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Review article

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

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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 in species that do not use inductive signaling to induce germ cell formation. The current evidence is consistent with the hypothesis that BMP signaling is ancestral in bilaterian inductive PGC specification. 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 resolu-

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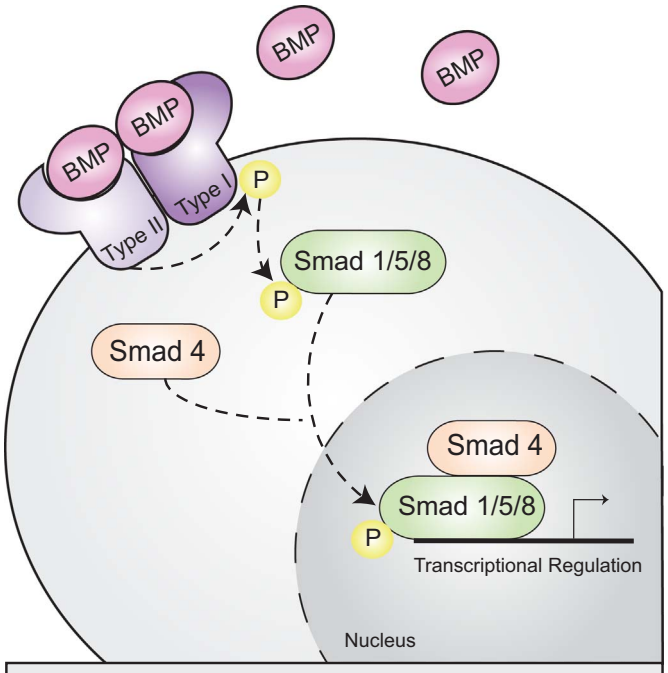

Mouse	Fly	Worm
Bmp2/4	*Dpp*	*Dbl-1*
Bmp5/6/7/8a/8b	*Gbb*	*Tig-2*
Bmp15	N/A	N/A
Bmpr1a (Alk3) *Bmpr1b (Alk6)*	*Tkv / Sax*	*Sma-6*
Bmpr2	*Put*	*Daf-4*
Smad1/5/8	*Mad*	*Sma-2/3*
Smad4	*Med*	*Sma-4*

Fig. 1. The Bone Morphogenetic Protein (BMP) signal transduction pathway across different model systems (*Mus musculus, Drosophila melanogaster, Caenorhabditis elegans*). Gene names for ligands (pink), type I receptors (purple), type II receptors (light purple), Receptor Smads (green), and Co-Smads (orange) from different organisms are indicated. A slash indicates distinct genes. For example, mice have both *Bmp2* and *Bmp4* genes, but in flies there is one ortholog for this pair (*dpp*). Phosphorylation is shown in yellow.

tion of this literature is highly variable, ranging, for example, from agricultural studies showing genetic associations of this pathway with different levels of fecundity among livestock, to molecular evidence indicating the requirement of BMP in the germ line stem cell (GSC) niche in *Drosophila melanogaster*. Here we aim to summarize and discuss the current literature in a phylogenetic context. Within this context, we propose specific evolutionary hypotheses about the evolution 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.

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), FGF 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, Blimp-1 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 Blimp-1 in PGC

