Jak/Stat signalling in niche support cells regulates $dpp$ transcription to control germline stem cell maintenance in the *Drosophila* ovary

Lourdes López-Onieva, Ana Fernández-Miñán* and Acaimo González-Reyes†

The existence of specialised regulatory microenvironments or niches that sustain stable stem cell populations is well documented in many tissues. However, the specific mechanisms by which niche support (or stromal) cells govern stem cell maintenance remain largely unknown. Here we demonstrate that removal of the Jak/Stat pathway in support cells of the *Drosophila* ovarian niche leads to germline stem cell loss by differentiation. Conversely, ectopic Jak/Stat activation in support cells induces stem cell tumours, implying the presence of a signal relay between the stromal compartment and the stem cell population. We further show that ectopic Jak/Stat signalling in support cells augments $dpp$ mRNA levels and increases the range of Dpp signalling, a Bmp2 orthologue known to act as a niche extrinsic factor required for female germline stem cell survival and division. Our results provide strong evidence for a model in which Jak/Stat signalling in somatic support cells regulates $dpp$ transcription to define niche size and to maintain the adjacent germline stem cells in an undifferentiated state.

**KEY WORDS:** Jak/Stat, Germline stem cells, Niche signalling, BMP, *Drosophila* oogenesis

**INTRODUCTION**

The generation, maintenance and repair of adult tissues and organs rely on populations of stem cells. In order to ensure an appropriate production of tissue cells during embryogenesis and adulthood, stem cells possess the ability to divide symmetrically or to undergo asymmetric divisions to self-renew and to produce differentiating progeny. The balance between stem cell proliferation and differentiation is brought about by regulatory microenvironments termed ‘niches’ in which a specialised cellular context provides signals and physical support to maintain stem cells. Thus, support (or stromal) cells of the niche play pivotal roles in organogenesis, in tissue homeostasis and repair, and in stem cell behaviour (Fuchs et al., 2004; Scadden, 2006; Spradling et al., 2001).

Germline stem cells (GSCs) are broadly conserved across animal species. Although the normal development of this type of stem cells is in some respects limited, as they normally give rise only to sexual gametes and accessory cells, they show a series of characteristics that make GSCs an important source of information useful for understanding stem cell behaviour (Wong et al., 2005). For instance, differentiating *Drosophila* germline cells have been shown to de-differentiate and to adopt a stem cell fate under certain experimental conditions, thus opening the possibility to find new sources of progenitor cells for tissue repair (Brawley and Matunis, 2004; de Rooij and Russell, 2000; Kai and Spradling, 2004). Similarly, a number of niches hosting GSCs have been defined in several experimental systems such as mice, flies and worms. The *Drosophila* germline has emerged as one of the best experimental systems in which to study the biology of stem cells and their niches. We have focused our investigations on the ovarian niche and on the behaviour of the GSCs contained within it. Ovarian GSCs are located in the anterior tip of the gerarium, a structure composed of germline cells – including GSCs, and differentiating cytoplasts and cystocytes – and a few somatic cell types, namely terminal filament cells (TFCs), cap cells (CpCs), escort stem cells (ESCs) and escort cells (ECs). These somatic cells have been shown to provide physical support and signals to the GSC population (Decotto and Spradling, 2005; Xie and Spradling, 2000).

Communication between support cells and stem cells is crucial to control ovarian niche formation and to avoid depletion of stem cells. *decapentaplegic* (*dpp*), *glass bottom boat* (*gbb*), *fs(1)Yb*, *piwi* and *hedgehog* are known to be expressed in somatic support cells and to control GSC numbers (Cox et al., 1998; Cox et al., 2000; King and Lin, 1999; King et al., 2001; Song et al., 2004; Xie and Spradling, 1998). Although it is well established that the activity of the two BMP-like molecules Dpp and Gbb is required for GSC maintenance by directly repressing transcription of the differentiation-promoting gene *bag of marbles* (*bam*) and by modulating the activity of the putative regulator of translation Pelota (Chen and McKearin, 2003; Song et al., 2004; Szakmary et al., 2005; Xi et al., 2005; Xie and Spradling, 1998), the mechanisms that ensure appropriate BMP signalling in the GSC niche remain unknown. In this work, we identify a signalling pathway that modulates BMP signalling in the niche. The evolutionarily conserved Janus kinase/Signal transducer and activator of transcription (Jak/Stat) signalling pathway has been identified as a key regulator of the *Drosophila* germline niches (Decotto and Spradling, 2005; Kiger et al., 2001; Tulina and Matunis, 2001). Here we show that this pathway acts upstream of *dpp* transcription in ovarian support cells to ensure the maintenance of the adjacent GSC population.

