Cease and desist: modulating short-range Dpp signalling in the stem-cell niche

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***Drosophila* ovarian germline stem cells (GSCs) are maintained by the extracellular BMP2/4 orthologue Dpp, which is produced from the surrounding somatic niche. The Dpp signal has a short range; it induces a response in GSCs within the niche, but is rapidly extinguished in their progeny only one cell-diameter away. To ensure the correct balance between stem-cell maintenance and differentiation, several regulatory mechanisms that modulate the Dpp signal at many stages of the pathway have been described. Here, we discuss the nature of the ovarian Dpp signal and review the catalogue of mechanisms that regulate it, demonstrating how the exquisite modulation of Dpp signalling in this context can result in precise and robust control of stem-cell fate. This modulation is applicable to other stem-cell environments that use BMPs as a niche signal, and the regulatory mechanisms are conceptually relevant to several other stem-cell systems.**

Keywords: BMP; Dpp; *Drosophila*; ovary; stem cells
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See Glossary for abbreviations used in this article.

Introduction

Following the proposal of a stem-cell niche over three decades ago (Schofield, 1978), the identification and characterization of these specialized microenvironments and their regulation of stem-cell behaviour, has progressed at a rapid rate. One of the first niches to be identified was that of the ovarian germline in *Drosophila*, which has since become a principal model system used to investigate stem-cell maintenance and regulation. This is due in part to its accessibility, its previous structural characterization and the variety of molecular markers that are available to label and visualize the cell types in the system. In addition, the lack of redundancy in the fly compared with mammalian systems, combined with the presence of a highly characterized signalling pathway that is necessary and sufficient for stem-cell maintenance, make the *Drosophila* ovary a valuable environment in which to study stem-cell behaviour. The functional unit of the ovary is called the germarium, and it generates a continuous supply of developing eggs (Fig 1). Each germarium contains 2–3 germline stem cells (GSCs) that give rise to the entire female germline. Adjacent to the GSCs are various specialized somatic cells that make

up the stem-cell niche. As a group, these somatic cells produce several self-renewing signals, including the key Dpp signal, and provide structural support to maintain the identity of the GSCs (Kirilly & Xie, 2007). When a GSC divides, the daughter cell moves out of the niche and away from these self-renewal signals, subsequently upregulating the crucial differentiation gene *bam*, and changing identity to a differentiating cystoblast. In the absence of the Dpp signal, the GSC population is lost to differentiation, while ectopic expression generates a tumorous abundance of GSCs (Xie & Spradling, 1998). Control of stem-cell number and identity is crucial for maintenance of the female germline, so the regulation of Dpp signalling is vital. In contrast to other developmental contexts that require Dpp signalling, such as the wing and embryo (O'Connor *et al*, 2006), the Dpp signal of the ovary is remarkably short range, being downregulated only one cell-diameter away from the niche in which it originates.

The extracellular Dpp ligand acts on neighbouring germline cells through the canonical signal transduction pathway involving the type I receptors Tkv and Sax, the type II receptor Punt and the signal transducer Smads, Mad and Med (Fig 2). Ligand-bound receptors phosphorylate Mad, which subsequently binds Med and translocates to the nucleus, where pMad/Med activate or repress target gene transcription. In the germline, the pMad/Med transcriptional complex binds directly to a discrete silencer element within the promoter of *bam* (Chen & McKearin, 2003), which encodes a protein essential for differentiation. In this way, Dpp signalling represses *bam* expression specifically within GSCs (Chen & McKearin, 2003; Song *et al*, 2004). Many elements that regulate the Dpp signal at all stages of the pathway have been discovered, and are discussed in this review.

The niche BMP signal: what and where?

