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Polo kinase Cdc5 is a central regulator of meiosis I

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During meiosis, two consecutive rounds of chromosome segregation yield four haploid gametes from one diploid cell. The Polo kinase Cdc5 is required for meiotic progression, but how Cdc5 coordinates multiple cell-cycle events during meiosis I is not understood. Here we show that CDC5-dependent phosphorylation of Rec8, a subunit of the cohesin complex that links sister chromatids, is required for efficient cohesin removal from chromosome arms, which is a prerequisite for meiosis I chromosome segregation. CDC5 also establishes conditions for centromeric cohesin removal during meiosis II by promoting the degradation of Spo13, a protein that protects centromeric cohesin during meiosis I. Despite CDC5’s central role in meiosis I, the protein kinase is dispensable during meiosis II and does not even phosphorylate its meiosis I targets during the second meiotic division. We conclude that Cdc5 has evolved into a master regulator of the unique meiosis I chromosome segregation pattern.

Polo kinases are central regulators of chromosome segregation and control multiple mitotic events (1). Budding yeast contains a single Polo kinase, CDC5. Unlike in higher eukaryotes, budding yeast CDC5 primarily regulates postmetaphase events, its essential function being to trigger exit from mitosis (2). CDC5 also contributes to the efficient inactivation of cohesins, the protein complexes that hold sister chromatids together until the onset of chromosome segregation. Cdc5 phosphorylates the cohesin subunit Mcd1/Scc1 to facilitate its cleavage by the protease separase (3).

CDC5 also regulates the specialized cell division that gives rise to gametes, known as meiosis (4). During meiosis, two consecutive rounds of chromosome segregation follow one round of DNA replication. During meiosis I, homologous chromosomes segregate; during meiosis II, sister chromatids separate (5). The chromosome segregation machinery is modified in three ways to facilitate the unusual meiosis I division. First, the combination of homologous recombination and cohesin complexes distal to the resulting cross-overs mediates the physical linkage of homologous chromosomes, which is essential for their accurate segregation during meiosis I. Second, sister chromatids of each homolog must be segregated to the same pole rather than to opposite poles, as they are during mitosis. The fusion of sister kinetochores by co-orientation factors (the monopolin complex in yeast) facilitates the attachment of microtubules emanating from one spindle pole. Third, cohesin complexes must be lost in a stepwise manner from chromosomes. During meiosis I cohesin complexes are lost from chromosome arms to bring about the segregation of homologous chromosomes (6). The residual cohesins at centromeres facilitate the accurate segregation of sister chromatids during meiosis II. Cdc5 has been implicated in the execution of all three meiosis I-specific events. CDC5 is required for the resolution of double Holliday junctions during homologous recombination (7, 8). Cdc5 also controls the co-orientation of sister chromatids by promoting the association of the monopolin complex with kinetochores (7, 9). Finally, CDC5 has been implicated in regulating the stepwise loss of cohesins (7, 9, 10). Phosphorylation of the cohesin subunit Rec8, a meiosis-specific cohesin subunit that replaces Scc1/Mcd1 in the meiotic cohesin complex, controls the stepwise loss of cohesins from chromosomes. Rec8 phosphorylation is critical for its proteolytic cleavage and removal from chromosome arms during meiosis I (10, 11). Maintaining Rec8 in a dephosphorylated form around centromeric regions protects it from cleavage. This is accomplished by Sgo1, a shugoshin/MEI-S332 family member that recruits protein phosphatase 2A to centromeric regions (12). Our studies have implicated Cdc5 as one, but not the only, protein kinase phosphorylating Rec8 to target it for proteolytic cleavage by separase (10).