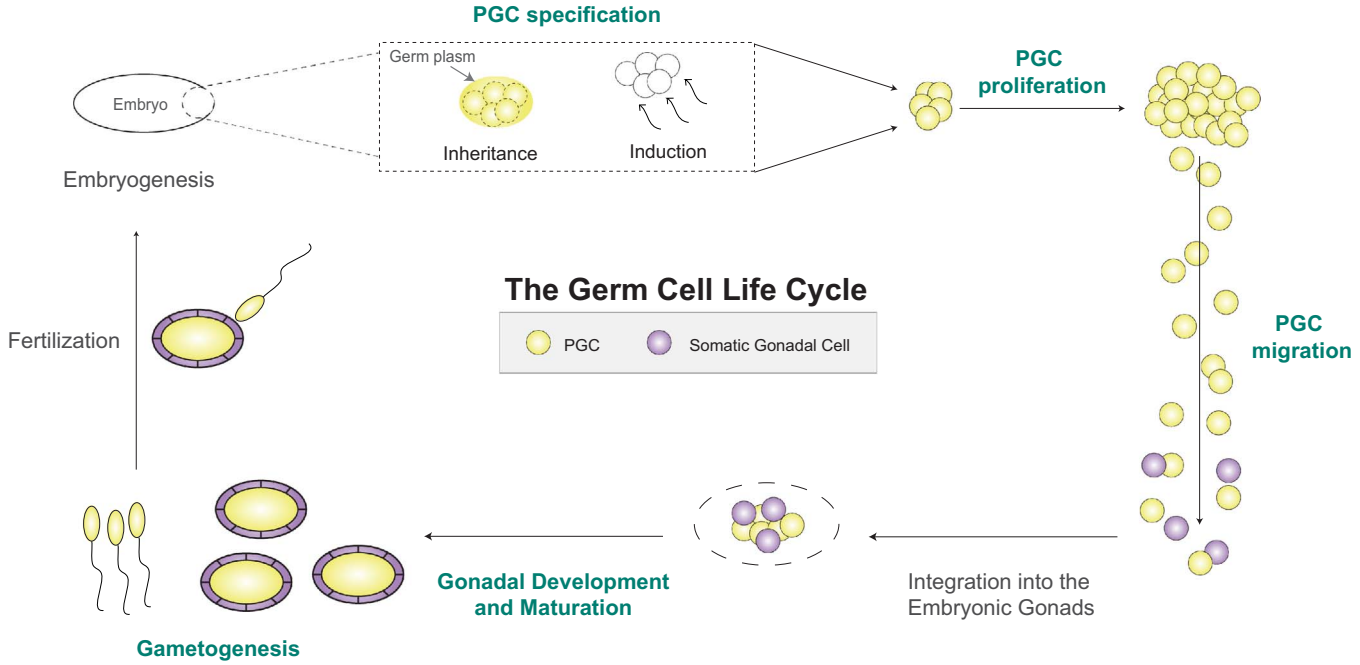

Fig. 2. The germ cell life cycle. Primordial germ cells (PGCs) are specified either by inheritance (germ plasm) or induction (extrinsic signals) during embryogenesis. After specification, PGCs may divide mitotically (PGC proliferation) and migrate to the final location of the gonads. They are then integrated into the developing gonads, together with somatic reproductive cells, and differentiate into mature germ cells. In the developing gonads, oogenesis and spermatogenesis begins and mature gametes undergo fertilization. We have found evidence for BMP signaling being involved in the processes indicated in green text, and we focus on these processes within this review.

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 *AP2γ* (*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 *AP2γ* 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 arthropod to date, including *G. bimaculatus*, where BMP-responsive *Blimp1* activity is required for PGC specification (Nakamura and 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 $Wt1^{R394W}$ mice also have lower *Bmp4*, *Smad5*, and *Smad8* transcript levels in the

Table 1
The role of BMP signal transduction in germ line-specific reproductive processes across the Metazoa.

