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REVIEW ARTICLE

Mating type switching in fission yeast: A unique model system of development and differentiation

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Homothallic strains of the fission yeast undergo a programmed process of switching from Plus to Minus and vice versa, which mimics the stem cell lineage in higher eukaryotes. The switching is brought about by transposition of two alternate alleles (*P* or *M*) from the silent donor loci to the recipient locus where they are expressed. The current investigations addressing the regulation of switching and silencing of the donor loci, focus on the multifarious protein–protein and protein–DNA interactions interfacing the processes of DNA replication, recombination and repair, that help in establishment and propagation of different epigenetic states. Current working models propose that these epigenetic states are regulated at the level of local and higher order chromatin structure. The ease of doing genetic analysis coupled with latest tools of molecular biology and cell biology should help decipher the molecular details of the mechanisms underlying these epigenetic events.

LIKE most eukaryotic species which reproduce sexually through differentiation of two alternate sexes, yeasts have also devised unique types of sexual differentiation mechanisms, whereby a single yeast cell can change, by the process of recombination, its mating type between two alternate choices. The best understood example is that of the commonly known baker's yeast, *Saccharomyces cerevisiae*, which has been studied in great detail over the last two decades. This yeast is referred to as a homothallic yeast as it can switch within the same population between two alternate mating types, namely, ***a*** and α. Thus, a cell with the ***a*** mating type can switch to α and vice versa, in a highly regulated manner, such that two out of four granddaughters, which are sister cells, switch to the opposite mating type. A similar mating type system exists in the fission yeast, *Schizosaccharomyces pombe*, which is evolutionally quite distant from *S. cerevisiae*. The name 'pombe' derives from the Swahili name of the yeast used for brewing a drink in the African continent. The fission yeast also exhibits two alternate mating types, namely Plus (*P*) and Minus (*M*), originally discovered by Leupold[1]. The basic rule governing the mode of switching was discovered by Miyata and Miyata who showed that a recently switched cell produces one switched granddaughter after two cell divisions – the so-called **one-in-four switching**

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rule[2]. This pattern of switching is different from that of *S. cerevisiae* in which case two out of four granddaughters undergo the switch. The two diverse patterns are depicted in Figure 1. Further work by Egel[3] and Klar *et al.*[4] led to definition of the other rules of switching in *S. pombe*. These are: the **single–sister rule**, the **consecutive switching rule** and the **directionality rule**. Since only one of the four granddaughters after two generations undergo the switch, it is implied that the first generation produces two developmentally distinct daughters, with only one daughter becoming competent to switch, designated *Ps* or *Ms*. The switching competent cell behaves like its mother cell: in the next generation it also produces a switching competent cell, *Ps* or *Ms* and another switched but switching incompetent cell *Mu* or *Pu*. It is inferred from the observed pattern of switching that a switchable cell *Ps*, produces a switched daughter *Mu*, and another switchable daughter *Ps*, like itself. This is referred to as the **single–sister rule**[1,4]; simultaneous switching of both the daughters is never observed. This pattern of switching implied that switching is a post-replication or G2 event, with only one of the two chromatids undergoing the switch. Furthermore, the switchable daughter *Ps*, borne by its switchable mother *Ps* inherits the switching competence such that it also produces a switched daughter *Mu* and a switchable daughter *Ps*. This is the so-called **consecutive switching rule**. This initiates a pedigree which closely resembles the stem cell lineage in higher eukaryotes. Lastly, in 80–90% of the cell divisions, the *Ps* cell divides to produce another *Ps* cell with the other daughter cell undergoing a switch to the opposite mating type *Mu*. This is referred to as the **directionality rule**, whereby switching occurs mostly to the opposite mating type. These rules are depicted in Figure 2.

Molecular and genetic characterization of mating type loci

Subsequent studies indicated that the mating type interconversion is dictated by the cassette model[5]. The cassette organization was characterized at the molecular level by Beach[6]. It consists of three loci, *mat1*, *mat2* and *mat3*, all of which contain the conserved homology boxes *H2* and *H1*, 135 and 59 bp in length, respectively, that are separated by about 1.1 kb region of allele-specific sequences encoding the (*P*) or (*M*)-specific transcripts. Thus, while that *mat2* contains the *P* alleles, *mat3* locus contains the *M* alleles. In addition, the *mat2* and *mat3* loci also contain another homology box called H3 box[7]. Paradoxically, despite containing the same genetic information, only the *mat1* locus is expressed, while *mat2* and *mat3* loci are not expressed. The *mat1* locus can express either *P* or *M* allele depending upon the resident allele present therein and dictate the phenotype of the cell as either *P* or *M*. The *mat2* locus is located 15 kb distal to *mat1* and the *mat3* locus is 11 kb distal to *mat2* on chromosome II (Figure 3).

The two major characteristics of the mating type loci are a well-regulated mechanism of switching and a highly complex mechanism of silencing, which are discussed next:

