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Dual Role of the Cdc7-regulatory Protein Dbf4 during Yeast Meiosis*[§]

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The Dbf4-dependent Cdc7 kinase (DDK) is essential for chromosome duplication in all eukaryotes, but was proposed to be dispensable for yeast pre-meiotic DNA replication. This discrepancy led us to investigate the role of the unstable Cdc7-regulatory protein Dbf4 in meiosis. We show that, when Dbf4 is depleted at the time of meiotic induction, cells enter the meiotic program but do not replicate their chromosomes. Surprisingly when Dbf4 is depleted after the initiation of DNA synthesis, S phase goes to completion, but most cells arrest before anaphase I. Deletion of the cohesin Rec8 suppresses this phenotype, suggesting a distinct role of DDK for meiotic chromosome segregation. As after Cdc5 depletion, a fraction of cells undergo a single equational division suggesting a failure to mono-orient sister kinetochores. Our results demonstrate that Dbf4 is essential for DNA replication during meiosis like in vegetative cells and provide evidence for an additional role in setting up the reductional division of meiosis I.

The initiation of eukaryotic DNA replication requires the activation of two Ser/Thr protein kinases, an S phase cyclin-dependent kinase (CDK)³ and a Dbf4-dependent kinase (DDK), named Cdc7 in budding yeast (1). The Cdc7-Dbf4 kinase, which is conserved from yeast to human (2), is responsible for a late step in replication origin firing, locally and throughout S phase (3, 4). Cdc7-Dbf4 is also a target and transducer of the ATR-dependent S phase checkpoint in yeast and vertebrates (5, 6). Besides its role in origin firing, Cdc7 has also been implicated in induced mutagenesis and meiotic recombination (2). Dbf4 is the Cdc7 kinase regulatory subunit, an unstable protein that accumulates in late G₁, binds to replication origins, and guides Cdc7 kinase activity toward specific subunits of the pre-initiation complex (7–10).

Despite its well characterized function for DNA replication in the mitotic cell cycle, little is known on the role of DDK during meiosis. Original experiments using a temperature-sensitive *cdc7* allele concluded that it was essential neither for pre-meiotic DNA replication nor for ARS1 origin firing (11, 12), leading to the idea that the mechanisms controlling DNA replication could be different in mitosis and meiosis. Hence several conserved initiation proteins (SpCdc18/Cdc6, Mcm2,

and Mcm4) were found dispensable for pre-meiotic S phase in fission yeast (13), but more recent results using tighter mutants did not confirm this conclusion (14–16). Furthermore, the same replication origins are generally used in mitosis and meiosis and acted upon by the same CDK complex in both cell types (17–21). In fact, Cdc7's dispensability for meiotic replication appeared as an exception and its precise function deserved further examination.

In this paper we reinvestigate the role of Cdc7-Dbf4 during meiosis. Contrary to initial reports, we show that Dbf4 is essential for pre-meiotic DNA replication and that no DNA synthesis occurs in its absence, as observed in mitosis. Furthermore we provide evidence that in addition to its role in origin firing, Dbf4 is also required after S phase to complete the first meiotic division, thus uncovering a novel and unexpected function of DDK for chromosome segregation during meiosis.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions—The strains used in this work are derived from SK1 background and are described in Table 1. The *TET-DBF4* integrative plasmid (ESD560 (10)) was cut at the unique EcoRV site to be integrated at *URA3* locus of strain E1414 containing one deleted copy of *DBF4*.

Cells were grown in YEP medium (1% yeast extract, 2% bacto-peptone) supplemented with 50 mg of adenine per liter and 2% dextrose (YEPD). Sporulation was performed as described (22). YPA was 1% yeast extract, 2% bacto-peptone, and 1% potassium acetate. Sporulation medium (SPM) contained 1% potassium acetate with the required amino acid. For synchronous liquid sporulation cells were grown at 30 °C in YEPD to late exponential stage ($0.8\text{--}1.2 \times 10^6$ cells/ml) and transferred overnight (no more than 12 h) at 30 °C in pre-sporulation medium (YPA) to obtain a concentration of $\sim 2 \times 10^7$ cells/ml. Then cells were washed twice in water and incubated in SPM with vigorous shaking at 30 °C. For depletion experiments of Fig. 2, a low concentration of doxycycline (1 $\mu\text{g/ml}$) was added into pre-sporulation medium (YPA) to reduce the level of the expression of the *Tet-DBF4* transcript to the wild-type level by the time cells were shifted to sporulation medium. The number of asci (tetrads and dyads) was measured by light microscopy 24 or 48 h after transfer in SPM; an average of 200 cells were counted for each experimental condition.

Centrifugal Elutriation—Cells were grown as described above into one liter of YPA to a concentration of $\sim 2 \times 10^7$ cells/ml. Cells were then resuspended in 50 ml of YPA, sonicated, and pumped into a 40-ml elutriation chamber at about 50 ml/min with a rotor speed of 3000 rpm. Cells were kept at 25 °C during the centrifugation and eluted at constant flow decreasing gradually rotor speed. Elutriated G₁ cells were collected, washed twice in water, and dissolved in SPM to a concentration of $\sim 3 \times 10^7$ cells/ml and incubated at 30 °C with vigorous shaking.

Analysis of Gene and Protein Expression—Total RNA isolation and Northern analysis were performed as described (23) with 20 μg of RNA per lane. RNAs were stained with SybrGold and visualized using an

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[§] The on-line version of this article (available at <http://www.jbc.org>) contains supplemental Figs. S1 and S2.

