Int. J. Dev. Biol. 50: 511-521 (2006)
doi: 10.1387/ijdb.052101xn

Review

BMP signalling in craniofacial development

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KEY WORDS: *growth/differentiation factor, signalling, craniofacial development, abnormality*

The head, comprising of the skull and the face, is the most anatomically sophisticated portion and evolutionary novelty in vertebrates. The most striking feature for the development of the vertebrate head is the emergence of the neural crest and its integration into the formation of craniofacial elements. The cranial neural crest, emerged from dorsal and lateral neural crest, contains multi-potent cells with high plasticity (Trainor *et al.*, 2003). After specification, these cells undergo well orchestrated migration and eventually populate the branchial arches. Once they have reached their destinations, these cells proliferate and differentiate into specific cell types and give rise to a number of craniofacial elements through epithelial-mesenchymal interactions. In subsequent development, facial primordia undergo a quick outgrowth and midline union and eventually form a continuous face. These developmental processes are controlled by a complex genetic cascade that involves in a number of critical developmental genes such as members of the *BMP* family.

The presence of BMP was initially implicated by the pioneering work of Urist who first discovered bone autoinduction by bone matrix (Urist, 1965). Thereafter, a number of BMPs were isolated and cloned (Wozney *et al.*, 1988). BMPs are now recognized as multifunctional growth factors that mediate a variety of biological functions essential for gastrulation, organogenesis and embryonic and postnatal growth. BMPs are also important participants in craniofacial development. How this conserved signal regulates the development of the phylogenetically new mammalian head remains an interesting and incompletely known issue. The use of mouse models and modern molecular techniques have greatly facilitated the process of craniofacial research and extended our understanding of craniofacial development in the past decade. The BMP signalling pathway has been shown to be involved in a number of developmental processes and critical for the formation of a variety of craniofacial elements including cranial neural crest, facial primordia, tooth, lip and palate. In the present review, the roles of BMP signalling in craniofacial development are discussed in detail, with a focus on recent advances.

Brief overview of the BMP gene family and the BMP signalling pathway

The *BMP* gene family belongs to *TGF-β* superfamily. Individually, members of this family are called either *BMPs*, osteogenic proteins (OP), cartilage derived morphogenetic protein (CDMP), or growth and differentiation factor (GDF). These members are divided into subfamilies based on phylogenetic analysis and sequence similarity. *BMP2* and *BMP4* are grouped as the *dpp* subfamily because of their similarity to the ancestral *dpp* gene

Abbreviations used in this paper: BMP, bone morphogenetic protein; CDMP, cartilage-derived morphogenetic protein; CNCC, cranial neural crest cell; GDF, growth and differentiation factor; NCC, neural crest cell; OP, osteogenic protein; r, rhombomere.

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0214-6282/2006/$25.00

Printed in Spain
www.intjdevbiol.com

(decapentaplegic) of *Drosophila*; *BMP5, -6, -7* and *-8* are grouped as the 60A subfamily; *BMP3* and *BMP3b* (*GDF10*) together constitute a unique subfamily; *GDF5, -6, -7* constitute another subgroup in this family; Nodal and Lefty are distant members of the *BMP* subfamily. So far, more than 20 members have been identified in the *BMP* family (Kishigami and Mishina, 2005). In Tables 1 and 2, most of the *Bmp* members are listed. Additional members are continued to be identified. One of the *BMPs*, *BMP1*, does not belong to the *TGF* superfamily. In mice, *Bmp1* encodes the most abundant protein of the bone extracellular matrix, a protease that releases the carboxy-terminal propeptide from the type I procollagen molecule. The members belong to the *dpp* (*BMP2, BMP4*) and 60A subfamilies (*BMP 5-8*) have been extensively investigated in embryonic and postnatal development. This review only focuses on the important participants of craniofacial development.

BMPs mainly function through BMP receptor (BMPR) type I and type II (BMPRI and BMPRII). Three type I (ALK2, ALK3 or BMPRIA and ALK6 or BMPRIB) and three type II receptors (BRII, ActRIIA and ActRIIB) known to mediate the signal have been identified (Nohe *et al.*, 2004). Generally, the type I receptors are the high-affinity binding receptors, whereas the type II receptors bind BMPs with low affinity. Binding of BMP to BMPR results in phosphorylation of downstream Smad proteins and thus triggers the intracellular signal cascade. The BMP signal activates Smad1, Smad5 and Smad8, which upon phosphorylation can associate with Smad4 (common Smad) into a heterodimeric complex that is further translocated to the nucleus where it activates transcription (Nohe *et al.*, 2004). Smad6 and Smad7, on the other hand, are inhibitory to this signal transduction (Nohe *et al.*, 2004). In addition to the Smad pathway, BMPs are believed to activate several different kinase pathways including MAPK, PI3 kinase and PKC (Kishigami and Mishina, 2005).

BMP signalling in the development of the cranial neural crest and facial primordia

Neural crest cells (NCCs) arise uniformly at the dorso-lateral edge of the closing neural folds along almost the entire length of the vertebrate embryo neuraxis, a region corresponds to the interface between the non-neural ectoderm and the neural plate. They are considered as stem-cell-like cells that show high plasticity (Trainor *et al.*, 2003). After neurulation, NCCs delaminate and migrate along defined pathways to differentiate into a variety of cells and tissues. NCCs that are originated from the anterior neural tube (forebrain, midbrain and anterior hindbrain), called the cranial neural crest cells (CNCCs), populate the facial primordia and the pharyngeal arches and eventually differentiate into typical neural crest derivatives such as skeletons, neurons, cranial ganglions and connective tissues.