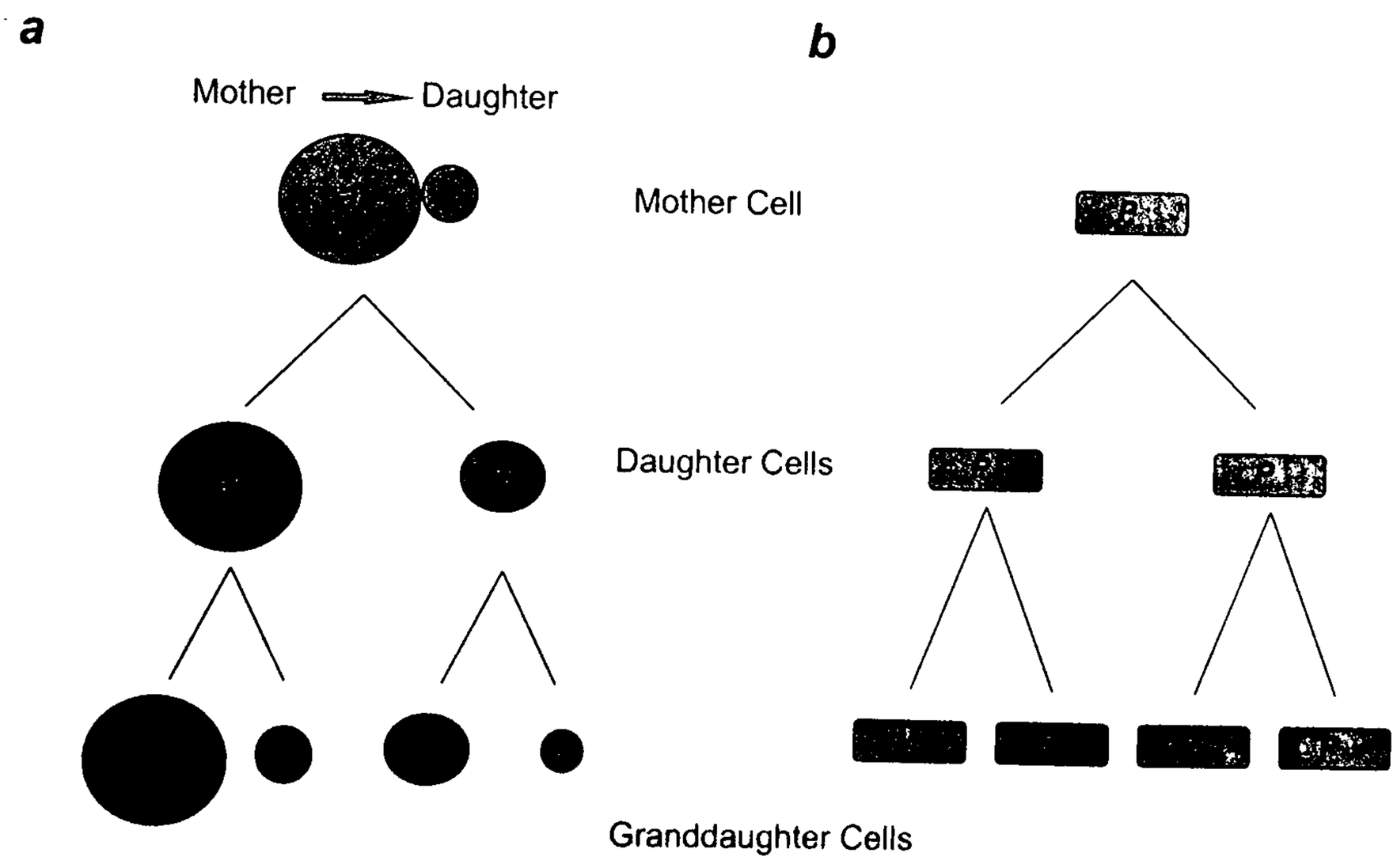

Figure 1. Pattern of switching in homothallic strains of ***a***, *S. cerevisiae* and ***b***, *Schizosaccharomyces pombe*. In (***a***) mother cells are shown on the left side of the figure and daughter cells on the right.

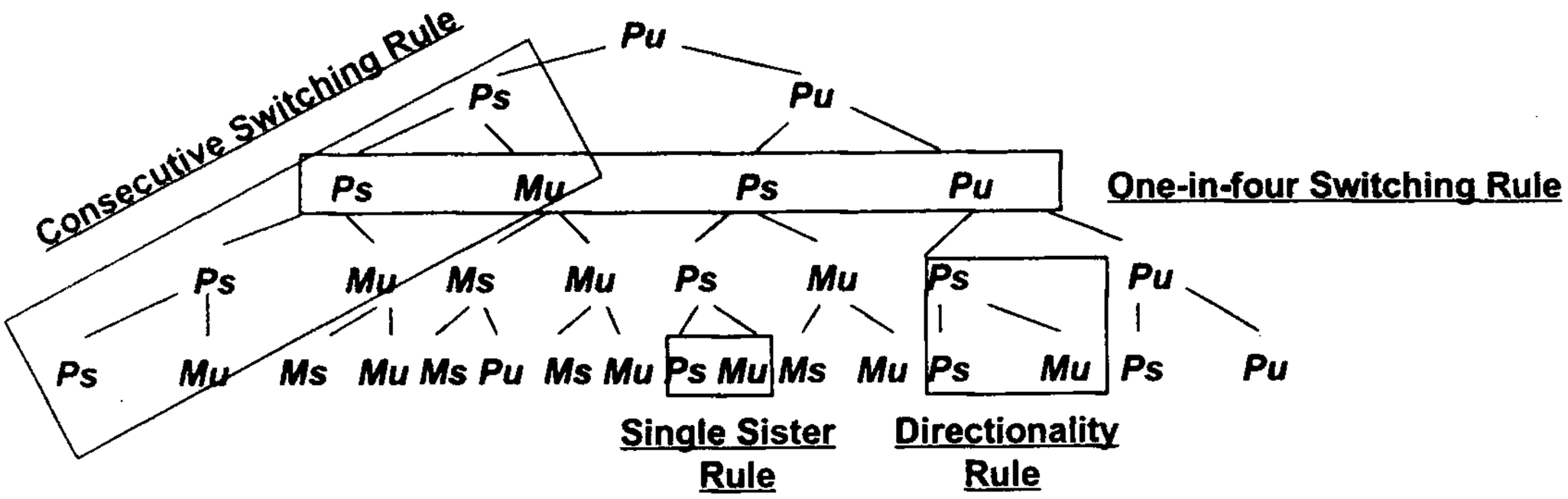

Figure 2. Cell lineage depicting the four rules of switching. *P* stands for Plus, *M* for Minus mating type, *u* for unswitchable, *s* for switchable.

