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Role of BMP family members during kidney development

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ABSTRACT Members of the Bone morphogenetic protein (BMP) family have been shown to be important signaling molecules throughout mouse development. Accordingly, many BMPs are also expressed during organogenesis of the metanephric kidney. However, only BMP7 has been shown to be absolutely required for proper formation of the kidney, thus the majority of information known involves this family member. BMP7 is expressed in both the ureteric epithelium and the mesenchyme throughout embryonic development and has been shown to function as a survival factor for the nephrogenic mesenchyme. However, there has been some controversy over the role of BMP7 as an inducing molecule for the metanephric mesenchyme. Recent studies have shown that BMP7 functions as an anti-differentiation factor for this mesenchyme cell population. The function of BMPs in the ureter and in the more differentiated epithelial structures of the nephron is less well understood.

KEY WORDS: *bone morphogenetic proteins, kidney, mouse*

Expression of BMP signaling components during kidney development

BMP family members display dynamic expression patterns during kidney development (Fig. 1; Dudley and Robertson, 1997). *Bmp7* transcripts are detected in the ureteric bud as it emerges from the Wolffian duct at 11.0 dpc and expression is maintained in derivatives of the bud throughout development. As the bud undergoes morphogenesis, *Bmp3* expression is observed in the trunk of the ureter and the collecting ducts, but not in the tips. From the initial contact until the cessation of nephrogenesis, the condensed mesenchyme surrounding the ureteric tips expresses *Bmp7* exclusively. Subsequently, the induced mesenchyme undergoes a process of nephrogenesis that involves several morphologically distinct stages proceeding from pretubular aggregate, comma- and S-shaped tubules, to nephrons which are fused with the collecting duct at the distal end and contain a glomerulus at the proximal end. BMP family members are expressed in a graded manner during this period. *Bmp2* expression in the kidney is first detected in the pretubular aggregates and is maintained in the distal part of the early tubules. By contrast, *Bmp7* is expressed uniformly throughout the aggregates and tubules. Expression of both *Bmp2* and *Bmp7* is downregulated in the distal part as the tubule matures, though *Bmp7* expression is maintained in the proximal part. In more developed tubules, *Bmp3, Bmp4* and *Bmp7* are all co-expressed in Bowman's capsule of the developing glomerulus. In addition, both *Bmp4* and *Bmp7* are expressed in the presumptive podocyte layer. BMP family members are also expressed in cells surrounding the epithelial components of the developing kidney that are generically considered stromal cells. Soon after ingression of the ureteric bud into the metanephric blastema, *Bmp4* transcripts are detected in a mesenchymal cell population adjacent to the ureter. Later, this cell population also expresses *Bmp5*, while loose stromal cells some distance from the ureteric tree express *Bmp6*. Interestingly, a population of *BF-2* positive stromal progenitor cells surrounding the condensed mesenchyme does not express detectable levels of any BMP family member examined to date.

Since BMP family members are secreted molecules, the expression patterns described above only provide information with regard to the source of the signal and not to the responding cell population. However, it is likely that members of the BMP family signal over relatively short distances and therefore the likely targets are either the expressing cells themselves or the immediate neighbors. In support of this idea, experiments in *Xenopus* demonstrate limited diffusion of BMPs from their source (Jones and Smith, 1998). Moreover, in most tissues, *Bmp7* mRNA expression and BMP7 protein are found colocalized (Vukicevic *et al.*, 1994). In addition, we have also demonstrated that when beads soaked in media containing BMP2 or BMP7 are added to explants of metanephric mesenchyme, responding cells are observed only within 150-200 μm of the bead, whereas FGF2 can signal throughout the

Abbreviations used in this paper: BMP, Bone morphogenetic protein; BMPRI and BMPRII, Bone morphogenetic protein receptors I and II; ActRI, ActRII, Activin receptors.

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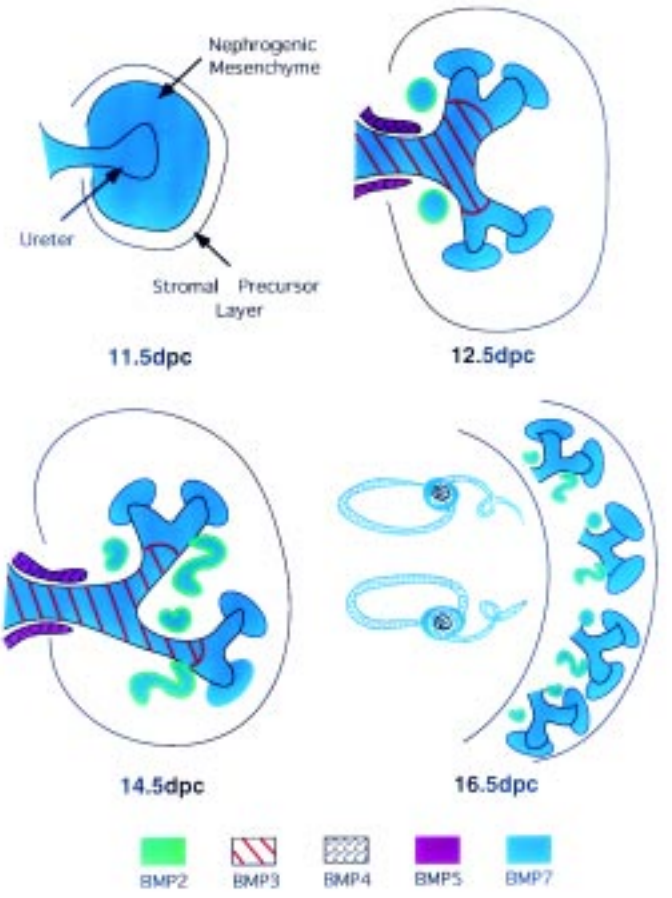