The formation of neural crest is a complex process that needs a cooperation of a number of critical signalling including the BMP, WNT and FGF pathways. The BMP signalling has long been recognized as a patterning signal for neural crest formation. It is found that *Bmp4* and *Bmp7* are present in the ectoderm and can substitute for non-neural ectoderm for neural crest cell induction (Liem *et al.*, 1995). Bmp4 is responsible for the maintenance of a variety of dorsal neural tube genes such as *Msx1, Msx2 and Slug* (Trainor *et al.*, 2003, Tribulo *et al.*, 2003) and a gradient of Bmp seems essential for specification of the neural plate and neural crest (Tribulo *et al.*, 2003). In addition to the inductive role, Bmp signalling is also required for migration of CNCCs to the facial primordia (Kanzler *et al.*, 2000, Knecht and Bronner-Fraser, 2002, Tribulo *et al.*, 2003). Blocking of Bmp2/Bmp4 in mouse cranial neural crest by Xnoggin leads to depletion of CNCCs from the targeted areas; as a consequence, the branchial arches, normally populated by NCCs, are hypomorphic and their skeletal and neural derivatives fail to development (Kanzler *et al.*, 2000).

During early craniofacial development, *Bmp4*, *Bmp2* and *Bmp7* are prominently expressed (Bennett *et al.*, 1995, Francis-West *et al.*, 1998, Francis-West *et al.*, 1994). In the early facial primordia high epithelial expression of *Bmp4* and *Bmp7* is associated with mesenchymal expression of *Bmp2*, *Msx1* and *Msx2* (Barlow and Francis-West, 1997, Bennett *et al.*, 1995, Francis-West *et al.*, 1998). Later, *Bmp4* is also expressed in the mesenchyme of facial primordia (Barlow and Francis-West, 1997). Ectopic application of recombinant BMP2 or BMP4 also activates the expression of *Msx* genes at sites where normally do not have *Msx* expression (Barlow and Francis-West, 1997). This signalling cascade is correlated with altered expression of *Fgf4* and *Shh* and leads to abnormal development of the facial primordia (Barlow and Francis-West, 1997).

Unfortunately, conventional knockout approach on *Bmps* does not reveal much information about the roles of *Bmps* in craniofacial development due to either early embryo death or functional redundancies among this family. For instance, deletion of *Bmp2* or *Bmp4*, the prototypes of *drosophila* homologue (decapentaplegic), leads to early embryonic death around gastrulation in mice (Winnier *et al.*, 1995, Zhang and Bradley, 1996), hampering the efforts to analyze their functions at late times; deletion of other members, however, does not show major defects in mouse craniofacial structures. Inactivation of *Bmprs* also leads to early lethality prior to craniofacial development (Beppu *et al.*, 2000, Mishina *et al.*, 1999, Mishina *et al.*, 1995). Conditional knockout and double knockout approaches are more useful than conventional knockout. Mice deficient of *Alk2* in the neural crest (*Alk2*$^{nc-/-}$) or *Bmpr1a* from the facial primordia (*Bmpr Ia*$^{fp-/-}$) show remarkable developmental anomalies in facial structures, implicating essential roles of Bmp signalling in a variety of developmental processes (Dudas *et al.*, 2004, Liu *et al.*, 2005). *Bmp5/Bmp7* double mutants show underdevelopment of the branchial arches (Solloway and Robertson, 1999). Expression profiles of some *Bmps* and knockout phenotypes in mice are summarized in Tables 1 and 2.

Orofacial union is an important mechanism for craniofacial development. The facial primordia, palate and tongue all undergo a midline union in early development. Perturbation of this process leads to various orofacial clefts such as cleft lips, cleft palate, oblique facial cleft, lateral facial cleft, mandibular cleft and cleft tongue. Bmp signalling is a critical mediator in this process as indicated by *Alk*-deficient mice, in which orofacial cleft is one of the major anomalies (Dudas *et al.*, 2004, Liu *et al.*, 2005). *Bmp4*, which is highly expressed in the putative orofacial epithelia prior to facial union, is implicated as a good candidate for this role (Gong and Guo, 2003). A recently generated mouse model with inactivation of *Bmp4* from facial primordia provides convincing evidence of this role (Liu *et al.*, 2005). In those mice, the lip development is delayed and *Fgf8*, a critical gene for early facial

