The JAK/STAT pathway positively regulates DPP signaling in the *Drosophila* germline stem cell niche

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The stem cell niche, formed by surrounding stromal cells, provides extrinsic signals that maintain stem cell self-renewal. However, it remains unclear how these extrinsic signals are regulated. In the *Drosophila* female germline stem cell (GSC) niche, Decapentaplegic (DPP) is an important niche factor for GSC self-renewal. The exact source of the DPP and how its transcription is regulated in this niche remain unclear. We show that *dpp* is expressed in somatic cells of the niche including the cap cells, a subtype of niche cells. Furthermore, our data show that the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway positively regulates *dpp* expression in the cap cells, suggesting that JAK/STAT activity is required in somatic niche cells to prevent precocious GSC differentiation. Our data suggest that the JAK/STAT pathway functions downstream/independently of cap cell formation induced by Notch signaling. JAK/STAT signaling may also regulate *dpp* expression in the male GSC niche, suggesting a common origin of female and male GSC niches.

Introduction

The maintenance of stem cells requires the concerted actions of extrinsic signals and intrinsic stem cell factors (Lin, 2002; Fuchs et al., 2004; Gilboa and Lehmann, 2004; Xie et al., 2005; Fuller and Spradling, 2007). The extrinsic signals are generated from the stem cell niche, a specialized microenvironment, and their functions are spatially restricted. The *Drosophila* ovary is a well-established system for studying stem cell biology in vivo (Lin, 2002; Xie et al., 2005). At the anterior region of the ovariole (the germarium), three types of stem cells, including germline stem cells (GSCs), escort stem cells (ESCs), and somatic stem cells (SSCs), are responsible for the continuous production of egg chambers. Both GSCs and ESCs are located at the anterior tip of the germarium and associated with each other and with the cap cells (Fig. 1 A), whereas SSCs are located at the middle of the germarium (Margolis and Spradling, 1995; Decotto and Spradling, 2005). GSC self-renewal is thought to be controlled by extrinsic signals generated from the niche, formed by somatic cells including terminal filament (TF) cells, cap cells, and ESCs (Fig. 1 A,) (Xie and Spradling, 2000; Decotto and Spradling, 2005). DPP, the fly homologue of vertebrate bone morphogenetic protein (BMP), is an important niche factor and acts as a short-range signal (Xie and Spradling, 1998). Only GSCs within the niche show high levels of DPP downstream signaling activity, repress the expression of the differentiation promoting factor *bag-of-marbles* (*bam*), and maintain stem cell identity, whereas cystoblasts (CBs) located outside the niche show low/no DPP signaling, de-repress *bam* expression, and initiate differentiation (Chen and McKearin, 2003a; Kai and Spradling, 2003; Song et al., 2004). DPP is believed to be produced by the niche cells; however, the exact source of active DPP and how its expression is regulated in these cells are unclear (Xie and Spradling, 2000; Song et al., 2007).

The conserved JAK/STAT cascade plays an important role in a wide spectrum of biological processes including immune response and stem cell maintenance (Hou et al., 2002; Arbouzova and Zeidler, 2006). The JAK/STAT pathway is activated upon binding of extracellular cytokines and growth factors to receptors with intrinsic or associated tyrosine-kinase activity and results in the phosphorylation of STAT, which enters the nucleus to regulate gene expression. The canonical JAK/STAT signaling cascade in *Drosophila* comprises extracellular ligands including Unpaired (Upd), Upd2, and Upd3, a transmembrane receptor Domeless (Dom), a single Janus tyrosine kinase Hopscotch (Hop), and the Stat92E transcription factor (Hombria and Brown, 2002). A recent study suggested that JAK/STAT signaling also plays a critical role in the female GSC niche function and acts within the escort cell lineage. The proposed role of the JAK/STAT pathway is to maintain the

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Abbreviations used in this paper: *bam*, *bag-of-marbles*; CB, cystoblast; Dad, daughters against DPP; DPP, Decapentaplegic; ESC, escort stem cell; GSC, germline stem cell; JAK/STAT, Janus kinase/signal transducer and activator of transcription; N, Notch; SSC, somatic stem cell; TF, terminal filament.

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anatomical structure of the niche; however, it is unclear whether this pathway might also play a role in cap cells (Decotto and Spradling, 2005).

Results and discussion

Ectopic activation of JAK/STAT pathway in ovary induces ectopic spectrosome-containing cells

DeCotto and Spradling (2005) had previously shown that ectopic expression of *upd* driven by c587-GAL4 in female germarium results in tumorous germarium, a phenotype similar to ectopic activation of DPP signaling, a key signaling component of the female GSC niche (Xie and Spradling, 1998). In a small-scale candidate-approach screen, we also found that ectopic expression of *upd* results in formation of ectopic GSC-like cells. We now investigate the nature of these tumors and consider a possible link of JAK/STAT signaling to DPP signaling.

