Pds1p is Required for Meiotic Recombination and Prophase I Progression in S. cerevisiae

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ABSTRACT

Sister chromatid separation at the metaphase-anaphase transition is regulated by a proteolytic cascade. Destruction of the securin Pds1p liberates the Esp1p separase which ultimately targets the mitotic cohesin Mcd1p/Scc1p for destruction. Pds1p stabilization by the spindle or DNA damage checkpoints prevents sister chromatid separation while mutants lacking PDS1 (pds1Δ) are temperature sensitive for growth due to elevated chromosome loss. This report examined the role of the budding yeast Pds1p in meiotic progression using genetic, cytological and biochemical assays. Similar to its mitotic function, Pds1p destruction is required for metaphase I-anaphase I transition. However, even at the permissive temperature for growth, pds1Δ mutants arrest with prophase I spindle and nuclear characteristics. This arrest was partially suppressed by preventing recombination initiation or by inactivating a subset of recombination checkpoint components. Further studies revealed that Pds1p is required for recombination both in double strand break formation and synaptonemal complex assembly. Although deleting PDS1 did not affect the degradation of the meiotic cohesin Rec8p, Mcd1p was precociously destroyed as cells entered the meiotic program. This role is meiosis-specific as Mcd1p destruction is not altered in vegetative pds1Δ cultures. These results define a previously undescribed role for Pds1p in cohesin maintenance, recombination, and meiotic progression.
INTRODUCTION

Meiosis generates haploid gametes through a specialized cell division process that consists of one round of DNA replication followed by two nuclear divisions. The first meiotic division is unique to meiosis for two reasons. First, during the extended prophase I, homologous chromosomes synapse and undergo high levels of genetic recombination that are essential for the correct chromosome alignment at metaphase I (KUPIEC et al. 1997). Second, following resolution of the recombination intermediates, the spindle makes monopolar attachments to the sister chromatids permitting the execution of meiosis I or the reductional division. Meiosis II resembles mitosis in that the replicated sister chromatids segregate to opposite poles.

Meiotic recombination establishes chromosome alignment essential for accurate segregation during the first meiotic division. It follows therefore that the first step in this process, i.e., the formation of double strand breaks (DSB's), is also a critical event (KEENEY et al. 1997). To date, in budding yeast, at least 10 proteins are required for this process (reviewed in (BAUDAT and KEENEY 2001); (BORDE 2007) (ARORA et al. 2004)). Some of these proteins are meiosis specific whereas others also have roles in mitotically dividing cells. Significantly, apart from Spo11p, that initiates DSB formation (KEENEY et al. 1997), little is known about the biochemical function of the individual components of this complex and how they are regulated.

The proper execution of recombination and other meiotic landmark events is governed by several checkpoint pathways (reviewed in (ROEDER 1997)). The DNA damage checkpoint senses broken DNA ends and transduces the signal through the Rad9p kinase (LYDALL et al. 1996; WEBER and BYERS 1992). The meiotic recombination checkpoint is more complex and can be divided into three different pathways depending on the signal that is generated (reviewed in (HOCHWAGEN and AMON 2006; ROEDER and BAILIS 2000). The rad50S checkpoint is triggered by unprocessed double strand breaks (DSB) generated by the endonuclease Spo11p. The recombination (or dmc1) pathway is activated by ressected, but not processed, DSB ends. Finally, the Zip1 checkpoint functions following strand invasion and is activated by an as yet undefined signal. Although the different checkpoint pathways monitor different steps in the recombination
process, they share many components. For example, the various recombination DNA lesions are recognized by the Rad17-Ddc1-Mec3 clamp loader. However, different proteins are recruited depending on the checkpoint signal. For example, Tel1p is recruited by the rad50S complex but not recombination pathway (USUI et al. 2001). Likewise, the chromosome structure proteins Red1p and Mek1p, are not required for the DNA damage checkpoint but are involved in all three arms of the meiotic recombination checkpoint (reviewed in Hochwagen, 2006 #2837). Mek1p is a meiotic kinase which upon activation phosphorylates Red1p, which is turn triggers a cascade of events that inhibits downstream effectors including the Cdc28p cyclin dependent kinase (LEU and ROEDER 1999) and the transcription factor Ndt80p (CHU and HERSKOWITZ 1998; HEPWORTH et al. 1998; LINDGREN et al. 2000; PAK and SEGALL 2002). Ndt80p activates the “middle” set of meiotic genes that encode proteins necessary for establishment of the meiotic I spindle (XIE et al. 1999; Xu et al. 1995).

Cohesion between sister chromatids is essential for proper chromosome disjunction during meiosis (REVENKOVA et al. 2004). Dissolution of cohesion requires the ubiquitin ligase termed the anaphase promoting complex/cyclosome (APC/C). The APC/C mediates the destruction of Pds1p (COHEN-FIX et al. 1996; YAMAMOTO et al. 1996a) thereby releasing Esp1p which in turn triggers sister chromatid separation by destroying the cohesin subunit Mcd1p/Scc1p (CHARLES et al. 1998; SHIRAYAMA et al. 1998; VISINTIN et al. 1997). Deleting PDS1 enables the cell to override the metaphase arrest imposed by apc mutations while a non-degradable form of Pds1p causes a metaphase arrest (COHEN-FIX et al. 1996; YAMAMOTO et al. 1996a). Upon entry into the meiotic program, Mcd1p provides connections between sister chromatids and helps establish domains for double strand break (DSB) initiation (KATENEVA et al. 2005). These domains are only accessible for interhomologue recombination, upon remodeling/removal of Mcd1p by Tid1p, a member of the SWI/SNF2 family of helicase-like chromatin remodeling proteins (ZHANG et al. 2005). Significantly, the removal of Mcd1p during meiotic prophase may be Esp1p independent (KATENEVA et al. 2005) and, unlike its role during mitotic divisions (GUACCI et al. 1997), is not essential for completion of the meiotic divisions (KLEIN et al. 1999). This non-essential role is probably due in part to the fact that sister chromatid cohesion is maintained by Mcd1p’s

In addition to sequestering its protease activity, Pds1p also escorts Esp1p into the nucleus and to its final destination on the spindle (Agarwal and Cohen-Fix 2002; Baskerville et al. 2008; Jensen et al. 2001). Here Esp1 protease activity is required for spindle elongation independent of Mcd1p cleavage (Baskerville et al. 2008). Pds1p is also required for mitotic exit following DNA damage (Agarwal and Cohen-Fix 2002; Cohen-Fix and Koshland 1997; Hwang et al. 2001; Sanchez et al. 1999; Yamamoto et al. 1996b) and for double strand break repair via single strand annealing following radiation damage (Demase et al. 2005). Despite these multiple functions, Pds1p is not essential for mitotic cell division although pds1 cells are temperature sensitive for growth and exhibit high chromosome loss rates (Yamamoto et al. 1996a). In this present study, we report that Pds1p is required for prophase I progression as mutants arrest prior to the nuclear divisions. This arrest is partially suppressed by eliminating DSB initiation suggesting a role for Pds1p in recombination. Further studies revealed that Pds1p is indeed required for efficient DSB formation and recombination. Surprisingly, these phenotypes appear independent of Rec8p function. However, we observed premature cleavage of Mcd1p as pds1 cultures entering meiosis. This effect appears specific for meiotic cells as Mcd1p cleavage kinetics is not altered in vegetative cultures lacking PDS1 (Alexandru et al. 1999). These findings indicate a meiosis-specific role for Pds1p in preventing Mcd1p destruction prior to meiotic prophase.

