Genes required for initiation and resolution steps of mating-type switching in fission yeast  
(DNA double-strand cut/DNA rearrangement)

RICHARD EGEL*, DAVID H. BEACH‡, AND AMAR J. S. KLAN‡

*Institute of Genetics, University of Copenhagen,  Oster Farimagsgade 2A, DK-1353 Copenhagen K., Denmark; and ‡Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724

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ABSTRACT  The fission yeast Schizosaccharomyces pombe switches mating type by transposition of a copy of DNA derived from either of the two storage cassettes, mat2-P and mat3-M, into the expression locus, mat1. The recombinational event of switching is initiated by a double-stranded DNA break present in approximately 20% of the molecules at mat1. Fifty-three mutants defective in switching of mating type have been isolated previously, and each has been assigned to 1 of 10 linkage groups. One group consists of cis-acting mutations at mat1, which reduce the amount of the DNA double-strand cut. The remaining nine groups are mutations in genes that are unlinked to the mating-type locus and are studied here. Three (swi7, -7, -7) are required for formation of the double-strand cut, whereas the others are not. Mutants of three genes (swid, -8, -8) undergo high-frequency rearrangement of the mating-type locus indicative of errors of resolution of recombinational intermediates. The remaining three (swi2, -5, -6) have normal levels of cut, do not make errors of resolution, and possibly are required either for efficient utilization of the cut or determining the directionality of switching. The data suggest that the switching process can be dissected into genetically distinguishable steps.

In wild-type (h⁰) strains of Schizosaccharomyces pombe, mating-type switching between h⁺ and h⁻ occurs approximately once in every three cell divisions (1, 2). Under conditions of nutritional deprivation, cells of opposite mating type arrest in G₁ phase and conjugate to form a temporary diploid zygote, which undergoes premeiotic S phase followed by meiosis to yield four haploid spores (3).

Genetic analysis (4–6) indicates that mating type is controlled by a tightly linked cluster of genes (known as the mating-type locus) located in the long arm of chromosome II. Isolation of the DNA of this region (7–9) has confirmed that mating-type switching occurs by copy-transposition of information contained in stores of unexpressed plus (mat2-P) or minus (mat3-M) information into the expression locus mat1 (Fig. 1A). The phenotype of the cell is determined by the temporary presence of the P or M allele of mat1, mat2-P, and mat3-M are referred to as cassettes, by analogy with the situation described in Saccharomyces cerevisiae (10–12). The distance between each cassette is approximately 15 kilobases (kb) (9). The cassettes consist of a 1.1-kb plus (P)- or minus (M)-specific region bounded distally with respect to the centromere by a 90-base-pair (bp) region (H₁) and proximally by a 190-bp region (H₂) of homology common to each (ref. 8; Fig. 1A). The existence of these blocks of homology suggests that mating-type switching could occur by a mechanism similar to that of mitotic or meiotic gene conversion. However, mating-type switching is distinguishable from conventional gene conversion by several features.

Mating-type switching occurs at very high frequency and shows extreme disparity. mat1 is always the recipient of a mating-type switch and never a donor.

The frequency and asymmetry of switching can be attributed to the presence of a double-stranded DNA cut within or close the H₁ region of mat1. It is presumed to initiate a switch by invading the H₂ region of either mat2-P or mat3-M (8). The site of the double-strand cut is defined genetically by a cis-acting mutation smt-S (switching of mating type) that maps at mat1 and reduces the rate of switching (13, 14). smt-S has a small deletion close to H₂ of mat1, which reduces the amount of the double-strand cut (8). The smt cut may play a role similar to that proposed for the YZ cut at MAT in S. cerevisiae (15), but, whereas the YZ cut is transiently formed and healed, in S. pombe the smt cut may be present throughout the cell cycle (8).

The importance of the smt cut for high-frequency switching is further confirmed by the observation that a strain carrying a mutation (smt5) unlinked to the mating-type locus has reduced switching frequency (5, 8, 14) and also reduced levels of smt cut. In this study, the level of smt cut has been assessed in strains carrying mutations in nine separate genes that are required for high-frequency switching. It has been possible to distinguish those trans-acting genes required for initiation as opposed to resolution steps of recombination.

