Partial Sequence of the Bovine (*Bos taurus coreanae*) Myogenic Factor Encoding Gene MyoD

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ABSTRACT : This experiment was carried out to isolate the partial bovine (*Bos taurus coreanae*) myogenic factor encoding gene, MyoD, using the rat myogenic factor (MyoD) gene sequence and to compare the gene sequence between another myogenic factor (Myf 5) and MyoD gene of the bovine. To make the probe and isolate the MyoD gene, PCR was performed to amplify rat and bovine MyoD gene including exon I, II and intron I. The homology between mouse and bovine MyoD is high; bovine MyoD gene shows 17 different gene sequence region compared to rat MyoD. Among those, two regions have significant differences; one is the exon I part between 2834 and 2850 bp, the other is intron part between 3274 and 3303 bp of the mouse. At this region homology was 40% in the former and 50% in the latter. Homology between bovine MyoD and Myf5 was 83% in the exon 1. Especially exon I in the Myf5 602-617 bp and 651-683 bp have significant differences. These results suggest that MyoD gene have a similar gene structure in mouse and bovine and MyoD and Myf5 of the bovine, at least in part, have a similar expression and activity. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 5 : 689-694*)

Key Words : Myogenic Factor (MyoD), *Bos taurus coreanae*, Sequence Homology

INTRODUCTION

The myogenic basic helix-loop-helix (bHLH) family including MyoD (Weintraub et al., 1991), MRF4, also called Myf6 or herculin (Rhodes and Konieczny, 1989; Braun et al., 1990; Miner and Wold, 1990), myogenin (Braun et al., 1989a; Edmondson and Olson, 1989; Wright et al., 1989) and Myf5 (Braun et al., 1989b) is believed to play an important regulatory role in the development of skeletal muscle and is exclusively expressed in skeletal muscle.

Vertebrate skeletal muscle is derived from cells in the somites and prechordal mesoderm that give rise to myoblast that form the skeletal muscle of the head, trunk, and limbs (reviewed by Miller, 1992).

Myogenic factor encoding gene, MyoD, is related to cell differentiation and expressed in the myoblast (Weintraub et al., 1991) and plays a significant role in the skeletal muscle of the mouse (Rudnicki et al., 1993). When the MyoD gene is transfected into the fibroblast cell, it will be changed into the myoblast cell (Choi et al., 1990).

In domestic livestock, myogenic factors encoding gene sequence of the chicken (Dechesne et al., 1994), sheep (Huynen, 1992), and swine (Chang et al., 1995) have been reported. In the bovine, MyoD gene sequence has not yet been reported, except for Myf5 (Barth et al., 1993).

The bHLH domains between sheep and mouse have almost same amino acid sequence, and sheep MyoD1 protein exhibits 90% amino acid identity to that of the mouse (Huynen et al., 1992). Myogenin and MyoD are different genes but they share the *myc* homology domains and thus form part of a gene family regulating myogenesis, and together interact to regulate the determination and differentiation of muscle cells (Wright et al., 1989).

Myf5 or MyoD is required for the determination of skeletal myoblasts, their propagation, or both during embryonic development and these factors play, at least in part, functionally redundant roles in myogenesis (Rudnicki et al., 1993). The expression and activity of the MyoD protein is regulated, in part, by interaction with members of a large family of proteins related to MyoD by sequence homology (Tapscott and Weintraub, 1991).

To isolate the bovine MyoD gene, we utilized the rat MyoD (Vaidya et al., 1992) and analyzed sequence of the bovine MyoD gene. Also we compared the gene sequence between MyoD and Myf5 gene of the bovine to confirm the sequence homology.

MATERIALS AND METHODS

DNA extraction and polymerase chain reaction

Genomic DNA was extracted from rat (Sprague Dawley) and bovine (Korean cattle) blood by Sambrook et al. (1989). The primer pairs (Forward; CCC AGG ACG CGA CTG CTT TC, Reverse; CCG CTG GAC GCC TCA CTG TA) was selected from complete rat MyoD sequence between Exon I (2401-3217bp) and Exon II (3667-3727bp) using Oligo 5.0 program (NBI).

The amplification of MyoD fragment was conducted by 2-step PCR (Primers 25 pmole, dNTP

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1.25 mM, $MgCl_2$ 1.5 mM in a volume of 50$\mu\ell$ reaction) using PCR machine (Perkin Elmer GenAmp 9600 Thermocycler). After denaturation for 5 min at 94℃ and annealing & extension for 8 min at 61.6℃, 35 cycles of two-step PCR (94℃ for 1min, 61.6℃ for 5 min) were fulfilled, and then post-elongation at 61.6℃ for 20 min.

