The Mechanism of Fission Yeast Mating Type Interconversion: Seal/Replicate/Cleave Model of Replication Across the Double-Stranded Break Site at matl

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ABSTRACT

The interconversion of cell type in the fission yeast, Schizosaccharomyces pombe, is initiated by a double-stranded break (DSB) found at the mating type locus (matl). A heritable site- and strand-specific DNA "imprinting" event at matl was recently hypothesized to be required to make the matl locus cleavable, and the DSB was suggested to be produced one generation before the actual switching event. It is known that only one cell among four granddaughters of a cell ever switches, and the sister of the recently switched cell switches efficiently in consecutive cell divisions. The feature of consecutive switching creates a major difficulty of having to replicate chromosomes possessing the DSB. The matl cis-acting leaky mutation, called smt-s, reduces the level of the DSB required for switching and is shown here to be a 27-bp deletion located 50 bp away from the cut site. Determination of the pattern and frequency of switching of the mutant allele by cell lineage studies has allowed us to conclude the following: (1) the chromosome with the DSB is sealed and replicated, then one of the specific chromatids is cleaved again to generate switching-competent cells in consecutive cell divisions and (2) the smt-s mutation affects DNA cleavage and not the hypothesized DNA imprinting step.

An interesting feature of the fission yeast mating type switching system is the specific pattern cells follow for spontaneously and efficiently switching one mating cell type to the other (Leupold 1950; Egel 1977; Miyata and Miyata 1981). The haploid cell exists as one of two alternate cell types, called P (for Plus) and M (for Minus) (Leupold 1950; Egel et al. 1980). These cell types are determined, respectively, by the alternate matl-P and matl-M alleles of the mating type locus (matl), which resides on linkage group II. Mating type interchange involves a gene conversion event in which a copy of unexpressed mating type information residing at the mat2-P or the mat3-M donor locus is transposed to matl, resulting in a switch of cell type (Figure 1). For recent reviews of the system, refer to Egel (1989) and Klar (1989).

The recombination event that mediates matl switching is initiated by a site-specific double-stranded DNA break (DSB) at the matl locus (Beach 1983; Beach and Klar 1984; Egel, Beach and Klar 1984). The cut site, designated smt [switching of mating type (Egel et al. 1980)], is situated near the junction of the matl allele-specific and H1 regions. The precise nature of the cut has been characterized for one strand of the DNA (Nielsen and Egel 1989) (Figure 1). Analysis of DNA isolated from exponentially growing cultures has shown that approximately 20–25% of matl DNA is cleaved and that the proportion of broken DNA is constant throughout the cell cycle (Beach 1983).

Three trans-acting switch genes are required to catalyze the DSB, and three matl cis-acting mutations are known to affect the rate of switching by reducing the level of DSB (Egel and Gutz 1981; Beach 1983; Beach and Klar 1984; Egel, Beach and Klar 1984; Engelke et al. 1987; Schmidt, Kapitza and Gutz 1987; Schmidt et al. 1989). The smt-s, also called C13-P11, is one such spontaneous cis-acting mutation (Egel and Gutz 1981).

Miyata and Miyata (1981) followed the pattern of matings between the progeny of a single cell growing under starvation conditions—conditions under which mating is induced. The remarkable result was that among four progeny (granddaughters) of a single cell, only one zygote was made in 72–94% of four related granddaughters. Thus, among the four progeny of a single cell, only one granddaughter had switched—the "one-in-four granddaughters switching rule." Recent studies have extended the pedigree analysis to more generations past the four-cell stage and identified a second rule of consecutive switching (Egel and Eie 1987; Klar 1990). That is, the sister of the recently switched cell will itself produce one switched cell (therefore, she was switchable) and one unswitched (but switchable) cell in 90% of cell divisions. In other words, chains of switching in consecutive cell divisions were observed—the "consecutive or
across the break to produce one daughter chromatid. The centromere is located about 0.5 centimorgans to the left of mat1. The homology boxes H1 (59 bp) and H2 (135 bp) are present in all cassettes and flank the allele-specific P (1113 bp, jagged line) and M (1127 bp, smooth line) sequences. The third homology region, H3 (57 bp), is present at mat2 and mat3 but not at mat1. The interval between mat1 and mat2 is 15 kb long and the interval between mat2 and mat3 is also ~15 kb long. DSB marks the site of the double-stranded break in mat1. Arrows indicate that mat1 switches occur by unidirectional transfer of DNA from mat2 and mat3 loci to mat1. Each mat1 allele encodes two transcripts. The figure is derived from data presented earlier (Egel and Gutz 1981; Beach 1983; Egel 1984a; Beach and Klar 1984; Kelly et al. 1988).

recurrent switching rule.” The observed pattern of switching is analogous to the mammalian “stem cell” lineages; that is, a cell produces one daughter like itself while the other daughter is advanced in its developmental program (Klar 1990).

