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Tetrad analysis shows that gene conversion is the major mechanism involved in mutation at the human minisatellite MS1 integrated in *Saccharomyces cerevisiae*

INGRID BERG, HÅKAN CEDERBERG* AND ULF RANNUG
Department of Genetic and Cellular Toxicology, Wallenberg Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

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Summary

Minisatellites are arrays of tandemly repeated DNA sequences which occur at thousands of locations in the human genome. They are frequently hypervariable with respect to allele length as a result of high rates of complex and incompletely understood recombination-based germline mutation events that alter the repeat copy number. MS1 is one of the most variable minisatellites so far isolated from the human genome. We have integrated MS1, flanked by synthetic markers, in the vicinity of a hot spot for meiotic double-strand breaks upstream of the *LEU2* locus in chromosome III of *Saccharomyces cerevisiae*. Here we present the first tetrad analysis of mutations at a human minisatellite locus. The data showed that mutant alleles occur as single mutants in one of the spores in a tetrad, also when the mutant structure was the result of a combination of intra- and inter-allelic rearrangements. The conversional transfer of repeat units from one allele to the other was associated with flanking marker conversion which always involved the same flank of the minisatellite. The results demonstrate that conversion is the predominant mechanism by which minisatellite alleles mutate to new lengths, and also support the assumption that *cis*-acting elements are involved in the regulation of the mutational process in humans.

1. Introduction

The human genome contains thousands of minisatellite loci of which several have been shown to be highly variable with respect to allele length as a result of germline mutation events that alter the repeat copy number (Jeffreys *et al.*, 1985, 1988). The germline length mutation rate can be as high as 13% per gamete (Vergnaud *et al.*, 1991), while the mitotic mutation rate is several hundred times lower (Jeffreys & Neumann, 1997). This implies that mutant minisatellite alleles are predominantly generated by mechanisms which operate specifically in meiosis.

The internal structure of alleles, i.e. the order of minisatellite variant repeats (MVRs), can be determined by MVR-PCR (Minisatellite Variant Repeat mapping by Polymerase Chain Reaction; Jeffreys *et al.*, 1991), which is an efficient tool for characterization of structural changes in *de novo* mutants to ascertain the nature of mutational mechanisms.

Conceivable mechanisms for minisatellite mutation are gene conversion, crossing-over, sister-chromatid exchange, replication slippage and intramolecular processes resulting in deletions and duplications. Studies of the minisatellite MS32 show that mutation in the human germline involves complex conversion events (Jeffreys *et al.*, 1994) which more frequently generate mutants with gains than with losses of repeat units. In contrast, mutation in mitotic cells involves simple duplications and deletions and gives rise to a smaller proportion of mutants with gains of repeat units (Jeffreys & Neumann, 1997).

The functions of minisatellites in the human genome are not known, but there are several reports which indicate that minisatellites might be involved in transcription regulation, as suggested by the association between certain alleles at minisatellites and an increased risk for insulin-dependent diabetes mellitus, progressive myoclonus epilepsy type 1 and several forms of cancer (Green & Krontiris, 1993; Krontiris *et al.*, 1993; Kennedy *et al.*, 1995; Virtaneva *et al.*, 1997).

To understand the genetic processes that play crucial roles in the onset of diseases associated with tandem repeat loci, as well as to clarify the organization, function and dynamics of non-coding

* Corresponding author.

building blocks of eukaryotic genomes, it is essential to obtain further knowledge about the molecular mechanisms by which minisatellite alleles mutate to new lengths in meiosis. To study these mutational mechanisms we have constructed a model system in the yeast *Saccharomyces cerevisiae* (Appelgren *et al*., 1997) by integrating human minisatellite alleles into chromosome III near a hot spot for meiotic double-strand breaks upstream of the *LEU2* locus (Cao *et al*., 1990; Baudat & Nicolas, 1997). We have previously shown that the human minisatellite MS205 in yeast provides a molecular environment which seems to favour *cis*-regulated induction of double-strand breaks in, or in the vicinity of, the repeat array (He *et al*., 1999). Yeast is very suitable for analyses of eukaryotic recombination mechanisms in both mitotic and meiotic cells. Tetrad analysis of mutant alleles generated during meiosis gives detailed information on molecular mechanisms of crossing-over and conversion which it is not possible to obtain with random samples of meiotic products, e.g. spermatozoa in human. The powerful combination of tetrad analysis and the hypervariable nature of minisatellites, with MVRs serving as internal markers, makes the system ideal for investigating previously unstudied components of the eukaryotic recombination machinery.

To gain further understanding of minisatellite variability in general, it is important to study loci with different mutational characteristics, such as mutation frequency, the ratio of gains and losses, mutation polarity and the proportion of inter- and intra-allelic events. Several minisatellites show a polar distribution of mutation events (Armour *et al*., 1993; Neil & Jeffreys, 1993; Jeffreys *et al*., 1994). Length-dependent intra-allelic mutation frequency has also been reported (Buard *et al*., 1998).

MS1 was one of the first human minisatellites to be cloned (Wong *et al*., 1987). It is a highly variable minisatellite with a degree of heterozygosity of 99·4% and a germline mutation rate of 5·2% (Jeffreys *et al*., 1988). MS1 is composed of repeat units with a length of 9 bp and the allele lengths vary from 2 to 20 kb (Wong *et al*., 1987). So far, 21 MVRs have been identified (Gray & Jeffreys, 1991; Maleki *et al*., 1997), which makes a detailed characterization of mutant structures possible. Unlike other minisatellites, MS1 has been found to be unstable in colorectal carcinoma cells (Hoff-Olsen *et al*., 1995). Since instability of microsatellites is commonly seen in tumour cells (Aaltonen *et al*., 1993; Kim *et al*., 1994), this might indicate that MS1 can mutate by similar pathways as microsatellites, as suggested by its fairly short repeat unit of 9 bp.

In the first study of a human minisatellite in yeast we found that MS1 mutates with high frequency to new lengths in haploid cells (Cederberg *et al*., 1993). In an investigation by Maleki *et al*. (1997) the length mutation frequency at MS1 in haploid yeast mitosis was shown to be related not only to allele length but also to the internal structure of alleles. In this report we present the first meiotic data on mutation at MS1 integrated in yeast which, furthermore, comprises the first tetrad analysis of mutations at a human minisatellite. It was found that the large majority of mutant alleles generated during meiosis occurred as single mutants in one of the spores in a tetrad. Most mutations involving inter-allelic transfer of repeats were accompanied by flanking marker exchange as a result of conversion which always occurred in the same flank of the allele, indicating that the mutation process is preferentially accomplished in one direction.

