Length and Sequence Heterozygosity Differentially Affect HRAS1 Minisatellite Stability During Meiosis in Yeast

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ABSTRACT

Minisatellites, one of the major classes of repetitive DNA sequences in eukaryotic genomes, are stable in somatic cells but destabilize during meiosis. We previously established a yeast model system by inserting the human Ha-ras/HRAS1 minisatellite into the HIS4 promoter and demonstrated that our system recapitulates all of the phenotypes associated with the human minisatellite. Here we demonstrate that meiotic minisatellite tracts are polar in nature (Capon et al. 1983). Additionally, in the human population there are four different common alleles of the minisatellite, designated a1–a4 (Krontiris et al. 1985), which consist of a particular combination of the four repeat types in a defined order. Alleles are considered common if they occur in >7% of the population (Krontiris et al. 1986). The common alleles differ from one another in the arrangement of the repeat types within each tract and the number of repeats in the tract.

Unlike microsatellites, which usually alter during the DNA synthesis stage of the mitotic cell cycle, minisatellites alter during meiosis, undergoing changes in overall length and repeat composition (Jarman and Wells 1989; Jeffreys et al. 1998). Minisatellite tracts have proven very useful for genomic mapping. Some minisatellites (MS1 and MS32, for example) exhibit very high rates of tract-length alterations; these types are often utilized for forensic DNA fingerprinting. Alterations in minisatellite tracts are often polar in nature (Armour et al. 1993; Jeffreys et al. 1994), occurring at one side of the tract preferentially. This polarity, and the meiotic timing of the alterations, has led to the hypothesis that alterations in minisatellite sequences result from a recombination event at the locus rather than during replication.

Identification of the meiotic factors underlying minisatellite stability has been complicated by the high level of variability exhibited by minisatellite sequences in the human population. We developed the yeast HIS4-HRAS1 model system to overcome these difficulties (Jauert et al. 2002). We replaced the Saccharomyces cerevisiae HIS4 promoter region with the a1 common allele of the HRAS1 minisatellite from humans and demonstrated that this model system recapitulated in yeast all of the phenotypes associated with the HRAS1 minisatellite in mammalian systems. In yeast the HRAS1 minisatellite tracts utilized the HIS4 locus. The tracts length altered at very high frequency during meiosis, but not during mitotic growth. The minisatellite also stimulated meiotic recombination at the HIS4 locus, a stimulation that was dependent on the meiotic double-strand break endonuclease Spol1p. Finally, we demonstrated that a meiotic DNA large loop repair activity requiring the RAD1 endonuclease (Kirkpatrick and Petes 1997; Kearney et al. 2001) acts specifically to

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generate expansions in the *HRAS1* minisatellite tract in *yeast* (Jauert et al. 2002).

These data were incorporated into the following model for *HRAS1* minisatellite tract-length alteration during meiosis on the basis of the double-strand-break repair model for meiotic recombination (Szostak et al. 1983). Meiotic recombination initiates by double-strand breaks (DSBs) adjacent to the repetitive tract (Jauert et al. 2002) (Figure 1a); other minisatellite tracts in yeast also generate DSBs (Debrauwere et al. 1999). Following processing (Figure 1b), a single-strand DNA tail invades the homolog, forming heteroduplex DNA (Figure 1, c and d). The presence of repetitive DNA in the heteroduplex allows possible misalignment of the repeats. If misalignment occurs, single-strand loops of an integral number of repeat units will be extruded, as shown in Figure 1d. These loops are substrates for meiotic DNA loop repair activities, one of which requires the RAD1 protein (Kirkpatrick and Petes 1997; Kearney et al. 2001). Depending on whether the loop is extruded from the template or invading strand, subsequent DNA repair will lead to expansion or contraction of the tract.

In this report, we examine structural factors that contribute to the stability of the *HRAS1* minisatellite during meiosis in *yeast*. We demonstrate that tracts alter during meiosis in units of 28 bases (the repeat length) and that length alteration is dependent on Spo11p. Further, we find that the primary sequence of the tract has a profound influence on stability when a diploid cell is heterozygous for minisatellite tract alleles. A strain that is heterozygous for tracts that differ by only two bases is twice as stable as a homozygous diploid. In contrast, a strain that is heterozygous for differing tract lengths exhibits an elevated level of detectable alterations relative to homozygous diploid strains. Most alterations are gene conversion events in which one parental tract-length allele is converted to the other parental allele’s tract length. Such tract conversion events would be undetectable in strains with homozygous tract-length alleles. In addition, tract-length alterations to a nonparental length are also observed. Thus, length and sequence heterozygosity have diametrically opposed influences on *HRAS1* minisatellite tract stability during meiosis.

