JAK-STAT Signaling in Stem Cells

14

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Abstract

Adult stem cells are essential for the regeneration and repair of tissues in an organism. Signals from many different pathways converge to regulate stem cell maintenance and differentiation while preventing overproliferation. Although each population of adult stem cells is unique, common themes arise by comparing the regulation of various stem cell types in an organism or by comparing similar stem cell types across species. The JAK-STAT signaling pathway, identified nearly two decades ago, is now known to be involved in many biological processes including the regulation of stem cells. Studies in *Drosophila* first implicated JAK-STAT signaling in the control of stem cell maintenance in the male germline stem cell microenvironment, or niche; subsequently it has been shown play a role in other niches in both *Drosophila* and mammals. In this chapter, we will address the role of JAK-STAT signaling in stem cells in the germline, intestinal, hematopoietic and neuronal niches in *Drosophila* as well as the hematopoietic and neuronal niches in mammals. We will comment on how the study of JAK-STAT signaling in invertebrate systems has helped to advance our understanding of signaling in vertebrates. In addition to the role of JAK- STAT signaling in stem cell niche homeostasis, we will also discuss the diseases, including cancers, that can arise when this pathway is misregulated.

Keywords

JAK-STAT signaling • Germ line stem cell • Neural stem cell • Intestinal stem cell • Hematopoietic stem cell

14.1 Overview of the JAK-STAT Pathway

Since its initial discovery nearly two decades ago, the Janus kinase-signal transducers and activators of transcription (JAK-STAT) signaling

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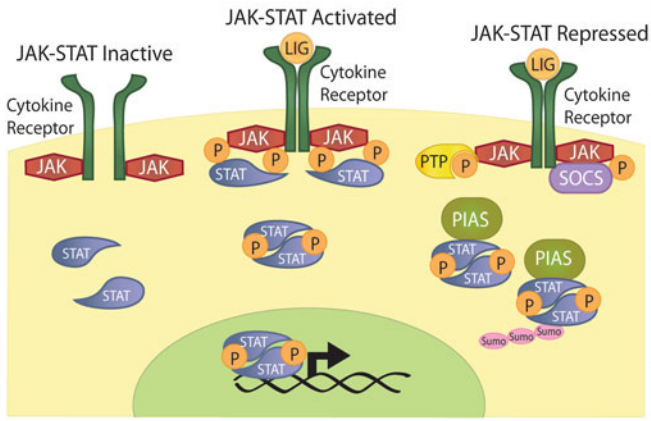

	Mammals	*Drosophila*
Ligand	Various	Upd, Upd2, Upd3
Receptor	Various	Dome, ET
JAK	JAK1-3, TYK2	Hop
STAT	STAT1-4, STAT5a STAT5b, STAT6	Stat92E
SOCS	CIS, SOCS1-7	Socs16E, Socs36E, Socs44A
PIAS	PIAS1-4	Su(Var)2-10
PTP	SHP-1, SHP-2 PTP1B, T-Cell PTP	PTP61F

Fig. 14.1 The JAK-STAT patway in mammals and *Drosophila*. JAK-STAT is inactive when no ligand is bound to a cognate cytokine receptor (*dark green*). Receptor subunits are disassociated and JAKs (*red*) are not phosphorylated. Inactive STATs (*bluish-purple*) remain in the cytoplasm. JAK-STAT is active when a ligand (*peach*) binds to its cognate cytokine receptor, causing receptor dimerization. JAKs are autophosphorylated (*light orange circles*) and recruit cytoplasmic STAT molecules, which are phosphorylated, dimerize and translocate into the nucleus to activate transcription of target genes. JAK-STAT is repressed when JAKs, STATs and/or receptors are dephosphorylated by protein tyrosine phosphotases (*PTPs*, *yellow*), JAKs are bound by SOCS proteins (*light purple*) or STATs are bound and/or SUMOylated by PIAS proteins (*green*). Components of the JAK-STAT signaling pathway in mammals and *Drosophila* are shown in the table

pathway has been implicated in diverse biological processes including immune response, hematopoiesis, neurogenesis, oncogenesis and control of many populations of stem cells [1]. Although the pathway was first identified in mammals, components are conserved through invertebrates including *Drosophila*, *C. elegans*, and even the slime mold *Dictyostelium* [2, 3]. Of these three invertebrate model systems, *Drosophila* has the most complete and well-conserved JAK-STAT pathway. Since the pathway was first described, JAK-STAT research in both the mammalian and *Drosophila* systems has progressed rapidly, leading to a greater understanding of both the normal function of the pathway and the pathobiology stemming from its misregulation.

JAK-STAT signaling is activated through a relatively simple mechanism that is well-conserved in both *Drosophila* and mammals (Fig. 14.1). Each cytoplasmic JAK associates with a cytokine receptor subunit. When a ligand activates its cognate receptor, the receptor dimerizes leading to a conformational change and, in some cases, the phosphorylation of the receptor. Activation of the receptor brings two associated JAKs into close proximity, allowing for their trans-phosphorylation. The phosphorylated JAKs then recruit inactive STAT molecules from the cytoplasm, activating them by phosphorylation. Two activated STAT molecules dimerize and translocate into the nucleus to activate the transcription of target genes. Additionally, several protein families can negatively regulate JAK/STAT signaling (reviewed by [4]). Protein tyrosine phosphatases inactivate signaling by directly removing activating phosphates on JAKs, STATs and/or cytokine receptors. Suppressors of Cytokine Signaling (SOCS) proteins bind to phosphorylated tyrosines on JAKs and their receptors, ultimately leading to their proteasomal degradation and preventing the activation of STATs. Protein Inhibitors of Activated STAT (PIAS) proteins bind to activated STAT molecules and prevent them from binding to DNA. PIAS proteins also function as SUMOylation E3 ligases, and STAT can be negatively regulated by PIAS-mediated SUMOylation, although this process has not been well studied [5–7]. Thus, the JAK-STAT signaling pathway can be regulated at several steps by the phosphorylation and dephosphorylation of multiple components.

14.1.1 JAK-STAT in Mammals

JAK-STAT was first discovered in mammals as a pathway capable of transducing signals from the surface of cells to genes in the nucleus. The Darnell lab was studying how extracellular glycoproteins released in response to infection (interferons or IFNs), lead to changes in gene expression in cultured cells. They discovered several proteins including STAT1 and STAT2 that were upregulated and phosphorylated in IFN-stimulated cells [8]. At around the same time, a novel family of kinases, called Janus kinases was identified and characterized by several labs as kinases that could be activated by IFN-dependent phosphorylation [9–12]. The relationship between the JAK and STAT families of proteins soon became apparent and JAK-STAT signaling was shown to bridge the gap between extrinsic IFN signaling and intrinsic changes in gene expression [13]. The pathway is now known to be activated by several families of ligands can including interferons, interleukins, growth factors and hormone factors. Various ligands can signal through different receptors or even heterodimeric combinations of receptors, allowing for diversity in the control of mammalian JAK-STAT signaling (reviewed by [14, 15]).

