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Turning Bone Morphogenetic Protein 2 (BMP2) on and off in Mesenchymal Cells

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ABSTRACT

The concentration, location, and timing of *bone morphogenetic protein 2* (BMP2, HGNC:1069, GeneID: 650) gene expression must be precisely regulated. Abnormal BMP2 levels cause congenital anomalies and diseases involving the mesenchymal cells that differentiate into muscle, fat, cartilage, and bone. The molecules and conditions that influence BMP2 synthesis are diverse. Understandably, complex mechanisms control *Bmp2* gene expression. This review includes a compilation of agents and conditions that can induce *Bmp2*. The currently known *trans*-regulatory factors and *cis*-regulatory elements that modulate *Bmp2* expression are summarized and discussed. Bone morphogenetic protein 2 (BMP2, HGNC:1069, GeneID: 650) is a classical morphogen; a molecule that acts at a distance and whose concentration influences cell behavior. In mesenchymal cells, the concentration of BMP2 influences myogenesis, adipogenesis, chondrogenesis, and osteogenesis. Because the amount, timing, and location of BMP2 synthesis influence the allocation of cells to muscle, fat, cartilage, and bone, the mechanisms that regulate the *Bmp2* gene are crucial. Key early mesodermal events that require precise *Bmp2* regulation include heart specification and morphogenesis. Originally named for its osteoinductive properties, healing fractures requires BMP2. The human *Bmp2* gene also has been linked to osteoporosis and osteoarthritis. In addition, all forms of pathological calcification in the vasculature and in cardiac valves involve the pro-osteogenic BMP2. The diverse tissues, mechanisms, and diseases influenced by BMP2 are too numerous to list here (see OMIM: 112261). However, in all BMP2-influenced pathologies, changes in the behavior and differentiation of pluripotent mesenchymal cells are a recurring theme. Consequently, much effort has been devoted to identifying the molecules and conditions that influence BMP2 synthesis and the complex mechanisms that control *Bmp2* gene expression. This review begins with an overview of the *Bmp2* gene's chromosomal neighborhood and then summarizes and evaluates known regulatory mechanisms and inducers. J. Cell. Biochem. 116: 2127–2138, 2015.

KEY WORDS: GENE REGULATION; TRANSCRIPTION; POST-TRANSCRIPTIONAL; MESENCHYMAl CELL; GROWTH FACTOR; MORPHOGEN; CALCIFICATION

GENE STRUCTURE AND CHROMOSOMAL CONTEXT

The *Bmp2* transcribed region is of moderate length with 3 exons and 2 introns as shown in Figure 1A. Most publicly annotated transcripts to date support only the three exons generating a mature transcript of about 3 kb (http://www.ncbi.nlm.nih.gov/projects/mapview/, GenBank accession no. NM_001200 [Feng et al., 1994]). Translation initiates in exon 2 and terminates in exon 3. The position of the second intron in the pro-domain of the protein-coding region is precisely conserved between the *Bmp2* gene and its close paralog *Bmp4,* and even the homologous *decapentaplegic* (*dpp*) genes in insects [Newfeld et al., 1997]. Alternative splicing of the *Bmp2* transcript has not been reported. However, one transcript variant that may be truncated at the 5′ and 3′ ends relative to the full-length transcript has been reported [Jiang et al., 2007]. In addition to the expected 3 kb RNA, a 1.5 kb BMP2 RNA was observed, but only in several cell types contaminated with mycoplasma. Some sequences in the public database may represent this variant transcript (e.g., GenBank accession no. M22489).

Considerable evidence supports two transcription start sites (TSS, Fig. 1). The proximal TSS (pTSS) is located 1,465 bp upstream of the mouse ATG translation initiation codon [Feng et al., 1994, 1997]. The distal TSS (dTSS) is 736 bp further upstream [Feng et al., 1997; Heller et al., 1999; Sugiura, 1999; Helvering et al., 2000; Ghosh-Choudhury et al., 2001; Abrams et al., 2004]. Transcription begins at both the distal and proximal promoters in many cell types including osteoblast- and fibroblast-type cells. However, the relative use of each promoter is differentially regulated. For example, only transcripts starting at the distal site were detected in human U2OS osteosarcoma and mouse F9 embryonal carcinoma cells [Heller et al.,

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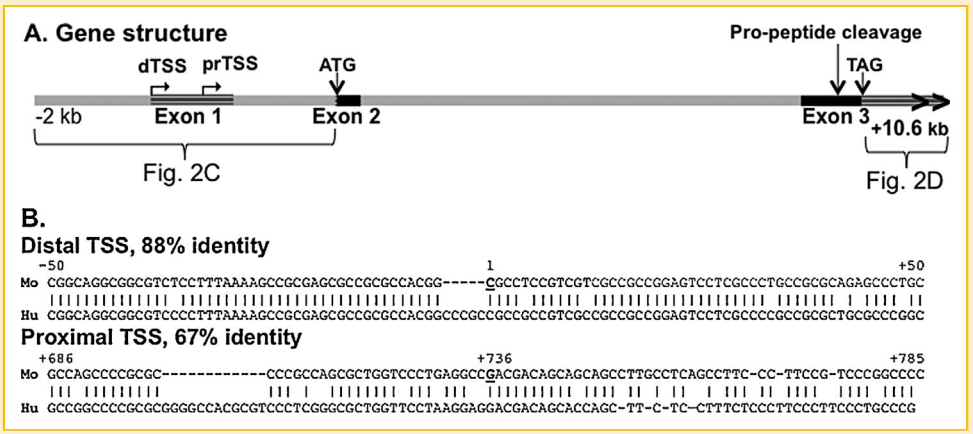

Fig. 1. *Bmp2* gene structure. A: Schematic of the mouse *Bmp2* gene structure showing the distal and proximal transcription start sites (dTSS and prTSS, respectively, indicated by ↱), the translation start codon (ATG), the site where the propeptide is cleaved to release the mature peptide, the translation termination codon (TAG), and the two alternate polyadenylation sites (>). Bmp2 has 3 exons and two introns (gray). The coding region is shown as a solid black bar. The 5′untranslated region (5′UTR) and 3′UTR are shown as horizontally striped bars. B: The 100 base pairs of mouse (Mo) sequence surrounding each transcription start site aligned to the human (Hu) start sites. Gencode 21 version, released October 2, 2014.

1999; Helvering et al., 2000]. Although both promoters are well conserved, the distal promoter is relatively more conserved as shown in Figure 1B [Abrams et al., 2004]. Two polyadenylation and cleavage sites are located 8,130 or 8,418 bp downstream of the start codon (10,330 and 10,618 bp relative to the dTSS) and are described in detail below (Fig. 2D).