In the wild-type germarium, there are 2–3 GSCs and 2–3 CBs, each of which contain a spectrosome, a spherical α-Spectrin–positive intracellular membranous organelle. During cyst formation, this structure branches to connect individual cystocytes and is referred to as a fusome (Fig. 1 B) (Lin et al., 1994). In germaria ectopically expressing *upd*, more than 90% ($n > 100$) of germaria exhibited extra spectrosome-containing cells (Fig. 1 C), consistent with a previous report (Decotto and Spradling, 2005). About 20% of these germaria ($n = 90$) contained hundreds of cells with a spectrosome (Fig. 1 D). We also noticed that a high proportion (> 50%, $n > 400$) of adult females were completely devoid of any ovary, suggesting that ectopic *upd* driven by *c587-GAL4* may have other effects during early stages of gonadal development. Indeed, enlarged ovaries with abnormal structure were often observed in late larval stages (Fig. S1, available at http://www/jcb.org/cgi/content/full/jcb.200711022/DC1). To investigate whether ectopic expression of *upd* can induce formation of ectopic GSC-like cells in the adult ovary, we used the "flip-out" cassette to induce ectopic *upd* expression in adult germaria (see Materials and methods) (Ito et al., 1997); these germaria also contained extra spectrosome-containing cells, albeit to a lesser extent (Fig. 1 E; see Fig. S2 for additional methods to confirm this result). Formation of these ectopic spectrosome-containing cells by *upd* overexpression was suppressed in the *stat92e^{TS}* background, suggesting that *upd* acts through the canonical JAK/STAT signaling pathway (Fig. 1, F and G). Together, these results show that ectopic activation of JAK/STAT signaling in the somatic cells of the adult ovary can induce the formation of ectopic spectrosome-containing cells.

The ectopic spectrosome-containing cells are GSC-like

To address the identity of these extra spectrosome-containing cells, we examined expression of *bam*, a key differentiation promoting factor that is turned off in GSCs but highly expressed in CBs and early differentiating cysts (McKearin and Ohlstein, 1995). As previously demonstrated by Chen and McKearin (2003b), in wild-type ovaries, *bam-GFP* is absent in GSCs but expressed in CBs and differentiating cysts (Fig. 1 H). However, in germaria with ectopic *upd* expression, most spectrosome-containing cells did not express *bam-GFP* (Fig. 1 I).

It has been shown that high levels of DPP signal reception within GSCs represses *bam* expression (Chen and McKearin, 2003a; Kai and Spradling, 2003; Song et al., 2004). We next assessed the status of DPP signal reception within these ectopic spectrosome-containing cells. In *Drosophila*, DPP binds and brings together type I and type II serine/threonine receptor kinases. The constitutively active type II receptor Punt phosphorylates type I receptor Tkv (Thick Vein) and/or Saxphone (Sax), which propagates signaling by phosphorylating and activating the R-Smad, Mad (Mothers against DPP). The phosphorylated Mad (p-Mad) forms a complex with Medea and translocates into the nucleus to regulate the expression of target gene such as *Dad* (*Daughters against DPP*) (Tabata and Takei, 2004). In wild type, only GSCs show high levels of Dad-lacZ (a lacZ insertion in Dad) (Tsuneizumi et al., 1997) and pMad expression (Fig. 2, A and C). However, in germaria ectopically expressing *upd*, most spectrosome-containing cells showed strong Dad-lacZ and pMad expression, indicating activation of DPP signaling (Fig. 2, B and D). These data show that ectopic activation of JAK/STAT signaling in germaria induces ectopic DPP signaling and excess GSC-like cells.

JAK/STAT signaling functions downstream/independently of Notch signaling for GSC self-renewal

DPP, presumably produced in niche cells, is the essential extrinsic signal required for GSC self-renewal and its activity is spatially restricted to the GSC niche (Xie and Spradling, 1998; Kai and Spradling, 2003). Recent studies suggest that ectopic cap cell formation induced by ectopic activation of Notch (N) pathway, another conserved signaling cascade, also supports formation of extra GSC-like cells (Ward et al., 2006; Song et al., 2007). So, it is possible that ectopic activation of JAK/STAT signaling may induce ectopic cap cells to support ectopic GSC-like cells. In wild type, there are 5.3 ± 0.7 ($n = 109$) cap cells at the base of the TF cell stack that associate with GSCs (Fig. 3 A) (Song et al., 2002, 2007; Ward et al., 2006). Germaria ectopically expressing *upd* also contained 5.8 ± 1.0 ($n = 39$) cap cells (Fig. 3 B), indicating that ectopic activation of JAK/STAT signaling does not induce ectopic cap cell formation. To address whether JAK/STAT signaling is required for cap cell formation under normal circumstances, we examined cap cell formation in *stat92e^{TS}* background. Consistent with Decotto and Spradling's (2005) result, *stat92e^{TS}* germaria contained 4.6 ± 1.4 ($n = 133$) cap cells, suggesting that JAK/STAT activity is not essential for the cap cell formation (Fig. 3 C). Together, these results indicate that ectopic activation of JAK/STAT signaling may function directly on DPP signaling rather than indirectly via cap cell formation to support ectopic GSC-like cells.

