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Authors	Hideo Tsubouchi, Bilge Argunhan, Hiroshi Iwasaki
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Biochemical properties of fission yeast homologous recombination enzymes

(**short title:** Biochemistry of fission yeast recombination)

Hideo Tsubouchi[1,2]*, Bilge Argunhan[1,3], Hiroshi Iwasaki[1,2]*

Affiliations

[1]Institute of Innovative Research, Tokyo Institute of Technology, Tokyo, Japan

[2]School of Life Science and Technology, Tokyo Institute of Technology, Tokyo, Japan

[3]Present address: Section of Structural Biology, Faculty of Medicine, Imperial College London, London, UK

*Corresponding authors: htsubouchi@bio.titech.ac.jp, hiwasaki@bio.titech.ac.jp

Summary

Homologous recombination (HR) is a universal phenomenon conserved from viruses to humans. The mechanisms of HR are essentially the same in humans and simple unicellular eukaryotes like yeast. Two highly diverged yeast species, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, have proven exceptionally useful in understanding the fundamental mechanisms of eukaryotic HR by serving as a source for unique biological insights and also complementing each other. Here, we will review the features of *S. pombe* HR mechanisms in comparison to *S. cerevisiae* and other model organisms. Particular emphasis will be put on the biochemical characterization of HR mechanisms uncovered using *S. pombe* proteins.

Key words:

Rad51, Dmc1, fission yeast, homologous recombination, DNA double-strand break

Introduction

One of the biggest threats to the genome are DNA double-strand breaks (DSBs), which strongly induce genome rearrangements. Homologous recombination (HR) is central to maintaining genome integrity because it provides the only means to accurately repair DSBs, which is in contrast to non-homologous end joining, an alternative DSB repair pathway known to be more error prone.

Generation of single-stranded DNA (ssDNA) at DSB ends promotes usage of HR for DSB repair [1]. This involves unwinding of duplex DNA and strand-specific degradation where 5'-ended strands are selectively resected. Exposed 3'-ended ssDNA serves as a loading site for homologous recombinases (homologues of bacterial RecA). Homologous recombinases cooperatively bind ssDNA and form a helical nucleoprotein filament [2]. This filament conducts homology search and invades duplex DNA once homology is identified, forming a displacement loop (D-loop). Repair synthesis begins from the 3'-end of the invading strand while the opposite end, the leading edge of strand exchange known as a branch, is subjected to regulation that determines D-loop stability [3]. If the branch moves toward the 3' end of the invading strand, the invading strand eventually dissociates from the target duplex, and having been extended, the released ssDNA can anneal to the other end of the DSB, sealing the break. Alternatively, the D-loop can be further stabilized if the other end of the DSB also anneals to its complementary strand of the target duplex (i.e., the displaced strand). This leads to a stable recombination intermediate called a double Holliday junction (HJ), which is the predominant species observed in the budding yeast *Saccharomyces cerevisiae*. Single HJs are more common in the fission yeast *Schizosaccharomyces pombe* [4]. HJs can be resolved by structure-specific endonucleases called resolvases (HJ resolution). Double HJs can also be disentangled by dissociating and decatenating the heteroduplexes (HJ dissolution) [3].

Two highly diverged yeast models, *S. cerevisiae* and *S. pombe*, have proven exceptionally useful in understanding the fundamental mechanism of eukaryotic HR Although they share many common characteristics, these two organisms also provide unique insights into the mechanisms of HR. Here, we review the features of

S. pombe HR in comparison to *S. cerevisiae* and other model organisms, with particular emphasis on the biochemical properties of HR enzymes.

Prelude to HR: exposing ssDNA at a DSB end

Like many eukaryotic species, *S. pombe* has a set of proteins involved in end resection of DSBs [5]. The most prominent factors among them are the Mre11-Rad50-Nbs1 complex (MRN) and its activator Ctp1 (CtIP in humans). In *S. cerevisiae*, the Ctp1 ortholog Sae2 is practically dispensable for general resection of DSB ends to produce 3'-ended ssDNA [6], while Ctp1 and CtIP are considered essential [5]. MRN-Ctp1 is primarily responsible for the initial short-range resection, which is succeeded by two long-range exonucleases, Exo1 and Dna2-Rqh1 [5]. Interestingly, Rad52, a major auxiliary factor of Rad51 (see below), restricts ssDNA formation by inhibiting the Dna2-Rqh1 pathway [7]. All Ctp1 orthologs (including Sae2) are indispensable for promoting the MRN-dependent processing of DSB ends that are covalently attached to topoisomerase-family proteins such as Top1, Top2, and the meiotic topoisomerase-like protein Spo11, as well as ends that are occluded by KU and RPA complexes [5].

Ctp1 is by and large a disordered protein with its conserved, α-helical N-terminus forming a homotetramer [8]. Multivalent interaction of Ctp1 with DNA allows tethering of two DNA molecules, or DNA bridging, which may contribute to efficient DSB repair. Recently, two mechanisms critical for stimulating the endonuclease activity of Mre11 by Ctp1 have been identified [9,10]. The first mechanism involves DNA damage-induced phosphorylation of Ctp1 and its binding to the Nbs1-FHA domain, triggering the association between MR and Ctp1. The second involves the very end of the C-terminus of Ctp1; a peptide consisting of only 15 amino acids of the C-terminus is essential and sufficient for stimulating the Mre11 endonuclease, and this mechanism is conserved in humans [10].

HR during the mitotic cell cycle: Rad51 and its many auxiliary factors

Overview

Consistent with observations in *S. cerevisiae*, *S. pombe* strains lacking Rad51 display severe sensitivity to DNA damaging agents but are nevertheless viable [11], which is in contrast to the embryonic lethality observed in mammals [12]. Furthermore, *Sp*Rad51 forms nucleoprotein filaments akin to *Sc*Rad51 and *Hs*Rad51 [13].