Although it has been well established that Dpp is essential for maintaining GSCs within the niche (Xie & Spradling, 1998), several important questions remain regarding this self-renewal signal. The first is the nature of the ligand, as Dpp is not the only BMP ligand expressed in the germarium (Fig 2). Alongside Dpp, cells of the niche also express an alternative BMP ligand: Gbb (Song *et al*, 2004), the homologue of BMP5/6/7/8 (Wharton *et al*, 1991). Loss of either ligand from the niche leads to derepression of *bam* in the germline and GSC differentiation, suggesting that both Dpp and Gbb are necessary for germline maintenance (Song *et al*, 2004; Xie & Spradling, 1998). However, ectopic expression of *gbb* is not sufficient to cause the tumorous GSC phenotype that is characteristic of *dpp* overexpression (Song *et al*, 2004), implying that Gbb alone is insufficient for effective signalling in the

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Fig 1 | The *Drosophila* ovarian stem cell niche structure. The ovary consists of ovarioles, each of which have a germarium at the tip. The germarium (enlarged) houses GSCs, from which the germline originates. Cap cells of the stem cell niche (yellow) produce the Dpp signal (blue) to maintain nearby GSC identity by repressing the *bam* gene. Following GSC division, one daughter cell moves away from the niche to become a cystoblast, leading to derepression of *bam*, which initiates the differentiation programme. Green shapes represent the fusome organelle, which allows identification of the different stages of differentiation. *bam*, bag of marbles; Dpp, Decapentaplegic; GSC, germline stem cells.

germline. These observations could be indicative of a requirement for a Dpp/Gbb heterodimer within the ovary, which is consistent with the evidence for such a heterodimer being required for wing-vein patterning (Shimmi *et al*, 2005a), as well as in the developing wing disc (Ray & Wharton, 2001) and a related Dpp/Scw heterodimer in the embryo (Shimmi *et al*, 2005b). Signalling by a Dpp/Gbb heterodimer in the germline is also supported by BMP-receptor use. Dpp and Gbb are thought to synergistically signal through the two receptors Tkv and Sax, with Gbb having a preference for Sax (Haerry *et al*, 1998). Both receptors are expressed and required in the germline, as is the type II receptor Punt (Iovino *et al*, 2009; Xie & Spradling, 1998). A second type II receptor, Wit, is also present in *Drosophila*, although its expression and requirement in the germline have not been studied. Research on the wing disc has suggested a model in which Sax can only mediate signalling when in complex with Tkv, whereas a Sax homomeric receptor sequesters Gbb to inhibit signalling (Bangli & Wharton, 2006). Loss of Sax from the germline leads

Glossary

Bam	Bag of marbles
BMP	bone morphogenetic protein
Brat	Brain tumour
Dad	Daughters against Dpp
Dally	Division abnormally delayed
dMyc	<i>Drosophila</i> Myc
Dpp	Decapentaplegic
dSmurf	<i>Drosophila</i> Smad ubiquitination regulatory factor
Gbb	Glass bottom boat
GSC	germline stem cell
Hh	Hedgehog
HSPG	Heparan sulphate proteoglycan
JAK-STAT	Janus kinase-signal transducer and activator of transcription
Lis-1	Lisencephaly-1
Mad	Mothers against Dpp
Med	Medea
miRNA	microRNA
mRNA	messenger RNA
Nos	Nanos
Ote	Otefin
Pent	Pentagone
pMad	phosphorylated (activated) Mad
Pum	Pumilio
Sax	Saxophone
Shn	Schnurri
Smad	SMA-Mothers against Dpp
Tkv	Thickveins
Vkg	Viking
Wit	Wishful thinking

to differentiation of GSCs (Xie & Spradling, 1998), indicating that Sax has a positive role in mediating the germline Dpp signal, and might therefore function primarily as a Tkv/Sax heteromeric receptor, rather than as a Sax homomeric receptor. Indeed, evidence from *Drosophila* tissue culture cells indicates that each type I receptor can coimmunoprecipitate with the other (Haerry, 2010), whereas signalling of a Dpp/Gbb heterodimer requires expression of both Tkv and Sax, and does not occur if one is absent (Shimmi *et al*, 2005a). Together, these data are consistent with the idea that BMP ligand in the germline is a Dpp/Gbb heterodimer that preferentially signals through a Tkv/Sax heteromeric receptor complex. However, for simplicity we will refer to the BMP signal as a Dpp signal.

