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Elevated Rad53 kinase activity influences formation and interhomolog repair of meiotic DNA double-strand breaks in budding yeast

Takehiko Usui *, Yoshiki Kanehara

Laboratory of Genome and Chromosome Functions, Institute for Protein Research, Osaka University, Suita, Osaka 565-0871, Japan

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ABSTRACT

Meiotic cells generate physiological programmed DNA double-strand breaks (DSBs) to initiate meiotic recombination. Interhomolog repair of the programmed DSBs by meiotic recombination is vital to ensure accurate chromosome segregation at meiosis I to produce normal gametes. In budding yeast, the DNA damage checkpoint kinase Rad53 is activated by DSBs which accidentally occur as DNA lesions in mitosis and meiosis; however, meiotic programmed DSBs which occur at ~160 loci per genome fail to activate the kinase. Thus, Rad53 activation appears to be silenced in response to meiotic programmed DSBs. In this study, to address the biological significance of Rad53's insensitivity to meiotic DSBs, we examined the effects of Rad53 overexpression on meiotic processes. The overexpression led to partial activation of Rad53, uncovering that the negative impacts of Rad53 kinase activation on meiotic progression, and formation and interhomolog repair of meiotic programmed DSBs.

1. Introduction

Meiotic cells generate physiological programmed DSBs, required for gamete production along with two serial cell divisions, meiosis I (MI) and meiosis II (MII), following a single round of DNA replication. The meiotic DSBs are produced by the topoisomerase-like protein Spo11 to initiate meiotic recombination [1]. A key feature of meiotic recombination is to repair the programmed DSBs, if not all, by interhomolog recombination with crossover (CO), orchestrated by two RecA-like DNA strand exchange proteins, Rad51 and meiosis-specific Dmc1 [2–4]. Interhomolog CO recombination makes a connection between homologous paternal and maternal chromosomes to ensure their accurate segregation at MI. On the other hand, in somatic cells, to maintain genome integrity, DSBs that accidentally occur as DNA lesions during S and G2 phases are repaired by intersister recombination [5].

Accidental and meiotic programmed DSBs are subjected to 5′ end processing to produce 3′ single-stranded DNA tails that not only serve substrates for homologous recombination but also elicit checkpoint signaling [6,7]. In budding yeast, checkpoint signaling pathways in response to accidental and meiotic DSBs are both initiated by the Rad17–Ddc1–Mec3 (i.e., 9-1-1) and Mec1–Ddc2 complexes [6,7]. Ddc2 binds to replication protein-A that coats the single-stranded DNA tails to recruit Mec1 kinase to DSB sites where Mec1 activation is mediated by 9-1-1 [8,9].

The distinct sets of proteins function at the signaling steps following Mec1 activation. The DNA damage checkpoint kinase Rad53 is activated by accidental DSBs in meiosis as well as in mitosis [10,11]. Programmed DSBs activate the meiosis-specific Rad53 paralog Mek1/Mre4 (hereafter Mek1) [12,13]. Rad53 and Mek1 kinases, whose activation requires Rad9 and Hop1 mediators, are important for DNA damage checkpoint and meiotic recombination checkpoint, respectively [6,7]. These checkpoints coordinate progression of cell cycle with chromosome metabolisms [6,7,12]. In addition, the Mek1-dependent recombination checkpoint creates interhomolog bias to promote interhomolog repair of meiotic DSBs [14,15].

Rad53 kinase appears to be unresponsive to meiotic programmed DSBs. Meiotic DSBs that occur at least 160 loci per cell [16] fail to activate Rad53 in wild type and even in *dmc1* deletion cells [11]. Since Dmc1 is required for interhomolog repair, the *dmc1* mutant accumulates unrepaired meiotic DSBs with extensive single-stranded DNA tails, thus activating the Mek1-dependent recombination checkpoint [3,12,17,18]. In contrast, even a single unrepaired DSB activates Rad53 in mitosis [10].

In this study, to address why Rad53 fails to respond to meiotic physiological programmed DSBs, we examined the influences of Rad53 overproduction on meiotic processes. The data suggest that Rad53 activation has negative impacts on meiotic progression and formation/repair of the programmed DSBs.

Abbreviations: DSBs, DNA double-strand breaks; CO, crossover; NCO, non-crossover; UTR, untranslated region; TCA, trichloroacetic acid; ISA, in situ autophosphorylation; KD, kinase dead; MI, meiosis I; MII, meiosis II; DAPI, 4′,6-diamidino-2-phenylindole.

* Corresponding author. Address: Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0871, Japan. Fax: +81 6 6879 8626.
E-mail address: takeusu@protein.osaka-u.ac.jp (T. Usui).

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2. Materials and methods

2.1. Strains and plasmids

All strains described here are derivatives of SK1 diploids (Table S1). About 0.5 kb DNA fragment containing 5′ untranslated region (UTR) of *DMC1* gene fused with C terminal 6× FLAG-tagged *RAD53* or *rad53-kinase dead* (*KD*) was cloned in pRS406 to make plasmids pUS13 and pUS14, respectively. To integrate *DMC1pr-FLAG-RAD53* or *DMC1pr-FLAG-rad53-KD* in the *ura3* locus, a SK-1 wild type was transformed with PstI-digested pUS13 or pUS14. *RAD51* cDNA was fused with the *DMC1* 5′ UTR fragment was cloned in pBluescript SKII+ to make plasmids pUS29. A SacI fragment containing KANMX6 was cloned in pUS29 to make pUS30. To integrate *DMC1pr-RAD51* in the *RAD51* locus, a SK-1 wild type was transformed with EcoRI-digested pUS30 and grown on YPD with 100 μg/ml G418. The resulting strain carried two copies of *RAD51* expressed from the native or *DMC1* promoters. *DMC1* and *RAD54* genes were deleted by a PCR-based method [19] using the primer sets shown in Table S2.