Recombinational DNA repair in *S. pombe* is dependent on a variety of evolutionarily conserved auxiliary factors that function with Rad51 [14]. As is the case in *S. cerevisiae*, both Rad52 and the Swi2/Snf2-family DNA translocase Rad54 are essential for Rad51-dependent DNA repair in *S. pombe*, and Rad52 also has additional roles in Rad51-independent DNA repair [15,16]. Rad55-Rad57 are both Rad51 paralogues and Rad51-dependent DNA repair is reduced, but not abolished, in their absence [17,18]. A similar reduction in Rad51-dependent DNA repair is observed in the absence of Swi5-Sfr1, and based on the observation that the *rad57*Δ *sfr1*Δ double mutant phenocopies *rad51*Δ, it was proposed that Rad55-Rad57 and Swi5-Sfr1 function in parallel to promote Rad51-dependent DNA repair [19]. Other than the genetic analysis suggesting that the Shu complex functions in an early stage of HR [20], perhaps in collaboration with Rad55-Rad57 [21], little is known about its function.

There are two interesting differences in Rad51 regulation that sets *S. pombe* apart from *S. cerevisiae*. The first concerns the Swi5-Sfr1 complex. Although first identified in *S. pombe* [19], Swi5-Sfr1 orthologues have been shown to promote HR in a variety of organisms including *S. cerevisiae*, mice, and humans, firmly establishing Swi5-Sfr1 as a widely conserved Rad51 activator [22]. However, while *S. pombe* Swi5-Sfr1 potentiates both Rad51 and Dmc1, the meiosis-specific RecA orthologue [23], the *S. cerevisiae* orthologue Mei5-Sae3 is meiosis-specific and only stimulates Dmc1 (discussed below) [22]. By contrast, mammalian SWI5-SFR1 promotes RAD51-dependent DNA repair like its *S. pombe* orthologue [22]. The second difference of note is the regulation of *Sp*Rad51 by Fbh1, an F-box containing DNA helicase [24]. F-box proteins are the specificity-conferring E3 ubiquitin ligases of the Skp1–Cullin–F-box protein (SCF) complex, which ubiquitylates proteins to target them for proteasomal destruction [16]. While Fbh1 is conserved in humans

[25] and has been shown to regulate *Hs*RAD51 through similar mechanisms [26,27], *S. cerevisiae* apparently lacks an Fbh1 homologue.

Biochemical characterization of SpRad51 and its regulation

Biochemical reconstitutions demonstrated that *Sp*Rad51 drives the pairing of ssDNA with dsDNA and subsequent strand exchange in a homology-dependent manner [13]. Bacterial RecA drives branch migration over an ssDNA-dsDNA junction in a process known as four-strand exchange, leading to the formation of HJ recombination intermediates [28]. *Sp*Rad51 was the first eukaryotic homologous recombinase shown to possess this activity, except the reaction proceeded with opposite polarity (3'-5' direction) to RecA; similar observations were also made with *Hs*RAD51 [29]. Furthermore, the analysis of *Sp*Rad51-driven strand exchange by real-time fluorescence resonance energy transfer (FRET)-based techniques provided strong evidence for the existence of two distinct recombination intermediates: a paranemic joint, in which the ssDNA is complexed with the homologous dsDNA but does not stably pair with the complementary strand of the duplex; and a plectonemic joint, in which the ssDNA is stably base paired and intertwined with the complementary strand of the duplex [30,31].

The most extensively characterised auxiliary factor in *S. pombe* is Swi5-Sfr1. Initial biochemical reconstitutions demonstrated that Swi5-Sfr1 stimulates Rad51-driven DNA strand exchange by stabilising Rad51-ssDNA filaments and potentiating the Rad51 ATPase [23,32]. Kinetic analysis of DNA strand exchange suggested that Swi5-Sfr1 stimulates maturation of the paranemic intermediate into the plectonemic intermediate, as well as the ATP hydrolysis-dependent release of the non-complementary strand that signifies completion of DNA strand exchange [30]. Mechanistically, circular dichroism spectroscopy experiments indicated that Swi5-Sfr1 elicits a change in configuration of the nucleotides in the Rad51 filament [33], and FRET analysis suggested that Swi5-Sfr1 induces the extended form of the filament that represents the active state [31]. Swi5-Sfr1 has a modular structure: the intrinsically disordered N-terminal half of Sfr1 (Sfr1N) is responsible for interacting with Rad51 via two distinct sites, while the C-terminal half of Sfr1 in complex with Swi5 (Swi5-Sfr1C), which forms a parallel coiled-coil heterodimer, functions as the

Rad51 activator [34,35]. It was proposed that Sfr1N plasters along the side of the Rad51-ssDNA filament, which allows the complementary geometry of Swi5-Sfr1C to insert into the grooves of the filament, leading to its stabilisation [36]. This stabilisation antagonises the disruption of Rad51 filaments by RPA [32], arguing for its physiological importance. This is bolstered by the fact that cells lacking Sfr1 show a reduction in DNA damage-induced Rad51 foci [37,38], which are cytological manifestations of nucleoprotein filaments at sites of ongoing DNA repair.

Much like its *S. cerevisiae* orthologue, *Sp*Rad52 possesses ssDNA annealing activity [39] and can promote Rad51 filamentation on RPA-coated ssDNA [32], indicating that it is a bonafide recombination mediator. Although the intrinsic mediator activity of *Sp*Rad52 is relatively weak in comparison to *Sc*Rad52, this basal activity is substantially enhanced by the presence of Swi5-Sfr1, pointing towards synergism between the two auxiliary factors [32].

Rad51 paralogues are notorious for their biochemical intractability and there are currently no studies detailing the biochemical characterisation of *Sp*Rad55-Rad57. However, we recently reported that a Rad55-Rad57–dependent mechanism suppresses the DNA damage sensitivity associated with defects in the physical interaction of Swi5-Sfr1 with Rad51. Indeed, partially purified Rad55-Rad57 interacted with purified Swi5-Sfr1, suggesting that, while capable of functioning independently [19], the two auxiliary factors collaborate to promote Rad51-dependent DNA repair [35]. As for *Sp*Rad54, it was recently shown to interact with Rad51 and stimulate its strand exchange activity [38], mirroring results obtained with orthologous systems [14]. Notably, an evolutionarily conserved protruding acidic patch (PAP) of Rad51 was shown to be essential for the interaction with Rad52 and important for the interactions with both Rad55-Rad57 and Rad54, indicating that multiple auxiliary factors utilise the same motif to interact with Rad51 in *S. pombe* [38]. Mutation of the PAP in *Sc*Rad51 also sensitises cells to DNA damage (B.A., unpublished data), arguing that this motif has an evolutionarily conserved role in promoting HR.

