**Bmp7 Regulates Germ Cell Proliferation in Mouse Fetal Gonads**

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**Key Words**
Bmp7 · Germ cells · Gonad development · Proliferation

**Abstract**
Relatively little is known regarding the signals that regulate the proliferation and sex-specific development of germ cells during mammalian fetal gonad differentiation. Members of the bone morphogenetic protein (BMP) family have been identified as key regulators of germ cells in the *Drosophila* gonad. Here we show that in mice *Bmp7* is expressed in gonads of both sexes and is required for germ cell proliferation during a narrow window of development between 10.5–11.5 days post coitum (dpc). The proliferation defect is more severe in male than in female embryos suggesting that there are sexually dimorphic compensatory pathways. BMP signaling appears to be an evolutionarily conserved pathway regulating embryonic germ cell proliferation in vertebrate and invertebrate species.

In the mouse, a population of primordial germ cells (PGCs) is first detectable at approximately 7.25 days post coitum (dpc) in a region posterior to the primitive streak [Ginsburg et al., 1990]. PGCs migrate through the hindgut into the urogenital ridges and populate the gonads between 10.0 and 11.0 dpc. Molecular signals have been identified that are required for the proliferation or survival of PGCs during their migratory phase, including Kit ligand and stromal cell derived factor 1 (*Sdf1*) [Loveland and Schlatt, 1997; Ara et al., 2003]. In addition, numerous factors have been shown to stimulate PGC proliferation and/or survival in vitro, including Kit ligand, FGFs, leukemia inhibitory factor (LIF), tumor-necrosis factor (TNF) and interleukin-4 (IL4) [Matsui et al., 1992; Resnick et al., 1992; Kawase et al., 1994; Cooke et al., 1996]. However, it is not clear what roles these factors have in regulating germ cell proliferation in fetal gonads in vivo.

The developmental fate of germ cells is dependent upon their somatic environment, and not on their chromosomal composition [Adams and McLaren, 2002]. When PGCs initially enter the urogenital ridges, the gonads are bipotential, with the ability to form either an ovary or a testis. Sexually divergent development begins between 10.5 and 12.0 dpc, when the Y-linked gene *Sry* is expressed in somatic cells of the XY gonad and triggers testis development. Germ cells entering an XX or XY gonad proliferate until approximately 14.0 dpc, when germ cells in XX gonads enter prophase of meiosis and germ cells in XY gonads arrest in mitosis and initiate differentiation as prospermatogonia. Recently, differences in gene expression and motility of germ cells in XX and XY gonads have been identified that are required for the regulation of germ cell proliferation in vivo.

This work was supported by grants from the NIH to A.R. (HD41317-02) and B.C. (HD39963).
gonads have been demonstrated as early as 11.5 dpc, suggesting that sex-specific germ cell development initiates shortly after expression of Sry begins [Takasaki et al., 2000; Molyneaux et al., 2001; Siggers et al., 2002; Menke et al., 2003; DiNapoli et al., 2006].

Currently, there is limited information regarding the signals that regulate the proliferation and differentiation of germ cells once they have populated embryonic gonads. Bone morphogenetic proteins (BMPs) are members of the TGFβ superfamily of secreted signaling molecules that have numerous roles in the regulation of germ cell development in both invertebrate and vertebrate species. In Drosophila, two homologs of mammalian BMPs, decapentaplegic (dpp) and glass bottom boat (gbb, also called 60A), are required for the maintenance and proliferation of germline stem cells [Xie and Spradling, 1998; Shivdasani and Ingham, 2003; Kawase et al., 2004]. In the mouse, BMPs have been shown to regulate germ cell development both during embryogenesis and in the adult. Bmp2, Bmp4, and Bmp8b are all involved in the regulation of PGC specification in the epiblast, and targeted inactivation of any of these genes results in reduced numbers of PGCs [Lawson et al., 1999; Ying et al., 2000; Ying and Zhao, 2001]. Mice homozygous for null mutations in either Bmp8a or Bmp8b or heterozygous for a mutation in Bmp4 display defects in spermatogenesis and germ cell survival in the adult testis [Zhao et al., 1996, 1998; Hu et al., 2004]. Two other members of the BMP family, growth differentiation factor 9 (GDF9) and BMP15, are required for normal oocyte development [Dong et al., 1996; Yan et al., 2004]. Two other members of the BMP family, growth differentiation factor 9 (GDF9) and BMP15, are required for normal oocyte development [Dong et al., 1996; Yan et al., 2004]. However, no functional roles for this family of signaling molecules during fetal gonad development have previously been described.