MATERIALS AND METHODS

Strains, plasmids and media: The genotype, source and background of the strains used in this study are listed in Table 1. Our wild-type parent strain (RSY335) is derived from SK1 and W303 parents. This strain sporulates to high levels similar to SK1 but does not enter meiosis prematurely. However, this strain background executes meiosis
more slowly and asynchronously. The double strand break experiments and \textit{arg4} recombination measurements were performed in diploids KCY427 and KCY428 that were derived from haploid SK1 strains ORD7238-15C, ORD7238-26A, and ORD7246-2B, ORD7237-27A respectively (a gift from A. Nicolas, Curie Institute, Paris). Diploids harboring the \textit{pds1}\^\text{Δ} allele (KCY429 and KCY430) were generated by first deleting \textit{PDS1} in the haploid parents. Recombination at the \textit{his7} locus was monitored in strains RSY1343 and RSY1344 derived from REE223 and REE218 (a gift from R. E. Esposito, Univ. Chicago). The \textit{pds1}\^\text{Δ} version of this strain was constructed by first deleting \textit{URA3} in REE223 using the marker swap plasmid M2660 (a gift from D. Stillman, Univ. Utah). Thereafter, \textit{PDS1} was deleted using pOC80 (a gift from O. Cohen-Fix, National Institute of Health) in both haploids and crossed to form RSY1343. RSY1433 was made by using marker swap plasmids (pUL9 and pUT11 (CROSS 1997)) to change \textit{pds1::ura3} haploid strains to \textit{pds1::ura3::LEU2} and \textit{pds1::ura3::TRP1} respectively. RSY1433 was made by mating these strains. \textit{ndt80}\^\text{Δ} (RSY1453 and RSY1456) and \textit{pch2}\^\text{Δ} strains (RSY1536 and RSY1537) were made by integrating pTP89 or pSS53, respectively (gifts from S. Roeder, Yale Univ.). The \textit{BUB2}, \textit{MAD2}, \textit{MEK1}, \textit{SPO11}, \textit{SPO13} and \textit{UBR1} disruptions were generated using oligonucleotide directed recombination as described previously (LONGTINE \textit{et al.} 1998). The \textit{RED1} disruption was made by transferring the \textit{red1::KAN} allele from the Research Genetics strain collection into our genetic background. Disruption of \textit{RAD17} or \textit{RAD9} was accomplished using pAAA19 or pAAA83, respectively (gifts from T. Weinert, Univ. Arizona). Successful integrations were verified by PCR analysis of genomic DNA. \textit{REC8-3HA} strains were made as previously described (SHONN \textit{et al.} 2002). The HA-tagged Pds1p expression construct pOC40 (CEN, \textit{URA3}) was provided by O. Cohen-Fix. The \textit{PDS1-3FLAG} expression plasmid was constructed by inserting the \textit{SalI/MfeI} fragment from pCD11 into the \textit{SalI/EcoRI} sites of YCplac111 (GIETZ and SUGINO 1988) forming pKC7000. Wild-type and a destruction box mutant of \textit{PDS1} were placed under the control of the \textit{AMA1} promotor by PCR amplification of the \textit{PDS1} ORF and subsequent substitution into the \textit{AMA1} gDNA (details available upon request) to form pMSC13. The \textit{PDS1-3HA} destruction box mutation (RXXL → AXXA) contained in pVG279 under control of \textit{AMA1} promotor and the \textit{CYC1} terminator in pRS426 (CHRISTIANSON \textit{et al.} 1992) to form
pMSC14. The Rec8-HA high-copy plasmid (pKC7001) contains the REC8-3HA allele amplified from KCY447 inserted into YEplac181 (Gietz and Sugino 1988). The ESP1 high-copy plasmid (pKC7003) contains the KpnI/SacI fragment from pJN1 (ESP1-CEN plasmid, gift from Christine Pratt, Carneige Inst., Baltimore) into YEplac181. The ZIP1-GFP plasmid (Scherthan et al. 2007) was a gift from D. Kaback (UMDNJ). Yeast transformations were performed as described (Schiestl and Gietz 1989). Rich medium for yeast growth was either YPDA medium (2% dextrose, 2% peptone, 1% yeast extract supplemented with 10 mg/L adenine or YPA which contained K acetate (1%) substituted for dextrose in YPDA. Minimal medium for plasmid selection was either SD medium (0.17% yeast nitrogen base without amino acids, 0.5% ammonium sulfate, 2% dextrose or synthetic acetate (K acetate 1% substituted for D-glucose, 1% Phthalic acid, pH 5.5) supplemented with appropriate amino acid supplements. Liquid sporulation medium (SPM) for meiotic timecourses contains 2% K acetate supplemented with uracil. Solid sporulation medium contains 2% K acetate, 0.1% dextrose, 0.25% yeast extract, and 1.5% agar. Both sporulation media were brought to pH 7.0 by addition of KOH.

Meiotic Timecourse Experiments: Meiotic timecourse experiments were conducted at 23° unless otherwise stated. Meiotic timecourse experiments for Northern analysis, recombination assays (both physical and return to growth assays), chromatin pelleting, original pds1-1 and pds1Δ arrest characterization and FACS analysis were conducted by growing cultures to 1 x 10^7 or 5 x 10^6 cells/mL for YPA and synthetic acetate cultures, respectively. Cells were harvested by centrifugation and washed in one-half culture volume sterile water. The resulting cell pellet was then resuspended in 1/5 original culture volume of SPM. Samples were collected by centrifugation, washed in 1/10 vol sterile water, and the resulting cell pellet flash frozen in liquid nitrogen or a dry ice/ethanol bath. Portions of each sample were removed prior to freezing and fixed in 70% ethanol (DAPI staining) or 3.7% formaldehyde (tubulin indirect immunofluorescence) for nuclei and spindle visualization, respectively. Total RNA was prepared as previously described (Cooper et al. 1997) for Northern blot analysis. Western blot analysis was conducted with 100µg total cell extract. Flag and HA-tagged proteins were visualized using α-Flag (M2 antibody, Sigma) and α-HA (Roche) mouse
monoclonal antibodies respectively at a final concentration of 2µg/ml concentration. Tub1p (tubulin) was detected using α-Tub1p rabbit polyclonal antibody at a final concentration of 0.1µl/ml. Western blot signals were detected using goat anti-mouse secondary antibodies conjugated to alkaline phosphatase (Jackson ImmunoResearch Laboratories, Inc.) and the CDP-Star chemiluminescence kit (Tropix, Bedford, MA). Blots were stripped and reprobed according to manufactures instructions (Millipore).

**Cellular analysis protocols:** Quantitation of meiosis I and II was achieved by analyzing 4’, 6-diamidino-2-phenylindole (DAPI) stained cells as described (COOPER and STRICH 2002). At least 200 cells were counted per timepoint. For determining percent sporulation of the various mutant strains, at least three independent isolates were sporulated as described above. After 24 hours at 23°C, the cells were resuspended in one ml 70% ethanol for one hr, washed in one ml sterile water, and then stained with DAPI (0.05%) for 15 min. Cells were washed with water as before and examined by fluorescent microscopy. Cells were scored as follows: prophase I, spindle smaller than nucleus; metaphase I, spindle the same width as nucleus; pseudo-anaphase, spindle masses separate with elongated, but not separated nuclei; fragmented nuclei, more than four DAPI staining bodies, irregular sized DAPI staining bodies, and/or mis-oriented (more than two poles) spindle formation. The results presented are the mean of the three strains with standard deviation. FACS analysis was performed as previously described (COOPER et al. 2000). Chromatin pelleting was conducted as described (GUACCI et al. 1997). Spindle morphology was determined using indirect immunofluorescence as previously described (COOPER et al. 2000). Likewise, indirect immunofluorescence was used to visualize the nucleolus using anti-Nop1 antibodies (Abcam). An Olympus PROVIS AX70 fluorescence microscope was used for all experiments except Zip1p localization where a Nikon eclipse 90i microscope was used. Z stacks were performed at 0.6 micron intervals using NIS elements software (Nikon). All microscopy is presented at 1000X magnification except Fig. 5B (600X).