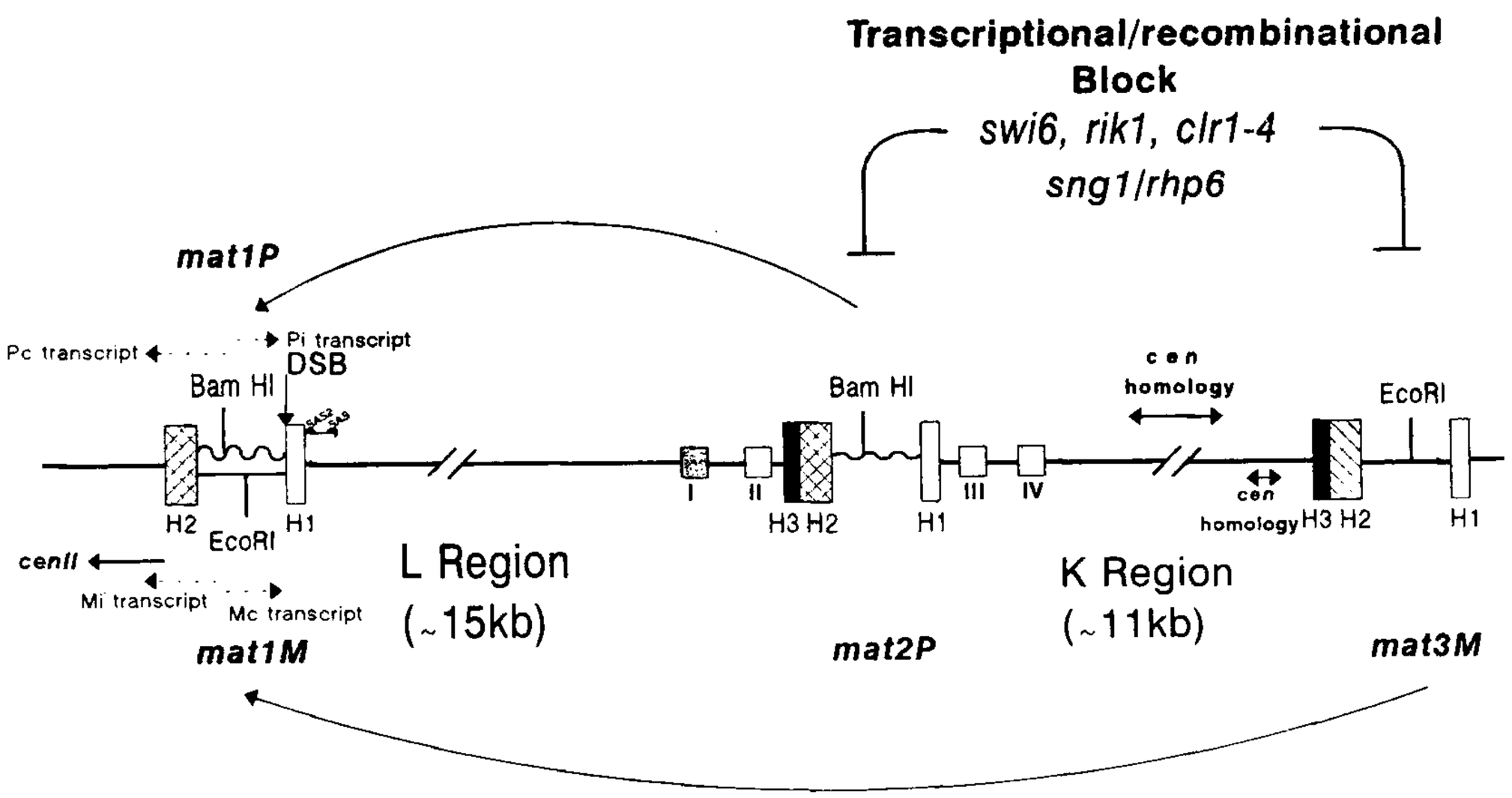

Figure 3. Molecular organization of the mating type loci in *S. pombe*. The three loci *mat1*, *mat2* and *mat3* are located distal to centromere on chromosome II. The regions that share homology at these loci are *H2* (135 bp) and *H1* (59 bp), while *mat2* and *mat3* share the *H3* (57 bp) region as well. *H2* and *H1* are separated by sequences corresponding to *P* (wavy line) or *M* (straight line) alleles. The short inverted arrow indicates the site of DSB at the junction of the H1 box and allele-specific sequences, while the long, curved arrows indicate the transfer of information during switching from *mat2* or *mat3* to the *mat1* locus. Immediately distal to the H1 box of *mat1* locus is indicated the presence of SAS1 and SAS2, two *cis*-acting sites required for generation of DSB. Also indicated are the physical distances in kb as well as genetic distances in *cM* between *mat1* and *mat2* and between *mat2* and *mat3*. *cen* indicates the direction in which the centromere is located on chromosome II. Also shown are the four regions flanking the *mat2* locus required for silencing. The region between *mat2* and *mat3* is subject to the transcriptional/recombinational block imposed by the action of several *trans*-acting factors, as indicated. Also shown in the *K* region are the areas that show homology to the *cen* sequences. The transcripts encoded by *P* and *M* alleles are indicated as *Pc*, *Pi* and *Mc*, *Mi*, respectively, where *c* stands for constitutive and *i* stands for inducibility by nitrogen starvation.

Mating type switching

The current level of understanding of the mechanism of switching has been made possible by a unique assay for switching. The cells of opposite mating type generated by a homothallic, efficiently switching strain, mate with each other under conditions of starvation and the resulting zygote immediately undergoes sporulation[8] (Figure 4). The spores contain a starch-like compound, which gives dark staining with iodine vapours. Thus, the level of iodine staining gives a qualitative measure of the level of switching. In wild type strains, the rate of switching is determined by pedigree analysis to be 75–90% (hence the strains are termed as h^{90}) and the colonies give a uniformly dark staining. Mutants that are defective in switching exhibit a much reduced level of

