Meiotic Deletion at the BUF1 Locus of the Fungus Magnaporthe grisea Is Controlled by Interaction With the Homologous Chromosome

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Manuscript received August 3, 2000
Accepted for publication October 9, 2001

ABSTRACT

The Magnaporthe grisea BUF1 gene suffers high-frequency mutation in certain genetic crosses, resulting in buff-colored progeny. Analysis of 16 buf1 mutants arising from a cross with a mutation frequency of 25% revealed that, in every case, the BUF1 gene was deleted. The deletions occurred in only one of the parental chromosomes and were due to intrachromosomal recombination. Tetrad analysis revealed that deletions occurred in 44% of meioses and usually affected both chromatids of the mutable chromosome. This suggests that they happen before the premeiotic round of DNA synthesis. However, they were also almost entirely restricted to heteroallelic crosses. This, together with the discovery of numerous repetitive elements that were present only in the mutable BUF1 locus, suggests that the deletion process is sensitive to pairing interactions between homologous chromosomes, such that only unpaired loci are subject to deletion. Given that karyogamy is not supposed to occur until after premeiotic DNA replication in Pyrenomycetous fungi such as M. grisea, this latter observation would place the time of deletion during, or after, DNA synthesis. These conflicting results suggest that karyogamy might actually precede DNA replication in Pyrenomycetous fungi or that parts of the genome remain unreplicated until after karyogamy and subsequent chromosome pairing have taken place.

Our current knowledge of the types of mutations that can result from erratic behavior of the meiotic machinery has come largely from studies of recombination in Saccharomyces cerevisiae. Along with a number of other ascomycetes, S. cerevisiae is well suited to such studies because the meiotic tetrad is retained in an ascus, which allows each product of meiosis to be analyzed. This can provide information on the frequency, timing, and strand specificity of mutational events and, hence, may provide clues to their underlying mechanisms. For example, analyses of meiotic tetrads obtained from a number of different specially constructed S. cerevisiae diploids reveal that most mutations arising during the S. cerevisiae sexual cycle occur after DNA replication and are a result of ectopic crossovers between duplicated genes or repeated DNA sequences (Peters and Hill 1988). Ectopic recombination also appears to be a common reason for mutation during gametogenesis of other organisms and has been associated with a number of mutations occurring in Drosophila (Goldberg et al. 1983) and in humans (Palau et al. 1993; Horstemke et al. 1996). In some organisms, the induction of a sexual cycle results in the appearance of mutant progeny at a frequency that is much greater than that expected from aberrations of normal meiotic recombination. In Drosophila, certain loci exhibit extremely high levels of meiotic instability as a symptom of the hybrid dysgenesis syndrome. The mutational events associated with hybrid dysgenesis are often premeiotic (Engels 1989). However, it is arguable that the processes causing hybrid dysgenesis are not integral to the sexual cycle per se,
because they occur as a result of germline-specific activation of transposons, which cause various chromosome aberrations during their excision and reinsertion (Preston et al. 1996). In a number of filamentous fungi, there are also mutational mechanisms that operate in the premeiotic phase of the sexual cycle. These are repeat-induced-point mutation (RIP), which occurs in Neurospora crassa (Cambareri et al. 1989); methylation induced premeiotically (MIP) in Asperobulus immersus (Rhounim et al. 1992) and Coprinus cinereus (Freeman and Pukkila 1993); and changes in nucleolar organizer region (NOR) size, also found in N. crassa (Butler and Metzenberg 1989). RIP and MIP operate on sequences that have become duplicated either spontaneously or by genetic transformation. The action of RIP causes G to A and C to T mutations and is associated with de novo methylation of cytosine residues (Selker et al. 1987). MIP, on the other hand, causes only DNA methylation (Rhounim et al. 1992). Unlike hybrid dysgenesis, these processes appear to be more intimately tied into the mechanics of sexual development. This is because they occur in specialized dikaryotic cells that are formed after fertilization and they operate prior to the premeiotic round of DNA replication (premeiotic S-phase). In addition, at least two of the processes (RIP and MIP) involve genome-wide searches for sequence duplications, a procedure that appears to represent a monitoring of meiotic eligibility. Another mutagenic process in N. crassa is temporally associated with RIP and affects tandemly duplicated sequences. Tandem repeats are not only particularly sensitive to the effects of RIP but are also frequently deleted (Selker et al. 1987). This loss of DNA also occurs before premeiotic S-phase (Selker et al. 1987), as do nonreciprocal size changes in the N. crassa NOR (Butler and Metzenberg 1989). In both cases, intrachromosomal recombination events are responsible for the associated deletion events.

