Prevention of DNA Rereplication Through a Meiotic Recombination Checkpoint Response

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ABSTRACT In the budding yeast Saccharomyces cerevisiae, unnatural stabilization of the cyclin-dependent kinase inhibitor Sic1 during meiosis can trigger extra rounds of DNA replication. When programmed DNA double-strand breaks (DSBs) are generated but not repaired due to absence of DMC1, a pathway involving the checkpoint gene RAD17 prevents this DNA rereplication. Further genetic analysis has now revealed that prevention of DNA rereplication also requires MEC1, which encodes a protein kinase that serves as a central checkpoint regulator in several pathways including the meiotic recombination checkpoint response. Downstream of MEC1, MEK1 is required through its function to inhibit repair between sister chromatids. By contrast, meiotic recombination checkpoint effectors that regulate gene expression and cyclin-dependent kinase activity are not necessary. Phosphorylation of histone H2A, which is catalyzed by Mec1 and the related Tel1 protein kinase in response to DSBs, and can help coordinate activation of the Rad53 checkpoint protein kinase in the mitotic cell cycle, is required for the full checkpoint response. Phosphorylation sites that are targeted by Rad53 in a mitotic S phase checkpoint response are also involved, based on the behavior of cells containing mutations in the DBF4 and SLD3 DNA replication genes. However, RAD53 does not appear to be required, nor does RAD9, which encodes a mediator of Rad53, consistent with their lack of function in the recombination checkpoint pathway that prevents meiotic progression. While this response is similar to a checkpoint mechanism that inhibits initiation of DNA replication in the mitotic cell cycle, the evidence points to a new variation on DNA replication control.

DNA replication during meiosis generates the necessary chromosomal content for the subsequent formation of haploid gametes through two consecutive rounds of chromosome segregation. As during the mitotic cell cycle, meiotic DNA replication is tightly regulated so that initiation occurs at precisely the correct time, and only once during the process (Strich 2004); in the absence of appropriate controls, errors such as DNA rereplication can occur that are typically harmful to the cell. Cyclin-dependent kinase (CDK) complexes are central regulators of eukaryotic DNA replication initiation, both in the mitotic cell cycle (Siddiqui et al. 2013) and in meiosis (Dirick et al. 1998; Stuart and Wittenberg 1998; Benjamin et al. 2003). We have shown in Saccharomyces cerevisiae that expression of a stabilized form of the B-type cyclin-CDK inhibitor Sic1 during meiosis can lead to extra rounds of DNA replication (Sawarynski et al. 2009). This observation is consistent with the well-established role of CDK, particularly Clb5-Cdk1, in preventing DNA rereplication during the mitotic cell cycle through several mechanisms that serve to inhibit reformation of the prereplicative complex (Nguyen et al. 2001; Ikui et al. 2007; Siddiqui et al. 2013). As in most eukaryotic organisms, meiotic DNA replication in S. cerevisiae is followed by programmed recombination between homologous chromosomes during prophase of the first meiotic division. The physical interaction of homologs afforded by recombination is important for accurate chromosome segregation during this division, and allows for transfer of genetic information between the parental...
Table 1 Yeast strains

| Strain       | Relevant Genotype                                                                 | Designation |
|--------------|----------------------------------------------------------------------------------|-------------|
| Diploids     |                                                                                  |             |
| YGB495       | ura3-1::HOP1pr-SIC1ΔMyc-URA3                                                      | SIC1αΔA     |
| YGB535       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::kanMX4/"                                    | SIC1αΔA     |
| YGB604       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::natMX/"                                     | SIC1αΔA     |
| YGB679       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::natMX/"                                     | SIC1αΔA     |
| YGB687       | swe1Δ::kanMX4/"                                                                  | swe1Δ       |
| YGB689       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 swe1Δ::kanMX4/"                                    | SIC1αΔA     |
| YGB697       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::natRX/"                                     | SIC1αΔA     |
| YGB700       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::natRX/"                                     | SIC1αΔA     |
| YGB703       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 pch2Δ::kanMX4/"                                    | SIC1αΔA     |
| YGB712       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 hop1Δ::kanMX4/"                                    | SIC1αΔA     |
| YGB713       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 /" dmc1Δ::natR/" hop1Δ::kanMX4/"                    | SIC1αΔA     |
| YGB721       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 red1Δ::kanMX4/"                                    | SIC1αΔA     |
| YGB722       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::natR/" red1Δ::kanMX4/"                       | SIC1αΔA     |
| YGB758       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 rad9Δ::kanMX4/"                                    | SIC1αΔA     |
| YGB759       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 rad5Δ::kanMX4/"                                    | SIC1αΔA     |
| YGB760       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 rad5Δ::kanMX4/"                                    | SIC1αΔA     |
| YGB761       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 rad5Δ::kanMX4/"                                    | SIC1αΔA     |
| YGB785       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 sum1Δ::kanMX4/"                                    | SIC1αΔA     |
| YGB786       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 sum1Δ::kanMX4/"                                    | SIC1αΔA     |
| YGB788       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 mec1Δ::LEU2/"                                      | SIC1αΔA     |
| YGB807       | SIC1ΔMyc::kanMX6/"                                                               | SIC1ΔMyc    |
| YGB808       | SIC1ΔMyc::kanMX6/"                                                               | SIC1ΔMyc    |
| YGB809       | SIC1ΔMyc::kanMX6/"                                                               | SIC1ΔMyc    |
| YGB814       | rad5Δ::HIS3/" sml1-1/"                                                          | rad5Δ::sml1-1F |
| YGB866       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 mcm5-bob1::HIS3/"                                   | SIC1αΔA     |
| YGB867       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 mcm5-bob1::HIS3/"                                   | SIC1αΔA     |
| YGB934       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::nat/R/"                                      | SIC1αΔA     |
| YGB938       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::nat/R/"                                      | SIC1αΔA     |
| YGB966       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::nat/R/"                                      | SIC1αΔA     |
| YGB976       | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::nat/R/"                                      | SIC1αΔA     |
| YGB1012      | ura3-1::HOP1pr-SIC1ΔMyc-URA3 rad5Δ::TRP1/"                                      | SIC1αΔA     |
| YGB1014      | ura3-1::HOP1pr-SIC1ΔMyc-URA3 rad5Δ::TRP1/"                                      | SIC1αΔA     |
| YGB1075      | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::nat/R/"                                      | SIC1αΔA     |
| YGB1241      | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::nat/R/"                                      | SIC1αΔA     |
| YGB1255      | ura3-1::HOP1pr-SIC1ΔMyc-URA3 dmc1Δ::nat/R/"                                      | SIC1αΔA     |
| YGB502       | SIC1ΔMyc::kanMX6/"                                                               | SIC1ΔMyc    |

All strains listed were constructed in the W303 background (Thomas and Rothstein 1989; diploid wild type = MATα ade2-1 ura3-1 leu2-3,112 his3-11,15 trp1-1 can1-100; haploid wild type = MATa ade2-1 ura3-1 leu2-3,112 his3-11,15 trp1-1 can1-100).

a Sawanywongse et al. (2009).
b This SIC1ΔαΔA dmc1Δ strain was used only for the experiment shown in Figure S2.

c Derived from YGB760.

chromosomes. Meiotic recombination initiates from a DNA double-strand break (DSB) generated by Spo11, a topoisomerase-like enzyme with DNA transesterase activity that functions in cooperation with several other proteins (Keeney et al. 1997; Maleki et al. 2007). It is estimated that Spo11 catalyzes formation of 140–170 DSBs per meiosis in S. cerevisiae (Buhler et al. 2007; Pan et al. 2011), with a number of controls in place to ensure that each of the 16 chromosomes sustains at least one event (Youds and Boulton 2011). Each DSB is initially processed to generate 3’-single-stranded DNA overhangs that can invade the homologous duplex chromosome (Cao et al. 1990; Sun et al. 1991).
In the absence of the meiosis-specific DNA recombinase Dmc1, strand invasion cannot proceed and extensive DNA resection results, leading to activation of a meiotic recombination checkpoint response that prevents exit from the pachytene stage of prophase I (Bishop et al. 1992; Xu et al. 1997).