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³ The abbreviations used are: CDK, cyclin-dependent kinase; DDK, Dbf4-dependent kinase; SPM, sporulation medium; DAPI, 4',6-diamidino-2-phenylindole; SPB, spindle pole body; PFGE, pulsed-field gel electrophoresis; FACS, fluorescence-activated cell sorter.

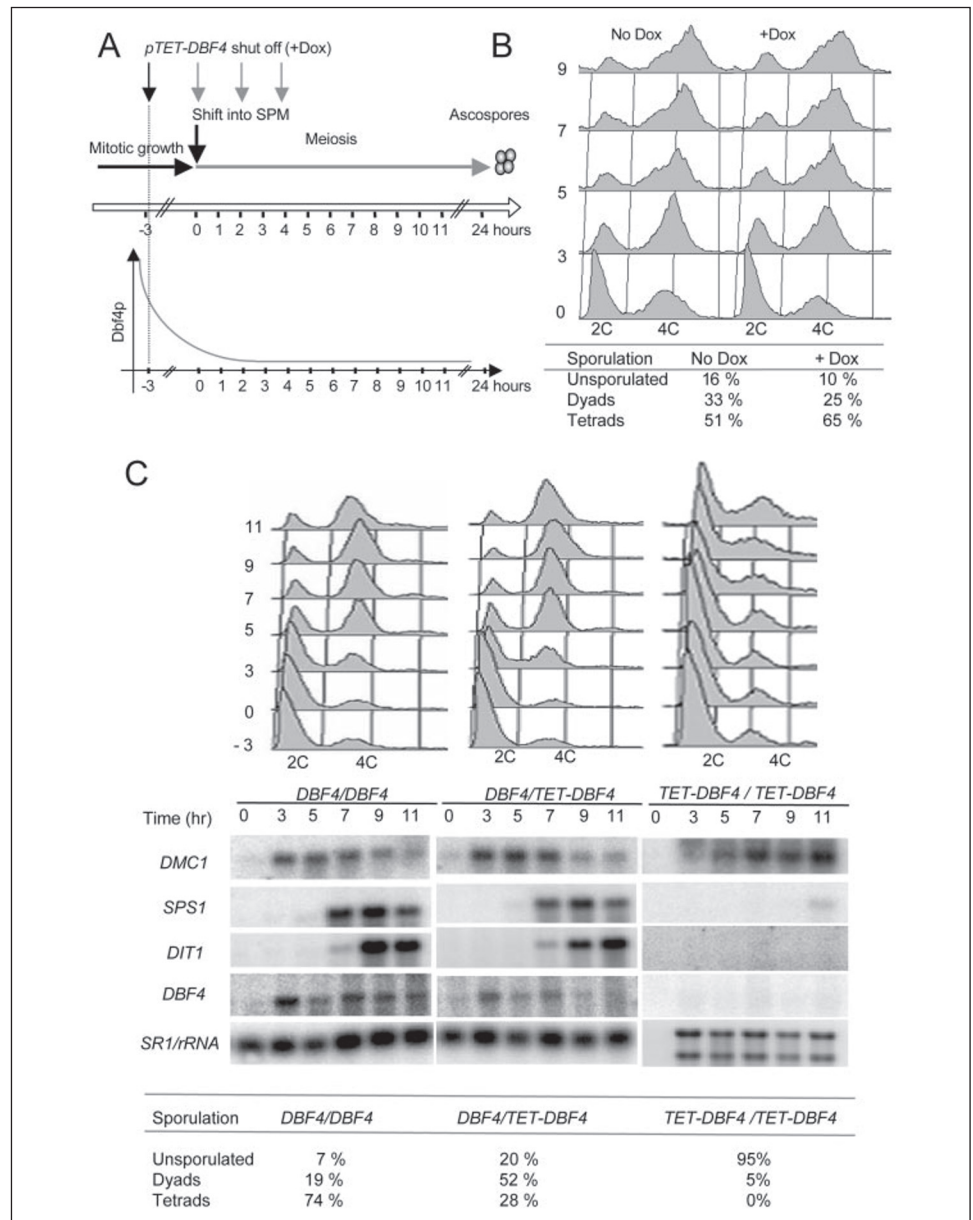


FIGURE 1. *DBF4* is required for pre-meiotic DNA replication. *A*, experimental approach: cells were grown vegetatively and doxycycline was added at a final concentration of 20 μ g/ml, 3 h before shifting to SPM or during meiotic progression (delayed *Dbf4* depletion) as indicated by arrows. *Dbf4* depletion is effective 3 h after doxycycline addition. *B*, doxycycline does not alter replication kinetics and sporulation efficiency in the wild-type strain E1180 grown in the presence of 20 μ g/ml of doxycycline (+Dox). *C*, meiosis time course of *DBF4/DBF4* (E1180), *DBF4/pTET-DBF4* (E1415) *pTET-DBF4/pTET-DBF4* (E1418) strains in the presence of doxycycline (*DBF4_{off}*). Sporulation efficiency was measured after 24 h. Northern blot membranes were sequentially hybridized with probes recognizing specific meiotic early (*DMC1*), middle (*SPS1*), and late (*DIT1*) transcripts to monitor meiotic progression. *SR1* transcript and rRNA densitometry were used as loading control.

image plate scanner (Typhoon, Amersham Biosciences). DNA probes were prepared by random priming of open reading frame PCR products, amplified from genomic DNA, and labeled according to the NEBlot protocol (New England Biolabs). Protein extracts were prepared from trichloroacetic acid-fixed cells, and analyzed by Western blot as described previously (10).

