebrafish Renio*, have been utilized to examine the in vivo function of the JAK/STAT signal transduction pathway. The structure and function of the JAK/STAT pathway have been extensively investigated in mammalian systems, particularly through mouse gene knockout studies. The mammalian results have recently been reviewed in depth elsewhere (see Decker, 1999; Levy, 1999; Mui, 1999; Yeh and Pellegrini, 1999; Imada and Leonard, 2000; O'Shea et al., 2002). Here we focus on what is known about these pathways in the other model systems.

Structure of the JAK/STAT Pathway in Model Organisms

STAT-like molecules have been found in *C. elegans*, *Dictyostelium*, *Drosophila*, and zebrafish (see Table 1). However, *Drosophila*, and most likely zebrafish, are the only model systems in which STAT transduces signals through a classic JAK/STAT pathway.

In *Dictyostelium*, four STAT proteins, Dd-STATa, b, c, and d have been identified; and so far, only Dd-STATa

[3]Correspondence: shou@mail.ncifcrf.gov

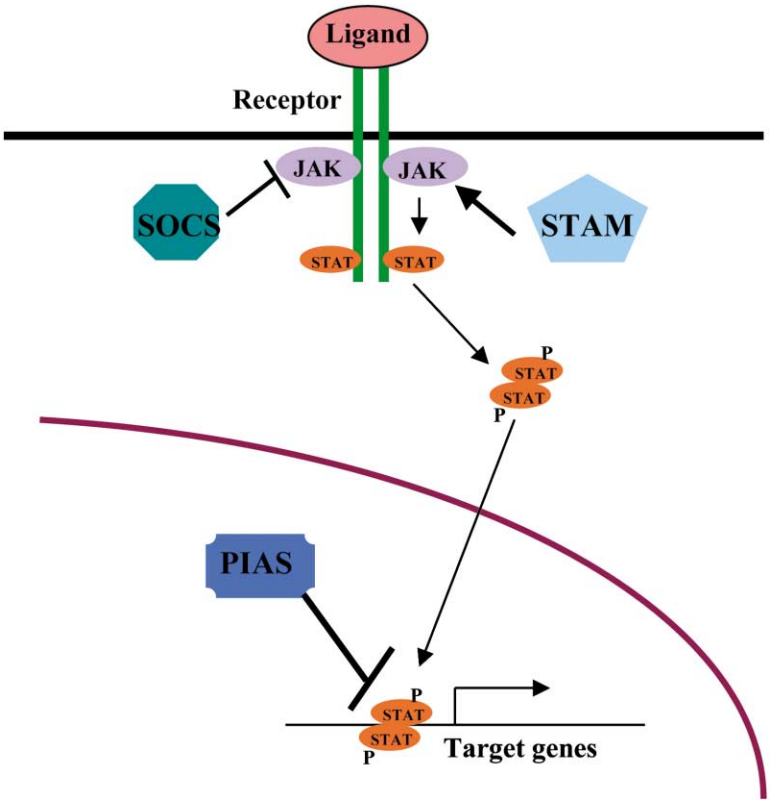

Figure 1. The Canonical JAK/STAT Pathway
See text for details.

and Dd-STATc have been studied in detail (Kawata et al., 1997; Mohanty et al., 1999; Fukuzawa et al., 2001; Jeffrey G. Williams, personal communication). In contrast to most isoforms of mammalian STATs, Dd-STATa and Dd-STATc lack a potential transcriptional activation domain at their C terminus and function as transcriptional repressors. Interestingly, Dd-STATa translocates to the nucleus in response to extracellular cAMP (Araki et al., 1998), a process that depends on cAR1, the major cAMP receptor (a serpentine receptor) present during early development (Araki et al., 1998). Dd-STATc is activated by a completely novel kind of STAT inducer, DIF (a chlorinated hexaohenone), through an unknown receptor (Table 1; Fukuzawa et al., 2001). The site of tyrosine phosphorylation is, in both cases, just a few amino acids from the C terminus. The tyrosine kinase that phosphorylates Dd-STATs is unknown (Williams, 1999).

One (possibly two) STAT has been found in the nematode *C. elegans* (Liu et al., 1999); however, no functional studies have yet been reported. Furthermore, there is no evidence for a JAK in the *C. elegans* genome sequence (Liu et al., 1999). Thus, the canonical JAK/STAT pathway probably does not exist in *C. elegans*, and how STAT is regulated in this organism remains unclear.

In *Drosophila*, the JAK/STAT pathway was originally identified through its role in embryonic segmentation (Binari and Perrimon, 1994). The four main components of this pathway are the ligand, Unpaired (UPD), the receptor Domeless/Master of marelle (DOME/MOM), the JAK, Hopscotch (HOP), and the STAT, STAT92E (also known as Marelle). The presence of a relatively simple JAK/STAT pathway, together with the wealth of sequence data and genetic techniques available, makes *Drosophila* an excellent system to study this pathway.

UPD is a secreted glycoprotein of 47 kDa that associates with the extracellular matrix (ECM), and can be released by heparin (Harrison et al., 1998). It activates the JAK/STAT pathway in a *Drosophila* cell line as measured by the activation of HOP tyrosine phosphorylation. Furthermore, *upd* mutations display the same distinctive

Table 1. Components of the JAK/STAT Pathway in Model Organisms

	Mouse	*Drosophila*	*C. elegans*	*Dictyostelium*	Zebrafish
Ligand	Cytokines	UPD	ND	cAMP, DIF	ND
Receptor	Cytokine receptors	DOME/MOM	ND	Serpentine receptor, ND	ND
Tyrosine kinase	JAK1, 2, 3 TYK	HOP	ND	ND	JAK1, 2a, 2b
STAT	STAT1–6	STAT92E/MRL	STAT	Dd-STATa, b, c, d	STAT1, 3, 5
PIAS	PIAS1, 3, xα, xβ, y	DPIAS	ND	ND	ND
SOCS	CIS, SOCS1–7	SOCS16D, SOCS36E, SOCS44A	ND	ND	ND
STAM	STAM1, STAM2A/Hbp, STAM2B, EAST	DSTAM	ND	ND	ND

See text for a full description of the components. ND, not yet determined.

embryonic segmentation defects as mutations in either *hop* or *stat92E* (Harrison et al., 1998), and UPD activates JAK/STAT signaling in the eye (Zeidler et al., 1999b). UPD has a signal sequence and several potential N-linked glycosylation sites, and is extremely basic with a predicted pI of nearly 12. In contrast to many soluble cytokines, UPD is associated with ECM, which may help it to bind to the receptor and limit its range of activity (Zeidler et al., 1999b). Besides UPD, three additional potential ligands (UPD homologs CG5988, CG5963, and CG15062) have been predicted from the genome analysis (Castelli-Gair Hombría and Brown, 2002).

DOME (Brown et al., 2001) or MOM (Chen et al., 2002) encodes the receptor activated by UPD. *dome/mom* mutations are associated with segmentation defects identical to those of either *upd*, *hop*, or *stat92E* mutations. *dome/mom* encodes a predicted protein of 1,282 amino acids that is distantly related to the mammalian gp130 subfamily. The predicted primary sequence of DOME/MOM contains the conserved cytokine receptor family domains that consist of four fibronectin type III (FN3) repeats and a YXXQ consensus motif for STAT binding. Physical interactions between DOME/MOM and UPD, and HOP and STAT92E, as well as the ability of DOME/MOM to activate HOP, were observed in S2 *Drosophila* cells (Chen et al., 2002). One additional gp130-like molecule (CG14225) exists in the *Drosophila* genome sequence and may also function in the HOP/STAT92E signal transduction pathway (Castelli-Gair Hombría and Brown, 2002).

HOP is a 120 kDa protein of 1,177 amino acids most similar to mammalian JAK2 (27% identity; Binari and Perrimon, 1994). The protein has seven conserved JAK homology (JH1–JH7) domains, with the tyrosine kinase catalytic domain (JH1) at the C terminus. Although the JH2 domain has all the structural features of a bona fide tyrosine kinase, it lacks catalytic activity, and its function is not well understood. The amino-terminal JH3–JH7 domains have been implicated in receptor association and appear to play an important role in determining the specificity of this binding. This region constitutes a FERM (*f*our-point-one, *e*zrin, *r*adixin, *m*oesin) domain that mediates association with receptors (Girault, et al., 1999; O'Shea et al., 2002). Interestingly, there are two hyperactivating mutations of *hop* that cause leukemia-like defects in flies. *Tumorous-lethal* (*hop*$^{Tum\text{-}l}$) contains an amino acid substitution (G341E) in the JH4 region (Harrison et al., 1995; Luo et al., 1995, 1997). *Hop*^{T42} is slightly stronger than *hop*$^{Tum\text{-}l}$, and also contains a single amino acid substitution (E695K) in the kinase-like domain; this represents mutation of a residue conserved in all known JAK homologs (Luo et al., 1997).

STAT92E encodes a 761 amino acid STAT protein that is most closely related to mammalian STAT5 (37% identity; Hou et al., 1996; Yan et al., 1996). HOP can phosphorylate STAT92E at tyrosine residue 711, which is required for STAT92E DNA binding activity (Yan et al., 1996). The DNA binding sequence for STAT92E, TTCNNNGAA, is similar to the binding sequences of mammalian STATs (Yan et al., 1996). In addition, STAT92E is also negatively regulated by a naturally occurring N-terminally truncated STAT92E protein (Henriksen et al., 2002).

In addition to these four components, three classes of proteins that modulate JAK/STAT signal transduction in mammals have homologs in flies. A single *Drosophila pias* gene (*dpias*), also termed *Su(var)2-10* and *zimp*, has been identified. In an in vitro protein association assay, DPIAS was shown to interact with tyrosine-phosphorylated STAT92E. DPIAS negatively regulates the HOP/STAT92E pathway in vivo and is required for blood cell and eye development (Betz et al., 2001; Hari et al., 2001). DPIAS colocalizes with nuclear lamin during interphase, and analysis of *dpias* mutants suggests that DPIAS may control multiple aspects of chromosome structure and function by establishing/maintaining chromosome organization in interphase nuclei (Hari et al., 2001). SOCS homologs are also present in *Drosophila* (Callus and Mathey-Prevot, 2002; Karsten et al., 2002). Although no loss-of-function mutations have been reported in any of the *Drosophila* SOCS genes, SOCS36E was shown to suppress the activity of both the HOP/STAT92E and *Drosophila* EGF receptor pathway in an overexpression assay (Callus and Mathey-Prevot, 2002). Finally, there is a *Drosophila* STAM (DSTAM; Mesilaty-Gross et al., 1999), which is strongly expressed as a maternal message. The fly STAM protein lacks the ITAM domain that is used by the mammalian STAMs to bind JAKs (Lohi and Lehto, 2001).

