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BMP and BMP receptor expression during murine organogenesis

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ABSTRACT

Cell–cell communication is critical for regulating embryonic organ growth and differentiation. The Bone Morphogenetic Protein (BMP) family of transforming growth factor β (TGFβ) molecules represents one class of such cell–cell signaling molecules that regulate the morphogenesis of several organs. Due to high redundancy between the myriad BMP ligands and receptors in certain tissues, it has been challenging to address the role of BMP signaling using targeting of single *Bmp* genes in mouse models. Here, we present a detailed study of the developmental expression profiles of three BMP ligands (*Bmp2*, *Bmp4*, *Bmp7*) and three BMP receptors (*Bmpr1a*, *Bmpr1b*, and *BmprII*), as well as their molecular antagonist (*noggin*), in the early embryo during the initial steps of murine organogenesis. In particular, we focus on the expression of Bmp family members in the first organs and tissues that take shape during embryogenesis, such as the heart, vascular system, lungs, liver, stomach, nervous system, somites and limbs. Using in situ hybridization, we identify domains where ligand(s) and receptor(s) are either singly or co-expressed in specific tissues. In addition, we identify a previously unnoticed asymmetric expression of *Bmp4* in the gut mesogastrium, which initiates just prior to gut turning and the establishment of organ asymmetry in the gastrointestinal tract. Our studies will aid in the future design and/or interpretation of targeted deletion of individual *Bmp* or *Bmpr* genes, since this study identifies organs and tissues where redundant BMP signaling pathways are likely to occur.

Morphogenesis of embryonic tissues and initiation of organogenesis begins post-gastrulation around embryonic day 8 (E8.0) in the mouse, with the onset of heart and blood vessel development. Over the next 24 h, the embryonic endoderm transforms from a single cell layer sheet into an open cylinder which rapidly zippers up into the gastrointestinal tract and associated umbilical cord. Coordinately, the embryo undergoes 'turning', as it twists on its axis and acquires its characteristic fetal shape. In addition, during this time, most organs appear along the anteroposterior axis, including the budding pituitary, thyroid, salivary glands, lung, liver and pancreas. During the next 48 h these organs continue to develop and take shape, and in the strikingly short span of approximately 2 days (E8.0–E10.5), the principal embryonic organs have become specified and emerged from relatively simple germ layers (ectoderm, mesoderm and endoderm), and undergone morphogenesis resulting in complex, multi-cellular organs. This dynamic process is ultimately the result of step-wise cell differentiation that involves a busy crosstalk of cell–cell signaling between growing tissues and the interplay of numerous different intrinsic gene pathways. A number of extrinsic factors have been shown to interact and drive organ and tissue formation during embryonic development, including the Wnt, hedgehog (Hh), fibroblast growth factor (Fgf), Notch, and transforming growth factor β (TGFβ/bone morphogenetic protein (BMP)) families of signaling molecules. Our studies focus on the BMP growth factor family and the expression of Bmps during organogenesis.

Bone morphogenetic proteins (BMPs) are part of the TGF-β superfamily (Kingsley, 1994) and comprise a large, evolutionarily conserved family of secreted signaling molecules that are required for numerous developmental processes. BMPs were originally isolated because of their capacity to promote bone and cartilage formation (Urist, 1965). However, they have also been shown to participate in the establishment of the initial vertebrate body plan, somite and neural tube patterning, as well as the development of a large number of structures and organs, such as kidney, lung, liver, limb, amnion, eye, teeth, pituitary, and testes (reviewed in Hogan, 1996; Zhao, 2003). The importance of the development function of these BMP factors is highlighted by the fact that deletion of many Bmp genes (including *Bmp2* and *Bmp4*) and their receptors (including *Bmpr1a* and *II*) results in early embryonic lethality (prior to E9.5) when most gastrointestinal organs are just beginning to initiate development (reviewed in Zhao, 2003). Although conditional ablation of *Bmpr1a* has demonstrated its specific requirement in a

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number of tissues (Eblaghie et al., 2006; Park et al., 2006), the specific roles of BMP ligands and other BMP receptors have yet to be described.

