Note

A 160-bp Palindrome Is a Rad50·Rad32-Dependent Mitotic Recombination Hotspot in Schizosaccharomyces pombe

Joseph A. Farah,* Edgar Hartsuiker,† Ken-ichi Mizuno,‡ Kunihiro Ohta† and Gerald R. Smith*†

*Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109-1024; †Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton BN1 9RR, United Kingdom; and ‡Genetic Dynamics Research Unit-Laboratory, RIKEN Institute, Hirokawa 2-1, Wako, Saitama 351-01, Japan

Manuscript received October 1, 2001
Accepted for publication February 8, 2002

ABSTRACT

Palindromic sequences can form hairpin and cruciform structures that pose a threat to genome integrity. We found that a 160-bp palindrome (an inverted repeat of 80 bp) conferred a mitotic recombination hotspot relative to a control nonpalindromic sequence when inserted into the ade6 gene of Schizosaccharomyces pombe. The hotspot activity of the palindrome, but not the basal level of recombination, was abolished by a rad50 deletion, by a rad50S “separation of function” mutation, or by a rad32D25A mutation in the nuclease domain of the Rad32 protein, an Mre11 homolog. We propose that upon extrusion of the palindrome the Rad50·Rad32 nuclease complex recognizes and cleaves the secondary structure thus formed and generates a recombinogenic break in the DNA.

DNA sequences that can adopt secondary structures can be unstable when present in the genome (Leach 1994). Mini-satellites such as CTG repeats that can adopt hairpin-like structures as well as palindromic sequences are unstable in the bacterium Escherichia coli, the yeast Saccharomyces cerevisiae, and humans (Gordenin et al. 1993; Henderson and Petes 1993; Ruskin and Fink 1993; Sarkar et al. 1998; Richard and Paques 2000; Bzymek and Lovett 2001; Edelmans et al. 2001). Instability of such structures can be deleterious, as observed in E. coli and humans (Leach 1994; Edelmans et al. 2001).

Depending on their size and their location in the genome, palindromic sequences display different degrees of stability and recombination stimulation. This behavior is thought to be dependent on their propensity to extrude and thereby form hairpin loops or cruciform structures. In S. cerevisiae, short palindromes (26 bp) appear not to extrude during vegetative growth and are infrequently repaired in heteroduplex DNA formed during meiotic recombination (Nag et al. 1989). Palindromes of 60–160 bp (hereafter called middle-sized palindromes, or M-pals) are frequently excised from the genome during mitotic growth (Gordenin et al. 1993; Henderson and Petes 1993; Ruskin and Fink 1993). This reaction depends on the presence of small (4–9 bp) direct repeats in the vicinity of the M-pals and on the replication machinery. A 140-bp M-pal is also a site of a DNA double-strand break (DSB) during meiosis in S. cerevisiae (Nag and Kurst 1997). Although longer palindromes (L-pals, or palindromes >600 bp) are mitotic recombination hotspots in S. cerevisiae, M-pals have not been reported to display such an activity (Gordenin et al. 1993; Lobachev et al. 1998, 2000; Nasar et al. 2000). L-pal-dependent recombination hotspot activity in S. cerevisiae likely stems from the propensity of these sequences to extrude into hairpins or cruciforms and from their subsequent cleavage or processing by the Rad50·Mre11·Xrs2 complex (Lobachev et al. 2002). M-pal mitotic instability as well as M-pal-dependent mitotic DSB formation in S. cerevisiae argue that these sequences do extrude during mitotic growth as well as during meiosis. These observations suggest that, in S. cerevisiae, an extruded M-pal either is not detected by the mitotic recombination machinery (including the Rad50·Mre11·Xrs2 complex) or is recognized and processed by a nonrecombinogenic pathway.

