The Cdc14 Phosphatase and the FEAR Network Control Meiotic Spindle Disassembly and Chromosome Segregation

Adele L. Marston, Brian H. Lee,
and Angelika Amon*
Center for Cancer Research
Howard Hughes Medical Institute
Massachusetts Institute of Technology
E17-233
40 Ames Street
Cambridge, Massachusetts 02139

Summary

During meiosis, DNA replication is followed by two consecutive rounds of chromosome segregation. Cells lacking the protein phosphatase *CDC14* or its regulators, *SPO12* and *SLK19*, undergo only a single meiotic division, with some chromosomes segregating reductionally and others equationally. We find that this abnormal chromosome behavior is due to an uncoupling of meiotic events. Anaphase I spindle disassembly is delayed in *cdc14-1*, *slk19Δ*, or *spo12Δ* mutants, but the chromosome segregation cycle continues, so that both meiotic chromosome segregation phases take place on the persisting meiosis I spindle. Our results show that Cdc14, Slk19, and Spo12 are not only required for meiosis I spindle disassembly but also play a pivotal role in establishing two consecutive chromosome segregation phases, a key feature of the meiotic cell cycle.

Introduction

All sexual reproduction in diploid organisms relies on the generation of haploid gametes from a diploid progenitor cell. This reduction in chromosome number is achieved through a specialized cell cycle, the meiotic cell cycle, during which two consecutive chromosome segregation phases, meiosis I and meiosis II, follow a single round of DNA replication. Meiosis I is characterized by a unique reductional pattern of chromosome segregation in which homologous chromosomes segregate; meiosis II is characterized by an equational division, which resembles mitosis.

The specialized meiotic chromosome segregation pattern is achieved by the generation of physical linkages between chromosomes (chiasmata), the stepwise loss of proteins that hold sister chromatids together, and differences in the way kinetochores attach to the meiosis I and meiosis II spindles (reviewed in Miyazaki and Orr-Weaver, 1994; Nasmyth, 2001; Figure 1A). During both meiotic divisions chromosome segregation is initiated by the degradation of the anaphase inhibitor securin (Pds1 in budding yeast), by the ubiquitin protein ligase APC/C (anaphase-promoting complex/cyclosome) bound to its activator, Cdc20 (reviewed in Harper et al., 2002; Lee and Amon, 2003). Degradation of Pds1 liberates the protease separase (Esp1 in budding yeast),

allowing it to cleave Rec8, a component of a protein complex called cohesin that holds sister chromatids together. Rec8 is lost from chromosome arms at the metaphase I-anaphase I transition, but centromeric regions retain Rec8 until the onset of anaphase II (Klein et al., 1999). Removal of cohesion from chromosome arms is required for the dissolution of chiasmata (the physical manifestation of crossover events), allowing homologs to segregate from each other at anaphase I (Buonomo et al., 2000). Retention of centromeric cohesion between sister chromatids beyond anaphase I, and its subsequent loss at the onset of anaphase II, is critical for proper segregation of sister chromatids during meiosis II (Tang et al., 1998; Toth et al., 2000). Differential attachment of kinetochores to the meiosis I and meiosis II spindle is also essential to bring about the meiotic chromosome segregation pattern. During meiosis I, sister chromatids attach to microtubules emanating from the same pole (coorientation). In meiosis II, sister chromatids attach to microtubules emanating from opposite poles (biorientation; reviewed in Miyazaki and Orr-Weaver, 1994; Lee and Amon, 2001; Nasmyth, 2001). The Mam1 protein is required to ensure coorientation of sister kinetochores during meiosis I (Toth et al., 2000). Its dissociation from kinetochores during early anaphase I is likely to be part of the events that allow sister kinetochores to biorient during meiosis II.

Over the last 23 years several mutations have been identified that appeared to transform the meiotic chromosome segregation pattern into a mitotic one, raising the possibility that the affected genes are critical for the establishment of the specialized meiotic cell division cycle. In 1980, Klapholz and Esposito discovered two mutations, *spo12-1* and *spo13-1*, from a natural variant of *S. cerevisiae* that caused cells to undergo a single meiotic division leading to the formation of two diploid spores (Klapholz and Esposito, 1980a). Subsequently, three other mutations, *cdc5-1*, *cdc14-3*, and *slk19Δ*, that led to a similarly abnormal meiosis were identified (Sharon and Simchen, 1990a, 1990b; Kamieniecki et al., 2000). Cells carrying any such mutation undergo a single meiotic division with a "mixed" chromosome segregation pattern, in which some chromosomes segregate predominantly in a reductional manner (meiosis I-like; homologs are separated) and others preferentially segregate equationally (mitosis-like; sister chromatids are separated; Sharon and Simchen, 1990a, 1990b; Kamieniecki et al., 2000). Furthermore, *slk19Δ* mutants generate meiotic cells with anaphase I spindles, but Rec8 is absent from centromeric regions (normally anaphase I cells should still retain Rec8 at centromeres), indicating that *SLK19* is a critical regulator of meiotic chromosome segregation (Kamieniecki et al., 2000). However, the molecular basis for the abnormal chromosome behavior in these mutants was unclear.

