The type I BMP receptor *BmprIB* is essential for female reproductive function

Soyun E. Yi*, Philip S. LaPolt†, Byeong S. Yoon‡, Jean Y.-C. Chen†, John K. H. Lu§, and Karen M. Lyons*‡¶‖

Departments of *Biological Chemistry, §Obstetrics and Gynecology, ‡Molecular, Cell, and Developmental Biology, and ¶Orthopedic Surgery, University of California, Los Angeles, CA 90095; and †Department of Biology and Microbiology, California State University, Los Angeles, 90032

Edited by Patricia K. Donahoe, Massachusetts General Hospital, Boston, MA, and approved April 20, 2001 (received for review January 3, 2001)

Maintenance of female reproductive competence depends on the actions of several hormones and signaling factors. Recent reports suggest roles for bone morphogenetic proteins (BMPs) in early stages of folliculogenesis. A role for the type I BMP receptor *BmprIB* as a regulator of ovulation rates in sheep has been described recently, but little is known about the roles of BMP signaling pathways in other aspects of reproductive function. We report here that BMPRIB is essential for multiple aspects of female fertility. Mice deficient in *BmprIB* exhibit irregular estrous cycles and an impaired pseudopregnancy response. *BmprIB* mutants produce oocytes that can be fertilized *in vitro*, but defects in cumulus expansion prevent fertilization *in vivo*. This defect is associated with decreased levels of aromatase production in granulosa cells. Unexpectedly, levels of mRNA for cyclooxygenase 2, an enzyme required for cumulus expansion, are increased. *BmprIB* mutants also exhibit a failure in endometrial gland formation. The expression of *BmprIB* in uterine linings suggests that these defects are a direct consequence of loss of BMP signaling in this tissue. In summary, these studies demonstrate the importance of BMP signaling pathways for estrus cyclicity, estradiol biosynthesis, and cumulus cell expansion *in vivo* and reveal sites of action for BMP signaling pathways in reproductive tissues.

Members of the transforming growth factor β (TGF-β) superfamily transduce their signals via one of two distinct pathways: the activin/TGF-β, or the bone morphogenetic protein (BMP) pathway. TGF-βs and BMPs have diverse effects because the TGF-β/activin and BMP pathways activate different subsets of genes (reviewed in ref. 1). The pathway activated by individual ligands is based on affinities for specific type I receptors. For example, activins and TGF-βs recognize type I activin and TGF-β receptors, respectively, and activate the intracellular mediators SMADs 2 and 3, whereas BMPs recognize one of three type I BMP receptors (BMPRIA/ALK3, BMPRIB/ALK6, and ActRI/ALK2) and activate SMADs 1 and 5 (1, 2).

Several members of the TGF-β superfamily play essential roles in folliculogenesis *in vivo*. For example, BMP15/GDF9b and growth differentiation factor 9 (GDF9) are expressed in oocytes, and loss of either of these genes leads to defective preantral follicle development (3, 4). It is not known whether BMP15 and GDF9 use the activin/TGF-β, BMP, or an as-yet-unknown pathway. Therefore, although the fundamental role of the activin pathway has been clearly established *in vivo* (5, 6), much less is known about the potential functions of the BMP signaling pathway in female reproduction.

A recent *in vitro* study has demonstrated the existence of a functional BMP system in the ovary, showing that BMP4 and BMP7 can have positive and negative effects on follicle-stimulating hormone (FSH)-induced steroidogenesis in granulosa cells (7). Although these studies strongly support a function for the BMP pathway in responsiveness of granulosa cells to FSH, they do not address other potential roles for this pathway in female reproduction. It has been shown recently that the *FecB* allele, which increases ovulation rate and litter size in sheep, carries a point mutation in *BmprIB* (8, 9). Whether the *FecB* allele encodes a receptor with increased or decreased signaling activity or altered specificity is unknown.

To investigate the role(s) of BMP signaling pathways in female fertility, we have examined the reproductive phenotype of mice lacking *BmprIB.* Unlike mice lacking either of the other two type I BMP receptors (10–12), *BmprIB*$-/-$ mice are viable. We show that *BmprIB*$-/-$ females are infertile due to a constellation of defects, including irregular estrus cyclicity, impaired pseudopregnancy responses, severe defects in cumulus cell expansion, and insufficient uterine endometrial gland development. Thus, our results provide *in vivo* evidence for functions for BMP pathways in ovarian and uterine physiology.

Materials and Methods

Mating, Superovulation, and *in Vitro* Fertilization. Generation of *BmprIB*-deficient mice was as described (13). *BmprIB*$-/-$ or wild-type (WT) littermate virgin 5- to 6-week-old mice were used in natural matings. Immature (22–23 days old) mice were superovulated with pregnant mare serum gonadotropin (5 units, Sigma) and with human chorionic gonadotropin (5 units, Sigma) 48 h later (14). Oocytes and embryos were recovered from the uterus or oviduct and classified as described (14). *In vitro* fertilization of eggs obtained from superovulated females was performed on oocytes without surrounding cumulus cells as described (14) by using sperm from CD-1 males.

Histology, Electron Microscopy, and Hoechst Staining. Paraffin sections (7 μm) of *BmprIB*$-/-$ and WT tissues were stained with hematoxylin and eosin. Oocytes were staged according to the system of Pedersen and Peters (15). Ultrastructural analysis was performed on ovaries from 4-week-old mice by the University of California, Los Angeles, Electron Microscopy Core Facility. Chromatin patterns in oocytes were evaluated by staining with Hoechst 33258 and classified as described (16).