BMPs, like other TGFβs, are first synthesized and folded in the cytoplasm and subsequently cleaved by proteases during secretion. BMPs form large, dimeric proteins, whose proper conformation is required for their receptor binding and biological action (Eimon and Harland, 1999). In mouse, a single BMP type II receptor subunit (*BMPRII*) has been identified (Beppu et al., 1997), while at least three type I receptors have been found (*BMPR1a*/Alk3, *BMPR1b*/Alk6 and ActRIa/Alk2) (ten Dijke et al., 1994). After BMP ligand binding, *BMPRII* heterodimerizes with a type I receptor, such as *BMPR1a* or *BMPR1b*, resulting in type II receptor phosphorylation and activation of the type I receptor. The type I receptor in turn phosphorylates cytoplasmic downstream target proteins, including Smad family proteins (see review Kretzschmar and Massague, 1998) which act as transcription factors and regulate many downstream pathways. Adding to BMP signaling complexity, BMPs can also interact with type II activin receptors ActRIIa and ActRIIb (Yamashita et al., 1995). Therefore, as BMPs display promiscuity in binding affinities and their receptors function as heterodimers, multiple possible signaling cascades exist and depend on the expression of specific BMP ligands and receptors in given tissues.

Bmp ligand and receptor expression profiles have been described in scattered reports in the literature (Bitgood and McMahon, 1995; Dewulf et al., 1995; Furuta et al., 1997; Jones et al., 1991; Lyons et al., 1995; Solloway and Robertson, 1999), however, a comprehensive comparative gene expression analysis has not been described for the decapentaplegic (*dpp*) subgroup of Bmp genes (*Bmp2*, *Bmp4*) and their receptors (*Bmpr1a*, *Bmpr1b* and *BmprII*) throughout organogenesis. Myriad reports have demonstrated the critical importance of both Bmp ligands and their receptors for embryonic organ and tissue development. In particular, elegant conditional deletion studies have shown the requirement for *Bmp4* (Kulessa and Hogan, 2002), *BmpRII* (Beppu et al., 2005) and *BmpR1a* (Mishina et al., 2002) in a number of tissues including the cardiovascular system (Kaneko et al., 2008; Park et al., 2006; Yu et al., 2005), lung (Eblaghie et al., 2006), limb (Ovchinnikov et al., 2006), central nervous system and many more. In this report, we analyze expression of *Bmp2*, *Bmp4*, *Bmp7* and their receptors *Bmpr1a*, *Bmpr1b*, and *BmprII*, and the BMP antagonist *noggin*, prior to and during organogenesis, with special focus on tissues where multiple ligands and receptors are co-expressed at distinct timepoints. These studies will help elucidate interpretations of genetic deletion studies that may be complicated by tissue specific BMP signaling redundancy. We employ in situ hybridization to examine and compare expression of transcripts of these genes, in postgastrulation embryos from stages E7.5 to E10.5. We aim to identify sites where single or multiple BMPs may play a role during organogenesis.

1. Results and discussion

1.1. Bmp2 – E7.25–E10.5

We initially assayed expression of Bmp genes using whole mount in situ hybridization. (For all descriptions of gene expression in embryonic organs and tissues refer to structure annotations found in top panels of each figure, either in schematic or overlaid on photographed embryo, and associated organ or tissue name in figure legends). Our analysis of Bmp gene expression began at E7.25, a time in development when embryonic tissues start to form following gastrulation. We find that at this stage, *Bmp2* is primarily expressed in the yolk sac (y) and allantois (a), in the pre-cardiac crescent (cc), just anterior to the anterior intestinal portal, or AIP (aip) (Fig. 1A1). Expression appears distinctly absent in the medial, open gut region (g) of the embryo, which includes pre-somitic mesoderm, endoderm, and neural tissues. By E8.25, we observe an increase in *Bmp2* expression in the linear heart tube (h) and allantois, while high levels of expression are observed in the constricting AIP and sinus venosus (sv), regions immediately ventro-posterior to the heart (Fig. 1A2). In addition, strong expression initiates in the dorsal most tip of the rostroanterior neural folds (nf) and the midline fusion point of the neural folds spanning the length of the embryo. Expression also appears robustly in the lateral plate mesoderm (lpm).