Fluorescence Microscopy—Cells were fixed 15 min at 25 °C in 4% paraformaldehyde added to the medium. Cells were washed twice with potassium phosphate buffer (KPO_4) and resuspended in 100 μ l of phosphate-buffered saline, pH 7.5, containing 4',6-diamidino-2-phenylindol (DAPI) and incubated for at least 30 min. Cells were subsequently washed in phosphate-buffered saline for microscopy. Epifluorescence microscopy was performed with a 63 \times objective on a Leica DMRA microscope equipped with a CoolSNAP HQ CCD camera (Roper Scientific). MetaMorph Imaging System v.4.5 (Universal Imaging Corp.) was used for image analysis.

Pulsed-field Gel Electrophoresis and DNA Quantification—Yeast cells were embedded in low melting agarose plugs (5×10^7 cells/plug),

and genomic DNA was extracted as described (24). Yeast chromosomes were separated by PFGE (Gene Navigator, AP Biotec). The gel was stained with SybrGold (1:10,000, Molecular Probes) and scanned with a FluorImager (Typhoon) and signals analyzed with ImageQuant (Amersham Biosciences). The amount of replicated DNA was estimated measuring the re-emergence from the gel wells of fully replicated chromosomes from nine representative chromosomal bands, and the average value was plotted on a graph considering the diploid DNA content as 100%.

RESULTS

***Dbf4* Is Required for Pre-meiotic DNA Replication**—We inactivated *Cdc7* during meiosis by depleting its unstable regulatory subunit, *Dbf4*, by shutting off its transcription from a repressible tetracycline promoter (*pTET-off*) (10). By adding doxycycline to the medium at different times, it is possible to inactivate *Cdc7* at different stages of meiotic progression (Fig. 1A). Because *Dbf4* degradation is gradual after transcriptional shut-off, doxycycline was added 3 h before shifting the cells to sporula-

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tion medium, to achieve full Dbf4 depletion at the beginning of meiosis. Any potential detrimental effect of doxycycline was ruled out by showing that a wild-type strain progresses through meiosis and sporulates

TABLE 1

Strain ^a	Relevant genotype
E1180	MATa/MAT α
E876	MATa/MAT α , <i>trp1:hisG</i> /"', <i>cdc7-1</i> /"'
E1414	MATa/MAT α , <i>dbf4::LEU2/DBF4</i>
E1415	MATa/MAT α , <i>dbf4::LEU2/DBF4</i> , URA3::TetDBF4 ^{myc6} /ura3
E1418	MATa/MAT α , <i>dbf4::LEU2</i> /"', URA3::TetDBF4 ^{myc6} /"'
E2377	MATa/MAT α , <i>dbf4::LEU2</i> /"', URA3::TetDBF4 ^{myc6} /"', <i>spc42Δ1::LEU2/SPC42</i> , TRP1::SPC42-GFP/ <i>trp1-1</i> , TUB1/TUB1-CFP::TRP1
E2382	MATa/MAT α , <i>dbf4::LEU2</i> /"', URA3::TetDBF4 ^{myc6} /"', <i>rad17::hisG:URA3</i> /"'
E2385	MATa/MAT α , <i>dbf4::LEU2</i> /"', URA3::TetDBF4 ^{myc6} /"', <i>rad24::LEU2</i> /"'
E2388	MATa/MAT α , <i>dbf4::LEU2</i> /"', URA3::TetDBF4 ^{myc6} /"', <i>mec1::TRP1</i> /"', <i>sml1::HIS3</i> /"'
E2391	MATa/MAT α , <i>dbf4::LEU2</i> /"', URA3::TetDBF4 ^{myc6} /"', <i>spo11::hisG-URA3-hisG</i> /"'
E2346	MATa/MAT α , <i>dbf4::LEU2</i> /"', URA3::TetDBF4 ^{myc6} /"', <i>mad2::kanMX</i> /"'
E2472	MATa/MAT α , Δ <i>ndt80::HIS3</i> /"', <i>spc42Δ1::LEU2/SPC42</i> , <i>trp1:hisG/TRP1::SPC42-GFP</i>
E2663	MATa/MAT α , <i>dbf4::LEU2</i> /"', URA3::TetDBF4 ^{myc6} /"', <i>spo11::hisG-URA3-hisG</i> /"', <i>rec8::kanMX4</i> /"'

^a All strains used are in the SK1 background and are homozygous for *ho:LYS2 leu2:hisG his4 ura3*.

normally whether in the presence or absence of the drug (Fig. 1B). We first determined the effect of shutting off one *DBF4* allele in a heterozygous *DBF4/pTET-DBF4* strain (Fig. 1C, middle). Northern blot analysis confirmed that *DBF4* expression was reduced compared with wild type. Despite this, the kinetics of pre-meiotic DNA replication and the induction of meiosis-specific genes were normal indicating that one copy of *DBF4* is sufficient for pre-meiotic DNA replication. However, the percentage of mature tetrads was decreased from 74% in the control strain to only 28%, suggesting that decreased *DBF4* dosage has a more pronounced effect on sporulation than on S phase.

When both *DBF4* alleles were shut off in a strain homozygous for the *pTET-DBF4* construct, *DBF4* mRNAs were no longer detectable, and importantly, no DNA synthesis was detected in these cells. This was not due to a failure to enter the meiotic program as early *DMC1* transcripts accumulated at normal levels in these cells (Fig. 1C, right, and data not shown). However, middle and late transcripts did not build up and only 5% of asci were formed, suggesting a block in meiotic progression. These data demonstrate that *DBF4* is required for pre-meiotic DNA replication.

