Review article

Bone Morphogenetic Protein (BMP) signaling in animal reproductive system development and function

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ARTICLE INFO

Keywords:
Germline
Bone Morphogenetic Protein (BMP)
Preformation
Epigenesis
Convergent evolution
Homology

ABSTRACT

In multicellular organisms, the specification, maintenance, and transmission of the germ cell lineage to subsequent generations are critical processes that ensure species survival. A number of studies suggest that the Bone Morphogenetic Protein (BMP) pathway plays multiple roles in this cell lineage. We wished to use a comparative framework to examine the role of BMP signaling in regulating these processes, to determine if patterns would emerge that might shed light on the evolution of molecular mechanisms that may play germ cell-specific or other reproductive roles across species. To this end, here we review evidence to date from the literature supporting a role for BMP signaling in reproductive processes across Metazoa. We focus on germ line-specific processes, and separately consider somatic reproductive processes. We find that from primordial germ cell (PGC) induction to maintenance of PGC identity and gametogenesis, BMP signaling regulates these processes throughout embryonic development and adult life in multiple deuterostome and protostome clades. In well-studied model organisms, functional genetic evidence suggests that BMP signaling is required in the germ line across all life stages, with the exception of PGC specification, herein termed induction. While BMP4 appears to be the most broadly employed ligand for the reproductive processes considered herein, we also noted evidence for sex-specific usage of different BMP ligands. In gametogenesis, BMP6 and BMP15 seem to have roles restricted to oogenesis, while BMP8 is restricted to spermatogenesis. We hypothesize that a BMP-based mechanism may have been recruited early in metazoan evolution to specify the germ line, and was subsequently co-opted for use in other germ line-specific and somatic reproductive processes. We suggest that if future studies assessing the function of the BMP pathway across extant species were to include a reproductive focus, that we would be likely to find continued evidence in favor of an ancient association between BMP signaling and the reproductive cell lineage in animals.

1. Introduction

In sexually-reproducing organisms, the survival of species is dependent upon their ability to segregate and maintain a population of germ cells that will produce gametes. This process is crucial for successful transmission of hereditary material to the next generation of a species. During embryogenesis, germ cell precursors known as primordial germ cells (PGCs) are specified. There are two main categories of mechanisms of PGC specification, herein termed inheritance and induction. Placing these mechanisms into a phylogenetic context supports the hypothesis that the inductive mode is ancestral among the Metazoa (Extavour and Akam, 2003). The inheritance mechanism relies upon maternally inherited factors that constitute a germ plasm that specifies PGCs, whereas induction relies upon signaling molecules to specify PGCs later in embryogenesis (Extavour and Akam, 2003).

Although induction appears to be more common in animals, the molecular mechanisms responsible for inductive PGC specification have only been elucidated in a few species, namely mice (reviewed by Saitou and Yamaji, 2012), salamanders (Chatfield et al., 2014; Johnson et al., 2003) and crickets (Donoughe et al., 2014; Nakamura and Extavour, 2016). All of these species employ the BMP signal transduction pathway (Fig. 1) for specifying the germ cell lineage. Furthermore, disparate literature shows that BMP signal transduction regulates reproductive processes from a variety of widely diverged animal species. The study systems and level of molecular mechanistic resol-
2. BMP signaling in primordial germ cell specification

While the inductive mode of PGC specification appears to be more common and is hypothesized to be ancestral among metazoans (Extavour, 2007; Extavour and Akam, 2003), a signaling pathway that can induce PGC specification has only been elucidated, to our knowledge, in three species, two vertebrates and one invertebrate. Studies in the mouse (Lawson et al., 1999; Ying et al., 2000, 2001; Ying and Zhao, 2001) and the axolotl (Chatfield et al., 2014; Johnson et al., 2003) have shown that BMP signal transduction is necessary for specifying PGCs via induction. BMP signaling is also required in the two-spotted cricket, Gryllus bimaculatus, for PGC specification (Donoughe et al., 2014). Interestingly, the knockdown of Gb-gbb (Bmp5/7) in crickets produces a more severe PGC loss phenotype than Gb-dpp1 (Bmp2/4) knockdown (Donoughe et al., 2014), even though BMP4 appears to be the most consistently used ligand in mammalian PGC fate induction (Table 1, Table S1). For example, BMP4 alone, but not BMP7 nor BMP8, can induce germ cell differentiation from human embryonic stem cells (ESCs) (Geens et al., 2011; Kee et al., 2006). Indeed, BMP4 promotes the in vitro differentiation of cultured pluripotent stem cells into PGC-like cells (PGCLCs) in human (embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs)) (Hiller et al., 2011; Irie et al., 2015; Kee et al., 2006; Sugawa et al., 2015), buffalo (ESCs) (Shah et al., 2015), cow (iPSCs) (Malaver-Ortega et al., 2016) and pig (epiblast stem cells) (Alberio et al., 2010) study systems.

While other signaling pathways have been implicated in PGC specification in some vertebrates (e.g., WNT3 in mice (Aramaki et al., 2013), GFG in axolotls (Chatfield et al., 2014)), BMP signaling is a common requirement across multiple species where at least some of the signaling proteins necessary for PGC specification have been identified. This includes cases of PGC specification in developing embryos, and differentiation of pluripotent cell types into cells with PGC-like properties. Taken together, these observations suggest that the role of BMP signaling in PGC specification could be widely conserved among animals.