We next asked whether ectopic N activation functions upstream of the JAK/STAT pathway to induce ectopic GSC-like cells. As previous reports (Ward et al., 2006; Song et al., 2007), ectopic N signaling activation in wild-type germaria induced extra cap cells (14.8 ± 7.4, $n = 52$) and extra GSC-like cells (10.4 ± 4.6, $n = 131$, Fig. 3 D). Interestingly, the formation of the ectopic

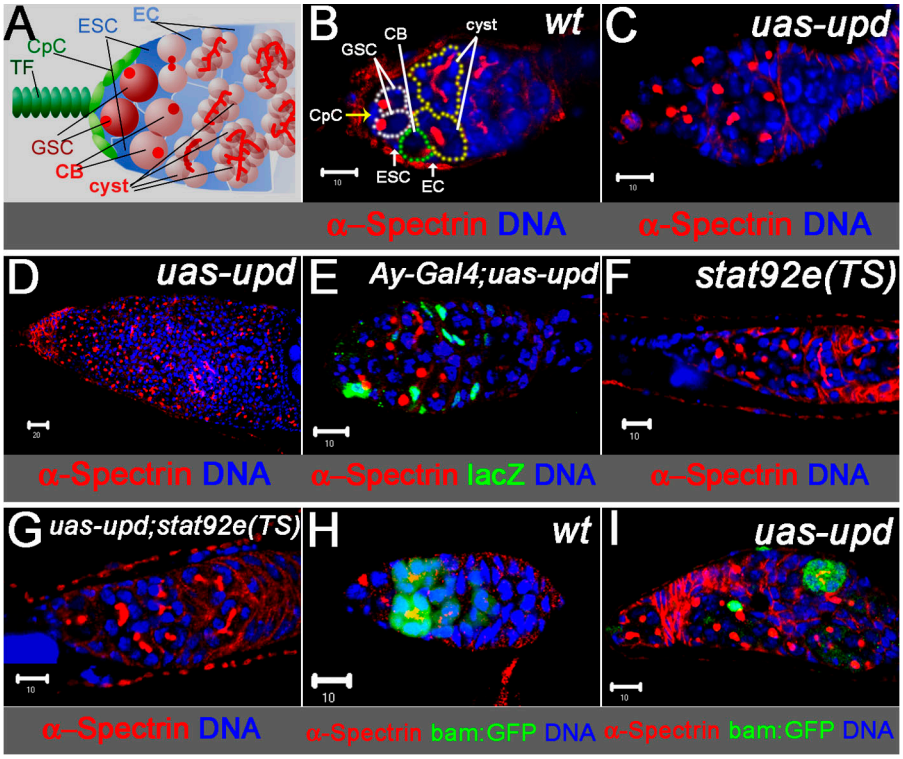

Figure 1. **Ectopic JAK/STAT signaling induces the formation of ectopic GSC-like cells.** (A) A cartoon represents GSCs and the niche. The niche is comprised of TFs, cap cells (CpCs), and ESCs. GSCs and CBs contain a specrtosome (spherical structure in red), and differentiating cysts contain a fusome (branched structure in red), (B) a wild-type germarium, (C and D) *upd* overexpressing germaria, and (E) a flp-out germarium contained extra spectrosome-containing cells; (F) a *stat92e*^{TS} germarium did not contain GSC, (G) *stat92e*^{TS} suppressed the formation of ectopic spectrosome-containing cells in *upd* overexpressing germarium, (H) bam:GFP is turn off in wild-type GSC, (I) spectrosome-containing cells in *upd* overexpressiong germarium did not express bam:GFP. Anterior toward left. Bars: 10 μm (B and C, and E–I): 20 μm (D).

cap cells (n = 13.3 ± 4.3, n = 77) induced by ectopic N signaling was not suppressed by compromising DPP signaling. However, the formation of ectopic GSC-like cells (4.8 ± 1.0, n = 45, Fig. 3 E) was suppressed by reduction of DPP signaling, suggesting that DPP signaling functions downstream of cap cell formation induced by N signaling to control GSC self-renewal. When JAK/STAT activity is compromised, ectopic N signaling still induced ectopic cap cells (12.5 ± 5.9, n = 38), in agreement with the notion that JAK/STAT activity is not required for cap cell formation. Interestingly, the formation of ectopic GSC-like cells induced by ectopic N signaling is suppressed in *stat92e*^{TS} background (6.8 ± 2.1, n = 50, Fig. 3 F), consistent with the notion that DPP signaling is compromised in these germaria. Together, these results indicate that JAK/STAT signaling functions downstream or in parallel of N signaling in the female GSC niche.

JAK/STAT signaling regulates *dpp* expression in the female GSC niche

The effects of ectopic expression of *upd* and *dpp* in somatic cells are similar. Both result in germ cell hyperplasia and arrest germ cells at a GSC-like stage with high levels of DPP signal reception and low levels of Bam expression (Fig. 2 G, and not depicted) (Xie and Spradling, 1998). These imply that JAK/STAT signaling and DPP signaling may function in a linear pathway to induce ectopic GSC-like cells. Consistent with this, reduction of DPP signaling in the *upd* overexpression background suppresses the ectopic GSC-like cell phenotype (Fig. 2 F and not depicted). Furthermore, DPP overexpression in somatic cells still induced ectopic GSC formation even in *stat92e*^{TS} background. These data suggest that DPP signaling may function downstream of JAK/STAT signaling (Fig. 2 H).

