Cdc7-dependent phosphorylation of Mer2 facilitates initiation of yeast meiotic recombination

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Meiosis ensures genetic diversification of gametes and sexual reproduction. For successful meiosis, multiple events such as DNA replication, recombination, and chromosome segregation must occur coordinately in a strict regulated order. We investigated the meiotic roles of Cdc7 kinase in the initiation of meiotic recombination, namely, DNA double-strand breaks (DSBs) mediated by Spo11 and other coactivating proteins. Genetic analysis using bob1-1 cdc7/H9004 reveals that Cdc7 is essential for meiotic DSBs and meiosis I progression. We also demonstrate that the N-terminal region of Mer2, a Spo11 ancillary protein required for DSB formation and phosphorylated by cyclin-dependent kinase (CDK), contains two types of Cdc7-dependent phosphorylation sites near the CDK site (Ser30): One (Ser29) is essential for meiotic DSB formation, and the others exhibit a cumulative effect to facilitate DSB formation. Importantly, mutations on these sites confer severe defects in DSB formation even when the CDK phosphorylation is present at Ser30. Diploids of cdc7/H9004 display defects in the chromatin binding of not only Spo11 but also Rec114 and Mei4, other meiotic coactivators that may assist Spo11 binding to DSB hot spots. We thus propose that Cdc7, in concert with CDK, regulates Spo11 loading to DSB sites via Mer2 phosphorylation.

Keywords: Cdc7; Mer2; meiotic recombination; Spo11; pre-DSB complex

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spatial and temporal controls. In fission yeast, cells entering into meiosis from the G2 phase of mitosis overlap premeiotic DNA synthesis and cause a fatal impairment in meiotic recombination [Watanabe et al. 2001]. In addition, Cln5–Cln6–cyclin-dependent kinase (CDK) activity has crucial roles in triggering both replication initiation and the activation of Spo11 in budding yeast meiosis [Dirick et al. 1998; Stuart and Wittenberg 1998; Smith et al. 2001; Henderson et al. 2006]. These studies suggest that cellular regulation coordinates DNA replication and DSB formation. Recently, it was demonstrated that CDK directly regulates DSB formation via the phosphorylation of Mer2 protein [Henderson et al. 2006]. The interplay of DNA replication and DSB formation has been also supported by other experiments. In budding yeast, the removal of all ARSs (autonomous replication sequences) from the left arm of chromosome III confers a left-arm-specific delay in DNA replication and meiotic DSB formation, suggesting the coordinated regulation of DNA replication and DSB formation at a chromosomal level. Such control may ensure that DSBs are not formed before the completion of DNA replication [Borde et al. 2000; Murakami et al. 2003].

To uncover such highly integrated regulation, we analyzed meiotic roles of Cdc7, which is required for the initiation of DNA replication and maintenance of replication fork stability. It has been previously reported that reduction of Cdc7 kinase activity in mice confers sterility with a testicular and ovary atrophy, indicating the reduction of Cdc7 kinase activity in mice confers sterility (Borde et al. 2000; Murakami et al. 2003). Moreover, analysis using chemical genetics of dbf4b mutant with the results in Figure 1C, in each locus, meiotic recombination hot spot, was severely impaired in bob1-1 cdc7Δ mutant (Fig. 1A). Microscopic analysis to monitor the meiotic nuclear division revealed that the bob1-1 cdc7Δ mutant arrested with one nucleus before meiosis I, which is consistent with the previously reported phenotype of cdc7Δ-dbfb4 [S. cerevisiae] and hsk1-dfp1/him1 [S. pombe] mutants (Fig. 1B, Ogino et al. 2006, Valentin et al. 2006). The proportion of asci with four spores is <7% of the total asci at 24 h in sporulation medium [Supplemental Fig. S1A], while prolonged incubation resulted in the formation of substantial dyads [Supplemental Fig. S1A,B]. Importantly, all the tetrad spores were inviable (0/60 viable/total spores in bob1-1 cdc7Δ). Meiotic progression, recombination frequency, and spore viability for the bob1-1 cdc7Δ single mutant were indistinguishable from those of the wild type [118/120 viable/total spores in bob1-1] (Fig. 1A,C,D).

In general, meiotic recombination-deficient mutants are known to confer very low spore viability. Thus, this result led us to examine the meiotic recombination in the bob1-1 cdc7Δ mutant during meiosis. Meiotic recombination at ARG4, a well-characterized meiotic recombination hot spot, was severely impaired in bob1-1 cdc7Δ (<0.01%; cf. bob1-1, 8.1%) [Fig. 1C]. We next analyzed meiotic DSB formation at CYSS and YCR048w, known as strong meiotic recombination hot spots [Wu and Lichten 1994; Vedel and Nicolas 1999]. Consistent with the results in Figure 1C, in each locus, meiotic DSBs were completely abolished in bob1-1 cdc7Δ, while the frequency of DSB formation in bob1-1 mutant was similar to that in wild type [Fig. 1D, top and middle panels]. Pulse field gel electrophoresis (PFGE) analysis to detect genome-wide meiotic DSBs revealed that no visible DSB is formed in the bob1-1 cdc7Δ mutant [Fig. 1D, bottom panel]. These results indicate that Cdc7 is essential for DSB formation and meiotic recombination. Because other DSB-deficient mutants such as spo11Δ are known to form four inviable spores (80%–90% of total asci contained four spores in spo11Δ), the defect in meiotic nuclear division observed in bob1-1 cdc7Δ is unlikely to be simply due to its defect in DSB formation. Thus, these results suggest that Cdc7 also participates in progression of meiosis I.

**Results**

The bob1-1 cdc7Δ mutation abolishes meiotic DSBs and arrests cells before MI after normal DNA replication

To examine the functions of the Cdc7 kinase in meiosis, we engineered an SK1 strain lacking CDC7 in the bob1-1 background, in which the defect of DNA replication in cdc7Δ is bypassed. The bob1-1 cdc7Δ diploid cells were larger than the wild type, but had a normal cell cycle in mitosis as shown previously [Hardy et al. 1997]. Premiotic DNA replication proceeded with only a slight delay in the bob1-1 cdc7Δ mutant [Fig. 1A]. Microscopic analysis to monitor the meiotic nuclear division revealed that the bob1-1 cdc7Δ mutant arrested with one nucleus before meiosis I, which is consistent with the previously reported phenotype of cdc7Δ-dbfb4 [S. cerevisiae] and hsk1-dfp1/him1 [S. pombe] mutants (Fig. 1B, Ogino et al. 2006, Valentin et al. 2006). The proportion of asci with four spores is <7% of the total asci at 24 h in sporulation medium [Supplemental Fig. S1A], while prolonged incubation resulted in the formation of substantial dyads [Supplemental Fig. S1A,B]. Importantly, all the tetrad spores were inviable (0/60 viable/total spores in bob1-1 cdc7Δ). Meiotic progression, recombination frequency, and spore viability for the bob1-1 single mutant were indistinguishable from those of the wild type [118/120 viable/total spores in bob1-1] (Fig. 1A,C,D).