Since meiotic recombination displays important differences in Schizosaccharomyces pombe and in S. cerevisiae (Fox and Smith 1998; Young et al. 2002), we have compared the behavior of an M-pal in S. pombe with that reported in S. cerevisiae. We found, as in S. cerevisiae, that an M-pal conferred a meiotic recombination hotspot and led to meiotic DSB formation (J. A. Farah, W. W. Steiner and G. R. Smith, unpublished data). We report here that the M-pal was also a strong mitotic recombinatio-
strain GP2638 to Ade6\(^-\) (red on limiting adenine EMM2 plates; Fox et al. 1997). To place the ade6-3034 and ade6-3036 alleles on an S. pombe replicative plasmid, SpeI-KpnI fragments of 3067 bp (ade6-3036 from plasmid pJF136) or 2987 bp (ade6-3034 from plasmid pJF134) were cloned into the BamHI-KpnI sites of vector pFY20 (noncompatible ends were blunted with the Klenow enzyme; Li et al. 1997) to give plasmids pJF138 and pJF141, respectively. The oligonucleotide corresponds to the mat-a-stk sequence from S. cerevisiae (positions 2044–2119 relative to GenBank sequence of the MATa locus; Ray et al. 1991). The inserted DNA is not drawn to scale. Both alleles are Ade\(^-\) .

Mitotic recombination associated with a 160-bp M-pal was measured both in a chromosome-by-chromosome system in diploid strains and in a plasmid-by-chromosome system in haploid strains. The alleles used are shown in Figure 1. Briefly, the ade6 alleles were constructed by inserting, at the unique BamHI site of the ade6 open reading frame, either one copy (the ade6-3034 control allele) or two copies in opposite orientation (the ade6-3036 M-pal allele) of an 80-bp oligonucleotide derived from the MATa locus of S. cerevisiae. These alleles were either integrated into the chromosomal ade6 locus or present on an S. pombe replicative plasmid. For scoring ade6\(^+\) recombinants these alleles were allowed to recombine with the ade6-469 allele present either on the pade6-469 plasmid in haploids (Szankasi et al. 1988) or on the homologous chromosome in diploids. Mitotic recombination rates were determined according to the method of the median (Lea and Coulson 1949).

We first determined mitotic recombination rates at ade6 in diploid rad\(^+\) strains. The ade6\(^+\) recombination rate in a strain containing the M-pal was 56-fold higher than that observed in a control strain: 280 recombination events per 10\(^6\) cell divisions compared to 5 recombination events per 10\(^6\) cell divisions for strains GP3486 (ade6-3036/ade6-469) and GP3484 (ade6-3034/ade6-469), respectively (Tables 1 and 2). Similarly, the ade6\(^+\) recombination rate in a haploid strain containing the M-pal on the chromosome was \(~\sim\)54-fold higher than that observed in a control strain: 700 \times 10\(^{-6}\) compared to 13 \times 10\(^{-6}\) for strains GP3019 (ade6-3036 pade6-469) and GP3017 (ade6-3034 pade6-469), respectively (Table 2). The latter recombination rate was comparable to the rate previously determined with equivalently spaced single-base-pair markers in ade6 (\(~\sim\)57 \times 10\(^{-6}\); Ponticelli et al. 1988). A chromosomal ade6 allele with two copies of the 80-bp fragment in a direct repeat orientation was also devoid of hotspot activity in the chromosome-by-plasmid recombination assay in a haploid strain (data not shown). Hence, a 160-bp M-pal in the ade6 gene was a strong mitotic recombination hotspot in S. pombe.

We next tested whether the M-pal-dependent hotspot activity was observed when the M-pal was present on a multicopy plasmid. Plasmids pJF138 (ade6-3036 M-pal) and pJF141 (ade6-3034 control) were introduced into strain GP2947 (with the ade6-469 allele on the chromosome; Table 1). Transformants with the control plasmid (pJF141) showed a recombination rate at ade6 (\(~\sim\)7 \times 10\(^{-6}\)) that was 4- to 19-fold lower than that of transformants with the M-pal-containing plasmid (pJF138; Table 3). M-pal transformant T1 gave a value of 135 \times 10\(^{-6}\), while M-pal transformant T2 gave a recombination rate of 26 \times 10\(^{-6}\).
The nature of the difference between these two transformant types is not clear, but the higher-frequency T1 type is more common. Among 12 additional transformants, 11 behaved like T1 and one like T2. Upon extraction and analysis of plasmids from the T1-like and T2-like transformants, no restriction site or sequence differences could be detected between the two (data not shown). Transformants of strain GP2947 with the plasmids extracted from the T1-like and T2-like transformants showed ade6 recombination frequencies similar to those of T1. Hence, the difference in the recombination rates between T1 and T2 is not a heritable property of the plasmid; it may stem from an epigenetic change in the plasmid or a genetic change in the host strain upon the initial transformation. Nevertheless, the plasmid-borne M-pal was a mitotic recombination hotspot when present on an extrachromosomal plasmid.