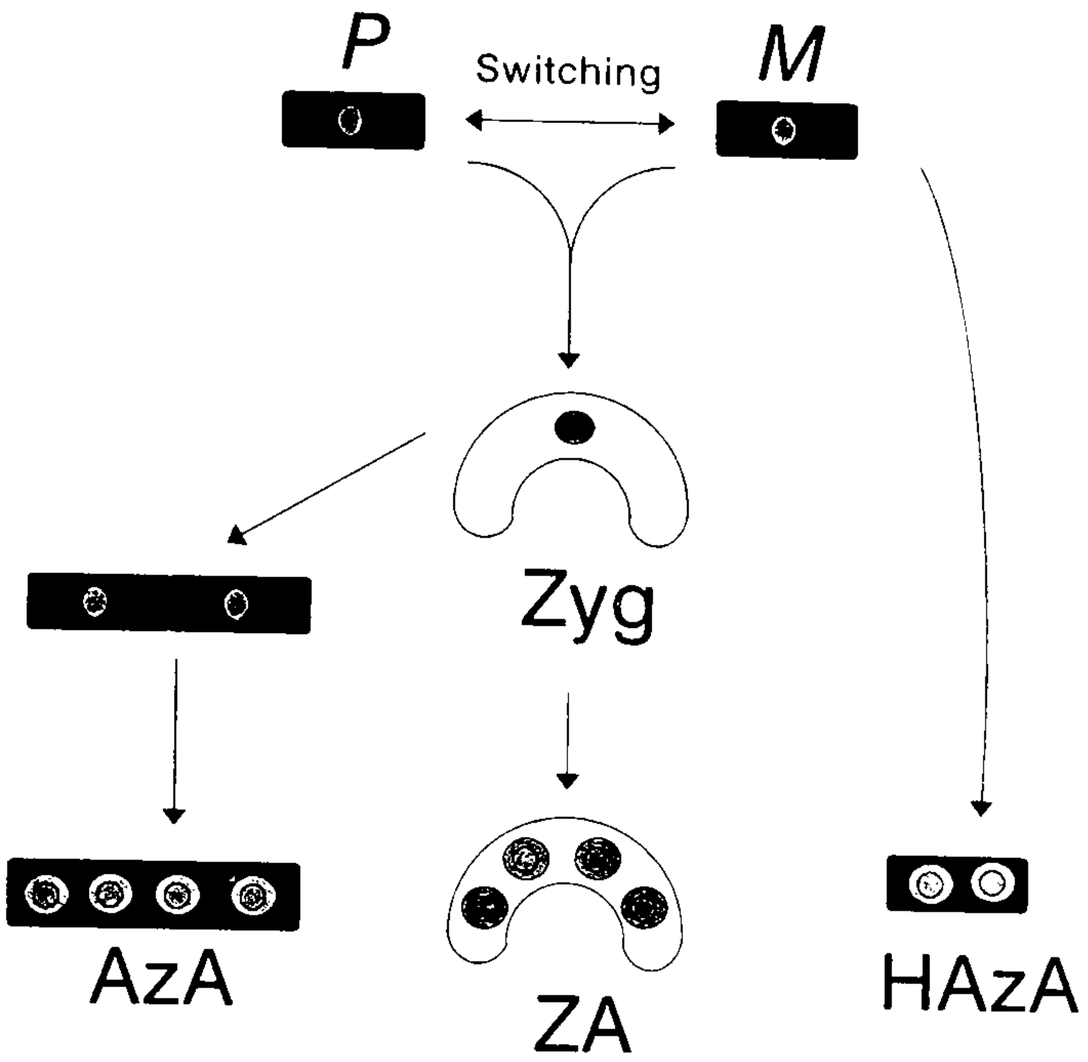

Figure 4. Sexual life cycle of *S. pombe*. *P* and *M* cells conjugate to form a diploid zygote (zyg) which quickly undergoes meiosis and sporulation resulting in the formation of a zygotic ascus (ZA). Diploids formed but grown in rich medium or by *ade6*$^-$ complementation grow vegetatively but upon transfer to sporulation medium, promptly undergo meiosis and sporulation to produce the Azygotic asci (AzA). Haploid cells in which the silent genes are derepressed undergo meiosis spontaneously in starvation medium and form the Haploid azygotic asci (HAzA) or haploid meiosis (hm).

iodine staining. Using this assay, 11 complementation groups of genes involved in switching have been identified[9]. These, in turn, are classified into 3 groups depending on the site of action. Class Ia mutants include *swi1*, *swi3* and *swi7*, and are defective in generation of DNA double-strand break (DSB) at the *mat1* locus. Generation of DSB is necessary for initiating mating type switching. Thus, these mutants are defective in initiating switching. Class Ib mutants include *swi2*, *swi5*, and *swi6*, which contain a normal level of DSB at the *mat1* locus but are defective in efficient utilization of DSB for switching. Of these, *swi6* is also involved in silencing, while *swi5* is involved in DNA repair[9]. Class II mutants include *swi4*, *swi8*, *swi9*, *swi10* and *rad22*. These mutants are characterized by increased rate of rearrangements in the mating type region and are thought to affect the resolution of the recombination intermediates formed during mating type switching[9]. Interestingly, these are also encoded by genes that participate in DNA repair. For example, *swi4* and *swi8* encode the *S. pombe* homologues of the human mismatch repair genes *msh3* (ref. 10) and *msh2* (ref. 11), respectively, *swi9* and *swi10* encode the homologues of *RAD1* and *RAD10*, respectively, from *S. cerevisiae*[12,13], while *rad22* is a pombe homologue of the *RAD52* gene of *S. cerevisiae*, which is involved in DSB repair[14]. These genes and their known functions are depicted in Table 1.

A site-specific break at mat1 initiates switching

Generation of site-specific DSB at *mat1* locus, as detected by Southern hybridization analysis, was shown to be required to initiate switching. Mutants that are defective in generation of DSB are also defective in switching (see here). This feature is similar to the mating type switching in *S. cerevisiae*[15]. In the latter yeast, DSB at the expressed *MAT* locus is made during G1, leading to switching of both the progeny after cell division, and the DSB is healed by the process of switching, such that DSB is not detectable in the switching population[15]. However, in *S. pombe*, a steady state level of 25% of total *mat1* DNA exists in the form of DSB as detected by Southern hybridization and this level does not change during the cell cycle[6]. In addition, genetic evidence also suggested that competence for switching during mitotic growth and meiotic gene conversion at *mat1* segregates in *cis* with *mat1* (ref. 16). Another unique feature of DSB in *S. pombe* is that strains carrying a deletion of the donor loci *mat2* and *mat3* are fully viable[17], despite carrying a break at *mat1*. This is unlike the DSB in *S. cerevisiae* where the DSB results in lethality if the donor loci are deleted[17]. Thus, it is possible that DSB is generated during S phase or soon after replication and utilized for switching during G2 of the following cell division. During the latter event, while one unbroken chromatid is utilized for switching, the other chromatid is probably healed and undergoes the break again during or after replication. These predictions were supported by elegant genetic experiments by Klar *et al.*[4], indicating that the generation of DSB appears to be mechanistically coupled to DNA replication. This notion is further strengthened by the finding that *swi7*, one of the three *trans*-acting genes involved in formation of the DSB at *mat1*, in fact, encodes the catalytic subunit of DNA polymerase α (ref. 18). Interestingly, the only viable double mutant among the class Ia mutants is *swi1*, *swi3*; both *swi1*, *swi7* and *swi3*, *swi7* double mutants are inviable[19], while the double mutant *swi1*, *swi3* shows the same level of residual switching as the single mutants *swi1* and *swi3* (ref. 19). These results suggest that *swi1* and *swi3* must perform some essential function in combination with *swi7* (DNA polα) and also in the generation of DSB. In addition to *swi1*, *3* and *7*, a *cis*-acting region of about 160 bp distal to the cut site at *mat1* is also required for generation of DSB (ref. 20). Deletion of SAS1 (which is bound by the switch-activating protein SAP1), located 140 pb distal to the cut site and SAS2, close to the H1 box drastically reduce the level of DSB and switching[20]. In fact, another deletion called *smto*, that extends distally from within the H1 box at

Table 1. Mutations affecting mating type switching in *Schizosaccharomyces pombe*

Class	Mutant	Defect			Homologues	Function	Ref.
		DSB	Switching	Resolution			
1a	*swi1*	–	+	+	Unknown		
	swi3	–	+	+	Unknown		
	swi7	–	+	+	DNA polα	DNA replication; lagging strand synthesis	18
1b	*swi2*	+	–	+	Unknown		
	swi5	+	–	+	Unknown		
	swi6	+	–	+	*HP1* (*Drosophila*)		51
					Human *m31*, *m32*	Heterochromatin	52
II	*swi4*	+	+	–	*msh3* (human)	Mismatch repair	10
	swi8	+	+	–	*msh2* (human)	Mismatch repair	11
	swi9	+	+	–	*RAD1* (Sc)	Excision repair	12
	swi10	+	+	–	*RAD10* (Sc)	Excision repair	13
III	*rad22*	+	+	–	*RAD52* (Sc)	DSB repair	14

Sc, *S. cerevisiae*.

31 pb from the cut completely abolishes the DSB and, hence, the switching[21]. Thus, the recognition sequence for precise cleavage at the H1 box in *S. pombe* is much longer than the analogous *HO*-cleavage site at the expressed *MAT* locus in *S. cerevisiae*[15]. It may be speculated that such a long sequence may be assembled into a nucleoprotein endonuclease complex involving *swi1*, *swi3*, *swi7* and SAP1. Mapping studies indicate DSB on the top strand but the site of cleavage on the bottom strand could not be mapped, possible because of covalent linkage of protein/s[22]. Using nuclei prepared by a gentler procedure, it has recently been shown that, instead of a DSB the *mat1* DNA undergoes a single-strand break at the top strand. The level of the nick remains unaltered during the cell cycle[23]. The activity responsible for the strand-specific break is yet to be identified.