We then asked how JAK/STAT signaling regulates DPP signaling. First, we examined the activation of JAK/STAT signaling in the germarium. Stat92E^{06346}, a lacZ enhancer trap insertion in *Stat92E*, is strongly expressed in somatic cells, including the cap and ESC cells (Baksa et al., 2002; Decotto and Spradling, 2005). A STAT92E-GFP transgene, which apparently reflects the activity of the JAK/STAT pathway in *Drosophila* (Bach et al., 2007), is also expressed in cap and ESC cells. These data suggest that JAK/STAT signaling is activated in the somatic cells associated with the GSC niche (Fig. 2 I). Next, we examined the status of DPP signaling in *stat92*^{TS} mutant germaria in which JAK/STAT signaling is compromised. In wild type, the 2–3 GSCs within the GSC niche show strong pMad expression, indicating that the DPP pathway is activated (Fig. 2 C). However, in *stat92e*^{TS} germaria, more than 50% (n = 120) of GSCs show markedly reduced pMad expression, indicative of low levels of DPP signaling (Fig. 2 J). As Decotto and Spradling's (2005) previous report, we also observed that *stat92e*^{TS} germaria showed progressive loss of GSCs as observed in *dpp* mutant germaria (Xie and Spradling, 1998; Decotto and Spradling, 2005). Together with the observation that JAK/STAT signaling is dispensable in the germline for GSC maintenance (Decotto and Spradling, 2005), these results suggest that there is a requirement for JAK/STAT signaling in the somatic cells of the niche for normal DPP signaling.

The observations that *dpp* mutants strongly suppress the ectopic GSC-like cell phenotype induced by *upd* overexpression suggest that JAK/STAT signaling may regulate *dpp* expression in the GSC niche. To address this, RNA in situ hybridization

