(CA/GT)_n microsatellites affect homologous recombination during yeast meiosis

Christiane-Gabrielle Gendrel,¹ Annick Boulet,² and Marie Dutreix^{1,3}

¹UMR-CNRS 2027, Institut Curie-Section de Recherche, Université Paris-Sud, F-91405 Orsay, France; ²UMR-CNRS 144, Institut Curie-Section de Recherche, F-75248 Paris Cedex 05, France

One of the most common microsatellites in eukaryotes consists of tandem arrays of the dinucleotide GT. Although the study of the instability of such repetitive DNA has been extremely fruitful over the last decade, no biological function has been demonstrated for these sequences. We investigated the genetic behavior of a region of the yeast *Saccharomyces cerevisiae* genome containing a 39-CA/GT dinucleotide repeat sequence. When the microsatellite sequence was present at the *ARG4* locus on homologous chromosomes, diploid cells undergoing meiosis generated an excess of tetrads containing a conversion of the region restricted to the region of the microsatellite close to the recombination-initiation double-strand break. Moreover, whereas the repetitive sequence had no effect on the frequency of single crossover, its presence strongly stimulated the formation of multiple crossovers. The combined data strongly suggest that numerous recombination events are restricted to the initiation side of the microsatellite as though progression of the strand exchange initiated at the *ARG4* promoter locus was impaired by the repetitive sequence. This observation corroborates *in vitro* experiments that demonstrated that RecA-promoted strand exchange is inhibited by CA/GT dinucleotide tracts. Surprisingly, meiotic instability of the microsatellite was very high (>0.1 alterations per tetrad) in all the spores with parental and recombinant chromosomes.

[Key Words: Microsatellite; homologous recombination; double-strand break repair; genome instability; meiosis; *Saccharomyces cerevisiae*]

Received November 19, 1999; revised version accepted March 28, 2000.

The genomes of all eukaryotic species contain tracts of DNA in which a single base or a small number of bases is repeated (microsatellites). Dinucleotide repeats are preferentially located in noncoding regions and display a high instability characterized by small changes in length occurring during cell growth. Tandem-repeat microsatellites have been conserved throughout evolution at an absolute frequency that is 30-fold higher than the expected random frequency; about one repetitive tract per 40 kb in *Homo sapiens* and *Saccharomyces cerevisiae* (Levinson and Gutman 1987; Debrauwère 1997). One of the most common microsatellites, the (CA/GT)_n microsatellite, consists of tandem arrays of the dinucleotides GT and CA. Such repetitive tracts are unstable (Levinson and Gutman 1987) (Wierdl et al. 1997), frequently undergoing changes in tract length. These changes result in polymorphisms that are useful in genetic mapping studies, and their high frequency is diagnosis of certain types of human tumors (for review, see de la Chapelle and Peltomäki 1995). Although the study of the instability of repetitive DNA has been fruitful over the last decade, no biological function has been demonstrated for these sequences. In this work, we propose that microsatellites

could play a fundamental role in genome stability by regulating recombination activity.

In mammalian cells (CA/GT)_n microsatellites have been shown to stimulate homologous recombination of transfected DNA (Bullock et al. 1986; Wahls et al. 1990), but do not influence chromosomal intramolecular recombination (Sargent et al. 1996). In yeast, repetitive sequences increase chromosomal recombination (Tresco and Arnheim 1986). In bacteria, these sequences stimulate RecA-independent intraplasmid recombination (Murphy and Stringer 1986). In a previous work we showed that *Escherichia coli* RecA, *H. sapiens* Rad51, and *S. cerevisiae* Rad51 recombination proteins bind with high affinity to repetitive single-stranded DNA carrying tracts of GT, CT, or CA dinucleotides (Biet et al. 1999). Moreover, in reactions promoted by the RecA protein, it has been observed that the presence of GT/CA repetitive tracts inhibits strand exchange between fully homologous DNA molecules and leads to accumulation of joint molecules that do not dissociate *in vitro* (Dutreix 1997). Although the evidence is indirect, it is likely that the inhibition of strand exchange is the consequence of a shifted alignment of repetitive sequences during pairing that is inefficiently corrected because of the tight binding of recombination proteins to these sequences. Because increased binding of recombination proteins is ob-

³Corresponding author.

E-MAIL marie.dutreix@curie.u-psud.fr; FAX 33 1 6986 9429.

served in the different species tested, inhibition of strand exchange by microsatellites could be a general process that may modify the recombination activity in the vicinity of the microsatellite. To test this hypothesis we analyzed the effect of a tract of 39 GT/CA dinucleotide repeats on meiotic recombination in *S. cerevisiae*. Because the amount of meiotic double-strand breaks (DSBs) is correlated with the frequency of gene conversion, it is assumed that meiotic breaks are the site of initiation of recombination events (Alani et al. 1990). Thus, during meiosis, it is possible to identify the site of initiation of one recombination event and to analyze strand-exchange progression and its resolution.