Fig. 1. BMP family members are expressed throughout kidney development from the initial inductive interactions at 11.5 dpc through later stages of tubule differentiation. *At 11.5 dpc, BMP7 is expressed in both the ureteric bud and the nephrogenic mesenchyme. No BMP family members have been found to be expressed in the stromal precursor layer. At 12.5 dpc, BMP3 is co-expressed with BMP7 in the ureter with the exception of the tips, the point of induction. BMP2 is co-expressed with BMP7 in the condensed mesenchyme. BMP4 and BMP5 are co-expressed in a population of stromal cells associated with the trunk of the ureter. At 14.5 dpc, BMP4 expression is now observed in the maturing tubules along with BMP2 and BMP7. At 16.5 dpc, stippled blue staining represents down regulation of BMP7 expression in the maturing tubules located in the medulla region but strong expression remains in the podocyte layer of the glomeruli. In the cortex, BMP expression patterns associated with forming nephrons is maintained. In addition to the BMP expression patterns shown above, BMP6 is expressed in stromal cells associated with the tubules and collecting ducts.*

explant (Dudley *et al.*, 1999). Restricted diffusion of BMP molecules may result from strong binding to basement membrane proteins (Vukicevic *et al.*, 1994) or heparin sulfate containing proteoglycans (Ruppert *et al.*, 1996).

Expression patterns of some components of the BMP signal transduction pathway have been described in the developing kidney. BMP ligands transduce their signal through the formation of heteromeric complexes of two serine/threonine kinase receptors. The type II receptors are capable of binding ligand independently, but require the type I receptor for signaling. To date, two type I BMP receptors and one type II receptor have been identified. BMPRIA is expressed at 12.5 dpc predominantly in the mesenchyme while BMPRIB expression is restricted to the epithelial components of the collecting ducts (Dewulf *et al.*, 1995). By 18 dpc, BMPRIA is expressed in the glomeruli and tubules derived from the nephrogenic mesenchyme (Ikeda *et al.*, 1996). BMPRII has been shown to be expressed in the kidney at 16.5 dpc, most strongly in the mesenchymal cells (Roelen *et al.*, 1997).

Signal transduction downstream of the receptors involves members of the Smad family of transcriptional co-factors. Smad1 and Smad5 have been shown to be specific for mediating BMP signals, while Smad2 and Smad3 are responsible for transducing TGFβ signals. To date, only limited description of the expression patterns of members of the Smad family have been described for the kidney. Both Smad1 and Smad2 are expressed in the developing kidney at 13.5 dpc-17.5 dpc in both the collecting ducts and tubules (Dick *et al.*, 1998). More recently, Smad5 has been shown to be expressed in the adult kidney by Northern blot analysis (Yang *et al.*, 1999). In addition, high expression of Smad5 is also reported in the kidney at 12.5 dpc (data not shown in Meersseman *et al.*, 1997). Collectively, these data suggest that most of the cells in the developing kidney are competent to respond to BMP signaling. However, it is unclear whether particular cell populations are responsive to only a subset of BMP ligands, since BMP receptors bind ligands promiscuously *in vitro*. For example, BMP2, BMP4 and BMP7 have all been shown to bind to the type I BMP receptors BMPRIA or BMPRIB in concert with the type two receptor BMPRII. In addition, BMP7 can bind and activate activin receptors ActRI, ActRII and ActRIIA. Northern blot analysis has shown that ActRI is expressed in the adult rat and human kidney (Attisano *et al.*, 1993; Tsuchida *et al.*, 1993) but embryonic expression has not been reported. In contrast, Activin type II receptors have been shown to be expressed in the 12.5 dpc kidney (Feijen *et al.*, 1994), but detailed analysis has not been performed. Analysis of BMP signaling in the kidney is also complicated by possible interactions with antagonists, which include noggin, follistatin and members of the DAN family. However, a detailed description of BMP antagonist expression in the developing kidney has not been published, though follistatin was shown not to be expressed in the 12.5 dpc kidney (Feijen *et al.*, 1994). In addition, BMP signals can be modulated by receptor protein tyrosine kinase signaling in the MEK/ERK pathway (Kretzschmar and Massague, 1998). Studies suggest that both epithelial and mesenchymal cell populations in the kidney express receptors that activate this pathway (Orr-Urtreger *et al.*, 1991).

Phenotypes of BMP null mice

All members of the BMP family (2-7) have been analyzed through gene targeting. BMP2 and BMP4 null embryos die early during gastrulation and, therefore, preclude analysis of their role during kidney development (Winnier *et al.*, 1995; Zhang and Bradley, 1996). However, more recently the BMP4 targeted allele was backcrossed onto the C57BL/6 genetic background. In this analysis, a haploinsufficent phenotype was observed at low penetrance that included defects in the kidney (Dunn *et al.*, 1997). Unilateral cystic kidneys were observed in 12% of adult animals. Affected kidneys present with hydronephrosis, atrophy of the kidney cortex and many cysts involving both the tubules and glomeruli. Thus, inadequate levels of active BMP4 in the comma and S-shaped tubules may result in uncontrolled growth resulting in cyst formation. In contrast to BMP2 and BMP4, mutations in

members of the 60A subfamily of BMPs (BMP5, BMP6, BMP7) result in much less severe phenotypes. BMP5 null animals are viable and display defects in specific bone elements and several soft tissues (Green and Green, 1942; King *et al.*, 1994). On the CBA/Ca genetic background, more than 50% of animals display hydronephrosis and/or hydroureter (Green, 1968). Often the ureter is increased in length and convoluted though not dilated. This defect is consistent with the expression of BMP5 in the stromal cells surrounding the ureter. Perhaps BMP5 in the stromal cells plays a role in the morphogenesis of the growing ureter. BMP6 mutant mice are also homozygous viable and display only a slight delay in the growth of the sternum, no kidney defects have been observed (Solloway *et al.*, 1998). Functional redundancy among BMP family members has long been an issue. Recently, BMP5/BMP7 double mutants have been generated (Solloway and Robertson, 1999). Unfortunately, these embryos die by 10.5 dpc and, therefore, are uninformative in regard to kidney development. BMP6/BMP7 double mutants have also been generated in our laboratory and are currently being analyzed.