2.2. Meiotic cell analyses

After SK-1 diploid cells were introduced into meiosis, meiotic cell cycle progression was monitored by 4′,6-diamidino-2-phenylindole (DAPI) staining, and immunostaining of meiotic spread nuclei was performed as described [20,21]. The DNA content of meiotic cells was examined by FACS Calibur flow cytometer (BD Biosciences). For Western blot analysis, trichloroacetic acid (TCA)-precipitated cell extracts were made as follows: 2×10^8 meiotic cells were fixed in 20% TCA and disrupted with glass beads using Bead Shocker (Yasui Kikai). TCA precipitates were collected and suspended in 0.24 ml of SDS-loading buffer supplemented with 0.33 M Tris–HCl (pH 8.0). The in situ autophosphorylation (ISA) assay was performed as described previously [10]. ^{32}P-incorporation to Rad53 was visualized with BAS2000 (Fujifilm). Anti-Flag (M2, Sigma), anti-alpha-tubulin (Serotec), anti-Rad51 [2], and anti-Dmc1 [21] were used for Western blot and immunostaining. Immunostained samples were observed as described previously [22]. Physical analyses of crossover/non-crossover (CO/NCO) recombinants at the *HIS4-LEU2* locus were performed as described [21,23].

3. Results

3.1. Meiosis-specific overexpression of Rad53 kinase delays cell cycle progression

A previous study showed that Rad53 activation by tethering the kinase to meiotic DSBs as a fusion to Ddc2 does not affect meiosis [11]. Here, we simply overexpressed Rad53 as a free (non-tethered) form in meiosis using a strong meiosis-specific promoter of

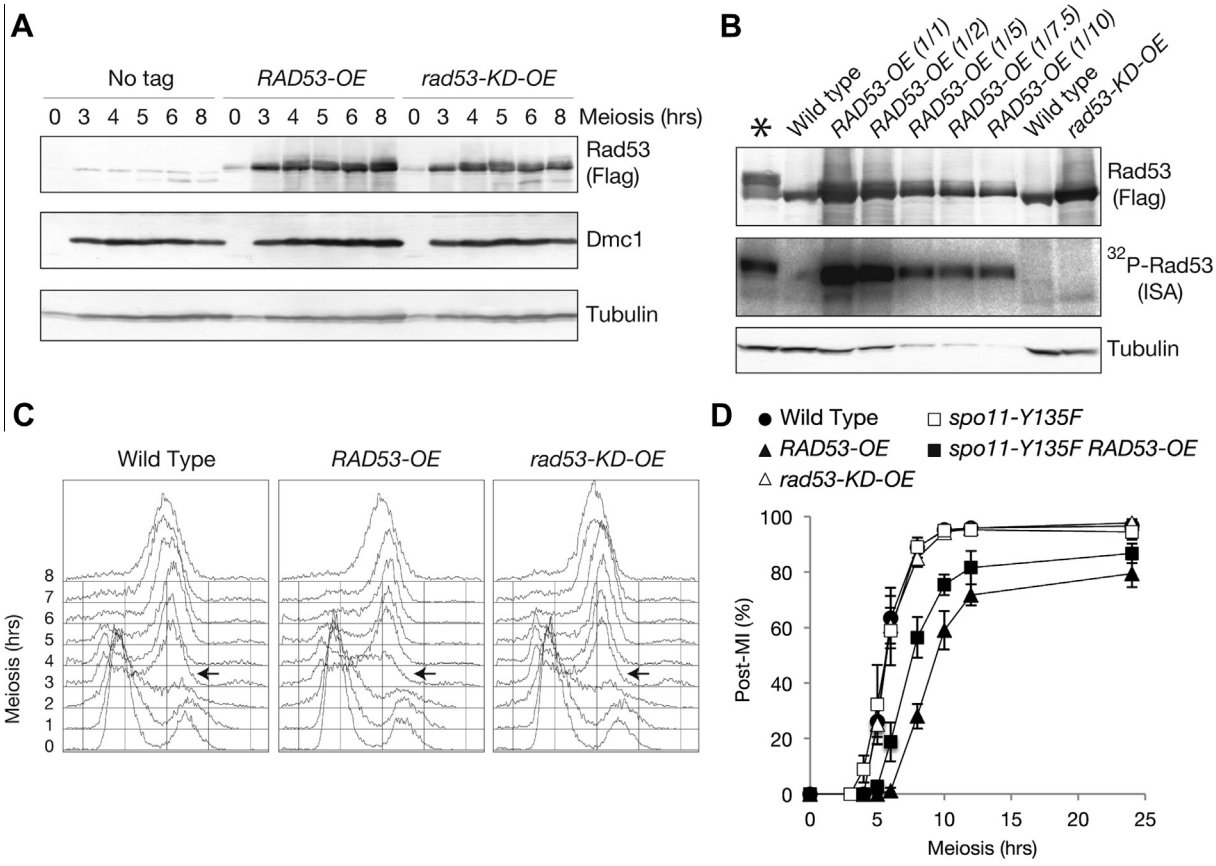


Fig. 1. Meiosis-specific Rad53 overproduction delays meiotic progression. (A) Western blot analysis of TCA-precipitated meiotic cell extracts of the indicated strains. (B) The Rad53 expression level and kinase activity were examined. A lane labeled with the asterisk shows fully activated Flag-Rad53. Wild type expressed Flag-Rad53 under the native *RAD53* promoter. All cell extracts were prepared from 4 h meiotic cells. Cell extracts (4×10^6 cells) were loaded for wild type, *RAD53-OE* (1/1) and *rad53-KD-OE*. Dilutions of *RAD53-OE* extracts were examined accordingly. ^{32}P-Rad53 represented ^{32}P incorporation to Rad53 in ISA assay. (C) DNA contents of the indicated strains were examined by FACS analysis. Arrows show that premeiotic DNA replication is not completed in *RAD53-OE*. (D) Meiotic nuclear divisions were monitored in the indicated strains by DAPI staining. *Y*-axis represents the percentage of cells that completed meiosis I and II (Post-MI). At least 200 cells were counted each time points. Error bars represent standard deviation (SD) calculated from at least three independent experiments.