Results

Introduction and analysis of a CA/GT microsatellite at the ARG4 locus

To analyze the effects on recombinational events around *ARG4* due to microsatellites, we introduced a sequence of 39 CA/GT dinucleotide repeats (MS) into the *ARG4* gene (Fig. 1) at a locus close to a well-characterized meiotic hotspot for recombination (Nicolas et al. 1989; Sun et al. 1989). This hot spot of meiotic recombination is located upstream of the *ARG4* coding sequences and is associated with meiosis-specific DSBs that occur in this area. DSBs are detected as transient and heterogeneous DNA fragments in wide-type *RAD50* strains, but accumulate as discrete bands in *rad50S-K181* mutant strains (Cao et al. 1990). The presence of a microsatellite sequence in the *ARG4* coding sequence does not affect the location or the frequency of the breaks induced during meiosis in the area (Fig. 2). Moreover, the processing of

DSBs at the *ARG4* promoter, estimated by the disappearance of the fragments during incubation, is similar in *RAD50* strains carrying or not carrying the microsatellite (data not shown).

To monitor recombination events induced at meiosis we introduced two genetic markers (*URA3* and *TRP1*), and two modifications of restriction sites in the flanking regions of the microsatellite (Fig. 1). During some constructions the microsatellite insertion on the chromosome carrying the *URA3* and *TRP1* markers lost one repeat. We used this opportunity to monitor the events of instability and recombination at the microsatellite locus. Two diploid strains carrying the microsatellite sequence were used: strains MS(38/39), carrying a one-repeat heterology in the microsatellites and strain MS(39/39), with fully homologous microsatellites. The meiotic products were analyzed for the expression of the *URA3* and *TRP1* genes on selective media and for the presence of restriction sites by digestion of PCR-amplified DNA fragments. Two kinds of recombination events were monitored: crossovers (CO) that give rise to a reciprocal exchange of homologous chromosome regions (new segregation 2:2) and conversions that result from the correction of the heteroduplex intermediate, which are characterized by three spores carrying the same marker (segregation 3:1). As a control, we inserted a random non-repetitive 50% GC-rich sequence (RS) in the *ARG4* locus with a length identical to that of the microsatellite and with no apparent secondary structure.

High frequency of short conversions do not pass the microsatellite sequence

Tetrad analysis shows that the total number of *Bst*Z171

Figure 2. Time-course detection of meiotic double-strand breaks (DSBs) in the *ARG4* region in *rad50S* diploid strains. (Black arrows) Positions of the *Sna*BI parental fragment (~12 kb) and the fragments generated by DSB in the *DED82–DED81* intergenic region (*DED* DSBs) and in the *ARG4* promoter region (*ARG4* DSBs). (Gray arrow) Position of the expected fragment generated by DSBs in the microsatellite sequences, if there were breaks.

and *Age*I conversion events, measured by digestion of PCR-amplified fragments, is similar in the two strains containing the microsatellite insert and in the strain with the random insert (Table 1). However, strains differ in the number of the short conversions that are restricted to the *Bst*Z171 marker and do not encompass the *Age*I marker. As a matter of fact, the number of short conversions is higher in MS diploids (11%–12%) than in RS diploids (3%). After statistical analysis of these values, we found that the difference between short conversion frequencies of the MS and RS diploids was highly signifi-

cant ($\chi^2 = 8.2$; $P \leq 0.002$; Table 1). Although the total number of conversions was not significantly higher in the MS strains ($\chi^2 = 0.32$; $P = 0.13$), we do not exclude that the extra short conversions observed in both MS strains result from rare new initiation events. Moreover, there was no apparent bias in the direction of the *Bst*Z171 conversion. Both chromosomes were donors or recipients for the converted sequence as expected if initiation can occur indifferently on each chromosome. The one-repeat heterology in the MS(38/39) diploid did not seem to have any specific effect on the conversions in the MS diploids.

The size of the microsatellite sequence for the spores of the converted tetrads in the MS(38/39) strain was analyzed by PCR amplification and sequencing gel electrophoresis. As expected, the microsatellite sequence was always converted with the *Age*I and *Bst*Z171 markers in the 18 spores with large conversions that we analyzed. In contrast, among the 11 spores with *Bst*Z171 short conversions that we analyzed, only 2 had converted the microsatellite. This reduction in the number of co-conversion of the *Bst*Z171 marker and the microsatellite suggests that some conversion events would be restricted to the region between the initiation site and the microsatellite and would not be able to extend across the repetitive sequence.