Species	PGC induction	PGC identity	Spermatogenesis	Oogenesis
CNIDARIA				
Hydra vulgaris	–	–	–	Smad1
CHORDATA				
Botryllus schlosseri	–	pSMAD1/5/8	pSMAD1/5/8	pSMAD1/5/8
Botryllus primigenus	**BMP2**[a], **BMP4**[a]	–	–	–
Danio rerio	–	–	**bmpr1b**, **bmpr1a**[a]**/b**[a], pSMAD1/5/8	**bmpr1b, BMP4**[a], **bmpr1a**[a]**/b**[a], **BMP4**[a], pSMAD1[a]/5[a]/8[a]
Ambystoma mexicanum	**BMP4**[a]	–	–	–
Gallus gallus	Alk2[a], Bmpr1a[a], BmprIb[a]	**BMP4**[a], pSMAD1/5/8, Alk2[a], Bmpr1a[a], Bmpr1b[a]	–	BMP15, Bmp15
Macropus eugenii	–	*BMP4*[a]	–	–
Homo sapiens	**BMP4**[a], **BMP2**[a], **BMP7**[a], **BMP8b**[a], *BMP4*[a], *BMP7*[a], *BMP8b*[a]	**BMP4**[a], BMP4, BMP7, BMPR1A, BMPR1B, BMPR2, BMP15	pSMAD1/5/8, BMPR1A, BMPR2	BMP15, Bmp15, BMP4, BMP7, BMPR1A, BMPR1B, BMPR2, Bmp15[a]
Macaca fascicularis	*BMP4*[a]	–	–	–
Oryctolagus cuniculus	Bmp4	–	–	–
Mesocricetus auratus	–	–	–	**BMP2**[a]
Rattus norvegicus	–	–	**BMP4**[a], BMP15, Bmp15, BMPR2, pSMAD1[a]/5[a]/8[a]	**BMP4**[a], Bmp4, Bmp7, Bmp15, Bmpr1a, Bmpr1b, Bmpr2, Bmp6
Mus musculus	**Bmp4, Bmp8b, Bmp2, Smad1/5, pSMAD1/5/8, Alk2, BMP4**[a]**, BMP2**[a]**, BMP8b**[a]**, Smad1**[a], Bmp4, *BMP8B*[a]	**Bmp4, Bmp7, Bmpr1a, BMP4**[a]	**Bmp4, Bmp8a, Bmp8b, Bmp7, BMP2**[a]**, BMP4**[a], BMP4, Bmp4, Bmp7, Bmp8a, Bmp8b, SMAD5, BMPR1A, Smad1/5/8/6/7, BMPR1A, pSMAD1[a]/5[a]/8[a], Bmpr1a[a]	**Bmp15, Bmp6, BMP6, Bmpr1b, BMP4**[a]**, pSMAD1**[a]**/5**[a]**/8**[a], BMP4, BMP15, Bmp15, pSMAD1/5/8, Smad1/5/8/4/6/7, Bmpr1a[a], BMPR1A[a], Bmpr1b[a], BMPR1B[a], Bmpr2[a], BMPR2[a], *Bmp6*
Canis lupus	–	–	SMAD2/4	–
Sus scrofa	**BMP4**[a]	–	–	BMPR1A, BMPR1B, BMPR2, Bmp2, Bmp15, Bmpr1a, Bmpr1b, Bmpr2, Bmp6, Bmp4[a], Bmp6[a], Bmp15[a], Bmpr1a[a], Bmpr1b[a], Bmpr2[a]
Ovis aries	–	–	Bmpr1b	**BMP4**[a], Bmp6, Bmp15, Bmpr1a, Bmpr1b, Bmpr2, Smad4, Bmp15[a]
Capra aegagrus	–	–	–	**BMP6**[a], BMP6, BMP15, Bmp15, Bmp6, Bmpr2, Bmpr1a, Bmpr1b
Bos taurus	**BMP4**[a]	–	Bmp15	**BMP15**[a], BMP6, BMPR1B, BMPR2BMP6, BMP7, BMPR1B, BMPR2, pSMAD1, ALK2, ACTR2, ACTR2B, Bmp7[a], Bmp2[a], Bmp4[a], Bmp6[a], Bmpr1a[a], Bmpr1b[a], Bmpr2[a], ActR2[a], Alk2[a]
Bubalus bubalis	**BMP4**[a]	–	–	Bmp15
NEMATODA				
Caenorhabditis elegans	–	–	–	**sma-2, daf-4, dbl-1, sma-3, sma-4, sma-6**
ARTHROPODA				
Gryllus bimaculatus	**gbb**, **dpp1**, **Mad**, tkv, put, mad	–	–	–
Apis mellifera	–	–	–	pMAD, dpp
Nasonia vitripennis	–	–	–	dpp, put2, tkv, sax, Mad1, Mad2
Pararge aegeria	–	–	–	dpp, gbb
Ceratitis capitata	–	–	–	dpp
Drosophila melanogaster	–	**dpp**, **gbb**, **tkv**, **Mad**, pMAD, *scw*	**dpp**, **gbb, tkv, put, sax, Mad, pMAD,** pMAD, dpp, gbb, *dpp*	**pMAD**, **dpp**, **sax**, **gbb**, **tkv, put, Mad, Med,** dpp, pMAD

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.

Table 2
The role of BMP signal transduction in non-germ line-specific reproductive roles across the Metazoa.