Vaginal Smears. Estrous cycles were monitored by daily vaginal smears over a period of 2 months. A small drop of water was flushed into the vagina and then dried on a slide. Cells were fixed in methanol for 10 s, stained with 1% methylene blue for 2 min, and rinsed in tap water to remove excess stain. Smears were taken daily between 2 and 4 p.m. Smears were evaluated by an experienced observer blinded to the genotype of the mice.

***In Situ* Hybridization, Semiquantitative Reverse Transcription–PCR, and Slot-Blot Analysis.** *In situ* hybridization was performed by using a previously described *BmprIB* antisense RNA probe (13)

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: BMP, bone morphogenetic protein; *BmprIB*, BMP receptor type IB; E2, estradiol; TGF-β, transforming growth factor β; GDF9, growth differentiation factor 9; FSH, follicle-stimulating hormone; WT, wild type; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; dpc, days postcoitum.

‖To whom reprint requests should be addressed at: University of California, 2641 MacDonald Research Laboratories, 675 Charles E. Young Drive South, Los Angeles, CA 90095. E-mail: klyons@mednet.ucla.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

 www.pnas.org/cgi/doi/10.1073/pnas.141002798

and protocol (13) except that [α-33P]UTP was used and exposure was for 5 days. Nonradioactive *in situ* hybridization was performed as described (13). Total RNA from ovaries of immature (P22–23) mice 48 h after FSH treatment was prepared by using TRIzol (GIBCO/BRL). Slot blot analysis was performed as described by using probes for *Cyp 19*, *Fshr*, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (17–19). *BmprIB*, *Cox-2*, and *EP2* levels were examined by semiquantitative reverse transcription–PCR on oligo(dT)-primed cDNA (Superscript, GIBCO/BRL) from ovarian total RNA using previously described primers for *BmprIB* (13), *Cox-2* and *EP2* (20), and GAPDH (21). Reactions were performed as described (13, 20, 21) for 18, 20, and 25 cycles. Quantitation of expression relative to GAPDH was performed by using IMAGEQUANT software. Mean values and standard errors were calculated by using Microsoft EXCEL 98.

Results

***BmrpIB*−/− Females Are Infertile.** In over 30 matings of *BmprIB*−/− females over a period of 60 weeks, only two litters of one pup and five pups were generated from two different mothers. No differences were observed in the onset of mating as assessed by the time of appearance of the first copulatory plug (age at first plug: WT, 33.5 ± 3.6 days, $n = 8$; *BmprIB* mutants, 36.1 ± 4.3 days, $n = 6$). We next examined *BmprIB* expression in reproductive tissues of WT mice (Fig. 1). In ovaries, *BmprIB* is expressed in oocytes of maturing (type 6) follicles (Fig. 1 *A* and *C*), and in oocytes and granulosa cells of antral follicles (Fig. 1 *B* and *C*). No *BmprIB* transcripts are detected in granulosa cells of resting, primordial, developing (types 1–5b), or atretic follicles, corporal lutea, or thecal cells (Fig. 1 *A–C*). Our results are thus in agreement with studies of *BmprIB* expression in the rat (1). *BmprIB* also is expressed in uterine endometrium (Fig. 1*D*). Therefore, *BmprIB* is expressed in a pattern consistent with roles in folliculogenesis, fertilization, and/or implantation. No *BmprIB* transcripts are detected in the pituitary (Fig. 2*A*), suggesting that *BmprIB* does not play a direct role in this tissue in the regulation of FSH release. Histological analysis (Fig. 2*B*) and transmission electron microscopy (Fig. 3*A*) of ovaries were performed to examine whether mutants exhibit impaired follicle development. No obvious abnormalities or differences in the numbers of developing follicles or corpora lutea were observed in mutant vs. WT adult mice (Fig. 2*B*).

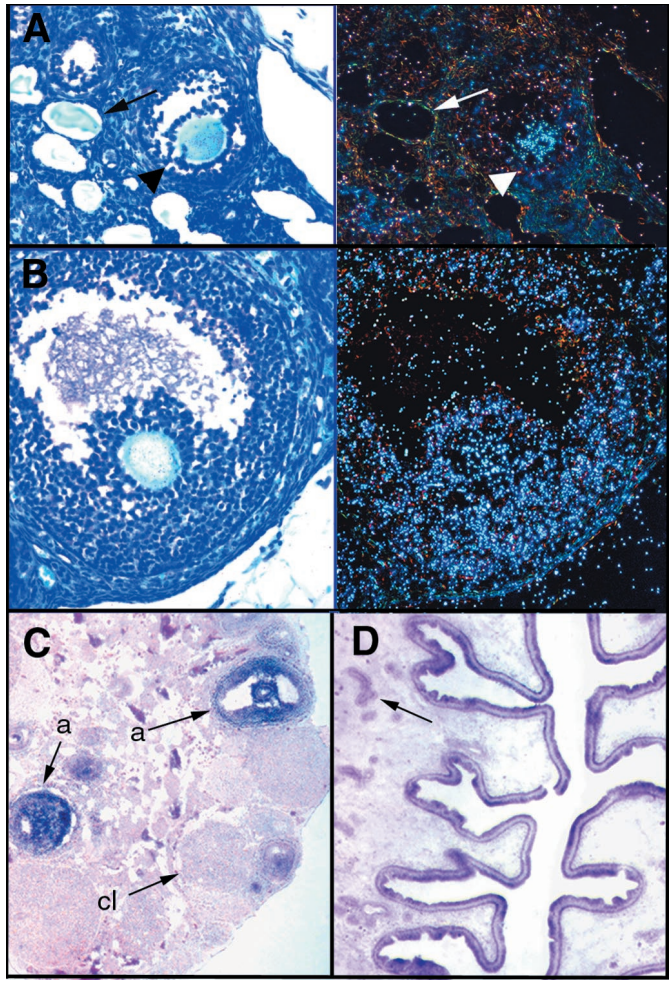

Fig. 1. Expression of *BmprIB* in adult ovary and uterus. (*A* and *B*) Bright-field (*Left*) and corresponding dark-field (*Right*) images of expression of *BmprIB* in (*A*) oocytes of maturing follicles (arrowhead) but not atretic follicles (arrow) and (*B*) antral follicles. (*C* and *D*) Nonradioactive *in situ* hybridization showing *BmprIB* expression in (*C*) antral follicles, but not in corpora lutea, and (*D*), in epithelium of the uterine endometrium and endometrial glands (arrow). *a*, antral follicle; *cl*, corpus luteum.