As might be expected, the meiotic recombination checkpoint pathway as defined by deletion of the DMC1 gene (dmc1Δ) shares many proteins with DNA damage checkpoint pathways that operate during the mitotic cell cycle (Lydall et al. 1996). Examples include the apical protein kinase Mek1, and its associated protein Ddc2, which are orthologs of human ATM- and Rad3-related protein kinase (ATR), and ATR-interacting protein (ATRIP), respectively, and the PCNA-like Ddc1-Mec3-Rad17 ("9-1-1") complex, which facilitates Mec1 function, and also has a human counterpart (Weinert et al. 1997; Lydall et al. 1996; Paciotti et al. 2000; Hong and Roeder 2002; Zou and Elledge 2003; Navadgi-Patil and Burgers 2011; Refolio et al. 2011). In addition, the Dot1 methyltransferase is involved in both (San-Segundo and Byers 1992; Cartagena-Lirola et al. 1996; Bailis and Roeder 2000). While Rad53 and Rad9 have been implicated in certain meiotic checkpoints, including the response to unprogrammed DNA damage (Weber and Byers 1992; Cartagena-Lirola et al. 1996; Paciotti et al. 2000), their absence in the recombination checkpoint can be explained by the existence of meiosis-specific proteins that operate specifically in the context of recombination intermediate structures (Hollingsworth and Ponte 1997; Xu et al. 1997; Bailis and Roeder 2000). These include Hop1, Red1, and Mek1, each of which is a component of the sister chromatid-derived axial elements that form during meiosis, and are critical for proper meiotic recombination. Hop1 and Red1 are structural in nature (Hollingsworth et al. 1990; Smith and Roeder 1997), whereas Mek1 is a protein kinase with sequence similarity to Rad53 (Rockmill and Roeder 1991; Leem and Ogawa 1992; Bailis and Roeder 2000). All three proteins help to enforce the proper bias of interhomolog recombination during unperturbed meiosis, thereby promoting faithful chromosome segregation (Hollingsworth and Byers 1989; Rockmill and Roeder 1991; Schwacha and Kleckner 1997; Thompson and Stahl 1999; Kim et al. 2010; Wu et al. 2010).

Ultimately, checkpoint-mediated prevention of pachytene exit and progression through the mitotic divisions is implemented in part through regulation of the NDT80 gene and its protein product, which is a meiosis-specific transcription factor required for proper expression of many "middle" sporulation genes (Chu and Herskowitz 1998; Hepworth et al. 1998; Lindgren et al. 2000; Tung et al. 2000; Pak and Segall 2002; Shubassi et al. 2003). These include CDC5, whose polo-like protein kinase product is required for pachytene exit, and also upregulates Ndt80 activation in a feedback loop (Sourirajan and Lichten 2008; Acosta et al. 2011), and CLB1, which encodes a B-type cyclin that is required for progression through meiosis I (Chu and Herskowitz 1998; Carlile and Amon 2008). Another target of the meiotic recombination checkpoint is the Swe1 protein kinase, which is activated to catalyze inhibitory phosphorylation of Cdk1 at tyrosine 19 (Leu and Roeder 1999). Early work in mitotic cells indicated that Swe1-catalyzed Cdk1 phosphorylation regulates the morphogenesis checkpoint (Lew and Reed 1995). However, it is now known that Swe1 is also a component of one of three Mec1-dependent mechanisms that operate in the S phase checkpoint to prevent cell cycle progression into mitosis (Palou et al. 2015).

In our previous studies, we found that deletion of DMC1 blocks the DNA rereplication induced by Sic1 stabilization (Sawarynski et al. 2009). In this report, we describe our further genetic investigation into constituents of the meiotic recombination checkpoint as they pertain to Sic1-induced DNA rereplication. We found that certain upstream components, including MEC1, were required to prevent DNA rereplication. However, we did not find evidence that particular downstream effectors that regulate meiotic progression were involved. We further examined...
processes that operate to prevent DNA replication in the mitotic cell cycle, and found overlap with respect to specific phosphorylation events, including those that are important for blocking late DNA replication origin firing in an S phase checkpoint response. Interestingly, these data suggest a pathway in which the effectors are phosphorylated through a Rad53-independent mechanism.

MATERIALS AND METHODS

Strains

Yeast strains used in this study are listed in Table 1. Construction of the HOP1pr-SIC1ΔPHA module, and its integration into the genome, were described previously (Sawarynski et al. 2009). In most cases, deletion mutations were generated in haploids by homology-directed site-specific replacement with selectable markers (Baudin et al. 1993). These markers were PCR-amplified from either genomic DNA of a deletion set mutant (Winzeler et al. 1999) (GE Dharmacon), or from a plasmid (Brachmann et al. 1998). Certain mutant progenitor strains in the W303 background were generously provided by other investigators: SKY2939 (h2a-S129A) (Downs et al. 2004) by Stephen Kron (University of Chicago), YFL234 (dot1Δ) (Giannattasio et al. 2005) by Marco Muzi-Falconi (Università degli Studi di Milano), U960 (rad53Δ sml1-1) (Zhao et al. 1998) by Stephen Elledge (Harvard University), and Y2359 and Y2573 (dbf4-4A, sld3-38A, and mcm5-bob1) (Zegerman and Diffley 2010) by Philip Zegerman (The Gurdon Institute, UK) and John Diffley (The Francis Crick Institute, UK). These mutations were then introduced into our cell system through crossing. Strain construction generally involved introduction of mutations into MATα cells and into MATα cells with the HOP1pr-SIC1ΔPHA module either present or subsequently added, followed by mating of the two cell types. (Note that the shorthand designation of SIC1ΔPHA used for diploids in the text and figures indicates the presence of a single copy of the HOP1pr-SIC1ΔPHA element, while other mutant allele designations indicate alteration of both gene copies.) All deletion mutations generated for this study were verified by PCR, and deletion of SWE1 was further confirmed by western blotting using antibody kindly provided by Doug Kellogg (University of California, Santa Cruz) (Sreenivasan and Kellogg 1999). DNA sequencing was used to validate the presence of certain point mutations in our strains. Epitope tagging of Sic1 (SIC113MYC) was performed as described (Longtine et al. 1998) in MATα and MATα cells, which were then mated to generate the diploid. An additional manipulation included 5-fluoroorotic acid-mediated counter-selection (Boeke et al. 1984) to isolate a rad53Δ sml1-1 diploid from a strain containing HOP1pr-SIC1ΔPHA.
Cell culture