Short conversions without associated crossovers are more frequent in the MS strains

During recombination, strands of homologous chromosomes exchange and form Holliday junctions that are resolved by specific resolvases. According to the junction resolutions, the resulting conversions are associated (+CO) or not (–CO) with an exchange of the adjacent regions. The chromosome reciprocal exchanges in the *ARG4* region were first monitored by the segregation of the *URA3* and *TRP1* markers. Few double crossover events that restore the parental segregation for these markers were also observed (Table 1). We compared the

Table 1. Number of conversion events in strains homozygous for the RS and MS sequences in the *ARG4* gene

Insert	Short conversion ^a				Long conversion ^b				Total number of tetrads analysed
	<i>Bst</i> ⁺ ^c conversion	<i>Bst</i> [–] ^d conversion	Total number	Total frequency	<i>Bst</i> ⁺ <i>Age</i> [–] ^c coconversion	<i>Bst</i> [–] <i>Age</i> ⁺ ^d coconversion	Total number	Total frequency	
RS	3 (1)	4 (2)	7 (3)	3%	7 (1)	33 (14)	40 (15)	21%	205
MS	12 (3*)	12 (3**)	24 (6)	11%	14 (8)	22 (8**)	36 (14)	17%	215
(38/39)									
MS	10 (2*)	12 (3*)	22 (5)	12%	7 (4)	21 (14)	30 (18)	16%	188
(39/39)									

Numbers in brackets correspond to conversion events associated with crossover.

^aShort tract conversion covering only the *Bst*Z171 site (*Bst*).

^bLong tract conversion encompassing the *Bst*Z171 (*Bst*) and *Age*I (*Age*) sites including the inserted sequence. The chromosome carrying the *URA3* and *TRP1* genetic markers and the *Age*I restriction site is either the recipient^c or the donor^d in *ARG4* gene conversion events.

*One conversion event is associated with a double crossover.

**Two conversion events are associated with a double crossover.

association of conversion events with a crossover in the MS and the RS strains. In all strains, nearly half of the large conversions encompassing the *AgeI* site were associated with a crossover (Table 1). Similarly, half of the short conversions restricted to the *BstZ171* site in the RS strain were associated with a crossover. However, in the MS strains only 23%–25% of the *BstZ171* conversions were found to be associated with crossovers (Table 1). This difference could indicate that part of the short conversions that accumulate in the presence of the microsatellite result from mechanisms different from those implicated in the RS strain.

Numerous MS tetrads with parental segregation of the URA and TRP markers have undergone two crossovers

We found that numerous spores that had a parental segregation for the *URA3* and *TRP1* distal markers display a reciprocal exchange of one or two central markers. These new marker arrangements are probably generated by two crossovers located on both sides of the exchanged region. We located the position of the crossovers in the different spores. They were classified as single and double crossovers according to their association with or without the reciprocal exchange of the distal *URA3* and *TRP1* markers. We compared the crossover locations in the RS and MS homozygous strains using the four markers (*URA3*, *TRP1*, *AgeI*, *BstZ171*; Table 2). In all strains, single crossovers occurred with roughly the same frequency in the three areas determined by the markers. In contrast, the total number of double crossovers differed significantly between the RS and MS strains. The MS strains produced about threefold more spores having undergone a double crossover than the RS strain ($\chi^2 = 7.44$; $P < 0.002$). Almost all the trends with a double crossover underwent a crossover in the region between *URA3* and *BstZ171*, the second event being located either between *BstZ171* and *AgeI* or between *AgeI* and *TRP1*. In the MS(39/39) strain, most of the double crossovers were located on the *URA* side of the *AgeI* site, and a significant number of double crossovers (41%) involved the four chromatids. These particular events were less frequent in the MS(38/39) strain. The difference in the location of the second crossover observed between the two

MS strains were highly significant ($\chi^2 = 12$; $P < 0.0002$). It is interesting to note that it was the only difference observed between the MS(39/39) and the MS39/38 strains.

