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The developmental fate of fission yeast cells is determined by the pattern of inheritance of parental and grandparental DNA strands

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A key feature for development consists of producing sister cells that differ in their potential for cellular differentiaton. Following two cell divisions, a haploid *Schizosaccharomyces pombe* cell produces one cell in four 'granddaughters' with a changed mating cell type, implying nonequivalence of sister cells in each of two consecutive cell divisions. The observed pattern of switching is analogous to the mammalian 'stem cell' lineage by which a cell produces one daughter like itself while the other daughter is advanced in its developmental program. It is tested here whether sisters differ because of unequal distribution of cytoplasmic and/or nuclear components to them or due to inheriting a specific parental DNA chain at the mating type locus. Only the DNA strand-segregation model predicts that those cells engineered to contain an inverted tandem duplication of the mating type locus should produce equivalent sisters. Consequently, two 'cousins' in four related granddaughter cells should switch. The results verified the prediction, thus establishng that all cells otherwise fully possess the potential to switch. Therefore, the program of cell type change in *S.pombe* cell lineages is determined by the pattern of DNA strand inheritance at the mating type locus. A specific DNA sequence present at the mating type locus is postulated to be the cause of developmental asymmetry between sister cells. A general model for cellular differentiation is proposed in which the act of DNA replication itself is hypothesized to produce developmentally nonequivalent sister genomes.

Key words: cell fate determination/fission yeast/mating type/strand segregation

Introduction

Discovering the mechanisms by which sister cells gain different developmental fates is central to an understanding of eukaryotic cell differentiation. This issue can be addressed in molecular terms by investigating the pattern of cell fate determination in the fission yeast *Schizosaccharomyces pombe*. Mitotically dividing cells of this ascomycete usually exist as single cells in a haploid state. The haploid cell exists as one of two alternate cell types (Leupold, 1950; Egel *et al.*, 1980), called *P* (for *P*lus) and *M* (for *M*inus). However, the *P* and *M* mating types are unstable and the cell types interchange spontaneously nearly every other generation (Egel, 1977). Consequently, a clonal population derived from a single cell contains a mixture of *P* and *M* cells in nearly equal proportion and the strain is designated *homothallic* (h^{90}). When the phenotype of individual cells is monitored, a remarkable pattern of cell type switching is observed. Miyata and Miyata (1981) found that among a pair of sister cells, one will divide to produce one changed cell and one unchanged cell in ~72–94% of cell divisions, while the other sister always produces an unchanged pair of cells. In other words, according to this so-called 'one-in-four rule', only one grandchild acquires the switched mating type among the four grandchildren of a cell.

The *P* and *M* cell types are controlled, respectively, by the alternate *mat1-P* and *mat1-M* alleles of the mating type locus (*mat1*), which resides on linkage group II (Egel *et al.*, 1980). 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 *mat1*, resulting in a switch of cell type (Figure 1). Previous studies have argued that the recombination event required for *mat1* switching is initiated by a site-specific double-stranded DNA break (DSB) in the *mat1* gene (Beach, 1983; Beach and Klar, 1984; Egel *et al.*, 1984). The cut site, designated *smt* [*s*witch of *m*ating *t*ype (Egel *et al.*, 1980)] is situated near the junction of the *mat1* allele-specific and H1 regions and has been partially defined (Nielsen and Egel, 1989; Figure 1). Analysis of DNA isolated from exponentially growing cultures showed that ~20–25% of the *mat1* DNA is cleaved and that the proportion of broken DNA is constant throughout the cell cycle. If it is assumed that the cut DNA at the receptor site is a necessary precondition for switching by gene conversion, then it follows that the one-in-four switching rule must be a consequence of regulation of the level of DSB *in vivo*.

The switching of one in four related cells must be the result of unequal distribution of developmental potential to daughter cells in each of two consecutive apparently asymmetric cell divisions. A strand segregation model was recently proposed (Klar, 1987a) to explain the developmental asymmetry in which a site- and strand-specific 'imprinting' of the *mat1* DNA is hypothesized to occur in each cell (Figure 2A). The term imprinting (after Crouse, 1960) refers to a non-mutational alteration of a chromosome that predetermines its behavior later in development. It was further proposed that at the time of DNA replication, only the chromatid that inherits the imprinted strand is cleaved by a hypothesized endonuclease. The cell inheriting the cleaved chromosome then generates one switched and repaired chromatid and one unswitched and cut chromatid. Their segregation will generate one switched and one nonswitched granddaughter cell. The recently switched cell will again imprint its DNA and will repeat the cycle to produce a single switched granddaughter cell. Thus, by this strand segregation model, cytoplasmic and nuclear competence of all yeast cells is suggested to be equivalent and the one-in-four rule is suggested to be the consequence of inheritance of different parental DNA strands by daughter cells. This follows from the fact that