In zebrafish both JAK and STAT proteins have been characterized. Three JAKs (JAK1, 2a, and 2b) and three STATs (STAT1, 3, and 5) have been reported (Conway et al., 1997; Oates et al., 1999a, 1999b; Yamashita et al., 2002). Zebrafish JAK2a and b are approximately 65% identical to their mouse homologs (Oates et al., 1999b), and zebrafish STAT1 and STAT3 have 63.9% and 86.5% identity to the mouse genes, respectively. Most functionally characterized domains are conserved between zebrafish and mammalian STATs, including the N-terminal 120 amino acid oligomerization domain, the DNA binding domain, the SH2 domain, the domain around the activating tyrosine, and the island around the serine phosphorylation site (Oates et al., 1999a). By comparison, zebrafish STAT1 has a higher rate of evolutionary change relative to STAT3. Nevertheless, zebrafish STAT1 rescues interferon-signaling functions in a STAT1-deficient human cell line, indicating that cytokine signaling mechanisms are conserved between fish and mammals (Oates et al., 1999a).). Besides JAKs and STATs, no other components (such as ligand, receptor, SOCS, PIAS, STAM, etc.) have been reported in zebrafish.

JAK/STAT Signaling Controls Cell Proliferation

Two dominant temperature-sensitive mutations that hyperactivate HOP, *hop*$^{Tum\text{-}l}$ and *hop*^{T42}, leads to melanotic or leukemia-like tumor formation (Corwin and Hanratty, 1976; Harrison et al., 1995; Luo et al., 1995, 1997). The blood phenotype associated with *hop*$^{Tum\text{-}l}$ and *hop*^{T42} mutations indicate that hyperactivation of JAK/STAT activity regulates cell proliferation in other tissues. This is consistent with loss-of-function studies, which revealed that activity of *upd* and *hop* is required for the proliferation and/or survival of eye imaginal cells (Luo et al., 1999; Chen et al., 2002). Strong alleles of *upd* are embryonic lethal, and weaker alleles such as *os*^{1} and *os*^{s} give rise to adult flies that have small eyes (Harrison et al.,

1998). Complete loss of *hop* activity results in the absence of proliferating diploid imaginal cells throughout the larva, and some transheterozygous combination of alleles give rise to adults that have a small eye or eyeless phenotype (Perrimon and Mahowald, 1986; Luo et al., 1999). Overexpression of *dpias* (the negative regulator of STAT92E) results in small and rough eyes (Betz et al., 2001). Further, the *os*[1] small eye phenotype could be partially suppressed by reducing the *dpias* gene dosage. Genetic interaction experiments suggest that the correct *dpias/stat92E* ratio is crucial for eye imaginal cell growth and differentiation (Betz et al., 2001).

The connection between STAT and cell proliferation has previously been observed in *stat5a/b* knockout mice (Moriggl et al., 1999). *stat5a/b* mutant peripheral T cells are profoundly deficient in proliferation; these cells are unable to enter the cell cycle and are accumulated at S/G2 phase following stimulation with anti-CD3 and anti-CD28 in the presence of IL-2. The inability to enter the cell cycle and proliferate is associated with a loss in the ability to express cyclin D2 and D3 as well as Cdk6 in *stat5a/b*-deficient T cells. Furthermore, the promoters of both cyclin D2 and D3 contain STAT5 binding sites, suggesting that STAT5 may directly regulate the expression of these genes.

Consistent with a role of the JAK/STAT pathway in the control of cell proliferation, overexpression of UPD in the eye under the control of the *glass* promoter causes dramatic overgrowth of the adult compound eye (Chen et al., 2002). Histological sections through the overgrown eyes revealed that most ommatidia have normal photoreceptor cells and regular cell size, indicating that the overgrown eye is caused by an increase in the number of ommatidia, and that UPD mainly regulates cell proliferation in the compound eye (Chen et al., 2002).

The JAK/STAT Pathway Participates in Various Cell Fate Determination Events

Th1/Th2 Cells

The JAK/STAT pathway has been implicated in a number of instances in the establishment of cell fate. An elegant example is illustrated by the phenotype of *stat4* and *stat6* gene mice knockouts (reviewed in Levy, 1999; Mui, 1999). Th1 and Th2 are two specialized T cell subsets and normally arise in just the right balance to orchestrate an attack against the invader. IL-12 promotes differentiation of naïve $CD4^+$ T cells to Th1 through activating STAT4. *stat4* knockout mice failed to respond to IL-12 in their bodies and consequently made almost no Th1 and almost exclusively Th2 cells. IL-4 activates STAT6 and promotes differentiation of $CD4^+$ cells to Th2 cells. *stat6* knockout mice have defective responses to IL-4 and Th2 cell differentiation. These results support a model of T helper lymphocyte differentiation in which the generation of Th2 cells requires STAT6, and the development of Th1 cells is augmented through the action of STAT4.

Polar/Stalk Cells

Another example where the JAK/STAT pathway controls cell fate is during *Drosophila* oogenesis, where it specifies stalk cell fate (McGregor et al., 2002). During the early stages of egg chamber development, a specialized group of follicle cells (FC) differentiate into stalks. Each egg chamber is attached to its neighbors by a stalk that forms as it leaves the germarium. The stalks contact the FC epithelium through two pairs of polar cells, situated at the anterior and posterior poles of the egg chamber (Spradling, 1993). A single precursor cell type gives rise to stalk cells and polar cells (Tworoger et al., 1999).

The HOP/STAT92E signal transduction pathway has recently been shown to specify stalk cell fate (McGregor et al., 2002). Reduction of HOP/STAT92E pathway activity results in the fusion of developing egg chambers due to the expansion of the polar cell population and concomitant loss of interfollicular stalk cells. Conversely, ubiquitous expression of *upd* throughout the ovary results in expansion of stalk cells. Concomitantly, polar cells are often missing from one pole of the developing egg chambers. Consistent with these phenotypes, *upd* is expressed specifically in the polar FC (Silver and Montell, 2001; McGregor et al., 2002).

Stem Cell Maintenance

Another unexpected finding was the discovery that the canonical JAK/STAT pathway is involved in male germ cell maintenance. *Drosophila* spermatogenesis takes place within the tubular testis (reviewed in Fuller, 1993; Figure 2A), and at the tip of the testis is a germinal proliferation center that is composed of a group of twelve nondividing somatic cells called the hub, and a small number of germline stem cells (GSCs; 16–18 in larvae, 5–9 in adult). Spermatogenesis is initiated by the asymmetric division of a GSC; one daughter remains at the hub and retains stem cell identity, while the other one is displaced and becomes a founder gonial cell (or gonialblast) that initiates differentiation. These GSCs are also flanked by somatic stem cells (SSCs) known as cyst progenitor cells, which maintain contact with the hub and divide to produce cyst cells that enclose each gonialblast.

Loss-of-function mutations in *hop* result in loss of GSCs and SSCs, indicating that signaling maintains stem cell fate or viability (Kiger et al., 2001). Also, GSCs null for *stat92E* can produce differentiating daughter cells but cannot maintain stem cell fate. Finally, ectopically activating the HOP/STAT92E pathway in testes produces ectopic cells with GSC and SSC features. These data suggest that HOP/STAT92E signaling instructs stem cell fate rather than maintains cell viability. The hub may define a stem cell niche in which localized activation of the HOP/STAT92E pathway instructs the self-renewal of GSCs and SSCs.

Hub cells express the ligand *upd*, which activates the JAK/STAT pathway in adjacent germ cells. This signaling ensures that GSCs and SSCs attached to the hub (niche) undergo self-renewal, while daughter cells that are displaced from the niche differentiate (Kiger et al., 2001; Tulina and Matunis, 2001). Perhaps cells displaced from the hub do not receive sufficiently high levels of HOP/STAT92E signaling to activate specific gene expression, and therefore lose self-renewing capacity. This decision to differentiate is further promoted by a MAP kinase signal that is relayed to the gonialblast from the surrounding cyst cell lineage (Kiger et al., 2000; Tran et al., 2000). The fly testis system is an intriguing parallel to the mammalian embryonic stem (ES) cells (Matsuda et al., 1999), in which JAK/STAT signaling is required for the maintenance of mammalian ES cells, while the

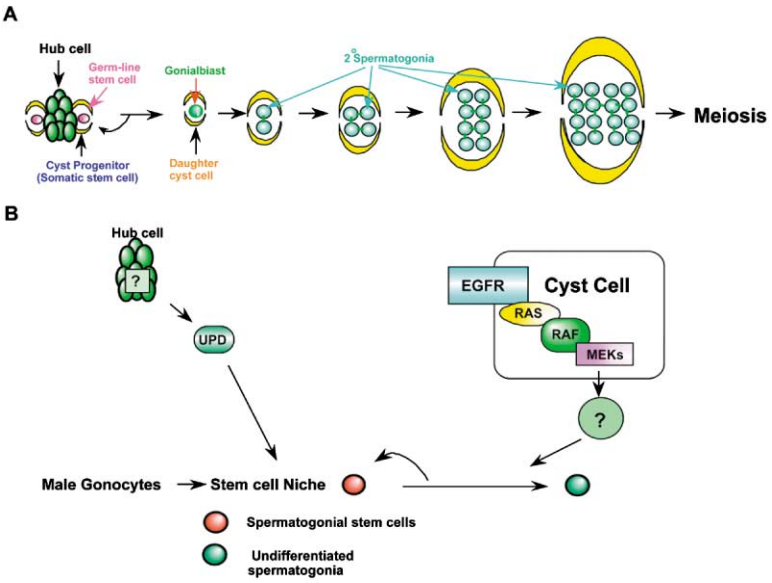

Figure 2. The HOP/STAT92E Pathway Regulates Male Germline Stem Cell Self-Renewal

(A) Five to nine germline stem cells (only two shown for clarity) and approximately twice as many somatic cyst progenitor cells are anchored around somatic hub cells at the apex of the *Drosophila* testis. The testis proliferation center comprises hub, germline, and somatic stem cells, gonialblasts, and 2° spermatogonia.