**MATERIALS AND METHODS**

**Strain construction:** All strains are derived from the haploid strains AS13 (MATa leu2 ura3 ade6 rme1) or AS4 (MATa trp1 arg4 tyr7 ade6 ura3 spc22) and are isogenic except for alterations introduced by transformation. The *HRAS1* minisatellite-containing strains are derived from DTK314 (Jauert et al. 2002) containing the 30-repeat *his4-A1* allele of the minisatellite tract. The *his4-H10*, *his4-h10m*, and *his4-H7* alleles were cloned from meiotic spore colonies of DTK314 that contained shortened tracts. PCR primers (cloning F and R) that anneal...
to the HIS4 DNA flanking the minisatellite tract were used to amplify the minisatellite tracts. These PCR products were transformed into PD57 (AS13 his4-H52) or PD63 (AS4 his4-H52), selecting for a His+ phenotype. The his4-lopc allele was then introduced into the AS13-derived strains (Jauert et al. 2002). To construct the his4-7m allele (the 7-repeat minisatellite tract containing the two base alterations), an oligo, his4-7m, that contained the HIS4 promoter sequence and the first two repeats of the minisatellite tract, was designed, but with a C-to-T change in the fourth position of the first repeat and an A-to-G change in the first position of the second repeat. The his4-7m oligo and the cloning R oligo were used to simultaneously amplify the his4-H7 allele and allow the bases in the repeats in repeats 1 and 2. This PCR product was transformed into PD63, selecting for a His+ phenotype. Correct integration and the primary sequence of the minisatellite tract were determined by sequencing. 

RAD1 deletions were introduced by transforming strains with BamHI-digested pDG18 (Kirpatrick and Peters 1997). PMS1 deletions were constructed by transformation of a PCR product with 5′ and 3′ homology to the PMS1 locus and internal sequence of the KANMX4 cassette encoding geneticin resistance (Wach et al. 1994).

**Strains generated:** Strains derived from AS13 were DTK608 (PD57 his4-lopc his4-H10), DTK669 (DTK608 Δrad1), DTK756 (DTK608 Δpms1), DTK810 (PD57 his4-lopc his4-H10m), DTK658 (DTK608 Δpms1), DTK794 (PD57 his4-lopc his4-H7); AS4-derived: DTK750 (PD63 his4-H10), DTK796 (DTK750 Δrad1), DTK757 (DTK750 Δpms1), DTK690 (PD63 his4-H10m), DTK635 (DTK607 Δrad1), DTK666 (DTK607 Δpms1), DTK774 (PD63 his4-H7), DTK798 (DTK774 Δrad1), and DTK818 (PD63 his4-H5m). The following strains were diploids: DTK751 (DTK608 × DTK750), DTK802 (DTK660 × DTK796), DTK758 (DTK756 × DTK757), DTK811 (DTK810 × DTK607), DTK611 (DTK608 × DTK607), DTK764 (DTK660 × DTK635), DTK763 (DTK658 × DTK666), DTK795 (DTK794 × DTK774), DTK777 (DTK776 × DTK774), DTK820 (DTK690 × DTK818), and DTK825 (DTK669 × DTK798).

**Meiotic protocols and tract-length analysis:** Strains were sporulated at 18° and dissected using protocols described in Jauert et al. (2002). Four-spored tetrads were scored for segregation of the his4-lopc allele on His− medium, and then whole-cell PCR was conducted (Jauert et al. 2002) to determine the tract length in each spore colony using tract-length diagnostic primers. Two independent diploids of each strain were examined and the data from each were summed over statistical analysis demonstrated that the two data sets were not statistically different. At least 200 complete tetrads were evaluated for recombination at HIS4 and 100 tetrads (400 spore colonies) were examined for tract-length alterations. Tract-length allele PCR products were purified using a High Pure PCR product purification kit (Roche Diagnostics) and then sequenced by the Advanced Genetic Analysis Center at the University of Minnesota, using the same primers as were used for tract-length analysis.

**Statistical analysis:** Comparisons were done with Instat 1.12 (GraphPad) for Macintosh, using either a chi-square or a Fisher’s exact variant test. Results were considered statistically significant if P was <0.05. For some analyses on data sets with a small sample size, groups were combined as appropriate (e.g., altered length vs. parental length).

**PCR oligonucleotides:**

Cloning: F, 5′-CTATTACACAGCCGCTGTGGCATATG; R, 5′-CTATTACACAGCCGCTGTGGCATATG; Tract-length diagnosis: 5′-CCTCTGGGAGGGGACTTG and R, 5′-GGGAGAGGTGGCATACTGCG; PMS1 knockouts: 5′-GAAGCGGAAAGAAAGAGCGGGCTCTC TCTTTAATAATCATTAGGATTAACCTAAGCTACGCTGA GGTCGAC and 5′-CTCCCTGTATATAATGTATTGTTTAA TTATATATAGAATATCAAAGATCGATGAATTC GAGCTCG

his4-7m oligo (lowercase letters are altered bases in repeats 1 and 2) 5′-AGGCCGTCGGCTGGCTGGCCAGGGCTGCA GGGACACTCCCCCCCTTCTGTCGCCAGGGGACCCCAACATCG GCCCTTCTCTCCAGGGGACCCGCcACTCCCCCT.