In addition to controlling meiosis I-specific events, CDC5 also regulates general cell-cycle functions during meiosis I that it does not affect during mitosis. During meiosis I, CDC5 controls separase activity. Degradation of the separase inhibitor securin (Pds1 in yeast) liberates separase to trigger anaphase (5). During meiosis I, but not during mitosis, CDC5 is required for Pds1 degradation (7, 9). How Cdc5 takes on new functions during meiosis I is not understood. Similarly, little is known about whether and how Cdc5 functions during meiosis II because cells depleted for Cdc5 arrest in metaphase I (7, 9).

Here we show that CDC5 controls cohesin removal in multiple ways. CDC5-dependent phosphorylation of Rec8 is essential for efficient Rec8 cleavage. Furthermore, Cdc5 triggers the degradation of Spo13, thereby contributing to the dismantling of the cohesin-protective domain around centromeres. Our data further show that despite its central role in meiosis I chromosome segregation, CDC5 is dispensable during meiosis II and does not phosphorylate its meiosis I targets during meiosis II. Our findings indicate that the evolution of additional CDC5 functions is a central aspect of establishing the unique meiotic chromosome segregation pattern.

Results

Phosphorylation of Rec8 is CDC5-Dependent. Others and we previously determined that phosphorylation of Rec8 is crucial for its cleavage during meiosis I (10, 11). Our studies showed that many of the sites important for Rec8 cleavage were phosphorylated in a CDC5-dependent manner and that CDC5 was required for Rec8 cleavage (10). Katis et al. (11) identified Cdc5 and two additional kinases, Hrr25 and DDK, to control Rec8 phosphorylation but came to the conclusion that CDC5-dependent phosphorylation of Rec8 did not contribute to Rec8 cleavage. This discrepancy prompted us to reinvestigate the role of CDC5 in Rec8 cleavage and cohesin removal.

We first verified that the phosphorylation sites in Rec8 that we previously determined to be CDC5-dependent by mass spectrometry (10) were indeed phosphorylated in a CDC5-dependent manner. We raised phospho-specific antibodies against three Rec8 phosphorylation sites, S136, S179, and S521. Consistent with our mass spectrometry analysis, S136 and S179 phosphorylation was

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The authors declare no conflict of interest.

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CDC5-dependent, whereas S521 phosphorylation was CDC5-independent. S136 and S179 were phosphorylated in cells depleted for the anaphase-promoting complex/cyclosome (APC/C) activator Cdc20, which arrests cells in metaphase I but not in cells depleted for Cdc5, which also arrests cells in metaphase I (Fig. 1A and Fig. S1 A–E) (9). Furthermore, expression of CDC5 was sufficient to induce S136 and S179 phosphorylation. We arrested cells in prophase I, when Cdc5 is normally not expressed, and then induced CDC5 expression from the copper-inducible CUP1 promoter. Both S136 and S179 phosphorylation were induced upon Cdc5 expression (Fig. 1B and Fig. S1 F and G). We conclude that CDC5 is necessary and sufficient for the phosphorylation of Rec8-S136 and Rec8-S179. Moreover, because the CDC5 dependence of S136, S179, and S521 was accurately defined by our mass spectrometry analysis, phosphorylation of other sites defined as CDC5-dependent by our mass spectrometry analysis most likely indeed depends on CDC5.

Phosphorylation of Rec8-S136 and Rec8-S179 Contributes to Cohesin Cleavage. By examining the effects of mutating phosphorylated residues to amino acids that mimic phosphorylation, Kats et al. (11) showed that phosphorylation of four sites, S136, S179, S197, and T209, is critical for cohesin removal. Three of these sites, S136, S179, and S197, were identified as CDC5-dependent by our mass spectrometry analysis (10), two of which, S136 and S179, were verified by phospho-specific antibodies (Fig. 1A and Fig. S1 A–E). If phosphorylation of these sites is important for cohesin cleavage, Rec8 mutants that mimic phosphorylation should no longer be protected from cleavage around centromeres and centromeric cohesins ought to be lost prematurely during meiosis I.

