Molecular characterization of a new human minisatellite that is able to form single-stranded loops in vitro and is recognized by nuclear proteins

Francisco Boán, Ana Isabel González, José Manuel Rodríguez, Jaime Gómez-Márquez*

Departamento de Bioquímica y Biología Molecular, Facultad de Biología, Universidad de Santiago, Santiago de Compostela, La Coruña, Galicia, Spain

Received 22 October 1997

Abstract We report the isolation of a new low polymorphic GC-rich human minisatellite locus (MsH42) that contains different recombination motifs and is homologous to sequences involved in immunoglobulin class-switching. Furthermore, we show that MsH42 undergoes slipped-strand mispairing during PCR indicating its ability to generate single-stranded loops. Specific DNA-protein complexes were detected in band-shifting experiments using nuclear extracts from mouse testes and human NC-37 cells. The possible implications of this minisatellite in recombination events is discussed.

Key words: Minisatellite; Homologous recombination; In vitro slippage; Band-shifting

1. Introduction

The mammalian genomes contain numerous dispersed minisatellites consisting of short DNA sequences (6–100 bp) tandemly repeated [1]. An interesting characteristic of many minisatellites is the high level of variability in the number of tandem repeats as well as the interspersion pattern of variant repeats along the tandem repeat array [2–7]. The mechanisms for generating new minisatellite variants are most likely replication slippage and recombination events such as unequal crossing-over between sister chromatids and gene conversion [8–11]. Recently, studies of the mutation processes in some human minisatellites indicate a mode of somatic mutation completely different from that seen in sperm cells [12].

In a previous work, we reported the sequencing of several mouse recombinant clones that were shown to hybridize with a GC-rich *Sma*I DNA fragment (*Sma*IA) of the *Herpes simplex* virus type 1 genome [13]. The analysis of these clones revealed the presence of tandemly repeated short DNA sequences closely related to immunoglobulin heavy chain sequences involved in class-switch recombination. In the present study, we have isolated from a human genomic library DNA sequences homologous to the *Sma*IA fragment. We found a new human DNA sequence containing a low polymorphic GC-rich minisatellite region with different recombination signals within and surrounding it. Comparison with other published sequences showed that this new minisatellite is quite similar to immunoglobulin regions involved in class-switch recombination. Furthermore, this minisatellite undergoes slipped-strand mispairing during PCR generating several slippage products and is recognized by mammalian nuclear proteins in band-shifting experiments. Altogether these findings suggest a role for this repetitive sequence in recombination events.

*Corresponding author. Fax: +34 (81) 596904.
E-mail: bnjgm@usc.es

Abbreviations: MsH42, human minisatellite 42; *Sma*IA, a 1.1 kb *Sma*I restriction fragment derived from the *Herpes simplex* type 1 genome

2. Materials and methods

2.1. Isolation and sequence analysis of H42

The screening of a human fetal liver DNA library, using the *Sma*IA DNA fragment as a probe, was carried out as described elsewhere [13]. One of the isolated clones, termed λ42, was digested with several restriction enzymes and the fragments separated in 1% agarose gels, blotted onto nitrocellulose and hybridized with the nick-translated ^{32}P-*Sma*IA. The probe hybridized strongly with an *Eco*RI-*Xba*I fragment of ~2 kb which was subcloned in the M13 vector. To obtain the complete nucleotide sequence of the insert, a library of *Bal*-31 deletion mutants was constructed [14] and the deletion fragments sequenced with the Sequenase system (Amersham).

2.2. Hybridization and PCR polymorphism analyses

DNA was extracted from peripheral blood samples of healthy individuals according to standard procedures [15]. DNAs (5–10 μg) were digested with *Bgl*II and electrophoresed through 1% agarose gels in TAE buffer. DNAs were transferred to a Hybond-N$^+$ nylon membrane (Amersham), baked at 80°C for 2 h and prehybridized at 47°C for 3 h in 3×SSC, 50% formamide, 10% dextran sulphate, 5×Denhardt's solution and 200 μg/ml of human denatured placental DNA. Hybridization was carried out overnight in the prehybridization solution with 4×10^6 cpm/ml of the plasmid pRep42 labeled by nick-translation (8×10^8 cpm/μg). After hybridization, membrane was washed 15 min at room temperature in 2×SSC, 0.5% SDS followed by 2 washes of 30 min in 1×SSC, 0.5% SDS at 65°C and another 2 washes of 30 min in 0.1×SSC, 0.5% SDS at 52°C. The membrane was exposed 20 h at −70°C using Kodak X-OMAT film with intensifying screens. PCR reactions of the MsH42 locus were performed with 250 ng of genomic DNA, 0.3 μM primers (P_1: 5′-CTTGGGCACTCTAGGACACC-3′, P_2: 5′-CACAGCTCTGGCTACAAGAG-3′), 1×Taq buffer, 1 unit of *Taq* DNA polymerase (Promega) and water up to 50 μl. An initial denaturing step at 95°C during 5 min was made prior adding the polymerase followed by 29 cycles (denaturing at 95°C, 1 min; annealing at 62°C, 40 s; extension at 72°C, 50 s) and a final cycle with an extension of 5 min. In all PCR experiments, a negative control without DNA template was made. For electrophoresis, 10 μl of PCR products were mixed with gel-loading buffer and run on a 3% Metaphor (FMC) agarose gel. The PCR products of 480 bp (short allele) and 626 bp (rare fragment) were purified using a QIAquick gel extraction kit (QIAGEN) and cloned using the pGemT cloning kit (Promega). The inserts were sequenced with the Sequenase system using [α-^{35}S]dATP and following the protocol for double-stranded DNA sequencing (Amersham).