(B) Schematic representation of the HOP/STAT92E and RAS/MAPK pathways, cooperatively regulating male germline stem cell self-renewal and differentiation. UPD expressed in the hub activates the HOP/STAT92E pathway in adjacent germ cells. This signaling ensures that GSCs and SSCs attached to the hub undergo self-renewal, while daughter cells that are displaced differentiate. The decision to differentiate is further promoted by a MAP kinase signal that is relayed to the gonialblast from the surrounding cyst cell lineage. This diagram is drawn based on information in Zhao and Garbers (2002).

MAP kinase pathway promotes ES cell differentiation (Smith, 2001). In both systems, the JAK/STAT signal is counterbalanced by the Ras/Raf/MAP kinase signal. The two signals may converge on some downstream targets to instruct cells to differentiate (Figure 2B).

JAK/STAT Pathway and Hematopoeisis

The JAK/STAT pathway plays fundamental roles during hematopoeitic development in the mouse, based on biochemical and in vivo analyses (Schindler, 2002). Targeting of the widely expressed *Jak2* gene resulted in embryonic lethality at day 12.5 due to failure of erythropoiesis (reviewed in O'Shea et al., 2002). *stat5a/b*-deficient hematopoietic progenitors have reduced ability to repopulate marrow, especially in competitive repopulation assays. Myeloid development is grossly normal in *stat5a/b* knockout mice, but in vitro cytokine-dependent proliferation, survival, and migration of myeloid cells to sites of inflammation in vivo are impaired (Kieslinger et al., 2000). Consistent with a role of JAK/STAT in hematopoeisis, a chromosomal translocation, [t(9;12)(p24;p13)], which creates a fusion protein comprising the dimerization domain of the transcription factor Tel and the kinase domain of JAK2, occurs in a subset of leukemias (Lacronique et al., 1997). Interestingly, lack of *stat5* activity abrogates transformation by Tel-JAK (Schwaller et al., 2000). Furthermore, many different tumors, including breast, prostate, melanoma, leukemia, lymphoma, and erythroleukemia cancers, express activated forms of *stat1*, *stat3*, and *stat5* (reviewed in Bromberg, 2002).

Findings in *Drosophila* suggest that the role of the JAK/STAT pathway in hematopoeisis may have been conserved throughout evolution. Four types of hemocytes (secretory cells, plasmatocytes, crystal cells, and lamellocytes), which are most comparable to myeloid cells in mammals and contribute to the host defense response, have been identified in *Drosophila* (Tepass et al., 1994; Dearolf, 1998; Lebestky et al., 2000; Bach and Perrimon, 2001; Lanot et al., 2001). The larval hematopoietic organ or lymph gland contains a pluripotent "stem cell" population or a number of undifferentiated prohemocytes, comparable to that of the mammalian bone marrow, which can differentiate into a given blood cell lineage when required (Lanot et al., 2001). Cell proliferation may occur predominantly in undifferentiated prohemocytes, either in the lymph glands or in circulation. Although differentiation into the secretory cells only occurs in the lymph gland, differentiation into the other three cell types can take place both in the hematopoietic organ and in circulation. The lamellocytes seldom appear in the hemolymph of *Drosophila* under unchallenged conditions. They differentiate massively after a specific immune challenge, such as infestation by parasitic wasp (Lanot et al., 2001). The plasmatocytes are responsible for immune surveillance in *Drosophila* larvae. The initial recognition of the intruder is ensured by the plasmatocytes, which rapidly attach to the chorion of the wasp egg upon massive release from the lymph gland, and a few hours later, lamellocytes appear in the hemolymph. It is possible that the succession of cellular events following wasp parasitization of *Drosophila* larvae may be mediated by a signal transduction pathway. A cytokine- and/or growth factor-related molecule may be emitted by the activated plasmatocytes to induce the differentiation of lamellocytes in the lymph gland (Lanot et al., 2001).

Consistent with this model, two dominant temperature-sensitive mutations that hyperactivate HOP, $hop^{Tum\text{-}l}$ and hop^{T42}, cause prohemocyte overproliferation and have an excess of circulating plasmatocytes (Corwin and Hanratty, 1976; Harrison et al., 1995; Luo et al., 1995, 1997). At the restrictive temperature, there is a large increase in lamellocyte production, which leads to blood cell aggregating into masses that become melanized (Dearolf, 1998; Luo et al., 2002). When transplanted into a wild-type host, $hop^{Tum\text{-}l}$ hypertrophied larval lymph glands retain the ability to cause overproliferation of prohemocytes and melanotic tumors. Interestingly, the lamellocyte differentiation and aggregation, but not the prohemocyte overproliferation associated with $hop^{Tum\text{-}l}$ and hop^{T42}, can be suppressed by a reduction in

STAT92E activity (Dearolf, 1998). In addition, *dpias* was recently shown to enhance melanotic tumor formation (Betz et al., 2001). Altogether, these results suggest that HOP may have two targets in hematopoeisis. First, it may regulate the expression of genes involved in lamellocyte differentiation and aggregation through STAT92E. Second, it may regulate prohemocyte cell proliferation through a novel STAT92E-independent pathway. The *Drosophila* Ras/MAP kinase pathway also participates in the $hop^{Tum\text{-}l}$-induced melanotic tumor formation (Luo et al., 2002) and has been proposed to affect the migration of blood cells from the lymph gland into the hemolymph, as well as the survival of circulating blood cells. Interestingly, the Ras/MAP kinase signal is not required for blood cell proliferation and differentiation in the lymph gland (Luo et al., 2002).

Another role of the JAK/STAT pathway in blood cells involves the immune response. Vertebrates defend themselves against infections by mobilizing both the innate and adaptive (acquired) immune systems. Adaptive immunity is mediated by B and T lymphocytes, which produce a variety of specific antigen receptors following somatic gene rearrangements. By contrast, innate immune responses depend on germline-encoded factors, and constitute the organism's first line of defense against infection and provide a rapid response to microbial and parasitic infections (Kimbrell and Beutler, 2001). One class of cytokines, encoded by the interferons (IFNs), plays a key role in defending the host from bacteria and other pathogens. IFNs help integrate early, innate responses by inducing immediate transcriptional responses through the JAK/STAT pathway (Decker et al., 2002).

As there is no evidence for adaptive immunity in *Drosophila*, this organism provides an opportunity to study the cellular and molecular mechanisms underlying innate immune reactions. Growing evidence indicates that JAK/STAT signaling is activated in insects in response to septic injury. First, in the mosquito *Anopheles gambiae*, STAT activation has been observed in response to bacterial challenge (Barillas-Mury et al., 1999). Second, in *Drosophila*, the JAK/STAT pathway regulates the expression of thiolester-proteases (Lagueux et al., 2000) and of small secreted proteins (Boutros et al., 2002), in the context of immune responses. The secretory cells may participate in the production of extracellular matrix and antifungal peptides following an immune challenge (Lanot et al., 2001). Thus, although still in its infancy, understanding the activation and function of the JAK/STAT pathway during pathogenesis in model organisms promises to reveal important insights into innate immunity.

STAT Activity Controls Cell Migration

Many cellular processes associated with cell migration involve regulation of STAT activity. For example, gastrulation, which in mammals requires cell migration to form the mesoderm, does not occur in stat3 mutants (Takeda et al., 1997). In addition, tissue-specific knockout of stat3 in keratinocytes results in defects in reepithelialization following wounding due to the epidermal cell migration defect (Sano et al., 1999). The central role of STAT regulation in cell migration processes is also apparent from other systems.

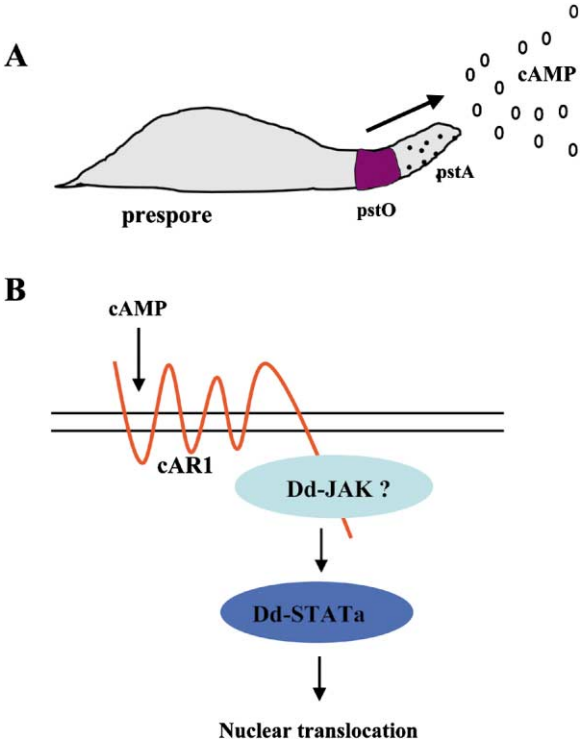

Figure 3. Serpentine Receptor-Dependent Activation of Dd-STATa in *Dictyostelium*

(A) pstA and pstO cells occupy and maintain their relative positions by differential chemotaxis (arrow) to cAMP.

(B) cAMP regulates Dd-STATa protein nuclear translocation through the major cAMP receptor cAR1. The kinase that phosphorylates Dd-STATa is unknown.

Prestalk Cell Movement in Dictyostelium

Dictyostelium discoideum grows as a unicellular organism that becomes multicellular in response to starvation. Starved cells aggregate and then differentiate, forming loose mounds ($\sim 10^5$ cells) with spatially dispersed populations of progenitor prestalk and prespore cells. Further cell movement results in the protrusion of a nipple-shaped tip at the apex of the mound, which elongates and gives rise to a slug-shaped structure that contains the precursors of the stalk cells in its front one-fifth and the precursors of the spores in its rear four-fifths (Figure 3A; Siegert and Weijer, 1997).

