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Autoregulation of Mouse BMP-2 Gene Transcription Is Directed by the Proximal Promoter Element

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Bone morphogenetic protein-2 (BMP-2) stimulates the commitment and differentiation of precursor mesenchymal cells to mature bone. We have isolated and sequenced 2712 base pairs (bp) of the 5′ flanking region of mouse BMP-2 gene. Using RNase protection assay we identified two transcription initiation sites within this 2712 bp region of the BMP-2 gene. The distal start site was mapped to −736 bp in relation to the proximal start site (+1). Recombinant BMP-2 preferentially stimulated transcription initiation from the proximal start site. To investigate the mechanism of transcription initiation from these two start sites, we identified two promoter elements upstream of the proximal and distal transcription initiation sites. Transfection of promoter-luciferase reporter constructs into cells of different organs demonstrated differential transcriptional activity of proximal and distal promoters, with highest activity in the osteoblast cell lineage. In osteoblasts, BMP-2 stimulated transcription from the proximal promoter only. Together our data provide the first evidence for the presence of two transcription initiation sites with two upstream promoter elements in mouse BMP-2 gene. Furthermore, we demonstrate for the first time that BMP-2 autoregulates its expression in osteoblasts through the proximal promoter-dependent transcriptional mechanism.

Key Words: **BMP-2 mRNA initiation sites; transcriptional regulation; BMP-2 autoinduction.**

The bone morphogenetic proteins (BMPs) represent a number of related peptides in the transforming growth factor β (TGFβ) superfamily which elicit pleotrophic effects in a range of tissues (1). The BMPs contribute to a variety of biological processes, including skin and hair formation, tooth development, neural cell differentiation, ventralization of mesoderm, kidney formation, heart development, cartilage and bone formation, and early and late patterning of the embryonic body (2–5). In *Drosophila*, the BMP homolog deccapentaplegic (*dpp*) acts as a morphogen (6). In mouse, BMP-2, a member of the BMP family of growth and differentiation factors, is also an important instructive molecule for a wide variety of tissues, including cranial and trunk neural crest cells, gut endodermal elements, heart, dermis, genital ridge primordia, as well as cartilage and bone elements (7). Deletion of the BMP-2 gene by homologous recombination is embryonically lethal (7).

BMP-2 plays a major role in osteogenic differentiation (8). We have previously shown that BMP-2 induces osteoblast differentiation *in vitro* to form mature bone nodules (9, 10). During this BMP-2-stimulated differentiation process, BMP-2 mRNA is induced, which maintains the sustained phenotype of the mature osteoblasts (10). However, the precise mechanism of BMP-2 mRNA expression during osteoblast differentiation has not been investigated. Here we report molecular cloning and analysis of 2.7-kb mouse BMP-2 5′ flanking sequence. We identified two transcription initiation sites in the BMP-2 mRNA in mouse osteoblasts. Recombinant BMP-2 stimulated mRNA initiation from the proximal site. Also we provide evidence for the presence of two promoter elements upstream of this two transcription start sites in the 5′ flanking sequence of BMP-2 gene. We demonstrate that BMP-2 gene transcription is autoregulated from the proximal promoter.

MATERIAL AND METHODS

Cell culture. Primary fetal rat calvarial cells were isolated from 19-day-old fetal rat calvariae by sequential digestion with trypsin and collagenase and propagated in αMEM containing 10% fetal

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calf serum and antibiotics essentially as described (11). Immortalized osteoblast cell line, 2T3, has been characterized and grown as described before (9). BNL mouse liver cells, JB6 epidermal cells, and kidney mesangeal cells (MC) were a gift of Dr. H. Abboud (University of Texas Health Science Center at San Antonio).

Molecular cloning of mouse BMP-2 5′ flanking sequence. 5×10^6 plaques were screened from a mouse genomic library purchase from Stratagene (San Diego, CA), using a 1.1-kb *Sma*I fragment of human BMP-2 cDNA probe containing most of the coding region. The BMP-2 genomic clones were sequenced by dideoxy chain termination method (12). All the fragments were sequenced at least twice and overlaps were established using the appropriate oligonucleotide primer. Primers were prepared on an Applied Biosystems Model 392 DNA Synthesizer.