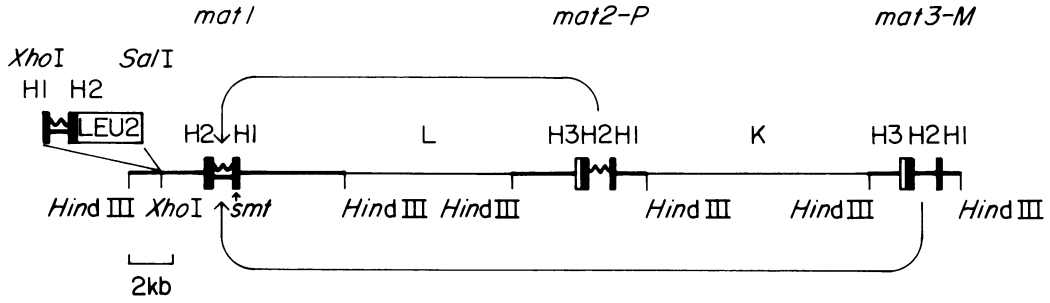

Fig. 1. The *mat* region on chromosome II. Thick lines show that 10.4, 6.3 and 4.2 kb *Hin*dIII fragments, respectively, containing *mat1*, *mat2-P*, and *mat3-M* cassettes. The centromere is located about 65 centiMorgans to the left of *mat1*. The homology boxes H1 (59 bp) and H2 (135 bp) are present in all cassettes and flank the allele-specific *P* (1113 bp, jagged line) and *M* (1127 bp, smooth line) sequences. The third homology H3 region (57 bp) is present at *mat2* and *mat3*. The interval between *mat1* and *mat2* is called L (~15 kb long) while the interval between *mat2* and *mat3* is called K (also ~15 kb in length). *smt* marks the site of the double-stranded cut in *mat1*. Arrows indicate that *mat1* switches occur by unidirectional transfer of DNA from *mat2* and *mat3* loci to *mat1*. The left *mat1* cassette was incorporated in the chromosome to construct the required duplication described earlier (Klar, 1987a). It involved insertion of the *mat1* cassette (1450 bp) in an inverted orientation plus *LEU2* (2.2 kb) sequences into the *mat1*-proximal *Xho*I site. Each *mat1* allele encodes two transcripts. The figure is derived from data presented earlier (Egel and Gutz, 1981; Beach, 1983; Beach and Klar, 1984; Egel, 1984a; Kelly *et al.*, 1988).

the two strands of DNA in the Watson–Crick model are different from each other, since they are complementary and not identical (Watson and Crick, 1953).

A key genetic prediction of the strand segregation/inheritance model is that two in four related cells should switch in strains containing an inverted tandem duplication of *mat1* (Figure 2B). This is predicted because, in a given cell (parent cell in Figure 2B), both strands can be imprinted—one strand in one cassette and the other in the second cassette. Their segregation will generate two developmentally equivalent sister chromatids and their inheritance should produce equivalent sister cells. Each of the equivalent sister cells may generate one switched and one unswitched progeny. The essence of this critical prediction is that two 'cousin' cells should switch.

By employing a single-cell assay, a test of the prediction is provided in this paper. It was observed that two (cousins) in four related cells indeed switch in strains containing an inverted tandem duplication of *mat1*. Consequently, I conclude that the pattern of switching in the fission yeast cell lineage is dictated by the pattern of inheritance of DNA strands and not by the unequal distribution of cytoplasmic or other nuclear components to daughter cells.

Results

The experimental rationale

Testing the prediction that two (cousins) in four cells would switch in strains containing duplicated mating type loci requires analysis by single-cell assays and is hindered by at least three experimental difficulties. First, cell type in *S.pombe* can be only tested by mating to cells of known mating type. The act of mating produces zygotic diploid cells which normally undergo meiosis and sporulation (Egel *et al.*, 1990). By this assay, the cell is lost in zygote formation and, therefore, testing the mating type of a cell and its progeny in consecutive generations in cell lineage studies is not possible. Miyata and Miyata (1981) developed a procedure for pedigree analysis that allowed the four progeny of a single cell to conjugate with each other to form zygotes. They observed that among the four cells, only two mated to form a zygote; thus, only one in four cells must have switched. This assay did not require determination of mating type of each cell and therefore could not be used in the present study. Second, in chromosomes where one *mat1* cassette is cleaved while the other is not (Figure 2B), the cut cassette might be healed by a gene conversion event using the adjoining uncut cassette as the donor, rather than the normally used *mat2* or *mat3* donor locus. Consequently, most (or all) of the switches could be homologous cassette replacements and would be undetectable. Third, the additional cassette in the duplication may not fully provide the *mat1* functions, and hence, its contents may not be phenotypically scorable.

To circumvent some of these problems, instead of testing mating, the ability of individual cells to sporulate was determined. To test whether the additional cassette in the duplication can provide *mat1* functions required for meiosis and sporulation, the ability of haploid cells containing the *mat1* inverted duplication (*Dup*) to undergo pseudomeiosis was examined afer growing cells in sporulation conditions. Two transcripts of each of the *P* and *M* alleles are required for meiosis and sporulation (Kelly *et al.*, 1988). Haploid *S.pombe* cells expressing both mating types can undergo pseudomeiosis to produce mostly inviable aberrant-looking and immature spores (Beach *et al.*, 1982). It was found that a significant fraction of haploid strain SP482 (*Dup*; see Table II for complete genotype) cells can indeed undergo pseudomeiosis. Thus, it is concluded that at least one or either of the *mat1* alleles, when present in the additional cassette, can provide the full complement of functions required for sporulation. Results presented later in this paper show that both *mat1* alleles in the duplication are functional. Determining the ability of individual haploid cells to sporulate in a cell lineage study was difficult because microscopic identification of immature spores contained in a cell was ambiguous. Therefore, the pedigree analysis of dipoid cells, which produce easily identifiable asci containing mature ascospores, was conducted.

Although one cannot predict how the problem of repairing the DSB from an adjoining cassette will affect the analysis, determination of the contents of the duplciated *mat1* cassettes