The prestalk region of the slug is composed of two prestalk cell subtypes, pstA and pstO (Figure 3A), which are defined by their relative levels of expression of the *ecmA* gene (Williams et al., 1989). pstA cells occupy the approximate front one-third of the prestalk region and pstO cells occupy the rear two-thirds of the prestalk region (Figure 3A; Early et al., 1995). In *Dd-STATa* null cells, aggregation is delayed and the segregation of pstA and pstO populations from one another seems to be significantly slower. In the wild-type, pstA and pstO cells occupy and maintain their relative positions by differential chemotaxis to cAMP at the tip (Figure 3A, arrow; Abe et al., 1994). cAMP regulates Dd-STATa protein nuclear translocation through the major cAMP receptor cAR1 (Figure 3B; Williams, 1999). *Dd-STATa* mutant cells

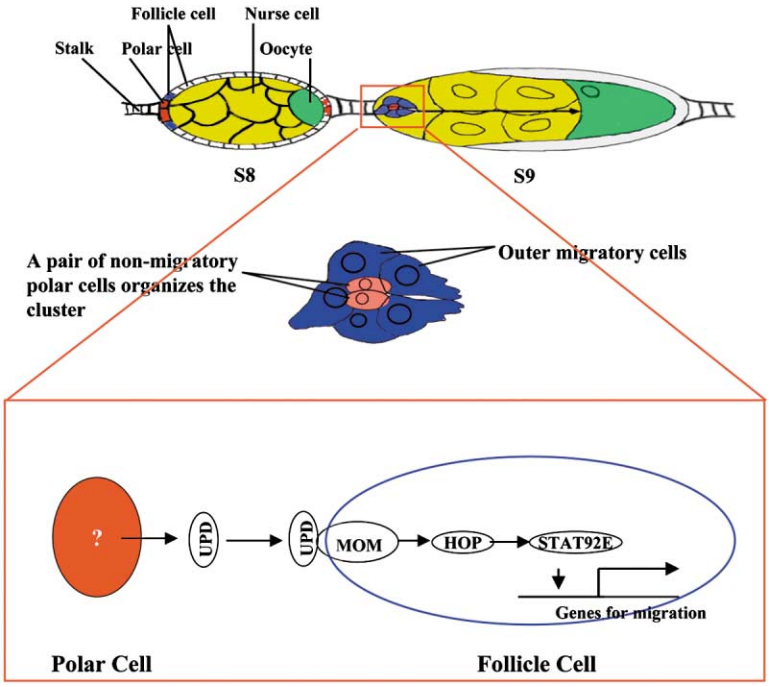

Figure 4. The Role of the JAK/STAT Pathway in Ovarian Development and Border Cell Migration

Schematic diagram of stage 8 and 9 egg chambers. The nurse cells (green yellow), oocyte (green), polar cells (red), and migrating follicle cells (blue) are indicated. The polar cell-expressed ligand UPD activates the HOP/STAT92E pathway in neighboring follicle cells to make them invasive. Part of the egg chamber drawings is provided by Dr. Denise J. Montell.

have a reduced ability to move to the tip. The delayed migration and segregation into discrete pstA and pstO regions of *Dd-STATa* mutant cells may be due to an inefficient chemotactic response to cAMP signals emanating from the tip.

The *Dd-STATa* null cell phenotypes are likely due to defects in *Dd-STATa*-regulated gene expression, because in a *Dd-STATa* mutant strain the prestalk cells precociously express *ecmB*, an early marker of stalk cell differentiation (Mohanty et al., 1999). Furthermore, two mutually redundant repressor elements within the *ecmB* promoter act to prevent precocious *ecmB* expression in prestalk cells, and Dd-STATa binds to both of these elements in vitro (Kawata et al., 1997).

Border Cell Migration

During the early stages of *Drosophila* egg chamber development, a specialized pair of follicle cells, known as polar cells, differentiates at each end of the chamber (Gupta and Schüpbach, 2001; Lehmann, 2001). The anterior polar cells then recruit four to eight follicle cells to form a specialized group of cells referred to as the border cells. During a 5–6 hr period thereafter, the border cells move as a cluster and invade the germline-derived nurse cells (Figure 4). When the border cells delaminate from the follicle cell layer, they undergo an epithelial to mesenchymal-like transition and extend large F actin-filled cytoplasmic protrusions and finer filopodia that reach between the nurse cells. Once the border cells reach the oocyte, they change direction and migrate along the oocyte/nurse cell border dorsally toward the oocyte nucleus. Here the polar cells, which have been carried along with the migrating border cells, form a pore in the micropyle, a special appendage of the egg through which the sperm enters (Gupta and Schüpbach, 2001; Lehmann, 2001).

Recent studies demonstrate that JAK and STAT are required to convert the border cells into migratory cells (Silver and Montell, 2001; Beccari et al., 2002; Figure 4). UPD is the major signal secreted by the polar cells that recruit adjacent follicle cells into the cluster and cause them to become migratory. Loss of *upd* in polar cells completely prevents border cell migration. Consistent with these observations, *upd* is specifically expressed in polar cells (Silver and Montell, 2001; Beccari, et al., 2002). Mutations in *stat92E* cause defects in migration and a reduction in the number of cells recruited to the cluster, while ectopic expression of either *upd* or *hop* is sufficient to induce ectopic expression of border cell markers, including *slbo* and extra epithelial cells, to migrate (Silver and Montell, 2001). *stat92E* null border cells lack SLBO protein (Silver and Montell, 2001), whereas partial loss of function of *stat92E* in border cells still causes migration defects without affecting expression of SLBO (Beccari et al., 2002). However, expression of focal adhesion kinase (FAK) and DE-cadherin is significantly reduced in *stat92E* mutant border cells (Silver and Montell, 2001), suggesting that they may be critical downstream targets of the JAK/STAT signal transduction pathway in regulating border cell migration.

The JAK/STAT Pathway and Convergent Extension

The JAK/STAT pathway has also been implicated in a more localized cell movement referred to as convergent extension (CE). CE describes the morphogenetic process by which an epithelial sheet changes shape as the result of mediolateral intercalation of the cells (Myers et al., 2002; Wallingford et al., 2002). Studies in both zebrafish and *Drosophila* have revealed an important role of the JAK/STAT pathway in this process.

JAK/STAT and Cell Movements during Zebrafish Gastrulation

During zebrafish gastrulation, four cell movements occur. First, blastomeres move down the surface of the

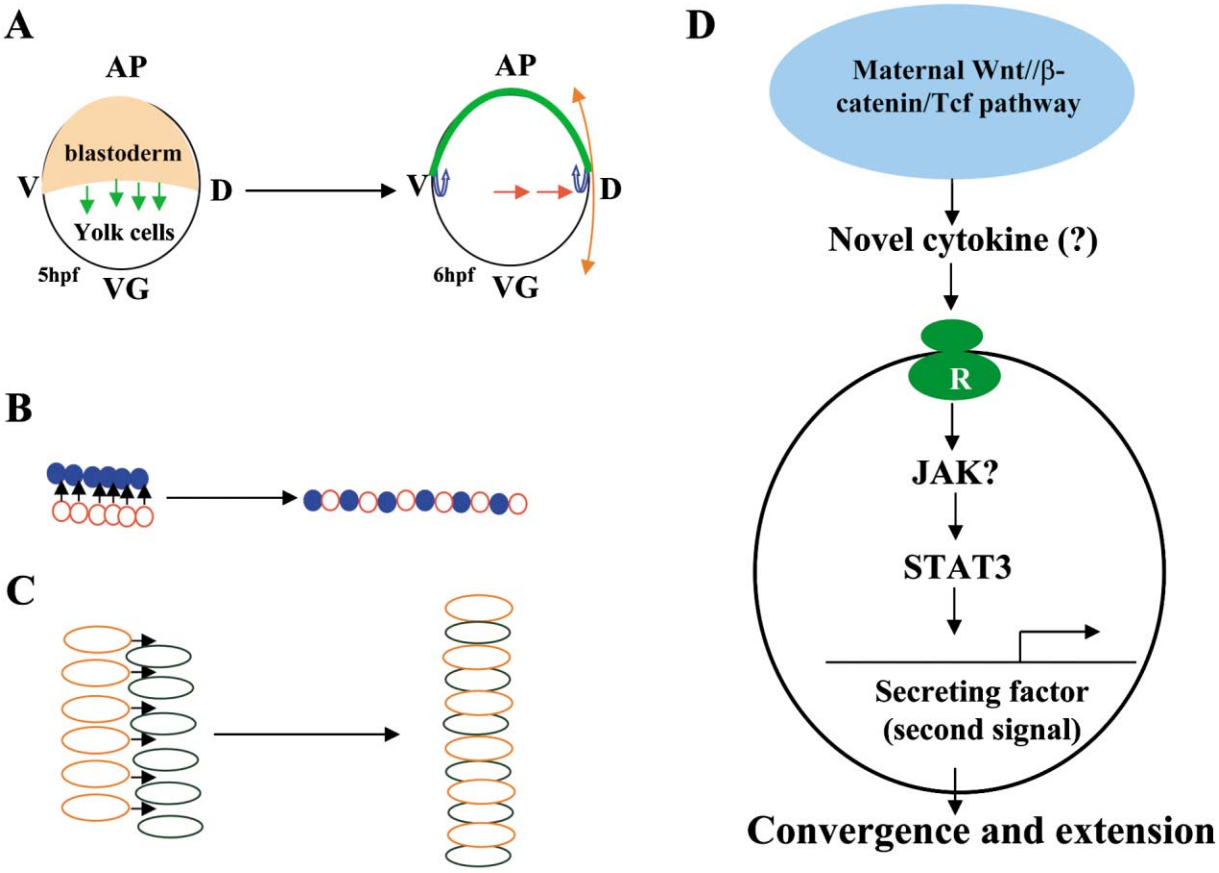

Figure 5. Gastrulation Movements in Zebrafish

(A) During early zebrafish development, four cell movements occur. First, blastomeres move down the surface of the centrally located yolk cell from the animal pole toward the vegetal pole (green arrows), a process called epiboly. Shortly after 6 hr of development, three additional cell movements begin. Internalization (blue arrows) results in the formation of a localized thickening of cells known as the germ ring. Convergence of cells (red arrows) toward the future dorsal side of the embryo creates the embryonic shield, and extension (orange arrows) causes an elongation of the embryonic shield.

(B) Epiboly is a radial intercalation process; deeper cells move up and insert themselves between more superficial neighbors, which leads to the spreading and thinning of the tissue.

(C) Cells intercalate along the D/V axis during the dorsal convergence of LME cells.