Oocytes from *BmprIB*−/− Mice Can Be Fertilized *in Vitro* but Not *in Vivo*. Because *BmprIB* is expressed in oocytes, we examined whether these were defective in mutants. Hoechst staining revealed normal chromatin configurations, germinal vesicle breakdown, and polar body formation in ovulated oocytes from mutants (data not shown). Ultrastructural analyses of oocytes within antral follicles revealed normal zona pellucidae and the presence of cortical granules (Fig. 3*A*). Therefore the infertility of *BmprIB*−/− females is unlikely to be attributable to a block in early stages of oocyte maturation. The recovery of similar numbers of oocytes from WT and mutant mice mated with fertile males at 0.5 days postcoitum (dpc) (Table 1), demonstrates that ovulation occurs in mutants. Moreover, mutants and WT mice display similar ovulatory responses to exogenous gonadotropins (Table 1). Therefore, events subsequent to ovulation were examined. At 1.5 dpc and 3.0 dpc, two-cell embryos and blastocysts, respectively, were recovered from reproductive tracts of WT females. However, only unfertilized oocytes were recovered from mutants (data not shown). Similar results were obtained at 0.5 dpc (Table 2). Therefore oocytes from *BmprIB* mutants fail to be fertilized *in vivo*, despite apparently normal responsiveness to gonadotropins.

We then performed *in vitro* fertilization experiments to determine whether oocytes from mutants are defective despite their apparently normal morphology and ultrastructure. Mutant oocytes can be fertilized *in vitro*, but not *in vivo* (Table 2). Eggs from *BmprIB* mutants fertilized *in vitro* progressed to the two-cell stage as efficiently as eggs from WT mice, providing further indication that oocyte quality is normal in mutants (data not shown). We therefore examined whether the viability or passage of sperm to the oviduct is defective in mutants. Examination of 0.5 dpc cumulus masses confirmed that normal numbers of motile sperm reach the isthmus of the oviduct in mutants (data not shown). Therefore, the absence of fertilization in mutants does not appear to be a result of an intrinsic defect in oocyte development or of a physical barrier preventing the union of eggs and sperm.

Defective Cumulus Expansion in *BmprIB*−/− Mice. *BmprIB* is expressed in cumulus cells (Fig. 1*B*). Cumulus expansion promotes oocyte maturation and is essential for sperm penetration, and hence for fertilization (22). Therefore, we examined the morphology of cumulus-oocyte complexes recovered from the oviducts of WT and *BmprIB* mutant females after superovulation (Fig. 3*B*). Complexes from WT mice were expanded, and abundant cumulus cells were arranged radially around each

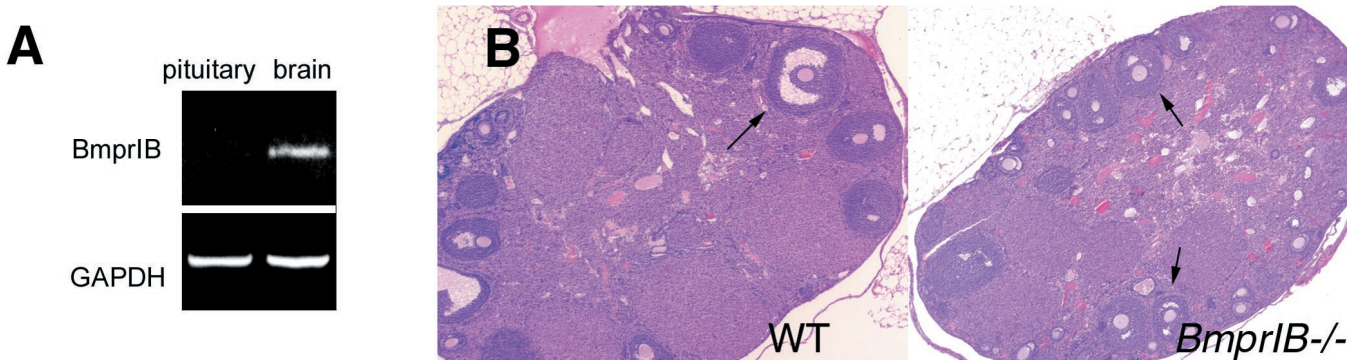

Fig. 2. Expression of *BmprIB* in pituitary and normal ovarian histology in *BmprIB* mutants. (*A*) Reverse transcription–PCR analysis of *BmprIB* expression in pituitary and brain of adult WT mice. (*B*) Sections through WT (*Left*) and mutant (*Right*) ovaries from 5-wk-old females. Ovaries from mutants and WT controls contain similar numbers of follicles at all stages of development, as well as corpora lutea, suggesting that ovulation takes place. Arrows point to antral follicles.

oocyte. In contrast, complexes from *BmprIB* mutants were aggregated and retained fewer cumulus cells, indicating that loss of *BmprIB* prevents cumulus cell expansion *in vivo*. These data suggest that a primary cause of infertility in *BmprIB* mutants is a failure in cumulus cell expansion. This is consistent with the ability of oocytes from *BmprIB* mutants to be fertilized *in vitro* but not *in vivo*; cumulus cells improve fertilization rates but are not essential *in vitro* (23), and cumulus cells were removed in our experiments.