A population of anterior cap cells, to which the GSCs are directly anchored (Xie & Spradling, 1998; Xie & Spradling, 2000), is probably responsible for the majority of the signal, although the extent of the *dpp* expression domain remains unclear. *dpp* mRNA has been detected in isolated populations of somatic cells, including cap cells, but not in the germline (Song *et al*, 2004), whereas *in situ* hybridization has shown that *dpp* is transcribed in the vicinity of these somatic niche cells (Liu *et al*, 2010; Wang *et al*, 2008a; Xie & Spradling, 2000). Dpp transcription in cap cells is regulated by the JAK-STAT signalling pathway (Lopez-Onieva *et al*, 2008; Wang *et al*, 2008a; Fig 2). Mutation of the JAK-STAT pathway components forces differentiation of GSCs (Lopez-Onieva *et al*, 2008; Wang *et al*, 2008a), whereas ectopic expression of an activated receptor increases the level of pMad in adjacent cells and the GSC population (Lopez-Onieva *et al*, 2008). In addition, the size of GSC population that can be supported is directly related to the number of cap cells (Song *et al*, 2007; Xie & Spradling, 2000; Fig 2), and increasing the number of

Fig 2 | The self-renewal signal of the niche. (1) The niche signal could consist of a Dpp/Gbb heterodimer, which could synergistically bind to a combination of Sax, Tkv and Punt receptors, and a Dpp homodimer, which would bind only to Tkv and Punt receptors, and might have less potency. Binding leads to phosphorylation of Mad, which, together with Med, represses *bam* transcription. As the source and range of Gbb is unknown, the BMP signal is depicted by Dpp in the main figure for simplicity. (2) Transcription of Dpp is activated by JAK–STAT in cap cells. (3) Notch positively regulates cap-cell numbers, and thereby Dpp levels. (4) The collagen Vkg binds to extracellular Dpp near to GSCs and prevents it from spreading further to the posterior. Vkg is also present surrounding somatic cells of the germarium as part of a basement membrane (not shown). (5) Similarly, Dally restricts Dpp distribution to GSCs in the niche. *bam*, bag of marbles; BMP, bone morphogenetic protein; Dally, Division abnormally delayed; Dpp, Decapentaplegic; Gbb, Glass bottom boat; GSC, germline stem cells; JAK–STAT, Janus kinase–signal transducer and activator of transcription; Mad, Mothers against Dpp; Med, Medea; Sax, Saxophone; Tkv, Thickveins; Vkg, Viking.

cap cells through ectopic Notch signalling results in the maintenance of many more GSCs, which behave similarly to wild-type GSCs and respond to the Dpp signal (Song *et al*, 2007). Finally, loss of Dpp function, specifically in cap cells and nearby terminal filament cells, leads to GSC loss (Liu *et al*, 2010). An important remaining question is whether there are additional processes that modulate the production of the ligand from cap cells, such as those described in other contexts; for example, regulation of Dpp activity by tissue-specific post-translational processing of the mature protein has been described in the embryo (Sopory *et al*, 2010). It remains to be established whether this also occurs in the ovary.

Regulation of the extracellular Dpp ligand

The distribution and activity of the BMP ligand in the extracellular niche space is crucial for regulating signalling in the germline. As direct visualization of the BMP ligands is technically challenging, expression of the Dpp target gene *Dad* has been used to indicate active signalling within GSCs and, to a lesser extent, cystoblasts. Expression of *Dad* correlates with the presence of pMad (Casanueva & Ferguson, 2004; Song *et al*, 2004), and is consistent with the extracellular ligand distribution being limited to these cells. Expression of the *Dad* reporter has been demonstrated in more-posterior somatic cells of the niche (Casanueva & Ferguson, 2004), but the significance of this expression is unclear. By contrast, several mechanisms have been described that support the idea of an anteriorly localized ligand. These mechanisms modify the distribution and signalling efficacy in the extracellular space, concentrating and promoting signalling specifically at the surface of GSCs. The type IV collagen Vkg is thought to be important in this process, as extracellular accumulation of Vkg occurs in the ovary, specifically