MATERIAL AND METHODS

All of the strains used are derived from Leupold’s original stocks of S. pombe, h⁰ (696), h⁻ (975), and h⁻ (972). Methods for culture, mutagenesis, crosses, and iodine staining of colonies were the same as described (5, 16, 17). DNA was isolated from 50-ml cultured yeasts as described in detail (9). Southern hybridization was performed as described by Strathern et al. (15).

RESULTS

Since homothallic (h⁰) strains of fission yeast switch mating type at a high frequency, mating and sporulation take place within a colony grown from a single cell. In strains that are heterothallic (nonswitching) because of rearrangement of the mating-type locus (e.g., h⁰; ref. 9), mating and sporulation is seen only at the line of contact between colonies of opposite mating type. Homothallic and heterothallic strains can be distinguished in a plate test by exposing the colonies to iodine vapor. Iodine specifically stains spore-containing colonies evenly black, whereas those not containing spores are stained yellow (16).

In a screen for further switching-defective mutants, an h⁰ strain was mutagenized with ethyl methanesulfonate, and 53 mutants that generate colonies staining internally with uneven streaks were isolated. Genetic analysis of the mutants based on linkage rather than complementation studies al-

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Abbreviations: kb, kilobase(s); bp, base pair(s).
Fig. 1. Diagrammatic representations of the mating-type locus. (A) Scale drawing of a portion of chromosome II, showing (thick lines) the 10.4-kb, 6.3-kb, and 4.2-kb HindIII fragments containing, respectively, mat1, mat2-P, and mat3-M. The regions of homology common to each cassette (H1 and H2) are marked as vertical blocks bounding the plus-specific («) or minus-specific (») regions. smt marks the site of the double-stranded DNA cut. The arrow (CenII) indicates the direction of the centromere. The location of the unique BamHI and unique EcoRI sites within the plus and the minus cassettes, respectively, allows the length of each HindIII fragment to be predicted in rearrangements of the mating-type locus. (B) Representation of the \( h^90 \), \( h^+N \), and mat2-P forms of the mating-type locus. The region normally between mat1 and mat2-P is referred to as L because its deletion is lethal, and that between mat2-P and mat3-M is referred to as K. Strains with the K region deletion are viable. The arrow marks the site of the smt cut in \( h^90 \) and \( h^+N \). The system of nomenclature of each cassette is as follows. The first number (e.g., in mat1:2) indicates the origin of the proximal flanking sequence, and the second number indicates the origin of the distal flanking sequence. Thus, mat1:2 has the proximal and distal sequences normally adjacent to mat1 and mat2-P, respectively. According to this system, mat1 of \( h^90 \) should strictly be referred to as mat1:1.

The clonal nature of the streaks of iodine stain in mutants of the nine swi genes (Fig. 2) suggests that these strains have low rates of mating-type switching. However, this pattern would also be obtained in genetically unstable mutants defective in any stage of mating or sporulation. To distinguish between these alternatives, each of the 46 swi mutants was restreaked and the phenotype of single colonies was assessed by iodine stain. Two types of results were obtained. Each isolate of swi1, -2, -3, -5, -6, and -7 (Table 1, class I) gave colonies, all of which had the parental streaky phenotype. However, each allele of swi4, -8 and -9 (class II) gave rise to some colonies with the parental phenotype (Fig. 2B), but the majority lacked any iodine stain (Fig. 2C). In matings with standard mating-type tester strains (\( h^+N \) and \( h^-S \)), the nonstaining colonies from swi4, -8 and -9 were shown to have an \( h^-S \) mating phenotype. However, the instability of the class II mutants is not due to loss of the original mutation because in out-crosses between non-iodine-staining swi colonies and a normal \( h^90 \) strain, segregants with the streaky iodine stain were always obtained. Southern hybridization experiments (see below) showed that the swi \( h^-S \) colonies carried a previously described rearrangement of the mating-type locus (Fig. 1B, \( h^-S \)), which reduces the rate of switching to a much greater degree than the swi mutations. It can be concluded that each of the swi mutations is stable and that the streaky phenotype of the swi \( h^90 \) colonies is due to lowered rates of switching of the mating-type cassettes.