2.3. PCR slippage assays

For in vitro slippage experiments, 1 ng of the inserts of pRep42, pB9M, pB20H (the long allele, the short allele and the rare fragment respectively), were amplified using the PCR conditions described above. Five μl of each PCR reaction were separated in 3% agarose gel and, in the case of pRep42 slippage products, 1/50 was employed for Southern hybridization. The PCR slippage fragments (480 bp, S1, S2, S3) were purified from the gel and sequenced with the Sequenase PCR product sequencing kit (Amersham). The influence of temper-

PII S0014-5793(97)01389-6

ature in the slipped-strand mispairing was tested carrying out the amplifications with the annealing temperature at 66°C.

2.4. Gel mobility shift assay

Nuclear extracts for band-shifting experiments were prepared from mouse testes and human NC-37 cells as described by Dignam et al. [16]. Binding reactions were performed essentially according to Wahls et al. [17]. Nuclear extract (10 μg) was incubated with 2 μg of poly(dI-dC)·(dI-dC) in 20 μl of binding buffer for 8 min at room temperature. End-labeled probe (0.5 ng, 50000 cpm) and competitor DNAs were added and the mixture incubated 15 min at room temperature. The DNA-protein complexes were separated on low ionic strength 4% polyacrylamide gels with low ionic strength electrode buffer, at 30 mA for 4 h with a thermostatic plate system to keep a constant temperature of 10°C. The gels were dried and exposed for 12–24 h at −80°C with intensifying screens. The probes used in the band-shifting assays came from the *Dde*I digestion of the pRep42 PCR fragment as mentioned above. These DNA fragments (260 bp, 220 bp and 105 bp) were end-labeled with T4 polynucleotide kinase using [γ-^{32}P]dATP and purified with a Sephadex-G50 column.

3. Results

3.1. Characterization of a new human GC-rich minisatellite sequence

To isolate human GC-rich DNA sequences, we carried out the screening of a human fetal liver DNA library [18], using the viral *Sma*IA DNA as a probe and the hybridization conditions described elsewhere [13]. Several positive clones were isolated and one of them, λ42, was used for the present study. Southern blot analysis of λ42 showed that the sequence homologous to the viral probe was located in an *Eco*RI-*Xba*I fragment of ~2 kb, named human 42 (H42), whose nucleotide sequence is shown in Fig. 1.

H42 contains a region of short tandem repeats with structure of minisatellite extended between nucleotide positions 1200 to 1543. The base composition of this region is 63.2% G+C, higher than the average (40–45%) in mammalian genomes, and has a strong purine-pyrimidine strand bias. This minisatellite, termed MsH42, is formed by 36 repeats of closely related decamers and nonamers and contains an internal duplication of 11 repeats. The sequences of repetitive units are the result of small changes in the repeats TGGGAGAGGC and TGGGAGAGC (>75% of the MsH42), a common characteristic of this type of sequences [4]. Flanking the MsH42 sequence there are two *Chi* motifs (GCTGGTGG) and at the beginning of the minisatellite there is a *Chi*-like sequence (GCTGGAGG). Moreover, two oligomers (TGTTTTTGG, CACAGTG) described as recombination signals in immunoglobulin VDJ joining [19] are also present at both sides of the minisatellite although neither the spacer between these sequences nor their orientation is conserved. Upstream there is a poly(CA), an abundant repetitive component of eukaryotic genomes.

Computer analysis showed that the portion of *Sma*IA involved in the homology with MsH42 is the reiteration II of viral fragment, a cluster of 18 repetitions of the unit CTGGGGCTGGGGAGGG [13]. In contrast, another GC-rich region of the *Sma*IA DNA, the reiteration III, composed by the unit GAGGGGGCGAGGGGCGG repeated 12 times, did not show relatedness with MsH42 in spite of its high GC content. Searches at the EMBL sequence database showed that this minisatellite is very similar to DNA sequences involved in immunoglobulin class-switch recombination [20]. The presence of the tandem repetitions (G)AGCT(G) and TGGG(G) in the immunoglobulin switch regions and the abundance of very similar motifs (GAGCT and TGGG) in MsH42, explain the similarity found between both loci. Other GC-rich sequences did not show significative scores with MsH42 indicating that the homology observed is not merely a consequence of GC richness.

