MOLECULAR AND CELLULAR BIOLOGY, July 2008, p. 4480–4493 Vol. 28, No. 14
0270-7306/08/$08.00+0 doi:10.1128/MCB.00375-08

Role of the *Saccharomyces cerevisiae* Rad53 Checkpoint Kinase in Signaling Double-Strand Breaks during the Meiotic Cell Cycle▽

Hugo Cartagena-Lirola,†‡ Ilaria Guerini,‡ Nicola Manfrini, Giovanna Lucchini, and Maria Pia Longhese*

Dipartimento di Biotecnologie e Bioscienze, P.zza della Scienza 2, Università di Milano-Bicocca, 20126 Milan, Italy

Received 5 March 2008/Returned for modification 21 April 2008/Accepted 12 May 2008

DNA double-strand breaks (DSBs) can arise at unpredictable locations after DNA damage or in a programmed manner during meiosis. DNA damage checkpoint response to accidental DSBs during mitosis requires the Rad53 effector kinase, whereas the meiosis-specific Mek1 kinase, together with Red1 and Hop1, mediates the recombination checkpoint in response to programmed meiotic DSBs. Here we provide evidence that exogenous DSBs lead to Rad53 phosphorylation during the meiotic cell cycle, whereas programmed meiotic DSBs do not. However, the latter can trigger phosphorylation of a protein fusion between Rad53 and the Mec1-interacting protein Ddc2, suggesting that the inability of Rad53 to transduce the meiosis-specific DSB signals might be due to its failure to access the meiotic recombination sites. Rad53 phosphorylation/activation is elicited when unrepaired meiosis-specific DSBs escape the recombination checkpoint. This activation requires homologous chromosome segregation and delays the second meiotic division. Altogether, these data indicate that Rad53 prevents sister chromatid segregation in the presence of unrepaired programmed meiotic DSBs, thus providing a salvage mechanism ensuring genetic integrity in the gametes even in the absence of the recombination checkpoint.

Chromosomal breaks can occur at unpredictable locations in the genome of eukaryotic cells as a result of ionizing radiation, radiomimetic chemicals, or DNA replication across nicked DNA. Moreover, they are introduced in a programmed manner to initiate meiotic recombination during meiosis, the specialized differentiation process in which two rounds of chromosome segregation follow one round of DNA replication (reviewed in references 21 and 26). In the first meiotic division (meiosis I) homologous chromosomes pair and separate, whereas sister chromatids segregate from each other in the second division (meiosis II). Both unprogrammed and programmed double-strand breaks (DSBs) can be repaired by homologous recombination. The primary function of homologous recombination in mitotic cells is to repair DSBs, whereas, during meiosis, it is essential to establish a physical connection between homologous chromosomes, thus ensuring their correct pairing and subsequent segregation at the first meiotic division. In any case, for crossovers to be functional in promoting meiosis I execution in *Saccharomyces cerevisiae*, they must occur in the context of a proteinaceous tripartite structure, the synaptonemal complex, which connects the axes of homologs along their entire lengths via a close-packed array of transverse filaments (reviewed in reference 36).

Programmed meiotic DSB formation requires the product of the meiosis-specific gene *SPO11*, which, together with several other factors, breaks both strands of a DNA molecule, creating a DSB with covalent linkages between the newly created 5′ DNA ends and a Spo11 catalytic tyrosine residue (18, 19). In *S. cerevisiae*, the highly conserved Sae2 protein and the MRX (Mre11-Rad50-Xrs2) complex catalyze the endonucleolytic cleavage of Spo11 from the 5′ DSB ends (11, 27, 28, 29, 32, 38). After Spo11 removal, one or more nucleases resect the break to generate 3′-ended single-stranded DNA (ssDNA) overhangs. The RecA-like strand exchange proteins Rad51 and Dmc1 bind such tails to form presynaptic nucleoprotein filaments, which engage in the search for homologous templates (reviewed in reference 31).

Both accidental and programmed DSB repair are coupled to cell cycle progression by surveillance mechanisms, named DNA damage checkpoint and recombination checkpoint, which delay mitotic and meiotic cell cycle progression, respectively, until DSB repair is achieved (reviewed in references 14, 21, and 22). Mechanistically, the two checkpoints are related to each other. In fact, DSB detection is accomplished in both cases by highly conserved protein kinases, among which mammalian ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and RAD3-related (ATR), as well as their *S. cerevisiae* orthologues Tel1 and Mec1. During the mitotic cell cycle, Tel1/ATM appears to bind unprocessed DSBs via the MRX/MRN complex, and its signaling activity is disrupted when DSB termini are resected (25, 30). By contrast, Mec1/ATR is thought to recognize ssDNA regions that arise after DSB processing (55).

During the meiotic cell cycle, unrepaired programmed DSBs are stably generated in *S. cerevisiae sae2*Δ or *rad50s* mutants, where Spo11 remains covalently attached to the DSB ends that therefore cannot be resected (27, 38). These meiotic aberrant intermediates activate a Tel1-dependent recombination checkpoint, which slows down meiosis I (47, 52). Unrepaired meiosis-specific DSBs with unusually long single-stranded tails are

* Corresponding author. Mailing address: Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca, P.zza della Scienza 2, 20126 Milan, Italy. Phone: 39-0264483425. Fax: 39-0264483565. E-mail: mariapia.longhese@unimib.it.

† Present address: Division of Hepatology and Gene Therapy, Universidad de Navarra, 31008 Pamplona, Spain.

‡ These two authors contributed equally to the work.

▽ Published ahead of print on 27 May 2008.

TABLE 1. *S. cerevisiae* strains used in this study[a]

Strain	Relevant genotype[b]
YLL1539/1C	*sae2*Δ::*KANMX4*/*sae2*Δ::*KANMX4 spo11*Δ::*hisG*/*spo11*Δ::*hisG*
YLL1573/2C	*MEK1-HA3*::*URA3*/*MEK1-HA3*::*URA3*
YLL1600/1A	*dmc1*Δ::*KANMX4*/*dmc1*Δ::*KANMX4 MEK1-HA3*::*URA3*/*MEK1-HA3*::*URA3*
YLL1602/2C	*sae2*Δ::*NATMX4*/*sae2*Δ::*NATMX4 MEK1-HA3*::*URA3*/*MEK1-HA3*::*URA3*
YLL1672/2C	*mek1*Δ::*HPHMX4*/*mek1*Δ::*HPHMX4*
YLL1672/6A	*dmc1*Δ::*KANMX4*/*dmc1*Δ::*KANMX4 mek1*Δ::*HPHMX4*/*mek1*Δ::*HPHMX4*
YLL1788/75B	*spo11*Δ::*NATMX4*/*spo11*Δ::*NATMX4 MEK1-HA3*::*URA3*/*MEK1-HA3*::*URA3*
YLL1970/2B	*tel1*Δ::*HPHMX4*/*tel1*Δ::*HPHMX4 sae2*Δ::*KANMX4*/*sae2*Δ::*KANMX4 MEK1-HA3*::*URA3*/*MEK1-HA3*::*URA3*
YLL1973/21B	*spo11*Δ::*NATMX4*/*spo11*Δ::*NATMX4 mek1*Δ::*HPHMX4*/*mek1*Δ::*HPHMX4*
YLL1986/13C	*mec1*Δ::*NATMX4*/*mec1*Δ::*NATMX4 sml1*Δ::*HPHMX4*/*sml1*Δ::*HPHMX4 sae2*Δ::*KANMX4*/*sae2*Δ::*KANMX4 MEK1-HA3*::*URA3*/*MEK1-HA3*::*URA3*
YLL2014/1B	*ndt80*Δ::*HPHMX4*/*ndt80*Δ::*HPHMX4 MEK1-HA3*::*URA3*/*MEK1-HA3*::*URA3*
YLL2014/11A	*sae2*Δ::*NATMX4*/*sae2*Δ::*NATMX4 ndt80*Δ::*HPHMX4*/*ndt80*Δ::*HPHMX4 MEK1-HA3*::*URA3*/*MEK1-HA3*::*URA3*
YLL2025	[*DDC2-rad53kd-3XFLAG*::*URA3*]
YLL2027	[*DDC2-RAD53-3XFLAG*::*URA3*]
YLL2032	*dmc1*Δ::*KANMX4*/*dmc1*Δ::*KANMX4* [*DDC2-rad53kd-3XFLAG*::*URA3*]
YLL2033	*dmc1*Δ::*KANMX4*/*dmc1*Δ::*KANMX4* [*DDC2-RAD53-3XFLAG*::*URA3*]
YLL2082/3B	*sae2*Δ::*HPHMX4*/*sae2*Δ::*HPHMX4 rad9*Δ::*KANMX4*/*rad9*Δ::*KANMX4*
YLL2082/12B	*rad9*Δ::*KANMX4*/*rad9*Δ::*KANMX4*
YLL2107/4C	*spo11*Δ::*NATMX4*/*spo11*Δ::*NATMX4 rad9*Δ::*HPHMX4*/*Rad9*Δ::*HPHMX4 MEK1-HA3*::*URA3*/*MEK1-HA3*::*URA3*
YLL2111/8C	*dmc1*Δ::*KANMX4*/*dmc1*Δ::*KANMX4 rad54*Δ::*NATMX4*/*rad54*Δ::*NATMX4*
YLL2111/13C	*dmc1*Δ::*KANMX4*/*dmc1*Δ::*KANMX4 rad54*Δ::*NATMX4*/*rad54*Δ::*NATMX4 mek1*Δ::*HPHMX4*/*mek1*Δ::*HPHMX4*

[a] All strains were from this study.
[b] Plasmids are indicated by brackets.

instead generated in *S. cerevisiae* cells lacking the strand exchange protein Dmc1 (5). These cells are competent to remove Spo11 from the DSB ends but are defective in strand invasion. Similarly to the DNA damage checkpoint, where generation of 3′-ended ssDNA results in Mec1 recruitment and Mec1-dependent checkpoint activation (55), activation of the recombination checkpoint in *dmc1*Δ mutants is dependent on Mec1 and its regulators Rad24 and Rad17 (23). Despite the persistence of unrepaired meiotic DSBs, *sae2*Δ and *rad50s* cells display only a transient Tel1-dependent delay of meiosis I, whereas *dmc1*Δ cells exhibit a permanent Mec1-dependent meiosis I block (47, 52), suggesting that Tel1 can sense and signal meiotic DSBs less efficiently than Mec1. Consistent with this hypothesis, Mec1 responds to a single DSB in mitosis, whereas Tel1 signaling activity becomes apparent only when multiple DSBs are generated in the absence of Mec1 (25). Interestingly, Sae2 undergoes Mec1- and Tel1-dependent phosphorylation during meiosis, with a peak at the time of DSB generation (8). Mutations altering the Sae2 [S/T]Q motifs preferred for phosphorylation by ATM/ATR-like kinases lead to the accumulation of unprocessed DSBs, as does the simultaneous absence of Mec1 and Tel1 (8), suggesting that the latter may allow DSB resection by phosphorylating Sae2.