TABLE 1

BMP/BMPR CRANIOFACIAL EXPRESSION AND KNOCKOUT PHENOTYPES IN MICE AND RELATED DISORDERS

	Expression profiles in developing craniofacial structures	Phenotype of Knockout or loss-function mutants and related disorders
Bmp2	Cranial neural crest, early facial primordia, skeletal precursor, hypertrophic chondrocytes, dental mesenchyme, odontoblasts, palate, tongue (Aberg et al., 1997, Bennett et al., 1995, Francis-West et al., 1994, Lu et al., 2000, Nie, 2005a, Nie, 2005c, Yamashiro et al., 2003)	Die around E7.5 to E10.5, defects in cardiac development (Zhang and Bradley, 1996)
Bmp4	Cranial neural crest, early facial primordia, skeletons, dental epithelia and mesenchyme, preodontoblasts, palate, tongue (Aberg et al., 1997, Bennett et al., 1995, Francis-West et al., 1994, Gong and Guo, 2003, Lu et al., 2000, Nie, 2005a, Nie, 2005c, Vainio et al., 1993, Yamashiro et al., 2003)	Die between E6.5 to E9.5, little or no mesoderm formation (Winnier et al., 1995) Conditioned knockout in craniofacial region: cleft lips (Liu et al., 2005) Related disorder: fibrodysplasia ossificans pregressiva (OMIM, 135100)
Bmp3 (osteogenin)	Osteoblast, cementoblasts and follicles of tooth, tongue, palate (Aberg et al., 1997, Nie, 2005a, Nie, 2005c, Yamashiro et al., 2003)	High bone density (Daluiski et al., 2001)
Bmp5	Skeleton, ear, epithelial ameloblasts, palate, tougue (Aberg et al., 1997, King et al., 1994, Lu et al., 2000, Nie, 2005a)	Sponteneous mutation: viable, short ears with skeleton defects (King et al., 1994, Kingsley et al., 1992) Related disorder: short ear phenotype in mice
Bmp6	Hypertrophic chondrocytes, osteoblasts, tooth pulp, tongue (Eames and Helms, 2004, Nakashima et al., 1998, Nie, 2005a)	Viable and fertile, mild defects in the sternum (Solloway et al., 1998)
Bmp7 (Osteogenic protein 1, OP1)	Early orofacial epithelial, skeleton, eyes, notochord, early tooth (Aberg et al., 1997, Barlow and Francis-West, 1997, Dudley and Robertson, 1997)	Die around birth, deformities in kidney and eyes, a hole in the basisphenoid bone and xyphoid cartilage (Dudley et al., 1995, Jena et al., 1997) Bmp5/7 double knockout mice: die at E10.5, reduced size of branchial arches (Solloway and Robertson, 1999)
Bmp8 (OP2)	Early embryogenesis, skeleton, tooth pulp (DiLeone et al., 1997, Nakashima et al., 1998, Ozkaynak et al., 1992)	Germ cell degeneration (Zhao et al., 1998)
Gdf1	Ventral neural tube, midbrain, paraxial mesoderm (Rankin et al., 2000, Wall et al., 2000)	Defects in left-right axis formation (Rankin et al., 2000)
Gdf5 (CDMP1)	Joint, tooth attachment apparatus and pulp (Nakashima et al., 1998, Sena et al., 2003, Settle et al., 2003)	Naturally occurred mutation in mice: brachypod syndrome, (Storm et al., 1994, Storm and Kingsley, 1996) Related disorders: loss function mutation associated with Brachychod in mice, chondrodysplasia Grebe type (OMIM, 200700) and Hunter-Thompson type of acromesomelic dysplasia (OMIM, 201250) in human. Haploinsufficiency mutation associated with brachydactyly type C (OMIM, 113100)
Gdf6 (Bmp13/CDMP2)	Coronal suture of the calvaria, middle ear cartilage, tooth attachment apparatus and pulp (Nakashima et al., 1998, Sena et al., 2003, Settle et al., 2003)	Defects in middle ear cartilage, absence of coronal suture (Settle et al., 2003) Related disorder: possibly related to carniosynostosis
Gdf7 (Bmp12/CDMP3)	Roof plate of the brain, tooth attachment apparatus (Lee et al., 1998, Nakashima et al., 1998, Sena et al., 2003, Settle et al., 2001)	In mutant mice, the D1A neurons is eliminated (Lee et al., 1998)
Gdf8 (myostatin)	Muscles (McPherron et al., 1997)	Large size and increased muscle mass (Byron et al., 2004, McPherron et al., 1997) Related disorders: gross muscle hypertrophy (Schuelke et al., 2004)
Gdf10 (Bmp3b)	Basicranium, nasal cavity, inner ear, palate, brain (Zhao et al., 1999)	No obvious abnormity (Zhao et al., 1999)
Gdf11 (Bmp11)	Branchial arch, nasal epithelium, retina, palate, odontoblasts, brain (McPherron et al., 1999, Nakashima et al., 1999)	Die shortly after birth, renal defect, anteriorly directed homeotic transformation of axis skeleton, palate anomaly (McPherron et al., 1999)
Alk2	Facial primordia, pharyngeal branches (Dudas et al., 2004)	Die before E9.5 (Gu et al., 1999, Mishina et al., 1999) Conditioned knockout in craniofacial region: survived, short head, hypotrophic and deformed mandible, incomplete zygomatic arch, Meckel cartilage abnormity, cleft palate (Dudas et al., 2004)
BmprIa (Alk3)	Broadly expressed in craniofacial region (Dewulf et al., 1995)	Die around E9, no mesoderm formation (Mishina et al., 1995) Conditioned knockout in craniofacial region: survived, bilateral cleft palate, tooth arrestment (Liu et al., 2005) Related disorder: Juvenile intestinal polypopsis (OMIM, 174900)
BmprIb (Alk6)	Skeleton, premuscle masses, blood vessels, central nervous system, developing ear and eye, and epithelium (Dewulf et al., 1995)	Viable, appendicular skeleton defects (Yi et al., 2000)
BmprII	Ubiquitously in early embryo (Beppu et al., 2000)	Die before E9.5, fail to form organized structure and mesoderm (Beppu et al., 2000) Related disorder: Pulmonary hypertension (OMIM, 178600)

development, fails to be upregulated at a crucial developmental stage and results in cleft lips. Those authors demonstrated that the Bmp4/BmprIa pathway is critical for the lip development (Liu *et al.*, 2005). This model also implies that *Bmp4* is a possible candidate gene related to isolated cleft lips. Normal orofacial union of the other parts in this model suggests distinct mechanisms of the orofacial union along the anterior-posterior axis. Expression of *Bmp4* is shown in the early developing facial primordia in Fig. 1.

Knockout of *Bmp7*, the other *Bmp* member expressed in early facial epithelia, shows no obvious defects in facial structures except for the eyes (Dudley *et al.*, 1995, Luo *et al.*, 1995, Solloway and Robertson, 1999), while *Bmp5/Bmp7* double knockout mice show early embryo death and deficiency of facial primordia development, suggesting functional redundancies among the 60A subgroup of the *Bmp* gene family. Functional redundancy is likely an evolutionary strategy for some of the crucial genes.

BMP signalling in craniofacial skeletogenesis

The BMP signal is critical for skeletogenesis throughout the body, regulating chondrogenesis and osteogenesis at multiple stages. The molecular mechanism of BMPs in these processes has been extensively investigated, producing a large body of literatures in the past decade. BMP signalling is involved in determination, migration, condensation, proliferation, differentiation and apoptosis of skeletal cells. In those processes, the Bmp signalling pathway interacts with Fgf, Hedgehog and Wnt signalling pathways and regulates the expression of several critical transcription factors such as Sox9, Cbfa1 and Msx (Karsenty, 1999, Karsenty, 2003, Minina *et al.,* 2002, Minina *et al.,* 2001, Naski *et al.,* 1998). In the Bmp gene family, Bmp2, -4, -7, -3, -5 and Gdf5 are critical in skeletogenesis. The vast majority of studies on skeletogenesis have been carried out using appendicular bones as a model system. A number of reviews concerning the roles of Bmps in skeletogenesis are available (Chen *et al.,* 2004, Karsenty, 1999, Karsenty, 2003, Yoon and Lyons, 2004). However, craniofacial skeletogenesis is not identical to that of appendicular bones (Eames and Helms, 2004, Helms and Schneider, 2003). This part of the review focuses on the roles of Bmp signalling in craniofacial skeletogenesis.