When studying de novo mutations that arise during the sexual cycle, it is important to consider that meiosis can be responsible for the associated deletion events. Of chromosome instability during meiosis.

**MATERIALS AND METHODS**

**Fungal strains:** The parental strains Guy11 and 2539 and 61 of their random ascospore progeny were used in the construction of a genetic map and have been described previously (Leung et al. 1988; Skinner et al. 1993).

**DNAs:** Overlapping cosmids A13-2-H and A22-7-E were kindly provided by S. Kang. These clones contain the BUF1 gene of M. grisea strain 4091-5-8, which is an ancestor of 2539 (Leung et al. 1988). Cosmids containing the BUF1 gene of strain 2539 and clones containing flanking DNA were isolated from a genomic library of 2539 DNA, constructed in vector pMLF2 (An et al. 1996).

**Genetic crosses:** Fungal strains were crossed by placing mycelial plugs 4 cm apart on oatmeal agar plates and growing them at 18° under constant fluorescent white light illumination. After 3–4 weeks, perithecial beaks emerged from within the zone of confluence between the colonies, indicating the onset of ascus development.

**Isolation of random ascospores:** With the aid of a stereomicroscope, a scalpel was used to excise perithecial bulbs from beneath the surface of the agar in the zone of confluence.
Bulbs were transferred to 4% water agar and cut open with a scalpel to release their contents. Aci were spread across the agar surface using a drawn-out and sealed glass capillary tube. After 16 hr, germinated aci were individually excised, transferred to fresh oatmeal agar plates, and grown until conidia were formed. The colonies were brushed lightly with a sealed Pasteur pipette to pick up conidia, which were then spread across the surface of water agar plates. After 16 hr, a single, germinated conidiuni was isolated from each ascus culture. Colonies derived from these single spore isolates represent independent meiotic products.

Isolation of tetrad progeny: Aci were spread across the surface of 4% water agar as described above. After 20 min, aci in which individual ascospores were discernible were massaged gently with a glass rod until they broke open. Using a drawn-out glass capillary tube, the eight ascospores were then spread out in a line. After 16 hr, ascospores containing the individual germinated ascospores were excised with a scalpel and transferred to fresh oatmeal agar plates. Most of aci did not yield eight viable ascospores. However, when at least seven ascospores were recovered, it was possible to infer the genotype of the eighth spore from its mitotic twin (assuming postmeiotic spores were recovered, it was possible to infer the genotype of BUF1 that exhibited a buff colony color. This phenotype would have resulted in a segregation ratio of 27:32.

Romao et al. (1990) was used to confirm that the four chosen spores represented a true tetrad. Analysis of RFLP haplotypes for several markers either side of the BUF1 locus and demonstrated that, in every case, the mutation was caused by deletion of the entire gene. It is important to note that the buf1 progeny did not show unusual segregation for >200 other RFLP markers, with the exception of CH3-24H, which maps distal to the BUF1 gene (Nitta et al. 1997).

Segregation analysis of the BamHI RFLP identified by the BUF1 probe (Figure 1) revealed that 32 progeny had inherited the 2539 allele, 11 possessed the Guy11 allele, and 2 had inherited both alleles. The genetic map location of BUF1 in the Guy11 × 2539 cross is also near the rDNA cluster on chromosome 2 (Figure 2A), at a distance of 11 cm.

Analysis of flanking markers revealed that 14 of the 16 buf1 progeny had inherited the RFLP markers on either side of the BUF1 gene from Guy11 (Figure 2B). A more detailed analysis of sequences flanking the deletion breakpoints revealed that this was also true for the two remaining progeny (results not shown). These results indicate that all the deletions had occurred in the Guy11 chromosome, suggesting that the BUF1 locus of Guy11 may have structural features that make it inherently unstable. The corresponding locus in 2539 appears to be relatively stable, as I have yet to recover any BUF1 mutations in the 2539 chromosome. If one assumes that the deleted loci should have been Guy11 alleles, this would have resulted in a segregation ratio of 27:32 (Guy11:2539 allele), which is not significantly different from 1:1 ($\chi^2 = 0.42, P = 0.50$), the ratio expected for single gene segregation in a haploid organism like M. grisea.

A possible explanation for absence of the BUF1 gene in ~25% of random ascospore progeny is that it resides...
on a chromosome segment that has been translocated to a different chromosome in one of the parental genomes. However, the distribution of BUF1 genotypes in the random progeny population provided evidence against an interchromosomal translocation. Specifically, the observed ratio of 11:32:2:16 (Guy11 allele:2539 allele:both alleles:null allele) is significantly different ($\chi^2 = 31.13; P \leq 0.001$) from the 1:1:1:1 ratio expected of this type of translocation. In particular, the number of duplication class progeny was smaller than expected and the progeny with the 2539 allele were too abundant.