**RESULTS**

**Base heterozygosity:** To examine the effect of sequence heterogeneity on the stability of the HRAS1 minisatellite tract, we constructed two diploid control strains, each homozygous for one of two HRAS1 minisatellite alleles. DTK751 is homozygous for the his4-H10 allele, which contains 10 copies of the 28-bp repeat sequence, while DTK811 bears the his4-H10m allele, which is identical to his4-H10 except for a C-to-T change in the fourth position of the third repeat (3.4 heterozygosity) and an A-to-G change in the first position of the fourth repeat (4.1 heterozygosity) (Figure 2). The minisatellites in DTK751 (10/10) and DTK811 (10m/10m) exhibit the same level of meiotic instability. In DTK751, 28% of the tetrads contained at least one spore with an altered tract, while 31% of the tetrads from DTK811 had alterations. The distribution of classes was also equivalent (Table 1). Therefore, in DTK811 the presence of the two sequence differences in repeats 3 and 4 of the HRAS1 minisatellite does not affect the stability of the minisatellite during meiosis when these differences are homozygous.

We constructed a diploid strain that was heterozygous for the base alterations (DTK611) and found a significant 50% reduction in the meiotic instability of the minisatellite tract compared to the two control homozygous strains. Only 15% of the tetrads exhibited meiotic tract-length alterations (P = 0.004 compared to DTK751). The distribution of classes was unaltered, but the percentage of tetrads in each class was reduced by approximately half (Table 1), indicating that the reduction in instability was not limited to a particular type of rearrangement.

The four minisatellite tracts in 22 tetrads of DTK611 (10/10m) were sequenced, including 8 tetrads that had no observable length alterations, 10 with a single tract-length deletion, and 4 with a single tract-length insertion. The segregation of the two heterozygous base-pair alterations [in repeat 3 at position 4 (3.4 heterozygosity) and in repeat 4 at position 1 (4.1 heterozygosity)] was determined for each of these tetrads. Of the 19 that could be scored, 13 (68%) exhibited aberrant segregation of the 3.4 and 4.1 heterozygosities. In 12 of the 13 aberrant segregations, the alterations occurred in the same direction at both the 3.4 and 4.1 position, a 25-bp separation, indicating coconversion of the two mismatches. Of these 12 tetrads, 6 converted the wild-type sequence to the mutant sequence, and 6 converted in the opposite direction, indicating no bias in directionality. Finally, 7 of the 8 tetrads that had no observable length alterations exhibited gene conversion of the 3.4
and 4.1 heterozygosities; no directional bias was observed. In conclusion, the base heterozygosities undergo a high level of gene conversion, but this high level of recombination is not reflected in the frequency of alteration of the minisatellite tract.

When we deleted the DNA mismatch repair gene \textit{PMS1} (Williamson et al. 1985) in DTK611 (10/10m), generating DTK763, the level of meiotic instability significantly increased \((P < 0.0001)\), becoming equivalent to that in DTK758, the \textit{pms1} derivative of DTK751 (10/10) (Table 1). This result indicates that the increased stability conferred by the sequence heterozygosity is dependent on DNA mismatch repair. Deletion of \textit{RAD1}, required for meiotic DNA loop repair and \textit{HRAS1} minisatellite stability (Jauert et al. 2002), resulted in a small increase in tract instability, from 15 to 21\%. This increase was not statistically significant \((P = 0.2;\) altered vs. unaltered in DTK611 and DTK764). However, given the small number of events observed, we cannot determine conclusively if loop repair influences overall stability when tracts contain sequence differences. The level of meiotic tract alteration was not significantly affected by \textit{pms1} or \textit{rad1} mutations in derivatives of DTK751 \([P = 0.35\text{ altered vs. unaltered with DTK802 (} \Delta \text{rad1 10/10)} \) and \(P = 0.18\) with DTK758 \((\Delta \text{pms1 10/10)}\). The frequency of recombination at the \textit{HIS4} locus can be determined by monitoring the aberrant segregation frequency of the heterozygous \textit{his4-lope} insertion allele in the coding sequence of \textit{HIS4}. DTK751 (10/10) had an altered tract instability, but this high level of recombination is not reflected in the frequency of alteration of the minisatellite tract.

Figure 2.—The structure of the \textit{HIS4-BIK1} locus in diploid strains. The rectangle with dark shading represents the \textit{BIK1} gene, while the box with light shading represents the \textit{HIS4} gene. One copy of the \textit{HIS4} gene carries the \textit{his4-lope} allele, indicated as a dark vertical line. The open ovals represent the TATAA box for \textit{HIS4}. Open rectangles represent the individual 28-bp repeats of the \textit{HRAS1} minisatellite. Thick dark lines adjacent to the repeats indicate the location of unique human DNA flanking the \textit{HRAS1} minisatellite. Dots within the repeats represent the two point mutations in \textit{his4-H10m} and \textit{his4-H7m}. Lightly shaded repeats have a C at the seventh position and a G at the fifteenth; open repeats have a G at the seventh and a C at the fifteenth; and dark shading indicates a G at both positions. The repeats are numbered in DTK751 to indicate orientation.