Primer extension. The transcription start site for BMP-2 gene was mapped by primer extension using the synthetic oligonucleotide primer 5′ CCCGGCAATTCAAGAAG 3′ in exon 1. Poly (A) RNA from the fetal rat calvarial cells were used to map the transcription start site using a Primer Extension kit from Promega (Madison, WI).

RNase protection assay. RNase Protection assay was essentially performed as described earlier (13). cRNA probe was synthesized using T7 polymerase in the presence of 40 mM Tris–HCl, pH 7.5, 6 mM $MgCl_2$, 10 mM NaCl, 2 mM spermidine, 40 mM dithiothreitol, and 0.5 mM each of ATP, GTP and CTP, 12 μM UTP, 40 U/ml RNasin, 50 μC_i of [^{32}P]UTP (800 C_i/mmol) and 10 μg/ml of linearized template plasmid containing BMP-2 genomic DNA fragment. The reaction was carried out for 1 h at 37°C, the template DNA was digested with DNaseI and the reaction was extracted with phenol-chloroform mixture followed by ethanol precipitation of the labeled RNA. The RNA (1×10^6 cpm) was hybridized to 5 μg of total RNA from 2T3 osteoblast cells. The hybridization reaction was done in a buffer containing 10 mM piperizine-*N*,*N*′-bis(2-ethanesulfonic acid), pH 6.4, 0.4 M NaCl and 1 mM EDTA in 80% formaldehyde for 12–18 h at 50°C. Following hybridization the sample was digested with 50 μg/ml RNase A and 2 μg/ml RNase T1 followed by 0.16 μg/ml proteinase K. The reaction was then extracted with phenol:chloroform and ethanol-precipitated. The products were then analyzed on 6% polyacrylamide gel containing 7 M urea. Gels were exposed to Kodak X-Omat-AR film.

Plasmid construction and transfection. The luciferase basic plasmid (pGL2 basic), the vector used for all our constructs, was purchased from Promega (Madison, WI). DNA fragments containing proximal and distal promoter from BMP-2 5′ flanking region were cloned at the multiple cloning sites of this plasmid, which is upstream of the firefly luciferase cDNA. For transient transfection assay, the cells were plated at 5×10^5 cells per 60 mm tissue culture dish, 18–24 h prior to transfection. Ten micrograms of reporter plasmid was transfected using calcium phosphate precipitation method. A pCMVRenilla luciferase construct was also used for normalization of transfection efficiency. The cells were harvested 48 h posttransfection. Luciferase activity in the cell lysates was measured using Dual Luciferase Assay system (Promega). Data are presented as ratio of firefly and Renilla luciferase activity.

RESULTS

Restriction Enzyme and DNA Sequence Analysis of BMP-2 5′ Flanking Region

We screened a mouse genomic library using a human BMP-2 cDNA probe. A 19-kb genomic clone was isolated. The transcription unit of the mouse BMP-2 gene contains one noncoding and two coding exons (14). Figure 1A shows the restriction enzyme map of the mouse BMP-2 gene with 2712-bp 5′ flanking sequence that extends up to the *Eco*RI site in the 5′ end. The DNA sequence of this fragment was determined using dideoxy method (Fig. 1B). Analysis of this DNA sequences shows the absence of any TATA or CAAT boxes in this 5′ flanking sequence of BMP-2 gene. However, several stretches of GC rich sequences, characteristic of TATA-less promoters, is found to be present in the 5′ flanking region of mouse BMP-2 gene.

Identification of Transcription Initiation Site of BMP-2 mRNA in Primary Fetal Rat Calvarial Cells

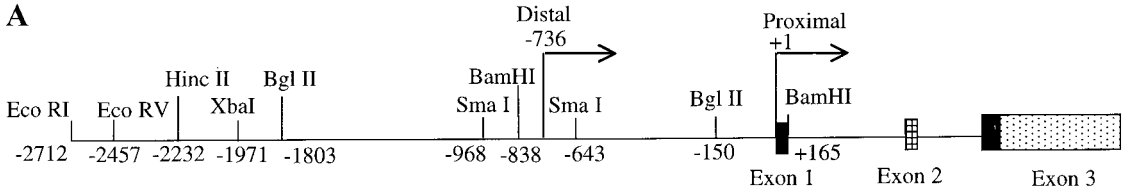
Primary fetal rat calvarial cells have been extensively used for studying osteoblast differentiation *in vitro.* We have previously shown that these cells express BMP-2 during osteoblast differentiation (10). To determine the transcription initiation site of BMP-2 mRNA, poly (A) RNA from these cells was used in a primer extension analysis with an 18-bp ^{32}P-labeled oligonucleotide in the exon 1. Analysis of the product by polyacrylamide gel electrophoresis shows the presence of a major extended product of 75 bp (Fig. 2, lane 1). We designated this boundary as the +1 site (Fig. 2, bottom). A 78 bp minor extended product (Fig. 2, lane 1, indicated by dotted arrow) is visible. This may be a minor adjacent start site, characteristic of TATA less promoters.