BMP2 and BMP4 are the mammalian BMPs most closely related to Drosophila Dpp, whereas Gbb is part of a subfamily of BMPs that also includes BMP5, BMP6, BMP7, BMP8a and BMP8b. Expression of Bmp2 has been reported previously specifically in murine XX gonads, whereas Gbb is part of a subfamily of BMPs that also includes BMP5, BMP6, BMP7, BMP8a and BMP8b. Expression of Bmp2 has been reported previously specifically in murine XX gonads,

11.5 dpc Testis and Ovary Histology

For whole mount immunostaining, samples were fixed over-night in 4% paraformaldehyde at 4 °C. After fixation, samples were dehydrated through a graded ethanol series, cleared with Histoclear (National Diagnostics, Atlanta, Ga, USA), and embedded in paraffin wax. Five μm sections were cut, stained using the Schiff’s Periodic Acid Staining Kit (Polysciences, catalog # 24200), following the kit instructions, and counterstained with hematoxylin.

Alkaline Phosphatase Staining

Samples were fixed in 4% paraformaldehyde at 4°C overnight. The tissue was washed twice briefly in PBS containing 0.1% Tween-20 then washed in 70% EtOH for several hours at room temperature. To detect alkaline phosphatase activity, samples were incubated in Fast Red stain (0.5 mg/ml Fast Red TR, 0.1 mg/ml naphthyl phosphate, 500 μM borax, 5 mM MgCl₂) for 15 min at room temperature, or until staining was visible, and washed twice in PBS to stop the staining reaction.

Immunohistochemistry

For whole mount immunostaining, samples were fixed over-night in 4% paraformaldehyde in PBS at 4°C. Tissue was blocked for 1 h at room temperature in blocking solution (10% heat inac-
activated goat serum, 0.1% Triton-X 100 in PBS). Primary antibody
incubations were carried out overnight at 4°C in blocking solu-
tion (1:200 dilution of rabbit anti-laminin antibody, provided by
Harold Erickson; 1:500 dilution of rat anti-PECAM-1 antibody,
Pharmingen; 1:250 dilution of rabbit anti-phosphorylated histone
H3, Upstate Biotechnology; 1:100 dilution of rabbit anti-active
caspase-3, Pharmingen). Gonads were washed 3 times in washing
solution (PBS/1% heat inactivated goat serum/0.1% Triton-X 100)
for 1 h each. Secondary antibody incubations were performed
overnight at 4°C with 1:500 dilution of fluorescein-conjugated
secondary antibodies (FITC- or Cy5-conjugated goat anti-rabbit
antibody and Cy3-conjugated goat anti-rat antibody, Jackson Im-
munocytokines). Samples were washed 3 times for 1 h each in
washing solution and mounted on glass slides in DABCO.

In situ Hybridization
Gonads were fixed in 4% paraformaldehyde at 4°C overnight.
Samples were washed in PBST (PBS with 0.1% Tween-20) and
treated with 10 μg/ml Proteinase K in PBST at 37°C for 15 min.
The tissue was fixed in 4% paraformaldehyde/0.1% glutaral-de-
hyde at room temperature for 15 min and washed twice with
PBST. Samples were incubated in hybridization buffer (50% for-
mamide, 2× SSC, 0.2% Tween-20, 1 mg/ml yeast tRNA, 50 μg/ml heparin, and 5 mM EDTA) for 1 h at 60°C. probe
was added to hybridization buffer and samples were incubated for
at least 24 h at 60°C. Samples were washed once for 30 min at 60°C
in 50% formamide; 2× SSC, 0.1% Tween-20, and twice for 30 min
at 60°C in 2× SSC, 0.1% Tween-20. Samples were washed in
MABT (100 mM Maleic acid, 150 mM NaCl, 0.1% Tween-20, pH
H3, Upstate Biotechnology; 1:100 dilution of rabbit anti-active
histone H3 and PECAM-1 labeling were counted
and divided by the total number of PECAM-1-positive germ
cells in the section. For statistical comparisons of total germ cell
numbers and percentages of mitotic cells, the results were ana-
yzed using unpaired Student’s t test and results were graphed
using MS Excel.