<table>
<thead>
<tr>
<th>Strain</th>
<th>\textit{HIS4} promoters</th>
<th>No. of repeats</th>
<th>Relevant mutation</th>
<th>Total tetrads</th>
<th>None altered</th>
<th>One spore increase</th>
<th>One spore decrease</th>
<th>Two altered</th>
<th>Three altered</th>
<th>Four altered</th>
<th>% altered</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTK751</td>
<td>\textit{his4-H10}/\textit{his4-H10}</td>
<td>10/10</td>
<td>Wild type</td>
<td>148</td>
<td>106 (72)</td>
<td>6 (4)</td>
<td>20 (14)</td>
<td>12 (8)</td>
<td>4 (3)</td>
<td>0</td>
<td>28</td>
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<tr>
<td>DTK802</td>
<td>\textit{his4-H10}/\textit{his4-H10}</td>
<td>10/10</td>
<td>( \Delta \text{rad1} )</td>
<td>117</td>
<td>77 (66)</td>
<td>2 (2)</td>
<td>22 (19)</td>
<td>11 (9)</td>
<td>5 (4)</td>
<td>0</td>
<td>34</td>
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<tr>
<td>DTK758</td>
<td>\textit{his4-H10}/\textit{his4-H10}</td>
<td>10/10</td>
<td>( \Delta \text{pms1} )</td>
<td>114</td>
<td>72 (63)</td>
<td>7 (6)</td>
<td>22 (19)</td>
<td>8 (7)</td>
<td>3 (3)</td>
<td>2 (2)</td>
<td>37</td>
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<td>DTK811</td>
<td>\textit{his4-H10m}/\textit{his4-H10m}</td>
<td>10m/10m</td>
<td>Wild type</td>
<td>118</td>
<td>82 (69)</td>
<td>4 (3)</td>
<td>18 (15)</td>
<td>11 (9)</td>
<td>3 (3)</td>
<td>0</td>
<td>31</td>
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<tr>
<td>DTK611</td>
<td>\textit{his4-H10}/\textit{his4-H10m}</td>
<td>10/10</td>
<td>Wild type</td>
<td>215</td>
<td>180 (85)</td>
<td>4 (2)</td>
<td>20 (9)</td>
<td>7 (3)</td>
<td>2 (1)</td>
<td>0</td>
<td>15</td>
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<tr>
<td>DTK764</td>
<td>\textit{his4-H10}/\textit{his4-H10m}</td>
<td>10/10</td>
<td>( \Delta \text{rad1} )</td>
<td>117</td>
<td>92 (79)</td>
<td>1 (1)</td>
<td>15 (13)</td>
<td>6 (5)</td>
<td>5 (3)</td>
<td>0</td>
<td>21</td>
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<td>DTK765</td>
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<td>10/10</td>
<td>( \Delta \text{pms1} )</td>
<td>115</td>
<td>72 (64)</td>
<td>4 (4)</td>
<td>25 (22)</td>
<td>8 (7)</td>
<td>4 (4)</td>
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<td>36</td>
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<td>\textit{his4-H7}/\textit{his4-H7}</td>
<td>7/7</td>
<td>Wild type</td>
<td>216</td>
<td>162 (75)</td>
<td>10 (5)</td>
<td>29 (15)</td>
<td>10 (5)</td>
<td>5 (2)</td>
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<td>25</td>
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</table>

* The first number represents the total number of four spore colony tetrads examined that exhibit the indicated alteration, and the number in parentheses is the percentage of the total number examined that exhibit the indicated alteration.
his4-H10/his4-H7m

DTK751 his4-H10/his4-H10 10/10 Wild type 235 112 14 12 35 35 10 17 52 38

DTK802 his4-H10/his4-H10 10/10 Δrad1/Δrad1 204 94 11 7 33 30 8 21 54 32

DTK758 his4-H10/his4-H10 10/10 Δpms1/Δpms1 208 99 15 9 34 31 8 12 52 44

DTK811 his4-H10m/his4-H10m 10/10 Wild type 249 92 13 8 54 45 18 19 63 34

DTK611 his4-H10/his4-H10m 10/10m Wild type 709 366 44 17 111 110 27 34 48 42

DTK764 his4-H10/his4-H10m 10/10m Δrad1/Δrad1 253 119 10 8 46 46 9 15 53 39

DTK763 his4-H10/his4-H10m 10/10m Δpms1/Δpms1 204 116 5 7 39 25 4 8 43 38

DTK795 his4-H7/his4-H7 7/7 Wild type 473 189 39 16 84 77 24 44 60 43

DTK777 his4-H10/his4-H7 10/7 Wild type 250 115 13 4 48 50 8 12 54 42

DTK825 his4-H10/his4-H7 10/7 Δrad1/Δrad1 219 81 12 8 45 48 12 13 63 40

DTK820 his4-H10/his4-H10m 10/7m Wild type 208 104 14 9 24 31 7 19 50 42

For all segregation patterns, the first number represents the wild-type allele and the second number represents the mutant his4-lopc allele. The segregation patterns include 4:4 (normal Mendelian segregation); 6:2 and 2:6 (gene conversion); 5:3 and 3:5 (tetrads with a single PMS event); aberrant 4:4 (one wild-type, one mutant, and two sectored colonies).

a DNY26 data are from Fan et al. (1995).

b Includes aberrant 6:2 and 2:6 tetrads as well as tetrads with three or four PMS or gene conversion events.

c Genetic map distance between LEU2 and HIS4.