DMC1 (hereafter *DMC1pr*) [3]. In addition to endogenous *RAD53* alleles, diploid cells with *FLAG-RAD53* or *FLAG-rad53-KD* (a kinase-negative allele of *RAD53*) under *DMC1pr* were made (referred to as *RAD53-OE* or *rad53-KD-OE*, respectively). *RAD53-OE* and *rad53-KD-OE* increased amounts of Flag-Rad53 and Flag-Rad53-KD like Dmc1 from 3 h in meiosis, respectively (Fig. 1A). When compared to the expression level of Flag-Rad53 under the native *RAD53* promoter, *RAD53-OE* expressed Flag-Rad53 5–10 times more at 4 h in meiosis (Fig. 1B). *RAD53-OE* showed shifted bands of Rad53 that were not observed in *rad53-KD-OE* and wild type, and were less extensive compared to activated Rad53 by DNA damage (Fig. 1B). The band shifts corresponding with phosphorylation [10] suggest Rad53 activation. Thus, we performed in situ autophosphorylation (ISA) assay to detect autophosphorylation activity indicative for Rad53 activation (Fig. 1B) [10]. *RAD53-OE* showed significant autophosphorylation activity, whereas overexpressed Flag-Rad53-KD and endogenous Flag-Rad53 did not. These data suggest that overexpression leads to partial Rad53 activation in meiosis.

To test whether Rad53 overexpression affects meiotic progression, we monitored premeiotic DNA replication and nuclear

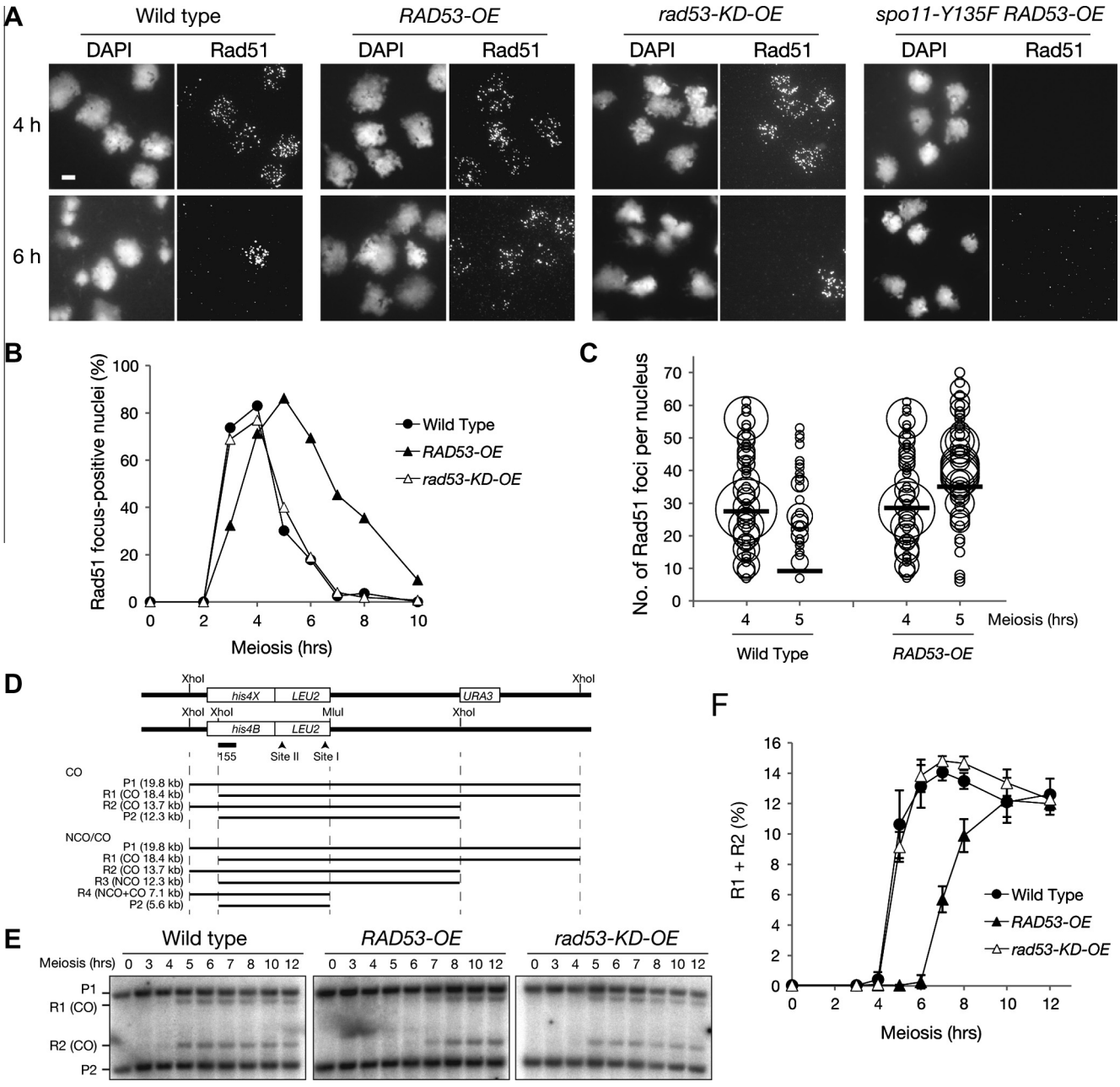


Fig. 2. Meiotic DSB repair is delayed in Rad53-overexpressing cells. (A) The representative images of Rad51 foci in spread nuclei of the indicated strains at 4 and 6 h in meiosis are shown. Bar equals 5 μm. (B) The percentage of nuclei of the indicated strains that had more than five Rad51 foci as "focus-positive nuclei" was plotted. The experiments were done at least three times, and the representative data are shown. (C) Distribution of nuclei according to Rad51 foci number per nucleus in wild type and *RAD53-OE* at 4 and 5 h in meiosis is presented. The size of the bubbles shows the percentage of nuclei which had the certain Rad51 foci number in total nuclei examined. Only nuclei which had more than five foci were plotted. Bars represent the average numbers of Rad51 foci per nucleus (27 at 4 h and 9 at 5 h in wild type, 29 at 4 h and 35 at 5 h in *RAD53-OE*). At least 100 nuclei were examined. (D) Schematic presentation of the *HIS4-LEU2* DSB site. (E) CO recombinants (R1 and R2) were detected by Southern blot using the probe pNKY155 after genomic DNA was digested with XhoI. (F) Quantitative data of R1 + R2 obtained from Southern blot are plotted. Error bars represent SD obtained from three independent cultures.

division by FACS and DAPI staining analyses, respectively. Compared to wild type, *RAD53-OE* delayed completion of premeiotic DNA replication by 1 h (Fig. 1C) and MI entry by about 3 h with 6 times more cells (20.5%) failing to enter MI than wild type (3.3%) (Fig. 1D). The delay in *RAD53-OE* depended on Rad53 kinase activity since *rad53-KD-OE* was indistinguishable from wild type (Fig. 1C and D). However, *RAD53-OE* maintained normal spore viability (98.2%, $n = 384$) comparable to wild type (99.0%, $n = 288$). These results indicate that Rad53 activation during meiosis impedes events in meiotic prophase I.