We monitored chromosome segregation using a tandem array of TetO sequences integrated close to the centromere of one copy of chromosome V. Cells also expressed a tetR–GFP fusion, allowing visualization of the tetO arrays. In wild-type cells carrying these heterozygous GFP dots, meiosis I yields two nuclei, with two GFP dots in one of the two nuclei. After meiosis II, two of the four nuclei contain one GFP dot each. If centromeric cohesins are lost prematurely, chromosome segregation will appear normal during meiosis I, but sister chromatids will segregate randomly during meiosis II. Whereas mutating S136 and S179 to aspartic acid alone had little effect on meiotic chromosome segregation, the two mutations significantly enhanced a S197D mutant, which by itself also had little effect on chromosome segregation. Meiosis II chromosome segregation was subtly affected in the rec8-S197D mutant (11.6% missegregation) but was close to random in the rec8-S136D S179D S197D mutant (27%; P = 0.016; Fig. 1C, Fig. S2A, and Table S1).

The premature loss of centromeric cohesin in the rec8-S136D S179D S197D mutant was not due to an inability to establish functional cohesion. Cells depleted for the APC/C activator Cdc20 (CDC20-mn) and lacking the double-strand break inducing endonuclease Spo11 arrest in metaphase II because they cannot degrade the separase inhibitor securin, thus preventing separase from cleaving cohesin (13). Replacing REC8 with the rec8-S136D S179D S197D allele did not affect the ability of CDC20-mn spo11Δ cells to arrest in metaphase II (Fig. 1D). Importantly, analysis of rec8-4D localization directly demonstrated that the protein loads normally onto chromosomes during prophase I but was lost prematurely in meiosis I (Fig. S2B). Only 53% of anaphase I/metaphase II cells exhibited Rec8 localization around centromeres. We conclude that Rec8-S136 and Rec8-S179 phosphorylation contributes to cohesin removal.

CDC5 Is Required for Rec8 Cleavage. Although the above phosphomutant analysis demonstrates that CDC5-dependent phosphorylation contributes to cohesin removal, the large number of CDC5-dependent phosphorylation sites within Rec8 makes it difficult to assess the degree to which CDC5 is needed for cohesin removal. We thus examined the consequences of depleting Cdc5 on cohesin cleavage by placing the CDC5 gene under the control of the mitosis-specific CLR2 promoter (CDC5-mn) (9).

CDC5-mn cells arrest in metaphase I. Securin is partially stabilized in this arrest, precluding us from looking directly at Rec8 cleavage (7, 9). To bypass the requirement of CDC5 for securin degradation, we deleted the mitosis-specific APC/C activator AMA1. In cells lacking AMA1, securin degradation is CDC5-independent (11). Both CDC5-mn and CDC5-mn ama1Δ cells arrest in metaphase I (Fig. S2C). However, whereas Pds1 was found in the nucleus of 80% of CDC5-mn metaphase I cells, this number was reduced to 50% in CDC5-mn ama1Δ cells (Fig. S2D). Rec8 cleavage was greatly delayed in the CDC5-mn ama1Δ double mutant even though at least half the cell population had

Fig. 1. CDC5 is required for Rec8 cleavage. (A) Cells depleted for CDC20 (A27808) or CDC5 (A27809) were induced to sporulate. Rec8-S179 phosphorylation and total Rec8-HA levels were determined at the indicated times. (B) Wild-type (A33368) and CUP-CDC5 (A32851) cells were arrested in prophase I by depleting Ndt80. CDC5 expression was induced by 50 μM CuSO4 addition 4 h after transfer into sporulation medium to examine Rec8-S179 phosphorylation and total Rec8-HA protein levels. A vegetative no-tag control (A4962) is shown. (C) HA-tagged REC8 phosphomimetic mutants (A30411, A30407, A30408, A32252, A30409, A32254, A32256, A32250, A32258, A30410, and A30406) were induced to sporulate and GFP dot distribution was analyzed. One hundred cells were counted for three experiments. Statistical analyses are shown in Table S1. (D) spo11Δ CDC20-mn (A33493, filled squares) or spo11Δ CDC20-mn rec8-S136D S179D S197D-HA (A33491, filled circles) were sporulated. The percentage of cells in metaphase II was quantified (n = 100 cells per time point). (E) Wild-type (A33293), ama1Δ (A33119), CDC5-mn (A33292), or ama1Δ CDC5-mn (A33118) cells were sporulated and Rec8-HA and Pgd1 (loading control) protein levels analyzed.

