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Sic1-induced DNA rereplication during meiosis

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Orderly progression through meiosis requires strict regulation of DNA metabolic events, so that a single round of DNA replication is systematically followed by a recombination phase and 2 rounds of chromosome segregation. We report here the disruption of this sequence of events in *Saccharomyces cerevisiae* through meiosis-specific induction of the cyclin-dependent kinase (CDK) inhibitor Sic1 mutated at multiple phosphorylation sites. Accumulation of this stabilized version of Sic1 led to significant DNA rereplication in the absence of normal chromosome segregation. Deletion of *DMC1* abolished DNA rereplication, but additional deletion of *RAD17* restored the original phenotype. Therefore, activation of the meiotic recombination checkpoint, which arrests meiotic progression at pachytene, suppressed DNA rereplication resulting from Sic1 stabilization. In contrast to deletion of *DMC1*, deletion of *NDT80*, which encodes a transcription factor required for pachytene exit, did not inhibit DNA rereplication. Our results provide strong evidence that CDK activity is required to prevent inappropriate initiation of DNA synthesis before the meiotic divisions.

checkpoint | cyclin-dependent kinase | premeiotic DNA replication | recombination

Meiosis is a specialized developmental process that leads to generation of haploid gametes from diploid precursors. Progression through the meiotic program includes a DNA replication phase, commonly referred to as "premeiotic S," followed by a reductional division (MI), in which homologous chromosomes segregate, and a subsequent equational division (MII), in which sister chromatids segregate. For most organisms, prophase of the meiotic divisions includes a period during which extensive recombination takes place. This process serves to increase variability by allowing for genetic exchange between parental chromosomes, and helps to ensure proper chromosome segregation during the meiotic divisions. When recombination intermediates accumulate, a checkpoint pathway is activated that delays meiotic progression at the pachytene stage of prophase until they disappear (1). Although considerable attention has been paid to meiotic recombination and chromosome segregation, including the checkpoints that govern these processes, much less is understood about the regulatory mechanisms that control premeiotic DNA replication.

The budding yeast *Saccharomyces cerevisiae* has proven to be an extremely useful model system for defining basic mechanisms underlying DNA replication control during the mitotic cell cycle. In this species, S phase entry depends on the B-type cyclin (Clb)/cyclin-dependent kinase (CDK) complexes Clb5 and −6/Cdk1(Cdc28) (2). A central regulatory component that controls Clb5,−6/Cdk1 activation at the G_1-S transition is the Clb/Cdk1 inhibitor Sic1 (3–5). During late G_1, G_1-cyclin/Cdk1 complexes catalyze Sic1 phosphorylation and promote its destruction by means of the ubiquitin/proteasome pathway (6–8). Accumulation of active Clb5,−6/Cdk1 enhances Sic1 destruction through the same mechanism and acts to advance cells into S phase. Once DNA synthesis has begun, Clb/Cdk1 in turn functions to prevent reinitiation of DNA replication by influencing various proteins involved in pre-replication complex (pre-RC) formation (9), including the origin recognition complex (ORC), the minichromosome maintenance (MCM) complex, and the Cdt1 and Cdc6 proteins that help ORC load the MCM complex. The effect of these phosphorylation reactions differs from protein to protein, as ORC is inactivated (10) while MCM and Cdt1 are exported from the nucleus (11, 12), and Cdc6 is degraded (13, 14). In addition to these enzymatic reactions, a Clb5-ORC physical interaction helps to inhibit reinitiation (15). The evolution of multiple mechanisms to prevent DNA rereplication indicates the importance of preventing this type of aberrant DNA metabolism, which in humans has been associated with genomic instability and cancer (16).

Deletion of the genes encoding Clb5 and Clb6 or inactivation of Cdk1 prevents premeiotic DNA replication (17–19), providing evidence that Clb5,−6/Cdk1 complexes also promote S phase entry during meiotic progression. Sic1 is present early in meiosis and disappears when cells enter premeiotic S phase (18, 19), suggesting that Sic1 destruction is an important event in meiosis as well. In fact, overexpression of Sic1 mutated at residues targeted by Cdk1 during the mitotic cell cycle stabilizes Sic1 and prevents premeiotic DNA replication in a dominant fashion (17, 20). Although these data suggest that the general mechanism of Clb5,−6/Cdk1 activation at the G_1-S transition is conserved, G_1-cyclin/Cdk1 complexes are not active during meiosis (18, 21), and Cdk1 activity is not required for Sic1 destruction accompanying premeiotic S phase entry (19). It has been proposed from genetic studies that the meiosis-specific protein kinase Ime2 directly replaces the G_1-cyclin/Cdk1 complexes (18); but recent results exploring the specificity of sites targeted by Ime2 have brought this hypothesis into question (20, 22–24). To our knowledge, whether Clb/Cdk1 activities are also involved in preventing DNA rereplication during meiosis as they are during the mitotic cell cycle had not been established before. Paradoxically, evidence exists that overexpression of various Clbs or prevention of Cdk1 inhibition during meiosis stimulates DNA rereplication (25, 26). This phenotype would not be expected if CDK activity were to act identically in preventing DNA rereplication during meiosis and the mitotic cell cycle.