We overproduced Rad53 in a *spo11* catalytic mutant, *spo11-Y135F*, which does not make meiotic DSBs [1]. The *spo11-Y135F RAD53-OE* cells entered MI 1.5 h earlier than *RAD53-OE* but still 1.5 h later than wild type and *spo11-Y135F* cells (Fig. 1D), suggesting that *RAD53-OE* delays MI entry in meiotic DSB-dependent and -independent manners.

3.2. RAD53-OE delays meiotic DSB repair

To examine the effects of Rad53 overexpression on meiotic recombination, we observed Rad51 focus formation on meiotic chromosomes as indicative of recombination intermediates [20]. In wild type, Rad51 focus-positive nuclei (more than 5 foci/nucleus) started to appear at 3 h in meiosis, peaked at 4 h, and disappeared afterwards (Fig. 2A and B). In *RAD53-OE*, Rad51 foci appeared at 3 h to a lesser extent than those in wild type, suggesting that DSB formation is delayed (Fig. 2B). Then Rad51 focus-positive nuclei reached the wild type level at 4 h but peaked at 5 h, and disappeared slower than those in wild type, suggesting delay in DSB repair after chromatin loading of Rad51 (Fig. 2A and B). Consistently, *RAD53-OE* nuclei harbored more steady level of Rad51 foci (35 ± 19 per nucleus at 5 h, $n = 102$) than wild type nuclei (27 ± 18 at 4 h, $n = 112$) at their peak ($P = 0.0005$, Wilcoxon rank sum test) (Fig. 2B and C). The kinetics of Dmc1 focus formation was similar to that of Rad51 focus formation in *RAD53-OE* meiosis (Fig. S1). The delayed Rad51/Dmc1 appearance and disappearance in *RAD53-OE* cells required Rad53 kinase activity since *rad53-KD-OE* mutant exhibited the similar kinetics to wild type (Fig. 2A and B).

Rad51 focus formation in *RAD53-OE* depended on Spo11-dependent DSBs since no Rad51 focus was observed in *spo11-Y135F RAD53-OE* (Fig. 2A). The data excluded a possibility that Rad53 overexpression causes random DNA damage to form Rad51 foci in meiosis.

We examined interhomolog crossover (CO) formation, the repair product of meiotic DSBs, at an artificial hotspot, *HIS4-LEU2*, by Southern blotting (Fig. 2D) [23]. Concomitant with disappearance of Rad51 foci, formation of CO recombinants became