**Determination of recombination frequencies:** Recombination frequencies in the return to growth assay were measured as described (SOUSTELLE et al. 2002). Samples
from the sporulation culture were taken at the times indicated in the text. The cells were lightly sonicated to disrupt clumps, and directly counted by hemocytometer. Dilutions were made in sterile water and plated onto either complete minimal medium (to determine total viable cells plated) or on minimal medium lacking arginine or histidine to identify recombinants. Recombination studies were done at least twice with two independent cultures each time. Plates were incubated for four days at 23° prior to cell count determinations. Only prototrophs observed above the 0 hr timepoint amount (indicative of mitotic recombination) were utilized in the calculations. Double strand break assays were performed essentially as described (VeDEL and NICOLAS 1999). Briefly, DNA was extracted in agarose plugs, digested with the appropriate restriction enzyme, separated on a 1% agarose gel, transferred to Hybond N membrane (Amersham), processed as described for Northern blot analysis (COOPER et al. 1997) and probed with sequences specific to YCR048W or CYS3. The YCR048W probe was made by amplifying wild-type DNA using PCR primers 5' GGA TTT GTT GCA AGA CGA AG 3' and 5' TAA TGA TAC TGG GCC CTG AA 3'. The CYS3 probe was made by amplifying wild-type DNA using PCR primers 5’ GCT GAA TTC TTG GCA GAC AAG 3’ and 5’ TCT TAG GCA GGT AAT ACC TCG 3’.

RESULTS

**Pds1p is required for progression through meiosis I:** Previous studies have found that non-degradable Pds1p, when expressed from “early” meiotic gene promoters, induces a prophase I arrest (OELSCHLAEGEL et al. 2005; SHONN et al. 2000). We confirmed these results by placing a non-degradable Pds1p mutant (PDS1dbΔ) under the control of the meiosis-specific AMA1 promoter (COOPER et al. 2000). Following 24 hrs in liquid sporulation medium (SPM), DAPI staining revealed that the wild-type diploid expressing the AMA1pro-PDS1 exhibited an 8-fold increase in tetranucleated cells compared AMA1pro-PDS1dbΔ expressing cells (Fig. 1A). To further analyze the role of Pds1p during meiosis, we also followed meiotic progression in a pds1-1 strain. As pds1-1 cells are inviable at 30°, we performed these experiments at the permissive temperature for growth (23°). Surprisingly, the majority of the pds1-1 culture (82%)
failed to execute either nuclear division compared to only 9% for the wild-type strain. Although normal bi- and tetra-nucleated cells were not observed (<0.5%), DAPI staining did reveal a significant number (18%) of cells demonstrating an abnormal fragmented nuclear morphology. These findings indicate that Pds1p is required for meiosis.

We further characterized the requirement of Pds1p for meiosis by constructing a homozygous null mutant (pds1Δ) diploid in the RSY335 strain background (see Table 1). All meiotic timecourse experiments were performed at the permissive temperature for vegetative growth (23°C) unless otherwise indicated. Log-phase cultures were harvested, washed and transferred to SPM. FACS analysis of samples taken at subsequent times following the shift revealed that pds1Δ cells completed meiotic S phase to the same extent as wild type, but did so with slower kinetics (Fig.1B). These findings indicate that the failure of pds1Δ mutants to execute the meiotic nuclear divisions was not due to a defect in meiotic entry.

To more precisely define the role of Pds1p in meiosis, spindle status of wild type and pds1Δ cultures following meiotic induction was determined by indirect immunofluorescence using Tub1p antibodies. The approach was the same as described for Fig. 1B except the timecourse was extended to 24 hrs. Following nine hrs in sporulation medium, 83% of the pds1Δ cells remained mononucleated with no recognizable spindle (Fig. 1C). Most of the remaining pds1Δ cells (13%) contained short thick spindles diagnostic of metaphase I. Conversely, more than half of the wild type culture had progressed past meiosis I or meiosis II by this timepoint (Fig. 1D). The 15 hr timepoint revealed a reduction in cells arrested with a prophase spindle and an increase in the metaphase-like morphology. By 24 hrs, 25% of the pds1Δ cells exhibited a morphology not normally observed during meiosis. These cells possessed a partially elongated spindle I containing a thin central region and elongated, but not segregated, nuclei. We classified this phenotype as pseudo-anaphase, since true anaphase I cells would have fully elongated spindles and completely separated DNA masses. The remaining 13% of pds1Δ cells exhibited multiple sub-nuclear size DAPI staining bodies with abnormal spindle formation suggesting the presence of fragmented nuclei. None of the pds1Δ cells exhibited a recognizable spore wall, even following 36 hrs in sporulation medium. 95% of the wild-type population had undergone at least one
nuclear division by 24 hrs. A similar terminal phenotype was also observed with the \textit{pds1-1} homozygous diploid also at permissive temperature for growth (data not shown). The \textit{pds1-1} and \textit{pds1}Δ strains were derived from A364A and SK1/W303 strains, respectively, demonstrating that this phenotype is not background specific.

The delayed appearance of the fragmented nuclei suggested two possibilities. They may represent nuclei that disintegrated in response to meiotic arrest. Alternatively the fragmented nuclei may be the result of aberrant attempts to execute the meiotic divisions. To distinguish between these two models, we examined the terminal phenotype of \textit{ndt80}Δ and \textit{ndt80}Δ \textit{pds1}Δ mutants. Ndt80p is a meiosis-specific transcription factor that is required for disassembly of the synaptonemal complex (SC) (reviewed in (Zickler and Kleckner 1999)). Importantly, \textit{ndt80} mutants arrest in late pachytene with synapsed chromosomes and are unable to enter into the meiotic divisions (Xu \textit{et al.} 1995). As seen in Fig. S1, \textit{ndt80}Δ \textit{pds1}Δ cells still display fragmented nuclei after 24 hours in sporulation medium suggesting that the “fragmented nuclei phenotype” is due to deteriorating prophase I cells. To conclude, the \textit{pds1}Δ fragmentation phenotype appears to be a secondary phenotype resulting from an extended prophase I arrest.

\textbf{Double strand break formation is required for the \textit{pds1}-dependent meiotic arrest:} To better define the execution point of Pds1p during prophase I, we took advantage of previous suppressor bypass studies employing \textit{spo11} or \textit{spo13} mutations (see Fig. 2F for summary). Spo13p prevents loss of centromere cohesion (Lee \textit{et al.} 2004) and overexpression can prevent meiotic progression (McCarroll and Esposito 1994) Therefore, aberrant activation of Spo13p could lead to meiotic arrest. Mutants defective for Spo11p, the protein that initiates DSB formation (Keeney \textit{et al.} 1997), bypass a meiotic arrest that occurs after recombination initiation (e.g. rad50S, mms4 (Alani \textit{et al.} 1990; De los Santos \textit{et al.} 2001; Malone and Esposito 1981)). As mentioned above, \textit{pds1}Δ cells arrest as both mono-nucleated as well as a fragmentation phenotype making the potential bypass scoring complicated. For clarification purposes, suppression was scored as the production of bi or tetra-nucleated cells and spore wall assembly. Double mutant \textit{pds1}Δ \textit{spo11}Δ and \textit{pds1}Δ \textit{spo13}Δ strains were constructed
and their ability to complete meiosis and spore formation tested (see Materials and methods for details). After 24 hours in liquid sporulation medium, partial suppression was observed with 39% of the spo11Δ pds1Δ mutants completing both meiotic divisions and producing spores indicating that DSB formation was required for at least a part of the pds1Δ-dependent arrest (Fig. 2B). As expected, micro-dissection of these asci revealed that the spores were inviable indicating that the requirement for recombination to allow normal meiosis I division is retained. A reduced, but reproducible suppression (12% versus <0.5% dyads) was observed when SPO13 was deleted in the pds1Δ mutant background (Fig. 2B). Taken together, the partial suppression by spo13 and spo11 alleles of the pds1Δ meiotic defect suggests that Pds1p might be involved in some aspect of recombination.