RNA Extraction and Reverse Transcription
Total RNA was extracted from 12.5-dpc CD-1 gonads using
TRIzol reagent (Invitrogen, cat# 15596-026) as directed by the
manufacturer, and eluted with 16 μl RNase-free water. To pre-
vent DNA contamination, RNA was treated with 2 μl DNase I and
2 μl 10× DNase I Reaction Buffer (Sigma, cat# AMP-D1) at room
temperature for 15 min. DNase was inactivated by treatment with
2 μl DNase I Stop Solution (Sigma, cat# AMP-D1) at 70°C for 10
min. RNA was reverse transcribed into cDNA in a 20-μl reaction
using the iScript™ cDNA Synthesis Kit (Bio-Rad, cat# 170-8890)
according to the manufacturer’s protocol.

Quantitative RT-PCR
Quantitative RT-PCR was used to determine relative expres-
sion levels of Bmp2, Bmp4, Bmp5, Bmp7, Bmp8a, and
Bmp8b in 12.5-dpc CD-1 gonads. Each analysis was performed in
triplicate in a total volume of 25 μl reaction mixture containing
1 μl cDNA template (1/20th of reverse transcription reaction),
12.5 μl 2× iQ™ SYBR® Green Supermix (Bio-Rad, cat# 170-
8890), 10.5 μl RNase free water, and 0.5 μl each gene specific for-
ward and reverse primer (500 nM final concentration each).
Quantitative PCR was performed on the iCycler™ Thermal Cy-
cycler (Bio-Rad, cat# 170-8720) with MyiQ™ Single-Color Real-
Time Detection System (Bio-Rad, cat# 170-9740). PCR cycling
conditions for all primers were as follows: 95°C for 3 min 30 s (one
cycle); 95°C for 30 s, 60°C for 45 s, 72°C for 45 s (40 cycles); and
72°C for 2 min (one cycle). β-actin primers: Forward (5'-GGCT-
GTATTCCCCCTCCATCG-3') and Reverse (5'-CCAGTGGTT-
AAGATGCGATGTG-3'). Bmp2 primers: Forward (5'-AAGG-
GTCAAAGCCAAACACAA-3') and Reverse (5'-GAGTGTTGAG-
GTTGTCAGAAC-3'). Bmp4 primers: Forward (5'-TTCC-
TGGGAACCAATGCTGA-3') and Reverse (5'-CTCGTGAAT-
TCGAGCGACTTTT-3'). Bmp5 primers: Forward (5'-ACGCA-
GGGGAAAGCACAC-3') and Reverse (5'-AACAGAAC-
ATTTCCCCGGCCAAA-3'). Bmp6 primers: Forward (5'-GAG-
CTTTGGTTGATGCTGA-3') and Reverse (5'-CTCAGGCTTGGAGA-
CTGTGA-3'). Bmp7 primers: Forward (5'-GTATT-
TGGTTGACGAC-3') and Reverse (5'-GGCTTTCCAGCCCAAA-
AA-3'). Bmp8a primers: Forward (5'-TTTCTGTTGGGACTCTC-
CAC-3') and Reverse (5'-TGCTGGCACTCTGGAAGAGC-3'). Bmp8b primers: Forward (5'-TTTG-
CTGCAGAAGCGCAGA-3') and Reverse (5'-TCCAG
GTGCAGCAGA-3'). Primer sets were tested for
efficiency and found to work optimally with the ΔCt method.
The Ct number, defined as the number of PCR amplification cycles
required to reach fluorescent intensity above threshold, was de-
termined for each Bmp and the housekeeping gene β-actin.
Normalized expression levels were obtained by subtracting the β-ac-

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tin Ct value from the Ct value obtained for each Bmp. Relative Bmp expression levels were obtained by comparing each normalized expression value to that of the lowest expressed Bmp in the set (in this case Bmp8b from the XY gonad).