### Table 2

<table>
<thead>
<tr>
<th>Strain</th>
<th>HIS4 promoters</th>
<th>No. of repeats</th>
<th>Relevant mutations</th>
<th>Total tetrads</th>
<th>4:4</th>
<th>6:2</th>
<th>2:6</th>
<th>5:3</th>
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<th>Aberrant 4:4</th>
<th>Other aberrant segregation</th>
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<td>67</td>
<td>15</td>
<td>33</td>
<td>51</td>
<td>31</td>
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<td>235</td>
<td>112 14 12 35 35 10 17 52 38</td>
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<td>94 11 7 33 30 8 21 54 32</td>
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<td>81 12 8 45 48 12 13 63 40</td>
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</table>

The majority (75%) of the length alterations in DTK777 are gene conversion events; the altered tract has a new length that is equivalent to the length of the other allele (Table 3). This “tract conversion” occurs in both directions (a 7-repeat tract increasing in length to a 10-repeat tract and vice versa). In 118 DTK777 tetrads examined, 27.1% were gain-of-length tract conversions, and 19.2% were loss-of-length tract conversions, while 15.9% involved alterations to a nonparental length or both gene conversions and nonparental length alterations (Table 3). In conversion events, gain-of-length alterations were more prevalent than loss-of-length events, but in nonparental alterations, loss-of-length events were more common.

We deleted RAD1 in DTK777 to determine the effect of the loss of loop repair on tract-length alteration when the strain contains heterozygous length alleles of the HRAS1 minisatellite. RAD1 has previously been shown to be involved in meiotic HRAS1 minisatellite tract expansions (Jauert et al. 2002). If RAD1 is involved in tract-length gene conversion events in a similar manner, loss of RAD1 should lead to a loss in gain-of-length tract conversions relative to the wild-type parental strain. In DTK825 (DTK777 Δrad1) the minisatellite tract was al-
Mechanism of tract-length alteration: To determine the precise alterations that occur in the HRAS1 minisatellite tracts during meiosis, we sequenced 160 minisatellite tracts from six different strains. The tract-length alteration was seen, while in nonparental alterations 23.4% of length alteration was seen, while in nonparental alterations of-length gene conversions, while 23.1% were loss-of-length gene conversions, while 23.1% were loss-of-length gene conversions, while 23.1% were loss-of-length gene conversions, while the remaining 23.4% involving alterations to a nonparental length or a mixture of nonparental length and gene conversion events. Among the gene conversion events, no bias in direction of alteration was seen, while in nonparental alterations loss-of-length events were more frequent than gain-of-length events (Table 3). Gain-of-length tract conversions occurred in 20.9% of the tetrads, while loss-of-length conversions occurred in 27.5%. This bias is the reverse of that seen in DTK777 (27.1 vs. 19.2%). Nonparental length alterations were also slightly biased toward loss events in DTK825. While this trend is consistent with RAD1 involvement in tract expansion, approximately twice as many tetrads would need to be evaluated for the difference to be statistically significant, so no conclusion can be drawn at this time. Aberrant segregation of the heterozygous his4-10m allele also increased in DTK825 relative to DTK777 (P = 0.05); other Δrad1 strains exhibited an elevation of his4-10m aberrant segregation, but the increase was not statistically significant in those strains (Table 2).

**Base vs. length heterozygosity:** To determine if the increase in stability due to base heterozygosity can influence the high level of tract conversion seen with length heterozygosity, we constructed a strain, DTK820, containing both base and length heterozygositites. To accomplish this, we constructed a version of his4-H7 that contains two point mutations (a C-to-T change in the fourth position of the first repeat and an A-to-G change in the first position of the second repeat) and then mated this his4-H7m strain with the his4-H10 strain. If we had used a diploid his4-10m/his4-H7 strain, the point mutations might have been contained in loops during heteroduplex formation and would therefore not have been present as base mismatches. In DTK820, the point mutations in the 7-repeat tract will be incorporated into heteroduplex DNA, rather than loops, during meiotic recombination, leading to separate base mismatches and loop formation. The point mutations in his4-7m are the same alterations as in his4-10m.

DTK820 (his4-7m/his4-H10) has a very high frequency of tetrads exhibiting tract alterations (70%, Table 3). DTK777, which is heterozygous for length alleles but lacks the base heterozygosities (his4-7/his4-H10), actually has a slightly lower level of tract alteration (63%), indicating that the presence of the point mutations in the his4-7m allele in DTK820 does not decrease the high level of tract conversion observed when the tracts are heterozygous in length. Thus, tract conversion appears to be dominant over base mismatch-dependent stabilization. In 112 DTK820 tetrads examined, 23.3% were gain-of-length gene conversions, while 23.1% were loss-of-length gene conversions, with the remaining 23.4% involving alterations to a nonparental length or a mixture of nonparental length and gene conversion events. Among the gene conversion events, no bias in direction of alteration was seen, while in nonparental alterations loss-of-length events were more frequent than gain-of-length events (Table 3).
alterations (13 insertions, 62 deletions) are shown in Figure 4, a and b. Among the 62 deletions sequenced, 4 tracts had a single repeat loss, 18 had two repeats deleted, 25 were missing three, 9 were missing four, 2 were missing five, 2 were missing six, and 1 lost nine repeats. Among the insertions, 3 had a single extra repeat, 5 had two extra, 2 had three extra, and 3 had four repeats added to the tract length. All tract-length alterations occurred in multiples of 28 bases with two exceptions as noted in Figure 4a. The alterations were conservative: no sequence rearrangements were detected in the tract surrounding the deletions, although one insertion had repeats that, while still 28 bp in length, had a number of internal sequence alterations that were not consistent with any of the standard repeat sequences (@ in Figure 4a).