3.2. Variability in the MsH42 locus

DNAs from several healthy individuals were prepared for Southern analysis. The probe, a 0.8 kb *Pst*I fragment of H42 subcloned into pUC18 (pRep42), includes the minisatellite region (MsH42 and flanking sequences) and excludes the poly(CA) to avoid cross-hybridization with the ubiquitous genomic CA repeats. We found a pattern consisting in two bands of 1.3 kb and 1.2 kb (Fig. 1A). A tenuous band (~2.6 kb) was always present when the individual has the 1.2 kb band, suggesting an association between these two DNA sequences. PCR analysis of genomic DNAs from individuals with the 1.3 kb band originated an amplified fragment of 585 bp (its sequence is shown in Fig. 1) whereas a 480 bp fragment was obtained after amplification of individuals with the 1.2 kb band (Fig. 1B). The 480 bp fragment was cloned into pGemT vector (clone pB9M). Sequence analysis revealed that the difference between both PCR fragments (105 bp) is inside the internal duplication of MsH42.

PCR analysis of three independent CEPH reference pedigrees (1347, 1362 and 1413) and 16 small families (the parents and a child) showed that the 585 bp (long allele) and the 480 bp (short allele) fragments were inherited codominantly. In the polymorphism analysis, we discovered an individual (20H) showing a longer PCR fragment absent in the other samples analyzed (Fig. 1C). This DNA fragment was cloned