Identification of Transcription Initiation Site of BMP-2 mRNA in Mouse Osteoblasts

Since we isolated and sequenced mouse BMP-2 5′ flanking region, we attempted to determine the transcription start site in mouse cells. We have established and extensively characterized an immortalized osteoblastic differentiation cell model, 2T3. 2T3 cells undergo complete osteoblastic differentiation to mineralized, matrix producing bone nodules, which is greatly enhanced by exogenously added recombinant BMP-2 (9, 15). To determine the transcription initiation site,

FIG. 1. (A) Cartoon showing partial restriction enzyme map of 5′ flanking sequence of mouse BMP-2 gene. Exons are shown by boxes. Few restriction enzyme sites are indicated. The proximal and distal transcription initiation sites, as determined in Fig. 3, are indicated by arrows. The numbers indicate the size of DNA fragments in bp as compared to the proximal start site. The positive and negative signs indicate positions downstream and upstream of the proximal start site, respectively. (B) DNA sequence of 5′ flanking region of mouse BMP-2 gene. Presence of a few transcription factor binding sites are shown. The arrows indicate the positions of two transcription initiation sites.

A



B

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. -2712 EcoR1
GAATTCATTTAAACTGGATTCACTTCTAGGTCCCATGCGTTTACACTCATTTCCACCACA -2653
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CTTGAGGAAAGTTCCTGGGAAAGGCTTTTTAGCAGCACCTCTCTGGGCTACAAAAAAGAA -2533
GCCAGCAGGCACCACCAAGGTGGAGTAACTGTCCAGAGGCATCCATTTTACCTCAGAGAC -2473
              . -2457 EcoRV
TTGATTACTAAGGATATCCTAAACGGCCAAACTCTCTCTTCTGGTGTTCCAGAGGCCCAA -2413
AGCTGCAAGGCATTGTTGATGTCATCACCAAAGGTTTCATTTTCATCTTTTCTTGGGGTT -2353
                    Homeobox BINDING SITE(HOX)
GGTCCAACAGCTGTCAGCTTTCTCTTCCTCATTAAAGGCAACTTTCTCATTTAAATCTCA -2293
TATAGGTTCGGAGTTTCTTGCTTTGCTCCTTCCGCCTCCGCGATGACAGAAGCAATGGTT -2233
. -2232 Hinc II
AACTTCTCAATTAAACTTGATAGGGAAGGAAATGGCTTCAGAGGCGATCAGCCCTTTTGA -2173
                          HOX
CTTACACACTTACACGTCTGAGTGGAGTGTTTTATTGCCGCCTTGTTTGGTGTCTCATGA -2113
TTCAGAGTGACAACTTCTGCAACACGTTTTAAAAAGGAATACAGTAGCTGATCGCAAATT -2053
GCTGGATCTATCCCTTCCTCTCCTTTAATTTCCCTTGTAGACAGCCTTCCTTCAAAAATA -1993