(D) An activation of STAT3 on the dorsal side is regulated by the maternal Wnt/β-catenin pathway and requires a Tcf transcription factor. An unidentified upstream molecule, possibly a cytokine or growth factor capable of activating STAT3 in early embryogenesis, is proposed to be one of the targets of the maternal Wnt/β-catenin pathway. No JAK has yet been shown to be involved in STAT3 activation. The JAK/STAT signal may regulate the expression of a second signal molecule in PCME cells. The LME cells possibly sense the local gradient of the second signal and converge dorsally. AP, animal pole; VG, vegetal pole; V, ventral; D, dorsal; hpf, hr postfertilization; LME, lateral mesendodermal; PCME, prechordal mesendodermal.

centrally located yolk cell from the animal pole toward the vegetal pole (Figure 5A, green arrows), eventually covering the entire surface of the yolk cell; this process is called epiboly and takes 10 hr to complete. Shortly after 6 hr of development, three additional cell movements begin. During internalization of mesendoderm, cells at the blastoderm margin curl underneath and migrate between the yolk cell (Figure 5A, blue arrows) and overlying cells, producing a localized thickening of cells around the equator of the embryo called the germ ring. As internalization and epiboly proceed, both internalized and noninternalized cells move toward the future dorsal side of the embryo in a process known as convergence (Figure 5A, red arrows), producing a thickening of the germ ring known as the embryonic shield. Cells in the embryonic shield undergo a fourth cell movement, known as extension (Figure 5A, orange arrows), causing an elongation of the embryonic shield. The four cell movements of gastrulation (epiboly, internalization, convergence, and extension) transform the initial ball of cells into an elongated structure with the major body axes (Conway et al., 1997; Myers et al., 2002).

Epiboly is a radial intercalation process in which deeper cells move up and insert themselves between more superficial neighbors (Figure 5B), leading to the spreading and thinning of the tissue. Eventually, the blastoderm cells spread over the yolk until they wrap up the entire egg. Injection of RNA-encoding dominant-negative JAK1 slows cell intercalation, and epiboly takes 16 hr to complete rather than the usual 10 (Conway

et al., 1997). Embryos injected with dominant-negative JAK1 show anterior defects, suggesting that the "epiboly signal" may be transduced through JAK1.

The other two cell movements, convergence and extension (CE), were recently shown to be regulated by STAT3 in zebrafish (Yamashita et al., 2002). Downregulation of STAT3 function with morpholino oligonucleotides impairs anterior movement of the axial mesoderm (prechordal mesendodermal or PCME cells) and dorsalward convergence of nonaxial mesoderm (lateral mesendodermal or LME cells), such that at the end of gastrula period the head is mispositioned and the embryonic axis is dramatically shortened. STAT3 is required in cells derived from the organizer (possibly in PCME cells) to drive PCME cells' anterior movement cell autonomously, and attract LME cells' dorsal convergence (Figure 5C) non-cell autonomously. Similar to mutations in components of the noncanonical Wnt signaling, reducing *stat3* function impairs LME cell CE without significant effect on cell fates in the gastrula.

Although *stat3* RNA is expressed ubiquitously at blastula stages and becomes confined to the anterior axial mesoderm in the gastrula, tyrosyl phosphorylation and presumed activation of STAT3 protein is observed exclusively in the dorsal side (including the future organizer) of the embryo. Activation of STAT3 on the dorsal side is regulated by the maternal Wnt/β-catenin pathway and requires a TCF transcription factor (Figure 5D). An upstream molecule (possibly an as yet unidentified cytokine or growth factor) capable of activating STAT3 in early embryogenesis is one of the targets of the maternal Wnt/β-catenin pathway (Figure 5D). No JAK has yet been shown to be involved in STAT3 activation. Among the three JAKs identified in zebrafish, *Jak1* and *Jak2b* are expressed before gastrulation, and *Jak2a* is expressed after gastrulation (Conway et al., 1997; Oates et al., 1999a, 1999b; Yamashita et al., 2002). As JAK1 is required for epiboly, JAK2b is a likely candidate for regulating STAT3.

The cell-autonomous requirement of the anterior migration of the PCME cells is similar to the requirement of the JAK/STAT signal transduction pathway in border cell migration during *Drosophila* oogenesis. These two migratory processes may be regulated similarly. In mouse, amphibian, and fly embryos, integrins, FAK, regulators of cytoskeletal reorganization (the small GTPases), and their activators have been implicated in the migration of mesodermal cells during gastrulation (Yang et al., 1993; Ilic et al., 1995; Barrett et al., 1997; Hacker and Perrimon, 1998). A recent report showed direct binding of STAT3 to the Rac1 GTPase (Simon et al., 2000). Thus, it is possible that a similar JAK/STAT/Rac/cytoskeleton pathway regulates the anterior migration of zebrafish LCME cells as well as border cell migration during *Drosophila* oogenesis.

The non-cell autonomous requirement of STAT3 in CE of LME cells is similar to the requirement for the JAK/STAT signal transduction pathway in *Drosophila* planar cell polarity (see below). The JAK/STAT signal may regulate the expression of a second signal in PCME cells. The LME cells could sense the local gradient of the second signal and converge dorsally. However, the upstream events of the JAK/STAT pathway in the *Drosophila* planar cell polarity and the zebrafish LME cell CE appear different. In zebrafish, the maternal Wnt pathway directly regulates the JAK/STAT signal transduction pathway, which may in turn regulate a second downstream signal. In the *Drosophila* eye disc, Wingless (Wg) and UPD are expressed in different positions and the two signals may converge on regulating a downstream second signal (Zeidler et al., 1999b; see below).

CE in Stigmatophore and Hindgut Morphogenesis in Drosophila

The posterior spiracles of *Drosophila* larvae are developed from two cell populations (Hu and Castelli-Gair Hombría, 1999). The inner cells give rise to the spiracle chamber by elongating into bottle-shaped cells. The surrounding cells give rise to a protruding stigmatophore by changing their relative positions. The morphogenetic mechanism used for formation of the stigmatophore of *Drosophila* posterior spiracles is similar to the CE movements during zebrafish gastrulation. The cells do not change shape very markedly, but rearrange their positions in the epithelium, resulting in elongation of the stigmatophore (Hu and Castelli-Gair Hombría, 1999). Strong *dome* mutations perturb stigmatophore formation (Brown et al., 2001). Furthermore, *stat92E* enhances the stigmatophore phenotype of the weak *dome* allele, suggesting that the whole HOP/STAT92E pathway regulates stigmatophore morphogenesis.

Elongation of the *Drosophila* hindgut has also been proposed to correspond to a CE mechanism. The developing hindgut becomes subdivided along the AP axis into three morphologically distinct regions: small intestine, large intestine, and rectum (Figure 6B). The small intestine is the most anterior region of the hindgut, and connects to the posterior midgut. At the anterior of the small intestine are two ureters, each of which drains a pair of Malpighian tubules; just posterior to the insertion of the ureters are the cells of the imaginal ring, which will develop into the anterior of the adult hindgut epithelium (Lengyel and Iwaki, 2002). Elongation of the internalized *Drosophila* hindgut primordium is driven by cell rearrangements similar to the events occurring in CE during zebrafish gastrulation, and analogous to the cell movements and intercalation seen in the elongating *Drosophila* stigmatophore. The HOP/STAT92E signal transduction pathway has been proposed to play an important role in hindgut cell rearrangements. STAT92E protein is expressed very early in the hindgut primordium (Figure 6A). *upd* is also expressed very early, and only in the small intestine (Lengyel and Iwaki, 2002; Figure 6B). Absence of *upd* results in a defect in both narrowing and elongation of the hindgut (Lengyel and Iwaki, 2002).

The JAK/STAT Pathway in Planar Polarity

Studies of the *Drosophila* compound eye have revealed a role of the JAK/STAT pathway in ommatidial rotation, a process that may be related to the cell rearrangement processes described above. In support of this, the signaling pathway that controls planar polarity in *Drosophila* also functions in vertebrate embryos during CE (Heisenberg et al., 2000; Adler, 2002; Myers et al., 2002; Wallingford et al., 2002).

The compound eye consists of ~800 individual facets, each formed by one photoreceptor cluster (an ommatidium) that is composed of only ten cell types: three types

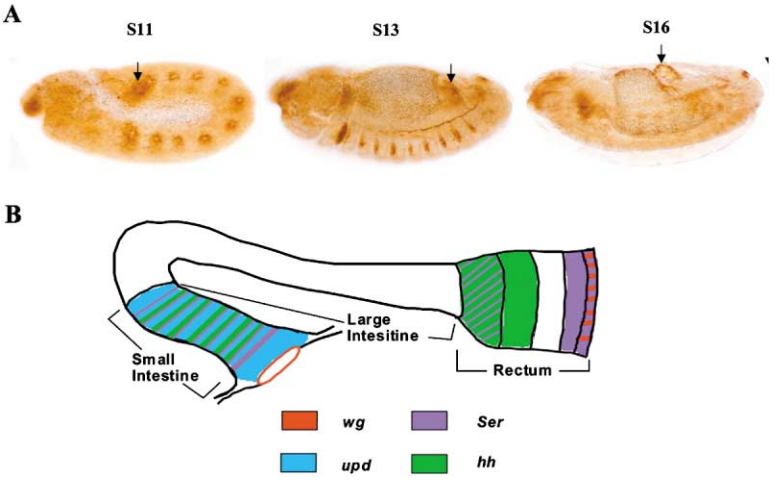

Figure 6. Patterning of the *Drosophila* Hindgut Epithelium

(A) STAT92E protein is expressed in the hindgut primordiums in stage 11, 13, and 16 embryos (arrows). The stainings are provided by Z.Z. in the S.X.H. laboratory.

(B) At stage 13, distinct domains within the hindgut epithelium are recognized by the localized expression of *upd*, *hh*, *wg*, and *Ser*. *upd*, which is only expressed in the small intestine but not in the large intestine, may play a role in the orientation of cell rearrangement. The lateral view of the hindgut is drawn based on information in Lengyel and Iwaki (2002).

of photoreceptor neurons (the basal central R8, six outer cells [R1–6], and the apical central R7), and seven types of non-light-sensitive accessory cells (Wolff and Ready, 1993). The eye develops during larval and pupal stages from the eye imaginal disc that is a monolayer epithelium of identical cells. Photoreceptor differentiation occurs in a wave that moves across the disc, posteriorly to anteriorly, over a period of ~2 days. As differentiation proceeds, photoreceptor cells are sequentially recruited into the nascent ommatidia. The ommatidia initially possess internal mirror image symmetry about the D/V axis and point posteriorly, but as differentiation progresses, the ommatidia rotate 90 degrees and become asymmetric. Clusters on either side of the D/V midline (subsequently known as the equator) rotate in opposite directions and take on opposite chiral forms. In the dorsal hemisphere of the right eye, the R3 cell is anterior to that of R4, and also dorsal to it. The ommatidia of the ventral hemisphere are inverted in the D/V direction so that R3 is ventral, but remains anterior. Thus, there are two mirror image fields containing two chiral forms of ommatidia.