2. Materials and methods

(i) *Strains*

The *Escherichia coli* strain DH5α (*supE44* Δ*lacU169* (*Φ*80*lacZ*Δ*M15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*) was used in transformations and for propagation of plasmids. The congenic haploid *Saccharomyces cerevisiae* strains W303-1B (*MAT*α *SUC2 ade2–1 can1–100 his3–11,15 leu2–3,112 trp1–1 ura3–1*) and W1134-1D (*MAT*a *SUC2 ade2–1 can1–100 his3–11,15 leu2–3,112 met 14δ ura3–1*) were used as recipients in transformations that selected for integration of MS1 alleles in chromosome III.

(ii) *Media, growth conditions, sporulation and tetrad dissection*

Bacteria were grown at 37 °C in liquid or solid LB medium with or without the addition of ampicillin (50 mg/l). Yeast strains were grown at 30 °C in liquid or solid YEPD or synthetic (SC) media prepared as described in Appelgren *et al*. (1997). Omission media lacking L-leucine (SC-LEU) or L-leucine, L-methionine and L-tryptophan (SC-LEU-MET-TRP) were used for selection of haploid transformants and selection of diploid strains, respectively. To determine the segregation of alleles at the *MET14* and *TRP1* loci, omission media lacking L-methionine (SC-MET) and L-tryptophan (SC-TRP) were used. SPS and sporulation media were prepared as described in Appelgren *et al*. (1997). Yeast cells were sporulated on solid sporulation medium in plates which were incubated at 30 °C for 72 h. Thereafter, tetrads were treated with zymolyase (50 mg/ml) on solid YEPD medium in plates which were incubated at room temperature for 10–15 min before dissection.

(iii) *DNA techniques*

Preparation of plasmids and genomic DNA was carried out as previously described (Appelgren *et al*.,

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1997). Transformation of *E. coli* was performed by treating cells with calcium chloride according to standard procedures (Sambrook *et al*., 1989). Yeast cells were transformed by electroporation using a Gene Pulser (Biorad).

(iv) *Construction of transminisatellitic yeast strains*

We have previously described the general procedure applied in the present study for constructing yeast strains with human minisatellites integrated in the *LEU2* region of chromosome III (Appelgren *et al*., 1997). Specific adaptations of the procedure to suit the construction of yeast strains carrying MS1 were as follows. Primers used for the amplification of MS1 from a human DNA fragment cloned in pUC13 were 1C and 1D, which are composed of the 3′ end 24 nt sequence of primers C and D, respectively (Maleki *et al*., 1997), and modified by adding a 28-nucleotide tag sequence at the 5′ end. The amplified fragments contained 48 bp and 51 bp of human flanking DNA on either side of MS1. Two alternative tag sequences were used for each of the primers giving a set of four primers designated 1CE, 1Ce, 1DR and 1Dr containing the flanking markers *Nhe*I, *Mlu*I, *Dra*I and *Stu*I, respectively, which henceforth are denominated E, e, R and r, respectively. *Sma*I was used as subcloning site in all primers. *Hind*III was used as cloning site in primers 1CE and 1Ce, and *Eco*RI in primers 1DR and 1Dr. The primer sequences were 1CE: 5′-TTGGTGTGTTAAGCTTCCCGGGGC-TAGCGCCACCCCAGCAAATTGAGAAATC-3′, 1Ce: 5′-TTGGTGTTGTAAGCTTCCCGGGAC-GCGTGCCACCCCAGCAAATTGAGAAATC-3′, 1DR: 5′-TTGAGTGGTGGAATTCCCCGGGTT-TAAAAGGACCACCCAATCTGGGCTCCCA-3′, 1Dr: 5′-TTAGTGTATTGAATTCCCCGGGAGG-CCTAGGACCACCCAATCTGGGCTCCCA-3′.

MS1 amplified with primers 1Ce and 1Dr was cloned and subcloned as described in Appelgren *et al*. (1997), while MS1 amplified with primers 1CE and 1DR was directly cloned into the *Hpa*I site in the 3 kb *Pst*I–*Hpa*I *LEU2* fragment cloned in pUC18 (Appelgren *et al*., 1997). Correct integration into chromosome III of the *LEU2* fragment containing MS1 was confirmed by separate Southern blot analyses in which a fragment of MS1 and *LEU2*, respectively, was used as probe. The genetic composition of haploid transminisatellitic strains is shown in Fig. 1. Since mutations at MS1 have been shown to frequently occur in haploid mitotic yeast cells (Maleki *et al*., 1997), haploid yeast strains were derived by isolating single cells from a colony of transformed cells to eliminate the presence of different MS1 alleles in the original isolate.

Diploid transminisatellitic strains heterozygous for MS1 alleles with lengths of 94 repeat units and 124

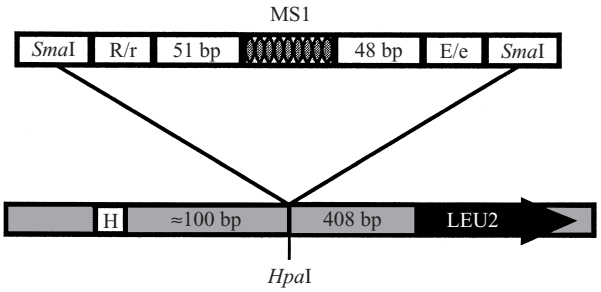

Fig. 1. Genetic composition of haploid yeast strains carrying MS1. A fragment containing the minisatellite with 48 and 51 bp of human flanking DNA, synthetic flanking markers E and R or e and r, and the cleaved *Sma*I site was integrated into chromosome III at the *Hpa*I site upstream of *LEU2*. H, hot spot for meiotic double-strand breaks. (From Cao *et al*. (1990).)

repeat units were produced by mating haploid strains on YEPD plates. After 2 days of growth, the cells were plated on SC-LEU-MET-TRP medium to select for diploid cells. Diploid strains were isolated and analysed by Southern blot hybridization to determine the length of MS1 alleles. The flanking marker combinations of alleles were determined by PCR. Southern blot hybridization and PCR analysis were carried out as described in Appelgren *et al*. (1997). Primers used for determination of flanking marker combinations were synthesized by Scandinavian Gene Synthesis AB by a procedure which generates primers with the same melting temperature regardless of DNA sequence. The primer sequences were FLK-E: 5′-AGTATTCCCACAGTTGGGGCTAGC-3, FLK-e: 5′-AGTATTCCCACAGTTGGGACGCG-T-3′, FLK-R: 5′-ATCTTGACCGCAGTTGGGTT-TAAA-3′, FLK-r: 5′-ATCTTGACCGCAGTTGG-GAGGCCT-3′.

