A GGCAGGG Motif in Minisatellites Affecting Their Germline Instability*

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Mouse and human genomes contain hypervariable DNA regions consisting of tandem repeats of a short sequence referred to as minisatellites. This variation is thought to arise through processes such as unequal crossover or replication slippage. A mo-1 minisatellite probe comprising a 14-base pair repeat sequence reveals many polymorphic fragments even in DNA of BALB/c sublines. Oligonucleotide probes with single base substitution in the mo-1 have been synthesized and used for assessing sequence involved in generation of polymorphisms. The results indicate that the loci containing mo-1 homologues with mutation in the GGCAGG sequence are monomorphic despite the other mutations showing polymorphism. Reciprocally, locus-specific polymorphic clones, Pc-1 and Pc-2, have been isolated with hybridization to mo-1, and both are shown to contain repeated sequence comprising the GGCAGG sequence. They reveal high mutation rates of 8.8% and 3.3% per gamete, respectively. These results strongly suggest that the motif contributes to the germline instability of minisatellites.

Mouse and human genomes contain hypervariable regions of DNA which are dispersed throughout chromosomes. The hypervariable regions consist of tandem repeats of a short sequence referred to as minisatellites (Jeffreys et al., 1985a, 1985b, 1986, 1987). Many of these minisatellites seem to be polymorphic due to variation in the number of repeats, presumably driven either by unequal recombination between misaligned minisatellites or by slippage at replication forks leading to the gain or loss of repeat units (Jeffreys, 1987). A probe consisting of a minisatellite detects multiallelic loci, each having considerable restriction fragment length polymorphisms, the complex pattern of which is called the DNA fingerprint (Jeffreys et al., 1985a). Some human minisatellite loci have been isolated and characterized (Wong et al., 1986, 1987; Jeffreys et al., 1988; Armeu et al., 1989).

Jeffreys (1985a) discovered and characterized a minisatellite composed of four repeats of a 33-bp sequence within the human myoglobin gene and its related minisatellites. On the basis of studying repeat unit sequences of the minisatellites, there is some similarity in repeat unit sequence between these minisatellites; they share a homologous "core" sequence of 16 nucleotides (GAGGTCAGGAGAG; R = A or G), present once per repeat unit (Jeffreys et al., 1985a; Wong et al., 1987).

Nakamura et al. (1987) have extended these studies to several other types of minisatellites and have proposed a similar but different core sequence (GGCGGTGGGG).

The mechanism(s) of generation and maintenance of these hypervariable minisatellite loci are unclear. Although minisatellites seem to comprise a family or families, it is unlikely that this set of core-containing minisatellites constitutes a family of evolutionally related sequences, because the sequence flanking the core in repeat units showed no similarity between different minisatellites. Instead, it is proposed that the core sequence assists minisatellite production, by promoting the initial duplication of a DNA segment containing a core sequence and/or by aiding the subsequent changes in repeat number driven by unequal exchange (Jeffreys, 1987). This model predicts that the majority of core-containing loci will contain core monomers or oligomers, and that only a minority of minisatellites will have fortuitously amplified until they have attained a high repeat copy number and extensive polymorphism.

We cloned a mouse minisatellite, mo-1, similar to the core sequence and characterized it (Kominami et al., 1988). The synthetic DNA fragment consisting of mo-1 consensus sequence (CTGGCCAGGGAGGA) hybridizes to the multiallelic loci in mouse DNA and can detect many polymorphic fragments even in DNA of BALB/c sublines. When, however, hybridization conditions are less stringent, a lot of monomorphic fragments hybridize to this probe, implying that the loci containing the mo-1 sequence or very closely related sequences are polymorphic and the loci harboring sequences having homology to the mo-1 but forming mismatches with unstable duplexes become monomorphic. The findings suggest that there is a specific sequence element(s) required for generation and maintenance of polymorphisms.

To investigate this question in more detail, oligonucleotide probes with single base substitution mutants of the mo-1 have been constructed. Using these probes, it was tested whether a sequence is involved in genetic polymorphisms of minisatellite loci. This paper shows evidence that the hexanucleotide GGCAGG sequence motif of mo-1 contributes to the germline instability of minisatellites. Besides, we describe the isolation and properties of two locus-specific polymorphic clones containing a repetitive sequence comprising the motif.