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Degraded Pds1 (Fig. 1E). The low levels of Rec8 cleavage observed in CDC5-mn anulΔ cells could be due to a partial activation of the CLB2 promoter. The CLB2 promoter used to drive CDC5 in this experiment is derepressed in anulΔ mutants (14) (Fig. S2E). We conclude that CDC5 is required for Rec8 cleavage.

Meiosis I Is Suppressed in Cells Overexpressing CDC5. If CDC5 is critical for cohesin removal, overexpressing CDC5 in meiosis I could lead to increased Rec8 phosphorylation at centromeric regions. To test this we reversibly arrested cells at the end of prophase I, by placing NDT80, the gene encoding a transcription factor required for entry into the meiotic divisions, under the control of the GAL1-10 promoter. Upon addition of β-estradiol to the medium, cells also containing a GAL4-estrogen receptor (GAL4-ER) fusion exit the prophase I block and progress through the meiotic divisions (15). Cells overexpressing CDC5 from the CUP1 promoter underwent a single meiotic division. This division was delayed compared with the first meiotic division in wild-type cells, with nuclei stretching for prolonged periods of time, ultimately resolving into two nuclei (Fig. 2A and Fig. S3A).

To determine whether the single meiotic division in CUP-CDC5 cells was meiosis I- or meiosis II-like we assessed the segregation of heterozygous GFP dots. At the same time we monitored Pds1 degradation using a Pds1-tdTomato fusion (16). In wild-type cells, GFP dots separated during meiosis II, 50 min after Pds1 degradation at the metaphase I–anaphase I transition (Fig. 2B and Fig. S3B). In CUP-CDC5 cells Pds1 degradation was significantly delayed (~80 min). However, once Pds1 was degraded, 48% of cells split sister chromatids immediately (Fig. 2B and Fig. S3B; median = 10 min after Pds1 degradation). Twenty percent of CDC5-overexpressing cells underwent meiosis I following Pds1 degradation and 32% never underwent chromosome segregation. This phenotypic heterogeneity could be due to differential overexpression from the CUP1 promoter. It is also possible that this “mixed segregation” reflects the true effect of high levels of CDC5 on chromosome segregation. Cells lacking SPO13, for example, exhibit a mixed chromosome segregation pattern (17–19). Irrespective of the origin of this heterogeneity, our results indicate that cells overexpressing CDC5 split sister chromatids prematurely. Consistent with premature sister chromatid segregation we found that Rec8 was absent around centromeres in 24% of binucleate cells (Fig. S3C). In 26% of binucleate cells we observed an unusual Rec8 staining pattern. Rec8 decorated chromosomes and the space around the DNA (Fig. S3C). This unusual Rec8 localization pattern likely reflects the high percentage of metaphase I-arrested cells with extremely stretched nuclei that we observe in CUP-CDC5 cultures. After spreading, such stretched metaphase I nuclei could be mis-categorized as binucleate cells. It is also possible that this mis-localization of Rec8 contributes to premature loss of centromeric cohesion. Our results suggest that overexpression of CDC5 induces premature loss of centromeric cohesion. Because approximately half of the CUP-CDC5 cells segregate sister chromatids during the single meiotic division, co-orientation in addition to stepwise loss of cohesion must at least be partially defective.

