New insights into donor directionality of mating-type switching in Schizosaccharomyces pombe

Maki, Takahisa; Ogura, Naoto; Haber, James E.; Iwasaki, Hiroshi; Thon, Genevieve

Published in:
PLOS Genetics

DOI:
10.1371/journal.pgen.1007424

Publication date:
2018

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Maki, T., Ogura, N., Haber, J. E., Iwasaki, H., & Thon, G. (2018). New insights into donor directionality of mating-type switching in Schizosaccharomyces pombe. PLOS Genetics, 14(5), 1-25. [e1007424].
https://doi.org/10.1371/journal.pgen.1007424
New insights into donor directionality of mating-type switching in *Schizosaccharomyces pombe*

Takahisa Maki¹, Naoto Ogura², James E. Haber³, Hiroshi Iwasaki¹,²*, Genevièве Thon⁴*

¹ Institute of Innovative Research, Tokyo Institute of Technology, Tokyo, Japan, ² School of Life Science and Technology, Department of Life Science and Technology, Tokyo Institute of Technology, Tokyo, Japan, ³ Department of Biology and Rosenstiel Basic Medical Science Research Center, Brandeis University, Waltham, Massachusetts, United States of America, ⁴ Department of Biology, University of Copenhagen, BioCenter, Copenhagen, Denmark

* hiwasaki@bio.titech.ac.jp (HI); gen@bio.ku.dk (GT)

Abstract

Mating-type switching in *Schizosaccharomyces pombe* entails programmed gene conversion events regulated by DNA replication, heterochromatin, and the HP1-like chromodomain protein Swi6. The whole mechanism remains to be fully understood. Using a gene deletion library, we screened ~3400 mutants for defects in the donor selection step where a heterochromatic locus, *mat2-P* or *mat3-M*, is chosen to convert the expressed *mat1* locus. By measuring the biases in *mat1* content that result from faulty directionality, we identified in total 20 factors required for donor selection. Unexpectedly, these included the histone H3 lysine 4 (H3K4) methyltransferase complex subunits Set1, Swd1, Swd2, Swd3, Spf1 and Ash2, the BRE1-like ubiquitin ligase Brl2 and the Elongator complex subunit Elp6. The mutant defects were investigated in strains with reversed donor loci (*mat2-M mat3-P*) or when the *SRE2* and *SRE3* recombination enhancers, adjacent to the donors, were deleted or transposed. Mutants in Set1C, Brl2 or Elp6 altered balanced donor usage away from *mat2* and the *SRE2* enhancer, towards *mat3* and the *SRE3* enhancer. The defects in these mutants were qualitatively similar to heterochromatin mutants lacking Swi6, the NAD⁺-dependent histone deacetylase Sir2, or the Clr4, Raf1 or Rik1 subunits of the histone H3 lysine 9 (H3K9) methyltransferase complex, albeit not as extreme. Other mutants showed clonal biases in switching. This was the case for mutants in the NAD⁺-independent deacetylase complex subunits Cir1, Cir2 and Cir3, the casein kinase CK2 subunit Ckb1, the ubiquitin ligase component Pof3, and the CENP-B homologue Cbp1, as well as for double mutants lacking Swi6 and Brl2, Pof3, or Cbp1. Thus, we propose that Set1C cooperates with Swi6 and heterochromatin to direct donor choice to *mat2-P* in M cells, perhaps by inhibiting the *SRE3* recombination enhancer, and that in the absence of Swi6 other factors are still capable of imposing biases to donor choice.
Effects of chromatin structure on recombination can be studied in the fission yeast *S. pombe* where two heterochromatic loci, *mat2* and *mat3*, are chosen in a cell-type specific manner to convert the expressed *mat1* locus and switch the yeast mating-type. The system has previously revealed the determining role of heterochromatin, histone H3K9 methylation and HP1 family protein Swi6, in donor selection. Here, we find that other chromatin modifiers and protein complexes, including components of the histone H3K4 methyltransferase complex Set1C, the histone H2B ubiquitin ligase HULC and Elongator, also participate in donor selection. Our findings open up new research paths to study mating-type switching in fission yeast and the roles of these complexes in recombination.

**Introduction**

The fission yeast *S. pombe* exists as two haploid cell types, plus (P) and minus (M), that differ at the *mat1* locus. When starved for nitrogen, haploid cells undergo sexual differentiation, mate with the opposite cell type and sporulate. These events are driven by master regulators expressed from the *mat1*-P and *mat1*-M alleles [1]. The regulators first drive sexual differentiation and mating and, when co-expressed in the zygote, meiosis and sporulation. Homothallic (*h⁰⁹*) colonies sporulate very efficiently because they contain equal proportions of P and M cells due to frequent gene conversions at *mat1*. The genetic information at *mat1* is replaced with genetic information copied from one of two silent loci, *mat2*-P or *mat3*-M [2]. The organization of the ~35 kb region of chromosome 2 that comprises *mat1*, *mat2*-P and *mat3*-M in *h⁰⁹* strains is depicted in Fig 1A. All three loci are flanked by short regions of sequence identity, the centromere-distal H1 box and the centromere-proximal H2 box. In addition, H3 homology boxes immediately adjacent to H2 are found exclusively at *mat2*-P and *mat3*-M. An alternative arrangement, known as *h⁰⁹*, has *mat2*-M and *mat3*-P cassettes [3].

Mating-type switching follows the so-called ‘Miyata’s rules’ inferred from pedigree analyses of dividing cells [4]. A single *h⁰⁹* cell produces both ‘unswitchable’ and ‘switchable’ cells. According to the ‘one-in-four-rule’, illustrated in Fig 1B, one of two sister cells originating from an ‘unswitchable’ cell becomes ‘switchable’. That cell produces one switched daughter upon cell division and one unswitched, but switchable, daughter. Thus, only one cell out of four cousins displays a switched mating-type (the ‘one-in-four’ rule [4]) and lineages of ‘switchable’ cells are observed (the ‘recurrent switching’ rule [5]). The known mechanisms of mating-type switching provide an explanation for these rules.

Mating-type switching is initiated by an imprint at *mat1* introduced during DNA replication [6, 7]. Replication stalls within *mat1* at the *MPS1* site and a nick or two ribonucleotides are incorporated into the lagging strand at the imprinting site situated nearby, at the junction of the *mat1* cell-type specific information and H1 box [6–15]. This imprint creates a ‘switchable’ cell. During the next round of DNA synthesis, the imprint is converted into a double-strand break (DSB) that triggers homologous recombination and mating-type switching [10, 14]. At least seven factors (Swi1, Swi3, Pol1 (Swi7), Sap1, Lsd1, Lsd2 and Mrc1) are required for efficient DSB formation. The Swi1-Swi3 complex and Mrc1 are necessary for imprinting by pausing replication forks at *MPS1* [7, 16]. The DNA primase Pol1 and a DNA element targeted by the essential DNA-binding protein Sap1 are not required for replication fork stalling at *MPS1*; hence Pol1 and Sap1 are believed to catalyze imprint formation downstream of the Swi1-Swi3 complex [7], while Lsd1 and Lsd2 are required upstream of Swi1-Swi3 [17, 18]. In addition, Swi1, Swi3 and Mrc1 block replication from the *mat1* distal side at the replication...
termination site RTS1 to optimize mating-type switching [7, 16]. Thus, replication proceeds unidirectionally when leading-strand synthesis reaches the imprint and the nick is converted into a one-ended DSB.

The DSB end initiates repair by recombining with one of the heterochromatic and transcriptionally silent cassettes mat2-P and mat3-M [10, 19, 20]. The free DNA end can invade either mat2-P or mat3-M, however mating-type information opposite to the information present at mat1 is chosen with a ~90% probability, leading to Miyata’s observation that switchable cells nearly always switch to the opposite mating-type [4, 9]. Surprisingly, this strongly biased...
donor selection relies on the heterochromatic state of the mat2-mat3 region [3, 21–24]. In the wild-type, histone H3K9 methylation deposited between the inverted repeat boundaries IR-L and IR-R permits the binding of the key switching factor Swi6, an HP1 homolog [25]. Defective donor choice in swi6 mutants biases h^90 cell populations towards the M mating-type due to preferred use of a mat3-M adjacent recombination enhancer over a mat2-P adjacent enhancer in the absence of Swi6 [22, 23, 26]. Also essential at this step of switching is the Swi2-Swi5 complex, capable of interacting with Swi6, and whose molecular role is inferred from the related Sfr1-Swi5 complex [27, 28]. Sfr1 shares sequence homology with the C-terminus of Swi2, in a domain that permits the interaction of either Swi2 or Sfr1 with the recombination mediator Swi5 and with the strand-exchange factor Rad51. The Sfr1-Swi5 complex stabilizes Rad51 filaments in vivo and promotes Rad51-mediated strand exchange in vitro [28–30]. Consistent with similar functions for the Swi2-Swi5 complex, Swi2-Swi5 interacts with Rad51 in two-hybrid assays [27]. Thus, mechanistically, the ability of Swi2-Swi5 to interact with Swi6 suggests that the complex participates in donor choice by biasing strand invasion [27]. This idea is supported by cell-type specific associations of Swi2 and Swi5 with the mating-type region [22]. Swi2 localizes to the mat2-P and mat3-M adjacent enhancers SRE2 and SRE3 (Swi2-dependent recombination enhancer element 2 and 3) in M cells, but only to SRE3 in P cells [22, 23]. In swi6 mutant cells, Swi2 localizes only to SRE3, as in P cells [22]. These observations indicate that the heterochromatin-mediated localization of Swi6 regulates Swi2-Swi5 localization to SRE2 to choose mat2-P [22, 23, 31].