The pds1Δ prophase I arrest is partially dependent on the recombination checkpoint signaling pathway: Recombination defect-induced meiotic arrest is mediated by three checkpoint pathways (rad50S, dmc1 and zip1) collectively referred to as the meiotic recombination checkpoint (see Fig. 2F). These pathways are abrogated by inactivating genes that sense the damage signal (e.g., RAD17, RAD24, MEC1, DDC1 and MEC3) or those mediating the arrest signal (e.g., RED1 and MEK1) (reviewed in (Hochwagen and Amon 2006)). To determine if the pds1Δ-induced arrest requires the checkpoint pathways, bypass experiments were performed with RAD17, a gene common to all three pathways (see Fig. 2F). Surprisingly, deleting RAD17 failed to suppress the pds1Δ arrest phenotype (data not shown). As this result was unexpected, we also determined whether the DNA damage checkpoint pathway was being evoked in pds1Δ cells (see Fig. 2F). However, a rad9Δ null allele did not suppress the arrest indicating that the DNA damage pathway was not involved (data not shown). As only a partial suppression was observed when SPO11 was deleted, this may suggest that multiple pathways are involved in the pds1Δ arrest. To test this possibility, a triple mutant diploid (rad9Δ rad17Δ pds1Δ) was constructed. This strain also displayed no suppression (Fig. 2C). Taken together these results suggest that neither the DNA damage or recombination checkpoint pathway is required for the pds1Δ-dependent meiotic arrest.
We next asked if mutations in the genes that regulate or generate the arrest signal (RED1 or MEK1) would bypass the terminal meiotic pds1Δ phenotype. As mentioned above, these proteins perform a different function to RAD17, providing a framework for the activation of the recombination checkpoint. Deleting either RED1 or MEK1 in a pds1Δ strain still resulted in about half of the population arresting prior to meiosis I (Fig. 2D). However, a significant fraction of the pds1Δ red1Δ or pds1Δ mek1Δ strains completed one or both divisions (41% and 32% bi and tetranucleated cells, respectively) and formed spore walls. In addition, the production of fragmented nuclei in these double mutants was also reduced. Although the appearance of bi and tetranucleated cells first occurred at a similar time in the red1 and red1 pds1 mutants (Fig. S2), the accumulation of these cells was slower in the double mutant. This observation suggests that Pds1p has an additional role in meiotic progression independent of Red1p. A similar result was obtained when PCH2, a gene required for the meiotic arrest in cells with defective SC or lacking DMC1 (SAN-SEGUNDO and ROEDER 1999) was deleted in combination with the pds1Δ null allele (Fig. 2E). To conclude, these data suggest that pds1Δ cells activate the recombination checkpoint. However, these results suggests that the pds1Δ-induced signal does not go through Rad17p as normally observed. Rather, this damage signal may more directly contact Mek1p and Red1p.

The pds1Δ prophase I arrest is not dependent on the spindle checkpoint signaling pathway: Our initial characterizations of the pds1Δ arrest revealed abnormal spindle formations including a pseudo-anaphase configuration and fragmented nuclei. These findings suggested either a spindle defect or a problem with chromosome cohesion. To test whether the kinetochore or spindle checkpoint pathways were responsible for the pds1Δ-associated arrest, each checkpoint was inactivated by mutating BUB2 (GARDNER et al. 2000) or MAD2 (SHONN et al. 2000). However, neither double mutant executed the first division indicating that these pathways were not solely responsible for the meiotic arrest (Fig. 2C and data not shown). In addition, a pds1Δ rad17Δ mad2Δ triple mutant also arrested prior to meiosis I suggesting that the recombination and spindle checkpoints were not performing redundant roles in mediating the pds1Δ arrest.
**Pds1p is required for the transcription of mid-late meiotic genes:** Our data indicate that the *pds1Δ* arrest is partially dependent on the signaling, but not sensing, components of the recombination checkpoint pathways. Activation of the recombination checkpoint results in meiotic arrest through inhibition of middle gene transcription (ROEDER and BAILIS 2000). One possible explanation of our results is that activation of Red1p and Mek1p in a *pds1Δ* mutant induces a similar loss in middle gene expression. To test this possibility, a meiotic timecourse with wild type and *pds1Δ* cultures was performed at 23° and total RNA preparations from the samples were subjected to Northern blot analysis. The blot was probed for transcripts from genes belonging to the “early” (*IME2*), “mid-early” (*NDT80*), “middle” (*SPS4, SPS2*) and “late” (*SPS100*) expression classes. This study revealed that the induction kinetics of the *IME2* mRNA appeared similar in both cultures (Fig. 3A) although the overall levels were reduced in the mutant strain. *NDT80* transcript levels were more significantly reduced and peak levels delayed in the *pds1Δ* mutant compared to wild type. Paradoxically, the transcript levels of *SPS2* and *SPS4*, two middle genes whose transcription require Ndt80p (HEPWORTH et al. 1998; LINDGREN et al. 2000), are similar to wild type although a delay in their induction of approximately three hrs was observed. These results suggest that sufficient Ndt80p is being synthesized to promote nearly normal levels of middle gene expression. Consistent with our suppression studies, the reduction, but not elimination, of *NDT80* transcription suggests that the *pds1Δ* arrest partially activates the recombination checkpoint.

Given the robust expression of the two middle genes in the *pds1Δ* mutant, it was surprising to find that the mRNA levels of the late gene *SPS100* were below the limits of detection. It is unlikely that this absence is due to the delay observed in *pds1Δ* strains as *SPS100* transcripts are present by 18 hrs in the control. These results suggested two possibilities. First, Pds1p may be directly required for late gene expression. Alternatively, the expression loss could be an indirect effect of the meiotic arrest associated with the *pds1Δ* allele. To test these possibilities, a meiotic timecourse was performed with a *pds1Δ spo13Δ* strain that allows partial suppression of the *pds1Δ* arrest (see Fig. 2C). In this experiment, the expression of early (*IME2*) and middle
(SPS2) was identical in both strains. However, similar to SPS100, DIT1 another late gene which is also required for spore wall assembly (BRIZA et al. 1990), transcripts were not detectable in the pds1Δ mutant (Fig. 3B). However, DIT1 was transcribed in the double mutant strain. These findings suggest that the defect in late gene expression is an indirect effect associated with the pds1Δ induced meiotic arrest.