Cumulus cell expansion is thought to require the combined activity of FSH and a soluble factor(s) produced by the oocyte (24). We therefore examined levels of *Fshr* mRNA in ovaries from WT and mutant hormone-primed immature mice (before the onset of estrous cycles). Mean *Fshr* mRNA levels did not differ significantly between WT and mutant littermates (Fig. 4*A*), suggesting that defective cumulus expansion in mutants is not primarily due to decreased levels of *Fshr* expression. Several studies have indicated an essential role for prostaglandin E_2 (PGE2) in cumulus expansion (20, 25), and this activity is mediated through the EP2 prostaglandin receptor (*Ptgerep2*) (23). Therefore, we examined the levels of expression of *Ptgerep2* and *cyclooxygenase 2* (*Cox2;Ptgs2*), the rate-limiting enzyme in the synthesis of prostaglandins. No significant differences in *EP2* levels were observed, but levels of *Cox2* mRNA were significantly increased in *BmprIB* mutants (Fig. 4*B*). This increase was unexpected given that prostanoids, the derivatives of COX-2 activity, are required for cumulus expansion *in vivo* (23). This result may reflect compensatory up-regulation of prostanoid signaling pathways due to the impairment in cumulus expansion and suggests that increased signaling through this pathway cannot compensate for loss of signaling through BMPRIB.

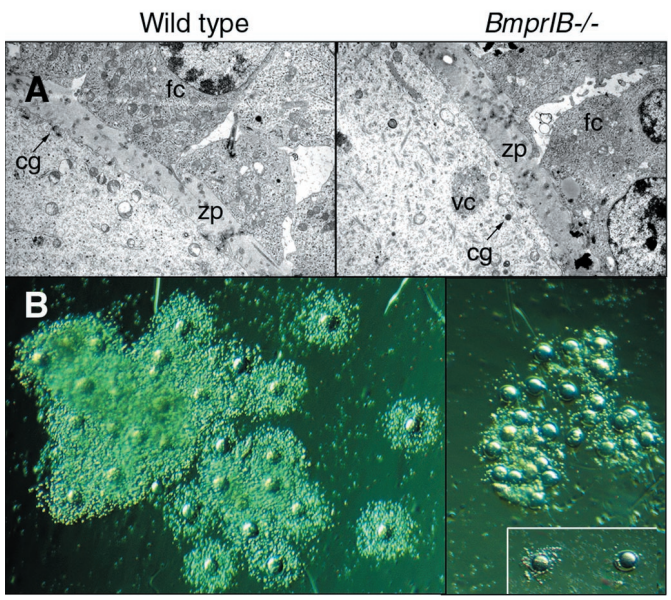

Fig. 3. Defective cumulus cell expansion in *BmprIB* mutants. (*A*) Transmission electron microscopy at the interface of the oocyte surface and granulosa cells in WT (*Left*) and *BmprIB* mutant (*Right*) mice. Cortical granules, a marker for oocyte maturation, are present in oocytes from mutants, and no differences were detected in the zona pellucida. (*B*) Cumulus cell-oocyte complexes from WT (*Left*) and mutant (*Right*) mice, photographed at the same magnification. (*Inset*) Two cumulus cell-oocyte complexes isolated from the cumulus mass. cg, cortical granules; g, granulosa cells; zp, zona pellucida.

Impaired Estrus Cyclicity and Pseudopregnancy Responses in *BmprIB*−/− Mutants. After mating with vasectomized males, females normally experience a pseudopregnancy response in reaction to stimulation of the cervix (26). During the subsequent 11–12 days, the females will not accept males for mating. Unexpectedly, *BmprIB* mutant females frequently mated over consecutive days (Fig. 4*C*), suggesting an impaired pseudopregnancy response. Moreover, monitoring of estrous cycles by vaginal smears revealed that 33% ($n = 2/6$) of *BmprIB* mutants exhibited an extended period of leukocytic smears, indicative of a prolonged di-estrous phase (data not shown).

The observation that mutant females display abnormal pseudopregnancy responses and ovulatory cycles characteristic of a prolonged di-estrous phase suggests delay or impairment in endogenous estradiol (E2) production. *BmprIB* and the gene product of the *Cyp 19* gene, P450 aromatase, which converts androgens into E, are coexpressed in granulosa cells, raising the possibility that E2 production is impaired in mutants. Direct

Table 1. Ovulation in WT and *BmprIB*−/− mice

Ovulation	WT	*BmprIB*−/−	*P* value
Spontaneous			
Mice ovulating	3/3 (100%)	4/4 (100%)	NS
Total eggs	19	25	
Eggs per mouse	6.3 ± 3.2	6.3 ± 4.0	NS
Hormonally induced			
Mice ovulating	12/12 (100%)	12/14 (86%)	NS
Total eggs	535	509	
Eggs per mouse	44.6 ± 19	36.4 ± 29	NS

Oocytes were recovered from spontaneously ovulating or hormonally induced females 0.5 days after mating to fertile males. Student's *t* test was used to assess statistical significance. NS, not significant.