(v) *Isolation of MS1 mutants in haploid mitotic cells*

Haploid yeast cells were cultured overnight in 5 ml of YEPD medium and plated on YEPD medium. Cells from single colonies were isolated and cultured in 24 well plates. The plates were arranged to give grid sizes of 12×12 wells and cells (0·7 ml) from each well in a row were pooled before DNA preparation, as were cells from each well in a 'column'. The DNA from pooled cells were digested with *Sau*3AI, and length mutated MS1 alleles were detected by Southern blot hybridization according to Appelgren *et al*. (1997).

(vi) *Isolation of mitotic and meiotic mutants in tetrads*

Cells from each growing spore from 96 tetrads with four viable spores were cultured in 2 ml liquid YEPD medium in 24 well plates. Cells from all four spores in

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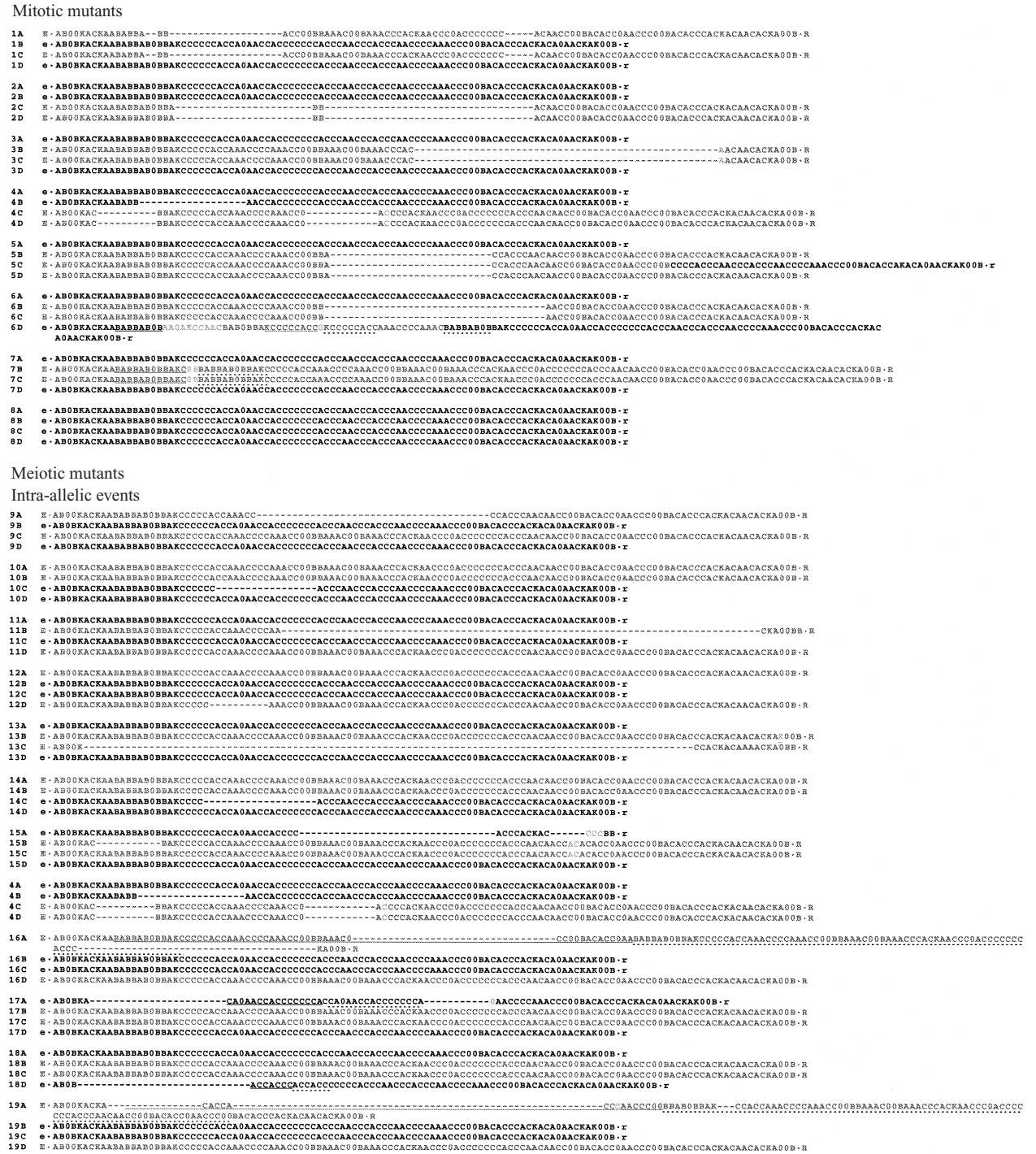

Fig. 2. For legend see opposite

a tetrad were pooled before DNA preparation. The genomic DNA from pooled cells was digested by *Sau*3AI, and the resulting fragments were detected by Southern blot hybridization which identified tetrads that contained alleles with new lengths. The flanking marker combination of all 384 (4×96) alleles was determined by PCR using marker-specific primers as described above. Some tetrads showed a 2:2 segregation of progenitor alleles of other sizes than 94 repeat units and 124 repeat units. Such mutants were considered to be of mitotic origin. The rest of the mutations were clearly generated during meiosis and occurred in tetrads with any progenitor alleles. All tetrads with four viable spores were examined for a 2:2 segregation of alleles at the *MET14* and *TRP1* loci.