surrounding GSCs and at the cap cell–GSC junction (Wang *et al*, 2008b). Investigation of various *vkg* mutants revealed an increase in GSCs consistent with a modest increase in Dpp signalling, but less severe than the tumorous phenotype associated with higher levels of Dpp expression (Xie & Spradling, 1998). As Vkg functions in the embryo to correctly distribute Dpp, while simultaneously promoting Dpp–receptor interaction (Wang *et al*, 2008b), these observations could suggest that Vkg binds to and sequesters the extracellular Dpp ligand specifically around GSCs, restricting its spread and potentially acting to promote signalling (Fig 2). This activity might be complemented by the function of an additional extracellular protein, a HSPG encoded by *dally*, which promotes GSC maintenance. Dally is a cell-surface glypican that regulates the Dpp gradient in the wing disc through physical interaction and stabilization of the ligand in the extracellular matrix, as well as augmenting Dpp signalling by preventing receptor-mediated ligand degradation (Akiyama *et al*, 2008; Fujise *et al*, 2003). In the ovary, *dally* is highly expressed in the cap cells of the niche (Guo & Wang, 2009; Hayashi *et al*, 2009) and downregulated in more-posterior somatic cells (Liu *et al*, 2010). Loss of niche *dally* activates *bam* expression in the germline and reduces expression of the Dpp responsive gene *Dad*, indicating that Dally regulates the niche Dpp signal (Guo & Wang, 2009; Hayashi *et al*, 2009; Liu *et al*, 2010). Mutational analyses reveal that it is required in somatic cells, but not in the germline. As the BMP signal is dispensable in somatic cells for germline maintenance (Bolivar *et al*, 2006; Xie & Spradling, 1998), this suggests that Dally acts *in trans* to the GSCs that it supports (Guo & Wang, 2009; Hayashi *et al*, 2009). Moreover, ectopic expression of *dally* in somatic cells outside the niche leads to an increase in BMP-responsive

Fig 3 | Intracellular regulation of the signal. (1) In GSCs (left), the Dpp signal is enhanced by stabilization of phosphorylated pMad by Lis-1, and (2) the inhibitory Smad Dad moderates the Dpp signal through competition with Mad. (3) In the cystoblast (right), miR-184 inhibits the translation of the Dpp receptor Sax. (4) Similarly, dSmurf targets the alternative Dpp receptor Tkv for degradation, and potentially the transducer Mad or its activated form. (5) The translational repressor Brat also negatively regulates Mad levels. Together, these processes enhance the Dpp signal in GSCs, and rapidly dampen it in the cystoblast to allow *bam* expression and thus differentiation. Although miR-184, Fused and dSmurf are only shown in the cystoblast, it is possible that they are expressed in both GSCs and cystoblasts, but are functionally limited to differentiating cells. *bam*, bag of marbles; Brat, Brain tumour; Dad, Daughters against Dpp; Dpp, Decapentaplegic; dSmurf, *Drosophila* Smad ubiquitination regulatory factor; Gbb, Glass bottom boat; GSC, germline stem cells; Lis-1, Lissencephaly-1; pMad, phosphorylated Mothers against Dpp; Sax, Saxophone; Tkv, Thickveins.

GSCs, potentially as a result of increased or redistributed Dpp ligand (Guo & Wang, 2009; Hayashi *et al*, 2009). Thus, Dally potentially acts to enhance Dpp signalling specifically at GSCs in the niche by either concentrating or stabilizing the Dpp ligand, or by sensitizing the GSCs to the ligand (Fig 2). This activity might also limit the proportion of signalling that occurs through endocytosis in favour of plasma-membrane-mediated pathway activation, as Dally in the wing disc has been suggested to disrupt liganded receptor endocytosis and degradation (Akiyama *et al*, 2008). The activities of Dally and Vkg promote signalling, but the way in which these factors interact to regulate extracellular Dpp requires further investigation. The activity of Dally itself was recently shown to be subject to regulation by an additional extracellular factor, Pent, which physically interacts with Dally in the wing disc. Pent promotes the Dpp-spreading activity of Dally, thereby enhancing the movement of Dally-bound Dpp at the expense of signalling. Whether Pent has a function in the female germline remains to be seen. However, the *pent* gene contains several functional Dpp silencer elements and is negatively regulated by Dpp signalling in the wing (Vuilleumier *et al*, 2010). The silencer elements would be predicted to repress *pent* expression in GSCs, thus limiting the spread of Dpp and preventing the negative impact of Pent on signalling. Together, the activities of Vkg and Dally, and perhaps the absence of Pent, function to concentrate the extracellular ligand around GSCs and augment the Dpp signal, simultaneously ensuring a ligand deficiency around more-posterior cells.