The mechanistic basis of switching competence – The strand imprinting model

The pedigree of switching observed by Miyata and Miyata[2] suggested that the switching competence segregates asymmetrically to one of the two daughter cells of a recently switched cell[2]. Subsequently, Egel and Eie[24] observed that in diploid cells the acquisition of switching competence in the two homologous chromosomes was random. This important finding ruled out the likelihood of a cytoplasmic factor and suggested a chromosomal basis for determination of switching competence. These observations led Amar Klar to propose a strand segregation or strand imprinting model, which hypothesized that only one of the two strands of the DNA double helix can acquire competence to undergo DSB and hence to switch after replication[25]. This hypothesis was tested by Klar in a simple and elegant experiment. He created an inverted duplication of the *mat1* locus and tested the pattern of switching. Interestingly, he observed that now two out of four granddaughters cells, which were cousin (but never sister) cells, underwent switching[25,26] (Figure 5). This result implied that unlike the normal pedigree where only one of the two daughter cells from the mother cell becomes switching competent, in a genetic set-up with an inverted duplication of the *mat1* locus wherein both the strands of the DNA double helix now have the potential genetic information for imprinting, both the daughter cells inheriting each imprinted strand now become switching competent[26]. This result was corroborated by Southern blot analysis data, which unequivocally demonstrated that the switching competence is dictated by the DNA strand and not a *trans*-acting diffusible cytoplasmic factor[25,26]. These novel results constitute the first experimental demonstration in any biological system that the double helical complementary nature of the DNA and the semiconservative mode of replication and segregation of DNA molecules confer development asymmetry to the daughter cells via the 'old' and 'new' DNA strands. Moreover, the finding that DNA polymerase α is required for generation of DSB and for imparting switching competence shows that the molecular machinery required for duplicating the DNA may catalyse DSB during or after DNA replication, thus coupling the process of DNA replication to advancing the developmental asymmetry programme[18]. The molecular basis of acquisition of switching competence remains to be elucidated.

Directionality of switching

One of the fascinating features of switching is its directionality; a cell with *P* allele at the *mat1* locus almost always switches to *M* using the *mat3* as the donor and a cell with *M* allele at the *mat1* locus almost always chooses the *P* allele from the *mat2* locus. In an interesting study, Thon and Klar investigated whether this bias

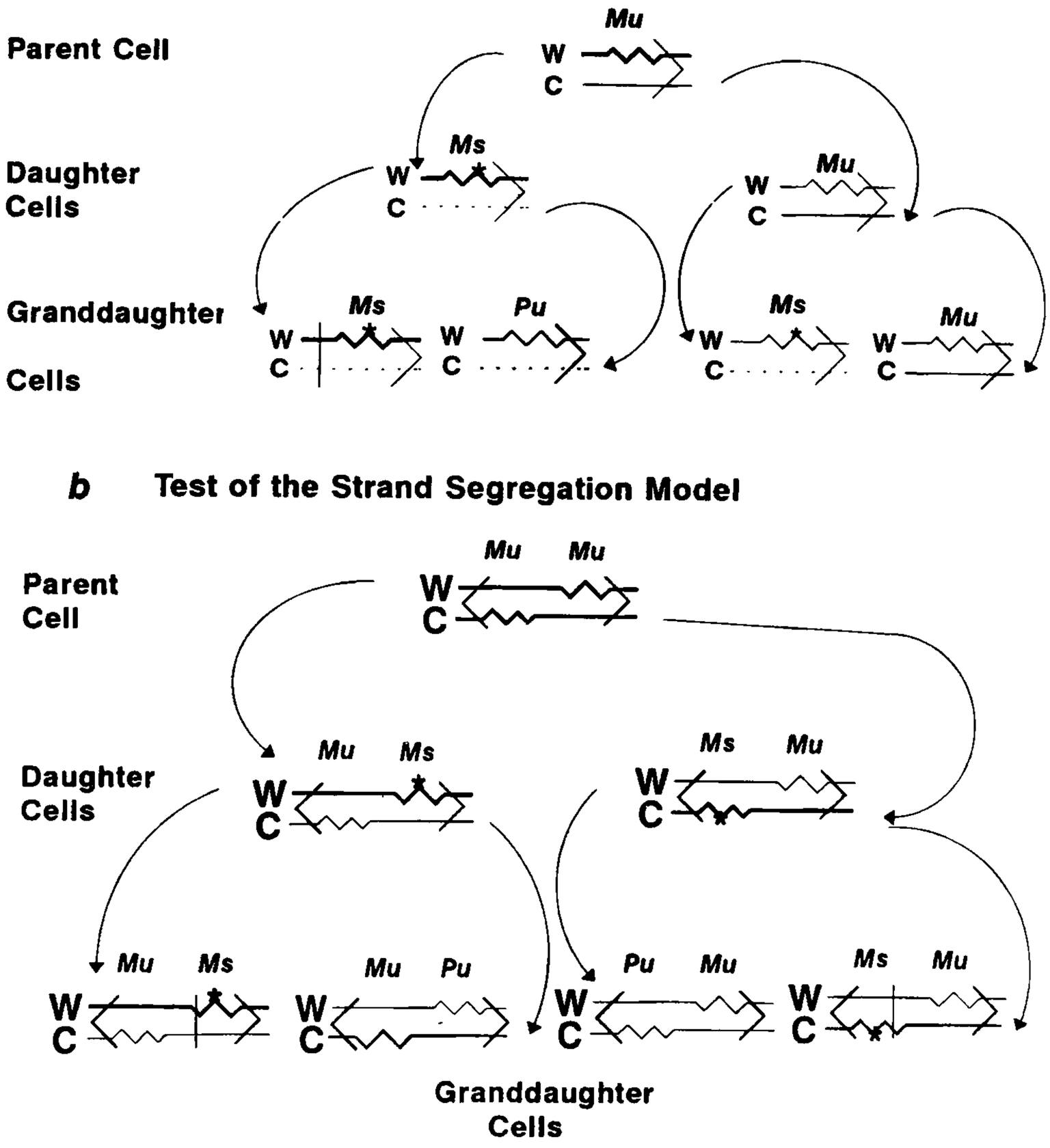

Figure 5. Strand segregation model proposed by Klar and its experimental test. *a*, The strand segregation model. During or following replication the Watson (W) strand of the mother (*Mu*) cell's *mat1* DNA, indicated by the bold jagged line, gets imprinted (*) and is transferred to one of the daughter cells which is denoted as switchable (*Ms*). During the second cell division the *Ms* daughter undergoes a single-strand break on one chromatid (indicated by the vertical line) which is utilized for initiating switching to the opposite mating type *Pu* using the donor cassette as a template, while the imprinted strand carrying the imprint (bold jagged line) can get recleaved after replication to produce the *Ms* cell. In the other cell of the first division the Crick (C) strand is inherited which does not carry the imprint. However, after the next round of replication its Watson (W) strand partner acquires the imprint and becomes switchable (*Ms*) like its aunt cell. *b*, Both the DNA strands become genetically identical in an inverted duplication of *mat1*, predicting that both the daughter DNAs will get the imprint and become switchable, producing in the next round of cell division, two cousin cells out of four granddaughters that switch.