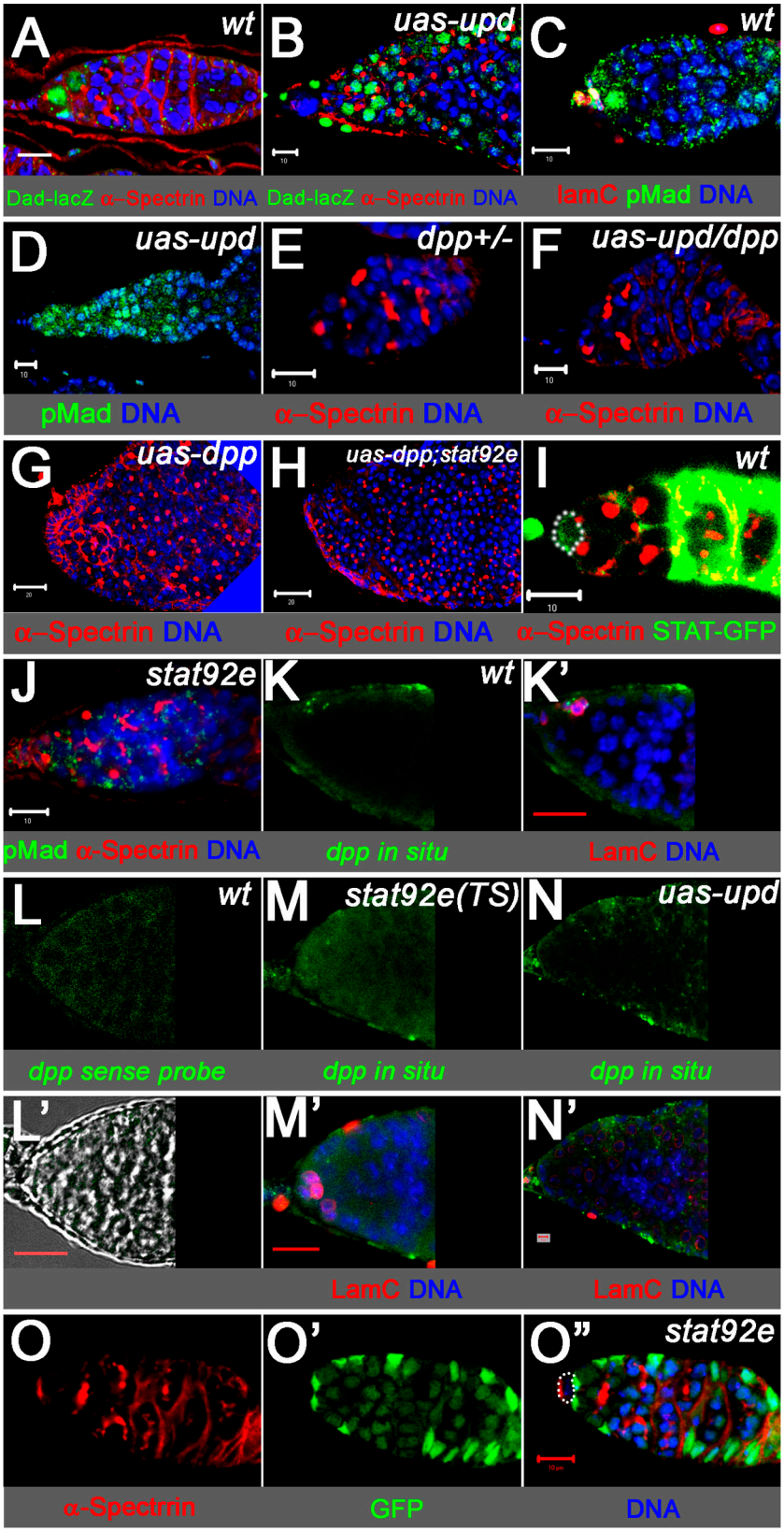

Figure 2. **JAK/STAT signaling regulates *dpp* expression in the GSC niche.** Most GSC-like cells in *upd* overexpressing germarium expressed high levels of Dad-lacZ (compare B with A) and pMad (compare D with C). (E) A $dpp^{+/-}$ germarium, (F) extra GSC-like cell phenotype in *upd* overexpressing germaria was suppressed by removing one copy of *dpp*. Ectopic *dpp* expression in wild type (G) and $stat92e^{TS}$ (H) resulted in the formation of ectopic GSC-like cells. (I) JAK/STAT signaling activity could be detected in cap cells using a STAT-GFP reporter. (J) GSCs in $stat92e^{TS}$ showed low pMad expression. (K) *dpp* transcripts were detected in cap cells marked by lamC, the punctuated signal likely reflects the *dpp* RNA in RNP granule or the nascent transcripts (L) sense probe as control, (L') phase-contrast image, (M) *dpp* transcripts were strongly reduced in $stat92e^{TS}$ germarium but up-regulated in *upd* overexpressing germarium (N), (O) compromising Stat92e activity in cap cells resulted in GSC loss, mutant clones lack GFP signal and were marked by white-dot circle. Anterior toward left. Bars: 10 μm (B–F, I–O); 20 μm (G and H).

experiments were performed. In the wild-type GSC niche, *dpp* transcripts were invariably detected in cap cells (Fig. 2, K–L, see Fig. S3 for more images; available at http://www/jcb.org/cgi/content/full/jcb.200711022/DC1). In many gemaria, lower levels of *dpp* expression were also detected in some somatic cells next to cap cells, presumably ESCs. It is worthy to note that even within the same germarium, the signal intensity varies among individual cap cells. This may reflect the dynamic nature

of *dpp* expression in cap cells. A similar observation was reported for the expression of Dad-lacZ (Casanueva and Ferguson, 2004). These results suggest that cap cells are likely a major source of *dpp* in the GSC niche and are consistent with the important role of cap cells in the GSC niche (Xie and Spradling, 2000; Song et al., 2007). We next examined *dpp* expression in germarium with compromised JAK/STAT activity. In *stat92e*^{TS} germaria, *dpp* transcripts were strongly reduced/absent from the cap cells (Fig. 2, M and M′; see Fig. S3 for more images) and presumably the ESCs. In a complementary experiment, in germaria with ectopic *upd* expression, *dpp* transcripts were strongly up-regulated (Fig. 2, N and N′). Furthermore, ectopic *upd* expression could activate luciferase reporters containing *dpp* regulatory regions in the S2 cell (Fig. S3). Decotto and Spradling (2005) had shown that JAK/STAT signaling functions in ESCs to affect GSC maintenance, the loss of GSCs in *stat92e*^{TS} germaria may reflect the collaborative roles of JAK/STAT signaling in cap cells and ESCs. We next examined whether JAK/STAT signaling is required in cap cells by generating cap cells mutant for STAT92E (see Materials and methods). Indeed, in 13% (n = 15) of these germaria, the GSC niche was occupied by a fusome-containing cyst, indicating precocious differentiation of GSCs (Fig. 2 O). Together, these data suggest that the JAK/STAT pathway positively regulates *dpp* expression in the GSC niche and DPP, in turn, acts to control GSC self-renewal.

The JAK/STAT pathway also appears to regulate *dpp* expression in the male GSC niche

The male GSC niche provides another well-established system to study stem cell biology (Gilboa and Lehmann, 2004; Yamashita et al., 2005; Fuller and Spradling, 2007). In the apical tip of the male testis, GSCs attach to a cluster of somatic hub cells, known as the niche. Upd is produced in the hub cells and functions as a short-range signal to activate downstream JAK/STAT signal reception within GSCs to maintain GSC self-renewal (Kiger et al., 2001; Tulina and Matunis, 2001). In addition to JAK/STAT signaling, DPP signaling is also implicated in the maintenance of GSC in male testis (Kawase et al., 2004). We examined whether JAK/STAT signaling may also regulate *dpp* expression in the male GSC niche.

In wild-type testis, GSCs undergo self-renewal division to generate a GSC daughter and a gonialblast, which initiates differentiation (Yamashita et al., 2005). pMad accumulates to high levels in GSCs located next to the hub cells and some gonialblasts, suggesting high levels of DPP/BMP signaling (Fig. 4, A and D) (Kawase et al., 2004). However, in *stat92e*^{TS} testes, most GSCs show low levels of pMad accumulation, consistent with the notion that DPP/BMP signaling is low when JAK/STAT activity is compromised (Fig. 4, B and E). However, testes with ectopic activation of JAK/STAT signaling contain hundreds of cells containing a spectrosome (Kiger et al., 2001; Tulina and Matunis, 2001). Interestingly, pMad also accumulates to high levels in these ectopic spectrosome-containing cells, indicating high levels of DPP/BMP signaling in these cells (Fig. 4, C and F). In wild-type testis, *dpp* is expressed at low levels (Fig. 4 G) (Kawase et al., 2004). Consistent with the notion that the JAK/STAT pathway can positively regulate *dpp* expression in the testis, *dpp* transcript is strongly up-regulated in testes ectopically expressing *upd* (Fig. 4, G and H). Together, these suggest that similar to the female germaria, JAK/STAT signaling may also regulate *dpp* expression in the male GSC niche.

