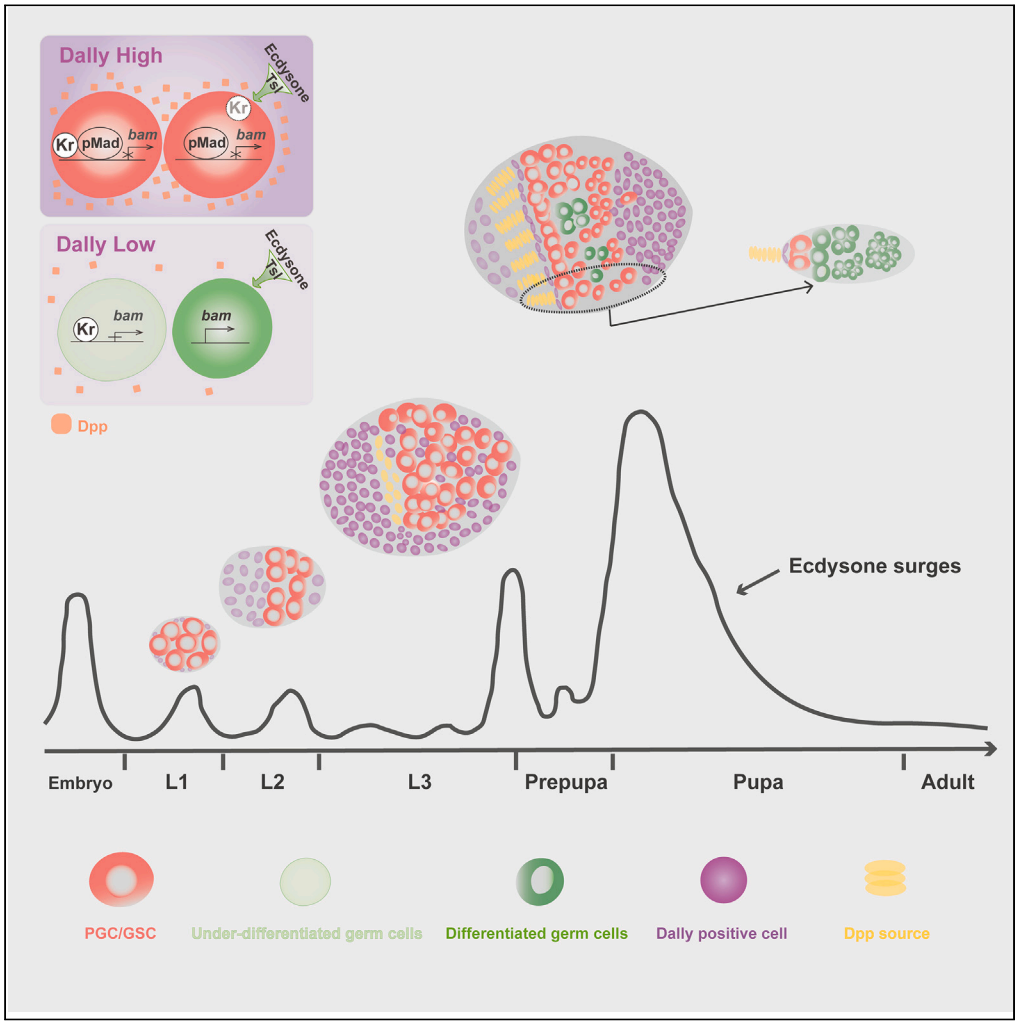
iScience

CellPress
OPEN ACCESS

Article

The spatiotemporal pattern of glypican coordinates primordial germ cell differentiation with ovary development



Xian Liu, Xin Li, Zhaohui Wang

zhwang@genetics.ac.cn

Highlights

Spatiotemporal pattern of glypican Dally regulates PGC differentiation

Dally modulates the BMP signaling to prevent precocious PGC differentiation

Dally increases the threshold of ecdysone to postpone PGC differentiation

Liu et al., iScience *27*, 108710
January 19, 2024
https://doi.org/10.1016/j.isci.2023.108710

iScience

CellPress
OPEN ACCESS

Article

The spatiotemporal pattern of glypican coordinates primordial germ cell differentiation with ovary development

Xian Liu,[1,2] Xin Li,[1] and Zhaohui Wang[1,2,3,*]

SUMMARY

The establishment, proliferation, and differentiation of stem cells are coordinated with organ development and regulated by the signals in the microenvironment. Prior to gonad formation, how primordial germ cells (PGC) differentiate spatiotemporally to coordinate with gonadogenesis is unclear. In adult ovary, drosophila extracellular glypican Dally in germline stem cell (GSC) niche promotes BMP signaling to inhibit germline differentiation. Here we investigated the relation between the fate of PGC and the spatiotemporal pattern of glypican during ovary development. We found that Dally in ovarian soma assisted BMP signaling to prevent PGC from precocious differentiation. Dally's presence raises the "hurdle" for ecdysone peaks to eventually remove the transcription factor Kr and de-repress pro-differentiation factor, temporally postponing PGC differentiation until GSC niche establishment. The spatiotemporal glypican in somatic matrix assists PGC to integrate the ovarian local BMP and organismal steroid signals that coordinate PGC's program with organ/body development to maximize reproductive potential.

INTRODUCTION

Stem cells are fundamental to regenerative tissues, and their establishment, proliferation, or differentiation has to be coordinated with organ development to attain the functions at birth. For germline stem cells (GSCs), it is even more complicated in that their precursors, the primordial germ cells (PGCs) formed in early embryo, migrate through different embryonic layers before reaching the gonadal platform. Prior to the settlement at the GSC niche, how PGCs maintain their naive state during the migratory journey or differentiate at the right time and right position during gonadogenesis is virtually unknown.

Since the generation of human primordial germ cell-like cells (hPGCLCs) from human pluripotent stem cells, finding the intrinsic and extrinsic factors to guide hPGC differentiation remains challenging. Though comparing the single-cell transcriptomes of humans, monkeys, and mice revealed primate-specific programs driving fetal oocyte development,[1] the only approach to generate oogonia from hPGCLCs is co-culturing with mouse embryonic ovarian somatic cells for months.[2] Co-culturing the common marmoset PGCLCs with mouse testicular somatic cells also produced an early prospermatogonia-like phenotype.[3] These observations demonstrate the functional conservation of external signals present in the gonads of primate and mouse, and provide the *in vitro* platforms to investigate the mechanism of hPGC differentiation.

Practically, murine models have been frequently used to decipher the spatiotemporal signaling that governs the precise differentiation series of mammalian PGC during development. Although the transcription factor networks specifying PGC are different between mouse and human, the networks in both are activated downstream of the extracellular BMP (bone morphogenic protein) family molecules,[4] which are conserved glycoproteins playing essential roles in multiple developmental processes such as embryogenesis, skeletal formation, hematopoiesis, and neurogenesis. To become gametogenesis-competent germ cells, DAZL (Deleted in Azoospermia Like, an RNA-binding protein and translational regulator in spermatogenesis) expression is required and is induced by the cues from somatic gonadal ridge where PGCs conclude their migration.[5] To induce germline sex differentiation, an *in vitro* reconstitution demonstrated that BMP and retinoic acid synergistically produced fetal primary oocytes from mouse PGCLCs.[6] However, the spatiotemporal scenario how PGCs differentiate during gonad formation remain largely unexplored *in vivo*.