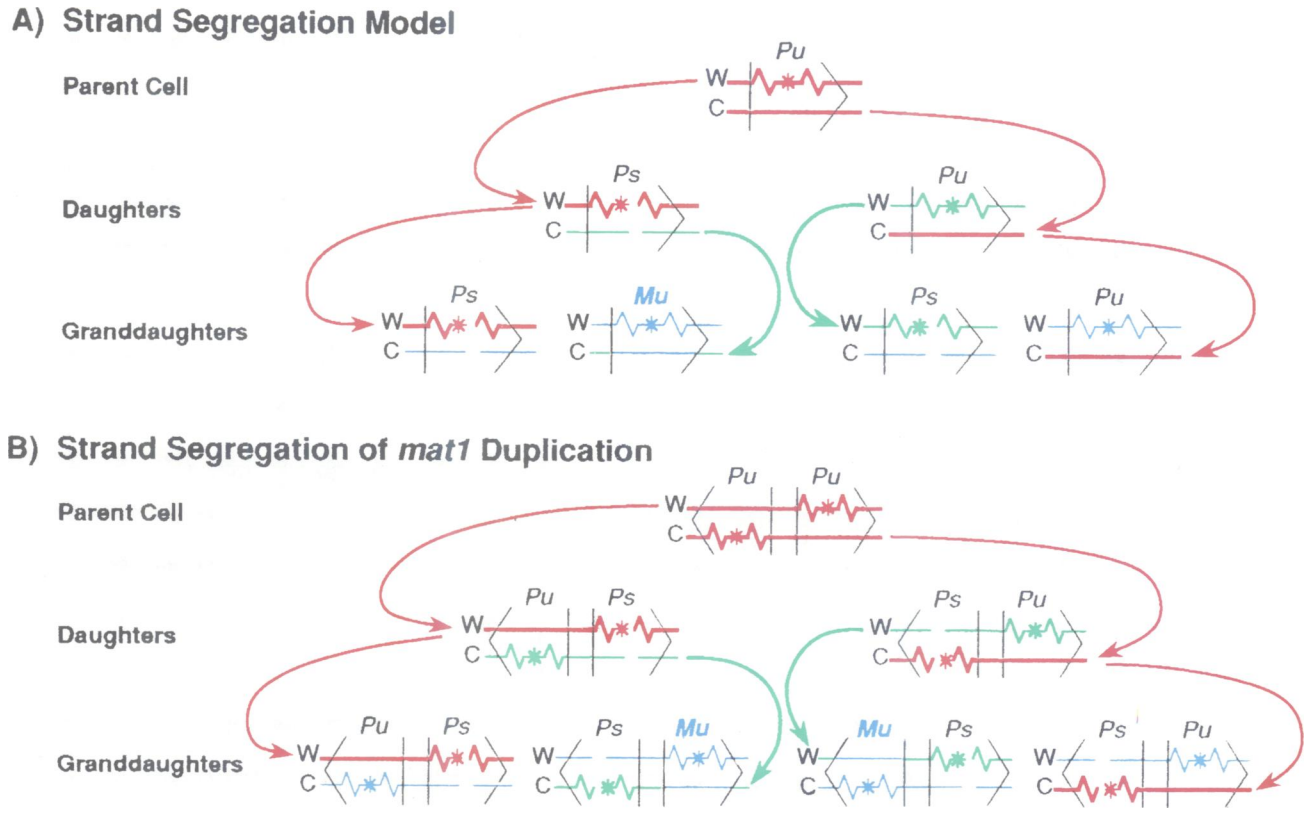

Fig. 2. The strand-segregation model and its tests. (**A**) The strand-segregation model. Imprinting (*) of the Watson (W) strand (only the jagged line is imprintable) at *mat1* occurs in the parent cell. It may have inherited the already imprinted chromosome from its parent that imprinted, say, in G2. During replication, the chromatid inheriting the imprinted W strand is cleaved by the hypothesized endonuclease, while the one inheriting the Crick (C) strand is not cleavable because its newly synthesized W strand is not yet 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 non-equivalent chromosomes, one inheriting the cut chromosome and its sister inheriting the chromosome without the cut. At or soon after the time of DNA replication, the daughter cell with the DSB generates one chromatid containing the switched and healed *mat1* allele and the other chromatid is cleaved and unchanged at the *mat1* allele. Furthermore, the switched chromatid is specified to contain the specific C strand. To help demonstrate the pattern of segregation to parental strands, those strands are represented by thick lines. Colored lines ending with small arrows indicate segregation of specific color-coded DNA chains to progeny cells. Wide arrows indicate cassette orientation in the chromosome. A recently switched allele is indicated by the boldface letter. A gap in the continuity of the chromosome denotes the presence of the DSB at the *smt* (cut) site. The *Pu* and *Mu* (*u* for *u*nswitchable) indicates cassettes that cannot switch during or soon after their replication, while the *Ps* and *Ms* (*s* for *s*witchable) can. (**B**) Segregation of DNA strands of the chromosome containing an inverted *mat1* duplication (see Figure 1 for a description of the duplication). The strand-segregation model predicts that (i) two cousin cells in four granddaughter cells would swtich in duplication containing strains and (ii) twice as many chromosomes should be cleaved as compared to the wild-type strains, and in a given chromosome either, but only one, cassette can ever be cleaved. The figure is modified from Klar (1987a).

by single-cell sporulation assays was attempted. Diploid cells expressing both *mat1-P* and *mat1-M* genes can undergo meiosis and sporulation, while those homozygous for either allele fail to do so (Egel *et al.*, 1980). By this procedure (Egel and Eie, 1987), but employing the meiosis and sporulation media used by Miyata and Miyata (1981), the pattern of *mat1* switching in a cell lineage was monitored. The capability of individual cells to sporulate was assayed by observing them microscopically while incubated on solid sporulation media. In the pedigree studies of diploid cells described below, switching of the *mat1* locus or loci in the duplication was possible only in one homologue. In each strain, the other chromosome carried a nonswitchable heterothallic allele, called h^{-L}, h^{+L}, h^{+N}, h^{-U}, or *smt-o*.

The pattern of switching of a single mat1 locus in diploid cells

As a control for the studies with strains containing an inverted duplication to be presented in the following section, the switching pattern of the diploid strain SP10 (h^{90}/h^{-L}; see Table II for complete genotype) was ascertained. This diploid contains wild-type *mat1* cassette (h^{90}) on one chromosome and h^{-L} (expressed *M* allele) rearrangement on the homologue. A diploid cell of genotype *mat1-M*/h^{-L} will not sporulate because of the lack of an expressed *P* allele and such a cell may divide on sporulation medium. However, when a *mat1-P*/h^{-L} cell is generated by switching on the h^{90} chromosome from *mat1-M* to *mat1-P*, it should stop growing and should sporulate, terminating that branch of lineage. By monitoring the pattern of sporulation of individual cells in a cell lineage study, the pattern of switching of the *mat1* allele on the h^{90} chromosome could, therefore, be determined. Figure 3a and Table I show results of one such pedigree. Likewise, the pattern of switching of the h^{90} chromosome from *mat1-P* to *mat1-M* in strain SP21 (h^{90}/h^{+L}) was examined (Figure 3b; Table I). Both alleles were found to switch by the usual rule of one-in-four even in diploid cells.

