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Double strand break repair and homologous recombination in *Schizosaccharomyces pombe*

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Short title: DSB repair in fission yeast

Keywords: double strand break repair; recombination; non-homologous end joining; schizosaccharomyces pombe; fission yeast

Abstract

In recent years our understanding of double strand break repair and homologous recombination in *Schizosaccharomyces pombe* has increased significantly, and the identification of novel pathways and genes with homologues in higher eukaryotes has increased its value as a model organisms for double strand break repair. We will review the *S. pombe* literature on double strand break repair, mainly focussing on homologous recombination in mitotic cells.

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Introduction

Double strand break (DSB) repair and homologous (HR) recombination are not only essential for the maintenance of genome stability and therefore survival and the prevention of cancer, but also for processes that depend on recombining genetic information, like meiotic recombination and the creation of antibody variation. The study of DSB repair and HR in the yeast *Saccharomyces cerevisiae* has provided the basis of our current understanding of these processes in other eukaryotes. The study of these mechanisms in the distantly related yeast *Schizosaccharomyces pombe* has unearthed many similarities. More interestingly, it has also led to many new insights that contribute to our understanding of DSB repair and HR and the identification of homologous proteins that are involved in these processes in higher eukaryotes. In this review we will give an overview of the literature on DSB repair in *S. pombe*, mainly focussing on HR in mitotic cells.

Occurrence of DNA double strand breaks

The repair of DNA damage is essential for the survival of an organism. DNA damage comes in different forms, and includes base damage, inter and intra strand DNA cross links and single and double strand DNA breaks. DNA double strand breaks (DSBs) are especially detrimental to the cell, as they can lead to the loss of large chromosomal fragments distal from the break site, resulting in loss of heterozygosity (often associated with cancer) and cell death.

It has been estimated that human cells suffer approximately 10 spontaneous DSBs per cell cycle, most of which are thought to arise during DNA replication, when the replication fork encounters a single strand DNA break (reviewed in [35]). A spontaneous DSB may also arise through the (abortive) action of topoisomerase II, which can change DNA topology and decatenate DNA through a DSB creation and rejoining mechanism.

Ionising radiation causes a wide spectrum of DNA damage, among which are single strand breaks (SSBs), DSBs, and damage to the base and sugar groups of the DNA. Many DNA damaging agents result in indirect DSB formation: excision repair pathways remove damaged base pairs, resulting in a SSB which can be transformed into a DSB when encountered by a replication fork. When two damaged base pairs are formed in close proximity on opposite DNA strands, excision repair might lead to DSB formation.

Although DSBs are often considered by the cell as DNA damage that needs to be repaired, they are also essential intermediates for important cellular processes that involve recombination of the DNA. Meiotic recombination contributes to genetic diversity of the offspring and is initiated when Spo11, a topoisomerase-like enzyme, creates a DSB. This DSB is subsequently repaired by homologous recombination (see below). Other examples of DSB mediated recombination reactions are V(D)J recombination, which is important for establishing the variable region of antigen receptor genes (for review see [39], and class switch recombination, which is responsible for the creation of IgG, IgA and IgE antibody isotypes (reviewed in [11]).

Repair of double strand breaks

Failure to repair a DSB or imprecise repair can lead to cell death and cancer. To protect cells and organisms from these deleterious consequences, several efficient repair pathways have evolved. DSB repair pathways can employ two general strategies to repair a DSB. The first strategy is to join the two ends of a DSB together. This leads to correct repair when DSB formation is not associated with base pair loss. However, a large fraction of DSBs is associated with the loss of one or more base pairs, and in these cases joining the ends together leads to deletions and loss of genetic information. End joining repair pathways are therefore often error prone. Examples of end joining pathways are non homologous end joining (NHEJ) and single strand annealing (SSA). These pathways will be discussed in more detail below.

A second strategy for DSB repair is to invade a homologous intact DNA strand (sister chromatid or homologous chromosome) and use this as a template to copy the DNA sequence that has been interrupted by the DSB. This strategy often leads to error free repair of a DSB. Pathways that use this mode of repair are classified as homologous recombination (HR). The different HR pathways have in common that they all use strand invasion to copy the missing information, but they differ in how the intermediate structure formed after strand invasion is resolved. Different HR pathways will be discussed below.

Early double strand break repair repair events: the Mre11 Rad50 Nbs1 complex

The MRN complex, composed of Mre11 (Rad32 in *S. pombe*), Rad50 and Nbs1 (Xrs2 in *S. cerevisiae*), is a highly conserved heterotrimeric complex involved in the early response to DNA damage. The complex has been implicated in DSB recognition, NHEJ, HR, telomere maintenance and activation of the DNA damage checkpoint, which delays