We have employed mainly genetic manipulations with fruit fly to reveal the relationship between the fate of PGC and the spatiotemporal pattern of glypican during ovary formation. Glypican is one of the two major families of heparan sulfate proteoglycans and plays a vital role in developmental morphogenesis. As one of the major components in extracellular matrix, glypican facilitates the distribution and receptor-binding of morphogens such as BMP.[7] In adult ovary, the drosophila glypican Dally in the somatic niche of germline stem cells promotes BMP signaling to inhibit germline differentiation.[8–10] In this report, we have found that glypican Dally in ovarian somatic cells is required

[1]State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, P.R. China
[2]The University of Chinese Academy of Sciences, Beijing 100049, P.R. China
[3]Lead contact
*Correspondence: zhwang@genetics.ac.cn
https://doi.org/10.1016/j.isci.2023.108710

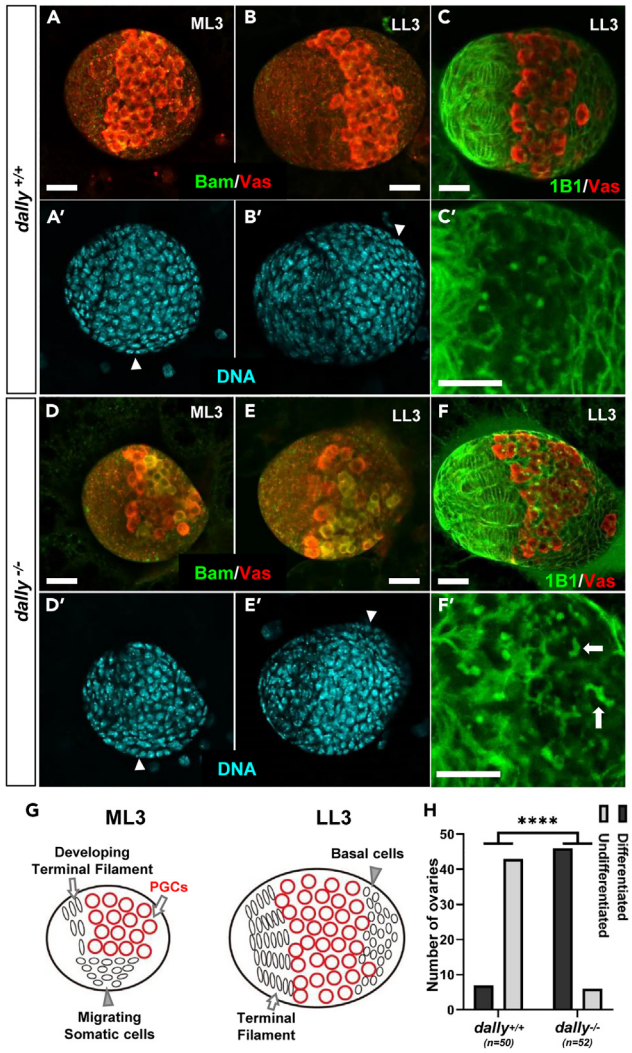


Figure 1. Glypican Dally prevents precocious PGCs differentiation

The ovaries of all images are orientated anteriorly to the left.

(A–G) PGCs are assessed for differentiation in wild-type (w^{1118}) and *dally* mutant ($dally^{Pz/80}$). Vas-positive cells are PGCs. Bam is a differentiation marker in germ cells. The dot-shaped 1B1 (stained by anti-Hts) implies undifferentiated PGCs, and the branch-shaped 1B1 (arrows in F′) indicates differentiation. C′ and F′ are magnified regions of C and F. Ovaries in ML3 (mid-larval3) are distinguished by migrating somatic cells (arrowheads in A′ and D′) and developing terminal filaments (TFs). Ovaries in LL3 (late-larval3) are distinguished by the established basal cells (arrowheads in B′ and E′) and TFs. ML3 and LL3 ovaries are schematically illustrated in G.

(H) Scoring ovaries based on the presence or absence of Bam in A, B, D, E. ****, $p < 0.0001$ (Chi-square). n, the total ovary number.

Scale bars: 20 μm.

to prevent the precocious differentiation of PGCs via BMP signaling. Transcription factor Kr has been found to regulate PGC differentiation in response to the organismal ecdysone surges.[11] Overexpressing Kr in PGCs partially rescued precocious differentiation in *dally* mutants, suggesting that glypican in the somatic matrix assists PGCs to integrate the ovarian local BMP and organismal steroid signals for a coordination of cell fate, organogenesis, and organismal development.

RESULTS

Dally in gonadal soma prevents precocious germline differentiation

At the end of drosophila embryogenesis, PGCs coalesce with some somatic cells to form the primordial gonad. To this point, PGC number was not apparently affected by Dally changes (Figure S1H). In female, PGCs remain undifferentiated until stem cell niche is established at the end of 3rd larval stage (Figure 1G cartoon, GSC niche is forming at the boundary between TFs and PGCs). Thus in wild-type larval ovaries, germline differentiation was not detected by either of the two protein markers, displaying very weak Bam and the dot-shaped 1B1

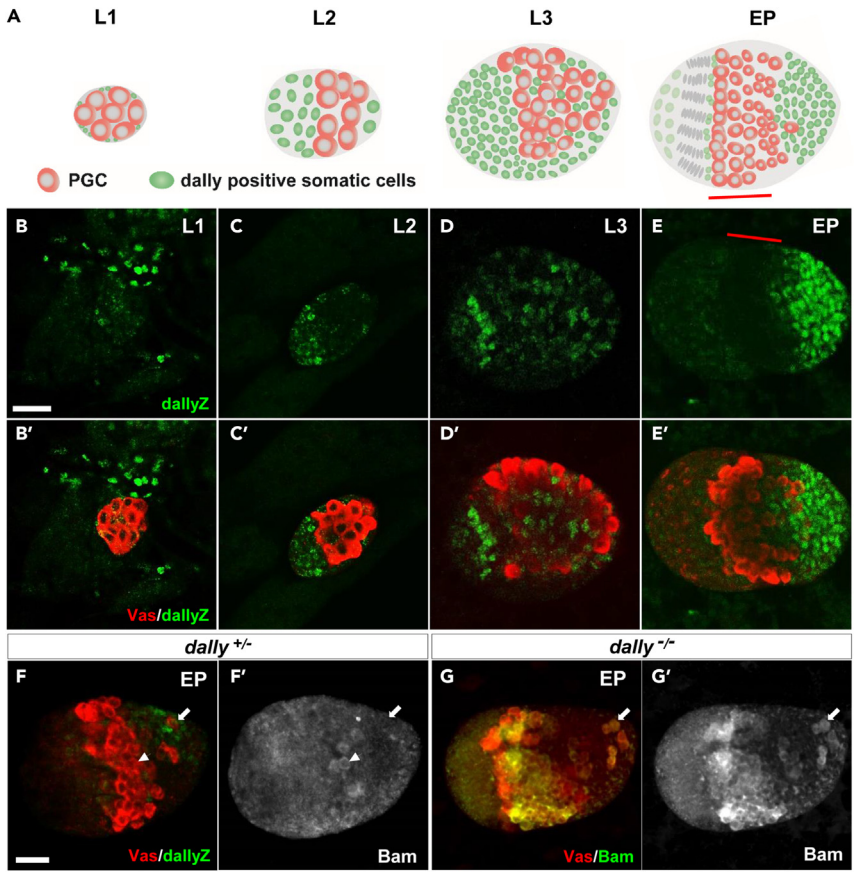


Figure 2. The relationship between PGC maintenance and the spatiotemporal pattern of Dally

(A) Schematic summary of Dally expression in ovaries of different developing stages. L1, L2, and L3 indicate 1st, 2nd, and 3rd larval instar. EP, early pupae. The red line marks the germ cell zone.