Unlike the auxiliary factors described above, Fbh1 functions as an anti-recombinase [40–42], negatively regulating Rad51 by disrupting the Rad51 nucleoprotein filament. Biochemical reconstitutions revealed that Fbh1 disassembles

Rad51 filaments and functions as a E3 ubiquitin ligase to facilitate ubiquitylation of Rad51; importantly, Fbh1-dependent filament destabilization is antagonized by Swi5-Sfr1 [43].

HR during meiosis: Rad51 and Dmc1

Overview

Dmc1 is the meiosis-specific RecA orthologue found in many (but not all) eukaryotes, including *S. pombe* [44]. However, unlike in *S. cerevisiae* where the absence of Dmc1 causes severe cell cycle arrest/delay at meiotic prophase, the meiotic cell cycle is only slightly delayed and spores are viable in the *S. pombe* *dmc1*Δ mutant [44–46]. The cell cycle arrest/delay phenotype of the *S. cerevisiae* *dmc1*Δ mutant is thought to be due to mechanisms that suppresses Rad51 during meiosis [46]. Thus, it is likely that *S. pombe* does not possess such mechanisms. Meiotic cell cycle arrest is not caused by the *dmc1* mutation in *Arabidopsis thaliana* while the absence of Dmc1 leads to meiotic prophase arrest and subsequent apoptosis in mice [47–49]. In contrast to *dmc1*Δ, the absence of Rad51 leads to a drastic reduction in spore formation viability, suggesting a predominant role for Rad51 in *S. pombe* meiosis [50]. The absence of both Rad51 and Dmc1 confers a further reduction in spore formation and viability, pointing towards some functional overlap between them.

Swi5-Sfr1 in *S. pombe* is the counterpart of *S. cerevisiae* Mei5-Sae3 (Sfr1 and Swi5 correspond to Mei5 and Sae3, respectively). While Mei5-Sae3 is meiosis-specific and functions exclusively with Dmc1, Swi5-Sfr1 also functions with Rad51 to promote HR in vegetative cells (discussed above). In *S. cerevisiae*, the localization of Dmc1, Mei5, and Sae3 to meiotic chromosomes as foci is mutually dependent [51,52], suggesting that Mei5-Sae3 promotes Dmc1 filament formation/stability. Based on biochemical reconstitutions (discussed below), similar results are expected in *S. pombe*.

The Hop2-Mnd1 complex (Meu13-Mcp7 in *S. pombe* but referred to as Hop2-Mnd1 hereafter) functions as another auxiliary factor [53,54]. The absence of this complex compromises Dmc1 functionality in both yeasts, leading to a reduction in homologous chromosome pairing [53,55]. In *S. cerevisiae*, *hop2*Δ/*mnd1*Δ mutants

show meiotic cell cycle arrest with a robust accumulation of unrepaired DSBs, reminiscent of *dmc1*Δ [55]. Importantly, the absence of these proteins leads to the chromosomal accumulation of Dmc1 in *S. cerevisiae*, suggesting a role downstream of Dmc1 nucleoprotein filament formation. Although Hop2-Mnd1 is meiosis-specific in both yeasts , HOP2-MND1 is produced in vegetative cells in mice, where it functions as an activator of RAD51 as well [56]. Mouse HOP2-MND1 also plays a role in telomere maintenance [57].

Unlike in *S. cerevisiae*, Rdh54, a homolog of Rad54, is meiosis-specific in *S. pombe,* although deleting *rdh54*$^+$ does not confer a major meiotic defect [58]. Deleting both *rad54*$^+$ and *rdh54*$^+$, however, causes a severe meiotic HR defect, suggesting some functional redundancy during meiosis. Yeast two hybrid experiments suggested preferential binding of Rad54 to Rad51 and Rdh54 to Dmc1 [58]. The much milder meiotic defect of *rad54*Δ compared to the *rad51*Δ mutant, as seen in *S. cerevisiae* [59,60], might imply that either Rdh54 can also function with Rad51 or Dmc1-Rdh54 can substitute for Rad51.

Biochemical characterization of meiotic HR

Dmc1 exhibits ATP-dependent ssDNA binding and forms nucleoprotein filament structures similar to Rad51 [13,23]. Dmc1 also drives ATP-dependent four-strand exchange, but the polarity (5'-3' direction) is opposite to Rad51 and the same as RecA [29]. Analogous to Rad51, Dmc1-driven strand exchange is greatly stimulated by the addition of its auxiliary factors, which include Swi5-Sfr1, Hop2-Mnd1, and Rdh54.

Swi5-Sfr1 stimulates Dmc1-driven DNA strand exchange by promoting Dmc1 nucleoprotein filament formation/stability and stimulating the Dmc1 ATPase [23,61]. Swi5-Sfr1 serves as a much better mediator to Dmc1 than to Rad51, efficiently facilitating the loading of Dmc1 onto RPA-coated ssDNA. Mei5-Sae3 also functions as a mediator for Dmc1 [62], suggesting that this function of Swi5-Sfr1 is evolutionarily conserved. In stark contrast to Rad51-driven DNA strand exchange, Rad52 appears to inhibit the activity of Dmc1 [61].