**The meiotic stability of the Guy11 BUF1 locus is dictated by the homologous chromosome:** A preliminary analysis of the physical organization of the two BUF1 loci revealed an extremely high level of restriction site polymorphism (see below), which led me to consider the possibility that instability at the Guy11 BUF1 locus may be due to improper pairing between the homologous BUF1 loci. This hypothesis was tested by determining if buf1 mutants appeared at a lower frequency in crosses that are homoallelic for BUF1$^{\text{Guy11}}$ (i.e., when both strains in the cross possessed the Guy11 BUF1 allele). For the crosses described below, none of the buf1 mutants were analyzed at the molecular level. Therefore, an assumption was made that the BUF1 gene was deleted in each mutant and, also, that only the BUF1$^{\text{Guy11}}$ allele was affected. However, this assumption is reasonable because, in related studies, >150 buf1 progeny have been analyzed at the molecular level and all of them suffered deletion of the Guy11 BUF1 allele (Y.-S. Kim and M. Farman, unpublished results).

Unfortunately, fertility in the F1 generation was sometimes very low and not all matings yielded viable progeny. However, in three cases, the fertility was sufficient to be able to use a strain carrying the BUF1$^{\text{Guy11}}$ allele as a common parent in both a homoallelic and a heteroallelic cross. In these cases, the BUF1$^{\text{Guy11}}$ allele was highly unstable when in a heteroallelic condition but was transmitted perfectly through the homoallelic crosses. For example, 16 mutants were found among 61 progeny of the original Guy11 $\times$ 2539 cross; there were 7 out of 38 in Guy11 $\times$ 6005; and 9 out of 64 from Guy11 $\times$ 2539.

**Figure 2:**—Map location of the BUF1 gene and inheritance of flanking markers. (A) The location of BUF1 on chromosome 2, relative to previously mapped RFLP markers. The horizontal line shows the distance in centimorgans. (B) The parentage of various portions of chromosome 2 in buf1 progeny, as determined by analysis of RFLPs. Deletion of the BUF1 gene is indicated by a solid vertical bar at the appropriate chromosome position (arrow). Another deletion in progeny 6099, encompassing marker CH2-90H, is shown as a black box over the affected marker. Open bar, inherited from Guy11; shaded bar, inherited from 2539.
6008 (Figure 3). However, when Guy11 was used in a backcross with strain 6077, both BUF1*arm alleles were transmitted faithfully through meiosis and no buf1 mutants were recovered among 80 progeny analyzed (Figure 3). Similar behavior was observed for the BUF1*arm allele in strains 6000 and 6089. Pooling of data for all crosses according to whether they were heteroallelic or homoallelic for BUF1*arm showed that 57 buf1 mutants were recovered from 170 heteroallelic meioses (33.5%), whereas 291 homoallelic meioses yielded just 1 (0.3%). Therefore, heteroallelism caused a 100-fold increase in meiotic instability. No buf1 mutants were recovered in crosses that were homoallelic for the BUF1*arm allele.

The recovery of a single buf1 mutant from the Guy11 × 6089 cross was atypical. It is possible that this was a result of mitotic deletion in a sector of one of the parent colonies. A preliminary determination of the mitotic deletion frequency of BUF1*arm yielded one mutant in 765 asexual conidiospores (0.13 ± 0.26%, 0.95 confidence interval). Thus, the conclusion that a mitotic event may have given rise to the mutant in the Guy11 × 6089 cross is a reasonable one. Regardless of its mechanism, the frequency of deletion in the Guy11 × 6089 cross was very low compared to crosses that were heteroallelic for BUF1.

Extensive structural polymorphism between the BUF1 loci of the parental strains Guy11 and 2539: Valent and Chumley (1994) previously reported that the mitotically and meiotically unstable BUF1 allele in strain O-137 was embedded in a chromosome region consisting largely of repetitive DNA. This seemed a likely explanation for the meiotic instability of the gene in Guy11. To initiate physical analysis of the BUF1 locus organization in strains Guy11 and 2539, I used cosmids from a contig spanning the 2539 BUF1 locus to perform Southern hybridization analysis of the parental genomic DNAs. The genomic organization of the cosmids is shown in Figure 4A. Cosmids A13-2-H and A22-7-E are overlapping clones that both contain the BUF1 gene of M. grisea strain 4091-5-8 and cosmids 11-11-N and 4-1-E were obtained by chromosome walking with a cosmid library of 2539 DNA. Strain 2539 is related to 4091-5-8 by descent and both strains possess the same BUF1 locus (M. Farman, unpublished results). Therefore, all four of the cosmids used as probes faithfully represent the BUF1 locus structure in the 2539 genome.