**MATERIALS AND METHODS**

**Staining procedures and microscopy**

Immunohistochemistry was performed at room temperature using standard procedures (detailed protocols are available upon request). Primary antibodies were used at the following concentrations: mouse anti-Hts [Developmental Studies Hybridoma Bank (DSHB), University of Iowa],

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Accepted 13 November 2007
Development 135 (3) 534 RESEARCH ARTICLE

1/50; rabbit anti-Upd (Harrison et al., 1998), 1/250; rabbit anti-β-galactosidase (Cappel), 1/10,000; rabbit anti-GFP (Molecular Probes), 1/500; rabbit anti-phosphorylated Mad (a gift from Ginés Morata, CBM-UAM, Madrid, Spain), 1/500. Cy2- and Cy3-conjugated secondary antibodies (Jackson ImmunoResearch) were used at 1/200. DNA staining was performed using the dyes TOPRO-3 (Molecular Probes) at 1/1,000, or Hoechst (Sigma) at 1/1,000. Images were captured with a Leica TCS-SP2 confocal microscope and processed using Adobe Photoshop.

Fly stocks
Flies were raised on standard Drosophila media at 25°C unless indicated otherwise. Star2EΔ12, domeΔ15, hop27, hop23, hop2 and updPMM26 (also known as ovoPMM) have been described elsewhere (Binari and Perrimon, 1994; Brown et al., 2001; Hou et al., 1996; Perrimon and Mahowald, 1986; Wieschaus et al., 1984). To express UAS-DsRed (Bloomington Stock Center) or UAST-dome27 (Brown et al., 2001) in somatic cells we used the bab1-Gal4 driver (Bolivar et al., 2006). In order to obtain adult females overexpressing upd2 (Hombria et al., 2005) or hop2m (Harrison et al., 1995) under the control of bab1-Gal4, we crossed w; tub-Gal80Δ/ CyO; bab1-Gal4/TM2 with yw; UAS-upd2 or yw; UAS-hop2m, respectively. The offspring were grown at 18°C and, upon eclosion, adult F1 flies were shifted to 31°C for 4 days.

Generation of somatic and germline clones
Germline mutant clones were generated using the FLP/FRT technique. The following chromosome combinations were used: y w hs-flp122, FRT2B Star2EΔ12, FRT19A domeΔ15 and FRT101 hop2; 72- to 96-hour-old larvae were heat shocked for 1 hour at 37°C; adult offspring were transferred to fresh food and kept at 25°C until dissection at the appropriate time. To generate somatic mutant clones we used the following chromosomes: FRT101 hop2 and FRT101 ubi-GFP; bab1-Gal4 UAST-flp.

Reverse transcriptase (RT) PCR
mRNA was isolated from ~100 ovary pairs from yw virgin females or from 12- to 24-hour-old yw embryos and purified using the QuickPrep Micro mRNA Purification Kit (Amersham Biosciences) according to the manufacturer’s instructions. One to two micrograms of mRNA was used as a template for first-strand cDNA synthesis together with 0.5 μg of oligo(dt) (Sigma Genosis) and the Superscript II RNase H Transcriptase (Invitrogen) in a final volume of 20 μl. Two microlitres of the ovarian or embryonic cDNA libraries served as templates for the subsequent RT-PCR amplification. The following primers (5’-3’) were used for cDNA detection:

<table>
<thead>
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<th>sense</th>
<th>antisense</th>
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<tr>
<td>frt</td>
<td>CGAGGAGACCTTGGGATCATGAGTTG</td>
</tr>
<tr>
<td>hh</td>
<td>CAACAGGGCAGCATCTCTTTCGCC</td>
</tr>
<tr>
<td>upd</td>
<td>TTCTCGGTCCTTCTGCTGCTCTT</td>
</tr>
<tr>
<td>upd2</td>
<td>AGCCGCGAACCCAGGACGAGTTAATC</td>
</tr>
<tr>
<td>upd3</td>
<td>ATGGCCCAAGGTGCTGCTTCTC</td>
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Real-time PCR
Approximately 200 ovary pairs of the genotype tub-Gal80Δ/++; bab1-Gal4/+ or ~500 ovary pairs from tub-Gal80Δ/UAST-upd2; bab1-Gal4/+ females were used for mRNA isolation and cDNA synthesis following the above protocol (both types of females were grown at 18°C and, upon eclosion, shifted to 31°C for 4 days). Per reaction, 100 ng of the different cDNA libraries were used as a template for the subsequent real-time PCR reactions. The relative quantification of dpp and gbb expression was carried out using the Comparative Cycle Threshold (Ct) method (Separate Tubes protocol, Applied Biosysystems User Bulletin no. 2 (1997), ABI Prism 7700 Sequence Detection System) and Fam dye-labelled TaqMan MGB probes. Primers and TaqMan probes for the different cDNAs were obtained from the Assays-by-Design Service (Applied Biosysystems) as follows (5’-3’):