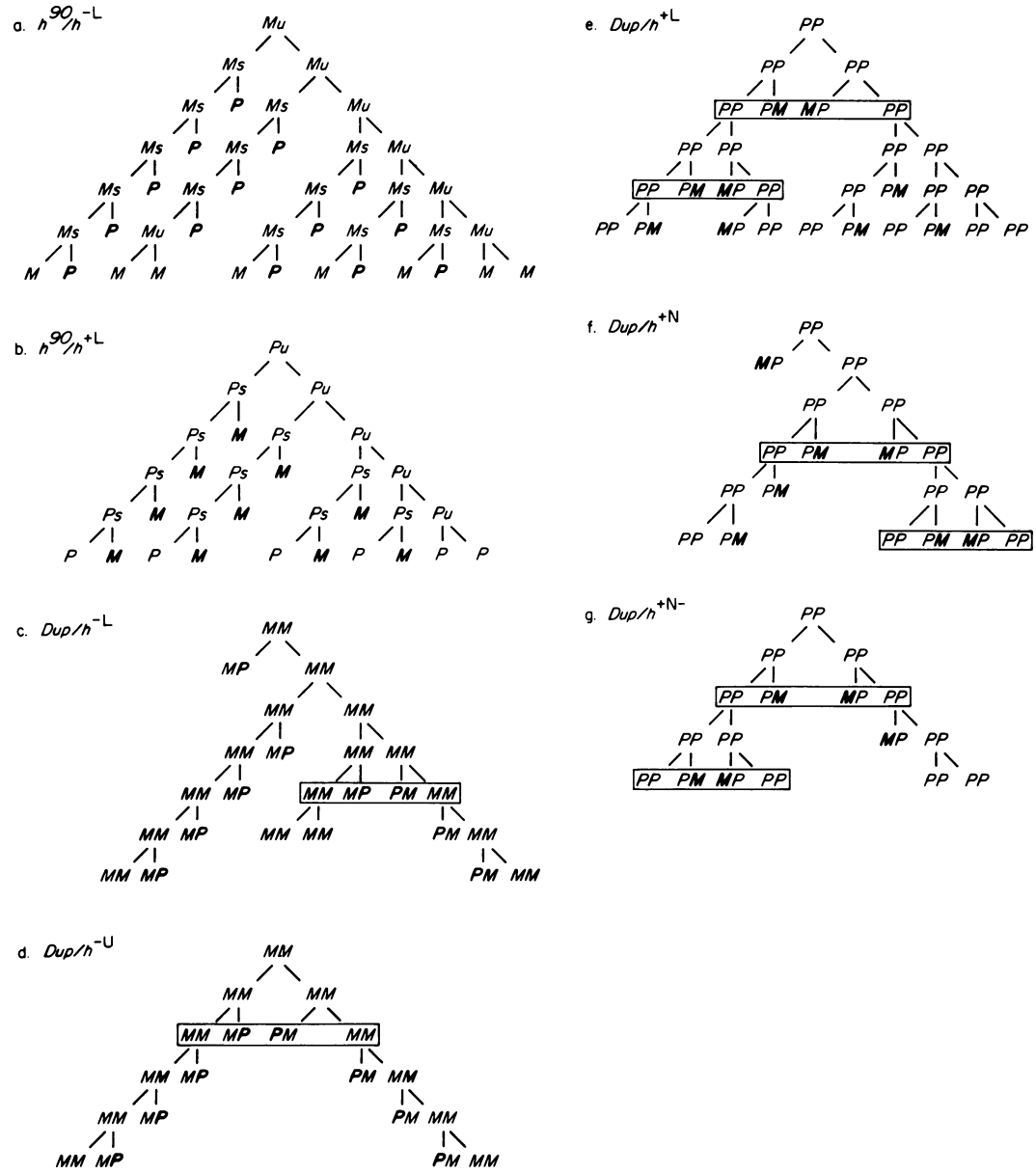

Fig. 3. Cell lineage study assays the ability of diploid cells to sporulate. **a**, SP10; **b**, SP21; **c**, SP559; **d**, SP563; **e**, SP481; **f**, SP556; **g**, SP557. A single representative lineage is presented from each strain A newly switched cassette is indicated in bold print. Switching of a particular cassette is arbitrarily assigned in *Dup*-containing strains, as it could not be determined which cassette had actually switched. Cases where two cousins have switched are boxed. To simplify the presentation, only the genotype of the switchable allele is presented in the drawings.

Table I. Efficiency of switching

Strain	Genotype	Direction of switching	Switched/4 cells			
			0/4	1/4	2/4[a]	% 2/4
A SP10	h^{90}/h^{-L}	M → P	23	129	0	0
B SP559	Dup/h^{-L}	M → P	13	37	3	6
C SP563	Dup/h^{-U}	M → P	10	29	31	44
D SP21	h^{90}/h^{+L}	M → P	25	275	0	0
E SP481	Dup/h^{+L}	M → P	35	167	28	12
F SP556	Dup/h^{+N}	M → P	6	48	15	22
G SP557	Dup/h^{+N-}	M ⇌ P	18	24	22	34
H SP752	h^{90}/h^{-u}	M → P	10	66	0	0
I SP824	$Dup\text{-}smt\text{-}o/h^{+N-}$	M → P	235	103	0	0

[a]2/4 column indicates that two cousins among four granddaughter cells have switched. Pedigrees of individual cells that did not produce an ascus at the 2-cell stage (i.e. those that behaved as parent cells; Figure 2) are presented in the table.