Only BMP7 has been shown to play a crucial role during early development of the metanephric kidney (Dudley *et al.*, 1995; Luo *et al.*, 1995). Mutant mice are born but most die within the first 24 h due to renal failure. One hundred per cent of these animals present bilateral dysplasia which is frequently accompanied by hydroureter. Other associated defects include micropthalmia or anopthalmia and hindlimb polydactyly with varying penetrance. The initial inductive interactions between the ureter and the mesenchyme are not affected as branching of the ureteric bud takes place and some glomeruli, derived from the nephrogenic mesenchyme, are formed. However, by 13.5 dpc there is a lack of condensed mesenchyme in the periphery, resulting in severely reduced size of the kidneys. Marker analysis of mesenchyme specific genes has shown that *Wnt4, Pax2, Pax8* and *WT1* are all initially induced but show a progressive loss of expression during development. This loss of gene expression most likely represents a loss of the metanephric mesenchyme due to apoptosis. Analysis of null alleles for BMP receptors, Smads and antagonists have not been informative in regard to kidney development, as all of these components are required much earlier in development and therefore result in lethality prior to the onset of metanephric development.

BMP7 as an inducer of metanephric mesenchyme?

Since BMP7 is initially expressed in the ureteric bud and it is a secreted signaling molecule, it has been predicted that BMP7 may function as an inducer of metanephric mesenchyme. However, direct tests of this hypothesis have led to controversy. The criteria for a molecule to function as an inducing factor for the metanephric mesenchyme has been described (Davies, 1996). Briefly: 1) the molecule must be expressed in the tips of the ureteric bud, the source of the inducing signal, 2) the molecule must be able to induce nephrogenesis in uninduced mesenchyme, and 3) inhibition of function must block the ability of the molecule to induce nephrogenesis. Based on the previous discussions, it is clear that BMP7 is expressed at the right time and in the right place to be considered a candidate inducing molecule. However, inhibition of *Bmp7* function via generation of a null mutation does not block nephrogenesis, suggesting that BMP7 is not an inducer of metanephric mesenchyme (Dudley *et al.*, 1995; Luo *et al.*, 1995).

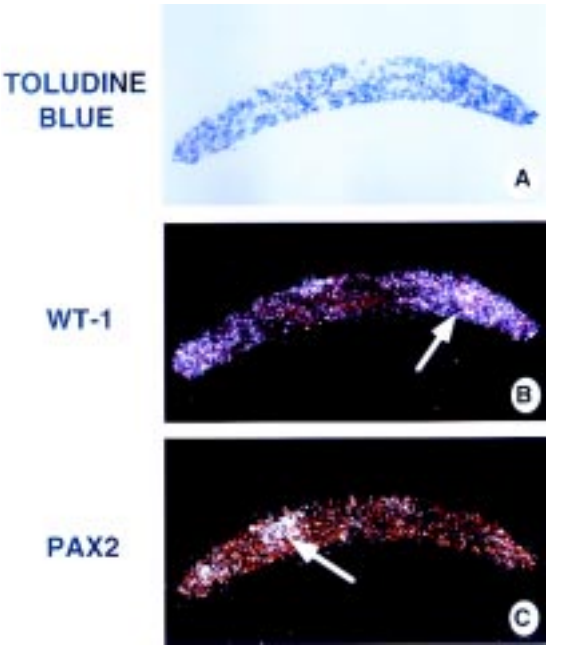

Fig. 2. Markers of induced cell types are expressed in untreated cultures of isolated metanephric mesenchyme. *Mesenchyme from 11.5 dpc embryos was separated from the ureteric bud following a brief trypsin treatment and explanted onto nuclepore rafts supported by a Trowell type device. After 24 h, explants were fixed in paraformaldehyde, embedded in paraffin, sectioned at 5 μm and subjected to in situ hybridization with radioactive RNA probes. Histological analysis of explants does not reveal evidence of mesenchymal condensation* **(A)***. However, subpopulations of cells expressWT-1* **(B)** *and Pax-2* **(C)***; two transcription factors upregulated in mesenchyme as a result of induction are observed (arrows).*

Vukicevic *et al.* (1996) demonstrated that antisense oligonucleotides directed against *Bmp7* transcripts and antibodies directed against BMP7 protein can block nephrogenesis in spinal cord-metanephric mesenchyme recombinant cultures. By contrast, we demonstrated that *Bmp7* mutant spinal cord can induce *BMP7* mutant mesenchyme in serum free culture, further supporting our contention that *Bmp7* is neither required for induction nor tubulogenesis (Dudley *et al.,* 1999). Since the spinal cord expresses other BMPs, it is possible that in this system induction is mediated by a functionally redundant family member. However, if this were the case, then Vukecevic *et al.* (1996) should have seen induction if their reagents are indeed specific to BMP7.

Although BMP7 signaling is not required for induction of metanephric mesenchyme, it is still important to ask whether BMP7 signaling can mediate an inductive response. Once again, conflicting data have been presented. Vukecevic *et al.* (1996) reported that recombinant BMP7 can induce expression of genes associated with induction in explants of metanephric mesenchyme. However, similar attempts have failed to replicate these results (Godin *et al.*, 1998; Dudley *et al.*, 1999). While it is impossible to conclude why these two groups obtained different results from the same experimental protocol, the data presented in these publications suggest one possible explanation. After 54 h in culture, explants treated with BMP7 by Vukicevic *et al.* (1996) display weak patches of cells expressing markers of induced mesenchyme, *Pax2* and *laminin α-1*, at levels similar to control cultures in which mesenchyme is induced by spinal cord. By contrast, Godin *et al.*