As described above, the tracts from all four meiotic spore products were sequenced in 22 tetrads of DTK611 (10/10m). Other than the gene conversions of the 3.4 and 4.1 base heterozygosities described above and the observed length alterations shown in Figure 4a, no alterations were detected in the minisatellite tracts in DTK611. This result indicates that meiotic alterations in the HRAS1 minisatellite are conservative: they do not appear to affect other homologs or sister chromatids in a detectable manner.

We previously demonstrated that Spo11p was required for the formation of DSBs at HIS4 and for the high frequency of aberrant segregation of the heterozygous his4-lope allele during meiosis in strains containing the 30-repeat his4-A1 allele of the HRAS1 minisatellite (Jauert et al. 2002). To determine if tract alterations in the minisatellite also are dependent on Spo11p, we compared the level of tract alteration in DTK633 (his4-A1 spo13) and DTK639 (his4-A1 Δspo11 spo13); these strains were previously used to investigate DSB formation and aberrant segregation of his4-lope (Jauert et al. 2002). Strains lacking Spo11p arrest during meiosis, but the spo13 mutation bypasses this arrest by eliminating the first meiotic division. Two diploid meiotic products are formed, rather than four haploid products (Klapolz and Esposito 1980). We dissected dyads from DTK633 and DTK639 and performed whole-cell PCR after colonies had formed from each diploid spore. In DTK633, 19/50 dyads (38%) exhibited an altered tract in at least one spore colony. In contrast, 0/48 dyads (0%) from DTK639 had an altered tract. Of the 19 altered tracts observed, 3 were a one-tract/spore increase in length, 8 were a one-tract/spore decrease in length, 5 were a two-tract/spore decrease in length, and 3 were dyads in which both spores had one of the two alleles decrease in length. These data indicate that, in the absence of Spo11p, alterations in tract length do not occur, but in the presence of Spo11p, a wild-type pattern of tract alterations is observed.

DISCUSSION

We previously introduced the HRAS1 minisatellite into the HIS4 locus of S. cerevisiae to allow us to identify structural and genetic factors that influence the stability of the minisatellite during meiosis (Jauert et al. 2002). In the human population there are a large number of HRAS1 minisatellite alleles that differ in total number of repeats (and thus in overall length) and in the primary sequence of the 28-bp repeat units. Individuals are therefore likely to be heterozygous at the HRAS1 minisatellite tract for both length and base composition. In this report we used our yeast model system to identify the relative influences of sequence heterozygosity and length heterozygosity on meiotic tract-length stability by constructing strains that vary in either length (repeat number) or composition (primary repeat sequence) and by comparing the frequency of tract-length changes when heterozygous and homozygous.

We find that diploid strains that are heterozygous for either point mutations or length alleles have dramatically different meiotic tract alteration frequencies. Length and sequence heterozygosity have diametrically opposed influences on HRAS1 minisatellite tract stability during meiosis. A strain that is heterozygous for tracts differing by three repeats has four times as many observable tract-length alterations as a strain with tracts that differ by only two bases.

Our data demonstrate that base sequence differences between the two alleles of the HRAS1 minisatellite tract in a diploid cell act to stabilize the minisatellite during meiosis. Further, we find that this stability increase is dependent on a functional DNA mismatch repair system. Three different models could account for this effect: (1) the base mismatches lead to a decreased level of heteroduplex formation and consequently a decreased level of loop extrusion; (2) the mismatches are detected by the DNA mismatch repair pathway and corrected, and nearby extruded loops are corrected incidentally;
Figure 4.—Analysis of deletions in minisatellite tracts. (a) The locations of insertions and deletions in the 10-repeat \textit{HRAS1} minisatellite tracts are shown. Insertions are depicted above the minisatellite tract; deletions are below the tract diagram. Each repeat is indicated by a shaded rectangle and numbered (1–10 or 1–7, with \textit{Bik} to the left and \textit{His4} to the right of the tract as diagrammed); repeats that have the same shade have the same nucleotides at the variable +7 and +15 positions as discussed in the Introduction. The extent and approximate position of each sequenced alteration is shown as a thin bar. Arrows attached to brackets indicate the repeat in which the insertion occurred. The exact deletion and insertion endpoints cannot be determined precisely due to the repetitive nature of the repeats. For deletions, the position in the figure is given relative to the first variable base that defines the left edge of the deletion. The strain from which each insertion/deletion was isolated is shown to the left. Bars indicating insertions/deletions from the same strain are shaded similarly to aid in distinguishing them. Numbers in parentheses to the right of the bars indicate the number of times that altered sequence was isolated independently. All insertions and deletions occurred in units of 28 nucleotides, except (*), which had a GGG trinucleotide insertion in the polyG tract within repeat 6 and (**), which had an additional A in repeat 7. The DTK611 insertion marked with “@” had base substitution alterations within the second and third repeats that caused them to deviate from the consensus sequence, although the overall length was not affected. (b) The locations of insertions and deletions in the seven-repeat \textit{HRAS1} minisatellite tract strain DTK795 are shown.
or (3) the presence of the mismatches stabilizes the repeats during strand annealing and heteroduplex formation, preventing loop extrusion.

