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Variations in the sequences of *BMP2* imply different mechanisms for the evolution of morphological diversity in vertebrates☆

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ABSTRACT

Bone morphogenetic protein 2 (*BMP2*) plays an important role in skeletogenesis, osteoblastic differentiation and limb patterning. Its protein coding region consists of the signal peptide, the pro-domain (that regulates post-translational control of synthesis) and the mature domain (that carries out gene function). This gene has been considered previously to be conserved. By re-analyzing the coding region of *BMP2 in 31 species of vertebrates, we found that the mature domain region is indeed conserved in mammals, but not among non-mammalian taxa. Moreover, compared to the mature domain, the signal peptide and pro-domain have experienced dramatic variation in all vertebrates. Six amino acid sites in the pro-domain were identified to be under diversifying Darwinian selection in mammals. These results indicate that the signal peptide and pro-domain of BMP2* may be involved in skeletal poly-morphology during mammal evolution and the mature domain may also contribute to this function in non-mammals. This supports the hypothesis that morphological variations in mammals result mainly from a change in post-translational control of synthesis, whereas in non-mammals they result mainly from gene functional change.

1. Introduction

BMP2 is a member of the transforming growth factor-β (TGF-β) superfamily. The coding region of this gene encodes an inactive precursor protein which consists of a short signal peptide (23 amino acids), a pro-domain (248–261 amino acids) and a mature domain (114–115 amino acids) (ten Dijke, 2006). The signal peptide directs the post-translational transport of the precursor protein and may regulate peptide transport or localization (von Heijne, 1998; Gao et al., 2005), and the mature bioactive dimeric protein encoded by the mature domain of *BMP2* is secreted after the pro-domain has been cleaved (ten Dijke et al., 2003). In this process, the pro-domain plays a regulatory role in protein synthesis (Constam and Robertson, 1999).

BMP2 plays an important role in skeletogenesis, osteoblastic differentiation and limb patterning (Peng et al., 2005; Bae et al., 2007; Robert, 2007). This gene can induce bone regeneration after fracture and is used in gene therapy (Tsuji et al., 2006; Gugala et al., 2007), and loss of *BMP2* can lead to limb malformation and can influence osteogenesis in transgenic mice (Bandyopadhyay et al., 2006). Up-regulated expression of this gene was found in the developing elongated forelimb of bats, which indicates its important role for bat flight (Sears et al., 2006). The expression pattern of *BMP2* also suggests a role in the developing fins of teleosts (Laforest et al., 1998).

Skeletal morphology is very variable among vertebrates. From aquatic teleostean fish to amphibians or terrestrial tetrapods, the corresponding parts of the skeleton are quite different (Kent and Carr, 2001). For example, bone density is low in bats and bones are hollow in birds, while fish have fin rays and tetrapods have evolved limbs with digits (Long et al., 2006; Shubin et al., 2006; Mercader, 2007). The shapes of limbs also vary, particularly in digit pattern (Opitz, 1996). Most tetrapods possess five digits, whereas birds, ungulates and some other mammals have a reduced digit number (Wagner and Gauthier, 1999; Laurin et al., 2000; Galis et al., 2002). As the only mammals capable of powered flight, bats have retained all digits that have become enormously elongated to support the wing (Sears, 2008).

Because *BMP2* is involved in osteogenesis, bone morphology and limb patterning, it may account for variations in the skeleton of vertebrates (Dahn and Fallon, 2000; Tickle, 2006). Previous studies focused mainly on the expression pattern of *BMP2* or the evolution of the non-coding region of this gene, as the coding region is usually considered to be conserved (Abrams et al., 2004; Fritz et al., 2004; Chandler et al., 2007). In this study, we re-analyze the coding region of *BMP2* with more species of vertebrates, based on three functional parts of this gene. This may detect the mechanism underlying morphological variation during the evolution of vertebrates.

☆ The *Bmp2* sequences from 10 mammalian species have been deposited in GenBank (accession numbers EU854579–EU854588).

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2. Materials and methods

2.1. Sequencing the whole coding region of BMP2

Total RNA was prepared from the whole brain of 10 species of mammals using the RNAiso kit (TakaRa, D312, Japan). These included five bat species (*Rousettus leschenaulti*, *Rhinolophus ferrumequinum*, *Taphozous melanopogon*, *Myotis ricketti* and *Miniopterus fuliginosus*), two carnivores (cat *Felis catus* and hog badger *Arctonyx collaris*), two artiodactyls (goat *Capra hircus and* pig *Sus scrofa*), and a perissodactyl (donkey *Equus asinus*). Then the total RNA was reversed to synthesize cDNA by SuperScript III Reverse Transcriptase (Invitrogen, 18080-051, US). The *BMP2* gene was amplified via the polymerase chain reaction (PCR) from the cDNA using two pairs of degenerate primers (Pair1: F1 [5′-TTAGACGGWCTGCGGTCTCCTAAAGGTC-3′] and R1 [5′-CAGTCATTCCACCCCACATC-3′]; Pair2: F2 [5′-GTGACAAGGTGGACxTGCACAGGGACTCG-3′] and R2 [5′-KTA TTTAWTYTTGCTGTRCTARCGACACCCAC-3′]) with an overlap of 302 bp. Finally, we sequenced the whole coding region (1188 bp) of *BMP2* from all the species, except for *T. melanopogon* and *A. collaris* in which we obtained only partial sequences. Furthermore, we used SMART RACE cDNA Amplification Kit (Clontech, 634914, US) and the gene-specific primer [5′-AAGGCGTGCTGTGTCCCAACGGAACTG-3′] to obtain the 3′ end UTR of *BMP2 in Myotis ricketti*. Thus the whole coding region was obtained in this species. We removed the 13 bp primer sequence at the end of the coding region in other species for evolutionary analysis. The nucleotide sequences are deposited in Genbank under accession numbers EU854579–EU854588.