Bones in craniofacial area are mostly derived from the CNCCs and formed through intramembranous ossification, in which process mesenchymal cells directly condense and differentiate into osteoblasts without any cartilage model. One exception is the basicranium, which is formed through endochondral ossification, in which process bone formation is guided by a cartilage template similar to that of the appendicular bones. The vertebrate basicranium evolutes from the ancient floor of the brain, with evolutionary new elements derived from the CNCCs in its anterior part (Nie, 2005b). During calvaria development, open fibrous sutures among intramembranous bones are formed as growth centres. In the endochondral basicranium, cartilaginous growth centres that are morphologically and functionally similar to the growth plates, termed synchondroses, are developed for this function. The facial bones are connected by relatively narrow fibrous sutures except for the mandible, which is a relatively independent and movable skeleton in the craniofacial area. In the mandible, secondary cartilage structures, the mandible condyles, are developed as growth centres for the posterior part of the mandible. Although the condyle cartilage basically contributes to mandible growth through endochondral bone formation as that of growth plate, it also displays unique features in development and regulation. The condyle cartilage differs to the primary cartilage in embryonic origin, histological organization, growth mode and regulation (Delatte *et al.,* 2004, Shibata *et al.,* 1996).

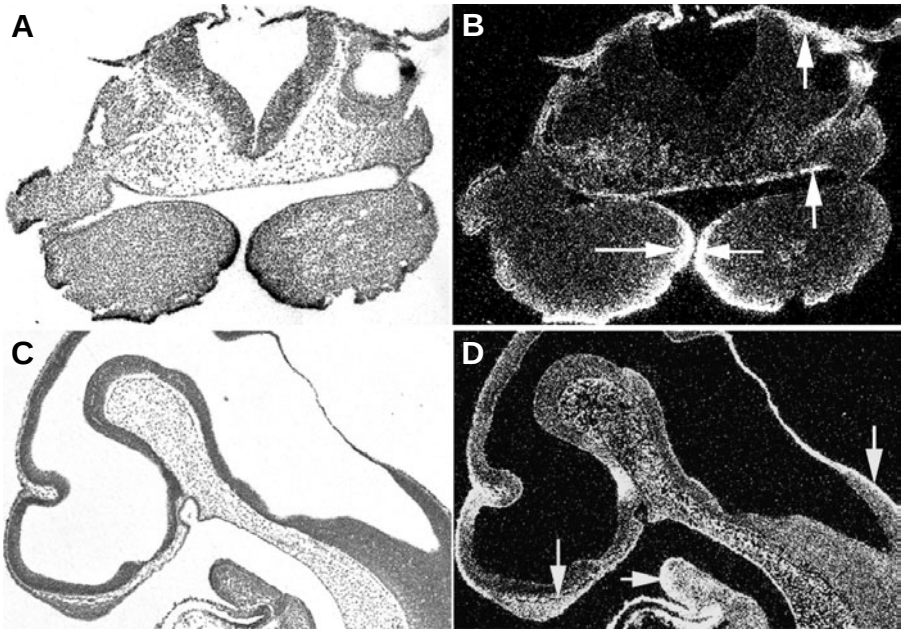

Fig. 1. *Bmp4* expression in the developing mouse head at embryo day 10 (E10) localized by radioactive *in situ* hybridization. *At E10,* Bmp4 *is intensely expressed in the epithelia of the first branchial arches, stomodeum and ectoderm of cranial neural crest (arrows).* **(A,C)** *bright field section;* **(B,D)** *dark field section.*

BMP signalling is conserved in craniofacial skeletons regulating both the endochondral and intramembranous bone formation (Fig. 2). It is crucial for the formation of skeletogenic precursor cells in the neural crest and their migration to the defined destinations (Kanzler *et al.,* 2000). Bmps are highly expressed in the migrating CNCCs and later in the early stage of cartilages and bones. Overexpression of Bmp or application of Bmp proteins changes the skeletal patterning process, resulting in altered size and morphology of the skeleton in both the face and limb (Barlow and Francis-West, 1997, Duprez *et al.,* 1996a). Moreover, deficiency of Bmp signalling in mouse cranial neural crest shows multiple defects in craniofacial skeletons (Dudas *et al.,* 2004). These data indicate a patterning role for craniofacial skeletogenesis.

After early development, Bmps maintain their expression in the skeletons and skeletal growth centres and play an important

role in regulating the activities of the growth centres. The cranial sutures are critical growth sites for the calvarias. Premature fusion of these sutures leads to a pathologic condition, known as craniosynostosis. The Bmp signal is an important player in regulating the sutural morphogenesis and function (Holleville *et al.,* 2003, Kim *et al.,* 1998). Both Bmp2 and Bmp4 are present in the osteogenic fronts of cranial sutures (Kim *et al.,* 1998). Application of Bmp4 protein increases the tissue volume in the suture and induces the expression of Msx genes (Kim *et al.,* 1998), which is also associated with craniosynostosis in mutated conditions. In avian suture, Bmp signalling induces and upregulates the expression of homeobox gene Dlx5 (Holleville *et al.,* 2003), a critical factor for development of craniofacial skeletons (Acampora *et al.,* 1999, Depew *et al.,* 1999). Gdf6 is another important signal in suture regulation. It was found in the coronal suture, whereas other members of this subgroup, Gdf5 and Gdf7, were not detected (Settle *et al.,* 2003). In Gdf6 mutant mice, the coronal suture of the skull, normally joining the frontal and parietal bones, is consistently missing (Settle *et al.,* 2003). This role of Gdf6 proposes it as a candidate gene of craniosynostosis. Bmpr1b is important for skeletogenesis. In the craniofacial region it is highly present in both developing and adult skeletal elements. Transgenic expression of truncated Bmpr1b in mouse osteoblasts using the type I collagen promoter leads to delayed ossification in the calvarias (Zhao *et al.,* 2002).

