MINIREVIEW

JAK/STAT signaling regulates tissue outgrowth and male germline stem cell fate in *Drosophila*

Shree Ram SINGH, Xiu CHEN, Steven X. HOU*

The Laboratory of Immunobiology,National Institutes of Health, National Cancer Institute at Frederick, Frederick, MD 21702, USA

ABSTRACT

In multicellular organisms, biological activities are regulated by cell signaling. The various signal transduction pathways regulate cell fate, proliferation, migration, and polarity. Miscoordination of the communicative signals will lead to disasters like cancer and other fatal diseases. The JAK/STAT signal transduction pathway is one of the pathways, which was first identified in vertebrates and is highly conserved throughout evolution. Studying the JAK/STAT signal transduction pathway in *Drosophila* provides an excellent opportunity to understand the molecular mechanism of the cell regulation during development and tumor formation. In this review, we discuss the general overview of JAK/STAT signaling in *Drosophila* with respect to its functions in the eye development and stem cell fate determination.

Keywords: cell signaling, JAK/STAT signal transduction pathway, cell regulation, *Drosophila*, stem cell fate, tissue outgrowth.

INTRODUCTION

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) cascade is a signal transduction pathway that was originally identified in the vertebrate system [1, 2] and has been extensively studied during the last decade in several model organisms, including *Drosophila* [1, 3, 4, 5]. This pathway was identified through studies on the transcriptional activation response to a variety of cytokines and growth factors [6, 7] and is highly conserved and capable of transmitting a multitude of signals for development and homeostasis in animals from flies to humans.

In the mammalian system, the JAK/STAT pathway plays a central role in transducing the cytokine signal and regulates various biological processes such as cell growth, differentiation, migration, apoptosis, fetal development, transformation, inflammation, and immune response. The constitutive activation of JAK/STAT is correlated with several oncogenic transformations, such as leukemia, lymphoma, and multiple myeloma, as well as some solid neoplasias, head, neck, brain, breast, lung, pancreas, prostate, and ovarian cancers [8, 9].

*Correspondence: Steven X. HOU
Tel: 301-846-1589; fax: 301-846-6145;
E-mail: shou@mail.ncifcrf.gov

JAK/STAT SIGNALING IN *DROSOPHILA*

The JAK/STAT pathway is also present in the *Drosophila* genetic model system [1, 4, 10]. The *Drosophila* JAK/STAT pathway has four main components: JAK, encoded by the *hopscotch* (*hop*) gene [11]; STAT, encoded by the *stat92E* gene [12, 13]; a ligand, encoded by the *unpaired* (*upd*) gene [14]; a receptor, encoded by the *domeless* (*dome* [15]) /*master of marelle* (*mom* [16]) gene. The current model of the Hop/Stat92E signal cascade is the following: The extracellular Upd protein binds and activates the receptor Dome/Mom, which in turn activates the intracellular Hop/JAK; Hop/JAK then phosphorylates and stabilizes Stat92E. The phosphorylated Stat92E dimerizes and translocates to the nucleus to activate the gene transcription [1, 3, 17, 18] (Fig. 1).

The JAK/STAT signal transduction pathway regulates various developmental processes in *Drosophila*, including embryonic segmentation, sex determination, eye formation, tracheal formation, male germline stem cell self-renewal, border cell migration, polar and follicular cell fate determination in the female germline, germ cell proliferation and invasive migration [1, 3, 17, 19, 20, 21]. Furthermore, hyperactivation of this pathway in *Drosophila* leads to melanotic- or leukemia-like tumor formation. In this review, we will focus on the pathway's functions in the eye development and male germline stem cell fate determination.