After strand invasion into the homologous sequence at the H1 homology box, the donor locus information is copied by polymerase extension until it reaches the H3 homology box, then H3 is believed to form a hairpin loop structure [32]. The mismatch repair Msh2 (Swi8)-Msh3 (Swi4) complex recognizes this conformation and DNA synthesis stops at the donor cassette. The Rad51 mediator Rad55-Rad57 has been suggested to work together with Msh2 (Swi8), because mutants in these factors tend to form h^+N rearrangements containing a duplication of the entire mat2-3 region at mat1 [33–36]. In addition to Rad55-Rad57, the homologous recombination factor Rad52 is required for DSB repair at mat1 [37, 38]. Presumably, Rad55-Rad57 and Rad52 are involved in annealing between two H2 boxes in mat1 and the donor cassette. The endonuclease Rad16 (Swi9)-Swi10 and its activator Pxd1 cleave the intermediate between the H2 and H3 boxes [37, 39]. This is followed by new DNA synthesis from H2 of mat1 to H1 to complete replication of this region and to thus switch mating-type.

This switching system has been utilized to study multiple aspects of replication, histone modification and recombination. Historically, the mating-type switching related genes were classified functionally by Southern blotting analysis of the effect of mutants on mat1 switching [40]. Class Ia genes (swi1, 3, and 7) are required for the imprinting step that leads to the DSB formation, hence mutants in these genes show no DSB in a Southern blot. Class Ib genes (swi2, 5 and 6) are not necessary for DSB formation, but required for efficient switching. The third group, Class II, (swi4, 8, 9, and 10) resolves the recombination intermediate, mutants in these genes contain frequent rearrangements of the mating-type region, in particular the h^+N duplication. Subsequent screens identified additional factors (Table 1) and suggested that yet more might exist. In particular, aspects of imprint formation and donor choice are still not understood.

To identify yet unknown regulators of mating-type switching, we combined the deletion of 3420 nonessential genes (Bioneer gene deletion library version 5) with an h^90 strain background. The strain also included a dual reporter system with CFP under the control of a P-specific promoter and YFP under the control of an M-specific promoter, so that the ratio of P-to-M cells was determined by comparing CFP and YFP fluorescence. As a secondary screening strategy, the genetic content at mat1 was quantified with multiplex PCR using genomic DNA
Table 1. Mating-type switching related genes.

| Systematic ID | Gene         | Gene description                | Ver5.0 position | reference |
|---------------|--------------|---------------------------------|-----------------|-----------|
| SPBC216.06c   | swi1         | replication fork protection complex subunit Swi1 | V5-P36-57       | [40]      |
| SPAC1142.03c  | swi2         | Swi5 complex subunit Swi2      | V5-P02-96       | [40]      |
| SPBC30D10.04  | swi3         | replication fork protection complex subunit Swi3 | V5-P21-55       | [40]      |
| SPAC8F11.03   | msh3 (swi4)  | MutS protein homolog 3         | V5-P05-64       | [40]      |
| SPBC409.03    | swi5         | Swi5 protein                   | V5-P30-21       | [40]      |
| SPAC664.01c   | swi6         | chromodomain protein Swi6      | V5-P05-31       | [40]      |
| SPAC3H5.06c   | pol1 (swi7)  | DNA polymerase alpha catalytic subunit | -              | [40]      |
| SPBC19G7.01c  | msh2 (swi8)  | MutS protein homolog 2         | V5-P31-52       | [40]      |
| SPCC970.01    | rad16 (swi9)| DNA repair endonuclease XPF     | V5-P23-76       | [40]      |
| SPBC4F6.15c   | swi10        | DNA repair endonuclease         | V5-P34-76       | [40]      |
| SPCC1672.02c  | sap1         | switch-activating protein Sap1  | -               | [41]      |
| SPAC30D11.10  | Rad52        | DNA repair protein Rad52       | V5-P10-40       | [38]      |
| SPBC2D10.17   | clr1         | cryptic loci regulator Clr1    | V5-P12-69       | [42]      |
| SPAC1B3.17    | clr2         | chromatin silencing protein Clr2 | V5-P10-18       | [43]      |
| SPBC800.03    | clr3         | histone deacetylase (class II) Clr3 | V5-P09-57      | [43]      |
| SPBC428.08c   | clr4         | histone H3 methyltransferase Clr4 | V5-P12-80       | [43]      |
| SPCC11E10.08  | rik1         | silencing protein Rik1         | V5-P08-03       | [43]      |
| SPAC18B11.07c | rhp6         | Rad6 homolog Rhp6              | V5-P03-66       | [44]      |
| SPAC17H9.20   | psc3         | mitotic cohesin complex, non-SMC subunit Psc3 | -              | [45]      |
| SPBC16D10.07c | sir2         | Sir2 family histone deacetylase Sir2 | V5-P13-87       | [46]      |
| SPAC3A11.08   | pcc4 (cul4)  | cullin 4                       | V5-P36-16       | [47]      |
| SPCC613.12c   | raf1 (clr8)  | Rik1-associated factor Raf1    | V5-P26-35       | [24]      |
| SPCC970.07c   | raf2 (clr7)  | Rik1-associated factor Raf2    | V5-P27-50       | [24]      |
| SPAC3C7.03c   | rad55        | RecA family ATPase Rad55      | V5-P13-53       | [35]      |
| SPAC2H4.07    | rad57        | RecA family ATPase Rad57      | V5-P25-35       | [36]      |
| SPAC644.14c   | rad51        | recombination Rad51           | V5-P05-30       | [36]      |
| SPAC15A10.03c | rad54        | Rad54 homolog Rad54           | V5-P11-89       | [36]      |
| SPAC1556.01c  | rad50        | DNA repair protein Rad50       | V5-P23-44       | [36]      |
| SPCC4G3.07c   | phf1         | PHD finger containing protein Phf1 | -              | [36]      |
| SPBC1105.04c  | chp1         | CENP-B homolog                 | V5-P09-19       | [48]      |
| SPAC343.11c   | msc1         | multi-copy suppressor of Chk1  | V5-P04-71       | [49]      |
| SPBC23G7.09   | mat1-mc      | Mating-type m-specific polypeptide Mc | -              | [26]      |
| SPBC106.09    | cut4         | platform subcomplex scaffold subunit Apc1 | -              | [50]      |
| SPAC6F12.15c  | cut9         | TPR lobe subcomplex subunit Cuc9/Apo6 | -              | [50]      |
| SPBC146.09c   | lsd1         | histone demethylase SWIRM1     | V5-P06-14       | [17]      |
| SPAC23E2.02   | lsd2         | histone demethylase SWIRM2     | -               | [17]      |
| SPCC1322.02   | pxd1         | sequence orphan                | V5-P11-47       | [39]      |
| SPAC694.06c   | mrc1         | mediator of replication checkpoint 1 | V5-P05-35      | [16]      |
| SPBC3H7.10    | elp6         | elongator homolog              | V5-P21-32       | This study |
| SPCC970.10c   | brl2         | ubiquitin-protein ligase E3    | V5-P08-48       | This study |
| SPAC1851.03   | clb1         | CK2 family regulatory subunit  | V5-P03-65       | This study |
| SPCC338.16    | po3          | F-box protein Pof3             | V5-P09-78       | This study |
| SPAC23H3.05c  | swd1         | COMPASS complex subunit Swd1   | V5-P13-45       | This study |
| SPBC18H10.06c | swd2         | COMPASS complex subunit Swd2   | V5-P06-59       | This study |
| SPCC594.05c   | spf1         | COMPASS complex subunit        | V5-P28-74       | This study |
| SPCC306.04c   | set1         | histone lysine methyltransferase Set1 | V5-P27-78       | This study |
| SPBC354.03    | swd3         | WD repeat protein Swd3         | V5-P22-71       | This study |
| SPBC13G1.08c  | ash2         | Ash2-trithorax family protein  | V5-P10-78       | This study |

https://doi.org/10.1371/journal.pgen.1007424.t001
isolated from the candidates that passed the initial screen. These extensive screens identified several new mating-type switching genes whose deletion results in a bias toward $M$ cells within $h^{+90}$ populations instead of the balanced $P:M$ ratio. In addition, analysis of $h^{+90}$ strains revealed that some strains showed clonal biases in independent colonies. As mentioned above, Swi6 is an essential directionality factor [3, 22, 23]. Epistasis analysis with $swi6\Delta$ suggests that Clr4, Sir2, Swd1, Clr3 work in the same pathway as Swi6, whereas Brl2, Pof3 and Cbp1 act via Swi6-dependent and -independent mechanisms. These observations provide new clues to understand the molecular mechanisms of mating-type switching.

Results and discussion
Identification of mating-type switching defective mutants

We conducted a genome-wide screen for factors required for mating-type switching. The screen used an $S. pombe$ gene deletion library (Bioneer) consisting of 3420 haploid strains, each of which lacks a non-essential gene. Mating-type switching occurs in $h^{+90}$ strains, yet the Bioneer library strains are heterothallic $h^{+N}$ strains for which a large duplication in the mating-type region abrogates mating-type switching. To construct $h^{+90}$ derivatives of the entire Bioneer collection, strain PG4045 was mated to the library. The $h^{+90}$ mating-type region of PG4045 could be selected in the progeny due to the linked LEU2 gene. In addition, PG4045 contains two fluorescent reporters specific for the $P$ (CFP controlled by the $map2$ promoter) and $M$ (YFP controlled by the $mfm3$ promoter) cell types, respectively. Both reporters are expressed from the $leu1$ locus where they were integrated together with the selectable $ura4^{+}$ gene. Thus, we selected $h^{+90}$ LEU2 ura4+ segregants (S1 Fig). We obtained 3298 $h^{+90}$ deletion strains in which we monitored expression of the fluorescent reporters (Fig 2A). Efficient mating-type switching results in rapid homogenization of $h^{+90}$ cell populations to equal proportions of $P$ and $M$ cells (Fig 1B). Here, screening specifically for mutants that displayed biased cell-type ratios, differing by > 3 standard deviations from the mean, we isolated 105 candidates with skewed proportions (Fig 2B, S2 Fig, S2 Table). In several deletion strains, we detected co-expression of CFP and YFP in a cell. This phenotype was most likely caused by derepression of the mating-type information at $mat2-P$ and $mat3-M$ in these mutants [42]. In addition, 568 strains that could not be evaluated due to low fluorescence intensity or poor growth were examined by iodine staining, a stain for $S. pombe$ spores that can be used as diagnostic for $mat1$ switching (Fig 2C, S3 Table). Wild-type $h^{+90}$ colonies are stained darkly by iodine vapors because of their high spore content while mutants with altered mating or sporulation are stained less. Here, 124 deletion strains among the strains tested showed a staining different from wild-type. They were analyzed by quantitative multiplex PCR for $mat1$ content alongside the 108 candidates that had passed the fluorescence microscopy screening.