<table>
<thead>
<tr>
<th>sense</th>
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<tr>
<td>dpp</td>
<td>GCCCAACACAGTGGGAAGTTTGA</td>
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<tr>
<td>gbb</td>
<td>CGCTGTCCTCGTGTAACA</td>
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RESULTS
Jak/Stat signalling in somatic niche cells in the germarium
Genetic studies in Drosophila have identified three cytokine-like ligands [Upd (also known as Outstretched – FlyBase), Upd2 and Upd3], one transmembrane receptor (Dome), one kinase (Hop) and one transcription factor (Stat92E) as the positive transducers of Jak/Stat signalling (Agaisse et al., 2003; Binari and Perrimon, 1994; Brown et al., 2001; Chen et al., 2002; Gilbert et al., 2005; Harrison et al., 1998; Hombria et al., 2005). In order to assess the role of the Jak/Stat pathway in the ovarian niche we analysed the expression of several of its components in the germarium (Fig. 1). We made use of an antibody that recognises the Upd ligand (Harrison et al., 1998) and found that this protein strongly accumulates in TFCs and CPCs (Fig. 1B). Secondly, utilising a Stat92E-lacZ line (the bacterial lacZ gene inserted into the Stat92E gene), we observed β-galactosidase (β-gal) expression in all of the somatic cell types present in the anterior half of the germarium: TFCs, CPCs, ESCs and ECs (Fig. 1C; see Fig. S1 in the supplementary material). In addition, the fact that the Jak/Stat pathway reporters xStat92E-GFP and 10xStat92E-GFP – consisting of tandem repeats of the Stat92E-binding sequence upstream of GFP (Bach et al., 2007) – are expressed in CPCs indicates that the pathway is at least active in these somatic cells (data not shown).

Next, we isolated ovarian mRNA from virgin females and performed RT-PCR analysis to confirm that the three known ligands of the pathway (upd, upd2 and upd3) are expressed in wild-type ovaries (Fig. 1D).

It has been reported that overexpression of the Upd ligand in ESCs and ECs using the c587-Gal4 line leads to disorganised germaria and to rare ovarioles (3.5%) filled with GSC-like cells (Decotto and Spradling, 2005; Kai and Spradling, 2003). Considering the importance of CPCs for niche function (Song et al., 2007), we wished to study the effect of Upd or Upd2 ectopic expression using the bab1-Gal4 line, which induces strong expression of reporter genes in TFCs and CPCs and weaker levels in ESCs and ECs (Fig. 1E) (Bolivar et al., 2006). Whereas the overexpression of Upd produced a mild increase in the number of GSC-like cells in experimental germaria (not shown), bab1-Gal4-driven expression of UAST-upd2 gave a very consistent phenotype, as it caused hyperplastic stem cell growth in all of the ovarioles examined (n=100; Fig. 1F). In these germaria, we never observed the gross organisational defects reported after c587-Gal4-driven Upd expression (Decotto and Spradling, 2005). Because it has been established that Gal4-mediated Upd2 overexpression results in ectopic activation of the pathway (Hombria et al., 2005), the above...
result demonstrates that strong Jak/Stat pathway overactivation in TFCs and CpCs, and at lower levels in ESCs and ECs, is sufficient to increase greatly the number of GSCs present in the niche.

**Jak/Stat signalling is required in the ovary for germline stem cell maintenance**

It has previously been shown that the ovarian niche requires Jak/Stat signalling (Decotto and Spradling, 2005). Using viable, hypomorphic conditions of the pathway [hop^{71}/hop^{27} and hop^{71} upd^{M55}/hop^{77}; analysed 2, 10 and 25 days after eclosion (AE); see Table S1 in the supplementary material] we were able to confirm that the Jak/Stat pathway is required in the ovary for GSC maintenance and cyst production (Fig. 2A,B,E). In contrast to the wild-type controls, which showed on average 2.57±0.5 GSCs per germarium 25 days AE (n=58), the average number of GSCs in germaria of the strongest mutant combination (hop^{71} upd^{M55}/hop^{77}; n=33) dropped to just 0.9±0.8 25 days AE. Furthermore, ~25% of these mutant ovarioles were devoid of germline cells (not shown).