Downstream of BMP signaling, the role of the transcription factor B-Lymphocyte-Induced Maturation Protein 1 (BLIMP1, also known as PRDM1) appears to be conserved in PGC specification as well. BLIMP1, induced by BMP signaling, is necessary for PGC specification in mice (Ohinata et al., 2005; Vincent et al., 2005). In this species, PGC precursors expressing BLIMP1 originate from the posterior proximal epiblast, as revealed by lineage tracing experiments (Lawson and Hage, 1994; Ohinata et al., 2005), in response to BMP4 and WNT3 signals originating from the extraembryonic ectoderm (Lawson et al., 1999; Ohinata et al., 2009). Moreover, BLIMP1 also acts downstream of BMP signaling to induce PGCs in crickets (Nakamura and Extavour, 2016), and for differentiation of human ESCs into PGCLCs (Irie et al., 2015). Expression data suggest a role for BMP-induced BLIMP1 in PGC specification of the germ line, its development and function in animals for which data are available.

Based on the available literature, we have organized this review into the involvement of BMP signaling in the regulation of germ line-specific and somatic reproductive roles across animals. In terms of germ line-specific roles, we discuss the processes of PGC specification, PGC proliferation, PGC migration, and gametogenesis (Fig. 2), as these are the processes for which we found evidence for a BMP-based mechanism in multiple animal species. We also discuss the involvement of BMP signaling in selected somatic reproductive processes. By including studies reporting that some BMP signaling pathway members are not involved in regulating one of these processes at a given stage, we uncover patterns of apparent sex-specificity among some BMP ligands. We have gathered the evidence for the involvement of BMP signaling in reproductive processes that we considered for this review in Tables 1 and 2. Tables S1 and S2 contain the literature references for each item in Tables 1 and 2, and are organized in the same way as the main tables. Table 1 contains evidence for BMP signaling in germ line-specific processes, while Table 2 is used to display evidence of this pathway’s function in somatic reproductive processes. We focus our discussion in the main text primarily on functional genetic evidence, with limited attention to purely expression-based and allele-association studies. However, we encourage interested readers to refer to these tables for this information, including data on ligand-specific and species-specific mechanisms. From the current evidence, we propose that a BMP-based mechanism was recruited early in metazoan evolution to specify PGCs, and was later recruited for other roles in the reproductive system.
specification in additional vertebrates. BLIMP-1 is expressed in the germ line of chicken embryos at various stages including in presumptive PGCs, migrating PGCs, and in gonadal germ cells (Wan et al., 2014). In rabbits, BLIMP1 is expressed in presumptive PGCs, while BMP2 and BMP4 are expressed in tissues adjacent to and at the site of PGC specification, suggesting that in this mammal as well, BLIMP1 may operate downstream of BMP signaling to affect PGC specification (Hopf et al., 2011). Blimp1 does not, however, appear to be necessary for PGC formation in axolotls (Chatfield et al., 2014) suggesting that its role in inductive PGC specification diverged in this species.

In the absence of cytokines, expression of Blimp1 and two other transcription factors, Prdm14 and AP2y (Tfap2c) is sufficient to induce a PGC-like state in mouse ESCs and iPSCs (Magnúsdóttir et al., 2013; Nakaki et al., 2013). Similar to Blimp1, Prdm14 is critical for PGC specification in mice (Yamaji et al., 2008), while AP2y is required for maintaining, but not specifying, PGCs (Weber et al., 2010). Prdm14 seems dispensable for human PGCLC induction (Irie et al., 2015), but is sufficient to drive PGC differentiation from mouse ESCs and iPSCs (Nakaki et al., 2013). A Prdm14 ortholog has not been described in any protostomes and deuterostomes (Extavour, 2016). Taken together it appears that Blimp1, but not Prdm14, may have a conserved role in PGC induction downstream of BMP signaling.

Having considered the regulatory network downstream of BMP signaling in PGCs when they are specified, in the following section we ask how PGCs are regulated during embryonic and post-embryonic development, and whether the same signaling factors involved in their specification are subsequently involved in their regulation.

3. BMP signaling can regulate PGC proliferation in protostomes and deuterostomes

Immediately following specification, PGCs and their descendants undergo a number of complex processes, including proliferation, migration, integration into the primordial gonads, and gametogenesis (Fig. 2). In this review, we consider three of these processes, for which the literature demonstrates consistent involvement of BMP signaling across multiple animal species: proliferation, migration, and gametogenesis.

PGC proliferation appears to be regulated by BMP signaling in the chicken (Whyte et al., 2015), mouse (Dudley et al., 2010, 2007; Fujiwara et al., 2001; Ross et al., 2007) and fruit fly (Deshpande et al., 2014; Gilboa and Lehmann, 2004; Sato et al., 2010; Zhu and Xie, 2003) (Fig. 3). In D. melanogaster, knockdown of the BMP2/4 orthologue decapentaplegic (dpp) in the somatic tissues of larval ovaries significantly reduces PGC numbers (Sato et al., 2010). In addition, PGCs homozygous for a loss of function allele of the type I BMP receptor thickveins (tkv) are never found to clonally populate a GSC niche (Zhu and Xie, 2003), suggesting a proliferation defect in PGCs that are unable to receive BMP signals. From late larval to early pupal stages in the fruit fly, dpp overexpression in somatic ovarian cells leads to an increase in PGC number (Sato et al., 2010; Zhu and Xie, 2003). The levels of cell death in these conditions are not significantly changed, but PGCs are mitotically active, suggesting that dpp specifically promotes PGC proliferation, as opposed to survival, in developing fly ovaries (Zhu and Xie, 2003). Ubiquitous dpp overexpression also induces increased PGC numbers in late embryos and first instar larvae, but cell death assays were not performed to rule out the possibility of improved PGC survival in this case (Sato et al., 2008). The Wnt family ligand wingless is also required for PGC mitosis, and may work with dpp to regulate this process (Sato et al., 2008).