The two cosmids that contain the BUF1 gene (A13-2-H and A22-7-E) revealed many RFLPs between the Guy11 and 2539 BUF1 loci (Figure 4B). In fact, these loci shared very few fragments of identical sizes, suggesting that the physical organization of the BUF1 loci in the two parental strains is very different. In addition, A13-2-H hybridized to fewer fragments in DNA of the parental strain Guy11, revealing the presence of an ancestral deletion in the Guy11 genome. This was con-
firmed by hybridization with the overlapping cosmid 11-11-N (Figure 4A), which did not hybridize to Guy11 DNA at all (Figure 4B). The next overlapping clone, 4-1-E, did hybridize to DNA of Guy11 (results not shown) and, therefore, appeared to define the left boundary of the region that is deleted in the Guy11 parent. However, detailed physical mapping studies have indicated that the sequences in the Guy11 genome that hybridized to 4-1-E are not contiguous with the sequences hybridizing to A13-2-H (results not shown), indicating that the deletion was not a precise excision and that a more significant rearrangement had taken place.

In addition to the obvious deletion, the molecular nature of many of the other polymorphisms between these two loci has been determined by sequencing a 100-kb region of DNA surrounding the BUF1 gene of Guy11. The region is peppered with transposon insertions, which constitute ~50% of the DNA at the locus (results not shown). In contrast, the 2539 locus is apparently devoid of repetitive DNA sequences, as evidenced by the simple hybridization patterns shown in Figure 4B. In addition, a 20-kb segment of the Guy11 locus is inverted with respect to the orientation of the same sequences within the 2539 locus (results not shown).

BUF1 deletions are de novo events: If most of the deletions occur in just one DNA duplex (i.e., in a single chromatid), the recovery of 25% buf1 would require a deletion event to occur in every meiosis. However, the actual frequency of deletion cannot be determined from random ascospores because it depends on the number of chromatids affected in each meiosis. Therefore, I isolated tetrads and calculated the frequency of deletion on a “per meiosis” basis, by dividing the number of tetrads containing buf1 progeny by the total number of tetrads analyzed.

Following meiosis in M. grisea, the four meiotic products undergo a mitotic division before the spores are delimitated, resulting in an ascus containing eight spores (an octad). Tetrad analysis can be performed as long as seven spores are recovered, because then one can be sure that each of the four meiotic products has been sampled. Seventy-one complete tetrads (containing at

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**TABLE 1**

Segregation of buf1 progeny in tetrads from the cross Guy11 × 2539

<table>
<thead>
<tr>
<th>Observed ratio (wt:BUF1)</th>
<th>Inferred ratio*</th>
<th>No. of tetrads</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:0 or 7:0</td>
<td>8:0</td>
<td>40</td>
<td>0.56</td>
</tr>
<tr>
<td>6:2 or 6:1 or 5:2</td>
<td>6:2</td>
<td>3</td>
<td>0.04</td>
</tr>
<tr>
<td>4:4 or 4:3 or 3:4</td>
<td>4:4</td>
<td>28</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* wt, wild type.
least seven ascospores) were isolated. Of these, 31 yielded buf1 ascospores (Table 1), indicating that the BUF1 gene was deleted in ~44% of meioses. However, in crosses between Guy11 and 2539, only 38% of the ascospores were viable. Consequently, only a small fraction (5%) of the total number of dissected asci contained seven or eight viable spores. Therefore, to be sure that the complete tetrads were representative of the majority of meioses with respect to BUF1 deletion, I also investigated the occurrence of buf1 mutants in a number of incomplete tetrads. The frequency of deletion in these tetrads was 48%. Thus, I conclude that the complete tetrads are truly representative of the majority of meioses, at least with respect to mutation of BUF1.

Although segregation ratios among random ascospores suggested that BUF1 deletion was not the result of a segregating translocation, it was possible that such an occurrence was being obscured by distorted segregation and/or differential spore viability. To address this issue, I used Southern hybridization analysis with the cDNA probe to monitor BUF1 segregation in genomic DNA of tetrad progeny. If the absence of the BUF1 gene was due to its residing on a translocated chromosome segment, the tetrads containing buf1 progeny should also contain “duplication class” progeny that inherited both alleles.