the cell cycle to allow repair. Deletions of MRN components are lethal in higher eukaryotes, whereas point mutations have been associated with disorders that predispose multicellular organisms to genome instability and cancer. The MRN complex has been extensively characterised in *S. cerevisiae* and higher eukaryotes (for reviews see [6, 45]). Mre11 displays both 3' to 5' exonuclease and endonuclease activities, and it is believed that these activities are important for the processing of DSB ends. The polarity of the exonuclease activity suggests that Mre11 is not directly responsible for the creation of 3' overhangs, and it has been suggested that another nuclease activity might be responsible for 5' to 3' DSB end resection (see also below). Rad50 shows similarity to SMC proteins, it consists of an N-terminal Walker A and C-terminal Walker B motif, separated by a long coiled coil region. It is thought that Rad50 folds back onto itself, allowing the Walker A and B motifs to form a globular ABC ATPase domain. The coiled coil region that connects the two domains forms an apex containing the Cys-X-X-Cys domain. Two of these domains can form a zinc hook, allowing Rad50 to form intermolecular dimers. Nbs1 is the least conserved member of the MRN complex. It can recruit the MRN complex to the DSB site through interaction of its FHA and BRCT domains with γ-H2AX. Nbs1 is a substrate of ATM, and in *S. cerevisiae* the Nbs1 homologue Xrs2 has been shown to interact with the ATM homologue Tel1 [61], suggesting that Nbs1 provides a link between the MRN complex and the checkpoint machinery (reviewed in [6, 45]).

The MRN complex has been conserved in *S. pombe*. The *mre11* homologue *rad32* has originally been identified as a radiation sensitive mutant and was cloned by complementation [84]. *S. pombe* Rad50 was identified using degenerate PCR based on existing homologies between the *S. cerevisiae*, *C. elegans* and human *rad50*

homologues [36]. *S. pombe* Nbs1 was independently identified in a screen for mutants that are synthetically lethal with *rad2Δ* and MMS sensitive [90] and by an *in silico* based approach [17]. Both Rad50 and Nbs1 were shown to interact with Rad32 [86, 90, 17]. Insofar assessed, deletions of *rad32*, *rad50* and *nbs1* show similar phenotypes, they result in slow growth, sensitivity to DNA damaging agents and reduced telomere length (see below). Whereas the MRN complex is needed in *S. cerevisiae* to promote NHEJ, *S. pombe rad50Δ* and *rad32Δ* deletions show no NHEJ defect in a plasmid based assay [51]. In *rad50Δ* cells, the recombination frequency between the homologous chromosomes is increased at the cost of recombination between the sister chromatids. Rad50, which shows similarity to SMC proteins (which are involved in sister chromatid cohesion), acts together with Rad21, the *S. pombe* homologue of the Scc1 cohesin protein, in the repair of MMS induced DNA damage, and it was proposed that Rad50 interacts with the cohesin complex to stimulate the use of the sister chromatid as a repair template for HR [36]. Two studies have reported that *S. pombe rad32Δ* and *rad50Δ* cells are not defective for the S-phase DNA damage checkpoint (MMS and HU) when cells are synchronised using nitrogen starvation [36, 52]. In a different study where cells were synchronised either by cdc10 arrest or by elutriation, it was found that both *rad32Δ* and *rad50Δ* were deficient for the S-phase DNA damage checkpoint in MMS treated cells, but did not show a defect for the G2 DNA damage checkpoint [17]. A link between the MRN complex and the DNA damage response has also recently been illustrated by the observation that the C-terminal region of Nbs1 interacts with Tel1, which is essential for the phosphorylation of histone H2A by Tel1 [102].

Several lines of evidence suggest that the nuclease activity of the MRN complex is important for the processing of "dirty" DSB ends. Meiotic DSBs are created by a topoisomerase-like enzyme called Spo11 (Rec12 in *S. pombe*) which remains covalently

linked to the DSB end and needs to be removed before recombinational repair is initiated. It remains unknown which activity is responsible for this removal in *S. cerevisiae*, but the fact that *rad50S* mutants are unable to remove covalently bound Spo11 implicates the involvement of the MRN complex (reviewed in [45]. Connelly et al. [19] showed that the bacterial SbcCD complex, the prokaryotic homologue of Rad50/Mre11, is able to remove covalently bound protein from DNA ends in vitro. *S. pombe rad50S* mutants are also unable to repair meiotic DSBs [103] and we have recently shown that Rec12 remains covalently linked to the DNA in *rad50S* and in a *rad32* nuclease dead mutant, suggesting that the (endo)nuclease activity of the MRN complex is directly responsible for Rec12 removal (Hartsuiker and Carr, unpublished observations). Farah et al. [26, 27] have shown that a 160 bp palindrome is a hotspot for both mitotic and meiotic recombination and that the hotspot activity is abolished in *rad50S* and a nuclease dead *rad32* mutation. In meiosis, palindrome recombination was accompanied by an early DSB (at the time of DNA replication), which was absent in *rad50S*, suggesting that the MRN complex is required for palindrome cleavage (during replication) and recombination [27].