To further understand the regulation of premeiotic S phase, we embarked on a series of experiments originally designed to elucidate the mechanism of Sic1 destruction. We used 2 versions of Sic1, one that is degraded normally during the mitotic cell cycle (referred to here as $Sic1^{HA}$), and one that is not degraded during the mitotic cell cycle due to mutation of multiple Cdk1-targeted phosphorylation sites ($Sic1\Delta P^{HA}$) (6). Through meiosis-specific expression of these Sic1 proteins, we found that stabilization of Sic1 during meiosis led to DNA rereplication. These data provide evidence that CDK activity is required to prevent DNA rereplication during meiotic progression before the meiotic divisions.

Results

Meiosis-Specific Expression of $Sic1^{HA}$ and $Sic1\Delta P^{HA}$. To gain insight into Sic1 regulation during meiosis, we placed the genes encoding $Sic1^{HA}$ and $Sic1\Delta P^{HA}$ (stable during the mitotic cell cycle; see ref. 6) downstream of the meiosis-specific *HOP1* promoter (*HOP1pr*).

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The authors declare no conflict of interest.

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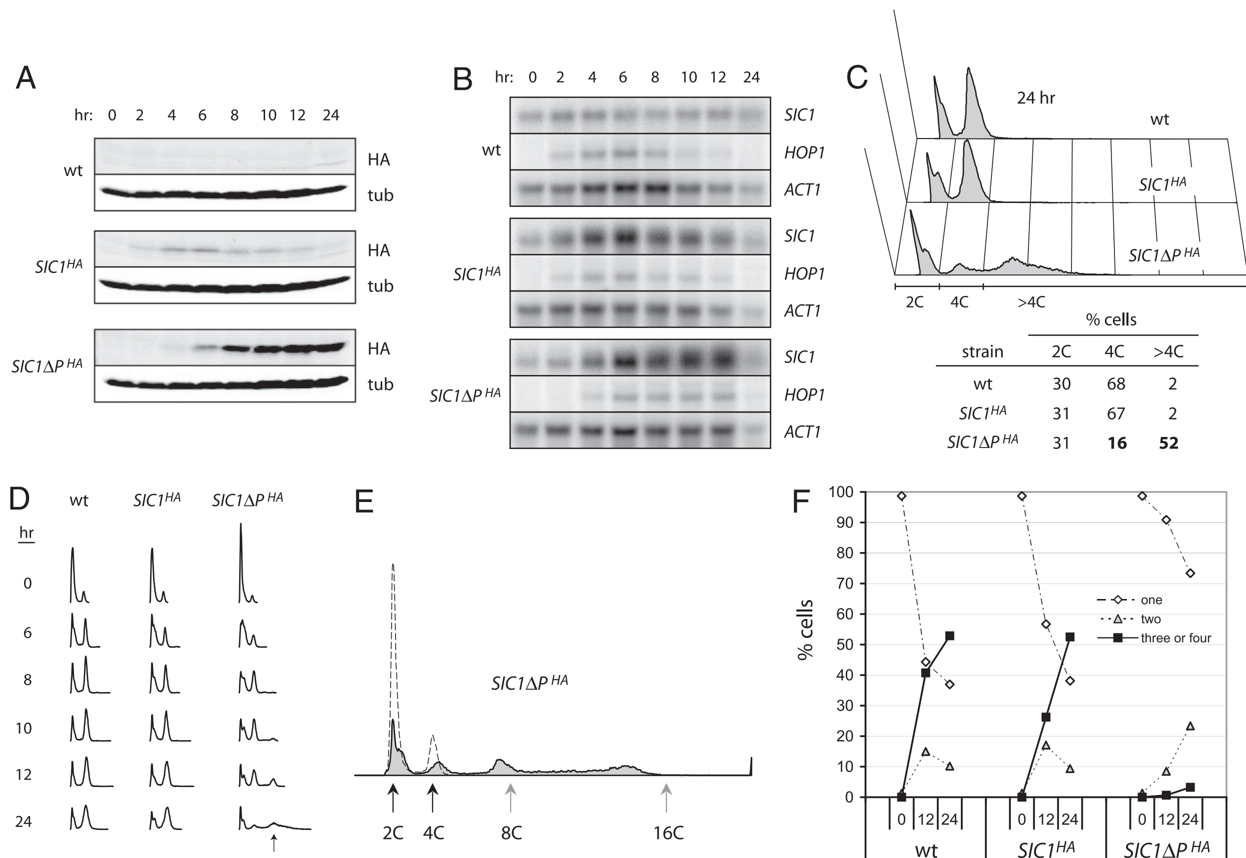