Regulation of signal reception and transduction

In addition to the extracellular mechanisms that enhance Dpp signalling at the GSCs, intracellular mechanisms that reinforce the signal within the GSC once it has been received have also been described (Fig 3). The Lis-1 protein acts to stabilize and enhance phosphorylation of activated Mad, specifically in GSCs (Chen *et al*,

2010). Lis-1 is related to the protein responsible for human lissencephaly disease, a condition primarily resulting from neuronal migration defects. Mouse knockout models confirm that Lis-1 is an essential gene, and demonstrate that it has an indispensable role in organizing brain tissue (Wynshaw-Boris & Gambello, 2001). In *Drosophila*, Lis-1 has also been linked to various cellular processes within neural systems (Liu *et al*, 2000; Siller & Doe, 2008) and during oogenesis (Chen *et al*, 2010; Liu *et al*, 1999). This includes a cell-autonomous requirement for GSC maintenance by promoting Dpp signalling and adhesion to the niche through E-cadherin (Chen *et al*, 2010). *Lis-1* mutant GSCs are less responsive to Dpp signalling and have less E-cadherin, causing them to differentiate faster than their wild-type neighbours (Chen *et al*, 2010). Tissue-culture studies in *Drosophila* cells have revealed a direct interaction between Lis-1 and Mad, which is enhanced by the presence of the Dpp receptors Tkv and Punt, resulting in stabilization of Mad levels and facilitation of its phosphorylation (Chen *et al*, 2010). However, this GSC-specific enhancement of signalling is balanced by the activity of the evolutionarily conserved inhibitory Smad, Dad. Dad directly competes with Mad for interaction with both type I receptors Tkv and Sax, preventing its further activation (Inoue *et al*, 1998; Kamiya *et al*, 2008). *Dad* is a Dpp target gene and is therefore strongly expressed in GSCs (Casanueva & Ferguson, 2004). Cell-autonomous loss of *Dad* is known to increase GSC lifespan (Xie & Spradling, 1998), and Dad might therefore act as a negative-feedback mechanism that moderates the Dpp signal in the germline. This negative regulation of Dpp transduction in GSCs seems counterintuitive; however, the activity of Dad could potentially limit the level of the self-renewal signal in GSCs to sensitize their response to perturbations in the Dpp signal, and could therefore be seen as a quality control mechanism that ensures their efficient turnover and replacement. This idea is supported by the observation that cellular fate in the

Fig 4 | Output of the signal, feedback and regulation. (A) (1) Shn acts with pMad/Med to repress *bam* expression. (2) Ote interacts with Med at the *bam* silencer element, recruits the locus to the nuclear periphery and silences its expression in GSCs. (3) A feedback loop ensures that, once it is removed from the niche, the Dpp signal is extinguished. Dpp signalling in GSCs is potentially enhanced by dMyc through increased ligand uptake. (4) Reduced signalling in the cystoblast leads to expression of *Brat*, which represses dMyc translation. This leads to further reduction in Dpp signalling, promoting differentiation. (B) miRNAs also control stem-cell fate. (1) A potential model is shown based on evidence from mammalian systems, in which Mad in the GSC stimulates DROSHA processing of miRNAs that promote self-renewal. Simultaneously, Dpp represses *bam* to allow Nos expression, which also contributes to self-renewal. (2) Mei-P26, which is related to Brat, could downregulate Mad in differentiating cells, resulting in a reduction of mature miRNA levels, and Bam represses Nos translation. Both effects prevent self-renewal in cystoblasts. This model would explain the previously described ability of Mei-P26 to affect miRNA levels and promote differentiation.

For simplicity, a single pool of miRNAs is depicted, but it is possible that distinct pools of differentially regulated miRNAs exist in GSCs and cystoblasts, for example, miR-184. *bam*, bag of marbles; Brat, Brain tumour; Dpp, Decapentaplegic; dMyc, *Drosophila* Myc; GSC, germline stem cells; Med, Medea; Nos, Nanos; Ote, Otefin; pMad, phosphorylated Mothers against Dpp; Shn, Schnurri.

germline is especially sensitive to genetic changes in Dpp-signalling components, as the loss of only one copy of either signal transducer can reverse the effect of Dpp ectopic expression (Xi *et al*, 2005).