All yeast incubations were conducted at 30°C. Meiosis was induced by starvation based on an established procedure for synchronous sporulation (Padmore et al. 1991). In this method, yeast cells were first grown on solid [2% (w/v) agar] YPG medium [1% (w/v) yeast extract, 2% (w/v) peptone, 3% (v/v) glycerol], or, alternatively, on solid YPD medium [1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) dextrose] for 3–4 d, and single colonies were used to inoculate YPD liquid cultures. The overnight YPD cultures were then used to inoculate YPA [1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) potassium acetate] at an OD600 of 0.2. Cells were incubated overnight, typically for 16 hr, and then resuspended at equivalent cell densities (based on OD600 values) for strains within an experiment in sporulation medium consisting of 0.3% (w/v) potassium acetate and 0.02% (w/v) raffinose supplemented with leucine, arginine, and histidine each at 250 μM, tryptophan at 100 μM, and uracil at 50 μM. Cells were returned to incubation at time 0, and aliquots were harvested at indicated time points for flow cytometry and protein analyses (see below). For most experiments, control strains SIC1 ΔPHA and SIC1 ΔPHA dmc1 Δ were included. Each experimental strain was analyzed at least twice in independent experiments, and in many cases more than twice (see Supplemental Material, Figure S1).

To conduct a synchronized mitotic cell cycle timecourse, MATα cells grown to saturation in YPD were brought to an OD600 of ~0.2 and incubated for 2 hr. The yeast mating pheromone α-factor (Zymo Research) was then added to a final concentration of 2.5 μM, and cells were incubated for an additional 2 hr to achieve G1 arrest. The cells were then washed with sterile water to remove α-factor, resuspended in fresh YPD, and further incubated. Aliquots were taken at 15-min intervals for flow cytometry and western blotting analyses (see below). For examining the response to inhibition of DNA replication, cells were arrested with α-factor as described above and then released into 0.8× YPD containing 0.2 M HU (MP Biomedicals).

DNA content

Cells were harvested by centrifugation, resuspended in 70% ethanol and stored at 4°C. Aliquots of the fixed cells were washed once with 50 mM Tris-HCl, pH 7.5, resuspended in 1 ml of the same buffer, and then treated with 250 μg RNase A for 1 hr at 37°C, followed by 250 μg proteinase K for 1 hr at 37°C. Digested samples were incubated with 10× SYBR Green I (Molecular Probes) at 4°C overnight, sonicated briefly, and analyzed with a FACSCanto II flow cytometer (BD Biosciences) (or, in one experiment, a BD LSR II flow cytometer) (Microscopy, Imaging, and Cytometry Resources Core at Wayne State University School of Medicine). DNA content histograms were generated using WinMDI freeware. DNA rereplication was quantified by using the gating function in WinMDI to determine the number of events (out of 20,000) that were recorded with > 4C DNA content. Gating was established based on the 4C DNA peak, and was held constant within each individual experiment.

Protein

Cells were harvested by centrifugation and stored at ~70°C. For most experiments, denatured whole-cell extracts were prepared based on an alkaline extraction method (Kushnirov 2000). In the case of Rad53...
analysis, a trichloroacetic acid bead beating method was used, as described (Foiani et al. 1994). Resulting samples were subjected to SDS-polyacrylamide electrophoresis. For western blotting, the separated proteins were transferred to nitrocellulose (GE Healthcare). Primary antibodies included rat anti-α-tubulin (Serotec), mouse anti-hemagglutinin (Covance), rabbit anti-yeast γ-H2A (generously provided by Christophe Redon and William Bonner, National Cancer Institute) (Nakamura et al. 2006), mouse anti-tyr (Santa Cruz), and rabbit anti-Rad53 (Abcam). Signals were generated with IRDye 800-conjugated goat anti-rat (Rockland), Alexa Fluor 680 goat anti-rabbit (Invitrogen), or Alexa Fluor 680 goat anti-mouse (Invitrogen) secondary antibodies. Reactive bands were visualized with an Odyssey infrared imaging system (Li-Cor). Flou 680 goat anti-mouse (Invitrogen) secondary antibodies. Reactive bands were visualized with an Odyssey infrared imaging system (Li-Cor).

RESULTS

The results are presented in the article and are supported by the data available. The article provides a comprehensive analysis of the findings, including details on the methodology and results. The data is presented in a clear and concise manner, allowing for easy understanding and interpretation.

MEC1 and associated genes

We further examined genes that operate downstream of MEC1 in the established response that prevents both interstitial repair and meiotic progression, including those that encode the axial proteins Mek1, Red1, and Hop1. As in the case of mec1Δ, deletion of any one of these genes restored DNA rereplication in SIC1ΔPHA dmc1Δ cells (Figure 2), indicating that they were required for prevention of DNA rereplication in response to the accumulation of unrepaired DSBs. In these cases, DNA rereplication was robust, as exhibited by the generation of cells with high DNA content in some cases reaching ~16C (Figure 2 and Figure S1).

It has been shown that dmc1Δ mek1Δ cells that are also rad54Δ, and therefore incapable of completing interstitial repair (Arbel et al. 1999) progress through meiosis, albeit with slower kinetics than wild-type or dmc1Δ mek1Δ cells (Cartagena-Lirola et al. 2008; Chuang et al. 2012), this phenotype illustrates the checkpoint function of MEC1. To determine whether the MEK1 function to suppress interstitial repair, or to prevent meiotic progression, was at play in our specialized case, we examined SIC1ΔPHA dmc1Δ mek1Δ cells. We found that the recovery of DNA rereplication observed in SIC1ΔPHA dmc1Δ cells was not observed with the addition of rad54Δ (Figure 2C). In each strain, we observed an increase in phosphorylated histone H2A (γ-H2A), which is generated through Mec1 (and related Tel1) catalysis in response to DSB formation, and leads to extensive regions of chromatin containing γ-H2A on either side of the DSB (Shroff et al. 2004). These data suggest a persistence of DSBs in our cells throughout the time course, regardless of DMC1 or RAD54 status. We further demonstrated that RAD54 itself was not required for the DNA rereplication phenotype (Figure S2). These data indicate that MEK1 inhibited DNA rereplication by preventing interstitial repair and maintaining the DSB-induced signal rather than by influencing DNA replication itself.
Studies have indicated that the AAA+-type ATPase Pch2 suppresses intersister repair to some extent, and helps to prevent meiotic progression when unrepaired DSBs accumulate (San-Segundo and Roeder 1999; Ho and Burgess 2011; Sanders et al. 2011; Chen et al. 2014). We found that deletion of PCH2 relieved the dmc1Δ-dependent inhibition of DNA rereplication in our SIC1ΔPHA system (Figure S3), indicating that PCH2 aided in preventing DNA rereplication. In this case, as in certain other mutants analyzed (see below), few cells with DNA content >~8C were observed. Extensive DNA rereplication was detected with SIC1ΔPHA pch2Δ cells (Figure S1 and Figure S3), indicating that PCH2 itself was not required for the DNA rereplication phenotype in SIC1ΔPHA cells.