The morphology of the spectrosome has previously been used as a marker to assess GSC division (de Cuevas and Spradling, 1998). Early interphase spectosomes display a characteristic ‘exclamation mark’ figure (Fig. 2C) in which the nascent cystoblast spectrosome on the basal side of the cytokinetic ring remains temporally linked to the apically-anchored GSC spectrosome via the cytokeratinic neck. This study also showed that GSC cytokinesis only occurs several hours later, after S phase of the following cycle is completed in both the GSC daughter and its sister cystoblast (de Cuevas and Spradling, 1998). Surprisingly, during our analysis of Jak/Stat hypomorphic mutant germaria, we found that a large proportion of GSCs undergoing cytokinesis exhibited a strikingly different spectrosome arrangement. In these cases, most of the GSC spectrosome lost its apical localisation and came to lie next to the cytokinetic ring, adopting – together with the future cystoblast’s spectrosome – a ‘dumbbell shape’. In addition, a small ‘scar’ of spectrosomal organisation are hereafter referred to as ‘anchorless’, the frequency of which depends on the severity of the mutant condition and on the age of the female (see Table S1 in the supplementary material). In fact, nearly 75% of GSCs of the strongest mutant combination analysed 25 days AE showed anchorless spectrosomes (Fig. 2D,F). A detailed study of control ovaries indicated that anchorless figures are also found in wild-type niches. We observed that until 10 days AE, a small percentage (11-13%) of control GSCs show anchorless figures. However, there was a noticeable increase in the frequency of anchorless figures 25 days AE (23.5%), as GSCs aged (Fig. 2F).

The increased frequency of GSCs containing anchorless spectrosomes in ageing wild-type niches and in Jak/Stat mutant niches raises the question of the significance for GSC niche function of the occurrence of anchorless spectrosomes. Wild-type GSCs are known to be lost from the niche as flies age (Xie and Spradling, 1998; Xie and Spradling, 2000). Similarly, we have shown that the average number of Jak/Stat mutant GSCs per germarium is greatly reduced in comparison to the controls, a phenotype that becomes more severe with time (Fig. 2E; see Table S1 in the supplementary material). Thus, there is a correlation between the rise in the frequency of anchorless figures in wild-type niches and in Jak/Stat mutant niches and the occurrence of GSC loss. Hence, it is possible that the high frequency of an anchorless spectrosome arrangement is generally related to stem cell loss. Both the experimental evidence provided below and the

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**Fig. 1. Components of the Jak/Stat pathway are expressed in the GSC niche. (A) Schematic of a Drosophila germarium. Terminal filament cells (TFCs), cap cells (CpCs), escort stem cells (ESCs) and escort cells (ECs) are of somatic origin. Germline stem cells (GSCs) are in close contact with CpCs and contain apical spectosomes that evolve into ‘exclamation mark’ figures in post-mitotic GSCs. Cystoblasts (CB) also contain spectosomes, but these do not maintain an apical localisation. CBs undergo four incomplete rounds of division giving rise to cysts of two, four, eight and sixteen cells interconnected by branched fusomes. (B) Wild-type germarium double stained to visualise spectosomes and fusosomes (anti-Hts, red) and the Upd ligand (anti-Upd, green). In addition, a weaker, spiky distribution is present in more posterior cells, possibly corresponding to ESCs and ECs, which are in contact with CpCs and contain apical spectosomes (Fig. 2C) in which the nascent spectrosome material is visible in TFCs and CpCs. In addition, a small ‘scar’ of spectrosomal organisation are hereafter referred to as ‘anchorless’, the frequency of which depends on the severity of the mutant condition and on the age of the female (see Table S1 in the supplementary material). In fact, nearly 75% of GSCs of the strongest mutant combination analysed 25 days AE showed anchorless spectrosomes (Fig. 2D,F). A detailed study of control ovaries indicated that anchorless figures are also found in wild-type niches. We observed that until 10 days AE, a small percentage (11-13%) of control GSCs show anchorless figures. However, there was a noticeable increase in the frequency of anchorless figures 25 days AE (23.5%), as GSCs aged (Fig. 2F).**
observation by Decotto and Spradling that the spectrosomes of the remaining GSCs present in Stat92E mutant germaria move away from CpCs (Decotto and Spradling, 2005), further support this possibility.

Jak/Stat signalling is required in the somatic cap cells

Next we set out to dissect the requirement for the Jak/Stat pathway in the different compartments of the GSC niche. To this end, we removed the activity of the pathway in GSCs by making hop<sup>−/−</sup>, Stat92E<sup>60-146</sup> or dome<sup>03068</sup> germline clones (n>70 for each genotype). We analysed mosaic germaria 2, 10 and 25 days AE and observed that the removal of any of the above components of the pathway had no detectable effect on germline development or on GSC maintenance (Fig. 3A). This observation is in agreement with recent results (Decotto and Spradling, 2005) confirming that the Jak/Stat pathway is not required in the germline. Furthermore, it indicates that the reduction in the number of GSCs in Jak/Stat mutant ovaries is probably a consequence of the activity of the pathway in the somatic cells of the niche.