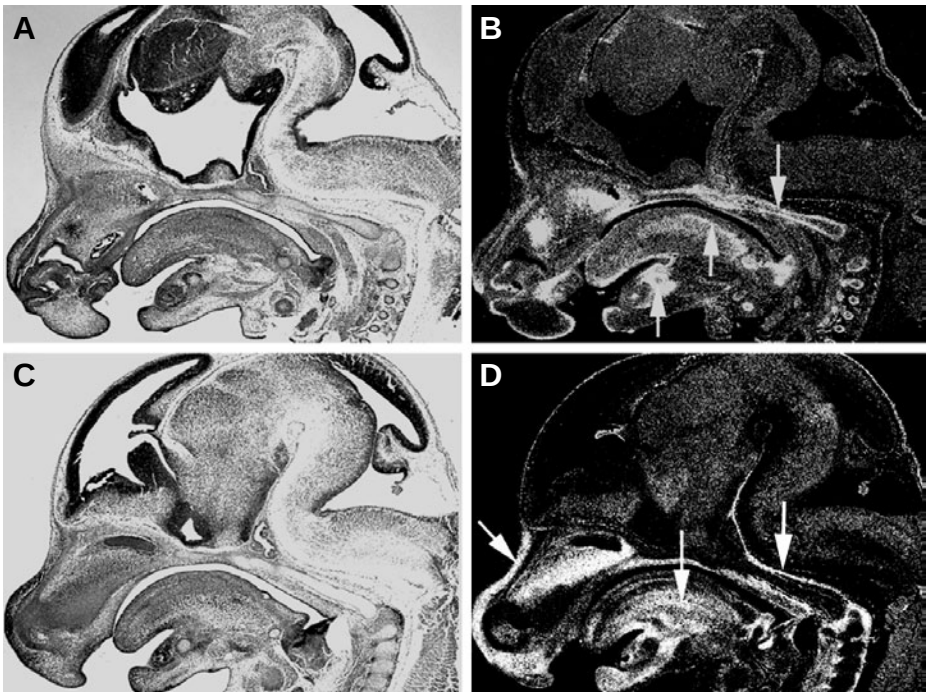

Fig. 2. Expression of *Bmp2* and *Bmp4* in the developing mouse head at E13. Bmp2 *is seen in the chondrocranium, myogenic mesenchyme of the tongue and nasal and mandibular cartilages* **(A,B)**. Bmp4 *is widespread in the perichondria of the chondrocranium, nasal cartilage, tongue and mesenchyme of the facial prominences* **(C,D)**. *Arrows indicate expression domains.*

During basicranium development, Bmp2 to -6 displays a spatiotemporal expression that largely conforms to that of other endochondral skeletons (Nie *et al.,* unpublished data). The effect of Bmp signalling on synchondrosis of basicranium was specifically analyzed using a serum-free organ culture system (Shum *et al.,* 2003). Bmp protein stimulates cartilage growth, matrix deposition and chondrocyte proliferation in a dose dependent manner. The regulation of synchondrosis by Bmp signalling appears largely similar or identical to that of its morphologic analogue - the growth plate.

BMP signalling is also important for mandible development. Inactivation of Alk2 in mouse neural crest results in a remarkable hypotrophic mandible (Dudas *et al.,* 2004). In such mice, the anterior cartilage derived from the distal extremity of the Meckel´s cartilage is absent; subsequently the mental symphysis, a critical growth site for anterior part of mandible, is not formed resulting in persistently separated mandible bones (Dudas *et al.,* 2004). In line with this, simultaneous abrogation of two Alk2 ligands, Bmp5 and Bmp7, leads to defects in proliferation and maintenance of branchial arch cells (Solloway and Robertson, 1999). Bmp/Msx signal loop is critical for alveolar bone formation of the mandible. In $Msx1^{-/-}$ mice, tooth development is arrested at the bud stage and associated with this is an absence of periodontal bone- the alveolar process. On the other hand, ectopic expression of Bmp4 driven by the mouse Msx1 promoter to the $Msx1^{-/-}$ dental mesenchyme not only partially restores tooth development, but also rescues alveolar bone formation (Zhao *et al.,* 2000). It was later demonstrated that Bmp4 mediates the function of Msx1 in controlling the development of the alveolar bone by regulating the expression of Dlx5 and Cbfa1 (Zhang *et al.,* 2003). The mechanism of temporomandibular joints (TMJ) development is poorly understood. Bmp2 and Bmp4 have been implied a role in determining the site of the TMJ by repressing Bapx1 (Wilson and Tucker, 2004). Gdf5, -6 and -7 subgroup is required for synovial joint formation (Settle *et al.,* 2003, Storm and Kingsley, 1996), but their roles in the development of TMJ remain to be specifically defined. How the Bmp signalling regulates the development of

TABLE 2

SOME ADDITIONAL MEMBERS OF THE *BMP* FAMILY: EXPRESSION PROFILES AND RELATED DISORDERS

	Expression profile
Bmp9 (*Gdf2*)	Central nervous system, Liver (Lopez-Coviella *et al.*, 2000, Miller *et al.*, 2000)
Bmp10	High in heart, lower in lung and liver (Neuhaus *et al.*, 1999)
Bmp15	Ovary (Dube *et al.*, 1998)
	Related disorder: Ovary dygenesis 2 (OMIM, 300510)
Gdf9	Ovary (Dube *et al.*, 1998, Teixeira Filho *et al.*, 2002)
	Related disorder: Polycystic ovary syndrome (Teixeira Filho *et al.*, 2002)
Gdf 3	Embryonal carcinoam cell line (Caricasole *et al.*, 1998)
Nodal	Early embryo, involved in establishing proximal-distal polarity (Kishigami and Mishina, 2005)
lefty	Notochord, presumptive floor plate, involved in left-right patterning (Kishigami and Mishina, 2005)