Crossovers resolved preferentially at the side of the microsatellite close to the initiation site

Diploid cells with the MS insert generated at meiosis an excess of tetrads containing reciprocally recombined product in the *BstZ171* and *AgeI* area: 9% in the MS(38/39) and 16% in the MS(39/39) tetrads had resolved at least one crossover between the *BstZ171* and *AgeI* restriction sites whereas only 3% of the RS tetrads displayed a crossover at this interval (Table 2). In the MS(38/39) strain, the 5 tetrads with a single crossover and 14 tetrads with a double crossover that had one crossover located in the insert region between the *BstZ171* and *AgeI* restriction sites were analyzed more precisely. We measured the length of the microsatellite associated to the exchange to locate the crossover in relation to the microsatellite sequence. All but two events resolved in the area limited by the *BstZ171* site and the microsatellite sequence corresponding to the side of the microsatellite close to the *ARG4* initiation DSB. Interestingly, the two crossovers that resolved at the other side of the microsatellite correspond to spores that have undergone double crossovers in which the second crossover is located in the *AgeI*–*TRP1* area. These events could result from repair of DSBs generated at the other side of the microsatellite. It is possible that a small number of meiotic DSBs could occur in the *TRP1* region (see Fig. 1).

Instability of the repetitive sequence is increased during meiotic divisions

During the study of the MS(38/39) tetrads we measured the length of the microsatellite to analyze the recombination events. We compared the frequency of microsatellite alterations in the different types of recombinant spores (Table 3). We found that dinucleotide repeat tract alterations were not more frequent in spores having un-

Table 2. Number of single and double crossover at different location in the *ARG4* region

Insert	Single crossover				Double crossover				Total number of tetrads analysed
	<i>URA</i> - <i>Bst</i>	<i>Bst</i> - <i>Age</i>	<i>Age</i> - <i>TRP</i>	Total	<i>URA</i> - <i>Bst</i> / <i>Bst</i> - <i>Age</i>	<i>URA</i> - <i>Bst</i> / <i>Age</i> - <i>TRP</i>	<i>Bst</i> - <i>Age</i> / <i>Age</i> - <i>TRP</i>	Total	
RS	17	1	24	42 (20%)	6	4	0	10 (5%)	205
MS (38/39)	15	5	20	40 (19%)	12**	14	2	28 (13%)	215
MS (39/39)	14	4	22	40 (21%)	22**	1	5*	28 (15%)	188

Values in brackets correspond to the percentage of recombinant tetrads. Some double crossovers involve more than two strands.

*Three-strand events (one tetrad).

**Four-strand events (three MS(38/39) tetrads and nine MS(39/39) tetrads).

dergone conversion or crossovers than in spores that displayed parental segregation. However, the rate of microsatellite meiotic instability was very high as 3 tetrads out of 32 contained at least one spore with an altered tract length. None of the tetrads displayed the same microsatellite changes on the two copies of one parental chromosome, as would be expected if instability had resulted from a mitotic event. Moreover, we found a frequency of 1/197 tract alterations in the MS diploid cells used for this study. The difference between the pre- and post-sporulation instability frequencies could indicate that, at the *ARG4* chromosome locus, the meiotic instability is high relative to the mitotic instability. As already observed in analysis of dinucleotide repeats instability (Henderson and Petes 1992), the changes in length were small (one or two repeats). The number of tract changes was underestimated as we eliminated changes that would generate a 3:1 segregation of the microsatellite, which cannot be distinguished from microsatellite gene conversion (Table 3).

Discussion

When the microsatellite sequence was introduced into homologous chromosomes at the *ARG4* locus, conversion and crossovers were induced in the area between the *Bst*Z171 site and the microsatellite sequence. With a random sequence inserted at the same position there were few events restricted to this small area (129 bp). Interestingly, this region corresponds to the side of the microsatellite close to the DSB initiation site. In MS strains, crossovers located near the microsatellite were mainly observed in tetrads having undergone two crossovers. The increase of multiple events induced by the microsatellite sequence has been already reported by Treco and Arnheim (1986). However, the diploid strain used did not further the precise location of the events. We found that most of the double crossovers in the MS(39/39) strain involved one resolution in the *URA3*–*Bst*Z171 interval and another in the *Bst*Z171–*Age*I. Because the site of the DSB was located in the *URA3*–*Bst*Z171 interval close to the *Bst*Z171 site (~330 bp) it is likely that these double crossovers result from the resolution of intermediates formed at each side of the DSB.

Table 3. Rates of instability and types of alterations for the microsatellite sequences in parental and recombinant spores

Genotype of spores	Rate of tract instability	Number of spores with additions or deletions of base pairs				Total
		–2	0	+2	+4	
Parental	1.3×10^{-2}	0	75	1	0	76
Recombinant	7.7×10^{-2}	0	48*	3	1	52

Recombinant spores include crossing-over and conversion events encompassing the *Bst*Z171 and/or *Age*I restriction sites. The asterix indicates that four of the recombinants classified as microsatellite conversions could be mutants with alterations of the repetitive sequence: Three spores (–2) and one spore (+2).