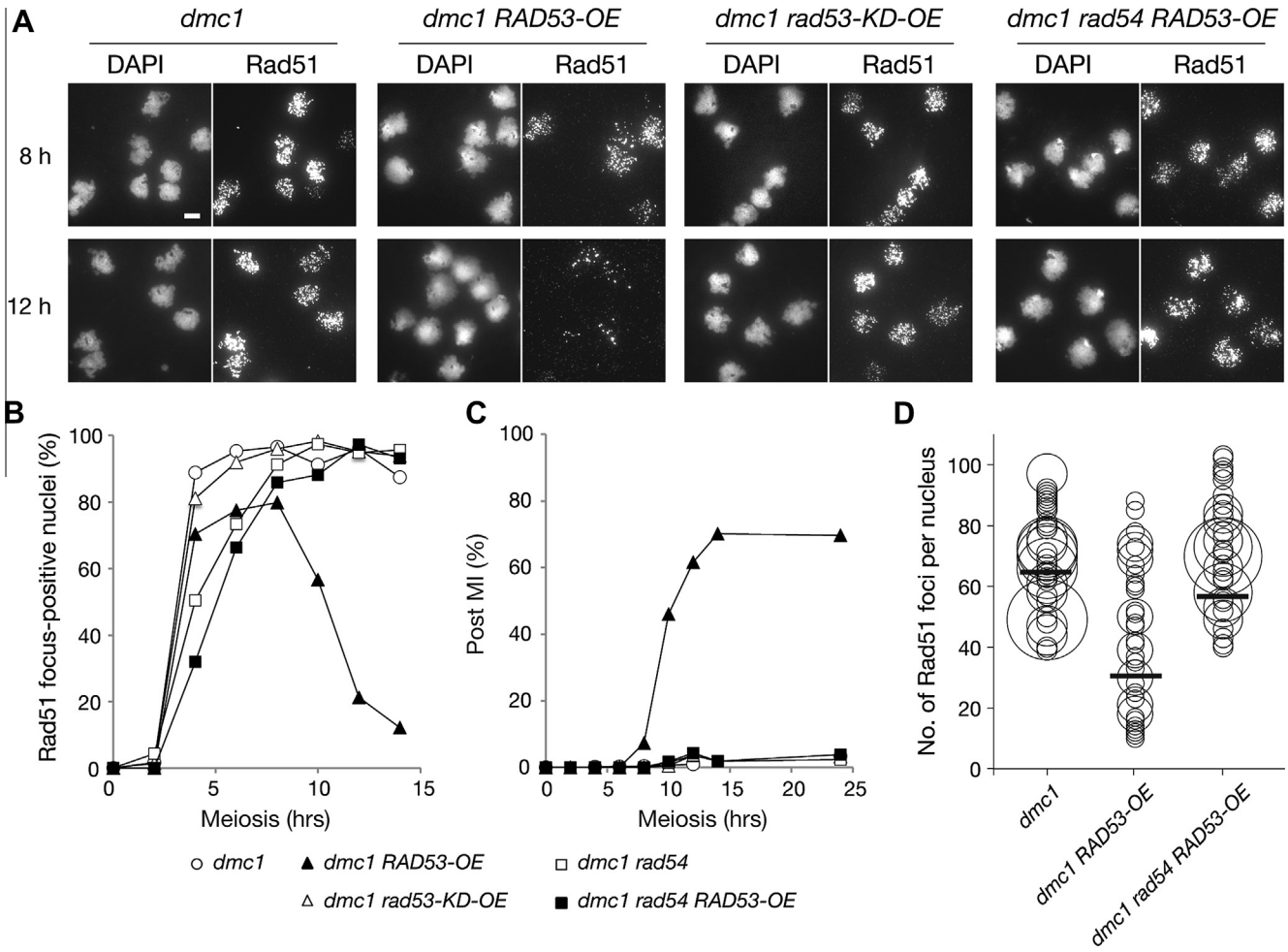


Fig. 3. Increased Rad53 kinase activity promotes meiotic DSB repair in *dmc1*. (A) The representative images of Rad51 foci in the indicated strains at 8 and 12 h in meiosis are shown. Bar equals 5 μm. (B) The percentage of the indicated strains' nuclei that had more than five Rad51 foci was plotted as in Fig. 2B. The experiments were done at least three times, and the representative data are shown. (C) Meiotic nuclear divisions were monitored as in Fig. 1D. (D) Distribution of nuclei according to Rad51 foci number per nucleus at 8 h in meiosis is presented as described in Fig. 2C. Bars represent the average numbers of Rad51 foci per nucleus (65 in *dmc1*, 30 in *dmc1 RAD53-OE*, 59 in *dmc1 rad54 RAD53-OE*). At least 50 nuclei were examined at each time point.

evident at 5 h and reached plateau at 7 h in wild type and *rad53-KD-OE* meioses whereas CO recombinant appearance delayed by 2 h and did not reached the wild type level until 10 h in *RAD53-OE* meiosis (Fig. 2E and F). These data suggest that the elevated level of Rad53 kinase activity delays interhomolog repair of meiotic DSBs.

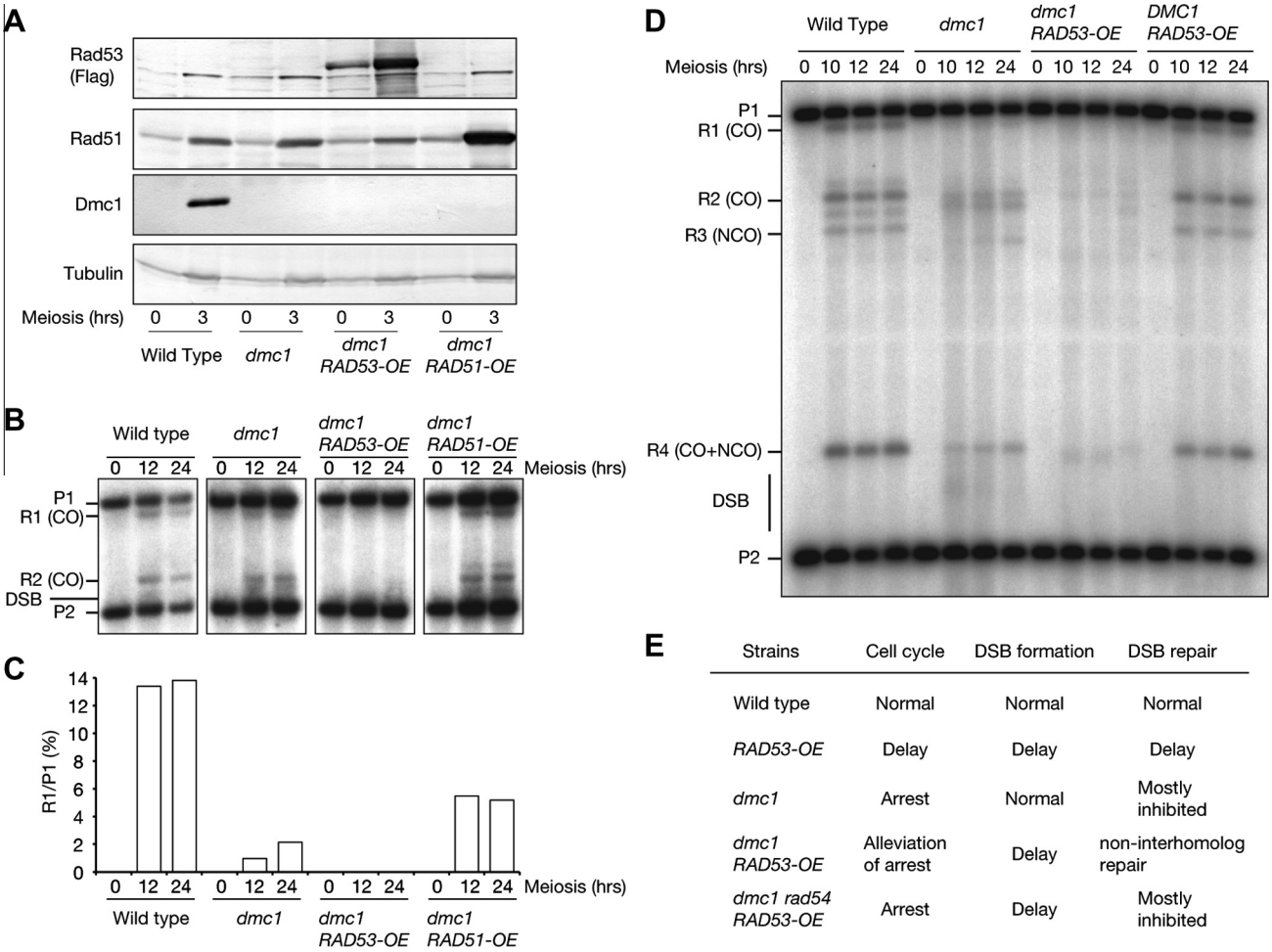
3.3. RAD53-OE potentiates Rad54-dependent homologous recombination repair of meiotic DSBs in dmc1