(1998) and Dudley *et al.* (1999) demonstrate significantly stronger expression of markers in mesenchyme co-cultured with spinal cord for 48 h. Although tubulogenesis is already apparent in these latter cultures, tubule formation is not evident in the cultures of Vukicevic *et al.* (1996). Moreover, the small, irregular patches of expressing cells in these cultures are inconsistent with the more regular, organized appearance of induced, condensed mesenchyme in explant culture. These data suggest that Vukicevic *et al.* (1996) were not observing inductive events. Why, then, are markers of induced cell types expressed? One possibility is that small groups of previously induced cells are maintained in these cultures in the presence of BMP7 signaling. Although, such cell populations were not observed in our experiments. Alternatively, the cells observed by Vukicevic *et al.* (1996) may not be nephrogenic mesenchyme cells. This is possible since none of the currently recognized markers for induced metanephric mesenchyme are specific for this event and cell type. For example, *Pax2*, *laminin α-1* and *Bmp7* are expressed in both the condensed mesenchyme and the epithelium. *Wnt4*, which is first expressed in the pretubular aggregates of nephrogenic mesenchyme, marks the onset of tubulogenesis not the initial inductive event, since it is not expressed in cultures of metanephric mesenchyme induced by lithium (Godin *et al.,* 1998). However, even *Wnt4* expression fails to unambiguously mark a specific mesenchyme cell population because as kidney development proceeds, *Wnt4* transcripts are detected in mesenchyme surrounding the renal pelvis and collecting ducts (Dudley *et al.*, 1995). Therefore, it is critical that positive signals for markers of induction are corroborated by the absence of definitive markers of collecting duct (*sonic hedgehog*, *Wnt7b*, *c-ret* and binding of the lectin *Dolichos Biflorus*) and stromal mesenchyme cell populations *(BF2*, *Bmp5* and *Bmp6*).

Even with specific markers, demonstrating that a factor can induce metanephric mesenchyme is technically very challenging. This is because the experiment is dependent on stimulating uninduced mesenchyme. An assumption has been made that since mesenchyme cultured in isolation dies, it is functionally uninduced. However, for most cultures, mesenchyme is obtained from 11-11.5 dpc kidney. At this stage, the mesenchyme cells have already received the inducing signal and markers for induction are already present in the isolated mesenchyme. Moreover, we have demonstrated that cultures of isolated mesenchyme continue to express markers of induction for up to 24 h (Fig. 2). Thus, the simple interpretation of experiments in which isolated mesenchyme treated with factors is seen to undergo nephrogenesis, is that the factor(s) either stimulated proliferation of already induced cells or enhanced the response to the inductive signal; not that the factor induced the mesenchyme. However, a newly developed assay can overcome this limitation. When isolated mesenchyme is cultured in the presence of FGF-2 and BMP-7 for 48 h, the resulting explant does not display any markers of induction, yet is competent to respond to induction by spinal cord or lithium ions (Dudley *et al.x*, 1999). This more rigorous assay should improve our understanding of the process of induction of metanephric mesenchyme.

Roles for BMP7 in early kidney development

Despite discrepancies in the results described above, data presented by these two groups demonstrates that BMP7 promotes survival of explants of metanephric mesenchyme that otherwise die in the absence of exogenously added factors. This has been demonstrated more directly using the fluorescent vital dye TOPRO-1, which is excluded from live cells. In explant culture, cells adjacent to beads soaked in BMP7 do not fluoresce but mesenchyme cells >150 μm from the bead display signal comparable to PBS treated control explants (Dudley *et al.*, 1999). Similar effects have been described for BMP2 (Godin *et al.*, 1998). A role for BMP7 in preventing cell death is consistent with the observation of apoptotic cells in the peripheral mesenchyme population of *Bmp7* null kidneys (Luo *et al.*, 1995; Dudley and Robertson, 1997). However, several growth factors have also been shown to rescue metanephric mesenchyme from apoptosis, including FGF2 and EGF (Weller *et al.*, 1991; Coles *et al.*, 1993; Perantoni *et al.*, 1995). Although many factors promote survival of explants, only mesenchyme cultured in the presence of FGF2 remains competent to undergo nephrogenesis in response to spinal cord (Dudley *et al.*, 1999). Since FGF2 signaling also maintains *Bmp7* expression in isolated mesenchyme (Godin *et al.*, 1998), it is possible that the ability to respond to inductive signals is mediated, in part, by BMP7. However, recent data suggest that BMP7 in addition to promoting survival also functions to inhibit differentiation of metanephric mesenchyme (Dudley *et al.*, 1999). Thus, when whole kidney explants are treated with BMP7, nephrogenesis is reversibly inhibited. Interestingly, this effect is upregulated by co-stimulation with FGF2. We have also observed that when a BMP7 soaked bead is placed in a mesenchyme explant that is subsequently induced by spinal cord, tubulogenesis does not take place adjacent to the bead, nor are markers of induction expressed. In contrast, cells further from the bead do respond to the inductive signal presented by the spinal cord (Fig. 3). Therefore, it seems that BMP7 can prevent apoptosis of the metanephric mesenchyme but in addition, actively functions to inhibit the differentiation of those cells. How do these proposed functions for BMP7 fit into the program of kidney development? One possibility is that following induction, mesenchyme cells are programmed to either differentiate into tubules or undergo apoptosis. *In vitro*, FGF2 signaling promotes survival of nephrogenic mesenchyme cells but does not maintain conditions necessary for tubulogenesis. Over time, the number of cells responsive to induction decreases, yielding a weak response after 48 h in culture. However, BMP7 signaling opposes further differentiation and therefore may prevent the nephrogenic mesenchyme cells from having to decide between tubulogenesis and death. Although BMP7 is produced by the nephrogenic mesenchyme cells, it may not function as an autocrine factor. Recent work demonstrates that BMP7 signaling, in conjunction with FGF2, promotes expansion of the stromal progenitor cell population adjacent to the nephrogenic mesenchyme (Dudley *et al.*, 1999). Since this growth occurs simultaneously with inhibition of tubulogenesis, it is intriguing to consider the possibility that these processes are connected. Thus, synergy between these signaling pathways expands the stromal precursor cell layer, as a result, there is an inhibition of nephrogenesis in the cortex and a decrease in the branching of the ureteric system. Inhibition of nephrogenesis can be released by placing the explants into fresh culture media lacking these factors. These results suggest that this treatment holds the nephrogenic cells in a stem cell state, thus one of the roles of the stromal cells is to maintain a population of undifferentiated cells at the periphery of the kidney. There is a growing body of evidence that suggests that the stromal cells play an important role