To explain the granddaughter switching rule and the chains of switching in consecutive cell divisions, a specific strand-segregation model has been proposed (Klar 1987, 1990). It was suggested that a sequence- and strand-specific imprinting event at mat1 is required for cleaving the DNA in vivo, and the cleavage perhaps occurring at the time of DNA replication. The DSB at mat1 persists throughout the cell cycle and is sealed by switching in one specific daughter chromatid during replication. The sister of the recently switched cell is switchable in 90% of cell divisions; therefore, it is presumed to possess the DSB. In other words, the parent cell possessing the DSB at mat1 produces one specific cleaved daughter chromatid, whose inheritance will produce a switching-competent daughter. Therefore, a major problem arises as to how a cut chromosome can be replicated across the break to produce one daughter chromatid again possessing the DSB. To fulfill this requirement, it has been proposed (Nielsen and Egel 1989) that a specific mechanism may exist which permits replication while preserving the DSB in one of the resultant chromatids, hence maintaining switching competence in one daughter cell in consecutive cell divisions. In other words, the DSB would act as a stable determinant of switching. Alternatively, we imagine that the cut ends are sealed at or immediately before replication. At or soon after replication, one of the chromatids is sealed by switching, while the other is recleaved, since the latter one has inherited the hypothesized imprinted event carried on it.

Determination of the pattern of switching of the mat1 smt-s allele has allowed us to differentiate between these models. We favor the idea of sealing the cut ends at or before the time of DNA replication.

MATERIALS AND METHODS

Strains: All strains are of Schizosaccharomyces pombe and their genotypes are presented in Table 1.

Culture conditions: Standard conditions for culture, sporulation, and tetrad analysis were employed according to Gutz et al. (1974). The mat1 switching efficiency of strains was roughly determined at the colony level by staining clones, growing and sporulating on sporulation media, with iodine vapors. Cells of opposite mating type in the clone mate, and the resulting zygotic cells undergo meiosis and sporulation. S. pombe produces a starchlike compound during sporulation that can be stained with iodine (Gutz et al. 1974). Clones of the wild-type strains stain black, while the leaky switching-deficient clones stain sparingly.

Pedigree analysis: Pedigree analysis was conducted according to Klar (1990; see also Egel and Eie 1987). Briefly, the procedure consists of assaying the ability of individual diploid cells to undergo meiosis and sporulation while growing on plates containing the semistarvation medium. Under such conditions, mat1-P/matl-P or mat1-M/matl-M cells grow, as they are incapable of sporulation because of the lack of expression of opposite mating type alleles. Their mitotic progeny containing a switched allele (i.e., mat1-P/matl-M) sporulate and therefore stop growing. Individual cells were separated by micromanipulation, and a record was kept of the lineage of each cell. In these experiments mat1 can only switch on one of the two chromosomes, while the mat1 allele present in the homolog is switching-deficient. Thus, the ascus formation in a cell lineage signals a switch of a particular mat1 allele to the opposite allele.

Biochemical techniques: DNA was isolated from 50-ml saturated cultures of yeast as described elsewhere (Beach and Klar 1984). Standard procedures for manipulating DNA in vitro were used (Manniatis, Fritsch and Sambrook 1982). For Southern blotting, ~1 μg of DNA was digested with HindIII and then subjected to electrophoresis through a 0.8% agarose gel. Transfer of DNA from the gel to nitrocellulose paper was done according to Southern (1975). The probe was nick translated in the presence of α-32P-labeled deoxyribonucleotide and then hybridized as described by Strathern et al. (1982). All enzymes were obtained from Boehringer Mannheim Biochemicals.