DNA Sequencing

Preparation of amplified DNA

Cloning of the PCR products was processed according to the procedures of the original TA cloning kit (Invitrogen). Ligated plasmid by TA cloning kit was transformed to *E.coli* ($DH_5\alpha$) by $CaCl_2$ method, and then amplified the rat and bovine MyoD gene. Plasmid DNA was prepared by Quiagen plasmid kit (Quiagen).

Nested deletion and DNA sequencing

To carry out the nested deletion, plasmid DNA was digested with restriction endonuclease *Kpn I* (3'-protruding) and then *BamH I* (5'-overtruding). And it was digested with Exo III nuclease every two minutes. After digestion with Exo III, digested plasmid DNA was re-ligated and then transformed into *E.coli* ($DH_5\alpha$) to amplify nested DNA. DNA sequence was analyzed by T7 sequencing kit (Pharmacia) and homology test was accomplished with the GenBank program.

RESULTS AND DISCUSSION

We obtained a PCR product of bovine MyoD fragment using rat MyoD sequence and digested with restriction endonuclease to confirm the bovine MyoD fragment (figure 1, 2). As expected, PCR products of the bovine MyoD showed a digestion pattern similar to the rat.

Figure 3 shows a partial bovine MyoD gene sequence including exon I, II and intron using Rat MyoD. Compared to the rat MyoD gene, it has a high homology except for two regions between 2834-2850 bp in exon I and 3274-3303 bp in intron of the mouse. At this region homology was 40% in the former (Rat; CGCGCGCCCAGCGGGC, Bovine; CCC GCCGCCCAGCGCG) and 50% in the latter (Rat; ACATTTCCAAGGCCCTTGGGGTGGGGGTGT, Bovine; CATTTC CAAGG CCCTTGGGGTGGGGGTG TC). In contrast, MyoD1 sequence between sheep and mouse was 51% homologous to the 5' untranslated region of mouse MyoD1 and 62% to the 3' untranslated region (Huynen et al., 1992). It is suggested that bovine and mouse MyoD gene in exon I, II and intron has a similar gene structure and may have a similar protein activity.

Among the bHLH family, MyoD gene showed a high homologous sequence compared to the Myf5 gene (Braun et al., 1989b). Myf5 expression is repressed by MyoD and it may partially substitute for the function of MyoD in mutant animals (Rudnicki et al.,1992).

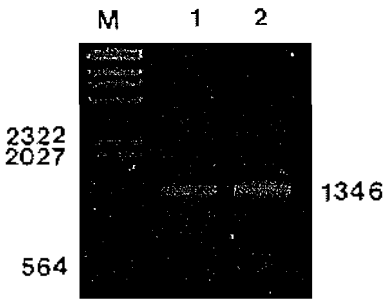

Figure 1. Electrophoresis of PCR products by primer MyoD3 (M:λ/*Hind* III, lane 1: Rat, 2: Bovine)

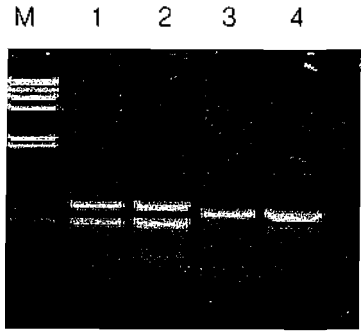

Figure 2. Electrophoresis of digested PCR products by *Mlu* I and *Pst* I (M:λ/*Hind* III, lane 1: Rat, *Mlu* I, 2: Bovine, *Mlu* I, 3: Rat, *Pst* I, 4: Bovine, *Pst* I)

Comparison of the bovine myogenic encoding gene sequence between MyoD and Myf5 shows that homology was 83% in the exon I of the Myf5 (Figure 4). And two regions have significant differences between 602-617 bp (MyoD; CTCTCGTGCGCGACC, Myf5; AGCTGCTT AGG GAAC) and 651-683 bp (MyoD; GCGACTCAGAC GCGTCCAGCCCGCGCTCCAACT, Myf5; GCTGCTCT GAGCCCACCAGCCCCACCTCAAGTT).

Herculin protein is closely similar to the myogenic regulators myogenin (82%), MyoD (81%), and Myf5 (79%). MyoD and Myf5 are the most closely related pair in the family, having 89% identity in the bHLH region of mouse (Miner and Wold, 1990).

There is a hierarchy in the cross activating efficiency of the various myogenic factors on the CMD1 promoter: CMD1> myogenin > MRF4 > and this difference is not dependent upon any known muscle specific transcriptional regulatory elements (Dechesne et al., 1994)

Sequence homology between MyoD and Myf5 of the bovine also indicate that these have a similar structure and partially interacte in the expression and activity between MyoD and Myf5.