Non Homologous End Joining

NHEJ, first discovered in mammalian cells, joins two DNA ends together without the need for extensive homology. NHEJ is initiated when the Ku70/80 heterodimer, which forms a ring structure, binds to the DSB ends. Once Ku70/80 is associated with the DNA ends, it forms the DNA-dependent protein kinase complex (DNA-PK) by recruiting the catalytic subunit DNA-PK$_{cs}$. Upon binding with DNA-PK$_{cs}$, Ku70/80 moves inwards onto the DNA. The assembled DNA-PK complex can now recruit the ligase IV/XRCC4 complex, which ligates the two DNA ends. A fraction of DSBs

contains ends that are not ligatable, and need processing before they can be repaired. Artemis is one of the proteins that plays a role in DSB end processing, it physically interacts with DNA-PK$_{cs}$ and contains exonuclease as well as endonuclease activities. For more extensive reviews of NHEJ in higher eukaryotes see [96, 37]. Recently a new NHEJ factor has been identified which interacts with XRCC4, called Xlf1 (or Cernunnos). Xlf1 displays weak sequence homology with and shows structural similarity to XRCC4 ([4, 12]. The NHEJ pathway has been conserved in *S. cerevisiae* where Ku70, Ku80, Ligase IV and XRCC4 homologues have been identified. No DNA-PK$_{cs}$ or Artemis [10] homologues have been found (for review see [25]).

NHEJ activity was detected in *S. pombe* using a linearised-plasmid rejoining assay [32]. The core NHEJ factors Ku70 (Pku70), Ku80 (Pku80) and Ligase IV (Lig4) have been conserved in *S. pombe*, but no clear DNA-PK$_{cs}$ and XRCC4 homologues have been identified. Using an *in vivo* plasmid based NHEJ assay, Baumann et al. [7] and Manolis et al. [51] found that NHEJ efficiency was reduced between 100 and 1000 fold in *pku70* and *lig4* deletions. Whereas it has been shown in *S. cerevisiae* that the Mre11/Rad50/Nbs1 complex is involved in NHEJ (reviewed in [25]), *S. pombe rad50Δ* and *rad32Δ* show no defect in NHEJ [51]. Xlf1 has been conserved in *S. pombe* [13] and analysis of a deletion mutant shows that it is involved in NHEJ (Hentges and Doherty, personal communication).

Homologous recombination

Homologous recombination can be accomplished by different pathways, that have in common that a DSB is repaired using a homologous DNA template (often on a sister chromatid or homologous chromosome) from which the missing sequence is copied. HR

is generally error free. In this review we will limit ourselves to a short overview of these pathways (which are covered in more depth in [69, 45] and concentrate on discussing some of the data relevant to our understanding of HR in *S. pombe*.

DSB repair through HR starts with the nucleolytic resection of the DNA ends to form 3' single stranded overhangs that are able to invade a homologous DNA strand and serve as a primer for copy synthesis. In the Double Strand Break Repair (DSBR) model proposed by Szostak et al. [82], this strand invasion leads to displacement and D-loop formation, leading to capture of the second 3' overhang and formation of a double Holliday junction. Resolution of this Holliday junction results in a gene conversion with or without associated crossovers. To explain the low number of associated crossovers in non-meiotic cells, the Synthesis Dependent Strand Annealing (SDSA) model was proposed. In this model, DSB repair is also initiated by strand invasion, but after copy synthesis, the newly synthesized strand is displaced from the template and returned to the broken DNA molecule, resulting in repair of the break without associated crossover (for review see [69].

The MRN complex has been implicated in the resection of the DSB ends to form the single strand overhangs (see above). However, Mre11 contains 3' to 5' exonuclease activity, whereas DSB ends are resected from 5' to 3'. It therefore seems likely that another exonuclease is responsible for resection. A deletion of *S. pombe exo1* [81] is not sensitive to ionising radiation, suggesting that it is not the only exonuclease responsible for DSB end resection. However, the double mutant *exo1Δ rad50Δ* is significantly more sensitive than either single mutant, suggesting that Exo1 can resect DSB ends independently of Rad50 [86]. The identity of the main (MRN dependent?) 5' to 3'

exonuclease responsible for DSB end resection remains unknown. Alternatively, it has been proposed that the Mre11 endonuclease activity in concert with a helicase is responsible for 5' to 3' resection [45].

The single strand DNA overhangs are subsequently coated with the heterotrimeric single strand binding protein RPA, which is thought to assist in the removal of secondary structures before the loading of Rad51 [45]. In *S. pombe*, *rad11* has been identified as the homologue of the large subunit of RPA. A deletion of the gene is lethal, whereas a temperature sensitive point mutation (*rad11-404*) shows sensitivity to DNA damaging agents [70].

Central to all HR pathways is the strand exchange reaction catalysed by Rad51, which is a homologue of the bacterial RecA protein. These proteins are able to bind to the single strand DNA overhangs forming a nucleoprotein filament and promote strand exchange. Rad51 has been conserved in *S. pombe*, (called Rhp51) and is extremely sensitive to ionising radiation when deleted [75, 57, 38]. Rhp51 has been shown to bind single stranded DNA forming a nucleoprotein filament, exhibit DNA-stimulated ATPase activity and promote strand exchange reactions with homologous duplex DNA [72].

RPA has a high affinity to ssDNA, and therefore competes with and prevents binding of Rad51. Recombination mediator proteins (reviewed in [79]) assist Rad51 in overcoming the RPA inhibition and binding to the ssDNA. One of these mediator proteins is Rad52, which interacts with Rad51. Loading of the Rad52-Rad51 complex on the ssDNA nucleates the formation of the Rad51 nucleoprotein filament. Another group of recombination mediator proteins are formed by the Rad55/Rad57 proteins (in *S.*

cerevisiae) and the Rad51 paralogues in mammalian cells (reviewed in [85]). They have in common that they show weak homology to Rad51 and show affinity to ssDNA. They are also thought to mediate Rad51 binding to the ssDNA overhangs [79]. Another protein that is thought to facilitate binding of Rad51 to ssDNA is Rad54, a member of the Swi2/Snf2 chromatin remodelling protein family [45].