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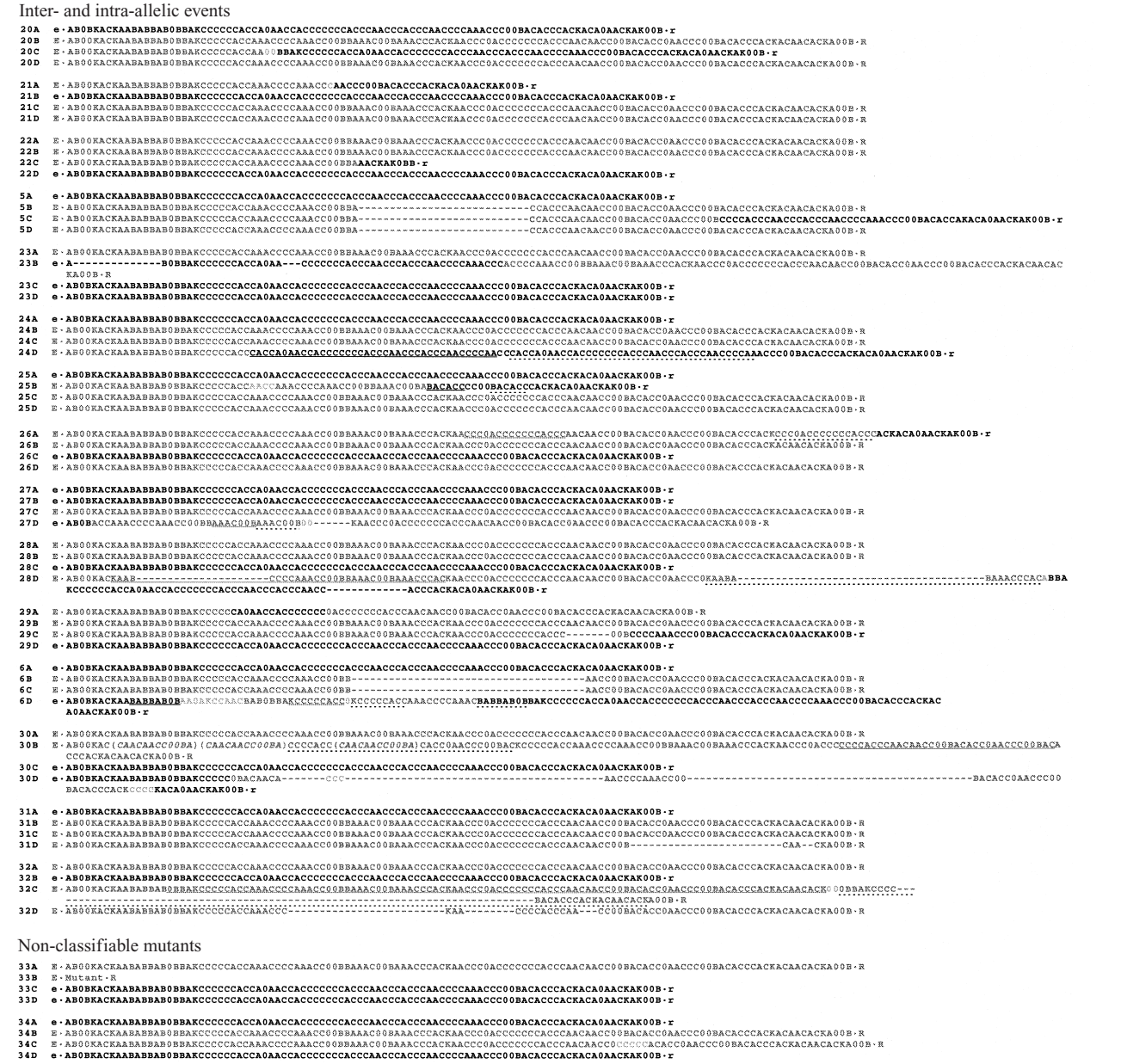

Fig. 2. MVR-PCR characterization of all four MS1 alleles in each of the 34 tetrads which contained mutant alleles. Alleles belonging to one tetrad are identified by a common tetrad number followed by A, B, C or D indicating from which of the four spores the allele was isolated. E, e, R and r are flanking markers between which the repeat units along the array are indicated by A, B, C, K and 0 for A-, B-, C-, K- and null-type repeats, respectively. Dashes represent deleted repeat units, single/dotted underlining represents duplications and italicized repeats within parentheses represent a triplication. Repeat units and flanking markers from the small (94 repeats) and the large (124 repeats) allele are represented in black and grey, respectively. Repeats in light grey represent repeat units whose origin is not obvious. Tetrads with both a mitotic and meiotic origin are listed twice.

(vii) *Determination of internal allele structure by MVR-PCR*

Internal mapping of variant repeats uses PCR amplification of fragments between a flanking primer and a tagged repeat specific primer (MVR-PCR) as described by Jeffreys *et al.* (1991). We have developed MVR-PCR for characterization of MS1 alleles. DNA samples from spores were subjected to MVR analysis using the primers 1Y and 1TAG-A plus TAG or 1TAG-B plus TAG or 1TAG-C plus TAG or 1TAG-K plus TAG. The primers 1TAG-A, 1TAG-B, 1TAG-C and 1TAG-K are specific for the repeat unit types which have been assigned the letters A, B, C and K, respectively, by Gray & Jeffreys (1991). Primer 1Y is the flanking primer which is located in the yeast DNA 260 bp outside the 5′ end of MS1. All PCR reactions contained 1 μM of the 1Y primer, 1 μM of the TAG

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primer, 0·025 U/μl *Taq* polymerase plus buffer and dNTPs according to Appelgren *et al.* (1997). The amount of template DNA and the concentration of repeat unit type specific primer were 50–100 ng DNA and 10 nM 1TAG-A in A-type repeat reactions, 50–100 ng DNA and 2·5 nM 1TAG-B in B-type repeat reactions, 25–50 ng DNA and 20 nM 1TAG-C in C-type repeat reactions, and 50–100 ng DNA and 2·5 nM 1TAG-K in K-type repeat reactions. The PCR conditions were 96 °C, 45 s; 50 °C, 45 s; 70 °C, 3 min and 20 s for 4 cycles and 14 cycles of 96 °C, 45 s; 67 °C, 45 s; 70 °C, 3 min and 20 s, followed by a chase at 70 °C, 10 min. The primer sequences were 1TAG-A: 5′-TCATGCGTCCATGGTCCGGATGGA(C/T)AGGGTGGA(C/T)AGGGTGGAT-3′, 1TAG-B: 5′-TCATGCGTCCATGGTCCGGATGGA(C/T)AGGGTGGA(C/T)AGGGTGGAG-3′, 1TAG-C: 5′-TCATGCGTCCATGGTCCGGATGGA(C/T)AGGGTGGA(C/T)AGGGTGGAC-3′, 1TAG-K: 5′-TCATGCGTCCATGGTCCGGAGTGGA(C/T)AGGGTGGA(C/T)AGGGTGGT-3′, 1Y: 5′-ATGAAAAGCCGGTTCCGCGGCTCTCA-3′, TAG: 5′-TCATGCGTCCATGGTCCGGA-3′. All DNA samples were amplified on a GeneAmp PCR System 2400 (Perkin Elmer). The PCR fragments were size separated on long (40 cm) 2% agarose gels by electrophoresis for 16 h at 150 V.