Although unlikely, it is conceivable that Dbf4 might function in pre-meiotic S phase independently of Cdc7. To verify that Cdc7 itself is necessary for this process, we sought for temperatures at which DNA replication would be affected in a homozygous *cdc7-1^{ts}* strain but not in wild type. Hence we monitored DNA synthesis and sporulation efficiency at 25, 30, and 34 °C, using the SK1 genetic background that is less heat-sensitive for meiosis. At 30 °C, the *cdc7-1* strain progressed more slowly through S phase compared with wild type, while at 34 °C pre-meiotic DNA replication was affected also in the wild-type strain (Fig. 2). This suggests that the *cdc7-1* strain is defective for DNA replication

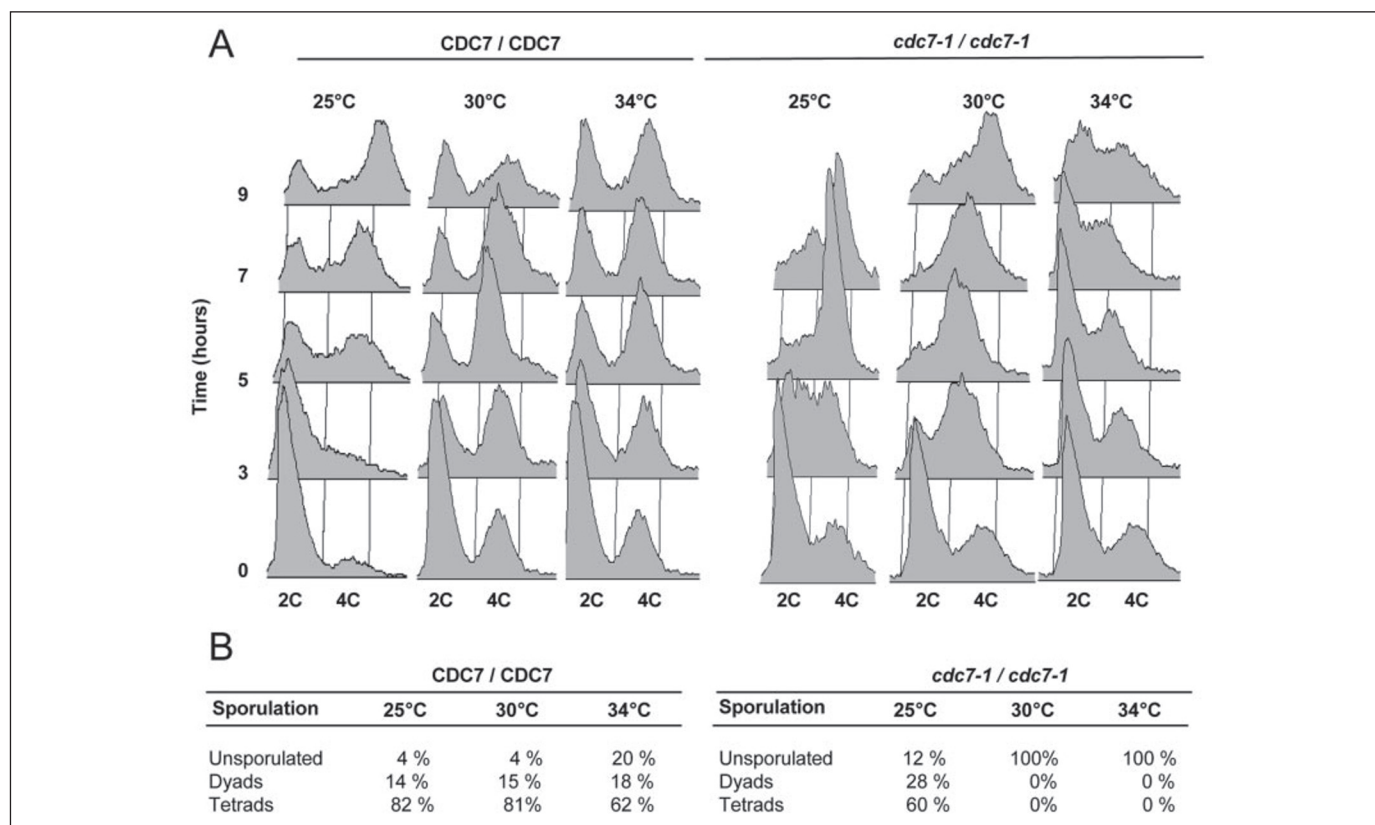


FIGURE 2. *cdc7-1* mutants have a defect in pre-meiotic DNA replication and sporulation. Wild-type (E51180) and *cdc7-1/cdc7-1* (E5876) diploid strains were grown at 25 °C, divided into three cultures, and shifted at 25, 30, and 34 °C, respectively, 1 h after sporulation induction. Replication profile was analyzed by FACS analysis and sporulation efficiency was measured 24 h later.

but retains some activity at 30 °C. Again, sporulation was more affected than DNA replication, being completely defective at 30 °C in the *cdc7-1^{ts}* strain. Thus both *DBF4* and *CDC7* seem necessary for DNA replication in meiosis, as during the vegetative cell cycle.