A mutation in one gene, swi3, had previously been shown to reduce the amount of double-stranded DNA smt cut at mat1 (8). Therefore, it was of interest to assess the amount of cut in strains carrying other swi mutations. One allele of each of \( h^90 \) swi1–swi9 was grown from a single colony to a saturated 50-ml culture, and DNA was prepared. A Southern transfer of HindIII-digested DNA was probed with a 10.4-kb HindIII fragment containing mat1-M (Fig. 1A). In DNA from normal \( h^90 \) strains, this probe hybridized with bands of 10.4 kb (mat1-M, mat1-P), 6.3 kb (mat2-P), and 4.2 kb (mat3-M)
and with the 5.0-kb and 5.4-kb products of the smt cut at mat1 (Figs. 1A and 3A).

The DNAs of swi2, swi5, and swi6 show wild-type levels of smt cut bands (5.0 kb and 5.4 kb, Fig. 3A), representing approximately 20% of the level of mat1 (8), whereas those of swi1, swi3, and swi7 show very much reduced levels. Thus, the class 1 mutants can be subdivided into those lacking the cut (class Ia) and those with normal levels of cut (class Ib) (Table 1). To test the combined effect of swi mutants of each class in the same cell, double mutants were constructed from class 1a × 1b crosses. These had rates of switching lower than either mutant alone. In a Southern blot (Fig. 3C), the smt cut was absent from each la/1b double mutant, indicating that the phenotype of class Ia is epistatic to that of class Ib.

In swi4, swi8, and swi9, the situation is complicated. During the growth of the culture, approximately half of the cells changed from the h^0 to the nonswitching h^+N form of the mating-type locus (Figs. 1B, 2C, and 3A). h^+N has a rearrangement, which occurs spontaneously in wild-type h^0 strains at 1 × 10^{-5} (4, 18), in which the DNA normally between mat2-P and mat3-M is copy-transposed into mat1 (9). This generates two cassettes at mat3:1 referred to as mat3:1 and mat3:1 (see the legend to Fig. 1 for the system of nomenclature). The left-hand cassette (6.7-kb band) determines the phenotype of the cell and switches only rarely (= 10^{-5}), whereas the right-hand cassette (8.2-kb band), in which switching continues, has no effect on the phenotype. In swi4, swi8, and swi9, h^+N either arises much more frequently than in wild-type cells or is under positive selection with respect to h^0 so that it overgrows the culture.

h^+N has approximately 25% of the level of smt cut in the mat3:1 cassette compared with that at mat1 of h^0 (9), and because half the culture of swi4, -8, and -9 is h^+N, this probably accounts fully for the reduced but discernable level of smt cut in the DNA preparations of these strains. Therefore, it is concluded that, in class II mutants, the amount of double-stranded DNA cut at mat1 of the h^0 chromosome is normal.

Class II mutants undergo rearrangement of the mating-type locus to form not only h^+N but also a deletion between mat1 and mat2-P, which yields an 18-kb circular minichromosome called mat2:1' (ref. 9; Fig. 1B). This is detected as a 9.5-kb HindIII band (Fig. 3A, lanes 7-9) and in undigested DNA as two weak bands, one above (open circle) and the other below (supercoiled) the chromosomal band (Fig. 3B). The epismal derivative of the mating-type locus is undetectable in normal h^0 DNA but has been described previously in the h^S strain, which has a deletion between mat2-P and mat3-M (9, 19).