in donor choice is dictated by the nature of the resident allele at the donor loci or by their chromosomal context. They engineered an exchange of the allele-specific region between the *mat2* and *mat3* cassettes such that the *mat2* locus now harbours the *M* alleles and *mat3* locus has the *P* alleles. Most interestingly, the resulting strain exhibited very much reduced level of switching (and hence named h^{09}, instead of the wild type h^{90}!), presumably because a *mat1 P* locus still switched to *mat3*, which harbours the homologous *P* allele instead of the normal *M* allele and, likewise, *mat1M* switches to *mat2*-resident *M* allele. These results showed that the donor choice or directionality is determined by the chromosomal context rather than content[27].

In addition, it has been found that the deletion of the *K* region spanning the *mat2* and *mat3* loci also restricts the donor choice to *mat3M* cassette, drastically reducing the switching to the *mat2P* locus[28,29]. It has been proposed that a unique chromatin structure adopted by the *K* region, which has considerable homology to the *cen* repeat sequences[29], may bring the proper *mat2P* donor cassette in apposition to the *mat1* locus and thus facili-

tate switching to the opposite mating type (see next section).

Position effect control

The hallmark of the cassette organization of mating type loci is that the cellular phenotype of *P* or *M* is dictated by the allele present at *mat1* since the donor loci *mat2* and *mat3* are transcriptionally silent. This phenomenon, wherein the donor loci with identical genetic information as *mat1* are kept in a repressed or silent state due to their chromosomal context, is variously referred to as position-effect control or silencing. These loci are flanked by *cis*-acting elements, whose deletion leads to loss of silencing[30]. This and several other aspects are similar to that in *S. cerevisiae*. Thus, the general logic of the mechanisms required for silencing may be quite similar in the two evolutionally distant species[16]. Particularly, as is the case in *S. cerevisiae*, the *cis*-acting silencer elements flanking the mating type loci can confer the ability to replicate and are referred to as autonomous replication sequences (ARS)[31]. In addition, mutations in several *trans*-acting factors namely, *swi6* (ref. 32), *rik1* (ref. 33), *clr1-clr4* (refs 34–36), *clr6* (ref. 37) and *rhp6* (ref. 38), also lead to loss of silencing (Table 2). The loss of silencing can be conveniently assayed by the haploid meiosis phenotype which is triggered by the simultaneous expression of the *P* and *M* alleles within a haploid cell[8]. Another convenient assay for silencing defect is the expression of marker gene like *ura4*$^+$ when placed at the *mat2* and *mat3* loci[34–36]. The marker gene is also subject to the position effect control and is kept transcriptionally repressed in wild type strains as indicated by their inability to grow on media lacking uracil. In the silencing mutants, the repressive effect of silencing is lifted and the *ura4*$^+$ gene is expressed as assayed by the cells' ability to grow on media lacking uracil[34–36].

Another interesting feature of the donor loci is that the region between *mat2* and *mat3*, referred to as the *K* region, acts as a cold spot for recombination and transcription. Although the physical distance between *mat2* and *mat3* is about 11 kb, no recombination is observed between *mat2* and *mat3* (ref. 38). Interestingly, both the repressive position effect control and the blockage of recombination are abrogated in the mutants that affect silencing, namely *swi6* (ref. 32), *rik1* (ref. 33), *clr1-4* (refs 34–36) and *clr6* (ref. 37), suggesting that a common mechanism may cause suppression of recombination and silencing. Thus, quite paradoxically, despite its important role in keeping the *mat2* and *mat3* interval recombinationally and transcriptionally silent, the *K* region is also required for proper switching through proper higher order chromatin folding, as mentioned earlier. Moreover, the effect of single mutations *swi6*, *clr1–clr4* on silencing is similar to that observed for various pairwise combinations between them or combination of *K* deletion with these *trans*-acting mutations. Therefore, the gene products of *swi6*, *clr1–clr4* may act as a complex primarily through the *K* region.

In view of the above, it is of central interest to understand how the *cis*-acting sequences (ARS elements and the centromere-like *K* region) and the *trans*-acting factors are involved in silencing. Conceivably, the *trans*-acting factors in combination with the *cis*-acting elements may lead to the formation of a heterochromatin structure at the donor loci *mat2* and *mat3* and the intervening *K* region, preventing the RNA polymerase and the transcription factors from gaining access to the promotor regions of either the donor loci *mat2* and *mat3*, or the marker genes placed in their vicinity. The loss of silencing in the *trans*-acting mutants *swi6*, *clr1-clr6*, *rik1* and *rhp6*, may be brought about by the loss of heterochromatin structure, thus allowing the RNA polymerase and transcription factors better access to *mat2* and *mat3* loci. This idea is supported by the recent finding that while the donor loci are inaccessible to methylation by the endogenously expressed *E. coli dam* methylase in wild type strains, these loci exhibit increased accessibility to *dam* methylase in *swi6* and *rhp6* (ref. 39), indicating that *swi6* (and probably *clr1–clr6*) and *rhp6* may participate, directly or indirectly, in assembling a heterochromatin structure at the donor loci. Here it would be of interest to briefly review the functions of the silencer elements and the *trans*-acting factors and visualize their individual as well as mutually interactive roles in silencing.

Table 2. Genes involved in silencing of the mating type loci in *Schizosaccharomyces pombe*

Mutant	Homologues	Functions	Ref.
swi6	*HP1*, *suvar* (*Drosophila*)	Heterochromatin	51
	m31, *m32* (human)		52
clr1	ND	ND	
clr2	ND	ND	
clr3	Histone deacetylase	Histone deacetylation	37
clr4	Chromodomain/ SET domain (suvar; *Drosophila*)	Heterochromatin	50
clr6	Histone deacetylases (HDAC's)	Histone deacetylation	37
rhp6	*RAD6* (*Sc*)	Post-replication repair	39

Sc, *S. cerevisiae*.