Hop2-Mnd1 is another major auxiliary factor of Dmc1. Hop2-Mnd1 facilitates the association of the Dmc1 nucleoprotein filament with dsDNA in a homology-

independent manner [63]. Hop2-Mnd1 greatly stimulates strand exchange by Dmc1 via a mechanism that is distinct from Swi5-Sfr1; Hop2-Mnd1 facilitates the initiation step of strand exchange while Swi5-Sfr1 stimulates subsequent strand transfer [63]. The stimulation of strand transfer by Swi5-Sfr1 might be related to stabilization of the Dmc1 nucleoprotein filament, consistent with its mediator function. Unlike Swi5-Sfr1, Hop2-Mnd1 does not display mediator activity, does not stabilize the Dmc1 nucleoprotein filament, and does not protect the Dmc1 filament from displacement by RPA. Consistent with these complementary attributes, simultaneous incubation of these two auxiliary factors with Dmc1 synergistically stimulates Dmc1-driven strand exchange. Finally, *Sp*Rdh54 has been shown to stimulate D-loop formation by Dmc1 in a ATP-dependent manner, which mirrors results obtained with *Sc*Rdh54 [2,64]. *Sp*Rdh54 also removes Dmc1 from dsDNA [64].

Maturation of recombinants: resolving intermediates

Branch migration is a critical determinant for formation of Holliday junctions (HJs). *S. pombe* has Fml1 and Fml2, orthologues of human FANCM, a tumour-suppressor gene product [65]. Fml1 is an ATP-dependent 3'-to-5' DNA helicase/translocase that preferentially destabilizes D-loop/HJ structures by dissociating the invading strand [66]. Thus, meiotic crossover formation is elevated in the absence of Fml1 [67].

In *S. cerevisiae*, double HJs, the major meiotic HR intermediates, can be separated into two dsDNA molecules through dissolution or resolution. In dissolution, Bloom-related helicases with the help of a type III topoisomerase dissolve double HJs without using endonucleolytic activity. There are three general eukaryotic HJ resolvases: Mus81-Eme1 (Mms4 in *S. cerevisiae*), Slx1-Slx4, and Gen1 [68]. *S. pombe* lacks Gen1 and *Sp*Slx1-Slx4 does not resolve HJs [69]. Furthermore, Mus81-Eme1 primarily resolves nicked HJs, which are supposedly major meiotic HR intermediates [4]. However, Mus81-Eme1 can also resolve intact HJs to the level comparable to the bacterial RuvC resolvase [70]. Thus, single intact HJs are likely to be primarily dealt with by Mus81-Eme1 in *S. pombe*.

Conclusions

Although both *S. cerevisiae* and *S. pombe* are very successful model organisms, each has its own unique features. In *S. pombe*, Ctp1 is indispensable for efficient DSB end resection and Rad51 is subjected to positive and negative regulation by Swi5-Sfr1 and Fbh1, respectively. These features are reminiscent of vertebrate HR. In the later step of HR, however, multiple endonucleases that act on HR intermediates, as seen in the human system, only exist in *S. cerevisiae*. Thus, the two yeasts complement each other with regards to HR research. Furthermore, *S. pombe* has the RNA interference and related heterochromatin formation mechanisms that *S. cerevisiae* lacks; it would be interesting to understand how such fundamental differences in DNA structure influence HR. As we are witnessing an ever-increasing number of DSB repair genes that only exist in higher eukaryotes, there is certainly a limitation to using these yeast models. Two prominent examples are BRCA1 and BRCA2, which are encoded by human tumor suppressor genes whose mutation dramatically increases the risk of breast and ovarian cancer [71]. Nonetheless, as seen in the recent advances reviewed here, *S. pombe* will remain a rich source of insight into the fundamental principles of HR mechanisms, which we are far from having understood completely.

Fig. 1 Homologous recombination steps and fission yeast recombination proteins

Homologous recombination steps represent the combined view of *S. cerevisiae* and *S. pombe* models. *S. pombe* proteins whose activity was validated using purified materials are shown in bold. *S. pombe* proteins whose involvement in a particular step is implicated based on genetic observations in *S. pombe* and/or biochemical reconstitutions with *S. cerevisiae* proteins are shown in regular font. *S. cerevisiae* protein names can be found in parentheses only when they are different from *S. pombe* names.

References

1. Symington LS, Gautier J: **Double-Strand Break End Resection and Repair Pathway Choice**. *Annu Rev Genet* 2011, **45**:247–271.
2. Greene EC: **DNA sequence alignment during homologous recombination**. *J Biol Chem* 2016, **291**:11572–11580.
3. Mehta A, Haber JE: **Sources of DNA Double-Strand Breaks and Models of Rec**. *Cold Spring Harb Perspect Biol* 2014, **6**:1–19.
4. Cromie GA, Hyppa RW, Taylor AF, Zakharyevich K, Hunter N, Smith GR: **Single Holliday junctions are intermediates of meiotic recombination.** *Cell* 2006, **127**:1167–78.
5. Reginato G, Cejka P: **The MRE11 complex: A versatile toolkit for the repair of broken DNA**. *DNA Repair (Amst)* 2020, **91–92**.
6. Shim EY, Chung WH, Nicolette ML, Zhang Y, Davis M, Zhu Z, Paull TT, Ira G, Lee SE: **Saccharomyces cerevisiae Mre11/Rad50/Xrs2 and Ku proteins regulate association of Exo1 and Dna2 with DNA breaks**. *EMBO J* 2010, **29**:3370–3380.
7. Yan Z, Xue C, Kumar S, Crickard JB, Yu Y, Wang W, Pham N, Li Y, Niu H, Sung P, et al.: **Rad52 Restrains Resection at DNA Double-Strand Break Ends in Yeast**. *Mol Cell* 2019, **76**:699-711.e6.
8. Andres SN, Appel CD, Westmoreland JW, Williams JS, Nguyen Y, Robertson PD, Resnick MA, Williams RS: **Tetrameric Ctp1 coordinates DNA binding and DNA bridging in DNA double-strand-break repair**. *Nat Struct Mol Biol* 2015, **22**:158–166.
9. Williams RS, Dodson GE, Limbo O, Yamada Y, Williams JS, Guenther G, Classen S, Glover JNM, Iwasaki H, Russell P, et al.: **Nbs1 Flexibly Tethers Ctp1 and Mre11-Rad50 to Coordinate DNA Double-Strand Break Processing and Repair**. *Cell* 2009, **139**:87–99.
10. Zdravković A, Daley JM, Dutta A, Niwa T, Murayama Y, Kanamaru S, Ito K, Maki T, Argunhan B, Takahashi M, et al.: **A conserved Ctp1/CtIP C-terminal peptide stimulates Mre11 endonuclease activity**. *Proc Natl Acad Sci* 2021, **118**:e2016287118.