In contrast to the enhancement of signalling in GSCs, mechanisms exist to ensure rapid downregulation of the signal after the cell has moved out of the niche and starts to differentiate. These mechanisms are numerous and diverse, and act on both the signal receptors and transducers (Fig 3). Both Tkv and Sax receptors are negatively regulated in the differentiating cell to prevent further signalling, albeit by

different mechanisms. The level of Sax receptor present in cystoblasts is regulated by miR-184, which is in contrast to the predominant role of miRNAs in promoting GSC self-renewal (Jin & Xie, 2007). Loss of miR-184 results in elevated expression and mislocalization of Sax, thus increasing Dpp signalling (Iovino *et al*, 2009). Interestingly, this loss of miR-184 seems to alter Tkv localization in a similar way to that of Sax, despite the fact that miR-184 does not directly regulate Tkv expression (Iovino *et al*, 2009); this provides further evidence for the existence of a Tkv/Sax heterodimer. Tkv itself is targeted for

ubiquitination and degradation by the ubiquitin ligase dSmurf and the Hh-pathway member Fused (Xia *et al.*, 2010). Experimental expression of a constitutively active Tkv receptor specifically in differentiating cells does not increase GSC number, and so a model was proposed in which degradation of specifically the active form of Tkv in differentiating cells blocks further signalling, causing the cells to become refractory to extracellular Dpp (Xia *et al.*, 2010). dSmurf also promotes proteasome-dependent degradation of activated Mad in various imaginal tissues and the embryo (Liang *et al.*, 2003; Podos *et al.*, 2001), and there is evidence that it could have a similar role in the germline (Casanueva & Ferguson, 2004). However, whether dSmurf-mediated degradation affects Mad or its activated form is unclear (Liang *et al.*, 2003; Zhu *et al.*, 1999).

The expression of Mad in cystoblasts is also regulated at the level of translation. The tumour suppressor Brat is a translational repressor in both the embryo—to regulate patterning (Sonoda & Wharton, 2001)—and the ovary, where it acts as part of a feedback system that controls stem-cell differentiation. Brat is expressed in cystoblasts, in which it functions with an additional translational repressor, Pum, to downregulate self-renewal-promoting proteins including Mad and dMyc (see below). The loss of Mad further dampens the cellular response to the Dpp signal and, together with the other mechanisms mentioned, enforces the differentiation programme in cells that have been removed from the niche (Harris *et al.*, 2011).

Regulation of signalling output

GSC maintenance is also regulated by epigenetic factors that modify the response of the GSC to the Dpp signal, including the DNA-associated factor Stonewall (Maines *et al.*, 2007) and the chromatin remodelling factor ISWI (Xi & Xie, 2005). ISWI is cell-autonomously required to maintain GSCs within the niche and regulate their proliferation by controlling the way in which the GSC interprets the Dpp signal. It is thought to do this by controlling the expression of a broad transcriptional response to niche Dpp within GSCs, which includes both activation and repression of the Dpp target genes *Dad* and *Bam*, respectively (Xi & Xie, 2005). A recent report has found that the steroid hormone ecdysone, which regulates many aspects of oogenesis, directly affects GSC self-renewal, and that this activity is through a functional interaction with ISWI (Ables & Drummond-Barbosa, 2010). A model has been proposed in which older follicles, which secrete significant levels of ecdysone, signal to earlier GSCs to positively reinforce their self-renewal and proliferation in physiologically favourable conditions. Loss of ecdysone, resulting from poor diet, would limit this process to slow oogenesis.

The transcriptional activity of Mad/Med is known to result in activation or repression of Dpp target genes in several contexts (Affolter *et al.*, 2001). The zinc-finger transcription factor Shn is recruited to the Mad/Med complex at silencer elements to repress Dpp target genes (Pyrowolakis *et al.*, 2004), and the Mad/Med/Shn complex has been shown to bind strongly to the *bam* silencer element in *Drosophila* cells (Chen & McKearin, 2003; Pyrowolakis *et al.*, 2004; Fig 4A). Although expression of *shn* has not been directly detected in the ovary, loss of *shn* from GSCs leads to their premature differentiation (Xie & Spradling, 2000), revealing the requirement for this particular cofactor in the germline, presumably to mediate Smad-dependent repression of genes such as *bam*. Repression of *bam* is also dependent on the nuclear lamin Ote, a membrane-associated protein that is present in the nuclear envelope (Jiang *et al.*, 2008; Fig 4A). *ote* clonal mutant GSCs cannot self-renew and are lost to differentiation, indicating a