In *S. pombe* two Rad52 homologues have been identified. Rad22, which is involved in both mating type switching and DNA repair [73], shows significant homology with *S. cerevisiae* Rad52 [68]. A *rad22* deletion is sensitive to ionising radiation [59]. Discrepancies in the level of sensitivity of *rad22Δ* between different publications (e.g see [59, 36, 24]) can be explained by the presence of a suppressor mutation [67]. The Rad22 protein was found to bind to DNA double strand breaks [43]. The second Rad52 homologue, Rti1, was isolated as a multicopy suppressor of the *rad22-H6* mutant [80] and was also identified on the basis of its sequence homology with *rad22* (also called *rad22B* [91]). An *rti1* deletion is only very slightly sensitive to DNA damaging agents, and slightly exacerbates the *rad22Δ* sensitivity. Both Rad22 and Rti1 have been found to interact with each other and with Rhp51 and Rpa [89, 43, 92]. *S. pombe* also contains a Rad54 homologue, called Rhp54, which interacts with Rhp51 and is sensitive to ionising radiation as well as to UV when deleted [58, 89].

Several recombination mediator proteins which show homology to Rad51 have been identified in *S. pombe*. The Rad55 homologue Rhp55 was identified on the basis of sequence homology. An *rhp55* deletion is highly sensitive against DNA damaging agents. Double mutant analysis suggested that *rhp55* acts in a pathway with *rhp51* and *rhp54*, but is in a different epistasis group than *rad22* for the repair of DNA damage

[40]. Rhp57, homologous to Rad57 (*S. cerevisiae*) and XRCC3 (vertebrates), has been identified through complementation of a mutant that showed hypersensitivity to MMS and was synthetically lethal with *rad2Δ* (the *S. pombe rad27*/FEN1 homologue). The phenotypes of an *rhp57* deletion are very similar to those of *rhp55Δ* [88]. Rhp55 and Rhp57 were found to strongly interact with each other. Two more Rad51-like proteins have been identified in *S. pombe*: Rlp1, which shows homology to the mammalian Rad51 paralogue XRCC2 [41], and the recently identified Rdl1, similar to the mammalian Rad51 paralogue Rad51D [53]. An *rlp1* deletion is mildly sensitive to MMS, ionising radiation and camptothecin, but not to UV. Rlp1 interacts weakly with Rhp57 in a two hybrid assay [41]. Rdl1 was found to interact with Sws1, which interacts with the Srs2 helicase (see below). Rdl1 also interacts with Rlp1 and the sensitivity of *rdl1Δ* to DNA damaging agents mimics that of *rlp1Δ* [53].

S. pombe Swi5 is involved in both mating type switching and HR [73]. Akamatsu et al. [5] found that Swi5 is conserved in higher eukaryotes and functions in Rhp51 dependent repair, but acts independently of Rhp57. They proposed that Swi5 and Rhp57 function in two parallel Rhp51 dependent sub pathways. Using Swi5 as a bait in a two hybrid assay, they identified Swi2, which in turn was found to interact with Swi5, Rhp51 and Swi6. Whereas the Rhp51/Swi2/Swi5/Swi6 complex probably plays a specific role in *S. pombe* mating type switching, Akamatsu et al [5] also identified a protein complex containing Rhp51, Swi5 and Sfr1, a protein which shows homology to and replaces Swi2. This complex is specifically involved in Swi5/Rhp51 mediated DNA repair.

Regulation of homologous recombination: preventing deleterious recombination outcomes

Whereas in meiosis crossovers (reciprocal recombination) between homologous chromosomes are essential to generate recombination and therefore increase genetic diversity of the offspring, in mitotic cells crossovers are often not beneficial to the cell. Crossovers between homologous DNA sequences that map to different positions on sister chromatids, homologous or even non-homologous chromosomes (ectopic recombination) can lead to abnormal chromosomal rearrangements. Also, a crossover between two homologous chromosomes in a mitotic G2 cell (when each chromosome consists of two sister chromatids) leads to loss of heterozygosity of the genes distal to the crossover point. Both chromosomal rearrangements and loss of heterozygosity can lead to cell death and cancer. For these reasons, reciprocal recombination (crossovers) is prevalent in meiotic cells, whereas mitotic cells prefer non-reciprocal recombination (gene conversion without associated crossover). Using artificially dispersed copies of the *ade6* gene [93] it was found that in meiotic *S. pombe* cells approximately 24 % of ectopic recombination is associated with a crossover, whereas in mitotic cells this was reduced to approximately 4 % [94].

In the Szostak et al. [82] DSBR model, crossovers are formed through resolution of double Holliday junctions. Cells can prevent crossovers by preventing HR and the formation of Holliday junctions (as in the SDSA model, see [69] for review) or by resolving Holliday junctions in such a way that they don't result in crossovers. In recent years several proteins and pathways have been identified that are involved in the regulation of HR and associated crossover formation in *S. pombe*.