Years of research have uncovered great complexity in the downstream components of the JAK-STAT pathway in mammals. Currently, a total of four JAKs (JAK1-3 and Tyk2) have been identified. Jak1 and Tyk2 respond mainly to Interferons and Interleukins, Jak2 responds to a host of ligands including erythropoietin, thrombopoietin, growth hormone and interleukins and Jak3 responds to interleukins [3]. The four JAKs activate seven different STATs (STAT1-4, STAT5a, STAT5b and STAT6) in response to ligand binding, leading to a variety of transcriptional outcomes. Even within the same tissue, multiple JAKs and STATs can be activated to induce different responses. Negative regulators of the pathway, including multiple protein tyrosine phosphatases, eight SOCS proteins and four PIAS proteins, have also been identified in mammals [4]. With so many components in the mammalian pathway, there are functional redundancies for many of the genes, making JAK-STAT signaling a complex area of research in mammals.

14.1.2 JAK-STAT Signaling in *Drosophila*

Because of the complexity of the mammalian pathway, the *Drosophila* pathway, which lacks redundancy, has been used extensively as a model system for studying JAK-STAT signaling within tissues. *Drosophila* encode a single JAK homologue called *Hopscotch* (*Hop*) and a single STAT homologue called *Stat92E* (reviewed by [16–18]). Only three cytokine ligands, Unpaired or Outstretched, (Upd), Upd2 and Upd3, are known to activate JAK/STAT signaling in flies [19, 20]. These ligands bind to a JAK-STAT receptor called Domeless (Dome) [21]. Recently, a second Dome-related receptor, Eye transformer (CG14225) was shown to negatively regulate signaling by heterodimerizing with Dome and antagonizing its activity [22, 23]. JAK-STAT conservation in *Drosophila* extends to negative regulators of the pathway. *Drosophila* have three identified *SOCS* orthologs as well as a *PIAS* ortholog, all of which have been shown to play a role in antagonizing JAK-STAT signaling [16]. While *Drosophila* have several protein tyrosine phosphatase genes, only one, *Ptp61F*, has been linked to JAK-STAT signaling. In addition to having conservation of orthologs, the JAK-STAT pathway is involved in immunity, cell proliferation and stem cell control in both mammals and *Drosophila* indicating that the pathway has functional conservation, the hallmark of a good model pathway.

14.2 JAK-STAT Signaling in Stem Cell Niches

JAK-STAT signaling plays an integral role in many different stem cell niches in both *Drosophila* and mammals. We will begin by discussing the role of JAK-STAT in *Drosophila* stem cell niches which are, in general, better understood than their mammalian counterparts. We will then discuss

JAK-STAT signaling in mammalian stem cell niches, drawing similarities to *Drosophila* when possible. Although there is relatively little known about JAK-STAT signaling in many mammalian stem cell populations, future research will likely uncover additional roles for this pathway in stem cell biology in mammals.

14.3 JAK-STAT Signaling in *Drosophila* Stem Cells

JAK-STAT was first implicated as an important stem cell niche factor in the *Drosophila* testis over a decade ago [24, 25]. Since then, the pathway has also been shown to control the homeostasis of the *Drosophila* intestinal stem cell niche, the maintenance of prohemocytes in the larval lymph gland and the maintenance of neuroepithelial stem cells in the larval optic lobe (reviewed by 26–35]). JAK-STAT signaling also plays a role in the maintenance of stem cells in the ovary and the Malpighian tubules. These examples will not be discussed since there is relatively little information about JAK-STAT function in these stem cells [36, 37]. The involvement of JAK-STAT signaling in multiple stem cell populations indicates that it is a generally important pathway for proper stem cell function.

14.3.1 JAK-STAT and *Drosophila* Spermatogonial Stem Cells

The *Drosophila* testis contains a niche that supports two different populations of stem cells: germline stem cells (GSCs) and somatic cyst stem cells (CySCs). At the apex of the testis, a cluster of non-mitotic somatic cells called the hub signals to both types of stem cells (Fig. 14.2a). GSCs broadly adhere to the hub and undergo stereotypically oriented asymmetric divisions to displace differentiating daughter cells, called gonialblasts, away from the hub [38]. Somatic CySCs are attached to the hub by thin cytoplasmic extensions and divide asymmetrically to produce cyst cell daughters. Two cyst cells wrap around each gonialblast and support its differentiation. The gonialblast will undergo four transit amplifying divisions with incomplete cytokinesis to produce a cluster of 16 interconnected spermatogonial cells that will further differentiate into spermatocytes and, eventually, sperm (reviewed by [39]). A comprehensive review of *Drosophila* spermatogenesis can be found in Chap. 4 of this volume.

JAK-STAT was the first signaling pathway shown to play an important role in the maintenance of testis stem cells [24, 25]. In addition to its involvement in the establishment of male germline stem cells in the embryo [40], JAK-STAT signaling is active in both the GSCs and CySCs in the adult testis niche. The secreted ligand *Upd* is expressed specifically in the hub and activates the JAK-STAT pathway in the GSCs and the CySCs [24, 25]. The daughter cells of both the GSCs and CySCs are displaced further from the hub and receive less Upd signal, leading to a decreased level of pathway activation in daughter cells compared to stem cells. In testes lacking JAK-STAT signaling, both GSCs and CySCs differentiate and are lost from the niche. Individual GSCs and CySCs in which *Stat92E* has been clonally removed are also quickly lost from the tissue [24, 25, 41, 42]. These observations indicate that JAK-STAT signaling is cell-autonomously required to maintain both GSCs and CySCs in the testis niche.

While JAK-STAT signaling is needed for the maintenance of GSCs and CySCs at the hub, additional experiments show that JAK-STAT signaling is required for different aspects of stem cell maintenance in these two lineages. Ectopic STAT activation in the cyst cell lineage alone is sufficient to induce self-renewal of both the CySC and GSC lineages away from the niche [43]. Additionally, in testes where *Stat92E* is expressed in the CySC lineage but removed from the GSC lineage, the GSCs lose adherence to the hub but remain at the testis apex and do not differentiate [44]. Under these conditions, CySCs cluster around the hub, and GSCs contact the CySCs and continue to divide and self renew. In contrast, ectopic activation of *Stat92E* in the GSC lineage alone does not induce ectopic self-renewal of GSCs away from the niche. Taken together, these data indicate that JAK-STAT signaling in CySCs is sufficient to induce self-renewal of both GSCs and CySCs. Two putative transcriptional targets of Stat92E,