REGULATION AT A DISTANCE

Bmp2 is located in a gene desert of over 1 megabase. The nearest protein coding genes are 645 kb upstream and 1,115 kb downstream respectively (Fig. 2A). *Bmp2* is typical of genes located in gene deserts, which often control critical developmental processes. The extensive cross-species synteny around *Bmp2* is consistent with selective pressure on regulatory elements that control *Bmp2* gene expression from a distance. Interestingly, genes located in gene deserts often have messenger RNAs (mRNAs) with 3′untranslated regions (UTRs) that are conserved between mammals and chick. As discussed below, *Bmp2* is no exception with the entire 3′UTR conserved to chicken and a large fraction conserved to fishes [Abrams et al., 2004; Liu et al., 2008b]. Such genes may require evolutionary preservation of both the DNA elements that influence transcription initiation and elongation, and the RNA elements involved in co- and post-transcriptional processes.

Long-range *cis*-regulatory elements around *Bmp2* were mapped with bacterial artificial chromosome (BAC) reporter vectors in transgenic mice (Fig. 2B [Chandler et al., 2007; Pregizer and Mortlock, 2015]). Exon 3 was replaced with a β-*geo* gene, which encodes a β-galactosidase-neomycin phosphotransferase fusion protein. An internal ribosome entry (IRES) initiated translation of the enzyme. Collectively, these BAC reporters revealed transcription-activating elements located 185 kb upstream to 207 kb downstream of the distal transcription start site. The two BACs that spanned this 392 kb region were expressed in many embryonic mesenchymal sites known to express *Bmp2*. For example, both BACs were expressed in the apical ectodermal ridge and mesenchyme of the mid-gestation mouse limb bud (E11.5). Therefore, regulatory elements driving limb bud expression are located in the 55.5 kb of common BAC sequence. This sequence includes elements that bind developmental regulators such as the HOXA13 homeodomain transcription factors described below.

Despite 55.5 kb of overlap (Fig. 2B), each BAC drove many patterns that were unique in mid-gestation embryos. The 5′BAC (−185 to +53 kb) was expressed in the heart mesoderm of day 9.5 embryos and later, in skeletal muscle, vascular cells, and synovial joints (E11.5 and E13.5). In contrast, the 3′BAC (−2.5 to +207 kb) was expressed in the dorsal limb bud mesenchyme (E11.5), and later in the interdigital mesenchyme of the developing limbs, tooth buds, and intervertebral discs.

In bones, the two BACs each drove a subset of the expected patterns. The 5′BAC was expressed in the hypertrophic chondrocytes of developing endochondral bones from the embryonic to the postnatal period (4 weeks) and in osteocytes [Chandler et al., 2007; Pregizer and Mortlock, 2015]. During fracture repair, the 5′BAC also was expressed in the smooth muscle and endothelial cells of the vasculature [Matsubara et al., 2012]. Significantly, both cell types may become osteoblasts: smooth muscle cells via *trans*-differentiation and endothelial cells following endothelial-mesenchymal transition. Pathological induction of *Bmp2* in these cell types via these 5′ regulatory elements may contribute to pathological calcification in the vasculature.

Curiously, a much smaller reporter gene with only sequence spanning the two transcription start sites also was expressed in chondrocytes (−1,977 to +900 relative to the dTSS, Fig. 2C [Feng et al., 2003]). This suggests that only promoter proximal regulatory elements control chondrocyte expression. However, despite containing all the sequence in the smaller reporter gene (−1.98 kb to