To locate the second crossover in relation to the microsatellite, we used the MS(39/38) strain, which contains microsatellites at the *ARG4* locus with a length differing by one repeat. Most of the crossovers resolved in the *Bst*Z171–*Age*I region were located on the DSB initiation side of the microsatellite. Surprisingly, whereas the high frequency of short conversions and the excess of double crossovers were also observed in this strain, half of the second crossovers were resolved in the *Age*I–*TRP1* intervals. This result suggests that the mismatched repeat could help the strand exchange to progress across the repeated sequence either by recruiting special proteins or by destabilizing the blocked recombination complex. The difference in crossover location was specific to the resolution of a second crossover as single crossovers showed the same distribution along the chromosome in the two MS strains. A large number of double crossovers occurring in the MS strains involved the four strands. Tetrads carrying three and four chromosome recombinations for a single interval have been already observed in chromosomes carrying microsatellite sequences (Treco and Arnheim 1986). In all the MS tetrads with four recombinant strands resulting from two double crossovers located on each chromosome, one crossover was located in the microsatellite region. In both MS strains, the high number of four-strand exchanges in the *URA*–*Bst*/*Bst*–*Age* regions can be explained simply by the random occurrence of two independent events that involve all four chromatids within the genetic interval monitored. As a matter of fact, double crossovers occurred at a frequency of 5.6% in MS(38/39) strain and 12% in the MS(39/39) strain so the two events should occur at frequencies of 0.3% and 1.3%, respectively. The observed frequencies of four-strand double crossovers were slightly higher for the two MS strains (1.4% and 4.8%). This difference between observed and calculated frequencies is not significant and could reflect the underestimation of the crossovers in this area. For example, recombination events leading to a double crossover *URA*–*Bst*/*Bst*–*Age* associated with a *Bst*Z171 conversion would be detected as a short conversion not associated with crossovers. We found an excess of short conversions that were not associated with single crossovers. Given the high frequency of double crossovers in this region, we cannot exclude that part of the short conversions were associated with undetectable double crossovers. Several mechanisms have been proposed to generate conversion events not associated with a reciprocal exchange of the flanking regions (Gilbertson and Stahl 1996). Resolution of DSB repair intermediates can involve the cleavage of two, one, or no Holliday junctions. In the first case, the intermediates should be processed by resolution of the two Holliday junctions, in the same orientation, either both vertically or both horizontally to avoid reciprocal exchange of the distal markers. This model implies that the microsatellite induces a distal constraint and influences the resolution of the Holliday junction formed at the other side of the initiation site. However, this model is attractive as it applies both to the double crossovers and to the conversion not associated to crossovers.

Whatever the resolution model, the high number of events that resolve before the repetitive sequence strongly suggest that progression of strand exchange is inhibited at this level. It may be inhibited by the binding of specific proteins or by a special effect of the sequence on the recombination proteins. This last hypothesis is in agreement with previous *in vitro* observations indicating that the recombination proteins do not exchange the strands of homologous DNA across such sequences (Duttreix 1997). We found that the highly affinity of recombination proteins for the repeated sequences could impair the reaction (Biet et al. 1999). A second possibility is that CA/GT sequences promote crossovers by presenting the meiotic recombination apparatus with a specific structure that could be a signal to initiate genetic exchange nearby. It could be a region where homologous DNA segments are drawn together as the initial step for the meiotic recombination apparatus to recognize homologous chromosomes. Because the analysis of DSBs induced at meiosis did not reveal breaks in the area of the microsatellite, it is unlikely that new breaks could be the initiators of the induced events. Moreover, the absence of a bias in chromatids acting as donors or recipients in genetic exchange between chromosomes that do not contain microsatellites of the same length (this work) or that contain microsatellite sequence on only one chromosome (in prep.) supports a model in which these sequences are not substrates for an endonuclease that nicks or cuts DNA to initiate.

The most important question raised is that of the role of this highly repetitive element in the evolution of eukaryotic genomes. If a CA/GT tract inhibits progression of the strand exchange, then do the 50 to 100 copies of this repetitive sequence that are normally found in the yeast genome serve similar functions? If so, then strand exchange would be limited to regions between such sequences, and the genome would be divided into recombination units. During meiosis the extent of strand exchange seems to be limited and such sequences would have an effect only if they are located close to a DSB. At mitosis, during repair of damage on chromosomes it has been observed that the exchanged regions are longer. Repetitive sequences could prevent their extension and the loss of heterozygosity in large regions of the genome. To support this hypothesis, the effect of microsatellite sequences on mitotic recombination has to be demonstrated. Alternatively, recombination may be an activity that microsatellite sequences influence by virtue of their structure, but this activity may be unrelated to some other function that the repetitive sequence performs within eukaryotic cells.