Cloning and sequence determination of the smt-s allele: The smt-s allele was isolated by the gap repair of a replicating plasmid that contains the 10.4-kb HindIII fragment spanning mat1 from which the internal cassette sequence from Xhol to PvuII (Beach 1983) was deleted. The linearized plasmid has a 1.7-kb sequence at one end and a 1.0-kb sequence at the other end; these sequences flank mat1 in the chromosome. The cut plasmid was introduced into yeast cells containing the smt-s mutant allele (strain SP109) by DNA-mediated transformation. Selection for the Saccharomyces cerevisiae LEU2 gene present in the plasmid was then performed as previously described (Beach and Nurse 1981). Gap repair placed the mat1 smt-s allele into the plasmid by the process of gene conversion (Orr-Weaver, Szostak and Rothstein 1981) using chromosomal information as the donor. The repaired plasmids were recovered by transforming Escherichia coli with DNA isolated from yeast transformants.

The dideoxy method of Sanger, Nickles and Coulson (1977) was used to determine the sequence of the HaellI/SphI fragment that spans both the cut site and the H1 region at mat1 (Kelly et al. 1988).

RESULTS

Sequence determination of the smt-s mutation: For the experiments presented here, we used the mat1 cis-
mat1 Interconversion

FIGURE 2.—Comparison of the C13-P11 (smt-s) mutation sequence with the wild-type allele shows a 27-bp deletion, situated about 50 bp away from the cut site. The autoradiogram (top) shows the sequence of the DNA flanking the deletion. Approximate position of the deletion with respect to H1 (horizontal arrow) and the DSB site (vertical arrow) are indicated at the bottom.

acting mutation, smt-s (also called C13-P11), which is known to reduce the steady state level of the DSB at mat1 and, consequently, reduces switching efficiency (Gutz and Fecke 1979; Egel and Gutz 1981; Beach 1983). The mutation is known to map distal to mat1, and since it cannot be “healed” by the act of switching, the mutation must lie outside the substituted sequences. Therefore, to molecularly determine the nature of the mutation, we determined the DNA sequence of the 254 bp mat1-P HaeIII-SspI fragment (Kelly et al. 1988) spanning the cut site and the H1 region (Figure 1). Earlier Southern analysis had shown the mutation to be due to a small deletion (Beach 1983). We found that in comparison with the sequence of the wild-type gene, the smt-s mutation is due to a 27-bp deletion removing 7 bp of the distal end of H1 extending distally to mat1 (Figure 2). The mutation deleted the region between a 6-bp sequence (5' GTTCGT-3') present as a direct repeat 27 bp apart, an observation suggesting that the deletion may have been generated by a replication error or by a recombination event within the repeat.

Cumulative effect of smt-s and swi1, swi3, or swi7 mutations on switching: There are three trans-acting switching-defective mutations known in swi1, swi3, and swi7 genes, which also reduce the level of the DSB. All of these mutations may be formally considered to be leaky, since switching is reduced but not abolished in mutant strains (Egel, Beach and Klar 1984; Gutz and Schmidt 1985).

To determine whether these trans-acting swi functions catalyze the DSB at mat1 by their interactions with the sequence deleted in the smt-s mutation, we constructed and analyzed double mutants. If a particular swi function acts only by interacting with the sequence deleted in the smt-s mutation, then a strain containing both mutations should exhibit the phenotype of the smt-s single mutant. Should the swi-encoded functions act elsewhere in the pathway of switching, then a cumulative reduction of switching is expected. It was found that smt-s and swi1, swi3, or swi7 double mutants (see Table 1 for complete genotype of strains) have a much reduced frequency of switching, as was indicated by the reduced level of iodine staining of colonies of such strains (Figure 3). The simplest interpretation of this result is that swi-encoded functions do not act through this site, or at least not solely via this site.

The smt-s mutation reduces the efficiency of first-time as well as consecutive switching: Pedigree analysis of diploids containing the smt-s mutation was conducted to explain how a chromosome containing the DSB for the entire length of the cell cycle can be replicated across the break, and also to determine whether the mutant is defective in imprinting or DNA cleavage steps hypothesized to be required to produce the DSB at mat1.