Role of silencer

The exact mechanism by which silencer elements bring about silencing is not fully understood. Association of silencer elements with ARS activity in *S. cerevisiae*[40] and *S. pombe*[31] suggested that replication may be important for silencing. The first key finding that replication

plays an important role in silencing dates back to the seminal work of Nasmyth showing that passage through S phase was essential for reestablishing silencing[41]. Interestingly, the *cis*-acting elements E and I, flanking the silent locus *HMRa* in *S. cerevisiae* also have the ARS activity, which has been shown to be required for silencing[40,42]. Lastly, mutations in the protein sub-units that are assembled into a complex called the origin recognition complex (ORC) also alleviate silencing[43–46]. These results have strongly suggested that replication is coupled to silencing in *S. cerevisiae* and a few studies suggest that the same may hold true for silencing of mating type loci in *S. pombe*.

Role of trans-acting factors

Recent genetic, molecular genetic and biochemical studies have provided a great deal of insight into how heterochromatin may be assembled at the mating type loci as well as telomeres in *S. cerevisiae*. Of the four known *trans*-acting factors (Silent Information Regulators, SIR1–4), SIR3 and SIR4 have been shown to interact with the N-terminal tails of histones H3 and H4 as well as with the repressor-activator protein RAP1, which is docked to its DNA binding site located in the *cis*-acting region flanking the mating type loci and telomeres. The *trans*-acting factors SIR3 and SIR4 are recruited to the site of RAP1 binding and interact with nucleosomal histones and these interactions may help in folding up of the chromatin fibre[47]. Furthermore, the lysine residues within the N-terminal tails of histones H3 and H4 remain underacetylated in the repressed state but become hyperacetylated in silencing defective mutants[48]. While underacetylation of histones may promote their interaction with the SIR proteins, hyperacetylation may disrupt this interaction leading to loss of chromatin folding and derepression of genes[47,48].

An understanding in such a molecular detail has not yet been achieved in *S. pombe*. Some of the *trans*-acting genes have been cloned in *S. pombe* (*swi6*, *clr1*, *clr3–clr4*, *clr6* and *rik1*). However, these genes do not share any sequence homology with the *SIR* genes of *S. cerevisiae*. On the other hand, it is striking that homologues of these silencing factors are found in higher eukaryotes. For example, *swi6* contains a highly conserved domain called the chromodomain[49] and *clr4* contains the chromodomain as well as the SET domain[50]. These domains have been found in several heterochromatin-associated proteins in different species including *Drosophila*[51] and mammals[52]. Thus, similar mechanisms of heterochromatin formation may exist in fission yeast and higher eukaryotes. Furthermore, like in *S. cerevisiae*[48] and mammals[53], where a correlation between hyperacetylation of histones and gene expression has been observed, mutations in two genes *clr3* and *clr6*, both of which encode histone deacetylases, have been shown to cause loss of silencing[37]. How do the chromodomain proteins like swi6 and clr4 as well as the histone deacetylases clr3 and clr6 get recruited to function at the silent loci is not yet understood.

Establishment and propagation of epigenetic states of switching and silencing

From the foregoing sections, it becomes clear as to which are some of the key molecular players participating in silencing. However, it is not clear in what distinct functional steps the silencer/ARS and the *trans*-acting functions are involved. It has been suggested that atleast two different pathways may exist[36], one in which replication machinery is involved and the other/s in which the components of heterochromatin generated by cooperative interactions between *trans*-acting factors and histones might be involved. With recent observations, however, an integrated view is emerging. Particularly, in *S. cerevisiae*, the ORC complex required for firing of replication origins has been shown to interact with the *trans*-acting factor SIR1 (ref. 54), which is known to play a role in establishment but not in maintenance of silencing[55]. Thus, the pathways of *trans*-acting factors including SIR proteins and the replication initiation may be mechanistically coupled.

Distinctive roles of two pathways have been suggested by a recent finding that the *K* region, located between the *mat2* and *mat3* loci, plays an important role in establishing an epigenetic state that is competent for switching: a strain carrying a deletion of the *K* region by insertion of a *ura4*$^+$ marker exists in two stable epigenetic states, one in which the stain is switching incompetent but expresses the *ura4*$^+$ marker gene and another state which is switching competent but its *ura4*$^+$ gene is repressed[28]. These two states interconvert very infrequently and are marked by a chromosomal imprint, which is stably inherited during mitosis and meiosis[28]. In addition, the switching incompetent state is fixed in a single mating type (*M*) rather than both *P* and *M* (ref. 29). It has been suggested that the *K* region may help in folding up of the chromatin state that brings the proper donor cassette in proximity of the recipient *mat1* locus during switching; the same process may also keep the region transcriptionally and recombinationally repressed. Thus, this region may be important for establishing the switching and silencing competent state of chromatin. Interestingly, this region shows considerable level of homology to the centromeric (*cen*) repeats[29]. Therefore, it is proposed that this region encompassing the ARS-like elements as well as *cen*-like regions may be folded up in a unique chromatin state which is different from the region near the normal expressed *mat1* locus. Further, it may be speculated that the *K* region, in