strain	2C	4C	>4C
wt	30	68	2
SIC1^{HA}	31	67	2
SIC1ΔP^{HA}	31	**16**	**52**

Fig. 1. Sic1ΔP^{HA} expression leads to DNA rereplication. Cells were induced to enter meiosis and analyzed for various parameters over time. Comparisons were made between the parental strain (wt; YGB138) and derivatives designed to express either *SIC1*^{HA} (YGB613) or *SIC1ΔP*^{HA} (YGB495) during meiosis (with the exception of *E*, data are shown for the same time course experiment). (*A*) Western blot analysis with antibodies directed against HA (for detection of ectopically generated Sic1) or tubulin. (*B*) Northern blot analysis with probes directed against *SIC1*, *HOP1*, or *ACT1*. The *SIC1* probe was designed to hybridize with either endogenous or ectopically expressed transcript. (*C*) DNA content analysis by flow cytometry. The 24-h histograms are directly compared, indicating the DNA rereplication phenotype. The table indicates the percentage of cells found in the 2C, 4C, and >4C ranges as defined under the histograms. (*D*) DNA content for multiple time points are shown. (*E*) DNA content of Sic1ΔP^{HA}-expressing cells at 0 h (dotted line) and 24 h (black line, filled) from a different time-course experiment. Positions corresponding to 2C and 4C DNA content for the 0-h sample are indicated. Estimated 8C and 16C positions are also shown, assuming a linear response of fluorescence intensity to DNA content. (*F*) Nuclear staining of cells at 0, 12, and 24 h. Percentages of cells containing the indicated number of DAPI-staining bodies are shown.

When subjected to sporulation conditions that induce meiosis, the *HOP1pr-SIC1*^{HA} cells behaved like wild-type cells with regard to efficiency of mature meiotic product formation (both achieving 45% ascus formation for the experiment shown in Fig. 1) and spore viability (>90% as determined by tetrad dissection). In contrast, the *HOP1pr-SIC1ΔP*^{HA} cells generated few asci, indicating a significant meiotic defect. We examined steady-state protein levels over time by Western blot analysis, and found that Sic1ΔP^{HA} protein accumulated to a much greater extent than Sic1^{HA} (Fig. 1*A*). Northern blot analysis revealed that both *SIC1*^{HA} transcripts were induced from the *HOP1pr* elements with patterns nearly identical to those of the *HOP1* transcripts [Fig. 1*B* and supporting information (SI) Fig. S1]. *SIC1*^{HA} exhibited a peak in expression, as would be expected from a *HOP1pr*-controlled transcript, but *SIC1ΔP*^{HA} did not. Note that recovery of 24-h transcripts was inefficient in all 3 strains. Inhibition of meiotic progression due to Sic1ΔP^{HA} accumulation (see further results below) most likely prevented the decline in *SIC1ΔP*^{HA} transcript at the later time points. On occasion, we observed a delay in *SIC1ΔP*^{HA} expression relative to *SIC1*^{HA}, reflected by a corresponding delay in *HOP1* expression as shown here (Fig. 1*B* and Fig. S1). Although it is unclear whether this type of delay was related to the transcript itself or was due to subtle experimental variation, the timing of Sic1^{HA} and Sic1ΔP^{HA} appearance coincided with the appearance of their respective transcripts (Fig. 1*A*). We conclude from the later time points that Cdk1-targeted residues help to control the steady-state level of Sic1 during meiosis, similar to what has been reported previously (17, 20), and consistent with the role of these residues during the mitotic cell cycle.

We further examined the cells in this time course for parameters related to meiotic progression. All 3 strains were capable of completing premeiotic DNA replication, as detected by increases in 4C DNA content over time (Fig. 1 *C* and *D*). Strikingly, a significant portion of the Sic1ΔP^{HA}-expressing cells underwent extra DNA replication. In this experiment, 1/2 of the cells accumulated >4C DNA content by 24 h, and 3/4 of the cells that proceeded through premeiotic S went on to rereplicate (Fig. 1*C*). To verify that the effect was due to Sic1ΔP^{HA} and not a spurious mutation, we used counterselection to isolate a spontaneous recombinant that had lost the *SIC1ΔP*^{HA} gene (see *Materials and Methods*). This strain did not exhibit DNA rereplication, indicating that the phenotype depended on the presence of *HOP1pr-SIC1ΔP*^{HA} (data not shown).

In the time course shown here, we first observed an accumulation of cells with DNA content approaching 8C (assuming a linear response of DNA content to fluorescence intensity in our flow cytometry analysis) at 10 h (Fig. 1*D*). Although we have observed a peak in this location routinely, on some occasions we have observed a second peak approaching 16C DNA content (Fig. 1*E*).