We took two experimental approaches to test the above hypothesis. First, we ectopically expressed a dominant-negative form of the receptor Dome (Dome<sup>ACCT</sup>) (Brown et al., 2001) in the somatic cells of the niche and analysed its effect(s) on GSC behaviour. Experimental females grown at 25°C for 25 days AE showed a small but significant decrease in the number of GSCs per germarium (control, 2.61±0.62 GSCs/germarium, n=61; bab1-Gal4/UAS-dome<sup>ACCT</sup>, 2.21±0.74 GSCs/germarium, n=39; P<0.05). This reduction in the number of GSCs populating the ovarian niche was accompanied by an increase in the frequency of anchorless figures 25 days AE, which rose from ~24% in controls to ~46.50% in experimental females. In fact, we observed germaria where all of the GSCs contained anchorless spectrosomes, a phenotype never encountered in wild-type niches (Fig. 3B).

Fig. 2. Jak/Stat signalling is required in the Drosophila ovary for GSC maintenance. (A,B) Wild-type (A) and hop<sup>−/−</sup>/hop<sup>−/−</sup> upd<sup>5055</sup> (B) germaria stained with anti-Hts to visualise the clear reduction in the number of GSCs and developing cysts in the mutant condition. (C,D) nanos-Gal4/UAS-Src-GFP (C) and hop<sup>−/−</sup>/hop<sup>−/−</sup>; nanos-Gal4/UAS-Src-GFP (D) germaria dissected 10 days after eclosion (AE). They were double stained with anti-Hts (red) and anti-GFP (green) to visualise spectrosomes and to outline the germline cells, respectively. The spectrosome in C displays the typical ‘exclamation mark’ shape (de Cuevas and Spradling, 1998); the spectrosome in D has lost its apical anchoring while still maintaining its connection with the cystoblast spectrosome and was therefore classified as an anchorless GSC spectrosome. The small ‘scar’ of spectrosomal material left on the apical side, adjacent to the CpCs, suggests that the GSC spectrosome has severed its apical connection prior to accumulating basally. (E) Bar chart representing the mean number of GSCs (±s.d.) per germarium in hop<sup>−/−</sup>/FM7 (control), hop<sup>−/−</sup>/hop<sup>−/−</sup> and hop<sup>−/−</sup>/hop<sup>−/−</sup> upd<sup>5055</sup> germaria. Ovaries were dissected 2, 10 and 25 days AE. Black triangles indicate a statistically significant difference between the given experimental condition and its control (Student’s t-test, P<0.01). (F) Bar chart showing the percentage of anchorless GSC figures in hop<sup>−/−</sup>/FM7 (control), hop<sup>−/−</sup>/hop<sup>−/−</sup> and hop<sup>−/−</sup>/hop<sup>−/−</sup> upd<sup>5055</sup> germaria dissected 2, 10 and 25 days AE. The number of germaria analysed for each experiment (n) shown in E,F is indicated in E. Asterisks, GSCs; CB, cystoblast. The white dashed lines delineate GSC-CB pairs. Scale bars: 10 µm.

CpCs mutant for a strong loss-of-function allele of the hop gene, hop<sup>−/−</sup>. To this end, we utilised a bab1-Gal4 UAS-flp chromosome to manipulate genetically the support cells of the GSC niche (Bolívar et al., 2006). Wild-type CpCs adopt a rosette-like arrangement at the base of the terminal filament and come to lie in close contact with the underlying GSCs (reviewed by González-Reyes, 2003; Spradling et al., 1997). The analysis of hop<sup>−/−</sup> clones revealed that the activity of the Jak/Stat pathway in CpCs is essential to prevent GSC differentiation. Where wild-type GSCs abutted both hop<sup>−/−</sup> and hop<sup>−/−</sup> CpCs, they appeared to be retained normally in the niche, as judged from possession of a normal-looking spectrosome 14 days AE (Fig. 3C). However, GSCs that made contact exclusively with hop<sup>−/−</sup> CpCs displayed characteristics of differentiating germline cells, as shown by the frequent appearance of anchorless GSCs and by the development of cysts directly abutting mutant CpCs 14 days AE (55% of cases; n=17; Fig. 3D,E). It is interesting to note that the presence of hop<sup>−/−</sup>-deficient CpCs did not affect the overall structure of the anterior gerarium, in contrast to Stat92E<sup>−/−</sup> ESCs (Decotto and Spradling, 2005). Finally, we assessed whether the removal of Jak/Stat signalling during gonadal development affects normal CpC specification. We analysed the pattern of expression of two CpC markers, the transcription factor Engrailed and nuclear Lamin C, in bab1-Gal4-induced hop<sup>−/−</sup> CpC clones (Forbes et al., 1996; Xie and Spradling, 2000) (Fig. 4). We found that the loss of hop activity did not affect the expression of either of these markers, strongly suggesting that GSC differentiation induced by the loss of hop from CpCs is not due to a failure of normal CpC development.