Pds1p is required for normal meiotic recombination: The ability of a spo11Δ mutation to suppress the pds1Δ meiotic arrest suggested a role for Pds1p in recombination. To test this possibility, heteroallelic recombination was measured in pds1Δ strains using return-to-growth assays (see Materials and methods). Wild type and pds1Δ SK1 strains containing arg4 heteroalleles (arg4-Erv and arg4-Ebg (NICOLAS et al. 1989) were induced to enter meiosis at 23°. Samples, taken at several timepoints following the shift to SPM, were assayed for the presence of Arg+ prototrophs. The wild-type strain exhibited a 100-fold increase in recombination frequency 8 hrs following shift to SPM reaching a final level of 6.2% (Fig. 4A). These values are similar but lower than those observed in other studies (10%) examining the same strain (SOUSTELLE et al. 2002). This may be due to the different temperatures (23° vs. 30°) under which these experiments were conducted. Significantly, approximately 100-fold fewer Arg+ prototrophs were observed for the pds1Δ diploid (Fig. 4A). This decrease in recombination efficiency was not the result of decreased pds1Δ diploid viability over the course of the experiment as determined by return to growth studies (data not shown).

The timing of recombination commitment occurs just prior to the appearance of bi- and tetra-nucleated cells observed in this strain at 23° (see KCY428, Fig. 4B). To determine if the defect in recombination was also observed at another locus, recombination was also monitored using his7 heteroalleles in the S288C strain background. This locus gives a significantly lower recombination frequency in the wild-type strain (Table S1). Similar to our findings with recombination at the arg4 locus, the number of His+ prototrophs was reduced at least two orders of magnitude in the pds1Δ strain. These results indicate that Pds1p is required for normal levels of meiotic recombination.
**Pds1p is required for normal double strand break formation:** To investigate the requirement of Pds1p for recombination, we first asked whether the mutant strain was able to form double strand breaks (DSB), the initiating step in this process. In wild-type diploid SK1, the *PDS1* gene was deleted and the resulting wild type and *pds1Δ* strains were induced to enter meiosis at 23°C. Samples were collected at various times and total DNA prepared. The appearance of DSBs was examined at the *YCR048W* locus (BAUDAT and NICOLAS 1997; LIU et al. 1995) by Southern blot analysis (see Materials and methods). In the wild type, the expected transient accumulation of DSB’s was detected peaking in the 9-12 hr timepoint (Fig. 5A). This is consistent with the commitment to recombination studies at this temperature. Significantly, little or no DSBs were detected in the *pds1Δ* mutant strain. This result is consistent with the approximately 100-fold reduction in recombination frequency.

This very low level of DSB’s in *pds1Δ* could be due to a decrease in DSB initiation, accelerated repair of the breaks, or both. To distinguish between these possibilities, we examined DSB accumulation in an SK1 strain harboring the *rad50S* allele that permits DSB accumulation but not their repair (ALANI et al. 1990). In the *rad50S* strain, the appearance of the DSB-induced fragment was observed 6 hours after exposure to sporulation medium (Fig. 5B) and reached an endpoint of 6.8% at 24 hrs (Fig. 5C) which is similar to previously reported values (BAUDAT and NICOLAS 1997). However, DSB formation in the *pds1Δ rad50S* strain was at the limits of detection prior to the terminal timepoint with a final level of 3%. Identical results were also obtained if a different restriction enzyme was used to monitor the same DSB locus (Fig. S3A) or at a different locus entirely (*CYS3*, Fig. S3B). These results indicate that Pds1p is required for normal DSB formation.

**Pds1p is required for synaptonemal complex formation:** The synaptonemal complex (SC) is a proteinaceous structure that forms along homologous chromosomes following normal DSB initiation and processing (ROEDER 1997). Therefore, if Pds1p plays a role in DSB formation, it would be predicted that *pds1Δ* strains would exhibit aberrant or no SC formation. To address this possibility, wild type (RSY335) and *pds1Δ* (RSY1433) cells were transformed with single-copy functional GFP-ZIP1 expression
plasmid (SCHERTHAN et al. 2007). Zip1, a component of the SC (SYM et al. 1993), is commonly used to monitor SC formation. Fluorescent microscopic analysis of GFP-Zip1p in live cells revealed the expected SC filament formation in wild-type cells (Fig. 6A). However, a bright crescent-shaped signal was observed in the pds1∆ mutants. This crescent shaped signal was further characterized using indirect immunofluorescence of fixed meiotic cells (Fig. 6B). These studies revealed that GFP-Zip1p formed a crescent shaped aggregate at the nuclear periphery but independent of the nucleolus as determined by Nop1p staining (Fig. 6C). This GFP-Zip1p aggregate may be similar to the polycomplex described in spo11 mutants using chromosome spreads (HENDERSON and KEENEY 2004). These data are consistent with a role for Pds1p in normal DSB formation and subsequent recombination.

**Pds1p does not associate with the chromatin during recombination:** Previous studies in vegetative cells revealed that Pds1p regulates sister chromatid cohesion indirectly via Esp1p activity (KOSHLAND and GUACCI 2000). The studies described above indicate a new role for Pds1p in DSB formation. To address whether Pds1p functioned at the chromatin or in an indirect manner, the presence of Pds1p was examined in chromatin pellets isolated through a sucrose cushion (see Materials and methods for details). A wild-type culture (RSY335) harboring epitope-tagged alleles of PDS1 (PDS1-FLAG) and REC8 (REC8-3HA) was induced to enter meiosis with timepoints taken 5 and 7 hrs following the switch to SPM. As this experiment was performed at 30°, these timepoints were chosen as 50% of this strain background (SK1/W303) completes the meiotic divisions by nine hrs (data not shown). Consistent with its meiosis-specific expression pattern, Rec8p-3HA was not detected in whole cell extracts (W) at T=0 but readily observed by 5 hrs (Fig. 7, bottom panel). Fractionation of this extract revealed that Rec8p-3HA was found primarily in the supernatant (S) indicating that it was not bound to the chromatin in the pellet (P). Similar to proteins required for DSB formation (KEE et al. 2004) or components of the SC, (DONG and ROEDER 2000; SMITH and ROEDER 1997), Rec8p-3HA localized almost exclusively with the chromatin fraction by seven hrs. Conversely, Pds1p-FLAG was not associated with the chromatin during mitotic cell division (0 hr) or in either of the meiotic samples (Fig. 7, top panel).
These results indicate that Pds1p most likely plays an indirect role in promoting recombination.

**Rec8p cleavage is not altered in the pds1Δ mutant:** In meiotic cells, Pds1p protects the cohesin Rec8p from separase dependent destruction thus preventing the onset of anaphase I (KLEIN et al. 1999). Similar to pds1 strains, rec8 mutants are also defective for recombination (KLEIN et al. 1999). Therefore, one possible explanation of our results is that deleting PDS1 causes premature cleavage of Rec8p. In this scenario, the pds1 and rec8 null alleles should share similar phenotypes. The analysis of a SK1/W303 rec8Δ diploid during meiosis revealed a similar, but not identical, phenotype compared to the pds1Δ strain. Although a similar level of mononucleated cells was found in the rec8Δ strain compared to the pds1Δ mutant, a significant percentage of the population was able to execute one or both meiotic divisions (Fig. 2A). This finding is similar to that reported for rec8 mutants in a SK1 background (KLEIN et al. 1999). Similar to pds1Δ strains, mutating SPO11 suppressed the rec8 meiotic defect. However, a spo13 mutation significantly suppressed the rec8 phenotype compared to pds1Δ (58% to 12% binucleates). Taken together, these results indicate that the pds1 and rec8 exhibit similar, but not identical, phenotypes.