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**Cdc7-dependent phosphorylation of Mer2**

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Induction of middle-late meiotic genes is dependent on Cdc7

To study the cause of the defect in meiotic progression in bob1-1 cdc7Δ, we examined the transcriptional profile of meiotically induced genes by Northern blotting. In the bob1-1 cdc7Δ mutant, early meiotic genes including IME2, SPO11, REC102, and DMC1 were almost normally expressed in the same fashion as in wild type and bob1-1 [Fig. 2A,B]. However, we found that NDT80 transcripts were not induced until 8 h in the bob1-1 cdc7Δ mutant. Ndt80 is known to play a role as a master transcriptional activator for >150 middle meiotic genes and to be required for meiotic nuclear division (Chu et al. 1998). At 6 h, the amount of NDT80 transcripts in bob1-1 cdc7Δ was reduced to only 8% of that in bob1-1 (Fig. 2A,B). Transcriptional induction of SPS4 and SPS22, which are controlled by Ndt80, were also abolished [Fig. 2A,B].

In order to compare the transcriptional profile of the bob1-1 cdc7Δ mutant with that of wild type in the whole genome, we performed transcriptome analysis using a microarray. At 8 h of meiosis in bob1-1 cdc7Δ, transcriptional induction of 344 genes, out of a total 404 genes that are clustered as “middle” and “middle-late” meiotic genes (Chu et al. 1998; Primig et al. 2000), was significantly lower than that in the bob1-1 mutant [Supplemental Fig. S2, top graph]. Among the 344 affected genes, meiotic induction of 199 genes was very severely impaired in bob1-1 cdc7Δ (>10-fold reduction), and 82 genes could be categorized as genes that are up-regulated by an ectopic expression of NDT80 in mitosis (Chu et al. 1998).
Less than 10% of these 82 genes were classified as “metabolic,” “early-middle meiotic,” and “unknown function” genes, although >90% of the 82 genes belonged to “middle” and “middle-late meiotic” genes, including YSW1, SPR28, and SPS2 [Supplemental Fig. S2]. These results indicate that Cdc7 facilitates meiotic progression via transcriptional induction of middle-late meiotic genes including NDT80 and some genes of the Ndt80 regulon.

**Mer2 protein is phosphorylated in a Cdc7-dependent manner**

We next focused on the role of Cdc7 in meiotic DSB formation. Recently, the Ser30 and Ser271 residues in Mer2 have been shown to be preferentially phosphorylated by CDK in meiosis [Henderson et al. 2006]. Cdc7 has been shown to preferentially phosphorylate a serine residue juxtaposed to the serine residue phosphorylated by CDK in the N-terminal region of Mcm2 and Mcm4 [Cho et al. 2006; Masai et al. 2006]. Therefore, we expected Mer2 protein to be a potential target of Cdc7 kinase. To examine the phosphorylation state of Mer2 in bob1-1 cdc7Δ, we conjugated Flag-tag sequences to the endogenous MER2 gene. The Mer2-3Flag protein expressed in diploid cells was specifically pulled down with anti-Flag antibody. After treating the immunoprecipitated fraction with or without calf intestine alkaline phosphatase (CIAP), we examined the mobility shift of Mer2 using SDS-PAGE.

The slower migrating bands of Mer2-3Flag were detected in the bob1-1 mutant at 4 h of meiosis. Such slower migrating bands were shifted to a faster migrating band by CIAP treatment but not by CIAP with phosphatase inhibitors. This indicates that the slower migration of Mer2-3Flag is due to phosphorylation [Fig. 3A [lanes 1–3], B [lanes 1–3]]. In contrast, Mer2-3Flag extracted from the bob1-1 cdc7Δ mutant (4 h of meiosis) migrated faster than that from bob1-1, and the former band was shifted to an even lower position after the CIAP treatment [Fig. 3A,B, cf. lanes 1 and 4]. These results indicate that Mer2 is phosphorylated depending on Cdc7.

The following observations suggest that CDK can phosphorylate Mer2 in the absence of Cdc7. First, in the bob1-1 cdc7Δ mutant, the slightly shifted mobility pattern of Mer2-3Flag could be detected at 4 and 8 h of meiosis, although there was no active Cdc7 [Fig. 3B, lanes 4,5]. Second, such a residual band shift in bob1-1 cdc7Δ was not detected when we introduced mer2S30, 271A-3Flag, an alanine substitution mutant of the CDK phosphorylation sites [Henderson et al. 2006]. Third, the Mer2S30, 271A-3Flag protein exhibited no mobility shift, and the band position was the same as that after the CIAP treatment. These results again suggest that the majority of the Mer2 phosphorylations other than the CDK-mediated ones are dependent on Cdc7 [Fig. 3C].

It has been reported that Mer2 formed a subcomplex with Rec114 and Mei4 in meiosis [Li et al. 2006]. Therefore, we also examined the phosphorylation states of Rec114 and Mei4 in meiosis [Li et al. 2006]. We also examined the phosphorylation states of Rec114 and Mei4. A faster-migrating band of Rec114-3Flag appeared after the CIAP treatment [Supplemental Fig. S3, arrow]. Protein bands derived from some of the degradation products of Rec114-3Flag [Supplemental Fig. S3, indicated by an asterisk] were lost by the CIAP treatment, indicating that Rec114 is also phosphorylated in meiosis [Supplemental Fig. S3, arrowheads]. In bob1-1 cdc7Δ, the putative phosphorylated, shifted forms decreased in intensity, but did not completely disappear.
In the case of Mei4-3Flag in meiosis, we did not detect a mobility shift in meiosis. Thus, we thereafter focused on the roles of phosphorylation of Mer2 mediated by Cdc7.

**Determination of Cdc7-dependent phosphorylation regions in Mer2 protein by TOF-MS analysis**

To determine the phosphorylation sites of Mer2 under physiological conditions, we analyzed by a TOF-MS the phosphorylated peptides of Mer2 extracted from meiotic cells (4 h). We detected Mer2-3Flag in immunoprecipitated Mer2-3Flag. Immunoprecipitated Mer2-3Flag was eluted with 3XFlag peptide. (Left panel) The silver-stained bands were excised from a gel for TOF-MS analysis. (Right panel) Western blotting with anti-Flag was done to detect Mer2-3Flag in the IP fraction. (E) Results of TOF-MS analysis. Black bold letters in the top amino acid sequence show the peptides identified by TOF-MS, and underlines correspond to the location of detected phosphopeptides. CDK-dependent phosphorylation sites (Ser30, Ser271) are indicated by large letters in the top sequence. One peptide in the C-terminal region (P1) and four peptides in the N-terminal region (P2–P5) were detected. “Modifications” in the right column present the detected number of phosphorylation sites in each peptide.