Jak/Stat activity regulates dpp transcription and signalling

The above results demonstrate that somatic Jak/Stat signalling has a specific effect on GSC maintenance and they strongly suggest that a signal is transmitted from the CpCs to the germline. To prove that
this is the case, we expressed a constitutively active form of the Janus kinase, \( \text{hop}^{\text{Tum}} \) (Luo et al., 1995), in support cells using the \( \text{bab1} \)-Gal4 driver. As shown in Fig. 5A, overexpression of \( \text{Hop}^{\text{Tum}} \) in support cells blocks cyst differentiation and induces ectopic GSCs. Since this gain-of-function form of Jak activates the pathway in support cells in a cell-autonomous manner, the effect observed on the germline demonstrates the existence of a signal relayed from the support cells to the GSCs that is regulated by Jak/Stat. In an attempt to determine the nature of this signal, we examined whether the Jak/Stat pathway was regulating the transcription of the vertebrate bone morphogenetic protein 2 (Bmp2) orthologue \( \text{dpp} \). This gene has been shown to encode an extrinsic signal required to prevent GSC differentiation in the germarial niche (Casanueva and Ferguson, 2004; Kai and Spradling, 2003; Song et al., 2004; Xie and Spradling, 1998; Xie and Spradling, 2000).

We analysed the level of \( \text{dpp} \) transcription after ectopic induction of the Jak/Stat pathway utilising the \( \text{bab1} \)-Gal4 driver. In our hands, the detection of \( \text{dpp} \) mRNA by in situ hybridisation on germaria did not yield consistent results. Thus, we resorted to performing real-time PCR on control ovaries and on experimental ovaries that had overexpressed the Upd2 ligand for 4 days, using primers that recognise all the predicted splice variants of \( \text{dpp} \) transcripts. We found that the ectopic activation of Jak/Stat in support cells led to a greater than 3-fold increase in the levels of \( \text{dpp} \) mRNA, strongly suggesting that \( \text{dpp} \) transcription in support cells is controlled by the activity of the Jak/Stat pathway (Fig. 5B).
In addition to dpp, another BMP-like ligand, Gbb, is also expressed in somatic cells of the gerarium and is required for GSC maintenance (Song et al., 2004). However, in contrast to dpp, we found that the ectopic activation of Jak/Stat signalling did not significantly affect gbb transcription, suggesting that it is probably not a downstream target of the Jak/Stat pathway (Fig. 5B). Importantly, because both dpp and gbb are expressed in support cells (Song et al., 2004), the fact that gbb mRNA levels are not increased upon Upd2 overexpression strongly suggests that the effect of Jak/Stat ectopic activation on dpp expression is not due to an increase in support cell numbers, but instead to transcriptional control. Finally, we wished to determine whether the ectopic GSC-like cells produced after bab1-Gal4-driven activation of Jak/Stat were transducing the dpp signal. We utilised the presence of phosphorylated Mad (pMad) as a reporter of an active dpp pathway (Tanimoto et al., 2000). In wild-type germaria, pMad is found at high levels only in GSCs (Fig. 5C) (Kai and Spradling, 2003). By contrast, the ectopic spectrosmes-containing cells in Upd2-induced tumourous germaria displayed strong pMad staining, even those located many cell diameters away from the CpCs (Fig. 5D). Taken together, our results strongly suggest that ectopic activation of the Jak/Stat pathway in support cells upregulates dpp expression in these cells, which consequently enlarges the GSC niche as witnessed by the expansion of Dpp signalling in the germline. Moreover, our results suggest that even though Dpp (or BMP) pathway activation is necessary and sufficient to prevent GSC differentiation, Jak/Stat signalling impinges on dpp in support cells to control the ovarian GSC niche.

**DISCUSSION**

The creation of unique ‘permissive zones’ by support cells is a general principle of stem cell niches. Niches are dynamic systems in which several signalling pathways are often integrated in order to coordinate different cell types and to respond to changing physiological conditions (Scadden, 2006). In this work, we have used the *Drosophila* ovarian germline niche to establish that Jak/Stat signalling in support cells regulates the production of the growth factor Dpp, an extrinsic signal transmitted from support cells and required for GSC division and perpetuation (Xie and Spradling, 1998; Xie and Spradling, 2000).