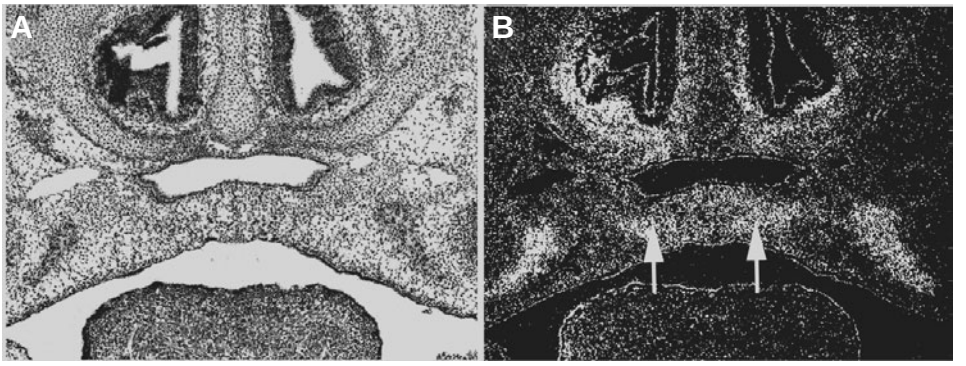

Fig. 3. Expression of *Bmp2* in palatogenesis. Bmp2 *is highly expressed in the palatal mesenchyme adjacent to the midline during palatal fusion (arrows).* **(A)** *Bright field section;* **(B)** *Dark field image.*

the secondary cartilage of the mandible, the condyles, also remains to be analyzed.

The BMP family is also found to be a heterogonous group in functions. BMP3 and BMP3b (GDF10) together represent a unique subgroup of this family. The role of BMP3 in skeletogenesis is controversial, owning to contradictory results (Bahamonde and Lyons, 2001, Han *et al.,* 2002). Now evidence is stronger in supporting an inhibitory role for bone formation. *Bmp3* knockout mice have increased bone density that are twice as much trabecular bone as wild type (Bahamonde and Lyons, 2001, Daluiski *et al.,* 2001). Bmp3 antagonized Bmp2-induced osteoblastic differentiation *in vitro* by a receptor independent way (Bahamonde and Lyons, 2001). Bmp3b has a similar antagonistic activity against Bmp2, but is differentially regulated (Daluiski *et al.,* 2001).

The inductive and regulatory roles of BMP signalling in skeletogenesis provide an important approach in skeletal tissue regeneration and engineering. Application of BMP proteins, BMP-induced or BMP-expressing stem cells, or BMP gene transfer could greatly facilitate the regeneration process of bone and cartilage (Chang *et al.,* 2004, Lieberman *et al.,* 1999, Lieberman *et al.,* 1998, Suzuki *et al.,* 2002). Skeletal tissue can even be produced *in vitro* by combination of stem cells with suitable matrix and inductive molecules such as Bmps. Breakthroughs in this field will undoubtedly provide a powerful armoury for craniofacial skeletal repair.

BMP signalling in palatogenesis

The development of palate is a complex process. Union of the primary palate of frontonasal process and paired lateral maxillary palatal shelves, which form the secondary palate, forms the mammalian palate. Formation of mammalian secondary palate is a multi-step process that includes mesenchymal cell proliferation, palatal shelf outgrowth, elevation, fusion and eventually disappearance of the midline epithelial seam. Disturbance of any events may lead to clefts in the palate. The most common mechanism of cleft is due to deficiency of mesenchymal proliferation or failure of epithelial fusion. Cleft palates with or without cleft lips occur either as isolated cases or simultaneous with human syndromes. Even though syndromic and isolated cleft palates are different in mechanisms, both types show high genetic background.

The Bmp signal is essential for palatogenesis. Bmp2, Bmp3, Bmp4 and Bmp5 are expressed in mouse palate in both the epithelia and mesenchyme prior to, during and after palatal shelf fusion (Lu *et al.,* 2000, Nie, 2005c, Zhang *et al.,* 2002). The expression is highly dynamic during palatogenesis and is differently regulated along the anterior-posterior axis (Nie, 2005c). The importance of Bmp signalling in palatogenesis is implied by a mice model of cleft palate. In such mice, cleft palate induced by retinoic acid is associated with decreased expression of Bmp2, -4 and -5 (Lu *et al.,* 2000). More convincing evidence is provided by studies of transgenic mice models with inactivation of Alk2 or BmprIa specifically in the neural crest or facial primordia, both of which consistently show oro-facial cleft (Dudas *et al.,* 2004, Liu *et al.,* 2005). This also implies that Bmp signalling mediated by Alk2 and BmprIa plays non-redundant roles in palatogenesis.

While inactivation of BmprIa in the facial primordia leads to palatal cleft, removal of BmprIa from the epithelia along does not show obvious affliction on palatogenesis in mice (Andl *et al.,* 2004). The secondary palate of BmprIa $^{fp-/-}$ mice is associated with diminished proliferation and elevated apoptosis and the palatal shelves of the mutants are competent to fuse when they are placed together in culture, suggesting that the cleft in BmprIa deficiency mice is due to lack of mesenchymal proliferation rather than failure of epithelial fusion (Liu *et al.,* 2005). In line with this, high expression of Bmp2 was observed in the mesenchyme adjacent to midline during palatal fusion (Fig. 3), whereas epithelial signalling mediated by Bmp4 and Bmp7 is either unessential or functional redundant with others as implicated by conditioned knockout models (Dudley *et al.,* 1995, Liu *et al.,* 2005, Nie, 2005c). Other members of the Bmp family do not appear crucial for palatogenesis, implicated by functional studies (Tables 1 and 2). Therefore, it is logical to speculate that Bmp2-mediated mesenchymal proliferation is a key event in palatogenesis. The Bmp signal has been proposed to be within the Msx1-induced signalling pathway in palatogenesis. Msx1 deletion leads to a complete cleft in the secondary palate in mice (Satokata and Maas, 1994), whereas transgenic expression of Bmp signal driven by Msx1 promoter rescues the clefts (Zhang *et al.,* 2002), suggesting that the Bmp signal is downstream of Msx1 in palatogenesis. Recently, Bmp11 (Gdf11) has been implicated a role in palatogenesis by transgenic approach (McPherron *et al.,* 1999). Unfortunately, detailed phenotype analysis is not available to date. Regarding to epithelial fusion, Tgf-β3 has been shown to be a pivotal factor (Gato *et al.,* 2002).