                    . -1971 XbaI
CCTTATTTGACCTCTACAGCTCTAGAAACAGCCAGGGCCTAATTTCCCTCTGTGGGTTGC -1933
TAATCCGATTTAGGTGAACGAACCTAGAGTTATTTTAGCTCCCCGACTGAAAAGCTAGCA -1873
CACGTGGGTAAAAAAATCATTAAAGCCCCTGCTTCTGGTCTTTCTCGGTCTTTGCTTTGC -1813
         . -1803 BglII
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                          SRF
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GCATCTTTTTATTAGATTCATTTATTTGAATATAAATGTATGAATATTTATAAAATGTAA -1213
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TAATGTGCTCTTACCTAAAAACTTCAAACTCAAGTTGAATATTGGCCCAATGAGGGAACT -1033
                                                  . -980 BamHI
CAGAGGCCAGTGGACTCTGGATTTGCCCTAGTCTCCCGCAGCTGTGGGCGCGGATCCAGG - 973
    . -968 SmaI
TCCCGGGGGTCGGCTTCACACTCATCCGGGACGCGACCCCTTAGCGGCCGCGCGCTCGCC - 913
  Sp1                 Sp1
CCGCCCCGCTCCACCGCGGCCGCCCCGTAGGGCGCGCCGTCCACACCCCTGCGCGCCGCT - 853
Sp1 SmaI-841.   . -838 BamHI
CCCGCCCGCCCGGGGATCCCCGGCGCGCTGCGCCTCCGAGGGGGAGGTGTTCGGCCACGG - 793
c-myc              NF-kB                Distal -736
CCGGGAGGGAGCCGGCAGGCGGCGTCTCCTTTAAAAGCCGCGAGCGCCGCGCCACGGCGC - 733
CTCCGTCGTCGCCGCCGGAGTCCTCGCCCTGCCGCGCAGAGCCCTGCTCGCACTGCGCCC - 673
                          . -643 SmaI
GCCGCCGCTGCGCTTCCCACAGCCCGCCCGGGATTGGCAGCCCCGGACGTAGCCTCCCCA - 613
                          E2F
GGCGACACCAGGCACCGGACGCCCTCCCGGCGAAAGACGCGAGGGTCACCCGCGGCTTCG - 553
AGGGACTGGCACGACACGGGTTGGAACTCCAGACTGTGCGCGCCTGGCGCTGTGGCCTCG - 493
GCTGTCCGGGAGAAGCTAGAGTCGCGGACCGACGCTAAGAACCGGGAGTCCGGAGCACAG - 433
TCTTACCCTCAATGCGGGGCCACTCTGACCCAGGAGTGAGCGCCCAAGGCGAGCGGGCGG - 373
AAGAGTGAGTGGACCCCAGGCTGCCACAAAAGACACTTGGCCCGAGGGCTCGGAGCGCGA - 313
GGTCACCCGGTTTGGCAACCCGAGACGCGCGGCTGGACTGTCTCGAGAATGAGCCCCAGG - 253
ACGCCGGGGCGCCGCAGCCGTGCGGGCTCTGCTGGCGAGCGCTGATGGGGGTGCGCCAGA - 193
                                              .-150 BglII
GTCAGGCTGAGGGATGCAGAGTGGCGGCCCGCCCGCCACCCAGATCTTCGCTGCGCCCTT - 133
GCCCGGACACGGCATCGCCCACGATGGCTGCCCCGAGCCATGGGTCGCGGCCCAGCTAAC -  73
GCAGAACGTCCGTCCCTCGCCCGGCGAGTCCCGGAGCCAGCCCCGCGCCCCGCCAGCGCT -  13
Proximal +1
GGTCCCTGAGGCCGACGACAGCAGCAGCCTTGCCTCAGCCTTCCCTTCCCGTCCCGGCCC +  48
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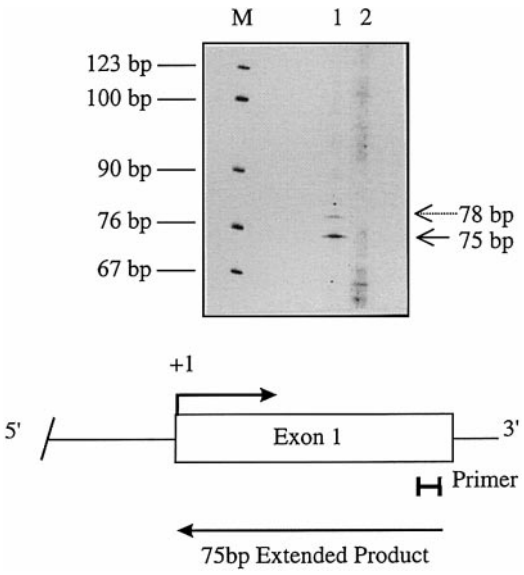