In an attempt to explain the rules of granddaughter switching and the chains of switching in consecutive cell divisions (see Introduction), a specific strand-segregation model has been proposed (Figure 4) (Klar 1987, 1990). In this model, a chromosome possessing the DSB at mat1 in switchable (Ps in Figure 4) cell is replicated to produce one switched (intact) chromatid while the unswitched sister chromatid still possesses the DSB. The problem then arises as to how a cut chromosome can be replicated across the break and still generate one daughter chromatid containing the cut. Two classes of models can be entertained. First, the cut chromosome is somehow replicated without sealing the break (model I in Figure 5). A second model can be envisioned in which the break, at least
TABLE 1

Experimental strains of *S. pombe*

<table>
<thead>
<tr>
<th>Strain</th>
<th><em>mat</em></th>
<th>Genotype</th>
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<tr>
<td>SP2</td>
<td><em>h</em>&lt;sup&gt;0&lt;/sup&gt;</td>
<td><em>leu</em>3-32 <em>his</em>3</td>
</tr>
<tr>
<td>SP105</td>
<td><em>h</em>&lt;sup&gt;0&lt;/sup&gt;</td>
<td><em>swi</em>3 <em>ade</em>6-M210 <em>his</em>2</td>
</tr>
<tr>
<td>SP109</td>
<td><em>h</em>&lt;sup&gt;0&lt;/sup&gt;</td>
<td><em>swi</em>1 <em>leu</em>1-32</td>
</tr>
<tr>
<td>SP110</td>
<td><em>h</em>&lt;sup&gt;0&lt;/sup&gt;</td>
<td><em>swi</em>7 <em>leu</em>1-32 <em>ade</em>6-M216</td>
</tr>
<tr>
<td>SP112</td>
<td><em>h</em>&lt;sup&gt;0&lt;/sup&gt;</td>
<td><em>swi</em>3 <em>leu</em>1-32</td>
</tr>
<tr>
<td>SP544</td>
<td><em>h</em>&lt;sup&gt;0&lt;/sup&gt;</td>
<td><em>swi</em>7</td>
</tr>
<tr>
<td>SP745</td>
<td><em>h</em>&lt;sup&gt;0&lt;/sup&gt;/<em>mat</em>1-<em>M</em> <em>smt</em>-s <em>mat</em>2,3Δ:<em>LEU</em>2</td>
<td><em>leu</em>1-32/<em>leu</em>1-32 <em>ade</em>6-M210/<em>ade</em>6-M216</td>
</tr>
<tr>
<td>SP745</td>
<td><em>h</em>&lt;sup&gt;0&lt;/sup&gt;/<em>mat</em>1-<em>P</em> <em>mat</em>2,3Δ:<em>LEU</em>2</td>
<td><em>leu</em>1-32/<em>leu</em>1-32 <em>ade</em>6-M210/<em>ade</em>6-M216</td>
</tr>
<tr>
<td>SP850</td>
<td><em>h</em>&lt;sup&gt;0&lt;/sup&gt;/<em>mat</em>1-<em>N</em> <em>smt</em>-o</td>
<td><em>leu</em>1-32/<em>leu</em>1-32 <em>ade</em>6-M210/<em>ade</em>6-M216</td>
</tr>
<tr>
<td>SP869</td>
<td><em>h</em>&lt;sup&gt;0&lt;/sup&gt; <em>smt</em>-s</td>
<td><em>swi</em>1 <em>leu</em>1-32 <em>ade</em>6-M216</td>
</tr>
<tr>
<td>SP870</td>
<td><em>h</em>&lt;sup&gt;0&lt;/sup&gt; <em>smt</em>-s</td>
<td><em>swi</em>7 <em>leu</em>1-32 <em>ade</em>6-M216</td>
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</table>

The *mat* region containing *mat*1, *mat*2 and *mat*3 loci is designated *h*<sup>0</sup>. The *smt*-s, *smt*-o, and *mat*2,3Δ:*LEU*2 mutant loci are defined in the text.

![Figure 3](image)

**Figure 3.**—Iodine staining of colonies of *smt*-s and *swi*<sup>7</sup> single and double mutants shows a cumulative reduction in switching of double mutants. Colonies growing from single cells of indicated genotype were allowed to grow, switch, mate and sporulate on solid media. Thereafter, the clones were stained by exposing them to iodine vapors according to GUTZ et al. (1974). The radial streaks of staining indicates mating in the adjoining small sectors of cells of opposite mating type. The observed level of staining reflects the efficiency of switching of a strain. The wild-type *mat*1 allele of respective strains is indicated by the *h*<sup>0</sup> designation (left vertical row) and the *smt*-s allele (right vertical row) contains a small deletion near *mat*1 (Figure 2). The genotype with respect to *swi* genes (horizontal rows) is also indicated. The complete genotype of appropriate strains is listed in Table 1.