Ommatidial rotation is cooperatively regulated by the activity of the JAK/STAT, Wg, and Notch (N) pathways (Zeidler et al., 1999b, 2000a). Ommatidia within *hop* and *Stat*92E loss-of-function clones contain all cell types and appear to differentiate correctly, and most rotate as anticipated. However, ommatidia situated close to the margin of large or broad mutant regions farthest from the equator often assume a 180° inverted orientation. Interestingly, ommatidial clusters at this boundary of *hop* mutant clones composed entirely of wild-type cells can still be inverted; this unexpected nonautonomous effect of *hop* mutant clones suggests that the HOP/STAT92E pathway is actually acting in a nonautonomous manner (on neighboring cells). This unexpected outcome is thought to result from a second diffusible molecule that is a downstream target of the JAK/STAT pathway. According to this model, the local gradient of the second signal is able to control the ommatidial rotation in the juxtaposed wild-type tissue.

The *four-jointed* (*fj*) gene may be a candidate for the second signal because it is expressed in a broad gradient across the developing eye (Zeidler et al., 1999a, 2000a, 2000b). The Fj protein is a putative type II transmembrane protein with an extracellular carboxyl terminus (Villano and Katz, 1995). The carboxyl terminus is likely to be cleaved and released as a secreted peptide that could act as a diffusible signaling molecule. Both *fj* gain- and loss-of-function clones produce ommatidia inversion phenotypes (Zeidler et al., 1999a). Furthermore, *fi* expression is regulated by the JAK/STAT pathway. *fj* is downregulated in *hop* mutant clones and upregulated in and around clones of cells that misexpress UPD. However, mutations in *fj* produce only modest polarity phenotypes, so if Fj is the second factor, it is likely to be redundant (Zeidler et al., 1999a, 2000a, 2000b). A recent study focusing on the role of the atypical cadherins *dachsous* (*ds*) and *fat* (*ft*) suggested that the establishment of this second signal is complex (Yang et al., 2002). The authors showed that *ft* promotes R3 development in a *frizzled* (*fz*)-dependent manner, while *ds* promotes R4 development in an *ft*-dependent manner. In addition, in mosaic ommatidia, *fj* promotes R3 development in a *ds*-dependent manner, suggesting that *fj* functions upstream of *ds* (Yang et al., 2002). A combination of *ft*, *ds*, and *fj* may define the proposed second signal.

Additional Function of the JAK/STAT Signal Transduction Pathway in *Drosophila* Development

Pair-Rule Gene Expression

Embryos mutant for *hop*, *stat92E*, *dome/mom*, or *upd* exhibit characteristic segmentation defects (Binari and Perrimon, 1994; Hou et al., 1996; Yan et al., 1996; Harrison et al., 1998; Brown et al., 2001; Chen et al., 2002). Proper embryonic segmentation in *Drosophila* is established by several sets of temporally expressed genes (gap, pair-rule, and segment polarity), and in *upd*, *hop*, and *stat92E* mutant embryos, the expression of several pair-rule genes, such as *even-skipped* (*eve*), *fushi tarazu*, and *runt* (Binari and Perrimon, 1994; Hou et al., 1996), is perturbed.

In *upd*, *hop*, and *stat92E* mutant embryos, expression of *eve* stripes 3 and 5 is significantly reduced (Binari and Perrimon, 1994; Hou et al., 1996; Yan et al., 1996; Harrison et al., 1998). Although the regulatory elements

controlling stripe 5 have not been identified, a 500 bp regulatory sequence in the *eve* promoter is sufficient to drive *eve* stripe 3 expression (Binari and Perrimon, 1994; Small et al., 1996). There are two consensus STAT binding sites, and several sites for the gap genes *Hunchback* (*Hb*) and *Knirps* (*Kni*) in the 500 bp sequence. Furthermore, in vitro assays indicate that tyrosine-phosphorylated, activated STAT92E directly bind to these sites. Moreover, the 500 bp sequence no longer drives *eve* 3 expression when the STAT binding sites are mutated in transgenic flies (Yan et al., 1996). Besides STAT92E, Hb and Kni also bind to the 500 bp sequence, and the anterior and posterior borders of *eve* 3 are defined through repression by Hb and Kni, respectively (Binari and Perrimon, 1994; Hou et al., 1996; Small et al., 1996; Yan et al., 1996; Hou and Perrimon, 1997). In conclusion, the HOP/STAT92E pathway directly regulates expression of pair-rule genes for segmentation, which is accomplished through cooperation with other gap genes.

Sex Determination

Somewhat surprisingly, the JAK/STAT pathway also plays a role in sex determination. In *Drosophila*, determination of sexual identity is controlled by the master gene *sex-lethal* (*sxl*). The initial expression of *sxl* is stimulated by positively acting factors located on the X chromosome and negatively regulated by the product of the autosomal gene *deadpan* (Cline and Meyer, 1996). In male embryos, the dose of positive X-linked regulators is not sufficient to overcome *deadpan* repression. However, extra doses of these activators in the female embryo can overcome the autosomal repression and drive *sxl* expression (Zeidler and Perrimon, 2000; Zeidler et al., 2000a). One of these X-linked activators has turned out to be *upd*, suggesting that the JAK/STAT pathway is involved in *sxl* transcription. Indeed, further studies using reporter genes demonstrated that the canonical JAK/STAT pathway is required for appropriate expression of *sxl* (Jinks et al., 2000; Sefton et al., 2000).

Trachea Formation in Drosophila

The embryonic tracheal system in *Drosophila* is an epithelial tubular network that is established from defined sets of ectodermal precursor cells (for a review, see Metzger and Krasnow, 1999). At stage 10 of embryonic development, segmentally repeated lateral clusters of ectodermal cells on both sides of the ten posterior parasegments (T2–A8) invaginate and form 20 tracheal placodes (sacs). They undergo two more rounds of cell division to generate ~80 cells in each placode. At stage 11, the clusters of cells invaginate to form tracheal pits and then carry out a stereotypical migration pattern. Subsequently, the branches of tracheal cells from the adjacent segments fused together to form a continuous tubular network. Finally, terminal tracheal cells send long extensions toward the target cells, forming blind-ended tubes that are connected to the main tracheal network. The trachea is then connected to the posterior spiracle, forming a functional tracheal system (Hu and Castelli-Gair Hombría, 1999; Metzger and Krasnow, 1999).

The *trachealess* (*trh*) gene, encoding a bHLH-PAS protein, selects the tracheal primordia in the embryonic ectoderm and drives the conversion of these planar epithelial regions into sacs. *trh* is expressed in the tracheal primordia 1 to 2 hr before sac formation, and TRH forms a complex with TANGO (Metzger and Krasnow, 1999), a broadly expressed bHLH-PAS protein homologous to mammalian ARNT. The TRH-TANGO heterodimer presumably regulates target genes encoding cytoskeletal and cell surface proteins responsible for sac formation. It also prepares the sacs for the branching events that follow by triggering expression of genes required for branching.

Tracheal formation was examined in *hop*, *mom*, and *stat92E* embryos (Brown et al., 2001; Chen et al., 2002) by using tracheal markers (Sutherland et al., 1996). Although *trh* expression is completely abolished and tracheal formation is completely blocked in *hop* null embryos, in paternally rescued *hop* embryos, residual *trh* transcripts were detected in tracheal pits and a defective tracheal system formed (Chen et al., 2002). These results suggest that the HOP/STAT92E pathway may control tracheal formation through regulating *trh* gene expression.

Emerging Themes on the JAK/STAT Signal Transduction Pathway

In the past few years, studies from model organisms have established that the JAK/STAT pathway is a central component of the signal transduction network that controls cell proliferation, fate, and movement. The outcome of JAK/STAT signaling in development depends on its collaboration with other signals and/or pathways. For example, during the control of cell proliferation, the JAK/STAT pathway likely cooperates with cell cycle regulators. In cell fate determination events, pathways such as the Ras/Raf/MAPK cassette counterbalances JAK/STAT signaling, as illustrated in regulation of *Drosophila* male germline stem cell self-renewal. During cell migration, the JAK/STAT signal may connect to the Rac/cytoskeleton pathway. Finally, in the establishment of planar polarity, the JAK/STAT pathway cooperates with other pathways such as Wnt and N to produce a secondary signal.

How can the same JAK/STAT pathway be used to produce completely different developmental outcomes in different tissue contexts? The answer to this question will require an understanding of the array of transcription factors and molecules expressed in specific cells (history of the cell), as well as a description of the multiple signals received by a single cell (combinatorial signaling). Characterization of the genes that are regulated by JAK/STAT, as well as the identification of signaling pathways that cooperate with JAK/STAT signaling, are promising avenues toward understand how this pathway can be involved in such a wide array of functions.

Acknowledgments

We thank Dr. Su-Wan Oh for helping in preparing figures, Dr. Hong Wang for helping in collecting information, Dr. Bernard Mathey-Prevot for careful reading the manuscript, Dr. Jeffrey G. Williams for communicating unpublished results and commenting on the manuscript, and Dr. Denise J. Montell for providing us with unpublished border cell drawings and commenting on the manuscript. This work is supported in part by a grant from the US Army (award number: DAMD17-00-1-0356) to S.X.H. N.P. is an Investigator of the Howard Hughes Medical Institute.

References

Abe, T., Early, A., Siegert, F., Weijer, C., and Williams, J.G. (1994). Patterns of cell movement within the *Dictyostelium* slug revealed by cell type-specific, surface labeling of living cells. Cell *77*, 687–699.

Adler, P.N. (2002). Planar signaling and morphogenesis in *Drosophila*. Dev. Cell *2*, 525–535.