MATERIALS AND METHODS

Minisatellite Probes: Synthetic and Labeling—Single-base substitution mutants of the mo-1 sequence with changes of A to T, T to A, C to G, or G to C at every position were made as three repeats of 14-mer using an automated DNA synthesizer (Applied Biosystems Inc.) and purified by polyacrylamide gel electrophoresis (Table 1). Each oligonucleotide (1 pmol) was labeled at the 5'-hydroxyl group with [*-32P]ATP (16 pmol at >5000 Ci mmol, Amersham) and T4 polynucleotide kinase (4 units; Takara) in 10 µl of kinase buffer (50 mM Tris-Cl (pH 7.5), 10 mM MgCl₂, 5 mM dithiothreitol, 0.1 mM spermidine, 0.1 mM EDTA). The kinase reaction was carried out at

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1 The abbreviations used are: bp, base pair(s); kb, kilobase(s); SDS, sodium dodecyl sulfate.
Sequences and polymorphism characteristics of oligonucleotide probes

P and M in the right column indicate the characteristics of the oligonucleotide probes showing polymorphism and monomorphism, respectively.

| chi       | GCTGTTGGG | P |
| core      | GCTGGCGAGGGGAGA | P |
| mo-1      | CTGGGCGAGGGGAGA | P |

The membrane was prehybridized in 5 x SSC, 1% SDS, and 50 pg/ml yeast RNA at 65 °C for 12 h. Then, the membrane was hybridized with 1 x 10^6 cpm/ml labeled oligonucleotide in 5 X SSPE (1 X SSPE = 10 mM sodium citrate) prior to use. The gel was directly hybridized with 1 x 10^6 cpm/ml labeled oligonucleotide in 5 x SSPE (1 X SSPE = 10 mM sodium citrate) to the 5'-terminally labeled probe with phenol/chloroform/isoamyl alcohol (25:24:1) and once with chloroform/isoamyl alcohol (24:1) and precipitated with ethanol.

**Dried Gel Hybridization**—Five µg of DNA from BALB/c sublines was digested with HindIII (Takara) under conditions recommended by the manufacturer. Digests were fractionated through 1.0% agarose gel in TAE buffer (40 mM Tris acetate (pH 7.5), 1 mM EDTA). The DNA was denatured in situ with 0.5 N NaOH, 0.015 M NaCl at room temperature for 30 min and neutralized with 0.5 M Tris-Cl (pH 8.0), 0.15 M NaCl at room temperature for 30 min. The gel was vacuum-dried on Whatman No. 3MM for 1 h at room temperature and 1 h at 60 °C (Ali et al., 1986). The dried gel was freed from the paper backing by wetting with 6 x SSC (1 x SSC = 0.15 M NaCl, 0.15 M sodium citrate) prior to use. The gel was directly hybridized with 1 x 10^6 cpm/ml labeled oligonucleotide in 5 x SSPE (1 x SSPE = 10 mM sodium phosphate (pH 7.0), 0.18 M NaCl, 1 mM EDTA), 0.1% SDS, 10 µg/ml sonicated denatured salmon sperm DNA at an appropriate temperature for 16 h. The gel was washed with 6 x SSC twice for 15 min each, followed by a 4-h wash at room temperature, and finally washed with 5 x SSPE at hybridization temperature for 1 min. After washing, the gel was exposed to x-ray film (Fuji, RX, Kodak, XAR) for 18 h with an intensifying screen at ~80 °C.