in the regulation of metanephric development. Recently, it has been shown that in RARα/β double mutants, the kidneys develop with reduced branching of the ureter, reduced numbers of nephrons and a loss of the nephrogenic zone (Mendelsohn *et al.*, 1999). Importantly, these receptors are expressed exclusively in the stromal precursor layer. Although BMP7 expression is maintained in these double mutant kidneys, it will be interesting to examine the possible relationship between RA and BMP7 signaling pathways in the nephrogenic and stromal cell populations of the kidney.

BMPs in epithelial morphogenesis and differentiation

Up to this point we have concentrated on roles for BMPs in the maintenance and differentiation of mesenchymal cell populations in the developing metanephros. As the kidney matures, however, most of the functional units are epithelial tissues derived from either the ureteric bud or by a mesenchyme to epithelial transformation of cells originating from the metanephric blastema. Considerably less attention has been paid to roles for BMPs in this context, however, once again, *Bmp7* figures prominently in this discussion.

All epithelial tissues of the pros-, meso- and metanephros express *Bmp7* at least transiently during development. However, a loss of *Bmp7* function does not block morphogenesis of epithelia of the mesonephros or metanephros. Defects in branching of the ureteric bud are observed in *Bmp7* null kidneys, but it is possible that these defects are the result of changes in the nephrogenic mesenchyme, a cell population that interacts reciprocally with the ureteric epithelium to promote branching. Unfortunately, no mesenchyme free system has been developed that allows study of ureteric bud morphogenesis. Several attempts utilizing heterologous mesenchyme have yielded buds with morphology inconsistent with that of an intact kidney (Sainio *et al.*, 1997; Kispert *et al.*, 1998). In these experiments, the ureteric bud assumes the shape of the endogenous epithelium and thus the mesenchyme appears to act instructively rather than passively. Despite this technical limitation, studies suggest that BMP signaling may play a role in ureteric bud morphogenesis. In whole kidney cultures, BMP7 has a dose dependent effect on both growth and branching of the ureteric bud. At low concentrations (<0.5 nM), BMP7 signaling promotes general growth of the explant and increased branching of the ureter while at higher doses there is a decrease in both parameters (Piscione *et al.*, 1997). In contrast, BMP2 is inhibitory to growth and branching. Moreover, using mIMCD3 cells as a model for tubule formation, it was shown that BMP7 and BMP2 have opposite effects on branching morphogenesis. BMP7 treated cells formed many branch points and were very thin displaying numerous filopodial extensions. In contrast, BMP2 treated cells formed shorter tubules with a wider diameter and very limited branching (Piscione *et al.*, 1997). Thus, BMP7 and BMP2 may play distinct, potentially opposing, roles in morphogenesis of developing tubules. This potential antagonism may prove important for morphogenesis in the distal part of the developing tubule where both *Bmp7* and *Bmp2* are transiently expressed. Moreover, it will be interesting to determine whether similar interactions extend to other members of the BMP gene family as different subsets of BMPs are expressed in regionally restricted patterns in the collecting ducts and proximal part of the early tubule. It is intriguing to consider that overlapping BMP signaling domains may contribute to regionalization of the epithelial tissues.

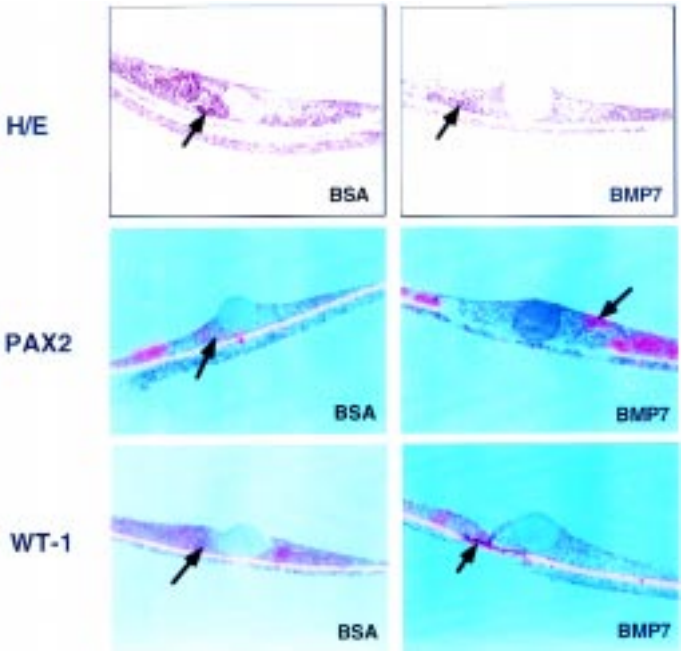

Fig. 3. BMP7 inhibits differentiation of metanephric mesenchyme. *Isolated mesenchyme grown in culture in the presence of spinal cord and a bead coated with either BSA or recombinant BMP7. Samples were then fixed, sectioned and processed for in situ hybridization. In the presence of BSA coated beads, tubules are formed and Pax2 and WT1 markers of induced mesenchyme are observed (arrows). In contrast, mesenchyme cells in the vicinity of a BMP7 coated bead are not induced, as tubules are not formed nor are markers of induced mesenchyme. However, further away from the bead, mesenchyme cells do respond to the inductive signals presented by the spinal cord (arrows).*