Of the 31 “mutant” tetrads that were analyzed, 28 contained four wild-type progeny and four that were buf1 (Table 1). Molecular analysis of progeny from 20 tetrads showed that the BUF1 gene probe did not hybridize to DNA of the buf1 progeny (Figure 5A). Moreover, none of the 20 tetrads that were analyzed contained progeny that had inherited both BUF1 alleles. Instead, the wild-type progeny all showed a single hybridization signal that corresponded to the BUF12539 allele (Figure 5A). The tetrads that did not yield buf1 mutants exhibited the expected 2:2 segregation of BUF1 alleles, as illustrated by tetrads T73 and T75 (Figure 5A). In addition, analysis of the tetrad DNAs with several unlinked RFLP markers revealed that other loci were inherited normally (Figure 5B). These results confirmed that the majority of deletions were not due to the presence of a translocation and were not the result of unequal crossing over between homologs or sister chromatids. Instead, they were de novo events and occurred by inter- or intrachromatid recombination.

In the case of the tetrads that exhibited octad ratios of 6:2 and 5:2 (gray:buff), deletion of BUF1 was apparently caused by unequal crossover, as duplication class progeny were also present in the tetrad and exchange of flanking markers was observed (results not shown). Also associated with these crossovers was a conversion of the BUF1 gene, as evidenced by the presence in these tetrads of three ascospores with the 2539 allele (i.e., tetrad T68, Figure 5). As shown in Table 1, most of the mutant meioses segregated buf1 mutants in 4:4 ratios (gray:buff spores). This is consistent with the hypothesis that the deletions occurred before the premeiotic round of DNA replication. This was further tested by molecular analysis of deletion breakpoints in tetrad progeny. Cosmid A22-7-E, which spans one of the deletion breakpoints, was used to probe Apal-digested DNAs of tetrad progeny. Genomic DNA of Guy11 revealed eight Apal fragments that hybridized to the probe (Figure 6). The hybridization patterns in the buf1 progeny revealed two types of deletion. Mutant progeny in tetrad T52 were missing three of the eight fragments, whereas the buf1 progeny in tetrad T54 had lost six fragments (Figure 6). In 14 out of the 20 mutant tetrads that were analyzed, the buf1 mutants exhibited identical types of deletion. This is consistent with the deletions having occurred prior to premeiotic S-phase. However, the hybridization patterns seen in the 6 remaining tetrads revealed that the sister
chromatids had experienced different-sized deletions (i.e., tetrad T76, Figure 6). This is indicative of deletions having occurred during or after replication. Meioses that did not result in the appearance of buf1 mutants showed normal (2:2) segregation of the Guy11 and 2539 RFLP patterns, as illustrated by tetrad T77 (Figure 6).

Deletion of BUF1 occurs after fertilization: In an attempt to define the earliest point at which deletion of BUF1 occurs, I examined the appearance of buf1 mutants in asci dissected from individual perithecia. In Pyrenomycetous fungi like M. grisea, all the asci in a peritheium are derived from a single pair of progenitor nuclei, one having come from each parent. The 200–300 ascospores are created through a series of mitotic divisions with one pair of nuclei from each division entering into meiosis (Figure 7). It can be seen that, if deletion of BUF1 occurred before fertilization (i.e., before step A in Figure 7), this would result in perithecia in which all the asci contain buf1 ascospores.

Small numbers of complete tetrads (seven or eight spores) were recovered from all five of the individual perithecia analyzed. Each peritheium yielded at least one complete tetrad, in which all ascospores were wild type (i.e., containing no buf1 ascospores). Two of the perithecia also yielded complete tetrads containing both gray and buff ascospores. In the remaining three perithecia, poor viability prevented recovery of complete tetrads with buf1 spores. However, in each case, incomplete tetrads with buf1 spores were obtained. These results revealed that all five perithecia contained asci that were wild type along with others that were mutant. Therefore, the deletion events must have occurred after fertilization.

DISCUSSION

All of the buf1 mutations were deletions in the chromosome of Guy11. This instability appears to be a meiotic phenomenon as deletions were much less frequent during mitosis. These results are similar to those of Chumley and Valent (1990), who found elevated instability of BUF1 during the sexual cycle in three different M. grisea strains.