Class I mutants lack a detectable amount of mat2:1'. How-

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**Table 1. The Ia, Ib, and II classes of swi mutants**

<table>
<thead>
<tr>
<th>Class</th>
<th>Gene</th>
<th>Isolates</th>
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<th>h^+N/mat2:1'</th>
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<tbody>
<tr>
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<td>15</td>
<td>-</td>
<td>-</td>
</tr>
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<td>la</td>
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<td>-</td>
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<td>lb</td>
<td>swi6</td>
<td>1</td>
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<tr>
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<td>+</td>
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<td>II</td>
<td>swi9</td>
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</table>

The table gives the number of alleles of each gene isolated, the presence (+) or absence (-) of double-stranded smt cut, and the generation (+) or the lack (-) of h^+N and mat2:1' rearrangements of the mating-type locus. The gene assignments into nine linkage groups are based on work in collaboration with H. Schmidt and H. Gutz. Complementation studies have not been done.

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**Fig. 2.** Photographs of yeast colonies stained by exposure to iodine vapors. (A) Composite colony grown from four spores of a tetrad that was formed by a self-mating of swi6^00. The internal streaks are due to mating-type switching within the colony. (B) Composite colony of four spores of a tetrad from a self-mating of swi4h^00. The heavy horizontal reaction line separates predominantly plus and minus halves of the clone. (C) Composite colony of four spores of a tetrad from a self-mating of swi8^00. Before mating, one partner must already have become h^+N, thus segregating two white/two streaky sectors in the tetrad. Note how the h^+N (white sectors) grow more rapidly than the h^0 sectors.
Fig. 3. Southern blot analysis of the mating-type locus in swi mutants; sizes are shown in kb. Each blot is probed with the 10.4-kb mat1-M HindIII fragment shown in Fig. 1A. (A) HindIII-digested DNA. Lanes: 1, h9°swi1; 2, h9°swi3; 3, h9°swi7; 4, h9°swi2; 5, h9°swi5; 6, h9°swi6; 7, h9°swi4; 8, h9°swi8; 9, h9°swi9; and 10, h9°(975). mat1 (10.4 kb), mat2 (6.3 kb), and mat3 (4.2 kb) are bands derived from the h9° chromosome. smt(P) and smt(D) are products of the smt cut at mat1. mat1:1 (8.2 kb) and mat1:2 (6.7 kb) are the bands derived from the h9° rearrangement of the mating-type locus. mat2:1 (9.9 kb) is derived from a circular minichromosome. (B) Undigested DNA. Lanes: 1, h9°(972); 2, smt2h8; 3, swi7h9; 4, smt8h5; 5, smt9h8; 6, smt10h5; and 7, smt11h8. The dark mass is the undigested chromosome below the open circular (o.c.) and above the weak supercoiled (s.c.) form of mat2:1. (C) HindIII-digested DNA. Lanes: 1, swilswi2h8; 2, swilswi5h8; 3, swi7h8; 4, swi10h5; 5, swilswi12h8; 6, swilswi6h8; 7, swilswi3h8; 8, swi12h8; 9, swi6h7h5h8; and 10, swi7h8. Each of these strains was obtained from single spores derived from class Ia × class Ib crosses.

ever, h90 swi6 DNA has a HindIII band of 8.2 kb (Fig. 3 A and B). This is not derived from the h9° form of the chromosome because the 6.7-kb counterpart is absent. It is, however, an anticipated product of mat3:1; a 34-kb minichromosome formed by fusion of mat1 and mat3-M. mat3:1 is not seen in the undigested DNA (Fig. 2B, lane 5) of swi6 possibly because its large size renders it difficult to isolate as an intact molecule.

DISCUSSION

Nine genes required for high-frequency mating-type switching of fission yeast have been described. Mutations in each gene have a leaky phenotype in which switching is not abolished but reduced approximately 10-fold. This leakiness may be essential for viability of some of the mutants and is a direct consequence of screening for mutant colonies displaying streaky iodine-staining reaction. No swi mutants have yet been identified among the large number of fully iodine-negative mutants screened.