Propagation of the checkpoint signals to the downstream targets occurs in two different ways, depending on whether the checkpoint response is elicited by accidental DSBs or by programmed meiotic DSBs. In fact, DNA damage checkpoint activation requires the effector kinase Rad53 and its adaptor Rad9 (12, 44). Rad9 first promotes Mec1-Rad53 interaction and Mec1-mediated Rad53 phosphorylation/activation (44) and then acts as a scaffold to facilitate in *trans* Rad53 autophosphorylation (12). Despite their essential role in activating the DNA damage checkpoint in response to mitotic DSBs, Rad9 and Rad53 do not appear to be involved in controlling meiosis I progression in response to meiotic programmed DSBs (23). This control instead requires the meiosis-specific proteins Mek1, Red1, and Hop1. In particular, meiotic DSB formation leads to Mec1- and Tel1-dependent Hop1 phosphorylation, which is required for Mek1 activation (6, 33, 34, 49). However, inactivation of *HOP1*, *RED1*, or *MEK1* in *dmc1*Δ cells leads to efficient repair of the breaks via intersister recombination, indicating that meiotic progression in these cells is a consequence of inappropriate repair rather than an arrest relief (33, 34, 52).

In this study, we investigated the role of Rad53 in responding to DSBs during the meiotic cell cycle. We show that Rad53 is not phosphorylated and activated as soon as programmed meiosis-specific DSBs occur, suggesting that such DSBs are hidden from the canonical Rad53-dependent DNA damage checkpoint machinery. However, Rad53 phosphorylation is triggered when unrepaired meiotic DSBs escape the recombination checkpoint-mediated prophase I arrest. This Rad53 phosphorylation and activation result in the slowing down of meiosis II.

MATERIALS AND METHODS

Yeast strains and media. Yeast strains used for this work are listed in Table 1. All the strains were SK1 derivatives that were isogenic with the NKY3000 (*MAT***a**/*MAT*α *HO*/*HO lys2*/*lys2 ura3*::*hisG*/*ura3*::*hisG leu2*::*hisG*/*leu2*::*hisG*) strain, kindly provided by N. Kleckner (Harvard University, Cambridge, MA) and R. Cha (Medical Research Council, London, United Kingdom). Heterozygous diploid strains carrying deletions of the *MEC1*, *TEL1*, *SML1*, *DMC1*, *SAE2*, *MEK1*, *RAD9*, *NDT80*, *RAD54*, or *SPO11* gene were obtained by one-step PCR disruption. Diploid strains homozygous for the above deletions were obtained after tetrad dissection of the corresponding heterozygous strains and self-diploidization of the spore carrying the desired deletion. Heterozygous diploid strains carrying the *MEK1-HA3* allele at the *MEK1* locus were generated by PCR one-step tagging, as previously described (8). Homozygous *MEK1-HA3* diploid strains were obtained by self-diploidization of spores with the desired tagged allele derived from the corresponding heterozygous strain. The *MEK1-HA3* allele was shown to be fully functional, since all the strains carrying it were undistinguishable from the isogenic untagged strains with respect to meiotic cell

cycle progression and meiotic DSB repair. pRS316 *DDC2-RAD53-3FLAG* (*DDC2-RAD53*) and pRS316 *DDC2-rad53K227A D339A-3FLAG* (*DDC2-rad53kd*) plasmids, used to transform wild-type (NKY3000) and *dmc1*Δ strains, were kindly provided by D. Stern (University of California, San Francisco) (20). The accuracy of all gene replacements and integrations was verified by Southern blot analysis or PCR.

Synchronous meiotic time course. The strains of interest were patched on YEPD (1% yeast extract, 2% Bacto peptone, 50 mg/liter adenine, 2% glucose) plates from a −80°C glycerol stock and incubated overnight at 30°C. To obtain synchronous G_1/G_0 cell population, overnight liquid YEPD cell cultures from these patches were diluted to a final concentration of 1×10^7 cells/ml in 200 ml YPA (1% yeast extract, 2% Bacto peptone, 1% potassium acetate) in a 2-liter flask and grown with vigorous shaking for 13 h at 30°C. Cells were then washed and transferred into the same volume of SPM (0.3% potassium acetate, 0.02% raffinose) to induce meiosis. Flow cytometric DNA analysis was determined on a Becton-Dickinson FACScan sorter. Nuclear division was scored with a fluorescence microscope in propidium iodide-stained cells.

Detection of meiotic DSB formation and processing. Genomic DNA was purified from cells collected from synchronized meiotic cultures, digested with EcoRI, and separated on native agarose gels. DSBs at the *THR4* hot spot were detected with a ^{32}P-labeled 1.6-kb fragment spanning the 5′ region of *THR4* as described in reference 16. This probe was obtained by PCR using oligonucleotides PRP686 (5′-GGG GTA CCC CCA AGG TAA AAT TTC ACC GCG-3′) and PRP687 (5′-GGG GTA CCC CGG CGT GCA ATA ATT GCA GAA-3′) as primers and genomic DNA as the template.

DSB end resection at the *THR4* hot spot was analyzed on alkaline agarose gels. The single-stranded probe used to detect DSB resection was obtained by in vitro transcription using Promega Riboprobe System-T7 and plasmid pML601 as the template. The latter was constructed by inserting in the pGEM-7Zf EcoRI site a 700-bp fragment containing part of the *THR4* locus (coordinates 212503 to 213199 on chromosome III), obtained by PCR by using yeast genomic DNA as template and PRP924 (5′-CGG AAT TCC ATG GAT GTT CTT GGG CTG GAT-3′) and PRP925 (5′-CGG AAT TCT GCA TGA AGA ACT GTG CCG TGA-3′) as primers.

Other techniques. The in situ autophosphorylation assay (ISA) was performed as described in reference 37. For Western blot analysis, protein extracts were prepared by trichloroacetic acid precipitation as previously described (35). Hemagglutinin-tagged proteins were detected with the monoclonal antibody 12CA5. FLAG-tagged proteins were detected with monoclonal anti-FLAG antibodies purchased from Sigma. Rad53 was detected using anti-Rad53 polyclonal antibodies kindly provided by J. Diffley (Clare Hall Laboratories, South Mimms, United Kingdom). Rad9 was detected using anti-Rad9 polyclonal antibodies kindly provided by N. Lowndes (National University of Ireland, Ireland).

RESULTS

Rad53 phosphorylation in response to accidental or programmed DSBs during meiosis. Mec1- and Tel1-dependent phosphorylation of Mek1 and Rad53 is required for their activation as protein kinases and can be detected as changes in their electrophoretic mobility (6, 12, 33, 39, 43, 44). It is known that Mek1 phosphorylation is induced by programmed DSBs that occur during meiosis (3, 6), whereas exogenous DNA lesions trigger Rad53 phosphorylation during mitosis (39). Thus, we asked whether treatment with DSB-inducing agents of cells undergoing meiosis in the absence of programmed DSBs, due to the lack of Spo11, could trigger Rad53 and Mek1 phosphorylation. Because meiotic programmed DSBs occur after completion of premeiotic DNA synthesis (time = 210 min), *spo11*Δ cells were treated for 30 min with the radiomimetic drug phleomycin 210 min after meiosis induction (Fig. 1A). As expected, due to their inability to generate meiotic DSBs, *spo11*Δ cells failed to phosphorylate Mek1 after meiosis induction in the absence of phleomycin (Fig. 1B, top). In contrast, both Rad53 and Mek1 were phosphorylated after phleomycin addition in *spo11*Δ cells (Fig. 1B), indicating that exogenous DNA damage during meiosis can trigger both Rad53 and Mek1 phosphorylation. Phleomycin-treated *spo11*Δ cells slowed down meiosis I compared to the untreated cells, and this delay was dependent on the recombination checkpoint (Fig. 1C). In fact, phleomycin-treated *spo11*Δ *mek1*Δ cells underwent meiosis I with kinetics similar to those of untreated *spo11*Δ *mek1*Δ cells and faster than those of phleomycin-treated *spo11*Δ cells (Fig. 1C). In contrast, when Rad53 phosphorylation was prevented by eliminating its regulator Rad9 (Fig. 1B, bottom), phleomycin-treated *spo11*Δ *rad9*Δ cells still slowed down meiosis I (Fig. 1C), indicating that Rad53 activation was not responsible for this delay. Thus, although Rad53 can be phosphorylated and activated in response to chemically induced DSBs, it does not induce arrest of meiosis I. Interestingly, although phleomycin-treated *spo11*Δ *mek1*Δ cells performed meiosis I with kinetics similar to those of the isogenic untreated cells, they still suffered a 60-min delay of meiosis II (Fig. 1C), suggesting that the phleomycin-induced meiosis II delay was not simply the consequence of the meiosis I delay.

The finding that Rad53 can be phosphorylated in response to chemically induced DSBs during the meiotic cell cycle prompted us to ask whether it can be phosphorylated/activated in response to meiosis-specific DSBs. Because the inability to repair meiotic DSBs is known to activate the recombination checkpoint, we analyzed the pattern of Rad53 and Mek1 phosphorylation not only during an unperturbed meiosis but also when DSB repair was prevented by the lack of Dmc1 (Fig. 2A). We also monitored in the same samples the kinetics of DSB formation at the *THR4* hot spot (Fig. 2B) by Southern blot analysis of EcoRI-digested genomic DNA run on a native agarose gel. As shown in Fig. 2, Mek1 was phosphorylated in both wild-type and *dmc1*Δ cells after transfer to sporulation medium, concomitantly with DSB formation (time = 210 min). Then, phosphorylated Mek1 decreased when DSBs were repaired in wild-type cells (time = 300 min), whereas it persisted until the end of the experiment in *dmc1*Δ cells (Fig. 2A, top), which remained arrested with undivided nuclei (data not shown) and accumulated unrepaired hyperresected DSBs (Fig. 2B). In contrast, Rad53 was not phosphorylated in wild-type cells and only slightly in *dmc1*Δ diploid cells after transfer to SPM medium (Fig. 2A, bottom). The inability to phosphorylate Rad53 was not due to competition with Mek1 for Mec1- and Tel1-mediated phosphorylation. In fact, Rad53 was only slightly phosphorylated even in meiotic *mek1*Δ cells (Fig. 2A, bottom). Thus, meiosis-specific programmed DSBs fail to trigger Rad53 phosphorylation both in the presence and in the absence of Mek1.