cell-autonomous requirement, whereas *ote* mutations genetically interact with the Dpp pathway. Biochemical and tissue culture studies show that Ote physically interacts with Med at the *bam* silencer elements, suggesting that silencing of *bam* might require recruitment of the locus to the nuclear periphery, which depends on the binding of Ote and Med. Therefore, Dpp-dependent transcriptional repression relies on factors that interact with the Mad/Med complex, but which contribute to repression in different ways. Similarly, distinct cofactors might also be required for Smad-dependent activation of genes such as *Dad*, and other direct target genes that have yet to be identified.

A final method of regulating Dpp signalling within the ovarian niche might be through feedback on the Dpp signal itself by the receiving cell. This feedback would involve cell competition, in which adjacent cells compete for limiting growth factors or survival signals such as Dpp, and is thought to be mediated by the transcription factor dMyc. *dMyc* is a potent oncogene that is expressed throughout *Drosophila* development (Johnston *et al.*, 1999), and experiments in the wing disc have shown that the expression level of *dMyc* can influence competitive interactions, whereby cells with higher relative *dMyc* levels out-compete adjacent cells at their expense (Moreno & Basler, 2004). Although the molecular nature of competition remains unclear, evidence suggests that endocytosis could be an important factor, possibly by acting to increase uptake of the Dpp ligand, which is known to increase Dpp target gene expression (Moreno & Basler, 2004). Within the ovary, *dMyc* is strongly expressed in GSCs (Neumuller *et al.*, 2008; Rhiner *et al.*, 2009), although initial experiments reported that it was dispensable for competition between GSCs within a niche; induction of strong *dMyc* mutant GSC clones failed to show competitive behaviour, and ectopic expression of *dMyc* at high levels in individual GSCs also did not result in competition (Jin *et al.*, 2008). However, another group has since found that experimentally increasing or decreasing the relative levels of *dMyc* in GSCs to a lesser extent, through hypomorphic alleles and weaker misexpression, results in an associated change in Dpp pathway activation, thereby enhancing their maintenance or loss (Rhiner *et al.*, 2009), similar to cells of the wing disc (Moreno & Basler, 2004). This suggests that dMyc-dependent competition can occur between neighbouring GSCs in the ovary, possibly as a mechanism to ensure that substandard stem cells are efficiently eliminated from the niche (Rhiner *et al.*, 2009). The translational repressor Brat negatively regulates dMyc levels specifically in cystoblasts, generating a large difference in dMyc levels between GSCs and cystoblasts (Harris *et al.*, 2011). Therefore, it is possible that competition also occurs naturally between these cell types (Fig 4A). Modelling data suggest that the Brat-mediated repression of dMyc in cystoblasts reduces the ability of the cell to further take up the Dpp ligand, leading to robust cell identities within the stem-cell niche. This is in addition to the role of Brat in inhibiting Dpp signal transduction by repressing Mad, as discussed previously. The modelling data further predicts that dMyc-mediated cell competition between a GSC and a cystoblast is more likely to occur through enhancement of Dpp ligand uptake, rather than an alternative model in which dMyc only enhanced general protein synthesis (Harris *et al.*, 2011)—a previously characterised function of dMyc (Grewal *et al.*, 2005). Therefore, both experimental observation and mathematical modelling lend significant support to the idea that dMyc-mediated modulation of Dpp ligand uptake, ultimately controlled by Brat, is an important factor in cell competition between GSCs and cystoblasts (Harris *et al.*, 2011). Together, this demonstrates an important feedback mechanism

in which Brat functions to link intracellular interpretation of the Dpp signal to extracellular regulation of the ligand.