Dbf4 Is Required for Completion of the First Meiotic Division—*CDC7* was originally reported to be dispensable for pre-meiotic DNA replication but required for synaptonemal complex formation and commitment to recombination, possibly indicative of a role after S phase (11, 25). To see whether such a function could be detected for *Dbf4*, we took advantage of the *pTET-DBF4* strain to deplete *DBF4* at different times along meiotic progression, which we describe hereafter as delayed *Dbf4* depletion (Fig. 1A). Because the kinetics of meiosis depend heavily on the history and environment of the cell, all following experiments started with the same preculture, which was split only at the time of meiotic induction. Hence the consequences of shutting off *DBF4* expression by adding doxycycline at time T_0 , T_{2h} , and T_{4h} after shift to sporulation medium were compared with a culture expressing *DBF4* constitutively (*No Dox*). Ectopic *DBF4* expression did not significantly alter pre-meiotic DNA replication and sporulation by 24 h was quite efficient, giving rise to 94% mature asci (37% dyads + 57% tetrads). When doxycycline was added at T_0 or later, *Dbf4* levels decreased but remained high enough to sustain efficient DNA replication in all cultures (Fig. 3, A and C). Sporulation, however, was seriously affected when doxycycline was added at T_0 but not at later times. *Dbf4* became undetectable by 3 h, DNA replication was completed by 4 h, but 63% of the cells nevertheless failed to form mature spores. Fluorescence microscopy showed that

most cells contained a single nuclear mass, indicative of an arrest before the first meiotic division (Fig. 3B). When doxycycline was added at T_{2h} , *Dbf4* levels decreased by 4 h, but this did not affect meiotic progression and sporulation. These results suggest that a critical level of *Dbf4* is still required after pre-meiotic DNA replication to complete meiosis I but might not be required after this stage.

Delayed Dbf4 Depletion Arrests Cells after Pachytene—To better characterize the arrest induced by delayed *Dbf4* depletion, we used a strain that in addition to *pTET-DBF4* expresses *SPC42-GFP* and *TUB1-CFP* enabling us to follow SPB separation and spindle morphology throughout meiosis. Doxycycline was added at T_0 , which allowed S phase to take place, albeit slightly slower than without depletion (Fig. 4A). As above, most cells arrested before meiosis I, and after 24 h in sporulation medium, only 18% of mature asci were scored (*DBF4_{off}*) compared with 88% when *Dbf4* was expressed (*DBF4_{on}*). The remaining cells could be subdivided in five classes depending on the presence of one, two, or four nuclear masses and on the extent of SPB separation (Fig. 4B). In the control culture (*DBF4_{on}*), 85% of the cells became tetranucleated (class V) indicating completion of the two meiotic divisions in most cells. Conversely 60% of the cells (classes II and III) failed to progress beyond anaphase I after *Dbf4* depletion (*DBF4_{off}*). Class II (24%) showed duplicated but coalescent *Spc42-GFP* spots, whereas class III (36%) exhibited separated SPBs with a short spindle characteristic of metaphase I. 16% of the cells (class I) showed a single nuclear mass with a single SPB, probably representing cells that did not replicate. Finally, only 24% of the cells reached the binucleated or tetranucleated stage

FIGURE 3. *Dbf4* is required for meiosis I. A, FACS profiles of *pTET-DBF4* diploid strain (E1418) grown at 30 °C in YPA and shifted to sporulation medium without (*No Dox*) or with 20 μ g/ml of doxycycline added at 0, 2, or 4 h after the shift. B, the proportion of cells having either one (●), two (■), or four (▲) masses of DNA was estimated by microscopic examination of propidium iodide stained cells. C, Western blot showing *Dbf4* depletion after doxycycline addition. *Pab1* was used as loading control. D, the sporulation efficiency of the different cultures after 24 h of incubation.