CDK
We considered the possibility that the checkpoint response might prevent CDK activation to inhibit DNA rereplication. One mechanism by which the meiotic recombination checkpoint prevents meiotic progression is through Sum1, a key transcription factor that represses expression of middle sporulation genes normally induced by the transcription factor Ndt80 (Lindgren et al. 2000; Pak and Segall 2002; Winter 2012). Included among these Ndt80-induced genes are those that encode the B-type cyclins Clb1, -3, -4, -5, and -6 (Chu et al. 2002; Winter 2012). Included among these Ndt80-induced genes are those that encode the B-type cyclins Clb1, -3, -4, -5, and -6 (Chu and Herskowitz 1998). We found that DNA rereplication occurred in SIC1ΔPHA sum1Δ cells, although with reduced efficiency for cells with DNA content >~8C, but not in SIC1ΔPHA dmc1Δ sum1Δ cells (Figure 3A and Figure S1), indicating that SUM1 was not involved in this checkpoint.

We also examined SWE1, whose product becomes activated in the meiotic recombination checkpoint response to catalyze inhibitory phosphorylation of Cdk1 at tyrosine 19 (Leu and Roeder 1999). We found that deletion of SWE1, like that of SUM1, did not prevent DNA rereplication in SIC1ΔPHA cells, nor did it reverse the dmc1Δ-dependent inhibition of DNA rereplication (Figure 3, B and C), suggesting that SWE1 was not required for prevention of DNA rereplication. It is noted that because Ndt80 levels and activity are downregulated by the meiotic recombination checkpoint response, thereby lowering B-type cyclin availability and CDK activity, we might not expect to see much of an effect by simply deleting SWE1 in our cells. However, dmc1Δ swe1Δ cells do progress into MI, although with delayed kinetics relative to wild type cells (Leu and Roeder 1999). During the course of these studies, we also examined a swe1Δ mutant without the SIC1ΔPHA allele. This experiment was prompted by the report that swe1Δ cells rereplicate their DNA during meiosis, a phenotype that is different from ours in that multispore asci are formed (Rice et al. 2005). We did not observe these phenotypes in our swe1Δ cells, perhaps due to differences in strain types or culture conditions (Figure 3B and data not shown).

To investigate another CDK regulator, Sic1, we generated strains in which Sic1 was tagged with MYC epitope repeats. In this way, we could distinguish endogenous Sic1 from the induced version lacking CDK-targeted phosphorylation sites, which is tagged with the HA epitope. We first examined a haploid strain during the cell cycle to ensure that dmc1Δ cells do progress into MI, although with delayed kinetics relative to wild type cells (Leu and Roeder 1999). During the course of these studies, we also examined a swe1Δ mutant without the SIC1ΔPHA allele. This experiment was prompted by the report that swe1Δ cells rereplicate their DNA during meiosis, a phenotype that is different from ours in that multispore asci are formed (Rice et al. 2005). We did not observe these phenotypes in our swe1Δ cells, perhaps due to differences in strain types or culture conditions (Figure 3B and data not shown).

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γ-H2A and DOT1

Mec1- and Tel1-catalyzed H2A phosphorylation at serine 129, which generates γ-H2A, is thought to be an important protective mechanism that promotes DSB repair to prevent genomic alterations (Downs et al. 2000; Redon et al. 2003), and functions in the G1 DNA damage checkpoint during the mitotic cell cycle (Javaheri et al. 2006; Hammet et al. 2007). Because MEC1 was required in the meiotic recombination checkpoint response that prevents DNA rereplication, we suspected that one of its major targets would be involved as well. We found that γ-H2A was generated in H2A (HTA1 and HTA2) cells regardless of DMC1 status (see Figure 2C). We generated a SIC1ΔPHA strain with HTA1 and HTA2 both mutated (h2a-S129A) so that the two H2A subunits could not be phosphorylated at serine 129, and confirmed through western blotting that these cells were devoid of γ-H2A (see Figure S2 and Figure S5). Importantly, absence of γ-H2A led to DNA rereplication in SIC1ΔPHA dmc1Δ cells, while not as extensive as in SIC1ΔPHA cells with regard to total number of cells exhibiting >4C DNA content and those reaching >8C, the DNA rereplication was obvious, and observed consistently (Figure 5 and Figure S1). SIC1ΔPHA h2a-S129A cells rereplicated their DNA with clear evidence of cells containing >8C DNA content (Figure S2).

We also examined the phenotype resulting from deletion of DOT1, which encodes a histone methyltransferase required for the meiotic recombination checkpoint response (San-Segundo and Roeder 2000). Dot1 catalyzes methylation of histone H3 at lysine K79 (H3meK79) (Lacoste et al. 2002; Ng et al. 2002; van Leeuwen et al. 2002), and, like γ-H2A, is important for the G1 DNA damage checkpoint in the mitotic cell cycle (Giannattasio et al. 2005; Wysocki et al. 2005). Similar to the case with h2a-S129A, we observed a modest degree of DNA rereplication in SIC1ΔPHA dmc1Δ dot1Δ cells, and combination of the dot1Δ and h2a-S129A mutations did not enhance this effect (Figure 5 and Figure S1). These data suggest that γ-H2A and Dot1 operated in the same pathway in preventing DNA rereplication in SIC1ΔPHA dmc1Δ cells.
RAD53
While RAD53 is not involved in the meiotic recombination checkpoint per se, it can be activated by genotoxic stress during meiosis (Weber and Byers 1992; Cartagena-Lirola et al. 2008; Blitzblau and Hochwagen 2013). Therefore, we elected to determine whether or not RAD53 was involved in the checkpoint that prevents DNA rereplication in our system. (As in the case of mec1Δ, rad53Δ cells are viable when SML1 is also defective (Zhao et al. 1998)). We were surprised to find that DNA rereplication did not occur in SIC1Δpha rad53Δ sml1Δ-1 cells, regardless of DMC1 status (Figure S6), even after 48 hr (data not shown). As an alternative genetic test, we turned to RAD9, which encodes a protein that mediates RAD53 activation in many circumstances (Sun et al. 1998; Vialard et al. 1998; Gilbert et al. 2001). By contrast to rad53Δ, rad9Δ did not prevent DNA rereplication in SIC1Δpha cells (Figure S6). Importantly, RAD9 was not required for suppressing DNA rereplication in SIC1Δpha dmc1Δ cells (Figure 6A), indicating that RAD9 was not involved in this checkpoint response.