Species	PGC induction	Maintenance	Testis function	Ovary function
CHORDATA				
Gallus gallus	–	–	BMP4	**BMP4**[a], **BMP6**[a], **BMP7**[a], **BMP15**[a], pSMAD1[a]5[a]/8[a], Bmp2, Bmp3, Bmp4, Bmp6, Bmp7, Bmpr1a, Bmpr1b, Bmpr2
Homo sapiens	–	–	**BMP4**[a], **BMP7**[a], BMP4, pSMAD1/5, BMPR1B, BMPR2, Bmp4[a]	**BMP2**[a], **BMP4**[a], **BMP6**[a], **BMP7**[a], **pSMAD1**[a]**/5**[a]**/8**[a], **BMP15**[a], **BMPR1A**[a], **BMPR1B**[a], **BMPR2**[a], **ALK4**[a], **ALK5**[a], **ALK7**[a], BMP6, Bmp2, Bmp15, Smad1[a]/5[a]/8[a], pSMAD1[a]/5[a]/8[a], Bmpr1a[a], Bmpr1b[a], Bmpr2[a], Alk2[a], Alk4[a], *BMP15*
Oryctolagus cuniculus	Bmp2	–	–	–
Mesocricetus auratus	–	**BMP2**[a], BMP2	–	**BMPR1A**[a], **ALK2**[a], BMP2
Rattus norvegicus	–	–	–	**BMP7, BMP2**[a], **BMP4**[a], **BMP6**[a], **BMP7**[a], **BMP15**[a], BMP4 pSMAD1/5/8, *Bmp8a*
Mus musculus	–	**BMP7**[a], Bmp4, Smad/5/8	**BMP7**[a], Bmpr2b[a], *Bmp15*	**BMP4**[a], **BMP6**[a], **BMP7**[a], **BMP15**[a], pSMAD1/5, Smad1/5/8/4/6/7, Bmpr1a[a], BMPR1A[a], Bmpr1b[a], BMPR1b[a], Bmpr2[a], BMPR2[a], *Bmp8a, Bmp6*
Sus scrofa	–	–	–	**BMP2**[a], **BMP6**[a], BMP2, BMP6
Ovis aries	–	–	–	**BMP6, BMP15, BMP2**[a], **BMP4**[a], **BMP6**[a], **BMP7**[a], **BMP15**[a], pSMAD1/5/8, Bmp4, Bmpr1a, Bmpr1b, Bmpr2, Bmp15, Smad4
Capra aegagrus	–	–	–	**BMP2**[a], **BMP4**[a], **BMP6**[a], **BMP7**[a], Bmp6, Bmp4, Bmp7, Bmpr2, Bmpr1b, Smad1/5/8/6, Bmp4[a], Bmp7[a], Smad5[a]/8[a]/7[a], Bmpr1a[a] *BMP6*[a]
Bos taurus	–	–	–	**BMP4**[a], **BMP7**[a], **BMP6**[a], **BMP15**[a], BMP7, BMP4, pSMAD1, Bmp7[a], Bmp2[a], Bmp4[a], Bmp6[a], Bmpr1a[a], Bmpr1b[a], Bmpr2[a], ActR2[a], Alk2[a]
Bubalus bubalis	**BMP4**[a]	–	–	Bmp15
NEMATODA				
Caenorhabditis elegans	–	–	**dbl-1**	–
ARTHROPODA				
Tribolium castaneum	pMAD	–	–	–
Ceratitis capitata	–	–	–	tkv, pMAD
Drosophila melanogaster	–	–	–	**dpp**, **wit (bmpr2),** tkv
Drosophila spp.	–	–	–	pMAD

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.
Drosophila spp. (18 species: D. borealis, D. busckii, D. erecta, D. ezoana, D. funebris. D. guttifera, D. littoralis, D. mercatorum, D. mojavensis, D. nasuta, D. nebulosa, D. phalerata, D. pseudoobscura, D. quinaria, D. tropicalis, D. virilis, D. willistoni, D. yakuba)

[a] Indicates that the experiment was conducted *ex vivo* (tissue explant or cell culture) and all unmarked data indicates *in vivo* evidence.