The high levels of microsatellite instability at meiosis was unexpected. Our results differ from data obtained with a 49-repeat-length tract (Wierdl et al. 1997) or a 15-repeat-length (Strand et al. 1993) microsatellite carried by a plasmid. It is unclear whether the lack of increase of instability at meiosis on plasmid-carried microsatellites represents a chromosome–plasmid difference or an effect of flanking DNA sequences. However, we favor the hypothesis of a plasmid effect because Strand et

al. (1993) have already observed that a 29-bp microsatellite has 3-fold higher instability and is 10-fold less sensitive to a defect in mismatch repair when carried by the chromosome rather than by the plasmid. The location and frequency of DSB initiation sites on these plasmids have not been studied and could play an important role in the meiotic instability of microsatellites. Two models have been proposed to explain the instability of simple repeats: unequal recombination (Smith 1973) or DNA polymerase slippage (Streisinger et al. 1996). The first model implies that instability would be higher in recombinant spores. Our data do not allow us to confirm this hypothesis, and a study of a higher number of recombinant tetrads would be necessary to confirm or exclude the role of the recombination in meiotic microsatellite instability. However, several studies in bacteria (Levinson and Gutman 1987) and yeast (Strand et al. 1993) indicate that slippage during replication seems to be the main mechanism of CA/GT repetitive tract instability. Our finding, that this process could be increased during meiosis, raises the questions of the fidelity of the replication complex formed and the efficiency of mismatch repair during meiotic divisions. Because expression of some genes involved in mismatch repair (*MSH5*, *MSH4*, and *MSH2*) and replication (*RPA*, *POL30*, *POL1*, *POL3*, and *POL4*) has been shown to be modified during meiosis, it is possible that one (or both) systems could change their fidelity and induce the instability of the microsatellite.

Materials and methods

Plasmids and strains

All plasmids were derived from the L1.1 plasmid. This plasmid is a derivative of the pt92 plasmid and contains the (poly1) substitution of the *ARG4* promoter sequence (–316 bp to –139 bp) described previously by de Massy and Nicolas (1993). Two types of large insert were used in this study: The MS (microsatellite) insert, *XbaI*–*PstI* fragment from the bacteriophage M13mp19[CA/GT]₃₉ containing a sequence of 39 CA/GT repeats (Duttreix 1997); the RS insert, a random sequence corresponding to the *PvuII*–*PstI* fragment of the puc18 plasmid. MS and RS sequences were inserted (out of frame) into the *EcoRV* (+262 bp) site in the *ARG4*-coding region of L1.1. In the MS constructions, the *BstZ171* and *AgeI* restriction sites were modified by insertion of 2 and 4 bp. The modified *ARG4* regions were introduced at the *ARG4* locus on chromosome VIII of the strains ORD11-4B and ORD17-47C. Sequences of the constructions were checked by PCR amplification and sequencing using a ABI PRISM system. The poly(CA/GT) tract was oriented such that the poly(CA) repeats were on the transcribed strand.

The *S. cerevisiae* strains used in this study were derived from the strains ORD11-4B [*Mata*; *ARG4Δ2060*; *ura3-52*; *trp1-28*; *leu2-3*; *ade2-10*; dup KV(*DED82*-Arg(*ΔHpaI*)-YSC83)] and ORD17-47C[*MATa*; *ARG4Δ2060*; *his3Δ1*; *DED82::URA3*; *ORF83::TRP1*; dupKV(*DED82*-Arg(*ΔHpaI*)-YSC83)] (Lichten et al. 1990). These strains are derivatives of the strain S288c (N. Schultes, unpubl.). *DED82::URA3* and *YSC83::TRP1* and correspond to a 1.5-kb *EcoRI* *TRP1* fragment and a 1.2-kb *HindIII* *URA3* fragment inserted into the *DED82* *BamHI* site and the *YSC83* *BglII* site, respectively. To complement deficiencies created upon disruption of the essential genes *DED82* and *YSC83*,

a 12-kb fragment was inserted into *ApaI*–*StuI* of the *URA3* gene at its normal chromosomal position on chromosome V. This insert, designed at dupKV (*DED82*-Arg(Δ HpaI)-YSC83), contains an *ApaI*–*SnaBI* fragment of the *ARG4* region deleted of a 2-kb fragment from –316 bp to +1745 bp that carries the *ARG4* gene (Δ 2060). All strains used in this study bear the poly(I) substitution. The *rad50sK181* mutation was introduced into various strains by crosses with the strains ORT329 (*MATa*; *ARG4* Δ 2060; *ura3-52*; *trp1-289*; *ade2-101*; *his3 Δ 1*; *rad50s::URA3*) and ORT324 (*Mat* α ; *ARG4* Δ 2060; *ura3-52*; *trp1-289*; *leu2-3*; *rad50s::URA3*) (de Massy and Nicolas 1993).