(B–E) Dally expression pattern in wild-type ovaries (*dally*$^{pz/+}$). dallyZ, a reporter of Dally transcription, stained by lacZ antibody. The red line corresponds to the red line in A. The scale bar is shared for B to E.

(F and G) Correlation between germline differentiation and the absence of Dally. The arrows point to posterior PGCs, which are undifferentiated in wild-type ovaries (*dally*$^{pz/+}$, F-F′) and differentiated in *dally* mutant (*dally*$^{pz/80}$, G-G′). Arrowheads point to the timely differentiated germ cells in wild-type EP ovaries. The scale bar is shared for F and G.

Scale bars: 20 μm.

(Figures 1A–1C and S1A). In contrast, we observed the germline differentiation signs, obvious Bam expression and branched 1B1 staining, in the 3rd instar larval ovaries of *dally* mutant (Figures 1D–1F, 1H, and S1B). Meanwhile, the somatic cells immediately surrounding PGCs did not appear different in number or distribution between wild-type and *dally* mutant (Figures S1C, S1D, and S1G).

To see the relationship between PGC maintenance and the spatiotemporal pattern of Dally during gonad development, we examined Dally expression using a reporter line in larval and early pupal ovaries (Figures 2A–2E, dallyZ, a LacZ enhancer trap line reflecting *dally* transcription). Prior to early pupal stage, dallyZ was observed around all PGCs (Figures 2A–2D), but became much decreased in the region where germ cells clustered and differentiated in pupal ovaries (Figures 2E and 2F, the arrowhead in F indicating one of the several differentiated germ cell). We noticed the stronger dallyZ signal in the posterior somatic cells that intermingled with some germ cells in pupal ovaries (Figure 2E). Correspondingly, those germ cells surrounded by stronger dallyZ signals did not have detectable Bam, the differentiation marker (Figure 2F, arrow). In *dally* mutants, however, high levels of Bam was easily detected in many germ cells including those scattered in the posterior part of ovary at this stage (Figure 2G).

To determine whether Dally exerts its function in somatic or in germline cells, we used cell-specific GAL4 lines to manipulate Dally levels in different cell types (Figures 3 and S2). Somatic-specific knockdown of Dally induced precocious Bam expression in germ cells (Figure 3A), whereas germline-specific knockdown of Dally did not (Figure 3B). Consistently, somatic-specific expression of Dally prevented precocious Bam expression in the germ cells of *dally* mutant (Figure 3C), whereas germline-specific expression of Dally did not (Figure 3D).

To further define in which group of somatic cells Dally is required, we examined three different GAL4 lines representing activation immediately surrounding PGCs (tjG4) or in the somatic parts outside PGC cluster of L3 ovaries (cv-2G4 and simG4) (Figure S2). Both cv-2G4 and

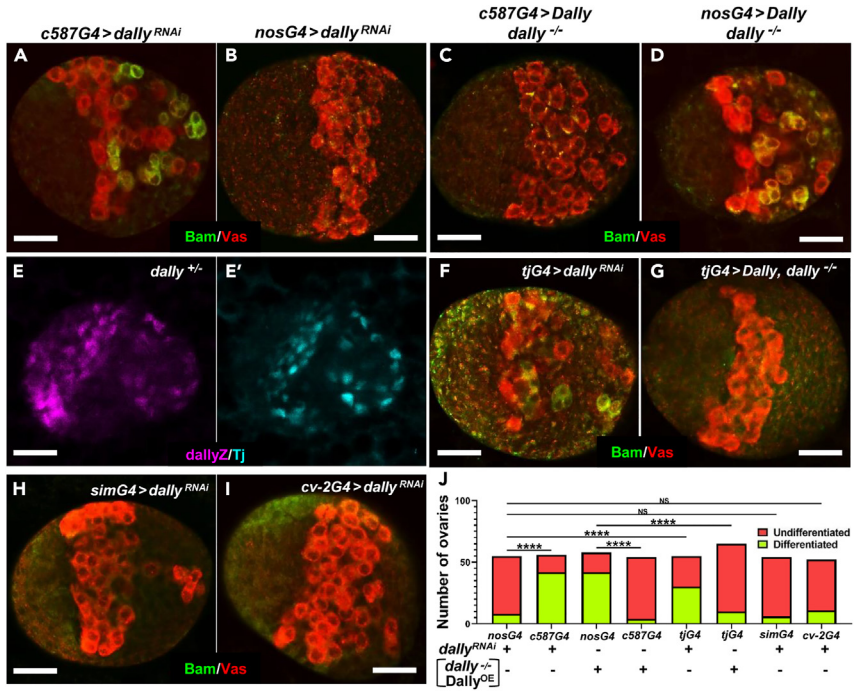


Figure 3. Dally is required in the Tj-active somatic cells to maintain PGC

(A–D) Dally RNAi and over-expression in somatic cells (*c587G4*) or germ cells (*nosG4*).

(E) Dally locates in Tj-positive cells of wild-type ovaries ($dally^{PZ/+}$). Tj indicates intermingled cells (ICs). The Dally reporter (dallyZ) colocalized with Tj at the posterior region of the ovaries.

(F–I) Dally RNAi and over-expression in different somatic cells. *tjG4*, *simG4* and *cv-2G4* expression are listed in Figure S2.

(J) Scoring ovaries based on the presence or absence of Bam in A-D and F-I. ****, $p < 0.0001$ (Chi-square). NS, no significance.

Scale bars: 20 μm.

simG4 covered some of those somatic cells migrating from gonad anterior to the posterior at early L3.[11] Signals of dallyZ and Tj immunostainings colocalized well around PGCs (Figures 3E and 3E′). Not surprisingly, reducing Dally by tjG4 led to evident germline differentiation (Figure 3F), whereas RNAi by either cv-2G4 or simG4 did not have any detectable effect (Figures 3H and 3I). Additionally, we also rescued the *dally* mutant PGCs from precocious differentiation by tjG4-expressed Dally (Figure 3G, comparing it with 3D). Using Bam as the differentiation indicator to score ovaries, the difference between wild-type/control and mutant/experimental was statistically significant (Figure 3J, $p < 0.0001$). In summary, Dally acts in gonadal somatic cells to prevent germline precocious differentiation.