In summary, the results of Tables 2 and 3 clearly showed that, in an otherwise wild-type background, an M-pal was a mitotic recombination hotspot in S. pombe whether present on the chromosome or on a plasmid, although the hotspot activity was lower in the latter situation than in the former. These results suggest that the secondary structure adopted by the 160-bp M-pal is responsible for the observed hotspot activity at ade6.

One possibility is that the M-pal forms a hairpin structure that is recognized and cleaved by a nuclease, thus generating a recombinogenic lesion such as a DSB. In E. coli, palindrome-dependent inviability is dependent on the SbcCD complex (Leach 1994). This complex cleaves hairpin loops in vitro (Connelly et al. 1998). A related complex in eukaryotes, Rad50•Mre11•Xrs2 (Nbs1), is involved in DNA-damage repair and meiotic recombination (Iohzuka and Ogawa 1995; Haber 1998). The human Rad50•Mre11•Nbs1 complex and the yeast Rad50•Mre11 complex are also nucleases that cleave hairpin DNA in vitro (Paul and Gellert 1999; Trujillo and Sung 2001). The overall architecture of these complexes involves the association of a structural-maintenance-of-chromosomes-type subunit (SbcC or Rad50) with a phosphoesterase enzyme (SbcD, Mre11, or Rad50). Transformants of strain GP2947 with the plasmids consists of transforming in vitro (Paul and Gellert 1999; Trujillo and Sung 2001). The overall architecture of these complexes involves the association of a structural-maintenance-of-chromosomes-type subunit (SbcC or Rad50) with a phosphoesterase enzyme (SbcD, Mre11, or Rad50). Transformants of strain GP2947 with the plasmids extracted from the T1-like and T2-like transformants showed ade6 recombination frequencies similar to those of T1. Hence, the difference in the recombination rates between T1 and T2 is not a heritable property of the plasmid; it may stem from an epigenetic change in the plasmid or a genetic change in the host strain upon the initial transformation. Nevertheless, the plasmid-borne M-pal was a mitotic recombination hotspot when present on an extrachromosomal plasmid.
M-pal-dependent recombination hotspot activity and rad gene dependence in diploid and haploid strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>ade6 chromosomal alleles</th>
<th>Genetic background</th>
<th>Recombination rate (events per 10^6 divisions)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Experiment 1</td>
</tr>
<tr>
<td>GP3017</td>
<td>ade6-3034</td>
<td>+</td>
<td>12 (10)</td>
</tr>
<tr>
<td>GP3019</td>
<td>ade6-3036</td>
<td>+</td>
<td>720 (10)</td>
</tr>
<tr>
<td>GP3219</td>
<td>ade6-3034 rad50S</td>
<td></td>
<td>10 (9)</td>
</tr>
<tr>
<td>GP3220</td>
<td>ade6-3036 rad50S</td>
<td></td>
<td>19 (9)</td>
</tr>
<tr>
<td>GP3125</td>
<td>ade6-3034 rad50::kanMX6</td>
<td></td>
<td>14 (9)</td>
</tr>
<tr>
<td>GP3127</td>
<td>ade6-3036 rad50::kanMX6</td>
<td></td>
<td>11 (9)</td>
</tr>
<tr>
<td>GP3285</td>
<td>ade6-3034 rad32-D25A</td>
<td></td>
<td>19 (9)</td>
</tr>
<tr>
<td>GP3287</td>
<td>ade6-3036 rad32-D25A</td>
<td></td>
<td>9 (9)</td>
</tr>
<tr>
<td>GP3216</td>
<td>ade6-3034 rad51::his3+</td>
<td></td>
<td>3 (9)</td>
</tr>
<tr>
<td>GP3259</td>
<td>ade6-3036 rad51::his3+</td>
<td></td>
<td>57 (9)</td>
</tr>
<tr>
<td>GP3484</td>
<td>ade6-3034</td>
<td>+/+</td>
<td>3 (9)</td>
</tr>
<tr>
<td>GP3486</td>
<td>ade6-3036</td>
<td>+/+</td>
<td>270 (9)</td>
</tr>
<tr>
<td>GP3600</td>
<td>ade6-3034 rad50S/rad50S</td>
<td></td>
<td>7 (7)</td>
</tr>
<tr>
<td>GP3601</td>
<td>ade6-3036 rad50S/rad50S</td>
<td></td>
<td>11 (7)</td>
</tr>
</tbody>
</table>