BMP signaling also promotes PGC proliferation in the chicken (Whyte et al., 2015) and the mouse (Ross et al., 2007). In the fetal gonads of mice, Bmp7 is required for maintaining PGC proliferation in both sexes (Ross et al., 2007). In Bmp7 homozygous null mice, the number of mitotically active PGCs is significantly reduced in gonads of both sexes at E10.5. By E11.5, however, this effect is only significant in male gonads (Ross et al., 2007). Further evidence for the involvement of BMP signaling in PGC proliferation comes from a study using mice with a point mutation (R394W) in the Wilms’ tumor suppressor (Wt1) gene, which have fewer proliferative PGCs in the genital ridges than control mice (Chen et al., 2013). These homozygous Wt1R394W mice also have lower Bmp4, Smad5, and Smad8 transcript levels in the
<table>
<thead>
<tr>
<th>Species</th>
<th>PGC induction</th>
<th>PGC identity</th>
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<th>Oogenesis</th>
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<td>dpp, gbb</td>
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Experimental evidence evaluating gene function is indicated with boldface, and negative data, suggesting the lack of a reproductive role for that gene/protein, is indicated in italics. Text that is not bolded indicates expression-based or allele-association studies. Proteins are indicated in all capitals. Transcripts are written in lowercase, or with the first letter capitalized, based on gene nomenclature for that species.

*a* Indicates that the experiment was conducted *ex vivo* (tissue explant or cell culture) and all unmarked data indicates *in vivo* evidence.
The role of BMP signal transduction in non-germ line-specific reproductive roles across the Metazoa.

<table>
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\( ^{*} \) Indicates that the experiment was conducted *ex vivo* (tissue explant or cell culture) and all unmarked data indicates *in vivo* evidence.
genital ridges at E11.5, where the PGCs reside at that point in time (Chen et al., 2013). These data suggest that BMP signaling may be involved in regulating mouse PGC proliferation during these stages. Moreover, in a cultured system of migratory chicken PGCs, addition of recombinant human BMP4 promotes PGC proliferation (Whyte et al., 2015). In summary, the evidence demonstrates a conserved role for BMP signaling in regulating PGC proliferation in both protostome and deuterostome species.

4. BMP signaling can regulate PGC migration in protostomes and deuterostomes

Following their specification, PGCs often migrate from their site of specification to the site of the future gonads. Although PGCs do not migrate in every species, they do so in three species where a role for PGC proliferation has been reported. In principle, BMP signaling could regulate migration either directly, as a chemoattractant or chemorepellent, or indirectly, by maintaining PGC identity and allowing them to respond to other signals in the environment that may be acting as guidance cues. At least in mice, it appears that BMPs do not act as chemoattractants for PGCs, as exogenous BMP4 does not affect the direction or speed of migrating PGCs in genital ridge tissue slices (Dudley et al., 2007). However, conditional knockout of the type I receptor Bmp receptor 1a (Bmpr1a), or addition of the BMP-antagonist Noggin, leads to fewer PGCs migrating along the correct route. Subsequently, fewer PGCs end up in the correct location within the genital ridges (Dudley et al., 2010, 2007), suggesting that they might require BMP signaling to recognize guidance cues. The loss of BMP4 from the extraembryonic tissue in mouse embryos causes migrating PGCs in the yolk sac to become more dispersed and be abnormally localized in this region (Fujiwara et al., 2001). Cultured migratory chicken PGCs actively transduce BMP signaling, suggesting that their

Fig. 3. BMP signaling is involved in germ line-specific reproductive processes across multiple Metazoan species. "PGC Integrity" includes PGC proliferation and PGC migration. Black triangles and circles indicate that we found experimental evidence of gene function in the literature for this species, while hollow triangles and circles indicate that we found expression-based or allele-association studies. Triangles indicate studies performed in vivo while circles represent ex vivo work (studies of tissue explants or cell culture). Categories without a triangle or circle are those for which we found no available evidence for BMP signaling regulating that germ line role in that species. For more details on the gene-specific information for each role, please see Table 1 and S1.
Inhibiting Bmp type I receptors in germ cell-derived cell cultures inhibits the growth and survival of female GSCs, but improves the survival of male spermatogonial stem cells (SSCs) (Wong and Collodi, 2013). This suggests that BMP signaling could be regulating apoptosis of SSCs during spermatogenesis, and promoting the proliferation and/or survival of female GSCs in zebrafish.

5.2. Evidence from nematodes and arthropods

Mutations in members of the Caenorhabditis elegans BMP pathway lead to changes in reproductive lifespan due to the improved oocyte quality of these BMP pathway mutants, as judged either by the proportion of oocytes that can be successfully fertilized and give rise to embryos that hatch (Luo et al., 2009), or by better maintenance of oocyte morphology (Luo et al., 2010). Mutants in the dpp orthologue db1-1, both type I and type II receptor genes sma-6 and daf-4, R-Smad genes sma-2 and sma-3, and a co-Smad daf-4 all show this phenotype (Luo et al., 2009). Although experiments have not been reported that assess sperm quality in these mutants, db1-1 loss-of-function mutants have defects in rays and spicules, which are male somatic copulatory structures (Suzuki et al., 1999). As we were unable to find reports of tests of a requirement for the BMP pathway in the male gonads of C. elegans, we are unable to make sex-specific comparisons in this species.