Molecular analysis of the BUF1 gene in mutant tetrads demonstrated unequivocally that loss of BUF1 was not due simply to segregation of a translocation but that its absence was caused by de novo deletion events. Surprisingly, de novo deletion of native loci is not often observed in the well-studied organism S. cerevisiae (Myung et al.)
but chromosomes exhibiting \textit{de novo} deletions have been identified in filamentous ascomycetes such as \textit{N. crassa} (Selker et al. 1987), \textit{Nectria haematococca} (Miao et al. 1991), \textit{Cochliobolus heterosporus} (Tzeng et al. 1992), and \textit{C. carbonum} (Pitkin et al. 2000). In these last two cases, the chromosomal instability also occurred in crosses where there was either structural heteroallelism or hemiallelism for the loci in question (Tzeng et al. 1992; Pitkin et al. 2000).

Chumley and Valent (1990; Valent et al. 1991) found that meiotically mutable \textit{BUF1} alleles did not suffer mutation in all crosses. On the basis of their observations, they intimated the existence of a \textit{BUF1} mutator locus. The correlation between instability and \textit{BUF1} heteroallelism revealed in the present study provides evidence that the “mutator” is in fact at the homologous \textit{BUF1} locus or is closely linked to this locus.

Southern hybridization analysis of the DNA region surrounding the \textit{BUF1} genes of the parental strains Guy11 and 2539 indicated that the structural organizations of the “homologous” \textit{BUF1} loci are very different (Figure 4). Nucleotide sequence analysis has shown that this is due to the presence of many repetitive elements in the Guy11 locus, none of which are present in the \textit{BUF1} locus of 2539 (M. Farman, unpublished results). The strikingly asymmetric distribution of repeated sequences explains the differential stability of the two loci. In addition, it indicates that deletion of \textit{BUF1} specifically in heteroallelic crosses is likely caused by mispairing between this locus and its homologous counterpart. If so, this mispairing would likely be during zygotene phase because observation of meiotic chromosomes in \textit{N. crassa} indicates that mispairing is resolved during pachytene by a process of “synaptic adjustment” (Bojko 1990).

Further evidence that mispairing is a factor in \textit{BUF1} deletions comes from the observation that deletions usually occur via intrachromatid exchanges. Such events are normally quite rare in \textit{S. cerevisiae}, but occur frequently when homolog pairing is suppressed (Petes 1980) or is completely absent (Wagstaff et al. 1985). These observations suggest that correct pairing of meiotic chromosomes may be critical, not only for proper recombination and chromosome segregation (Roeder 1995), but also for preventing potentially catastrophic intrachromatid exchanges.

To my knowledge, the \textit{BUF1} locus is the first example of a chromosomal region whose instability is strongly influenced by its homologous pairing partner. I have named the overall phenomenon \textit{meiosis-associated deletion in heteroallelic repeats} (MDHR). This acronym describes the salient features of the underlying processes, namely that they occur during the sexual cycle; they cause deletions; and they affect regions of heteroallelic, repetitive DNA.

The genomes of the parental strains used in this study differ widely in their repetitive DNA contents. Consequently, I expect that MDHR occurs at several \textit{M. grisea} loci. Indeed, examination of RFLP segregation in the Guy11 × 2539 mapping population (Nitta et al. 1997) revealed a number of \textit{de novo} deletions that occurred via intrachromatid recombination. Experiments are in progress to determine if these deletions exhibit the other hallmarks of MDHR.

The presence in a single perithecium of some asci containing \textit{buf1} progeny and others having only gray ascospores indicates that MDHR occurs sometime after fertilization. If MDHR occurs in the normal recombinogenic phase of the sexual cycle, only one chromatid should have been affected, resulting in two mutant ascospores (Figure 8A). Instead, the appearance of four mutant spores provides strong evidence that MDHR is initiated just before premeiotic DNA replication (Figure 8B). Otherwise, to explain the complete loss of the Guy11 allele from mutant tetrads, two independent deletions would have to occur, one in each of the “unstable” chromatids (Figure 8C).

The presence of identical deletion breakpoints in sister chromatids (Figure 6) provided additional evidence that they occurred prior to DNA replication. Even in the ascI that contained mutant ascospores with different-sized deletions, both chromatids were always affected, which suggests that these events were also initiated before premeiotic S-phase. In these cases, resolution of the recombination intermediate(s) appears to have occurred during or after replication, resulting in different-sized deletions in the sister strands.