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### The multiple phosphorylation of the N-terminal region of Mer2 contributes to spore viability and DSB formation

To identify the phosphorylation sites at the N-terminal region of Mer2 in detail, we constructed various mer2 mutants in which potential phosphorylation sites are substituted for alanine residues (Fig. 4A). Since it is known that serine rather than threonine or tyrosine is likely a preferred phosphorylation target by Cdc7, we focused on the serine residues of Mer2 (Jiang et al. 1999; Cho et al. 2006). Among the mer2 mutants, the mer2S11A mutant conferred almost wild-type levels of normal asc formation and spore viability.
(Fig. 4A, top panel). The mer2S11, 15A, mer2S11, 19A, and mer2S15, 19A double mutations slightly but significantly reduced spore viability, as compared with wild type [Fig. 4A, top panel]. Spore viability was reduced to ∼60% in the mer2S11, 15, 19A triple mutant. As shown in Figure 4B, electrophoretic mobility in this triple substitution mutant was faster than that in wild type or the mer2S11, 15A mutant, indicating that these three serines were, indeed, phosphorylated in vivo (Fig. 4B, lanes 1–4). On the other hand, the mer2S11, 15, 19A triple mutant protein seemed to be still phosphorylated in vivo [Fig. 4B, lanes 1–4]. The right graph of the panel shows the dissected spore viability of each mutant. (B) Electrophoretic mobility shift of Mer2-3Flag mutant proteins. Meiotic cells (5 × 10^9) were harvested at 4 h. The strains used were YHS35, YHS1009, YHS1015, YHS1019, YHS975, YHS979, and YHS775. The sample in lane 9 was treated with phosphatase prior to electrophoresis. The asterisks indicate the degradation products. (C) CDK-dependent phosphorylation of Ser30 on Mer2. Samples were the same as in B. Western blotting was performed with anti-phospho Ser30 Mer2 [top panel] or anti-Flag antibody [bottom panel] after samples were separated on a 4%–20% gradient polyacrylamide gel. (D) The mitotic phosphorylation of Mer2 by Cdc7 kinase. Mer2-expressed vectors under the ADH1 promoter were transformed into YHS5 (wild type) and YHS869 (bob1-1 cdc7Δ). Mer2-3Flag immunoprecipitated from log-phase cell extracts was detected with anti-Flag [top panel] and anti-phospho Ser30 antibody [bottom panel]. Meiotic Mer2-3Flag was loaded in lane 1 as a control.

| Figure 4. Cdc7-dependent phosphorylation of Mer2 is essential for spore viability. (A) Schematic drawings of various Mer2 alanine substitution constructs. Based on the results from TOF-MS analysis, nine untagged mer2 mutants were generated. “A” indicates a substitution of serine for alanine. The right graph of the panel shows the dissected spore viability of each mutant. (B) Electrophoretic mobility shift of Mer2-3Flag mutant proteins. Meiotic cells (5 × 10^9) were harvested at 4 h. The strains used were YHS35, YHS1009, YHS1015, YHS1019, YHS975, YHS979, and YHS775. The sample in lane 9 was treated with phosphatase prior to electrophoresis. The asterisks indicate the degradation products. (C) CDK-dependent phosphorylation of Ser30 on Mer2. Samples were the same as in B. Western blotting was performed with anti-phospho Ser30 Mer2 [top panel] or anti-Flag antibody [bottom panel] after samples were separated on a 4%–20% gradient polyacrylamide gel. (D) The mitotic phosphorylation of Mer2 by Cdc7 kinase. Mer2-expressed vectors under the ADH1 promoter were transformed into YHS5 (wild type) and YHS869 (bob1-1 cdc7Δ). Mer2-3Flag immunoprecipitated from log-phase cell extracts was detected with anti-Flag [top panel] and anti-phospho Ser30 antibody [bottom panel]. Meiotic Mer2-3Flag was loaded in lane 1 as a control. |
pressed under the control of ADH1 promoter in mitotic log-phase cells, we detected the small but significant amount of a slower migrating form of Mer2 (Fig. 4D, lane 2, asterisk). This form was abolished in bob1-1 cdc7A and in the mer2S11, 15, 19A mutant, indicating that the N-terminal region of Mer2 is phosphorylated in mitosis in a Cdc7-dependent manner. Interestingly, in comparison with meiotic Mer2-3Flag, we could not detect the Ser30 phosphorylation of Mer2 in mitosis, although levels of the S-phase cyclin remained high throughout DNA replication and G2 phase (Fig. 4D, bottom panel). This result suggests that the Ser30 phosphorylation by CDK is regulated in a meiosis-specific manner [see Discussion].

Cdc7-dependent phosphorylation of Mer2 is pivotal for meiotic recombination

As shown above, the Cdc7-dependent phosphorylation in the N-terminal region of Mer2 is critical for the spore viability. In addition, Cdc7 is required for the meiotic DSB formation and recombination. Thus, Cdc7-dependent phosphorylation of Mer2 is likely to be crucial for meiotic DSB formation and recombination. To test this notion, we examined the effects of the phosphorylation site mutations on meiotic DSB formation and recombi-

nation. The recombination frequency at the ARG4 locus [revealed by generation of Arg+ prototrophs] was reduced to various extents in the alanine-substituted mer2 mutants [Fig. 5A]. The recombination frequency at ARG4 was only slightly reduced in mer2S11A and mer2S11, 15A mutants [mer2S11A, 7.9%; mer2S11, 15A, 7.7%; wild type, 8.6%] [Fig. 5A]. The mer2S11, 15, 19A triple mutant showed a partial reduction in recombination frequency (4.4%, nearly half of the wild-type level) [Fig. 5A]. The mer2S11, 15, 19, 22A quadruple mutant conferred a more severe defect in meiotic recombination frequency (3.3%; i.e., 38% of the wild-type level) [Fig. 5A]. Importantly, both the mer2S29A single and the mer2S11, 15, 19, 22, 29A quintuple mutants showed almost complete loss of meiotic recombination frequency [Fig. 5A,B]. Consistent with these results, in the mer2S29A single and the mer2S11, 15, 19, 22, 29A quintuple mutants, meiotic DSB formation at YCR048w, CYS3, and GAT1 hot spots was almost completely abolished [Fig. 5B,C]. The mer2S11, 15, 19, 22A quadruple mutant exhibited a partial but severe defect [one of four at YCR048w and one of 10 at GAT1 hot spots of the wild-type level] in meiotic DSB formation at YCR048w and GAT1 hot spots [Fig. 5B,C], despite the fact that the high level of phosphorylation at Ser30 by CDK could be still detected in the mer2S11, 15, 19, 22A mutant