S. pombe Mus81, which interacts with Eme1, is an XPF-like endonuclease that has been found to interact with Cds1 and promotes crossover formation in meiosis. A *mus81* deletion is sensitive to UV irradiation, but only mildly sensitive to γ-irradiation. Mus81 is essential for survival of *rqh1Δ* cells. A *mus81* deletion shows a strongly reduced meiotic spore viability and crossover frequency, whereas gene conversion is not reduced [8, 9, 66, 76]. Based on these phenotypes, it was proposed that Mus81-Eme1 is responsible for Holliday junction resolution, a hypothesis which was supported by the rescue of the meiotic defects by overexpression of RusA, a bacterial Holliday junction resolvase, and the ability of the Mus81-Eme1 complex to cleave Holliday junctions in vitro [9]. However, as in *S. cerevisiae* meiotic spore viability was not dramatically reduced in *mus81Δ*, and (intact) Holliday junctions are a relatively poor substrate for Mus81-Eme1, alternative roles for Mus81-Eme1 have been proposed [22, 31] and references cited therein). Gaillard et al. [31] provided evidence that nicked Holliday junctions are efficiently resolved by Mus81-Eme1, but the activity that creates the first nick in the Holliday junction remains elusive. Osman et al. [66] proposed an alternative mechanism by which Mus81-Eme1 cleaves a junction that is formed in the transition from DSB to double Holliday junction, which results in the formation of a crossover without double Holliday junction formation (also reviewed in [98]. It has also been proposed that Mus81-Eme1 acts on stalled replication forks. Doe et al. [22] show that *mus81Δ* is hypersensitive to agents that cause replication fork stalling, and that this sensitivity is rescued by the overexpression of RusA. Also the inviability of a *rqh1Δ mus81Δ* double mutants is rescued by the overexpression of RusA. Mus81-Eme1 efficiently cleaves substrates resembling (stalled and reversed) replication forks *in vitro* [22, 97].

One of the proteins that is thought to be involved in preventing crossovers through alternative resolution of double Holliday junctions in *S. pombe* is Rqh1, which is a helicase and homologous to *E. coli* RecQ and the RecQ homologue family in humans, mutations of which have been associated with increased genomic instability. Rqh1 shows the highest similarity to BLM [78, 60]. BLM patients show an increase of sister chromatid exchange. BLM interacts with Rad51, RPA and Top3 and it has been proposed that the BLM helicase plays a role in the resolution of double Holliday junctions by stimulating reverse branch migration coupled to Top3 dependent resolution of the resulting hemi-catenane, avoiding formation of crossover products (reviewed in [18]. Data obtained in *S. pombe* are largely compatible with this model. *S. pombe rqh1Δ* cells are unable to recover from hydroxyurea (HU) induced S-phase arrest and show strongly increased levels of recombination after HU treatment [78]. Rqh1 has been implicated in a UV damage tolerance pathway together with Rhp51, probably functioning in recombination dependent bypass of UV damage during replication [60]. *S. pombe* Rqh1 displays helicase activity and forms a complex with Top3 [47, 1, 2]. A *top3* deletion is inviable, but viability is rescued by concomitant loss of *rqh1* [33, 49] or loss of recombination functions [63, 47]. These data are in agreement with the notion that Rqh1 creates a recombination intermediate that causes lethality in the absence of Top3 [33]. Overexpression of a bacterial Holliday junction resolvase partially rescues *rqh1Δ* phenotypes, suggesting that Rqh1 is involved in the non-recombinogenic resolution of Holliday junctions [21]. The terminal phenotype of a *top3* shut off strain shows an accumulation of aberrant DNA structures (intertwined chromosomes) in S-phase and subsequent aberrant mitosis [100]. *rqh1Δ* cells are delayed in anaphase progression and show lagging chromosomal DNA [101]. Using a system that induces replication fork blockage in between two direct repeats (the *ade6-L469* and *ade6-M375*

alleles; see below), Ahn et al. [3] showed that deletion of *rqh1* leads to a pronounced increase of recombination, associated with the detection of a one-sided DSB at or near the replication fork barrier, suggesting that Rqh1 prevents the collapse of blocked replication forks.

Srs2 is another helicase that has been implicated in anti-recombination pathways. In *S. cerevisiae*, Srs2 interacts with Rad51 and is able to displace Rad51 from the ssDNA, and it is believed that this activity is responsible for its anti-recombinogenic function (reviewed in [48]. In *S. pombe*, cells deleted for *srs2* display a mild sensitivity to DNA damaging agents, whereas spontaneous recombination rates were increased compared to WT [95, 23]. An *srs2* deletion shows complicated genetic interactions with mutants involved in HR. An *srs2Δ rhp54Δ* double mutant is synthetic lethal and this lethality is rescued by the concomitant deletion of *rhp51*. This result, and other genetic data not discussed here, is consistent with the idea that Srs2 removes Rhp51 from the nucleoprotein filament [50, 23]. Simultaneous deletion of both *srs2* and *rqh1* results in a dramatic growth defect [95, 50, 23]. Whereas Wang et al. [95] originally reported that this growth defect was not rescued by concomitant deletion of *rhp51*, both Maftahi et al. [50] and Doe and Whitby [23] reported that *rhp51Δ*, as well as as *rhp55Δ/rhp57Δ*, rescues the slow growth phenotype of the *srs2Δ rqh1Δ* double mutant. Recently, Srs2 was found to interact with Sws1 in a two hybrid screen. Sws1 in turn interacts with the Rad51-like proteins Rlp1 and Rdl1 (see above). Deletion of *sws1* as well as *rlp1Δ* and *rdl1Δ* suppresses various *rqh1Δ* and *srs2Δ* phenotypes, suggesting that the Sws1/Rlp1/Rdl1 complex, which is conserved in humans, is pro-recombinogenic [53].