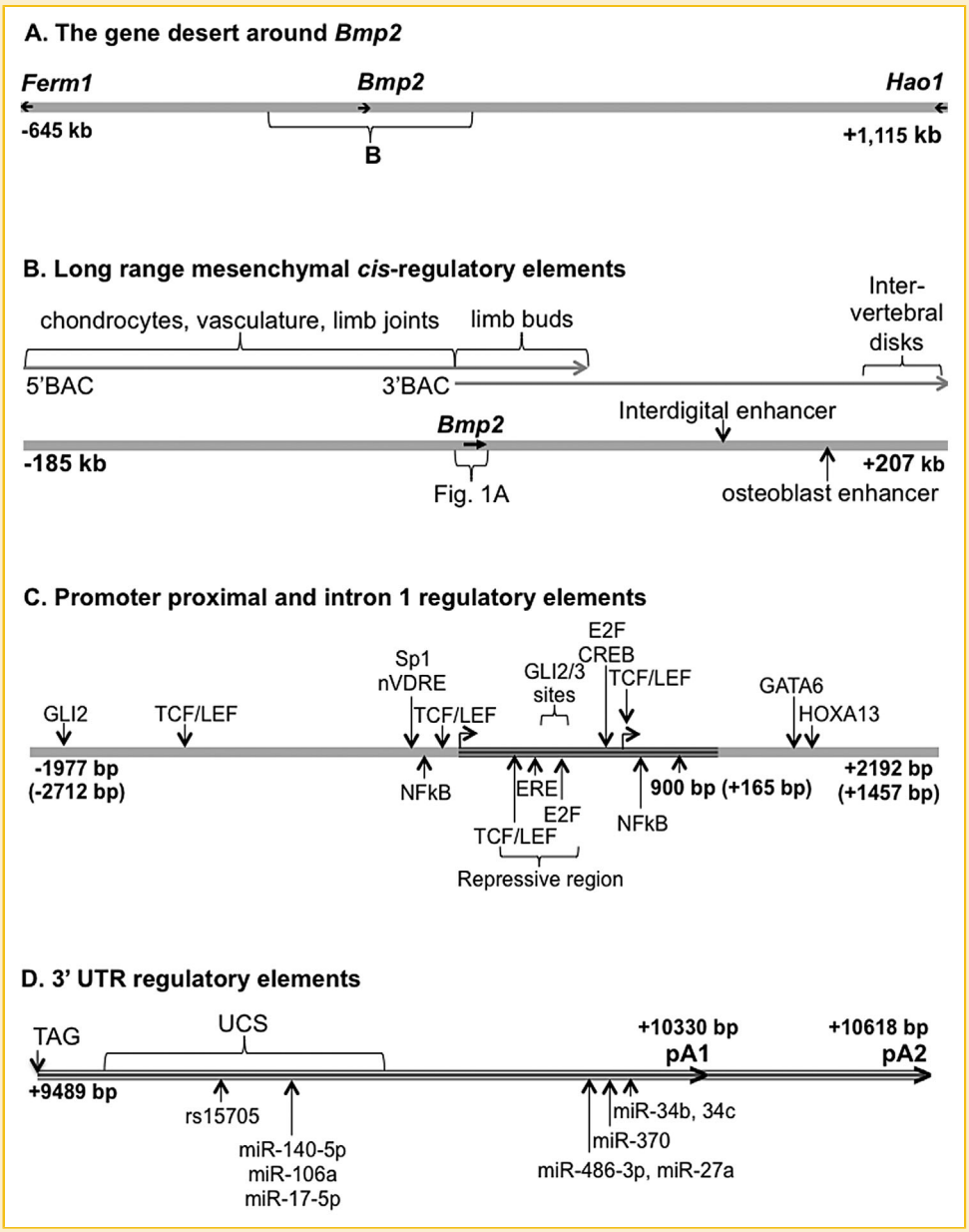

Fig. 2. *Bmp2* gene regulation. Schematic diagram of the chromosomal and *cis*-regulatory features surrounding the *Bmp2* gene. Gene features are plotted relative to the more widely expressed and conserved distal TSS (dTSS). However, because both start sites have been used to orient transcription factor binding sites, Fig. 2C indicates both numbers. Some papers have mapped binding sites relative to an outdated Genbank start located 826 nt downstream of the dTSS or the human mRNA Reference Sequence 5′ end (NM_001200.2) located 387 nt downstream of the human dTSS. These sites also have been plotted relative to the dTSS. See text for references supporting these features. A: The *Bmp2* gene relative to the nearest coding genes (fermitin family member 1, *Ferm1* and hydroxyacid oxidase 1, *Hao1*). Noncoding transcripts are not shown. Gencode 21 version, released October 2, 2014. B: The 392 kb of sequence flanking the mouse *Bmp2* gene that was surveyed by bacterial artificial chromosome (BAC) reporter clones in transgenic mice. Regions that are expressed in major mesenchymal cell types are indicated. C: Transcription factor binding sites upstream and downstream of the promoter region; distal and proximal TSS are indicated by (⇈). Only factors for which physical binding or reporter gene evidence is available are shown. Negative Vitamin D Response Element (nVDRE), Estrogen Response Element (ERE). D: Known regulatory features of the 3′UTR. Ultra Conserved Sequence (UCS) and two polyadenylation and cleavage sites (pA1 and pA2) are shown.

+207 kb), the 3′BAC failed to be expressed in hypertrophic chondrocytes [Chandler et al., 2007]. The mechanism that represses the 3′BAC in chondrocytes has yet to be discovered.

In contrast to the 5′BAC, the 3′BAC was expressed in osteoblast progenitors of both endochondral and intramembranous bones in embryos, but not after birth [Pregizer and Mortlock, 2015]. The sequence that directed expression in osteoblast progenitors was narrowed to a 4.5 kb fragment located 156 kb downstream of the distal promoter termed ECR1. Fibroblast Growth Factor 2 (FGF2) induces *Bmp2* in osteoblast progenitors. Consistent with ECR1

mediating the activating effect of FGF2 on *Bmp2,* reporter genes bearing ECR1 were activated by FGF2 in MC3T3-E1 pre-osteoblast cells [Jiang et al., 2010a].

The BAC studies also mapped enhancer activity that drove expression in the interdigital mesenchyme of developing limbs to between 94 and 132 kb downstream of *Bmp2* [Chandler et al., 2007]. Within this region, 4.5 to 5.5 kb tandem microduplications approximately 110 kb downstream of *BMP2* were identified in four families with autosomal-dominant brachydactyly type A2 (BDA2, OMIM: 112600). BDA2 is a congenital defect characterized by hypoplastic middle phalange of the second finger and occasionally other digits. A mouse genomic fragment (mouse Bmp2 homolog-enhancer region aka mBmp2-h-ER) corresponding to the human duplication was cloned into a *lacZ* reporter transgene. The resulting β-galactosidase staining recapitulated the *Bmp2* RNA pattern observed in the interdigital mesenchyme of limb buds and the developing phalanges [Dathe et al., 2009]. Normal digit formation requires tight integration of BMP signaling with other developmental signals. It is reasonable to expect that copy number variation in a regulatory element that controls BMP2 synthesis in the interdigital regions would disrupt digit patterning.

The ECR1 osteoblast enhancer is conserved from mammals to chickens [Chandler et al., 2007]. Portions of the interdigital mesenchyme enhancer region associated with BDA2 are conserved between mammals, chicken, and the frog *Xenopus tropicales* [Dathe et al., 2009]. Selective pressure to maintain distant enhancers like these two residing over 100 kb downstream of the transcribed region must contribute to maintaining the gene desert surrounding *Bmp2*.

REGULATION AT THE FRONT END—LINKS BETWEEN SIGNALING AND TRANSCRIPTION

BMP AND WNT/β-CATENIN SIGNALING

Feedback regulation of *Bmp2* is a recurring theme with enormous biological relevance. Indeed, BMP2 induces its own gene′s expression [Ghosh-Choudhury et al., 2001]. This auto-induction amplifies the effect of other inducing agents and mechanisms. The interactions between BMP and WNT/β-catenin signaling are an excellent example. In addition to the independent effects furthered by each pathway, a bewildering array of synergistic and opposing effects occur in different tissues and contexts. Similar complexity occurs in the homologous invertebrate *decaplentaplegic* (*dpp*) and *wingless* (*wg*) paths. BMP/WNT signaling controls the differentiation of mesenchymal cells into adipocytes, osteoblasts, and chondrocytes. Both physiological osteogenesis in bone and osteogenesis in pathological calcification of the cardiovascular system involve this cooperation.

The *Bmp2* gene is a direct downstream target of WNT signaling. In osteoblasts or preosteoblasts, BMP2 induces WNT signaling [Bain et al., 2003; Rawadi et al., 2003]. Conversely, WNT signaling induces BMP2 signaling [Cho et al., 2012; Nemoto et al., 2012; Zhang et al., 2013; Cho et al., 2014]. Canonical WNT signaling causes the translocation of β-catenin to the nucleus where it activates T-cell factor/lymphoid enhancer factor (TCF/LEF)-mediated transcription. Binding sites for TCF/LEF have been physically mapped to −1,085 bp upstream of the distal TSS [Zhang et al., 2013], flanking the distal TSS (−21 and +233 bp), and just downstream of the proximal TSS (+808 bp) [Cho et al., 2012; Cho et al., 2014]. The site 21 bp upstream of the distal TSS may be particularly important, because the local sequence is identical between rodents and primates (Figs. 1B and 2C [Abrams et al., 2004]).

Interestingly, *Bmp2* is more responsive to WNT signaling in mesenchymal cells that have traveled further down the osteogenic pathway (MC3T3-E1 pre-osteoblast, C3H10T1/2 mesenchymal progenitor cells, ST2 bone marrow stromal cells, and C2C12 pre-myoblasts) relative to mesenchymal cells that retain alternative potential (3T3-L1 pre-adipocytes or NIH3T3 fibroblasts) [Cho et al., 2014]. The highly GC-rich *Bmp2* promoter region (Fig. 1B) bears the repressive epigenetic chromatin marks of CpG methylation and reduced histone acetylation in the non-responsive cells. Agents that reduce CpG methylation (5-azacytidine) or increase the acetylation of histones H3 and H4 (trichostain A) stimulate the recruitment of LEF-1 to the *Bmp2* promoter [Cho et al., 2014]. This leads to increased *Bmp2* expression that enhances osteogenesis in non-permissive cells. The important finding that epigenetic control can restrain *Bmp2* expression begins to explain tissue specific BMP and WNT crosstalk. Furthermore, diseases involving excess BMP2 and subsequent pathological *trans*-differentiation, e.g., the conversion of vascular smooth muscle cells or valve interstitial cells into calcification-promoting osteoblasts, may respond to pharmaceutical approaches that alter chromatin modification.

