SHORT COMMUNICATION

PCR amplification and cloning of a mouse bone morphogenetic protein (BMP) cDNA

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Introduction

Up to the present, eight different species of bone morphogenetic proteins (BMP) have already been reported. They have a homologous structure except BMP-1 and are the members of a transforming growth factor- β (TGF- β) superfamily¹⁻³). Several reports suggest that TGF- β superfamily proteins may play important roles in development of bone and oral tissues⁴⁻⁷). The polymerase chain reaction (PCR) method has already become an important technique in molecular biology⁸). We attempted to amplify BMP cDNAs by this method. In this study, we succeeded in amplifying a BMP cDNA within several hours using this method. We cloned the DNA into a plasmid and identified as a messenger of mouse BMP-6 (Vgr-1).

Material and Methods

Template cDNA Preparation

Polyadenylated RNA was prepared from the whole tissue extract of a new born mouse⁹). cDNA was synthesized using a cDNA synthesis system (Amersham Co.).

Primer Synthesis

Oligonucleotide PCR primers were prepared

Fig. 1 Sequences of the oligonucleotide primers (V2 and V3) for mouse BMP-6 cDNA prepared for the PCR amplification. Hatched box at the left end indicates the signal peptide. The other hatched box at the right side indicates mature region of BMP-6 having about 100 amino acid residues until the C-terminal.

by a MilliGen DNA synthesis system using a Cyclone plus DNA Synthesizer according to the phosphoamidite method.

Amplification and Cloning

About 100 ng of the template cDNA prepared as described above was amplified in a 50 μ l reaction mixture containing two primers, V2 and V3 (Fig. 1), 100 ng each in 10 mM Tris-HCl pH 8.9, 1.5 mM MgCl₂, 80 mM KCl, 500 μ g/ml BSA, 0.1% sodium cholate,

Fig. 2 Polyacrylamide electrophoresis of DNA fragments. (A) lane 1: size markers of ϕ X174 Hae III digests, lane 2: the product DNA by PCR amplification. (B) lane 1: size markers of ϕ X174 Hae III digests. Lanes 2-9 are digests of the product DNA with restriction enzymes; 2: Kpn I, 3: Hind III, 4: Eco RI, 5: Pst I, 6: Sma I, 7: Bam HI, 8: Xba I, 9: Sac I.

0.1% TritonX-100, 0.2 mM dNTPs, using 2 units of Tth polymerase (TOYOBO Co.). Three steps of denaturation (1 min at 93°C), annealing (2.5 min at 53°C) and extension (2.5 min at 75°C) were repeated for 30 cycles. In order to make a blunt end and phosphorylation, amplified fragments were treated by T4 DNA polymerase and T4 polynucleotide kinase in a kinase buffer (TAKARA Co.).

Blunt-ended PCR fragments were ligated into a pBluescript SK (+) plasmid vector at 16°C overnight. Then the vectors were transfected into competent *E. coli* cells (JM 109). Random clones were isolated with color selection methods.

DNA Sequencing

Nucleotide sequence was determined by the dideoxynucleotide chain termination method (Sanger et al, 1977) with a SEQUENASE (US Biochem. Corp.).

Result and Discussion

According to the BMP-6 (Vgr-1) nucleotide sequence¹⁰, oligonucleotide PCR primers V2 and V3 were prepared (Fig. 1). V2 primer was designed to contain a single EcoRI restriction site. Using these two primers, a PCR product of about 470 bp was obtained (Fig.

Construction of BM5 Plasmid

Fig. 3 Construction of a BM5 plasmid. BMP-6 cDNA amplified by PCR was inserted into a pBluescript SK(+) plasmid vector. Three restriction enzyme sites (*) are contained in the inserted BMP cDNA at multi cloning site. Hinc II site was lost during the insertion procedure.

Table 1 Comparison of BM5 to other members of the mouse and human TGF- β superfamily.

		BM5	Ref.
mouse	BMP-6	98.9	(10)
	TGF- β 1	49.6	(12)
	GDF-1	51.3	(5)
human	BMP-2	(62.6)	(1)
	BMP-3	—	(1)
	BMP-4	59.6	(1)
	BMP-7	71.5	(3)

Homology of human BMP-2 (62.6%) indicates only in mature region. (—): No homology.

2A). This size corresponded to that of the expected BMP-6 cDNA. After ligation and transfection, several white colonies were cultured on a small scale and the inserts were checked by polyacrylamide gel electrophoresis.