Because our results precluded analysis of RAD53 function through gene deletion, we explored the possibility of a RAD53, or RAD53-like, function through different means. In response to replication fork stalling or DNA damage during S phase of the mitotic cell cycle, RAD53 is activated to catalyze phosphorylation of Dbf4 and Sld3, thereby preventing firing of late origins (Lopez-Mosqueda et al. 2010; Zegerman and Diffley 2010). We examined two different types of mutants to determine whether this process could be involved in our system. The first involved cells containing mutant alleles of both DBF4 and SLD3, encoding proteins with alterations in important RAD53-targeted phosphorylation sites. SIC1Δpha dmc1Δ cells containing the dbf4-4A and sld3-38A alleles exhibited DNA rereplication, indicating that phosphorylation of Dbf4 or Sld3, or both proteins, was required for full prevention of DNA rereplication (Figure 7 and Figure S1). We noticed that, as in the case of SIC1Δpha dmc1Δ h2A-S129A cells, these cells appeared to rereplicate their DNA less than SIC1Δpha cells, in that they did not exhibit >5C DNA content. In SIC1Δpha dbf4-4A sld3-38A cells, we did observe cells with >5C DNA content, indicating that the phosphorylation site mutations themselves were not responsible for limiting DNA rereplication (Figure S7). The second type of mutant cells contained the sld3-38A allele and mcm5-bob1, a mutant allele that bypasses the essential function of DBF4 (Hardy et al. 1997). DNA rereplication was not observed in SIC1Δpha dmc1Δ mcm5-bob1 sld3-38A cells (Figure 7). We confirmed that the mcm5-bob1 allele did not prevent DNA rereplication in our system (Figure S7). While it might be expected that cells with altered Dbf4 phosphorylation sites would behave similarly to cells with mcm5-bob1, as in the mitotic cell cycle studies, certain experimental factors may account for this discrepancy (see Discussion). These data suggest that Dbf4 phosphorylation was sufficient to prevent DNA rereplication, at least in the context of the mcm5-bob1 allele. It is noted that either Dbf4 or Sld3 phosphorylation alone can contribute to prevention of late origin firing in mitotic S phase (Lopez-Mosqueda et al. 2010; Zegerman and Diffley 2010). We conclude that sites normally phosphorylated by RAD53 in the mitotic cell cycle also functioned to prevent DNA replication during meiosis under our conditions that promote DNA rereplication.

DISCUSSION
When S. cerevisiae cells deleted for DMC1 are induced to enter the meiotic program, a response is activated that inhibits recombination between sister chromatids and prevents progression into the meiotic divisions (Bishop et al. 1992; Xu et al. 1997; Wan et al. 2004; Niu et al. 2005). We have observed that cells undergoing meiosis but engineered to rereplicate their DNA through expression of SIC1Δpha also respond to deletion of DMC1 by preventing extra DNA replication (Sawarynski et al. 2009). Our previous studies indicated that this response requires RAD17, suggesting a checkpoint mechanism. Our further genetic analysis shown here has confirmed that a checkpoint response is involved. We observed differences in the degree of DNA rereplication recovery in SIC1Δpha dmc1Δ cells depending on which additional gene was
A meiotic recombination checkpoint response can inhibit DNA rereplication. This diagram based on our genetic analysis depicts certain key protein components in a pathway that leads from accumulation of unrepaired DSBs to inhibition of DNA rereplication in the SIC1ΔPHA system. Also shown is an outline of the pathways that prevent intersister repair and meiotic progression in the normal checkpoint. See text for details.

The meiotic recombination checkpoint response that prevents DNA rereplication shares several components with cell cycle DNA damage response checkpoint can target the DNA replication machinery. checkpoint pathway that we have uncovered affects only a subset of genes encoding Mek1 and Rad17, and presumably the entire 9-1-1 complex that includes Rad17 and Mec1 function, were required in our system. It is interesting that a response in which a protein kinase that recognizes motifs in a similar manner to Rad53 is activated by conventional means (electrophoretic mobility shift or in situ autophosphorylation), this possibility seems remote. In fact, our results are consistent with the fact that the Rad9-Rad53 axis is not involved in the meiotic recombination checkpoint that serves to prevent pachytene exit and progression through the meiotic divisions (Bishop et al. 1992; Lydall et al. 1996; Bailis and Roeder 2000). Of particular interest, however, is that residues in Dbf4, and presumably Sld3 as well, that are phosphorylated through Rad53 catalysis in the mitotic cell cycle to prevent late origin firing (Lopez-Mosqueda et al. 2010; Zegerman and Diffley 2010), also function in the meiotic response to prevent DNA rereplication. By contrast to the mitotic cell cycle observation (Zegerman and Diffley 2010), we found that the mcm5-bob1 allele, which eliminates the requirement for the Dbf4-Cdc7 protein kinase in DNA replication initiation (Hardy et al. 1997), did not substitute for the dbf4-4A mutant allele as it did in the mitotic cell cycle studies. This distinction could reflect intrinsic differences in DNA replication regulation during the mitotic cell cycle and meiosis; alternatively, it could be due to the lowered CDK activity in our engineered cells.

Taken together, our results suggest a response in which a protein kinase that recognizes motifs in a similar manner to Rad53 is activated through a mechanism equivalent in many ways to the one that operates in response to DNA damage during the mitotic cell cycle (see Figure 8). One likely candidate for this kinase activity would be Mek1, given its structural similarity to Rad53 and its meiosis-specific expression (Rockmill and Roeder 1991; Leem and Ogawa 1992; Bailis and Roeder 2000). However, our data argue against this possibility because deleted or mutated. While experimental variability makes it difficult to be conclusive about these differences, we observed trends in particular circumstances. For example, removal of genes encoding certain proteins, such as Mek1, that serve to prevent both intersister repair and meiotic progression led to extensive DNA rereplication including cells containing >~8C DNA content. Our experiments further suggest that the MEC1 function to prevent intersister repair, and to thereby retain the checkpoint signal originating from unrepaired DSBs, is operative in preventing DNA rereplication in our cells. By contrast to these gene deletions, mutation of genes to abrogate H2A or Dbf4 and Sld3 phosphorylation led to less DNA rereplication, with few cells detected containing >~8C DNA content. This difference might indicate that the degree of DNA rereplication is limited by the presence of unrepaired and resected DSBs, although we did observe considerable H2A staining in cells, regardless of checkpoint status. In addition, more than a single checkpoint mechanism may be involved in preventing DNA rereplication, with only one being absent in certain cases such as SIC1ΔPHA dmc1Δ dbf4-4A sld3-38A cells. In this sense, perhaps the checkpoint pathway that we have uncovered affects only a subset of origins. Regardless, the data provide clear evidence that the meiotic recombination checkpoint can target the DNA replication machinery.

The meiotic recombination checkpoint response that prevents DNA rereplication shares several components with cell cycle DNA damage response checkpoint mechanisms. Genes encoding Mec1 and Rad17, and, presumably, the entire 9-1-1 complex that includes Rad17 and facilitates Mec1 function, were required in our system. It is interesting to note that DNA rereplication itself in the mitotic cell cycle initiates a checkpoint response dependent on MEC1 and RAD17 that restricts the extent of DNA rereplication (Archambault et al. 2005). In our case, we observed slightly less DNA rereplication upon deletion of MEC1 and SML1 in SIC1ΔPHA dmc1Δ cells when compared with SIC1ΔPHA cells deleted for genes such as MEK1, particularly with regard to >~8C DNA content cells. (The possibility is noted that deletion of MEC1 and SML1 might have had a minor effect on DNA rereplication in SIC1ΔPHA cells as well). It has been reported that dmc1Δ mec1-1 cells continue to progress through meiosis with unrepaired DSBs (Lydall et al. 1996); as suggested above, the presence of unrepaired DSBs may influence the degree of DNA rereplication in our system. We did not interrogate TEL1, which encodes a close relative of Mec1 that is involved in DNA damage response pathways, including the meiotic recombination checkpoint response (Greenwell et al. 1995; Morrow et al. 1995; Usui et al. 2001). Like Mec1, Tel1 catalyzes Hop1 phosphorylation, which is required for Mek1 activation (Car ballo et al. 2008). It is possible that the phenotypic difference between SIC1ΔPHA dmc1Δ mec1Δ sml1Δ and SIC1ΔPHA dmc1Δ mek1Δ cells (observed despite the fact that Mek1 functions downstream of Mec1) is due to the presence of Tel1. In addition, there might exist downstream effectors of Mec1, other than Mek1, that have an influence by promoting DNA rereplication.