HEDGEHOG AND FGF SIGNALING

Other major developmental paths that intersect with BMP signaling in vertebrates and invertebrates include the Hedgehog and FGF pathways [Lin and Hankenson, 2011]. Three GLI proteins transmit the Hedgehog signal in mammals. GLI1 and 2 mainly activate, while GLI3 is bifunctional, like its fly homolog *cubitus interruptus* (Ci), and can either repress or activate transcription. In flies, Hedgehog directly regulates the homolog of *Bmp2*,*decapentaplegic*, via a Ci binding site. Similarly, GLI binding sites have been mapped to the *Bmp2* promoter region. One site is centered 1,798 bp upstream of the distal TSS, while three sites are clustered between +422 and +580 (Fig. 2C [Garrett et al., 2003; Zhao et al., 2006; Zhao et al., 2009]). Hedgehog signaling prevents the proteolytic degradation of the activator GLI2 and the proteolytic processing of GLI3 to a repressor. GLI2 and full-length GLI3 can activate *Bmp2* expression using this cluster. However, after cleavage converts GLI3 to a repressor, these same sites mediate inhibition [Garrett et al., 2003].

The cluster of GLI binding sites resides between the proximal and distal promoters. This region represses *Bmp2* expression in different cell types [Jiang et al., 2010a]. In MC3T3-E1 preosteoblasts, this site completely blocked the ability of FGF2 to induce a *Bmp2* reporter gene bearing the ECR1 osteoblast progenitor enhancer (Fig. 2B). As described below, E2F transcription factors also can repress *Bmp2* via this inter-promoter region [Porlan et al., 2013]. Thus, agents that activate *Bmp2*, e.g., FGF and the ECR1 enhancer, must overcome several repressors that act between the promoters.

RETINOIDS

Relief of repression also may explain the observed activation of *Bmp2* by retinoic acid in cardiogenic mesoderm, mesenchymal stem cells, interdigital mesenchyme of developing limbs, and other cell types despite the lack of a classical retinoic acid response element (RARE) near the promoter [Rodriguez-Leon et al., 1999; Heller et al., 1999; Ghatpande et al., 2006; Helvering et al., 2000]. Sp1 and Sp3, members of the Krüpple-Like Factor (KLF) family of regulatory proteins, bind a site located 183 bp upstream of the distal promoter (Fig. 2C [Abrams et al., 2004; Xu and Rogers, 2007]). These transcription factors can either enhance or repress gene activity depending on their relative ratios within cells, the local binding site context, and cooperation with various transcription factors. Mithramycin, which blocks the binding of Sp1 to DNA, activates a *Bmp2*-driven reporter gene in the absence of retinoid treatment [Abrams et al., 2004]. Furthermore, Sp1 proteins and a non-ligand bound retinoic acid receptor (RARβ) assemble on the inactive *Bmp2* promoter. Retinoid-treatment induces disassembly and *Bmp2* expression [Abrams et al., 2004; Xu and Rogers, 2007]. Thus, activation of *Bmp2* by retinoids involves lifting a transcriptional barrier.

VITAMIN D

Intriguingly, the site bound by the RARβ/Sp1 complex can also function as a negative vitamin D response element (nVDRE). Elevated levels of the vitamin D receptor (VDR) disrupt calcium metabolism in genetic hypercalciuric stone-forming (GHS) rats. These rats have low bone mass, which may be partly due to repression of *Bmp2* by 1,25-dihydroxyvitamin D3 (1,25[OH] 2D3)—bound vitamin D receptor (VDR) [Fu et al., 2013]. In untreated or 1,25(OH) 2D3-treated rat Bone Marrow Stromal Cells (BMSCs) and UMR-106 rat osteosarcoma cells, *Bmp2* expression inversely correlated with the relative level of two chromatin marks typical of inactive genes, CpG methylation and methylation of histone H3 at lysine 9 (H3K9me2). Furthermore, drugs that reversed methylation or increased histone acetylation released this VDR-mediated repression and induced *Bmp2* [Fu et al., 2013]. Indeed, 1,25(OH) 2D3 has been shown to stimulate *Bmp2* in human aortic smooth muscle cells grown in high phosphate media that promotes calcification [Martinez-Moreno et al., 2012]. Pro-calcific conditions may promote a chromatin structure favorable to *Bmp2* transcription.

NF-κB ACTIVATION

Many stimuli trigger the NF-κB family of inducible transcription factors. A deficit in chondrocyte proliferation was observed in mice lacking both the p50 and p52 subunits of NFκB [Feng et al., 2003]. The abundance of the *Bmp2* transcript in the chondrocytes of the bone growth plate was reduced. Two conserved NFκB sites located −99 bp upstream and 863 bp downstream of the distal TSS are likely to mediate *Bmp2* induction by this regulatory factor (Fig. 2C). Curiously, *Bmp2* expression in the nearby articular chondrocytes was unaffected by the absence of NFκB [Feng et al., 2003]. In the context of this cell specific influence, it may be relevant that the upstream NFκB site is within the promoter proximal chromatin that is subject to epigenetic control [Fu et al., 2013; Cho et al., 2014] and just downstream of the repressive site bound by the vitamin D receptor [Fu et al., 2013] and the RARβ/Sp1 complex [Abrams et al., 2004; Xu and Rogers, 2007]. Multiple types of transcription factor assemblies are likely to be involved in the temporal and spatial patterns of BMP2 synthesis in the developing bone.

ESTROGEN

An effective strategy for preventing post-menopausal osteoporosis is estrogen supplementation, which has an anabolic effect on bones. A variant estrogen response element (ERE), located 409 bp downstream of the distal TSS, facilitates the ability of estrogen to induce the mouse *Bmp2* promoter [Zhou et al., 2003]. The binding of estrogen receptor α at this position also mediates the activating effect of resveratrol on *Bmp2* expression [Su et al., 2007]. Both estrogen and resveratrol clearly induce *Bmp2* in rodents [Zhou et al., 2003; Su et al., 2007]. However, the variant estrogen response element is poorly conserved even between mice and rats [Abrams et al., 2004]. Whether or not this region controls the human *Bmp2* gene remains to be established.

CYCLIC AMP (cAMP)

Intermittent exposure to parathyroid hormone (PTH) is another effective bone strengthening approach. PTH induces the second messenger cAMP, which activates the cAMP response element binding protein (CREB) and the coactivator CREB-binding protein (CBP). Both PTH and directly applied cAMP analogs are potent *Bmp2* inducers in mouse and rat cells [Rogers et al., 1992; Zhang et al., 2011]. One CREB/CBP binding site is centered 669 bp downstream of the distal TSS and only 66 bp upstream of the proximal TSS in the mouse gene [Zhang et al., 2011; Shim et al., 2012]. Other sites likely are located upstream because the sequence between −1.237 and +471 mediates responsiveness to dibutyryl cAMP [Abrams et al., 2004]. Mutations in the human *CBP* gene cause Rubinstein-Taybi syndrome, whose symptoms include skeletal dysplasia and an increased frequency of fractures (OMIM: 180849). BMP2 treatment alleviated the skeletal defects associated with *Cbp* mutations in mice [Shim et al., 2012]. A direct link between cAMP signaling and *BMP2* expression raises hope for new treatment strategies for skeletal conditions.