**Southern Hybridization**—A 5-µg sample of DNA was digested with HindIII, HaeIII, or Mbol (Takara) under the conditions recommended by the manufacturer. Digests were fractionated through 1.0% or 1.2% agarose gel in TAE buffer. The DNA was denatured in situ with 0.5 N NaOH, 1.5 M NaCl at room temperature for 30 min, neutralized with 1 M Tris-Cl (pH 8.0), 1.5 M NaCl at room temperature for 30 min, and transferred by blotting to a nylon membrane (Du Pont-New England Nuclear; GeneScreen Plus) (Southern, 1975). The DNA was fixed on a membrane by UV irradiation or baking at 80 °C for 2 h. The membrane was prehybridized in 5 x SSC, 1% SDS, and 50 µg/ml yeast RNA at 65 °C for 12 h. Then, the membrane was hybridized in the prehybridization buffer containing 1-2 x 10^6 cpm/ml probe labeled by the random primer method (Boehringer Mannheim: Random Primed Labeling Kit) (Feinberg and Vogelstein, 1984) at 65 °C for 16 h. The membrane was washed for 60 min twice in 1.5 x SSC, 0.5% SDS at 65 °C and in 0.1 X SSC, 0.5% SDS at 68 °C to detect DNA fingerprints and locus-specific bands, respectively.

**Screening of BALB/c Genomic Library**—Two hundred µg of DNA from BALB/cHeA was digested with Sau3AI completely and fractionated through 0.6% agarose gel. Fragments longer than 4 kb were recovered from agarose gel, ligated with 5 µg of chromaid 3-34 (Saito and Stark, 1986), linearized with BamHI (Takara: DNA ligation kit) and packaged in vitro (Strategene: Giga Pack Gold). 1 x 10^7 colonies per µg of fractionated genomic DNA were obtained. 3 x 10^8 colonies were screened by hybridization with a synthetic mo-1 sequence (Maniatis et al., 1982). Five strongly positive signals of colonies were detected and finally two clones were isolated. Sau3A inserts of the two clones, Pc-1 and Pc-2, were subcloned into the BamHI site of pUC19 and were subjected to Southern analysis and DNA sequencing.

**DNA Sequencing**—Pc-1 and Pc-2 inserts in pUC19 were transferred into the M13 vectors mp18 and mp19 for DNA sequencing (Meesing, 1985). Nucleotide sequences were determined by the dideoxy chain termination method (Sanger et al., 1977).

**RESULTS**

**DNA Fingerprint with mo-1**—Dried gel hybridization with a minisatellite, mo-1, was carried out for DNA of five BALB/c sublines. This method is a version of Southern blot hybridization using synthetic oligonucleotide probes. Under appropriate conditions, an oligonucleotide probe complementary to the mo-1 sequence will hybridize to the loci containing mo-1 and related sequences, and, accordingly, a specific hybridization pattern can be obtained consisting of stable duplexes (Ali et al., 1986; Ali and Wallace, 1988; Yam et al., 1987). The mo-1 was isolated from BALB/c mouse DNA by cross-hybridization to the human core sequence and consisted of a consensus sequence of repeat units, CTGGGCAGGGGAGA (Table I). When hybridization and washing were carried out at 55 °C using the mo-1 synthetic probe consisting of the consensus sequence, it revealed several larger (more than 3 kb) DNA fragments showing polymorphism and smaller monomorphic fragments among BALB/c sublines (Fig. 1). These fragments detected should contain a tandem repeat sequence homologous to mo-1, because the 5'-terminally labeled probe with the low specific activity of 2 x 10^6 cpm/µg fails to detect single copy DNA under the hybridization conditions. The

**TABLE I**

| No | Sample | Characteristics |
|----|--------|----------------|
| 1  | BALB/cArg | P |
| 2  | BALB/cSKI | P |
| 3  | BALB/cDAG | P |
| 4  | BALB/cHeA | P |
| 5  | BALB/cORNL | P |