CDC5 Regulates the Stability of Spo13. The phenotype of CUP-CDC5 cells resembles that of cells lacking SPO13. Spo13 is a meiosis I-specific protein required for preventing cohesin removal around centromeres during meiosis I and for sister kinetochore co-orientation (17, 18). spo13Δ cells, like CUP-CDC5 cells, undergo a single meiotic division in which some chromosomes segregate in a meiosis I-like and others in a meiosis II-like manner. Additionally, both CUP-CDC5 and spo13Δ strains exhibit a metaphase I delay (Fig. 3A). These findings raised the possibility that high levels of Cdc5 interfere with SPO13 activity, thereby affecting both centromeric cohesin protection and sister kinetochore co-orientation.

To determine whether Cdc5 affects SPO13 function we first analyzed Spo13 protein levels in CUP-CDC5 cells. In wild-type cells Spo13 is degraded at the metaphase I–anaphase I transition by the APC/C-Cdc20 (20) (Fig. 3B and Fig. S4A). In CUP-CDC5 cells, Spo13 levels declined prematurely (Fig. 3B and Fig. S4A and B). The decline in Spo13 levels was preceded by the appearance of slower migrating forms of Spo13, which is consistent with CDC5-dependent phosphorylation of Spo13 seen later during meiosis I (21). CDC5 was also required for the decline in Spo13 levels observed in anaphase I. Spo13 levels remained as high in the metaphase I arrest caused by the depletion of Cdc5 as they did in cells depleted for the APC/C activator Cdc20 (Fig. 3C and Fig. S4C). Furthermore, Spo13, but not another meiosis-specific protein, Mam1, was stable in Cdc5-depleted cells (Fig. S4D). We conclude that CDC5 controls Spo13 stability.

The observation that CDC5 regulates Spo13 stability raised the possibility that the premature loss of centromeric cohesion observed in CUP-CDC5 cells is a consequence of premature loss of Spo13. If CDC5 was regulating cohesin removal solely by affecting SPO13 function, deleting SPO13 should suppress the Rec8 cleavage defect of cells depleted for Cdc5. This was not the case (Fig. S4E and F). We conclude that CDC5 controls cohesin removal in at least three ways: CDC5 is required for securin degradation, Cdc5 phosphorylates Rec8, and CDC5 promotes the degradation of Spo13.

CDC5 Is Disposable During Meiosis II. Is CDC5, a central regulator of meiosis I, also a critical regulator of meiosis II? To address this question we used an inhibitor-sensitive allele of CDC5, cdc5-as1, which allowed us to inhibit Cdc5 kinase activity at any time during sporulation (22). We arrested wild-type and cdc5-as1 cells in prophase I and added inhibitor at various times after release from the block. As expected, addition of inhibitor to meiotic cultures before metaphase I (1 h after release from the prophase I block) resulted in a metaphase I arrest (Fig. S5A and B). Adding inhibitor 15 min later (1.25 h after release from the prophase I block) revealed that CDC5 is also required for exit from meiosis I. We observed an accumulation of anaphase I cells in

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**Fig. 2.** Cells overexpressing CDC5 undergo a mixed meiosis I. (A) Wild-type (A31020) or CUP-CDC5 (A32746) cells were sporulated. The percentage of wild-type or CUP-CDC5 cells with stretched nuclei (squares), two nuclei (circles), and four nuclei (triangles) is shown (n = 100 cells per time point). (B) Wild-type (A31020) and CUP-CDC5 (A32746) cells were sporulated and followed by live cell microscopy (n = 75 cells). A representative montage for wild type (Upper) and CUP-CDC5 (Lower) is shown.
these cultures (Fig. S5C). This finding is consistent with the observation that the Cdc14 early anaphase release (FEAR) network, in which CDC3 plays a critical role, is essential for exit from meiosis I (23–27).