Figure 5. Recombination frequency and DSB formation in mutants substituted for sites phosphorylated dependent on Cdc7. (A) Measurement of recombination frequency at the arg4 locus. Cells were plated on media lacking arginine and on complete media and incubated for 4 d at 30°C. (B) Detection of DSB formation around the YCR048w, CYS3, and GAT1 regions. All strains (YHS1198, YHS1196, and YHS1204) used here were of the sae2Δ::CgLEU background. The experiment was conducted twice and gave the same profile. (C) Quantification of band intensities of a prominent DSB site (indicated by black arrow in B) of YCR048w [top graph] and GAT1 [bottom graph] loci at 6 h of meiosis in the mutants shown in B.
Cdc7-dependent phosphorylation of Mer2

...protein (Fig. 4C). These data clearly demonstrate that the Cdc7-dependent phosphorylation of Mer2 plays crucial roles in meiotic DSB formation and recombination. Moreover, it is very likely that meiotic DSB formation and recombination require both the cumulative phosphorylations in the N-terminal region of Mer2 and a critical single phosphorylation at Ser29, which all depend on Cdc7.

As shown in Figure 2, the meiotic induction of NDT80-dependent transcription is possibly mediated by the Cdc7-dependent phosphorylation of Mer2. However, the mer2 mutants with alanine substitutions at the Cdc7-dependent phosphorylation sites could produce asci with four spores. Therefore, there may be alternative Cdc7 targets involved in the activation of the NDT80-dependent transcription.

Cdc7-dependent phosphorylation of Mer2 facilitates Spo11 binding to DSB sites

It has been demonstrated that Spo11 binds DSB hot spots when meiotic DSBs are formed and then dissociates from covalent Spo11-DNA intermediates by unknown exonucleases (Neale et al. 2005; Prieler et al. 2005; Sasnuma et al. 2007). We examined whether Cdc7 kinase activity is required for the Spo11 binding to DSB hot spots by conducting chromatin immunoprecipitation (ChIP) analyses. In the bob1-1 strain, Spo11-3Flag transiently bound the YCR048w hot spot, peaking at 3 h of meiosis [Fig. 6A, top right graph]. However, little association of Spo11-3Flag at the same DSB site was observed in bob1-1 cdc7Δ [Fig. 6A, top middle graph]. Moreover, we observed no association of Spo11-3Flag with DSB hot spot in the mer2S11, 15, 19, 22, 29A mutant [Fig. 6A, top right graph].

To understand the regulatory mechanism of Spo11 binding to DSB hot spots, we next examined the loading of Rec114-3Flag and Mei4-3Flag to the DSB site in the bob1-1 cdc7Δ mutant during meiosis. The Rec114-3Flag and Mei4-3Flag were shown to bind the YCR048w hot spot with kinetics similar to that of Spo11 in wild type. Using high-resolution genome tiling arrays, we confirmed the binding of Rec114-3Flag and Mei4-3Flag around the YCR048w hot spot at 4 h of meiosis [data not shown]. In the bob1-1 cdc7Δ mutant, Rec114-3Flag was loaded onto the YCR048w hot spot, but the binding was reduced to one-fourth of the wild-type level [Fig. 6B, middle]. The similar level of reduction in Rec114-3Flag binding was observed in mer2Δ [Fig. 6B, right]. These results suggest that the Cdc7-dependent phosphorylation of Mer2 may facilitate the loading of Rec114 to DSB hot spots. Mei4-3Flag efficiently bound the YCR048w hot spot in wild type, but not in bob1-1 cdc7Δ [Fig. 6C]. This result suggests that Mer2 phosphorylation may be required for Mei4 binding to DSB sites. We could detect the meiosis-specific ChIP signal of Mei4-3Flag and Rec114-3Flag to the YCR048w hot spot in spo11Δ, indicating that hot spot binding is independent of Spo11 protein [Fig. 6D]. Therefore, it is likely that the loading of Rec114 and Mei4 to DSB sites precedes the binding of Spo11 or Spo11-Rec102-Rec104-Ski8 subcomplex to DSB hot spots.

In fission yeast, the accumulation of Rec12-Flag [a homolog of Spo11 in fission yeast] at the mbs1 locus [a natural recombination hot spot] was detected in a rad50s background [Fig. 6A, bottom left graph]. On the other hand, in the hsk1Δ-89 mutant [a temperature-sensitive mutant of Cdc7 ortholog in fission yeast], no binding was detected at a restrictive temperature, consistent with the findings in budding yeast [Fig. 6A, bottom right panel, Ogino et al. 2006]. These results indicate that Cdc7 kinase has an essential role in Spo11 loading to recombination hot spots in both budding and fission yeasts.

Discussion

In this study, we showed that Cdc7 plays an essential role in the regulation of meiotic DSB formation via phosphorylation of the N-terminal region of Mer2. We also demonstrated that Cdc7 is required for the entry into meiosis I via the proper induction of sporulation genes. Thus, this study provides the evidence that Cdc7 kinase participates in the dual meiotic regulations for the initiation of meiotic recombination and later meiotic progression into meiosis I.

Role of Cdc7 in the progression of meiosis I

Our previous studies have indicated that fission yeast Hsk1 is essential for progression of meiosis, since hsk1Δ mutant cells uniformly arrested meiosis with one nucleus (Ogino et al. 2006). We show here that Cdc7 kinase of budding yeast is also critical for the progression of meiosis I. The cells lacking Cdc7 kinase arrested with one nucleus, and the induction of Ndt80, an essential regulator for the progression of meiosis I, is severely impaired [Figs. 1, 2]. Transcription of NDT80 is regulated by several pathways. Ndt80 is activated through the phosphorylation by Ime2, a meiotic kinase required for the initiation of replication [Mitchell et al. 1990; Hepworth et al. 1998]. Since replication proceeds normally in cells lacking Cdc7 kinase, Ime2 kinase is supposed to be almost fully activated [Benjamin et al. 2003]. Thus, the poor induction of NDT80 and other defects observed in bob1-1 cdc7Δ may not be due to the lack of Ime2 activity. Alternatively, it is likely that some unknown targets of Cdc7 kinase may play crucial roles in meiotic induction of middle and middle-late meiotic genes including Ndt80-regulon.