Two well-characterised extrinsic factors acting in the ovarian GSC niche are the BMP-like proteins Dpp and Gbb, which are known to block germline stem cell differentiation by repressing the transcription of the bam gene (Chen and McKearin, 2005; Song et al., 2004; Szakmary et al., 2005). In addition to its effect on bam expression, BMP signalling in the germline controls GSC maintenance through the activity of pelota, a putative regulator of translation that controls GSC self-renewal by repressing a Bam-independent differentiation pathway (Xi et al., 2005). The short-range signalling by Dpp and Gbb is restricted to GSCs and, albeit at lower levels, to cystoblasts (Kai and Spradling, 2003). In the absence of Dpp or Gbb signalling, Bam is expressed in GSCs and the repressor activity of Pelota is probably reduced. As a result, GSCs differentiate and the niche is emptied (Song et al., 2004; Xi et al., 2005; Xie and Spradling, 1998). There are however clear differences between the roles of Dpp and Gbb in the female GSC niche. Dpp overexpression prevents stem cell differentiation and induces the formation of large tumours of GSC-like cells, partly by de-differentiating ‘committed’ cystocytes and...
partly by inducing GSC division (Kai and Spradling, 2004; Xie and Spradling, 1998). Thus, the reception of Dpp in the germine is not only necessary to maintain a stable population of GSCs, but is also sufficient to specify stem cell fate. This conclusion points towards Dpp as the limiting factor that controls female GSC niche size and function. By contrast, Gbb is necessary but not sufficient to prevent female GSC differentiation (Song et al., 2004). In this work, we have demonstrated that the Jak/Stat pathway is required in support cells to preserve GSCs, most probably by regulating dpp transcription and by determining the extent of BMP pathway activation in the germine. Therefore, considering the significance of Dpp in the proper functioning of the GSC niche, our results strongly suggest that the activity of the Jak/Stat pathway defines the GSC niche in the female ovary. Interestingly, Jak/Stat signalling in the Drosophila testis constitutes another extrinsic factor essential for GSCs to retain self-renewing potential, even though in this case the transduction of the pathway is required cell-autonomously in the germine (Kiger et al., 2001; Tulina and Matunis, 2001). It would thus appear that the same signalling pathway defines both male and female GSC niches. This conclusion correlates with previous results suggesting that male and female germline niches are governed by common signals (Decotto and Spradling, 2005; Gilboa and Lehmann, 2004).

The mechanism(s) by which Jak/Stat signalling modulates dpp transcription remain to be elucidated. Sequence analysis shows the existence of several consensus binding sites for the Stat transcription factor in the dpp gene (data not shown) (Bach et al., 2003), but their functionality has not been tested. Alternatively, or in addition, the control of dpp mRNA levels by Jak/Stat might be indirect. Whatever the situation, it is unlikely that the increase in dpp mRNA after ectopic Jak/Stat signalling is a non-specific effect of the global disruption of heterochromatic gene silencing that occurs in Drosophila larvae and adults upon Jak overactivation (Shi et al., 2006). First, gbb transcription is not affected under the same experimental conditions that cause an increase in dpp mRNA levels. Second, it has been suggested that dpp might function downstream of, or in parallel to, Jak/Stat signalling in Drosophila testes (Singh et al., 2006). Finally, the ectopic expression of Usp in eye discs results in a slight enhancement of dpp mRNA levels (Bach et al., 2003). Altogether, these and our observations strongly suggest that Jak/Stat activation in support cells specifically regulates dpp transcription.

Our analysis of GSC spectroscomes has revealed a new organisation of the spectrosome that might constitute a useful tool to analyse niche function. Anchorless figures are formed during post-mitotic (early interphase) stages and are observed in a small percentage of wild-type GSCs, suggesting that either this organisation of the spectrosome is very dynamic and lasts for a short period of time in GSCs undergoing cytokinesis, or that only a few of the GSCs present in an ovary develop it. In any case, because a significant increase in the frequency of these figures is associated with stem cell loss when Jak/Stat signalling is impaired, the rise in the frequency of anchorless spectroscomes might reflect the existence of defective niche signalling. We propose that Jak/Stat pathway activation in support cells prevents premature GSC loss by regulating the production of the relay signal Dpp. Thus, mutant niches might not achieve the right balance of survival factors, including Dpp, required to maintain a wild-type population of GSCs during the female’s lifetime.

Given the importance of BMP signalling to avoid depletion of GSCs and to control their proliferation, the production of BMP ligands ought to be tightly regulated. In this context, CpCs and ESCs seem to act as a signalling centre where several signalling pathways might be integrated. In addition to dpp and gbb, other extrinsic factors with defined roles in the control of populations of GSCs and/or follicle stem cells, such as fs(1)Yb, piwi, wingless and hedgehog, are known to be expressed in CpCs (Cox et al., 1998; Cox et al., 2000; King and Lin, 1999; King et al., 2001; Song et al., 2004; Song and Xie, 2003; Xie and Spradling, 1998; Zhang and Kalderon, 2001). Our observations add the transduction of the Jak/Stat signal(s) to the complex network of signalling pathways that co-exist in the CpCs. Similarly, the Jak/Stat pathway is required in ESCs to maintain GSCs (Decotto and Spradling, 2005). Altogether, this evidence emphasises the contribution of support cells in direct contact with GSCs (CpCs and ESCs) in the determination of GSC niche size and function (Song et al., 2007). The signals that regulate Jak/Stat pathway activation in the niche are at present unknown, but clear candidates are any of the signalling molecules present in CpCs. In this regard, it is interesting to note that the expression of piwi and hedgehog in these cells is controlled by fs(1)Yb (King et al., 2001). In addition, systemic signals such as the neural-derived insulin-like peptides, utilised in the ovary to sense nutritional input and to impinge on GSC niche activity to coordinate nutrient availability with egg production (LaFever and Drummond-Barbosa, 2005), might play a role. Deciphering the mechanism(s) that modulates Jak/Stat activity in ovarian support cells and determining the generality of Jak/Stat regulation of BMP signalling in other well-established niches are interesting questions that await further investigation.