In the species discussed to this point, we cannot always make complete comparisons regarding sex-specific BMP signaling roles, as all aspects of the pathway have not been interrogated in each sex for a reproductive phenotype. In D. melanogaster, however, comprehensive functional analyses of this pathway are available for both sexes, allowing for robust comparisons to be made. In the adult gonads of both sexes, BMP signaling is required to maintain the germ line stem cell (GSC) population, as determined by tracking the phenotypes of individual GSC clones mutant for receptor (punt (put), tkv, saxophone (sax)) and downstream effector (Medea (Med), Mothers against dpp (Mad)) genes of the BMP pathway in the ovaries (Xie and Spradling, 1998) and the testes (Kawase et al., 2004). GSCs are derived from PGCs (Bhat and Schedl, 1997; Zhu and Xie, 2003), and they function as stem cells, producing oogonia and spermatogonia respectively. There are sex-specific differences in the requirement for specific BMP ligands for GSC maintenance. In the ovary, dpp is required for GSC maintenance (Xie and Spradling, 1998), but dpp is dispensable for this role in the testis (Kawase et al., 2004; Shivdasani and Ingham, 2003). In contrast, gbb is required for GSC maintenance in both the testes (Kawase et al., 2004; Shivdasani and Ingham, 2003), and the ovaries (Song et al., 2004). GSCs are lost as a result of dpp homozygous loss-of-function in a gbb heterozygous loss-of-function mutant background (Kawase et al., 2004), but neither dpp homozygotes nor gbb heterozygotes display a GSC loss phenotype. This suggests that dpp and gbb function cooperatively to maintain GSCs in the testis (Kawase et al., 2004).

The testis GSC loss phenotype due to defective BMP signaling occurs due to premature differentiation of spermatogonia, rather than apoptosis (Kawase et al., 2004; Xie and Spradling, 1998). BMP signaling represses the transcription of the differentiation factor bag-of-marbles (bam) (McKearin and Ohlstein, 1995; Ohlstein and McKearin, 1997) in GSCs of the ovaries (Chen and McKearin, 2003a; Song et al., 2004) and the testes (Bunt and Hime, 2004; Kawase et al., 2004; Schulz et al., 2004). In the ovary, Med and Mad directly repress bam by binding to a bam silencer element (Chen and McKearin, 2003a; Song et al., 2004), but it is unclear if this repression is direct in the testis (Bunt and Hime, 2004; Kawase et al., 2004; Schulz et al., 2004). Conversely, bam also represses BMP signaling in more differentiated germ cells (Casamueva and Ferguson, 2004). As a result, there are opposite patterns of bam transcription (low versus high) and BMP signal transduction (high versus low) in early versus mature germ cells. In both sexes, dpp but not gbb is sufficient to repress bam transcription in GSCs (Kawase et al., 2004; Song et al., 2004). In the ovary, both dpp...
and gbb are required non-cell autonomously for bam repression (Song et al., 2004), but in the testis, only gbb is essential for this role (Kawase et al., 2004). In addition to repressing the differentiation of GSCs, BMP signaling also actively promotes their division, as both dpp and gbb are required in ovaries and the testes to promote GSC mitosis (Shvidasani and Ingham, 2003; Xie and Spradling, 1998). BMP signaling is also required for the clonal expansion of a GSC to populate a GSC niche in the adult ovary (Zhu and Xie, 2003), and for spermatogonial mitotic divisions in the testes (Shvidasani and Ingham, 2003). In summary, it is clear that BMP signaling is essential for germ cell maintenance in the adult gonads of both sexes in D. melanogaster, with subtle sex-specific differences.

6. BMP signaling in folliculogenesis

Until this point we have kept our discussions focused on germ line-specific reproductive processes, but there is also considerable evidence suggesting the involvement of BMP signaling in somatic reproductive tissues. This pathway can thus regulate sexual reproduction both directly (in the germ line) and indirectly (in the soma). Much of this evidence comes from studies of female fertility in sexually mature animals, and thus we will consider it here. As noted above for gametogenesis, sex-specificity occurs among some BMP ligands, including BMP15 and BMP6, which appear to be primarily ovary-specific (but see Nicholls et al., 2009; Sun et al., 2014). In vertebrates, many of these ovary-specific roles involve regulating folliculogenesis via hormone signaling. In Drosophila species, active BMP signaling in follicle cells also suggests a role for this pathway in folliculogenesis (Niepielko et al., 2011, 2012).

During folliculogenesis and oocyte maturation in mammals, BMP signaling affects female fertility by regulating various traits, including ovarian hormone secretion, gonadotropin receptor gene expression and oocyte quality. BMP pathway members have also been associated with ovulation rate and normal estrous cycling, processes that are tightly regulated by hormone signaling in the ovary. In humans, rats, sheep, and cows, progesterone secretion by granulosa cells is generally increased in the presence of BMP2 levels in sheep (McNatty et al., 2005). Conversely, estradiol secretion signaling also actively promotes their division, as both that may be caused by their abnormal serum progesterone concentration (Miyoshi et al., 2007; Otsuka et al., 2001), levels are reduced, and these rats have a lower ovulation rate (Lee et al., 2000). When BMP7 is injected into rat ovaries, serum progesterone synthesis by granulosa cells (Miyoshi et al., 2007; Otsuka et al., 2001), appears to be primarily ovary-specific (but see Nicholls et al., 2009; Sun et al., 2014). In vertebrates, many of these ovary-specific roles involve regulating folliculogenesis via hormone signaling. In Drosophila species, active BMP signaling in follicle cells also suggests a role for this pathway in folliculogenesis (Niepielko et al., 2011, 2012).