For *mat*1 cleavage. Since a switchable cell produces one switchable daughter in 90% of cell divisions (Klar 1990), it can be imagined that the imprinted event is stably inherited by the switchable daughter in most or all of the subsequent cell divisions (EGEL and EIE 1987; Klar 1990).

Pedigree analysis of the leaky cis-acting *smt*-s mutant was used to determine whether the mutation affects imprinting or the DNA cleavage step. If the mutation affects imprinting function, then it should reduce the rate of first-time switching (one-in-four cases), but once the DNA strand is imprinted (i.e., in lineages where initial switching has occurred), the rate of consecutive switching should be similar to that found in wild-type strains (Figure 4). Pedigree analysis of such an imprinting defect will not allow us to differentiate between the models presented in Figure 5.

If, however, the *smt*-s mutation affects DNA cleavage and not imprinting, then these two models can be differentiated by the following rationale. According to the model I in Figure 5, a function for catalyzing the DSB will be required only to start the chain of switching, that is, for the one-in-four switching. Subsequent switching-competent cells should be produced efficiently, as the break is postulated to be preserved and delivered to one specific switching-competent daughter cell. Consequently, the rate of consecutive switching should be as high as that of wild-type strains. In contrast, according to the seal/replicate/cleave model (model II in Figure 5), the rate of recurrent switching should remain low similar to the rate of first-time switching, since a new cut is required for recurrent switching. These predictions are summarized in Table 2. Thus, by the pedigree analysis of a strain containing the *smt*-s mutation, 1) we can determine whether the mutation affects imprinting or the DNA cleavage step, and 2) if the cleavage step is affected, then we can differentiate between models I and II (Figure 5).

To determine the pattern and the efficiency of
**FIGURE 4.**—The Strand-Segregation model. Imprinting (*) of the Watson (W) strand (only the jagged line is imprintable) at mat1 occurs in the parent Pu (U for unswitchable) cell. It may have inherited the already imprinted chromosome from its parent, which may have imprinted it, say, in G2. During replication, the Ps (U for switchable) chromatid inheriting the imprinted parental ("old") W strand is cleaved. Its sister, the Pu chromatid inheriting the parental Crick (C) strand, or alternatively, the newly synthesized W strand, is not cleavable because its newly synthesized W strand is yet not imprinted. It will be imprinted by the time it is to be replicated in the next cell cycle. Thus, daughters of the parent cell inherit developmentally nonequivalent chromosomes, one inheriting the cut chromosome (Ps) and its sister inheriting the chromosome without the cut (Pu). At or soon after the time of DNA replication, the daughter cell with the DSB (Ps) generates one chromatid containing the switched and healed mat1 allele (Mu) and one chromatid that is cleaved and unchanged at the mat1 locus (Ps). Furthermore, the switched chromatid is specified to contain the specific C strand. Thus, each of the Ps cells will mostly produce one switched (Mu) and one switchable (Ps) daughter in consecutive cell divisions resulting in chains of recurrent switching. The cells with the newly switched mat1-M allele will switch back to mat1-P by following the same pattern of switching. Wide arrows indicate mat1 cassette orientation in the chromosome, while small arrows indicate the segregation of specific DNA chains to progeny cells. The DNA chains of the parental cell are represented by thicker lines to help illustrate their inheritance by progeny cells. A recently switched allele is indicated by the boldface letter. A gap in the continuity of the chromosome denotes the presence of the DSB. The figure is adapted from Klare (EMBO J., 1990).

**FIGURE 5.**—Two possible models for processing the DSB at mat1 during chromosome replication. By either model, the cut chromosome generates one cut daughter chromatid during replication. Model I: the mat1 DNA is replicated without sealing the cut, and both parental strands with the DSB are conservatively delivered to the switching-competent daughter cell (Nielsen and Egel 1989). Model II: the imprinted (top) strand is sealed, replicated, and cleaved again (perhaps during replication) to generate the switching-competent chromosome. The other strand is suggested to be repaired by switching in an unspecified manner. All other designations are defined in the legend to Figure 4.