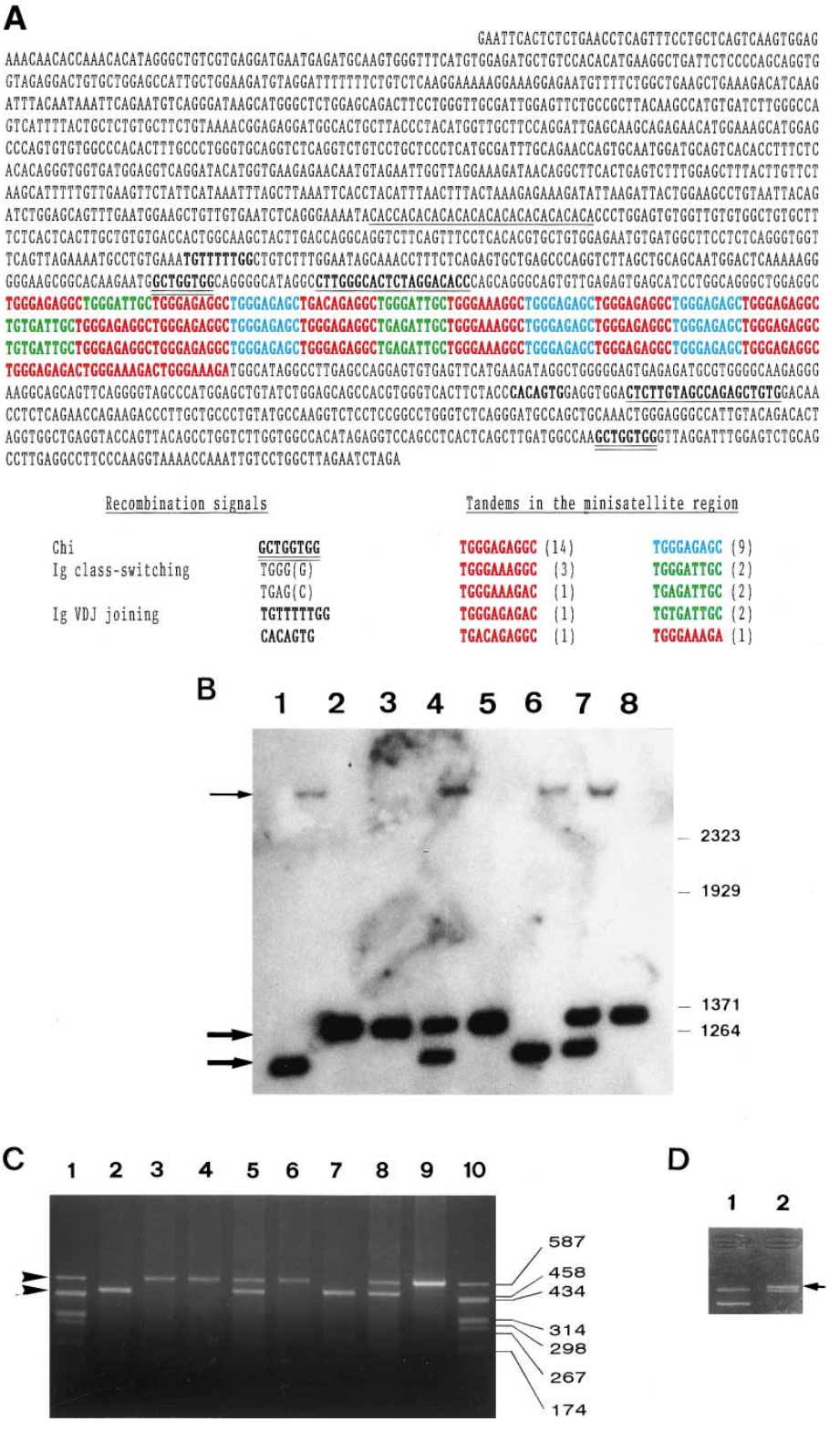
→

Fig. 1. Complete DNA sequence of H42 and polymorphism analysis. A: The MsH42 region is extended between the nucleotide positions 1200–1543; colours represent identical or very similar repetitions. The CA stretch is underlined. *Chi* and Ig VDJ joining motifs are double-underlined and in bold-face type, respectively. The primers employed in the PCR analysis are in bold-face type and underlined. Table below the sequence represents the different recombination signals occurred in H42 and the abundance of the repeats (identified by the colour code) in MsH42. B: Southern blot analysis of human genomic DNAs digested with *Bgl*II, separated in 1% agarose gel and hybridized with the pRep42 probe. Big arrows signal the 1.2 kb and the 1.3 kb fragments corresponding to the short and the long alleles. Samples 1 and 6 are homozygous for the 1.2 kb fragment; samples 2, 3, 5 and 8 are homozygous for the 1.3 kb fragment; samples 4 and 7 are heterozygous. Thin arrow indicates the 2.6 kb fragment linked to the 1.2 kb fragment. The sizes of the molecular weight marker (λ DNA digested with *Bst*EII) are given in bp (right column). C: PCR analysis of MsH42 locus. The same DNAs used for Southern analysis were employed for the PCR analysis. Arrowheads indicate the 585 bp and 480 bp amplified fragments. Samples 2–9 are identical to the 1–8 in A. Samples 2 and 7 are homozygous for the 480 bp fragment; samples 3, 4, 6 and 9 are homozygous for the 585 bp fragment and samples 5 and 8 are heterozygous. Lanes 1 and 10, molecular weight marker (pGem3Z digested with *Hae*III) in bp. D: Electrophoretic analysis of the sample 20H. Lane 1 corresponds to the PCR product of a heterozygous sample and lane 2 represents the PCR product of the individual 20H consisting in the 585 bp fragment (long allele) and the 627 bp fragment (small arrow). These sequence data have been submitted to the EMBL database under the accession number X87174.

in the pGemT vector (clone pB20H). Sequence analysis showed a duplication of 41 bp between the C inside the *Chi*-like motif and the first A included in the fourth repeat of MsH42. Further analysis would be needed to determine the possible inheritance and frequency of this rare fragment in the human population.

3.3. *In vitro slippage behaviour of MsH42*

Short tandem organization facilitates misalignments, creating loop structures with single-stranded conformation, which are important in recombination models. These misalignments probably cause replication slippage that may generate detectable mutations in the repetitive sequences. To search for the