BMP ligands may also regulate hormone signaling by affecting expression of the gonadotropin receptors Follicle Stimulating Hormone Receptor (Fshr) and Luteinizing Hormone Receptor (Lhr) in the ovary to promote cell survival. BMP2, BMP4, BMP7 and BMP6 all induce Fshr expression in mammals (Frota et al., 2011; Lee et al., 2004; Shi et al., 2009, 2011, 2010; Zhu et al., 2013) and in the hen (Ocon-Grove et al., 2012). In contrast to the effect on Fshr expression, Lhr expression tends to be downregulated by BMP signaling (Shi et al., 2011, 2010; Zhu et al., 2013), except in the case of BMP6, which upregulates Lhr expression in goat granulosa cells (Zhu et al., 2013). Interestingly, Fshr knockdown induces granulosa cell apoptosis, suggesting the possibility that BMP signaling could be indirectly contributing to cell survival in the ovary by stimulating Fshr expression. In cultured developing hamster ovaries, BMP2 reduces the overall levels of apoptosis in the ovary (Chakraborty and Roy, 2015). In bovine ovaries BMP-4 and BMP-7 suppress apoptosis in granulosa cells (Kayamori et al., 2009; Shimizu et al., 2012), while BMP-6, BMP-7 and BMP-15 all reduce the levels of cumulus cell apoptosis (Hussein et al., 2005). In sum, the BMP pathway regulates the function of reproductive hormone signaling, at the levels of both hormone production and hormone reception, which in turn regulates the survival and differentiation of gametogenic cells.

We can expand the evidence for the role of BMP signaling in folliculogenesis to dipterans, but most of the evidence for this comes from a single genus of flies. In 19 Drosophila species including D. melanogaster (Table 2, S2), follicle cells around the entire circumference of the egg chamber at the border between nurse cells and oocytes actively transduce BMP signaling in stage 10 egg chambers (Niepielko et al., 2011, 2012). Across these Drosophila species, the pattern of pMAD activation is similar during early stages (stage 10), but varies between species at later stages of oogenesis (stages 11 and 12), in ways that correspond to the variations in final eggshell pattern among these species (Niepielko et al., 2011). In D. melanogaster, the type II receptor wishful thinking (uit) is required for BMP signal transduction in these follicle cells, and this receptor is required for normal eggshell morphology (Marmion et al., 2013). Roles for BMP signaling in regulating the somatic cells in closest association with developing oocytes, are thus present in both vertebrate and insect systems.

7. Ovary-specific BMP ligands in vertebrates: BMP15 and BMP6

In considering the roles of BMP signaling in vertebrate germ cell development and function, the ligands BMP6 and BMP15 have clear roles in the ovaries but not in the testes. Bmp15 is a novel BMP family member that is found only in vertebrates (Monestier et al., 2014). This gene is thought to be the product of a gene duplication event at the locus of a TGFβ superfamily member called Growth Differentiation Factor 9 (GDF9), and has oocyte-specific expression in vertebrates (Dube et al., 1998). Interestingly, mutations in BMP15 are associated with many aspects of female fertility in mammals, including ovarian failure in women (Di Pasquale et al., 2004; Dixit et al., 2006; Laissue et al., 2006), and ovulation and fertilization rates leading to differences in fecundity in various breeds of sheep (Bodin et al., 2007; Chu et al., 2007; Galloway et al., 2000; Hanrahan et al., 2004; Martinez-Royo et al., 2008; Monteagudo et al., 2009). Bmp15−/− female mice display defective ovulation and fertilization rates leading to reduced fertility, while male fertility is unaffected (Yan et al., 2001). Bmp15 and Bmp1r1b may interact to regulate fertility in some animals, as suggested by the observation that sheep with a mutation in BMPRIB is associated with higher ovulation rate also tend to have lower transcript levels of BMP15 in oocytes (Crawford et al., 2011). As Bmp15 is only present in vertebrate lineages (Monestier et al., 2014), the sex-specificity of this ligand’s reproductive role may be an example of the BMP pathway being co-opted in the lineage leading to vertebrates to regulate reproductive processes.

Bmp6 also appears to have female-specific gonadal roles. Given that it is required for similar roles to those played by Bmp15, it is possible that these two ligands may have partially redundant roles in female reproduction. For example, Bmp6 has also been shown to increase the rate of antral follicle maturation (Wang et al., 2015), and appears to be regulating gonadotropin hormone release in the ovaries of rats and sheep (Campbell et al., 2009; Otsuka et al., 2001). In mice being superovulated with chorionic gonadotropins, injecting BMP6 leads to better oocyte quality, as measured by the competency of those oocytes to give rise to healthy embryos, compared to controls injected with chorionic gonadotropins but without BMP6 (Park et al., 2012). However, female mice described as homozygous null for Bmp6 have been reported either as having normal litter sizes (Solloway et al., 2008; Monteagudo et al., 2009). In contrast to the e
1998) or significantly reduced litter sizes (Sugiura et al., 2010) in comparison to their wild-type littermates, depending on the nature of the null allele. Solloway et al. (1998) deleted a portion of the second exon and several hundred base pairs downstream, while the strain used by Sugiura et al. (2010) lacked exons 5–7 of Bmp6. It is possible that normal fecundity was observed by Solloway et al. (1998) because targeting only a portion of the second exon may not have completely nullified BMP6 activity.