Results

Expression of Bmp7 during Early Gonad Development

Expression of Bmp7 was detected by ISH in the indifferent gonads as early as 10.5 dpc (fig. 1A). Bmp7 was expressed in both XX and XY gonads through 11.5 dpc, after which stage it was detected only in XY gonads (fig. 1B, C, D). Bmp7 expression was maintained in XY gonads and was localized to testis cords at 13.5 dpc (fig. 1E) and all later stages examined, from 15.5 dpc until birth (not shown). Bmp7 was also expressed in the mesonephros, within the mesonephric duct and tubules (fig. 1D, red arrows).

Two cell types are found within testis cords, germ cells and Sertoli cells, the somatic supporting cells of the testis. The expression of Bmp7 within testis cords suggests that these genes are expressed by either germ cells, Sertoli cells, or both. To gain information regarding which cell type(s) express Bmp7, we used gonads from busulfan-treated embryos, which lack germ cells, for in situ hybridization (fig. 1F). Bmp7 expression was very strong in testes lacking germ cells, demonstrating that Bmp7 is expressed by Sertoli cells. Importantly, this result does not rule out the possibility that Bmp7 is also expressed by germ cells. Germ cells have been shown to express Bmp7 at later developmental stages; specifically, in spermatogonia and spermatocytes in the adult testis [Zhao et al., 2001].

Reduced Germ Cell Numbers in Bmp7 Mutant Gonads

To determine if Bmp7 plays an important role during gonad development, we examined mice homozygous for a null mutation of Bmp7. As Bmp7 mutants die perinatally, we examined testes and ovaries from homozygous mutants at a stage shortly before birth. At 18.5 dpc, the gross morphology of Bmp7–/– testes and ovaries appeared similar to wild-type controls (not shown). Sections of ovaries and testes from Bmp7 mutants were also examined (fig. 2). No obvious defects were apparent in the mutant ovaries, and germ cells displayed the characteristic features of meiosis (fig. 2A–D). By contrast, a defect in Bmp7 mutant testes was immediately detected. Testes from Bmp7 mutants had tubules that contained few or no
prospermatogonia, although the testes did not completely lack germ cells (fig. 2E–H). Other than the reduction in germ cell number, the organization and morphology of the mutant testes appeared normal.

Earlier stages of gonad development were examined to determine when the reduction in germ cells originates in the Bmp7 mutants. Gonads from 10.5 through 13.5-dpc mutant and control embryos were stained for alkaline phosphatase activity, a marker of germ cells in both sexes at these stages (fig. 3A–F). There was no obvious visible difference in the appearance of XX or XY mutant gonads at 11.5 dpc (fig. 3A, B). By 12.5 dpc, however, a pro-
Fig. 3. The germ cell defect in Bmp7 mutants occurs during early stages of gonad development. A–F Gonads from Bmp7 mutants embryos and controls from 11.5 to 13.5 dpc were stained for alkaline phosphatase activity (reddish-brown staining) to label germ cells. C, D A reduction in germ cell numbers is clearly visible in XY Bmp7 mutants by 12.5 dpc, whereas XX mutant gonads are slightly smaller in size than controls, but not as severely affected. E, F Similar differences were observed at 13.5 dpc, with both XX and XY mutant gonads smaller than controls. G–J Immunostaining for PECAM-1 (red, labels germ cells and elongated vascular endothelial cells) and laminin (green, outlines testis cords, white arrows) on gonads from 13.5-dpc Bmp7 mutants and controls. G, I XY Bmp7–/– gonads are morphologically similar to controls, but contain a lower density of germ cells. K Total germ cell numbers in 10.5-dpc mutant and control genital ridges and germ cells per longitudinal gonad section in embryos from 11.5 to 13.5 dpc. A statistically significant difference is first observed between mutants and controls at 11.5 dpc, and is detected at all later stages. Wild type and heterozygotes, blue bars; homozygous Bmp7 mutants, red bars. Values represent mean ± s.e.m. * Statistically significant (p < 0.05, Student’s t test). 11.5-dpc XY controls (n = 21 gonads), XY mutants (n = 25), XX controls (n = 14), XX mutants (n = 15); 12.5-dpc XY controls (n = 24), XY mutants (n = 21), XX controls (n = 12), XX mutants (n = 12); 13.5-dpc XY controls (n = 18), XY mutants (n = 17), XX controls (n = 20), XX mutants (n = 20).