Intriguingly, the bob1-1 cdc7Δ diploids formed some asci with dyads after prolonged incubation (>30% asci had dyads) [Supplemental Fig. S1]. Microscopic analysis using chromosome tagging by the chromosome tagging system has revealed that the homologous chromosomes segregate equatorially in this situation [Valentin et al. 2006; T. Fukuda, H. Sasnuma, and K. Ohta, unpubl.]. This result raises the possibility that Cdc7 kinase has other targets to facilitate reductional segregation of homologous chromosomes. Alternatively, the delayed induction of NDT80 may cause the equational segregation.
It is intriguing to investigate how Cdc7 regulates modes of chromosome segregation.

Cdc7-dependent phosphorylation of Mer2 is crucial for meiotic DSB formation

We showed that Cdc7 is pivotal for meiotic DSB formation and recombination (Fig. 1). The TOF-MS analysis revealed that the N-terminal region of Mer2 harbors multiple phosphorylation sites regulated by Cdc7 kinase (Fig. 3). The alanine substitution of all serine residues (Ser11, Ser15, Ser19, Ser22, Ser29) in this region reduced Mer2 phosphorylation to the bob1-1 cdc7/H9004 level and conferred severe defects in spore viability, meiotic DSB formation, and recombination, indicating that the Mer2 phosphorylation dependent on Cdc7 kinase is vital.

Figure 6. ChIP of Spo11, Rec12 (in fission yeast), Rec114, and Mei4 at recombination hot spot. (A) The binding of Spo11/Rec12 with the hot spots in bob1-1 cdc7Δ, mer2S111, 15, 19, 22, 29A, and hsk1-89 mutants. The top graphs show the kinetics of Spo11-3Flag binding to the YCR048w (hot spot) and the SMC1 (cold spot) regions during meiosis. The bottom graphs indicate Rec12-Flag binding to mbs1 (hot spot) and fbp1 (cold spot). In fission yeast, the accumulated Rec12-3Flag association is detected by introducing the rad50S mutation. G1 arrest and meiotic induction of the pat1-114 strain were carried out as described previously (Ogino et al. 2006). The strains used were YHS916 (bob1-1 SPO11-3FLAG), YHS896 (bob1-1 cdc7Δ SPO11-3FLAG), YHS1222 (mer2S111, 15, 19, 22, 29A SPO11-3FLAG), PKH50 (rec12-flag), PHK253 (hsk1-89 rec12-flag), YHS866 (bob1-1 cdc7Δ REC114-3FLAG), YHS747 [REC114-3FLAG mer2Δ], and YHS777 (bob1-1 cdc7Δ MEI4-3FLAG). The experiment was conducted at least twice and gave the same profile. (B–D) The association of Rec114 and Mei4 with the YCR048w and SMC1 loci. (B) Rec114-3Flag binding in the wild-type [left], bob1-1 cdc7Δ [middle], and mer2Δ [right] background. (C) Mei4-3Flag binding in wild-type [left] and bob1-1 cdc7Δ [right]. (D) Rec114-3Flag [left] and Mei4-3Flag [right] binding in spo11Δ. ChIP was performed as for A.
for meiotic DSB formation. In addition, we found that the Ser29 residue is a critically important phosphorylation site, since the substitution at this site to alanine exhibited totally impaired DSB formation [cf. the accompanying study by Wan et al. 2008]. Moreover, we showed that Ser11, Ser15, Ser19, and Ser22 residues are important for DSB formation as well. These results suggest two modes of phosphorylation in the N-terminal region of Mer2: a critical single phosphorylation site at Ser29 and cumulative phosphorylation sites. In vitro kinase assay revealed that purified Mer2 can be directly phosphorylated by Cdc7–Dbf4 (data not shown).

The N-terminal regions of Mcm2 and Mcm4, which are required for DNA replication, are also known to be directly phosphorylated by Cdc7 [Cho et al. 2006; Masai et al. 2006; Montagnoli et al. 2006]. Cdc7 can phosphorylate substrates containing a minimum consensus sequence [Ser/Thr-Pro] (Ubersax et al. 2003). Cdc7 may preferentially phosphorylate the first serine residue of the “Ser–Ser/Thr–Pro” consensus sequence that has been subjected to prior phosphorylation by CDK. The coordinating sufficient levels of N-terminal phosphorylations of Mcm proteins by Cdc7 kinase and CDK facilitate the assembly of replication complex to initiate DNA replication [Masai et al. 2006; Sheu and Stillman 2006]. In the case of Mer2, the “Ser–Ser/Thr–Pro” consensus sequences are located in the N-terminal region [Fig. 3E]. Ser29 and Ser30 of Mer2 protein correspond to the first and second serine residues of this consensus sequence, respectively. The latter is known to be phosphorylated by CDK [Henderson et al. 2006]. Therefore, these results implicate that Mer2 may be also phosphorylated in a stepwise manner by Cdc7 and CDK, as in the case of Mcm proteins [cf. the accompanying study by Wan et al. 2008].

Furthermore, the present results lead us to speculate a role of cumulative phosphorylations of the remaining N-terminal Ser11, Ser15, Ser19, and Ser22. After the stepwise phosphorylation at Ser29 and Ser30, the N-terminal region may be converted to more preferred targets for Cdc7 kinase due to an increase in negative charge, leading to further phosphorylation of this N-terminal segment by Cdc7. Alternatively, the Ser11, Ser15, Ser19, Ser22, and Ser29 residues may be phosphorylated independently of CDK. However, the latter possibility is unlikely, since the phosphorylations at Ser11, Ser15, and Ser19 on Mer2 were substantially reduced in mitotic cells, in which the phosphorylation of Ser30 is absent [Fig. 4D]. Therefore, it is most likely that the Cdc7-dependent phosphorylation in the Mer2 N-terminal region progressively occurs after the priming phosphorylation by CDK. It is intriguing to investigate the coordinated fashion of Mer2 phosphorylation by Cdc7 and CDK kinases [cf. the accompanying study by Wan et al. 2008].

It is possible that other targets of Cdc7-dependent phosphorylation may be involved in DSB formation. For example, Rec114 protein is also phosphorylated depending on Cdc7 in meiosis [Supplemental Fig. S3]. Moreover, it has been reported that Rec104, a factor interacting with Spo11, is also phosphorylated in meiosis [Kee et al. 2004]. These results raise the possibility that the establishment and activation of the pre-DSB complex at DSB hot spots is under integrated regulation via phosphorylations on various factors. This notion is very similar to the regulation of the prereplicative complex (pre-RC) at replication origins that involves multiple layers of phosphorylation. Our results demonstrate that two sets of kinases, CDK and Cdc7–Dbf4 [DDK], are commonly involved in both meiotic DSB formation and replication. It is an interesting future study to investigate the common molecular basis of the activation of pre-DSB and pre-RC complex by combined actions of CDK and Cdc7.