Targeting Rad53 to Mec1 results in Rad53 phosphorylation in response to meiotic DSB formation in both wild-type and *dmc1*Δ cells. Because Mec1 and Tel1 are required to phosphorylate and activate Mek1 in response to meiotic programmed DSBs, whereas chemically induced DSBs trigger Rad53 phosphorylation by the same kinases, the choice of using Mek1 instead of Rad53 in the recombination checkpoint cannot likely be exerted at the levels of Mec1- and/or Tel1-mediated DSB recognition. One possibility is that the DSB signals can be easily transduced to Mek1, Red1, and Hop1, because they are structural components of the meiosis-specific chromosome structure, whereas Rad53 activation might be prevented by its inability to detect Mec1/Tel1 signaling at meiotic DSBs. If this were the case, artificial targeting of Rad53 to

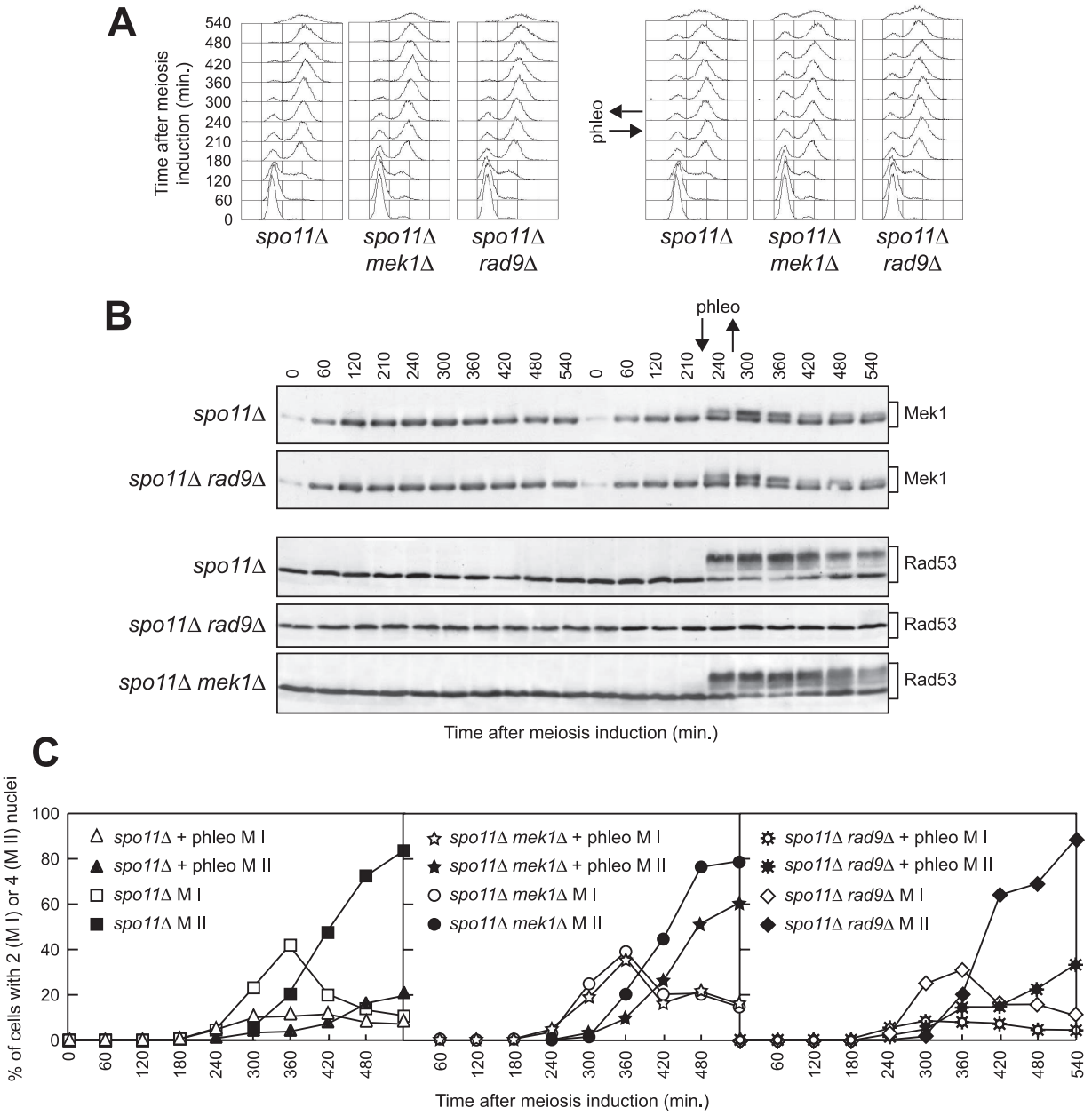

FIG. 1. Rad53 phosphorylation in response to chemically induced DSBs during meiosis I. *spo11*Δ and *spo11*Δ *rad9*Δ diploid cells expressing Mek1-HA3 from the *MEK1* promoter, as well as *spo11*Δ *mek1*Δ diploid cells, were grown to stationary phase in YPA medium and then resuspended in SPM medium at time zero. At 210 min after transfer to SPM, half of each cell culture was incubated for 30 min in the presence of 5 μg/ml of phleomycin. Cell samples were collected at the indicated time points after transfer to SPM to analyze DNA content by fluorescence-activated cell sorting analysis (A); the phosphorylation pattern of Mek1 (B, top) and Rad53 (B, bottom) by Western blot analysis with anti-HA and anti Rad53 antibodies, respectively; and the percentages of binucleate (completed meiosis I [M I]) and tetranucleate (completed meiosis II [M II]) cells (C) by fluorescence microscope analysis of propidium iodide-stained cells. In all Western analysis, the same quantity of total protein extracts was loaded in each lane according to Coomassie blue staining.

Mec1 by fusing it with the Mec1 regulatory subunit Ddc2 (35) should result in Rad53 phosphorylation/activation in response to meiotic DSB formation. In order to analyze this possibility, wild-type and *dmc1*Δ cells were transformed with a plasmid carrying a *DDC2-RAD53*-FLAG in-frame fusion, whose expression was driven by the *RAD53* upstream regulatory sequences (20). The encoded Ddc2-Rad53 fusion protein was shown to be activated in a Mec1- and Tel1-dependent manner upon DNA damage and to circumvent the Ddc2, Rad24, Rad17, Mec3, and Rad9 requirement for Rad53 activation (20). It is known that Rad53 activation requires Mec1- and Tel1-dependent phosphorylation, which allows Rad53 molecules to undergo in *trans* autophosphorylation, thus completing the Rad53 activation process (12, 37, 44). We therefore monitored the activation of the Ddc2-Rad53 chimera by evaluating its electrophoretic mobility by Western blot analysis, as well as

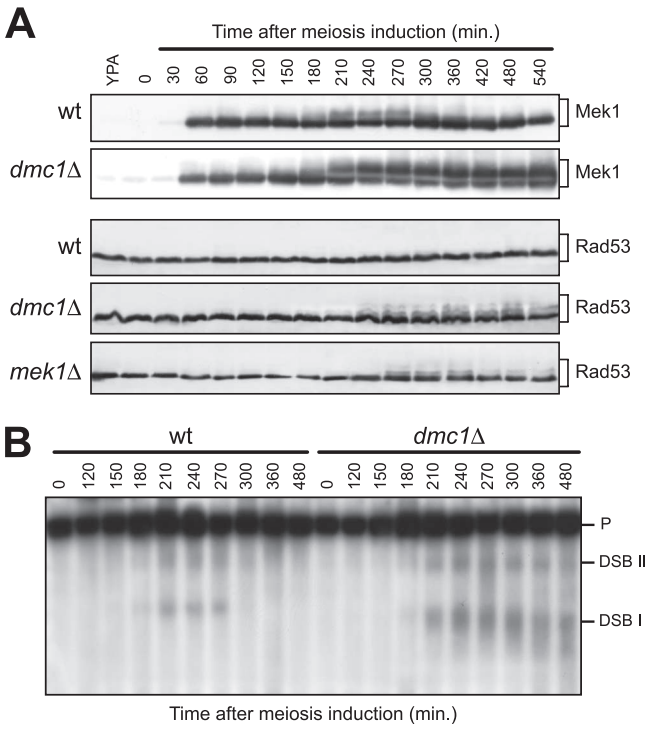

FIG. 2. Rad53 phosphorylation in response to programmed DSBs during meiosis I. Wild-type (wt) and *dmc1*Δ diploid cells expressing Mek1-HA3 from the *MEK1* promoter and *mek1*Δ diploid cells were grown to stationary phase in YPA medium and then resuspended in SPM at time zero. Cell samples were collected at the indicated time points after transfer to SPM to analyze phosphorylation of Mek1 (A, top) and Rad53 (A, bottom) as in Fig. 1B and meiotic DSB formation by Southern blot analysis (B). Southern blotting was performed on EcoRI-digested genomic DNA run on a native agarose gel, and the filter was hybridized with a probe complementary to the 5′ noncoding region of the *THR4* gene. This probe reveals an intact parental EcoRI fragment (P) of 7.9 kb and two bands of 5.7 and 7.1 kb corresponding to the two prominent meiotic DSB sites (DSB I and DSB II).

its autophosphorylation activity by an ISA (37). As a control for Rad53 activation, we analyzed also isogenic cells expressing a Ddc2-Rad53kd fusion, where Ddc2 was fused to the kinase-defective Rad53K227A D339A variant (20). As shown in Fig. 3A, wild-type and *dmc1*Δ cells expressing either the Ddc2-Rad53 or the Ddc2-Rad53kd chimeras initiated and completed premeiotic S phase with similar kinetics. Strikingly, slowly migrating forms of the Ddc2-Rad53 fusion (indicated as Ddc2-Rad53), presumably due to phosphorylation events, appeared in both wild-type and *dmc1*Δ cells (Fig. 3B) concomitantly with meiotic DSB formation (time = 210 min; Fig. 3C). Then, they decreased in wild-type cells when DSBs were repaired (time = 360 min), whereas they were detectable as the predominant forms until the end of the experiment in *dmc1*Δ cells (Fig. 3B), where DSBs could not be repaired (data not shown). Consistent with the finding that Rad53 phosphorylation was not induced by meiotic DSB formation (Fig. 2A), the endogenous Rad53 protein (indicated as Rad53) did not show significant changes in electrophoretic mobility in either wild-type or *dmc1*Δ cells (Fig. 3B).

When the same extracts were tested by ISA, a phosphorylated form corresponding to the Ddc2-Rad53 fusion protein was detected in both wild-type and *dmc1*Δ cells (time = 210 to 240 min) (Fig. 3D), and the corresponding phosphorylation reaction was dependent on Rad53 kinase activity. In fact, no phosphorylated forms were detectable by ISA in either wild-type or *dmc1*Δ cells expressing the Ddc2-Rad53kd fusion protein (Fig. 3D). Moreover, the simple proximity of Rad53 to Ddc2 in the Ddc2-Rad53 fusion was not sufficient to activate Rad53 in the absence of meiotic DSBs. In fact, similarly to the Ddc2-Rad53 mobility shift, Rad53 autophosphorylation activity became detectable in wild-type cells at the time of DSB formation (time = 210 to 240 min) and it decreased when DSBs were repaired (time = 360 min) (Fig. 3C and D), while it persisted until the end of the experiment in *dmc1*Δ cells that failed to repair meiotic DSBs (Fig. 3D) and arrested with undivided nuclei due to the presence of Mek1 (data not shown). Thus, targeting Rad53 to Mec1 through its fusion with Ddc2 triggers Rad53 activation in response to meiotic programmed DSBs. Consistent with our previous observation that Rad53 activation by exogenous DSBs did not result in meiosis I delay, wild-type cells expressing the Ddc2-Rad53 or the Ddc2-Rad53kd fusion protein progressed through meiosis with similar kinetics (data not shown).