Mec1 activation involves the interaction of its partner Ddc2 with single-stranded-DNA bound replication protein A (Zou and Elledge 2003), which, in the case of dmc1Δ cells would be formed readily due to the highly resected Spo11-generated DSBs (Bishop et al. 1992). In turn, Mec1 can catalyze formation of γ-H2A, which we found contributed to prevention of DNA rereplication, as did DOT1, encoding the enzyme that generates H3meK79. Because γ-H2A was abundant in SIC1ΔPHA cells that underwent DNA rereplication, and H3meK79 was likely to be abundant as well (van Leeuwen et al. 2002), these two histone modifications are necessary for the full checkpoint response, but not sufficient. In the mitotic cell cycle, particularly with respect to the G1 DNA damage checkpoint response, these modifications (as well as Rad6-Bre1 mediated histone H2B ubiquitylation required for H3meK79 generation) are important for Rad9 recruitment and Rad53 activation (Giannattasio et al. 2005; Javaheri et al. 2006; Hammet et al. 2007). In the checkpoint response that prevents DNA rereplication, neither RAD9 nor RAD53 appeared to be involved. While it is theoretically possible that Rad53 can be activated by a mechanism that cannot be detected by conventional means (electrophoretic mobility shift or in situ autophosphorylation), this possibility seems remote. In fact, our results are consistent with the fact that the Rad9-Rad53 axis is not involved in the meiotic recombination checkpoint that serves to prevent pachytene exit and progression through the meiotic divisions (Bishop et al. 1992; Lydall et al. 1996; Bailis and Roeder 2000). Of particular interest, however, is that residues in Dbf4, and presumably Sld3 as well, that are phosphorylated through Rad53 catalysis in the mitotic cell cycle to prevent late origin firing (Lopez-Mosqueda et al. 2010; Zegerman and Diffley 2010), also function in the meiotic response to prevent DNA rereplication. By contrast to the mitotic cell cycle observation (Zegerman and Diffley 2010), we found that the mcm5-bob1 allele, which eliminates the requirement for the Dbf4-Cdc7 protein kinase in DNA replication initiation (Hardy et al. 1997), did not substitute for the dbf4-4A mutant allele as it did in the mitotic cell cycle studies. This distinction could reflect intrinsic differences in DNA replication regulation during the mitotic cell cycle and meiosis; alternatively, it could be due to the lowered CDK activity in our engineered cells.

Taken together, our results suggest a response in which a protein kinase that recognizes motifs in a similar manner to Rad53 is activated through a mechanism equivalent in many ways to the one that operates in response to DNA damage during the mitotic cell cycle (see Figure 8). One likely candidate for this kinase activity would be Mek1, given its structural similarity to Rad53 and its meiosis-specific expression (Rockmill and Roeder 1991; Leem and Ogawa 1992; Bailis and Roeder 2000). However, our data argue against this possibility because...
SIC1ΔΔRSA dmc1Δ cells devoid of both MEK1 and RAD54, designed to examine MEK1 checkpoint function specifically, did not display DNA rereplication. Furthermore, while a peptide-based investigation into the phosphorylation site specificity of yeast protein kinases has placed Rad53 and Mek1 into the same largest group of five clusterings, the two kinases are not closely related in this regard, and exhibit a considerable difference in their degree of specificity (Mok et al. 2010). Another kinase with some physical similarity to Rad53 is Dun1, which, in response to genotoxic stress, operates downstream of Rad53 to regulate nucleotide pool levels and transcription (Allen et al. 1994; Zhao and Rothstein 2002). However, biochemical studies suggest that the two enzymes have different specificities (Zheng et al. 1993; Sanchez et al. 1997; Sidorkova and Breeden 2003; Uchiki et al. 2004; Chen et al. 2007; Mok et al. 2010), and a comprehensive analysis of transcriptional regulation upon DNA damage indicates different targeting by Rad53 and Dun1 (Jaechnig et al. 2013). Therefore, evidence does not exist to indicate that Mek1 or Dun1 would likely catalyze phosphorylation of the same sites in Dbf4 and Sld3 as Rad53 does, suggesting that a different Rad53-like enzyme is present in meiosis that can influence initiation of DNA replication.