Recently, an additional anti-recombinogenic helicase, Fbh1, was identified in *S. pombe*

which seems to counteract the role of the Rad22 and Rhp55/Rhp57 mediator proteins and shows similarity to Srs2. Fbh1 was independently identified as a suppressor of *rad22Δ* [67] and in a screen for mutants which are MMS sensitive and synthetic lethal with *rad2Δ* [56]. An *fbh1* deletion is sensitive to DNA damaging agents and is epistatic with *rhp51Δ* for DNA repair. *fbh1Δ* is synthetically lethal with *rqh1Δ* and shows a synthetic growth defect with *srs2Δ*. These phenotypes are rescued by the concomitant deletion of *rhp51*, *rhp55* or *rhp57* [67, 56]. Fbh1 co-localises with Rhp51 after DNA damage [67]. Suppression of *rad22Δ* slow growth and sensitivity to DNA damaging agents depends on the presence of Rhp51, suggesting that Fbh1 inhibits the action of Rhp51 in the absence of Rad22 [67].

The role of homologous recombination in meiosis

HR is not only involved in the repair of DSBs in vegetative cells, but is also responsible for meiotic recombination, which is initiated when Spo11 creates a meiosis specific DSB. The detailed study of meiosis in *S. cerevisiae* has made an important impact on our current understanding of DSB repair by HR. Also in *S. pombe* meiotic recombination is initiated by meiotic DSBs that are dependent on the Spo11 homologue Rec12 [16, 103, 77]. Proteins involved in HR in vegetative *S. pombe* cells have also been implicated in meiotic recombination.

Maybe surprisingly, deletions of *rhp51*, *rad22* and *rhp54*, which are largely defective for HR in mitotic cells, show only a mild impact on meiotic recombination. This is due to the existence of meiosis specific homologues of these genes, deletions of which have little or no effect on the sensitivity to DNA damaging agents in mitotic cells. Deletion of *rhp51* shows only a relatively mild reduction in meiotic recombination [59, 34] whereas

concomitant deletion of *dmc1*, the meiosis specific *rhp51* homologue, shows a several

hundred fold reduction [30, 34]. A similar relation is observed between *rhp54Δ* and

rdh54Δ, the meiosis specific *rhp54* homologue [59, 15]. Also a *rad22* deletion does not

show a reduction in meiotic recombination [59]. The *rad22* homologue *rti1* shows a

mild reduction of meiotic recombination when deleted. Although spore viability in the

r*ad22Δ rti1Δ* double mutant its strongly reduced, meiotic recombination does not seem

to be affected. However, Rti1 expression is strongly induced during meiosis suggesting

it might have a specialised meiotic function [91, 92].

Deletions of *rhp55* and *rhp57* show a mild reduction in meiotic recombination, similar

to that of the double mutant. Combination of *rhp51Δ* with either *rhp55Δ*, *rhp57Δ* or the

r*hp55Δ rhp57Δ* double mutant does not reduce the meiotic recombination frequency

below *rhp51Δ* levels, suggesting that they function in the same pathway. An *rlp1*

deletion decreases the meiotic crossover frequency, but increases meiotic gene

conversion [34].

The role of homologous recombination in replication

HR has been implicated in the bypass of replication fork barriers and restart of the

replication fork [54]. Also in *S. pombe*, the mutation of genes involved in HR often

leads to problems in DNA replication (e.g. see [58, 40, 36]. Using two dimensional gel

electrophoresis, Segurado et al. [74] showed that DNA replication in *S. pombe* is

associated with structures that probably represent joint DNA molecules or Holliday

junctions. These structures were absent in *rad22Δ*, *rhp51Δ* and *rhp54Δ*. These results

suggest that recombination intermediates are commonly associated with DNA

replication in *S. pombe*. Two recent studies used the Replication Termination Sequence

RTS1, which is normally found in the mating type region, as a replication fork barrier. Lambert et al. [46] integrated RTS1 on either side of *ura4*, and confirmed with two dimensional gels that this induces replication fork stalling. Upon fork stalling, recombination mutants (*rad50Δ* and *rhp51Δ*) but not checkpoint mutants display a slow growth phenotype and lose viability. Fork stalling also induces Rhp51 and Rad22 foci formation and Rad22 association (detected using chromatin IP) with the region of fork collapse and results in an increase in recombination and gross chromosomal rearrangements. Using a system where RTS1 is integrated in between two direct repeats (the *ade6-L469* and *ade6-M375* alleles), Ahn et al. [3] also found that replication fork blockage results in an increase in *rhp51* and *rad22* dependent recombination between the repeats.