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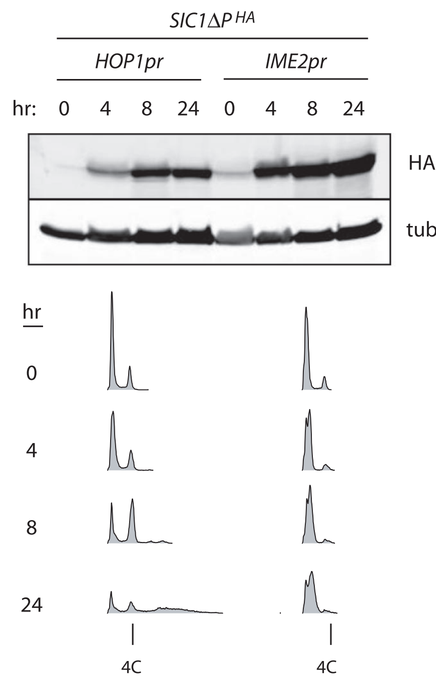

Fig. 2. The effect of *SIC1ΔP*^{HA} expression depends on the meiosis-specific promoter element. Cells containing *SIC1ΔP*^{HA} under the control of the *HOP1pr* (YGB586) or the *IME2pr* (YGB602) were induced to enter meiosis and compared for Sic1ΔP^{HA} protein level by Western blot analysis, and for DNA content by flow cytometry.

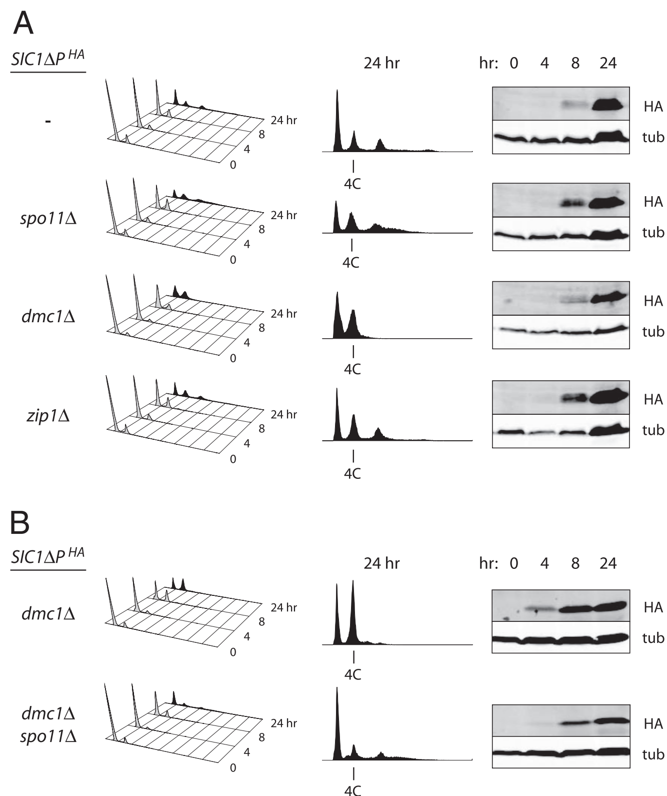

Fig. 3. Deletion of *DMC1* abolishes DNA rereplication. (*A*) DNA content and Western blot analyses were conducted on cells induced to enter meiosis. Sic1ΔP^{HA}-expressing cells contained either intact meiotic recombination machinery (YGB495) or specific recombination defects due to gene deletions: *spo11Δ* (YGB532), *dmc1Δ* (YGB535), or *zip1Δ* (YGB533). Progression through meiotic S phase can be assessed from the composites on the left, whereas the degree of DNA rereplication can be assessed from the enlarged 24-h histograms. (*B*) Dependence of the *dmc1Δ* phenotype on DNA-double-strand break formation was tested by comparison of *dmc1Δ* (YGB604) with *dmc1Δ spo11Δ* (YGB595).

These data suggest that some cells expressing Sic1ΔP^{HA} were capable of reduplicating their entire genomes once and possibly twice. However, further characterization will be required to determine whether all DNA sequences were rereplicated or specific regions were preferentially amplified.

We also examined progression into the meiotic divisions through nuclear staining (Fig. 1*F*). For both control strains, ~50% of the cells contained 3 or 4 DAPI-staining bodies by 24 h, consistent with progression through MII. In contrast, a majority of the Sic1ΔP^{HA}-inducing cells contained a single DAPI-staining body even at 24 h, with <5% containing 3 or 4 DAPI-staining bodies. This type of meiotic phenotype would be expected with Sic1 accumulation, at least after prophase, because Clb/Cdk1 activities are required for progression through the meiotic divisions (27, 28).

Previous studies have shown that cells induced to express Sic1ΔP on meiotic entry are incapable of completing premeiotic DNA replication (17, 20). We reasoned that factors such as the strain background or the promoter element used in our experiments led to a condition that permitted premeiotic DNA replication despite expression of stabilized Sic1. To explore this issue, we repeated our experiment with the *IME2* promoter (*IME2pr*), which was used in the previous studies. In this case, we found that expression of Sic1ΔP^{HA} prevented completion of premeiotic DNA replication, as previously reported (Fig. 2). Therefore, the phenotype resulting from Sic1ΔP^{HA} expression depended on the particular promoter element that was used. Based on comparison of Sic1ΔP^{HA} protein levels, it appears that *IME2pr* directed stronger, and perhaps earlier, expression than *HOP1pr* in our system.