The loading mechanism of Spo11 on meiotic recombination hot spot

Given that Mer2 protein is regulated by Cdc7 and CDK via phosphorylation, the next question is how Mer2 regulates DSB formation. We demonstrated that the mer2S11, 15, 19, 22, 29A mutant or the cells lacking Cdc7 kinase fail to load Spo11 to meiotic DSB sites, indicating that the defect of DSB formation is caused by the lack of the Spo11 binding to DSB hot spots [Fig. 6]. Previous studies had revealed that the binding of Spo11 to DSB hot spots requires at least three proteins: Rec102, Rec104, and Rec114 [Prieler et al. 2005]. Rec102 and Rec104 can form a subcomplex with Spo11 [Kee et al. 2004]. Recently, it has been reported that Rec114 interacts with Mei4 and Mer2 to form another type of subcomplex in meiosis [Li et al. 2006]. In this study, we demonstrated that Rec114 and Mei4 bind to DSB hot spots with kinetics similar to that of meiotic DSB formation. In addition, we found that Rec114 and Mei4 bound to DSB hot spots in the spo11Δ mutant [Fig. 6D]. Moreover, the chromatin binding of Rec114 occurs independently of Spo11 and most of the other DSB factors [Malecki et al. 2007]. All these results suggest that Rec114 or Rec114–Mer2–Mei4 complex acts as a regulatory subunit for the Spo11 loading to DSB sites [Fig. 7]. Importantly, the chromatin binding of both Rec114 and Mei4 was impaired in bob1-1 cdc7Δ. Therefore, Cdc7-dependent phosphorylation of Mer2 may contribute to stabilize or enhance the assembly of the Rec114–Mer2–Mei4 complex on chromatin around DSB sites [Fig. 7].

Temporal regulation of DNA replication and DSB formation by CDK and DDK

We demonstrated the essential role of Cdc7 in meiotic DSB formation. Taken together with previous studies, it is most likely that the activities of both CDK and Cdc7 are needed for the phosphorylation of Mer2 during meiosis, and thereby facilitate meiotic DSB formation. Given such dual regulation by distinct entities of kinases, we speculate about the physiological significance of the regulation. The activity of CDK is required for various meiotic events such as meiotic chromosome segregation, premeiotic DNA replication, or DSB formation in concert with other kinases [Benjamin et al. 2003; Brar et
The Spo11 complex at the DSB hot spot. Finally, an activated Mei4 complex possibly serves as a scaffold that retains Mer2, Rec104, and Ski8, is loaded onto hot spot (Step II). The Rec114 phosphorylation of Mer2, which may stabilize the complex on the region (Step I), and undergoes CDK- and Cdc7-dependent phosphorylation of Mei4 is assembled at the DSB hot spot. Subsequently, the replication fork and opening of the chromatin structure, the CDK- and Cdc7-dependent Mei4 phosphorylation (Ser11, 15, 19, 22, 29, 30, 271) might be regulated so that it occurs strictly during meiosis.

On the other hand, Borde et al. (2000) suggested that DSB formation is also regulated to occur only in the regions where the DNA replication forks have passed through. Thus, some unknown mechanisms modulate DSB formation at the chromosomal level. DDK has important roles in S-phase checkpoint response in addition to the conserved system for regulation of meiotic recombination in fission yeast. Therefore, we speculate that other meiotic regulator(s) or functional homologs of Mer2 may be involved in Hsk1-mediated regulation of meiotic recombination in fission yeast. Further elucidation of the regulatory mechanism of DSB formation by Cdc7 kinase would shed light on the conserved system for regulation of meiotic recombination initiation.

Materials and methods

Yeast strains and plasmid construction

The MCM5 gene was cloned in pRS306 carrying a URA3 marker (Sikorski and Hieter 1989), and the mutations (cytosine to thymine at base 248 and adenine to guanine at base 246 from the start codon, called the bob1-1 mutation) were introduced to generate the bob1-1 mutant (P83L) and a SacI cutting site. The resulting plasmid pRS306-bob1-1 was linearized by SacI treatment and transformed in wild-type haploid cells. The transformant carrying two copies of the MCM5 gene and URA3 marker were streaked on the plate containing 5-FOA to pop out a wild-type copy of the MCM5 gene, and the bob1-1 mutant was obtained. The CDC7 gene was deleted with a kanMX4 maker in the bob1-1 background. To construct Flag-tagged strains, we used the standard PCR-mediated integration method (De Antoni and Gallwitz 2000). The resulting REC114-3FLAG and MEI4-3FLAG diploid strains showed normal spore formation and spore viability, but the MER2-3FLAG diploid strain showed a slight reduction in spore viability compared with wild type. The MER2 gene containing the promoter region [upstream 500 base pairs [bp]] was cloned in pRS306, and the various point mutations were introduced. To construct tagged mer2 mutants, the 3xFlag sequence was conjugated at the end of the mer2 ORF by PCR. The resultant plasmid pURS306-U500mer2-3FLAG was linearized by EcoRI treatment, integrated into a haploid cell, and further popped out by counterselection on the plate containing 5-FOA. The cMER2 gene was amplified by PCR from a cDNA library generated from an RNA sample of 4 h in meiosis and cloned in the KpnI/SacI site of pAUR123 (TaKaRa-Bio Co. Ltd.). The BamHI fragment containing the ADH1 promoter region upstream 500 bp was cloned in pRS306, and a terminator cassette was swapped to the Ycp50 vector to express in yeast.

Immunoprecipitation (IP), Western blotting, and antibodies

IP was performed as described previously (Sasanuma et al. 2007), with a slight modification. IP was conducted using anti-Flag M2 monoclonal antibody-conjugated Sepharose beads (Sigma). To treat the IP fraction with CIAP (TaKaRa-Bio Co. Ltd.), the immunoprecipitates were washed well with NEB buffer #3 [50 mM Tris-Cl at pH 7.9, 10 mM MgCl2, 100 mM NaCl, 1 mM DTT] to ChIP analysis indicating that the distribution of Spo11 along the chromosomes gradually spreads from early-replicating to late-replicating regions (K. Kugou, T. Fukuda, H. Sasanuma, S. Mori, Y. Katou, T. Itoh, K. Matsumoto, T. Shibata, K. Shirahige, and K. Ohta, unpubl.). Therefore, we propose that Cdc7 functions as a coordinator for the interplay between replication and regional control of meiotic chromosomal events, which may ensure the strict temporal order of meiotic events.

We also showed that Hsk1 kinase is vital for the recruitment of Spo11 to a DSB site in the distantly related fission yeast, suggesting that this regulatory mechanism may be conserved in other eukaryotes. In fission yeast, the obvious counterpart of Mer2 has not been found yet. Thus, we speculate that other meiotic regulator(s) or functional homologs of Mer2 may be involved in Hsk1-mediated regulation of meiotic recombination in fission yeast. Further elucidation of the regulatory mechanism of DSB formation by Cdc7 kinase would shed light on the conserved system for regulation of meiotic recombination initiation.