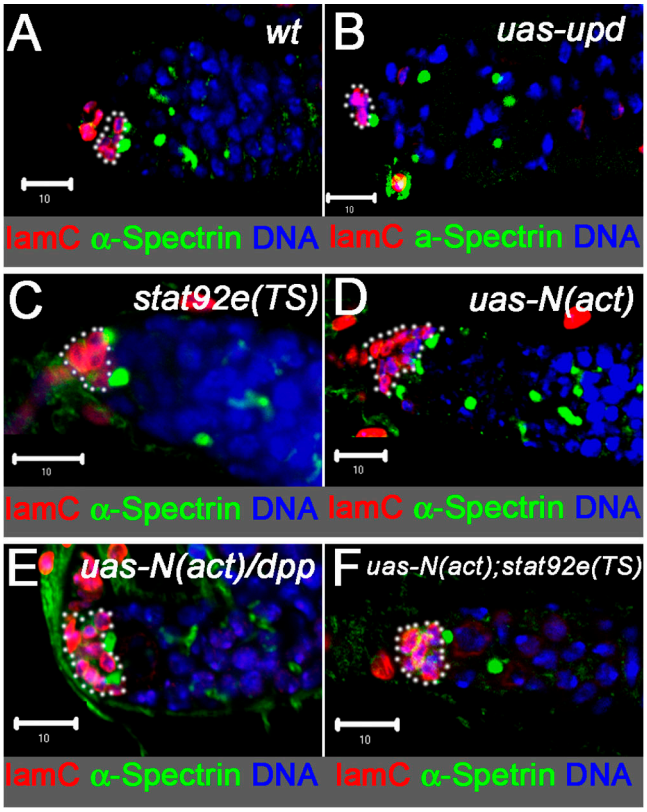

Figure 3. **JAK/STAT signaling functions downstream or in parallel of N pathway.** Similar number of lamin C–positive cap cells were present in (A) wild-type, (B) *c587-GAL4; uas-upd*, and (C) *stat92e*^{TS} germaria. Note that there are more lamin C–positive cap cells when N signaling was ectopically activated in wild-type (D), *dpp*$^{+/-}$ (E), and *stat92e*^{TS} (F) background. Anterior toward left. Bars, 10 μm.

A model for JAK/STAT signaling in GSC niche

In this study, we show that in female GSC niche, *dpp* expression is mainly detected in the cap cells, supporting previous observations that the number of cap cells correlates closely with the number of GSCs (Xie and Spradling, 2000; Song et al., 2007). A recent report showed that in the female GSC niche, JAK/STAT signaling also involves in GSC maintenance via its requirement in ESC lineage for the anatomical structure of the niche (Decotto and Spradling, 2005). Our results further extend this conclusion and importantly define a new role for JAK/STAT signaling. Our data suggest that in female GSC niche, JAK/STAT signaling acts downstream/independently of the N pathway but upstream of DPP signaling to regulate GSC self-renewal by regulating *dpp* expression. Consistent with this, compromising JAK/STAT activity in niche cells causes GSC to undergo precocious differentiation, whereas ectopic activation of JAK/STAT activity in these cells results in expansion of the activity of the GSC niche and subsequent expansion of the GSC-like population.