Table 2. Fertilization in WT and *BmprIB*−/− mice

Fertilization	WT	*BmprIB*−/−	*P* value
In vivo			
Eggs fertilized (spontaneous ovulation)	11/25 (44%) ($n = 3$)	0/38 (0%) ($n = 3$)	<0.001
Eggs fertilized (hormonally induced, 0.5 dpc)	73/144 (51%) ($n = 4$)	0/167 (0%) ($n = 4$)	<0.001
In vitro			
Eggs fertilized	84/274 (30.6%) ($n = 12$)	35/173 (20.2%) ($n = 14$)	NS

Oocytes or one-cell embryos were recovered from spontaneously ovulating or hormonally induced females 0.5 days after mating to fertile males. For *in vitro* fertilization, oocytes were collected from the oviducts of hormonally induced animals 19 hr after human chorionic gonadotrophin treatment. Cumulus cells were removed by treatment with hyaluronidase. In all experiments, oocytes had undergone germinal vesicle breakdown and extrusion of the first polar body. One-cell embryos (0.5 dpc) were classified as having two polar bodies and/or two pronuclei. Student's *t* test was used to assess statistical significance. NS, not significant.

comparisons of E2 levels in WT and mutant mice cannot be made because mutants exhibit irregular cycles. Therefore, *Cyp 19* expression was monitored in ovaries of immature mice in response to exogenous gonadotropin stimulation. Although mean *Cyp 19* levels were not statistically different owing to variation among individuals of each genotype, mutants exhibited a clear trend toward decreased expression relative to WT littermates (Fig. 4*A*). This result suggests that *BmprIB* is required in granulosa cells for maximal induction of aromatase, and hence, for normal levels of E2 biosynthesis.

***BmprIB* Is Required for Endometrial Gland Formation.** During each estrous cycle, endometrial cells proliferate in response to increasing E2 levels (e.g., ref. 27). If E2 remains low, the endometrial lining fails to thicken, uterine glands are underdeveloped, and implantation cannot take place. When uterine horns from WT and mutant females were compared 0.5 days after mating, striking differences were observed (Fig. 5). The uterine linings were thin, and endometrial glands were absent or underdeveloped in 89% (8/9) of the mutants (Fig. 5). Therefore, *BmprIB* is required for endometrial gland formation. There is wide variation in the extent of endometrial development among mutants, ranging from a failure in gland formation (Fig. 5*B*) to inability to develop beyond a simple epithelium (Fig. 5*C*). The basis for the variable phenotype is unknown, but may reflect in part the effects of modifier genes segregating in the hybrid 129Sv × C57BL/6 background. *BmprIB* may have both direct and indirect actions on endometrial development. A direct action for BMPRIB signaling is supported by the observation that *BmprIB*, and its potential ligands are expressed in the endometrium and endometrial glands (Fig. 1*D*, refs. 28 and 29). An indirect action due to diminished E2 levels resulting from decreased aromatase expression is also possible; however, no morphological defects were seen in other E2-sensitive tissues, such as the oviducts (Fig. 1*A*, and data not shown).

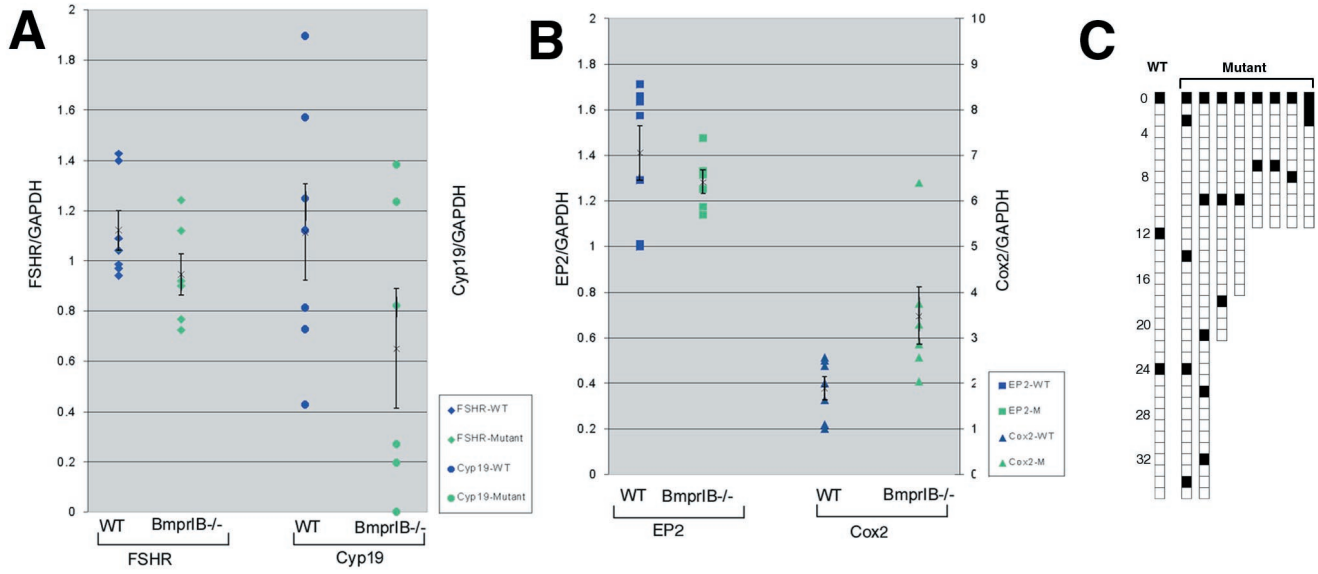

Fig. 4. Expression of *Fshr*, *Cyp 19* (aromatase), prostaglandin receptor *EP2*, and *Cox2*, and pseudopregnancy responses. (*A*) Slot blot analysis of *Fshr*, and *Cyp 19* (aromatase) expression normalized to *GAPDH* in ovaries from immature noncycling (P22–23) WT and *BmprIB* mutant mice 48 h after treatment with FSH. (*B*) Reverse transcription–PCR analysis of *Ptgs2* (*Cox2)* and *EP2* expression normalized to *GAPDH* in ovaries from immature noncycling (P22–23) WT and mutant mice 48 h after treatment with FSH. Mean values and standard errors are represented by an X and bars, respectively. Each square or triangle represents a measurement from an individual mouse. (*C*) Impaired pseudopregnancy responses in *BmprIB*−/− mice. Mating frequency in *BmprIB*−/− mice as assessed by the appearance of copulatory plugs. *BmprIB*−/− (mutant) and WT females housed individually with vasectomized males were checked daily for the presence of a vaginal plug, indicated by a filled box. Data for a single WT female are shown.