Execution of meiosis I with unrepaired meiosis-specific DSBs triggers Rad53 phosphorylation. If the chromosome structure specifically formed during meiosis I inhibits Rad53 access to the meiotic DSB signals, unrepaired meiotic DSBs might be capable of inducing Rad53 phosphorylation once homologous chromosomes have separated from each other and cells enter meiosis II. Because the lack of Sae2 allows meiotic cells to perform meiosis I in the presence of unprocessed DSBs (Fig. 4C and D) (47, 52), we monitored Rad53 phosphorylation in *sae2*Δ cells after meiosis induction (Fig. 4A and B). Rad53 phosphorylation was detectable in *sae2*Δ cells about 300 min after meiosis induction, and it was DSB dependent, because it was prevented in *spo11*Δ *sae2*Δ cells (Fig. 4B, top). Rad53 phosphorylation in *sae2*Δ cells occurred concomitantly with homologous chromosome segregation (time = 300 min) (Fig. 4B, top, and C), well after DSB formation (Fig. 4D), whereas Mek1 phosphorylation became detectable in the same *sae2*Δ cells at the time of meiotic DSB formation (time = 210 min) (Fig. 4B, bottom, and D). This suggests that unrepaired meiotic DSBs become capable of activating Rad53 after homologous chromosome segregation, whose inhibition might therefore prevent Rad53 phosphorylation in meiotic *sae2*Δ cells. To address this point, we monitored Rad53 phosphorylation in *sae2*Δ cells lacking the meiosis-specific transcription factor Ndt80, which is required to activate transcription of middle meiosis genes (9) and whose lack causes meiotic cells to arrest at the pachytene stage of meiosis I (51). We found that Rad53 was not phosphorylated in *sae2*Δ *ndt80*Δ cells (Fig. 4B, top), which, as expected, failed to divide nuclei (Fig. 4C). The inability of *sae2*Δ *ndt80*Δ cells to phosphorylate Rad53 was not due to the failure to generate meiotic DSBs, because both these cells and *sae2*Δ cells phosphorylated Mek1 and accumulated unrepaired meiotic DSBs with similar kinetics (Fig. 4B, bottom, and D). Thus, unrepaired meiotic DSBs seem to induce Rad53 phosphorylation only after homologous chromosome segregation.

In order to further investigate this possibility, we asked whether Rad53 underwent phosphorylation in *dmc1*Δ cells that

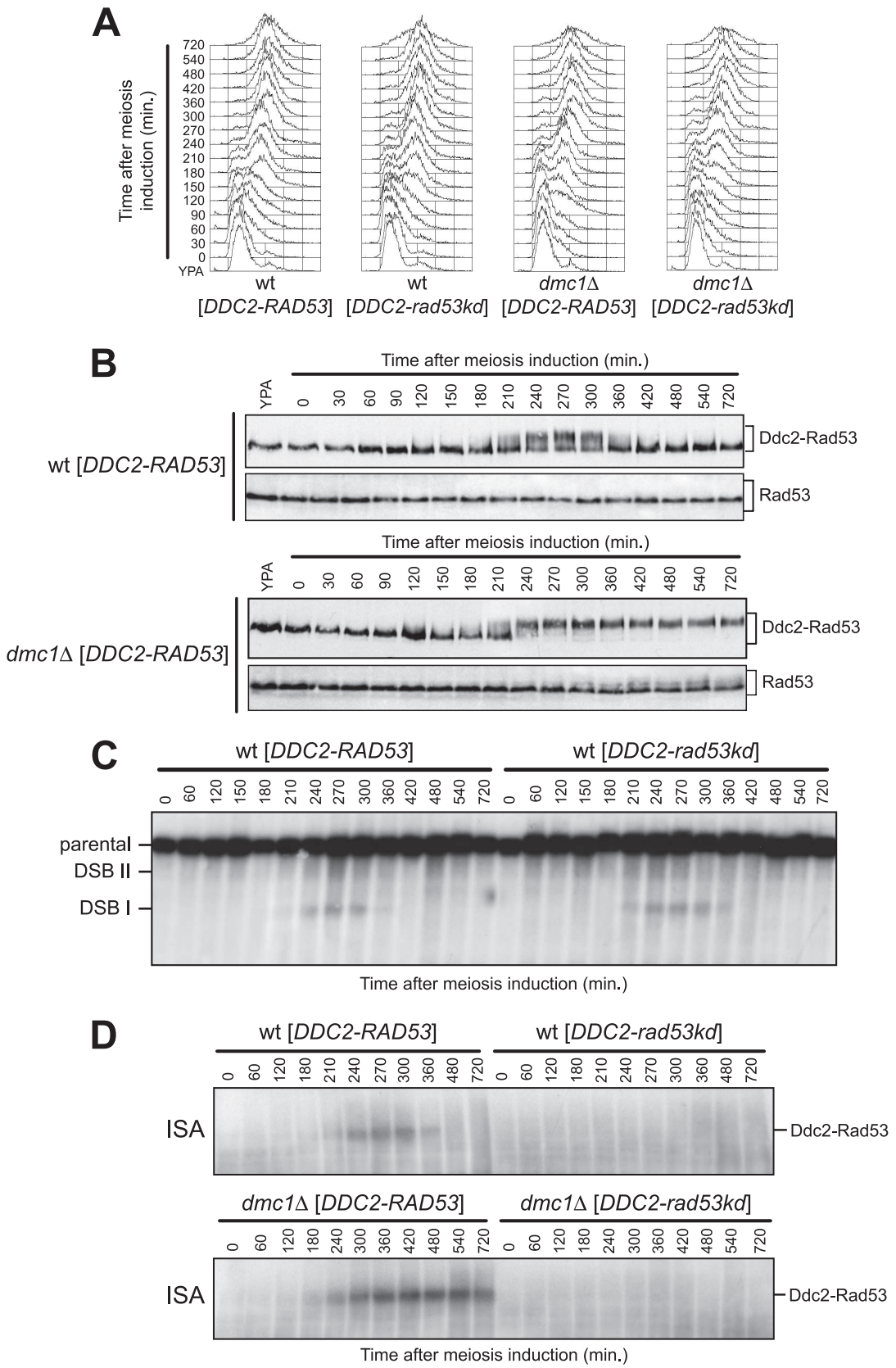

FIG. 3. Targeting Rad53 to Mec1 results in Rad53 activation in response to meiotic DSB formation. Wild-type (wt) and *dmc1*Δ diploid cells, carrying the pRS316 *DDC2-RAD53-3FLAG* plasmid (*DDC2-RAD53*) or the pRS316 *DDC2-rad53K227A D339A-3FLAG* plasmid (*DDC2-rad53kd*), were grown to stationary phase in YPA and then resuspended in SPM at time zero. Cell samples were collected at the indicated time points after transfer to SPM to analyze DNA content by fluorescence-activated cell sorting analysis (A) and DSB formation (C) by Southern blot analysis on EcoRI-digested genomic DNA as described for Fig. 2B. Total protein extracts were prepared from the indicated strains and subjected to Western blot analysis with anti-FLAG and anti-Rad53 antibodies (B) and to ISA (D).

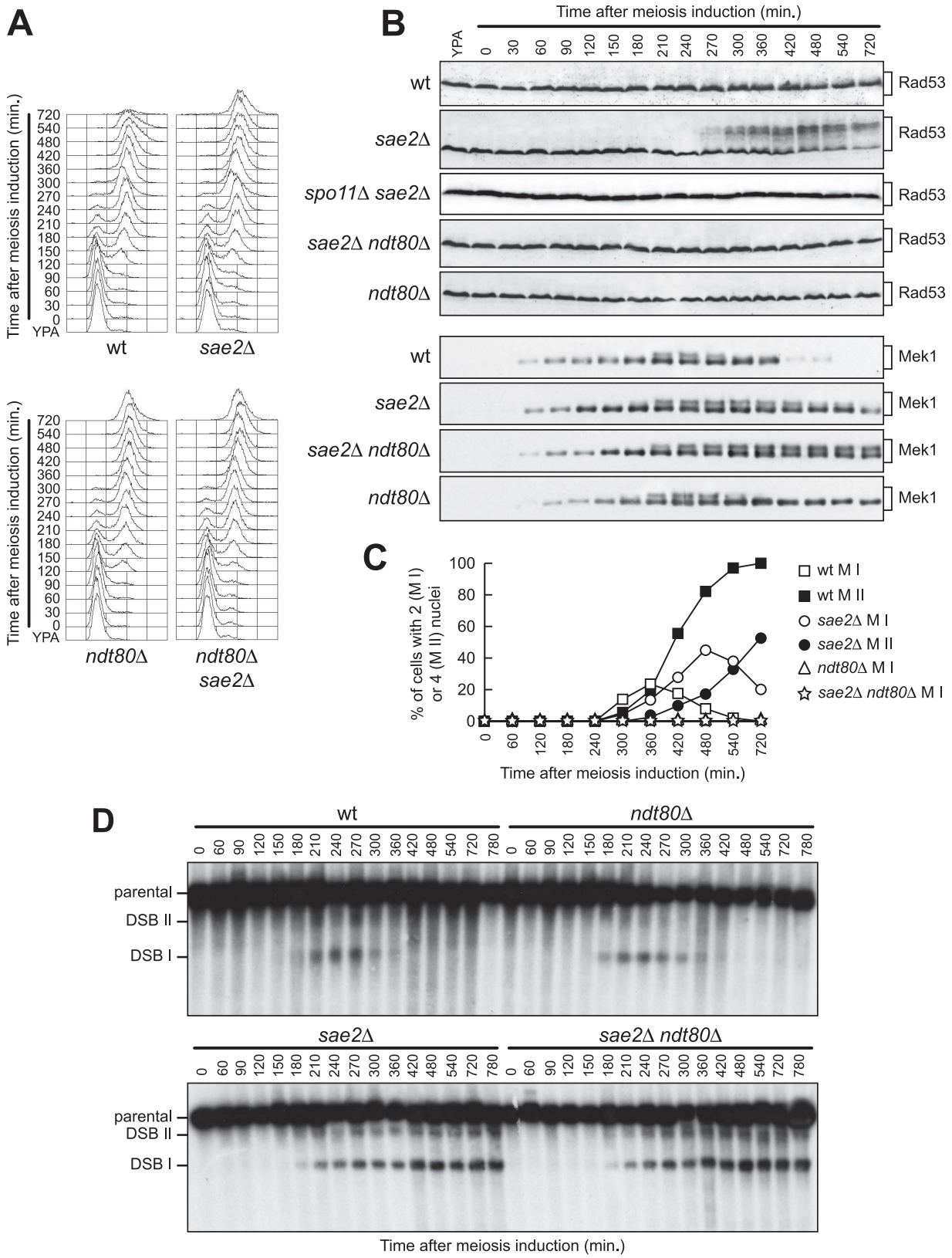

FIG. 4. Rad53 phosphorylation in *sae2*Δ meiotic cells requires homologous chromosome segregation. Wild-type (wt), *sae2*Δ, *sae2*Δ *spo11*Δ, *ndt80*Δ, and *ndt80*Δ *sae2*Δ diploid cells, all expressing Mek1-HA3 from the *MEK1* promoter, were grown to stationary phase in YPA and then resuspended in SPM at time zero. Cell samples were collected at the indicated time points after transfer to SPM to analyze DNA content (A); phosphorylation of Rad53 (B, top) and Mek1 (B, bottom); the percentages of binucleate (M I) and tetranucleate cells (M II) (C), as described for Fig. 1C; and DSB formation (D) as described for Fig. 2B.