11. Muris DFR, Vreeken K, Carr AM, Broghton BC, Lehmann AR, Lohman PHM, Pastink A: **Cloning the RAD51 homologue of Schizosaccharomyces pombe**. *Nucleic Acids Res* 1993, **21**:4586–4591.
12. Tsuzuki T, Fujii Y, Sakumi K, Tominaga Y, Nakao K, Sekiguchi M, Matsushiro A, Yoshimura Y, MoritaT: **Targeted disruption of the Rad51 gene leads to lethality in embryonic mice.** *Proc Natl Acad Sci* 1996, **93**:6236–6240.
13. Sauvageau S, Stasiak AZ, Banville I, Ploquin M, Stasiak A, Masson J-Y: **Fission Yeast Rad51 and Dmc1, Two Efficient DNA Recombinases Forming Helical Nucleoprotein Filaments**. *Mol Cell Biol* 2005, **25**:4377–4387.
14. Daley JM, Gaines WA, Kwon Y, Sung P: **Regulation of DNA Pairing in Homologous Recombination**. *Cold Spring Harb Perspect Biol* 2014, **6**:1–15.
15. Muris DFR, Vreeken K, Schmidt H, Ostermann K, Clever B, Lohman PHM, Pastink A: **Homologous recombination in the fission yeast Schizosaccharomyces pombe: different requirements for the rhp51 + , rhp54 + and rad22 + genes**. *Curr Genet* 1997, **31**:248–254.
16. Doe CL, Osman F, Dixon J, Whitby MC: **DNA repair by a Rad22-Mus81-dependent pathway that is independent of Rhp51.** *Nucleic Acids Res* 2004, **32**:5570–5581.
17. Khasanov FK, Savchenko G V, Bashkirova E V, Korolev VG, Heyer WD, Bashkirov VI: **A new recombinational DNA repair gene from Schizosaccharomyces pombe with homology to Escherichia coli RecA.** *Genetics* 1999, **152**:1557–72.
18. Tsutsui Y, Morishita T, Iwasaki H, Toh H, Shinagawa H: **A recombination repair gene of Schizosaccharomyces pombe, rhp57, is a functional homolog of the Saccharomyces cerevisiae RAD57 gene and is phylogenetically related to the human XRCC3 gene.** *Genetics* 2000, **154**:1451–61.
19. Akamatsu Y, Dziadkowiec 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* 2003, **100**:15770–15775.

20. Martín V, Chahwan C, Gao H, Blais V, Wohlschlegel J, Yates JR, McGowan CH, Russell P: **Sws1 is a conserved regulator of homologous recombination in eukaryotic cells**. *EMBO J* 2006, **25**:2564–2574.
21. Khasanov FK, Salakhova AF, Chepurnaja O V., Korolev VG, Bashkirov VI: **Identification and characterization of the rlp1+, the novel Rad51 paralog in the fission yeast Schizosaccharomyces pombe**. *DNA Repair (Amst)* 2004, **3**:1363–1374.
22. Argunhan B, Murayama Y, Iwasaki H: **The differentiated and conserved roles of Swi5-Sfr1 in homologous recombination**. *FEBS Lett* 2017, **591**:2035–2047.
23. Haruta N, Kurokawa Y, Murayama Y, Akamatsu Y, Unzai S, Tsutsui Y, Iwasaki H: **The Swi5-Sfr1 complex stimulates Rhp51/Rad51 - and Dmc1-mediated DNA strand exchange in vitro**. *Nat Struct Mol Biol* 2006, **13**:823–830.
24. Park JS, Choi E, Lee S-H, Lee C, Seo Y-S: **A DNA Helicase from Schizosaccharomyces pombeStimulated by Single-stranded DNA-binding Protein at Low ATP Concentration**. *J Biol Chem* 1997, **272**:18910–18919.
25. Kim J, Kim J-H, Lee S-H, Kim D-H, Kang H-Y, Bae S-H, Pan Z-Q, Seo Y-S: **The Novel Human DNA Helicase hFBH1 Is an F-box Protein**. *J Biol Chem* 2002, **277**:24530–24537.
26. Simandlova J, Zagelbaum J, Payne MJ, Chu WK, Shevelev I, Hanada K, Chatterjee S, Reid DA, Liu Y, Janscak P, et al.: **FBH1 Helicase Disrupts RAD51 Filaments in Vitro and Modulates Homologous Recombination in Mammalian Cells**. *J Biol Chem* 2013, **288**:34168–34180.
27. Chu WK, Payne MJ, Beli P, Hanada K, Choudhary C, Hickson ID: **FBH1 influences DNA replication fork stability and homologous recombination through ubiquitylation of RAD51**. *Nat Commun* 2015, **6**:5931.
28. Cox MM: **Motoring along with the bacterial RecA protein**. *Nat Rev Mol Cell Biol* 2007, **8**:127–138.
29. Murayama Y, Kurokawa Y, Mayanagi K, Iwasaki H: **Formation and branch migration of Holliday junctions mediated by eukaryotic recombinases**. *Nature* 2008, **451**:1018–1021.