SIGNALING LEADING TO AUTO-INDUCTION

The ability of BMP2 to induce the transcription of its own gene is an important regulatory feature of both the mammalian *Bmp2* genes and the fly homolog, *decapentaplegic* [Ghosh-Choudhury et al., 2001]. Self-activation amplifies the effect of BMP2 inducers. As described above, BMP2 induces WNT signaling which then induces *Bmp2* itself. Another feedback mechanism involves activated intracellular kinases. BMP2 induces phosphatidylinositol-3-kinase (PI-3-kinase)/Akt signaling which then induces *Bmp2* RNA and protein levels. The transcription factor Myocyte Enhancer Factor 2A (MEF-2A), which influences myogenesis, is activated by PI-3-kinase in BMP2 treated cells. MEF-2A may directly target *Bmp2*, because both PI-3-kinase activation and MEF-2A overexpression stimulate a luciferase reporter driven by the *Bmp2* promoter (−1,977 bp to +900 relative to the dTSS [Ghosh-Choudhury et al., 2003, 2013]).

OTHER TRANSCRIPTION FACTORS

E2F TRANSCRIPTION FACTORS

The E2F gene family encodes essential regulators of cell proliferation that may function as repressors or activators. Association with members of the pRB (retinoblastoma) family facilitates repression. Phosphorylation of Rb frees the E2F factors to activate transcription. E2F1, 2, and 3 can strongly activate *Bmp2* transcription [Muller et al., 2001]. The p21 cyclin-dependent kinase (CDK) inhibitor controls whether or not the E2F factors up- or down-regulate transcription. Repression in cells with active p21 occurs when E2F factors bind between the two *Bmp2* promoters (+561 to +626, Fig. 2C) [Porlan et al., 2013]. Differentiation is tightly linked to cell cycle decisions. A direct link with key cell cycle regulators is an essential aspect of controlling this key morphogen.

GATA-6

Only three transcription factors have been reported to bind sites within the first intron; the developmentally critical GATA-6 and two homeodomain proteins (Fig. 2C). The GATA-6 transcription factor is required for forming the extraembryonic endoderm. GATA-6 also promotes the high levels of BMP2 observed in visceral extraembryonic endoderm [Rong et al., 2012]. An antibody to GATA-6 precipitates chromatin sequence beginning 319 bp into the first intron (1,444–1,635 relative to the dTSS) [Rong et al., 2012]. Two sequences conforming to a GATA consensus sequence of (T/A)GATA (A/G) were reported within the mouse sequence; however, this region is poorly conserved between mice and humans.

HOXA13 AND HOXD13

Hoxa13 and *Hoxd13* encode homeodomain transcription factors expressed in the developing limb. *Hoxa13* and *Bmp2* are co-expressed in the interdigital mesenchyme and developing joints of mid-gestation mouse embryos [Knosp et al., 2004]. Loss-of-HOXA13 function reduces *Bmp2* RNA levels and causes digit deformities. The malformations can be partially rescued by exogenous application of BMP2. Four sites with a core consensus sequence of TAAT between 1,610 and 1,982 relative to the dTSS can bind a peptide bearing the HOXA13 DNA binding domain [Knosp et al., 2004]. A separate ChIP-on-chip assay for HOXD13-interacting genes identified *Bmp2* as a potential target. A gain-of-HOXD13 function strategy in chick limbs confirmed that this homeodomain protein also can stimulate *Bmp2* [Salsi et al., 2008]. HOX-mediated induction of *Bmp2* would be unsurprising because homeodomain proteins induce the fly homolog *decapentaplegic*.

THE YIN AND YANG OF TRANSCRIPTION

In Chinese philosophy, yin and yang describes the interdependence of opposing forces that contribute to a necessary balance. The whole panoply of transcription regulators described above and those as yet undiscovered must work in combination to precisely control *Bmp2* transcription initiation. To a large degree, this comes down to whether the chromatin structure in the promoter region is closed and repressive or open and favorable for the recruitment of RNA polymerase II and associated factors. A repressive chromatin structure mediates the effect of vitamin D on *Bmp2* expression [Fu et al., 2013]. It also blocks WNT-activation of *Bmp2* in non-osteogenic mesenchymal cell types [Cho et al., 2014]. The changes in chromatin structure instigated by repressors such as the truncated GLI3 [Garrett et al., 2003] or the RARβ/Sp1 complex [Abrams et al., 2004; Xu and Rogers, 2007] or E2F [Porlan et al., 2013] remain to be described. However, activating *Bmp2* undoubtedly requires overcoming a locally repressive promoter context.

THE BACK END

TRANSCRIPT PROCESSING

Transcription regulation is only part of the equation that controls the timing, the location, and quantity of BMP2. Indeed, transcription elongation is influenced by co-transcriptional RNA processing: removal of introns and choice of polyadenylation and cleavage sites. In addition, alternative splicing and polyadenylation can yield messenger RNAs (mRNA) with potentially distinct coding sequences and/or distinct binding sites for post-transcriptional regulatory factors. Non-coding segments located upstream of the translation initiation codon, the 5′UTR, or downstream of the stop codon, the 3′UTR, contain important *cis*-regulatory motifs that control translational efficiency, mRNA localization, and mRNA stability.

Alternative splicing has not been observed for the *Bmp2* RNA. However, the distal and proximal transcription start sites described above yield distinct 5′ untranslated regions (UTRs) of 1,475 or 740 nt. respectively (Fig. 1). The role of the *Bmp2* 5′UTRs has not been studied directly. However, it should be pointed out that mRNAs generated by reporter genes bearing both promoters; e.g., the widely used vector developed by Ghosh-Choudhury et al. [2001], should bear the extended 5′UTR. Consequently, some compounds inferred to transcriptionally activate *Bmp2* via sites downstream of the distal promoter may also or instead act post-transcriptionally.

Experimentally validated polyadenylation sites yield two 3′ UTRs of ∼875 and ∼1,185 nt respectively in various human and mouse cell types [Fritz et al., 2004; Fukui et al., 2006; Liu et al., 2008b]. A study using Rapid Amplification of cDNA Ends (3′ RACE) suggested that another site may yield a 3′UTR of 3,736 nt in human chondrocytes, but not in mouse cells [Fukui et al., 2006]. Sequence examination suggests this putative polyadenylation site may be a false positive. First, the cleavage position is poorly conserved. Second, a stretch of 15 adenines occurs in the human genome, but not the mouse genome. Contaminating genomic DNA may have bound the oligo(dT) primer and led to amplification of a downstream sequence. It should be noted that the BAC reporters described above had a strong SV40 polyadenylation signal inserted between the coding sequence and the 3′UTR. The BAC-generated transcripts lack the natural *Bmp2* 3′UTR [Kruithof et al., 2011a]. Consequently the BAC expression patterns do not reflect any control mechanisms involving RNA elements in the *Bmp2* 3′UTR. However, the transcripts would be initiated at the natural *Bmp2* promoters and be spliced at the same exon-intron junctions as *Bmp2*. Accordingly, post-transcriptional elements in the 5′UTR and the introns may function normally.