To test the effect of Rad53 overexpression on meiotic DSB metabolism in a *dmc1* mutant which accumulates DSBs with extensive resection but fails to activate Rad53 [3,11], Rad51 focus formation was examined. Rad51 focus-positive nuclei in *dmc1* reached a plateau by 6 h and never decreased afterwards (Fig. 3A and B). In contrast, Rad51 focus-positive nuclei in *dmc1 RAD53-OE* reached the maximum level by 8 h (79.9%), but started decreasing at 10 h (56.7%) (Fig. 3A and B). At 14 h, 12.1% of nuclei were Rad51 focus-positive in *dmc1 RAD53-OE* whereas almost nuclei were still positive in *dmc1* (Fig. 3B). Thus, the data suggest that Rad53 overexpression promotes Rad51 disassembly in *dmc1*. The Rad51 disassembly depended on the kinase activity since Rad51 foci did not disappear in *dmc1 rad53-KD-OE* (Fig. 3A and B). *dmc1* arrests in prophase I of meiosis permanently due to accumulation of unrepaired DSBs [3]. Thus, the effect of *RAD53-OE* on *dmc1*-induced cell cycle arrest was examined. Unlike *dmc1* and *dmc1 rad53-KD-OE*, 70% of *dmc1 RAD53-OE* cells finished MI at 14 h (Fig. 3C). Thus, these data suggest that meiotic DSBs are repaired in *dmc1 RAD53-OE*.

We examined whether disassembly of Rad51 foci in *dmc1 RAD53-OE* requires the Rad54 helicase since Rad54 promotes Rad51-dependent homologous recombination [18,24]. Even after 10 h in meiosis, more than 90% of *dmc1 rad54 RAD53-OE* cells sustained Rad51 focus-positive nuclei which are indistinguishable to *dmc1*, *dmc1 rad53-KD-OE*, and *dmc1 rad54* (Fig. 3A and B). Accordingly, *dmc1 rad54 RAD53-OE* exhibited permanent cell cycle arrest (Fig. 3C). At 8 h, an average number of Rad51 foci per nucleus in *dmc1 RAD53-OE* (30 ± 28, $n = 65$) was smaller than that in *dmc1* (65 ± 19, $n = 57$) ($P = 4.9 \times 10^{-10}$, Wilcoxon rank sum test), whereas *dmc1 rad54 RAD53-OE* nuclei ($n = 59$) retained 59 ± 28 foci of Rad51 which is comparable to that in *dmc1* ($P = 0.4$) (Fig. 3D), suggesting that Rad54-dependent Rad51 disassembly occurs even at 8 h in meiosis in *dmc1 RAD53-OE*. Collectively, these data suggest that elevated Rad53 kinase activity potentiates the Rad51/Rad54-dependent homologous recombination to repair meiotic DSBs in *dmc1*.

3.4. RAD53-OE inhibits interhomolog repair in dmc1

Previous studies showed that overexpression of Rad51 or Rad54 repairs meiotic DSBs by interhomolog CO recombination in *dmc1*



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Strains	Cell cycle	DSB formation	DSB repair
Wild type	Normal	Normal	Normal
RAD53-OE	Delay	Delay	Delay
dmc1	Arrest	Normal	Mostly inhibited
dmc1 RAD53-OE	Alleviation of arrest	Delay	non-interhomolog repair
dmc1 rad54 RAD53-OE	Arrest	Delay	Mostly inhibited

Fig. 4. Increased Rad53 kinase activity inhibits interhomolog repair of meiotic DSBs in *dmc1*. (A) Western blot analysis of TCA-precipitated cell extracts at 0 and 3 h in meiosis. (B) CO recombinants were detected at 0, 12 and 24 h in meiosis as in Fig. 2E. (C) Quantitative data of R1 from B are plotted. Since unrepaired DSB signals overlapped with R2, only R1 was quantified. (D) CO/NCO recombinants (as illustrated in Fig. 2D) were detected by Southern blot using the probe pNKY155 after genomic DNA was digested with XhoI and MluI. (E) Summary of meiotic phenotypes of the indicated strains.

to produce viable spores [25–27]. Since DSB repair in *dmc1 RAD53-OE* appeared to require Rad51 and Rad54, we examined whether *dmc1 RAD53-OE* generates CO recombinants, compared to *dmc1 RAD51-OE* where Rad51 was overexpressed form *DMC1pr* (Fig. 4A). CO recombinants were detected in *dmc1 RAD51-OE* but not in *dmc1 RAD53-OE* (Fig. 4B and C), suggesting that Rad53 overexpression channels DSB repair in *dmc1* into non-interhomolog CO pathway. Consistently, *dmc1 RAD51-OE* (79.9%, $n = 288$) but not *dmc1 RAD53-OE* (0.7%, $n = 288$) produced viable spores. We also checked the formation of interhomolog non-crossover (NCO), at *HIS4-LEU2* (R3 in Fig. 2C) in *dmc1*. NCO recombinants were not detected in *dmc1 RAD53-OE* as well as *dmc1* whereas *DMC1 RAD53-OE* produced NCO at a similar level to wild type (Fig. 4D). Collectively, in *dmc1 RAD53-OE*, meiotic DSBs did not appear to be repaired by interhomolog recombination.

4. Discussion

To get insights into the biological significance of Rad53's insensitivity to meiotic DSBs, we examined the influences of meiosis-specific overexpression of Rad53. Our data suggest that increased kinase activity of Rad53 has negative impacts on meiotic progression and the formation and repair of programmed DSBs.

In this study, meiosis-specific overexpression of Rad53 reveals the impacts of Rad53 kinase activation on the meiotic processes. The previous study shows that Rad53 fusion proteins with Ddc2 that binds to DSBs [8,28] are activated by meiotic DSBs, but do not affect meiotic processes [11]. Given the mobile nature of Rad53 [28,29], Rad53 tethered to DSBs by Ddc2 may lose target accessibility even though the kinase is activated. Consistently, the Ddc2-Rad53 fusion does not fully function in mitosis [30]. Overexpression may facilitate trans autophosphorylation of Rad53 required for Rad53 activation [29], providing mobile activated kinase in cells.