**FIG. 1.** DNA fingerprint patterns of BALB/c sublines with mo-1 under various conditions. DNA of five BALB/c sublines was isolated, digested with HindIII and subjected to dried gel hybridization. Hybridization and washing were performed at the temperature indicated below each autoradiogram. Arrowheads indicate the polymorphic fragment of a locus, whose band intensity reflects different hybridization efficiency. 1, BALB/cArg; 2, BALB/cSKI; 3, BALB/cDAG; 4, BALB/cHeA; 5, BALB/cORNL.
FIG. 2. DNA fingerprints with each mutant probe. Hybridization and washing were carried out at 55 °C.
All the autoradiograms except that of mutant 4 were obtained after overnight exposure. The autoradiogram of mutant 4 was obtained after a 3-day exposure. The DNA sample in each lane is the same as that in Fig. 1. A, mutants which show DNA fingerprints having polymorphic bands in addition to the DNA fingerprint with mo-1; B, mutants which detect some of the polymorphic bands of the mo-1; C, mutants which detect only monomorphic bands that are different from the bands of the mo-1 DNA fingerprints; D, a mutant which reveals polymorphic fragments that are not detected by mo-1. E, the DNA fingerprint obtained with mutant 6'. Mutant 6' is a 42-mer oligonucleotide having the same repeat sequence as mutant 6 but the mutation is located in the first position. The sizes of marker DNA fragments (bacteriophage λ-DNA digested with EcoT14I) are shown.
BALB/c mouse strain has been separately maintained in different laboratories for more than 20 to 60 years after proper inbreeding and constitutes sublines with some different phenotypes (Potter, 1985).

When, however, the hybridization and washing became less stringent, at 50 °C or at 42 °C, many additional bands common to all BALB/c sublines appeared (Fig. 1). These results suggest that the DNA fragments forming stable hybrids with the mo-1 probe under the stringent conditions are highly variable depending on their size, but that the DNA fragments having many mismatches to the mo-1 probe and hence forming unstable duplexes are genetically stable during breeding. The findings point toward the possibility that the mo-1 sequence contains a specific sequence element involved in genetic variability.

**Mutant Analysis**—In order to answer the specific structural question, mutant analysis was carried out. 14 kinds of DNA fragments with single-base substitution were used; the rule of change was G to C and A to T or reverse (Table I). To minimize the position effect of mutation on hybridization efficiency, we synthesized 42-mer oligonucleotides consisting of three tandem repeats of 14-bp core sequence. Using each mutant as a probe, hybridization was carried out for DNA of the BALB/c sublines under the stringent conditions (Fig. 2). The DNA fingerprints were distinguishable from each other, consistent with the assumption that this method detects specific hybrids formed with each probe. The complex patterns of DNA fingerprints can be classified into four types. First, mutants 1, 2, 10, 13, and 14 hybridized to the polymorphic bands of the mo-1 DNA fingerprint with some additional bands (Fig. 2A). Second, mutants 3, 11, and 12 hybridized to some of the polymorphic bands of the mo-1 (Fig. 2B). Third, mutants 4, 6, 7, and 8 detected only monomorphic bands. Mutant 5 failed to detect any bands. Last, mutant 9 revealed mainly monomorphic bands and a polymorphic fragment that was not detected by the mo-1 (Fig. 2D).

The mutant probes which failed to hybridize to polymorphic mo-1 fragments appear to have mismatches in the middle of the core sequence (Table I), and hence the location of base pair mismatches may affect the prevention of hybridization. To rule out this possibility, we synthesized a new probe of mutant 6 (mutant 6'), where the mutation was placed in the first nucleotide of the repeat unit, and tested whether this probe is capable of hybridizing to polymorphic mo-1 sequences or not. This probe also failed to hybridize to mo-1 although the mismatch was located at the edge of the repeat sequence (Fig. 2F). The result indicates that the duplexes formed under the hybridization conditions depend on sequence and are not affected by the position of mutation in the repeat unit.

It is clearly demonstrated that the mutants containing a base substitution in a sequence GGCAGG do not hybridize to the polymorphic bands homologous to the mo-1 sequence but the other mutants can hybridize to all or some of the mo-1 polymorphic bands (summarized in Table I). The results indicate that a GGCAGG sequence participates in stable duplex formation with polymorphic fragments but not with monomorphic fragments, suggesting that a sequence motif GGCAGG plays an important role in assisting generation and maintenance of polymorphisms of mo-1 minisatellites.