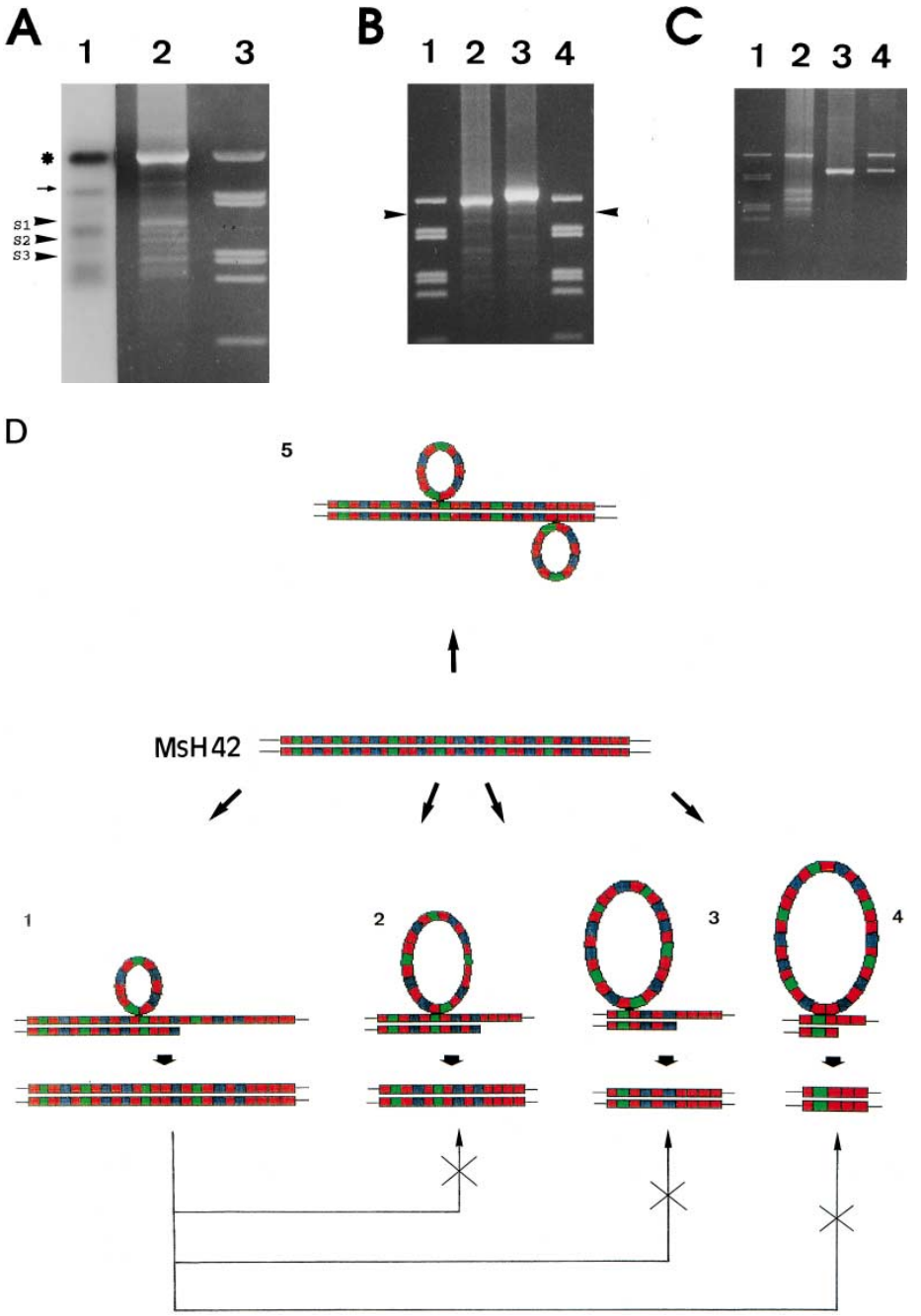


Fig. 2. Slippage behaviour of MsH42. A: In vitro slippage of the long allele. pRep42 slippage products generated during PCR were fractionated in an agarose gel (lane 2) and hybridized with the pRep42 insert as probe (lane 1); lane 3, molecular weight marker (pGem3Z digested with *Hae*III). The 585 bp fragment (long allele) is indicated by an asterisk and the PCR-generated short allele is marked by a small arrow; arrowheads mark the ladder fragments S1, S2 and S3. B: Comparison between the slippage products generated in vitro by the long allele (lane 2) and the rare fragment of the person 20H (lane 3). Note the difference in the size of the slippage products (arrowheads). Lanes 1 and 4, molecular weight marker. C: Differential slippage behaviour of the long (lane 2) and short (lane 3) alleles of MsH42. Note the absence of slippage bands below the 480 bp (short allele) fragment. Lane 1, molecular weight marker (pGem3Z cut with *Hae*III). Lane 4, internal marker (an heterozygous sample of MsH42). D: Slippage model of MsH42. Colour boxes represent the different tandem repeats (colour code as in Fig. 1). Central drawing corresponds to the long allele of MsH42. Drawings 1–4 show the hypothetical slipped-strand mispairings that underwent the long allele during PCR; these drawings explain the in vitro generation of the short allele (drawing 1) and the S1, S2 and S3 ladder bands (drawings 2, 3 and 4, respectively). According to this model, the slippage generates a loop in the template strand during replications and the resulting fragments are deleted in the stretch involved in the loop formation. Drawing 5 represents the long allele undergoing a hypothetical simultaneous slippage in both strands, generating two loops with the same sequence.

ability of MsH42 to undergo slipped-strand mispairing, the insert of pRep42 was subjected to PCR under the same conditions employed for genomic DNA amplifications. In addition to the expected 585 bp fragment, we observed several extra bands including a 480 bp fragment (the size of the short allele) and a ladder of three prominent bands (S1, S2, S3)