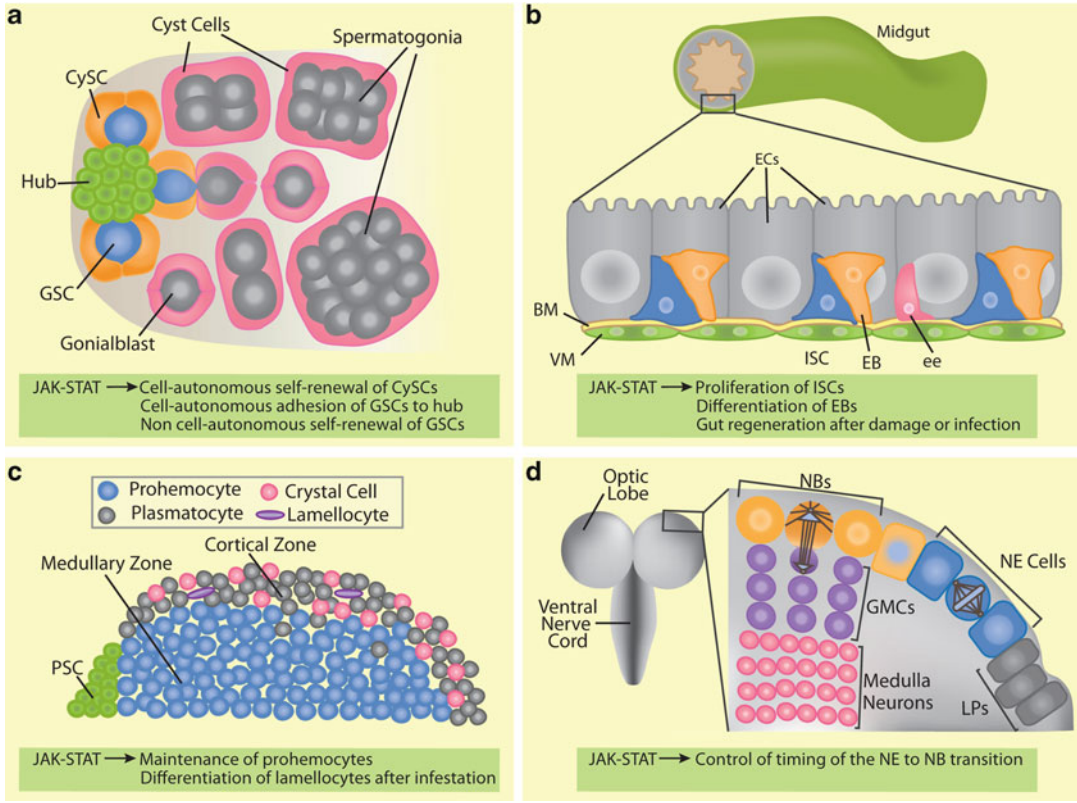

Fig. 14.2 JAK-STAT signaling in *Drosophila* stem cell niches. (**a**) At the testis tip, somatic cyst stem cells (*CySCs*, *Orange*) and germline stem cells (*GSCs*, *blue*) adhere to a cluster of somatic hub cells (*green*). GSCs divide asymmetrically to produce gonialblast daughters (*gray*, single cells) that amplify to produce spermatogonia (*gray*, clusters). CySCs divide asymmetrically to produce cyst cell daughters (*pink*) that wrap around the developing spermatogonia. (**b**) Intestinal stem cells (*ISCs*, *blue*) are scattered down the length of the midgut tube along the basement membrane (*BM*, *cream*) which is lined with visceral muscle (*VM*, *green*). ISCs divide asymmetrically to produce enteroblasts (*EBs*, *orange*). EBs directly differentiate into either enteroendocrine cells (*ee*, *pink*) or enterocytes (*ECs*, *gray*). (**c**) One primary lobe of the lymph gland contains a posterior signaling center (*PSC*, *green*) which signals to prohemocytes in the medullary zone. Prohemocytes differentiate into plasmatocytes, crystal cells and occasionally lamellocytes as they move into the cortical zone. (**d**) The optic lobe of the *Drosophila* brain (*light gray* structure) contains the outer proliferation center (*boxed area*, magnified). Neuroepithelial cells (*NE* cells, *blue*) divide symmetrically to expand in number before transitioning into asymmetrically dividing neuroblasts (*NBs*, *orange*) at the medial edge. NB cells give rise to ganglion mother cells (*GMCs*, *purple*), which undergo an amplifying division and differentiate into medulla neurons. At the lateral edge of the outer proliferation center, NE cells transition into lamina precursors (*LPs*, *gray*)

zinc finger homeodomain 1 (*zfh1*) and *chronologically inappropriate morphogenesis* (*chinmo*), can also induce self-renewal of the CySCs and GSCs away from the niche when they are ectopically expressed in CySC lineage [43, 45]. Although they are both required cell-autonomously for CySC but not GSC maintenance, they do not appear to genetically interact with one another, indicating that the two genes are working in parallel pathways in the CySCs [45]. It appears that JAK-STAT signaling in the CySCs has an important role in maintaining the self-renewal of both of the testis stem cell populations.

Although JAK-STAT signaling is not autonomously required in the GSCs for their self-renewal, JAK-STAT is required cell-autonomously for DE-cadherin-mediated adhesion of GSCs to the hub (reviewed by [39, 44]). In Stat92E-depleted testes, DE-cadherin, which is normally enriched at the contacts between hub cells and GSCs

begins to delocalize, indicating that adhesion of the GSCs to the hub is compromised [44]. This decreased adhesion explains why individual GSCs lacking Stat92E are not maintained in the tissue. CySC-mediated self-renewal of GSCs is mediated by the Bone Morphogenic Protein (BMP) pathway, which is known to be required for self-renewal in GSCs in both the *Drosophila* ovary and testis [46–50]. Hub cells release BMP ligands that activate BMP signaling in GSCs and GSCs with cell-autonomous loss of BMP signaling are lost from the niche [46, 47]. CySCs also express BMP ligands, which appear to be indirectly activated via JAK-STAT signaling, leading to activation of BMP signaling in the adjacent GSCs [44]. However, ectopic BMP signaling in the GSC lineage is not sufficient to induce GSC self-renewal away from the niche [46, 47, 50], indicating that a combination of ectopic Stat92E activation in the CySC lineage and a resulting increase in BMP signaling leads to increased self-renewal of the germ cell lineage away from the niche. While JAK-STAT signaling can regulate adhesion levels in the GSCs, its role in GSC self-renewal remains less clear.

Epigenetic factors also affect stem cell maintenance through JAK-STAT signaling in the testis. The chromatin remodeling factor Nurf301/BPTF, which functions as a negative regulator of JAK-STAT signaling in the *Drosophila* innate immune system, is specifically required in both GSCs and CySCs for their maintenance in the niche [51, 52]. In contrast to its role in innate immunity, *Nurf301* acts as positive regulator of JAK-STAT signaling in the testis. GSCs lacking *Nurf301* have decreased levels of Stat92E. In addition, *Nurf301* mutant GSCs show upregulation of a differentiation factor called Bag of Marbles (Bam). This indicates that GSCs lacking *Nurf301* begin to differentiate prematurely. Other epigenetic factors including members of the Polycomb suppressor family of proteins inhibit the JAK-STAT pathway in eye imaginal discs [53]. Although Polycomb proteins have not yet been implicated in *Drosophila* testis stem cell homeostasis, they do play roles in other *Drosophila* and mammalian niches [54], and further investigation is likely to yield insight into the connection between JAK-STAT and epigenetic regulation of stem cells.

With two types of stem cells residing in the same niche, maintaining the proper numbers of each cell type is important for proper niche function. This is accomplished, in part, by the action of Suppressor of Cytokine Signaling at 36E (Socs36E), which controls cell competition in the niche by modulating levels of cell adhesion [41]. Socs36E can dampen the activation of JAK-STAT signaling in the CySCs, leading to a balanced ratio of GSCs and CySCs surrounding the hub [41, 42]. While both populations of stem cells require DE-cadherin to adhere to the niche [55], CySCs also use integrin-mediated adhesion, and Socs36E can modulate integrin levels. If Socs36E levels are decreased, integrin levels in the CySCs increase, and they overtake the niche, displacing the GSCs [41]. Therefore, *Socs36E* mutant testes have a decreased number of GSCs and a higher ratio of CySCs to GSCs surrounding the hub. Additionally, individual CySCs lacking *Socs36E* can displace wildtype CySCs and GSCs from the niche due to higher levels of integrin expression. Although JAK-STAT signaling is required in both populations of stem cells, CySCs require a lower level of signaling than GSCs, and Socs36E specifically modulates JAK-STAT signaling levels in the CySCs to maintain the correct ratio of cell types. Maintaining this ratio allows the stem cell niche to function efficiently since two CySC daughters are needed to support the differentiation of each GSC daughter.