Unlike the *Bmp2* 5′UTR and introns, the 3′UTR has been actively studied. The *Bmp2* 3′UTRs are 2 to 3 times longer than the median

3′UTR length of 385 nt in mouse and are extensively conserved between chicken and mammals [Fritz et al., 2004; Tian et al., 2005; Hu et al., 2006]. In contrast, the closely related *Bmp4* has a relatively short and poorly conserved 3′UTR even between mice and humans [Hu et al., 2006]. Conservation of the two polyadenylation sites shown in Fig. 1D suggest that the need for alternative 3′UTRs has been retained for at least the ~310 million years since the mammalian and avian lineages separated [Fritz et al., 2004; Liu et al., 2008b]. In both human and murine cells, the upstream polyadenylation site closest to the stop codon (pA1) is used preferentially over the downstream site (pA2). Like many strong polyadenylation signals, a conserved U-rich motif located between the consensus pA1 signal (AAUAAA) and the cleavage and polyadenylation site confers the relatively greater strength of pA1. Although the human and mouse polyadenylation signals are very similar, the precise ratio of endogenous *Bmp2* transcripts ending at pA1 or pA2 differs between mouse and human cells. A higher affinity of mouse *cis*-regulatory elements for the polyadenylation factor CstF-64 accounted for this species-specific difference, rather than species-specific *trans*-regulatory factors [Liu et al., 2008b]. Thus, both broadly conserved and species-specific mechanisms regulate alternative polyadenylation of *Bmp2* mRNAs [Liu et al., 2008a]. The common regions of the two 3′UTRs will interact with the same set of regulatory proteins and microRNAs (miRNAs). However, factors that bind only the longer transcript may influence the temporal and spatial patterns of BMP2 synthesis in healthy or diseased tissues.

REGULATORY ELEMENTS IN THE 3′UTR

More striking sequence conservation occurs in the first half of the 3′UTR, where a 265-nucleotide stretch is 73% identical between mammals, birds, frogs, and fishes (Fig. 2D, [Abrams et al., 2004; Fritz et al., 2004]). Indeed, motifs within this ultra-conserved sequence (UCS) align with the 3′UTR of the *AmphiBMP2/4* gene in Amphioxus, a chordate cousin whose family branched 650 million years ago [Fritz et al., 2004]. Only the BMP2 amino acid sequence has changed less than the 3′UTR over evolutionary time. This remarkably slow pace of evolutionary change relative to the 5′UTR, introns, and most intergenic sequences strongly supports ancient regulatory mechanisms necessary for controlling BMP2 synthesis.

Single stranded RNAs can form a plethora of folded structures. Thus, the primary sequence and its nucleotide composition and the resulting secondary conformation of the UCS will influence the binding of regulatory proteins and miRNAs. The UCS harbors multiple copies of AU-rich elements (AREs), which are post-transcriptional motifs that affect the polyA tail length, translation efficiency, and mRNA stability. The regulatory factors that bind AREs can facilitate or prevent the recruitment of mRNAs to ribosomes or the degradation apparatus, thus altering protein synthesis more rapidly than transcription factors. Typically, genes with AU-rich 3′UTRs encode proteins such as cytokines and growth factors that are rapidly induced by local conditions and then equally rapidly cleared. BMP2 is a classic example. Specifically, the short-lived transcript forms highly dynamic and precise patterns, for example, in the developing heart valve and immediately after bone fracture.

The number of AREs influences the rate of synthetic RNA decay in cell extracts in vitro and the half-life of reporter gene RNAs in living cells. The full-length UCS consistently decays more rapidly than segments bearing fewer AREs [Abrams et al., 2004; Fritz et al., 2004; Fritz et al., 2006; Fukui et al., 2006]. However, the absolute rate of decay in extracts or in cells, depends on cell type. Cells stimulated to express more BMP2 relative to partially stimulated cells produced reporter gene RNAs with an increased half-life [Abrams et al., 2004; Fritz et al., 2004; Fritz et al., 2006; Fukui et al., 2006]. Evidently, the UCS can influence BMP2 synthesis by controlling transcript degradation. Translational regulation also may occur as suggested by discordance between reporter gene activity and RNA levels observed in lung cells [Jiang et al., 2010b]. However, whether or not the UCS also influences recruitment to ribosomes and translation rate has yet to be determined in mesenchymal cells.

Clearly, some level of transcription is needed to produce an mRNA that is subject to co-transcriptional or post-transcriptional regulation. In this two-step activation process, RNA polymerase II must be recruited to the promoter and initiate transcription. Subsequently, regulatory factors can bind the growing transcript to choose the polyadenylation and cleavage site, or the mature transcript to control the rate of translation or decay. A complete block to synthesis can occur at this level. For example, the UCS and the 3′UTR can only activate reporter genes when the *Bmp2* promoter is independently activated, such as by differentiation status [Abrams et al., 2004; Fukui et al., 2006]. The first hint that some cells may initiate *Bmp2* transcription but then remain poised to make BMP2 was the demonstration that *Bmp2* transcripts are synthesized in the nuclei of differentiated chondrocytes. TNF-α treatment leading to p38 signaling was required to finally produce BMP2 [Fukui et al., 2006]. Subsequent reporter gene studies in cells and transgenic mice revealed that the UCS may hold BMP2 synthesis at bay in many cell types, including most mesenchymal cells; e.g., primary mouse calvarial cells, C3H10T½ pluripotent mesenchymal cells, vascular smooth muscle cells, perivascular fibroblasts, and heart valve cells [Kruithof et al., 2011a,b]. Clinically severe calcification pathologies involving abnormal levels of BMP2 such as calcific aortic valve disease, atherosclerosis, and medial artery calcification occur in mesenchymal tissues [Bostrom et al., 1993; Yutzey et al., 2014]. A reasonable hypothesis is that the first step, transcriptional activation, has occurred but that BMP2 synthesis is blocked at a later post-transcriptional stage in these cells. Physiological conditions that promote cardiovascular calcification such as diabetes, kidney disease, or hyperlipidemia may weaken this barrier to BMP2 synthesis. If so, then understanding how the UCS represses BMP2 synthesis may reveal novel therapeutic strategies for diseases involving adverse BMP2 synthesis.