Two cousins in four related cells switched in mat1 duplicated strains

To test the genetic prediction of the strand segregation model, pedigree analysis was undertaken with diploid cells containing the *mat1* duplication in one of their chromosomes. Figure 3 and Table I show the results of studies with strains SP559 (Dup/h^{-L}) and SP563 (Dup/h^{-U}) for testing switches of *mat1-M* to *mat1-P*, and with strains SP481 (Dup/h^{+L}) and SP556 (Dup/h^{+N}) for testing switches of *mat1-P* to *mat1-M*. The most significant finding was that two cousins in four related cells indeed switched in a considerable proportion of progeny. These cases were found at varying rates (Table I, last column). Perhaps strain differences or statistical fluctuations, given the small number of pedigrees analysed in some cases, contributed to the observed variation (see also Discussion).

About 10% of the cells derived from Dup/h^{-U} and

Table II. Experimental strains of *S.pombe*

Strain	*mat1*[a]	Genotype	Source
SP21	h^{90}/h^{+L}	*leu1.32/leu1.32 ade6-M216/ade6-M210*	Beach and Klar, 1984
SP481	Dup/h^{+L}	*leu1.32/leu1.32 ade6-M216/ade6-M210*	SP21 derivative
SP482	*Dup*	*leu1.32 ade6-M210*	SP481 segregant
SP10	h^{90}/h^{-L}	*swi3/+ ade6-M210/+ +/leu1.32 +/his2*	This study
SP559	Dup/h^{-L}	*leu1.32/leu1.32 ade6-M216/ade6-M210 his3(?)/+*	This study
SP563	Dup/h^{-U}	*leu1.32/leu1.32 ade6-M210/ade6-M216*	This study
SP556	Dup/h^{+N}	*leu1.32/leu1.32 his3/+ +/his2 ade6-M210/ade6-M216*	This study
SP557	Dup/h^{+N-}	*leu1.32/+ his3/+ ade6-M210/ade6-M216 +/mat2-P-B102*	This study
SP562	h^{-U}	*ade6-M216*	This study
SP752	h^{90}/h^{-u}	*XhoI::LEU2/XhoI leu1.32/+ ade6-M216/ade6-M210*	This study
SP824	$Dup\text{-}smt\text{-}o/h^{+N-}$	*+/mat2-P-B102, +/his3, ade6-M216/ade6-M210 leu1.32/leu1.32*	This study

[a]The wild-type *mat1* is designated h^{90}. The heterothallic alleles h^{-L} and h^{-U} express *M* information, while the h^{+L} and h^{+N} provide *P* function. These are rearrangements of the *mat1* locus and switch rarely ($<1 \times 10^{-5}$; Egel *et al.*, 1980; Beach annd Klar, 1984). The h^{+N-} allele was derived from a strain kindly provided by D.Beach. This strain has a mutant *mat1* that causes meiosis and sporulation failure. *Dup* indicates inverted tandem *mat1* duplication. The *smt-o* mutation is defined in the text.

Dup/h^{+N} strains produced asci rarely (at $<1 \times 10^{-3}$). DNA analysis demonstrated (data not shown) that these cells had lost the *mat1* duplication and probably had become homozygous for the heterothallic h^{-U} or h^{+N} *mat1* allele. Such cases were disregarded while compiling the data presented in Table I and are presently under further study.

Cassettes in the duplication switch individually

From the above analyses, it was not possible to determine whether one or both cassettes of the duplication had switched in any given cell. This was determined by analysis of the pedigree of strain SP557 (Dup/h^{+N-}). This strain contains the h^{+N-}-defective heterothallic allele, which does not provide sporulation functions. Therefore, any cell that sporulated must have expressed simultaneously both *P* and *M* information from the duplicated cassettes, an arrangement that must be created by an individual cassette switching event. Here again, two cousins in four related cells sporulated in 34% of cases (Figure 3g and Table I). It therefore seems probable that such single switch events generate the meiosis data in Figure 3 and Table I, and that cousin cells that formed asci switched single and presumably different cassettes.

The insertion of LEU2 alone at the mat1 proximal XhoI site does not change the pattern of switching

As a control to test whether the altered pattern of switching of strains containing the inverted duplication was simply due to the insertion of any sequence next to *mat1*, the pattern of switching of a strain containing only the 2.2 kb *SalI/XhoI LEU2* fragment incorporated into the *mat1*-proximal *XhoI* site (Figure 1) was investigated. The pedigree analysis of such a strain, SP752 ($XhoI::LEU2\ h^{90}/h^{-u}$), demonstrated that only one in four grandchildren switched (Table I), a pattern similar to that found in wild-type strains.

The inverted mat1 cassette also switches by following the one-in-four rule

The observed two-in-four switching pattern of duplication containing strains is suggested by the model to be the result of switching of both *mat1* loci, but individually and in different portions of the lineage, each cassette follows the on-in-four rule (Figure 2B). Thus, as another control, the pattern of switching of the inverted *mat1* cassette alone was determined. To make this control as analogous as possible to the experimental situation, strain SP824 was constructed in which only the single inverted cassette could switch (left one in Figure 1). The adjoining (original) cassette in the duplication contains the nonswitchable *mat1-M* allele with the *smt-o* mutation. The switching-deficient *smt-o* mutation (Engelke *et al.*, 1987) is a *cis*-acting small deletion of the *mat1*-distal sequence (O.Nielson and R. Egel, personal communication). The single cassette with the *smt-o* mutation alone, or contained in the duplication, is not cleaved and consequently fails to switch (Engelke *et al.*, 1987; unpublished observations). The SP824 strain was constructed by conventional genetic crosses employing a strain containing wild-type cassettes in the duplication and a *smt-o* mutant strain. The other chromosome in this diploid contains the nonswitchable h^{+N-} allele (see Table II for complete genotype of SP824). Therefore, in this strain only switching of the left inverted cassette occurs, which can be assayed by pedigree analysis. As the right cassette contains an unswitchable *M* allele, ony switches from *M* to *P* in the left cassette will make the cells competent to sporulate and thus can be assayed.