Previously, the differentiation of stem cells into more specialized progeny was thought to be a unidirectional process. We now know that this process is, at least to some extent, reversible. We call this "reverse differentiation" dedifferentiation. Manipulating JAK-STAT signaling in the testis stem cell niche can induce dedifferentiation [56]. If JAK-STAT signaling is conditionally ablated in the niche, the GSCs can no longer undergo self-renewing divisions and differentiate into spermatogonia. However, if JAK-STAT signaling is restored, spermatogonial cysts break down, or dedifferentiate, and germline cells regain contact with hub, resuming their self renewing divisions and regaining their stem

cell morphology. Dedifferentiation can also be induced by ectopically overexpressing the differentiation factor *Bam* and then restoring normal Bam levels [57]. Although dedifferentiation has now been demonstrated in mammalian systems, the mechanism is still not well understood [58]. JAK-STAT signaling seems to be required for spermatogonial dedifferentiation. Stat92E expression, which is normally restricted to GSCs and their daughters, is upregulated in some spermatogonial cysts near the hub in testes that are undergoing dedifferentiation [57]. Furthermore, spermatogonia overexpressing the JAK-STAT inhibitor *Socs36E* cannot dedifferentiate as efficiently as spermatogonia with uninhibited JAK-STAT signaling. It is not clear whether JAK-STAT mediates adhesion of dedifferentiating germ cells to the hub or allows the cells to re-establish self-renewing divisions, but just as JAK-STAT is important for maintaining homeostasis under normal conditions, the pathway plays an important role in re-establishing homeostasis after the niche has been disrupted.

14.3.2 JAK-STAT and *Drosophila* Intestinal Stem Cells

In addition to its role as a maintenance factor in the testis stem cell niche, JAK-STAT also controls stem cell homeostasis in the *Drosophila* posterior midgut. Gut tissue is subject to constant insults from bacteria and digestive by-products that can cause cellular damage. Intestinal stem cells (ISCs) divide to replenish and repair the tissue, and this replacement process must be carefully coordinated with the level of damage and loss (reviewed by [59]). The intestinal stem cell niche is unique in that ISCs are not clustered in a distinct location but rather scattered down the length of the posterior midgut, making contact with the basement membrane (Fig. 14.2b). ISCs divide asymmetrically to give rise to self-renewing ISC daughters and differentiating enteroblast (EB) daughters. EBs differentiate directly into one of two functionally and morphologically distinct cell types: absorptive enterocytes or secretory enteroendocrine cells [59, 60]. Notch is a major pathway controlling stem cell behavior in the ISC niche [61]. Although Notch is not active in ISCs, ISCs express the Notch ligand Delta, activating Notch signaling in the EBs. Transcription of Notch targets leads to the repression of self-renewal capability in the EBs. EBs with higher levels of Notch signaling become enterocytes, which are large, polyploid cells that make up the bulk of the midgut. EBs with lower levels of Notch signaling become enteroendocrine cells, small, diploid cells that are found in pairs interspersed throughout the midgut. In addition to Notch, many pathways interact to ensure homeostasis in the ISC stem cell niche of *Drosophila*. While this chapter focuses on the role of JAK-STAT signaling, a more comprehensive review of *Drosophila* intestinal stem cells can be found in Chap. 5 of this volume.

JAK-STAT signaling controls both ISC proliferation and EB differentiation in the *Drosophila* midgut. Signaling is activated via the Upd ligands, mainly Upd3. It is a matter of some controversy exactly which cells in the tissue produce Upd ligands, with different groups reporting that either the muscle cells surrounding the gut or the ISCs and EBs are the source [27, 28]. JAK-STAT signaling is activated in both the ISCs and EBs. Although clonal analysis indicates that JAK-STAT is not required for ISC self-renewal, ISCs lacking JAK-STAT signaling components are under proliferative [27–29]. Additionally, overexpression of *Upd* in ISCs and EBs leads to an increased division rate in these cells. On the other hand, upregulation of Notch signaling represses ISC division rates. When Notch signaling is repressed in the midgut, the effects of *Upd* overexpression on ISC division rates are enhanced [27, 29]. Whether Notch functions upstream, downstream or in parallel to JAK-STAT signaling is a matter of controversy in the literature [28, 29]. While Liu et al. report that Notch is required to repress JAK-STAT signaling, Beebe et al. show that JAK-STAT is required for proper Notch activation in EBs. Further experiments are needed to clarify the relationship between JAK-STAT and Notch signaling in the midgut. In addition to its role in ISC proliferation, JAK-STAT is necessary

for the multilineage differentiation of EB cells. Individual ISCs and EBs which lack components of the JAK-STAT pathway are not able to differentiate into enteroendocrine cells or enterocytes but rather form morphologically abnormal cells resembling EBs. Regardless of Notch signaling levels, EBs lacking JAK-STAT signaling fail to form mature midgut cell types, indicating that along with Notch signaling, JAK-STAT is required for proper EB differentiation [29]. JAK-STAT plays a complex role in the *Drosophila* intestinal niche, mediating both proliferation of ISCs and differentiation of EB progenitors in conjunction with other signaling pathways to maintain homeostasis in the midgut.

Because environmental conditions can change, a stem cell niche must be able to respond to external cues in order to maintain tissue homeostasis following insult. In response to infection or stress, JAK-STAT signaling induces a proliferative response to stimulate tissue regeneration in the *Drosophila* midgut. If the gut experiences cellular stress, large scale apoptosis caused by death-inducing transgenes, or infection with certain bacteria, expression of JAK-STAT signaling pathway members including the three *Upd* ligands are significantly upregulated [30, 31]. Following JAK-STAT upregulation, the midgut undergoes rapid proliferation and differentiation of ISCs. The Hippo pathway is believed to play a role in JAK-STAT activation in response to damage in the midgut [62–65]. Upon injury, the Hippo target Yorkie is upregulated, leading to the induction of *Upd* expression in enterocytes and increased JAK-STAT activation. In response to stress and insult, a niche must be able to respond to its environment, and in multiple stem cell niches, it is clear that JAK-STAT mediates this response. The pathway stimulates both the regeneration of damaged midguts and the dedifferentiation of germline cells in testes that have been depleted of stem cells.