Those data also indicates differential requirement of Bmp signalling in palate and lip formation and thus potential different mechanisms underling these processes. For instance, Bmp4 are critical for the fusion process of lip development, whereas in palatogenesis Bmp signalling is implicated to be a signal for mesenchymal proliferation (Liu *et al.,* 2005). The development and fusion of palate is also relatively a late event in embryogenesis and is critically influenced by development of related structures. All these aspects make palatogenesis a very fragile

process that is easily affected by genetic and environmental factors.

BMP signalling in craniofacial skeletal muscle development

The Bmp signal is not essential for the early initiation of myogenesis. On the contrary, repression of the Bmp signal is required for early development of craniofacial muscles (Tzahor *et al.,* 2003). During muscular regeneration, the Bmp signal is also absent (Zhao and Hoffman, 2004). An inhibitory role in myogenesis is implicated for Bmp signalling by many studies (Duprez *et al.,* 1996b, Huang *et al.,* 2001, Inada *et al.,* 1996, Musgrave *et al.,* 2001, Tzahor *et al.,* 2003). Of note, the myostatin (Gdf8) was identified as a key regulator in skeletal muscle development and growth (McPherron *et al.,* 1997). Gdf8 is specifically expressed in developing and adult skeletal muscles. Gdf8 null mutant mice are significantly larger than wild type mice and show a dramatic and widespread increase in skeletal muscle mass (McPherron *et al.,* 1997). Significantly, heterozygous mice are also affected, although in a less degree, suggesting that the effect of myostatin is dose dependent (McPherron *et al.,* 1997).

The craniofacial muscles are mostly derived from mesoderm and formed within a surrounding environment of neural-crest-derived elements (Noden, 1983, Noden, 1988). Their development is characterized of early expression of myogenic regulatory factors (MRFs) and thus early maturation. Bmp signalling is present during craniofacial muscle development. The tongue is a good model for studying craniofacial myogenesis, for it is basically a muscle organ. Many Bmps have been localized in muscles of developing tongue (Bennett *et al.,* 1995, Huang *et al.,* 2001, Nie, 2005a). Application of Noggin, an antagonists of Bmps, to the avian tongue results in thickening of the tongue (Huang *et al.,* 2001), conforming to the negative role of Bmp signalling in muscle development in general. In myostatin-deficient mice, the temporalis mass becomes significantly large (Byron *et al.,* 2004). Therefore, Bmp signalling plays a role in controlling the pace of myogenic proliferation and differentiation of craniofacial muscles.

BMP4 mutation is associated with fibrodysplasia ossificans progressiva (FOP, OMIM no.135100), characterized by progressive postnatal heterotopic ossification in muscles. The fibroproliferative lesions evolve through endochondral ossification into a mature laminar bone with marrow elements. Transgenic mice overexpressing Bmp4 develop a similar phenotype like FOP (Kan *et al.,* 2004). In a child with gross muscle hypertrophy, loss function mutation of myostatin gene was detected (Schuelke *et al.,* 2004), suggesting the inactivation of myostatin has similar effects in mice and human.

BMP signalling in tooth development

Tooth morphogenesis initiates from thickening of stomodeal epithelium. This dental lamina gives rise to epithelium buds for further development. Thereafter early tooth bud coupled with underlining mesenchyme undergoes successive morphological changes known as cap stage and bell stage. The Bmp signal is also a fundamental regulator of tooth development, as evidenced by the arrestment of tooth development in Bmpr1a deficient mice (Andl *et al.,* 2004, Liu *et al.,* 2005). At the early tooth initiation stage, Bmp4, Bmp2 and Bmp7, expressed in dental epithelia, play an important role in epithelial-mesenchymal interactions in inducing tooth formation (Aberg *et al.,* 1997, Vainio *et al.,* 1993). During the bud to cap transitional stage, Bmp4 induces the expression of Msx1 and Msx2 in the mesenchyme. The latter signalling further induces Bmp4 expression in the mesenchyme. Ectopic Bmp4 expression driven by mouse Msx1 promoter partially rescues tooth development in the Msx1-deficient mice (Zhao *et al.,* 2000).

Therefore, Bmp/Msx signal loop mediates reciprocal interactions between the epithelium and mesenchyme during tooth initiation and crown morphogenesis.

In the developing mouse tooth germ Bmp4 regulates the expression of another critical patterning gene, the Shh (Zhang *et al.,* 2000). Inhibition of Bmp4 activity by Noggin resulted in repression of Shh and Bmp2 in wild-type dental epithelium (Zhang *et al.,* 2000), while ectopic expression of human BMP4 to the dental mesenchyme driven by mouse Msx1 promoter restored Shh expression in the Msx1 mutant dental epithelium (Zhang *et al.,* 2000, Zhao *et al.,* 2000). In subsequent development, Bmp4 is expressed in preodontoblasts and its expression is sharply downregulated after their differentiation, whereas Bmp2 expression is upregulated during the terminal differentiation of odontoblasts (Nakashima, 1994, Nakashima and Reddi, 2003, Yamashiro *et al.,* 2003). These data suggests that the Bmp signal is inductive to odontoblastic differentiation. In Fig. 4, mRNA expression of Bmp2 in the developing mouse incisor is shown. Additionally, Bmp3 was localized in cementoblasts and dental follicles (Yamashiro *et al.,* 2003); Bm6, Bmp8 and Gdf7 were identified in dental pulp (Nakashima *et al.,* 1998); Gdf11 was observed in the terminally differentiated odontoblasts (Nakashima *et al.,* 1999); while Gdf5, -6 and -7 were detected in the periodontium (Sena *et al.,* 2003).