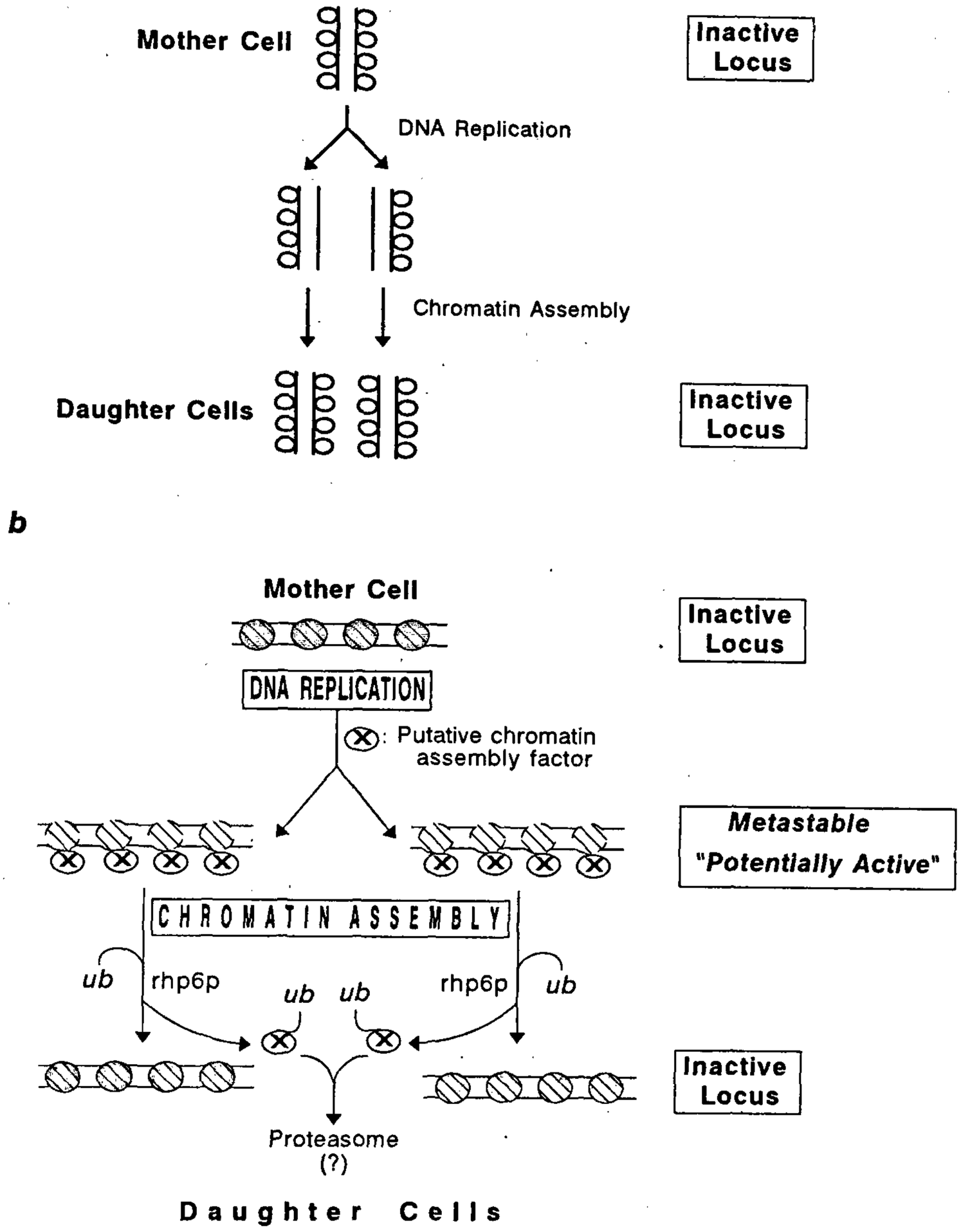

Figure 6. Two alternate models for propagation of chromatin states. *a*, Chromatin replication model[28], wherein a residual structure segregating with daughter chromatids may reassemble into a new structure after replication reestablishing the parental chromatin structure; *b*, Chromatin remodelling model with the active participation of a hypothetical mediator of *rhp6* action (protein X). The *rhp6* target protein X may interact with histones and TBP and this interaction may be modulated by ubiquitination. A transient interaction during a narrow time window followed by the removal of the ubiquitinated *uhp1* may be critical for assembly of the inactive structure through nucleosome positioning. Thus regulation may be lost in the *rhp6*$^-$ mutant wherein the protein X may stay bound to the nucleosomes and generate a perturbed chromatin structure.

problems yet to be deciphered are how DNA replication is mechanistically connected with the assembly of chromatin and how the chromatin states are propagated following replication, which constitute the fundamental problems in biology and are central to our understanding the molecular basis of development and differentiation. Some relatively unexplored areas like prion phenomenon, involving propagation of variant protein structures by self-templating, may turn out to be critical for epigenetic regulation. Thus, mating type system may yet sanctify a marriage between the fields of protein biochemistry and developmental genetics.

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RESEARCH ARTICLE

Hydrographic characteristics of the Indian sector of the Southern Ocean

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Oceanographic studies in the Indian sector of the Southern Ocean (58–61°S and 30–40°E) carried out during December 1995 and March 1996 indicate a 3-layer structure typical of summer in the oceanic domain south of the Antarctic Polar Front. The upper 300 m water column consists of three distinctive thermohaline characteristics. The surface layer (50 m) of summer surface water formed by seasonal warming with temperatures (0–2°C) and low salinity (< 33.8 PSU), the intermediate layer (50–150 m) of winter reminiscent water with negative temperatures (0 to –2°C) and moderate salinity (33.8–34.3 PSU) and the deeper layer (below 150 m) of Circumpolar Deep Watermass (CDW) with positive temperatures (0–2°C) and higher salinity (34.3–34.8 PSU). The influence of the strong Antarctic Divergence between the east and west drifts is noticed at 61°S, 34°E marked by phytoplankton and zooplankton patches.

The dissolved oxygen (DO) shows high concentrations (7.6 to >8.0 ml l^{-1}) in the upper layer. These higher levels of DO sometimes extend down to the intermediate layer and gradually decrease further downwards. The observed low temperature and high DO in the subsurface water (50–75 m) are typical signatures of Antarctic Winter Watermass (AWW) formed during winter. Calcium, magnesium and boron show significant spatial variations with higher values of Ca and Mg along 59°S and 60°S relative to 61°S. Ca exhibits large variations (398–421 mg kg^{-1} while Mg shows least variations with concentrations close to the average value for normal sea water (1294 mg kg^{-1}). Boron depicts high values along 59°S and 61°S compared to 60°S, with higher values in the subsurface layer (25–75 m) which are attributed to biological characteristics and the frontal systems in the region.

Phytoplankton cell counts remain high (between 1.92 and 22.9×10^4 per litre) while zooplankton biomass shows variations from 9.8 to 303.62 ml 100 m^{-3}. A marked drop of both phytoplankton and zooplankton in the region of krill swarm (between 60°40'–61°13'S and 33°34'–34°14'E), however, indicates active feeding by the latter on the planktonic communities.

THE Southern Ocean is characterized by large seasonal variability in its environmental conditions which influence the global climate. This has led to a growing interest in the exploration of the Southern Ocean to understand various physical, chemical and biological processes and their driving mechanisms operating in this region. Compared to the Atlantic and Pacific sectors of the Southern Ocean, its Indian Ocean sector has remained largely unexplored.

The area under investigation lies in the vicinity of the Antarctic Divergence Zone. The oceanic frontal systems in the Indian Ocean sector of the Southern Ocean have been described in detail by several researchers[1–7]. Lutjeharms[7] described in detail the nature and geographic distribution of major oceanic fronts south of Africa. The circulation south of 52°S extending up to 60°E corresponds to the eastern boundary of the Weddell Gyre[8]. This is consistent with the results of Fine Resolution Antarctic Model (FRAM)[9].

The present study on the physical, chemical and biological characteristics of waters in the region between 58°–61°S and 30°–40°E was carried out during the Indian Antarctic Krill Expedition on-board FORV *Sagar Sampada* from December 1995 to March 1996 (Figure 1). This expedition was aimed at exploiting the marine living resources in the Antarctic region and the stations for hydrographic sampling are broadly grouped into three zonal sections corresponding to 59°, 60°, 61°S latitudes, respectively as shown in Figure 1.

Materials and methods

A total of 90 sea water samples were collected from the upper 300 m water column from 16 stations (Figure 1) in the region (58 to 61.3°S and 30 to 40°E) at standard depths (surface, 50, 100, 150, 200, 250 and 300 m) using Sea-Bird CTD Rossette system provided with Niskin samplers. The collected samples were preserved in the deep freeze for analysis of major elements (such as calcium, magnesium and boron) at the shore laboratory. Temperature, salinity and dissolved oxygen (DO) data (after calibration) were obtained from the CTD on-board the ship. The calibration of CTD DO values was done

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