**TABLE 2**

<table>
<thead>
<tr>
<th>Possible smt-s switching defects and predictions of models of Figure 5</th>
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<tr>
<td><strong>Defect</strong></td>
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<tr>
<td>DNA imprinting</td>
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<td>DNA cleavage model I</td>
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<td>DNA cleavage model II</td>
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</table>

switching of the smt-s allele from mat1-M to mat1-P, pedigree analysis of the diploid strain SP850 (mat1 smt-s, mat2, mat3/mat1-M smt-o, mat2, mat3) was conducted by following the competence of single cells to sporulate as described in MATERIALS AND METHODS. The tight switching-deficient cis-acting smt-o mutation (Engelke et al. 1987) is a 262-bp deletion of the mat1 distal sequences encompassing the smt-s site (O. Nielsen and R. Egel, personal communication). Cells containing the mat1-M smt-s/mat1-M smt-o constitution at mat1 will not sporulate, but when the mat1-M smt-s allele switches to a mat1-P, then that cell will form an ascus. Thus, in this strain the efficiency of switching of mat1-M smt-s to mat1-P smt-s can be determined by the cell lineage studies. As shown in Figure 6, the mat1-M smt-s switched inefficiently (12.4%) by following the rule of one switch in four granddaughters. An additional and more telling observation is that the rate of consecutive switching was also low at 13%.

Similarly, to determine the pattern and efficiency of switching of the mat1-P smt-s allele to mat1-M, strain SP754 was subjected to pedigree analysis. The diploid strain contains the mat1 smt-s, mat2-P, mat3-M genotype in one chromosome and the mat1-P mat2,3Δ::LEU2 genotype in the homolog. The mat2,3Δ::LEU2 is a deletion of mat2, mat3, and sequences bracketed by these loci, and the S. cerevisiae LEU2 gene is inserted in their place (Klare and Migliore 1986). In this strain, switching only in cis occurs as the donor loci present in the homolog failed to donate information to the wild-type mat1-P locus located in the donor deleted chromosome (A. Klare, unpublished results to be reported elsewhere). As shown in Figure 6, one in four granddaughter cells switched in 16% of the cases. Additional pedigrees were analyzed to determine the rate of consecutive switching. Only 4 among 60 cells (7%) switched in consecutive cell divisions.

As a control for these studies, pedigree analysis of strain SP743 (mat1, mat2, mat3/mat1-M smt-s, mat2,3Δ::LEU2) was conducted. The mat1-M smt-s failed to switch altogether because it lacks the donor information present in the same chromosome (data not shown). The wild-type mat1 allele switched from
developmental asymmetry of both cell divisions is due to produce one switch in four related cells. The cell lineages of fission yeast is strictly controlled by switching rate was similarly low.

DNA replication generates nonequivalent genomes at the locus and not to other cellular differences (KLAR seems that generation of the DSB as well as utilization the regulation of the DSB at (KLAR 1990). If the cell already contains the switchable daughter. If the cell contains intact matl DNA, then after replication one chromatid will be unswitchable, as it lacks the break, while the other chromatid will acquire the break and, hence, will generate a switchable daughter. If the cell already contains the DSB at matl, then after replication one specific chromatid is mostly switched, while its sister chromatid contains the DSB, whose inheritance will produce a switching-competent daughter cell (Figure 4) suggesting that developmental asymmetry as compared to wild-type cells, cells containing inverted tandem duplication of matl cassettes contained twice as many cleaved chromosomes and both cassettes in the same chromosome were not simultaneously cleaved (KLAR 1987). This result contrasts with the wild-type allele, which switched by the one-in-four rule in 92% of the pedigrees and also switched efficiently (96%) in consecutive cell divisions (this paper; EGEI and EIE 1987; KLAR 1990). Thus, the sequences defined by the matl-P mutation are equally required for both primary and consecutive switching. The major conclusion drawn from this result is that the cut is made anew in each cell undergoing switching in consecutive cell divisions. If the cut was preserved as such and inherited by the switching-competent specific daughter as recently proposed (NIelsen and EGEL 1989), then the rate of consecutive switching was predicted to be high. Nielsen and EGEL (1989) model proposed that both strands of the parental cut molecule are conservatively segregated to the unswitched (but switching-competent) daughter cell, thereby preserving the DSB. It was proposed that a special kind of mechanism replicates the cleaved chromosome at matl resulting in unilateral transmission of high frequency state of mating type switching. Our results suggest that the DSB must be sealed before the site could be replicated, thus arguing against preserving the DSB model. This conclusion solves the enigmatic problem of having to replicate the chromosome across the long-lived break (KLAR 1987, 1990). The second conclusion is that sequences deleted by the matl mutation are required directly for cleaving the DNA and not for the imprinting step hypothesized to be required for making the matl DNA competent to be cleaved (KLAR 1987, 1990).