The delay of *RAD53-OE* in premeiotic DNA replication completion and DSB formation may be related to the finding that timing of meiotic DSB formation is tightly linked to the regional completion of DNA replication in budding yeast [31]. The recent studies show that Rad53 inhibits late origin firing in response to DNA damage in mitosis by phosphorylating Sld3 and Dbf4, the binding partner of Cdc7 kinase, both of which are required for origin firing [32,33]. Therefore, delay in DSB formation may be due to the inhibitory effect of Rad53 overexpression on genome-wide origin firing mediated by Sld3 and Cdc7-Dbf4. Alternatively, Rad53 may inhibit Cdc7-Dbf4 kinase activity to disturb the axis-loop interaction of meiotic chromosomes important for DSB formation [34]. A meiosis-specific protein Mer2, involved in the axis-loop interaction [34], is phosphorylated by Cdc7 to promote DSB formation [35]. Interestingly, Cds1, the fission yeast Rad53 ortholog, downregulates Mde2 required for the axis-loop interaction although there is no Mde2 ortholog in budding yeast [36,37].

RAD53-OE interrupted interhomolog repair of meiotic DSBs but eventually produced the wild type level of interhomolog CO/NCO recombinants, suggesting that meiotic cells overcome the negative effects of Rad53 overexpression. In contrast, when programmed DSBs were not repaired by Dmc1 deficiency, increased Rad53 kinase activity in turn promoted DSB repair. In *dmc1 RAD53-OE*, Rad54-dependent homologous recombination that promotes intersister repair with Rad51 [18] repaired meiotic DSBs, whereas residual interhomolog repair was inhibited. Thus, interhomolog interaction function of Dmc1 [15,17,18] may antagonize the effect of Rad53 overexpression on meiotic DSB repair. Interestingly, the biochemical study using human proteins shows that the D-loop structure of Dmc1 is more resistant to dissociation by Rad54 than that of Rad51 [38].

Nevertheless, we cannot conclude that meiotic DSBs in *dmc1 RAD53-OE* are repaired by intersister recombination. We found that spore inviability of *dmc1 RAD53-OE* was not suppressed by a *spo13* mutation (data not shown). Since *spo13* bypasses either MI or MII [39], viable spores are formed in a mutant (i.e., *dmc1 mek1 spo13*) where intersister recombination repairs meiotic DSBs [40]. The data raise the possibility that *dmc1 RAD53-OE* repairs meiotic DSBs by non-intersister recombination. Alternatively, given that cell cycle arrest by recombination checkpoint is alleviated when unrepaired meiotic DSBs decrease to a certain level [41], *dmc1 RAD53-OE* may promote intersister recombination to repair DSBs enough to form spores, but fail to repair overall meiotic DSBs, thus producing inviable spores. To define the molecular mechanisms of Rad53-mediated DSB repair, the search of Rad53 targets is underway.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.10.111.

References

[1] S. Keeney, C.N. Giroux, N. Kleckner, Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family, Cell 88 (1997) 375–384.
[2] A. Shinohara, H. Ogawa, T. Ogawa, Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein, Cell 69 (1992) 457–470.
[3] D.K. Bishop, D. Park, L. Xu, N. Kleckner, *DMC1*: a meiosis-specific yeast homolog of *E. coli recA* required for recombination, synaptonemal complex formation, and cell cycle progression, Cell 69 (1992) 439–456.
[4] M.J. Neale, S. Keeney, Clarifying the mechanics of DNA strand exchange in meiotic recombination, Nature 442 (2006) 153–158.
[5] M.E. Moynahan, M. Jasin, Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis, Nat. Rev. Mol. Cell Biol. 11 (2010) 196–207.
[6] K.A. Nyberg, R.J. Michelson, C.W. Putnam, T.A. Weinert, Toward maintaining the genome: DNA damage and replication checkpoints, Annu. Rev. Genet. 36 (2002) 617–656.
[7] A.J. MacQueen, A. Hochwagen, Checkpoint mechanisms: the puppet masters of meiotic prophase, Trends Cell Biol. 21 (2011) 393–400.
[8] L. Zou, S.J. Elledge, Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes, Science 300 (2003) 1542–1548.
[9] J. Majka, A. Niedziela-Majka, P.M. Burgers, The checkpoint clamp activates Mec1 kinase during initiation of the DNA damage checkpoint, Mol. Cell 24 (2006) 891–901.
[10] A. Pellicioli, S.E. Lee, C. Lucca, M. Foiani, J.E. Haber, Regulation of *Saccharomyces* Rad53 checkpoint kinase during adaptation from DNA damage-induced G2/M arrest, Mol. Cell 7 (2001) 293–300.
[11] H. Cartagena-Lirola, I. Guerini, N. Manfrini, G. Lucchini, M.P. Longhese, Role of the *Saccharomyces cerevisiae* Rad53 checkpoint kinase in signaling double-strand breaks during the meiotic cell cycle, Mol. Cell. Biol. 28 (2008) 4480–4493.
[12] L. Xu, B.M. Weiner, N. Kleckner, Meiotic cells monitor the status of the interhomolog recombination complex, Genes Dev. 11 (1997) 106–118.
[13] J.A. Carballo, A.L. Johnson, S.G. Sedgwick, R.S. Cha, Phosphorylation of the axial element protein Hop1 by Mec1/Tel1 ensures meiotic interhomolog recombination, Cell 132 (2008) 758–770.
[14] K.P. Kim, B.M. Weiner, L. Zhang, A. Jordan, J. Dekker, N. Kleckner, Sister cohesion and structural axis components mediate homolog bias of meiotic recombination, Cell 143 (2010) 924–937.
[15] S. Hong, Y. Sung, M. Yu, M. Lee, N. Kleckner, K.P. Kim, The logic and mechanism of homologous recombination partner choice, Mol. Cell 51 (2013) 440–453.
[16] J. Pan, M. Sasaki, R. Kniewel, H. Murakami, H.G. Blitzblau, S.E. Tischfield, X. Zhu, M.J. Neale, M. Jasin, N.D. Socci, A. Hochwagen, S. Keeney, A hierarchical combination of factors shapes the genome-wide topography of yeast meiotic recombination initiation, Cell 144 (2011) 719–731.