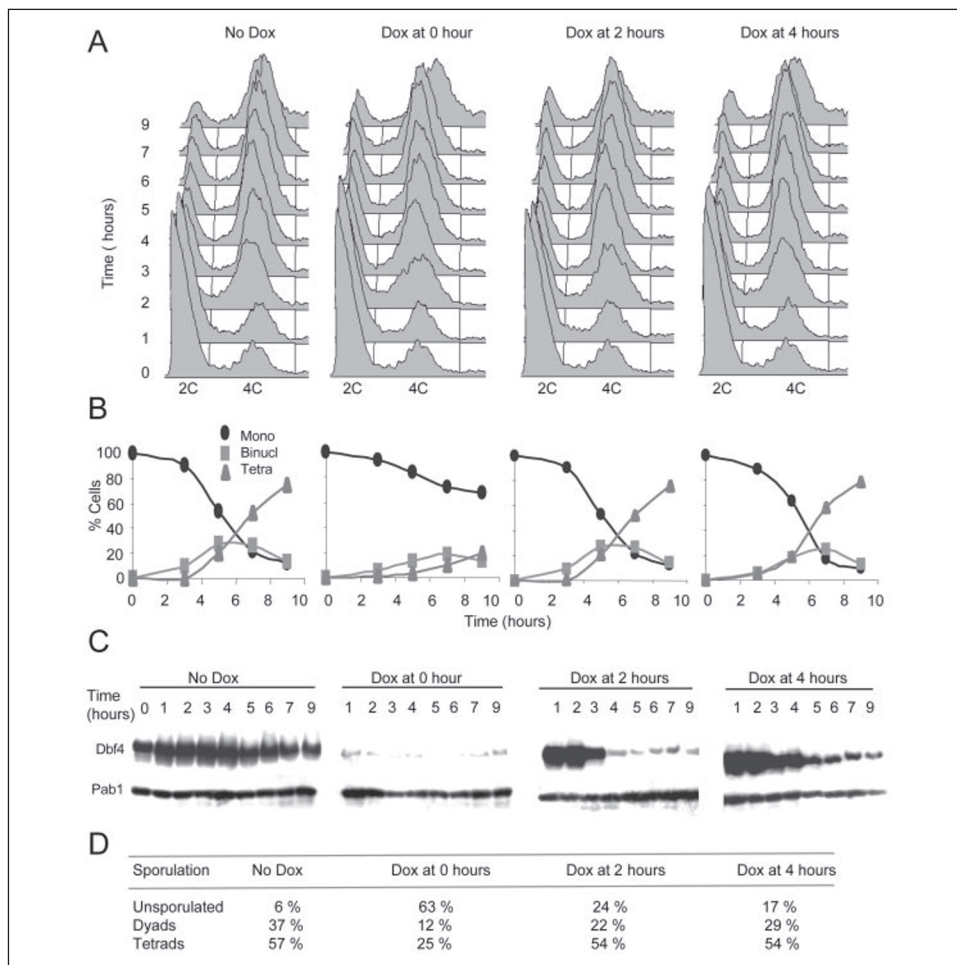
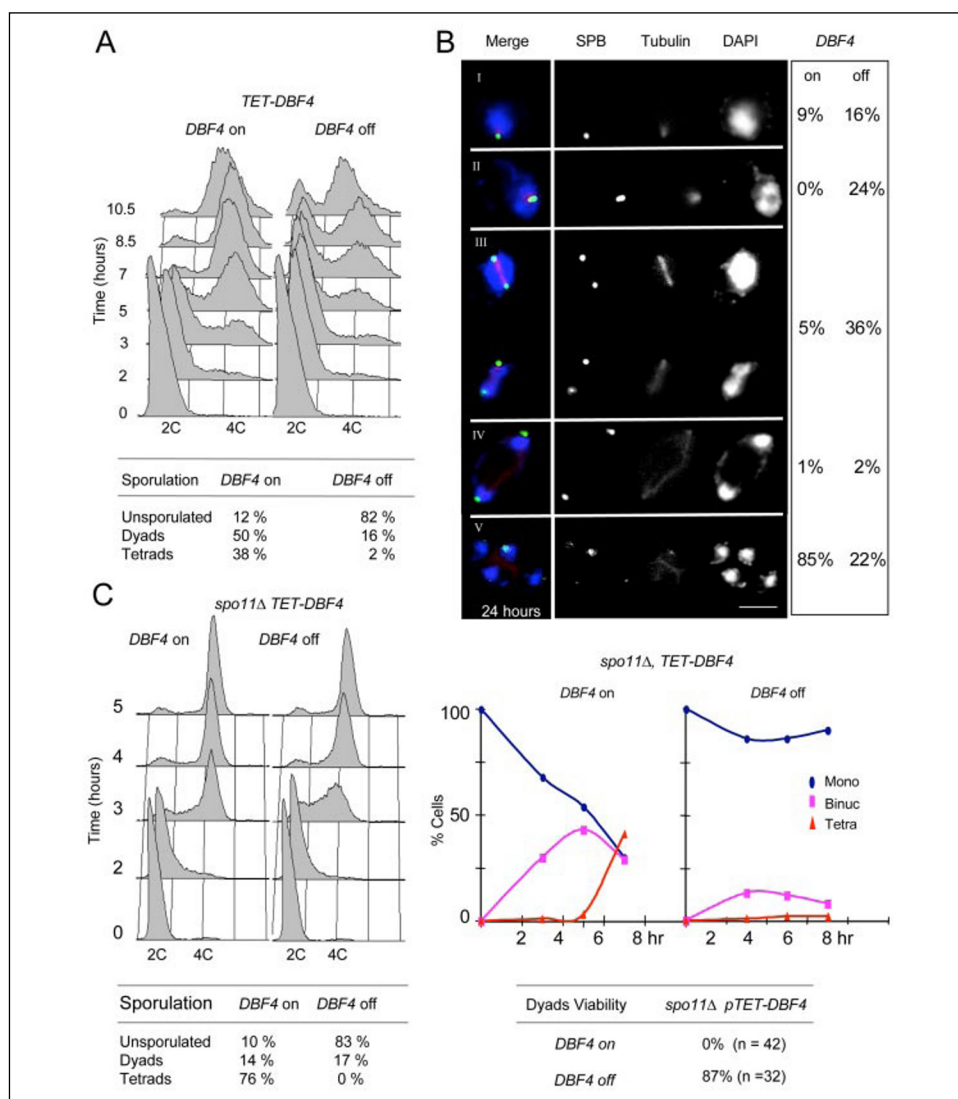


FIGURE 4. *Dbf4* depletion arrests cells after pachytene independently of recombination. *pTET-DBF4*, *SPC42-GFP*, *TUB1-CFP* diploid strain (E2377) was synchronized by centrifugal elutriation and put in SPM without (*DBF4_{on}*) or with 20 μ g/ml doxycycline (*DBF4_{off}*). **A**, FACS analysis and sporulation efficiency. **B**, cytological classification of arrest phenotypes after 24 h in SPM, without (*DBF4_{on}*) or with 20 μ g/ml doxycycline (*DBF4_{off}*). Cells were scored according to SPBs, spindle and nuclear morphologies as: mononucleated cells with unduplicated SPB (I), mononucleated cells with duplicated, but coalesced, SPBs (II), mononucleated cells with short spindles and two separated SPBs (III), binucleated cells with elongated spindle (IV), and tetranucleated cells (V). *Bar* = 2 μ m. **C**, the proportion of cells having either one (●), two (■), or four (▲) masses of DNA was estimated by microscopic examination of DAPI-stained cells. Spore viability was measured by dissecting isolated dyads on YPD plates and scoring colony formation after 1 week. The number of dissected dyads is indicated in parentheses.



(classes IV and V) presumably representing cells in which *Dbf4* depletion occurred too late. Thus the majority of cells (60%) depleted of *Dbf4* after DNA synthesis arrest with separated SPBs (class II and III). This corresponds to a post-pachytene stage as *ndt80Δ* mutants, which arrest at pachytene with duplicated but side-by-side spindle pole bodies (26), show only a single *Spc42-GFP* spot (data not shown).