The swi genes fall broadly into three categories referred to as class Ia, Ib, and II, suggesting the existence of genetically distinguishable steps in the recombination event of switching. Mutants of class Ia have very much reduced levels of smt double-strand cut but display no other obvious phenotype. High-frequency switching clearly requires the smt cut, whose formation has been presumed to be an initiating event, allowing invasion of the cut end into the H1 regions of either mat2-P or mat3-M (8). While formal proof that the cut is a recombination initiator rather than resulting from a resolution step is lacking in S. pombe, in S. cerevisiae the YZ cut cannot be a result of resolution because it is formed in strains carrying total deletions of both donor cassettes (20).

The three genes required for formation of the smt cut need not each be involved directly in generation of the cut but might, for example, control the accessibility of the chromosome to cutting. In fission yeast, switching does not occur at random but with an inherited pattern (1, 2, 8). Experiments on switching of diploid cells suggest that this pattern is controlled not by asymmetric segregation of a cytoplasmic factor but by a mechanism of chromosomal predetermination (21). In S. cerevisiae it has been found that each of five identified swi genes is required for expression of the HO gene (22), which is active in switching mother cells but is unexpressed in nonswitching daughter cells (23).

The class Ib mutants present a puzzle. They contain normal levels of smt cut and yet switch infrequently. Thus, the smt cut can be formed and presumably healed without leading necessarily to a switch of mating type. The class Ib mutants could be defective for either of two different types of function: (i) utilization of the smt cut or (ii) control of the directionality of switching. Plus → plus or minus → minus switching could be occurring at high frequency but would not be detected as a change of mating type. Since there is no assay for homologous switching in fission yeast, the alternatives of nonutilization of the cut, as opposed to incorrect utilization of the cut, cannot be distinguished at present.

The class II genes are required for the correct resolution of recombinational intermediates of switching. In strains carrying class II mutations, the level of smt cut is normal in the h9° chromosome, but the mating-type locus rearranges to give aberrant resolution events, h9° and mat2:1. mat2:1 arises during a switch between mat2-P and mat1 in which resolution of a presumed Holliday structure results in a cross-over. This yields mat2:1 and a fusion between mat1 and mat2-P. Loss of the DNA between mat1 and mat2-P is lethal to the cell (9, 18), and the poor viability of the class II mutants probably reflects mitotic instability of the mat2:1' episteme (unpublished data). Tight class II mutants would be expected to be lethal.

Resolution associated with cross-over occurs approximately once in 500 cell divisions in normal h9° strains (24). While the rate at which it occurs has not been directly determined in the class II mutants, the level of unstable mat2:1' episteme is higher in the mutants than in wild-type h9° strains in the diploid in which it has not been detected (Fig. 3 A and B; Ref. 9). h9° arises from h90 as a consequence of a quite different defect of switching resolution. If during the plus → minus switch, resolution fails to occur between the H2 regions of mat1-P and mat3-M but instead copy synthesis continues through the mat3-M cassette, the next possible site of resolution is between the H2 regions of mat1-P and mat2-P. Resolution between these sites results in transposition of mat2-P and mat3-M cassettes along with the intervening sequence.
(K) into \textit{matl} (Fig. 1B). It is not possible to state whether \(h^{+N}\) arises at a higher frequency than normal in class II mutants. The predominance of \(h^{+N}\) in cultures of class II mutants might merely reflect selection for \(h^{+N}\). This form of the chromosome contains less double-strand cut than normal (9) and would be expected to give some protection against the lethal formation of \textit{mat2:1}\. The tetrad shown in Fig. 2C illustrates the overgrowth of \textit{swi9} by \textit{swi4}.

The phenotypes of \textit{swi} mutants described here suggest that the recombination event of mating-type switching can be subdivided into three distinguishable steps: (i) formation of the \textit{smt} cut, (ii) utilization of the \textit{smt} cut, and (iii) resolution of recombinational intermediates. The role of the \textit{swi} genes in recombination events other than mating-type switching is largely unexplored, but it has been found that \textit{swi5} and \textit{swi9} strains are abnormally sensitive to UV irradiation (H. Gutz, personal communication). The action of some \textit{swi} gene products may be restricted to switching of mating-type cassettes, whereas others are required for general DNA recombination and repair processes.

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