We thank J. C. Hombría, S. Noselli, T. Schupbach, A. Spradling, the Developmental Studies Hybribroma Bank (University of Iowa) and the Bloomington Stock Centre for fly stocks and reagents. F. Casares, M. D. Martín-Bermudo, J. Pearson and P. Rojas-Rios provided useful comments on the manuscript. Research in our laboratory is funded by the Spanish Ministerio de Educación y Ciencia (BMC2003-01512 and BFU2006-10934) and by the Junta de Andalucía (CVI-280 and P06-CVI-01592). L.L.-O. and A.F.-M. were supported by FPI studentships from the Spanish Ministerio de Ciencia y Tecnología and by an IFP-CSCIS contract (to A.F.-M.). The institutional support from the Junta de Andalucía to the CABD is acknowledged.

Supplementary material
Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/135/3/533/DC1

References
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Table S1. Average number (±s.d.) of germline stem cells (GSCs) per germarium in different genetic backgrounds and scored 2, 10 and 25 days after eclosion (AE)

<table>
<thead>
<tr>
<th>Genotype</th>
<th># GSCs/germ (n)</th>
<th># GSCs analysed</th>
<th>% Postmitotic GSCs</th>
<th>% 'Exclamation mark'/ 'anchorless' spectrosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>hop(^{-})/FM7 (2 d)</td>
<td>2.79±0.51 (60)</td>
<td>165</td>
<td>50.9</td>
<td>86.9/13.1</td>
</tr>
<tr>
<td>hop(^{-})/FM7 (10 d)</td>
<td>2.45±0.66 (67)</td>
<td>159</td>
<td>50.9</td>
<td>88.9/11.1</td>
</tr>
<tr>
<td>hop(^{-})/FM7 (25 d)</td>
<td>2.57±0.52 (58)</td>
<td>149</td>
<td>38.9</td>
<td>76.5/23.5</td>
</tr>
<tr>
<td>hop(^{-})/hop(^{+}) (2 d)</td>
<td>2.52±0.58 (51)</td>
<td>129</td>
<td>44.9</td>
<td>91.4/8.6</td>
</tr>
<tr>
<td>hop(^{-})/hop(^{+}) (10 d)</td>
<td>2.53±0.58 (71)</td>
<td>181</td>
<td>43.1</td>
<td>90.6/7.7</td>
</tr>
<tr>
<td>hop(^{-})/hop(^{+}) (25 d)</td>
<td>2.11±0.94 (44)*</td>
<td>92</td>
<td>31.5</td>
<td>62.1/37.9</td>
</tr>
<tr>
<td>hop(^{-})/hop(^{+}) upd(^{Y55}) (2 d)</td>
<td>2.10±0.70 (57)*</td>
<td>122</td>
<td>50.8</td>
<td>58.1/41.9</td>
</tr>
<tr>
<td>hop(^{-})/hop(^{+}) upd(^{Y55}) (10 d)</td>
<td>1.79±0.94 (95)*</td>
<td>167</td>
<td>57.5</td>
<td>69.8/30.2</td>
</tr>
<tr>
<td>hop(^{-})/hop(^{+}) upd(^{Y55}) (25 d)</td>
<td>0.87±0.79 (53)*</td>
<td>49</td>
<td>27.6</td>
<td>25.0/75.0</td>
</tr>
</tbody>
</table>

Post-mitotic GSCs were identified according to de Cuevas and Spradling (1998). The mutant combinations analysed in this study showed an occurrence of post-mitotic GSCs 2 days and 10 days AE, similar to controls (~45-50%; n>270). However, 25 days AE this frequency dropped to ~30% (n>70), indicating that mutant GSCs have a tendency to slow down entry into mitosis or mitosis itself. 'Exclamation mark' spectrosomes and 'anchorless' spectrosomes are described in the text.

*Statistically significant differences with respect to the appropriate hop\(^{-}\)/FM7 controls (Student’s t-test, P<0.05).