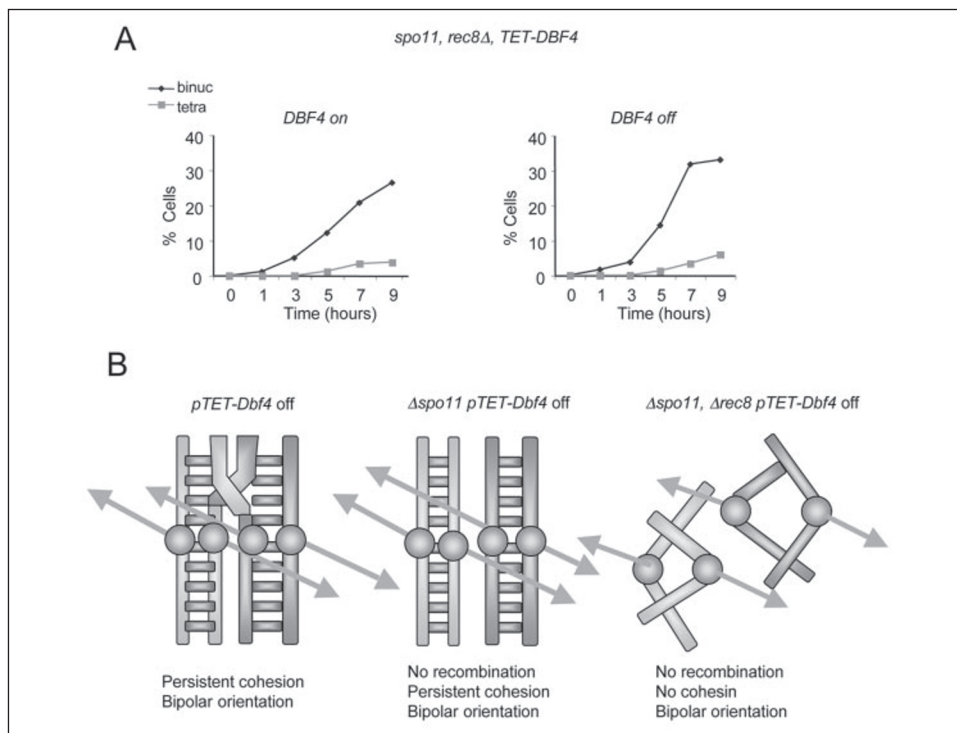
Arrest Is Not Due to Incomplete DNA Replication or Faulty Recombination—Partial or defective replication is detected during meiosis by a replication checkpoint that stops cell division (21). Since S phase was slightly extended under conditions of delayed *Dbf4* depletion, we envisaged the possibility that the meiosis I arrest was a consequence of activating the S phase or recombination checkpoints. Hence we tested whether this arrest was dependent on the DNA damage checkpoint genes *RAD17*, *RAD24*, and *MEC1* (supplemental Fig. S1) or on the spindle checkpoint gene *MAD2* (E2346, data not shown) and found that deletion of neither of these genes bypassed the arrest. To check that DNA replication went to completion, we quantified the amount of chromosomal DNA by fluorography using the more sensitive technique of pulse field gel electrophoresis (24). By this approach, only fully replicated chromosomes migrate in the gel, while replication and recombination intermediates remain trapped in the well. To circumvent the recombination problem we used a strain deleted for *SPO11*, which

encodes the trans-esterase required for double-strand break formation and recombination (27). Chromosomes from *spo11* mutants do not undergo meiotic recombination (28), allowing their separation by PFGE. The quantification of individual chromosomes resolved in PFGE showed a precise doubling, indicating that S phase was completed after delayed *Dbf4* depletion, albeit slightly later than in the control culture (supplemental Fig. S2). Furthermore, the deletion of *SPO11* did not bypass the meiosis I arrest induced by *Dbf4* depletion, indicating that it is also not caused by defective or delayed recombination or subsequent DNA repair (Fig. 4C, right).

Delayed *Dbf4* Depletion in the *spo11Δ* Strain Produces Viable Spores—Despite the lack of recombination, *spo11* mutants are able to complete meiosis and produce tetrads, even though the spores are non-viable due to random segregation of homologues at the first division (28, 29). Depletion of *Dbf4* in *spo11* mutants produced only 17% of dyads, but surprisingly 87% of spores from these dyads were viable (Fig. 3C, right). Remarkably these spores turned out to be MATa/MAT α diploids, indicating that they were the product of an equational division. This result suggests that delayed *Dbf4* depletion prevents mono-orientation of sister kinetochores at meiosis I, a phenotype reminiscent of that produced by the depletion of the polo-like kinase *Cdc5* (30, 31).

Deletion of *REC8* Suppresses *DBF4*-dependent Arrest—The absence of *Cdc5*, like that of *Dbf4*, leads to a normal progression of the early

FIGURE 5. Deletion of *REC8* bypasses the arrest caused by *Dbf4* depletion. *A*, *spo11Δ, rec8Δ, pTET-DBF4* strain (E2663) was shifted to SPM with (*DBF4_{on}*) or without doxycycline (*DBF4_{off}*). Cells having two (●) or four DNA masses (■) were scored by DAPI staining. *B*, proposed model for chromosome segregation and kinetochore attachments (arrows) following *Dbf4* depletion alone (left), in combination with *spo11Δ* (middle), or in combination with *spo11Δ* and *rec8Δ*. In the absence of *Dbf4* (left panel) and *Spo11* (middle panel) bipolar orientation of sister kinetochores and persistent cohesion impairs chromosome segregation. Deletion of *REC8* (right panel) allows sister chromosomes to separate.