Bovine: 1
CCCAGGACGCGACTGCTTTCTTCACCACACCTCTGACAGGACAGGACAGGGAGGGAAGGT
CCCAGGACGCGACTGCTTTCTTCACCACACCTCTGACAGGACAGGACAGGGAGGGAAGGT
Rattus: 2401

Bovine: 61
AGAGGACAGCAGGTGTGCATTCCAACCGTCAGAACCATTGTCATTGTACTATTGGGGTTC
AGAGGACAGCAGGTGTGCATTCCAACCGTCAGAACCATTGTCATTGTACTATTGGGGTTC
Rattus: 2461

Bovine: 121
AGGAGTGACAGAAAGTTAAGACGACTCTTCAGGCTTGGGTTGAGCGAGAAGCAGGACCCA
AGGAGTGACAGAAAGTTAAGACGACTCTTCAGGCTTGGGTTGAGCGAGAAGCAGGACCCA
Rattus: 2521

Bovine: 181
GAACTGGGACATGGAGCTACTATCGCCGCCACTCCGGGACACAGACTTGCTAGGCCCCGA
GAACTGGGACATGGAGCTACTATCGCCGCCACTCCGGGACACAGACTTGCTAGGCCCCGA
Rattus: 2581

Bovine: 241
CGGCTCTCTCTGCTCCTTTGCGACAGCCGATGACTTCTATGATGATCCGTGTTTCGACTC
CGGCTCTCTCTGCTCCTTTGCGACACGCGATGACTTCTATGATGATCCGTGTTTCGACTC
Rattus: 2641

Bovine: 301
ACCAGACCTGCGCTTTTTTGAGGACCTGGACCCGCGCCTGGTGCACGTGGGAGCCCTCCT
ACCAGACCTGCGCTTTTTTGAGGACCTGGACCCGCGCCTGGTGCACGTGGGAGCCCTCCT
Rattus: 2701

Bovine: 367
GGAGGACGCACACTTCCCCACTACGGTGCACCCAGGCCCAGGCGCTCGCGAGGATGAGCA
GGAGCACGCACACTTCCCCACTACGGTGCACCCAGGCCCAGGCGCTCGCGAGGATGAGCA
Rattus: 2770

Bovine: 427
TGTGCCCGCCGCCCAGCGCGACCACCAGGCGGGTCGCTGCTTGCTGTGGGCCTGCAAGCG
TGTGCGCGCGCCCAGCGGGCACCACCAGGCGGGTCGCTGCTTGCTGTGGGCCTGCAAGGC
Rattus: 2830

Bovine: 487
TCTCAAGCGCAAGACCACTAACGCTGATCGCCGCAAGGCAGCCACCATGCGTGA 540
GTGCAAGCGCAAGACCACTAACGCTGATCGCCGCAAGGCAGCCACCATGCGTGA 2943
Rattus: 2890

Bovine: 542
GCGGCGCCGCCTGAGCAAAGTGAACGAGGCCTTCGAGACCCTTAAGCGCTGCACGTCCAG
GCGGCGCCGCCTGAGCAAAGTGAACGAGGCCTTCGAGACCCTTAAGCGCTGCACGTCCAG
Rattus: 2944

Bovine: 602
CAACCCGAACCAGCGGATACCCAAGGTGGAGATCCTGCGCAACGCCATCCGCTACATTGA
CAACCCGAACCAGCGGCTACCCAAGGTGGAGATCCTGCGCAACGCCATCCGCTACATTGA
Rattus: 3004

Bovine: 662
AGGTCTGCAGGCTCTCGTGCGCGACCAGGACGCCGCTCCCCCTGGCGCCGCTGCCTTCTA
AGGTCTGCAGGCTCTGCTGCGCGACCAGGACGCCGCTCCCCCTGGCGCCGCTGCCTTCTA
Rattus: 3064

Bovine: 722 **CGCACCTGGCCCGCTGCCCCCAGGCCGTGGCAGTGAGC** 759
Rattus: 3124 CGCACCTGGCCCGCTGCCCCCAGGCCGTGGCAGTGAGC 3161

Bovine: 761 Exon I ←
ACTACAGCGGCGACTCAGACGCGTCCAGCCCGCGCTCCAACTGCTCTGATGGCATGGTAA
ACTACAGCGGCGACTCAGACGCGTCCAGCCCGCGCTCCAACTGCTCTGATGGCATGGTAA
Rattus: 3162

Bovine: 821 TGCTGTGACCAAGGAGGATGACAAGTGGAGGCAGCGC 857
Rattus: 3222 GGCTGTGACCAAGGAGGATGACAAGTGGAGGCAGCGC 3258

Bovine: 858 CTAGAGTATCTGCAAAACATTTCCAAGGCCCTTGGGGTGGGGGTGTCCTT 907
CTAGAGTATCTGCAAAACATTTCCAAGGCCCTTGGGGTGGGGGTGTCTTT 3307
Rattus: 3258

