Molecular Cloning of a Bovine Minisatellite DNA Homologous to Mouse Minisatellite

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Abstract We have isolated a genomic DNA clone containing a bovine homologue of mouse minisatellite. Southern blot analysis and sequencing revealed that a 6.5 Kb *Bam*HI fragment within the clone contained repetitive DNA consisting of tandem repeats with a core sequence GGCTGTGTGT. The cloned bovine minisatellite DNA detected high restriction fragment length polymorphism (RFLP) among individuals when used as a probe, indicating that the probe could be a useful for DNA fingerprinting.

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Minisatellite DNA regions of the genome exhibit high length-polymorphism due to a variable number of tandem repeats of a short sequence. These DNA regions were first found in the human genome⁹⁾ and subsequently in the genomes of other higher eukaryotic species^{1, 6, 7, 13)}. Jeffreys *et al.*¹⁰⁾ demonstrated that a DNA 'polycore' probe based on a myoglobin gene minisatellite can simultaneously detect, by hybridization to human genomic DNA, a large number of dispersed hypervariable loci containing tandem repeats of a similar sequence. The complex restriction fragment pattern resulting from such hypervariable loci has been shown to produce a DNA fingerprint specific to an individual. DNA fingerprints can be obtained from a wide range of vertebrates and have many applications including

individual identification, paternity testing^{3, 4, 11, 13)} and linkage analysis^{2, 5, 14)}. Such hypervariable loci might also be useful markers in genetic analysis of domestic animals. Previously, we reported the fingerprint analyses of bovine DNA using mouse minisatellite DNA as a probe⁸⁾. Here, we report the cloning and structural analysis of a bovine minisatellite DNA, which is consisted of 17 tandem repeats of core sequence GGCTGTGTGT, homologous to the mouse minisatellite.

Materials and Methods

Construction of genomic DNA library :

Bovine blood DNA prepared according to the method described by Kunkel *et al.*¹⁵⁾, was partially digested with *Sau*3AI. DNA ranging

マウスのミニサテライトに相同なウシのミニサテライト DNA のクローニング : 池田和博・堤 賢一・木村 茂・江尻慎一郎・高橋寿太郎・安田泰久 (岩手大学農学部, 盛岡市 020)

Fig. 1. Restriction map (A), DNA fingerprinting (B), and nucleotide sequence (C) of a bovine minisatellite DNA clone.

(A) Nucleotide length of the *Bam* HI fragments of the clone are shown in Kb below the map. The cross-hatched box represents fragment containing a repetitive sequence homologous to mouse minisatellite. Open box which represents a fragment containing a repetitive sequence, was subjected to nucleotide sequencing. Symbols are: B, *Bam* HI; H, *Hin* dIII; K, *Kpn* I; P, *Pst* I; S, *Sal* I; Sm, *Sma* I; X, *Xba* I; Xh, *Xho* I. (B) DNA fingerprinting using 6.5 Kb *Bam* HI fragment as a probe. DNAs from half-sib pedigrees of Japanese Black cattle were digested with *Hin* fI, separated on agarose gel, blotted, and hybridized with a labeled 6.5 Kb *Bam* HI fragment. f denotes the father, m1 to m5 are mothers and o1 to o5 are corresponding offsprings. Size markers are given in Kb.

(C) Nucleotide sequence of an 1.6 Kb *Sma* I fragment (open box in a). Highly repeated core sequence which built up the sequence is shown. Variations observed in the core sequence are indicated by small letters.

from 9 Kb to 23 Kb was ligated to phage vector EMBL3, packaged *in vitro* using GIGAPACK II GOLD PACKAGING EXTRACT (STRATAGENE) and infected into *E. coli* LE 392.

The bovine genomic DNA library was screened by hybridization with mouse minisatellite I-3-2 as a probe. The mouse clone I-3-2 was obtained by hybridization with multimerized minisatellite core DNA in an intron of human myoglobin gene (ref. 16 and Kominami and Oyanagi, unpublished data).

Southern blot analysis and DNA fingerprinting :

Individual bovine DNA was digested with *Bam*HI, electrophoresed on an 1.2% agarose gel, and blotted overnight onto a nylon membrane. The filter was prehybridized for about 2 hr at 65°C in 4×SSC containing 1% SDS, 10 mM Tris-HCl, pH 7.5 and 10 µg/ml *E. coli* tRNA, and then hybridized with the labeled probe overnight at 65°C. Washing was performed twice at 65°C in 1×SSC containing 0.2% SDS for 30 min (low stringency) and further washed twice at 65°C in 0.1×SSC containing 0.2% SDS for 30 min (high stringency).

For DNA fingerprinting, 3 µg of genomic DNA were digested with *Hin*fl. Hybridization conditions were the same as above, except that washing was carried out under low stringency condition.

Results and Discussion

From a bovine genomic DNA library, we isolated several clones which hybridized with mouse minisatellite DNA I-3-2. One clone containing an insert of approximately 17 Kb was analyzed in detail. Figure 1 A shows the restriction map of the clone. To detect regions showing repetitive sequences in the clone, Southern blot analyses were performed under low and high stringency conditions using 6.5, 2.5, 3.1 and 3.5 Kb *Bam*HI fragments (designated as probes 6.5, 2.5, 3.1 and 3.5, respectively; see Fig. 1 A) as probes. Under low stringency condition, probes 6.5, 2.5 and 3.5

strongly hybridized to multiple fragments of the genome. In contrast, when hybridization was carried out under high stringency, probes 2.5 and 3.5 hybridized only to fragments of comparable sizes expected from the map in Fig. 1 A. On the other hand, the probe 6.5 hybridized to multiple bands even under high stringency condition. Thus, it appears that repetitive sequence (s) within the 6.5 Kb fragment is dispersed throughout the genome and therefore this fragment is suitable for a multi-locus probe. Indeed, the 6.5 Kb fragment gave typical DNA fingerprints when used as a probe (Fig. 1 B).

To detect repetitive region in the 6.5 Kb fragment, Southern blot hybridization experiments were carried out using various probes obtained from the 6.5 Kb fragments (Fig. 1 A). An 1.6 Kb *Sma*I fragment within the 6.5 Kb DNA (open box in Fig. 1 A) gave multiple bands on Southern blots when used as a probe, showing that this DNA contains a repetitive sequence which is dispersed throughout the genome (data not shown). Part of the nucleotide sequence of the 1.6 Kb *Sma*I fragment is shown in Fig. 1 C. The sequence is comprised of tandemly repeated core sequence GGCTGTGTGT. The observed variations of the core sequence are indicated by small letters in Fig. 1 C. It has been reported that minisatellites tend to consist of a G-rich sequence^{12, 16, 18-21}. In this context, the sequence presented here should be the bovine homologue of minisatellite.

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