Examination of HIS4 recombination levels, as measured by the frequency of aberrant segregation at HIS4, argues against the first model. We find that aberrant segregation of the his4-lope heterozygous insertion allele is not significantly different between DTK611 (10/10m) and DTK751 (10/10) (Table 2). Deletion of the PMS1 gene in DTK611 increases tract instability (DTK611 vs. DTK763, Table 1), but aberrant segregation of his4-lope actually decreases, the opposite of the result expected if decreased heteroduplex formation is responsible for the decreased level of tract alteration observed in DTK611 (Table 2). In support of these results, a high level of gene conversion of the 3.4 and 4.1 heterozygositites (68%) was detected by sequence analysis in 19 tetrads from DTK611, indicating that these mismatches are incorporated into heteroduplex DNA at a high level, even though the tracts show a significant reduction in tract-length alterations. As the base heterozygositites have no readily scorable phenotype, the sequenced DTK611 tetrads represent a random sampling with respect to aberrant segregation of the 3.4 and 4.1 base heterozygositites. Sample bias could have arisen through choice of the tetrads that were sequenced (for example, due to the selection of tetrads in which at least one spore colony exhibited an altered tract length). However, 7 of the 8 tetrads that were chosen for sequencing specifically because they did not have any detectable tract alterations had gene conversion events at the base heterozygositites. These genetic data do not, however, rule out the possibility that recombination-initiating DSB formation differs in the heterozygous and homozygous strains.

Previous analysis of DTK314, containing the 30-repeat HRAS1 minisatellite allele, indicated that meiosis-specific DSBs formed 5' and 3' of the minisatellite insertion (Jauert et al. 2002). If, in the heterozygous strain, the frequency of the 5' BKI-proximal breaks was reduced while the normal frequency of the 3' HIS4-proximal breaks was maintained, minisatellite tract alterations could be reduced while his4-lope aberrant segregation would remain relatively unaltered, explaining the reduction in tract alteration without a reduction in his4-lope aberrant segregation.

The second model predicts that the repeats adjacent to the mismatches are less likely to be involved in an alteration event, as these repeats would be corrected efficiently if extruded as a loop. However, sequencing of altered tracts indicated that the distribution of alteration events is similar in DTK611 and DTK751 (Figure 4a). The locations of deletions in DTK751 and DTK611 are distributed across the entire minisatellite tract; only DTK758 (10/10 Δpms1) may exhibit a bias in deletions. Insertions are also found adjacent to the 3.4 and 4.1 heterozygositites in DTK611 (Figure 4a). If the mismatches were directing loop repair, one would expect that the intervals adjacent to the 3.4 and 4.1 heterozygositites would exhibit less tract-length alteration. The high level of gene conversion of the 3.4 and 4.1 heterozygositites (68%) indicates that these mismatches are recognized and repaired at a high level. This repair may include adjacent loops, but the repair would require a bias in direction such that two 7-repeat and two 10-repeat tracts are maintained, but with no directionality in gene conversion of the 3.4 and 4.1 heterozygositites. While the evidence does not support the second model, we cannot rule it out completely, as the number of
events in DTK611 and DTK751 that are directly relevant is relatively small.

Our data are consistent with the third model, in which loop formation is repressed in some manner by the presence of the base heterozygosities. The exact mechanism by which this suppression occurs is not evident from our data. However, DNA mismatch repair has been implicated in suppression of homeologous recombination—recombination between diverged sequences (reviewed in Borts et al. 2000; Harfe and Jinks-Robertson 2000). The role of PMS1 in homeologous recombination is complex, as PMS1 has been shown to be involved directly in some studies of homeologous recombination (Chambers et al. 1996), but in other studies it had no influence (Selva et al. 1995).

Our results demonstrate that a high level of tract conversion is detectable when there are length differences between the two alleles of the HRAS1 minisatellite tract. This finding is predicted by our model for tract alteration (Figure 1), which involves loop formation during heteroduplex DNA; the sequencing and Δspo11 data presented here further support this model. In strains heterozygous for tract length, heteroduplex formation involving the minisatellite tract should lead to loop formation, with loop size dependent on the difference in tract-length size. Thus, it might be expected that a strain heterozygous for length alleles would have an elevated level of tract alteration, observable as tract-length gene conversion events. Such gene conversions are not detectable in strains homozygous for a tract-length allele, but can be seen in heterozygous strains. We observed a high level of aberrant segregation of minisatellite tract length in DTK777 (10/7), DTK820 (10/7m), and DTK825 (10/7 Δrad1), with a majority of these events being gene conversions. If we eliminate those tetrads exhibiting only gene conversion events, considering only tetrads that altered to a nonparental length (Table 3), the frequency of alteration is reduced to 16% in DTK777, 19% in DTK820, and 23% in DTK825. In comparison, the alteration frequency in DTK751 (10/10) was 28% and in DTK795 (7/7) was 25% (Table 1). The only statistically significant deviation is between DTK777 and DTK751 (P = 0.02; altered vs. unaltered only). While a trend toward a lower frequency of nonparental length alterations can be seen in strains with homozygous length alleles, this result is not generally significant statistically, due to the small number of tetrads of the appropriate classes available for analysis. Thus, the high level of meiotic tract alterations in the heterozygous strains is the sum of the random formation of loops seen in homozygous length strains (which can lead to nonparental length tracts) plus the extrusion of loops during heteroduplex formation due to alignment of the unique DNA sequences that flank the heterozygous minisatellite tracts (which leads to parental tract-length gene conversion events).