Analyzing the content of $mat1$ permitted us to pinpoint mutants for which biased cell-type expression or poor sporulation is likely to result from switching defects. A number of candidates failed to show a biased $mat1$ content by multiplex PCR according to the chosen thresholds of $P$ band intensity (Fig 2A and 2D). Indeed, many mutations might result in biased reporter gene expression or altered sporulation without affecting mating-type switching, for example genes located in the I. region of the $mat$ locus were eliminated from this screening. In addition, 35 deletion mutants that were diploid and/or heterothallic ($h^{+N}$) were eliminated from the list of candidates (S4 Table). A bias in $mat1$ content was detected for 32 mutants, in all cases towards $mat1-M$, suggesting an increased use of $mat3-M$. The identity of the deleted gene in these mutants was confirmed using published barcode sequences or gene specific primers. Surprisingly, 9 genes encoding ribosomal proteins are in the list, possibly as a consequence of protein synthesis defects. We did not pursue the investigation of these mutants, but
focused instead on the remaining 23 mutants. These included nearly all known non-essential switch-related genes, 16 of which were identified in total. A few switch-related genes (swi1, 5 or 10) were not deleted in the Bioneer library and were therefore not tested by the screen. A few other mutants might have escaped detection due to clonal variation, alternative switching phenotype with low frequency as for msc1Δ [49] or due to a switching bias close to the set thresholds, as for mrc1Δ [16]. Globally though the screening strategy was strongly validated by
the identification of known switch-related factors alongside novel factors. The 7 newly identified factors include the F box protein Pof3, the CK2 family regulatory subunit Ckb1, the elongator complex subunit Elp6, the E3 ubiquitin protein ligase Brl2 and three subunits of the Set1/compass complex (Set1C), Swd1, Swd2 and Spf1 (Fig 2D, S2 Fig, Table 1). A protein interaction network analysis regrouping novel and previously known factors showed a high degree of connectivity (see below).

The fluorescence microscopy and multiplex PCR analyses for known and newly identified factors (S2 Fig) were confirmed by Southern blot analysis of mat1 content with the DdeI restriction enzyme (S3 Fig).

**Classification of genes related to mating-type switching by Southern blot**

Southern blots can be used to detect the imprint at mat1 and to determine whether rearrangements have occurred in the mating-type region. A DSB results from breakage at the fragile imprint site during DNA preparation [40]. As mentioned in the Introduction, the mating-type switching factors can be subdivided into three groups by Southern blot analysis of mutants, reflecting the molecular function of each factor. Class Ia is required for DSB formation at mat1, Class Ib is involved in donor selection for mating-type switching or other steps in the use of the break, and Class II is required for processing the gene conversion intermediates [40]. The 23 strains selected for analysis were assayed by Southern blot (Fig 3). The analysis confirmed previous conclusions in the case of known factors, for instance swi3Δ abolished DSB, and rad57Δ, msh2Δ, msh3Δ, rad16Δ, and pxd1Δ caused high frequencies of rearrangements of the h^+N type as expected for resolution-defective mutants [40].

The newly identified mating-type switching genes were assigned to Class Ib; elp6Δ, swd1Δ, spf1Δ, brl2Δ, pof3Δ and ckb1Δ strains were in that category together with swi2Δ, cbp1Δ, swi6Δ, sir2Δ and deletions of the Clr4 methyltransferase complex (CLRC), clr4Δ, rik1Δ and rad1Δ, or Snf/Hdac-containing repressor complex (SHREC), clr1Δ, clr2Δ and clr3Δ, subunits. As previously noticed for the swi6Δ mutant [51] a rearrangement producing an 8.2 kb HindIII fragment could be detected in several Class Ib mutants. The rearrangement could be a mat3:1 circle or a duplication of the mating-type region creating a mat3:1 cassette: mat1-L-mat2-P-K-mat3:1-L-mat2-P-K-mat3-M. The mat3:1 cassette would not be amplified by the primers used to detect mat1 content by PCR. The swd2Δ strain differed from the other mutants by showing a 9.9 kb HindIII fragment hybridizing to the mat1 probe, however reconstruction of the strain produced a Class Ib mutant lacking this additional band and the reconstructed deletion allele was used in further analyses.

**Investigations of directionality defects in h^09 cells and in cells with mutated recombination enhancers**

In the h^09 mating-type region, the contents of the silent cassettes are swapped to mat2-M mat3-P (Fig 4, S4 Fig). This arrangement results in inefficient heterologous switching and in a mat1 content biased towards mat1-M [3]. How mutations affect this bias provides insights into the directionality of mating-type switching. For example, deletion of swi6 biases the mat1 content towards mat1-P in h^09 cells, and towards mat1-M in h^09 cells, consistent in both cases with preferred selection of mat3 as a donor [3]. This likely reflects a preferential use of the SRE3 recombination enhancer in swi6Δ cells [23]. We created h^09 strains to test whether the newly identified Class Ib factors contribute to mating-type switching in the same or similar way as Swi6. Each deletion mutant was crossed with the h^09 strain PG4048. After the selection of recombinants, we analyzed four independent colonies of each h^09 deletion strain by multiplex PCR for mat1 content. As a control, colonies originating from spores of self-mating PG4048
cells were analyzed. As expected, PG4048 contained a greater proportion of M cells than P cells (a mean of 86% M cells; Fig 4). All mutations tested affected this ratio. The effects varied. During these analyses, we created a fresh deletion of \textit{swd2} to eliminate the rearrangement detected in the Bioneer mutant (9.9 kb band in Fig 3), as mentioned above, and confirmed that the observed directionality defects were not a result of the rearrangement.

A first group of factors comprised Swi6, CLRC subunits (Clr4, Rik1 and Raf1) and the histone deacetylase Sir2 (Fig 4). Mutations affecting CLRC or Sir2 produced nearly identical
values (around 70% P cells), similar to the loss of Swi6 (77% P cells). These mutants were also very similar to each other in the h90 background (around 20% P cells; Fig 2D). In the mating-type switching process, CLRC and its catalytic Clr4 subunit are believed to work by catalyzing the methylation of H3K9 in the mat2-mat3 heterochromatic domain and creating binding sites for Swi6 [24, 52–55]. The role of Sir2 in the process might be to remove acetyl groups from H3K9 [46], thus facilitating heterochromatin formation [46, 56–58]. Indeed, the methylation of H3K9 and Swi6 association are reduced several fold in the mating-type region of sir2Δ cells [46]. This is consistent with Sir2 acting upstream of CLRC and Swi6 through H3K9 deacetylation, without excluding that other actions of Sir2, e.g. H3K4 deacetylation [59], might also be relevant to directionality.

The second group of mutants affecting mating-type switching in h09 cells were deletions of the genes for the ubiquitin E3 ligase Brl2, the Set1C subunits Swd1, Swd2 and Spf1, and the Elongator subunit Elp6 (Fig 4). These mutants resulted in a consistent increase in mat1-P content in h09 cells, from ~20% in wild-type background to ~35% in each of the three mutants. While not as pronounced as for Swi6 or CLRC mutants, the increase occurred to the same degree in all four isolates examined in each case. These mutants were unexpected because it has been reported that set1Δ has no effect on mating-type switching [60]. We examined each component of Set1C (Set1, Swd1, Swd2, Spf1, Ash2, Shg1 and Sdc1 [61]) by iodine staining and multiplex PCR of mutants (Fig 5A–5D). Consistent with the previous report [60], deletion of set1 or other subunit genes showed little effect on iodine staining of either h09 or h90 colonies (Fig 5A and 5B). However, monitoring mat1 content clearly showed that individual gene deletions biased switching toward mat3-P in h09 cells and resulted in a correlated
increased use of mat3-M in \( h^{90} \) cells for six Set1C subunits (Fig 5C and 5D). This trend is similar to mutations in the H3K9 methylation pathway, although not to the same amplitude (Fig 4). Interestingly, the iodine staining level of \( set1 \Delta \) colonies differed between \( h^{90} \) and \( h^{09} \) in spite of similar cell-type ratios (39% P cells in \( h^{90} \) and 36% in \( h^{09} \)). Due to a different switching pattern, P and M cells might be less evenly mixed in \( h^{09} \) colonies compared with \( h^{90} \). This would lead to less efficient mating and spore formation in \( h^{09} \) even though the cell-type bias is only a little more pronounced than in \( h^{90} \). To investigate the functionality of each SRE element in \( set1 \Delta \) cells, we analyzed directionality in SRE element mutants (Fig 5E–5H). Deletion of \( set1^{+} \) did not significantly affect \( mat1 \) content in 2×SRE2 cells (where the SRE3 element is replaced Fig 5).
with SRE2) (Fig 5E) or in SRE3Δ cells (Fig 5G). However, in populations of 2×SRE3 cells (where the SRE2 element is replaced with SRE3) deletion of set1 caused a small increase in M cells (31% P cells in 2×SRE3 set1Δ compared with 36% P cells in 2×SRE3 set1+) (Fig 5F). In the case of SRE2Δ, donor choice was more strongly biased towards mat3-M in set1Δ cells (6% P cells) than in set1+ cells (13% P cells) (Fig 5H). This suggests that Set1C normally inhibits the choice of mat3-M-SRE3 in M cells. It has similarly been observed that deletion of swi6 causes virtually no change in donor choice in the 2×SRE2 and SRE3Δ backgrounds, where SRE2 keeps being used, but decreases use of SRE3 in SRE2Δ cells (5% P cells in SRE2Δ swi6Δ compared with 16% P cells in SRE2Δ swi6+). Loss of the Brl2 ubiquitin ligase resulted in phenotypes similar to mutations compromising Set1C (Figs 4 and 5). Thus, like Swi6 and CLRC, Set1C and Brl2 appear to favor use of the SRE2 recombination enhancer over SRE3 when both enhancers are present, perhaps by inhibiting the use of SRE3, to result in balanced switching.