Thus far, no premeiotic phenomena have been documented in the intensively studied yeast \textit{S. cerevisiae}. In contrast, filamentous fungi such as \textit{N. crassa} (Selker et al. 1987; Butler and Metzenberg 1989), \textit{A. immersus} (Rhounim et al. 1992), \textit{C. cinereus} (Freedman and Pukkila 1993), and \textit{N. haematococca} (Miao et al. 1991) all exhibit unusual behaviors that occur in the premeiotic phase of the sexual cycle. Most relevant to the present studies is \textit{N. crassa}, which experiences frequent premeiotic deletion within the tandemly arrayed ribosomal RNA genes in the nucleolar organizer region, as well as in tandem duplications of nonribosomal genes (Selker et al. 1987). These deletions also occur via intrachromatid exchanges (Butler and Metzenberg 1989) and, like MDHR, a small proportion happen during, or after, premeiotic S-phase (Selker et al. 1987; Butler and Metzenberg 1989). Thus, it appears that these phenomena may be mechanistically related, although it should be noted that there is no evidence that premeiotic deletions in \textit{N. crassa} are affected by allelic status (Irelan et al. 1994).

MDHR usually occurs before the DNA replication phase of meiosis, as indicated by the tetrad and deletion breakpoint analyses. However, its dependence on heteroallelism and the large differences in \textit{BUF1} locus structure suggests that deletion is triggered by mispairing between the homologous \textit{BUF1} loci. These data are
Figure 8.—Models for 4:4 BUF1 gene deletion ratios in octads. A general scheme for Pyrenomycete meiosis is outlined to show the development of an eight-spored ascus. The final premeiotic round of DNA replication is thought to occur prior to karyogamy. Once replication has occurred, the nuclei fuse and enter immediately into meiosis. Meiosis I results in segregation of homologous centromeres. Meiosis II results in the separation of sister chromatids yielding the four meiotic products. A single mitotic division ensues, followed by the delimitation of eight spores, which consist of four pairs of mitotic twins. Deletion of BUF1 from one chromosome prior to replication will result in four ascospores with a deletion (A). Deletion during meiosis would result in only two mutant spores (B), unless parallel deletions happened in both chromatids (C).

clearly in conflict with the current model of Pyrenomycete meiosis (Raju 1980), in which chromosome pairing does not take place until after karyogamy and, hence, after premeiotic DNA replication has taken place (Figure 7). Consequently, it is hard to explain how the allelic status of the BUF1 genes could be detected prior to replication, as the homologous chromosomes should not yet be in the same nucleus.

A number of models could provide a satisfactory resolution to this paradox. One interpretation of the data is that the current model of meiosis in Pyrenomycetous fungi is incorrect and that karyogamy precedes replication, as it does in S. cerevisiae (Weiner and Kleckner 1994). The evidence that replication precedes karyogamy in fungi like N. crassa, Sordaria fimicola, and Neottiella rutlandt is based on microspectrophotometric quantitation of DNA in premeiotic nuclei (Rossen and Westergaard 1966; Iyengar et al. 1976; Bell and Therrien 1977). However, it should be noted that this technique is potentially subject to artifacts, and absorbance readings may be affected by the shape and spatial positions of nuclei, which may become distorted during sample preparation. One study of ascus development in M. grisea suggested that karyogamy does precede replication (Tanaka et al. 1979). However, the authors did not state how this conclusion was reached and the only kind of data that were presented (diameter of nuclei) is not an accurate way to measure nuclear DNA content. Thus, the timing of premeiotic S-phase in M. grisea and other Pyrenomycetes remains questionable and will be the subject of further investigation.

Another explanation for the disparity in apparent timing of MDHR is that despite their initial appearance as premeiotic occurrences, BUF1 deletions actually happen after DNA replication (as illustrated in Figure 8C). However, analysis of the buf1 progeny in three tetrads suggests that this is not the case because, within each tetrad, the four buf1 spores exhibited identical mismatch repair patterns at the deletion breakpoints (M. Farman, unpublished results). This result is consistent with recombination having occurred prior to premeiotic S-phase.

A further possibility is that some genomic regions in M. grisea are not yet replicated when karyogamy occurs. This hypothesis is attractive, as it does not require too wide a divergence from the regnant model of the choreography of sexual development and meiosis in the Pyrenomycetes (Raju 1980). Furthermore, the presence of stalled replication forks could explain the high frequency of MDHR, because replication arrest often results in recombination at the site of the block (Kuzminov 1995). In some organisms biochemical evidence suggests that certain chromosomal regions do replicate much later in the meiotic cycle than others (Chiu and Hastings 1973; Stern and Hotta 1973). For example, ~0.3% of the lily genome is not replicated during premeiotic S-phase, with its synthesis being completed after chromosome pairing has taken place (Hotta et al. 1966; Stern and Hotta 1973). Thus, the paradoxical genetic behavior of the BUF1 locus may be an indication that it too is replicated late in the meiotic cycle.