The results presented in Table I (line I) show that the inverted cassette itself switches, as predicted by the strand-segregation model, by following the rule of one-in-four switching, and no case of two-in-four switching was observed. The observed level of one-in-four switching in

30% of the pedigrees compares well with the 34% value of two-in-four switching of an analogous strain but containing both wild-type *mat1* alleles in the duplication (SP557, *Dup/h*$^{+N-}$, Table I, line G). As concluded in a previous section, each cell of the SP557 duplication-containing strain that switched must have resulted from single cassette switches. Therefore, those two-in-four cases in duplication-containing strains are most certainly due to the switching of both cassettes, but only one at a time in a particular cell, with each cassette following the on-in-four rule. Since the inverted cassette can switch, the switching is obviously independent of the *mat1* orientation in the chromosome.

Discussion

There must be two consecutive asymmetric cell divisions occurring in fission yeast cells to produce only a single switched cell among four related granddaughter cells. Whether the asymmetry is due to cellular differences or due to the pattern of segregation of DNA chains is tested here. Several conclusions can be drawn from these studies.

The developmental asymmetry of progeny of Pu and Mu cells is due to specific DNA strand imprinting and its inheritance

The major result is the verification of the key genetic prediction of the strand-segregation model. It was observed that at a frequency varying from 6 to 44%, two cousins in four granddaughter cells switched in strains containing an inverted tandem duplication of *mat1* (see Figure 2B for predictions and Figure 3 and Table I for results). Those two-in-four cases were most likely due to switching of both cassettes in the duplication, but only one in a given cell, with each cassette individually following the one-in-four rule. Overall, the observed frequency (~20%) of two-cousins-in-four switches was less than the expected 69% (0.83 × 0.83), which was calculated from the observed frequency (0.83) of switching of a single *mat1* cassette in diploids (this paper). Perhaps the reduced rate was due to the healing of some of the DSBs by using the adjoining *mat1* cassette in the duplication as the donor, resulting in futile homologous switches. Moreover, the additional cassette may not have efficiently interacted with the opposite allele donor. Indeed, the inverted cassette itself was found to switch from *mat1-M* to *mat1-P* at a reduced level of 30%, thus fully accounting for the reduced frequency of two-in-four cases found in duplicated strains. Thus, the observation of two cousins switching among four cells is conclusive evidence for the strand-segregation model, since in the pedigrees with control strains (Table I, lines A and D) such cases were not found.

As an alternative interpretation, one can imagine that the observed switching of two cousins in four related cells (this paper) is not due to strand segregation but it is due to some chromatin disturbance(s) introduced by DNA insertion next to *mat1* in the *mat1*-proximal *Xho*I site. A possible control to test this notion is to determine the pattern of switching of cells containing the direct duplication. Unfortunately, this is technically impossible, since in efficiently switching strains the direct duplication is extremely unstable (Klar, 1987a). Clearly, the DSB found in the duplication promotes efficient deletion formation, leaving only one cassette in place of the duplication. Secondly, maintaining the duplication by selecting for *LEU2* presence in the duplication during pedigree analysis of (*LEU2*/−) diploids is experimentally prohibitive because of the very weak expression of the *Saccharomyces cerevisiae LEU2* gene in *S.pombe*. As an alternative control, the pattern of switching of a strain containing only *LEU2* at the *mat1*-proximal *Xho*I site (Sp752, *H*^{90} *Xho*I::*LEU2*/*h*$^{-u}$) was attempted. This *Xho*I site is the same site where *mat1* duplications were constructed (Figure 1). It was found that the *mat1* allele present in *cis* with the *LEU2* insert switched by the usual pattern of one-in-four. This finding argues against overt chromatin changes induced by DNA inserts placed next to *mat1* for affecting the pattern of switching. As another control, it was observed that the left (inverted) cassette in the duplication itself switches by the one-in-four rule. Furthermore, only a single cassette at a time switches in the duplication in a given cell. Therefore, the two-in-four switches in duplication-containing strains must be due to switches of both cassettes, each following the one-in-four on its own in related cell lineages. These conclusions exactly satisfy detailed features of the prediction in Figure 2B. Thus, analysis of these controls makes the analysis of the impossible direct duplication control rather unnecessary.

The observations that two cousins in four granddaughter cells indeed switch demonstrate that both parents (themselves sisters of each other) of the four granddaughter cells have become developmentally equivalent. Thus, one of the sister cells that never produced switched progeny in wild-type strains is now shown to acquire the competence to do so. These results significantly extend the earlier molecular observations which suggested that two among four cells should switch since twice as many chromosomes are cleaved in duplication containing strains. Earlier molecular results favouring the strand-segregation model consisted of assaying the level of DSB of both *mat1* loci in the inverted duplication. It was found that (i) each cassette in the duplication was cleaved at the wild-type rate; (ii) twice as many chromosomes were cleaved; yet (iii) both sites were not simultaneously cleaved in the same chromosome (Figure 2B for predictions; Klar 1987a). Since twice as many chromosomes were found to be cleaved, and as each site was cleaved at the wild-type rate, cleavage at one site might not have interfered with the cleavage at the adjoining site (see Russell, 1987). Thus, developmentally different sister cells are generated because they inherit chromosomes containing different strands of parental DNA. Therefore, the two-stranded structure of DNA (Watson and Crick, 1953), and its mode of semi-conservative replication and segregation (Meselson and Stahl, 1958), can potentially provide developmental asymmetry in any given cell division.

More specifically, to generate nonequivalent sister chromatids I argue that the strand differences in *S.pombe* are due to DNA imprinting at *mat1* which is proposed to be site-specific and also strand-specific. Egel (1984b) had earlier found in an interesting observation that in a diploid asymmetrically segregating competence for switching is determined separately for the two homologues, indicating a chromosomal basis for segregation of developmental asymmetry. This paper provides a basis for why some chromosomes are enabled to switch while others are prohibited by arguing for the existence of a strand-specific sequence at *mat1* whose replication produces developmentally nonequivalent chromatids. Although different in detail,

similar models proposing DNA strand differences to explain cellular differentiation (Holliday and Pugh, 1975), for perpetuating stem cells while protecting them against mutation (Cairns, 1975), and for fission and budding yeast mating type interconversion (Egel, 1984b; Williamson and Fennell, 1981; Nasmyth *et al.*, 1987b; Klar, 1987b) have been considered in the past. Only the results with *S.pombe* provide strong evidence for generating developmental asymmetry by segregating parental DNA strands.