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LITERATURE CITED

Acosta, I., D. Ontoso, and P. A. San-Segundo, 2011 The budding yeast polo-like kinase Cdc5 regulates the Ndt80 branch of the meiotic recombination checkpoint pathway. Mol. Biol. Cell 22(18): 3478–3490.
Allen, J. B., Z. Zhou, W. Siede, E. C. Friedberg, and S. J. Elledge, 1994 The SAD1/RAD53 protein kinase controls multiple checkpoints and DNA damage-induced transcription in yeast. Genes Dev. 8(20): 2401–2415.
Arbel, A., D. Zenvirth, and G. Simchen, 1999 Sister chromatid-based DNA repair is mediated by RAD54, not by DMC1 or TID1. EMBO J. 18(9): 2648–2658.
Archambault, V., A. E. Ikui, B. J. Drapkin, and F. R. Cross, 2005 Disruption of mechanisms that prevent rereplication triggers a DNA damage response. Mol. Cell. Biol. 25(15): 6707–6721.
Balits, J. M., and G. S. Roeder, 2000 Pachytene exit controlled by reversal of the axial element protein Hop1 by Mec1/Tel1 ensures meiotic interhomolog recombination. Cell 132(5): 758–770.
Buhler, C., V. Borde, and M. Lichten, 2007 Mapping meiotic single-strand DNA reveals a new landscape of DNA double-strand breaks in Saccharomyces cerevisiae. PLoS Biol. 5(12): e324.
Cao, L., E. Alani, and N. Kleckner, 1990 A pathway for generation and processing of double-strand breaks during meiotic recombination in S. cerevisiae. Cell 61(6): 1089–1101.
Carballo, J. A., A. L. Johnson, S. G. Sedgwick, and R. S. Cha, 2008 Phosphorylation of the axial element protein Hop1 by Mec1/Tel1 ensures meiotic interhomolog recombination. Cell 132(5): 758–770.
Carli, T. M., and A. Amon, 2008 Meiosis I is established through division-specific translational control of a cyclin. Cell 133(2): 280–291.
Cartagena-Lirola, H., I. Guerini, N. Manfrini, G. Lucchini, and M. P. Longhese, 2008 Role of the Saccharomyces cerevisiae Rad53 checkpoint kinase in signaling double-strand breaks during the meiotic cell cycle. Mol. Cell. Biol. 28(14): 4480–4493.
Chen, C., A. Jomaa, J. Ortega, and E. E. Alani, 2014 Pch2 is a hexameric ring ATPase that remodels the chromosome axis protein Hop1. Proc. Natl. Acad. Sci. USA 111(1): E44–E53.
Chen, S. H., M. B. Smolka, and H. Zhou, 2007 Mechanism of Dun1 activation by Rad53 phosphorylation in Saccharomyces cerevisiae. J. Biol. Chem. 282(2): 986–995.
Chu, S., and I. Herskowitz, 1998 Gametogenesis in yeast is regulated by a transcriptional cascade dependent on Ndt80. Mol. Cell 1(5): 685–696.
Chuang, C. N., Y. H. Cheng, and T. F. Wang, 2012 Mek1 stabilizes Hop1-Thr318 phosphorylation to promote interhomolog recombination and checkpoint responses during yeast meiosis. Nucleic Acids Res. 40(22): 11416–11427.
Dirick, L., L. Goetsch, G. Ammerer, and B. Byers, 1998 Regulation of meiotic S phase by Ime2 and a Cbk6,6-associated kinase in Saccharomyces cerevisiae. Science 281(5384): 1854–1857.
Downs, J. A., N. F. Lowndes, and S. P. Jackson, 2000 A role for Saccharomyces cerevisiae histone H2A in DNA repair. Nature 408(6815): 1001–1004.
Downs, J. A., S. Allard, O. Jobin-Robitaille, A. Javelieri, A. Auger et al., 2004 Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. Mol. Cell 16(6): 979–990.
Eichinger, C. S., and S. Jentsch, 2010 Synaptonemal complex formation and meiotic checkpoint signaling are linked to the lateral element protein Red1. Proc. Natl. Acad. Sci. USA 107(25): 11370–11375.
Foiani, M., F. Marini, D. Gamba, G. Lucchini, and P. Plevani, 1994 The B subunit of the DNA polymerase alpha-primase complex in Saccharomyces cerevisiae executes an essential function at the initial stage of DNA replication. Mol. Cell. Biol. 14(2): 923–933.
Giannattasio, M., F. Lazzaro, P. Plevani, and M. Muzi-Falconi, 2005 The DNA damage checkpoint response requires histone H2B ubiquitination by Rads8/Bre1 and H3 methylation by Dot1. J. Biol. Chem. 280(11): 9879–9886.
Gilbert, C. S., C. M. Green, and N. F. Lowndes, 2001 Budding yeast Rad9 is an ATP-dependent Rad53 activating machine. Mol. Cell 8(1): 129–136.
Greenwell, P. W., S. L. Kronmal, S. E. Porter, J. Gassenhuber, B. Obermaier et al., 2005 TEL1, a gene involved in controlling telomere length in S. cerevisiae, is homologous to the human ataxia telangiectasia gene. Cell 82(5): 823–829.
Hammet, A., C. Magill, J. Heierhorst, and S. P. Jackson, 2007 Rad7 BRCT domain interaction with phosphorylated H2AX regulates the GI checkpoint in budding yeast. EMBO Rep. 8(9): 851–857.
Hardy, C. F., O. Dryga, S. Seematter, P. M. Pahl, and R. A. Sclafani, 1997 cme5/cdc46-bool bypasses the requirement for the S phase activator Cdc7p. Proc. Natl. Acad. Sci. USA 94(7): 3151–3155.
Hepworth, S. R., H. Friesen, and J. Segall, 1998 NDT80 and the meiotic recombination checkpoint regulate expression of middle
soporulation-specific genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 18(10): 5750–5761.

Ho, H. C., and S. M. Burgess, 2011 Phc2 acts through Xrs2 and Tel1/ATM to modulate interhomolog bias and checkpoint function during meiosis. PLoS Genet. 7(11): e1002351.

Hollingsworth, N. M., and B. Byers, 1989 *HOP1*: a yeast meiotic pairing gene. Genetics 121(3): 445–462.

Hollingsworth, N. M., and L. Ponte, 1997 Genetic interactions between *HOP1*, *RED1* and *MEK1* suggest that *MEK1* regulates assembly of axial element components during meiosis in the yeast *Saccharomyces cerevisiae*. Genetics 147(1): 33–42.

Hollingsworth, N. M., L. Goetsch, and B. Byers, 1990 *The HOP1* gene encodes a meiosis-specific component of yeast chromosomes. Cell 61(1): 73–84.

Hong, E. J., and G. S. Roeder, 2002 A role for Ddc1 in signaling meiotic double-strand breaks at the pachytene checkpoint. Genes Dev. 16(3): 363–376.

Ikui, A. E., V. Archambault, B. J. Drapkin, V. Campbell, and F. R. Cross, 2007 Cyclin and cyclin-dependent kinase substrate requirements for preventing recombination reveal the need for concomitant activation and inhibition. Genetics 175(3): 1011–1022.

Jaehning, E. J., D. Kuo, H. Hombauer, T. G. Ideker, and R. D. Kodolny, 2013 Checkpoint kinases regulate a global network of transcription factors in response to DNA damage. Cell Reports 4(1): 174–188.

Javaheri, A., R. Wysocki, O. Jobin-Robitaille, M. Altfa, J. Cote et al., 2006 Yeast G1 DNA damage checkpoint regulation by H2A phosphorylation is independent of chromatin remodeling. Proc. Natl. Acad. Sci. USA 103(37): 13771–13776.

Kato, R., and H. Ogawa, 1994 *An essential gene, ESRI*, is required for mitotic cell growth, DNA repair and meiotic recombination in *Saccharomyces cerevisiae*. Nucleic Acids Res. 22(15): 3104–3112.

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(3): 375–384.

Kim, K. P., B. M. Weiner, L. Zhang, A. Jordan, J. Dekker et al., 2010 Sister cohesion and structural axis components mediate homolog bias of meiotic recombination. Cell 143(6): 924–937.

Kushnirov, V. V., 2000 Rapid and reliable protein extraction from yeast. Yeast 16(9): 857–860.

Lacoste, N., R. T. Ulely, I. M. Hunter, G. P. Poirier, and J. Cote, 2002 Disruptor of telomeric silencing-1 is a chromatin-specific histone H3 methyltransferase. J. Biol. Chem. 277(34): 30421–30424.

Leem, S. H., and H. Ogawa, 1992 *The MRE4* gene encodes a novel protein kinase homologue required for meiotic recombination in *Saccharomyces cerevisiae*. Nucleic Acids Res. 20(3): 449–457.

Leu, J. Y., and G. S. Roeder, 1999 *The pachytene checkpoint in S. cerevisiae* depends on Swel-mediated phosphorylation of the cyclin-dependent kinase Cdc28. Mol. Cell 4(5): 805–816.

Lew, D. J., and S. I. Reed, 1995 A cell cycle checkpoint monitors cell morphogenesis in budding yeast. J. Cell Biol. 129(3): 739–749.

Lindgren, A., D. Bungard, M. Pierce, J. Xie, A. Vershon et al., 2000 The pachytene checkpoint in *Saccharomyces cerevisiae* requires the Sum1 transcriptional repressor. EMBO J. 19(23): 6489–6497.

Longtine, M. S., A. McKenzie, III, D. J. Demarini, N. G. Shah, A. Wach et al., 1998 Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast 14(10): 953–961.

Lopez-Mosqueda, J., N. I. Maas, Z. O. Jonsson, L. G. Defazio-Eli, J. Wohlschlegel et al., 2010 Damage-induced phosphorylation of Sld3 is important to block late origin firing. Nature 467(7314): 479–483.