Media, culture conditions, and genetic analysis

Growth and sporulation of yeast cells were performed by standard methods (de Massy and Nicolas 1993). Cells were grown in YPD media. For sporulation, cells were grown at 30°C in presporulation media (SPS) to a concentration of 2×10^7 to 4×10^7 cells/ml, washed in water, and incubated at the same density in sporulation medium (1% potassium acetate supplemented with the required amino acids) at 30°C. The distribution and frequencies of meiotic recombination implicating the marker genes (*URA3* and *TRP1*) and gene conversion events in the *ARG4* gene were studied by tetrad analysis. The segregation of *ARG4*, *URA3*, *TRP1* and at least four additional markers (*LEU2*, *HIS3*, *ADE2*, and the mating type locus *MAT*) were examined. All tetrads showing non-Mendelian segregation for *URA3*, *TRP1*, or *ARG4* were tested for all the markers, using larger patches of cells.

Analysis of the segregation of the restriction sites

The segregation of the *Bst*Z171 and *AgeI* sites was studied by the polymerase chain reaction with primers complementary to positions +8 bp and +730 bp. DNA was amplified directly from colonies arising from individual spores. The PCR products were digested with the *Bst*Z171 or *AgeI* restriction enzymes and analyzed by agarose gel electrophoresis on 2% NuSieve GTG (FMS BioProducts, Rockland, Maine) agarose gels.

Analysis of the length of repetitive tracts

The lengths of the tracts in the spores were determined by PCR amplification directly on colonies with primers that flank the repeated tracts (positions +198 bp and +432 bp). The PCR products were run on 6% denaturing polyacrylamide gels with control DNA samples containing poly(GT) tracts of 38 and 39 bp and transferred onto a nylon membrane (Amersham, N+). Membranes were prehybridized for 2 hr and hybridized for 12 hr at 42 °C with a 20-bp poly(CA) label probe. Probes were labeled by 3' end-labeling (terminal transferase; Boehringer Mannheim) with 50 μ Ci of [α -³²P]dCTP, 3000 mCi/mmol for 100 ng of DNA fragment. Unincorporated nucleotides were separated by filtration on a ProbeQuant G-50 Micro column (Amersham). Exposure times were from 20 to 50 min.

Detection of meiotic DSBs and quantification

Cells (200 ml) were grown in sporulation medium and, at the appropriate times (0, 4, 8, 10, and 24 hr), 25-ml aliquots were mixed with 25 ml of ethanol (100% at –20°C) and 1.25 ml of 0.5 M EDTA and kept at –20°C. Chromosomal DNA was extracted and 1.5 μ g of DNA was digested by *SnaBI* restriction enzyme (5 U/ μ g DNA; twice for 3 hr at 37°C), separated on a 1.2% agarose gel, and transferred under vacuum (LKB 2016 VACUGENE; Pharmacia) onto a nylon membrane (Amersham, Hybond N+).

Membranes were prehybridized for 2 hr and hybridized for 24 hr at 65°C with the labeled probe (20 ng/ml). Probes were labeled by random priming (Readyprime Kit; Amersham) with [α -³²P]dCTP, 3000 mCi/mmol, 110 TBq/mmol (Amersham). Unincorporated nucleotides were separated by filtration on a ProbeQuant G-50 Micro column. The probe used in the hybridization as the 707-bp PCR product (primers complementary to positions +8 bp and +715 bp) internal to the *ARG4*-coding region. Quantification of DSB signals were performed as described previously (de Massy and Nicolas 1993) by use of a PhosphorImager (Molecular Dynamics, STORM 860) and the ImageQuant program.

Statistical analysis

All results were tested by statistical analysis. Fisher's exact variant of the chi-square test was used for most comparisons and a *P* value of <0.05 was considered to be statistically significant.

Acknowledgments

We thank S. Ganglov, A. Nicolas, and F. Fabre for helpful advice and S. Yacine and N. Thiercelin for their efficient technical assistance. We are grateful to A. Nicolas for providing parental yeast strains. This work was supported by the Institut Curie (Program of Genotoxicology).