Thus far in mesenchymal cells, the UCS has been reported to be purely inhibitory. In contrast, the UCS up-regulates *Bmp2* in differentiated embryonal carcinoma cells and transformed lung cells. In these cells, which express high levels of *Bmp2*, the UCS activates expression by 3 to 5 times the level driven by the *Bmp2* promoter alone [Abrams et al., 2004; Jiang et al., 2010b]. Mesenchymal cell repression is independent of the promoter, coding sequence, and polyadenylation signal [Fukui et al., 2006; Devaney et al., 2009; Kruithof et al., 2011a,b]. In contrast, we have only observed reporter gene activation in vectors driven by the *Bmp2* promoter itself [Abrams et al., 2004; Jiang et al., 2010b]. It may be

TABLE I. Molecules and Conditions That Induce *Bmp2* Expression

	Effector	Assay	Cell type	References[a]
Growth factors	BMP2	RNA, reporter gene activity	2T3 pre-osteoblasts, BNL mouse liver cells, JB6 epidermal cells and kidney mesangial cells	[1, 2] and many other reports
	tumor necrosis factor (TNF) α	RNA, reporter gene activity	fibroblast-like synoviocytes, mouse ATDC5 chondrogenic cells, primary cultured adult human articular chondrocytes, coronary arterial endothelial cells	[3–5]
	fibroblast growth factor (FGF2)	RNA	MC3T3-E1 pre-osteoblast cells, mouse or chick calvarial cells, primary human osteoblasts	[6–9]
	calcitonin gene-related peptide (CGRP)	RNA	female osteoporotic rat-derived bone mesenchymal stem cells	[10]
	parathyroid hormone (PTH)	RNA, protein, reporter gene activity	C2C12, 2T3, UMR106, and MC3T3-E1 osteoblastic cell lines, primary calvarial cells	[11]
	interleukin-1β (IL-1β)	RNA, protein	fibroblast-like synoviocytes, periodontal ligament cells	[3, 12]
	interleukin-6 (IL-6)	RNA	vascular smooth muscle cells	[13]
Physical conditions	stress fracture	RNA, protein, reporter gene activity	bones	[30–32]
	mechanical cyclic tensile stretch	RNA	human intraoral mesenchymal stem and progenitor cells	[33]
	biomechanical stimulation	RNA	articular chondrocyte alone or co-cultured with osteoblasts	[34]
	Hypoxia (3% for 6 hours)	RNA, reporter gene activity	human fetal osteoblasts, human MG-63 osteoblast-like cells, mouse M2-10B4 bone marrow stromal cells	[35]
	Enamel matrix derivative (EMD), mechanical stretch	RNA, protein	periodontal ligament cells	[12]
	laser irradiation	RNA, protein	MC3T3-E1 pre-osteoblasts	[36]
Cellular signals and pathways	WNT/β-catenin signaling	RNA, reporter gene activity	C3H10T1/2 pluripotent mesenchymal, MC3T3-E1 pre-osteoblasts, and 2T3 cells; ST2 bone marrow stromal cells, C2C12 pre-myoblasts (3T3-L1 pre-adipocytes and NIH3T3 fibroblasts only after drug-mediated derepression of chromatin modifications)	[14–17]
	Cysteine-rich protein 61 (CYR61) integrin ligand	RNA, protein	MC3T3-E1 pre-osteoblasts	[18]
	prostaglandin E2	RNA	human mesenchymal stem cells	[19]
	estrogens	RNA, reporter gene activity	mouse bone marrow mesenchymal stem cells from ovariectomized mice, C3H10T1/2 cells expressing exogenous estrogen receptors	[20, 21]
	E2F transcription factors	RNA	U2OS human osteosarcoma cell line, c17.2 mouse multipotent neural stem-like cells	[22, 23]
	all trans retinoic acid (ATRA)	RNA, reporter gene activity	F9 embryonal carcinoma cells, chick limb bud, quail embryonic heart	[24–26]
	ATRA, 9 cis RA, 13 cis RA	RNA, reporter gene activity	U2OS human osteosarcoma, UMR-106 rat osteosarcoma, C2C12 myoblastic cells	[27]
	all trans retinaldehyde (Rald)	RNA, mice and cells unable to convert Rald to ATRA	primary Aldh1a1 null mesenchymal stem cells	[28]
	1,25 dihydroxyvitamin D3 in high phosphate media	RNA	human aortic smooth muscle cells (1,25 dihydroxyvitamin D3 repressed Bmp2 in rat bone marrow stromal and UMR-106 rat osteosarcoma cells grown in DMEM, see text)	[29]
	reactive oxygen species (ROS)	RNA, reporter gene activity	2T3 pre-osteoblasts	[2]
	hydrogen peroxide	RNA	coronary arterial endothelial cells	[5]
Bacterial	Mycoplasma species	RNA, protein, reporter gene activity	C3H10T1/2 pluripotent mesenchymal, A549 lung adenocarcinoma, MCF7 breast cancer, HeLa cervical cancer, BEAS-2B immortalized bronchial epithelial cells	[37]
	Helicobacter pylori	RNA	human gastric carcinoma cell line AGS	[38]

TABLE I. (*Continued*)

	Effector	Assay	Cell type	References[a]
Drugs and other small molecules	bisphosphonates	RNA	MG-63 osteoblast-like cells	[39]
	Statins	RNA, protein, reporter gene activity	2T3 pre-osteoblasts, MG-63 osteoblast-like cells, mouse calvarial cells	[40]
	Microtubule assembly inhibitors: TN16, colchicine, nocodazole	RNA, reporter gene activity	2T3 pre-osteoblasts	[41]
	proteasome inhibitors	RNA, protein, reporter gene activity	MG63 osteoblast-like cells, 2T3 pre-osteoblasts, Hu09 human osteoblastic cells, and fetal rat calvarial cells	[42]
	Mithramycin (blocks Sp1 binding)	reporter gene activity	F9 embryonal carcinoma cells	[43]
	5-aza-Cytidine (prevents cytosine methylation), trichostatin A (TSA, prevents histone deacetylation)	RNA, reporter gene activity	WNT activated 3T3-L1 pre-adipocytes and NIH3T3 fibroblasts	[17]
	Resveratol	RNA, protein, reporter gene activity	MC3T3-E1 pre-osteoblasts, MG-63 osteoblast-like cells, rat primary osteoblasts, MCF-7 and MDA-MB-231 breast cancer	[21]
Botanical compounds	Botanical extracts (quercetin, licorice, eleuthero, rehmannia, sophora)	RNA	MG-63 osteoblast-like cells, 2T3 pre-osteoblasts	[44]
	Osthole	RNA, protein	primary mouse calvarial cells	[45]
	Fucoidan	RNA, protein	human alveolar bone marrow-derived mesenchymal stem cells	[46]
	Daidzein	RNA, protein	rat primary calvarial cells	[47]

[a]References are in supplemental material.

that co-transcriptional processes involving an interaction with the transcription machinery, and/or upstream transcript regions are required for the UCS to positively affect *Bmp2* expression.

Surprisingly, reporter genes and RNAs bearing the zebrafish UCS can act similarly to the mammalian UCS in biochemical decay assays and reporter gene assays in mammalian cells [Fritz et al., 2004]. This might lead one to conclude that only the most conserved 73% of the UCS is sufficient to interact appropriately with cellular decay factors. As expected for a sequence that has changed little over evolutionary time, genetic variation is limited. However, an A to C transversion (rs15705) occurs within the UCS with a frequency of 0.22. Individuals heterozygous for the C allele occur in about 4% of the population and exhibit no apparent developmental anomalies. Significantly, this single nucleotide polymorphism (SNP) disrupts an ARE motif. Altering just this one ARE within the UCS exerts a measurable effect on the binding of regulatory proteins such as HuR and nucleolin, on decay in vitro, and on reporter gene expression [Fritz et al., 2006; Devaney et al., 2009; Jiang et al., 2010b]. Furthermore, in healthy men, this SNP alone explained 2% to 4% of inter-individual variability in baseline subcutaneous fat volumes ($P = 0.0030$) and in muscle volume gain following resistance training ($P = 0.0060$, [Devaney et al., 2009]). In women, an association with change in bone volume was observed ($P = 0.0099$). BMP2 is a potent morphogen whose concentration influences myogenesis, adipogenesis, chondrogenesis, and osteogenesis. Therefore, modest variation in *cis*-regulatory elements within the Bmp2 3′UTR may impact BMP2 influenced pathologies in mesenchymal cells. Indeed, rs15705 SNP and other non-coding SNPs near *Bmp2* have been associated with disorders of mesenchymal tissues such as osteoporosis and osteoarthritis (referenced in [Devaney et al., 2009]; http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=650&chooseRs=all).