Glypican Dally assists bone morphogenic protein/Drosophila homolog of bone morphogenic protein signaling to maintain primordial germ cells prior to ovary formation

We and others have demonstrated that Dally in the GSC niche of adult ovary promotes BMP signaling to inhibit germline differentiation.[8–10] During ovary development, where is Dpp (Drosophila homolog of BMP) expressed and how does Dally affect Dpp signaling? Dpp mRNA has been observed in the anterior somatic cells of early pupal ovary.[12] We examined Dpp expression pattern by a lacZ reporter in larval ovaries (Figures 4A–4C, dppZ).[13] DppZ activities were not detected in primordial ovaries until early L3, and became very strong at the posterior end of terminal filament (TF) at late L3 (Figures 4C and S3A′). Consequentially, immunostaining of phosphorylated Mad, the downstream effector of BMP/Dpp signaling, was intense in a row of PGCs adjacent to TFs which is also the high-dppZ zone (Figure S3A); whereas Bam, a target repressed by pMad of Dpp signaling and normally derepressed in germ cells only 1-cell distant from TFs in adult ovary,[14,15] was not detected in any PGCs of L3 ovaries regardless of their position (Figure 4D). In *dally* mutant L3 ovaries, Bam was derepressed though Dpp was present at the GSC niche (Figures 4E and 4K), and pMad staining next to Dpp source remained positive though appeared weaker and in less cells than that in the wild-type (Figures 4E′ and S3B). In contrast to that in adult ovary where niche-localized Dally only secure the germ cells immediately adjacent to Dpp source, widely expressed Dally facilitated Dpp signaling to reach all PGCs in larval ovary (Figures 4F–4I).

To see if the activation of pMad or the repression of Bam is indeed dependent on Dpp in L3 ovaries, we used a combination of two weak *dpp* alleles, and observed dramatic decrease of pMad as well as Bam upregulation (Figure 4L). Conversely, when we expressed Dpp in the ovarian matrix of *dally* mutant, we successfully activated Mad and repressed Bam in L3 ovaries (Figures 4J and S3C), similar to the effect of Dally expression in the same genetic settings (Figure 3C). Thus, glypican Dally assists BMP/Dpp signaling to maintain PGCs prior to the GSC niche establishment during ovary formation, even in the PGCs distant from the Dpp source.

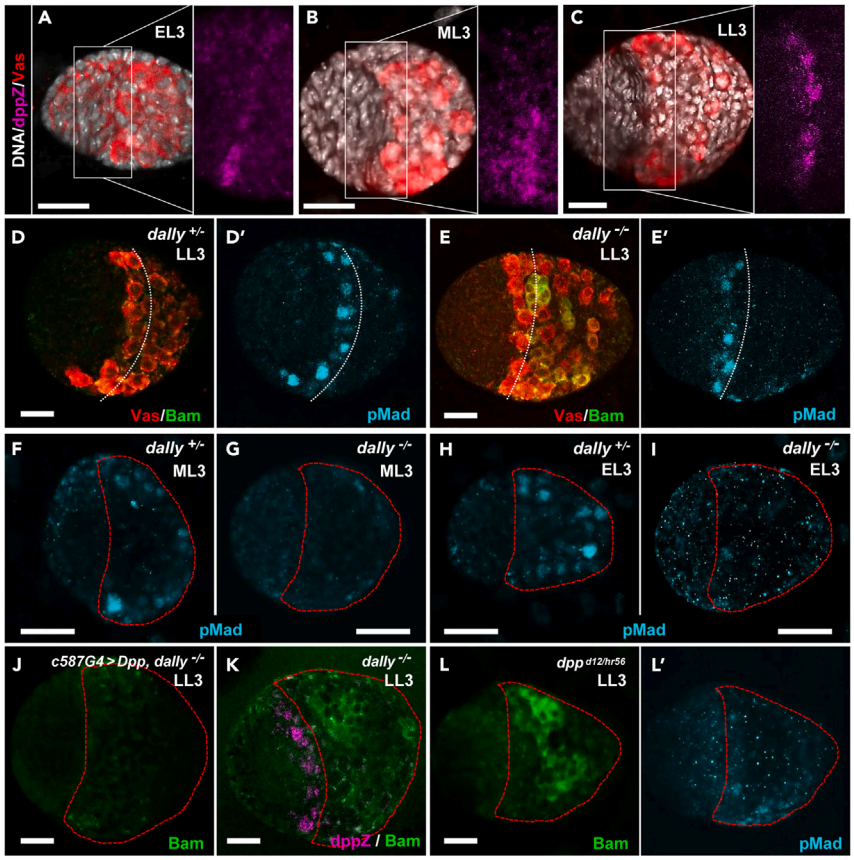


Figure 4. Glypican Dally modulates the spatiotemporal pattern of BMP signaling

(A–C) The Dpp expression during ovary development. dppZ (dpp-P4-lacZ, stained by lacZ antibody), a reporter of Dpp (one of the BMP ligands). DNA staining shows the characters of ovaries at EL3-LL3 (early larval3, mid-larval3, and late-larval3). And the developing terminal filaments (TFs) at each stage framed by white lines are magnified in the right panels.

(D–I) BMP/Dpp signaling in EL3-LL3 wild-type (*dally*$^{pz/+}$) and *dally* mutant (*dally*$^{pz/80}$) ovaries. pMad, a downstream effector of BMP signaling.

(J) The *dally* mutant's (*dally*$^{pz/80}$) ovary with somatically expressed Dpp shows no differentiation compared with E.

(K) Dpp expression (shown as dppZ) in the *dally* mutant (*dally*^{gem}) ovary. Bam shows the differentiated germ cells apart from Dpp.

(L) PGC differentiation (Bam positive) is measured in the *dpp* mutant, and pMad shows the down-regulated BMP signaling compared with the wild-type in D′. The red dashes outlined the areas of germ cells. Scale bars: 20 μm.

Glypican Dally coordinates local bone morphogenic protein and organismal steroid signals to regulate primordial germ cells differentiation

In wild-type L2 ovaries, pMad was present in all PGCs though dppZ reporter was not detectable (Figures 5A′ and 5C). In *dally* mutant, however, differentiation factor Bam was still repressed even in the absence of pMad (Figure 5, compare A&B and A′&B′). To see if the lack of Bam detection was due to the sensitivity of Bam antibody, we used *bam*-promoter driven GFP as another reporter to assay Bam expression. Indeed it was a more sensitive Bam marker in that we detected Bam transcriptional activities in EL3 ovaries of *dally* mutant (Figures S4C, S4D, and S4F, *bam*P-GFP). However, it remained negative in L2 ovaries (Figures S4A, S4B, and S4E).

Is there a redundant mechanism to secure PGCs on the correct differentiation track during ovary development? The transcription factor Kr has been found to inhibit PGC differentiation in response to the steroid ecdysone.[11] Ecdysone peaks high enough at L3 stage via Torso signaling to relieve the Kr-mediated repression of *bam* gene in PGCs.[11] We speculate that Kr, the downstream factor of Torso pathway, represents another mechanism to regulate PGC differentiation. When we reduced only Kr levels in germ cells at mid L3 stage, we could not see the de-repression of *bam* gene (Figure 5D). In general, Bam protein was not detectable in early L3 germ cells of *dally* mutants (Figure 5E), but was observed when we combined Kr and Dally reductions (Figure 5F). Conversely, Bam expression was usually obvious in late L3 germ cells of *dally* mutants (Figure 1E), but disappeared in the presence of Kr (Figures 5G1 and 5H). However, such repression of *bam* gene was not absolute, and we occasionally found the germ cells positive for strong signals of both Kr and Bam (Figures 5G2 and 5G2′).