14.3.3 JAK-STAT and *Drosophila* Hematopoietic Stem Cells

Hematopoiesis in *Drosophila* is a well-conserved process that generates blood cells (hemocytes) which are released into the circulating hemolymph. In larvae, hematopoiesis takes place in the lymph gland, a multilobular structure in which two, larger primary lobes each contain a hematopoietic niche. In each primary lobe, a cluster of cells called the posterior signaling center (PSC) signals to a pool of prohemocyte progenitor cells in the adjacent medullary zone (Fig. 14.2c, reviewed by [26, 66]). The prohemocytes move toward the outer cortical zone as they differentiate into mature hemocytes including plasmatocytes which are phagocytosing cells similar to monocytes, and crystal cells which are important for melanization and would healing. The PSC is required to maintain prohemocytes in the medullary zone [32, 67]. Signals in the PSC lead to JAK-STAT activation in the prohemocytes, and JAK-STAT signaling prevents premature prohemocyte differentiation [32]. JAK-STAT mediates this role, in part, by upregulating its target gene *u-shaped*, a Friend of GATA protein that regulates prohemocyte potency [68]. The mechanism by which the PSC activates JAK-STAT signaling in the prohemocytes is still under investigation; *Upd3* expression is detected in both the PSC and the medullary zone during the larval hematopoiesis but removal of *Upd3* transcription from the PSC has little effect on prohemocyte maintenance [22]. This indicates that another signal from the PSC is likely inducing JAK-STAT activation in the prohemocytes. The Notch pathway is active in the PSC, leading to the expression of the transcription factor *Collier*, which is required for PSC maintenance [32]. The PSC also expresses the secreted ligand *Hedgehog* [67]. Since the signal(s) activating JAK-STAT in the prohemocytes must be acting at long range, *Hedgehog* is a likely candidate. Downstream components of the Hedgehog pathway including *Patched* and *Cubitus interruptus* are expressed in the medullary zone. Furthermore, Hedgehog and JAK-STAT signaling are both required for prohemocytes maintenance. Since Hedgehog signaling has been shown to activate *Upd* expression in *Drosophila* eye discs [69], it would be interesting to determine if JAK-STAT is activated at long range by Hedgehog signaling in the larval lymph gland. Additionally, since the signal activating *Upd* expression in the *Drosophila* testis is unknown, *Hedgehog*, which is expressed

in hub cells, is also a good candidate for JAK-STAT signaling activation in the testis [70].

JAK-STAT signaling serves a similar role in both the larval hematopoietic and testis stem cell niches. In both tissues, niche cells are required to activate JAK-STAT signaling in the stem or precursor cells. JAK-STAT activation is required for the maintenance of CySCs and GSCs in the testis and prohemocytes in the medullary zone. However, in the testis, the niche secretes Upd directly, leading to local activation of JAK-STAT signaling, whereas in the lymph gland, *Upd3* expression appears to be activated by a signal from the PSC [24, 25, 32]. One reason for this difference is that stem cells in the *Drosophila* testis are in direct contact with the hub and so JAK-STAT is only activated in cells at a short range. In contrast, JAK-STAT is activated in all prohemocytes in the medullary zone, some of which are a larger distance from the PSC. A secondary signal may be necessary to relay or amplify signaling from the PSC and activate JAK-STAT in all prohemocytes. An additional difference between these two niches is that at the onset of metamorphosis, the lymph gland bursts and the remaining prohemocytes in the medullary zone are released into the hemolymph [71]. In contrast, spermatogenesis occurs throughout the life of the organism, and a decline in JAK-STAT signaling components in the testis with age leads to a decline in stem cell number [39, 72]. In both niches, JAK-STAT is an important maintenance signal under homeostatic conditions.

Under parasitized conditions (e.g. infestation with wasp eggs), hematopoiesis shifts towards the production of lamellocytes, cells which engulf parasites that cannot be destroyed by plasmatocytes. This shift requires major changes in signaling. JAK-STAT signaling in prohemocytes is repressed following infestation. This triggers a wave of mitoses in the prohemocyte pool [73]. The sudden abrogation of JAK/STAT signaling is mediated by Eye transformer (CG14225), a short, Dome-related receptor, which dimerizes with Domeless and antagonizes its function [22]. As JAK-STAT signaling abates, the expanded prohemocyte pool terminally differentiates. There is a marked increase in lamellocyte number while the levels crystal cells and plasmatocytes decrease significantly. Lineage tracing shows that plasmatocytes are capable of differentiating into lamellocytes in infested lymph glands or in circulation, and JAK-STAT signaling is known to be required for this transition [74]. Following infestation, the prohemocyte population is almost completely depleted, and few or no prohemocytes are released when the lymph gland bursts. This mechanism to increase lamellocyte production in response to infection allows the organism respond to its environment, keeping immune precursors for adulthood if conditions are favorable or using them to survive infestation. As it does in the damaged midgut and the depleted testes, JAK-STAT mediates a niche response to an environmental change in the larval lymph gland.

14.3.4 JAK-STAT and *Drosophila* Neuronal Stem Cells

JAK-STAT signaling plays an important role in controlling the balance of neural stem cell self-renewal and differentiation in *Drosophila*. The *Drosophila* larval brain consists of the ventral nerve cord and central brain, to which connect two optic lobes (Fig. 14.2d). The outer edge of each optic lobe contains the outer proliferation center (OPC) where neuroepithelial (NE) stem cells reside [75]. These cells divide symmetrically and expand throughout late embryogenesis and early larval development until around the onset of the third instar larval stage when a proneural wave of signaling triggers the NE cells to convert to asymmetrically dividing neuroblasts (NBs) [33]. This conversion from NE cells into NBs occurs in a well-controlled manner, beginning at the medial edge of the NE cell population in the OPC. The timing of this conversion is important to ensure the correct balance of cell types. NBs divide asymmetrically to produce one ganglion mother cell and one self-renewing NB [76]. The ganglion mother cell usually divides once more to produce two terminally differentiated, non-dividing neurons. The NE cells at the lateral edge differentiate to become precursors to the lamina, the outer layer of the optic lobe, and by the onset of pupation, the NE population is completely depleted [75].

JAK-STAT signaling is required in the NE cells to prevent premature onset of the proneural wave of gene expression that triggers the NE to NB transition. The JAK-STAT ligand *Upd* is expressed in the laminal furrow adjacent to the NE cells, while the receptor *Dome* and *Stat92E* are expressed in both the laminal precursors and the NE cells themselves [34]. Loss of JAK-STAT signaling leads to precocious differentiation of NE cells and fewer NE and NB cells overall [33–35]. This decrease in cell number is due to the fact NE cells are stimulated to differentiate before they can undergo the proper level of mitotic expansion through symmetric divisions. Notch signaling is also required to prevent premature transition of NE cells into NBs [35, 77]. Notch loss of function mutations in the optic lobe phenocopy JAK-STAT mutations, and epistasis analysis places JAK-STAT signaling upstream of Notch signaling in this tissue [35, 77]. Regulation of JAK-STAT signaling in the optic lobe is critical for the correct timing of the transition from NE cells to NBs.

While JAK-STAT signaling blocks the premature start of the proneural wave, it also indirectly promotes NB fate. Under normal conditions, the transition from NE to NB cell fate is induced by the expression of the gene *lethal of scute* (*L(1)sc*). While *L(1)sc* is not required for the induction of NB cell fate, it does help to control proper timing of the NE to NB transition, and overexpression of *L(1)sc* causes NE cells to prematurely convert into NBs. *L(1)sc* is activated at the medial edge of the NE cells at the onset of the proneural wave and deactivates Notch signaling [78]. Its expression slowly moves laterally as the medial most NE cells begin to transition into NBs [33]. Expression of *L(1)sc* depends on EGFR signaling, and JAK-STAT signaling is required for induction of EGFR signaling during the NE to NB transition [79]. Therefore JAK-STAT signaling indirectly controls NB cell fate through EGFR signaling, allowing JAK-STAT to finely regulate and balance the timing of the proneural wave. Correct timing of the proneural wave ensures that NE cells are not prematurely depleted and excess NBs are not produced. JAK-STAT function in the optic lobe is similar to its function in the ISC niche, where the pathway interacts with several different signals to control both the proliferation rates of ISCs and the differentiation of EBs to maintain a proper balance of progenitors and mature midgut cells.