We next tested whether Rec8p cleavage was altered in meiotic cultures lacking Pds1p. The chromosomal allele of REC8 was tagged with three repeats of the HA epitope (see Material and methods). This allele was functional as determined by sporulation assays (data not shown). Rec8p-3HA cleavage was monitored in a strain background mutated for the ubiquitin ligase Ubr1p to allow accumulation of the cleaved intermediates (BUONOMO et al. 2000). This strain, and the isogenic pds1Δ ubr1Δ double mutant, were induced to enter meiosis at 23° and timepoints taken for Western blot analysis. Rec8p-3HA accumulation was observed in both strains by four hrs following the shift to SPM although the steady state levels appear lower in the pds1Δ strain (Fig. 8A). The appearance of the Rec8p cleavage products also occurred with similar timing (six hrs). Taken together, these studies indicate that Pds1p is not essential for proper Rec8p cleavage but may play a subtle role in its total accumulation (see below). To investigate this issue further, REC8 was placed on a high-copy plasmid and introduced
into the \textit{pds1}\textsuperscript{Δ} SK1 strain used to measure recombination at the \textit{arg4} locus. The rationale for this experiment was that if the small reduction in Rec8p full-length levels in the \textit{pds1}\textsuperscript{Δ} mutant was sufficient to cause a recombination phenotype, this defect should be rescued by increasing gene dosage. However, no difference in recombination was observed with the \textit{pds1}\textsuperscript{Δ} strain harboring the \textit{REC8} high-copy plasmid (Fig. 8B) even though Rec8p levels were increased above vector control levels (Fig. 8C). These findings suggest that the requirement for Pds1p during recombination is not linked to the timing of Rec8p cleavage or its overall levels.

Pds1p also ensures proper localization of Esp1 to the nucleus and spindle in mitotic cells (JENSEN \textit{et al.} 2001). This latter role may also be critical as overexpression of \textit{ESP1} is able to partially bypass the temperature sensitive growth phenotype associated with \textit{pds1-1} alleles (Ciösk \textit{et al.} 1998). To address whether \textit{ESP1} overexpression can also relieve the meiotic requirement for Pds1p, a \textit{pds1}\textsuperscript{Δ} diploid (KCY472) containing either a high-copy \textit{ESP1} plasmid (pKC7003) or a control vector was induced to enter the meiotic program. Unlike the vegetative cultures, no differences in the terminal phenotype of \textit{pds1}\textsuperscript{Δ} cells were observed between the \textit{ESP1} overexpression construct and a vector control (data not shown) even at the permissive temperature (23°). Introduction of pKC7003 was able to suppress the \textit{pds1-1} vegetative lethality at restrictive temperature (Fig. S3) indicating that our over-expression system was functioning as anticipated. Finally, we monitored Rec8p destruction in a wild type and \textit{pds1}\textsuperscript{Δ} strain over-expressing Esp1p. No change in Rec8p degradation kinetics was observed in these two strains (Fig. 8D) although in this experiment the kinetics of Rec8p cleavage was faster than experiments performed in the \textit{ubr1}\textsuperscript{Δ} background (Fig. 8A). These data indicate that Esp1p-dependent destruction of Rec8p is not altered in a \textit{pds1}\textsuperscript{Δ} mutant and are consistent with a model that Pds1p performs a role during meiosis independent of Rec8p function.

\textbf{Pds1p prevents the premature destruction of Mcd1p during meiosis:} During meiosis, it has been proposed that Mcd1p co-localizes with sites of DSB formation. These domains remain refractory for DSB initiation until Tid1p is recruited to these domains. This model suggest that the loading of Tid1p leads to the separation, rather
than alignment, of sister chromatids, thereby promoting inter homologue exchange (KATENEVA et al. 2005). As Pds1p forms a complex with Esp1 and in turn Esp1 cleaves Mcd1p during mitotic cell division, the kinetics of Mcd1p cleavage was examined in wild type and pds1Δ strains upon entry into the meiotic program. As previously described (KLEIN et al. 1999), Mcd1p levels are maintained in the wild-type culture until meiS phase at which point Rec8p is synthesized and loaded onto the chromosome (Fig. 8E). However, Mcd1p is prematurely destroyed within two hrs following shift to sporulation medium, approximately the start of meiS. These data indicate that Pds1p is required to maintain Mcd1p on the chromosomes as cells enter the meiotic program. Interestingly, this role occurs only in meiotic cells at Mcd1p levels are not affected in vegetative cells, (T = 0) in these experiments.

**DISCUSSION**

This study reports new roles for Pds1p in meiotic progression and recombination. Similar to mitotic cells, Pds1p destruction is required for the metaphase-anaphase transition at meiosis I. However, mutants lacking Pds1p arrest in prophase I although extended exposure to sporulation medium produces cells with abnormal DNA masses and spindle formation. This meiotic defect is partially suppressed by preventing DSB formation or inactivating the signaling portion of the recombination checkpoint system (Red1p and Mek1p). Mutants lacking Pds1p exhibit reduced DSB initiation resulting in lower recombination efficiency and defective SC formation. The role of Pds1p in recombination is independent of its function prohibiting Rec8p cleavage as the expression profile of this meiotic cohesin appears normal. However, Pds1p prevents Mcd1p destruction as the cells enter meiosis. Significantly, this role is meiosis specific, as the kinetics of Mcd1p cleavage is not affected in pds1 during mitotic cell division (ALEXANDRU et al. 1999). Taken together, these data indicate that Pds1p has additional functions during meiosis independent of its role as an anaphase inhibitor. In addition, these studies suggest an early role for chromosome cohesion for normal recombination and meiotic progression.
Our data indicate that Mcd1p is prematurely destroyed in a pds1 mutant background. However, this finding is unlikely to be the sole cause of all the pds1Δ-associated phenotypes as strains lacking Mcd1p/Scc1p execute both meiotic divisions (KLEIN et al. 1999). However, an important role has been reported in several species for Mcd1p and cohesion in DSB repair (SONODA et al. 2001; STROM et al. 2004; UNAL et al. 2004). Therefore, it is possible that the recombination defect observed in pds1Δ strains may be a consequence of early Mcd1p destruction. Upon entry into the meiotic program, Mcd1p not only maintains cohesion between newly replicated sister chromatids, it has also been hypothesized to serve as a guide for recruiting Tid1p onto chromosomes (KATENEVA et al. 2005). In the absence of Pds1p, premature cleavage of Mcd1p may reduce or delay Tid1p loading onto chromosomes. As a consequence, Tid1p, and potentially Dmc1p which has been shown to interact with Tid1p (SHINOHARA et al. 2000), may not be able to effectively promote homolog-directed repair. If this model is correct, then one prediction is that pds1 phenotypes would mimic those of tid1Δ strains. This is indeed the case with respect to some tid1Δ phenotypes. Both pds1 and tid1 strains arrest primarily as mononucleates that is partially suppressed by also deleting spo11 or spo13. In addition, recombination in tid1 mutants appears reduced and delayed, similar to the pds1 strain. However, many important differences exist. For example, tid1 mutants display normal SC assembly which is lacking in the pds1 mutant. Therefore, Pds1p appears to have functions independent of Tid1p in directing meiotic recombination that may not involve Mcd1p regulation.

This model is consistent with an additional recombination phenotype associated with loss of Pds1p activity. We demonstrate that in pds1Δ strains, DSB’s are decreased only 2.5 fold whereas interhomologue exchange is decreased 100 fold. These findings suggest the possibility that DSB are being repaired by sisters rather than homologs. In support of this idea is the observation that deletions of Red1p and Mek1p, two checkpoint proteins that prevent sister exchange (reviewed in (HOCHWAGEN and AMON 2006)), weakly bypass the requirement for Pds1p during meiosis. Previous studies have found that Dmc1p is required for the interhomologue bias observed for recombination (SCHWACHA and KLECKNER 1997). However, we found that Dmc1p is able to load onto chromosomes with similar efficiency (Fig. S5) suggesting that loss of
homolog bias is not due to a defect in Dmc1p function. It is possible that Dmc1p does not load properly or in a productive manner in the \textit{pds1} mutant that would not be discerned by chromatin spreads. Further study into Dmc1p activity in the \textit{pds1} mutant strain would be required to distinguish between these models.