FIG. 2. Primer extension analysis of fetal rat calvarial cell RNA. Poly adenylated (Poly A) RNA was isolated from the fetal rat calvarial cells. A ^{32}P-labeled oligonucleotide primer (5′ CCCGGCAAGTTCAAGAAG 3′) in the exon 1 was used in the primer extension analysis, as described in the Methods. The extended product was separated on a polyacrylamide gel and labeled band was visualized by autoradiography. The arrow indicates the extended product of 75 bp. Dotted arrow indicates a 78-bp minor extended product. Radiolabeled pBR322 DNA digested with MspI was used as the DNA size marker in lane M. In the lower panel, a cartoon shows the extended product and the transcription initiation site designated as +1.

we analyzed BMP-2 mRNA expression in 2T3 cell line, undergoing differentiation in the presence and in the absence of recombinant BMP-2, using RNase protection assays. An *in vitro* transcribed 391-bp ^{32}P RNA probe containing 315 bp BMP-2 sequence spanning a region thought to contain the transcription start site was used based on the primer extension analysis (Fig. 2). The results show two protected RNA fragments of sizes 165 and 315 bp (Fig. 3A). These data indicate the presence of at least two major transcription start sites. The 165-bp protected fragment correlated with the transcription start site determined by primer extension analysis (Fig. 2). It was designated as the proximal transcription initiation site (+1) (Fig. 3A, bottom). The 315-bp fragment represents the completely protected BMP-2 sequence within the 391 bp probe (containing 315 bp BMP-2 sequence plus 76 bp of plasmid sequence) indicating the presence of a distal transcription start site in the BMP-2 gene. Furthermore, BMP-2 stimulated the mRNA initiation preferably from the proximal +1 site (165-bp protected fragment in Fig. 3A, compare lane 2 with lane 1). This result was confirmed by using an *in situ* RNase protection assay, in which a house keeping gene 36B4 probe was used, along with the 315-bp BMP-2-specific probe. Quantitation of the protected 165-bp fragment by phosphoimager analysis showed a 1.9-fold stimulation of tran-

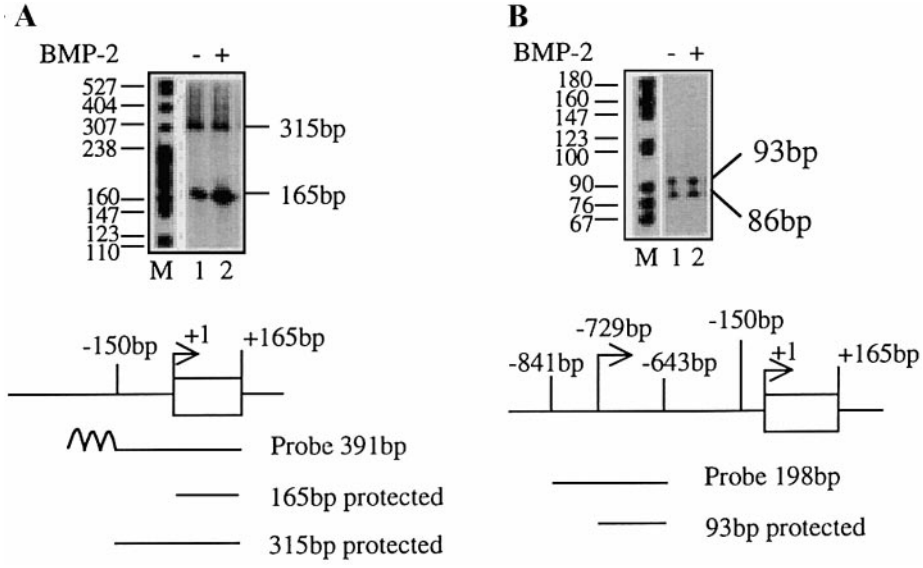

FIG. 3. Identification of transcription start sites of BMP-2 mRNA in mouse osteoblasts. (A) RNase protection assay of mouse osteoblast RNA. Total RNA was isolated from control (−) and BMP-2-treated (+) 2T3 osteoblasts. A 391 bp *in vitro* transcribed RNA probe spanning −150 to +165 bp of BMP-2 gene was used in RNase protection assay as described in the Methods. The protected fragments were separated by polyacrylamide gel electrophoresis. Protected fragments of 315 and 165 bp are shown in the right margin. 'M' indicates pBR322 DNA digested with MspI, used as DNA size marker. Lower panel shows the cartoon demonstrating line drawing of probe and protected fragments. The wavy line in the probe indicates the plasmid sequence in the probe. The transcription start site is shown by arrow. (B) RNase protection analysis to identify second transcription start site. RNA was isolated from control (−) and BMP-2-treated (+) 2T3 cells and used in RNase protection assay with a 198 bp RNA probe spanning −841 bp and −643 bp of BMP-2 gene. The protected fragments were analyzed as described in A. The size of the protected fragments are shown in right margin. 'M' indicates size marker (pBR322 DNA digested with *Msp*I). Lower panel shows line drawing of the probe and protected fragments. The RNA initiation sites are indicated by arrows.