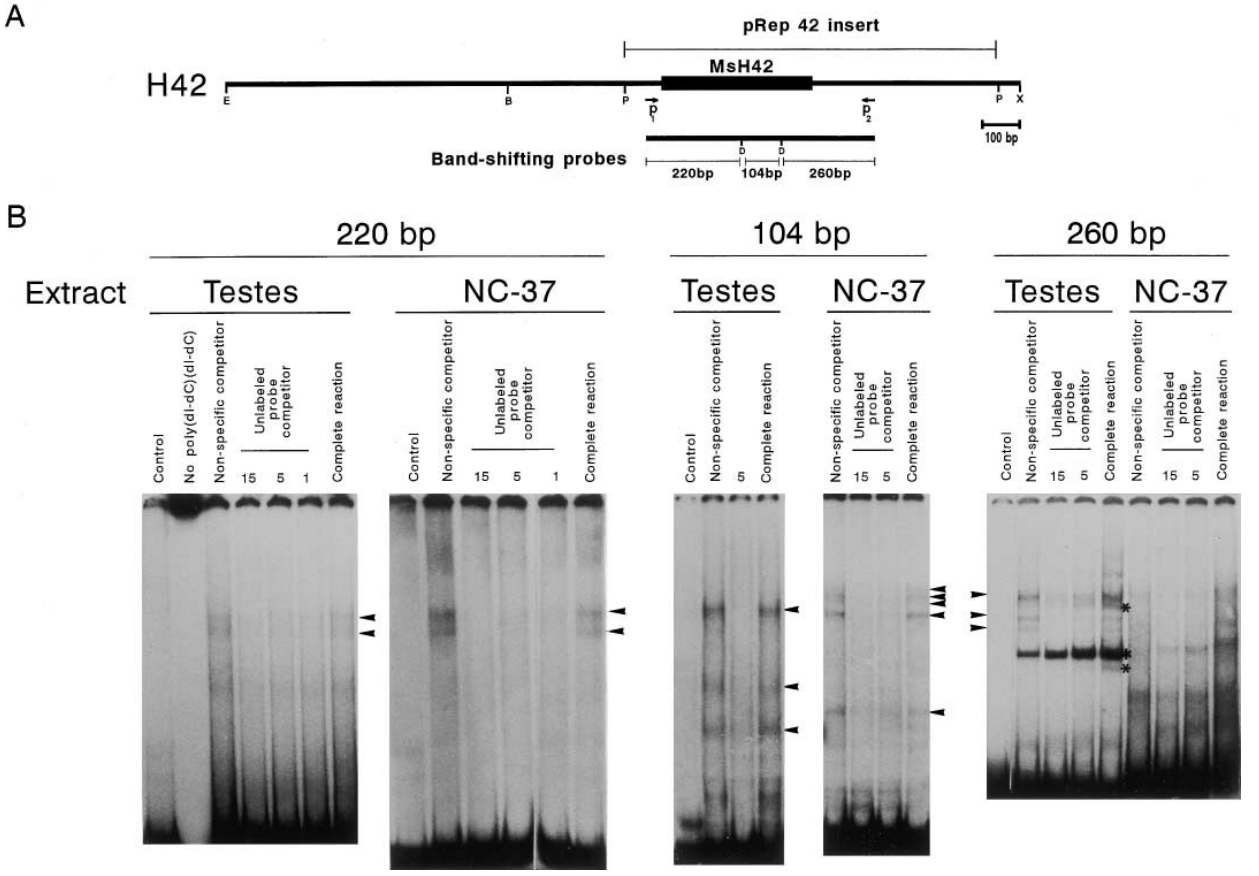


Fig. 3. Detection of MsH42 DNA binding proteins by gel mobility shift assay. A: Schematic representation of H42 restriction map showing the probes derived from the *Dde*I digestion of the 585 bp PCR fragment. B: Band-shifting experiments with different nuclear extracts. Control represents a reaction carried out without nuclear extract. The numbers in competition reactions with unlabeled probe indicate the molar excess with respect to the labeled probe. Arrowheads show the retarded bands due to specific DNA-protein complexes. Asterisks indicate unspecific DNA-protein complexes.

intercalated with other three weaker bands (Fig. 2A). Similarly, amplification of pB20H generated a set of ladder bands slightly bigger than those found in the PCR of the long allele (Fig. 2B). In contrast, amplification of pB9M insert, that corresponds to the short allele, did not generate any extra fragments (Fig. 2C) indicating that the deleted sequence is essential for the slippage behaviour. Therefore the inserts of pRep42, pB20H and pB9M have different ableness for producing mispairing. Interestingly, direct sequencing of the 480 bp fragment synthesized by in vitro slippage, confirmed that the short allele was indeed originated from the long allele during amplifications. The other major slippage products (S1, S2, S3) were also purified, reamplified and sequenced. The S1 band has a deletion of 210 bp (repeats 7 to 28) with respect to the long allele, the S2 band a deletion of 248 bp (repeats 3 to 28) and the S3 band a deletion of 296 bp (repeats 3 to 33). In the case of pB20H, the fragments originated by slippage (Fig. 2B) are bigger than those generated from the long allele due to the presence of a 41 bp duplication in the rare fragment.

It has been reported that an elevated reaction temperature enhances the slipped-strand mispairing [8,21]. In this case, using higher annealing temperatures in the amplification of pRep42 insert provoked that the extra amplification fragments were more abundant (not shown). Fig. 2 shows a model explaining the slippage behaviour of MsH42.

3.4. Specific binding of nuclear proteins to MsH42

To detect if there are specific MsH42 DNA binding proteins, we employed three distinct DNA probes derived from the *Dde*I digestion of the 585 bp PCR DNA fragment (Fig. 3A). This approach allows to examine separately the binding ability of different MsH42 regions. Nuclear extracts prepared from either mouse testes or human NC-37 cells were preincubated with poly(dI-dC)·(dI-dC) to avoid non-specific interactions and band-shifting reactions were carried out as indicated in Section 2. We found several bands that migrated retarded with respect to the native DNA (Fig. 3B). To determine the binding specificity, increasing amounts of unlabeled probes were added to the reactions. A 5-fold molar excess of unlabeled probe provoked an important decrease in the intensity of some shifted bands which were titrated out with a 15-fold molar excess. A 500-fold molar excess of unlabeled non-specific competitor GC-rich DNA did not compete with some of the DNA-protein complexes demonstrating their specificity. In contrast, the intensity of some bands diminished with the addition of the non-specific competitor, indicating that such DNA-protein interaction is due to unspecific binding (Fig. 3B). The presence of multiple shifted bands could be the result of different proteins interacting with the *Dde*I fragments, several binding positions along the probe, or the existence of protein-protein interactions that may induce the formation of several bands. The pattern obtained with the 220 and 260

bp probes was very similar among the two nuclear extracts. However, it was clearly distinct when the shifting experiments were done with the 104 bp probe, suggesting the existence of different proteins binding to the MsH42 sequence.