Double strand break repair and telomere length maintenance

Telomeres protect the DNA at the end of the chromosomes. A common perception is that DSB repair mechanisms need to be repressed at the telomeres to prevent inappropriate chromosome fusions and recombination. It might therefore seem paradoxical that several studies have implicated both NHEJ and HR to be involved in telomere maintenance in a wide range of organisms.

In *S. pombe*, both NHEJ and HR have been shown to play a role in survival and alternative telomere maintenance in cells that are deleted for proteins involved in telomere maintenance/elongation and protection. Deletion of the *S. pombe* telomere reverse transcriptase Trt1, the catalytic subunit of the telomerase which is responsible for telomere elongation, leads to a complete loss of telomeric DNA and viability. However, a proportion of cells can survive through two different mechanisms. On

plates, a small subpopulation of cells circularise their chromosomes [62]. A *trt1Δ pku70Δ* double mutant is still able to circularise its chromosomes, but shows an increase in telomere degradation, suggesting that Pku70 is not necessary for circularisation, but protects the telomeres (in *trtΔ* cells) against degradation, possibly by preventing access of nucleases to the telomeres [7]. In liquid culture *trtΔ* cells can survive through an alternative lengthening pathway, maintaining linear chromosomes [62]. In a *trtΔ rad22Δ* strain, survivors circularise their chromosomes, suggesting that the maintenance of linear chromosomes in *trt1Δ* cells is dependent on recombination. Another protein that is important for telomere maintenance is the Myb domain containing protein Taz1. In *taz1Δ* strains, the telomeres are dramatically elongated [20]. When *taz1Δ* cells are starved for nitrogen (which arrests cells in G1 and promotes NHEJ) they loose viability and show an increase of telomeric chromosome fusions, which are dependent on the presence of Ku70 and Lig4 [28].

Whereas these studies show that NHEJ and HR are involved in telomere metabolism in cells that are deleted for the telomere maintenance proteins Trt1 and Taz1, NHEJ and HR have also been implicated in telomere maintenance in cells that are WT for these proteins. Deletion of *pku70* in *S. pombe* leads to shortened telomeres [7, 51] and rearrangements of telomere associated sequences [7]. These rearrangements were not observed in a *pku70Δ rad22Δ* double mutant [7] or in *pku80Δ rhp51Δ* [42]. In *pku80* deleted cells Rhp51 is found at the telomeres using chromatin IP [42]. These data suggest that the Pku70/Pku80 complex prevents Rhp51 from loading and thus prevents (inappropriate) recombination at the telomeres.

Tomita et al. [86] found that *taz1Δ* cells contain extensive 3' G-rich strand overhangs,

and that *taz1Δ rad50Δ* and *taz1Δ rad32Δ* double mutants lack this G-rich strand overhang. As the telomere length in these double mutants was not affected compared to the *taz1Δ* single mutant, they concluded that the MRN complex was unlikely to be involved in elongation of the G-rich strand but is probably responsible for degradation of the C-rich strand. In a *taz1Δ rad50Δ pku70Δ* triple mutant the G-rich strand overhang is restored again. Maybe surprisingly, the nuclease dead *rad32-D25A* mutation in combination with *taz1Δ* still possesses G-rich overhangs. These data suggest that the nuclease activity responsible for C-rich strand resection is dependent on but not provided by the MRN complex [86]. Tomita et al. [87] identified the nuclease Dna2 as being responsible for the generation of G-rich overhangs in *taz1Δ* as well as in WT cells: at semi-permissive temperature the *dna2-C2* mutant shows reduced G-rich overhang. Dna2 was localised at the telomeres using chromatin IP and the mutant shows reduced telomere length at semi-permissive temperature [87]. These data lead to a model in which the MRN complex recruits and/or regulates the Dna2 nuclease activity to create G-rich strand overhangs. The MRN complex enables Dna2 to resect the C-rich DNA strand when the Pku70/Pku80 heterodimer is present, but is not needed in the absence of Pku70/Pku80. Creation of the G-rich strand overhang allows the loading of Trt1 and therefore elongation of the telomeres [87]. As Dna2 binds to RPA in *S. cerevisiae*, which binds to telomeric DNA in both *S. cerevisiae* and *S. pombe*, it was proposed that Dna2 might be involved in the loading of the telomerase complex onto the telomere [64, 87]. This might also explain the shortened telomeres in *rad11-D223Y*, a mutant in the large subunit of RPA in *S. pombe* [64].

Shortened telomere length has also been reported in *rhp51Δ* [99]. The role of Rhp51 in telomere length maintenance in cells that are otherwise proficient for telomere

elongation pathways is not fully understood. It is possible that the shortened telomeres in *rhp51Δ* cells are related to problems in genomic DNA replication or alternatively it might suggest that recombination between telomeres also contributes to telomere elongation independent of other telomerase elongation pathways (reviewed in [83]).

The action and interplay of both HR and NHEJ factors on the telomere is highly complex. Overall, these data suggest that rather than hiding the chromosome ends from HR and NHEJ pathways, the telomeres strictly regulate and modify these repair pathways to prevent treatment of the chromosome ends as "ordinary" DSBs that need repair.