(viii) *Positioning of mutation events within progenitor alleles*

The location of each mutation event was basically determined as described by May *et al.* (1996) and expressed as a fraction of the possible positions from the extreme 5′ to 3′ end of the progenitor alleles. Events at the extreme 5′ and 3′ end were given scores of 0 and 1, respectively. Mutants involving exchange of flanking markers led to scores in both progenitor alleles at the position of exchange. Positions of multiple events were scored as separate events in the relevant progenitor allele.

3. Results

We have integrated two alleles (94 and 124 repeat units) of the human minisatellite MS1 upstream of *LEU2* in yeast chromosome III, in order to study mechanisms involved in length mutations at this minisatellite. The alleles are flanked by synthetic markers E/e and R/r (see also Fig. 1).

(i) *Mutation frequencies*

The MS1 mutant frequency in haploid mitotic cells was 1·5% (3 of 204 alleles) and 7·0% (14 of 201 alleles) in the strains carrying the 94 repeat unit and 124 repeat unit allele, respectively. All 3 mutant alleles of the shorter allele had lost repeat units, whereas 5 of the 14 mutants of the long allele had gained repeat units. Of the 384 alleles recovered after sporulation and dissection of 96 tetrads with four viable spores, 8·6% were length mutants of meiotic origin and 4·2%, i.e. 8 of the 192 progenitor alleles in the sporulated diploid cells, were mutants of mitotic origin. The proportion of mutant alleles which had gained repeat units was 45·5% (15 of 33 alleles) and 12·5% (1 of 8 alleles) in meiosis and mitosis, respectively. The proportions were not significantly different.

(ii) *Internal structure and flanking marker combinations of mutant alleles*

The MS1 allele lengths in all 384 spores from the 96 dissected tetrads were measured by Southern blot hybridization. The flanking markers of all alleles was determined by PCR. Alleles with new lengths were observed in 34 tetrads. All four alleles in these tetrads were analysed by MVR-PCR to determine the internal structures which are presented in Fig. 2. Flanking marker transfers without generation of a new allele length were not found in any tetrad. Of 29 tetrads with mutants of meiotic origin, the majority (25) contained only one spore with a mutated allele. None of the 29 tetrads contained more than two mutants of meiotic origin. This was the case for mutants generated by intra-allelic as well as inter-allelic events. The proportion of mutant alleles in which mutation involved inter-allelic transfer of repeats was 12·5% (1 of 8 alleles) and 51·6% (16 of 31 alleles) in mitosis and meiosis, respectively. The corresponding figures for duplications were 12·5% (1 of 8 alleles) and 38·7% (12 of 31 alleles), and for deletions 75·0% (6 of 8 alleles) and 67·7% (21 of 31 alleles). Although no significant differences between the proportions of mutation types could be established, there is a tendency for a higher contribution of duplications and a strong tendency for a higher contribution of inter-allelic transfers of repeats in mutation during meiosis compared with mitosis. In meiosis, duplications were always accompanied by an additional mutation event which was either an insertion of repeats from the other allele or a deletion. For a few of the mutant alleles of meiotic origin (33B and 34C) the type of mutation event could not be determined with certainty. These alleles are therefore not included in the analysis. Allele 33B could not be analysed by MVR-PCR since it was too large. The insertion of C-repeats in allele 34C has no obvious origin and therefore gave no information regarding the type of mutation event.

Thirteen of the 384 alleles in the 96 tetrads showed inter-allelic transfers of repeats and the associated

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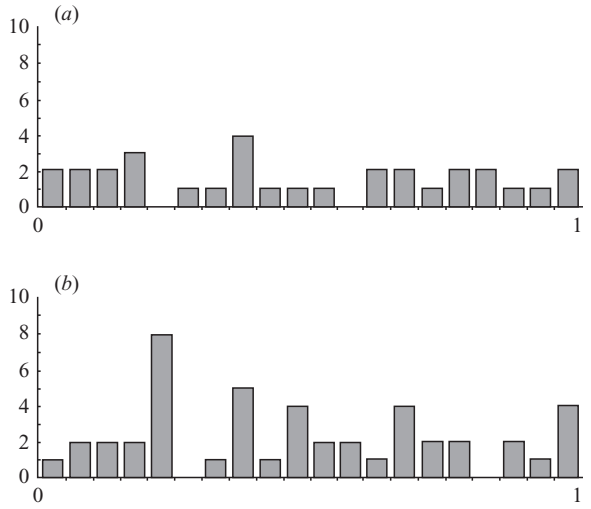

Fig. 3. Distribution of meiotic mutation positions along the MS1 array normalized for progenitor allele lengths. (*a*) Mutation events in the 94 repeat unit allele. (*b*) Mutation events in the 124 repeat unit allele. Distributions in (*a*) and (*b*) are not significantly different from each other or from a random distribution.

flanking marker. As pointed out above, all 13 alleles were length mutated. Eleven of the 13 alleles showed inter-allelic transfer of the flanking marker adjacent to *LEU2*. Of these 11 tetrads, 9 (nos. 5, 20, 21, 22, 24, 25, 26, 28 and 29) contained a mutant allele with the flanking marker combination E/r and 2 (nos. 23 and 27) had a mutant allele with the flanking marker combination e/R. Two tetrads (nos. 31 and 32) had 3 alleles of large progenitor origin. Consequently, none of the 13 tetrads above contained a pair of alleles with reciprocal flanking marker combinations as would be expected from a crossing-over event within the MS1 region. The tetrads with mutated alleles with flanking marker combination E/r always contained two alleles with the parental combination E/R, while tetrads containing alleles with flanking marker combination e/R contained two alleles with the parental combination e/r. Only 1 (no. 29) of the 13 tetrads in which inter-allelic transfer of repeats was involved in length mutation contained more than one mutant allele with repeats from both progenitor alleles. A small proportion of inter-allelic transfers were inserts (tetrads nos. 6, 29 and 30), i.e. the mutant allele did not show exchange of flanking markers. In addition, 13 of these 16 alleles showed conversion in favour of the larger parental allele. The conversion disparity is statistically significant ($\chi^2_c = 5{\cdot}06$, $P = 0{\cdot}024$).

(iii) *Mutation position distribution*

As shown in Fig. 3, the relative positions of mutation events were randomly distributed along the MS1 repeat array and thus showed no indication of polarity towards the 5′ or 3′ ends in either of the progenitor alleles. Neither was there a significant difference between the distributions of different mutation types, nor a tendency for polarity for any of the mutation types.