A final model supposes that the triggering of deletion events does not actually require physical interaction of the BUF1 loci at all. Instead, a factor encoded by a gene in the 2539 genome may enter the Guy11 nucleus and induce deletions at the BUF1 locus. In this case, the
factor should be encoded by a gene linked to the 2539 BUF1 gene, so as to explain the dependence of deletions on heteroallelism at the BUF1 locus. Current experiments are seeking to test the above models.

The mechanism by which MDHR leads to deletion of BUF1 appears to be quite different from other causes of frequent meiotic instability such as hybrid dysgenesis (Engels 1989) and unequal crossing over (Petes and Hill 1988). Unlike MDHR, hybrid dysgenesis is not sensitive to allelic status, and meiotic crossovers occur at a later stage in the sexual cycle than MDHR, being initiated by double-strand breaks, which are formed after DNA replication (Cao et al. 1990; Borde et al. 2000; Davis et al. 2001; Smith et al. 2001; reviewed in Baudat and Keeney 2001). In addition, MDHR is much more frequent than normal crossovers. Therefore, it seems likely that MDHR represents the work of a system that efficiently activates a repair process upon detection of mispaired DNA (and/or stalled replication forks), eventually leading to eviction of the BUF1 gene. Whether these repair processes serve a beneficial purpose remains to be seen, for it is also possible that MDHR represents inappropriate behavior of the M. grisea meiotic machinery, as a result of its not yet having “learned” how to handle chromosomes with such divergent organizations.

There are indications that processes like MDHR may operate elsewhere. Meiotic instability in other fungi is frequently associated with repetitive DNA and is correlated with the existence of structural differences between homologous chromosomes (Pitkin et al. 2000) or the lack of a homolog altogether (Tzeng et al. 1992). Structural heteroallelism also appears to induce aberrant recombination in S. cerevisiae (Welch et al. 1991). Human chromosome instability has been associated with the presence of repetitive DNAs, leading to genome rearrangements such as tandem duplications, inversions, and translocations (Yi et al. 2000). In some cases, chromosome instability in humans has also been associated with mispairing (Chandly 1989).

Even if MDHR occurs in other eukaryotes, there are reasons why it might not have yet been discovered. First, detection of heteroallelism-induced chromosome instability based on phenotype would be difficult in organisms that do not produce large numbers of progeny or in those for which controlled crosses are not experimentally feasible. Second, if the inherent deletion frequency of a particular locus is much lower than that of BUF1, it is unlikely that pairing-dependent instability would be detected, even in the most genetically tractable organisms. In this case, mutations would appear to be stochastic events, suggesting an indeterminate genetic basis. Another hallmark of the operation of MDHR is its apparent occurrence before DNA replication. For most organisms, it is not possible to establish the timing of recombination events because the four meiotic products are not retained in a way that they may be analyzed as a unit. Thus, meiotic chromosome instability in a number of organisms may also be initiated in the prereplication phase of the meiotic cycle but, in many cases, this would also be difficult to detect.

In conclusion, the M. grisea BUF1 locus, by virtue of its peculiar genetic behavior, may provide important insights, not only into chromosome instability during the sexual cycle, but also into the mechanics of meiosis itself. First and foremost, the indication that homolog pairing plays a role in the maintenance of chromosome integrity proffers a new paradigm for meiotic chromosome instability. Due to its unusual hallmarks, it is possible that MDHR or related processes operate in many organisms without having been discovered. If true, it is possible that some of the de novo mutations that arise in the germline of higher eukaryotes do so because the meiotic machinery operates inappropriately on mispaired and/or unreplicated chromosome regions. Alternatively, these mutations could reflect the activity of repair processes that deal specifically with these occurrences. In any case, this notion is quite different from the commonly held belief that de novo germline mutations arise by chance or are the results of chemical or environmental insults to gametogenic tissues or their products (Chandly 1991). If MDHR does operate in other organisms, the potential implications are far reaching and, consequently, the role of homolog pairing in chromosome stability deserves wider investigation.

I acknowledge M. Olmstead and D. Thornbury for technical assistance with parts of this work. I thank S. Kang for cosmids clones; L. Engels, S. Hill, B. C. Jensen, A. D. Budde, S. Taura and M. L. Farman for critical reading of the manuscript; and W. Engels, R. Aramayo, R. Metzenberg, E. Selker, and M. E. Zolan for stimulating discussions. I also thank the manuscript reviewers for providing very useful insights. This work was supported in part by a March of Dimes Birth Defects Foundation grant FY98-0749 and by HATCH grant KY012009 from the Kentucky Agricultural Experiment Station (KAES). This is KAES publication 00-12-138.

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