Single strand annealing

When a DSB is formed between two (nearly) homologous repeats, it can be repaired by an error prone pathway called Single Strand Annealing (SSA), resulting in the deletion of the sequence between the homologous sequences flanking the DSB. SSA has first been discovered in mammalian cells but has most extensively been studied in *S. cerevisiae* (for review see [69]). SSA starts with the resection of the DSB ends by an exonuclease which produces long single stranded DNA overhangs. When two complementary (homologous) sequences are exposed, they can anneal, leaving long single stranded non-homologous DNA flaps. Removal of these flaps is dependent on the Rad1/Rad10 flap endonucleases (that are also involved in nucleotide excision repair). SSA is independent of Rad51. When a DSB is formed between two homologous repeats, SSA is the predominant repair pathway. SSA is very efficient in *S. cerevisiae* when the homologous sequences are separated by at least 400 bp (and up to 15 kb), but is inefficient when the sequences are separated by only 60 bp (reviewed in [69]).

Information available on the role of SSA in *S. pombe* is limited. Using a system where two *ade6* alleles were separated by a region of unique DNA containing a recognition site for the HO endonuclease, Osman et al. [65] found that expression of the HO endonuclease resulted in loss of the unique region and restoration of the *ade6* ORF. As the cut in unique DNA precludes HR (when both sister chromatids are cut in G2) they concluded that these events most likely represent SSA. Werler and Carr (personal communication) designed a similar system in *S. pombe* where two partial *LEU2* genes are separated by a 12 kb region containing a *ura4* gene with an HO recognition site. They were able to show that induction of the HO-endonuclease leads to a high frequency of *ura4* marker loss associated with the creation of a functional *LEU2* gene. These events were dependent on *rad16* and *swi10* (homologues of *S. cerevisiae rad1* and *rad10* genes) and *rad22*, but not dependent on *rhp51*, suggesting that these events represent SSA.

It is worth noting that SSA annealing has mainly been studied in artificial constructs, and its relative contribution to DSB repair in both *S. cerevisiae* and *S. pombe* remains unknown. However, the fact that *S. cerevisiae rad1* and *rad10* mutations show no X-ray sensitivity [55], and a *S. pombe swi10* (*rad10* homologue) mutant is only very slightly sensitive to γ irradiation [73] suggests that SSA does not play an important role in the natural response of these organisms to DSBs.

DSB repair pathway choice and prevalence

DSBs can be repaired through NHEJ, HR and SSA. NHEJ is a major repair pathway in mammalian cells and leads to significant sensitivity to ionising radiation when disrupted. *S. pombe* spends most of its time in G2, during which a sister chromatid is

available as a repair template. HR is the dominant repair pathway in asynchronously dividing *S. pombe* cells, and deletions of HR genes show a high sensitivity to DNA damaging agents, whereas *pku70Δ* and *lig4Δ* show no sensitivity [51]. As *S. pombe* is a haploid organism, no repair template is available in G1, and therefore HR is not possible in G1. Using a system in which a DSB can be induced in (both sister chromatids of) a non-essential minichromosome, Prudden et al. [71] showed that the majority of the breaks is repaired by HR (gene conversion) with the homologous chromosome. Surprisingly, deletion of *pku70* led to a decrease of minichromosome loss. Gene conversion levels were not increased in this background, but were dependent on Rhp51. Loss of Pku70 was also associated with an increase of DSB-induced chromosomal rearrangements. These data suggest that NHEJ and HR might compete with each other for DSB repair [71]. Tomita et al., [86] reported that *pku70Δ* rescues the sensitivity to MMS of *rad50Δ* and that this rescue is dependent on the presence of Exo1. This suggests that in the absence of the MRN complex Pku70 inhibits DSB end processing (and probably the repair of the DSB by HR) by Exo1. Two studies suggest that HR and NHEJ are regulated in a cell cycle dependent manner. Ferreira and Cooper [29] showed that NHEJ and HR are reciprocally regulated in *S. pombe* G1 and G2 cells. In G1 arrested cells NHEJ is 7 to 10 times more efficient than in asynchronously dividing cells, and *ku70Δ* cells show significant sensitivity to γ-irradiation. Caspari et al. [14] characterised a temperature sensitive mutation in cyclin B (*cdc13-245*) that confers sensitivity to ionising radiation. They showed that this mutation reduces Cdc2 kinase activity and Rad51 foci formation in response to ionising radiation and proposed a model in which CDK activity positively regulates an early step in HR.

Outlook

The characterisation of HR in *S. cerevisiae* meiosis has provided important information about the mechanisms of HR. However, it has become clear in recent years that the recombination model(s) resulting from these studies are only partially applicable to HR in mitotic cells, where HR is accomplished through as yet poorly characterised mechanisms that prevent crossover formation. Although many HR proteins have been identified and characterised in *S. pombe*, the mechanisms that are utilised for HR in mitotic or in meiotic cells remain obscure. A number of systems/methodologies have now been developed that allow a more detailed study of HR in *S. pombe* meiosis (e.g. see [16, 103, 77], mitosis [71], replication [46, 3] and SSA (Werler and Carr, personal communication) and hopefully will allow the physical detection of recombination intermediates in the future, contributing to our understanding of HR mechanisms and choice and interplay between different HR pathways in *S. pombe* mitosis and meiosis.

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