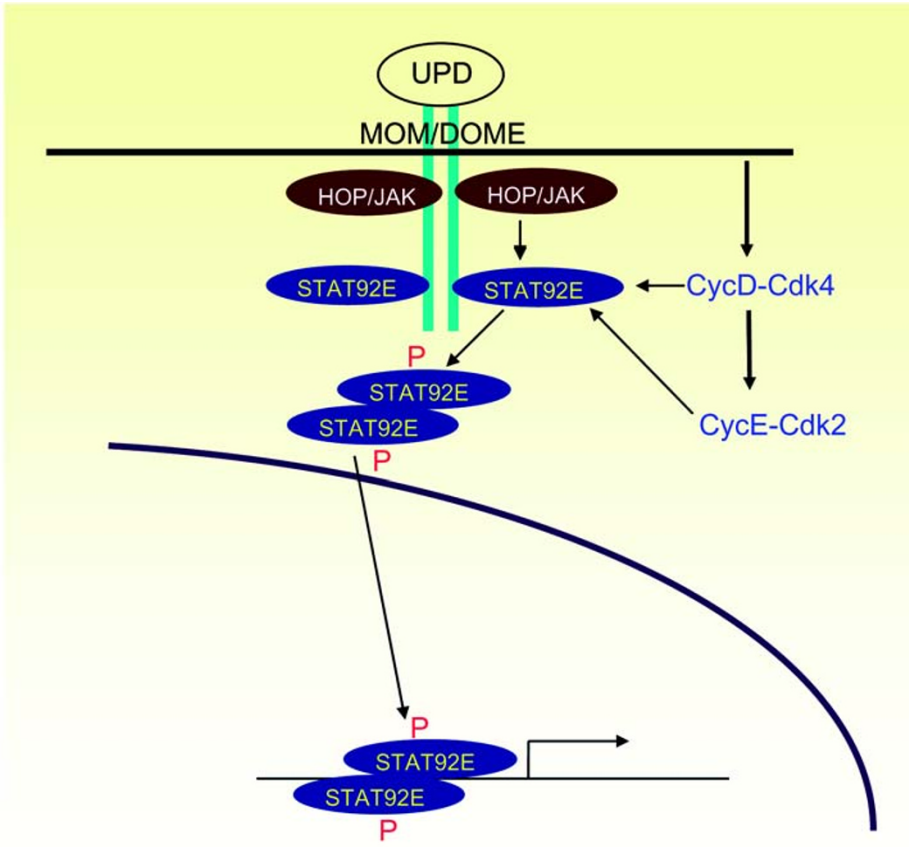

Fig. 1 The *Drosophila* JAK/STAT pathway.

Role in cell proliferation during eye development

In both mammalian and *Drosophila* systems, the JAK/STAT signaling pathway plays a crucial role in controlling organ or tissue size. The *Drosophila* eye is a complex neural tissue with precise cellular architecture and composed of approximately 800 subunits called ommatidia, each of which contains eight photoreceptors and 12 accessory cells arranged in an invariant pattern [22]. The role of the JAK/STAT pathway in the eye development of *Drosophila* has been studied genetically [10, 18]. The activity of *upd* and *hop* is required for the proliferation and/or survival of eye imaginal cells [10, 16]. 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 [14]. Loss of *hop* activity results in the absence of proliferating diploid imaginal cells throughout the larva, and some transheterozygous combinations of alleles give rise to adults that have a small eye or eyeless phenotype [10, 23]. Overexpression of *dpias* (the negative regulator of Stat92E) results in small and rough eyes [24]. 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 [24]. Upd is expressed in the center of the posterior margin of the eye disc [25]. Loss-of-function *upd* mutations result in a small eye size, and ectopic misexpression of *upd* causes enlargement of the eye [16, 24, 25]. *upd* regulates eye size through the Mom/Hop/Stat92E signaling pathway to promote cell proliferation, by accelerating the cell cycle in the undifferentiated cells from anterior to the MF (morphogenetic furrow).

In the eye, excess Hop/Stat92E signaling induces cell overproliferation, and excess CycD-Cdk4 activity blocks differentiation and induces overgrowth. Our group recently demonstrated that excess Hop/Stat92E signaling can synergize with both CycD-Cdk4 and CycE-Cdk2 in melanotic tumors, but specifically synergizes with CycD-Cdk4, not CycE-Cdk2, to promote the formation of an enlarged eye with extra ommatidia [19]. These results demonstrate that the JAK/STAT pathway and Cyclin-Cdk cooperatively regulate tissue outgrowth and tumor formation in *Drosophila*.