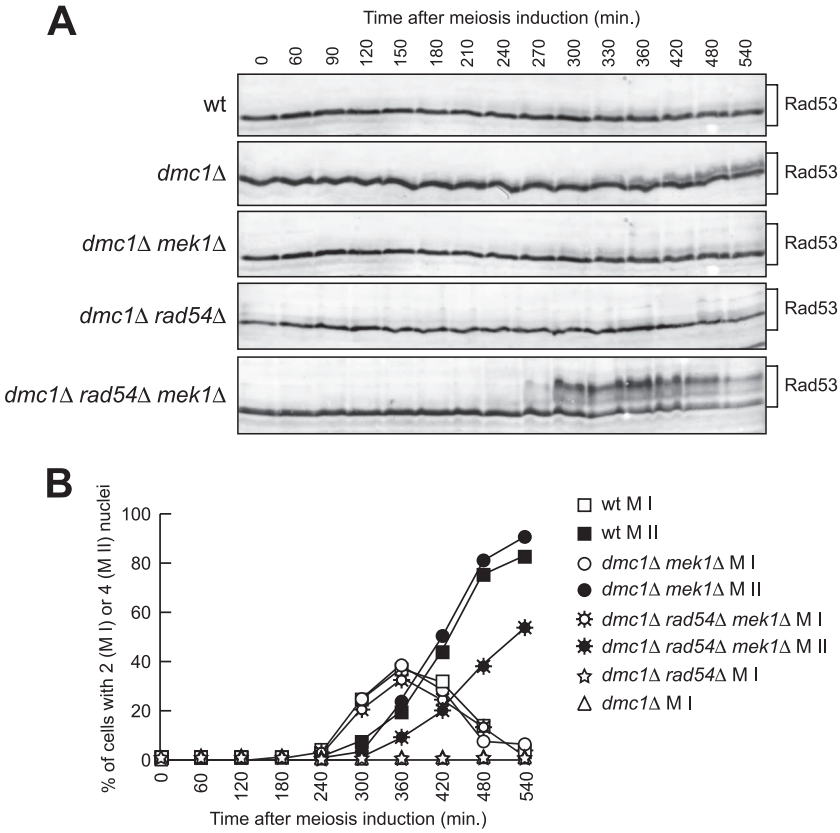

FIG. 5. Rad53 phosphorylation after segregation of homologous chromosomes carrying unrepaired meiotic DSBs. Wild-type (wt), *dmc1*Δ, *dmc1*Δ *mek1*Δ, *dmc1*Δ *rad54*Δ, and *dmc1*Δ *mek1*Δ *rad54*Δ diploid cells were grown to stationary phase in YPA and then resuspended in SPM at time zero. Cell samples were collected at the indicated time points after transfer to SPM to analyze Rad53 phosphorylation (A) and the percentages of binucleate (M I) and tetranucleate cells (M II) (B) as described for Fig. 1C.

were allowed to segregate their homologous chromosomes due to the lack of Mek1. Because *MEK1* deletion allows repair of meiotic DSBs in *dmc1*Δ cells by using sister chromatids (34), we also deleted the *RAD54* gene that is required for this repair process in *dmc1*Δ *mek1*Δ cells (34). As shown in Fig. 5, Rad53 phosphorylation became detectable in *rad54*Δ *dmc1*Δ *mek1*Δ cells at the time of homologous chromosome segregation (time = 300 min). In contrast, it was under the detection level in *dmc1*Δ cells (Fig. 5A), which failed to complete meiosis I (Fig. 5B), and in *dmc1*Δ *mek1*Δ cells (Fig. 5A), where meiotic DSBs were repaired (34; data not shown). Therefore, unrepaired meiotic DSBs induce Rad53 phosphorylation after execution of meiosis I.

The meiosis II delay in *sae2*Δ cells depends on Rad53 activation. Both *sae2*Δ cells and *dmc1*Δ *mek1*Δ *rad54*Δ cells exhibit a delay in segregating sister chromatids during meiosis II (Fig. 4C and 5B). Since meiosis II is functionally equivalent to mitosis, we asked whether Rad53 activation was responsible for the *sae2*Δ-induced meiosis II delay. We were unable to synchronize *rad53*Δ *sml1*Δ meiotic cell cultures, because the lack of Rad53 impairs cell viability and mitotic cell cycle progression even in the absence of Sml1. We therefore inactivated Rad53 by deleting the *RAD9* gene, whose product is required for Rad53 activation in response to DNA damage during the mitotic cell cycle (12, 44), while it is not involved in the recombination checkpoint (23). Rad9 is known to undergo phosphorylation by cyclin-dependent kinases in the mitotic cycle (46) as well as Mec1- and Tel1-dependent hyperphosphorylation in response to DNA damage (48). We found that Rad9 was hyperphosphorylated about 240 min after meiosis induction in *sae2*Δ cells (asterisk in Fig. 6A), similarly to Rad53 in the same cells (Fig. 6C). Furthermore, meiotic *sae2*Δ *rad9*Δ cells did not phosphorylate Rad53 (Fig. 6B and C), confirming that Rad53 activation after segregation of unrepaired homologous chromosomes depends on Rad9. Consistent with the knowledge that the Mek1-dependent checkpoint is responsible for the meiosis I delay of *sae2*Δ cells (52), both *sae2*Δ and *sae2*Δ *rad9*Δ cells showed similar delays in meiosis I execution compared to wild-type and *rad9*Δ cells (Fig. 6D). In contrast, meiosis II started earlier in *sae2*Δ *rad9*Δ cells than in *sae2*Δ cells (Fig. 6D), although it was still delayed compared to wild-type cells, due to the Mek1-dependent slowing down of meiosis I. Thus, Rad9-dependent activation of the Rad53 kinase is likely responsible for the meiosis II delay in *sae2*Δ cells.

Both Mec1 and Tel1 promote Rad53 phosphorylation after execution of meiosis I. The *sae2*Δ mutation prevents Spo11 removal from meiotic DSB ends, leading to the accumulation of unresected DSBs, which in turn triggers a delay of both meiosis I and meiosis II (1, 8). This delay is dependent on both Mec1 and Tel1, with Tel1 playing the major role (47). In contrast, Tel1 is dispensable for the meiosis I arrest of *dmc1*Δ cells, which are known to accumulate unrepaired DSBs with

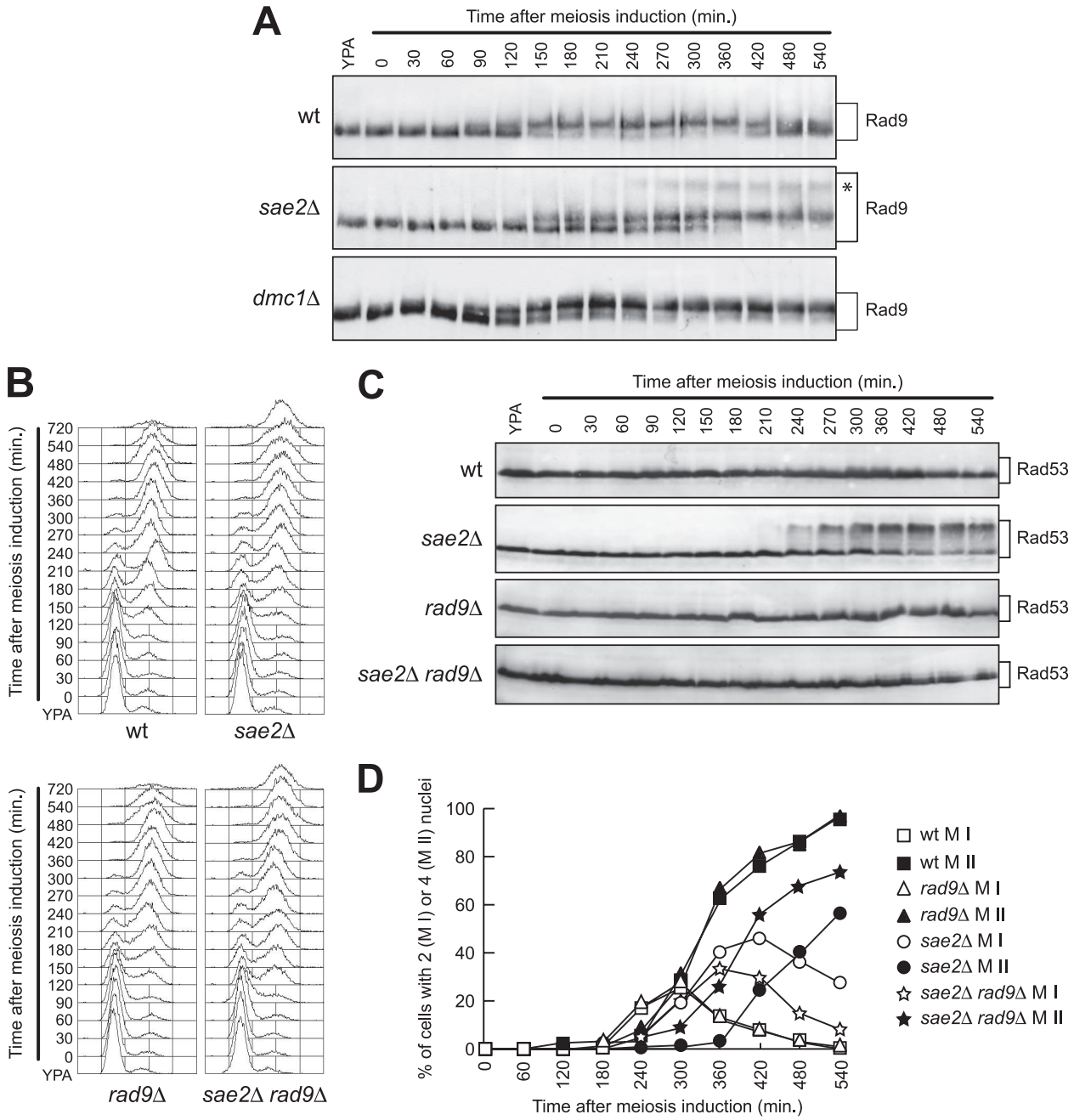

FIG. 6. Progression through meiosis in *sae2*Δ cells lacking Rad9. (A) Wild-type (wt), *sae2*Δ, and *dmc1*Δ diploid cells were grown to stationary phase in YPA and then resuspended in SPM at time zero. Total protein extracts were prepared from the indicated strains and subjected to Western blot analysis using anti-Rad9 antibodies. The asterisk points out hyperphosphorylated Rad9. (B to D) Wild-type, *sae2*Δ, *rad9*Δ, and *sae2*Δ *rad9*Δ diploid cells were grown to stationary phase in YPA and then resuspended in SPM at time zero. Samples were taken at the indicated time points for fluorescence-activated cell sorting analysis of DNA content (B), Western blot analysis of protein extracts with anti-Rad53 antibodies (C), and determination of the percentages of binucleate (M I) and tetranucleate (M II) cells (D).

unusually long single-stranded tails that are monitored by Mec1 (5, 23).