30. Ito K, Murayama Y, Takahashi M, Iwasaki H: **Two three-strand intermediates are processed during Rad51-driven DNA strand exchange**. *Nat Struct Mol Biol* 2018, **25**:29–36.
31. Ito K, Murayama Y, Kurokawa Y, Kanamaru S, Kokabu Y, Maki T, Mikawa T, Argunhan B, Tsubouchi H, Ikeguchi M, et al.: **Real-time tracking reveals catalytic roles for the two DNA binding sites of Rad51**. *Nat Commun* 2020, **11**:1–7.
32. 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* 2008, **6**:836–848.
33. Fornander LH, Renodon-Cornière A, Kuwabara N, Ito K, Tsutsui Y, Shimizu T, Iwasaki H, Nordén B, Takahashi M: **Swi5-Sfr1 protein stimulates Rad51-mediated DNA strand exchange reaction through organization of DNA bases in the presynaptic filament**. *Nucleic Acids Res* 2014, **42**:2358–2365.
34. Kuwabara N, Murayama Y, Hashimoto H, Kokabu Y, Ikeguchi M, Sato M, Mayanagi K, Tsutsui Y, Iwasaki H, Shimizu T: **Mechanistic insights into the activation of Rad51-mediated strand exchange from the structure of a recombination activator, the Swi5-Sfr1 complex**. *Structure* 2012, **20**:440–449.
35. Argunhan B, Sakakura M, Afshar N, Kurihara M, Ito K, Maki T, Kanamaru S, Murayama Y, Tsubouchi H, Takahashi M, et al.: **Cooperative interactions facilitate stimulation of Rad51 by the Swi5-Sfr1 auxiliary factor complex**. *Elife* 2020, **9**:1–28.
36. Kokabu Y, Murayama Y, Kuwabara N, Oroguchi T, Hashimoto H, Tsutsui Y, Nozaki N, Akashi S, Unzai S, Shimizu T, et al.: **Fission yeast Swi5-Sfr1 protein complex, an activator of Rad51 recombinase, forms an extremely elongated dogleg-shaped structure**. *J Biol Chem* 2011, **286**:43569–43576.
37. Akamatsu Y, Tsutsui Y, Morishita T, Siddique MDSP, Kurokawa Y, Ikeguchi M, Yamao F, Arcangioli B, Iwasaki H: **Fission yeast Swi5/Sfr1 and Rhp55/Rhp57 differentially regulate Rhp51-dependent recombination outcomes**. *EMBO J* 2007, **26**:1352–1362.

38. Afshar N, Argunhan B, Palihati M, Taniguchi G, Tsubouchi H, Iwasaki H: **A novel motif of Rad51 serves as an interaction hub for recombination auxiliary factors**. *Elife* 2021, **10**.
39. de Vries FAT, Zonneveld JBM, de Groot AJ, Koning RI, van Zeeland AA, Pastink A: **Schizosaccharomyces pombe Rad22A and Rad22B have similar biochemical properties and form multimeric structures**. *Mutat Res Mol Mech Mutagen* 2007, **615**:143–152.
40. Morishita T, Furukawa F, Sakaguchi C, Toda T, Carr AM, Iwasaki H, Shinagawa H: **Role of the Schizosaccharomyces pombe F-Box DNA Helicase in Processing Recombination Intermediates**. *Mol Cell Biol* 2005, **25**:8074–8083.
41. Osman F, Dixon J, Barr AR, Whitby MC: **The F-Box DNA Helicase Fbh1 Prevents Rhp51-Dependent Recombination without Mediator Proteins**. *Mol Cell Biol* 2005, **25**:8084–8096.
42. Lorenz A, Osman F, Folkyte V, Sofueva S, Whitby MC: **Fbh1 Limits Rad51-Dependent Recombination at Blocked Replication Forks**. *Mol Cell Biol* 2009, **29**:4742–4756.
43. Tsutsui Y, Kurokawa Y, Ito K, Siddique MSP, Kawano Y, Yamao F, Iwasaki H: **Multiple Regulation of Rad51-Mediated Homologous Recombination by Fission Yeast Fbh1**. *PLoS Genet* 2014, **10**.
44. Fukushima K, Tanaka Y, Nabeshima K, Yoneki T, Tougan T, Tanaka S, Nojima H: **Dmc1 of Schizosaccharomyces pombe plays a role in meiotic recombination.** *Nucleic Acids Res* 2000, **28**:2709–16.
45. Shimada M, Nabeshima K, Tougan T, Nojima H: **The meiotic recombination checkpoint is regulated by checkpoint rad+genes in fission yeast**. *EMBO J* 2002, **21**:2807–2818.
46. Brown MS, Bishop DK: **DNA Strand Exchange and RecA Homologs**. *Cold Spring Harb Perspect Biol* 2014, doi:10.1101/cshperspect.a016659.
47. Couteau F, Belzile F, Horlow C, Grandjean O, Vezon D, Doutriaux MP: **Random chromosome segregation without meiotic arrest in both male and female meiocytes of a dmc1 mutant of Arabidopsis**. *Plant Cell* 1999, **11**:1623–1634.

48. Pittman DL, Cobb J, Schimenti KJ, Wilson LA, Cooper DM, Brignull E, Handel MA, Schimenti JC: **Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmc1, a germline-specific RecA homolog**. *Mol Cell* 1998, **1**:697–705.

49. Yoshida K, Kondoh G, Matsuda Y, Habu T, Nishimune Y, Morita T: **The mouse RecA-like gene Dmc1 is required for homologous chromosome synapsis during meiosis**. *Mol Cell* 1998, **1**:707–718.

50. Grishchuk AL, Kohli J: **Five RecA-like Proteins of Schizosaccharomyces pombe Are Involved in Meiotic Recombination**. *Genetics* 2003, **165**:1031–1043.

51. Tsubouchi H, Roeder GS: **The budding yeast Mei5 and Sae3 proteins act together with Dmc1 during meiotic recombination**. *Genetics* 2004, **168**:1219–1230.

52. Hayase A, Takagi M, Miyazaki T, Oshiumi H, Shinohara M, Shinohara A: **A protein complex containing Mei5 and Sae3 promotes the assembly of the meiosis-specific RecA homolog Dmc1**. *Cell* 2004, **119**:927–940.

53. Nabeshima K, Kakihara Y, Hiraoka Y, Nojima H: **A novel meiosis-specific protein of fission yeast, Meu13p, promotes homologous pairing independently of homologous recombination**. *EMBO J* 2001, **20**:3871–3881.