	Primer	V3 →
*BM5	5'- <u>TTCATGGTGGCCTTCTTCAAGGTGAGC</u>	
	868 878 888	
Mouse BMP-6	5'-CCCTTCATGGTGGCCTTCTTCAAGGTGAGC	
	ProPheMetValAlaPhePheLysValSer	
*GAGGTCCACGTGCGCACCACCAGGTACAGCTCCAGTCGGCGGCGGCAGCAGAGTCGCAAC		
	898 908 918 928 938 948	
GAGGTCCACGTGCGCACCACCAGGTACAGCTCCAGTCGGCGGCGGCAGCAGAGTCGCAAC		
GluValHisValArgThrThrArgSerAlaSerSerArgArgGlnGlnSerArgAsn		
	<u>Sma I</u>	
*CGGTCCACCCAGTCGACGACGTGTCCCGGGGCTCC-GTTCTTCAGACTACAACGGCAGT		
	958 968 978 988 1008	
CGGTCCACCCAGTCGACGACGTGTCCCGGGGCTCCGGTTCTTCAGACTACAACGGCAGT		
ArgSerThrGlnSerGlnAspValSerArgGlySerGlySerSerAspTyrAsnGlySer		
	<u>Sac I</u>	
*GAGTTAAAAACAGCTTGCAAGAAGCATGAGCTCTATGTGAGCTTCCAGGACCTGGGATGG		
	1018 1028 1038 1048 1058 1068	
GAGTTAAAAACAGCTTGCAAGAAGCATGAGCTCTATGTGAGCTTCCAGGACCTGGGATGG		
GluLeuLysThrAlaCysLysLysHisGluLeuTyrValSerPheGlnAspLeuGlyTrp		
*CAGGACTGGATCATGTGCACCCAAAGGCTACGCTGCCAACTAC-GTGATGGAGAGTGTTC		
	1078 1088 1098 1108 1118 1128	
CAGGACTGGATCATGTGCACCCAAAGGCTACGCTGCCAACTACTGTGATGGAGAGTGTTC		
GlnAspTrpIleIleAlaProLysGlyTyrAlaAlaAsnTyrCysAspGlyGluCysSer		
*TTCCCACTCAACGCACACATGAATGCCACCAACCACGCCATTGTACAGACCTTGGTCCAC		
	1138 1148 1158 1168 1178 1188	
TTCCCACTCAACGCACACATGAATGCCACCAACCACGCCATTGTACAGACCTTGGTCCAC		
PheProLeuAsnAlaHisMetAsnAlaThrAsnHisAlaIleValGlnThrLeuValHis		
*CTTATGAATCCCGAGTACGTCCCAAAACCATGCTGCGCACCAACCAAACTGAATGCCATC		
	1198 1208 1218 1228 1238 1248	
CTTATGAATCCCGAGTACGTCCCAAAACCATGCTGCGCACCAACCAAACTGAATGCCATC		
LeuMetAsnProGluTyrValProLysProCysCysAlaProThrLysLeuAsnAlaIle		
*TCGGTTCTTTACTTCGATGATAACTCCAATGTCATCTTGAAAAAGTACAGGAATATGGTC		
	1258 1268 1278 1288 1298 1308	
TCGGTTCTTTACTTCGATGATAACTCCAATGTCATCTTGAAAAAGTACAGGAATATGGTC		
SerValLeuTyrPheAspAsnSerAsnValIleLeuLysLysTyrArgAsnMetVal		
← Primer	V2	
3'-TCTCGAACADDAACGGTAATTCTTAAG-5'		
	<u>Eco RI</u>	
*GTGAGAGCTTGTGGTTGCCATTAAAGATTC		
	1318 1328 1338	
GTGAGAGCTTGTGGTTGCCATTAAAGTTGAAGC		
ValArgAlaCysGlyCysHis***		

Fig. 4 DNA sequence of BM5 plasmid. Compared to the mouse BMP-6 (Vgr-1), two nucleotides are defected (—) in BM5. Three restriction enzyme sites and primer sequence are indicated by over and underlines.

One clone named BM5 was selected and the orientation of the cloned DNA was determined by digestion with several restriction enzymes (Fig. 2B). The direction was such that transcription was under control of the T3 RNA polymerase promoter (Fig. 3). Two nucleotides were defected but the nucleotide sequence of BM5 was 98.9% identical to corresponding mouse BMP-6 (Fig. 4 and Table 1). Similar cases of nucleotide misincorporation by thermostable DNA polymerase have already been

reported¹¹⁾. We concluded that BM5 is mature region of mouse BMP-6 cDNA. The result shows that we succeeded in amplifying BMP-6 cDNA selectively from a mouse cDNA mixture by the PCR method in relatively short hours.

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