8. Discussion

The evidence for BMP signaling in germ cell-specific and reproductive roles that we have compiled here suggests that this pathway plays conserved roles in animal germ cell induction, and has been recruited to regulate other reproductive roles across multiple metazoan species. Specifically, these data support the hypothesis that BMP signaling was used to specify PGCs in a last common bilaterian ancestor, and was later co-opted in various lineages to regulate additional germ line processes, including proliferation, migration and gametogenesis. In addition to germ line-specific processes, the requirement for BMP signaling in a number of somatic reproductive processes suggests that this pathway was likely recruited for these roles as well.

8.1. Broader evolutionary questions in animal reproduction

A number of outstanding questions on the degree of convergence, homology and pleiotropy of BMP signaling in animal reproduction remain. Addressing these will require significant additional studies, especially in taxa where these questions have yet to be addressed. For example, do all of the species that show a requirement for BMP signaling in a reproductive role have a conserved molecular module of upstream activators and downstream targets that originated with the evolution of the use of this pathway for germ cell segregation? Or rather, are these roles the result of independent instances of co-option of BMP signaling? Is there something intrinsic about BMP signaling that makes it more likely than other signaling pathways to become co-opted for reproductive roles? For example, the fact that its ligands may have multiple ways to traverse distances of several cell diameters (see for example Hamaratoglu et al., 2014; Kruse et al., 2004; Schwank et al., 2011) could make it a particularly flexible signal to control germ cell fate and behavior in multiple tissue contexts during development and adulthood. Or, if the germ line was already competent to receive BMP signals during specification, this pathway could have been a good candidate for continual regulation of this cell lineage throughout development and adulthood. Current knowledge suggests that the TGFβ pathway, of which the BMP pathway is a specific subtype, originated coincidentally with the origin of metazoans (Huminiecki et al., 2009). A germ line-soma separation, however, is not limited to Metazoa, but instead is seen in multiple independent eukaryotic lineages (discussed by Buss (1987), Kirk (2005); Michod and Roze (2001)). A potential ancestral role for BMP signaling in segregating the germ line from somatic lineages is thus apparently a metazoan-specific mechanism. This implies that the mechanisms governing the germ line-soma divide that accompanied the evolution of multicellularity in other eukaryotic lineages are likely determined by other signaling processes. In other words, the convergent evolution of the germ line-soma divide likely involved distinct molecular mechanisms in different lineages.

8.2. BMP signaling as a candidate pathway in germ line regulation

Among traditional model organisms that have been heavily studied, including the mouse and fruit fly, there is functional in vivo evidence indicating that BMP signal transduction has a role in PGC proliferation, PGC migration and gametogenesis. In mice, where PGCs are specified inductively, there is additionally a requirement for BMP signaling in this process. Notably, the earliest stage of D. melanogaster germ cell precursors, known as pole cells, are actively responding to BMP signaling (Deshpande et al., 2014; Dorfman and Shilo, 2001), despite the fact that they acquire their fate by maternal inheritance rather than inductive signaling (Illmensee and Mahowald, 1974, 1976; Illmensee et al., 1976). Specifically, without BMP signaling, pole cells show defects in the formation of a germ cell specific organelle called the spectrosome (Lin and Spradling, 1995), and fail to localize to the embryonic gonads, suggesting that their germ cell identity cannot be maintained without BMP signal transduction (Deshpande et al., 2014). This suggests that BMP signaling can have an early germ cell-specific role even in an organism that does not employ the inductive method of germ cell segregation. For many species where evidence for reproductive roles of BMP signaling has been reported, there are some stages of the organism’s life cycle where a role for this pathway in germ cell regulation has not been addressed. If, as we suggest, a BMP-based mechanism was recruited for multiple germ line-specific regulatory roles early in metazoan evolution, then we would expect many extant species to show a nearly continuous requirement for BMP signaling in germ cell regulation and gametogenesis throughout life. For example, in G. bimaculatus, in addition to its requirement for PGC induction, we hypothesize that the BMP pathway has a role in maintaining PGC identity in juvenile stages, and in regulating gametogenesis in adults.

8.3. BMP signaling in the germ lines of bilaterian outgroups

In the bilaterian outgroups Ctenophora and Cnidaria, germ cells are likely specified by induction (reviewed by Extavour and Akam, 2003), but a molecular basis has yet to be determined. We believe it is a reasonable possibility that BMP or TGFβ signaling is involved in specifying their germ cell precursors. If this prediction is borne out by experimental testing, this would be consistent with an ancestral role for BMP signaling in germ cell segregation among metazoans. It is difficult to ask these questions in Ctenophores at the moment, given that the data currently available do not probe gene function. However, expression data begin to provide some testable hypotheses regarding the possible involvement of BMP signaling in germ cell establishment and function in these groups. In ctenophores, gene products that are often germ line markers in metazoans (e.g. vasa, piwi, and nanos orthologues) are clearly expressed in both germ cells and somatic cells (Alié et al., 2010; Reitzel et al., 2016). The classical literature suggests that ctenophore germ cells are derived from the endoderm of the meridional canal (Extavour and Akam, 2003). A number of TGFβ ligands and receptors are expressed in tissues that could include the canal anlage and the positions of the adult gonads (Pang et al., 2011). It therefore seems plausible, although far from definitive, that BMP or TGFβ signaling could be involved in ctenophore germ cell specification and function.