54. Saito TT, Tougan T, Kasama T, Okuzaki D, Nojima H: **Mcp7, a meiosis-specific coiled-coil protein of fission yeast, associates with Meu13 and is required for meiotic recombination**. *Nucleic Acids Res* 2004, **32**:3325–3339.

55. Tsubouchi H, Roeder GS: **The importance of genetic recombination for fidelity of chromosome pairing in meiosis**. *Dev Cell* 2003, **5**:915–925.

56. Chi P, San Filippo J, Sehorn MG, Petukhova G V., Sung P: **Bipartite stimulatory action of the Hop2-Mnd1 complex on the Rad51 recombinase**. *Genes Dev* 2007, **21**:1747–1757.

57. Cho NW, Dilley RL, Lampson MA, Greenberg RA: **Interchromosomal homology searches drive directional ALT telomere movement and synapsis**. *Cell* 2014, **159**:108–121.

58. Catlett MG, Forsburg SL: **Schizosaccharomyces pombe Rdh54 (TID1) Acts with Rhp54 (RAD54) to Repair Meiotic Double-Strand Breaks**. *Mol Biol Cell* 2003, **14**:4707–4720.
59. Game JC, Mortimer RK: **A genetic study of x-ray sensitive mutants in yeast.** *Mutat Res* 1974, **24**:281–92.
60. Shinohara M, Shita-Yamaguchi E, Buerstedde JM, Shinagawa H, Ogawa H, Shinohara A: **Characterization of the roles of the Saccharomyces cerevisiae RAD54 gene and a homologue of RAD54, RDH54/TID1, in mitosis and meiosis**. *Genetics* 1997, **147**:1545–1556.
61. Murayama Y, Kurokawa Y, Tsutsui Y, Iwasaki H: **Dual regulation of Dmc1-driven DNA strand exchange by Swi5- Sfr1 activation and Rad22 inhibition**. *Genes Dev* 2013, **27**:2299–2304.
62. Ferrari SR, Grubb J, Bishop DK: **The Mei5-Sae3 protein complex mediates Dmc1 activity in Saccharomyces cerevisiae**. *J Biol Chem* 2009, **284**:11766–11770.
63. Tsubouchi H, Argunhan B, Ito K, Takahashi M, Iwasaki H: **Two auxiliary factors promote Dmc1-driven DNA strand exchange via stepwise mechanisms**. *Proc Natl Acad Sci* 2020, **117**:12062–12070.
64. Chi P, Kwon Y, Moses DN, Seong C, Sehorn MG, Singh AK, Tsubouchi H, Greene EC, Klein HL, Sung P: **Functional interactions of meiotic recombination factors Rdh54 and Dmc1**. *DNA Repair (Amst)* 2009, **8**:279–284.
65. Whitby MC: **The FANCM family of DNA helicases/translocases**. *DNA Repair (Amst)* 2010, **9**:224–236.
66. Sun W, Nandi S, Osman F, Ahn JS, Jakovleska J, Lorenz A, Whitby MC: **The FANCM Ortholog Fml1 Promotes Recombination at Stalled Replication Forks and Limits Crossing Over during DNA Double-Strand Break Repair**. *Mol Cell* 2008, **32**:118–128.
67. Lorenz A, Osman F, Sun W, Nandi S, Steinacher R, Whitby MC: **The Fission Yeast FANCM Ortholog Directs Non-Crossover Recombination During Meiosis**. *Science (80-)* 2012, **336**:1585–1588.

68. Matos J, West SC: **Holliday junction resolution: Regulation in space and time**. *DNA Repair (Amst)* 2014, **19**:176–181.
69. Coulon S, Gaillard P-HL, Chahwan C, McDonald WH, Yates JR, Russell P: **Slx1-Slx4 Are Subunits of a Structure-specific Endonuclease That Maintains Ribosomal DNA in Fission Yeast**. *Mol Biol Cell* 2004, **15**:71–80.
70. Gaskell LJ, Osman F, Gilbert RJC, Whitby MC: **Mus81 cleavage of Holliday junctions: A failsafe for processing meiotic recombination intermediates?** *EMBO J* 2007, **26**:1891–1901.
71. Roy R, Chun J, Powell SN: **BRCA1 and BRCA2: different roles in a common pathway of genome protection.** *Nat Rev Cancer* 2011, **12**:68–78.

Ref #7 •

Yan Z, Xue C, Kumar S, Crickard JB, Yu Y, Wang W, Pham N, Li Y, Niu H, Sung P, et al.: **Rad52 Restrains Resection at DNA Double-Strand Break Ends in Yeast**. *Mol Cell* 2019, **76**:699-711.e6.

Rad52 is a major auxiliary factor of Rad51. This study identified a role for *Sp*Rad52 in regulating DSB end resection, especially in the choice of long-range resection mechanisms. Two major long-range mechanisms operate: Exo1-dependent and Dna2-Rqh1–dependent. Exo1-dependent is predominant in *S. pombe*. In *rad52* mutants, end resection becomes promoted in an Rqh1-dependent manner. Thus, Rad52 likely functions as a negative regulator of the Dna2-Rqh1 pathway.

Ref #8 ••

Andres SN, Appel CD, Westmoreland JW, Williams JS, Nguyen Y, Robertson PD, Resnick MA, Williams RS: **Tetrameric Ctp1 coordinates DNA binding and DNA bridging in DNA double-strand-break repair**. *Nat Struct Mol Biol* 2015, **22**:158–166.

This paper identified key architectural features of Ctp1: an N-terminal helical domain supporting the tetrameric form of Ctp1, an internal unstructured region, and a C-terminal DNA-interaction domain. The structure of the tetrameric form of N-terminal $Ctp1_{5\text{-}60}$ was determined. These structural features are the basis for the multivalent DNA-binding and DNA-bridging activities, likely relevant to efficient DSB repair.

Ref #10 ••

Zdravković A, Daley JM, Dutta A, Niwa T, Murayama Y, Kanamaru S, Ito K, Maki T, Argunhan B, Takahashi M, et al.: **A conserved Ctp1/CtIP C-terminal peptide stimulates Mre11 endonuclease activity**. *Proc Natl Acad Sci* 2021, **118**:e2016287118.