Slightly later, at E8.75, expression remains strong in the sinus venosus and in the fusing dorsal neural tube (Figs. 1A3, 2A1, and 3A1). Strikingly, rather significant expression is also detected in the heart, shortly after the heart takes shape and starts to loop. Expression is particularly strong in the region that joins the left ventricle and the atria, which will later give rise to the atrioventricular canal region, or AVC (avc) (arrow in Fig. 1A3), a region previously shown to express both *Bmp2* and *Tbx2* (Christoffels et al., 2004). Expression is also high in the pericardium, with declining expression in the 'seam' of the dorsal brain, which represents the region of the midline fusion of the anterior neural folds.

Later, at E9.0, *Bmp2* remains strongly expressed in the AVC, but has declined from the midline seam of the telencephalon (ms) (Figs. 1A4, 2A2, and 3A2). In addition, expression has decreased in the constricting base of the yolk sac (ysc) (or the future umbilical cord), and associated lateral plate mesoderm, as embryonic turning proceeds and the gut tube forms (Fig. 1A4). Expression is low in the head and branchial arches, as well as the dorsal neural tube at this stage of development. Expression remains strong in the liver diverticulum (l) (Figs. 1A4 and 2A2). *Bmp2* also increases expression in the developing forelimbs (lb) (Figs. 2A3 and 3A3), the rostral dorsal aortae (ao) of the trunk and the anterior tip of the developing mesonephros (m) (arrowheads in Fig. 1A4).

At E9.5, *Bmp2* has also begun to be expressed in the apical ectodermal ridge, or AER (aer), of the limb bud, a region long noted for its organizing activity in driving limb development, and the ventral portion of the developing limb bud, as previously noted (Ahn et al., 2001; Lyons et al., 1995) (Figs. 1A5, 2A3 and 3A3). Notably, expression in the heart AVC peaks at this point. In addition, expression is prominent in subpopulations of cells between the branchial arches (Fig. 1A5). By E10.5, expression levels have generally declined throughout the head and tail, and are low throughout the gut tube (Figs. 2A4 and 3A4–5); however, there is still detectable expression in the constricting yolk sac (Fig. 1A6). In addition, expression remains relatively high in the heart AVC (inset, Fig. 2A5), otic vesicle (ov), and branchial arches. Expression is robust in the AER at this stage and appears in an ectodermal patch on the posterior aspect of the forelimbs. Additionally, expression in the ventromedial somites (s) increases, especially in the anterior trunk (Figs. 1A6 and 2A6).

1.2. Bmp4 – E7.5–E10.5

Bmp4 is expressed in a pattern initially similar to that of *Bmp2*; however, it subsequently varies dynamically throughout early embryogenesis. At E7.25, *Bmp4* like *Bmp2*, is strongly expressed in the yolk sac and allantois, and is concentrated in the anterior part of the embryo, including the cardiac crescent and early neural folds (Fig. 1B1). At E8.25, *Bmp4* remains expressed in the allantois, and strong expression initiates in the posterior lateral plate mesoderm, the AIP, and the sinus venosus (Fig. 1B2). However, yolk sac expression is slightly lower than that of *Bmp2*.

After turning, around E8.75, expression continues to increase in the posterior lateral plate and remains relatively strong in the sinus venosus, the allantois and the caudal most mesoderm of the