To investigate whether phosphorylation of Mek1 and Rad53 in meiotic *sae2*Δ cells had the same genetic requirements, we monitored such phosphorylation events after meiosis induction in *sae2*Δ *tel1*Δ and *sae2*Δ *mec1*Δ cells, the latter being kept viable by *SML1* deletion (54). The absence of Mec1 or Tel1 did not affect the kinetics of either premeiotic DNA replication (data not shown) or DSB accumulation (Fig. 7B). Although inactivation of either Mec1 or Tel1 affected both Mek1 and Rad53 phosphorylation in *sae2*Δ cells, their effects were quantitatively different. In fact, Mek1 phosphorylation was dramatically reduced in *sae2*Δ *tel1*Δ cells compared to *sae2*Δ cells, whereas it was only slightly affected in *sae2*Δ *mec1*Δ cells under the same conditions (Fig. 7A, left). In contrast, Rad53 phosphorylation was undetectable in *sae2*Δ *mec1*Δ cells, whereas its

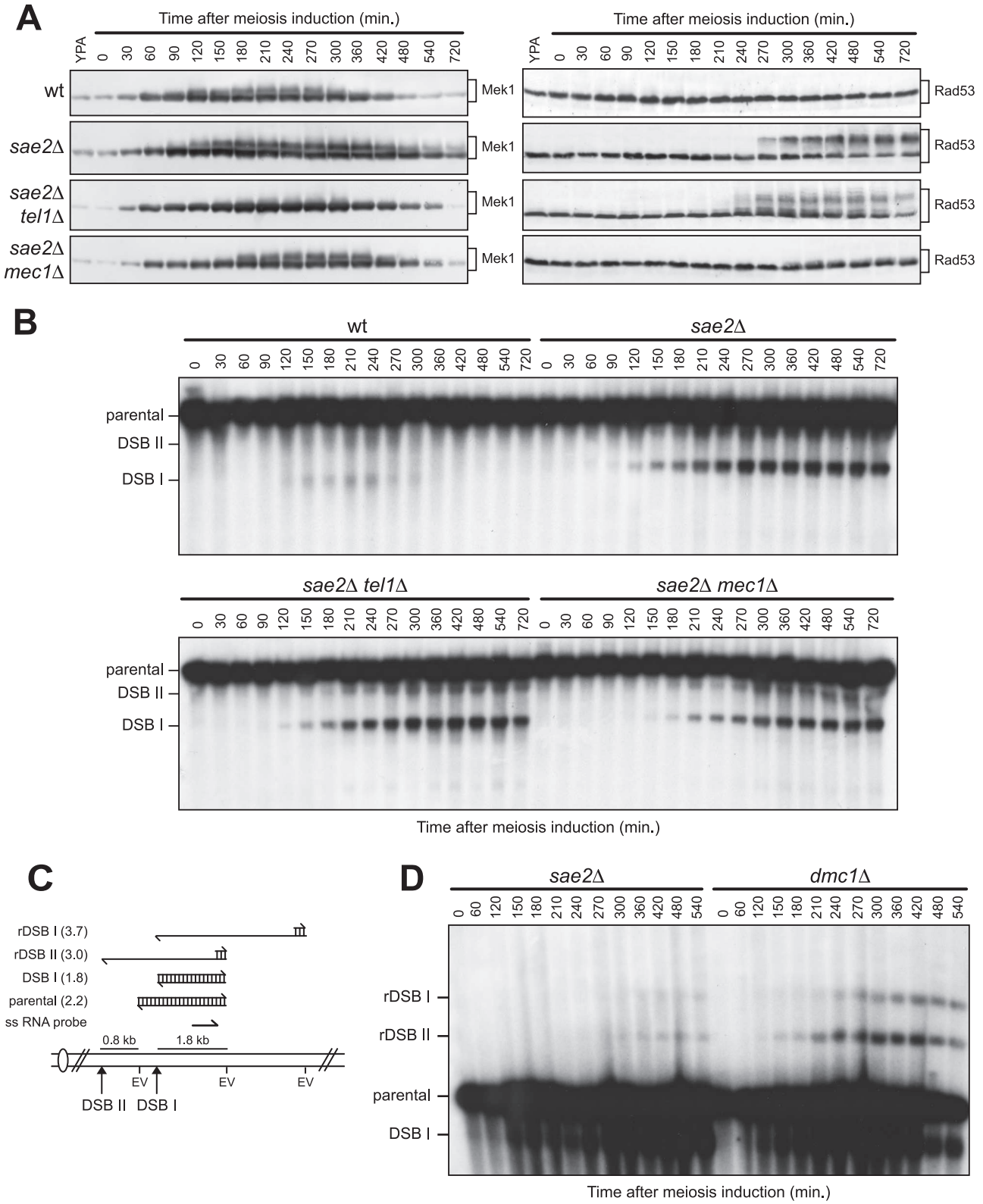

FIG. 7. Rad53 phosphorylation in meiotic *sae2*Δ cells requires the checkpoint kinases Mec1 and Tel1. (A and B) Wild-type (wt), *sae2*Δ, *sae2*Δ *tel1*Δ, and *sae2*Δ *mec1*Δ diploid cells, all expressing Mek1-HA3 from the *MEK1* promoter, were grown to stationary phase in YPA and then resuspended in SPM at time zero. Cell samples were collected at the indicated time points after transfer to SPM to analyze phosphorylation of Mek1 (A, left) and Rad53 (A, right) as described for Fig. 1B and DSB formation (B) as described for Fig. 2B. (C and D) Diploid cells carrying the *sae2*Δ or *dmc1*Δ allele were grown to stationary phase in YPA and then resuspended in SPM at time zero. (C) 5′-to-3′ resection eliminates EcoRV sites located 0.8 kb centromere-distal from DSB II and 1.8 kb centromere-distal from DSB I, producing larger EcoRV fragments (rDSB II and rDSB I) of 3 kb and 3.7 kb, respectively, detected by the probe. (D) Genomic DNA prepared from aliquots taken at the indicated times after transfer in SPM was digested with EcoRV and separated on an alkaline agarose gel. Gel blots were hybridized with a single-stranded RNA probe specific for the 5′ noncoding region of the *THR4* gene, which reveals Spo11-cut and uncut fragments of 1.8 kb and 2.2 kb, respectively.

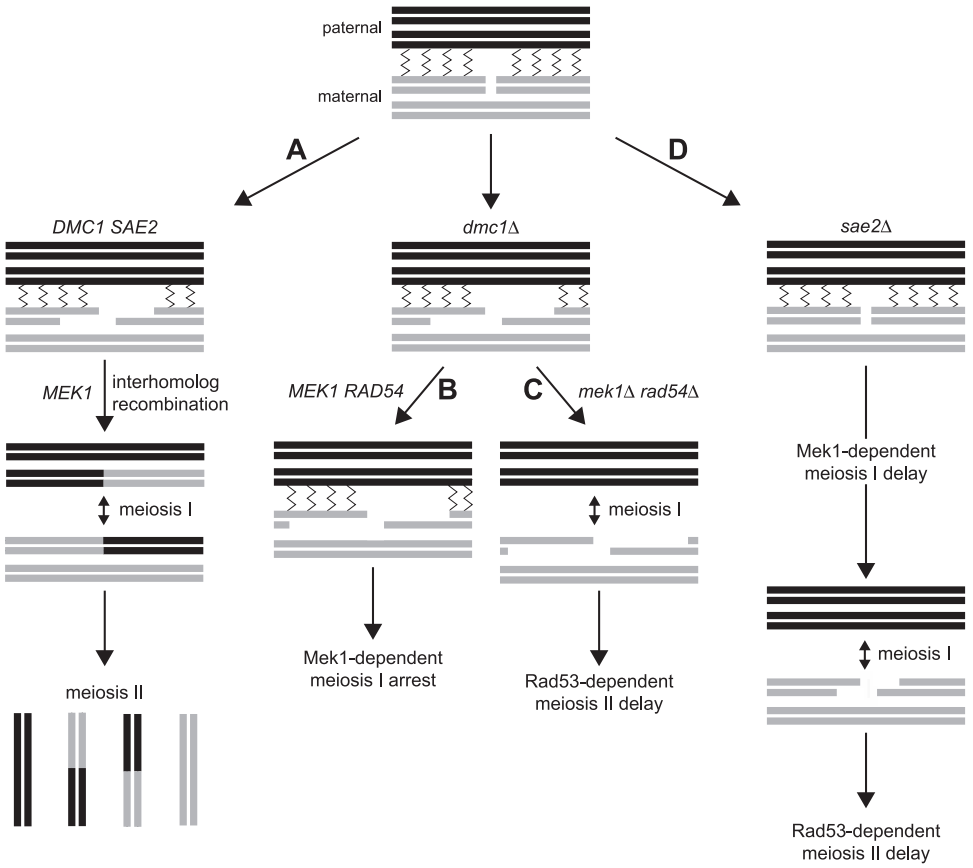

FIG. 8. Detection of meiotic DSBs by the checkpoint machineries. Homologs are indicated in black (paternal) and gray (maternal). Zigzag lines represent the meiosis-specific chromosome structure(s). In wild-type cells, DSB repair is accomplished via interhomolog recombination (A). In *dmc1*Δ cells, the inability to repair meiotic DSBs leads to Mek1 phosphorylation and a meiosis I arrest (B). Unprocessed meiotic DSBs in *sae2*Δ cells lead to a Mek1-dependent slowing down of meiosis I (D). When homologous chromosomes with unrepaired meiotic DSBs segregate from each other, these DSBs elicit a Rad53-dependent checkpoint that delays meiosis II (C and D).

amount was reduced in *sae2*Δ *tel1*Δ cells compared to *sae2*Δ cells (Fig. 7A, right). Thus, Tel1 has a major role in triggering Mek1 phosphorylation in *sae2*Δ cells, while Rad53 phosphorylation in the same cells is primarily dependent on Mec1, suggesting that the signals eliciting Mek1 and Rad53 phosphorylation in *sae2*Δ cells undergoing meiosis I and meiosis II, respectively, are different.