In cnidarians, there are a number of relevant data points available in the literature. First, the upregulation of the Smad1 homolog (HySmad1) in Hydra vulgaris during oogenesis suggests a potential adult reproductive role of BMP signaling in this cnidarian (Hobmayer et al., 2001). Functional genetic work to assess germ cell and reproductive phenotypes following HySmad1 knockdown would be highly valuable to test the hypothesis that BMP signaling specifically has a conserved role in hydrozoan oogenesis. In anthozoans, data from Nematostella vectensis indicates that BMP signaling might be active at the eight reproductive mesenteries (Finnerty et al., 2004), which is where adult gonads are located, and potentially where germ cells first arise (Extavour et al., 2005). This could mean that BMP signaling is involved in N. vectensis PGC specification, or in some other aspect of this anthozoan’s germ line life cycle. Like all evidence based exclusively on gene expression patterns, these reports along are not strong enough to definitively indicate a role for BMP signaling in N. vectensis PGC specification. For this reason, we have not included these studies in Fig. 3. However, we note that, given the suggestive expression patterns described above, functional analyses of BMP function in these clades
would be valuable contributions to this field.

8.4. The I cell problem in germ line specification

Consideration of the Cnidaria also raises the tricky issue of the relationship between germ cells and pluripotent stem cells, as BMP signaling may also regulate the functions of the latter cell type in some systems (Srouji and Extavour, 2010). In many cnidarians, PGCs do not appear to be specified during embryogenesis, but instead arise during adult reproductive life as the product of divisions of stem cells that are capable of generating both somatic and gametogenic cells; these stem cells are called I cells. The question of PGC specification in such organisms, therefore, is reduced to one of understanding first, the specification of I cells, and second, the presumably inductive signaling event that converts some I cell progeny into PGCs. In the hydrozoan Clytia hemisphaerica, the I cell lineage is associated with a specialized, asymmetrically localized cytoplasm that is detectable from the one cell stage onwards, and contains many of the same molecules found in metazoan germ plasm (Leclère et al., 2012). However, this special cytoplasm is not required for the animal to specify I cells or germ cells as an adult. In this animal and others, this has been called a “two-step” model for PGC determination (Juliano et al., 2010; Rebscher et al., 2012, 2007). It seems likely that if there is indeed an ancestral germ plasm-like substance associated with the germ line, inductive events may also be needed for that inherent material to have the effect of first specifying a pluripotent cell lineage. We note that the idea of the germ line arising from a pre-existing “somatic” cell lineage, which is competent to give rise to PGCs but must receive some additional stimulus to realize this potential, is essentially the idea of the “germ track” as described by August Weismann in his treatise on the immortality of the germ line (Weismann, 1885).

8.5. Conclusions

To conclude, we suggest the hypothesis that during early metazoan evolution, BMP signaling was deployed to inductively specify PGCs, and later recruited for several other germ line specific and somatic reproductive roles. If this view is correct, we expect that future research will uncover reproductive roles for this pathway in more metazoan species. While we propose that the deployment of BMP signaling for PGC specification may have predated its use in other reproductive processes, further data on the degree of conservation of BMP signaling in other reproductive roles will be required to test this prediction. If BMP signaling was recruited for other reproductive roles shortly after being recruited for PGC induction, then we expect that the role of this pathway will not be limited to PGC specification, but that it will be involved in all or most germ cell stages across most metazoan phyla.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ydbio.2017.03.002.

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A.K. Loehab, C.G. Extavour


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Zhu, G., Cui, Y., Qinling, W., Yonggang, K., Yanzhi, L., Wang, J., Song, Y., Cao, B., 2013. Bone morphogenetic proteins (BMP) 2, 4, 6 and 7 affect ovarian follicular development through regulation of follicle-stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHHR) expression in goat granulosa cells. J. Cell Biol. 3. 14–21.
Table S1. Referenced literature suggesting germ cell-specific roles for BMP signal transduction across the Metazoa. Experimental evidence evaluating gene function is in **bold**, and negative data, suggesting the lack of a reproductive role for that gene/protein, is indicated in *italics*. Text that is not bolded indicates expression-based or allele association studies. Proteins are capitalized and transcripts are indicated in lowercase. * indicates that the experiment was conducted *ex vivo* (tissue explant or cell culture) and all non-starred data indicates *in vivo* evidence.

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<th>Species</th>
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<td>SMAD2/4 (Zhang et al., 2011)</td>
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**Canis lupis**