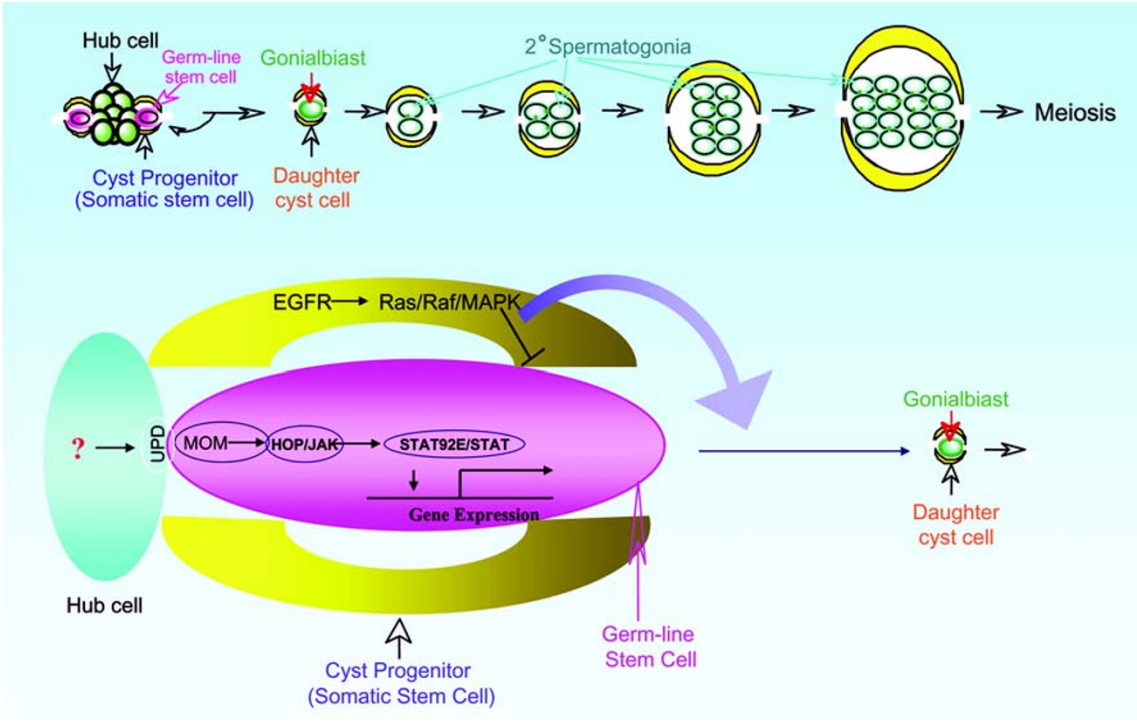

Fig. 2 The HOP/STAT92E pathway regulates male germline stem cell self-renewal and differentiation. (A) Five to nine germline stem cells (only two GSCs are shown in this scheme) are surrounded by approximately twice as many cyst progenitor cells (somatic stem cell), and both are anchored around somatic hub cells at the tip of the *Drosophila* testis. The testis proliferation center is composed of hub, germline, and somatic stem cells, gonialblasts, and 2° spermatogonia. (B) Schematic diagram showing the cooperative regulation of the male germline stem cell self-renewal and differentiation by HOP/STAT92E and RAS/MAPK pathways.

Although it remains to be seen whether cooperation of CycD-Cdk4 and the JAK/STAT pathway in regulating tissue growth is a general phenomena in all species, abundant evidence shows that CycD-Cdk4 and the JAK/STAT pathway regulate similar biological processes in both flies [1] and mammals [2, 26]. Just as overexpressing CycD-Cdk4 in the fly wing or eye causes hyperplasia, targeted overexpression of CycD1 in mice can promote epidermal, mammary, and thymic hyperplasia. These parallels are also evident in loss-of-function studies. Both CycD1 and Cdk4 knock-out mice are smaller than their littermates and exhibit decreased growth rates. Cdk4 mutant flies are also small. Both male and female Stat5a/b-deficient mice are small with reduced size of fat pads and reduced levels of insulin-like growth factor-1. As discussed above, reduction of the Hop/STAT92E signaling gives rise to adult flies that have a small eye or eyeless phenotype. Taken together, all of these observations suggest that both CycD-Cdk4 and the JAK/STAT pathway regulate tissue growth. Further, in the fly, two dominant temperature-sensitive mutations that hyperactivate Hop, $hop^{Tum\text{-}l}$ and hop^{T42} lead to melanotic- or leukemia-like tumor formation. Germline mutations in human Cdk4, a result of the Arg24Cys substitution, have been identified in familial melanoma [27, 28]. These mutations abrogate the inhibition of Cdk4 activity by its inhibitor, $P16^{INK4a}$ [27, 28]. Mice with R24C mutation display an increased weight of 5%–10%, compared with control littermates. However, $Cdk4^{R24C/R24C}$ mice did not develop melanoma similar to that observed in humans carrying the same R24C mutation in *Cdk4* [29]. It is likely that predisposition to melanoma requires a second cooperative signal. The JAK/STAT signaling may identify the second signal. The collaboration of *upd* and *CycD-Cdk4* in the fly eye may somehow mimic signal cooperation during tumor formation. *CycD-Cdk4* promotes cell growth and blocks cell differentiation; likewise, *upd* stimulates cell proliferation. Cooperation of the two signals results in a dramatically outgrown tumor-like eye.