Because Mec1 is known to detect and signal ssDNA (55), we wondered whether *sae2*Δ unresected DSB ends might undergo some processing after homologous chromosome segregation, thus allowing their detection by Mec1 and subsequent Rad53 phosphorylation. To test this hypothesis, we monitored ssDNA formation at two meiosis-specific DSBs within the *THR4* locus (Fig. 7C). As a control for DSB end resection, we also analyzed *dmc1*Δ cells, which are known to accumulate hyperresected DSBs. As shown in Fig. 7D, 3′-ended ssDNA resection products could be detected in meiotic *sae2*Δ cells at both DSBs (rDSB I and rDSB II), although they appeared later and in lower amounts than in *dmc1*Δ cells under the same conditions. The ssDNA regions appeared in *sae2*Δ cells about 270 to 300 min after meiosis induction, at the time of Rad53 phosphorylation, when most cells had completed meiosis I (data not shown). Therefore, segregation of the homologous chromosomes containing unrepaired DSBs in *sae2*Δ cells may allow some DSB processing by unknown mechanisms, thus generating ssDNA regions that can be detected by Mec1 and induce Rad53 phosphorylation.

DISCUSSION

Accumulation of unrepaired meiosis-specific DSBs is known to activate the recombination checkpoint that arrests the meiotic cell cycle prior to meiosis I (Fig. 8B). On the other hand, the DNA damage checkpoint senses and signals DSBs that arise at unpredictable locations as a consequence of DNA damage, thus delaying the mitotic G_2/M transition (reviewed in reference 21). Although these two checkpoint mechanisms share the sensor kinases Mec1/ATR and Tel1/ATM, the meiosis-specific Mek1 kinase is the effector of the recombination checkpoint, while the Rad53 kinase is known to be essential for transducing the DNA damage checkpoint signals during mitosis.

Here, we provide evidence that Rad53 can be phosphorylated during the meiotic cell cycle after generation of chemically induced DSBs, indicating that Rad53 can be activated in response to DNA damage also during meiosis. However, Rad53 activation does not result in meiosis I delay, suggesting that the regulators of meiosis I progression

cannot be targeted by Rad53. Interestingly, neither Rad53 nor its activator Rad9 is phosphorylated and activated when programmed meiosis-specific DSBs arise during meiosis I, even when their repair is prevented by the lack of Dmc1. However, targeting Rad53 to Mec1 by a Ddc2-Rad53 chimera allows its phosphorylation and activation in response to meiotic programmed DSBs. Thus, the reason why Rad53 activation by meiotic programmed DSBs is prevented may be that Rad53 and/or Rad9 is not reachable by Mec1 signaling from the meiotic recombination sites. On the other hand, a Rad53-dependent checkpoint response, which causes a delay of the second meiotic division, is elicited when unrepaired meiosis-specific DSBs escape the recombination checkpoint-mediated prophase I arrest (Fig. 8C and D). In fact, Rad53 is phosphorylated in *dmc1*Δ *mek1*Δ *rad54*Δ cells, which fail to repair meiotic DSBs due to the absence of Rad54, but are allowed to segregate homologous chromosomes containing hyperresected DSBs due to the absence of Mek1. Moreover, Rad53 is phosphorylated in *sae2*Δ cells, which are known to perform meiosis I in the presence of unprocessed DSBs. This phosphorylation requires DSB formation and causes a delay of the second meiotic division. Unlike Mek1, whose phosphorylation is detectable concomitantly with meiotic DSB generation, Rad53 phosphorylation in both *sae2*Δ and *dmc1*Δ *mek1*Δ *rad54*Δ cells occurs at the time of meiosis I completion. Moreover, Rad53 phosphorylation is prevented in *sae2*Δ cells by eliminating Ndt80, which causes a meiosis I arrest at late prophase. This suggests that unrepaired meiosis-specific DSBs can elicit a Rad53-dependent checkpoint only when homologous chromosomes segregate from each other, and this checkpoint causes a delay in sister chromatid separation at meiosis II (Fig. 8C and D). The Rad53-dependent delay of meiosis II is transient even if meiotic DSBs are not repaired. Because meiotic cells progress through meiosis and form spores even if a DSB remains unrepaired (24), one possibility is that the checkpoint mechanism is less responsive to DNA damage during meiosis than during mitosis.

Tel1 is thought to recognize unprocessed DSBs, whereas Mec1 senses and signals ssDNA regions that arise after DSB processing and are covered by the replication protein A complex (25, 55). Consistent with previous data showing that the lack of Sae2 impairs DSB processing (10), Tel1 has the major role in triggering Mek1 phosphorylation in meiotic *sae2*Δ cells. In contrast, Mec1 has a more critical role than Tel1 in triggering Rad53 phosphorylation in the same cells. These results suggest that a subset of meiotic DSBs are processed after homologous chromosome segregation in *sae2*Δ cells, thus generating ssDNA regions that are detected by Mec1.

How can Rad53 activation in response to programmed meiotic DSBs be prevented? Inhibition of Rad53 phosphorylation during meiosis I recalls the ability of meiotic cells to generate the so-called "barrier to sister chromatid repair" (33). In fact, one of the differences between mitosis and meiosis is that meiotic DSBs are repaired using an intact homologous nonsister chromatid, whereas mitotic recombination occurs preferentially between sister chromatids (15, 40). The Mek1, Hop1, and Red1 proteins, which are structural components of the meiosis-specific chromosome structures that favor the association between homologous chromosomes, are essential to establish the correct meiotic recombination partner choice (6, 33, 34, 41, 49). Thus, one possibility is that this meiosis-specific structure (or some specific components) may not only suppress intersister DSB repair but also hide programmed meiosis-specific DSBs from being signaled as DNA damage to the Rad53 kinase, thus preventing activation of the Rad53-dependent DNA damage checkpoint during meiosis I. When homologous chromosome segregation takes place and interhomolog bias is abolished, meiotic DSBs that are not yet repaired could then be monitored as DNA damage by the canonical Rad53-dependent DNA damage checkpoint machinery. Exogenous DSBs during meiosis I may in turn cause a local disruption of the meiosis-specific chromosome structure, thus allowing Rad53 to be phosphorylated and activated.

Given that DSB-induced Mek1 activation is required to ensure the formation of interhomolog crossovers (34), the meiosis-specific propagation of the checkpoint signals through Mek1, Red1, and Hop1 instead of Rad53 is likely critical for the formation of genetically balanced gametes. In fact, reduced Mek1 phosphorylation would allow meiosis to proceed without the correct repair partner choice and formation of chiasmata, which are critical for proper meiotic chromosome segregation.

The meiosis-specific large-scale structure does not prevent sensing and signaling of meiotic programmed DSBs by Mek1, Red1, Hop1, Mec1, and Tel1, possibly because they are part of the normal recombination machinery. In fact, Mek1, Red1, and Hop1 proteins are structural components of the meiotic chromosome axes (3, 50, 52). Moreover, Mec1 loss of function leads to a number of meiotic defects, including aberrant chromosome synapsis, reduced recombination frequency and spore viability, and loss of interhomolog bias and of crossover control (reviewed in reference 7). In higher eukaryotes, both ATR and ATM associate with different sites along meiotically pairing chromosomes (17), and ATM-deficient mice show aberrant synapsis with unpaired axial cores and fragmented synaptonemal complexes (4, 53). Finally, mutations in the *RAD17*, *RAD24*, or *MEC3* gene, encoding regulators of Mec1 activity, reduce meiotic interhomolog recombination frequency, while increasing the frequency of ectopic recombination events and of illegitimate repair from the sister chromatids (2, 13, 42, 45).

In conclusion, whereas accidental DSBs induce a Rad53-dependent DNA damage response during both mitosis and meiosis, meiotic DSB repair is monitored by a meiosis-specific checkpoint mechanism involving integral components of the chromosomal structures specifically formed during meiosis. On the other hand, when meiosis I takes place despite unrepaired meiotic DSBs, the latter can trigger a Rad53-dependent DNA damage checkpoint slowing down the second meiotic division, which is functionally equivalent to mitosis. The possibility of activating this checkpoint might provide a salvage mechanism preventing chromosome rearrangements and/or loss in the gametes even in the absence of the recombination checkpoint, thereby further protecting the offspring from birth defects and cancer predisposition.

ACKNOWLEDGMENTS

We thank R. Cha, J. Diffley, N. Kleckner, N. Lowndes, and D. Stern for yeast strains and plasmids; M. Foiani for critical reading of the

manuscript; and all the members of our laboratory for useful discussions and criticisms.

This work was supported by grants from Associazione Italiana per la Ricerca sul Cancro and Cofinanziamento MIUR/Università di Milano-Bicocca to M.P.L. H. Cartagena-Lirola was supported by an EC Research Training Network Grant (HPRN-CT-2002-00238).