A particular mat1 locus in diploid cell switches by following the one-in-four rule

As a control for studies with the *mat1*-duplicated strains, it was found that h^{90}/h^{-L} and h^{90}/h^{+L} diploids exhibit switches of the single *mat1* locus in the h^{90} chromosome at a comparable frequency and by the same pattern of one-in-four cells as was earlier described for h^{90} haploids by Miyata and Miyata (1981). Egel and Eie (1987) have recently described the pattern of switching of *mat1* in the h^{90} chromosome of genotypically different h^{90}/h^{-U} and h^{90}/h^{+N} diploids. They observed a reduced rate of switching in diploids, possibly due to differences in the sporulation conditions employed for testing switching. Both studies, however, tested switches of the single h^{90} chromosome in diploid cells, and two types of 'stem cell lineages' were observed. *Mu* and *Pu* cells efficiently generate *Ms* + *Mu* and *Ps* + *Pu* cells, respectively (Figure 3a,b). In turn the *Ms* and *Ps* cells efficiently switch (>80%) to generate *P* (switched) + *Ms* and *M* (switched) + *Ps* cells, respectively. That is, chains of consecutive switches are found, suggesting that the imprinted event is mostly stable and that it is passed on generation after generation to one of the unswitched progeny cells in >80% of cell divisions. Also, since the *Ps* cells produce an *M* and *Ps* pair, the recently switched *M* cell must be *Mu*. Were it not so, it would violate the Miyata's one-in-four rule. By the same argument, a cell recently switched to *P* must possess a *Pu* cassette. Also, the inverted cassette in the duplication switches by following the one-on-four rule, demonstrating that switching is independent of *mat1* cassette orientation.

The developmental asymmetry of progeny of Ps and Ms cells is also due to specific strand inheritance

The strand-segregation model only suggests that the basis of the first asymmetric cell division of *Pu* and *Mu* cells is due to differences in the segregation of a premarked DNA strand (Figure 2A). However, I argue that the second asymmetric cell division, i.e. those of *Ps* and *Ms* cells, is also due to specific strand segegation for the following reasons. Consider those cells that contain the inverted duplication. A cell containing *Pu* (unbroken but imprinted on one particular strand) and the adjoining *Ps* (cleaved) cassette generates a pair of *PM* and *PP* cells (Figure 2B). If the imprinted strand, originally inherited from the parent cell, is always inherited by the nonswitched *PP* cells, then *PuPs* and *PsMu* pairs will be generated (see bottom left of Figure 2B). Each cell will have a cut at one or the other cassette, but never simultaneously at both—a prediction borne out by earlier results (Klar, 1987a). On the other hand if the imprinted strand is inherited randomly or always by a recently switched cell, then *PuMu* and *PsPs* pairs of sister cells could be generated. The *PsPs* cell should have the DSB at both *smt* sites. However, this was not observed. Whether a *PuPs* cell can generate a *PuMu* and *PsPs* pair of cells can also be determined by pedigree analysis. The hypothesized *PsPs* daughter cell should generate *PM* and *MP* cells. Consequently, the *PuMu* daughter and *PM* and *MP* progeny of the *PsPs* daughter should form asci in the assay system employed here. Such pedigrees with 'three asci' were not found. Thus, the *PsPs*, and by analogy the *MsMs*, cells are not generated. Therefore, it seems certain that the switched granddaughter cell always inherits the nonimprintable specific chromosomal strand from its immediate parent cell.

The specific strand segregation model extended to two consecutive cell divisions

By combining genetic observations discussed in this paper with the molecular results presented earlier (Klar 1987a), a specific strand-segregation model can be proposed. It has the following features (Figure 2A). (i) In the O generation ('parent' cells with *Pu* or *Mu* cassettes in Figure 2A) the arbitrarily chosen Watson (W) strand (and only the W strand can ever be imprinted) was imprinted at *mat1*, as it possibly inherited the already imprinted chromosome from its parent cell which imprinted that chromatid in G2. (ii) At the time of chromosome replication in the parent cell, (a) the chromatid inheriting the imprinted W strand is cleaved at *mat1*, while (b) the chromatid inheriting the Crick (C) strand is not cleavable, since its recently synthesized complementary W strand is not yet imprinted. It will be imprinted later in that cell cycle, possibly in G2. The cell inheriting the uncut chromatid acts as a stem cell and behaves exactly like the parental *Pu* or *Mu* cell. (iii) The cleaved chromatid will maintain the cut for the entire length of the cell cycle and will participate in switching in the first generation in *Ps* and *Ms* cells at the time of their DNA replication. (a) The cut C strand of *Ps* and *Ms* leads to a switched, uncut duplex whose segregation generates, respectively, the recently switched cells *Mu* and *Pu* in the second generation. (b) The other cut W strand of *Ps* and *Ms* cells mostly leads to a cut but unswitched cell (*Ps* and *Ms*), which can again produce switched progeny in the next cell cycle. In other words, if the W strand is imprinted, only the C strand leads to a changed mating type. Perhaps at the time of replication of the cut chromosome, the C strand is healed by a switching (gene conversion) event due to DNA repair synthesis primed at the 3′-end of the break. The other strand may be sealed without switching just before replication, but the resulting chromatid can be efficiently cleaved at the time of DNA replication, perhaps because that strand still has the imprinted event present on it. Yeast cells clearly have the capacity to seal the break without switching, since strains containing deletions of both *mat2* and *mat3* donor loci exhibit normal levels of the break and inviable progeny are not generated. In addition, recombination-deficient mutant cells that contain the DSB but are defective in utilizing the DSB for switching do not produce inviable progeny, again suggesting the capability to seal the break even without switching (Klar and Miglio, 1986). Similarly, those cases where sisters of the recently switched cells apparently skipped a switching event and behaved as *Pu* and *Mu* cells could be ascribed to the capacity of those cells or their daughters to heal without switching. However, homologous cassette replacements, which do not lead to phenotypic changes, could also explain why switches are not observed in every generation of *Ps* and *Ms* cells.