Factors that control BMP7 expression

The signals required for initiation of *Bmp7* transcription are currently unidentified. Our analysis of expression in embryonic kidney suggests two classes of molecules may be involved. Expression of *Bmp7* in isolated mouse metanephric mesenchyme is stimulated by co-culture with spinal cord or addition of lithium chloride. Lithium ions also promote condensation of metanephric mesenchyme without subsequent tubulogenesis. Although lithium ions are likely to act pleiotropically in biological systems, strong evidence exists for lithium acting to stimulate the Wnt signaling pathway. Thus, it is possible that Wnt molecules initiate *Bmp7* expression in condensed mesenchyme, cells fated to form epithelial tubules. Alternatively, *Bmp7* expression may be immediately downstream of a cell-cell adhesion event. It is interesting to note that in most cases, cells that express *Bmp7* in the kidney are either part of a tight epithelial sheet or in a condensed mesenchyme in which the cells display a semi-ordered appearance. Even in explant cultures treated with BMP7 and FGF2 prior to co-culture with spinal cord, cells expressing *Bmp7* that lie outside obvious epithelial structures appear in small aggregates. One exception to this is in similar cultures that were stimulated with lithium chloride rather than co-cultured with spinal cord. In this case we occasionally observe individual cells expressing *Bmp7*. Thus, it is possible that two distinct pathways can function to initiate *Bmp7* expression.

Once *Bmp7* expression is established in epithelial cells it appears to be maintained by a mechanism that is not dependent on specific mesenchyme derived signals. Expression in the ureteric bud is maintained in the presence of lung or limb mesenchyme, even though growth and branching fails to occur in the latter. One possibility is that *Bmp7* expression is maintained by an autoregulative mechanism. However, since expression of a *Bmp7*^{lacz} allele is not altered in a null environment, this possibility is unlikely. Since several *Wnt* family members are expressed in the ureter, it is possible that Wnt signaling plays a role in the maintenance of *Bmp7* expression as implied above. However, the addition of sodium chlorate, which disrupts Wnt signaling, to cultures of whole kidney does not affect expression of *Bmp7* in epithelial tissues. Moreover, *Wnt* gene family members are not expressed in all epithelial tissues of the kidney that express *Bmp7*. Therefore, an alternative mechanism is likely to exist. In each case, strong correlation exists between cells expressing *Bmp7* and the expression of *Pax2*. Thus it is possible that either Pax2 maintains *Bmp7* expression or that the expression of both genes is regulated by a common mechanism. Interestingly, although *in situ* hybridization analysis of the developing kidney reveals ubiquitous expression of *Bmp7* in epithelial tissues, analysis of staining patterns in *Bmp7*^{lacz} embryos suggests that BMP7 may not be produced by all epithelial cells throughout development. For example, analysis of LacZ staining patterns suggests a rapid downregulation of *Bmp7* expression in the distal part of the early tubule. Likewise, as the ureteric bud branches, LacZ activity is robust in all cells at the tips of the ureter but only in certain cells in the trunk region. It will be interesting to explore the mechanisms that downregulate expression. What is the significance of the small number of LacZ positive cells remaining in the collecting ducts and why do they persist into adulthood? One characteristic of mature epithelium is the ability to replace cells that are damaged or shed through normal function or following injury. BMP7 signaling may be an important component of this process. Following ischemic injury to the adult rat kidney, there is an apparent upregulation of *Bmp7* expression in the tubules as well as an increase in the number of expressing cells (Simon *et al.*, 1999). Consistent with this observation, intravenous administration of BMP7 post insult improved both the histological appearance and function of the kidney (Vukicevic *et al.*, 1998). Although the target population of BMP7 signaling has not been defined in the adult kidney, it is possible that these cells represent a BMP7-dependent "stem cell"-like population that maintains the integrity of the collecting ducts postnatally.

BMPs and other cell types later in development

To date, much of the focus on metanephric development concerns interactions between three cell populations: the ureteric epithelium, the nephrogenic mesenchyme and the stromal progenitor cell mesenchyme. However, function of the mature kidney also relies on several additional tissues, two of which are vasculature and neurons. Both endothelial and neural progenitors are present at or soon after the first induction event in kidney development (Sariola *et al.*, 1988; Pinson-Hyink *et al.*, 1996). In both cases, the majority of cells are found in close proximity to epithelial structures. In these environments the progenitors are surrounded by populations expressing several BMPs and depending on migration of the progenitors within the kidney, these cells may experience signaling by different subsets of BMP family members (Fig. 1). Interestingly, in neural crest cultures, BMP2, BMP4 and BMP7, as well as constitutively active type I BMP receptor, can stimulate formation of cells displaying characteristics of adrenergic neurons (Varley *et al.*, 1995, 1996, 1998). BMP6, however, does not produce this effect (Varley *et al.*, 1996). Furthermore, BMP2 can bind to receptors on endothelial cells (Iwasaki *et al.*, 1995) and a loss of Smad5 function, a downstream component of the BMP signaling pathway, results in defects in angiogenesis (Yang *et al.*, 1999).

Summary

Although many BMP family members are expressed during organogenesis of the kidney, the majority of analysis has focused on the function of BMP7. The data discussed above clearly shows that BMP7 functions as a survival factor for the metanephric mesenchyme. However, there is conflicting data for BMP7 functioning as an inducing molecule. Although one report suggests that recombinant BMP7 can induce markers of induction and tubule formation, we have not been able to replicate these results. Moreover, recombination experiments using BMP7 null tissue in which tubule formation is observed and the phenotype of three independently derived lines of BMP7 null mice, all of which show initial tubule formation and more mature glomeruli, strongly suggest that BMP7 is not required for induction of the metanephric mesenchyme. According to the guidelines established for an inducing molecule, this rules out BMP7 as an inducer of the metanephric mesenchyme.