REFERENCES

1. **Alani, E., R. Padmore, and N. Kleckner.** 1990. Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell **61:**419–436.
2. **Aylon, Y., and M. Kupiec.** 2003. The checkpoint protein Rad24 of *Saccharomyces cerevisiae* is involved in processing double-strand break ends and in recombination partner choice. Mol. Cell. Biol. **23:**6585–6596.
3. **Bailis, J. M., and G. S. Roeder.** 1998. Synaptonemal complex morphogenesis and sister-chromatid cohesion require Mek1-dependent phosphorylation of a meiotic chromosomal protein. Genes Dev. **12:**3551–3563.
4. **Barlow, C., M. Liyanage, P. B. Moens, M. Tarsounas, K. Nagashima, K. Brown, S. Rottinghaus, S. P. Jackson, D. Tagle, T. Ried, and A. Wynshaw-Boris.** 1998. Atm deficiency results in severe meiotic disruption as early as leptonema of prophase I. Development **125:**4007–4017.
5. **Bishop, D. K., D. Park, L. Xu, and N. Kleckner.** 1992. *DMC1*: a meiosis-specific yeast homolog of *E. coli* recA required for recombination, synaptonemal complex formation, and cell cycle progression. Cell **69:**439–456.
6. **Carballo, J. A., A. L. Johnson, S. G. Sedwick, and R. S. Cha.** 2008. Phosphorylation of the axial element protein Hop1 by Mec1/Tel1 ensures meiotic interhomolog recombination. Cell **132:**758–770.
7. **Carballo, J. A., and R. S. Cha.** 2007. Meiotic roles of Mec1, a budding yeast homolog of mammalian ATR/ATM. Chromosome Res. **15:**539–550.
8. **Cartagena-Lirola, H., I. Guerini, V. Viscardi, G. Lucchini, and M. P. Longhese.** 2006. Budding yeast Sae2 is an in vivo target of the Mec1 and Tel1 checkpoint kinases during meiosis. Cell Cycle **5:**1549–1559.
9. **Chu, S., and I. Herskowitz.** 1998. Gametogenesis in yeast is regulated by a transcriptional cascade dependent on Ndt80. Mol. Cell **1:**685–696.
10. **Clerici, M., D. Mantiero, G. Lucchini, and M. P. Longhese.** 2006. The *Saccharomyces cerevisiae* Sae2 protein negatively regulates DNA damage checkpoint signalling. EMBO Rep. **7:**212–218.
11. **Furuse, M., Y. Nagase, H. Tsubouchi, K. Murakami-Murofushi, T. Shibata, and K. Ohta.** 1998. Distinct roles of two separable in vitro activities of yeast Mre11 in mitotic and meiotic recombination. EMBO J. **17:**6412–6425.
12. **Gilbert, C. S., C. M. Green, and N. F. Lowndes.** 2001. Budding yeast Rad9 is an ATP-dependent Rad53 activating machine. Mol. Cell **8:**129–136.
13. **Grushcow, J. M., T. M. Holzen, K. J. Park, T. Weinert, M. Lichten, and D. K. Bishop.** 1999. *Saccharomyces cerevisiae* checkpoint genes *MEC1*, *RAD17* and *RAD24* are required for normal meiotic recombination partner choice. Genetics **153:**607–620.
14. **Hochwagen, A., and A. Amon.** 2006. Checking your breaks: surveillance mechanisms of meiotic recombination. Curr. Biol. **16:**217–228.
15. **Kadyk, L. C., and L. H. Hartwell.** 1992. Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae*. Genetics **132:**387–402.
16. **Kee, K., and S. Keeney.** 2002. Functional interactions between *SPO11* and *REC102* during initiation of meiotic recombination in *Saccharomyces cerevisiae*. Genetics **160:**111–122.
17. **Keegan, K. S., D. A. Holtzman, A. W. Plug, E. R. Christenson, E. E. Brainerd, G. Flaggs, N. J. Bentley, E. M. Taylor, M. S. Meyn, S. B. Moss, A. M. Carr, T. Ashley, and M. F. Hoekstra.** 1996. The Atr and Atm protein kinases associate with different sites along meiotically pairing chromosomes. Genes Dev. **10:**2423–2437.
18. **Keeney, S., C. N. Giroux, and N. Kleckner.** 1997. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:**375–384.
19. **Keeney, S., and N. Kleckner.** 1995. Covalent protein-DNA complexes at the 5′ strand termini of meiosis-specific double-strand breaks in yeast. Proc. Natl. Acad. Sci. USA **92:**11274–11278.
20. **Lee, S. J., J. K. Duong, and D. F. Stern.** 2004. A Ddc2-Rad53 fusion protein can bypass the requirements for *RAD9* and *MRC1* in Rad53 activation. Mol. Biol. Cell **15:**5443–5455.
21. **Longhese, M. P., I. Guerini, V. Baldo, and M. Clerici.** 2008. Surveillance mechanisms monitoring chromosome breaks during mitosis and meiosis. DNA Repair **7:**545–557.
22. **Longhese, M. P., D. Mantiero, and M. Clerici.** 2006. The cellular response to chromosome breakage. Mol. Microbiol. **60:**1099–1108.
23. **Lydall, D., Y. Nikolsky, D. K. Bishop, and T. Weinert.** 1996. A meiotic recombination checkpoint controlled by mitotic checkpoint genes. Nature **383:**840–843.
24. **Malkova, A., L. Ross, D. Dawson, M. F. Hoekstra, and J. E. Haber.** 1996. Meiotic recombination initiated by a double-strand break in rad50 delta yeast cells otherwise unable to initiate meiotic recombination. Genetics **143:**741–754.
25. **Mantiero, D., M. Clerici, G. Lucchini, and M. P. Longhese.** 2007. Dual role for *Saccharomyces cerevisiae* Tel1 in the checkpoint response to double-strand breaks. EMBO Rep. **8:**380–387.
26. **Marston, A. L., and A. Amon.** 2004. Meiosis: cell-cycle controls shuffle and deal. Nat. Rev. Mol. Cell Biol. **5:**983–997.
27. **McKee, A. H. Z., and N. Kleckner.** 1997. A general method for identifying recessive diploid-specific mutations in *Saccharomyces cerevisiae*, its application to the isolation of mutants blocked at intermediate stages of meiotic prophase and characterization of a new gene *SAE2*. Genetics **146:**797–816.
28. **Moreau, S., J. R. Ferguson, and L. S. Symington.** 1999. The nuclease activity of Mre11 is required for meiosis but not for mating type switching, end joining, or telomere maintenance. Mol. Cell. Biol. **19:**556–566.
29. **Nairz, K., and F. Klein.** 1997. *mre11S*—a yeast mutation that blocks double-strand-break processing and permits nonhomologous synapsis in meiosis. Genes Dev. **11:**2272–2290.
30. **Nakada, D., K. Matsumoto, and K. Sugimoto.** 2003. ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. Genes Dev. **16:**1957–1962.
31. **Neale, M. J., and S. Keeney.** 2006. Clarifying the mechanics of DNA strand exchange in meiotic recombination. Nature **442:**153–158.
32. **Neale, M. J., J. Pan, and S. Keeney.** 2005. Endonucleolytic processing of covalent protein-linked DNA double-strand breaks. Nature **436:**1053–1057.
33. **Niu, H., X. Li, E. Job, C. Park, D. Moazed, S. P. Gygi, and N. Hollingsworth.** 2007. Mek1 kinase is regulated to suppress double-strand break repair between sister chromatids during budding yeast meiosis. Mol. Cell. Biol. **27:**5456–5467.
34. **Niu, H., L. Wan, B. Baumgartner, D. Schaefer, J. Loidl, and N. M. Hollingsworth.** 2005. Partner choice during meiosis is regulated by Hop1-promoted dimerization of Mek1. Mol. Biol. Cell **16:**5804–5818.
35. **Paciotti, V., M. Clerici, G. Lucchini, and M. P. Longhese.** 2000. The checkpoint protein Ddc2, functionally related to *S. pombe* Rad26, interacts with Mec1 and is regulated by Mec1-dependent phosphorylation in budding yeast. Genes Dev. **14:**2046–2059.
36. **Page, S. L., and R. S. Hawley.** 2004. The genetics and molecular biology of the synaptonemal complex. Annu. Rev. Cell Dev. Biol. **20:**525–558.
37. **Pellicioli, A., C. Lucca, G. Liberi, F. Marini, M. Lopes, P. Plevani, A. Romano, P. P. Di Fiore, and M. Foiani.** 1999. Activation of Rad53 kinase in response to DNA damage and its effect in modulating phosphorylation of the lagging strand DNA polymerase. EMBO J. **18:**6561–6572.
38. **Prinz, S., A. Amon, and F. Klein.** 1997. Isolation of *COM1*, a new gene required to complete meiotic double-strand break-induced recombination in *Saccharomyces cerevisiae*. Genetics **146:**781–795.
39. **Sanchez, Y., J. Bachant, H. Wang, F. Hu, D. Liu, M. Tetzlaff, and S. J. Elledge.** 1999. Control of the DNA damage checkpoint by Chk1 and Rad53 protein kinases through distinct mechanisms. Science **286:**1166–1171.
40. **Schwacha, A., and N. Kleckner.** 1994. Identification of joint molecules that form frequently between homologs but rarely between sister chromatids during yeast meiosis. Cell **76:**51–63.
41. **Schwacha, A., and N. Kleckner.** 1997. Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. Cell **90:**1123–1135.
42. **Shinohara, M., K. Sakai, T. Ogawa, and A. Shinohara.** 2003. The mitotic DNA damage checkpoint proteins Rad17 and Rad24 are required for repair of double-strand breaks during meiosis in yeast. Genetics **164:**855–865.
43. **Sun, Z., J. Hsiao, D. S. Fay, and D. F. Stern.** 1998. Rad53 FHA domain associated with phosphorylated Rad9 in the DNA damage checkpoint. Science **281:**272–274.
44. **Sweeney, F. D., F. Yang, A. Chi, J. Shabanowitz, D. F. Hunt, and D. Durocher.** 2005. *Saccharomyces cerevisiae* Rad9 acts as a Mec1 adaptor to allow Rad53 activation. Curr. Biol. **15:**1364–1375.
45. **Thompson, D. A., and F. W. Stahl.** 1999. Genetic control of recombination partner preference in yeast meiosis. Isolation and characterization of mutants elevated for meiotic unequal sister-chromatid recombination. Genetics **153:**621–641.
46. **Ubersax, J. A., E. L. Woodbury, P. N. Quang, M. Paraz, J. D. Blethrow, K. Shah, K. M. Shokat, and D. O. Morgan.** 2003. Targets of the cyclin-dependent kinase Cdk1. Nature **425:**859–864.
47. **Usui, T., H. Ogawa, and J. H. J. Petrini.** 2001. A DNA damage response pathway controlled by Tel1 and the Mre11 complex. Mol. Cell **7:**1255–1266.
48. **Vialard, J. E., C. S. Gilbert, C. M. Green, and N. F. Lowndes.** 1998. The budding yeast Rad9 checkpoint protein is subjected to Mec1/Tel1-dependent hyperphosphorylation and interacts with Rad53 after DNA damage. EMBO J. **17:**5679–5688.
49. **Wan, L., T. de los Santos, C. Zhang, K. Shokat, and N. M. Hollingsworth.** 2004. Mek1 kinase activity functions downstream of *RED1* in the regulation of meiotic double strand break repair in budding yeast. Mol. Biol. Cell **15:**11–23.
50. **Woltering, D., B. Baumgartner, S. Bagchi, B. Larkin, J. Loidl, T. de los Santos, and N. M. Hollingsworth.** 2000. Meiotic segregation, synapsis, and recombination checkpoint functions require physical interaction be-

tween the chromosomal proteins Red1p and Hop1p. Mol. Cell. Biol. **20:**6646–6658.
51. **Xu, L., M. Ajimura, R. Padmore, C. Klein, and N. Kleckner.** 1995. *NDT80*, a meiosis-specific gene required for exit from pachytene in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **15:**6572–6581.
52. **Xu, L., B. M. Weiner, and N. Kleckner.** 1997. Meiotic cells monitor the status of the interhomolog recombination complex. Genes Dev. **11:**106–118.
53. **Xu, Y., T. Ashley, E. E. Brainerd, R. T. Bronson, M. S. Meyn, and D. Baltimore.** 1996. Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. Genes Dev. **10:**2411–2422.
54. **Zhao, X., E. G. D. Muller, and R. Rothstein.** 1998. A suppressor of two essential checkpoint genes identifies a novel protein that negatively affects dNTP pools. Mol. Cell **2:**329–340.
55. **Zou, L., and S. J. Elledge.** 2003. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science **300:**1542–1548.