Tsl (Torso-like) and Tll (Tailless) are respectively the ligand and the transcription factor of Torso pathway, and the expression of either led to Bam de-repression.[11] To confirm the connection between glypican-modulated BMP and Torso-Kr programs on PGC differentiation, in *dally*

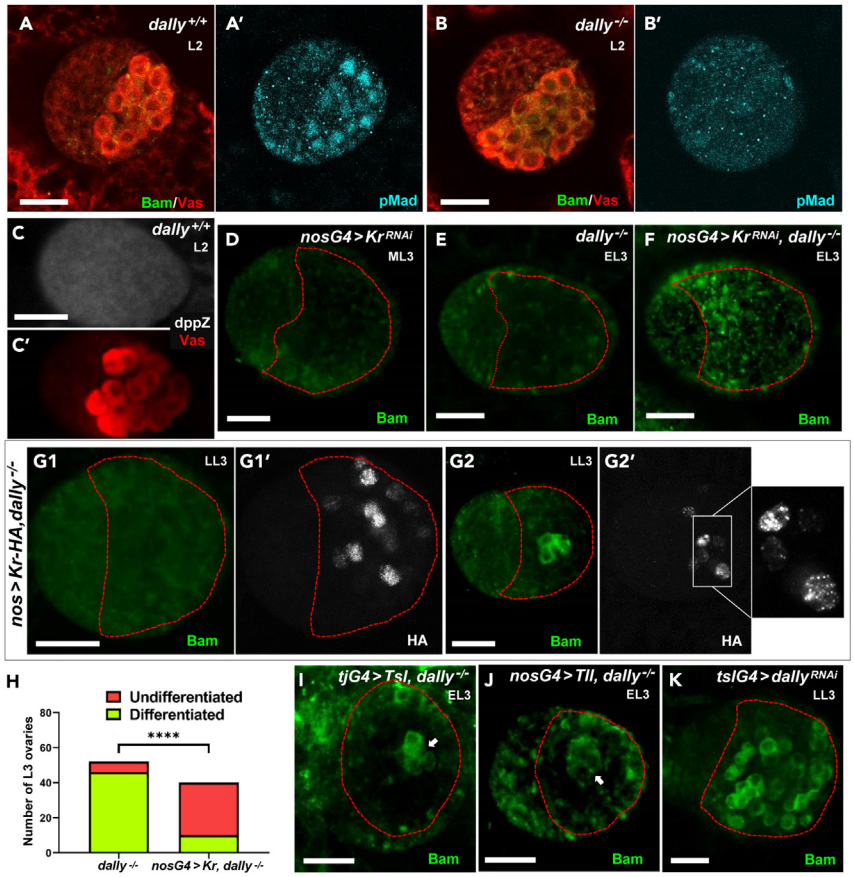


Figure 5. The dual regulation of PGC maintenance

(A and B) PGC maintenance of L2 ovaries in the wild-type (w^{1118}, A) and *dally* mutant ($dally^{pz/80}$, B). pMad, is a BMP effector.

(C) Dpp expression (shown as dppZ) in the wild-type (w^{1118}) L2 ovary.

(D–F) Bam expression is regulated by both Kr and Dally. $dally^{pz/80}$ was used as the mutant.

(G1-G2) Over-expression of Kr (with HA tag) in *dally* mutant ($dally^{pz/80}$) PGCs.

(H) Scoring ovaries based on the presence or absence of Bam in G1-G2 and the *dally* mutant ($dally^{pz/80}$). ****, $p < 0.0001$ (Chi-square).

(I–J) PGCs differentiate earlier with up-regulated Torso signaling in the *dally* mutant (compared with E). The arrows point to the differentiated germ cell clusters. Tsl is the ligand of the Torso pathway. Tll is a downstream effector of Torso signaling.

(K) PGC differentiated in the *tsl*-driven *dally* RNAi.

The red dashes outlined the areas of germ cells. Scale bars: 20 μm.

mutant background we expressed Tsl in somatic cells where it is normally released, or expressed Tll in germ cells (Figures 5I and 5J). Either manipulation brought up Bam levels in a few germ cells of early L3 ovaries. Additionally, reducing Dally in Tsl-active cells efficiently upregulated Bam in most germ cells (Figure 5K).

When glypican Dally decreases during early development, what is the consequence once gonad is formed? Because PGCs differentiated prematurely in *dally* mutants, we wonder whether or not this would shrink the GSCs pool at the beginning of adulthood. We checked the germarium, the initiative segment of an egg-assembly line (*aka* "ovariole"), to see if it contained germ cells or not in the ovarioles of 1-day virgins. In general, a germarium on the first day of adulthood would contain no Vas-positive cells if no GSC has ever occupied the GSC niche prior to the formation of germarium or ovarian assembly line. In wild-type 1-day ovaries, therefore, most germaria were full of Vas-positive cells (Figures 6B and 6D); whereas in *dally* mutant ovaries, there were completely empty germaria and significantly less germaria filled with Vas-positive germ cells (Figures 6C and 6D). Apparently, insufficient Dally during ovarian development eventually compromised the capacity of egg production at the beginning of adulthood.

DISCUSSION

In this study, we have found that the spatiotemporal pattern of glypican Dally is required to guide germ cell differentiation in the right space and at the proper stage in coordination with ovary development (Figures 6A1–6A4). The transcription promoter of the germline differentiation

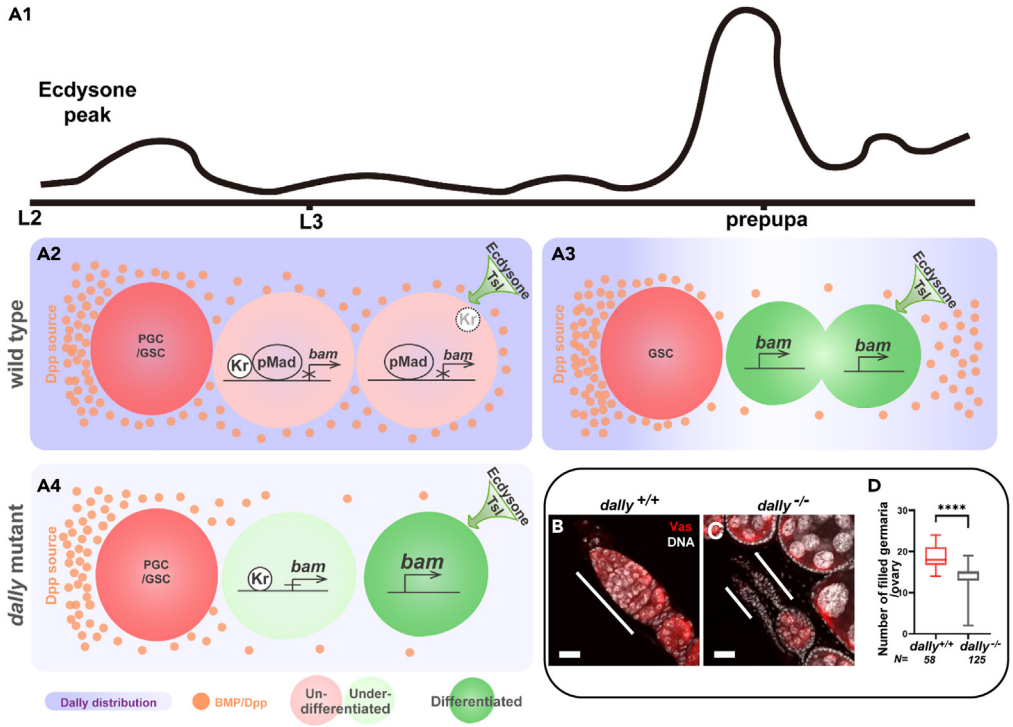


Figure 6. Glypican Dally coordinates PGC differentiation with ovary development

(A1) The timing and titer of ecdysone pulses from 2nd larval to pupal stages (modified from[17]).