Interestingly, when we added inhibitor after cells had completed meiosis I (1.5 h after release from the prophase block) we only observed a subtle delay in meiosis II progression (Fig. 4 A and B and Fig. S5D). This was not owing to the inhibitor’s being ineffective when added at later times. Cdc5 activates the protein phosphatase Cdc14 by promoting its release from the nucleolus during mitotic anaphase as well as during anaphase I and II (9, 23–25). Cdc14 release from the nucleolus did not occur during anaphase II in cdc5-as1 cells when the kinase was inhibited 1.5 h after release from the prophase I block (Fig. S5 E and F). We conclude that CDC5 is largely dispensable for meiosis II. Furthermore, our results show that, unlike during mitosis and meiosis I, release of Cdc14 from the nucleolus is not required for exit from meiosis II.

Many Cdc5 Substrates Are Only Phosphorylated During Meiosis I. Although present during meiosis II (Fig. S6 B and C), CDC5 seems largely dispensable during meiosis II. Analysis of two CDC5-dependent phosphorylation sites in Rec8, S136 and S179, further showed that CDC5 is not only dispensable for cohesin removal during meiosis II, but that residues in Rec8 known to be phosphorylated in a CDC5-dependent manner during meiosis I are in fact not phosphorylated during meiosis II (Fig. S4 and Fig. S6A). Because Rec8 levels are much lower in metaphase II than in metaphase I, differences in Rec8 phosphorylation are most clearly seen when amounts of Rec8 from meiosis I and meiosis II time points were compared (Fig. 5 B and C). Lrs4, a component of the monopolin complex, is also phosphorylated in a CDC5-dependent manner, which is detected as slower migrating forms on SDS PAGE (7, 28). Slower migrating forms of Lrs4-Myc were present during prophase I and metaphase I but absent during meiosis II (Fig. S6 D and E). The B-type cyclin Clb1 also undergoes dramatic CDC5-dependent changes in mobility during meiosis I but not meiosis II (15) (Fig. S5D and Fig. S6 F and G). We conclude that phosphorylation of at least some Cdc5 substrates is high in meiosis I but largely absent during meiosis II.

Rec8 Phosphorylation Is Dispensable for Anaphase II Entry. Our results indicate that despite Cdc5’s central role in cohesin removal during meiosis I, it is dispensable for this process during meiosis II. This difference could be due to other kinases (i.e., Hrr25 or DDK) bringing about phosphorylation-dependent removal of Rec8 from chromosomes. Alternatively, Rec8 phosphorylation may not be essential for centromeric cohesin removal and hence meiosis II chromosome segregation. To distinguish between these possibilities we examined the effects of mutating 29 phosphorylation sites in Rec8 required for efficient cleavage to alanine (rec8-29A) (10) on meiosis II chromosome segregation. The rec8-29A mutant exhibits a prophase I delay, owing to delayed double-strand break repair and a metaphase I delay owing to impairment of Rec8 cleavage (Fig. 5 E) (29). It was nevertheless clear that rec8-29A mutants did not exhibit a metaphase II delay (Fig. 5F).

To further address potential meiosis II effects of the rec8-29A mutant, we circumvented the meiosis I delay of the rec8-29A mutant by deleting SPO11. Cells deleted for SPO11 progressed through the first meiotic division more slowly than wild-type cells but, remarkably, rec8-29A spo11A double mutants did not exhibit a metaphase II delay (Fig. 5G). This finding indicates that although it is critical for anaphase I onset Rec8 phosphorylation is dispensable for anaphase II onset. Taken together, our results

![Fig. 3. Cdc5 regulates Spo13 stability. (A) Wild-type (A31020), CUP-CDC5 (A32746), or spo13Δ (A30960) cells were sporulated and the percentage of metaphase I cells in wild type, spo13Δ, and CUP-CDC5 was determined (n = 100 cells per time point). (B) Wild-type (A33501) or CUP-CDC5 (A33467) cells were sporulated using the Ndt80 block-release system and Spo13 levels examined. The peaks of meiosis I (MI) and meiosis II (MII) are indicated. Meiotic progression is shown in Fig. 5 A and B. (C) Wild-type (A23405), CDC5-mn (A23757), and CDC20-mn (A23664) cells were sporulated using the Ndt80 block-release system. Spo13 levels were analyzed. Meiotic progression is shown in Fig. S4C.