Figure 7. A schematic model of establishment of pre-DSB complex at meiotic DSB hot spot. Following the progression of the replication fork and opening of the chromatin structure, the Rec114–Mer2–Mei4 complex is assembled at the DSB hot spot region (Step I), and undergoes CDK- and Cdc7-dependent phosphorylation of Mer2, which may stabilize the complex on the chromatin. Then the Spo11 complex, including Rec102, Rec104, and Ski8, is loaded onto hot spot (Step II). The Rec114–Mer2–Mei4 complex possibly serves as a scaffold that retains the Spo11 complex at the DSB hot spot. Finally, an activated complex would stimulate Spo11-catalyzed DSB formation.

Sasanuma et al.
remove inhibitors against CIAP in lysis buffer completely. After eluting with 150 μg/mL 3xFlag peptide (Sigma), the IP fraction was divided into three aliquots. The first aliquot was added to buffer [untreated], the second was treated with 36 U of CIAP, and the third was treated with the phosphatase plus inhibitors [20 mM NaVO₃, 100 mM NaF, 50 mM EDTA]. Each aliquot was incubated for 2 h at 37°C. SDS-PAGE was performed by low electrical current to detect the subtle mobility shift of phosphorylated Mer2. Anti-phosphorylated Mer2 antibody (anti-S305, Abcam) was used at 1/5000 dilution.

TOF-MS analysis
For TOF-MS analysis to identify the phosphorylation site of Mer2, meiotic cell lysate expressing Mer2-3Flag was prepared from 6 L of SPM culture (YHS35, the diploid MER2-3FLAG strain) [-1.2 x 10⁶ cells] and incubated with anti-Flag agarose beads for 5 h at 4°C. After washing several times with lysis buffer, Mer2-3Flag was eluted with 3xFlag peptide. After the IP fraction was separated by a 4%–20% gradient polyacrylamide gel [Dai-ichikagaku], the gel was stained with a silver staining KIT (Wako). The band corresponding to Mer2-3Flag protein was excised from several lanes, and TOF-MS analysis was performed by Aproscience Co. Ltd.

Northern blotting
Total RNA was prepared from ~2 x 10⁸ cells according to the method described in Elder et al. [1983]. For Northern analysis, 10 µg of total RNA was denatured with formamide, separated on 1 % agarose gels containing formaldehyde, and blotted on a charged nylon membrane [BioDyne B membrane, PALL, EA]. Probes were described previously for IME2, SPS4, SPS22/YCI048w, DMC1, and CMD1 [Kugou et al. 2007]. The probes for SPO11 and REC102 were prepared from DNA fragments using a random-primering kit (GE Healthcare). The DNA fragment of full-length SPO11 ORF was used to detect the SPO11 transcript. The 406-bp EcoRV fragment from a cloned REC102 gene in pCR2.1 [Invitrogen] was gel-purified and used to detect the REC102 transcript.

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References
Arora, C., Kee, K., Maleki, S., and Keeney, S. 2004. Antiviral protein Ski8 is a direct partner of Spo11 in meiotic DNA break formation, independent of its cytoplasmic role in RNA metabolism. Mol. Cell 13: 549–559.
Benjamin, K.R., Zhang, C., Shokat, K.M., and Herskowitz, I. 2003. Control of landmark events in meiosis by the CDK Cdc28 and the meiosis-specific kinase Ime2. Genes & Dev. 17: 1524–1539.
Borde, V., Goldman, A.S., and Lichten, M. 2000. Direct coupling between meiotic DNA replication and recombination initiation. Science 290: 806–809.
Brar, G.A., Kiburz, B.M., Zhang, Y., Kim, J.E., White, F., and Amon, A. 2006. Rec8 phosphorylation and recombination promote the step-wise loss of cohesins in meiosis. Nature 441: 532–536.
Cho, W.H., Lee, Y.J., Kong, S.I., Hurwitz, J., and Lee, J.K. 2006. CDC7 kinase phosphorylates serine residues adjacent to acidic amino acids in the minichromosome maintenance 2 protein. Proc. Natl. Acad. Sci. 103: 11521–11526.
Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P.O., and Herskowitz, I. 1998. The transcriptional program of sporulation in budding yeast. Science 282: 699–705.
De Antoni, A. and Gallwitz, D. 2000. A novel multi-purpose cassette for repeated integrative epitope tagging of genes in Saccharomyces cerevisiae. Gene 246: 179–185.
Dirick, L., Goetsch, L., Ammerer, G., and Byers, B. 1998. Regulation of meiotic S phase by Ime2 and a Clb5,6-associated kinase in Saccharomyces cerevisiae. Science 281: 1854–1857.
Elder, R.T., Loh, E.Y., and Davis, R.W. 1983. RNA from the yeast transposable element Ty1 has both ends in the direct repeats, a structure similar to retrovirus RNA. Proc. Natl. Acad. Sci. 80: 2432–2436.
Hardy, C.F., Dryga, O., Seematter, S., Pahl, P.M., and Sclafani, R.A. 1997. mcm5/cdc46-bob1 bypasses the requirement for the S phase activator Cdc7p. Proc. Natl. Acad. Sci. 94: 3151–3155.
Henderson, K.A., Kee, K., Maleki, S., Santini, P.A., and Keeney, S. 2006. Cyclin-dependent kinase directly regulates initiation of meiotic recombination. Cell 125: 1321–1332.
Hepworth, S.R., Friesen, H., and Segall, J. 1998. NDT80 and the meiotic recombination checkpoint regulate expression of middle sporulation-specific genes in Saccharomyces cerevisiae. Mol. Cell. Biol. 18: 5750–5761.
Jiang, W., McDonald, D., Hope, T.J., and Hunter, T. 1999. Mammalian Cdc7–Dbf4 protein kinase complex is essential for initiation of DNA replication. EMBO J. 18: 5703–5713.
Kee, K., Protacio, R.U., Arora, C., and Keeney, S. 2004. Spatial organization and dynamics of the association of Rec102 and Rec104 with meiotic chromosomes. EMBO J. 23: 1815–1824.
Keeney, S. 2001. Mechanism and control of meiotic recombination initiation. Curr. Top. Dev. Biol. 52: 1–53.
Kim, J.M. and Masai, H. 2004. Genetic dissection of mammalian Cdc7 kinase: Cell cycle and developmental roles. Cell Cycle 3: 300–304.
Kim, J.M., Takemoto, N., Arai, K., and Masai, H. 2003a. Hypomorphic mutation in an essential cell-cycle kinase causes growth retardation and impaired spermatogenesis. EMBO J. 22: 5260–5272.
Kim, J.M., Yamada, M., and Masai, H. 2003b. Functions of mammalian Cdc7 kinase in initiation/monitoring of DNA replication and development. Mutat. Res. 532: 29–40.
Kugou, K., Sasanuma, H., Matsumoto, K., Shiraighe, K., and Ohta, K. 2007. Mre11 mediates gene regulation in yeast spore development. Genes Genet. Syst. 82: 21–33.
Li, J., Hooker, G.W., and Roeder, G.S. 2006. Saccharomyces cerevisiae Mer2, Mei4 and Rec114 form a complex required for meiotic double-strand break formation. Genetics 173: 1969–1981.
Maleki, S., Neale, M.J., Arora, C., Henderson, K.A., and Keeney, S. 2007. Interactions between Mei4, Rec114, and other proteins required for meiotic DNA double-strand break formation in Saccharomyces cerevisiae. Chromosoma 116: 471–486.
Sasanuma et al.