The presence in DTK611 (10/10m) of HRAS1 mini-satellite tract-length alterations that are gene conversion events indicates that the factors acting on the extruded single-strand loop are not capable of discriminating an alteration repair event from a restoration event or, if capable, preferentially repair the loops to yield gene conversions. This finding is similar to other observations of gene conversion vs. restoration repair at the HIS4 locus. Gene conversion of base mismatches at the HIS4 locus exhibits a polarity gradient, with those at the 5'-end of the gene being preferentially repaired as gene conversion events and an increasing fraction being repaired as restoration events when the mismatch occurs farther from the site of initiation (reviewed in Nicolas and Petes 1994).

Our data demonstrate that length differences between the two alleles of the HRAS1 minisatellite tract lead to a high level of observable alteration during meiosis. The high level of alteration is not likely to be the result of an increase in the overall frequency of heteroduplex formation: the amount of aberrant segregation of his4-lopc is not statistically different in DTK751 (10/10: 52%), DTK795 (7/7: 60%), and DTK777 (10/7: 54%) (Table 2), and the heterozygous strain DTK777 does not exhibit the highest amount of aberrant segregation. A similar high level of tract-length alteration was detected in another yeast minisatellite model system, using 31- and 51-repeat alleles of the human MS205 tract, although there was significant internal repeat variation between the 31- and 51-repeat alleles that may have influenced stability (He et al. 2002) and complicates interpretation of the data. Additionally, the authors reported a high level of spore inviability that they attributed to uncorrected double-strand breaks. We observed no alteration in spore viability in any of our strains (data not shown).

In strains that are heterozygous for minisatellite tract-length alleles, the level of detectable tract-length alterations (tract conversions plus nonparental length alterations) (Table 3) is higher than the aberrant segregation of his4-lope in those same strains (Table 2). This difference may be due to the difference in distance between the recombination initiation site(s) and the minisatellite tract and the initiation site and the his4-lope insertion, with his4-lope being farther down the gradient of heteroduplex formation (Figure 1). It is possible that aberrant segregation events at his4-lope may also be generated only by heteroduplex tracts initiating from HIS4-proximal DSBs, while the minisatellite alterations may be generated by heteroduplex from both HIS4-proximal and BIK1-proximal DSB regions, depending on the length of the heteroduplex DNA tracts resulting from the different initiation events.

In conclusion, our data indicate that during meiosis the length and sequence heterogeneity of the two HRAS1 minisatellite alleles will have competing influences on the final structure of the tract that is included in a gamete’s genome. Human cells are likely to contain alleles that are hetero-
zygous for both length and base composition, as the most common allele, a1, is found only in ~50% of the population (Garrett et al. 1993). Factors that influence minisatellite tract length or sequence composition may potentially affect essential genes, including genes important in oncogenesis or development, as minisatellite tracts have been shown to influence the transcription of surrounding genes. For example, rearranged alleles of the HRAS1 minisatellite have been correlated with cancers, including primary tumors of the brain (Vega et al. 2001), lung (Rosell et al. 1999), ovaries (Weitzel et al. 2000), colon (Krontiris et al. 1993), bladder (Krontiris et al. 1993), and breast (Krontiris et al. 1993; Ding et al. 1999). Some studies also show a positive correlation between BRCA1 mutations, rare alleles of the HRAS1 minisatellite, and ovarian cancer incidence (Phelan et al. 1996). A link between the HRAS1 minisatellite and transcriptional activity of the HRAS1 gene has been demonstrated (Cohen et al. 1987; Spandidos and Holmes 1987). Different HRAS1 minisatellite alleles have differing enhancer activities, with some of the rare alleles having a stronger transcription enhancement in vitro than the common forms (Green and Krontiris 1993), impairing the rare alleles in cancer formation (Krontiris et al. 1985) possibly by H-ras protein level elevation through enhanced HRAS1 transcription. Our results identify DNA sequence elements that may contribute to the formation of rearranged and potentially oncogenic minisatellite tracts. Finally, our findings may be applicable to other minisatellites in the human genome, including some that have been associated with diseases, such as progressive myoclonus epilepsy (LaFreniere et al. 1997; Vrhtaneva et al. 1997) and insulin-dependent diabetes mellitus (Kennedy et al. 1995).

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