The diverse combinations of RNA-binding proteins and miRNAs that mediate the effects of the Bmp2 3′UTR are only beginning to be deciphered. Nucleolin and the stabilizing ARE-binding protein HuR are among the dozens of proteins that can bind the UCS [Fritz et al., 2006; Devaney et al., 2009; Jiang et al., 2010b]. In addition, hundreds of miRNAs are strongly predicted to bind the *Bmp2* 3′UTR (http://www.microrna.org/microrna/getMrna.do?gene=650&utr=21822&torganism=9606). Several of these miRNAs have been experimentally validated (Fig. 2D). For example mir-140-5p, mir-106a, mir-17-5p, mir-27a, and mir-370 inhibit the differentiation of mesenchymal cells into osteoblasts by directly targeting *Bmp2* [Itoh et al., 2012; Li et al., 2013; Gong et al., 2014; Hwang et al., 2014]. Additional miRNAs that target *Bmp2* include mir-34b, mir-34c-3p, and miR-486-3p [Fotinos et al., 2014]. *Bmp2*-targeting miRNAs also inhibit effectors of BMP2 signaling, such as the BMP receptor BMPR1A (miR-27a [Gong et al., 2014]), the signaling intermediary SMAD1 (miR-486 [Lin et al., 2009]), and the osteogenic transcription factor RUNX2 (mir-34c [Zhang et al., 2011]). The ability of these proteins and miRNAs to modulate the synthesis of both BMP2 and downstream effectors can precisely control where, when, and how much BMP2 is synthesized and activated.

DOES MY FAVORITE MOLECULE/CONDITION INDUCE *BMP2*?

Many molecules and conditions have been reported to induce *Bmp2* gene expression. Inducers that can activate by 10 fold or more

include small molecules such as the vitamin A-derivative retinoic acid (RA) [Rogers et al., 1992; Francis et al., 1994; Helvering et al., 2000; Hallahan et al., 2003] and proteins such as the proinflammatory cytokines IL-1β and TNF-α [Fukui et al., 2003; Lories et al., 2003]. Other biomedically relevant agents thought to directly regulate *Bmp2* expression include estrogen [Zhou et al., 2003], statins [Mundy et al., 1999], proteasome inhibitors [Garrett et al., 2003], and bisphosphonates [Im et al., 2004]. Physical forces, such as fracture, also induce *Bmp2* [Gerstenfeld et al., 2003].

One of the most effective and universal activators deserves a strong cautionary note. Mycoplasma infection sharply induces BMP2 synthesis in many cell types, including mesenchymal cells [Jiang et al., 2007]. Both the expected 3 kb *Bmp2* transcript and an unusual 1.5 kb transcript are present in infected cells [Jiang et al., 2007]. The smaller transcript that may be unique to contaminated cells can be revealed by Northern blot techniques. Between 10 and 30% of cell cultures are mycoplasma-contaminated. Unsuspected BMP2 synthesis resulting from mycoplasma contamination will confound experimental results both in tissue culture and in vivo, for example, in experimental or clinical orthopedic studies.

Finally, the BMP2 protein itself stimulates *Bmp2* expression [Ghosh-Choudhury et al., 2001]. Consequently, the effect of an agent that induces *Bmp2* expression will be amplified by *Bmp2* auto-induction. To answer a question we are frequently posed "Does this _____ induce *Bmp2*?" we have compiled a table of reported inducers (Table I). Several issues hamper the approaches used to identify compounds that induce *Bmp2*. Reverse transcription PCR, the most commonly used method of detecting the relatively low abundance *Bmp2* transcript, is often non-quantitative and may not differentiate between the normal and mycoplasma-associated RNAs. The extreme conservation of the BMP2 protein and its similarity to BMP4 has hindered raising good, selective antibodies that distinguish BMP2 and BMP4. A luciferase reporter vector with 2.7 kb of sequence spanning the two transcription start sites (−1,977 bp to +900 relative to the dTSS, Fig. 2C, [Ghosh-Choudhury et al., 2001] has been a workhorse of many *Bmp2* gene regulatory studies and searches for activating compounds. However, all published reporter constructs, even the large BAC constructs, lack specific transcriptional or post-transcriptional *cis*-regulatory regions that may activate or repress expression. For Table I, we have chosen compounds or conditions that up-regulate the *Bmp2* RNA or protein by at least threefold or have multiple lines of evidence supporting induction.

CONCLUSIONS

Many gaps remain in our understanding of *Bmp2* gene regulation. The role of conserved elements located in introns has not been investigated. What is the significance of the extreme conservation of long distance elements (the gene desert) and the highly conserved 3′UTR? Does this reflect necessary co-transcriptional regulation? What *cis*-regulatory elements mediate the effects of other vital signaling pathways; for example, the repression of *Bmp2* by Hey1 and Hey2 that follows Notch signal activation in the heart [de la Pompa and Epstein, 2012]? Can cell specific activators be harnessed to stimulate BMP2 synthesis and thus increase bone mass or facilitate fracture repair? Can repressors be exploited to control synthesis in the calcification-prone vasculature and valves?

Numerous factors that up-regulate *Bmp2* expression have been described (FGF2 via ECR1, WNT signals via TCF/LEF sites, Hedgehog via GLI binding sites, cAMP via CREB binding sites, NFκB, E2Fs, MEF2A, GATA6, and HOXA13 and HOXD13). However, negative regulation of *Bmp2* expression and function is pervasive. At the signaling level, various highly conserved extracellular antagonists such as Noggin and intracellular repressors such as inhibitory SMADs block BMP2 signal transduction. At the transcriptional level, *Bmp2* expression is down-regulated by many repressors that facilitate repressive epigenetic chromatin modifications (truncated GLI3, RARβ complexed with Sp1, vitamin D receptor, and E2F factors in p21-activated cells). Finally, extraordinarily conserved post-transcriptional processes act via the 3′UTR to modulate BMP2 synthesis. The 3′UTR activates expression in some cells. But, especially in mesenchymal cells, the 3′UTR frequently restrains *Bmp2*. Animals have evolved a plethora of means to limit BMP2 signaling. Balancing the positive and negative factors that regulate *Bmp2* expression is the first step needed for this morphogen to function precisely in the right place, at the right time, and to the right extent.

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