Effects of Recombination Defects on DNA Rereplication. Programmed recombination immediately follows premeiotic S during prophase of the meiotic divisions. To investigate the impact of recombination on the DNA rereplication phenotype, we generated mutants defective for 1 of 3 recombination proteins that act at different stages during meiotic recombination: Spo11, a transesterase that catalyzes DNA double-strand break formation (29); Dmc1, a meiosis-specific recombinase that catalyzes strand exchange (30); and Zip1, a critical component of the synaptonemal complex that promotes cross-over events (31, 32). All recombination-deficient strains accumulated Sic1ΔP^{HA} when allowed to enter meiosis (Fig. 3). Deletion of *SPO11* or *ZIP1* did not prevent expression of the Sic1ΔP^{HA}-induced phenotype, but DNA rereplication was inhibited in the *dmc1Δ* strain (Fig. 3*A*), even at an extended incubation time of 48 h (data not shown). To confirm that *dmc1Δ*-mediated inhibition of DNA rereplication resulted from the recombination defect associated with the absence of Dmc1, we combined *dmc1Δ* and *spo11Δ* in our Sic1ΔP^{HA}-expressing cells. As expected, we found that inhibition of DNA rereplication was relieved (Fig. 3*B*), indicating that Spo11-catalyzed DNA double-strand break formation was required for the *dmc1Δ* phenotype. Assuming that strain-dependent variations in Sic1ΔP^{HA} expression were not involved (see *Discussion*), we conclude that the recombination intermediates generated in a *dmc1Δ* mutant, which are characterized by DNA double-strand breaks with long 3′-single-stranded DNA extensions (30), inhibited the DNA rereplication associated with Sic1ΔP^{HA} expression. Although we might have expected *zip1Δ*-induced recombination intermediates to inhibit DNA rereplication in a similar fashion, *zip1Δ* cells exhibit different degrees of checkpoint-mediated delay, depending on strain background and incubation temperatures (32). Therefore, we focused further efforts on the *dmc1Δ* effect in this study.

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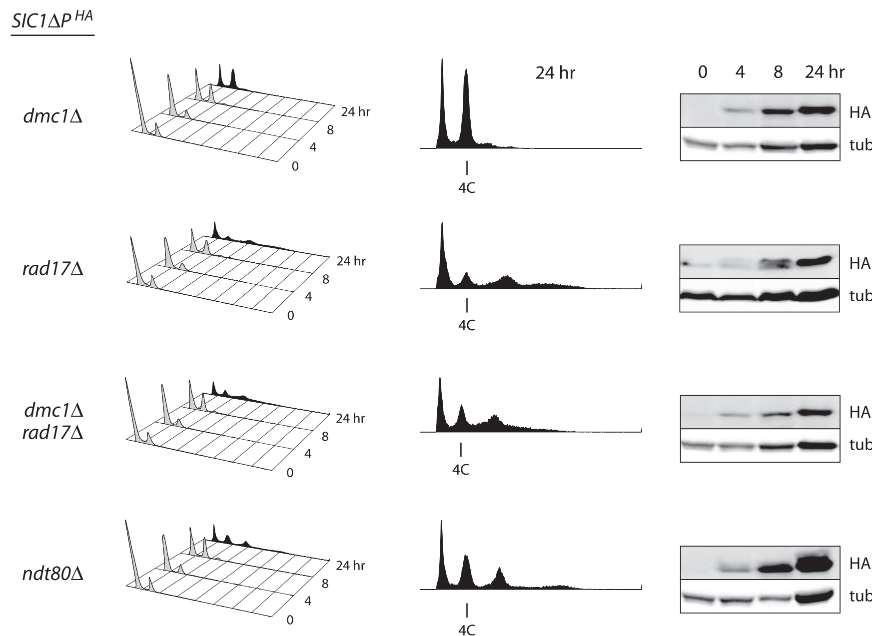

Fig. 4. A Rad17-mediated checkpoint prevents Sic1ΔP^{HA}-induced DNA rereplication. DNA content and Western blot analyses were conducted on cells induced to enter meiosis. Sic1ΔP^{HA}-expressing cells contained the following mutations: *dmc1Δ* (YGB535), *rad17Δ* (YGB553), *dmc1Δ rad17Δ* (YGB554), or *ndt80Δ* (YGB583).

The Recombination Checkpoint and DNA Rereplication. Because Dmc1 recombinase activity itself was not required for DNA rereplication (Fig. 3*B*), we considered 2 other possibilities that could explain the lack of DNA rereplication specifically in the absence of Dmc1: (*i*) recombination intermediates generated chromosomal structures that were physically impossible to replicate, or (*ii*) a checkpoint signal was induced that prevented DNA replication. To address these possibilities, we analyzed the role of Rad17. This protein is a member of a PCNA-like clamp that encircles DNA (33), and is required for initiation of many DNA metabolism-related checkpoint responses, including the meiotic recombination checkpoint (1). We found that Sic1ΔP^{HA}-induced DNA rereplication reappeared when both Dmc1 and Rad17 were absent (Fig. 4). These data argue against a restriction of DNA rereplication due to recombination intermediates, which are generated in *dmc1Δ rad17Δ* cells (1), and suggest instead that the *dmc1Δ* mutation induced a checkpoint signal that prevented DNA rereplication when Sic1ΔP^{HA} was expressed.