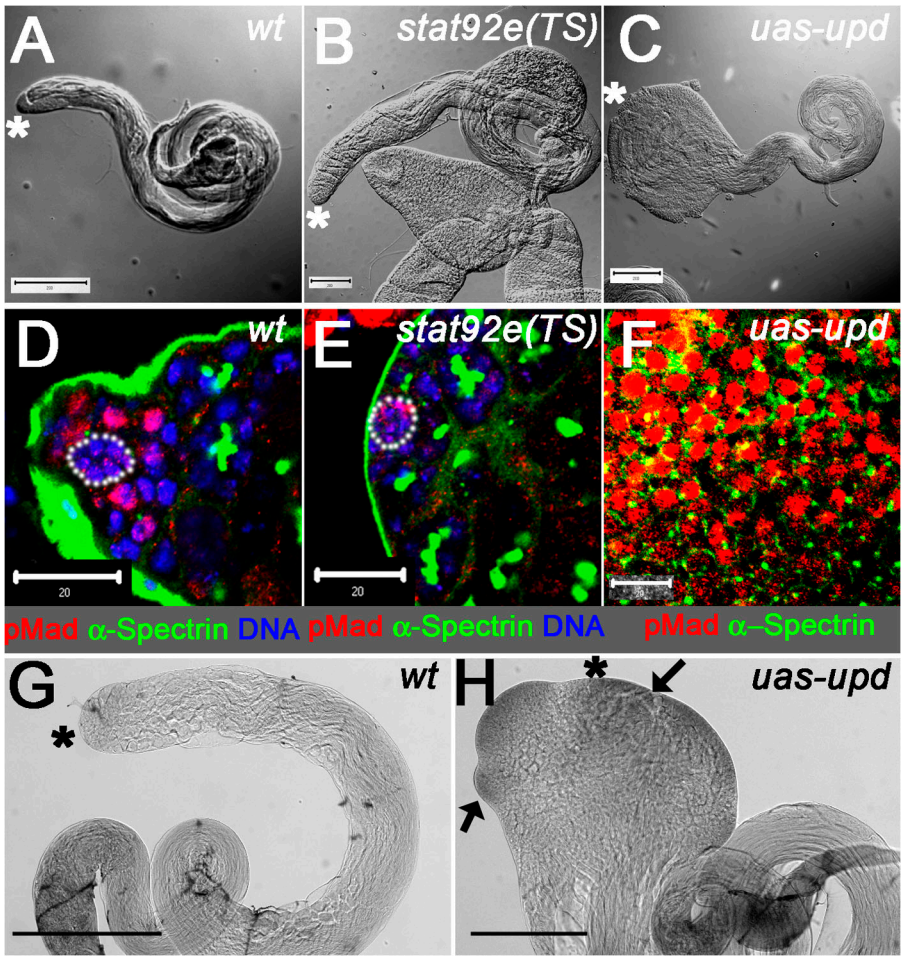

Figure 4. **JAK/STAT signaling may regulate *dpp* expression in male testis.** (A–C) phase-contrast images of adult (A) wild-type, (B) *stat92e*^{TS} (2 d after temperature shift in 31°C), and (C) *c587-GAL4; uas-upd* testes. Note there is drastic enlargement of anterior tip. pMad expression was down-regulated in *stat92e*^{TS} (compare E with D) but up-regulated in *upd* overexpressing germarium (compare F with D). Hub cells are outlined with white dots in D and E. *dpp* expression was not detected in wild-type testis (G) by RNA in situ, but was highly expressed in *upd* overexpressing testis (H, arrows). Anterior toward left (except in H) and marked by asterisk. Bars: 200 μm (A–C and G–H); 20 μm (D–F).

Thus, the activity of JAK/STAT signaling contributes to the spatial activity of the niche. Furthermore, in the male GSC niche, JAK/STAT signaling also appears to regulate DPP/BMP signaling. These data are consistent with previous findings showing that overexpression of *dpp* can partially rescue the *stat92e* loss-of-function phenotypes in the male testis (Singh et al., 2006). Thus, our findings highlight the similarity between the female and male GSC niche and suggest a possible common origin for these two stem cell niches. However, it is interesting to note that the requirement for JAK/STAT signaling and DPP signaling are different in these niches. Cell-autonomous JAK/STAT activity is required in male GSC for self-renewal, but dispensable for female GSC self-renewal (Decotto and Spradling, 2005; Yamashita et al., 2005). Although the JAK/STAT pathway might be a common ancestor shared by these two niches, clear differences exist in the manner in which it is used. Thus, our data further suggest that the similarity and difference between these two stem cell niches might provide an excellent opportunity to understand how the diversity of niches could evolve from a common ancestor.

Materials and methods

Drosophila stocks

All strains were maintained at room temperature on standard cornmeal-agar medium. Information about strains used in this study was described in the text or in FlyBase. The genotypes of the mutant lines used in this study were: y^1,w^{1118} (used as wild-type control), *uas-upd* (gift of Toshie Kai, TLL, Singapore), c587-GAL4 (Kai and Spradling, 2003), *stat92e*^{06346} (Hou et al., 1996), *stat92e*^{F} (Baksa et al., 2002), *stat92e*^{j6C8} (Spradling et al., 1999), *dpp*^{hr54} (Bangi and Wharton, 2006), *dpp*^{hr4} (Wharton et al., 1996), *dpp*^{e90} (Wharton et al., 1993), dpp-lacZ (Jiang and Struhl, 1995), *mad*^{12} (Sekelsky et al., 1995), *med*^{13} (Xu et al., 1998), *sax*^{P} (Nellen et al., 1994), *put*^{135} (Ruberte et al., 1995), dad-lacZ (Tsuneizumi et al., 1997), *uas-N*^{act}, *uas-DPP* (Tracey et al., 2000), 10XSTAT-GFP (Bach et al., 2007), bam:GFP (Chen and McKearin, 2003b), Ay-gal4 (Ito et al., 1997), hs-flp (Xu and Rubin, 1993), and uas-flp (Exelixis, Inc).

All crosses (except for *stat92e*^{TS} and *uas-N*^{act} experiments) were maintained at room temperature. Ovaries were dissected and examined from 4-d-old females (unless otherwise specified). For *stat92e*^{TS} (stat92e^{06346}/stat92e^{F}) experiments, crosses were set up at 18°C (permissive temperature). Larvae at mid-L3 stage were shifted to 31°C (nonpermissive temperature) until early pupal stage (cap cell formation period), and then shifted back to 20°C. Newly hatched females were shifted to 31°C and examined within 2–4 d after temperature shift (unless otherwise specified). For *c587-GAL4; uas-N*^{act}*; stat92e*^{TS} experiments, crosses were set up at 20°C, mid-L3 stage larvae were shifted to 31°C until early pupal stage, then shifted back to 20°C. Newly hatched females were shifted to 31°C and were examined within 4 d.

Generation of flp-out clone using Ay-GAL4 system

This technique combines the Flippase(flp)/FRT system and the GAL4/UAS system (Ito et al., 1997). Before heat-shock treatment, the Act5C promoter-GAL4 fusion gene is interrupted by a FRT cassette containing yellow (y^+) gene. Upon heat-shock treatment, *flp* gene is activated. Consequently, FLP excises the FRT cassette from the Act5C promoter-GAL4 region. As a result, the Act5C-GAL4 activity is reconstituted and drives uas-upd and uas-lacZ expression.