14.4 JAK-STAT Signaling in Mammalian Stem Cell Niches

In addition to its role in regulating stem cells in *Drosophila*, JAK-STAT is important for proper function of several mammalian stem cell populations. Purified mammalian embryonic, hematopoietic and neural stem cells express high levels of JAK-STAT pathway components, suggesting that this pathway is generally required in multiple types of stem cells in mammals, just as it required in many types of stem cells in *Drosophila* [80]. However, since mammalian tissues are significantly more complex than those of invertebrates, the signals controlling mammalian stem cell niches is often poorly understood. Although there are fewer examples of JAK-STAT signaling controlling stem cells in mammalian tissues, the pathway is known to be involved in the control of stem cells during hematopoiesis in the bone marrow and neurogenesis in the developing cortex (reviewed in [81, 82]).

14.4.1 JAK-STAT Signaling and the Mammalian Hematopoietic Stem Cell Niche

Although Schofield first described the concept of a hematopoietic stem cell niche in 1978 [83], details of exactly how the niche functions to maintain HSCs are still not completely understood (reviewed in [81]). HSCs reside in two locations in the bone marrow: adjacent to osteoblasts in a region termed the endosteal niche and adjacent to small sinusoidal vessels in a region termed the vascular niche (Fig. 14.3a). Long-term quiescent HSCs are located in the endosteal niche and remain dormant while short-term HSCs, which are mitotically active, reside in regions closer to the vasculature [84]. Short-term HSCs give rise to multipotent progenitors that

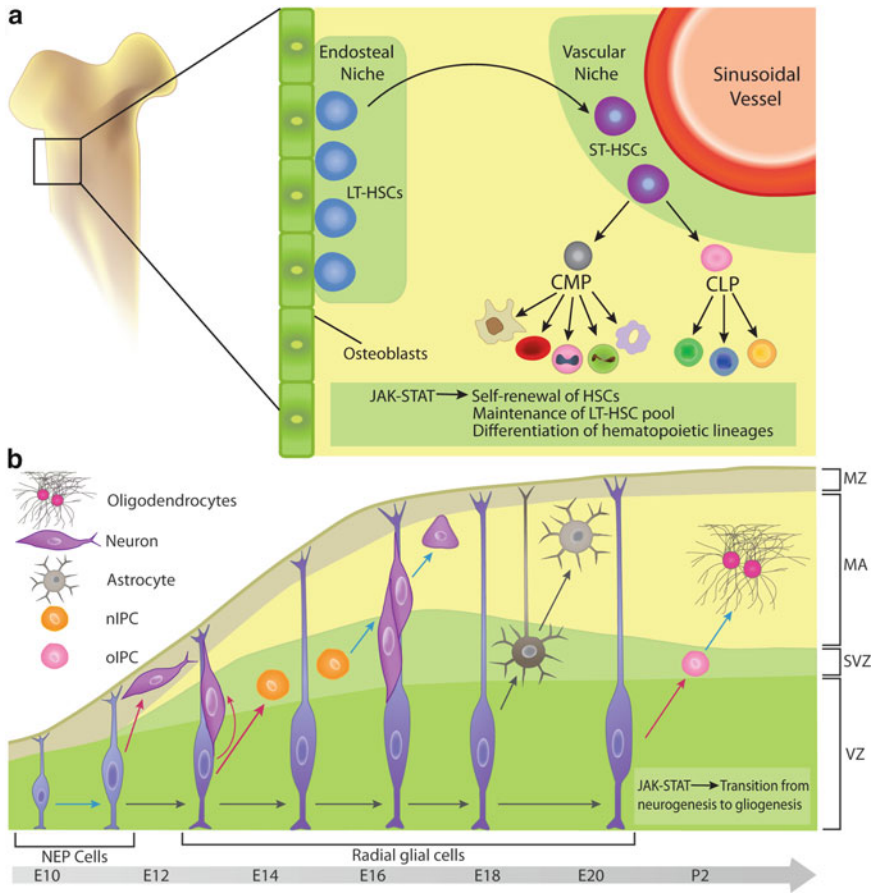

Fig. 14.3 JAK-STAT in mammalian stem cell niches. (**a**) In the bone marrow, quiescent long-term hematopoietic stem cells (*LT-HSCs*, *blue*) reside in the endosteal niche adjacent to osteoblasts (*green rectangles*). More active short-term HSCs (*ST-HSCs*, *purple*) are found in the vascular niche in regions close to vessels. ST-HSCs readily divide to form hematopoietic progenitor cells including common myeloid progenitors (*CMPs*, *gray*) and common lymphoid progenitors (*CLPs*, *pink*), each of which can give rise to many types of blood cells. (**b**) In the developing embryonic cortex, neuroepithelial cells (*NEP* cells) divide symmetrically (*blue arrows*) to amplify the NEP population, some dividing asymmetrically (*pink arrows*) to generate early neurons. As the brain epithelium develops and becomes thicker, NEP cells elongate and directly transition (*gray arrows*) into radial glial cells. Radial glial cells divide asymmetrically to give rise directly to neurons or produce intermediate progenitor cells (*nIPCs*) which will differentiate into neurons. At the onset of gliogenesis, some radial glial cells begin to detach from the apical floor and convert into astrocytes. Radial glial cells also give rise to intermediate progenitor cells (*oIPCs*) that generate oligodendrocytes as development continues. *MA* mantle, *MZ* marginal zone, *SVZ* subventricular zone, *VZ* ventricular zone

can differentiate into at least 10 different types of mature blood cells including erythrocytes (red blood cells), megakaryocytes (platelet-producing cells), and cells of the immune system [85]. Although many signaling pathways are required for the maintenance, differentiation and control of HSCs in the bone marrow, this chapter will focus specifically on the role of JAK-STAT signaling. The JAK-STAT pathway has been implicated in both the self-renewal of HSCs and their differentiation into certain lineages including erythrocytes and megakaryocytes. For a more comprehensive overview of HSC signaling, see Chap. 11 of this volume.

The activation of STAT5 in the bone marrow hematopoietic stem cell niche is important for the self-renewal of HSCs. Thrombopoietin (TPO), a glycoprotein hormone, binds to its receptor c-Mpl

on HSCs and activates JAK2, leading to the activation of both STAT5a and STAT5b. Although mice lacking *Stat5ab* can produce relatively normal numbers of HSCs, these mice are deficient in multipotent hematopoietic progenitors and several differentiated blood cell lineages [86]. *Stat5ab* stem cells cannot effectively repopulate the bone marrow of an irradiated host in a competitive transplantation assay, indicating that STAT5 plays a role in HSC self-renewal [86, 87]. TPO signaling in HSCs is further modulated by an adaptor protein, LNK, which interacts with JAK2 to negatively regulate STAT5 activity. Loss of LNK leads to over-active JAK-STAT signaling and over-proliferative HSCs, in addition to defects in B-lymphopoiesis, erythropoiesis and generation of megakaryocyte lineages [88–90]. Interestingly, HSCs isolated from mice mutant for the TPO receptor *c-mpl* can repopulate irradiated host bone marrow more effectively than *Stat5ab* –/– HSCs, indicating that STAT5 is likely activated by multiple ligands in addition to TPO in HSCs. Constitutively active STAT5 promotes HSC self-renewal and leads to an expansion of the multipotent progenitor cells that arise from HSC differentiation [91]. STAT5 signaling is also involved in maintaining long-term or dormant HSCs in their quiescent state in the bone marrow [92]. In mice, loss of STAT5 activity in long-term HSCs induces them to exit their quiescent state and transition into short-term HSCs, leading to a gradual depletion of the long-term HSC pool in the bone marrow. The mammalian hematopoietic stem cell niche is complex, and the activity of even a single JAK-STAT component, STAT5, is regulated at multiple levels to control both HSC self-renewal and quiescence.