- SMAD2/4 (Zhang et al., 2011) -
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<td>Bubalus bubalis</td>
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<td>Ceratitis capitata</td>
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| Drosophila melanogaster    | dpp (Deshpande et al., 2014; Gilboa and Lehmann, 2004; Sato et al., 2008, 2010; Zhu and Xie, 2003), gbb (Deshpande et al., dpp (Bunt and Hime, 2004; Kawase et al., 2004; Michel et al., 2011; Schulz et al., 2004; Shivdasani and Ingham, 2003), pMAD (Dolezal et al., 2015; Kai and Spradling, 2003), dpp (Chen and McKearin, 2003; Chen et al., 2010; Kai and Spradling, 2003, pMAD (Wilson et al., 2011), dpp, put2, tkv, sax, mad1, mad2 (Ozuak et al., 2014), pMAD, dpp (Wilson et al., 2011), dpp, gbb (Carter et al., 2013), dpp (Vreede et al., 2013), dpp (Chen and McKearin, 2003; Chen et al., 2010; Kai and Spradling, 2003,
tkv (Deshpande et al., 2014; Gilboa and Lehmann, 2004; Zhu and Xie, 2003), Mad (Sato et al., 2010), pMAD (Dorfman and Shilo, 2001; Gilboa and Lehmann, 2004), scw (Deshpande et al., 2014) gbb (Kawase et al., 2004; Shivdasani and Ingham, 2003; put (Kawase et al., 2004; Matunis et al., 1997; Shivdasani and Ingham, 2003), sax (Schulz et al., 2004), mad (Shivdasani and Ingham, 2003), pMAD (Chang et al., 2013), pMAD (Zheng et al., 2011), dpp, gbb (Kawase et al., 2004), dpp (Kawase et al., 2004; Shivdasani and Ingham, 2003)

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development and homeostasis. Genes Dev. 18, 1482–1494.


cDNA library of buffalo follicular oocytes. Animal 7, 446–454.


primordial germ cell commitment in vitro associates with a unique PRDM14 expression profile. EMBO J. 34, 1009–1024.


Zhao, G.-Q., and Hogan, B.L.M. (1996). Evidence that mouse Bmp8a (Op2) and Bmp8b are duplicated genes that play a role in spermatogenesis and placental development. Mech. Dev. 57, 159–168.


Table S2. Referenced suggesting roles for BMP signal transduction in non-germ cell-specific reproductive roles across the Metazoa. Experimental evidence evaluating gene function is in **bold**, and negative data, suggesting the lack of a reproductive role for that gene/protein, is indicated in *italics*. Text that is not bolded indicates expression-based or allele association studies. Proteins are capitalized and transcripts are indicated in lowercase.
* indicates that the experiment was conducted ex vivo (tissue explant or cell culture) and all non-starred data indicates in vivo evidence.

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<th>Species</th>
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<td><strong>BMP6</strong> (Ocon-Grove et al., 2012), BMP4*, BMP7* (Onagbesan et al., 2003), <strong>BMP15</strong>, pSMAD1* (Elis et al., 2007; Ocon-Grove et al., 2012) pSMAD5*/8* (Ocon-Grove et al., 2012), Bmp3 (Carré et al., 2011), Bmp2, Bmp4, Bmp7 (Carré et al., 2011; Onagbesan et al., 2003), Bmp6, Alk3 (Bmpr1A), Alk6 (Bmpr1B), Bmp2 (Onagbesan et al., 2003)</td>
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<td><em>Homo sapiens</em></td>
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<td>BMP7 (Lee et al., 2001), BMP4* (Juengel et al., 2006; Nilsson and Skinner, 2003; Shimasaki et al., 1999), BMP7* (Juengel et al., 2006; Miyoshi et al., 2007; Shimasaki et al., 1999), BMP6* (Juengel et al., 2006; Miyoshi et al., 2007), BMP2* (Juengel et al., 2006), BMP15* (Otsuka and Shimasaki, 2002; Otsuka et al., 2000), BMP4 (Nilsson and Skinner, 2003), pSMAD1/5/8 (Miyoshi et al., 2007), Bmp4, Bmp7, Alk3 (Bmpr1a), Alk6 (Bmpr1b), Bmpr2 (Erickson and Shimasaki, 2003), Bmp2, Bmp3, Bmp3b (Erickson and Shimasaki, 2003)</td>
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<td>Mus musculus</td>
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| Ovis aries             | -                                                   | BMP6 (Campbell et al., 2009), BMP15 (Juengel et al., 2002, 2011), BMP4* (Juengel et al., 2006; Pierre et al., 2004), BMP2* (Juengel et al., 2006; Kumar et al., 2014; Souza et al., 2002), BMP6*, BMP7* (Juengel et al., 2006), BMP15* (McNatty et al., 2005), pSMAD1/5/8 (Hogg et al., 2010), Bmp4 (Baillet et al., 2008; Xu et al., 2010), Bmpr2 (Xu et al., 2010), Alk6 (Bmpr1B) (Chu et al., 2007;
Mulsant et al., 2001; Souza et al., 2001), Bmp15 (Bodin et al., 2007; Chu et al., 2007; Galloway et al., 2000; Hanrahan et al., 2004; Martinez-Royo et al., 2008; Monteagudo et al., 2009; Xu et al., 2010), Smad4, Alk3 (Bmpr1A) (Xu et al., 2010).

**Capra aegagrus**

- - -

**Bos taurus**

- - -

**NEMATODA**

*Caenorhabditis elegans* - - Dbl-1 (Suzuki et al., 1999)

**ARTHROPODA**

*Trichoplusia ni*  pMAD (Schröder, 2006; Sharma et al., 2013)

*Ceratitis capitata* - - - tkv, pMAD (Vreede et al., 2013)
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Erickson, G.F., and Shimasaki, S. (2003). The spatiotemporal expression pattern of the bone morphogenetic protein family in rat ovary cell types during the


Hogg, K., Etherington, S.L., Young, J.M., McNeilly, A.S., and Duncan, W.C. (2010). Inhibitor of differentiation (Id) genes are expressed in the steroidogenic cells of the ovine ovary and are differentially regulated by members of the transforming growth factor-?? family. Endocrinology 151, 1247–1256.