**Core-like Mutants**—In addition to one-point mutants, a two-base substitution mutant, C1, resembling a core minisatellite, was synthesized. There are two base substitutions and...
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FIG. 5. Mutations detected in one litter of F2 mice crossed between BALB/c and SM/J. DNA was isolated from F2 mice, digested with HaeIII, and subjected to Southern hybridization with PC-1 (A) and PC-2 (B) probes. The parental germline fragments of PC-1 and PC-2 see BALB/c and SM/J are shown with bars. New mutations are indicated by arrowheads. Arrows mark three mutated bands with the same size, which resulted from a mutated fragment in the F1 germline cells or possibly from a new mutation in a F1 germline cell.

FIG. 6. DNA Sequencing of PC-1 (A) and PC-2 (B) minisatellites. The sequence of the 5'- and 3'-flanking regions is shown together with the repeat unit sequences in each DNA fragment. The Ncol restriction site of PC-1 is underlined. Some of the tandem repeat region of PC-2 is not sequenced, although clearly a tandem repeat of the consensus sequence (AGGCAGG) is present by inspection of sequencing autoradiograms.

two base deletions (or additions) between the mo-1 and core sequences (Table I). Mutant C1 has AG instead of GA at positions 10 and 11 in the core sequence. C1 detected polymorphic bands for the BALB/c sublines that completely differed from those of the mo-1 and constituted another type of fingerprint (Fig. 3A). Another mutant, C2, that had an additional base substitution in the GGCAGG motif, a C to G change in position 6 was made. C2 did not give the DNA fingerprint showing polymorphism (Fig. 3B). These results agree that the GGCAGG motif is important for promoting instability of minisatellites even if the fingerprint pattern is different from that of the mo-1.

Locus-specific Polymorphic Clones—A reciprocal experiment was carried out to know whether the GGCAGG motif actually contributes to genetic instability of minisatellites. A chromid library of Sau3AI-digested fragments of BALB/c was constructed and screened by hybridization to a synthetic mo-1. Five strongly positive clones were obtained and shown to contain a Sau3AI insert of 2.7 kb, 2.0 kb, 1.4 kb, 2.1 kb, and 1.7 kb, but the first three were derived from a fragment, PC-1, and the rest from PC-2. Since these inserts were shorter than the PC-1 band (3.7 kb) and the PC-2 band (3.9 kb) detected in the Southern blots (see Fig. 4), part of each insert had been lost during propagation in Escherichia coli. The 2.0-kb and 2.1-kb inserts of PC-1 and PC-2 clones, respectively, were subcloned in pUC19 and used as probes for Southern hybridization. For DNA of the BALB/c sublines, PC-1 detected a locus with allelic variation, which was actually hybridized with the mo-1 probe (Fig. 4A). PC-2 also revealed a polymorphic pattern, although some bands due to cross-hybridization remained. Two BALB/c sublines (lanes 1 and 2) showed two bands though inbred, implying that the PC-2 locus was heterozygous at least in the mouse, which probably resulted from hypermutation.

To determine mutation rates to new length alleles, PC-1 and PC-2 each hybridized to HaeIII-digested DNA of 66 F2 mice crossed between BALB/c and SM/J. Examples of the new mutations consisting of a litter are shown in Fig. 5. For the PC-1 locus, 13 mutations were detected of the 148 alleles (66 F2 and 8 F1) and apparently clustered in only four of the eight litters obtained. Besides, in one F2 mouse (lane 2 in Fig. 5), mutation occurred at both alleles. The mutation rate was 8.8% per gamete. PC-2 detected five mutations and hence the mutation rate was 3.3% per gamete. These results demonstrate the hypervariability of the PC-1 and PC-2 loci where mo-1 minisatellites exist.

Locus-specific Polymorphic Clones Contain Repeated Sequence with the GGCAGG Motif—2.0-kb and 2.1-kb inserts of PC-1 and PC-2, respectively, were recloned in either of the M13 vectors mp18 and mp19 for sequencing. The region of PC-1 determined with mp19 spanned a minisatellite and a Ncol site (Fig. 6A). Since the synthetic mo-1 probe hybridized to the EcoRI-Ncol 0.4-kb fragment of PC-1 but not to the other Ncol-HindIII fragment, the homologous sequence is present only in this region (data not shown). The minisatellite of PC-1 contains 28 homogeneous repeat units consisting of a short sequence, GGGCA. The original PC-1 fragment should contain more repeat units, because the fragment sequenced undergoes partial deletion of repeat units. Two contiguous repeat sequences provide the GGCAGG motif and a flanking sequence, GCAG.