(A2–A4) The relative level of Dally in ovarian soma is indicated by different intensities of purple color in the background.

(A2) During wildtype ovary development, Dally is expressed in somatic cells to facilitate the signaling of Dpp that is transcriptionally restricted at the anterior border between somatic and germ cells. Pro-differentiation factor Bam is repressed by pMad and Kr under the control of Dpp and Ecdysone respectively. pMad is strong in the germ cells adjacent to the Dpp source (deep pink "PGC/GSC"). The ecdysone signal is relayed by Tsl released from the posterior somatic cells. Even when Kr is removed by Ecdysone/Tsl signals, the BMP signaling sustained by Dally in the germ cells more distant from the Dpp source can still repress Bam and thus inhibit PGC differentiation (light pink cells).

(A3) In early pupal ovary, downregulated Dally in somatic cells around PGCs and Kr relieved by Ecdysone/Tsl signals jointly de-repress Bam by removing pMad and Kr, and PGCs start to differentiate (green). The germ cells adjacent to the Dpp source remain undifferentiated (GSC).

(A4) In *dally* mutant, Dally in the somatic cells is insufficient to maintain pMad in PGCs distant from the Dpp source (two different green cells). The precocious differentiation initiates when Kr is also removed by ecdysone surges before GSC-niche to be formed at pupal stage.

(B and C) Germaria of adult Day-1 ovaries. The white lines indicate the germaria. The scale bars: 20 μm.

(D) Scoring the number of functional germaria (germ cell-containing) shown in B but not the empty ones in C. N: number of ovaries counted in each genotype (w^{1118} and $dally^{pz/80}$). ****, $p < 0.0001$ (t-test).

factor Bam integrates the inputs of the local signal BMP/Dpp and the global signal steroid/ecdysone via the promotor-binding by pMad and Kr respectively (Figure 6A2 and 6A3). By enhancing Dpp signaling, Dally ensures the repression of Bam in PGCs, especially in the region distant from where Dpp is expressed to achieve the spatial assistance (Figure 2); Dally's presence also matches ecdysone surges to eventually remove Kr and de-repress Bam, i.e., temporally postponing PGC differentiation until the establishment of GSC-niche (Figures 5 and 6A3). Dally's reduction would lower the "hurdle" overcome by the ecdysone peak required for PGC differentiation which is consequently precocious in *dally* mutant (Figure 6A4). The two interacting regulatory pathways secure the accurate program of PGC differentiation in such a way to coordinate cell fate with organ and body development to maximize the reproductive potential (Figures 6B–6D).

What maintained PGCs in L2 ovaries when both Dally and Kr were compromised? In either *dally* mutant or Kr-RNAi, precocious PGC differentiation was observed in mid-to late L3 ovaries (Figures 1, 5D, and 5G2). When both Dally and Kr were reduced, we did not see PGC differentiation earlier than L3 (Figure 5F), while pMad was already diminished in L2 ovaries of *dally* mutant (Figure 5B'). There could be at least three possibilities: first, the Kr-RNAi line we used in this study was not very efficient; secondly, because we mainly relied on the immunostaining of Bam to reveal PGC differentiation, this Bam assay was probably not sensitive enough; thirdly, a Mad-independent mechanism may exist to safeguard PGCs at earlier stages. Other signaling pathways have been found in the GSC regulation of adult gonad. For example, Tu et al. demonstrated in adult ovaries that glypican-mediated Hh and Wnt signaling interdependently operates in the niche to promote GSC progeny differentiation by preventing BMP signaling.[16]

What makes the difference in Dally's spatial function between larval and adult ovary? Apparently, it relies on the different spatial expression of glypican Dally at these two stages. As one of the major components in extracellular matrix, Dally facilitates the distribution and receptor-binding

of the morphogen BMP.[7] In adult ovary, Dally is strictly expressed in the cap cells, the somatic niche of GSCs, and restricts BMP signaling to inhibit GSC differentiation in a 1-cell range adjacent to niche-localized Dally.[8–10] In contrast, Dally is widely expressed (notably strong in the posterior region of prepupal ovary) and facilitates Dpp signaling to reach all PGCs in larval ovary (Figure 2). Thus, when Dally was reduced in mutant ovaries and though Dpp expression at GSC-niche remained present, the germ cells not close enough to the Dpp source were prone to differentiation (Figure 4).

Is Kr by itself sufficient to repress Bam? Unlikely, because we observed strong Bam activities in the cells over-expressing Kr in the nuclei of germ cells (Figures 5G2 and G2′). As a result, the rescuing effect of Kr over-expression on *dally* mutant was significant but not complete (Figure 5H), though this level of Kr-overexpression seemed much higher than the endogenous Kr normally present in PGC. Notably, the endogenous Kr was easily detectable in the nuclei of fat cells surrounding larval gonads, but very weak in the PGCs either by immunostaining (data not shown) or by RNA *in situ*.[11] There may be a synergy between pMad and Kr to ensure the transcriptional repression of Bam.

In male flies, we did not observe apparent abnormalities in larval testes of *dally* mutant (data not shown). Notably in wild-type, GSC-niche is established and the PGCs outside the niche start to differentiate as soon as testes formed at the end of embryogenesis. Seemingly, at least zygotic Dally is not required for the GSC-niche formation and maintenance in testis development.

In mammals, what could be the parallel mechanisms regulating PGC differentiation in accordance with organogenesis and organism development? Mammalian *in vivo* studies have mostly been done in mouse. Discovering the intrinsic and extrinsic factors which guide human PGC differentiation heavily relies on iPS techniques to generate hPGCLC. From fruit fly to human, TGF-β family proteins (including BMP/Dpp, GDF, and and so forth) have been found to play a central role in specifying and/or maintaining the cell fate of PGC. Whether glypicans modulate BMP or GDF signaling in mammalian gonad development remains to be explored. Further, generation of oogonia from hPGCLCs can be accomplished by co-culturing with mouse embryonic ovarian somatic cells, suggesting the existence of conserved signaling molecules relayed by the somatic cells in gonad.[2] This relay seems similar to Tsl-release from gonadal soma in response to organismal steroid surges during drosophila development.[11] The local/gonadal and global/organismal signaling pathways that coordinately regulate germline differentiation remain to be identified in the mammalian systems.

Limitations of the study

We found the spatiotemporal expression of Dally coordinates the PGC development with organ/body development, but the upstream regulator of Dally was not identified. Although some evidence showed that EGFR could be upstream of Dally, further investigations of the factors regulating Dally's expression need to be executed, especially the signals in the developing ovary.