One of the known targets of the meiotic recombination checkpoint response is Cdk1 itself, which becomes phosphorylated in a Swe1-dependent reaction that helps to prevent Clb/Cdk1 activation and consequent pachytene exit (34). A second target of the meiotic recombination checkpoint is Ndt80, which is a transcription factor that activates expression of many so-called "middle" genes, including those that encode 5 different Clbs (35). In normal meiotic progression, Ndt80 function is required for pachytene exit (36), and activation of the recombination checkpoint inhibits Ndt80-mediated transcriptional activation to facilitate prevention of pachytene exit (35, 37, 38). Based on the requirement of Clb/Cdk1 activities for the meiotic divisions (27, 28), we suspected that DNA rereplication induced in our system by the Sic1 Clb/Cdk1 inhibitor did not require pachytene exit. The defective meiotic progression of our Sic1ΔP^{HA}-expressing cells provided additional evidence that DNA replication could occur before the meiotic divisions (Fig. 1*E*). To further explore this possibility, we analyzed *ndt80Δ* cells and found that Sic1ΔP^{HA}-induced DNA rereplication still occurred in the absence of Ndt80 (Fig. 4). This result confirmed that pachytene exit was not required for DNA rereplication in our system, and indicated that down-regulation of Ndt80 was not involved in the meiotic recombination checkpoint that prevented reinitiation of DNA synthesis when Sic1ΔP^{HA} was expressed.

Discussion

During the mitotic cell cycle, Clb/Cdk1 activities promote S phase entry and prevent DNA rereplication (2, 9). These enzymes are also required for premeiotic DNA replication (17–19), but their role in preventing DNA rereplication during meiosis had not been defined previously. Through ectopic expression of a mutant form of the Clb/Cdk1 inhibitor Sic1, we have now successfully induced meiotic cells to undergo DNA rereplication. The version of Sic1 that we used is not properly degraded during the mitotic cell cycle due to mutation of multiple residues targeted by Cdk1 (6). Based on previously published results, we anticipated that expression of Sic1ΔP^{HA} would inhibit progression of cells through premeiotic S phase (17, 20). However, we used a different early meiosis-specific promoter (*HOP1pr* vs. *IME2pr*), which accounted for this disparity due to timing and/or strength of induction. Despite the difference in phenotype, we have reached the same conclusion of these earlier studies that the sites targeted by Cdk1 during mitosis also have a role in destabilizing Sic1 during meiosis. Importantly, our data provide evidence that Clb/Cdk1 activity is required to prevent DNA rereplication during meiosis. From our genetic analysis, we conclude that this mechanism is activated before cells enter the meiotic divisions. This timing would suggest involvement of Clb5,-6/Cdk1 complexes, which are required for initiation of both premeiotic DNA replication (17–19) and recombination (39, 40), as opposed to the Clb1,−3, and −4/Cdk1 complexes, which are required for the meiotic divisions (Fig. 5) (27, 28).

Sic1 overexpression has been successfully used to induce DNA rereplication during the mitotic cell cycle in *S. cerevisiae*. However, this phenotype requires induction of Sic1 to allow for pre-RC establishment at origins followed by reduction of Sic1 to provide sufficient CDK activity for origin firing (9). The DNA rereplication that we induced during meiosis did not require experimental modulation of Sic1 levels, and is reminiscent of the DNA rereplication that is observed in fission yeast *Schizosaccharomyces pombe* mitotic cells when CDK inhibitors are overexpressed (41, 42). We have consistently observed 1 peak approaching 8C DNA content, corresponding to 1 round of DNA rereplication, and have occa-

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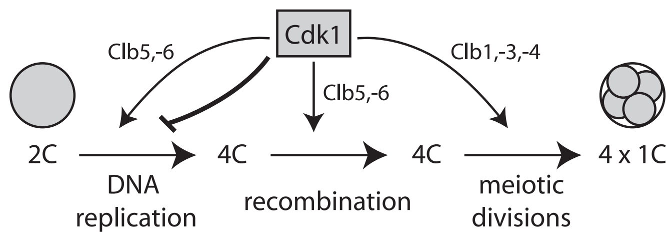

Fig. 5. Meiotic CDK-mediated pathways. Cdk1 is activated by the indicated Clb subunits during meiosis to promote proper development of a single diploid (2C DNA content) progenitor into 4 haploid (1C) spores. The bold line represents the inhibition of DNA rereplication characterized in this study. See text for further details and references.