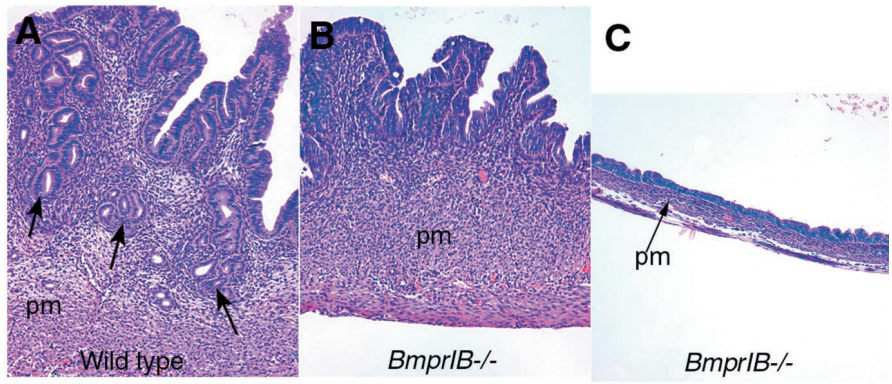

Fig. 5. Defective endometrial development in *BmprIB* mutants. Uteri from (*A*) WT and (*B* and *C*) mutant adult mice 0.5 days after mating. Numerous glands can be seen in the endometrium of the WT uterus in *A* (arrows). (*B*) Uterus from a mutant female, exhibiting some thickening of the endometrial stroma but no gland formation. The muscular layer and perimetrium appear normal. (*C*) A severely underdeveloped uterus from a mutant female. The epithelial endometrium is present, but has a flattened, cuboidal appearance. The stromal perimetrial and muscular layers are thinner than in WT mice. *pm*, perimetrium.

Discussion

The BMPs comprise the largest subgroup of the TGF-β superfamily, yet little is known about the potential roles of BMPs in reproductive function. Studies by Shimisaki *et al.* (7) demonstrate that granulosa cells respond to exogenous BMPs *in vitro*, raising the possibility that BMPs play an essential role in ovarian function. Our studies confirm that BMP signaling pathways are required for normal ovarian function *in vivo*. The prolonged di-estrus phase and decreased *Cyp 19* expression observed in mutants suggests that circulating E2 levels may be insufficient for normal reproductive function. These results are consistent with those of Shimasaki *et al.* (7), showing that BMPs stimulate E2 production in granulosa cells *in vitro*, and indicate that BMPRIB is an essential receptor for the regulation of E2 production by BMPs *in vivo*. Our results contrast with those reported for sheep carrying the *FecB* allele of *BmprIB. FecB* acts in a dose-dependent manner to increase ovulation rate (8, 9), whereas the prolonged ovulatory cycles seen in *BmprIB*$-/-$ mice imply a decreased ovulation rate. Moreover, *FecB* increases litter size (8, 9), whereas the two litters we have obtained from *BmprIB* females consisted of one pup and five pups, respectively (average litter size for WT littermates is $\approx$8–10). One interpretation for the different phenotypes is that the *FecB* allele represents an activating mutation in *BmprIB*, leading to increased signaling capacity. Consistent with this hypothesis, granulosa cells from *FecB* sheep exhibit increased *Cyp 19* (aromatase) activity at a smaller follicular diameter than in WT sheep (30), whereas we observed decreased levels of *Cyp 19* mRNA in *BmprIB*$-/-$ mice. It will be interesting in future studies to examine whether the onset of *Cyp 19* expression is delayed in follicles from *BmprIB*$-/-$ mice.

Our results show that loss of *BmprIB* is associated with defective cumulus cell expansion, decreased levels of *Cyp 19* (aromatase), and increased *Ptgs2* (Cox2*)* expression. The expression of *BmprIB* in ovaries, but not in the pituitary, suggests that these defects result from insufficient BMP signaling in ovarian tissues. The expression of *BmprIB* in granulosa cells is consistent with a direct role for BMP signaling pathways in cumulus cell expansion. However, we cannot exclude the possibility that *BmprIB* acts in the oocyte, because *BmprIB* is expressed in oocytes, and factors secreted by oocytes are required for cumulus cell expansion (31). The molecular basis for the defective cumulus expansion in *BmrpIB* mutants is currently unknown. Cumulus expansion requires the activity of FSH, prostaglandins, and soluble oocyte factor(s); TGF-β can substitute for the oocyte-derived factor(s) (23, 24). *BmprIB* mutants do not exhibit decreased *Fshr* mRNA expression, suggesting that decreased activation of FSH receptor does not contribute to defective expansion. COX-2 is the rate-limiting enzyme responsible for the production of prostanoids, and mutations in the prostanoid receptor *EP2* cause defective cumulus expansion (23). Although levels of *EP2* receptor mRNA are unaffected, *Cox-2* levels are significantly increased in *BmprIB* mutants. Therefore, defective cumulus expansion in *BmprIB* mutants is not a result of decreased expression of prostanoids or their receptors. BMPRIB may act in a parallel signaling pathway, and/or downstream of prostanoid action.