According to our observation, the expression of Dally at the very anterior of the L3 ovary and the very posterior of the EP ovary was much higher than in other parts of the ovary. However, these Dally expressing cells are not adjacent to PGC. The function of Dally expressed in the somatic anterior has not been investigated in this study.

We demonstrated that glypican Dally modulates the BMP signaling to maintain PGCs. Although glypicans or BMP signaling are evolutionarily conserved, whether or not they play similar roles in mammals remain to be investigated.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
- METHOD DETAILS
  - Transgenic flies
  - Immunofluorescence
  - qPCR analysis
  - bamP-GFP analysis
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.108710.

ACKNOWLEDGMENTS

We are deeply grateful to Dahua Chen, Hiroshi Nakato, Rongwen Xi, Ruth Lehmann, Shaowei Zhao, Ting Xie, Xinhua Lin for sharing research materials; to Tsinghua Fly Center, Vienna Drosophila Resource Center, FlyORF, and Bloomington Drosophila Stock Center for providing fly strains. This work is funded by National Key R&D Program of Chian (Grant No. 2018YFC1003300).

AUTHOR CONTRIBUTIONS

X.Liu & Z.Wang proposed and designed this research. X.Liu. was responsible for conducting experiments and statistical analysis; X.Liu & X.Li prepared experimental materials and collected samples; X.Liu conducted bioinformatic analysis and produced figures; Z.Wang drafted this article and revised this article. All authors have read and approved this article to be published.

DECLARATION OF INTERESTS

On behalf of all authors, we declare that there is no conflict of interests.

Received: June 29, 2023
Revised: October 18, 2023
Accepted: December 8, 2023
Published: December 12, 2023

REFERENCES

1. Mizuta, K., Katou, Y., Nakakita, B., Kishine, A., Nosaka, Y., Saito, S., Iwatani, C., Tsuchiya, H., Kawamoto, I., Nakaya, M., et al. (2022). Ex vivo reconstitution of fetal oocyte development in humans and cynomolgus monkeys. EMBO J. *41*, e110815.
2. Yamashiro, C., Sasaki, K., Yokobayashi, S., Kojima, Y., and Saitou, M. (2020). Generation of human oogonia from induced pluripotent stem cells in culture. Nat. Protoc. *15*, 1560–1583.
3. Seita, Y., Cheng, K., McCarrey, J.R., Yadu, N., Cheeseman, I.H., Bagwell, A., Ross, C.N., Santana Toro, I., Yen, L.H., Vargas, S., et al. (2023). Efficient generation of marmoset primordial germ cell-like cells using induced pluripotent stem cells. Elife *12*, e82263.
4. Hancock, G.V., Wamaitha, S.E., Peretz, L., and Clark, A.T. (2021). Mammalian primordial germ cell specification. Development *148*, dev189217.
5. Hu, Y.C., Nicholls, P.K., Soh, Y.Q.S., Daniele, J.R., Junker, J.P., van Oudenaarden, A., and Page, D.C. (2015). Licensing of primordial germ cells for gametogenesis depends on genital ridge signaling. PLoS Genet. *11*, e1005019.
6. Miyauchi, H., Ohta, H., Nagaoka, S., Nakaki, F., Sasaki, K., Hayashi, K., Yabuta, Y., Nakamura, T., Yamamoto, T., and Saitou, M. (2017). Bone morphogenetic protein and retinoic acid synergistically specify female germ-cell fate in mice. EMBO J. *36*, 3100–3119.
7. Yan, D., and Lin, X. (2009). Shaping morphogen gradients by proteoglycans. Cold Spring Harbor Perspect. Biol. *1*, a002493.
8. Guo, Z., and Wang, Z. (2009). The glypican Dally is required in the niche for the maintenance of germline stem cells and short-range BMP signaling in the *Drosophila* ovary. Development *136*, 3627–3635.
9. Hayashi, Y., Kobayashi, S., and Nakato, H. (2009). *Drosophila* glypicans regulate the germline stem cell niche. J. Cell Biol. *187*, 473–480.
10. Liu, M., Lim, T.M., and Cai, Y. (2010). The Drosophila female germline stem cell lineage acts to spatially restrict DPP function within the niche. Sci. Signal. *3*, ra57.
11. Banisch, T.U., Slaidina, M., Gupta, S., Ho, M., Gilboa, L., and Lehmann, R. (2021). A transitory signaling center controls timing of primordial germ cell differentiation. Dev. Cell *56*, 1742–1755.e4.
12. Zhu, C.H., and Xie, T. (2003). Clonal expansion of ovarian germline stem cells during niche formation in Drosophila. Development *130*, 2579–2588.
13. Li, X., Yang, F., Chen, H., Deng, B., Li, X., and Xi, R. (2016). Control of germline stem cell differentiation by Polycomb and Trithorax group genes in the niche microenvironment. Development *143*, 3449–3458.
14. Chen, D., and McKearin, D. (2003). Dpp signaling silences bam transcription directly to establish asymmetric divisions of germline stem cells. Curr. Biol. *13*, 1786–1791.
15. Song, X., Wong, M.D., Kawase, E., Xi, R., Ding, B.C., McCarthy, J.J., and Xie, T. (2004). Bmp signals from niche cells directly repress transcription of a differentiation-promoting gene, bag of marbles, in germline stem cells in the Drosophila ovary. Development *131*, 1353–1364.
16. Tu, R., Duan, B., Song, X., and Xie, T. (2020). Dlp-mediated Hh and Wnt signaling interdependence is critical in the niche for germline stem cell progeny differentiation. Sci. Adv. *6*, eaaz0480.
17. Thummel, C.S. (2001). Molecular mechanisms of developmental timing in C. elegans and Drosophila. Dev. Cell *1*, 453–465.
18. Li, C.Y., Guo, Z., and Wang, Z. (2007). TGFbeta receptor saxophone non-autonomously regulates germline proliferation in a Smox/dSmad2-dependent manner in Drosophila testis. Dev. Biol. *309*, 70–77.
19. Gancz, D., and Gilboa, L. (2017). RNA Isolation from Early Drosophila Larval Ovaries. Methods Mol. Biol. *1463*, 75–83.
20. Van Doren, M., Williamson, A.L., and Lehmann, R. (1998). Regulation of zygotic gene expression in Drosophila primordial germ cells. Curr. Biol. *8*, 243–246.
21. Lee, P.T., Zirin, J., Kanca, O., Lin, W.W., Schulze, K.L., Li-Kroeger, D., Tao, R., Devereaux, C., Hu, Y., Chung, V., et al. (2018). A gene-specific T2A-GAL4 library for Drosophila. Elife *7*, e35574.
22. Nakato, H., Futch, T.A., and Selleck, S.B. (1995). The division abnormally delayed (dally) gene: a putative integral membrane proteoglycan required for cell division patterning during postembryonic development of the nervous system in *Drosophila*. Development *121*, 3687–3702.
23. Han, C., Belenkaya, T.Y., Wang, B., and Lin, X. (2004). Drosophila glypicans control the cell-to-cell movement of Hedgehog by a dynamin-independent process. Development *131*, 601–611.
24. Qiao, H.H., Wang, F., Xu, R.G., Sun, J., Zhu, R., Mao, D., Ren, X., Wang, X., Jia, Y., Peng, P., et al. (2018). An efficient and multiple target transgenic RNAi technique with low toxicity in Drosophila. Nat. Commun. *9*, 4160.
25. Spencer, F.A., Hoffmann, F.M., and Gelbart, W.M. (1982). Decapentaplegic: a gene complex affecting morphogenesis in Drosophila melanogaster. Cell *28*, 451–461.
26. Blackman, R.K., Grimaila, R., Koehler, M.M., and Gelbart, W.M. (1987). Mobilization of hobo elements residing within the decapentaplegic gene complex: suggestion of a new hybrid dysgenesis system in Drosophila melanogaster. Cell *49*, 497–505.
27. Xiao, H., Hrdlicka, L.A., and Nambu, J.R. (1996). Alternate functions of the single-minded and rhomboid genes in development of the Drosophila ventral neuroectoderm. Mech. Dev. *58*, 65–74.
28. Furriols, M., Ventura, G., and Casanova, J. (2007). Two distinct but convergent groups of cells trigger Torso receptor tyrosine kinase activation by independently expressing torso-like. Proc. Natl. Acad. Sci. USA *104*, 11660–11665.
29. Bischof, J., Björklund, M., Furger, E., Schertel, C., Taipale, J., and Basler, K. (2013). A versatile platform for creating a comprehensive UAS-ORFeome library in Drosophila. Development *140*, 2434–2442.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
mouse anti-Bam (1:2000)	a gift from Prof. Dahua Chen	N/A
rabbit anti-Bam (1:2000)	a gift from Prof. Dahua Chen	N/A
mouse anti-beta-galactosidase (1:100) for lacZ (product name 40-1a)	DSHB	Cat# 40-1a; RRID: AB_528100
rat anti-GFP (1:1000)	Santa Cruz Biotechnology	Cat# sc-101536; RRID: AB_1124404
mouse anti-Hts (1:100) (product name 1B1)	DSHB	Cat# hts RC; RRID: AB_528289
rabbit anti-Tj (1:1000)	Our lab made	N/A
rabbit anti-pSmad3 (1:200) for pMad	Abcam	Cat# ab52903; RRID: AB_882596
guinea pig anti-Vasa (1:4000)	Our lab made	N/A
rabbit anti-Vasa (1:4000)	Our lab made	N/A
Experimental models: Organisms/strains		
bamP-GFP	a gift from Prof. Dahua Chen	N/A
c587GAL4	Van Doren et al.[20]	N/A
cv-2GAL4	Lee et al.[21]	BDSC #67491
$dally^{pz}$ (also known as $dally^{06464}$, a *dally*-reporter)	Nakato et al.[22]	BDSC #11685
$dally^{80}$	Han et al.[23]	N/A
$dally^{gem}$	Nakato et al.[22]	N/A
UAS-$dally^{RNAi}$	Ni et al.[24]	Tsinghua Fly Center #TH201501181.S
UASt-Dally-HA	this study	N/A
UASz-Dally	this study	N/A
dpp^{d12},	Spencer et al.[25]	BDSC #2070
dpp^{hr56}	Blackman et al.[26]	BDSC #36528
UAS-Dpp-GFP	a gift from Dahua Chen	N/A
dpp-P4-lacZ (dppZ)	Li et al.[13]	N/A
UAS-Kr^{RNAi}	Vienna Drosophila Resource Center (VDRC)	VDRC #v104150
UASz-Kr-HA	this study	N/A
UAS-nlacZ	Zhaohui Wang Lab stock	N/A
nosGAL4	Zhaohui Wang Lab stock	N/A
simGAL4,	Xiao et al.[27]	BDSC #9150
tjGAL4,	Zhaohui Wang Lab stock	N/A
tslGAL4,	Furriols et al.[28]	N/A
UAS-Tsl	Furriols et al.[28]	N/A
UAS-Tll	FlyORF[29]	FlyORF #F000061
w^{1118}	Zhaohui Wang Lab stock	N/A
Oligonucleotides		
bam fw (for qPCR)	TGGAACCGGAAGAGTAAAGG	N/A
bam rv (for qPCR)	TTAGGCATCGTGCTCTTCAC	N/A
Kr fw (for qPCR)	CGAGGCATCCAGGAATAGAT	N/A
Kr rv (for qPCR)	CACTGGGTACGTGAGGGATT	N/A
ND-42 fw (for qPCR)	TGAGGTTGTGGTGGAGGA	N/A
ND-42 rv (for qPCR)	CATCACGCCAGACTTTGC	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Zhaohui Wang (zhwang@genetics.ac.cn).