Both regions of PC-2 determined with mp18 and mp19 contained part of a minisatellite and the flanking sequence. The results are compiled in Fig. 6B. PC-2 contains a minisatellite consisting of repeats of the AGGCAGG sequence, which also comprises the GGCAGG motif. The result that either of the two polymorphic clones hybridized with mo-1 contain the GGCAGG motif supports the previous implication that the motif affects the meiotic instability of minisatellites.

Mo-1 Minisatellites Are Rather Stable in Somatic Cells—
The Pc-1 and Pc-2 probes hybridized to MboI digests of DNA from 23 independent clones of FM3A to determine instability during mitosis. Three cell clones were cultured randomly for 2 months and then eight clones each (one DNA sample missed) were obtained again. The difference in history and passage time between the original clones is unclear. As shown in Fig. 7, A and B, no new length allele resulting from mutation was observed for either the Pc-1 or Pc-2 locus. The filter rehybridized to the mo-1, revealing one band (marked by arrowheads in Fig. 7C) different between the three original cell clones. These results suggest that mo-1 minisatellites are relatively stable during mitosis.

**DISCUSSION**

**Genetic Instability of mo-1 Minisatellites**—In general, tandemly repeated sequences are genetically unstable because the homologous recombination system is active in meiosis. Especially, minisatellites consisting of tandem repeats of a short sequence show a high degree of instability and comprise hypervariable regions of chromosomal DNA (Jeffreys et al., 1985a). The minisatellite probe hybridizes to many multiallelic loci and provides individual specific DNA fingerprints in human, mouse, and other eukaryotes (Jeffreys et al., 1985a, 1985b, 1985c, 1987; Burke and Bruford, 1987; Wetton et al., 1987; Gill et al., 1985; Jeffreys and Morton, 1987; Dallas, 1988). An individual pattern of a DNA fingerprint is somatically stable, and almost all of the bands in the pattern transmit from parent to offspring according to the Mendelian law. However, mutations to new length alleles are sometimes detected. Jeffreys and co-workers found a consensus sequence "core" shared by the myoglobin minisatellite and related minisatellites and suggested that since the core sequence is similar to χ, the cross-over hot spot initiator sequence (GCTGGTGG) of E. coli (Smith et al., 1981), it might serve as a recombination signal in human DNA (Table I). The idea is supported by evidence that large scale duplication/deletion of minisatellite fragments was observed, which can be easily explained by unequal crossover rather than DNA replication slippage. This hypothesis, however, requires an experimental test. Alternatively, it is possible that some trans-acting factors and cis-acting elements are present to suppress homologous recombination and maintain a stable copy number of repeats (Christman et al., 1988). The minisatellites showing polymorphism may lose such cis-elements.

We isolated a mo-1 minisatellite clone from the BALB/c mouse DNA that had sequence homology to the core sequence of minisatellites present in the human genome (Kominami et al., 1988). The mo-1 has a characteristic of being genetically very unstable (Fig. 1); most larger fragments homologous to mo-1 differ in size among BALB/c sublines which have been separately maintained for 50 to 250 generations after inbreeding. The level of instability is greater than that of the core minisatellites (data not shown). Therefore, a combination of mo-1 and BALB/c sublines provides a proper system in which to examine the sequence responsible for producing genetic instability of minisatellites.

**GGCAGG Motif**—Mutant analysis revealed that in the loci containing the mo-1 homologues with an intact hexanucleotide GGCAGG motif, at least some show polymorphisms, but that the loci containing mo-1 relatives with mutations in the motif do not show any polymorphisms (Fig. 2). It indicates that there are two distinct types of mo-1-related minisatellites; one can be polymorphic and the other not (Table I). The present data demonstrate that one determinant of generating and maintaining the polymorphism is the GGCAGG motif of minisatellite repeats. This is consistent with the result of a core-like minisatellite probe. Since the C2 probe with an additional mutation at position 6 in the motif did not detect any polymorphic fragment, its GGCAGG motif is also important for variability of the minisatellites. Reciprocally, two isolates, Pc-1 and Pc-2, that hybridized to mo-1 show polymorphic fragments in BALB/c sublines and contain repeat sequences with the motif. Compilation of these results strongly suggests that the GGCAGG motif of minisatellites plays an important part in generating and maintaining their polymorphism.