![Fig. 4. Cdc5 is dispensable during meiosis II. Wild-type (A22132) or cdc5-as1 (A33513) cells were sporulated using the Ndt80 block-release system and meiotic progression was assessed (n = 100 cells per time point). The cdc5-as1 inhibitor CMK (chloromethylketone), 5 μM, was added at the following times: wild type: 1 h after release into the meiotic divisions and cdc5-as1: 1.5 h after release into the meiotic divisions. Percentage of metaphase II (A) and anaphase I (B) cells is shown (n = 100 cells per time point). Detailed meiotic progressions are shown in Fig. S5 A–D.](image-url)
show that meiosis I and meiosis II chromosome segregation are regulated in fundamentally different ways and that CDC5 is a significant contributor to shaping the unique meiosis I chromosome segregation pattern.

Discussion

Cdc5 has evolved into a master regulator of the unique meiosis I chromosome segregation pattern. Control of cohesion removal exemplifies this. Cdc5 has acquired new roles in this process during meiosis I. CDC5 regulates securin degradation during meiosis I, CDC5 regulates securin degradation during meiosis I, CDC5 regulates securin degradation during meiosis I, and CDC5 regulates securin degradation during meiosis I, CDC5 regulates securin degradation during meiosis I, CDC5 regulates securin degradation during meiosis I, CDC5 regulates securin degradation during meiosis I, CDC5 regulates securin degradation during meiosis I, CDC5 regulates securin degradation during meiosis I, CDC5 regulates securin degradation during meiosis I, CDC5 regulates securin degradation during meiosis I.

CDC5-mn ama1 mutant in which S136, S179, S197, and T209 were mutated to aspartic acid caused premature loss of centromeric cohesion. Three of these four sites (S136, S179, and S197) were found to be CDC5-dependent by our studies. The fact that several sites in Rec8 were mutated to aspartic acid to cause premature loss of centromeric cohesion is consistent with the previous proposal that bulk Rec8 phosphorylation is required for its cleavage. The conclusion that CDC5-dependent phosphorylation of Rec8 is important for its cleavage and removal from chromosomes is not only supported by our phospho-mutant analysis but also by the observation that Rec8 cleavage is greatly delayed in CDC5-mn ama1 mutants. A previous study examined this very strain and concluded that CDC5 was not required for Rec8 cleavage (11). We suspect that the dynamic range of the assay used to assess the role of CDC5 in Rec8 cleavage, the presence of Rec8 in the nucleus, was limited, precluding the observation of the Rec8 cleavage defect of CDC5-mn ama1 mutants. It is important to note that Cdc5 is clearly not the only kinase that promotes Rec8 cleavage. Mutating a subset of the CDC5-dependent phosphorylation sites in Rec8 to residues that can no longer be phosphorylated does not lead to a delay in anaphase I onset (10). Katis et al. (11) identified DDK and Hrr25 as kinases phosphorylating Rec8, and these kinases certainly contribute to cohesion cleavage and removal during meiosis I.