sionally observed a second peak approaching 16C, corresponding to 2 rounds of DNA rereplication (see Fig. 1*E*). Accumulation of cells with ≈2^n DNA contents (>4C) suggests that initiation of DNA rereplication was periodic, similar to the natural endoreplication process that serves to increase ploidy in various cell types (43). Because DNA rereplication in our system was observed in the absence of Spo11 (Fig. 3), we suspect that reinitiation did not proceed through a recombination-mediated process, but was likely to be origin-dependent as in the case of normal premeiotic DNA replication. Based on quantitative models of CDK-mediated endoreplication (44), and evidence that higher CDK activity is required for the meiotic divisions than premeiotic DNA replication (19), we suggest that meiosis-specific induction of Sic1ΔP^{HA} led to a decrease in CDK activity that was below the threshold required for normal chromosome segregation, but periodically above the threshold required for origin-dependent initiation of DNA synthesis. Temporal oscillation of the reduced CDK activity would allow for a second phase of pre-RC formation (lower CDK activity) followed by origin-firing and immediate prevention of reinitiation (higher CDK activity). An additional wave of CDK inactivation/activation would give rise to an additional peak of cells with 16C DNA content. It is possible that the strength of promoters used in *S. cerevisiae* mitotic studies has prevented expression of the same phenotype through simple *SIC1* overexpression.

In considering the Sic1-induced meiotic DNA rereplication that we report here, it is interesting to note that overexpression of Clb1 or Clb5 during meiosis is reported to stimulate DNA rereplication (25), as is deregulation of Cdk1 (26). These results have suggested that DNA rereplication prevention is accomplished differently in meiotic and proliferating cells, because neither Clb overexpression nor prevention of Cdk1 inhibition stimulates DNA rereplication during the normal cell cycle. These results also seem to be at odds with our conclusion that Clb/Cdk1 complexes help to prevent DNA rereplication. However, Clb/Cdk1 activation leads to formation of viable multads that contain >4 haploid products, and in some cases as many as 20. Therefore, the nature of the DNA rereplication induced by increased CDK activity is clearly different from the type that we observed. It is possible that differences in the timing of CDK deregulation are at play, leading to phenotypes that in both cases involve inappropriate DNA replication but with substantially different end results.

In testing recombination-deficient mutants, we discovered that loss of Dmc1 prevented DNA rereplication. This result on its own might suggest that the recombinase activity provided by Dmc1 was required for inappropriate DNA replication in our system. However, loss of Dmc1 combined with loss of either Spo11 or Rad17 overrode the block, disproving direct involvement of Dmc1 and suggesting that the absence of Dmc1 activated a checkpoint that prevented DNA rereplication. We cannot formally rule out the possibility that gene deletions indirectly influenced the DNA rereplication phenotype by altering Sic1ΔP^{HA} levels. However, expression of the DNA rereplication phenotype within the context of these mutations did not appear to correlate with either enhanced or reduced Sic1ΔP^{HA} expression (see Sic1ΔP^{HA} levels in Figs. 3 and 4). Therefore, we propose that the meiotic recombination checkpoint influenced DNA rereplication through 1 of 2 possible mechanisms. In the first scenario, activation of the checkpoint lowered the residual CDK activity below the threshold required for initiation of DNA replication. This decrease might occur through Swe1-mediated Cdk1 inactivation, a mechanism that helps to prevent chromosome segregation in the meiotic recombination checkpoint response (34). In the second scenario, activation of the meiotic recombination checkpoint targeted the DNA replication machinery more directly to inhibit reinitiation. This possibility is based on the knowledge that the Rad53 checkpoint protein is required for delay of initiation at late origins during S phase of the mitotic cell cycle (45). Therefore, the checkpoint machinery in mitotic cells can inhibit firing of certain origins despite the presence of adequate CDK activity. Further studies will be required to precisely define the nature of Sic1-induced DNA rereplication during meiosis and the means by which the recombination checkpoint prevents its occurrence.