ND, not done.

a Isolated colonies were inoculated into 5 ml of Difco nitrogen-base minimal liquid medium with the appropriate additives (for the haploid strains) or yeast extract liquid (for the diploid strains). The cultures were incubated for 2 or 3 days at 30°C, at which point 1-ml aliquots were washed twice with water and appropriate dilutions plated on Difco yeast extract agar (YEA) for total viable cell counts and on YEA + guanine (80 μg/ml) for Ade^+ recombinant counts (Cummins and Mitchison 1967). Alternatively, cells were plated on nitrogen-base minimal agar (NBA) with appropriate additives for total viable cell counts and on NBA-adenine + guanine for Ade^+ recombinant counts. Plates were incubated at 32°C. Typically, the total number of cells in the cultures ranged between 10^7 and 10^8, depending on the strain. Experiments 1 and 2 were done on different days. Recombination rates were measured according to the method of the median (Lea and Coulson 1949) with the number of cultures noted in parentheses; for even numbers of cultures, the median was the mean of the two medial values.

b Mean of experiment 1 and experiment 2. When only one experiment was performed, the values obtained were reported in that column.

c The fluctuation tests were based on independent cultures of one transformant for each strain.

The indicated haploid strain was transformed with plasmid pade6-469, which carries the ade6 allele as the selection marker (Szankasi et al. 1988; see Figure 1).

The ade6-469 allele was present on the second homolog.

tray et al. 2001). On the three-dimensional structure of the Pyrococcus furiosus Rad50 ATP-binding domain, the rad50S mutations cluster to a region of the protein that may interact with other proteins (Hopfner et al. 2000).

An attractive view is that the S. pombe Rad50•Rad32 complex is directly responsible for the cleavage of the hairpin formed by the extrusion of the M-pal. Although a complex between Rad50 and Rad32 has not been reported in S. pombe, we infer such a complex by analogy to the S. cerevisiae and human homologs. We first tested whether the M-pal-dependent mitotic recombination hotspot was dependent on the Rad50 protein in S. pombe and found it to be (Table 2). In the M-pal haploid strain GP3127 (ade6-3036 rad50Δ pade6-469) the ade6^+ recombination rate (13 × 10^-6) was very close to those of the control strains GP3017 (ade6-3034 rad50^+ pade6-469; 13 × 10^-6) and GP3125 (ade6-3034 rad50Δ pade6-469; 16 × 10^-6) with the nonpalindromic insertion at ade6. Hence, in the absence of the Rad50 protein, the hotspot activity of the M-pal was eliminated but the basal recombination rate was not greatly affected.

To test whether the M-pal-dependent hotspot was dependent on particular functions of the Rad50•Rad32 complex, we measured ade6^+ recombination rates in the presence of the non-null alleles rad50S and rad32-D25A (with an Asp-to-Ala change at the highly conserved position 25 in esterase motif I). The M-pal-dependent hotspot effect, but not the basal recombination level, was abrogated in these two mutant backgrounds. The M-pal haploid strains GP3290 (ade6-3036 rad50S pade6-469) and GP3287 (ade6-3036 rad32-D25A pade6-469)
showed ade6+ recombination rates of 19 × 10⁻⁴ and 11 × 10⁻⁴, respectively, which are not very different from the basal rates measured in the respective control strains GP3219 (ade6-3034 rad50 S pade6-469; 10 × 10⁻⁶) and GP3285 (ade6-3034 rad32-D25A pade6-469; 15 × 10⁻⁶) without M-pal. Similar results were also observed with diploid strains homozygous for the rad50 S allele (Table 2). The M-pal-dependent recombination hotspot was eliminated in strain GP3601 (ade6-3034/ade6-469 rad50 S/rad50 S) with a recombination rate (11 × 10⁻⁴) similar to that of the control strain GP3600 (ade6-3034/ade6-469 rad50 S/rad50 S; 8 × 10⁻⁶).