Fig. 4. Reduced proliferation of germ cells in Bmp7−/− gonads until 11.5 dpc. A–H Immunostaining of gonads with antibodies against phosphorylated histone H3 (red) to label mitotic cells and PECAM-1 (green), which labels germ cells (round) and vascular endothelial cells (elongated). Mitotic germ cells were identified as cells outlined in green with red nuclei (A, inset). A, B At 11.0 dpc, numerous mitotic germ cells (white arrows, round cells with both green and red staining) are observed in XX and XY gonads from wild-type embryos. C, D Few or no mitotic germ cells are observed in most sections from XX or XY Bmp7 mutant gonads at the same stage, but numerous mitotic somatic cells are still present (blue arrowheads), including PECAM-1-positive cells that can be identified as vascular endothelial cells based on their morphology (purple arrowhead). E–H At 12.5 dpc, there are fewer mitotic germ cells (white arrows) in XY mutant gonads as compared to controls, but there are also fewer total germ cells. Similar numbers of mi-
Mitotic germ cells were observed in XX mutants and controls at this stage. Percentage of germ cells positive for phospho-histone H3. A statistically significant reduction in the percentage of mitotic germ cells was observed in mutant gonads (XX and XY) as compared to controls at 10.5 dpc (10.5-dpc mutants, n = 8 gonads, controls n = 10). At 11.5 dpc, a small but significant reduction in mitotic germ cells was observed in mutant XY gonads, but not in XX gonads (11.5-dpc XY mutants, n = 7 gonads, XY controls n = 8). At 12.5 and 13.5 dpc, rates of mitosis are similar in mutants and controls of both sexes. Values represent mean ± s.e.m. * Statistically significant (p < 0.05, Student’s t test). J, K Immunostaining for active caspase-3 (green) to label apoptotic cells (white arrowheads), and PECAM-1 (red) in 11.5-dpc XY Bmp7 mutant gonad and control. Few dying germ or somatic cells were detected in either mutant or control samples at any stage examined.
ounced defect was observed in XY $Bmp7^{-/-}$ gonads. At this stage, the XY mutant gonads were smaller than controls and showed reduced levels of alkaline phosphatase staining (fig. 3C). However, two markers of Sertoli cell development, $Sox9$ and $Dhh$, were expressed normally in 12.5-dpc XY gonads, suggesting that differentiation of the somatic gonad was normal (ISH data not shown). By 13.5 dpc, the mutant testes were significantly smaller than controls and appeared to contain fewer germ cells, but were otherwise morphologically normal (fig. 3E). $Bmp7$ mutant XX gonads appeared to contain a similar density of germ cells as controls, but were slightly reduced in size (fig. 3D, F).

To confirm these results and enable better quantification of germ cell numbers, immunostaining for PECAM-1 (platelet/endothelial cell adhesion molecule 1) labels both germ cells and vascular endothelial cells in the embryonic gonads, but the two cell types can easily be distinguished based on shape and location. Sections of mutant gonads stained for PECAM-1 contained a reduced number of germ cells, but appeared otherwise normal (fig. 3G–J). The phenotype was more striking when the extent of the germ cell reduction was quantified. Germ cell counts were performed on sections of mutant and control gonads stained with PECAM-1 at different developmental stages (fig. 3K). Of note, no statistical difference was found in germ cell number between wild-type and heterozygous gonads at any stage, so these genotype classes were combined and used as controls in these studies. At 10.5 dpc, there was no significant difference in germ cell numbers in the gonads of mutants and controls, suggesting that the initial specification and migration of germ cells to the urogenital ridge occurs normally. By 11.5 dpc, however, a small but significant ($p < 0.05$) reduction in germ cell numbers was found in both XX and XY mutants (Germ cells per section: XY controls, mean = 67 ± 5, n = 21 gonads; XY mutants, mean = 49 ± 3, n = 25; XX controls, mean = 98 ± 4, n = 14; XX mutants 66 ± 5, n = 15) (fig. 3). [Note that this result does not mean that total germ cell numbers are higher in XX than XY gonads, as XY gonads are larger than XX gonads at these stages.] Similar reductions in germ cell numbers were observed in the mutants at both 12.5 dpc and 13.5 dpc (fig. 3K). The effect was more pronounced in XY gonads than in XX. By 13.5 dpc, XX mutant gonads had an average of 78% the number of germ cells/section as compared to controls (362 ± 9, n = 20 versus 462 ± 16, n = 20), whereas XY mutant samples contained an average of 58% the number of germ cells per section as controls (152 ± 17, n = 17 versus 260 ± 14, n = 18). This is consistent with the qualitative results described above, in which the germ cell defect was more clearly visible in XY mutants than XX at both 13.5 dpc (fig. 3E, F) and 18.5 dpc (fig. 2). Given that the mutant gonads are smaller than wild type and have fewer sections, the total numbers of germ cells present are further reduced.