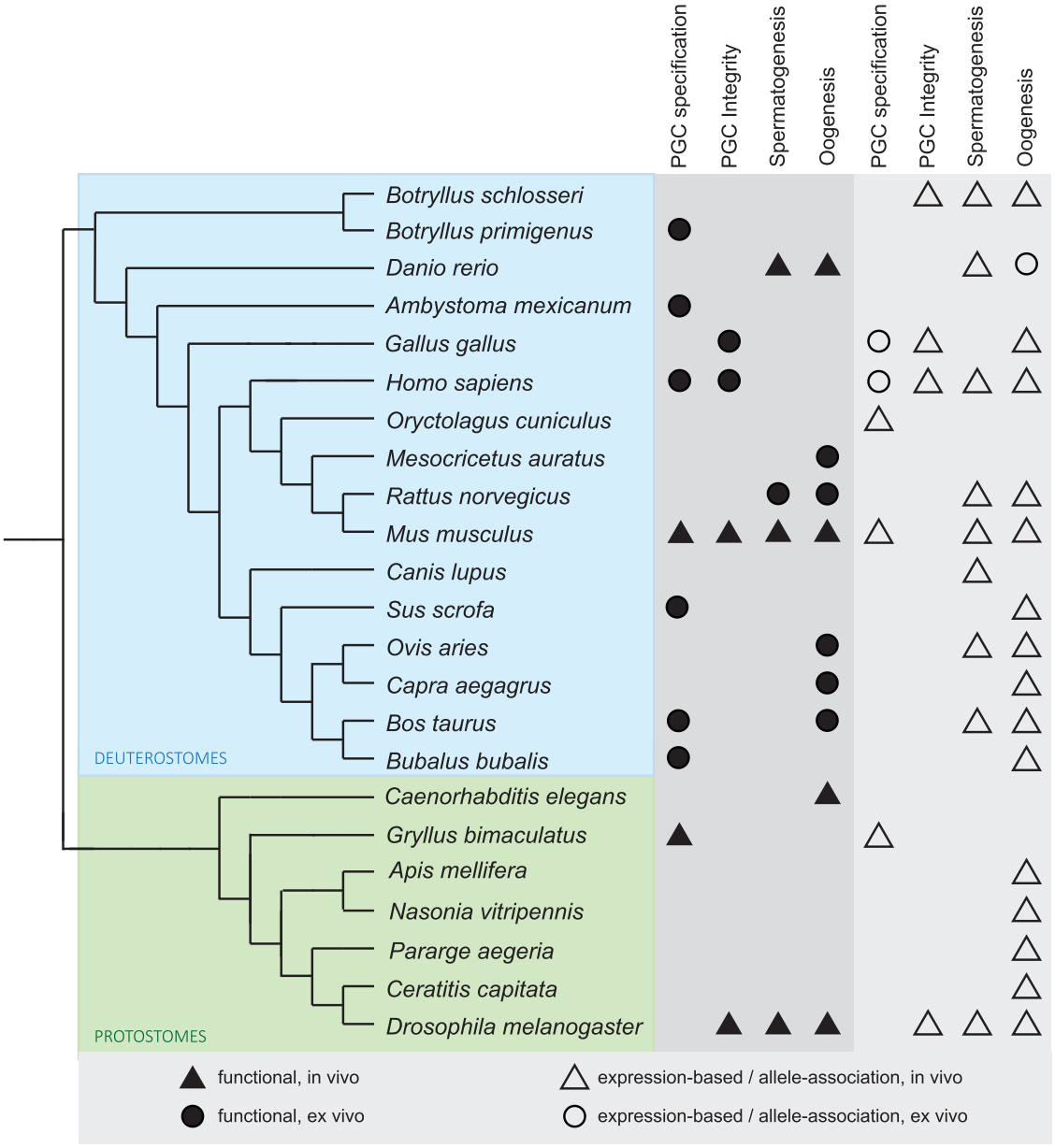

Fig. 3. BMP signaling is involved in germ line-specific reproductive processes across multiple Metazoan species. "PGC Integrity" includes PGC proliferation and PGC migration. Black triangles and circles indicate that we found experimental evidence of gene function in the literature for this species, 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.

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

migration in embryos may be regulated by this pathway as well (Whyte et al., 2015).

Among the arthropods, to our knowledge, the possible involvement of BMP signaling during PGC migration has only been explored in *D. melanogaster* (Deshpande et al., 2014). Overexpression of *dpp* in the mesoderm during the migratory phase of PGCs in *D. melanogaster* leads to their scattering and mislocalization (Deshpande et al., 2014). While patterning defects are also evident in embryos with mesodermal *dpp* overexpression, PGCs at this stage are transducing Dpp signals (Deshpande et al., 2014). This suggests that the mislocalization of these PGCs may be due to a true migration defect, rather an indirect effect on PGC localization as a consequence of abnormal somatic patterning (Deshpande et al., 2014). Taken together, a conserved role for BMP signaling in regulating PGC migration may exist in both protostome and deuterostome lineages.