Taken together, the above results suggest that a nuclease-proficient Rad50×Rad52 complex is necessary for the recombination hotspot activity of the M-pal inserted in the ade6 gene of S. pombe. Although the S. pombe Rad32-D25A polypeptide was not tested directly for nuclease activity in vitro, the S. cerevisiae Mre11-D16A polypeptide (with the same amino-acid change at the homologous position as in Rad32-D25A) shows no in vitro nuclease activity despite wild-type affinity for DNA binding (FURUHE et al. 1998).

If the recombination hotspot is due indeed to nuclease cleavage of the M-pal and DSB formation at that site, one prediction, according to two DSB repair models (RESNIK 1976; SZOSTAK et al. 1985), is that the M-pal allele should be a recipient of wild-type information when recombining nonreciprocally with the ade6-469 allele. Because the 102 ade6+ recombinants analyzed from strains GP3484 and GP3486 (experiments 1 of Table 4) segregated red colonies upon sporulation (data not shown), we conclude that these recombinants were heterozygous diploids (ade6+/ade6−). Since the majority of these had lost the insertion (see below), it is reasonable to assume that ade6+ recombinants derive from nonreciprocal recombination (gene conversion). We determined the frequency of conversion of the ade6-3036 (M-pal) and the ade6-3034 (control) alleles in rad6+ diploid strains (Table 4). In strain GP3486 (ade6-3036/ade6-469), the M-pal allele was converted to wild type with a frequency of ~98%, significantly higher than the conversion of the ade6-3034 control allele in strain GP3484 (ade6-3034/ade6-469, 70%, contingency χ² = 28, P ≤ 0.001). The ade6+ conversion frequency in strain GP3484 (ade6-3034/ade6-469, 70%) was higher than 50%, the expected value if there were no bias for conversion between the two recombining alleles. One explanation for this bias could be due to the nature of the ade6-3034 allele, an insertion, that could be recognized and eliminated more efficiently than a point mutation in heteroduplex DNA by the mismatch repair or the nucleotide-excision repair machinery of the cell. Hence, the M-pal had a tendency to favor its own conversion to wild type as predicted.

A second prediction is that in a rad mutant that abolishes the hotspot activity of the M-pal, the conversion frequency of both the M-pal and the control allele should be similar, with no preference for either being converted to wild type. This was indeed the case when conversion frequencies were determined in the diploid strains homozygous for rad50 S (Table 4). Strain GP3601 (ade6-3036/ade6-469 rad50 S/rad50 S) converted the M-pal

### TABLE 4

<table>
<thead>
<tr>
<th>Diploid strain</th>
<th>ade6 chromosomal alleles</th>
<th>Genetic background</th>
<th>Conversion of ade6-3034 and ade6-3036 (% of total Ade+ tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP3484</td>
<td>ade6-3034</td>
<td>+/+</td>
<td>34/51 (67)</td>
</tr>
<tr>
<td>GP3486</td>
<td>ade6-3036</td>
<td>+/+</td>
<td>50/51 (98)</td>
</tr>
<tr>
<td>GP3600</td>
<td>ade6-3034</td>
<td>rad50 S/rad50 S</td>
<td>29/50 (58)</td>
</tr>
<tr>
<td>GP3601</td>
<td>ade6-3036</td>
<td>rad50 S/rad50 S</td>
<td>31/50 (62)</td>
</tr>
</tbody>
</table>

*The ade6-469 allele was present on the second homolog.

*Mean percentage from experiment 1 and experiment 2.