**Reduced Levels of Germ Cell Proliferation in Bmp7 Mutants**

The reduction in germ cell number observed in $Bmp7$ mutants suggests that there is a defect in germ cell proliferation and/or survival. To investigate these possibilities, we examined the expression of markers of cell proliferation and cell death in gonads of $Bmp7$ mutants at different developmental stages. During mitosis, histone H3 is phosphorylated on its amino terminus (serine 10), and this phosphorylation event correlates with mitotic chromosome condensation [Hendzel et al., 1997; Wei and Allis, 1998]. We stained gonads from $Bmp7$ mutants and controls at stages from 10.5 dpc to 13.5 dpc with antibodies against PECAM-1 and phosphorylated histone H3 to identify proliferating germ cells (fig. 4A–H). Gonads from $Bmp7$ mutants at 10.5 or 11.0 dpc displayed a striking reduction in the number of mitotic germ cells as compared to controls, as few or no germ cells positive for phosphorylated histone H3 were observed in mutant gonads (fig. 4A–H). The percentage of mitotic germ cells relative to total germ cell numbers was calculated, and it was discovered that there was a statistically significant reduction ($p < 0.05$) in the mitotic index until 11.5 dpc (fig. 4I). At 10.5 dpc, the average percentage of germ cells positive for phospho-histone H3 staining in control genital ridges was more than three times the amount observed in mutants (9.0 ± 1%, n = 10 gonads versus 3.0 ± 0.3%, n = 8). By 11.5 dpc, there was no significant difference in the percentage of mitotic germ cells in mutant XX gonads versus control XX gonads. However, there was still a small but statistically significant reduction in the percentage of mitotic germ cells in XY mutant gonads as compared to controls at this stage (XY controls: 6.8 ± 0.7%, n = 8 gonads; XY mutants: 4.9 ± 0.3%, n = 7, p = 0.013). At 12.5 or 13.5 dpc, the mitotic index was similar to controls in both XX and XY mutants (fig. 4I). Thus, there is a transient requirement for $Bmp7$ for germ cell proliferation in embryonic gonads until approximately 11.5 dpc, lasting slightly longer in XY than XX gonads.
Marketers of cell death were also examined to determine if there was a germ cell survival defect in the mutants. Bmp7 mutant gonads were labeled with antibodies against the active form of caspase-3, an early marker of apoptotic death, at stages from 10.5 to 12.5 dpc. Few apoptotic cells were observed in either mutants or controls at 11.5 dpc (fig. 4J, K) or other stages examined (not shown). Similar results were obtained using Lysotracker dye to label dying cells (not shown). Therefore, the reduced germ cell numbers in Bmp7 mutants appear to result from a proliferation defect manifest just as germ cells arrive in the urogenital ridge.

Expression of Dpp/Gbb Family Members in Fetal Gonads

The observation that the germ cell deficiency is more pronounced in XY than XX mutant gonads suggests that another BMP is compensating for loss of BMP7 in the XX mutant gonad. To investigate this possibility, we quantified the expression of Bmp7 and various other Dpp and Gbb family members in 12.5-dpc wild-type gonads by quantitative RT-PCR (Q-RT-PCR). All have previously been found to be expressed in fetal gonads by microarray analysis [Nef et al., 2005; Small et al., 2005; Bouma et al., 2006].