TABLE 1

Autoregulation of BMP-2 Gene Transcription

Ratio of protected fragments	Control	BMP-2	Fold change
165-bp fragment/36B4 (Proximal)	0.96	1.8	1.9
315-bp fragment/36B4 (Distal)	0.96	0.93	1.1

Note. BMP-2 transcripts were analyzed by RNase protection assay in 2T3 osteoblasts treated with 40 ng/ml BMP-2 for 48 h as described in Fig. 3A. 36B4 housekeeping gene transcript was used *in situ* in the assay to normalize the loading. The protected transcripts were analyzed by phosphorimager. The quantitation is presented as the ratio of BMP-2 transcript and 36B4 transcript for both the proximal and distal transcription start sites. Fold change is calculated for the BMP-2 transcripts obtained from untreated (control) and treated (BMP-2) 2T3 cells.

scription initiation from the proximal +1 site (Table 1). Quantitation of the 315-bp protected fragment showed no significant stimulation by BMP-2 (Fig. 3A and Table 1).

To determine the location of the distal initiation site, we used an RNA probe spanning −841 bp and −643 bp of the BMP-2 5′ flanking sequence in the RNase protection assay. Two protected fragments of 93 and 86 bp were detected both in the presence and in the absence of BMP-2 (Fig. 3B). These data define two closely located distal transcription start sites in this region which map to −736 bp and −729 bp respectively compared to the proximal initiation site (Fig. 3B, bottom). These data indicate that at least two promoter elements, upstream of these transcription start sites, one proximal and one distal, may initiate transcription of BMP-2 gene in mouse 2T3 cells and BMP-2 preferably stimulates transcription initiation from proximal site (+1).

Identification of Two Promoter Elements in BMP-2 5′ Flanking Region

To test the promoter activity of the BMP-2 5′ flanking sequences present upstream of these proximal and distal transcription initiation sites, we analyzed the transcriptional activity of two DNA fragments containing the two transcription start sites. The DNA fragments spanning −150 to +165 bp and −643 to −841 bp contain the proximal and distal start sites respectively. These DNA fragments were cloned upstream of firefly luciferase gene to construct the reporter plasmids. Transient transfection of these plasmids into primary fetal rat calvarial osteoblasts showed significant transcriptional activity from both these fragments, as measured by the luciferase activity in the cell lysates (Fig. 4A). These data indicate the presence of a proximal and a distal promoter in the BMP-2 5′ flanking sequence upstream of the respective transcription initiation sites. To further characterize these two promoters, we measured their transcriptional activity in different cell lines derived from various organs along with mouse osteoblasts. Transfection of proximal and distal promoter-luciferase constructs into osteoblasts (2T3), hepatocytes (BNL), epidermal cells (JB6) and kidney mesangial cells (MC) showed that both these promoters are active in these cells to different extent (Figs. 4B and 4C). Transcriptional activity of both the promoters were highest in 2T3 osteoblasts than that of any other cell lines tested. However, the distal promoter was more active in JB6 epidermal cells and kidney mesangial cells than the proximal promoter (Figs. 4B and 4C). These data indicate that flanking sequences upstream of proximal and distal transcription initiation sites containing two distinct promoter elements are transcriptionally active in different cell lineages.

BMP-2 Regulates Transcription from Proximal Promoter

We showed above that BMP-2 stimulates the transcription from the proximal initiation sites in mouse osteoblasts (Fig. 3A) suggesting that the proximal promoter would be responsive to BMP-2. To test the effect of BMP-2 on the transcriptional activity of both proximal and distal promoters, the luciferase reporter constructs were transfected into 2T3 osteoblasts. Transiently transfected cells were incubated with recombinant BMP-2 and luciferase activity in the lysates were determined. The results show that BMP-2 stimulated transcription from the proximal promoter in a dose-dependent manner (Fig. 5A). No transcriptional activation in response to BMP-2 was observed from the distal promoter element (Fig. 5B). These data further confirm that transcription from the proximal initiation site of the BMP-2 gene (Fig. 3) is responsive to BMP-2.