One of the main questions unanswered to date is the mechanism of BMP signaling. For example, do different BMP family members form heterodimers? *In vitro,* these molecules have been produced and have shown to be more potent than the individual homodimers. However, such heterodimers have not been identified *in vivo*. Functional redundancy amongst members of the BMP family also remains an open question. However, detailed analysis of the expression patterns and creation of double mutants suggests that this may be the case. We are currently investigating this possibility further through targeted gene swap experiments. These experiments will directly test, *in vivo*, the ability of BMP family members to functionally substitute for one another.

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References

ATTISANO, L., CARCAMO, J., VENTURA, F., WEIS, F.M.B, MASSAGUE, J. and WRANA, J.L. (1993). Identification of human activin and TGFB type I receptors that form heteromeric kinase complexes with type II receptors. *Cell 75:* 671-680.

COLES, H.S.R., BURNE, J.F. and RAFF, M.C. (1993). Large-scale normal cell death in the developing rat kidney and its reduction by epidermal growth factor. *Development 118:* 777-784.

DAVIES, J.A. (1996). Mesenchyme to epithelium transition during development of the mammalian kidney tubule. *Acta. Anat. 156:* 187-201.

DEWULF, N., VERSCHUEREN, K., LONNOY, O., MOREN, A., GRIMSBY, S., VANDE SPIEGEL, K., MIYAZONO, K., HUYLEBROECK, D. and TEN DIJKE, P. (1995). Distinct spatial and temporal expression patterns of two type I receptors for bone morphogenetic proteins during mouse embryogenesis. *Endocrinology 136:* 2652-2663.

DICK, A., RISAU, W. and DREXLER, H. (1998). Expression of Smad 1 and Smad 2 during embryogenesis suggests a role in organ development. *Dev. Dynamics 211:* 293-305.

DUDLEY, A.T. and ROBERTSON, E.J. (1997). Overlapping expression domains of bone morphogenetic protein family members potentially account for limited tissue defects in BMP7 deficient embryos. *Dev. Dynamics 208:* 349-362.

DUDLEY A.T., GODIN, R.E and ROBERTSON, E.J (1999). Interaction between FGF and BMP signaling pathways regulates development of metanephric mesenchyme. *Genes Dev. 13:* 1601-1613.

DUDLEY, A.T., LYONS, K.M. and ROBERTSON, E.J. (1995). A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev. 9:* 2795- 2807.

DUNN, N.R., WINNIER, G.E., HARGETT, L.K., SCHRICK, J.J., FOGO, A.B. and HOGAN, B.L.M. (1997). Haploinsufficient phenotypes in BMP4 heterozygous null mice and modification by mutations in Gli3 and Alx4. *Dev. Biol. 188:* 235-247

FEIJEN, A., GOUMANS, M.-J. and VAN DEN EIJNDEN-VAN RAAIJ, A.J.M. (1994). Expression of activin subunits, activin receptors and follistatin in postimplantation mouse embryos suggests specific developmental functions for different activins. *Development 120:* 3621-3637.

GODIN, R.E., TAKAESU, N., ROBERTSON, E.J. and DUDLEY, A.T. (1998). Regulation of Bmp7 expression during kidney development. *Development 125:* 3473-3482.

GREEN, E.L. and GREEN, M.C. (1942). The development of three manifestations of the short ear gene in the mouse. *J. Morphol. 70:* 1-19.

GREEN, M.C. (1968). Mechanism of the pleiotropic effects of the short ear mutant gene in the mouse. *J. Exp. Zool. 167:* 129-150.

IKEDA, T., TAKAHASHI, H., SUZUKI, A., UENO, N., YOKOSE, S., YAMAGUCHI, A. and YOSHIKI, S. (1996). Cloning of rat type I receptor cDNA for bone morphogenetic protein -2 and bone morphogenetic protein-4, and the localization compared with that of the ligands. *Dev. Dynamics 206:* 318-329.

IWASAKI, S., TSURUOKA, N., HATTORI, A., SATO, M., TSUJIMOTO, M. and KOHNO, M. (1995). Distribution and characterization of specific cellular binding proteins for bone morphogenetic protein 2. *J. Biol. Chem. 270:* 5476-5482.

JONES, C.M. and SMITH J.C. (1998). Establishment of a BMP4 morphogen gradient by long range inhibition. *Dev. Biol. 194:* 12-17.

KING, J.A., MARKER, P.C., SEUNG, K.S. and KINGSLEY, D.M. (1994). BMP5 and the molecular, skeletal and soft tissue alterations in short ear mice. *Dev. Biol. 166:* 112-122.

KISPERT, A., VAINIO, S. and MCMAHON, A.P. (1998). Wnt4 is a mesenchymal signal for epithelial transformation of metanephric mesenchyme in the developing kidney. *Development 125*: 4225-4234.

KRETZSCHMAR, M. and MASSAGUE, J. (1998). Smads: mediators and regulators of TGF-beta signaling. *Curr. Opin. Genet. Dev. 8:* 103-111.

LUO, G., HOFMANN, C., BRONCKERS, A.L.J.J., SOHOCKI, M., BRADLEY, A. and KARSENTY, G. (1995). BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. *Genes Dev. 9:* 2808-2820.

MEERSSEMAN, G., VERSCHUEREN, K., NELLES, L., BLUMENSTOCK, C., KRAFT, H., WUYTENS, G., REMACLE, J., KOZAK, C.A., TYLZANOWSKI, P., NIEHRS, C., and HUYLEBROECK, D. (1997). The C-terminal domain of mad like signal transducers is sufficient for biological activity in the Xenopus embryo and transcriptional activation. *Mech. Dev. 61:* 127-140.

MENDELSOHN, C., BATOURINA, E., FUNG, S., GILBERT, T. and DODD, J. (1999). Stromal cells mediate retinoid-dependent functions essential for renal development. *Development 126:* 1139-1148.

ORR-URTREGER, A., GIVOL, D., YAYON, A., YARDEN, Y. and LONAI, P. (1991). Developmental expression of two murine fibroblast growth factor receptors, flg and bek. *Development 113:* 1419-1434.