A third group of mutants displayed a variegated phenotype (clr1Δ, clr2Δ, clr3Δ, ckb1Δ, pof3Δ and cbp1Δ; Fig 4), with the proportion of P cells varying between independent cultures. In some isolates, the proportion of P cells was nearly wild-type whereas in others it was similar to the swi6Δ mutant. These phenotypes were not caused by rearrangements in the mating-type region (S4 Fig). Clr1, 2 and 3 are subunits of SHREC, the Snf2-histone deacetylase repressor complex [62]. Clr3 participates in the recruitment of Clr4 to the mating-type region, but in its absence a Clr3-independent, RNAi-dependent pathway accomplishes this function to some extent [63]. Clr3 localizes to three regions in the mating-type region, which are close to mat2-P (REII), cenH and mat3-M (REEIII), respectively [62, 64]. In the cenH region, the heterochromatin platform is likely established by RNAi-mechanisms; on the other hand, at the REIII site, it is established by an RNAi-independent mechanism [63, 65, 66]. The clonal variations observed in clr1, clr2 and clr3 h99 mutants might reflect these distinct pathways of heterochromatin establishment similar to position effect variegation [67]. Heterochromatin would be partially formed and inherited in some clonal populations of the mutant strains but not in others, leading to populations differentially proficient for switching. It is also known that the CK2-dependent phosphorylation of Swi6 mediates Clr3 recruitment to centromeric regions [68], possibly accounting for the Ckb1 defects observed here.

Swi6-dependent and independent pathways of mating-type switching

To further address the mechanisms of mating-type switching, we analyzed the protein-protein interaction network linking newly identified and previously known mating-type switching factors in the STRING database (Table 1) [69]. The obtained interaction network correlates strongly with categories established by Southern blotting and phenotypic classification (Fig 6A). Among the newly identified factors, six subunits of Set1C and Brl2 are connected to Class Ib factors. While Pof3, Ckb1 and Elp6 show no direct interaction with Class Ib factors in the STRING analysis (Fig 6A), several studies have reported that Pof3 plays a role in heterochromatin silencing [70–72] and Ckb1 phosphorylates Swi6 [68]. Elp6 is an orthologue of a part of the six-subunit Elongator complex (Elp1–6) in S. cerevisiae [73, 74]. Elp3 also passed the initial screening (S2 Table), however the other subunits of Elongator complex did not. The main cellular function of Elongator is thought to be in tRNA modification, but Elongator has also been proposed to acetylate histones [75–77].

We tested the hypothesis that some factors might act through Swi6 by performing an epistasis analysis. Double mutants combining swi6Δ with each candidate gene deletion were constructed and four independent colonies were analyzed by multiplex PCR for each of them (Fig 6B and 6C).
The mating-type switching phenotypes of the swi6Δ clr4Δ and swi6Δ sir2Δ double mutants were quite similar to the single deletions of swi6, clr4 or sir2 (Figs 2D, 4, 6B and 6C). This is consistent with CLRC and Sir2 being required for heterochromatin establishment and Swi6 recruitment at the mat locus (Fig 7).

Double mutants combining swi6Δ with swd1Δ, lacking a Set1C subunit, or clr3Δ, lacking a SHREC subunit, also displayed a phenotype quite similar to the swi6Δ single mutant (Fig 6B and 6C). This indicates that Set1C and SHREC work in the Swi6 pathway. A previous study has shown that set1Δ does not affect Swi6 localization or silencing of a ura4+ marker gene at cenH in the mating-type region [60] but other assays have found that Set1C subunits
participate in the repression of heterochromatic loci including the mating-type region \[78\]. This latter effect might be related to the occurrence of switching defects in Set1C mutants. Set1C may control Swi6 localization in a site-specific manner such as at SRE3 (Fig 5).

Donor preference in swi6Δ ckb1Δ cells indicated that swi6Δ is also epistatic to ckb1Δ even though phenotypes could only be assigned in the h\(^{00}\) background due to apparently high rates of rearrangement in the h\(^{09}\) background. Nevertheless, the data suggest that Swi6 phosphorylation by Ckb1 \[68\] is important for switching directionality controlled by Swi6.

More complex epistatic relationships were observed for the remaining mutants, brl2Δ, po3Δ, cbp1Δ, elp6Δ, and swi2Δ, when these mutations were combined with swi6Δ (Fig 6B and 6C). The predominant mating-type had a tendency to vary between isolates, particularly with the h\(^{09}\) mating-type region, and rearrangements occurred. Double mutants combining brl2Δ
and swi6Δ showed a mat1-M bias apparently even more pronounced than for the swi6Δ single mutant in h90, while in h90 two swi6Δ brl2Δ isolates were similar to swi6Δ and two had more balanced mat1 contents. Populations of h90 swi6Δ pof3Δ cells showed biases similar to the h90 swi6Δ single mutant for three isolates, whereas the fourth isolate was biased towards P cells rather than M cells. Populations of h90 swi6Δ pof3Δ cells had varied ratios of P and M cells, and two isolates were rearranged. The switching bias for swi6Δ cbp1Δ was similar to swi6Δ with the h90 mating-type region (Fig 6B), but two strains in four differed from swi6Δ with the h09 mating-type region (Fig 6C). This phenotype may be caused by Swi2 expression level, which is controlled by Cbp1 [26, 31]. Rearrangements in h90 swi6Δ elp6Δ and h09 swi6Δ elp6Δ mutants precluded analysis. These observations suggest that both Swi6-dependent and -independent pathways control switching directionality by Brl2, Pof3 and Cbp1. Finally, swi2Δ swi6Δ double mutants differed from swi6Δ in both h90 and h09. It has been reported that Swi2 can localize to SRE3 in the absence of Swi6 [22, 23, 27]. The more balanced cell populations in swi6Δ swi2Δ mutants are probably caused by loss of Swi6-independent function of Swi2.

In summary, our observations suggest that the factors, Clr4, Sir2, Swd1 and Clr3 probably work in the same pathway as Swi6, but Brl2, Pof3 and Cbp1 and Swi2 have an effect on donor selection through Swi6-dependent and -independent mechanisms. Frequent DNA rearrangements in Class Ib mutants (Fig 3) and in double mutants with swi6Δ indicate that histone modifications not only direct donor choice, but also facilitate resolution steps or prevent unequal sister chromatid exchanges between cassettes.

### Mating-type switching model by regulation of Swi6 association with the mating-type region

We propose a model summarizing how each factor identified in this study might participate in the donor selection mechanism (Fig 7). In this model, the heterochromatin structure in M cells favors the cassette adjacent to SRE2 as a donor while structural changes in mutants and in P cells favor SRE3 [23]. In addition, chromatin structure prevents selection of the cassette adjacent to SRE3 in M cell (Fig 7A). It has been reported that SRE2 can facilitate donor choice efficiently not only in M cells but also in P cells whereas SRE3 is more active in P cells than in M cells [23]. These data indicate that the inhibition of donor choice does not affect SRE2.

Mating-type switching is initiated by a site-specific imprint during replication. In the replisome, Swi1-Swi3, Pol1 and Mrc1 are required for the imprint [16, 40]. One of the novel switch factors identified here is the ubiquitin ligase component Pof3. Pof3 interacts with the replisome, in an Mrc1- and Mcl1-dependent manner [71, 79]. However, rather than participating in imprint formation, we found that Po3 affects donor selection. Po3 is also required for heterochromatic silencing near mat3 [70]. We speculate that both effects are brought about by the Po3-mediated degradation of replisome components [80] or of Ams2, a cell cycle-regulated transcription factor for histone genes [81] that also mediates long range chromosomal interactions [82] and interacts with Raf1, a component of CLRC [83]. Thus, Po3 would couple the deposition of new histones and their modification by CLRC. During S phase, partly as a result of new histones deposition onto replicated DNA, Swi6 and H3K9me2 levels decrease at silenced loci [84, 85]. A wave of H3K9 acetylation, observed in other organisms in front of the replication fork [86], might further weaken heterochromatin. The current search expands on previous work to show that enzymatic complexes required for the restoration of heterochromatin, both NAD+-dependent and -independent HDACs and CLRC, are necessary for donor selection. Remarkably, lack of Sir2 or of a CLRC subunit phenocopied the swi6Δ deletion in both h90 and h09 cells (Figs 2D and 4) while the loss of SHREC components resulted in variegated phenotypes. We take these differences as reflecting the different substrate specificities.
and recruitment mechanisms of the two HDACs to heterochromatic regions [57, 87]. In both cases, our epistasis analysis with the Swi6 mutant points to defects in Swi6 recruitment. Our search also identified Cbp1 and CK2, both of which are thought to recruit Clr3 to heterochromatin regions [64, 68]. Cbp1 also controls expression of the swi2 gene and the cell-type specific protein isoform it produces, together with the M-specific protein Mc [26, 31].