4. Discussion

Studies on minisatellite mutation in humans suggest that conversion events during meiosis are involved in the generation of new length alleles (Buard & Vergnaud, 1994; Jeffreys *et al*., 1994). In this report we present the first tetrad analysis of mutations at a human minisatellite locus. The segregation and structures of mutant alleles in tetrads from a yeast strain carrying the human minisatellite MS1 integrated in chromosome III demonstrated that conversion is the major mutation mechanism by which inter-allelic transfer of repeats occur. Accordingly, in tetrads which contained a length-mutated allele with flanking marker exchange the reciprocal allele structure and flanking-marker combination that would be expected from a crossing-over event were not found. Instead, a 3:1 distribution was observed both for the transferred block of repeats and the flanking marker associated with that block. It was always the marker in the flank adjacent to *LEU2* locus that was converted, which indicates that recombination events involved in inter-allelic transfer preferentially include the corresponding end of the repeat array. Hence, the data suggest that the mutation process is preferentially accomplished in the direction of *LEU2*. Similar inter-allelic mutants of the minisatellite MS32 in humans have been analysed by Jeffreys *et al*. (1998). Although the authors could not exclude the possibility that mutants were generated by conversional transfer of repeats and flanking DNA, they concluded that the mutants were the result of unequal crossover events since a large majority showed exchange of all markers within a 832 bp segment of the flank upstream of the array. In addition, these mutants showed a simple exchange structure compared with the more complex structure of convertants. In contrast, the present study shows that conversion events can give rise to both simple and complex mutant structures. Furthermore, studies in yeast have shown that the average length of conversion tracts is 1·5 kb (Borts & Haber, 1987) and that single-stranded DNA tails produced at double-strand breaks can be up to 800 bp (Sun *et al*., 1991). Consequently, without knowledge of reciprocity of events in humans, it is not possible unequivocally to discriminate between crossing-over and conversion in minisatellite mutation.

The mutation rate at MS1 during meiosis and the MS1 mutant frequency in diploid mitotic cells was 8·6% and 4·2%, respectively. Since the mutant frequency in mitotic cells is the result of mutants

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which have accumulated during many cell divisions, it is likely that the mutant frequency is an overestimation of the actual mutation rate per cell division in mitosis. Consequently, the results show that the mutation rate during meiosis is at least twice as high as in mitosis. The frequency of mutant alleles that had gained repeat units was higher in meiosis (45·5%) than in mitosis (12·5%). The mutation data are consistent with results from a study of MS32 in humans which shows that the frequency of mutant alleles in blood cells is 250-fold lower than in sperm, and that 37% of the new alleles in blood cells had gained repeats compared with 78% in sperm (Jeffreys & Newmann, 1997). In a mutation study of MS32 integrated in yeast at the same chromosomal location as MS1 in the present study no mutations were detected in diploid mitotic cells, whereas the mutation rate in meiosis was about 10% (Appelgren *et al*., 1997). Hence, length mutation events at minisatellites in yeast as well as in humans are mainly the result of mechanisms operating during meiosis. Thus, in both organisms, mitotic mutants are not only less frequent but also simpler in structure than meiotic mutants.

The positions of mutation events at MS1 were randomly distributed along the alleles and, consequently, showed no polarity (Fig. 3). There are no human data available on the distribution of mutation events at MS1, but polarity has been reported for other minisatellites, such as MS32 and MS205 (Jeffreys *et al*., 1994; Armour *et al*., 1993). Polarity has also been observed at these minisatellite loci integrated in yeast at the same chromosomal location as MS1 in this study (Appelgren *et al*., 1997; He *et al*., 1999). The absence of polarity might be related to the size of human flanking DNA. This is shorter in yeast strains carrying MS1 (48 and 51 bp) compared with strains carrying MS32 (205 and 348 bp) or MS205 (128 and 185 bp). Since each flank makes up the distance between the repeat array and the yeast DNA, a *cis*-regulating yeast element would, most likely, act differently upon the three minisatellites in the strains above. In the yeast strains carrying MS1 the centre of the repeat array might be positioned at about the middle of the range for recombination events, which would result in a fairly random distribution of mutation positions, particularly in relatively short alleles such as the ones used in this study.

The double-strand break repair model for meiotic recombination in yeast presented by Szostak *et al*. (1983), or the modified version of that model presented by Sun *et al*. (1991), could be applied to explain the inter-allelic mutation events at MS1 in yeast, by analogy with what was originally suggested by Jeffreys *et al*. (1994) in a model for minisatellite mutation in humans. This model provides an explanation, without the involvement of crossing-over, for the generation of new length-alleles with or without exchanged flanking markers. A typical tetrad containing one mutant allele resulting from an inter-allelic event could be generated as shown in Fig. 4. First, a double-strand break is induced in the repeat array (Fig. 4*a*). Single-strand invasion out of register with the double-strand break position results in a gap which is repaired using the invaded homologous chromatid as a template (Fig. 4*b*, *c*). Thereafter, one Holliday junction