The hybridization experiments with mo-1 probes and the sequence analysis of Pc-1 and Pc-2 may indicate that the polymorphic loci do not contain any repeat copy with mutated motives. In fact, the original mo-1 locus which contains mutant motives does not show polymorphism (data not shown). If the sequence of even one repeat having an impaired motif in a minisatellite acts as suppressor for variability resulting from unequal crossover. However, since mo-1 itself has an impaired motif, GGGAGG, in position 8 to 13 as well, that possibility remains unclear. There is an alternative explanation; even if a repeat unit with a mutated motif emerges in a hypervariable minisatellite, the repeat unit tends to disappear in the minisatellite, since it has no or little ability to amplify through unequal homologous recombination.

Presumably, some minisatellites with the motif fortuitously amplified and have attained a repeat copy number and extensive polymorphism. It is not clear, however, why there are
many monomorphic minisatellites having the motif. There must be other factors affecting the instability of minisatellites. Recently, Ali and Wallace (1988) suggested that some factors other than the DNA sequence of a minisatellite locus is involved in the mechanism(s) generating the polymorphism, since not all of the minisatellite loci are polymorphic, despite the fact that they share the core sequence. It may be the nature of flanking sequences or chromatin conformation which gives stability to a minisatellite locus. The structures of Pc-1 and Pc-2 loci are different from that of the nonpolymorphic mo-1 locus with regard to repeat unit length. They contain multiple repeats of short sequences, 5 and 7 base pairs, respectively. Therefore, the length of the repeat unit in minisatellites may be another determinant in affecting the efficiency of recombination between repeats.

Base substitution of the two nucleotides flanking GGCAGC in the mo-1 results in detecting another type of minisatellite; Cl synthetic probe (AG instead of GA at positions 10 and 11) resembling the human core sequence hybridized to polymorphic fragments in DNA of human individuals as found in mammalian genomes. Taking into account the results of PC-1 and PC-~, they contain the GGCAGG motif and flanking sequences GCAG and A, respectively. Each of them provides distinct polymorphic DNA fingerprints in BALB/c sublines (data not shown).

Mutant 6 of mo-1 does not show polymorphism between BALB/c sublines whose genetic differences are presumably small, but it can detect some polymorphic bands between three mouse strains of BALB/c, C57BL/6J, and C57BL/10Q (data not shown). It therefore indicates that the loci carrying mo-1 homologues with mutation in the GGCAGG motif are variable, but the variability is small. The motif is present in the core sequence and the mouse MHC (Kobori et al., 1986; Steinmetz et al., 1986), among polymorphic minisatellites (Bell et al., 1982; Capon et al., 1983; Goodburn et al., 1983; Stoker et al., 1985; Jarman et al., 1986; Knott et al., 1986; Silva et al., 1987). The mo-1 probe also hybridizes to human DNA and provides polymorphic DNA fingerprints among individuals. Besides, two mutants at positions 6 and 8 that do not show any polymorphism for BALB/c sublines can detect polymorphic fragments in DNA of human individuals as effectively as the mo-1 (data not shown). Although genetic distances of the two samples are different, in the human genomes the mo-1 homologues with mutated motifs may serve as an efficient hot spot for recombination.

**Hypervariable Loci**—We have obtained locus-specific clones, Pc-1 and Pc-2, homologous to mo-1, which are both prone to mutation. The Pc-1 locus showed a higher mutation rate, 8.8% per gamete, in the examination of 66 F2 mice crossed between BALB/c and SM/J (Fig. 5). Since the highest mutation rate that has been observed for minisatellite loci is 5.2% at the human MS1 locus (Jeffreys et al., 1988; Armour et al., 1983; Goodburn et al., 1983; Gil, 1985; 1986) Nature 318, 577-579

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