4. Discussion

Homologous recombination is a process of genetic information exchange between stretches of homologous DNA that generates new combinations of alleles which favour the plasticity and, therefore, the evolution of genomes. The rate of genetic exchange between two loci during homologous recombination is roughly proportional to the physical distance among them. Nevertheless, some chromosomic regions are prone to undergo a much higher recombination rate. These sites, that originate a disparity between genetic and physical maps, are known as recombination hotspots. Several findings indicate that some minisatellite sequences can function as specific sites for the initiation of homologous recombination. Thus, the similarity between the consensus core sequence of some minisatellites [1] and the well-known prokaryotic *Chi* recombination hotspot [22,23], the presence of hypervariable minisatellite sequences at the meiotic recombinational hotspot within the mouse major histocompatibility complex locus [24] as well as in the chiasmata of meiotic human chromosomes [25], the stimulation of homologous recombination shown by a synthetic hypervariable minisatellite sequence [26], and the existence of hypervariable minisatellites whose polymorphism is generated by complex recombination events [10,11], strongly support a role of these sequences in recombination.

We have isolated a new human DNA sequence containing a GC-rich low polymorphic minisatellite with two alleles that differ in a block of 11 repeats. The MsH42 region fulfils three main requirements for recombination: the existence of many recombination and recombination-like signals (Fig. 1), its ableness to assume single-stranded conformation (slippage capacity, Fig. 2), and its specific interaction with nuclear proteins (Fig. 3).

Flanking the minisatellite there are two prokaryotic recombination *Chi* motifs and at the 5′ end there is a nearly complete *Chi* sequence. MsH42 also contains oligomers similar to those involved in immunoglobulin class-switching and VDJ joining recombination processes. The presence of *Chi* and *Chi*-related sequences is common in minisatellites [27], immunoglobulin genes [28] and at the breakpoints of oncogene translocations apparently created by aberrant recombinase activity [29]. Related to these sequences is the motif GGCAGG, which contributes to germline instability of minisatellites [30]. Noteworthy, the iteration of the consensus sequence AGAGGTGGGCAGGTGG, resembling a fusion of *Chi* and immunoglobulin class-switch sequences, stimulates homologous recombination in human cells [26].

An important requirement of recombination models [23,31–33] is the ability of DNA sequences to form loop structures with single-stranded conformation that would invade homologous double-stranded DNA. According to this, another indication that the MsH42 region may promote recombination comes from its ability to generate single-stranded loops in the minisatellite during PCR replications. It was reported that some minisatellites form misaligned structures in vitro with single-stranded loops [34], similar to those shown in drawing 5 (Fig. 2), and that these structures are linked to recombination during DNA replication [35]. DNA sequences containing short tandem repeats can form transitory single-stranded regions [8], which can produce parallel DNA triplexes or quadruplexes that would promote recombination in such regions [36,37]. Finally, in agreement with the existence of slippage events in MsH42, production of the short allele and ladder bands is improved when the PCR reaction was carried out at higher annealing temperatures. Alternatively, the synthesis of the slippage products from MsH42 could be also explained by the generation of incompletely extended fragments during PCR. However, the same fragments were obtained increasing the extension step in the PCR cycles ruling out this possibility.

A main requirement of eukaryotic recombinational models is that the DNA target should interact specifically with nuclear proteins. Previous works have detected minisatellite binding proteins from several biological sources [38–40]. Our band-shifting results indicate the existence of proteins that specifically bind to the MsH42 region (Fig. 3). Interestingly, the central portion of the MsH42 region (the 104 bp fragment) showed a different band-shifting pattern between both nuclear extracts. In fact, it has been proposed that homologous recombination may be regulated by discrete DNA sites and proteins that interact with those sites [41]. Perhaps in the sequence within the 104 bp *Dde*I fragment and in the proteins that bind to this site lies the hypothetical recombinogenic activity of the MsH42 region.

Acknowledgements: We thank Dr. Xavier Estivill for supplying DNAs from CEPH families.