The ligands that activate BMPRIB in the ovary are currently unknown. To date, reproductive phenotypes have been associated with mutations in the genes encoding two BMP family members, *Gdf9*, and *Bmp15* (3, 4). The receptors for these ligands have not been identified. GDF9 is capable of stimulating cumulus expansion *in vitro* (32), raising the possibility that this oocyte-specific ligand binds to BMPRIB *in vivo*. However, GDF9 is structurally divergent from ligands known to bind to BMPRIB, and folliculogenesis is blocked at an early stage in *Gdf9*$-/-$ mice (3, 33, 34), but not in *BmprIB* mutants. Moreover, GDF9 increases *Cox2* mRNA levels *in vitro*, whereas we observe increased levels of *Cox2* in the absence of signaling through BMPRIB. In sheep, BMP15 affects follicle development in a dose-dependent manner, but does not elicit cumulus expansion *in vitro* (7, 32). Therefore, although BMPRIB may act as a receptor for GDF9 or BMP15 *in vivo*, these ligands are unlikely to mediate the effects of BMPRIB on cumulus expansion and *Cox2* expression. It is possible that BMPRIB regulates levels of expression of these ligands and/or their receptor(s) in follicle cells. Other potential ligands for BMPRIB include BMPs 2, 4, and 7, which are expressed in thecal cells and are known to signal through BMPRIB *in vitro* (2, 7, 34, 35). GDF5 is the most potent stimulator of signal transduction through BMPRIB known to date (34). Although reproductive functions have not been described for GDF5, the related family members GDF6 and GDF7 may play roles in female fertility.

Recently, it has been shown that BMPRIB can act *in vitro* as a receptor for Müllerian inhibiting substance (MIS) (36). MIS is the TGF-β-related molecule responsible for the regression of the Müllerian duct (which develops into the uterus in females) in males. Although *BmprIB* males exhibit reduced fertility due to defective growth of seminal vesicles (unpublished data), we observe no remnants of the Müllerian duct in *BmprIB* mutant males (37). Our *in vitro* and *in situ* hybridization studies suggest that ALK2 rather than BMPRIB is a receptor for MIS *in vivo* (37). Although we find no evidence for a role for BMPRIB in regression of the Müllerian duct in males, our results do indicate

that BMPRIB is required for normal uterine function in females. Although it is not possible at present to determine whether the uterine defects in *BmprIB* mutants are secondary to ovarian insufficiency, expression of *BmprIB* and BMP ligands in uterine epithelium (28, 29) argues for a primary role for BMPRIB in the preparation of the uterus for successful fertilization and implantation. Future studies examining cycle-specific expression of *BmprIB* in the uterus should help to clarify the role of BMP signaling in endometrial gland formation. It would also be of interest to examine endometrial gland formation in *FecB* sheep.

The targets of BMP signaling pathways in follicular and endometrial cells are unknown. The similar defects in cumulus expansion and endometrial development in mice lacking *BmprIB, Ptgs2 (Cox-2)* (38), or the prostanoid receptor *EP2* (39, 40) suggest a potential link between BMP and prostanoid signaling pathways. However, despite increased *Cox-2* expression, *BmprIB* mutants exhibit impairments in cumulus expansion and endometrial development. This finding suggests that BMPRIB may act in a parallel pathway in conjunction with prostaglandin pathways and/or downstream of prostanoid action in reproductive tissues. The infertility of *BmprIB*-deficient mice is reminiscent of female reproductive senescence. Estrous cycle length increases toward the end of reproductive life as a result of diminishing E2 production (41–43). Vaginal smear data indicated a prolonged estrous phase in a fraction of *BmprIB* mutants. Therefore, examination of *BmprIB* levels in granulosa cells from aging females will be warranted in future studies.

We thank K. Williams for assistance with hormone-priming and J. Lengyel and L. Birnbaumer for comments on the manuscript. This work was supported by National Institutes of Health Grant AR44528 to K.M.L.