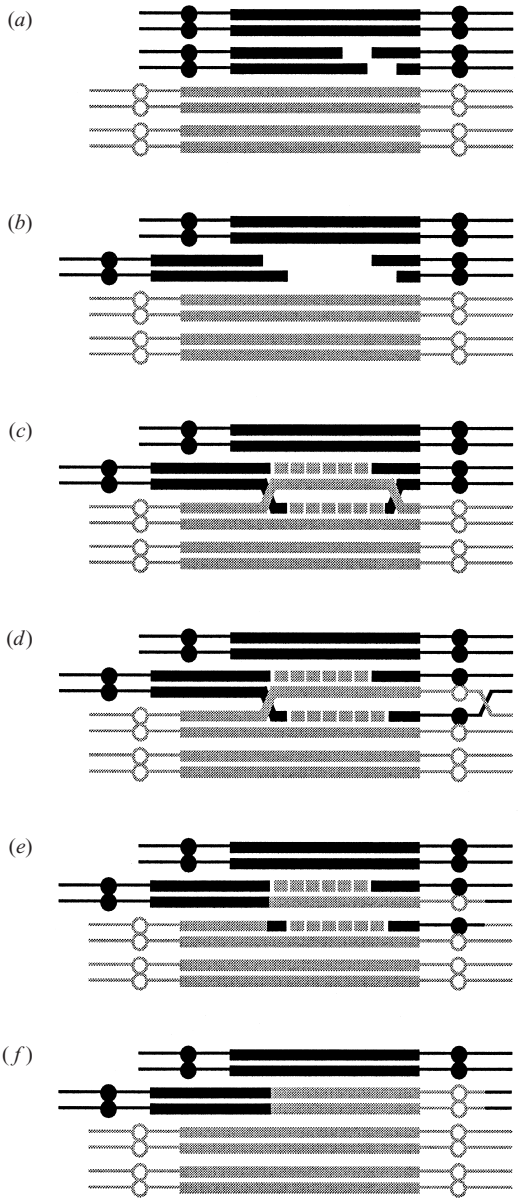

Fig. 4. Explanation based on the double-strand break model for recombination in yeast (Sun *et al*., 1991), for the generation of a typical tetrad with one mutant allele resulting from inter-allelic transfer both of a block of repeats and of a flanking marker associated with that block. Thick lines represent the repeat array. Thin lines represent flanking DNA. Flanking markers are indicated by filled and open circles. Chromatids carrying the smaller allele are represented in black, and chromatids carrying the larger allele are represented in grey. Dashes indicate DNA synthesis. For an explanation of (*a*)–(*f*) see the text.

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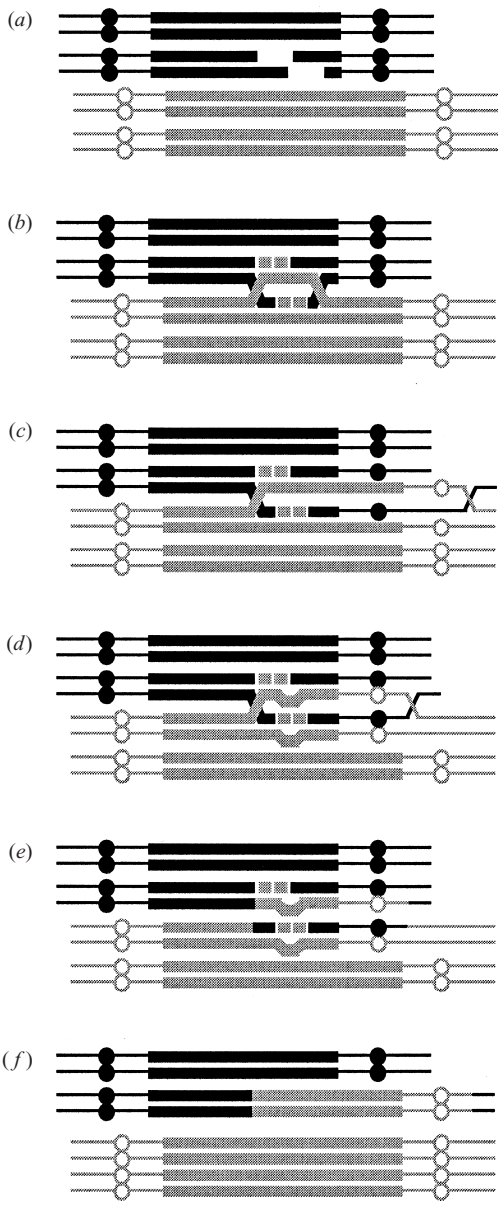

Fig. 5. Explanation based on the double-strand break model for recombination in yeast (Sun *et al.*, 1991), modified by including heterologous branch migration, for the generation of mutant alleles resulting from inter-allelic transfer both of a block of repeats and of a flanking marker associated with that block. Thick lines represent the repeat array. Thin lines represent flanking DNA. Flanking markers are indicated by filled and open circles. Chromatids carrying the smaller allele are represented in black, and chromatids carrying the larger allele are represented in grey. Dashes indicate DNA synthesis. For an explanation of (*a*)–(*f*) see the text.

migrates to the other side of the marker in the 3′ flank (Fig. 4*d*). Finally, the Holliday junctions are resolved (Fig. 4*e*) and the heteroduplex regions in both chromatids are repaired using the donor sequence as a template, which results in a 3:1 distribution of the flanking marker and the 3′ end of the repeat array associated with that marker (Fig. 4*f*). This model also explains the mutants seen in tetrads 31 and 32, where the large progenitor allele and its flanking markers had been transferred to the homologous chromosome. To generate these mutants, each Holliday junction must migrate beyond the flanking marker on either side prior to conversion.

Another model which would explain the mutants observed in this study involves heterologous branch migration (Fig. 5). First, a double-strand break is introduced in the repeat array (Fig. 5*a*), followed by single-strand invasion and DNA repair synthesis (Fig. 5*b*), branch migration through heterologous DNA (Fig. 5*c*) and re-establishment of homologous branch migration (Fig. 5*d*). Thereafter the Holliday junctions are resolved (Fig. 5*e*) and the heteroduplices are repaired resulting in conversion (Fig. 5*f*). The initiating break could be located outside the repeat array in order to create mutants found in tetrads 31 and 32, whereas it is unlikely that the other inter-allelic events were initiated outside the repeat array, since branch migration into the aligned ends of the alleles would not result in new allele lengths. In yeast, branch migration has been suggested to be dependent on the absence of mispaired bases (Alani *et al.*, 1994). However, an *in vitro* study has shown that the branch migration enzyme complex of RuvAB in *E. coli* can pass through a heterology of 300 bp during four-strand branch migration (Adams & West, 1996).

None of the models above provides an explanation for the duplications seen in tetrads nos. 26 and 28. One of the spores carries a mutant allele with a structure showing that a duplication in one chromatid had been transferred to a homologous chromatid. The other three spores in these tetrads were non-mutants. Two of them carried an allele identical to the progenitor allele which must have been involved in the intra-allelic mutation event that generated the duplication in the mutant allele, and one of them carried the other progenitor allele. If the duplication is the result of double-strand break repair during a recombination event between sister chromatids prior to the inter-allelic event which transferred the duplication to a homologous chromatid, then the duplication present in the mutant allele would be accompanied by a mutant structure in one of the other spores, all of which were non-mutants. Consequently, this suggests that a double-strand break repair process of recombination might not always be involved in intra-allelic events. As shown in Fig. 6, one possible explanation for the tetrads above is that a single-strand nick (Fig. 6*a*) results in the generation of a duplication in the nicked strand of the chromatid (Fig. 6*b*, *c*). The 3′ end could copy information either from the complementary strand following slippage as shown in Fig. 6*b*, or from the sister chromatid following strand invasion. A double-strand break in a homologous chromatid is then repaired by using the