Bovine: 872 AAACATTTCCAAGGCCCTTGGGGTGGGGGTGTCCTTTATGCCCAGATGCTCATGGTATCT
AAAACATTTCCAAGGCCCTTGGGGTGGGGGTGTCTTTATGCCCAGATGCTCATGGTATCT
Rattus: 3271

Bovine: 946 TTTGGAGACCCAGGGCATCTATGATTCTGCCAATTAAAGGTGTTATTAGAACC 998
TTTGGAGACCCAGGGCATCTATGATTCTGCCAATTAAAGGTGTTATTAGAACC 3395
Rattus: 3343

Bovine: 995 AACCTGCTGCGCAGACCCTGGGATACGCTTTTCCTCCTCAAATCCTTATTACCCTAATGC
ACCCTGCTGCGCAGACCCTGGGATACGCTTTTCCTCCTCAAATCCTTATTACCCTAATGC
Rattus: 3393

Bovine: 1055 AGATTATTGTTCTTGGGTGACTGTCCACTCTCAGTTTGGCTCTGGATGGGACAGCTTCCA
AGATTATTGTTCTTGGGTGACTGTCCACTCTCAGTTTGGCTCTGCATGGGACAGCTTCCA
Rattus: 3453

Bovine: 1115 ATGAGTGCTGGCTCCTACCTCCTAAATGGGGCTGCACCAGTCCTTGACCCGGCAGCTAAC
ATGAGTGCTGGCTCCTACCTCCTAAATGGGGCTGCACCAGTCCTGGACCCGGCAGCTAAC
Rattus: 3513

Bovine: 1175 ACTAAGGGAGTGAGGGAGGGGTGATGACAAGGAGTCTTGCTTGAGACCCACTCGGGCCCT
ACTAAGGGAGTGAGGGAGGGGTGATGACAAGGAGTCTTGCTTGAGACCCACTCGGGCCCT
Rattus: 3573

Bovine: 1235 ATAGCCTGACTTCGTC 1250
Rattus: 3633 ATAGGCTGACTTCGTC 3648

Bovine: 1250 →Exon II
CGTCCTTGCTATTCGCAG**ATGGATTACAGCGGCCCCCCAAGGCGGCCCCCG** 1300
CGTCCTTGCTATTCGCAGATGGATTACAGCGGCCCCCCAAGCGGCCCCCGG 3699
Rattus: 3649

Bovine: 1294
GCCCCCGGCGGGCAGAATGGCTACGACGCCGCCTACTACAGTGAGGCGTCCAGCGG
GGCCCCCGGCGGCAGAATGGCTACGACGCCGCCTACTACAGTGAGGCGTCCAGCGG
Rattus: 3691

Figure 3. Comparison of DNA sequence between bovine and rat MyoD gene

BMyoD: 404
CCAGGCGCTCGCGAGGATGAGCATGTGCCCGCCGCCCAGCGCGACCACCAGGCGGGTCGC
CAAGGCTCAGACGAGGACGAGCACGTGCGAGCACCCACGGGCCACCACCAGGCCGGCCAC
BMYF5: 335

BMyoD: 464
TGCTTGCTGTGGGCCTGCAAGCGTCTCAAGCGCAAGACCACTAACGCTGATCGCCGCAAG
TGCCTCATGTGGGCCTGCAAAGCATGCAAAAGGAAGTCCACCACCATGGATCGGCGGAAG
BMYF5: 395

BMyoD: 524 **GCAGCCACCATGCGTGA** 540
BMYF5: 455 GCGGCCACCATGCGCGA 471

BMyoD: 542
GCGGCGCCGCCTGAGCAAAGTGAACGAGGCCTTCGAGACCCTTAAGCGCTGCACGTCCAG
GCGGAGACGCCTGAAGAAGGTCAACCAGGCTTTCGACACGCTCAAGCGGTGCACCACGAC
BMYF5: 472

BMyoD: 602
CAACCCGAACCAGCGGATACCCAAGGTGGAGATCCTGCGCAACGCCATCCGCTACATTGA
CAACCCTAACCAGAGGCTGCCCAAGGTGGAGATCCTCAGGAATGCCATCCGCTACATTGA
BMYF5: 532

BMyoD: 662 **AGGTCTGCAGGCTCTCGTGCGCGACCAGG** 690
BMYF5: 592 GAGTCTGCAGGAGCTGCTTAGGGAACAGG 620

BMyoD: 770 **GCGACTCAGACGCGTCCAGCCCGCGCTCCAACTGCTCTGATGGCATGGTAA**
GCTGCTCTGAGCCCACCAGCCCCACCTCAAGTTGCTCTGATGGCATGGTAA
BMYF5: 651

Figure 4. Comparison of DNA sequence between bovine and rat Myf5 gene

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