meiotic events but causes a failure at anaphase I. *cdc5* mutants accumulate hypophosphorylated forms of the meiotic cohesin Rec8, which fails to be degraded (30, 31) and prevents the proper resolution of chiasmata and the separation of homologues during meiosis I (32). In addition, Cdc5 depletion prevents the association of the monopolin Mam1 with kinetochores, causing an abnormal bi-orientation of sister chromatids at meiosis I. Considering the similar phenotypes induced by Cdc5 and *Dbf4* depletion, we investigated whether a persistent cohesion could be responsible for the *Dbf4*-dependent arrest. If, as for Cdc5, loss of *Dbf4* interfered with both cohesin cleavage and chromatid mono-orientation, we would expect chromosomes to fully segregate in *Dbf4*-depleted *spo11Δ rec8Δ* cells. Deletion of *REC8* should allow cells to undergo a first division, but not efficient sporulation, as seen previously for *rec8* mutants (33). This prediction was entirely satisfied by the experiment. The formation of bi-nucleated cells (meiosis I) in *spo11Δ rec8Δ* cells was no longer affected by the depletion of *Dbf4* (Fig. 5A). This indicates that cells depleted for *Dbf4* after S phase arrest before anaphase I mainly because they bi-orient their sister chromatids without being able to dissolve the cohesive links between them (Fig. 5B).

DISCUSSION

The meiotic division is a differentiation program precisely controlled by the action of protein kinases (34). Regulatory circuits check the occurrence of the specific meiotic division (meiose I) that should be coordinated with DNA replication and recombination to produce the correct segregation of homologous chromosomes into the gametes (35).

The present study demonstrates that Cdc7-*Dbf4* is required during the early stages of meiosis to initiate DNA replication. These data contrast with earlier findings that suggested that *CDC7* was not required for pre-meiotic DNA replication. This discrepancy could be due to the fact that initial investigations on meiotic functions of *CDC7* used temperature-sensitive alleles (11). As meiosis itself is inhibited at elevated temperatures, the results obtained were likely to suffer a strong experimental bias. To avoid this caveat we inactivated Cdc7 by depleting its unstable regulatory subunit *Dbf4*. Using this approach we demonstrate

that pre-meiotic DNA replication depends on Cdc7/*Dbf4* activity (DDK). In budding yeast, pre-meiotic DNA replication is triggered by the same S phase CDKs that act in mitosis (18, 20, 21). These data combined with our results strongly suggest that mitosis and meiosis share a common mechanism for the activation of the origins of DNA replication.

In addition to the replication function, we show that *Dbf4* has a second role during meiosis. Indeed, in the absence of *Dbf4* cells arrest before the first meiotic division suggesting a possible role of *Dbf4* in setting up the specific chromosome segregation during meiosis. Two experimental observations confirmed this hypothesis. First, although in the absence of *Dbf4* most of the cells did not carry out anaphase, a few cells eventually divided their nuclei. However, they produced only two diploid spores instead of four haploid gametes. This indicated that an equational, instead of the normal reductional division, occurred. Second, deletion of *Rec8* restored the nuclear division despite the absence of *Dbf4*, suggesting that the loss of *Dbf4* may cause persistence of *Rec8* cohesion. On the basis of these observations, we propose the following model (Fig. 5B). The absence of *Dbf4* causes both kinetochore bipolar orientation and cohesion persistence between sister chromatides. Therefore, spindle fibers pull chromosomes to the opposite cell poles, while *Rec8* cohesion persists, causing an arrest of chromosome separation. This arrest is bypassed by the deletion of *REC8* that allows chromosomes to separate. This model can account for the arrest observed in the absence of *Dbf4* and the failure of the subsequent anaphase. Our results thus give evidence that *Dbf4* plays an unexpected but important role for the first meiotic division.

This new meiotic function for *Dbf4* is in agreement with literature reports as paralogs and orthologs of *DBF4* and *CDC7* have been reported to play distinct biological functions in other organisms. In fission yeast, a second Cdc7-*Dbf4*-related complex (*Spo4-Spo6*) is required for chromosome segregation during the second meiotic division but dispensable in mitosis (36). A role distinct to replication initiation has been ascribed to the fission yeast Cdc7 ortholog Hsk1, specifically for the formation of centromeric heterochromatin and sister

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chromatid cohesion (37, 38). A Dbf4-related protein, Drf1, involved in the progression of both S and M phases as well as in checkpoint responses, was isolated in human cells (39, 40) and in *Xenopus* (41). It has also been proposed that the *Xenopus* homolog of Dbf4 is required for heart development independently of its function in DNA replication (42). No Cdc7 or Dbf4 paralogs have been described in budding yeast so far, making plausible a dual role for Dbf4 in meiosis.

The phenotypes that we described for the loss of Dbf4 function (*i.e.* persistent cohesion and kinetochore bi-orientation) show a strong resemblance with *cdc5/polo* mutant phenotypes during meiosis (30, 31). We propose that Dbf4 might be necessary for Cdc5/Polo function. Polo-like kinases are characterized by the presence of two phospho-dependent substrate-targeting modules, the polo boxes, in their non-catalytic C-terminal domain (43). Polo boxes recognize S-[pS/pT]-P/X motifs in pre-phosphorylated substrates, and by doing so, both unmask the catalytic domain and recruit the kinase to specific subcellular structures. Thus the biological activity of polo kinases is believed to rely on “priming kinases” that pre-phosphorylate their substrates. The Cdc7 kinase is known to preferentially phosphorylate S-S/T-P motifs. Because the meiotic phenotypes of depleting Cdc5 and Dbf4 are so similar, it is tempting to speculate that Cdc7-Dbf4 may act as the priming kinase for Cdc5 providing a link between DNA replication and chromosome segregation during meiosis.

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