A novel and intriguing outcome of our study is that multiple subunits of Set1C and the E3 ubiquitin ligase Brl2 are required for accurate donor selection. Set1C catalyzes the methylation of H3K4. A role in heterochromatin appears paradoxical, given that methylated H3K4 is strongly associated with expressed genes. One possibility is that Set1C regulates the expression of Swi2, central effector of switching directionality, or of other switching factors. RNA profiling analysis has revealed that switching genes are expressed to similar levels in wild type and individual Set1C mutants [87], however more subtle effects such as shifts in transcription initiation have not been ruled out. In addition, S. pombe Set1C appears directly required for silencing at various locations [78]. In the mating-type region, all subunits except for Shg1 are required for silencing of the cenH repeat. Evidence has also emerged for roles in meiotic recombination [88, 89] even though the effects of H3K4 methylation on recombination have been hard to unravel due to the prevalence of that modification genome-wide [90]. Here, we favor a simple model where Set1C affects donor choice directly. This could be through local, possibly temporally restricted methylation of H3K4 at SRE3 that would either act as such or by preventing H3K4 acetylation. Brl2 is part of HULC that catalyzes the ubiquitylation of histone 2B (H2Bub) [61, 91, 92]. H2Bub stabilizes the interaction of Set1 with chromatin in vitro [93]. The single brl2Δ deletion affected mat1 content similar to the deletion of Set1C components (Figs 2, 4 and 5). These connections and phenotypic similarities indicated that Set1C and HULC might co-operate to choose a correct donor. (Figs 4 and 6B). However, the phenotypes of two in four independent colonies of h^90 swi6Δ brl2Δ strain differed from h^90 swi6Δ swd1Δ (Fig 6C). Brl2 is also known to interact with Nse5, which is a part of the structural maintenance of chromosome 5/6 (Smc5-6) holocomplex [94, 95]. It may have multiple functions in mating-type switching.

Following the cell-type specific deposition of Swi6, Swi2-Swi5 localizes to SRE2 by interaction with Swi6 in M cells [22, 23, 27]. Together with the inhibited use of SRE3, this regulated recruitment of Swi2-Swi5 effectively directs the Rad51 strand-exchange protein to initiate homologous recombination at the proper cassette, providing an increasingly well understood model for the effects of chromatin structure on recombination.

Materials and methods

Yeast strains, strain construction, and strain manipulations

S. pombe strains were generated and propagated according to standard protocols [96]. They were manipulated with a Singer RoToR plate-handling robot (Singer Instruments) for high throughput screens. To test for mating-type switching defects by fluorescence analysis, a query strain (PG4045: h^90 (Blp1)::LEU2 leu1::ura4^+ [mfn3p-YFP]-[map2p-CFP] ura4-D18 ade6-M216) was mated to the Bioneer gene deletion library (h^+ leu1-32 ura4-D18 ade6-210 or 216, ORFΔ::kanMX4). Mating was performed on SPA plates supplemented with 200 mg/l leucine, 100 mg/l uracil, and 100 mg/l adenine. Cells were allowed to mate and sporulate at 30˚C for two days. The mating plates were then moved to 42˚C for three days to eliminate vegetative cells. Following heat treatment, spores were transferred onto YES plates with 100 mg/l G418 and allowed to germinate and divide for three days at 30˚C. To select for h^90 progeny, cells were then transferred from the YES plates to MSA plates with 100 mg/l G418 and 100 mg/l adenine and grown for a further three days at 30˚C. This scheme selects for (Blp1)::LEU2,
tightly linked to the h^{90} region, for ura4+, tightly linked to the fluorescent reporters, and for kanMX4 that marks each ORF deletion. Single colonies were isolated from bulk recombinants by streaking cells onto MSA plates containing G418 and adenine, incubated for three days at 30˚C. The sdc1 deletion strain (h′ sdc1::Kan′ ade6-M21? leu1-32 ura4-D18) was obtained from the National BioResource Project (NBRP ID: FY23769, strain name: P1-1G).

**Fluorescence microscopy**

Single colonies of recombinants obtained as described above were inoculated into 50 μL MSA medium supplemented with 100 mg/l adenine and grown for two days at 30˚C in 96 well plates. Cells were then diluted 24 times into MSA medium with adenine and grown for ~20 hr at 30˚C. Sixty μl cell suspensions diluted 15 times with MSA medium with adenine were transferred to 384 well microplates with clear bottom (CellCarrier-384 ultra, Perkin Elmer). The fluorescence of cells was measured using an Opera high-content screening microscope (Perkin Elmer). The following settings were used [Filter sets: Camera 1: 475/50 for CFP signals, Camera 2: 540/75 for YFP signals, Camera 3: 690/50 for bright field, Light source: 405/488/635]. Twelve images were taken for each well, for a total of ~200 cells per strain. The proportion of P cells was then calculated using the Acapella software program (PerkinElmer). Selected strains were imaged again using a Delta Vision Elite microscope (GE Healthcare).

**Iodine staining**

Cells were plated onto MSA medium supplemented with 100 mg/l adenine, allowed to form single colonies, and exposed to iodine vapors.

**Multiplex PCR**

*S. pombe* cells were propagated in 2 mL liquid YES cultures at 30˚C to saturation. Genomic DNA was prepared from wild-type and mutant cells as described [97]. The genomic DNA concentration was measured using QuantiFluor One dsDNA Dye System (Promega) and 4 μL genomic DNA (1.25–5.0 ng/μL) in TE was added to 16 μL PCR reaction reagent (total 20 μL) to perform multiplex PCR to determine the genetic content of the mat1 or mat3 locus. The primers used were FAM-MT1 (5’-AAATAGTGGGTTAGCCGT GAAAGG-3’) at 400 nM, MP1 (5’-ATCTATCAGGAGATTGG GCAGGTG-3’) at 200 nM and MM1(5’-GGGAACCCG CTGATAATTCTTTG-3’) at 200 nM (S3 Fig). The 5’ end of FAM-MT1 and FAM-MT3 were modified with 6-carboxyfluorescein (FAM). To reduce non-specific PCR products, 400 nM heat-stable RecA protein from a thermophilic bacterium, *Thermus thermophiles*, and 400 μM ATP were included in the PCR reaction buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 2.5 mM MgCl2) [98]. The following amplification program was used: 2 min at 94˚C—27 x [30 s at 94˚C —30 s at 55˚C—1 min at 72˚C]—5 min at 72˚C. PCR fragments corresponding to mat1-P and mat1-M alleles were resolved on 5% polyacrylamide gels. Fluorescence was detected and quantified using Typhoon FLA9500 (GE Healthcare) and ImageQuant (GE Healthcare).

**Barcode sequencing**

Each gene knockout in the Bioneer collection contains ‘up-tag’ and ‘down-tag’ sequences that provide a unique barcode for each knockout. To confirm the identity of the mutants identified in our screen, we amplified the KanMX4 region using the U1 primer (5’-CGCTCCCGCCT TACTTGCAGC-3’) and D1 primer (5’- TTGCGTTGCGTAGGAGGGGAT). The PCR products were then sequenced using cpn1 (5’-CGTCTGTGAGGGGAGC GTTT-3’) to read the up-tag
and cpc300 (5′-AGACCGATACCAGGATCTTGCC-3′) to read the down-tag. The results were compared with the barcode list.

**Southern blots**

*S. pombe* cells were propagated in 16 mL liquid cultures (YES) at 30°C to pre-saturation and genomic DNA for Southern blots was prepared as described above. Genomic DNA was digested with *Hin*III to classify the mating-type switching defective genes according to DSB formation or presence of rearrangements in the mating-type region (Fig 3), or with *Dde*I to assay *mat1* content ([S3 Fig](#fig3)). The digested samples were electrophoresed in 0.7% agarose gels. The probe to analyze *mat1* content was a PCR product made with GTO-1369 (5′-GAGCCTACTGTTAATATAACATTATG-3′) and GTO-1370 (5′-CCTTCAACTACTCTCTTCTTCTTTACCC-3′), corresponding to the centromere-proximal *Dde*I-*Nsi*I fragment [23]. The probes to classify the mutants were 10.4 kb *Hin*III fragments containing *mat1*-P or *mat1*-M.

**Supporting information**

[S1 Fig](#fig1). **Outline of screen.** The Bioneer collection of *S. pombe* haploid deletion strains (V5) was mated with PG4045 on SPA plates in an arrayed format using a high-throughput robot (Singer Inst.). Spores were selected, germinated, and G418-resistant progeny that were also prototrophic for leucine (selecting for *h*90 mating-type region) and uracil (selecting for cell-type specific fluorescent reporters) were obtained, colony purified, re-arrayed, and examined by a combination of fluorescence microscopy with a high throughput Opera microscope (all strains), iodine staining of colonies (subset of strains with unclear fluorescence output), and multiplex PCR (subset of strains with potential mating-type switching defects obtained in the first screens).

[S2 Fig](#fig2). **Analysis of mating-type switching related genes by fluorescence microscopy and iodine staining assays.** (A) Merged channels for wild-type *h*90 strain (cntl) and two mutants. The 15 mutants shown in (B, C) were identified by fluorescence microscopy or iodine staining analysis in the screen outlined in [S1 Fig](#fig1). (B) Repeat of cell-type quantification using a DeltaVision Elite microscope (GE Healthcare). (C) Iodine staining of strains with the indicated gene deletions.

[S3 Fig](#fig3). **Effects of culture conditions and detection method (multiplex PCR versus Southern blot) on mat1 content measurements.** (A, B) Representation of the *mat1* region showing (A) priming sites used for multiplex PCR and (B) the restriction sites and probe (*Dde*I-*Nsi*I fragment) used for the Southern blots in (E). (C) Location of primers and probe complementarity on *h*90, *h*90, duplicated mating-type region (*mat1*-L-*mat2*-P-*K*-mat3:1-*L*-mat2-P-*K*-mat3-M), and *mat2:1* or *mat3:1* circles. (D) 30 μL of saturated YES pre-cultures (2 ml each) were used to inoculate 20 mL YES cultures that were then propagated until late exponential phase. DNA was extracted from both the 2 ml pre-cultures and the 20 ml cultures and analyzed by multiplex PCR. (E) DNA preps from 20 mL YES cultures were analyzed by Southern blot taking advantage of the size difference between a *mat1*-P and *mat1*-M fragment in *Dde*I digests. Measurements by multiplex PCR and Southern blots are compared.