*Number of convertants among the number of Ade+ recombinants analyzed. Conversion of the ade6-3034 and ade6-3036 alleles was determined by PCR amplification of DNA from Ade+ colonies using primers qF57 and qfF185 (Figure 1). The PCR fragments from the mutant alleles were distinguished from each other and from the wild-type fragment by electrophoresis on a 1.5% agarose gel.
cleavage or processing ensues (block of steps 2 and 3). In the rad51 deletion background, the major pathway for strand exchange (step 4, thick arrow) is abrogated, but minor rad51-independent pathways (thin arrow) allow lower efficiency recombination. In blocking steps 1 or 2 lagging strand DNA synthesis is expected to be halted at the secondary structure and could resume either when the hairpin unfolds or when the replication machinery “slips” past it. In the latter case, the M-pal is expected to be deleted from the genome (but see text).

67% of the time, a frequency similar to that of the control allele in strain GP3600 (ade6-3034/ade6-469 rad50S/rad50S, 53%, contingency $\chi^2 = 3.5$, 0.05 < $P < 0.1$). Hence, in the absence of hotspot activity the M-pal allele was not converted preferentially to wild type. These results strongly suggest that the Rad50•Rad32 complex recognizes and cleaves the extruded M-pal. The DSB ends thus formed are subsequently processed (by trimming the nonhomologous extremities) and recombined with a homologous sequence with concomitant loss (conversion) of the M-pal insertion. In the rad50S mutant, the Rad50S•Rad32 complex cannot cleave the extruded M-pal, thus eliminating both the hotspot activity and the preferential conversion of that allele.

The involvement of the nuclease activity of Mre11 in mitotic DNA repair and recombination has been questioned on the basis of results obtained with certain S. cerevisiae esterase motif mutants. Some of these mutants with an Mre11 polypeptide devoid of detectable nuclease activity in vitro have no defect in the mating-type conversion reaction and are significantly more resistant to ionizing radiation than mre11Δ strains (Moreau et al. 1999). However, we favor a direct role of the nuclease activity of the Rad50•Rad32 complex in the M-pal-dependent hotspot effect. The nuclease of the Rad50•Rad32 complex might be active on DNA substrates with secondary structures such as palindromes or microsatellites that might be rare in the genome but could form accidentally upon replication slippage or illegitimate recombination (Moore et al. 1999; Richard and Paques 2000). Perhaps such sequences are processed by the nuclease activity of the Rad50•Rad32 complex in an attempt to overcome their deleterious effects (Richard et al. 2000). In our system, where an artificial M-pal was introduced into the cell, this processing would result in the formation of a DSB at the M-pal and the recombination hotspot effect. Hence, one function of the Rad50•Rad32 complex could be to protect the genome from sequences that can form secondary structures known to cause genome instability.

The hotspot observed above could, however, be due to a less direct action of the Rad50•Rad32 complex on the M-pal. For instance, recombinogenic lesions could arise by a Rad50•Rad32-independent mechanism at the same rate on M-pal-containing and nonpalindromic alleles, but the subsequent processing of the lesion could favor recombination only with the M-pal-containing allele, thereby giving a higher recombination rate at ade6. In this case, it is reasonable to assume that the hotspot activity of the M-pal would be dependent on gene products acting at steps subsequent to the initial lesion. We therefore determined whether the hotspot effect of the M-pal depended on the rad51+ gene product (also called rhp51+; Table 2). The Rad51 protein is an S. pombe homolog of the S. cerevisiae Rad51 protein involved in DNA pairing and strand exchange between recombining DNA molecules, a step subsequent to the initial lesion (Muris et al. 1993; Sung 1994). The recombination rate in haploid strain GP3259 (ade6-3036 rad51Δ pade6-469; 50 × 10⁻¹¹) was 14-fold lower than that in strain GP3019 (ade6-3036 rad51Δ pade6-469; 700 × 10⁻¹¹; Table 2) but still significantly higher than that in strain GP3216 (ade6-3034 rad51Δ pade6-469) with the nonpalindromic substrate, $3 \times 10^{-11}$, near the limit of reliability. Hence, despite the dramatic decrease in the ade6 recombination rates in the rad51 deletion strains, an M-pal-dependent hotspot activity of at least 17-fold was still
present in this genetic background. These results reinforce the notion that the Rad50•Rad32 complex acts directly on the secondary structure of the M-pal, perhaps by generating a lesion that is subsequently processed to a DSB.