5. BMP signaling in gametogenesis

After germ cells have integrated into the gonads, gametogenesis begins. Gametogenesis must be stringently regulated to ensure the successful production of mature gametes. Different BMP pathway members have been implicated in adult male and female fertility across various species, and there appear to be instances of sex-specificity for different ligands. There is strong functional evidence for a role of the BMP pathway in regulating spermatogenesis and oogenesis in mouse, fly, and rat species. Additionally, functional evidence for the involvement of the BMP pathway in oogenesis has been reported for hamster, sheep, goat and nematode species. In the following sections, we discuss the differential use of certain ligands for gametogenesis among sexes, and the broader relationships we observe both within and across the chordate, nematode and arthropod lineages.

5.1. Evidence from chordates

Different Bmp ligands appear to regulate gametogenesis in a sex-specific manner. For example, in mice, *Bmp15* and *Bmp6* predominantly have roles in oogenesis.[1] In contrast, *Bmp8a* and *Bmp8b* are both necessary for the maintenance of spermatogenesis in mice and *Bmp8b* is involved in the initiation of spermatogenesis (Zhao and Hogan, 1996; Zhao et al., 1996, 1998). Moreover, *Bmp8* family ligands were never implicated in female reproductive roles across our literature search in any species. Other ligands, including BMP4 and BMP7, are neither sex-specific nor restricted to a single role across multiple species, but appear more broadly used in regulating the germ line. This suggests that certain BMP ligands, including BMP6, BMP15, and BMP8 have specialized in sex-specific reproductive roles while others are more broadly applicable.

Among closely related species, the same ligands sometimes, but not always, have similar functions. For example, in both rats and mice, BMP4 promotes the differentiation of spermatogonial cells (Carlomagno et al., 2010; Nagano et al., 2003; Pellegrini et al., 2003). In contrast, BMP2 appears to promote the proliferation of spermatogonial cells within *ex vivo* cultured testis fragments of mice (Puglisi et al., 2004), while in the hamster, *Mesocricetus auratus*, BMP2 regulates germ cell apoptosis in developing ovaries, and increases number of oocytes in meiotic prophase at the time of assay, relative to the total number of germ cells in the developing ovary (Chakraborty and Roy, 2015).

In zebrafish, the type I receptor *bmp receptor 1b* (*bmpr1b*) is necessary for gametogenesis in both sexes, and *bmpr1b* transcripts are expressed in early oocytes and spermatogonia (Neumann et al., 2011). 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 *dbl-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 *sma-4* all show this phenotype (Luo et al., 2009). Although experiments have not been reported that assess sperm quality in these mutants, *dbl-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, 2003b; 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 (Casanueva 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*

[1] To our knowledge, the only reported exception to this are findings that *Bmp15* expression is observed in pachytene spermatocytes and gonocytes of rats (Nicholls et al., 2009), and the association of *BMP-15* with sperm motility in bulls (Sun et al., 2014).

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 (Shivdasani 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 (Shivdasani 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 suppressed by BMP ligands (Glister et al., 2004; Juengel et al., 2006; Lee et al., 2001; Miyoshi et al., 2007, 2006; Otsuka et al., 2000; Pierre et al., 2004; Shimasaki et al., 1999; Yamashita et al., 2011; Zhang et al., 2015). In contrast, however, BMP15 has no effect on granulosa cell progesterone levels in sheep (McNatty et al., 2005). Conversely, estradiol secretion by granulosa cells is generally increased in the presence of BMP2 (Kumar et al., 2014; Souza et al., 2002), BMP4, BMP7 (Glister et al., 2004; Miyoshi et al., 2007; Shimasaki et al., 1999), and BMP6 (Campbell et al., 2009; Ebeling et al., 2011; Glister et al., 2004; Wang et al., 2015) in mammals. An exception to this is found in rats, where neither BMP6 nor BMP15 appear to affect the levels of estradiol synthesis by granulosa cells (Miyoshi et al., 2007; Otsuka et al., 2001, 2000). When BMP7 is injected into rat ovaries, serum progesterone levels are reduced, and these rats have a lower ovulation rate (Lee et al., 2001). Sheep immunized against BMP15 have irregular estrous cycling that may be caused by their abnormal serum progesterone concentrations (Juengel et al., 2002).

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 (wit)* 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 *Bmpr1b* may interact to regulate fertility in some animals, as suggested by the observation that sheep with a mutation in *BMPR1B* that 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.,

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 inherited material to have the effect of first specifying a pluripotent cell linage. 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 life 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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