DISCUSSION

Expression of BMP-2 is tightly regulated during osteoblast differentiation. Towards better understanding of transcriptional regulation of this osteogenic growth and differentiation factor, we provide the first evidence of the presence of two transcription initiation sites in mouse osteoblasts. We demonstrate that BMP-2 gene is transcribed from two promoter elements present upstream of these two transcription start sites. Furthermore we show for the first time that BMP-2-induced autoregulation of BMP-2 expression is due to the transcription from the proximal promoter element.

During embryogenesis, BMP-2 plays a role of true morphogen (6). Therefore, precise timing of expression of BMP-2 is critical for normal embryonic patterning. The correct expression of this ligand in a tissue deter-

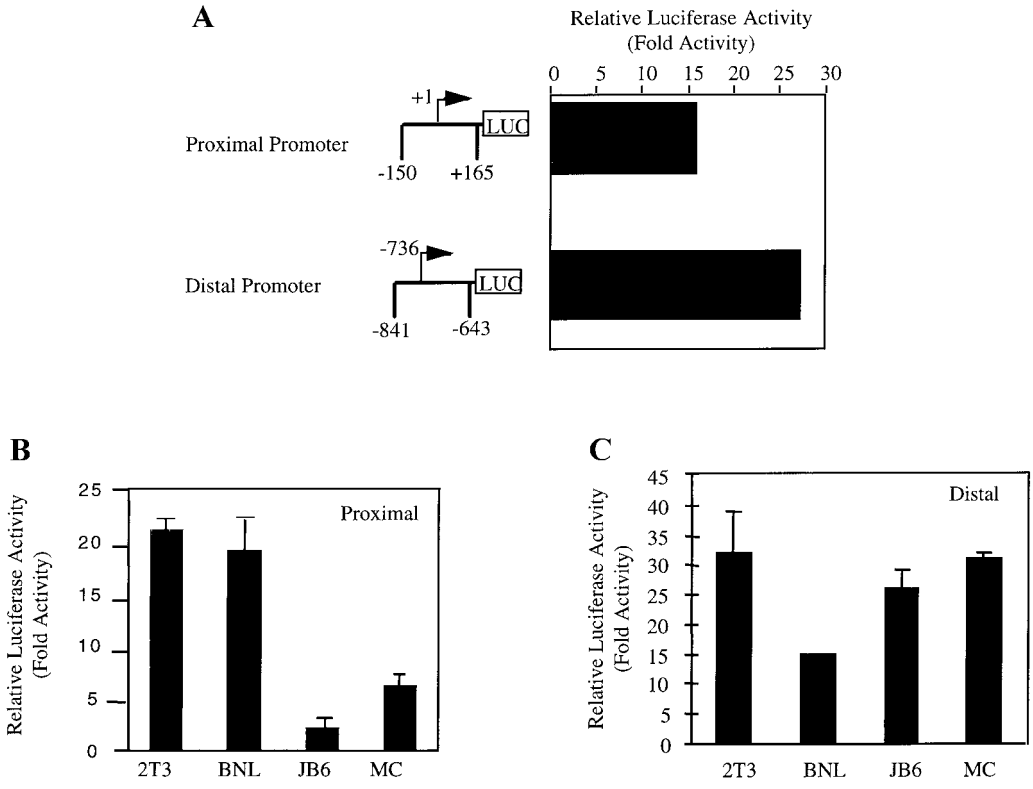

FIG. 4. The BMP-2 gene contains two promoters upstream of each transcription start sites. (A) Identification of two promoters. The proximal DNA fragment spanning −150 to +165 bp and the distal fragment spanning −841 to −643 bp were cloned into pGL2 basic upstream of the luciferase cDNA as described in the Methods. These two reporter constructs were transfected into 2T3 cells. The lysates were assayed for luciferase activity as described in the Methods. The data are presented as fold activity as compared to pGL2basic transfected cells. (B and C) Activation of proximal and distal BMP-2 promoters in cells of different organ. The proximal and distal promoter constructs were transfected into 2T3 osteoblasts, BNL mouse liver cells, JB6 epidermal cells and kidney mesangial cells (MC), respectively. Cell lysates were assayed for luciferase activity as described above.