Pds1p is required for the normal transcript accumulation of the mid-early gene (\textit{NDT80}) and two loci (\textit{SPS100} and \textit{DIT1}) that are expressed late in meiosis. Both of these results may be instructive for understanding the meiotic defect associated with loss of Pds1p activity. \textit{NDT80} transcription is under control of the recombination/DNA damage checkpoint pathways. When activated, these pathway inhibit \textit{NDT80} transcription thus preventing meiotic progression. In the \textit{pds1}\textsubscript{\textDelta} mutant, we observed a reduced, but not eliminated, \textit{NDT80} transcription. These findings may suggest that partial activation of the checkpoint pathway(s) are occurring. Although eliminating the checkpoint systems did not restore normal meiosis and sporulation to \textit{pds1} mutant strains, a partial rescue was observed (eg., \textit{mek1} or \textit{red1} mutants). These results may suggest that Pds1p has multiple roles in meiosis that are manifested in a complex phenotype in the mutant strain. The requirement of Pds1p in late meiotic gene transcription was more surprising. Two models could explain this result. Either Pds1p is directly required for the transcription of this late expression class or the defect was due to the earlier meiotic arrest. The finding that late gene transcription was restored in the \textit{pds1 spo13} double mutant suggests that the latter explanation is more likely. If late gene transcription is dependent on the execution of earlier events, then a system must exist that permits the execution of late events only after the successful completion of earlier ones. As Pds1p is involved in several early processes, the nature of this signal is not clear. However, these results do suggest that the checkpoint pathways linking meiotic progression to spore morphogenesis may be more complex than previously appreciated.

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References


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Table 2. Yeast strains used in this study.  "Asterisk denotes strains also harboring cyh2-z ho::LYS2 leu2::hisG lys2 trp1::hisG ura3 alleles.  "All alleles listed are homozygous unless indicated otherwise.  All strains in this table were derived in this study except RSY335 (COOPER et al. 1997).

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"Table 2. Yeast strains used in this study.  "Asterisk denotes strains also harboring cyh2-z ho::LYS2 leu2::hisG lys2 trp1::hisG ura3 alleles.  "All alleles listed are homozygous unless indicated otherwise.  All strains in this table were derived in this study except RSY335 (COOPER et al. 1997)."
Figure Legends

Figure 1. Pds1p regulates meiotic progression. (A) Pds1p destruction is required for meiotic progression. Wild-type culture (RSY335) harboring either wild type or non-degradable Pds1p mutant (PDS1^Δdb) plasmids (pMSC13 and pMSC14, respectively) under the control of the meiosis-specific AMA1 promoter, and pds1-1 cells (KCY348) were induced to enter meiosis at 23°C. After 24 hrs, nuclear divisions and spore formation were analyzed by DAPI staining (bottom panels) or Nomarski imaging (upper panels), respectively. Percentage of the culture containing one, two or three + four, or fragmented nuclei are indicated. Fragmented nuclei were scored as described in Materials and methods. (B) Pds1p is not required for pre-meiotic S phase. Wild type (RSY335) and pds1Δ (RSY787) diploid strains were induced to enter meiosis at 23°C and samples taken at the times indicated (hrs) then analyzed by FACS. 2C (unreplicated) and 4C (replicated) peaks are indicated. (C and D) Terminal meiotic arrest phenotypes of pds1Δ mutants. Wild type (RSY335, panel D) and pds1Δ (RSY787, panel C) diploid strains were induced to enter meiosis at 23°C and samples were taken at the times indicated. Nuclear and spindle morphologies were determined by DAPI staining (top panels) and indirect immunofluorescence of tubulin (bottom panels). Cells were scored as described in Materials and methods. For all morphology quantitations presented, the standard deviations were ≤ 6% for all values. 1000X magnification. (D) All strains used (except KCY348 which is derived from the A364A background) are isogenic to RSY335, our standard SK1/303 wild type.

Figure 2. Recombination initiation is required for the pds1Δ-induced meiotic arrest. (A) The meiotic progression and spore wall assembly of wild type (RSY335), spo11Δ (KCY198), spo13Δ (RSY767), pds1Δ (RSY787) and rec8Δ (KCY385) strains were assayed after 24hrs at 23°C by DAPI analysis (bottom panels) and Nomarski imaging (upper panels), respectively. Population percentages of mono-, bi-, tri- and tetraneucleated cells as well as irregular nuclei (scored as fragmented nuclei) in each culture are indicated. (B) Same as (A) except that spo11Δ pds1Δ (KCY207), spo13Δ
*pds1Δ* (RSY795), *spo11Δ rec8Δ* (KCY398) and *spo13Δ rec8Δ* (KCY399) strains were assayed for nuclear division and spore formation. (C) Same as (A) except that *rad9Δ rad17Δ* (KCY450), *rad9Δ rad17Δ pds1Δ* (KCY453) *mad2Δ* (RSY740) and *mad2Δ pds1Δ* (RSY864) strains were assayed for nuclear division and spore formation. (D) Same as (A) except that *red1Δ* (RSY1355), *red1Δ pds1Δ* (RSY1358), *mek1Δ* (RSY1356), and *mek1Δ pds1Δ* (RSY1359) strains were examined. (E) Same as (A) except that *pch2Δ* (RSY1536), *pch2Δ pds1Δ* (RSY1537) strains were examined. For all morphology quantitations presented, the standard deviations were ≤9% for all values. 1000X magnification. All the strains used are isogenic to RSY335, the SK1/W303 parent. (F) Diagram depicting the meiotic checkpoints tested with the bypass function of the genes assayed indicated.

**Figure 3.** The *pds1Δ* prophase arrest does not inhibit middle meiotic gene expression. (A) Wild type (RSY335) and *pds1Δ* (RSY787) SK1/W303 cells were induced to enter meiosis at 23°C and samples were taken at the times indicated (hrs). Total RNA was prepared and the transcript expression profiles of *IME2, NDT80, SPS2, SPS4* and *SPS100* were analyzed by Northern analysis. The approximate times of meiosis I (MI) and meiosis II (MII) as determined by DAPI analysis are indicated in the wild-type strain. *ENO1* serves as a loading control. (B) Northern analysis of *IME2, SPS2* and *DIT1* mRNA expression in *pds1Δ* (RSY787) and *pds1Δ spo13Δ* (RSY795) SK1/W303 strains. Bottom panel; Ethidium bromide stained rRNA is shown as a loading control.

**Figure 4.** Pds1p is required for meiotic recombination. (A) Three individual cultures of wild type (KCY257) and *pds1Δ* (KCY274) SK1 strains containing heteroalleles at the *arg4* locus were induced to enter meiosis at 23°C. Samples were taken at the timepoints indicated following meiotic induction, serial diluted and plated onto SD medium lacking arginine or complete SD medium. The resulting colonies were counted and the percentage of *Arg*⁺ prototrophs recovered was calculated (see Table S1). Plating dilutions for all experiments were adjusted based on known recombination frequencies in wild-type strains at these loci. (B) Appearance of bi and tetraneucleated cells in WT cells in the SK1 background at 23°C.
Figure 5. Pds1p is required for normal double strand break formation. (A) Physical detection of DSB at the YCR048W recombination hotspot in wild type (KCY428) and pds1Δ (KCY430) SK1 derivatives. The strains were induced to enter meiosis at 23°C and samples taken at the timepoints indicated (hrs). DNA extracts were digested with AseI and probed with sequences specific to YCR048W as described (BORDE et al. 2004). Arrows indicate predicted recombination-restriction enzyme double strand break fragments. The asterisk denotes a non-specific cross-hybridizing band. (B) Same as (A) except that rad50S (KCY427) and rad50S pds1Δ (KCY429) SK1 strains were examined. (C) Graphic representation of quantified DSB percentages with respect to parental signal in (B).