More than 80% of cancers have detectable lesions in one component of the Cdk4 complex (Cdk4, INK4a, D1, and RB) [30]. Many growth factors and components of signal transduction are oncogenes. Our recent results show that CycD-Cdk4 and the Hop/Stat92E pathway collaboratively induce tissue overgrowth and melanotic tumor formation. We suggest that this relationship between coordinated STAT and Cyclin-Cdk signaling could regu-

late cell fate, the cell cycle, and/or tumor progression in mammals as well. Thus, the powerful genetic manipulations available in *Drosophila* may make this an ideal system to study cancer.

Role in male germline stem cell

In last few years, several findings demonstrated that the canonical JAK/STAT pathway in the fly testis regulates germline stem cell self-renewal, maintenance, and differentiation [31, 32, 33]. Spermatogenesis in *Drosophila* takes place within the tubular testis [34], and at the tip of the *Drosophila* testis is a germinal proliferation center composed of a cluster of twelve nonmitotic somatic cells called the hub, and a small number of germline stem cells (GSCs; 16–18 in larvae, 5–9 in adult; Fig. 2A). It is reported that stem cells reside in niches, which actually determine their capacity to self-renew or differentiate [35]. An asymmetric or stereotypically oriented division of stem cells controls spermatogenesis in *Drosophila*, whereby one daughter cell 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 [36]. 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. The gonialblast divides further to produce interconnected spermatogonia, while SSCs grow without further division and enclose the spermatogonial clusters.

The ligand Upd, which is exclusively expressed in the hub cells, maintains the male germline stem cell self-renewal by activation of the JAK/STAT pathway in GSCs (Fig. 2B). Upd normally instructs GSCs to undergo self-renewal through the Mom/Hop/Stat92E pathway; Stat92E then enters the nucleus to activate expression of genes that instruct the self-renewal of GSCs and SSCs.

Overexpression of the Hop/Stat92E pathway through overexpressing *upd* leads to unrestricted stem cell division, and loss-of-function mutations in the Hop/Stat92E pathway lead to loss of GSCs, suggesting that signaling maintains stem cell fate or viability [31, 32]. Furthermore, GSCs null for *stat92E* can produce differentiating daughter cells but cannot maintain stem cell fate. These data suggest that Hop/Stat92E signaling instructs stem cell fate rather than maintaining cell viability [31]. 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. Very recently, Brawley and Matunis [33] found that early spermatogonia with limited mitotic divisions can repopulate the niche and can be reverted to stem cell identity by conditionally manipulating the Hop/Stat92E signaling. Further, their results provide a mechanism for the replacement of lost stem cells in an intact stem cell niche and also aid in better understanding the mechanism of tissue regeneration.

In addition to the JAK/STAT signal transduction pathway, the epidermal growth factor receptor (EGFR) / MAP kinase (MAPK) pathway also regulates germline stem cell fates in the fly testis [37, 38] (Fig. 2B). The *Drosophila* EGFR (DER) through Raf/MAPK pathway functions in SSCs. The DER/Raf/MAPK pathway mediates an SSC to GSC signal that restricts self-renewal and promotes differentiation of the GSCs. If the cyst cells do not send the signal to GSCs through DER/Raf/MAPK pathway, both daughters of the GSCs will retain stem cell identity. Loss of function of *der* or *raf* in SSCs will break the balance of self-renewal/differentiation and result in an excess number of GSCs and gonialblasts at the expense of differentiated cell types. These data suggest that SSCs play a guardian role to ensure the balance between the self-renewal and differentiation of GSCs [37, 38] (Fig. 2B). The *Drosophila* testis system is an intriguing parallel to mouse embryonic stem (ES) cells [39], in which the JAK/STAT signaling is required for the maintenance of the ES cells, while the MAP kinase pathway promotes the ES cell differentiation [40]. In both systems, the JAK/STAT signal is counterbalanced by the Ras/Raf/MAPK signal. The two signals may converge on some downstream targets to regulate cell fate determination (Fig. 2B).

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