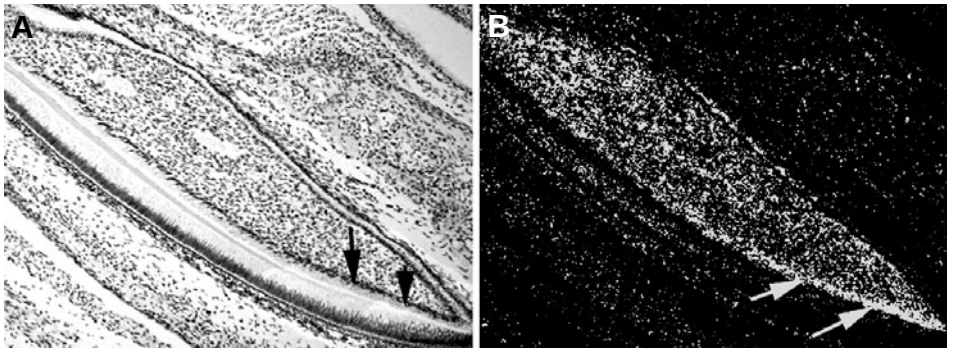

Fig. 4. *Bmp2* expression in the mouse incisor in the lower jaw at newborn stage. *Intense expression is seen in odontoblasts near the tooth root. Arrows indicate expression domains.* **(A)** *Bright field image;* **(B)** *dark field image.*

The role of inducing odontoblastic differentiation provides potential application for dental tissue engineering and regeneration. Recently, dental pulp stem cells was induced into odontoblasts in a culture system by Bmp2, as indicated by marker genes like dentin sialophosphoproteinn (Iohara *et al.,* 2004). Based on the *in vitro* experiments, Bmp-treated pellet culture

of pulp stem cells transplanted to amputated pulp markedly stimulated dentin formation (Iohara *et al.,* 2004). These advances provide potential clinical use of stem cells in the regeneration of tooth tissue or even replacement of missing tooth with engineered tooth primordia.

BMP signalling involved in apoptosis in craniofacial development

Bmp signalling is involved in apoptosis in developing limb (Macias *et al.,* 1997, Omi *et al.,* 2000, Yokouchi *et al.,* 1996). Msx2 was further demonstrated to be the mediator of Bmp-induced apoptosis in a *in vitro* system (Marazzi *et al.,* 1997). In the developing head, Bmp signalling is also associated with apoptosis during development of the cranial neural crest, facial primordia and eye (Barlow and Francis-West, 1997, Graham *et al.,* 1994, Guha *et al.,* 2002). In chicken cranial neural crest, Bmp4 induces apoptosis in rhombomeres (r) 3 and r5 through upregulating the expression of Msx2 (Graham *et al.,* 1994). Addition of recombinant BMP4 protein to explant cultures of r3 or r5, which produce neural crest when isolated from their neighbouring rhombomeres, upregulates Msx2 expression and reinstates apoptosis in the neural crest population (Graham *et al.,* 1994). Further more, overexpression of Msx2 along the cephalic neural tube also results in increased apoptosis (Takahashi *et al.,* 1998). Apoptosis in the neural crest is suggested as a mechanism of eliminating those cells that are not necessary for differentiation and results in the sculpting of discrete migratory streams of neural crest in chicken.

In contrast to chicken, mice do not show the rhombomere-specific cell death. Although consistent and reproducible cell death is observed in the hindbrain, there is no specific pattern that could be attributed to either odd or even rhombomeres (Kulesa *et al.,* 2004). Mouse Bmp4 is not expressed in the r3 and r5 and is actually absent from the dorsal neural tube (Kulesa *et al.,* 2004). Moreover, Msx2 is expressed uniformly in the dorsal edges of the neural tube along the entire anterior-posterior axis during the period of neural crest formation and migration, differing markedly from the patterns of the chicken homologue (Kulesa *et al.,* 2004). Therefore, Bmp4/Msx2 mediated apoptosis of neural crest cells in odd-numbered rhombomeres is not responsible for patterning the pathways of the neural crest migration in mice. This also holds true in zebrafish and *Xenopus*. These data suggest that apoptosis in r3 and r5 of chicken is an evolutionary species-specific feature.

Bmp signalling is also associated with apoptosis in early facial development. The expression of Bmp2 and Bmp4 overlaps with domains of cell death in the frontonasal mass and regions of union between primordia (Barlow and Francis-West, 1997). Moreover, exogenous Bmp protein induces cell death in facial primordia (Barlow and Francis-West, 1997). Of note, in Bmp5/Bmp7 double mutant mice apoptosis pattern in the branchial arch and dorsal midline is altered (Solloway and Robertson, 1999). Collectively, these results support an apoptotic role of Bmp signalling in some processes of craniofacial development.

Concluding remarks

In summary, functions of BMP signaling have been under revealing in a variety of craniofacial structures. The BMP signal is conserved in many developmental processes, but mediates distinct functions in different tissues and stages. Its specific roles and mechanisms in the development of craniofacial elements clearly need further elucidation. Crosstalk between the BMP and other signalling pathways remains to be clarified. Particularly the downstream factors of the BMP signal are not completely identified in many processes. These factors are usually site-specific. Therefore, unraveling the BMP signalling pathway within the genetic cascade of a certain developmental process is important for understanding of the overall mechanism. The developmental roles of BMPs also provide an important approach in tissue engineer and regeneration. Therefore, the BMP signalling pathway will undoubtedly continue to attract attention in future studies.

Summary

The BMP signalling pathway is conserved throughout evolution and essential for mammalian embryonic and postnatal development and growth. In the vertebrate head, this signal is involved in the development of a variety of structures and shows divergent roles. During early head development, BMP signalling participates in the induction, formation, determination and migration of the cranial neural crest cells, which give rise to most of the craniofacial structures. Subsequently, it is also important for patterning and formation of facial primordia. During craniofacial skeletogenesis, BMP signalling is an early inductive signal required for committed cell migration, condensation, proliferation and differentiation. Thereafter, BMP signalling maintains regulatory roles in skeletons and skeletal growth centres. For myogenesis, BMP signalling is a negative regulator. Importantly, myostatin has been identified as a key mediator in this process. During palatogenesis, the crucial role of BMP signalling is demonstrated by mouse models with *Alk2* or *Alk3* (BMP type I receptors) deletion from the neural crest or craniofacial region, in which cleft palate is one of the major anomalies. BMP signalling is also an important participant for tooth development, regulating early tooth morphogenesis and subsequent odontoblast differentiation. In this review these aspects are discussed in detail with a focus on recent advances.

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Received: September 2005
Reviewed by Referees: November 2005
Modified by Authors and Accepted for Publication: March 2006
Online Publication: April 2006