2.2. BMP2 sequences from databases

Nineteen *BMP2* (alias: *BMP2A*) sequences, respectively from *Homo sapiens*, *Pan troglodytes*, *Macaca mulatta*, *Mus musculus*, *Rattus norvegicus*, *Oryctolagus cuniculus*, *Canis familiaris*, *Bos taurus*, *Dama* sp., *Equus caballus*, *Monodelphis domestica*, *Ornithorhynchus anatinus*, *Gallus gallus*, *Trachemys scripta*, *Xenopus laevis*, *Xenopus tropicalis*, *Ambystoma mexicanum*, *Astyanax mexicanus* and *Danio rerio* were obtained from Genbank, accessions NM_001200, XM_514508, XM_001115987, NM_007553, NM_017178, AF041421, XM_534351, XM_866011, AJ001817, XM_001493895, XM_001374465, XM_001514514, NM_204358, AY327846, X63424, NM_001015963, EU339232, DQ915171 and NM_131359, respectively. The putative coding region of *BMP2* of *Callithrix jacchus and Pongo pygmaeus* were obtained from their genomes from a public database website (http://genome.ucsc.edu).

2.3. Phylogenetic construction

In total, 31 species of vertebrates were studied and a phylogenetic tree was constructed based on the topology of published studies (Fig. 1) (Goodman et al., 1998; Kumar and Hedges, 1998; Murphy et al., 2001; Hernandez Fernandez and Vrba, 2005; Teeling et al., 2005). These species belonged to 5 classes and 15 orders were used for the evolutionary analysis of *BMP2*. All the mammals except for *Monodelphis domestica* and *Ornithorhynchus anatinus* in the tree belong to clade Boreoeutheria which is comprised of the sister taxa Laurasiatheria and Euarchontoglires (Supraprimates). They have included nearly all the orders of Boreoeutherian mammals and their *BMP2* sequences were used for detecting positively selected sites in this clade.

2.4. Sliding widow

To characterize variation in the rate of molecular evolution over the *BMP2* gene in different taxa (mammals, non-mammals and amphibians), we performed sliding window analyses (window = 30 bp, step = 12 bp) of Nei-Gojobori estimates of synonymous and non-synonymous substitutions per site (dS and dN, respectively) and the dN/dS (omega, ω) in SWAAP1.0.2. To get the right alignment for

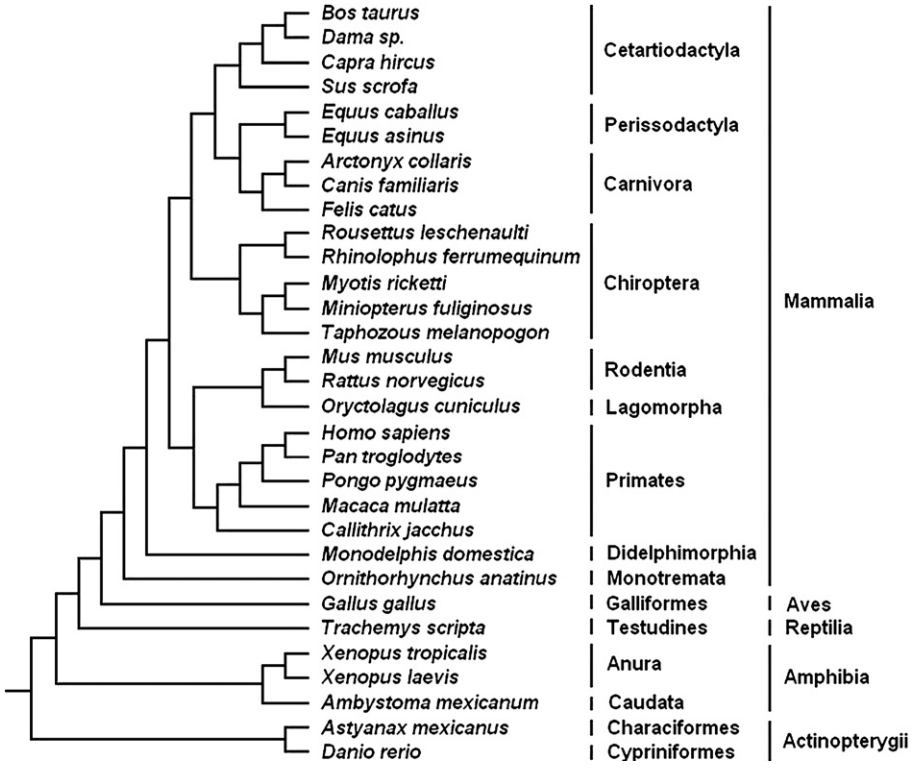

Fig. 1. Phylogenetic tree of vertebrates studied in this paper. This tree is constructed based on the topology of published studies (Goodman et al., 1998; Kumar and Hedges, 1998; Murphy et al., 2001; Hernandez Fernandez and Vrba, 2005; Teeling et al., 2005). These species have covered five main classes of vertebrates and nearly all the orders of Boreoeutherian mammals.