1. Massagué, J. (1998) *Annu. Rev. Biochem.* **67,** 753–791.
2. ten Dijke, P., Yamashita, H., Sampath, T. K., Reddi, A. H., Estevez, M., Riddle, D. L., Ichijo, H., Heldin, C.-H. & Miyazono, K. (1994) *J. Biol. Chem.* **269,** 16985–16988.
3. Dong, J., Albertini, D. F., Nishimori, K., Kumar, T. R., Lu, N. & Matzuk, M. M. (1996) *Nature (London)* **383,** 531–535.
4. Galloway, S. M., McNatty, K. P., Cambridge, L. M., Laitinen, M. P. E., Juengel, J. L., Jokiranta, T. S., McLaren, R. J., Luiro, K., Dodds, K. G., Montogmery, G. W., *et al*. (2000) *Nat. Genet.* **25,** 279–283.
5. Matzuk, M. M., Kumar, T. R. & Bradley, A. (1995) *Nature (London)* **374,** 356–360.
6. Knight, P. G. (1996) *Front. Neuroendocrinol.* **17,** 476–509.
7. Shimasaki, S., Zachow, R. J., Li, D., Kim, H., Iemura, S., Ueno, N., Sampath, K., Chang, R. J. & Erickson, G. F. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 7282–7287.
8. Wilson, T., Wu, X.-Y., Juengel, J. L., Ross, I. K., Lumsden, J. M., Lord, E. A., Dodds, K. G., Walling, G. A., McEwan, J. C., O'Connell, A. R., *et al*. (2001) *Biol. Reprod.* **64,** 1225–1235.
9. Mulsant, P., Lecerf, F., Fabre, S., Schibler, L., Monget, P., Lanneluc, I., Pisselet, C., Riquet, J., Monniaux, D., Callebaut, I., *et al*. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 5104-5109.
10. Mishina, Y., Suzuki, A., Ueno, N. & Behringer, R. R. (1995) *Genes Dev.* **9,** 3027–3037.
11. Mishina, Y., Crmbie, R., Bradley, A. & Behringer, R. R. (1999) *Dev. Biol.* **15,** 314–326.
12. Gu, Z., Reynolds, E. M., Song, J., Lei, H., Feigen, A., Yu, L., He, W., MacLaughlin, D. T., van den Eijnden-van Raaij, J., Donahoe, P. K., *et al*. (1999) *Development (Cambridge, U.K.)* **126,** 2551–2561.
13. Yi, S. E., Daluiski, A., Pederson, R., Rosen, V. & Lyons, K. M. (2000) *Development (Cambridge, U.K.)* **127,** 621–630.
14. Hogan, B. L. M., Beddington, R., Constatini, F. & Lacy, E. (1994) *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
15. Pedersen, T. & Peters, H. (1968) *J. Reprod. Fertil.* **17,** 555–557.
16. Mattson, B. A. & Albertini, D. F. (1990) *Mol. Reprod. Dev.* **25,** 374–383.
17. Lyons, K. M., Graycar, J. L., Lee, A., Hashmi, S., Lindquist, P. B., Chen, E. Y., Hogan, B. L. M. & Derynck, R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4554–4558.
18. Hickey, G. J., Krasnow, J. S., Beattie, W. G. & Richards, J. S. (1990) *Mol. Endocrinol.* **4,** 3–12.
19. LaPolt, P. S., Tilly, J. S., Aihara, T., Nishimori, K. & Hsueh, A. J. W. (1992) *Endocrinology* **130,** 1289–1295.
20. Elvin, J. A., Yan, C. & Matzuk, M. M. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 10288–10293. (First Published August 15, 2000, 10.1073/pnas.180295197)
21. Bostrom, K., Tintut, Y., Kao, S. C., Stanford, W. P. & Demer, L. L. (2000) *J. Cell Biochem.* **78,** 210–221.
22. Eppig, J. (1991) *BioEssays* **13,** 569–574.
23. Hizaki, H., Segi, E., Sugimoto, Y., Hirose, M., Saji, T., Ushikubi, F., Matsuoka, T., Noda, Y., Tanaka, T., Yoshida, N., *et al*. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 10501–10506.
24. Tirone, E., D'Alessandris, C., Hascall, V. C., Siracussa, G. & Salustri, A. (1997) *J. Biol. Chem.* **272,** 4787–4794.
25. Eppig, J. J. (1981) *Biol. Reprod.* **25,** 191–195.
26. Wuttke, W. (1976) *Rev. Physiol. Biochem. Pharmacol.* **76,** 59–101.
27. Safranski, T. J., Lamberson, W. R. & Keisler, D. H. (1993) *Biol. Reprod.* **48,** 669–673.
28. Ozkaynak, E., Jin, D. F., Jelic, M., Vukicevic, S. & Oppermann, H. (1997) *Biochem. Biophys. Res. Commun.* **234,** 242–246.
29. Zhao, R., Lawler, A. M. & Lee, S.-J. (1999) *Dev. Biol.* **212,** 68–79.
30. Montgomery, G. W., McNatty, K. P. & Davis, G. H. (1992) *Endocr. Rev.* **13,** 309–328.
31. Eppig, J. J., Chesnel, F., Hirao, Y., O'Brien, M., Pendola, F. L., Watanabe, S. & Wigglesworth, K. (1997) *Hum. Reprod.* **12,** 127–132.
32. Elvin, J. A., Clark, A. T., Wang, P., Wolfman, N. M. & Matzuk, M. M. (1999) *Mol. Endocrinol.* **13,** 1035–1048.
33. Elvin, J. A., Yan, C., Wang, P., Nishimori, K. & Matzuk, M. M. (1999) *Mol. Endocrinol.* **13,** 1018–1034.
34. Nishitoh, H., Ichijo, H., Kimura, M., Matsumoto, T., Makishima, F., Yamaguchi, A., Yamashita, H., Enomoto, S. & Miyazono, K. (1996) *J. Biol. Chem.* **271,** 21345–21352.
35. Liu, F., Ventura, F., Doody, J. & Massagué, J. (1995) *Mol. Cell Biol.* **15,** 3479–3486.
36. Gouedard, L., Chen, Y. G., Thevenet, L., Racine, C., Borie, S., Lamarre, I., Josso, N., Massague, J. & di Clemente, N. (2000) *J. Biol. Chem.* **275,** 27973–27978.
37. Clarke, T. R., Hoshiya, Y., Yi, S. E., Liu, X., Lyons, K. M. & Donahoe, P. K. (2001) *Endocrinology*, in press.
38. Lim, H., Paria, B. C., Das, S. K., Dinchuk, J. E., Langenbach, R., Trzaskos, J. M. & Dey, S. K. (1997) *Cell* **91,** 197–208.
39. Tilley, S. L., Audoly, L. P., Hicks, E. H., Kim, H. S., Flannery, P. J., Coffman, T. M. & Koller, B. H. (1999) *J. Clin. Invest.* **103,** 1539–1545.
40. Kennedy, C. R., Zhang, Y., Brandon, S., Guan, Y., Coffee, K., Funk, C. D., Magnuson, M. A., Oates, J. A., Breyer, M. D. & Breyer, R. M. (1999) *Nat. Med.* **5,** 217–220.
41. Lu, J. K., Anzalone, C. R. & LaPolt, P. S. (1994) *Neurobiol. Aging* **15,** 541–544.
42. Thung, P. J., Boot, L. M. & Muhlbock, O. (1956) *Acta Endocrinol.* **23,** 8–32.
43. Nelson, J. F., Felicio, L. S., Randall, P. K., Sims, C. & Finch, C. E. (1982) *Biol. Reprod.* **27,** 327–339.