[S4 Fig](#fig4). **Effect of Class Ib mutants identified in the screen on mating-type switching in the *h*90 strain.** (A) Multiplex PCR analysis of the mutants shown in [Fig 4](#fig4). The content of *mat1* was
estimated by quantification of P- and M-specific band intensities. (B) Southern blot analysis using HindIII digests of genomic DNA and a 10.4 kb mat1 HindIII fragment as probe.

S5 Fig. Multiplex PCR analysis of mating-type switching directionality in Set1C subunit mutants and SRE element mutants. (A, B) Multiplex PCR analysis of the mutants shown in Fig 5C and 5D and quantification of mat1 content estimated from P- and M-specific band intensities. The relative P band intensity in each lane (P/(P+M)) was calculated from (A) h<sup>90</sup> and (B) h<sup>69</sup>. (C-F) Epistasis analysis by multiplex PCR analysis for the Set1 and SRE elements mutants shown in Fig 5E–5H. The relative P band intensity (P/(P+M)) was calculated for each lane. (C) 2×SRE2 (mat2-P-SRE2 mat3-M-SRE2) strains were derived from strain TP126; (D) 2×SRE3 (mat2-P-SRE3 mat3-M-SRE3) strains were derived from strain TP303; (E) SRE3Δ from TP75 and (F) SRE2Δ from TP8.

S6 Fig. Multiplex PCR analysis of double mutants. Multiplex PCR analysis of the mutants shown in Fig 6B and 6C. The content of mat1 was estimated by quantification of P- and M-specific band intensities. N.D. = Not-detected.

S1 Table. List of strains used in crosses. (DOC)

S2 Table. Bioneer mutants showing potentially biased mating-type ratios in fluorescence screen and their characterization by multiplex PCR for mat1 content and by barcode sequencing. (XLSX)

S3 Table. Bioneer mutants with altered iodine staining phenotypes and their characterization by multiplex PCR for mat1 content and by barcode sequencing. (XLSX)

S4 Table. Bioneer mutants excluded from the analysis. (XLSX)

Acknowledgments

We thank N. Steen, P. Brøgger, M. M. Jørgensen, S. Brown, A. E. Chavez, B. Argunhan, H. Tsubouchi and Y. Murayama for valuable discussions and suggestions, J. Verhein-Hansen and Y. Umamoto for technical assistance. We are thankful to the National BioResource Project for providing strains. Imaging data from the Opera microscope were collected at the Center for Advanced Bioimaging (CAB) Denmark, University of Copenhagen, with the help of Nynne Christensen. We thank the Biomaterials Analysis Division, Tokyo Institute of Technology for sequence analysis.

Author Contributions

Conceptualization: Takahisa Maki, Hiroshi Iwasaki, Geneviève Thon.

Data curation: Takahisa Maki.

Formal analysis: Takahisa Maki, James E. Haber, Hiroshi Iwasaki, Geneviève Thon.

Funding acquisition: James E. Haber, Hiroshi Iwasaki, Geneviève Thon.
Investigation: Takahisa Maki, Naoto Ogura.
Methodology: Takahisa Maki, Geneviève Thon.
Project administration: Hiroshi Iwasaki, Geneviève Thon.
Resources: Hiroshi Iwasaki.
Supervision: Hiroshi Iwasaki, Geneviève Thon.
Validation: Takahisa Maki.
Visualization: Takahisa Maki.
Writing – original draft: Takahisa Maki.
Writing – review & editing: James E. Haber, Hiroshi Iwasaki, Geneviève Thon.

References
1. Kelly M, Burke J, Smith M, Klar A, Beach D. Four mating-type genes control sexual differentiation in the fission yeast. EMBO J. 1988; 7(5):1537–47. PMID: 2900761.
2. Klar AJ, Ishikawa K, Moore S. A Unique DNA Recombination Mechanism of the Mating/Cell-type Switching of Fission Yeasts: a Review. Microbiol Spectr. 2014; 2(5). https://doi.org/10.1128/ microbiospec.MDNA3-0003-2014 PMID: 26104357.
3. Thon G, Klar AJ. Directionality of fission yeast mating-type interconversion is controlled by the location of the donor loci. Genetics. 1993; 134(4):1045–54. PMID: 8375648.
4. Miyata H, Miyata M. Mode of Conjugation in Homothallic Cells of Schizosaccharomyces-Pombe. Journal of General and Applied Microbiology. 1981; 27(5):365–71. https://doi.org/10.2323/jgam.27.365
5. Egel R, Eie B. Cell lineage asymmetry in Schizosaccharomyces pombe: unilateral transmission of a high-frequency state for mating-type switching in diploid pedigrees. Current Genetics. 1987; 12(6):429–33. https://doi.org/10.1007/bf00434820
6. Dalgaard JZ, Klar AJ. Orientation of DNA replication establishes mating-type switching pattern in S. pombe. Nature. 1999; 400(6740):181–4. https://doi.org/10.1038/22139 PMID: 10408447.
7. Dalgaard JZ, Klar AJ. swi1 and swi3 perform imprinting, pausing, and termination of DNA replication in S-pombe. Cell. 2000; 102(6):745–51. https://doi.org/10.1016/S0092-8674(00)00063-5 PMID: 11030618
8. Klar AJ. Differentiated parental DNA strands confer developmental asymmetry on daughter cells in fission yeast. Nature. 1987; 326(6112):466–70. https://doi.org/10.1038/326466a0 PMID: 3561486.
9. Klar AJ. The Developmental Fate of Fission Yeast-Cells Is Determined by the Pattern of Inheritance of Parental and Grandparental DNA Strands. Embo Journal. 1990; 9(5):1405–15. PMID: 2328720
10. Holmes AM, Kaykov A, Arcangioli B. Molecular and cellular dissection of mating-type switching steps in Schizosaccharomyces pombe. Mol Cell Biol. 2005; 25(1):303–11. https://doi.org/10.1128/MCB.25.1.303-311.2005 PMID: 15601851.
11. Vengrova S, Dalgaard JZ. The wild-type Schizosaccharomyces pombe mat1 imprint consists of two ribonucleotides. EMBO Rep. 2006; 7(1):59–65. https://doi.org/10.1038/sj.embor.7400576 PMID: 16299470.
12. Vengrova S, Dalgaard JZ. RNase-sensitive DNA modification(s) initiates S. pombe mating-type switching. Genes Dev. 2004; 18(7):794–804. https://doi.org/10.1101/gad.289404 PMID: 15059961.
13. Arcangioli B. A site- and strand-specific DNA break confers asymmetric switching potential in fission yeast. EMBO J. 1998; 17(15):4503–10. https://doi.org/10.1093/emboj/17.15.4503 PMID: 9887516.
14. Arcangioli B. Fate of mat1 DNA strands during mating-type switching in fission yeast. EMBO Rep. 2000; 1(2):145–50. https://doi.org/10.1093/embo-reports/kvd023 PMID: 11265754.
15. Kaykov A, Arcangioli B. A programmed strand-specific and modified nick in S. pombe constitutes a novel type of chromosomal imprint. Curr Biol. 2004; 14(21):1924–8. https://doi.org/10.1016/j.cub.2004.10.026 PMID: 15530393.
16. Zech J, Godfrey EL, Masai H, Hartsuiker E, Dalgaard JZ. The DNA-Binding Domain of S. pombe Mrcl (Claspin) Acts to Enhance Stalling at Replication Barriers. PLoS One. 2015; 10(7):e0132595. https://doi.org/10.1371/journal.pone.0132595 PMID: 26201080.
17. Holmes A, Roseaun L, Schurra C, Waxin H, Lambert S, Zaratiegui M, et al. Lsd1 and lsd2 control programmed replication fork pauses and imprinting in fission yeast. Cell Rep. 2012; 2(6):1513–20. https://doi.org/10.1016/j.celrep.2012.10.011 PMID: 23260662.

18. Kaykov A, Holmes AM, Arcangioli B. Formation, maintenance and consequences of the imprint at the mating-type locus in fission yeast. Embo Journal. 2004; 23(4):930–8. https://doi.org/10.1038/sj.emboj.760099 PMID: 14765111.

19. Raimondi C, Jagla B, Proux C, Waxin H, Gangloff S, Arcangioli B. Molecular signature of the imprintosome complex at the mating-type locus in fission yeast. Microb Cell. 2018; 5(4):169–83. https://doi.org/10.15698/mic2018.04.623 PMID: 29610759.

20. Yamada-Inagawa T, Klar AJ, Dalgaard JZ. Schizosaccharomyces pombe switches mating type by the synthesis-dependent strand-annealing mechanism. Genetics. 2007; 177(1):255–65. https://doi.org/10.1534/genetics.107.076315 PMID: 17660548.

21. Grewal SIS, Klar AJS. A recombinationally repressed region between mat2 and mat3 loci shares homology to centromeric repeats and regulates directionality of mating-type switching in fission yeast. Genetics. 1997; 146(4):1221–38. PMID: 9258669.

22. Jia S, Yamada T, Grewal SI. Heterochromatin regulates cell type-specific long-range chromatin interactions essential for directed recombination. Cell. 2004; 119(4):469–80. https://doi.org/10.1016/j.cell.2004.10.020 PMID: 15373537.

23. Jakociunas T, Holm LR, Verhein-Hansen J, Trusina A, Thon G. Two portable recombination enhancers direct donor choice in fission yeast heterochromatin. PLoS Genet. 2013; 9(10):e1003762. https://doi.org/10.1371/journal.pgen.1003762 PMID: 24204285.