In the model in Figure 2, opening of the DNA helix during DNA replication allows extrusion of the M-pal on the less processively synthesized lagging strand (Trinh and Sinden 1991). Such a structure, which could stall the replication machinery and lead to breakage of the replication fork, could be processed by structure-specific nucleases (Leach 1994). The Rad50•Rad32 complex may accomplish that task by first binding (step 1) and then cleaving (steps 2 and 3) the secondary structure. A DSB that is repaired by recombination with a sister chromatid with retention of the M-pal ensues, as has been inferred in E. coli (step 4; Leach et al. 1997).

Alternatively, the DSB can be repaired by recombination at high rate with a homologous plasmid or chromosome, thus displaying the hotspot activity described above. In the rad50Δ background, we propose that the Rad50•Rad32 complex is not properly targeted or bound to the hairpin or is not active on it (block at step 1). Because rad50Δ cells show near normal vegetative growth in contrast to rad50 deletion strains (Hartsuiker et al. 2001; E. Hartsuiker, unpublished observations), the Rad50•Rad32 complex appears to fulfill most of its other tasks in the cell. Only when special DNA features such as M-pals or special recombination substrates are present in the genome does a rad50Δ strain display a noticeable phenotype during vegetative growth (this work and Rattray et al. 2001). Alternatively, the Rad50 protein might control the nuclease activity of Rad32, and in the rad50Δ background a partial deficiency in that control might inhibit the activation of the nuclease at the M-pal, thus abrogating the hotspot. In the rad32Δ-d25α background, the Rad50•Rad32 complex may bind to the hairpin but be unable to cleave or process it (block at step 2), thereby leaving this structure intact. Finally, in the rad51 deletion background, the main pathway for DNA pairing and strand exchange is abolished (step 4), but minor Rad51-independent pathways still allow some recombination to occur without affecting the hotspot activity that is dependent on earlier events (steps 2 and 3).

An additional issue is the stability of M-pals in S. pombe. In the budding yeast S. cerevisiae, M-pals are unstable during mitotic growth and are excised from a plasmid or from the chromosome at a high rate (Henderson and Petes 1993; Ruskin and Fink 1993). The excision rate is increased in the presence of temperature-sensitive alleles of POL1 (encoding Polα; Ruskin and Fink 1993) or POL3 (encoding Polβ) at the semirestrictive temperature (Gordenin et al. 1993), suggesting that M-pal excision is intimately linked to replication on the lagging strand (Morrison et al. 1990).

To determine M-pal excision in S. pombe, we measured ade6Δ reversion rates. Strains GP3017 (ade6-3034) and GP3019 (ade6-3036), with no plasmid present, were plated on Ade+ selective plates. For both strains, the ade6Δ reversion rate was <1.3 × 10⁻⁸ (95% confidence limit). Hence, the M-pal seemed stable when present in the chromosome, although 4-bp direct repeats flanked the M-pal, and DNA polymerase slippage at these repeats was expected to restore a wild-type ade6Δ sequence (Ruskin and Fink 1993). These 4-bp repeats might not be long enough, however, to allow polymerase slippage.

Although some biological processes are conserved between budding yeast and fission yeast, it is becoming increasingly clear that others are regulated differently despite conservation of the proteins involved (Forsburg 1999). The behavior of the M-pal may be an example of this difference: although the 160-bp M-pal is a meiotic hotspot and a site of meiotic DSB (J. A. Farah, W. W. Steiner and G. R. Smith, unpublished data), as expected from work in S. cerevisiae, a mitotic recombination hotspot at an M-pal has not been reported in the budding yeast.

We thank Sue Amundsen, Luther Davis, Harmit Malik, Walt Steiner, and Andrew Taylor for critical reading of the manuscript, and R. Krachenbuehl and Jürg Kohli for strains. This work was supported by National Institutes of Health grant GM-32194 to G. R. Smith.

LITERATURE CITED


Hartsuiker, E., E. Vaessen, A. M. Carr and J. Kohli, 2001 Fission...
yeast Rad50 stimulates sister chromatid recombination and links cohesion with repair. EMBO J. 20: 6660–6671.


Communicating editor: L. S. Symington