Based on Q-RT-PCR analysis, expression of Bmp7 was found to be slightly higher in female than in male gonads (fig. 5). This finding is inconsistent with the in situ results. There are several possible explanations for this discrepancy which we are currently investigating: (1) perhaps different spliceforms are detected by the in situ probe and the Q-RT-PCR primers; or (2) perhaps mRNA for Bmp7 is masked by RNA-binding proteins in XX but not XY gonads (we would argue that ISH might not detect these RNAs whereas they are detectable after Trizol extraction). Both Dpp family members, Bmp2 and Bmp4, were expressed at significantly higher levels in XX gonads than XY. The expression levels of other Gbb family members, Bmp5, Bmp6, Bmp8a, and Bmp8b, were not significantly different between XX and XY gonads. Any or all of these BMPs may partially compensate for loss of BMP7 during fetal gonad development.

Discussion

Functions for BMPs have been well characterized in the Drosophila testis and ovary, where Dpp and Gbb are required for the self-renewal of germline stem cells. We provide the first in vivo evidence that BMP signaling is required for the early rounds of PGC proliferation in mammalian fetal gonads. This finding is especially interesting, as it suggests that BMPs are conserved signals that regulate germ cell proliferation in very diverse organisms during multiple developmental stages.

Bmp7 mutants display a reduction in germ cell number in both XX and XY embryonic gonads, first detectable at about 11.5 dpc. The reduction results from a defect in germ cell proliferation that occurs between 10.5 and 11.5 dpc. This phenotype was observed in both sexes; however, the germ cell deficiency was more severe and the proliferation defect lasted slightly longer in XY mutant gonads than in XX. Thus, there are different requirements for BMP7 signaling in germ cells after the divergence of gonad development in XX and XY embryos, supporting the idea that sex-specific germ cell development initiates shortly after PGCs migrate into the gonads.

There may be different Bmps in XX and XY gonads that compensate for loss of Bmp7, with different efficiencies. Bmp2 is more highly expressed in XX gonads than in XY gonads at very early stages, starting between 11.0 and 11.5 dpc. Both BMP2 and its close relative BMP4 have mitogenic effects on germ cells in culture [Pellegrini et al., 2003; Puglisi et al., 2004]; thus, it is possible that BMP2 and/or BMP4 compensate for loss of Bmp7 in XX gonads during the period of time that their expression overlaps. A recent study showed that BMP4 was able to fully substitute for loss of BMP7 in the developing kidney, although these molecules share minimal sequence simi-
larity [Oxburgh et al., 2005]. This could explain why the germ cell loss observed in XX Bmp7 mutant gonads is less severe than germ cell loss in XY mutants. Analysis at the protein level will be required to resolve this question; however, currently available BMP7 antibodies lack the specificity to obtain a clear result.

Proliferation of germ cells in XY gonads may be dependent on Bmp7 for a longer period of time. Bmp5, Bmp6, Bmp8a, and Bmp8b are also expressed in gonads of both sexes at 12.5 dpc. These members of the Gbb family are very closely related to Bmp7 and could be functionally redundant with Bmp7, as has been shown in other cases. For example, mice that are double homozygous mutant for Bmp6 and Bmp7 display cardiac defects not observed in either of the single mutants [Kim et al., 2001]. Analysis of mice heterozygous or homozygous for various combinations of these genes would be required to address this possibility and reveal phenotypes not observed in the single mutants.

Bmp7 is only required for germ cell proliferation during a short developmental window. Interestingly, a recent study working with aggregates of neural stem cells in culture discovered that BMP7 was expressed during the early proliferative expansion of these cells [Deleyrolle et al., 2006]. However, a switch to expression of BMP4 and BMP6 occurred as the cells differentiated. Perhaps a similar switch occurs in the gonads, with BMP7 stimulating the early expansion of the germ cell population, and other BMPs regulating subsequent proliferation and/or differentiation. It is likely that multiple pathways synergize to regulate PGC proliferation and safeguard fertility. Future work will hopefully elucidate the coordinate roles of BMPs and other molecular signals in the regulation of germ cell development in the early gonad.

Acknowledgements

The authors would like to thank the following colleagues for generously providing antibodies or plasmids: Harold Erickson, Brigid Hogan, and David Page. We would also like to thank Humphrey Yao, Doug Coveney, Leo DiNapoli, Yuna Kim and Hao Tang for their helpful comments during the course of this work.

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