24. Thon G, Hansen KR, Altes SP, Sidhu D, Singh G, Verhein-Hansen J, et al. The Ctr7 and Ctr8 directionality factors and the Pcu4 cullin mediate heterochromatin formation in the fission yeast Schizosaccharomyces pombe. Genetics. 2005; 171(4):1583–95. https://doi.org/10.1534/genetics.105.048298 PMID: 16176522.

25. Thon G, Bjerling P, Bunner CM, Verhein-Hansen J. Expression-state boundaries in the mating-type region of fission yeast. Genetics. 2002; 161(2):611–22. PMID: 12072458.

26. Matsuda E, Sugioka-Sugiyama R, Mizuguchi T, Mehta S, Cui B, Grewal SI. A homolog of male sex-determining factor SRY cooperates with a transposon-derived CENP-B protein to control sex-specific directed recombination. Proc Natl Acad Sci U S A. 2011; 108(46):18754–9. https://doi.org/10.1073/pnas.1109988108 PMID: 22042869.

27. Akamatsu Y, Dziaikowicz D, Ikeguchi M, Shinagawa H, Iwasaki H. Two different Swi5-containing protein complexes are involved in mating-type switching and recombination repair in fission yeast. Proc Natl Acad Sci U S A. 2003; 100(26):15770–5. https://doi.org/10.1073/pnas.2632890100 PMID: 14663140.

28. Akamatsu Y, Tsutsui Y, Morishita T, Siddique MS, Kurokawa Y, Ikeguchi M, et al. Fission yeast Swi5/Str1 and Rhp55/Rhp57 differentially regulate Rhp51-dependent recombination outcomes. EMBO J. 2007; 26(5):1352–62. https://doi.org/10.1038/sj.emboj.7601582 PMID: 17304215.

29. Haruta N, Kurokawa Y, Murayama Y, Akamatsu Y, Unzai S, Tsutsui Y, et al. The Swi5-Str1 complex stimulates Rhp51/Rad51- and Dmc1-mediated DNA strand exchange in vitro. Nat Struct Mol Biol. 2006; 13(9):823–30. https://doi.org/10.1038/nsmb1136 PMID: 16921379.

30. Kurokawa Y, Murayama Y, Haruta-Takahashi N, Urabe I, Iwasaki H. Reconstitution of DNA strand exchange mediated by Rhp51 recombinase and two mediators. PLoS Biol. 2006; 4(4):e88. https://doi.org/10.1371/journal.pbio.0060088 PMID: 18416603.

31. Yu C, Bonaduce MJ, Klar AJ. Going in the right direction: mating-type switching of Schizosaccharomyces pombe is controlled by judicious expression of two different swi2 transcripts. Genetics. 2012; 193(3):977–87. https://doi.org/10.1534/genetics.111.137109 PMID: 22209903.

32. Rudolph C, Kunz C, Parisi S, Lehmann E, Hartsuiker E, Farthmann B, et al. The msh2 gene of Schizosaccharomyces pombe is involved in mismatch repair, mating-type switching, and meiotic chromosome organization. Molecular and Cellular Biology. 1999; 19(1):241–50. PMID: 9858548.

33. Tsutsui Y, Khasanov FK, Shinagawa H, Iwasaki H, Bashkirov VI. Multiple interactions among the components of the recombinational DNA repair system in Schizosaccharomyces pombe. Genetics. 2001; 159(1):91–105. PMID: 11560889.

34. Fleck O, Rudolph C, Albrecht A, Lorentz A, Schar P, Schmidt H. The mutator gene swi8 effects specific mutations in the mating-type region of Schizosaccharomyces pombe. Genetics. 1994; 136(3):621–32. PMID: 7851760.

35. Vagin DA, Khasanov FK, Bashkirov VI. [The role of recombinational repair proteins in mating type switching in fission yeast cells]. Genetika. 2006; 42(4):487–93. PMID: 16756067.
36. Roseaulin L, Yamada Y, Tsutsui Y, Russell P, Iwasaki H, Arcangioli B. Mus81 is essential for sister chromatid recombination at broken replication forks. Embo Journal. 2008; 27(9):1378–87. https://doi.org/10.1038/emboj.2008.65 PMID: 1838861

37. Schmidt H, Kapizafecke P, Stephen ER, Gutz H. Some of the Swi Genes of Schizosaccharomyces-Pombe Also Have a Function in the Repair of Radiation-Damage. Current Genetics. 1989; 16(2):89–94. https://doi.org/10.1007/BF00393400 PMID: 2598273

38. Ostermann K, Lorentz A, Schmidt H. The Fission Yeast Rad22 Gene, Having a Function in Mating-Type Switching and Repair of DNA Damages, Encodes a Protein Homolog to Rad52 of Saccharomyces-Cerevisiae. Nucleic Acids Research. 1993; 21(25):5940–4. https://doi.org/10.1093/nar/21.25.5940 PMID: 8290356

39. Zhang JM, Liu XM, Ding YH, Xiong LY, Ren JY, Zhou ZX, et al. Fission yeast Pxd1 promotes proper DNA repair by activating Rad16XP and inhibiting Dna2. PLoS Biol. 2014; 12(9):e1001946. https://doi.org/10.1371/journal.pbio.1001946 PMID: 25203555

40. Egel R, Beach DH, Klar AJ. Genes required for initiation and resolution steps of mating-type switching in fission yeast. Proc Natl Acad Sci U S A. 1984; 81(11):3481–5. PMID: 6587363.

41. Arcangioli B, Klar AJ. A novel switch-activating site (SAS1) and its cognate binding factor (SAP1) required for efficient mat1 switching in Schizosaccharomyces pombe. EMBO J. 1991; 10(10):3025–32. PMID: 1915277.

42. Thon G, Klar AJ. The clr1 locus regulates the expression of the cryptic mating-type loci of fission yeast. Genetics. 1992; 131(2):287–96. PMID: 1644273.

43. Ekwall K, Ruusala T. Mutations in rik1, clr2, clr3 and clr4 genes asymmetrically derepress the silent mating-type loci in fission yeast. Mol Cell Biol. 1998; 18(9):5511–22. PMID: 9710635.

44. Lawrence RJ, Volpe TA. Msc1 links dynamic Swi6/HP1 binding to cell fate determination. Proc Natl Acad Sci U S A. 2009; 106(4):1163–8. https://doi.org/10.1073/pnas.0811161106 PMID: 19164572.

45. Dubey RN, Nakwal N, Bisht KK, Saini A, Haldar S, Singh J. Interaction of APC/C-E3 ligase with Swi6/HP1 and Clr4/Suv39 in heterochromatin assembly in fission yeast. Mol Biol. 2009; 34(12):2975–85. https://doi.org/10.1016/j.molbi.2009.09.016 PMID: 19975911.

46. Zhang K, Mosch K, Fischle W, Grewal SI. Roles of the Clr4 methyltransferase complex in nuclear spreading and maintenance of heterochromatin. Nat Struct Mol Biol. 2005; 12(4):381–8. https://doi.org/10.1038/nsmb.1406 PMID: 18345014.
57. Buscaino A, Lejeune E, Audergon P, Hamilton G, Pidoux A, Allshire RC. Distinct roles for Sir2 and RNAi in centromeric heterochromatin nucleation, spreading and maintenance. EMBO J. 2013; 32(9):1250–64. https://doi.org/10.1038/embj.2013.72 PMID: 23572080.

58. Freeman-Cook LL, Gomez EB, Spedale EJ, Marlett J, Forsburg SL, Pillus L, et al. Conserved locus-specific silencing functions of Schizosaccharomyces pombe sir2+. Genetics. 2005; 169(3):1243–60. https://doi.org/10.1534/genetics.103.032714 PMID: 15545655.

59. Xhemalce B, Kouzarides T. A chromodomain switch mediated by histone H3 Lys 4 acetylation regulates heterochromatin assembly. Genes Dev. 2010; 24(7):647–52. https://doi.org/10.1101/gad.188170 PMID: 20299449.

60. Noma K, Grewal SIS. Histone H3 lysine 4 methylation is mediated by Set1 and promotes maintenance of active chromatin states in fission yeast. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:16438–45. https://doi.org/10.1073/pnas.182436399 PMID: 12193658.

61. Roguev A, Schaft D, Shevchenko A, Aslaiand R, Shevchenko A, Stewart AF. High conservation of the Set1/Rad6 axis of histone 3 lysine 4 methylation in budding and fission yeasts. J Biol Chem. 2003; 278(10):8487–93. https://doi.org/10.1074/jbc.M209562200 PMID: 12488447.

62. Sugiyama T, Cam HP, Sugiyama R, Noma K, Zofall M, Kobayashi R, et al. SHREC, an effector complex for heterochromatin transcriptional silencing. Cell. 2007; 128(3):491–504. https://doi.org/10.1016/j.cell.2006.12.035 PMID: 17289569.

63. Yamada T, Fischle W, Sugiyama T, Allis CD, Grewal SI. The nucleation and maintenance of heterochromatin by a histone deacetylase in fission yeast. Mol Cell. 2005; 20(2):173–85. https://doi.org/10.1016/j.molcel.2005.10.002 PMID: 16246721.

64. Cam HP, Noma K, Ebina H, Levin HL, Grewal SI. Host genome surveillance for retrotransposons by transposon-derived proteins. Nature. 2008; 451(7177):431–6. https://doi.org/10.1038/nature06499 PMID: 18094683.

65. Hall IM, Shankaranarayana GD, Noma KI, Ayoub N, Cohen A, Grewal SIS. Establishment and maintenance of a heterochromatin domain. Science. 2002; 297(5590):2232–3. https://doi.org/10.1126/science.1076466 PMID: 12193653.