In summary, both divisions of wild-type cells are inherently asymmetric because of segregation of DNA chains and not because of other cellular differences such as those attributed to nonequivalent gene expression or distribution or stability of their gene products. Specifically, it can be stated that a particular grandchild cell that does not receive atoms from the DNA of the mating type region of the grandparental chromosome inherits the switched allele (Figure 2).

Previous results (Klar, 1987a) and those of this paper show that cells with an inverted duplication have twice the number of cleaved chromosomes when compared two wild-type strains, and also they switch two (cousins) in four cells, compared to the one-in-four in wild-type strains. Collectively, these results strongly support the strand segregation model. One interesting observation is perplexing, however. According to the strand-segregation model, nearly 40% of the chromosomes of wild-type cells should be cleaved (Figure 2A, assuming 0.8 probability of cleavage of the *smt* site in 50% of the cells), and by the same token ~80% of the chromosomes should be cleaved (but only at one or other *smt* site) in cells containing an inverted duplication (Figure 2B). The observed level of DSB in strains of each genotype is about one-half of the expected level (Beach, 1983; Klar, 1987a). Perhaps the demonstrated ability of cells to seal a DSB even without switching (Klar and Miglio, 1986) contributes to this apparent paradox. Another simpler explanation is that the switching pattern of cells was determined when they were growing in poor medium with a generation time of ~6 h, but the level of DSB was determined from cells growing in rich medium with a generation time of ~2 h. The different growth conditions may affect the level of DSB found at *mat1*. This possibility is being investigated.

Recently, the possibility of heritable epigenetic changes in chromosomes and chromosome imprinting have drawn considerable attention and have been implicated in many diverse systems (for reviews, see Holliday, 1987; Monk, 1988). Although the *S.pombe* style of generating asymmetric cell divisions is simple to imagine in principle as being inherent in any given cell division (two chromatids with DNA strands of different age), the idea for segregation of DNA chains of a particular chromosome has been tested only in one other system. In budding yeast *Saccharomyces cerevisiae*, the *HO* gene required for mating type switching is expressed in mother (older) cells and not in daughter cells (Nasmyth, 1983). The possibility that this is due to strand segregation of the *HO* gene has been ruled out, since cells containing an inverted *HO* gene switch with the same pattern exhibited by the wild-type cells (Klar, 1987c). In this system, asymmetric segregation of a positive regulatory element encoded by the *SWI5* gene is proposed to be essential for restricting *HO* gene expression in mother cells (Nasmyth *et al.*, 1987a; Sternberg *et al.*, 1987). It should be pointed out that the results presented here do not address whether nonrandom segregation of DNA chains to progeny cells occurs in *S.pombe*. In other words, whatever daughter cell inherits a chromatid containing a specific parental strand, that particular cell will exhibit the developmental program based on whether or not that chromatid was enabled to switch.

A general model for cellular differentiation

In essence, the most important conclusion of general significance from this study is that by the process of DNA replication developmentally non-equivalent sister chromatids are produced (Figure 2A). One can generalize this model for other systems by proposing that the act of replication modulates acivities of the developmentally important genes. For example, a precedence for repressing previously active genes by DNA replication (albeit on both sister chromatids) is found in the regulation of silent mating type genes of *S.cerevisiae* (Miller and Nasmyth, 1984). In a more general and simpler version of this model, it is not essential to propose that strand-specific imprinting is required or that a specific parental strand must be inherited by a particular chromatid. All that is required is that during replication developmentally different sister genomes are generated. This model particularly stresses the specific role of DNA replication for generating developmental asymmetry required for cellular differentiation and it is equally applicable to both prokaryotes and eukaryotes.

Materials and methods

Strains and culture conditions

All strains are of *S.pombe* and their genotypes are presented in Table II. Standard conditions for cell culture were employed according to Gutz *et al.* (1974).

Pedigree analysis

Determining the pattern of switching by assaying for the ability of individual cells to sporulate is highly problematic since nutritional conditions required for growth are very different from those needed for meiosis and sporulation. In the standard growth medium sporulation is prohibited; conversely, in the sporulation medium, growth of cells is prohibited due to nutrient limitation. Therefore, it was essential to define conditions in which those cells incapable of sporulation (e.g. *mat1-P/mat1-P* and *mat1-M/mat1-M*) should grow, but their mitotic progeny containing a switched allele (e.g. *mat1-P/mat1-M*) should be starved enough to sporulate. The following conditions proved appropriate.

Before pedigree analysis, the cells were starved by growing in YM medium for 15 h, where sporulation competent cells undergo meiosis and sporulation. Starved cells were then transferred to plates containing the sporulation SP1 medium supplemented with the auxotrophic requirements of a strain under study for pedigree analysis. The media and sporulation conditions have been described by Miyata and Miyata (1981). Thin strips of media (12 ml in a standard plate) were used for analysis. Individual cells were separated by micromanipulation according to Egel and Eie (1987), and a record was kept of the lineage of each cell. At the temperature of incubation (25°C), the sporulation-incompetent diploid cells (e.g. *mat1-P/mat1-P*) divided with a generation time of ~6 h. On SP1 medium, isolated cells from the pedigree that failed to sporulate divided for more than 10 generations. Those cells heterozygous at *mat1*, however, stopped growth and formed asci in ~12 h. In ~10% of cell divisions in all diploid strains, both members of a pair of sister cells formed asci. These cases most probably resulted from an extra cell division of a recently switched cell that was heterozygous at the *mat1* locus. Since sporulation is induced by starvation (Gutz *et al.*, 1974), perhaps the medium that is used sometimes allows an extra division of sporulation-competent cells. Consistent with this interpretation, when pedigree analysis was conducted on thicker strips of medium, an increased frequency of such cases was observed. Therefore, the mother of the two asci was scored as a recently switched cell (Figure 3 and Table II).

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