Araki, T., Gamper, M., Early, A., Fukuzawa, M., Abe, Y., Kawata, T., Kim, E., Firtel, R.A., and Williams, J.G. (1998). Developmentally and spatially regulated activation of a *Dictyostelium* STAT protein by a serpentine receptor. EMBO J. *17*, 4018–4028.

Bach, E.A., and Perrimon, N. (2001). The role of the JAK-STAT in hematopoeisis and immune responses in *Drosophila*. In The JAK-STAT Pathway in Hematopoeisis, A. Ward, ed. (Georgetown, TX: Landes Bioscience), eurekah.com.

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.

Barrett, K., Leptin, M., and Settleman, J. (1997). The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in *Drosophila* gastrulation. Cell *91*, 905–915.

Beccari, S., Teixeira, L., and Rφrth, P. (2002). The JAK/STAT pathway is required for border cell migration during *Drosophila* oogenesis. Mech. Dev. *111*, 115–123.

Betz, A., Lampen, N., Martinek, S., Young, M.W., and Darnell, J.E. (2001). A *Drosophila* PIAS homologue negatively regulates *stat92E*. Proc. Natl. Acad. Sci. USA *98*, 9563–9568.

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.

Boutros, M., Agaisse, H., and Perrimon, N. (2002). Sequential activation of signaling pathways during innate immune responses in *Drosophila*. Dev. Cell *3*, 711–722.

Bromberg, J.F. (2002). Stat proteins and oncogenesis. J. Clin. Invest. *109*, 1139–1142.

Bromberg, J.F., Wrzeszczynska, M.H., Devgan, G., Zhao, Y., Pestell, R.G., Albanese, C., and Darnell, J.E., Jr. (1999). STAT3 as an oncogene. Cell *98*, 295–303.

Brown, S., Hu, N., and Castelli-Gair Hombría, J. (2001). Identification of the first invertebrate interleukin JAK/STAT receptor, the *Drosophila* gene *domeless*. Curr. Biol. *11*, 1700–1705.

Callus, B.A., and Mathey-Prevot, B. (2002). SOCS36E, a novel *Drosophila* SOCS protein, suppresses JAK/STAT and EGF-R signaling in the imaginal disc. Oncogene *21*, 4812–4821.

Castelli-Gair Hombría, J., and Brown, S. (2002). The fertile field of *Drosophila* jAK/STAT signaling. Curr. Biol. *12*, R569–R575.

Chen, H.W., Chen, X., Oh, S.W., Marrinissen, M.J., Gutkind, J.S., and Hou, S.X. (2002). *mom* identifies a receptor for the *Drosophila* JAK/STAT signal transduction pathway and encodes a protein distantly related to the mammalian cytokine receptor family. Genes Dev. *16*, 388–398.

Cline, T.W., and Meyer, B.J. (1996). Vive la différence: males vs. females in flies vs. worms. Annu. Rev. Genet. *30*, 637–702.

Conway, G., Margoliath, A., Wong-Madden, S., Roberts, R.J., and Gilbert, W. (1997). Jak1 kinase is required for cell migrations and anterior specification in zebrafish embryos. Proc. Natl. Acad. Sci. USA *94*, 3082–3087.

Corwin, H.O., and Hanratty, W.P. (1976). Characterization of a unique lethal tumorous mutation in *Drosophila*. Mol. Gen. Genet. *144*, 345–347.

Dearolf, C.R. (1998). Fruit fly "leukemia." Biochim. Biophys. Acta *1377*, M13–M23.

Decker, T. (1999). Introduction: STATs as essential intracellular mediators of cytokine responses. Cell. Mol. Life Sci. *55*, 1505–1508.

Decker, T., Stockinger, S., Karaghiosoff, M., Muller, M., and Kovarik, P. (2002). IFNs and STATs in innate immunity to microorganisms. J. Clin. Invest. *109*, 1271–1277.

Early, A.E., Abe, T., and Williams, J.G. (1995). Evidence for positional differentiation of prestalk cells and for a morphogenetic gradient in *Dictyostelium*. Cell *83*, 91–99.

Fukuzawa, M., Araki, T., Adrian, I., and Williams, J.G. (2001). Tyrosine phosphorylation-independent nuclear translocation of a *Dictyostelium* STAT in response to DIF signaling. Mol. Cell *7*, 779–788.

Fuller, M.T. (1993). Spermatogenesis. In The Development of *Drosophila melanogaster*, M. Bate and A. Martinez-Arias, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 71–147.

Girault, J.A., Labesse, G., Mornon, J.P., and Callebaut, I. (1999). The N-termini of FAK and JAKs contains divergent band 4.1 domains. Trends Biochem. Sci. *24*, 54–57.

Gupta, T., and Schüpbach, T. (2001). Two signals are better than one: border cell migration in *Drosophila*. Dev. Cell *1*, 443–445.

Hacker, U., and Perrimon, N. (1998). DRhoGEF2 encodes a member of the Dbl family of oncogenes and controls cell shape changes during gastrulation in *Drosophila*. Genes Dev. *12*, 274–284.

Hari, K.L., Cook, K.R., and Karpen, G.H. (2001). The *Drosophila Su(var)2-10* locus regulates chromosome structure and function and encodes a member of the PIAS protein family. Genes Dev. *15*, 1334–1348.

Harrison, D.A., Binari, R., Nahreini, 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.

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.

Heisenberg, C.P., Tada, M., Rauch, G.J., Saude, L., Concha, M.L., Geisler, R., Stemple, D.L., Smith, J.C., and Wilson, S.W. (2000). Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. Nature *405*, 76–81.

Henriksen, M.A., Betz, A., Fuccillo, M.V., and Darnell, J.E. (2002). Negative regulation of STAT92E by an N-terminally truncated STAT protein derived from an alternative promoter site. Gens Dev. *16*, 2379–2389.

Hou, X.S., and Perrimon, N. (1997). The JAK-STAT pathway in *Drosophila*. Trends Genet. *13*, 105–110.

Hou, S.X., 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.

Hu, N., and Castelli-Gair Hombría, J. (1999). Study of the posterior spiracles of *Drosophila* as a model to understand the genetic and cellular mechanisms controlling morphogenesis. Dev. Biol. *214*, 197–210.

Ilic, D., Furuta, Y., Kanazawa, S., Takeda, N., Sobue, K., Nakatsuji, N., Nomura, S., Fujimoto, J., Okada, M., and Yamamoto, T. (1995). Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature *377*, 539–544.

Imada, K., and Leonard, W.J. (2000). The Jak-STAT pathway. Mol. Immunol. *37*, 1–11.

Jinks, T.M., Polydorides, A.D., Calhoun, G., and Schedl, P. (2000). The JAK/STAT signaling pathway is required for the initial choice of sexual identity in *Drosophila melanogaster*. Mol. Cell *5*, 581–587.

Karsten, P., Häder, S., and Zeidler, M.P. (2002). Cloning and expression of *Drosophila SOCS36E* and its potential regulation by the JAK/STAT pathway. Mech. Dev. *117*, 343–346.

Kawata, T., Shevchenko, A., Fukuzawa, M., Jermyn, K.A., Totty, N.F., Zhukovskaya, N.V., Sterling, A.E., Mann, M., and Williams, J.G. (1997). SH2 signaling in a lower eukaryote: a STAT protein that regulates stalk cell differentiation in *Dictyostelium*. Cell *89*, 909–916.

Kieslinger, M., Woldman, I., Moriggl, R., Hofmann, J., Marine, J.C., Ihle, J.N., Beug, H., and Decker, T. (2000). Antiapoptotic activity of Stat5 required during terminal stages of myeloid differentiation. Genes Dev. *14*, 232–244.

Kiger, A.A., White-Cooper, H., and Fuller, M.T. (2000). Somatic support cells restrict germline stem cell self-renewal and promote differentiation. Nature *407*, 750–754.

Kiger, A.A., Jones, D.L., Schulz, C., Rogers, M.B., and Fuller, M.T. (2001). Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. Science *294*, 2542–2545.

Kimbrell, D.A., and Beutler, B. (2001). The evolution and genetics of innate immunity. Nat. Rev. Genet. *2*, 256–267.

Lacronique, V., Boureux, A., Valle, V.D., Poirel, H., Quang, C.T., Mauchauffe, M., Berthou, C., Lessard, M., Berger, R., Ghysdael, J., and Bernard, O.A. (1997). A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. Science *278*, 1309–1312.

Lagueux, M., Perrodou, E., Levashina, E.A., Capovilla, M., and Hoffmann, J.A. (2000). Constitutive expression of a complement-like protein in toll and JAK gain-of-function mutants of *Drosophila*. Proc. Natl. Acad. Sci. USA *97*, 11427–11432.

Lanot, R., Zachary, D., Holder, F., and Meister, M. (2001). Postembryonic hematopoiesis in *Drosophila*. Dev. Biol. *230*, 243–257.

Lebestky, T., Chang, T., Hartenstein, V., and Bannerjee, U. (2000). Specification of *Drosophila* hematopoietic lineage by conserved transcription factors. Science *288*, 146–149.

Lehmann, R. (2001). Cell migration in invertebrates: clues from border and distal tip cells. Curr. Opin. Genet. Dev. *11*, 457–463.

Lengyel, J.A., and Iwaki, D.D. (2002). It takes guts: the *Drosophila* hindgut as a model system for organogenesis. Dev. Biol. *243*, 1–19.

Levy, D.E. (1999). Physiological significance of STAT proteins: investigations through gene disruption in vivo. Cell. Mol. Life Sci. *55*, 1559–1567.

Liu, X., Quinn, A.M., Chin, Y.E., and Fu, X.Y. (1999). STAT genes found in *C. elegans*. Science *285*, 167a.

Lohi, O., and Lehto, V.P. (2001). STAM/EAST/Hbp adapter proteins-integrators of signaling pathways. FEBS Lett. *508*, 287–290.

Luo, H., Hanratty, W.P., and Dearolf, C.R. (1995). An amino acid substitution in the *Drosophila hop*$^{Tum\text{-}l}$ JAK kinase causes leukemia-like hematopoietic defects. EMBO J. *14*, 1412–1420.

Luo, H., Rose, P., Barber, D., Hanratty, W.P., Lee, S., Roberts, T.M., D'Andrea, A.D., and Dearolf, C.R. (1997). Mutation in the JAK kinase JH2 domain hyperactivates *Drosophila* and mammalian JAK-STAT pathways. Mol. Cell. Biol. *17*, 1562–1571.