This study identified the C-terminal region of Ctp1 as being essential for activation of the Mre11 endonuclease. Remarkably, a synthetic peptide of the 15 amino acids at the extreme C-terminus of Ctp1 was shown to be sufficient for stimulation of the Mre11 endonuclease, highlighting the importance of this region for DNA end

resection. The authors also showed that the corresponding region of CtIP activates human MRN, demonstrating that this mechanism is evolutionarily conserved.

Ref #30 ••

Ito K, Murayama Y, Takahashi M, Iwasaki H: **Two three-strand intermediates are processed during Rad51-driven DNA strand exchange**. *Nat Struct Mol Biol* 2018, **25**:29–36.

It had been suggested that DNA strand exchange by bacterial RecA involves two distinct three-stranded intermediates. However, it was not known whether Rad51-driven strand exchange occurred via a similar mechanism. By applying FRET-based strand exchange assays, this study demonstrated that DNA strand exchange driven by *Sp*Rad51 involves two three-stranded intermediates. Swi5-Sfr1 was shown to promote three-strand intermediate maturation and the subsequent release of the non-complementary ssDNA to complete strand exchange.

Ref #31 •

Ito K, Murayama Y, Kurokawa Y, Kanamaru S, Kokabu Y, Maki T, Mikawa T, Argunhan B, Tsubouchi H, Ikeguchi M, et al.: **Real-time tracking reveals catalytic roles for the two DNA binding sites of Rad51**. *Nat Commun* 2020, **11**:1–7.

Through the application of numerous different FRET-based real-time assays, this study uncovered distinct roles for the different DNA binding domains of *Sp*Rad51 in DNA strand exchange. The authors also provided evidence that Swi5-Sfr1 induces an extended form of the Rad51-ssDNA nucleoprotein filament, which is thought to be the active state.

Ref #35 •

Argunhan B, Sakakura M, Afshar N, Kurihara M, Ito K, Maki T, Kanamaru S, Murayama Y, Tsubouchi H, Takahashi M, et al.: **Cooperative interactions facilitate stimulation of Rad51 by the Swi5-Sfr1 auxiliary factor complex**. *Elife* 2020, **9**:1–28.

This study identified two distinct regions within the intrinsically disordered N-terminal domain of Sfr1 that are important for the binding of Swi5-Sfr1 to Rad51. Importantly,

Rad55-Rad57 suppressed defects in DNA repair associated with mutations in these interaction sites and was shown to interact with Swi5-Sfr1, suggesting that the two auxiliary factor complexes collaboratively promote Rad51-dependent DNA repair.

Ref #38 ••

Afshar N, Argunhan B, Palihati M, Taniguchi G, Tsubouchi H, Iwasaki H: **A novel motif of Rad51 serves as an interaction hub for recombination auxiliary factors**. *Elife* 2021, **10**.

Little was known about the motif(s) of Rad51 responsible for binding auxiliary factors. By examining structural models of the *Sp*Rad51-ssDNA nucleoprotein filament, this study identified a prominent acidic patch on the filament exterior. Mutation of this patch completely abolished Rad51-dependent DNA repair, despite the mutant Rad51 protein being completely functional. This acidic patch of Rad51 was shown to be critical for the binding of Rad52 to Rad51, and important for the binding of Rad55-Rad57 and Rad54 to Rad51, demonstrating that multiple auxiliary factors bind the same motif of Rad51 to potentiate its activity.

Ref #63 ••

Tsubouchi H, Argunhan B, Ito K, Takahashi M, Iwasaki H: **Two auxiliary factors promote Dmc1-driven DNA strand exchange via stepwise mechanisms**. *Proc Natl Acad Sci* 2020, doi:10.1073/pnas.1917419117.

In this study, biochemical reconstitutions were performed with *S. pombe* Dmc1 and its two key auxiliary factors: Swi5-Sfr1 and Hop2-Mnd1. Whereas Swi5-Sfr1 was found to foster Dmc1-ssDNA nucleoprotein filament formation and promote filament stability, Hop2-Mnd1 had little effect on Dmc1 filaments. Hop2-Mnd1 could nevertheless strongly stimulate the ability of Dmc1-ssDNA filaments to engage homologous dsDNA molecules. Combining the two auxiliary factors led to a synergistic stimulation of Dmc1-driven strand exchange, indicating that Swi5-Sfr1 and Hop2-Mnd1 promote Dmc1-driven DNA strand exchange through distinct yet complementary mechanisms.

Ref #66 ••

Sun W, Nandi S, Osman F, Ahn JS, Jakovleska J, Lorenz A, Whitby MC: **The FANCM Ortholog Fml1 Promotes Recombination at Stalled Replication Forks and Limits Crossing Over during DNA Double-Strand Break Repair**. *Mol Cell* 2008, **32**:118–128.

In this paper, pro- and anti-recombinogenic roles of Fml1, a *S. pombe* FANCM orthologue, were identified. Fml1 was found to promote Rad51-dependent gene conversion at stalled replication forks and suppress crossing over in mitotic DSB repair. Purified C-terminally truncated Fml1 exhibited HJ branch migration, D-loop dissociation, and DNA replication fork reversal activities, implicating their involvement in HR regulation by Fml1.

Ref #70 ••

Gaskell LJ, Osman F, Gilbert RJC, Whitby MC: **Mus81 cleavage of Holliday junctions: A failsafe for processing meiotic recombination intermediates?** *EMBO J* 2007, **26**:1891–1901.

How *S. pombe* produces crossovers during meiosis is not well known. It was proposed that Mus81-Eme1 primarily resolves nicked HJ, which explains predominant formation of crossovers in meiotic DSB repair. This paper identified the robust activity of resolving intact HJs by Mus81-Eme1, which is comparable to the archetypal HJ resolvase RuvC. This versatility of Mus81-Eme1 may not only be relevant to meiotic recombination but also to mitotic recombination and DNA replication.