Masai, H., Taniyama, C., Ogino, K., Matsui, E., Kakusho, N., Matsumoto, S., Kim, J.M., Ishii, A., Tanaka, T., Kobayashi, T., et al. 2006. Phosphorylation of MCM4 by Cdc7 kinase facilitates its interaction with Cdc45 on the chromatin. J. Biol. Chem. 281: 39249–39261.

Matsumoto, S., Ogino, K., Noguchi, E., Russell, P., and Masai, H. 2005. Hsk1–Dip1/Him1, the Cdc7–Dbf4 kinase in Schizosaccharomyces pombe, associates with Swi1, a component of the replication fork protection complex. J. Biol. Chem. 280: 42536–42542.

Mitchell, A.P., Driscoll, S.E., and Smith, H.E. 1990. Positive control of sporulation-specific genes by the IME1 and IME2 products in Saccharomyces cerevisiae. Mol. Cell. Biol. 10: 2104–2110.

Montagnoli, A., Valsasina, B., Brotherton, D., Troiani, S., Rainoldi, S., Tenca, P., Molinari, A., and Santocanale, C. 2006. Identification of Mcm2 phosphorylation sites by S-phase-regulating kinases. J. Biol. Chem. 281: 10281–10290.

Murakami, H., Borde, V., Shibata, T., Lichten, M., and Ohta, K. 2003. Correlation between premeiotic DNA replication and chromatin transition at yeast recombination initiation sites. Nucleic Acids Res. 31: 4085–4090.

Nacal, M.J., Pan, J., and Keeney, S. 2005. Endonucleolytic processing of covalent protein-linked DNA double-strand breaks. Nature 436: 1053–1057.

Ogino, K., Hirota, K., Matsumoto, S., Takeda, T., Ohta, K., Arai, K., and Masai, H. 2006. Hsk1 kinase is required for induction of meiotic dsDNA breaks without involving checkpoint kinases in fission yeast. Proc. Natl. Acad. Sci. 103: 8131–8136.

Ohta, K., Shibata, T., and Nicolas, A. 1994. Changes in chromatin structure at recombination initiation sites during yeast meiosis. EMBO J. 13: 5754–5763.

Prieler, S., Penkner, A., Borde, V., and Klein, F. 2005. The control of Spo11’s interaction with meiotic recombination hotspots. Genes & Dev. 19: 255–269.

Primig, M., Williams, R.M., Winzeler, E.A., Tevzadze, G.G., Conway, A.R., Hwang, S.Y., Davis, R.W., and Esposito, R.E. 2000. The core meiotic transcriptome in budding yeasts. Nat. Genet. 26: 415–423.

Sasanuma, H., Murakami, H., Fukuda, T., Shibata, T., Nicolas, A., and Ohta, K. 2007. Meiotic association between Spo11 regulated by Rec102, Rec104 and Rec114. Nucleic Acids Res. 35: 1119–1133.

Schlissel, D. and Byers, B. 1978. Meiotic effects of DNA-defective cell division cycle mutations of Saccharomyces cerevisiae. Chromosoma 70: 109–130.

Sheu, Y.J. and Stillman, B. 2006. Cdc7–Dbf4 phosphorylates MCM proteins via a docking site-mediated mechanism to promote S phase progression. Mol. Cell 24: 101–113.

Sikorski, R.S. and Hieter, P. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 122: 19–27.

Smith, K.N., Penkner, A., Ohta, K., Klein, F., and Nicolas, A. 2001. B-type cyclins CLB5 and CLB6 control the initiation of recombination and synaptonemal complex formation in yeast meiosis. Curr. Biol. 11: 88–97.

Sommariva, E., Pellny, T.K., Karahan, N., Kumar, S., Huberman, J.A., and Dalgaard, J.Z. 2005. Schizosaccharomyces pombe Swi2, Swi3, and Hsk1 are components of a novel S-phase response pathway to alkyltion damage. Mol. Cell. Biol. 25: 2770–2784.

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

Ubersax, J.A., Woodbury, E.L., Quang, P.N., Paraz, M., Bledow, J.D., Shah, K., Shokat, K.M., and Morgan, D.O. 2003. Targets of the cyclin-dependent kinase Cdk1. Nature 425: 859–864.

Valentini, G., Schwob, E., and Della Seta, F. 2006. Dual role of the Cdc7-regulatory protein Dbf4 during yeast meiosis. J. Biol. Chem. 281: 2828–2834.

Vedel, M. and Nicolas, A. 1999. CYS3, a hotspot of meiotic recombination in Saccharomyces cerevisiae. Effects of heterozygosity and mismatch repair functions on gene conversion and recombination intermediates. Genetics 151: 1245–1259.

Wan, L., Zhang, C., Shokat, K.M., and Hollingsworth, N.M. 2006. Chemical inactivation of cdc7 kinase in budding yeast results in a reversible arrest that allows efficient cell synchronization prior to meiotic recombination. Genetics 174: 1767–1774.

Wan, L., Niu, H., Futcher, B., Zhang, C., Shokat, K.M., Boulton, S.J., and Hollingsworth, N.M. 2008. Cdc28–Clb5 (CDK-S) and Cdc7–Dbf4 (DDK) collaborate to initiate meiotic recombination in yeast. Genes & Dev. (this issue), doi: 10.1101/gad.1626408.

Watanabe, Y., Yokohayashi, S., Yamamoto, M., and Nurse, P. 2001. Pre-meiotic S phase is linked to reductive chromosome segregation and recombination. Nature 409: 359–363.

Wu, T.C. and Lichten, M. 1994. Meiosis-induced double-strand break sites determined by yeast chromatin structure. Science 263: 515–518.
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