66. Kim HS, Choi ES, Shin JA, Jang YK, Park SD. Regulation of Swi6/HP1-dependent heterochromatin assembly by cooperation of components of the mitogen-activated protein kinase pathway and a histone deacetylase Ctr6. J Biol Chem. 2004; 279(41):42850–9. https://doi.org/10.1074/jbc.M407259200 PMID: 15292231.

67. Hansaen KR, Hazan I, Shanker S, Watt S, Verhein-Hansen J, Bahler J, et al. H3K9me-independent gene silencing in fission yeast heterochromatin by Ctr5 and histone deacetylases. PLoS Genet. 2011; 7(1):e1001268. https://doi.org/10.1371/journal.pgen.1001268 PMID: 21255711.

68. Shimada A, Dohke K, Sadaie M, Shinmyozu K, Nakayama J, Urano T, et al. Phosphorylation of Swi6/HP1 regulates transcriptional gene silencing at heterochromatin. Genes Dev. 2009; 23(1):18–23. https://doi.org/10.1101/gad.1708009 PMID: 19136623.

69. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res. 2015; 43(Database issue):D447–52. https://doi.org/10.1093/nar/gku1003 PMID: 25352553.

70. Jahn LJ, Mason B, Brogger P, Toteva T, Nielsen DK, Thon G. Dependency of Heterochromatin Domains on Replication Factors. G3 (Bethesda). 2017. https://doi.org/10.1534/g3.117.300341 PMID: 28187422.

71. Mamnun YM, Katayama S, Toda T. Fission yeast Mcf1 interacts with SCFPof3 and is required for centromere formation. Biochemical and Biophysical Research Communications. 2006; 350(1):125–30. https://doi.org/10.1016/j.bbrc.2006.09.024 PMID: 16997270.

72. Katayama S, Kitamura K, Lehmann A, Nikaido O, Toda T. Fission yeast F-box protein Pof3 is required for genome integrity and telomere function. Mol Biol Cell. 2002; 13(1):211–24. https://doi.org/10.1091/mbc.01-07-0333 PMID: 11809834.

73. Otero G, Fellows J, Li Y, de Bizemont T, Dirac AMG, Gustafsson CM, et al. Elongator, a multisubunit component of a novel RNA polymerase II holoenzyme for transcriptional elongation. Molecular Cell. 1999; 3(1):109–18. https://doi.org/10.1016/S1097-2765(00)80179-3 PMID: 10024864.

74. Krogan NJ, Greenblatt JF. Characterization of a six-subunit holo-elongator complex required for the regulated expression of a group of genes in Saccharomyces cerevisiae. Mol Cell Biol. 2001; 21(23):8203–12. https://doi.org/10.1128/MCB.21.23.8203-8212.2001 PMID: 11689709.

75. Wittschieben BO, Otero G, de Bizemont T, Fellows J, Erdjument-Bromage H, Obha R, et al. A novel histone acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. Molecular Cell. 1999; 4(1):123–3. https://doi.org/10.1016/S1097-2765(00)80194-X PMID: 10445034.
76. Esberg A, Huang B, Johansson MJ, Bystrom AS. Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis. Mol Cell. 2006; 24(1):139–48. https://doi.org/10.1016/j.molcel.2006.07.031 PMID: 17018299.

77. Glatt S, Seraphin B, Muller CW. Elongator: transcriptional or translational regulator? Transcription. 2012; 3(6):273–6. https://doi.org/10.4161/trns.21525 PMID: 2288844.

78. Mikhailova IV, Grady PJ, Tamburini FB, Lorenz DR, Cam HP. Multifaceted genome control by Set1 Dependent and Independent of H3K4 methylation and the Set1C/COMPASS complex. PLoS Genet. 2014; 10(10):e1004740. https://doi.org/10.1371/journal.pgen.1004740 PMID: 25356590.

79. Morohashi H, Maculins T, Labib K. The Amino-Terminal TPR Domain of Dia2 Tethers SCFDia2 to the Replisome Progression Complex. Current Biology. 2009; 19(22):1943–9. https://doi.org/10.1016/j.cub.2009.09.062 PMID: 19913425.

80. Roseauin LC, Noguchi C, Martinez E, Ziegler MA, Toda T, Noguchi E. Coordinate degradation of Replisome Components Ensures Genome Stability upon Replication Stress in the Absence of the Replication Fork Protection Complex. Plos Genetics. 2013; 9(1). https://doi.org/10.1371/journal.pgen.1003232 PMID: 23349636.

81. Takayama Y, Mamnun YM, Trickey M, Dhut S, Masuda F, Yamano H, et al. Hsk1-and SCFPof3-Dependent Proteolysis of S. pombe Ams2 Ensures Histone Homeostasis and Centromere Function. Developmental Cell. 2010; 18(3):385–96. https://doi.org/10.1016/j.devcel.2009.12.024 PMID: 20230746.

82. Kim KD, Tanizawa H, Iwasaki O, Noma K. Transcription factors mediate condensin recruitment and global chromosomal organization in fission yeast. Nat Genet. 2016; 48(10):1242–52. https://doi.org/10.1038/ng.3647 PMID: 27548313.

83. Chen ES, Zhang K, Nicolas E, Cam HP, Zofall M, Grewal SI. Cell cycle control of centromeric repeat transcription and heterochromatin assembly. Nature. 2008; 451(7179):734–7. https://doi.org/10.1038/nature06561 PMID: 18216783.

84. Kloc A, Zaratiegui M, Nora E, Martienssen R. RNA interference guides histone modification during the S phase of chromosomal replication. Curr Biol. 2008; 18(7):490–5. https://doi.org/10.1016/j.cub.2008.03.016 PMID: 18394897.

85. Bar-Ziv R, Voichek Y, Barkai N. Chromatin dynamics during DNA replication. Genome Res. 2016; 26 (9):1245–56. https://doi.org/10.1101/gr.201244.115 PMID: 27225843.

86. Abhishe N, Rajan RE, Verreault A, Thibault P. Unraveling Site-Specific and Combinatorial Histone Modifications Using High-Resolution Mass Spectrometry in Histone Deacetylase Mutants of Fission Yeast. J Proteome Res. 2016; 15(7):2132–42. https://doi.org/10.1021/acs.jproteome.5b01156 PMID: 27223649.

87. Sollier J, Lin WK, Soustelle C, Suhrke K, Nicolas A, Gelli V, et al. Set1 is required for meiotic S-phase onset, double-strand break formation and middle gene expression. Embo Journal. 2004; 23(9):1957–67. https://doi.org/10.1038/sj.emboj.7600204 PMID: 15071505.

88. Sommermeyer V, Beneut C, Chaplais E, Serrentino ME, Borde V. Spp1, a member of the Set1 Complex, promotes meiotic DSB formation in promoters by tethering histone H3K4 methylation sites to chromosome axes. Mol Cell. 2013; 49(1):43–54. https://doi.org/10.1016/j.molcel.2012.11.008 PMID: 23246437.

89. Tischfield SE, Keeney S. Scale matters: The spatial correlation of yeast meiotic DNA breaks with histone H3 trimethylation is driven largely by independent colocalization at promoters. Cell Cycle. 2012; 11 (8):1496–503. https://doi.org/10.4161/cc.19733 PMID: 22433953.

90. Tischfield SE, Keeney S. Scale matters: The spatial correlation of yeast meiotic DNA breaks with histone H3 trimethylation is driven largely by independent colocalization at promoters. Cell Cycle. 2012; 11 (8):1496–503. https://doi.org/10.4161/cc.19733 PMID: 22433953.

91. Zofall M, Grewal SIS. HULC, a histone H2B ubiquitinating complex, modulates heterochromatin independent of histone methylation in fission yeast. Journal of Biological Chemistry. 2007; 282(19):14065–72. https://doi.org/10.1074/jbc.M700292200 PMID: 17363370.

92. Tanny JC, Erdjument-Bromage H, Tempst P, Allis CD. Ubiquitylation of histone H2B controls RNA polymerase II transcription elongation independently of histone H3 methylation. Genes Dev. 2007; 21 (7):835–47. https://doi.org/10.1101/gad.1516207 PMID: 17374714.

93. Racine A, Page V, Nagy S, Grabowski D, Tanny JC. Histone H2B ubiquitylation promotes activity of the intact Set1 histone methyltransferase complex in fission yeast. J Biol Chem. 2012; 287(23):19040–7. https://doi.org/10.1074/jbc.M112.356253 PMID: 22507222.

94. Pebernard S, Wohlschlegel J, McDonald WH, Yates JR 3rd, Boddy MN. The Nse5-Nae6 dimer mediates DNA repair roles of the Smc5-Smc6 complex. Mol Cell Biol. 2006; 26(5):1617–30. https://doi.org/10.1128/MCB.26.5.1617-1630.2006 PMID: 16478864.
95. Prudden J, Pebernard S, Raffa G, Slavin DA, Perry JJP, Tainer JA, et al. SUMO-targeted ubiquitin ligases in genome stability. Embo Journal. 2007; 26(18):4089–101. https://doi.org/10.1038/sj.emboj.7601838 PMID: 17762865

96. Ekwall K, Thon G. Setting up Schizosaccharomyces pombe Crosses/Matings. Cold Spring Harb Protoc. 2017; 2017(7):pdb prot091694. https://doi.org/10.1101/pdb.prot091694 PMID: 28679701.

97. Roguev A, Xu J, Krogan N. DNA Preparation from Schizosaccharomyces pombe. Cold Spring Harb Protoc. 2017. https://doi.org/10.1101/pdb.prot091959 PMID: 28733411.

98. Shigemori Y, Mikawa T, Shibata T, Oishi M. Multiplex PCR: use of heat-stable Thermus thermophilus RecA protein to minimize non-specific PCR products. Nucleic Acids Res. 2005; 33(14):e126. https://doi.org/10.1093/nar/gni111 PMID: 16087733.