PERANTONI, A.O., DOVE, L.F. and KARAVANOVA, I**.** (1995). Basic fibroblast growth factor can mediate the early inductive events in renal development. *Proc. Natl. Acad. Sci. USA 92:* 4696-4700.

PINSON-HYINK, D., TUCKER, D.C., ST JOHN, P. T., LEARDKAMDKARN, V., ACCAVITTI, M. A., ABRASS, C.K. and ABRAHAMSON, D.R. (1996)Endogenous origin of glomerular endothelial and mesangial cells in grafts of embryonic kidneys. *Am. J. Physiol. 270:* F886-899.

PISCIONE, T.D., YAGER, T.D., GUPTA, I.R., GRINFIELD, B., PEI, Y., ATTISANO, L., WRANA, J.L. and ROOSENBLUM, N.D. (1997). BMP2 and OP1 exert direct and opposite effects on renal branching morphogenesis. *Am. J. Physiol. 273:* F961-F975.

ROELEN, B.A.J., GOUMANS, M.-J., VAN ROOIJEN, M.A. and MUMMERY, C.L. (1997). Differential expression of BMP receptors in early mouse development. *Int. J. Dev. Biol. 41:* 541-549.

RUPPERT, R., HOFFMANN, E. and SEBALD, W. (1996). Human bone morphogenetic protein 2 contains a heparin binding site which modifies its biological activity. *Eur. J. Biochem. 237:* 295-302.

SAINIO, K., SUVANTO, P., DAVIES, J., WARTIOVAARA, J., WARTIOVARA, K., SAARMA, M., AMMAE, U., MENG, X., LINDAHL. M., PACHNIS, V. and SARIOLA, H. (1997). Glial cell line derived neurotrophic factor is required for bud initiation from the ureteric epithelium. *Development 124:* 4077-4087.

SARIOLA, H., HOLM, K. and HENKE-FAHLE, S. (1988). Early innervation of the metanephric kidney. *Development 104*: 589-599.

SIMON, M., MARESH, J.G., HARRIS, S.E., HERNANDEZ, J.D., ARAR, M., OLSON, M.S. and ABBOUD, H.E. (1999). Expression of bone morphogenetic protein-7 mRNA in normal and ischemic adult rat kidney. *Am. J. Physiol. 276*: F382-389.

SOLLOWAY, M.J. and ROBERTSON, E.J. (1999). Early embryonic lethality in BMP5;BMP7 double mutant mice suggests functional redundancy within the 60A subgroup. *Development 126*: 1753-1768.

SOLLOWAY, M.J., DUDLEY, A.T., BIKOFF, E.K., LYONS, K.M., HOGAN, B.L. and ROBERTSON, E.J. (1998). Mice lacking BMP6 function. *Dev. Genet. 22:* 321-339.

TSUCHIDA, K., MATHEWS, L.S. and VALE, W.W. (1993). Cloning and characterization of a transmembrane serine kinase that acts as an activin type I receptor. *Proc. Natl. Acad. Sci. USA. 90:* 11242-11246.

VARLEY, J.E. and MAXWELL, G.D. (1996). BMP-2 and BMP-4, but not BMP-6, increase the number of adrenergic cells which develop in quail trunk neural crest cultures. *Exp. Neurol. 140:* 84-94.

VARLEY, J.E., MCPHERSON, C.E., ZOU, H., NISWANDER, L. and MAXWELL, G.D. (1998). Expression of a constitutively active type I BMP receptor using a retroviral vector promotes the development of adrenergic cells in neural crest cultures. *Dev. Biol. 196:* 107-118.

VARLEY, J.E., WEHBY, R.G., RUEGER, D.C. and MAXWELL, G.D. (1995). Number of adrenergic and islet-1 immunoreactive cells is increased in avian trunk neural crest cultures in the presence of human recombinant osteogenic protein-1. *Dev. Dynamics 203*: 434-447.

VUKICEVIC, S., BASIC, V., ROGIC, D., BASIC, N., SHIH, M.S., SHEPARD, A., JIN, D., DATTATREYAMURTY, B., JONES, W., DORAI, H., RYAN, S., GRIFFITHS, D., MALIAKAL, J., JELIC, M., PASTORCIC, M., STAVLJENIC, A. and SAMPATH, T.K. (1998). Osteogenic protein-1 (bone morphogenetic protein-7) reduces severity of injury after ischemic acute renal failure in rat. *J. Clin. Invest. 102:* 202-214.

VUKICEVIC, S., KOPP, J.B., LUYTEN, F.P. and SAMPATH, T.K. (1996). Induction of nephrogenic mesenchyme by osteogenic protein 1 (bone morphogenetic protein 7). *Proc. Natl. Acad. Sci. USA 93:* 9021-9026.

VUKICEVIC, S., LATIN, V., CHEN, P., BATORSKY, R., REDDI, A.H. and SAMPATH, K. (1994). Localization of osteogenic protein-1 (Bone morphogenetic protein 7) during human embryonic development: high affinity binding to basement membranes. *Biochem. Biophys. Res. Commun 198:* 693-700.

WELLER, A., SOROKIN, L., ILLGREN, E.M. and EKBLOM, P. (1991). Development and growth of mouse embryonic kidney in organ culture and modulation of development by soluble growth factors. *Dev. Biol. 144:* 248-261.

WINNIER, G., BLESSING, M., LABOSKY, P.A. and HOGAN, B.L.M. (1995). Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev. 9:* 2105-2116

YANG, X., CASTILLA, L.H., XU, X., LI, C., GOTAY, J., WEINSTEIN, M., LIU, P.P. and DENG, C.-X. (1999). Angiogenesis defects and mesenchymal apoptosis in mice lacking smad 5. *Development 126:* 1571-1580.

ZHANG, H. and BRADLEY, A. (1996). Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. *Development 122:* 2977-2986.