mines the fate of development of specific organ system such as heart (7). Also expression of BMP-2 is tightly associated with dorsal-ventral axis formation (16) and apoptosis of interdigital cells during limb development. Thus a central biological question is how BMP-2 is expressed in its proper temporal and spatial patterns. We identified two transcription initiation sites for BMP-2, which is functional in mouse osteoblasts (Fig. 3). It is possible that during embryonic development and differentiation of osteogenic precursor cells to

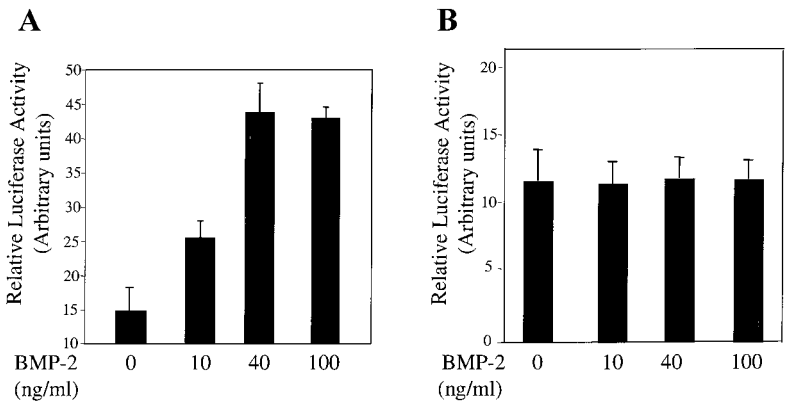

FIG. 5. Autoregulation of BMP-2 transcription. The proximal (A) and distal (B) promoter luciferase constructs were transfected into 2T3 osteoblasts. Serum-deprived transfected cells were incubated with different concentrations of recombinant human BMP-2 for 24 h. The lysates were assayed for luciferase activity as described in the Methods.

bone, these transcription initiation sites are differentially used for BMP-2 expression. In fact, we have previously shown that BMP-2 autoregulates its mRNA expression during osteoblast differentiation (10). Our data that BMP-2-induces transcription initiation from the proximal transcription start site confirms this notion (Fig. 3).

The *dpp* gene encoding *Drosophila* homolog of BMP-2 has three major and several minor transcripts (17). Also BMP-4, a closely related homolog of BMP-2, has two transcripts which are expressed in a cell type and differentiation-dependent manner (18). In the present study, presence of two transcription initiation sites (Fig. 3) suggested that the expression of mouse BMP-2 gene involves complex transcriptional regulation. Thus we identified two promoter elements upstream of the two respective transcription initiation sites in the 5′ flanking sequence of BMP-2 gene (Fig. 4). In *Drosophila*, multiple promoters are responsible for expression of different transcripts of *dpp* (17). These results explain stage specific expression of *dpp* during embryonic development. In fact, different promoters for *dpp* gene transcribe at different developmental stages and in different tissues. Similarly presence of two promoters for expression of mouse and human BMP-4 gene, the closest homolog of BMP-2, has been reported (18, 19). Both promoters in mouse BMP-4 are present in the 5′ flanking sequence of its gene (19). In contrast, the proximal promoter in human BMP-4 is present in intron 1 of its gene (18). We identified both BMP-2 promoters in mouse in the 5′ flanking sequence of its gene (Fig. 4A). These data indicate that the transcriptional regulation of BMP family of growth and differentiation factors is extremely complex not only in *Drosophila*, but also in the vertebrates. We also demonstrate that the proximal and distal promoters of mouse BMP-2 gene are differentially activated in cells of different organ (Figs. 4B and 4C). Expression of *dpp* in *Drosophila* is under the control of different upstream signaling (20). Also *dpp* itself positively regulates its own transcription (21). This regulation of BMP is also conserved in vertebrate. Thus we demonstrate that BMP-2 stimulates BMP-2 gene transcription from the proximal promoter (Fig. 5). In summary, these data provide a mechanism of differential activation of BMP-2 gene expression from the proximal and distal transcription initiation sites during osteoblastic differentiation of precursor mesenchymal cells.

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