Lydall, D., Y. Nikolsky, D. K. Bishop, and T. Weinert, 1996 *A meiotic recombination checkpoint controlled by mitotic checkpoint genes*. Nature 383(6603): 840–843.

Małek, S. M., J. J. Neale, C. Arora, K. A. Henderson, and S. Keeney, 2007 Interactions between Me4, Rec114, and other proteins required for meiotic DNA double-strand break formation in *Saccharomyces cerevisiae*. Chromosoma 116(5): 471–486.
Schwacha, A., and N. Kleckner, 1997  Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. Cell 90(6): 1123–1135.

Shroff, R., A. Arbel-Eden, D. Pilch, G. Ira, W. M. Bonner et al., 2004  Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. Curr. Biol. 14(19): 1703–1711.

Shubassi, G., N. Luca, J. Pak, and J. Segall, 2003  Activity of phosphoforms and truncated versions of Ndt80, a checkpoint-regulated sporulation-specific transcription factor of Saccharomyces cerevisiae. Mol. Genet. Genomics 270(4): 324–336.

Siddiqui, K., K. F. On, and J. F. Diffley, 2013  Regulating DNA replication in eukarya. Cold Spring Harb. Perspect. Biol. 5(9): a012930.

Sidorova, J. M., and L. L. Breeden, 2003  Rad53 checkpoint kinase phosphorylation site preference identified in the Swi6 protein of Saccharomyces cerevisiae. Mol. Cell. Biol. 23(10): 3405–3416.

Smith, A. V., and G. S. Roeder, 1997  The yeast Red1 protein localizes to the cores of meiotic chromosomes. J. Cell Biol. 136(5): 957–967.

Smith, A. V., and G. S. Roeder, 1997  The yeast Red1 protein localizes to the cores of meiotic chromosomes. J. Cell Biol. 136(5): 957–967.

Sourirajan, A., and M. Lichten, 2008  Polo-like kinase Cdc5 drives exit from pachytene during budding yeast meiosis. Genes Dev. 22(19): 2627–2632.

Sreenivasan, A., and D. Kellogg, 1999  The Elm1 kinase functions in a mitotic signaling network in budding yeast. Mol. Cell. Biol. 19(12): 7983–7994.

Stracker, T. H., T. Usui, and J. H. Petrini, 2009  Taking the time to make important decisions: the checkpoint effector kinases Chk1 and Chk2 and the DNA damage response. DNA Repair (Amst.) 8(9): 1047–1054.

Stuart, D., and C. Wittenberg, 1998  CLB5 and CLB6 are required for premeiotic DNA replication and activation of the meiotic S/M checkpoint. Genes Dev. 12(17): 2698–2710.

Sun, H., D. Treco, and J. W. Szostak, 1991  Extensive 3’-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the ARG4 recombination initiation site. Cell 64(6): 1155–1161.

Sun, Z., D. S. Fay, F. Marin, M. Foiani, and D. F. Stern, 1996  Spkl/Rad53 is regulated by Mec1-dependent protein phosphorylation in DNA replication and damage checkpoint pathways. Genes Dev. 10(4): 395–406.

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(5374): 272–274.

Thomas, B. J., and R. Rothstein, 1989  Elevated recombination rates in transcriptionally active DNA. Cell 56(4): 619–630.

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(2): 621–641.

Tung, K. S., E. J. Hong, and G. S. Roeder, 2000  The pachytene checkpoint prevents accumulation and phosphorylation of the meiosis-specific transcription factor Ndt80. Proc. Natl. Acad. Sci. USA 97(22): 12187–12192.

Uchiki, T., L. T. Dice, R. L. Hettich, and C. Dealwis, 2004  Identification of phosphorylation sites on the yeast ribonucleotide reductase inhibitor Smn1. J. Biol. Chem. 279(12): 11293–11303.

Usui, T., H. Ogawa, and J. H. Petrini, 2001  A DNA damage response pathway controlled by Tel1 and the Mre11 complex. Mol. Cell. 7(6): 1255–1266.

van Leeuwen, F., P. R. Gafken, and D. E. Gottschling, 2002  Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell 109(6): 745–756.

Verma, R., R. S. Annan, M. J. Huddleston, S. A. Carr, G. Reynard et al., 1997  Phosphorylation of Sic1p by G1 Cdk required for its degradation and entry into S phase. Science 278(5337): 455–460.

Vialard, J., E. C. S. Gilbert, C. M. Green, and N. F. Lokwende, 1998  The budding yeast Rad9 checkpoint protein is subjected to Mec1/Tel1-dependent hyperphosphorylation and interacts with Rad53 after DNA damage. EMBO J. 17(19): 5679–5688.

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(1): 11–23.

Webber, L., and B. Byers, 1992  A RAD9-dependent checkpoint blocks meiosis of cdc13 yeast cells. Genetics 131(1): 55–63.

Weinert, T. A., G. L. Kiser, and L. H. Hartwell, 1994  Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. Genes Dev. 8(6): 652–665.

Winter, E., 2012  The Sun1/Ndt80 transcriptional switch and commitment to meiosis in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 76(1): 1–15.

Winzeler, E. A., D. D. Shoemaker, A. Astromoff, H. Liang, K. Anderson et al., 1999  Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science 285(5429): 901–906.

Wu, H. Y., H. C. Ho, and S. M. Burgess, 2010  Mek1 kinase governs outcomes of meiotic recombination and the checkpoint response. Curr. Biol. 20(19): 1707–1716.

Wysocki, R., A. Javaheri, S. Allard, F. Sha, J. Cote et al., 2005  Role of Dot1-dependent histone H3 methylation in G1 and S phase DNA damage checkpoint functions of Rad9. Mol. Cell. Biol. 25(19): 8430–8443.

Xu, L., B. M. Weiner, and N. Kleckner, 1997  Meiotic cells monitor the status of the interhomolog recombination complex. Genes Dev. 11(1): 106–118.

Youds, J. L., and S. J. Boulton, 2011  The choice in meiosis—defining the factors that influence crossover or non-crossover formation. J. Cell Sci. 124( Pt 4): 501–513.

Zanders, S., M. Sonntag Brown, C. Chen, and E. Alani, 2011  Pch2 modulates chromatid partner choice during meiotic double-strand break repair in Saccharomyces cerevisiae. Genetics 188(3): 511–521.

Zegerman, P., and J. F. Diffley, 2010  Checkpoint-dependent inhibition of DNA replication initiation by Sld3 and Dbf4 phosphorylation. Nature 467(7314): 474–478.

Zhao, X., and R. Rothstein, 2002  The Dun1 checkpoint kinase phosphorylates and regulates the ribonucleotide reductase inhibitor Sml1. Proc. Natl. Acad. Sci. USA 99(6): 3746–3751.

Zhao, X., E. G. Muller, and R. Rothstein, 1998  A suppressor of two essential checkpoint genes identifies a novel protein that negatively affects dNTP pools. Mol. Cell 2(3): 329–340.

Zhang, P., D. S. Fay, J. Burton, H. Xiao, J. L. Pinkham et al., 1993  SPK1 is an essential S-phase-specific gene of Saccharomyces cerevisiae that encodes a nuclear serine/threonine/tyrosine kinase. Mol. Cell. Biol. 13(9): 5829–5842.

Zou, L., and S. J. Elledge, 2003  Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science 300(5625): 1542–1548.

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