The key feature of the strand-segregation model (Figure 4) suggesting that developmental asymmetry for switching is conferred through the inheritance of parental DNA chains by progeny cells was borne out by our earlier published work. It was observed that, as compared to wild-type cells, cells containing inverted tandem duplication of matl cassettes contained twice as many cleaved chromosomes and both cassettes in the same chromosome were not simultaneously cleaved (KLAR 1987). Secondly, the duplication containing cells switched two in four granddaughter cells, to be contrasted with the one-in-four pattern.
followed by wild-type cells, and the additional inserted cassette itself switched by the conventional one-in-four rule (KLAR 1990). These results established only the key strand segregation aspect and do not address other hypothesized more specific details of the model. In particular, the existence of the imprinted event required for cleaving the DNA was only hypothesized and a published evidence for or against it is lacking. Clearly, our earlier results demonstrated that the switching system does differentiate between the "old" W (Watson) strand inherited by the cleaved chromatid and the newly synthesized W strand inherited by the uncut sister chromatid. It is not unreasonable to postulate the presence of an imprinted event segregating with the older strand to differentiate between old and new chains. Secondly, the process of replication through matl produces only one daughter chromatid that is cleaved even though both chromatids are present together in the same nucleus. Again, an imprinted event at matl can be imagined to make the replication across matl somewhat special as it is certainly different from replication of other sequences in the genome which most likely do not produce one cut daughter chromatid. This paper addresses a specific detail of the model as to how a broken chromosome can be replicated across the break. The results provided here do not test whether or not imprinted event exists on DNA.

Mutations in the swi1, swi3, and swi7 trans-acting functions, functions also known to be required for the formation of the DSB (EGEL, BEACH and KLAR 1984), further reduce the rate of switching of the smt-s mutation. We have recently defined another cis-acting switch activating sequence (SAS1), located 140 bp distal to the cut site; deletions of this sequence also show a cumulative reduction in switching when combined with mutations in these swi genes (B. ARCANGIOLI and A. KLAR, unpublished observations). The simplest interpretation of cumulative reduction in switching is that swi functions do not act through the sequences defined by both of these cis-acting mutations. An alternative possibility is that swi functions in fact act through both of these sequences to perform complementary and overlapping processes, both of which are required for efficient switching. A third possibility regarding smt-s is that it is a "spacing" mutation that affects the level of the DSB by affecting the matl chromatin structure or by changing the distance of the cut site from some other required cis-acting component. Further studies are required to resolve these issues. Another more complicated but formal possibility is that the smt-s mutation actually affects stability of the imprinted event, and consequently reduces the rate of consecutive switching. This possibility cannot be ruled out, however, it will not affect the major conclusion of this paper of producing a fresh DSB by a recent DNA cleavage event during recurrent switching. At this stage, it should be stated that as yet we have no evidence indicating that the DSB is produced by an endonuclease-like activity. It is equally possible that the act of DNA replication in an unspecified manner generates one broken chromatin. Should the second possibility to be true, then this system will be drastically different from the way the DSB is generated and repaired by switching mating type in G1 phase of the cell cycle of S. cerevisiae (STRATHERN et al. 1982).

While considering processing of the DSB, it is particularly interesting to note that strains deleted for matl2 and matl3 have the normal level of break, maintain completely stable mating type, and also do not produce inviable cells. Thus, the machinery must exist to seal the break even without switching (KLAR and MIGLIO 1986). In contrast, such a cut at MAT in S. cerevisiae cannot be healed without switching, since the donor deleted strains die when trying to switch (KLAR, STRATHERN and ABRAHAM 1984).

The conclusion that cut ends are sealed and replicated and then one of the chromatids is cleaved again raises the question as to why the DNA is cut in the first place if it is to be sealed before replication. We can propose a model in which matl switching is a novel kind of gene conversion event mediated by the DNA replication process itself. The replication machinery may only seal one specific strand to allow it to replicate (Figure 5), while the other strand is healed by matl switching at the time of DNA replication. Clearly, further studies are needed to understand additional details of the mechanism of switching.

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