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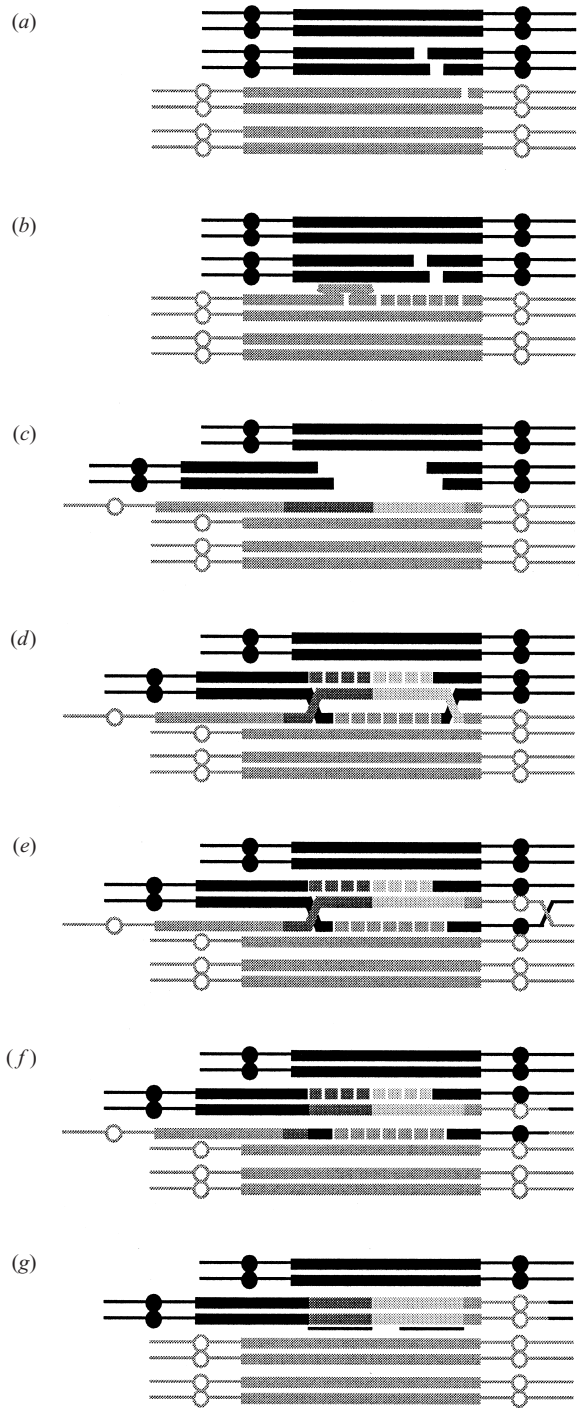

Fig. 6. Explanation based on combining the repair process of a single-strand nick with the double-strand break model for recombination in yeast (Sun *et al.*, 1991), for the generation of a tetrad with one mutant allele resulting from inter-allelic transfer both of a block of repeats containing a duplication and of a flanking marker associated with the duplication. Thick lines represent the repeat array. Thin lines represent flanking DNA. Flanking markers are indicated by filled and open circles. Chromatids carrying the smaller allele are represented in black, and chromatids carrying the larger allele are represented in grey. Dashes indicate DNA synthesis. The original duplication is indicated by dark grey and light grey lines. The final duplication in the mutant allele is underlined. For an explanation of (*a*)–(*g*) see the text.

duplicated strand as a template (Fig. 6*d–f*). The non-duplicated strand is used as a conversion template to restore the progenitor allele, and the duplicated strand is used as a template to convert the recipient chromatid, which results in a tetrad with one mutant allele showing inter-allelic transfer of a duplication (Fig. 6*g*).

Of the 16 alleles in which mutation involved an inter-allelic event, 13 showed conversion in favour of the larger parental allele. In yeast, the conversion outcome is thought to be dictated by the chromatid in which the recombination event is initiated (Leung *et al.*, 1997; reviewed by McKee, 1996) in such a way that it acts as the recipient of information from the non-initiated chromatid. If this is the case also for the integrated MS1, the results indicate that recombination events are preferentially induced in the smaller progenitor allele.

Several factors suggest that duplication events occur prior to events which involve inter-allelic transfer of repeats. In tetrad no. 30 one spore has an allele containing parts of a structure resulting from a duplication followed by a triplication within the region that was inserted in the homologous chromosome. The original mutant structure is present in another spore, which shows that this allele served as donor in the inter-allelic recombination event. Furthermore, in 5 of 6 tetrads with a mutant allele in which mutation involved a duplication and inter-allelic transfer of repeats, the duplication starts at the exchange point between homologous chromatids. This seems unlikely if the duplications had occurred after the inter-allelic transfer. In addition, no allele has been found in which the exchange point between homologous chromatids was included in a duplication.

Deletion is the most common mutation type observed at MS1 in yeast. The deletion events could be explained by the single-strand annealing model presented by Lin *et al.* (1984). However, according to the single-strand annealing model, several double-strand breaks in one allele would be necessary for the generation of mutants with multiple deletions. A more likely explanation is that these mutants are the result of single-strand loop formations that have been deleted during the recombination process. Mutant alleles in tetrads nos. 16, 28 and 32 show that duplicated regions can have different deletions, which demonstrates that, in these alleles, the duplication occurred before the deletions.

The meiotic mutation rate at MS1 in this study, and at MS32 in a study by Appelgren *et al.* (1997), was approximately 5- to 6-fold higher than the frequency of double-strand breaks determined at the hot spot in the vicinity of the minisatellite integration site upstream of *LEU2* (Baudat & Nicholas, 1997). The mutation rate would correspond to the frequency of double-strand breaks if all mutations were generated

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according to the double-strand break repair model of recombination. As pointed out above, the present study suggests that minisatellite mutations might not be exclusively dependent on the induction of double-strand breaks. However, according to present knowledge of recombination in yeast, inter-allelic mutation events can be assumed to be induced by double-strand breaks. Even if these events are solely taken into account, the frequency of mutant alleles is still twice as high as the reported double-strand break frequency at the *LEU2* region. Although it cannot be excluded that the integration *per se* has modified double-strand break activity in the region, a possible explanation for this observation is that the repetitive nature of minisatellite DNA attracts double-strand break induction. Alternatively, a fraction of inter-allelic mutation events do not result from double-strand breaks. Further studies are therefore necessary to elucidate the role of single- and double-strand breaks in the minisatellite mutation process. The yeast model system has considerable potential to dissect further the molecular basis of minisatellite mutation and will therefore contribute important information which is relevant to studies on minisatellite mutation in humans. Furthermore, this minisatellite-based yeast model is a powerful tool for analysing previously unknown actions of components of general eukaryotic recombination processes.

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