Generation of cap cell clone

C587-GAL4 is expressed in most, if not all, somatic cells including TFs, cap cells during niche formation in L3 stage (Song et al., 2007). C587-GLA4 was

recombined onto uas-flp chromosome. C587-GAL4-uas-flp/Fm7; FRT82B-ubi-GFP/Tb stock was established and crossed to FRT82B-stat92e^{i6c8} to generate cap cell clones. Females were examined 7–10 d after hatching.

In situ hybridization

Primers CAAGGAGGCGCTCATCAAG and TAATACGACTCACTATAGGGAGACACCAGCAGTCCGTAGTTGC were used to amplify *dpp* antisense in situ template from a cDNA pool generated from ovaries and confirmed by DNA sequencing. Primers for sense control in situ template are TAATACGACTCACTATAGGGAGACAAGGAGGCGCTCATCAAG and CACCAGCAGTCCGTAGTTGC. Probes are labeled using Roche DIG RNA labeling kit (SP6/T7) following manufacturer's instructions.

Protocol for fluorescent in situ hybridization for Fig. 2 follows Wilkie et al. (1999) and Vanzo and Ephrussi (2002).

Protocol for in situ hybridization for Fig. 4 was modified from conventional method used in embryos with DIG-labelled probes followed by alkaline phosphatase (AP)–based histochemical detection. Testes were dissected in PBS, then fixed in 4% paraformaldehyde, 0.1M Hepes (pH 7.4) in PBS for 20 min, washed 5 times with PBT (0.1% Tween20), washed with 1:1 PBT/hybridization solution (50% formamide, 4x SSC, 1x Denhardts, 250 ug/ml tRAN, 250 ug/ml ssDNA, 50 ug/ml heparin, 0.1% Tween 20, and 5% dextran sulfate), rinsed 3x with PBT, prehybridized at 52°C for 1 h, hybridized overnight with DIG-labelled RNA probe at 52°C, rinsed with wash buffer (50% formamide, 2x SSC, and 0.1% Tween 20), washed overnight with wash buffer, rinsed with PBT, incubated with anti-DIG-AP (1:2,000; Roche) in PBT (with 3% BSA) for 2 h, washed with PBT for 1 h, rinsed with AP buffer (100 mM Tris, pH 9.5, 100 mM Nacl, 50 mM $MgCl_2$, and 0.1% Tween 20), incubated in 0.3 ml AP buffer containing 2.7 μl NBT/X-gal (Roche) until desired signal appears and then stopped with PBT, removed PBT, and mounted in Vectashield mounting medium (Vector Laboratories).

Immunostaining

Antibody stainings of ovaries were modified from (Tomancak et al., 2000) as follows. Females were dissected in PBS, ovaries were collected and then fixed in 4% paraformaldehyde, 0.1M Hepes (pH 7.4) in PBS for 20 min, blocked with PBS + 0.1% Triton X-100 + 3% BSA for 1 h, probed with primary antibody for 4 h at room temperature, washed 3x with PBS + 0.1% Triton X-100, probed with fluorescence-labeled secondary antibody for 2 h at room temperature, washed 3x with PBS + 0.1% Triton X-100, stained with ToPro-3 for 10 min, and then mounted in Vectashield mounting medium (Vector Laboratories). The following antibodies were used: rabbit anti-β-galactosidase (1:5,000; Cappel), rabbit anti-PS1 (1: 1,000; Tanimoto et al., 2000), rat anti-BamC (1:500; McKearin and Ohlstein, 1995), rabbit anti-α-Spectrin (1:500; Byers et al., 1987), mouse anti-α-Spectrin (3A9, 1:50; DSHB), mouse anti-β-galactosidase (1:1,000; Promega), mouse anti-lamin C (LC28.26, 1:50; DSHB), and Alexa 555/643/488 conjugated goat anti–mouse and rabbit secondary antibodies were used to detect primary antibodies (1:500; Molecular Probes).

Microscopy

Samples were mounted in Vectashield mounting medium (Vector Laboratories). Images were collected using a microscope (Axioplan 2) with an upright confocal system (LSM510 META; both from Carl Zeiss, Inc.) at room temperature. The objective lens used was a Plan NEOFLUAR 40×/1.3 oil and the imaging software used was Zeiss LSM510 (both from Carl Zeiss, Inc). The confocal images were extracted with LSM510 browser software (Carl Zeiss, Inc.) then processed in Adobe Photoshop 7.0.1 with adjustments of brightness and contrast. Bars are indicated in each individual image.

Online supplemental material

Fig. S1 shows abnormal ovary development in larval stage (L3) when *upd* is ectopically expressed. Fig. S2 shows additional data to show that ectopic activation of JAK/STAT signaling induces the formation of ectopic GSC-like cells. Fig. S3 contains additional data show that *dpp* is mainly expressed in cap cells of female GSC niche, and this expression depends on JAK/STAT signaling. In addition, ectopic *upd* expression in S2 culture cells could activate luciferase reporters containing regulatory regions of *dpp* locus. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200711022/DC1.

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