Although Cdc5 is critical for cohesion removal at the metaphase I–anaphase I transition, it is not needed for the equivalent transition during meiosis II. In fact, Rec8 phosphorylation in general is not needed for meiosis II chromosome segregation. spo11Δ rec8-29A cells do not exhibit a delay in entry into anaphase II, even though spo11Δ cells harbor, in addition to centromeric cohesins, a substantial amount of arm cohesins in meiosis II (31). Rec8 phosphorylation may be required to make Rec8 a better substrate for separase. If separase activity is low at the metaphase I–anaphase I transition, possibly owing to low APC/C activity, but high at the metaphase II–anaphase II transition, Rec8 phosphorylation may not be needed in meiosis II. It is also possible that centromeric cohesin differs from chromosome arm cohesin. Perhaps cohesins loaded onto chromosomes during homologous recombination require phosphorylation for their removal but cohesins loaded during S phase do not. In this scenario, eliminating homologous recombination would eliminate the need for Rec8 phosphorylation in its cleavage.
Although CDC5 activity is not needed during meiosis II, Cdc5 is likely to affect meiosis II chromosome segregation by functions it executes during meiosis I. By triggering Spo13 degradation, Cdc5 eliminates meiosis I-specific chromosome properties: a cohesin protective domain around centromeres and co-oriented sister chromatids. Thus, Cdc5 ensures that the meiosis I chromosome characteristics are eliminated before meiosis II. We do not yet know whether Cdc5 activity is not only dispensable for meiosis II chromosome segregation but also for exit from meiosis II. This is, however, due to the remarkable fact that Cdc41 release from the nucleus is dispensable for exit from meiosis II. Cdc41 brings about exit from mitosis by triggering the inactivation of B-type cyclin (Clb)–cyclin-dependent kinases (CDKs) (2). Perhaps CDK inactivation at the end of meiosis II does not require Cdc41 activity because the bulk of Clb-CDK inactivation occurs before exit from meiosis II.

Cdc5 sets itself apart from most other cell cycle kinases through its ability to phosphorylate different substrates at different cell-cycle stages. Several Cdc5 substrates, Rec8 S136 and S179, Lsr4, and Cibl, are phosphorylated in meiosis I but not in meiosis II. We do not yet know whether this reflects a general down-regulation in Cdc5 activity during meiosis II or whether Cdc5’s ability to phosphorylate a subset of substrates is decreased during meiosis II. Prior phosphorylation of substrates by CDKs can target Polo kinases to their substrates (32). It is thus possible that lowered CDK activity in meiosis II could lead to a general decrease in CDC5-dependent phosphorylation in meiosis II. We favor the idea that Cdc5’s ability to phosphorylate a subset of substrates changes during meiosis. Cdc5 is needed for the release of Cdc41 from the nucleus during meiosis II, suggesting that some Cdc5 activity is present during meiosis II. The critical Cdc5 targets in Cdc41 activation have not yet been identified, precluding us from directly comparing Cdc5 activity in mitotic exit network regulation with its ability to phosphorylate Rec8, Lsr4, and Cibl. However, support for the idea of substrate selectivity comes from observations in mitosis. There, it is clear that Cdc5 phosphorylates different substrates at different times (3, 33). During meiosis, Polo-like kinases have been implicated in mitotic regulation in many species. Our results identify a dramatic differential requirement for Polo kinases between meiosis I and meiosis II. It will be interesting to determine whether a similar differential requirement also exists in other eukaryotes and how it is established.

**Materials and Methods**

All yeast strains used in this study are derivatives of SK1 and listed in Table S2.

**Fluorescence Microscopy.** Indirect in situ immunofluorescence was performed as described previously (16). Anti-tubulin antibodies were used to stain spindle microtubules, and DAPI was used to stain DNA. Cdc14-3HA and Pds1-18Myc immunofluorescence was performed as described in ref. 23. Cells were imaged with a Zeiss Axioplan 2 microscope and a Hamamatsu ORCA-ER digital camera. Live cell imaging analysis and chromosome spreads were described previously (16).

**Other Techniques.** Western blot analyses were performed as described in ref. 15. Quantification was performed using ImageQuant software. Rec8-3HA, Rec8-17A-3HA, and Rec8-3VS were immunoprecipitated as described previously (10). Samples were subjected to immunoblot analysis with antibodies recognizing Rec8-pS136 (1:500) or Rec8-pS179 (1:1,000). Anti-HA (1:1,000) and anti-V5 (1:2,000) antibodies were used to recognize total Rec8.

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