[17] A. Schwacha, N. Kleckner, Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway, Cell 90 (1997) 1123–1135.
[18] A. Arbel, D. Zenvirth, G. Simchen, Sister chromatid-based DNA repair is mediated by *RAD54*, not by *DMC1* or *TID1*, EMBO J. 18 (1999) 2648–2658.
[19] M.S. Longtine, A. McKenzie 3rd, D.J. Demarini, N.G. Shah, A. Wach, A. Brachat, P. Philippsen, J.R. Pringle, Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*, Yeast 14 (1998) 953–961.
[20] D.K. Bishop, RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis, Cell 79 (1994) 1081–1092.
[21] A. Shinohara, S. Gasior, T. Ogawa, N. Kleckner, D.K. Bishop, *Saccharomyces cerevisiae recA* homologues *RAD51* and *DMC1* have both distinct and overlapping roles in meiotic recombination, Genes Cells 2 (1997) 615–629.
[22] M. Shinohara, S.L. Gasior, D.K. Bishop, A. Shinohara, Tid1/Rdh54 promotes colocalization of Rad51 and Dmc1 during meiotic recombination, Proc. Natl. Acad. Sci. U S A 97 (2000) 10814–10819.
[23] A. Storlazzi, L. Xu, L. Cao, N. Kleckner, Crossover and noncrossover recombination during meiosis: timing and pathway relationships, Proc. Natl. Acad. Sci. U S A 92 (1995) 8512–8516.
[24] G. Petukhova, S. Stratton, P. Sung, Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins, Nature 393 (1998) 91–94.
[25] H. Tsubouchi, G.S. Roeder, The importance of genetic recombination for fidelity of chromosome pairing in meiosis, Dev. Cell 5 (2003) 915–925.
[26] M. Shinohara, K. Sakai, A. Shinohara, D.K. Bishop, Crossover interference in *Saccharomyces cerevisiae* requires a *TID1/RDH54*- and *DMC1*-dependent pathway, Genetics 163 (2003) 1273–1286.
[27] D.K. Bishop, Y. Nikolski, J. Oshiro, J. Chon, M. Shinohara, X. Chen, High copy number suppression of the meiotic arrest caused by a *dmc1* mutation: *REC114* imposes an early recombination block and *RAD54* promotes a *DMC1*-independent DSB repair pathway, Genes Cells 4 (1999) 425–444.
[28] M. Lisby, J.H. Barlow, R.C. Burgess, R. Rothstein, Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins, Cell 118 (2004) 699–713.
[29] C.S. Gilbert, C.M. Green, N.F. Lowndes, Budding yeast Rad9 is an ATP-dependent Rad53 activating machine, Mol. Cell 8 (2001) 129–136.
[30] S.J. Lee, J.K. Duong, D.F. Stern, A Ddc2-Rad53 fusion protein can bypass the requirements for *RAD9* and *MRC1* in Rad53 activation, Mol. Biol. Cell 15 (2004) 5443–5455.
[31] V. Borde, A.S. Goldman, M. Lichten, Direct coupling between meiotic DNA replication and recombination initiation, Science 290 (2000) 806–809.
[32] P. Zegerman, J.F. Diffley, Checkpoint-dependent inhibition of DNA replication initiation by Sld3 and Dbf4 phosphorylation, Nature 467 (2010) 474–478.
[33] J. Lopez-Mosqueda, N.L. Maas, Z.O. Jonsson, L.G. Defazio-Eli, J. Wohlschlegel, D.P. Toczyski, Damage-induced phosphorylation of Sld3 is important to block late origin firing, Nature 467 (2010) 479–483.
[34] S. Panizza, M.A. Mendoza, M. Berlinger, L. Huang, A. Nicolas, K. Shirahige, F. Klein, Spo11-accessory proteins link double-strand break sites to the chromosome axis in early meiotic recombination, Cell 146 (2011) 372–383.
[35] H. Sasanuma, K. Hirota, T. Fukuda, N. Kakusho, K. Kugou, Y. Kawasaki, T. Shibata, H. Masai, K. Ohta, Cdc7-dependent phosphorylation of Mer2 facilitates initiation of yeast meiotic recombination, Genes Dev. 22 (2008) 398–410.
[36] K. Ogino, H. Masai, Rad3-Cds1 mediates coupling of initiation of meiotic recombination with DNA replication. Mei4-dependent transcription as a potential target of meiotic checkpoint, J. Biol. Chem. 281 (2006) 1338–1344.
[37] T. Miyoshi, M. Ito, K. Kugou, S. Yamada, M. Furuichi, A. Oda, T. Yamada, K. Hirota, H. Masai, K. Ohta, A central coupler for recombination initiation linking chromosome architecture to S phase checkpoint, Mol. Cell 47 (2012) 722–733.
[38] D.V. Bugreev, R.J. Pezza, O.M. Mazina, O.N. Voloshin, R.D. Camerini-Otero, A.V. Mazin, The resistance of *DMC1* D-loops to dissociation may account for the *DMC1* requirement in meiosis, Nat. Struct. Mol. Biol. 18 (2011) 56–60.
[39] S. Klapholz, R.E. Esposito, Recombination and chromosome segregation during the single division meiosis in *SPO12-1* and *SPO13-1* diploids, Genetics 96 (1980) 589–611.
[40] H. Niu, L. Wan, B. Baumgartner, D. Schaefer, J. Loidl, N.M. Hollingsworth, Partner choice during meiosis is regulated by Hop1-promoted dimerization of Mek1, Mol. Biol. Cell 16 (2005) 5804–5818.
[41] S. Zanders, M.S. Brown, C. Chen, E. Alani, Pch2 modulates chromatid partner choice during meiotic double-strand break repair in *Saccharomyces cerevisiae*, Genetics 188 (2011) 511–521.