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Alani, E., R. Padmore, and N. Kleckner. 1990. Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. *Cell* **61**: 419–436.
- Biet, E., J. Sun, and M. Dutreix. 1999. Conserved sequence preference in DNA binding among recombination proteins: An effect of ssDNA secondary structure. *Nucleic Acids Res.* **27**: 596–600.
- Bullock, P., J. Miller, and M. Botchan. 1986. Effects of poly[d(pGpt)·d(pApC)] and poly[d(pCpG)·d(pCpG)] repeats on homologous recombination in somatic cells. *Mol. Cell. Biol.* **6**: 3948–3953.
- Cao, L., E. Alani, and N. Kleckner. 1990. A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. *Cell* **61**: 1089–1101.
- de la Chapelle, A. and P. Peltomäki. 1995. Genetics of hereditary colon cancer. *Annu. Rev. Genet.* **29**: 392–348.
- de Massy, B. and A. Nicolas. 1993. The control *in cis* of the position and the amount of the *ARG4* meiotic double-strand break of *Saccharomyces cerevisiae*. *EMBO J.* **12**: 1459–1466.
- Debrauwère, H., C.G. Gendrel, S. Lechat, and M. Dutreix. 1997. Differences and similarities between various tandem repeat sequences: Minisatellites and microsatellites. *Biochimie* **79**: 577–586.
- Dutreix, M. 1997. (GT)_n repetitive tracts affect several stages of RecA-promoted recombination. *J. Mol. Biol.* **273**: 105–113.
- Gilbertson, L.A. and F.W. Stahl. 1996. A test of the double-strand break repair model for meiotic recombination in *Saccharomyces cerevisiae*. *Genetics* **144**: 27–41.
- Henderson, S.T. and T.D. Petes. 1992. Instability of simple sequence DNA in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*

Gendrel et al.

- 12:** 2749–2757.
- Levinson, G. and G.A. Gutman. 1987. High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in *Escherichia coli* K-12. *Nucleic Acids Res.* **15:** 5323–5338.
- Lichten, M., C. Goyon, N.P. Schultes, D. Treco, J.W. Szostak, J.E. Haber, and A. Nicolas. 1990. Detection of heteroduplex DNA molecules among the products of *Saccharomyces cerevisiae* meiosis. *Proc. Natl. Acad. Sci.* **87:** 7653–7657.
- Murphy, K.E. and J.R. Stringer. 1986. RecA independent recombination of poly[d(GT)-d(CA)] in pBR322. *Nucleic Acids Res.* **14:** 7325–7340.
- Nicolas, A., D. Treco, N.P. Schultes, and J.W. Szostak. 1989. An initiation site for meiotic gene conversion in the yeast *Saccharomyces cerevisiae*. *Nature* **338:** 35–39.
- Sargent, R.G., R.V. Merrihew, R. Nairn, G. Adair, M. Meuth, and J.H. Wilson. 1996. The influence of a (GT)₂₉ microsatellite sequence on homologous recombination in the hamster adenine phosphoribosyltransferase gene. *Nucleic Acids Res.* **24:** 746–753.
- Smith, G.P. 1973. Unequal crossover and the evolution of multigene families. *Cold Spring Harbor Laboratory Symp. Quant. Biol.* **31:** 507–513.
- Strand, M., T.A. Prolla, R.M. Liskay, and T.D. Petes. 1993. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* **365:** 274–276.
- Streisinger, G., Y. Okeda, Y. Emrich, J. Newton, A. Tsugita, E. Terzaghi, and M. Inouye. 1996. Frameshift mutations and the genetic code. *Cold Spring Harbor Symp. Quant. Biol.* **31:** 77–84.
- Sun, H., D. Treco, N.P. Schultes, and J.W. Szostak. 1989. Double-strand breaks at an initiation site for meiotic gene conversion. *Nature* **338:** 87–90.
- Treco, D. and N. Arnheim. 1986. The evolutionarily conserved repetitive sequence d(TG·AC)_n promotes reciprocal exchange and generates unusual recombinant tetrads during yeast meiosis. *Mol. Cell. Biol.* **6:** 3934–3947.
- Wahls, W.P., L.J. Wallace, and P.D. Moore. 1990. The Z-DNA motif d(TG)₃₀ promotes reception of information during gene conversion events while stimulating homologous recombination in human cells in culture. *Mol. Cell. Biol.* **10:** 785–793.
- Wierdl, M., M. Dominska, and T.D. Petes. 1997. Microsatellite instability in yeast: Dependence on the length of the microsatellite. *Genetics* **146:** 769–779.

(CA/GT)_n microsatellites affect homologous recombination during yeast meiosis

Christiane-Gabrielle Gendrel, Annick Boulet and Marie Dutreix

Genes Dev. 2000, **14**:

Access the most recent version at doi:[10.1101/gad.14.10.1261](https://doi.org/10.1101/gad.14.10.1261)

References

This article cites 20 articles, 5 of which can be accessed free at:
<http://genesdev.cshlp.org/content/14/10/1261.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).