Materials availability

The Kr and Dally over-expression fly lines generated in this study are available from the lead contact on a collaborative basis.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

The flies (*Drosophila melanogaster*) were raised on the cornmeal food medium and kept at 25°C. Flies with GAL4-UAS tools and *dpp* temperature-sensitive mutants were crossed at 25°C, and the larvae were transferred to 29°C. All genotypes used in this study were listed in key resources table. Staged ovary samples were collected after 12 hours cross and aged for 1 day (first larval instar), 2 days (second larval instar), 3 days (early 3rd larval instar), 4 days (mid-3rd larval instar), 5 days (late-3rd larval instar), or 6 days (white early pupa instar). Embryos were collected after 4 hours cross and aged for 20 hours.

METHOD DETAILS

Transgenic flies

cDNA of Dally was cloned from the Drosophila larvae, and inserted into pUASt-3xHA-attB and pUASz-attB vectors. cDNA of Kr was cloned from the Drosophila larvae, and inserted into the pUASz-3xHA-attB vector. The constructs were inserted into p51D flies to generate *UASt-dally-3xHA*, *UASz-dally*, or *UASz-Kr-3xHA* transgenic flies.

Immunofluorescence

All ovary samples were dissected in PBS, fixed in 4% FA/PBS. The fixed samples were immunostained as described,[18] except that the Bam, pMad and lacZ antibodies were incubated for 2 days at 4°C for better effect. Primary antibodies are listed in the antibody table above. Alexa-Fluor-conjugated secondary antibodies were used at 1:4000 (Molecular Probes, Invitrogen). DAPI was used to stain DNA at 0.1 ng/μL. Fluorescent images were collected by the OLYMPUS FV1000 Confocal micro-imaging system.

qPCR analysis

Larva dissections were carried out following previous method.[19] For high RNA yield, 30-40 L3 gonads were used for one biological repeat and were repeated 3 time. RNA extraction was done using TRIzol (Invitrogen) and followed by cDNA synthesis with QuantiTect Reverse Transcription Kit (QIAGEN). mRNA levels of ND-42 (the gene NADH dehydrogenase (ubiquinone) 42 kDa subunit) served as a control. qPCR analysis was performed on a TIANLONG Gentier 96E system.

bamP-GFP analysis

The ovaries of different genotype were dissected, stained and imaged in parallel under the same conditions/settings. For confocal imaging, the focal plane with the most germ cells was captured. The ten brightest germ cells per gonad were determined visually and the relative GFP levels were obtained by ImageJ. For each genotype, at least 8 gonads were measured.

QUANTIFICATION AND STATISTICAL ANALYSIS

All experiments were performed with at least three biological repeats. For all ovary samples, over 50 ovaries were examined. Statistical significance was calculated using Welch's t-test or Chi-square. Error bars represent standard deviation (SD).