Luo, H., Asha, H., Kockel, L., Parke, T., Mlodzik, M., and Dearolf, C.R. (1999). The *Drosophila* JAK kinase Hopscotch is required for multiple developmental processes in the eye. Dev. Biol. *213*, 432–441.

Luo, H., Rose, P.E., Roberts, T.M., and Dearolf, C.R. (2002). The hopscotch Jak kinase requires the Raf pathway to promote blood cell activation and differentiation in *Drosophila*. Mol. Genet. Genomics *267*, 57–63.

Matsuda, T., Nakamura, T., Nakao, K., Arai, T., Katsuki, M., Heike, T., and Yokota, T. (1999). STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. EMBO J. *18*, 4261–4269.

McGregor, J.R., Xi, R., and Harrison, D.A. (2002). JAK signaling is somatically required for follicle cell differentiation in *Drosophila*. Development *129*, 705–717.

Mesilaty-Gross, S., Reich, A., Motro, B., and Wides, R. (1999). The *Drosophila STAM* homolog is in a tight gene cluster, and its expression correlates to that of the adjacent gene *ial*. Gene *231*, 173–186.

Metzger, R.J., and Krasnow, M.A. (1999). Genetic control of branching morphogenesis. Science *284*, 1635–1639.

Mohanty, S., Jermyn, K.A., Early, A., Kawata, T., Aubry, L., Ceccarelli, A., Schaap, P., Williams, J.G., and Firtel, R.A. (1999). Evidence that the *Dictyostelium* Dd-STATa protein is a repressor that regulates commitment to stalk cell differentiation and is also required for efficient chemotaxis. Development *126*, 3391–3405.

Moriggl, R., Topham, D.J., Teglund, S., Sexl, V., McKay, C., Wang, D., Hoffmeyer, A., van Deursen, J., Sangster, M.Y., Bunting, K.D., et al. (1999). STAT5 is required for IL-2-induced cell cycle progression of peripheral T cells. Immunity *10*, 249–259.

Mui, A.L.F. (1999). The role of STATs in proliferation, differentiation, and apoptosis. Cell. Mol. Life Sci. *55*, 1547–1558.

Myers, D.C., Sepich, D.S., and Solnica-Krezel, L. (2002). Convergence and extension in vertebrate gastrulae: cell movements according to or in search of identity? Trends Genet. *18*, 447–455.

Oates, A.C., Wollberg, P., Pratt, S.J., Paw, B.H., Johnson, S.L., Ho, R.K., Postlethwatt, J.H., Zon, L.I., and Wilks, A.F. (1999a). Zebrafish *stat3* is expressed in restricted tissues during embryogenesis and *stat1* rescues cytokine signaling in a *STAT1*-deficient human cell line. Dev. Dyn. *215*, 352–370.

Oates, A.C., Brownlie, A., Pratt, S.J., Irvine, D.V., Liao, E.C., Paw, B.H., Dorian, K.J., Johnson, S.L., Postlethwatt, J.H., Zon, L.I., and Wilks, A.F. (1999b). Gene duplication of zebrafish *JAK2* homologs is accompanied by divergent embryonic expression patterns: only *jak2a* is expressed during erythropoiesis. Blood *94*, 2622–2636.

O'Shea, J.J., Gadina, M., and Schreiber, R.D. (2002). Cytokine signaling in 2002: new surprises in the JAK/STAT pathway. Cell *109*, S121–S131.

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.

Sano, S., Itami, S., Takeda, K., Tarutani, M., Yamaguchi, Y., Miura, H., Yoshikawa, K., Akira, S., and Takeda, J. (1999). Keratinocyte-specific ablation of STAT3 exhibits impaired skin remodeling, but does not affect skin morphogenesis. EMBO J. *18*, 4657–4668.

Schindler, C.W. (2002). JAK-STAT signaling in human disease. J. Clin. Invest. *109*, 1133–1137.

Schindler, C.W., and Darnell, J.E., Jr. (1995). Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. Annu. Rev. Biochem. *64*, 621–651.

Schwaller, J., Parganas, E., Wang, D., Cain, D., Aster, J.C., Williams, I.R., Lee, C.K., Gerthner, R., Kitamura, T., Frantsve, J., et al. (2000). Stat5 is essential for the myelo- and lymphoproliferative disease induced by TEL/JAK2. Mol. Cell *6*, 693–704.

Sefton, L., Timmer, J.R., Zhang, Y., Beranger, F., and Cline, T.W. (2000). An extracellular activator of the *Drosophila* JAK/STAT pathway is a sex-determination signal element. Nature *405*, 970–973.

Shuai, K. (2000). Modulation of STAT signaling by STAT-interacting proteins. Oncogene *19*, 2638–2644.

Siegert, F., and Weijer, C. (1997). Control of cell movement during multicellular morphogenesis. In *Dictyostelium*: A Model System for Cell and Development Biology, Y. Maeda, K. Inouye, and I. Takeuchi, eds. (Tokyo, Japan: Universal Academy Press), pp. 425–436.

Silver, D.L., and Montell, D.J. (2001). Paracrine signaling through the JAK/STAT pathway activates invasive behavior of ovarian epithelial cells in *Drosophila*. Cell *107*, 831–841.

Simon, A.R., Vikis, H.G., Stewart, S., Fanburg, B.L., Cochran, B.H., and Guan, K.L. (2000). Regulation of STAT3 by direct binding to the Rac1 GTPase. Science *290*, 144–147.

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.

Smith, A.G. (2001). Self-renewal and differentiation of pluripotent embryonic stem cells. Dev. Biol. *235*, 289–290.

Spradling, A.C. (1993). Developmental genetics of oogenesis. In The Development of *Drosophila melanogaster*, M. Bate and A. Martinez Arias, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 1–70.

Starr, R., and Hilton, D.J. (1999). Negative regulation of the JAK/STAT pathway. Bioessays *21*, 47–52.

Sutherland, D., Samakovlis, C., and Krasnow, M. (1996). *Branchless* encodes a *Drosophila* FGF homologue that controls tracheal cell migration and the pattern of branching. Cell *87*, 1091–1101.

Takeda, K., Noguchi, K., Shi, W., Tanaka, T., Matsumoto, M., Yoshida, N., Kishimoto, T., and Akira, S. (1997). Targeted disruption of the mouse STAT3 gene leads to early embryonic lethality. Proc. Natl. Acad. Sci. USA *94*, 3801–3804.

Tepass, U., Fessler, L.I., Aziz, A., and Hartenstein, V. (1994). Embryonic origin of hemocytes and their relationship to cell death in *Drosophila*. Development *120*, 1829–1837.

Tran, J., Brenner, T.J., and DiNardo, S. (2000). Somatic control over

the germline stem cell lineage during *Drosophila* spermatogenesis. Nature *407*, 754–757.

Tulina, N., and Matunis, N. (2001). Control of stem cell self-renewal in *Drosophila* spermatogenesis by JAK-STAT signaling. Science *294*, 2546–2549.

Tworoger, M., Larkin, M.K., Bryant, Z., and Ruohola-Baker, H. (1999). Mosaic analysis in the *Drosophila* ovary reveals a common hedgehog-inducible precursor stage for stalk and polar cells. Genetics *151*, 739–748.

Villano, J.L., and Katz, F.N. (1995). Four-jointed is required for intermediate growth in the proximal-distal axis in *Drosophila*. Development *121*, 2767–2777.

Wallingford, J.B., Fraser, S.E., and Harland, R.M. (2002). Convergent extension: the molecular control of polarized cell movement during embryonic development. Dev. Cell *2*, 695–706.

Williams, J.G. (1999). Serpentine receptors and STAT activation: more than one way to twin a STAT. Trends Biochem. Sci. *24*, 333–334.

Williams, J.G., Duffy, K.T., Lane, D.P., McRobbie, S.J., Harwood, A.J., Traynor, D., Kay, R.R., and Jermyn, K.A. (1989). Origins of the prestalk-prespore pattern in *Dictyostelium* development. Cell *59*, 1157–1163.

Wolff, T., and Ready, D.F. (1993). Pattern formation in the *Drosophila* retina. In The Development of *Drosophila melanogaster*, M. Bate and A. Martinez Arias, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 1277–1326.

Yamashita, S., Miyagi, C., Carmany-Rampey, A., Shimizu, T., Fujii, R., Schier, A.F., and Hirano, T. (2002). STAT3 controls cell movements during zebrafish gastrulation. Dev. Cell *2*, 363–375.

Yan, R., Small, S., Desplan, C., Dearolf, C.R., and Darnell, J.J. (1996). Identification of a Stat gene that functions in *Drosophila* development. Cell *84*, 421–430.

Yang, J.T., Rayburn, H., and Hynes, R.O. (1993). Embryonic mesodermal defects in α 5 integrin-deficient mice. Development *119*, 1093–1105.

Yang, C.H., Axelrod, J.D., and Simon, M.A. (2002). Regulation of frizzled by fat-like cadherins during planar polarity signaling in the *Drosophila* compound eye. Cell *108*, 675–688.

Yeh, T.C., and Pellegrini, S. (1999). The Janus kinase family of protein tyrosine kinases and their role in signaling. Cell. Mol. Life Sci. *55*, 1523–1534.

Zeidler, M.P., and Perrimon, N. (2000). Sex determination: co-opted signals determine gender. Curr. Biol. *10*, R682–R684.

Zeidler, M.P., Perrimon, N., and Strutt, D.I. (1999a). The four-jointed gene is required in the *Drosophila* eye for ommatidial polarity specification. Curr. Biol. *9*, 1363–1372.

Zeidler, M.P., Perrimon, N., and Strutt, D.I. (1999b). Polarity determination in the *Drosophila* eye: a novel role for unpaired and JAK/STAT signaling. Genes Dev. *13*, 1342–1353.

Zeidler, M.P., Bach, E.A., and Perrimon, N. (2000a). The roles of the *Drosophila* JAK/STAT pathway. Oncogene *19*, 2598–2606.

Zeidler, M.P., Perrimon, N., and Strutt, D.I. (2000b). Multiple roles for four-jointed in planar polarity and limb patterning. Dev. Biol. *228*, 181–196.

Zhao, G.-Q., and Garbers, D.L. (2002). Male germ cell specification and differentiation. Dev. Cell *2*, 537–547.