Materials and Methods

Strains and Plasmids. Yeast strains used in this study were congenic with W303 (46), and are listed in Table S1. Plasmids encoding meiosis-inducible Sic1 proteins were generated from plasmids kindly provided by Raymond Deshaies (California Institute of Technology, Pasadena, CA). These plasmids encode galactose-inducible Sic1 derivatives with HA and 6xHis tags at the C termini. One protein version, referred to here as Sic1^{HA}, is degraded properly during vegetative growth, whereas the other, referred to here as Sic1ΔP^{HA}, is resistant to degradation during vegetative growth due to mutations of multiple Cdk1-targeted phosphorylation sites (6). Both proteins contain a T2A mutation, whereas Sic1ΔP^{HA} contains additional T5GP, T33A, and S76A mutations. Approximately 1 kb *HOP1pr* and *IME2pr* regions were PCR amplified with BamHI-tailed primers from pNH59–2 (47), kindly provided by Jacqueline Segall (University of Toronto, Toronto, Canada), and yeast genomic DNA, respectively. These PCR products were inserted individually upstream of *SIC1^{HA}* and *SIC1ΔP^{HA}* at the BamHI site in the 2 plasmids, and the sequences of cloned promoters were verified. The resulting plasmids were integrated into the genomes of various strains at the *ura3* locus after digestion with Sse8387I (Amersham) or its isoschizomer SbfI (New England Biolabs). All insertions were verified by PCR. A spontaneous revertant of the diploid strain containing *HOP1pr-SIC1ΔP^{HA}* (YGB495) to uracil auxotrophy (indicating loss of the *HOP1pr-SIC1ΔP^{HA}* element) was isolated by counterselection with fluoro-orotic acid (Toronto Research Chemicals) (48). Deletion mutations were generated in haploids by site-specific integration of markers PCR-amplified from the genomic DNA of previously characterized deletion mutants (49). Where necessary, *kanMX4* (G418 resistance) markers were switched to *natR* (nourseothricin resistance) markers by using the p4339 plasmid kindly provided by Charles Boone (University of Toronto) (50). All deletions were verified by PCR.

Synchronous Sporulation. All yeast incubations were conducted at 30 °C. Meiosis was induced by starvation, based on an established procedure for synchronous sporulation (51). In this method, cells were grown in YPA (1% yeast extract/2% peptone/2% potassium acetate) liquid culture for 15½ −16 h and then switched to a liquid sporulation medium consisting of 0.3% potassium acetate and 0.02% raffinose supplemented with leucine and histidine both at 250 μM, tryptophan at 100 μM, uracil at 50 μM, and adenine at 19 μM. With the exception of the *SIC1ΔP^{HA} rad17Δ* time course included in Fig. 4, comparisons are shown between strains that were grown and sporulated with common media prepared for use in a single experiment.

Whole-Cell Lysate Preparation and Western Blot Analysis. Denatured whole-cell extracts were prepared as previously described (52, 53), with minor modifications. Cells (2 mL) were harvested by centrifugation, resuspended in 1 mL of ice-cold water, and treated with 150 μL of ice-cold 2 N NaOH/8% ß-mercaptoethanol. Proteins were precipitated by the addition of 150 μL of 50% trichloroacetic acid and harvested by centrifugation. Protein pellets were washed twice in cold acetone, dried completely, and resuspended in 50 μL of SDS/PAGE sample buffer. Samples were vortexed to completely dissolve the pellets, and then heated at 95 °C for 5 min. Samples were then subjected to SDS/PAGE, and the separated proteins were transferred to nitrocellulose membranes (GE Healthcare) in 25 mM Tris/192 mM glycine/20% methanol. Immunoblotting was conducted by using the fluorescence-scanning Odyssey system and its associated software (LiCor). Pri-

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mary antibodies used were mouse anti-hemagglutinin monoclonal (HA-11, Covance) and rat anti α-tubulin polyclonal (Serotec). Signals were generated with Alexa Fluor 680 goat anti-mouse (Invitrogen) and IRDye 800-conjugated goat anti-rat (Rockland) secondary antibodies.

Northern Blot Analysis. Total RNA (5 μg) was isolated by using a kit from Epicentre and subjected to electrophoresis through a 1.2% agarose gel in 20 mM Mops, pH 7.0/5 mM sodium acetate/1 mM EDTA/0.74% formaldehyde. The separated RNA was transferred to Hybond-N^+ nylon membrane (Amersham) in 10× SSC buffer by capillary elution. Probes specific to *SIC1* (ORF nucleotides 305–783), *HOP1* (ORF nucleotides 269–778), and *ACT1* (ORF nucleotides 277–870) were generated by PCR amplification with genomic DNA as template and then labeled with [α-^{32}P]dCTP (PerkinElmer) by using a random primer DNA labeling kit (Roche). Hybridization was conducted with individual probes overnight at 65 °C. Radioactivity was detected through PhosphorImager (GE Healthcare) analysis.

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 of RNase A for 1 h at 37 °C, followed by 250 μg of proteinase K for 1 h at 37 °C. The digested samples were incubated with 10× SYBR Green I (Molecular Probes) at 4 °C overnight, sonicated briefly, and analyzed with a FacsCalibur flow cytometer (BD Biosciences). DNA content histograms were generated and analyzed by using WinMDI software.

Nuclear Staining. Cells were initially treated for 1 h at room temperature with 3.7% formaldehyde, washed with water, and stored overnight at 4 °C in 1 M sorbitol. The fixed cells were then resuspended in a freshly prepared 4% formaldehyde fixative (54), and incubated at room temperature for 2 h, washed with water, and stored in 1.2 M sorbitol at 4 °C. Aliquots were resuspended in 50% glycerol containing 1 μg/mL DAPI, applied to polylysine-coated slides, and analyzed by fluorescence microscopy by using an Olympus IX71 inverted microscope equipped with a Hamamatsu ORCA-ER Model 1394 camera for capturing digital images. At least 150 cells were counted for each sample (range, 150 to 167 cells).

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