References

[1] Jeffreys, A.J., Wilson, V. and Thein, S.L. (1985) Nature 314, 67–73.
[2] Nakamura, Y., Leppert, M., O'Connel, P., Wolff, R., Holm, T., Culver, M., Martin, C., Fujimoto, E., Hoff, M., Kumlin, E. and White, R. (1987) Science 235, 1616–1622.
[3] Ali, S. and Wallace, R.B. (1988) Nucleic Acids Res. 16, 8487–8496.
[4] Jeffreys, A.J., Neuman, R. and Wilson, V. (1990) Cell 60, 473–485.
[5] Jeffreys, A.J., MacLeod, A., Tamaki, K., Neil, D. and Monckton, D. (1991) Nature 354, 204–209.
[6] Armour, J.A.L., Harris, P.C. and Jeffreys, A.J. (1993) Hum. Mol. Genet. 2, 1137–1145.
[7] Neil, D.L. and Jeffreys, A.J. (1993) Hum. Mol. Genet. 2, 1129–1135.
[8] Levinson, G. and Gutman, G.A. (1987) Mol. Biol. Evol. 4, 203–221.
[9] Charlesworth, B., Sniegowski, P. and Stephan, W. (1994) Nature 371, 215–220.
[10] Jeffreys, A.J., Tamaki, K., MacLeod, A., Monckton, D.G., Neil, D.L. and Armour, J.A. (1994) Nature Genet. 6, 136–145.
[11] Bouard, J. and Vergnaud, G. (1994) EMBO J. 13, 3203–3210.
[12] Jeffreys, A.J. and Neumann, R. (1997) Hum. Mol. Genet. 6, 129–136.
[13] Gómez-Márquez, J., Puga, A. and Notkins, A.L. (1985) J. Biol. Chem. 260, 3490–3495.
[14] Poncz, M., Solowiejczyk, D., Ballantine, M., Schwartz, E. and Surrey, S. (1982) Proc. Natl. Acad. Sci. USA 79, 4298–4302.
[15] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[16] Dignam, J., Lebowitz, R. and Roder, R. (1983) Nucleic Acids Res. 11, 1475–1489.
[17] Wahls, W.P., Swenson, G. and Moore, P.D. (1991) Nucleic Acids Res. 19, 3269–3274.
[18] Lawn, R., Fritsch, E.F., Parker, R.C., Blake, G. and Maniatis, T. (1978) Cell 15, 1157–1174.

[19] Sakano, H., Kurosawa, Y., Weigert, M. and Tonegawa, S. (1981) Nature 290, 562–565.
[20] Dunnick, W., Hertz, G., Scappino, L. and Gritzmacher, C. (1993) Nucleic Acids Res. 21, 365–372.
[21] Litt, M. (1992) in: PCR of TG Microsatellites (Mcpherson, M.J., Quirke, P. and Taylor R.R., Eds.) PCR, A Practical Approach, pp. 85–99, IRL Press, Oxford, UK.
[22] Smith, G.R., Kunes, S., Schultz, D., Taylor, A. and Triman, K.L. (1981) Cell 24, 429–436.
[23] Smith, G.R. and Stahl, F.W. (1985) BioEssays 2, 244–249.
[24] Steinmetz, M., Stephan, D. and Lindahl, K.F. (1986) Cell 44, 895–904.
[25] Chandley, A.C. and Mitchell, A.R. (1988) Cytogenet. Cell Genet. 48, 152–155.
[26] Wahls, W.P., Wallace, L.J. and Moore, P.D. (1990) Cell 60, 95–103.
[27] Vogt, P. (1990) Hum. Genet. 84, 301–336.
[28] Kenter, A.L. and Birshtein, B.K. (1981) Nature 293, 402–404.
[29] Krowczynska, A., Rudders, R. and Krontiris, T.G. (1990) Nucleic Acids Res. 18, 1121–1127.
[30] Mitani, K., Takahashi, Y. and Kominami, R.A. (1990) J. Biol. Chem. 265, 15203–15210.
[31] Meselson, M.S. and Radding, C.M. (1975) Proc. Natl. Acad. Sci. USA 72, 358–361.
[32] Radding, C.M. (1982) Annu. Rev. Genet. 16, 405–437.
[33] Szostak, J.W., Orr-Weaver, T.L., Rothstein, R.J. and Stahl, F.W. (1983) Cell 33, 25–35.
[34] Coggins, L.W., O'Prey, M. and Akhter, S. (1992) Gene 121, 279–285.
[35] Bi, X. and Liu, L. (1996) Prog. Nucleic Acids Res. Mol. Biol. 54, 253–292.
[36] Sen, D. and Gilbert, W. (1988) Nature 334, 364–366.
[37] Camerini-Otero, R.D. and Hsieh, P. (1993) Cell 73, 217–223.
[38] Collick, A., Dunn, M.G. and Jeffreys, A.J. (1991) Nucleic Acids Res. 19, 6399–6404.
[39] Yamazaki, H., Nomoto, S., Mishima, Y. and Kominami, R. (1992) J. Biol. Chem. 267, 12311–12316.
[40] Shinder, G., Manam, S., Ledwith, B. and Nichols, W. (1994) Exp. Cell Res. 213, 107–112.
[41] Wahls, W.P. and Smith, G.R. (1994) Genes Dev. 8, 1693–1702.