Figure 6. Pds1p is required for normal SC formation. (A) Live cell overlay images (GFP and Nomaski) of wild type (RSY335) and pds1Δ (RSY1433) strains expressing GFP-Zip1p 6 hours after transfer to SPM. The finger like structure present in the wild-type cells represent individual chromosomes with formed SC’s. (B) Fixed pds1Δ cells (RSY1433) harboring the GFP-ZIP1 expression plasmid 7.5 hrs. transfer to SPM stained with DAPI (blue) examined under the fluorescent microscope. An expanded region indicated by the box is provided on the right (600X magnification). (C) The cells described in (B) were subjected to indirect immunofluorescence staining for the nucleolar protein Nop1p. The images shown represent de-convoluted Z-stacks (0.6 µM slices) of Nomarski, GFP-Zip1p (green), Nop1p (red) and nuclei (blue). 1000X magnification.

Figure 7. Pds1p does not associate with chromatin during recombination. REC8-HA tagged SK1/W303 strain (KCY392) harboring the Pds1p-FLAG expression plasmid (pKC7000) was grown to mid-log phase at 30°C in synthetic acetate medium then transferred to SPM and incubated at the same temperature. Samples were taken at the timepoints indicated (hrs) and whole cell lysates (W) fractionated through a sucrose cushion to generate a chromatin bound pellet (P) and unbound supernatant (S) were analyzed by Western blotting and probed for the presence of Pds1p-FLAG (arrows, top
Figure 8. Mcd1p is cleaved prematurely in \textit{pds1}\(\Delta\) strains. (A) \textit{ubr1}\(\Delta\) \textit{REC8-3HA} (KCY447) and \textit{ubr1}\(\Delta\) \textit{pds1}\(\Delta\) \textit{REC8-3HA} (KCY448) SK1/W303 strains were induced to enter meiosis at 23° and samples taken for Western taken at the timepoints indicated (hrs). Full length Rec8p-3HA and cleavage products are indicated by arrows. Asterisks indicate non-specific cross-reactive bands observed with the HA antibody. The blot was striped and re-probed for the presence of Tub1p for a loading control. (B) Return to growth recombination experiments as described in Fig. 3 were performed on SK1/W303 \textit{pds1}\(\Delta\) culture (KCY274) at 23° harboring either Rec8p-HA expressed from a high-copy plasmid (pKC7001) or vector control (YEplac181). (C) Increasing \textit{REC8} gene dosage increases Rec8p production. Western blot analysis of Rec8p in a \textit{pds1}\(\Delta\) strain harboring either a single-copy (REC8 int) or multi-copy (REC8 OE) HA epitope tagged \textit{REC8} allele following transfer to SPM medium as indicated (hrs). Tub1p serves as a loading control. (D) Increased gene dosage of \textit{ESP1} does not alter Rec8p degradation. Wild-type (RSY392) and \textit{pds1}\(\Delta\) (RSY448) SK1/W303 diploid strains containing the high-copy \textit{ESP1} plasmid (pKC7003) were induced to enter meiosis at 23°C and samples were taken at the times indicated (hrs). Rec8p-3HA levels were monitored by Western blot analysis of total protein extracts. Tub1p serves as loading control. (E) Mcd1p is prematurely destroyed in \textit{pds1}\(\Delta\) cells. Wild type (RSY392) and \textit{pds1}\(\Delta\) (RSY448) SK1/W303 diploid strains were induced to enter meiosis at 23° and samples taken for Western taken at the timepoints indicated (hrs). Full length Mcd1p was visualized by Western blot probing with antibodies directed against Mcd1p. The blot was striped and re-probed for Tub1p for a loading control.

Supplemental Figure 1. The meiotic progression and spore wall assembly of wild type, \textit{ndt80}\(\Delta\), \textit{pds1}\(\Delta\) and \textit{ndt80}\(\Delta\) \textit{pds1}\(\Delta\) cells (RSY335, RSY1456, RSY1433 and RSY1453, respectively) strains were assayed after 24hrs at 23°C by DAPI staining and Normarski imaging.
Supplemental Figure 2. Appearance of bi or tetranucleated cells in wild type, red1Δ and red1Δ pds1Δ SK1 cultures. Samples were analyzed by DAPI staining and fluorescent microscopy for the times indicated following transfer to SPM.

Supplemental Figure 3. Pds1p is required for DSB formation at the YCR048W locus. DSB formation at YCR048W (arrow heads) was monitored using a different restriction enzyme (BglII) as described (Borde et al. 2004) in rad50S (RSY427) and rad50S pds1Δ (RSY429) SK1 strains. Meiotic timecourses were conducted at 23°. (B) DSB formation (arrow heads) was examined at the CYS3 locus (VeDel and Nicolas 1999) in rad50S and rad50S pds1Δ strains as described in panel A. Quantitation of DSB accumulation revealed a reduction in the pds1Δ mutant (0.5%) versus the PDS1 culture (4%). Asterisks indicate background bands.

Supplemental Figure 4. Overexpression of Esp1 partially relieves the requirement for Pds1p during mitotic division. Mid-log phase pds1-1 cultures (AY673-6-4) containing either vector (pRS202), 2µ ESP1 (p7003) or 2µ PDS1 (pT1) were serially diluted (1:10) and spotted onto medium selecting for plasmid maintenance at 23°C (permissive) and various restrictive temperatures as indicated. The plates were incubated for 72 hrs prior to image capture.

Supplemental Figure 5. Wild type (RSY335) and pds1Δ (RSY787) W303/SK1 diploid strains were induced to enter meiosis at 23° and timepoints taken at 6 and 12 hrs. Chromatin spreads were prepared as described in Materials and methods and the presence of Dmc1p determined by indirect immunofluorescence. The number of Dmc1p cells in these samples were similar although the signal was weaker in the mutant culture. Nuclei are indicated by DAPI staining.
### FIGURE 1

#### A

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#### B

- **Left Column**: Wild type
- **Right Column**: pds1Δ

#### C

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hrs. in SPM

iME2

NDT80

SPS4

SPS2

SPS100

ENO1

B

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hrs. in SPM

IME2

SPS2

DIT1

rRNA
FIGURE 5

A

Hrs. in SPM

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- parental strand
- DSB (YCR048W)

PDS1

pds1Δ

B

Hrs. in SPM

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- parental strand
- DSB (YCR048W)

rad50S

rad50S pds1Δ

C

% DSBs

Time (hrs)

rad50S

rad50S pds1Δ

0 5 10 15 20 25

0 2 4 6 8
**FIGURE 6**

A. GFP-Zip1p, six hrs in SPM

WT  |  pds1Δ (view1)  |  pds1Δ (view2)

B. GFP-Zip1p, 7.5 hrs in SPM

pds1Δ

C. T=7.5

WT  |  pds1Δ
FIGURE 7

The figure shows a protein blot analysis with different proteins and their molecular weights indicated on the left and right side. The blot is labeled with Pds1p-FLAG and Rec8p-3HA. The samples are labeled with W, S, and P, and the time points are 0 hr, 5 hr, and 7 hr in SPM.