The Dpp signal and stem-cell fate

In summary, three principal requirements are essential to maintain GSC identity: (i) transcriptional repression of the *bam* gene; (ii) translational repression of differentiation-promoting genes by two repressors, Pum and Nos; and (iii) the expression of miRNAs, which potentially silence targets that contribute to the differentiation programme, as the miRNA pathway acts in favour of self-renewal overall, although these targets remain to be identified (Jin & Xie, 2007). We speculate that the Dpp signal might coordinate these processes to ensure that GSCs are maintained in a proliferating, self-renewing state within the niche. As the dominant signal from the niche, Dpp is strictly regulated to ensure that pathway activation occurs in only those GSCs most proximal to the niche. Within these cells, the signal represses the key differentiation gene *bam*. This is mediated in part by repression of the essential downstream translational repressor Nos by Bam (Li *et al*, 2009). Thus, the Dpp signal acts to indirectly maintain Pum-Nos translational repression in GSCs (Fig 4B). Finally, the Dpp pathway might have an additional, uncharacterized function in maintaining stem-cell fate through the upregulation of miRNAs in GSCs. In mammalian cells, the early processing and maturation of miRNAs by the DROSHA microprocessor complex is stimulated by interaction of the p68 subunit (encoded by *Rm62* in *Drosophila*) with a BMP-specific Smad (Davis *et al*, 2008). As miRNAs generally act in favour of GSC self-renewal (Jin & Xie, 2007), an equivalent interaction between the *Drosophila* BMP signal in the ovary and miRNA maturation could exist. Interestingly, the level of mature miRNAs in the germline was shown to be negatively regulated in differentiating cells by the Brat-related protein, Mei-P26 (Neumuller *et al*, 2008). As Brat can repress Mad levels in the germline (Harris *et al*, 2011), if Mei-P26 performs a similar function, this would lead to the intriguing possibility that Mei-P26 in cystoblasts represses Mad-mediated stimulation of DROSHA, thus reducing the levels of mature miRNAs and promoting differentiation (Fig 4B). However, further investigation of the relevance of the BMP–DROSHA interaction in the *Drosophila* ovary—as well as into the role of Mei-P26—are required to test this model. This matter and other important questions remain to be answered (Sidebar A).

Overall, the Dpp signal coordinates several processes that are required for stem-cell maintenance within the niche, and loss of this signal leads to a change in cell fate due to simultaneous activation of the differentiation programme by these individual processes. This idea is relevant to other stem-cell niches, as evidence suggests that BMPs have an important role in many mammalian stem-cell systems, and that this role is context-dependent. Consistent with the *Drosophila* germline, the function of BMPs in regulating GSCs is partly conserved, as BMP4 and BMP8b (orthologues of Dpp and Gbb, respectively) are required to induce primordial GSCs in the early mouse embryo (Lawson *et al*, 1999; Ying *et al*, 2000). Similarly, BMP4 is known to block differentiation and promote pluripotency of cultured mammalian embryonic stem cells (Ying *et al*, 2003), although this is probably a result of a carefully balanced system in which BMPs that promote non-neural cell fate oppose factors that induce neural cell fate. When expressed in the absence of these factors, BMPs can alter stem-cell identity to generate non-neural cell identity, and thus can also be considered as a differentiation factor (Xu *et al*, 2002). Even within one stem cell, BMPs can elicit different responses, such as the induction of mesenchymal stem-cell differentiation into either

Sidebar A | In need of answers

- (i) What is the nature of the BMP ligand?
- (ii) To what extent is the ligand present in the extracellular space?
- (iii) Which other directly regulated Dpp target genes, aside from *bam*, have a role in the system?
- (iv) Does the transient low pMad level in cystoblasts have a transcriptional effect distinct from the high level in GSCs, and does the Dpp signal in the ovary therefore act as a morphogen?
- (v) Does Dpp regulate miRNA levels, and how do miRNAs contribute to maintaining GSC fate?

osteoblast or adipocyte cells, which is dependent on BMP-receptor use (Chen *et al*, 1998). Other functions of BMPs include the limitation of stem-cell population size through control of cell regeneration in the intestinal system (He *et al*, 2004) or by regulating niche size in the haematopoietic system (Zhang *et al*, 2003). The regulation of the BMP signal in each of these contexts is probably also complex, due in part to the genetic redundancy found within mammalian systems, and the BMP pathway elements themselves. However, mammalian orthologues of many, if not all, of the factors described here that regulate the *Drosophila* ovarian Dpp signal have been characterized, and many have been shown to have comparable roles in BMP signal regulation, including within stem-cell systems. Therefore, the continued study of Dpp signal regulation within the *Drosophila* germline can yield vital insight into vertebrate stem-cell behaviour.

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