STAT3 is also active in the bone marrow niche although, unlike STAT5, STAT3 is dispensable in vivo for normal HSC and progenitor function under homeostatic conditions [91]. STAT3 does, however, play a role in HSC self-renewal during the initial phase of hematopoietic regeneration following competitive transplantation. HSCs with constitutively active STAT3 are better able to reconstitute a niche than HSCs with normally functioning STAT3 when transplanted into a lethally irradiated host, whereas HSCs with a dominant negative STAT3 mutation are not as competitive in transplantation assays [93, 94]. After the initial phase of regeneration, STAT3 becomes dispensable for HSC self-renewal [93]. Its function will again be required if these HSCs are used in serial transplantation assays. These experiments show that while both STAT3 and STAT5 play a role in HSC self-renewal, they function non-redundantly in the hematopoietic niche.

In addition to a role in self-renewal, JAK-STAT signaling can also modulate the differentiation of many hematopoietic cell lineages. JAK-STAT signaling is required for the differentiation of myeloid progenitors into all types of myeloid derived cells including erythrocytes and megakaryocytes (reviewed by [95]). For example, TPO-mediated STAT5 activity is required for the proper differentiation of megakaryocytes, the cells that give rise to platelets [96]. Additionally, the hormone erythropoietin can activate JAK-STAT signaling through JAK2 in proerythroblasts, red blood cell progenitors, leading to STAT5 activation [97]. This promotes the proliferation and differentiation of red blood cell progenitors and explains why *Stat5ab* –/– mice are anemic and deficient in erythropoiesis. Just as JAK-STAT signaling is required for the proper differentiation of *Drosophila* midgut progenitors, JAK-STAT signaling is required for the proper differentiation of multiple hematopoietic lineages. In both the ISC niche in *Drosophila* and the HSC niche in mammals, levels of JAK-STAT signaling, in combination with levels of additional signaling pathways including Notch, determine which fate a precursor cell will adopt. Small changes in signaling levels can cause a shift in differentiation from one cell fate to another, or cause a disruption in differentiation altogether.

In addition to its role in differentiation, JAK-STAT signaling also modulates HSC proliferation upon infection, leading to the increased production of mature immune cells [98]. Interferons (IFNs), cytokines which are produced by the immune system in response to viral or bacterial infection or tumor formation, activate the JAK-STAT pathway in long-term hematopoietic stem cells. IFNs α and β bind to

their receptors to activate JAK1 and TYK2 leading to the phosphorylation of STAT1 and STAT2. IFNγ can activate JAK1 and JAK2, leading to signaling through STAT1 [99]. Each interferon signal leads to the activation of specific target genes, ultimately causing long-term HSCs to transition from a dormant to an active state. This process is tightly controlled to allow response to insult without complete depletion of dormant HSC stores. As it does in the *Drosophila* midgut and in the larval lymph gland, JAK-STAT signaling regulates stem cell function within the mammalian hematopoietic niche, causing a proliferative response following environmental changes like infection. This conserved function of JAK-STAT signaling is particularly important in the hematopoietic niche since, in response to infection, JAK-STAT signaling directly increases the production of immune cells.

14.4.2 JAK-STAT Signaling in the Mammalian Neural Stem Cell Niche

Neurogenesis in mammals, like in *Drosophila*, requires JAK-STAT signaling for the formation of the proper cell types at the proper time in the nervous system. During the development of the embryonic central nervous system, neuroepithelial (NEP) cells contact the walls of the lateral ventricles in the developing brain and divide symmetrically to expand the NEP population (Fig. 14.3b, reviewed by [100, 101]). NEPs then elongate and transform directly into radial glial cells, which remain in contact with the ventricle wall but extend a projection through the ventricular and subventricular zones to make contact with blood vessel walls. Radial glial cells function as neural precursor cells, dividing asymmetrically to produce neurons, astrocytes or oligodendrocytes depending on the signals they receive. Radial glial cells often give rise to intermediate progenitor cells, transit amplifying cells that will form neurons or oligodendrocytes. The timing of this developmental process is carefully controlled and occurs sequentially, beginning with the generation of neurons, followed by the generation of astrocytes (also called astroglia) and then oligodendrocytes (reviewed by [82]).

The transition from the production of neurons to glial cells is carefully regulated by several signaling pathways including JAK-STAT [102]. As neurons are born, they produce the gliogenic cytokine cardiotrophin-1 (CT-1) [103]. As CT-1 accumulates, it binds to gp130 and LIF receptor β to activate multiple JAKs, leading to the phosphorylation of STAT1 and STAT3. Although the initial activation of JAK-STAT signaling is small, the signal increases over time to drive the transition from neurogenesis to gliogenesis. An autoregulatory loop gradually amplifies JAK-STAT signaling, directing precursor cells toward a gliogenic cell fate [104]. The role of JAK-STAT signaling in controlling the onset of gliogenesis in mammals is similar to its role in controlling the timing of the proneural wave in *Drosophila*, causing the progenitor cells in the optic lobe to transition from one cell type to another at a specific time in development.

The correct timing of gliogenesis requires interactions between JAK-STAT and several other signaling pathways. Near the onset of gliogenesis, the BMP signaling pathway cooperates with JAK-STAT to activate target genes. Initially, low levels of STAT1 and STAT3 are activated resulting in modest transcription of target genes including Bone morphogenic protein 2 (BMP2). BMP2 can then activate the transcription factor Smad1, which complexes with activated STAT1/3 along with the co-activators p300 and CBP, leading to increased transcription of the target genes important for the onset of gliogenesis [105, 106]. In this way, BMP signaling helps to amplify an initially small JAK-STAT signaling response to promote the transition from neurogenesis to gliogenesis in the embryonic cortical zone. The EGFR pathway amplifies JAK-STAT signaling through a different mechanism than BMP signaling. EGFR signaling mediates progenitor cell sensitivity to an additional cytokine ligand, Leukemia Inhibitory Factor (LIF). As EGFR signaling increases, LIF becomes competent to stimulate JAK-STAT signaling and activate gliogenesis-inducing genes [107]. Notch signaling also helps to boost JAK-STAT through its effector

HES proteins during gliogenesis [108]. HES proteins, basic helix-loop-helix factors, act as scaffolds between JAK and STAT to mediate more efficient STAT phosphorylation and activation. In the *Drosophila* testis, intestinal and neuronal stem cell niches, the JAK-STAT pathway interacts with the BMP, Notch and EGFR pathways to maintain niche homeostasis. In the complex mammalian cortical zone, JAK-STAT interacts with each of these pathways to regulate the stem cell niche homeostasis. This suggests that similar interactions may be occurring in multiple stem cell niches. For example, in the mammalian intestinal stem cell niche, both BMP and Notch signaling are known to be important regulators of stem cell homeostasis [109]. While JAK-STAT has not yet been shown to play a role in the mammalian intestinal niche, further investigation may uncover a role for this signaling pathway.

JAK-STAT signaling is also controlled at the epigenetic level in the mammalian cortical zone to ensure the proper of timing events during neurogenesis [110]. In precursor cells that are not yet fated to give rise to astrocytes, promoters of gliogenic genes become methylated and bound by methyl CpG binding proteins, which prevent STAT-mediated gene activation [110, 111]. This ensures that gliogenic genes will not be activated, even if cytokines are precociously expressed and the JAK-STAT pathway is stimulated. Later in development, chromatin modifications help to trigger the onset of gliogenesis. Fibroblast growth factor stimulation leads to an increase in H3-K4 methylation, an activating histone mark, and a decrease in H3-K9 methylation, a repressing histone mark, at the promoters of gliogenic genes [112]. This chromatin remodeling increases the ability of JAK-STAT to upregulate expression of these genes and induce gliogenesis in early neuronal progenitors. Just as the chromatin remodeling factor Nurf301 can modulate JAK-STAT signaling in multiple *Drosophila* tissues [51, 52], chromatin remodelers are important regulators of JAK-STAT function in mammals. With an increasing number of high-throughput epigenetic data sets entering the public domain, it will be of great interest to look for more cases of potential epigenetic regulation of JAK-STAT signaling.

14.5 JAK-STAT and Disease

While JAK-STAT signaling is important in many different stem cell niches in both invertebrates and vertebrates to maintain homeostasis, misregulation of this pathway can lead to dire pathological consequences. Constitutive activation of JAK-STAT in both *Drosophila* and mammals can lead to tumors. For example, in *Drosophila*, the Hop^{TumL} mutation, a constitutively active mutation in JAK, leads to neoplasia of the blood lineages and melanotic tumors [113]. Removal of one copy of the JAK-STAT inhibitor *PIAS* (*Su*(*var*)*2-10*) enhances the Hop^{TumL} phenotype [114]. Similarly, overactivation of JAK-STAT in mammals leads to overproliferation of various cell lineages including hematopoietic and neuronal progenitor cells [71, 115]. A variety of blood disorders arise from JAK- STAT hyperactivity, and JAK-STAT upregulation also plays a role in the initiation of several types of cancers.

14.5.1 JAK-STAT and Cancer Stem Cells

While the idea of stem cells in niches replenishing healthy tissues was proposed in the late 1970s [83], the concept of tumor initiating cancer stem cells and their niches is a controversial, though growing area of interest in cancer biology [116, 117]. With several types of cancer stem cells now isolated [118–121], these cells are believed to play a role in the initiation and maintenance of tumors. Pathways that are critical to the function of normal stem cells remain to be explored in many of these cancer stem cell populations. Perhaps unsurprisingly due to its role in the maintenance of normal stem cells, JAK-STAT signaling, when misregulated, can alter the behavior of cancer stem cells and promote tumor initiation and maintenance.

In several types of cancer stem cells, JAK-STAT signaling is deregulated. Breast cancer stem cell-like populations show upregulation of the JAK/STAT signaling pathway, and inhibition of STAT3 decreases the stem cell-like population [122]. Similarly, transcriptional profiling of prostate

cancer stem cells shows that components of the JAK-STAT pathway are upregulated in these cells, consistent with a role for JAK-STAT signaling in prostate cancer [123–125]. Glioma-initiating cells (GICs), cancer stem cells that give rise to glial-derived gliomas, and glioblastoma-stem-like cells (GBSCs), cancer stem cells that give rise to astrocyte-derived glioblastomas, also require JAK-STAT signaling (reviewed by [126–128]). Both GICs and GBSCs require JAK-STAT signaling for self-renewal while GBSCs also need it for proliferation [129, 130]. Interestingly, if STAT3 signaling becomes deregulated in astrocytes in vivo, it can lead to the formation of glioblastomas in humans [131], and JAK-STAT upregulation in glioblastomas is a clinical indicator of poor prognosis [132]. Leukemia-initiating cells that can lead to various cancers of the blood have also been identified [133]. Upregulation of JAK-STAT expands leukemia-initiating cells in the bone marrow. Many patients with acute myeloid leukemia have mutations leading to constitutive activation of JAK-STAT signaling, some correlating with decreased disease-free survival [134, 135]. Upregulation of JAK-STAT signaling is becoming a common theme in cancer stem cells and cancer initiation. This highlights the pathway as a potential target for treatment. There has been some success in clinical trials using JAK inhibitors to treat leukemias, lymphomas and other cancers, although resistance has been an issue [136–138].

14.5.2 Myeloproliferative Disorders

Because JAK-STAT signaling regulates the differentiation of HSCs into myeloid progenitor cells as well as progenitor proliferation, misregulation of JAK-STAT in HSCs can lead to blood disorders linked to abnormal production of certain blood lineages. Collectively termed myeloproliferative disorders (or myeloproliferative neoplasms), these diseases include polycythemia vera, idiopathic primary myelofibrosis, and essential thrombocythemia and are defined by the overproduction of blood cells [139, 140]. The disorders are caused by the often clonal overexpansion of hematopoietic precursors and can lead to leukemia [139, 141–144].

Several lines of evidence point to a critical role of JAK/STAT signaling in polycythemia vera. Polycythemia vera patients exhibit several defects in JAK-STAT signaling including constitutive activation of STAT3 and JAK2 in the absence of any stimulating ligand [139, 145–147]. A *JAK2* gain-of-function mutation (V617F) is present in the HSCs of greater than 65 % of polycythemia vera patients [147]. This mutation is also detected in 30 % patients with essential idiopathic myelofibrosis and thrombocytopenia, but not in healthy controls [139, 144, 148]. This suggests a common role for constitutively active JAK/STAT signaling in myeloproliferative disorders, which may be therapeutically targeted. Inhibiting JAK2 in cell culture blocks spontaneous erythroid terminal differentiation [149], while *JAK2* targeting siRNA inhibits spontaneous erythroid differentiation and colony formation [147]. A Jak2 inhibitor has now been shown to prevent polycythemia vera development in a mouse model, while several human trials using targeted JAK2 inhibitor therapy to treat myeloproliferative disorders are showing great promise [150–152].

14.6 Conclusions

JAK-STAT, with its diverse array of transcriptional targets, is now recognized as an integral signaling pathway for the regulation of many types of stem cells. Like many major pathways, JAK-STAT can interact with other signals including Notch, EGFR and BMP to finely control the regulation of stem cells. While much has already been discovered about JAK-STAT signaling in *Drosophila* stem cell niches, more remains to be learned about JAK-STAT signaling in stem cells, especially in mammalian niches. Will additional lessons from *Drosophila* hold true in mammalian systems? Will JAK-STAT regulate mammalian spermatogonial or intestinal stem cells? Preliminary studies regarding mammalian spermatogonial stem cells have indicated that STAT3 is expressed in the murine male germline

and may regulate stem cell differentiation [153, 154]. Additionally, knocking down short-type PB-cadherin in male spermatogonial stem cells decreases both self-renewal and JAK-STAT signaling [155]. While these results do not clarify the exact role of JAK-STAT signaling in the mammalian male germline, they do indicate that JAK-STAT signaling is likely involved in stem cell regulation in this tissue. Also, similar to *Drosophila*, JAK-STAT signaling is upregulated in the mammalian intestine in response to infection or inflammation, although it has not been implicated as a factor important for the normal regulation of mammalian intestinal stem cells at this point [156]. Perhaps as JAK-STAT research continues, we will discover a place for this pathway in additional stem cell niches. Misregulation of JAK-STAT can often lead to tissue overproliferation and disease progression. As we understand more about this pathway and the tissues that it regulates, we can hope to discover ways to modulate it and prevent the progression of JAK-STAT-based diseases.

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