The recent finding that *SLK19, SPO12*, and *CDC5* are components of a regulatory network, the FEAR network, that controls the release of the protein phosphatase Cdc14 from the nucleolus during mitosis (Stegmeier et al., 2002, Pereira et al., 2002; Yoshida et al., 2002)
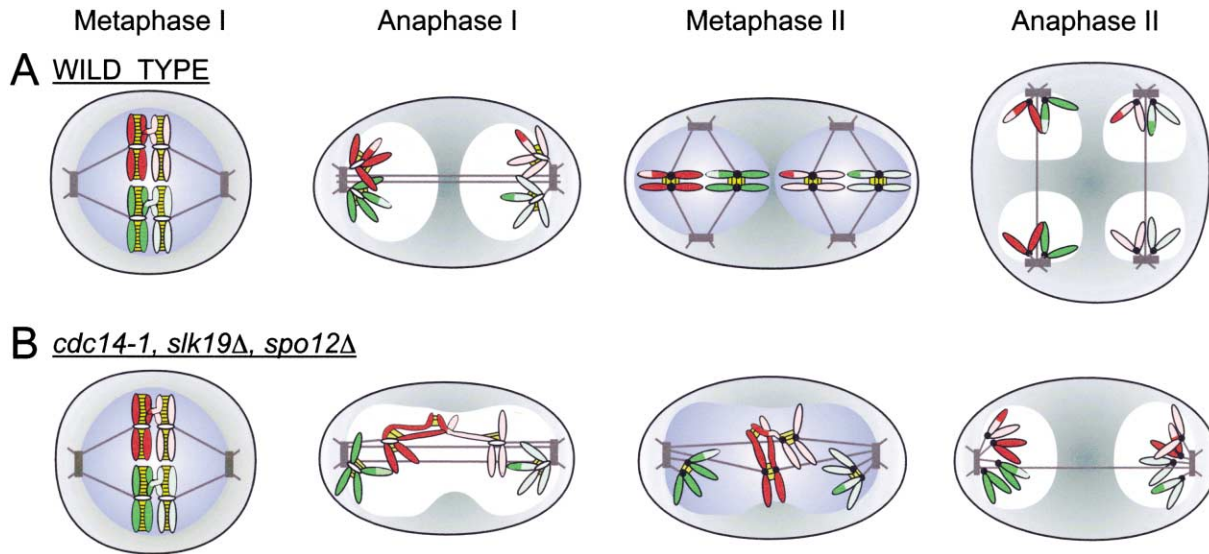
*Correspondence: angelika@mit.edu

Figure 1. A Model for Meiotic Chromosome Segregation in *cdc14-1*, *slk19Δ*, and *spo12Δ* Mutants

Two yeast chromosomes (pairs of homologs or bivalents) are shown, one in red and one in green. The dark and pale colors represent maternal and paternal chromosomes, respectively. Cohesion, yellow and black stripes; Securin/Pds1, blue; cooriented kinetochores, white ovals; bioriented kinetochores, black circles.

(A) The meiotic chromosome segregation phases in wild-type cells. See text for details.

(B) Uncoupling of meiotic events and its consequences in *cdc14-1* and FEAR network mutants. See text for details.

prompted us to investigate the possibility that the FEAR network regulates meiotic chromosome segregation through activating Cdc14. Cdc14 plays an essential role in promoting the inactivation of mitotic CDKs (also called Clb-CDKs) and exit from mitosis. The activity of this phosphatase is regulated by an inhibitor, Cfi1/Net1, which holds Cdc14 inactive in the nucleolus during the G1, S, G2, and early M phase. The release of Cdc14 from its inhibitor during anaphase (Shou et al., 1999; Visintin et al., 1999) is promoted by the FEAR network during early anaphase (Stegmeier et al., 2002). The mitotic exit network, a GTPase signaling cascade, maintains Cdc14 in the released state during late anaphase and telophase (reviewed in McCollum and Gould, 2001; Bardin and Amon, 2001).

Here we show that two of the FEAR network components, *SLK19* and *SPO12*, promote Cdc14 release from the nucleolus during anaphase I and are necessary for the timely disassembly of the meiosis I spindle. Our findings also provide an explanation for the abnormal chromosome segregation pattern observed in *cdc14-1* and FEAR network mutants (Figure 1B). We find that, despite a delay in anaphase I spindle disassembly, meiosis II events occur. As a result, both meiotic chromosome segregation phases take place on the same meiosis I spindle. Finally, we propose that the delay in anaphase I spindle disassembly and recombination are responsible for the fact that some chromosomes undergo meiosis II on the meiosis I spindle. Our results show that, although the anaphase I spindle persists, meiosis II events are not inhibited in the absence of *CDC14* or its activators, indicating that these genes are critical coordinators of the meiotic chromosome segregation program.

Results

CDC14 Is Required for Meiotic Spindle Disassembly

Cells carrying a temperature-sensitive *cdc14-3* allele form two (dyads), instead of four (tetrads), spores, with some chromosomes having segregated in an equational manner and others in a reductional manner (mixed segregation; Sharon and Simchen, 1990a, 1990b). To determine the function of *CDC14* in meiotic cell cycle progression in more detail, we analyzed a temperature-sensitive *cdc14-1* mutant progressing through the meiotic cell cycle at the restrictive temperature. The *cdc14-1* mutant was delayed in meiosis I spindle disassembly, as judged by the persistence of cells with anaphase I/telophase I spindles (Figure 2B). Meiosis II spindles did not form, and *cdc14-1* mutants underwent a single meiotic division, as previously reported (Figure 2A, Table 1; Sharon and Simchen, 1990a, 1990b). We also noted that nuclear division was impaired in *cdc14-1* mutants, since the DNA of anaphase I cells had frequently not separated into two distinct masses (Table 2). We conclude that *CDC14* is required for spindle disassembly and efficient chromosome segregation during meiosis.

Cdc14 Release from the Nucleolus during Anaphase I Is Controlled by the FEAR Network

Cdc14 exhibited a localization pattern during meiosis that is similar to that observed during mitosis. Cdc14 resided in the nucleolus throughout meiotic prophase I and metaphase I (Figures 2C and 2D). Upon entry into anaphase I, Cdc14 was completely released (spread throughout the nucleus or nucleus and cytoplasm) in 64% of cells and partially released (Cdc14 was present
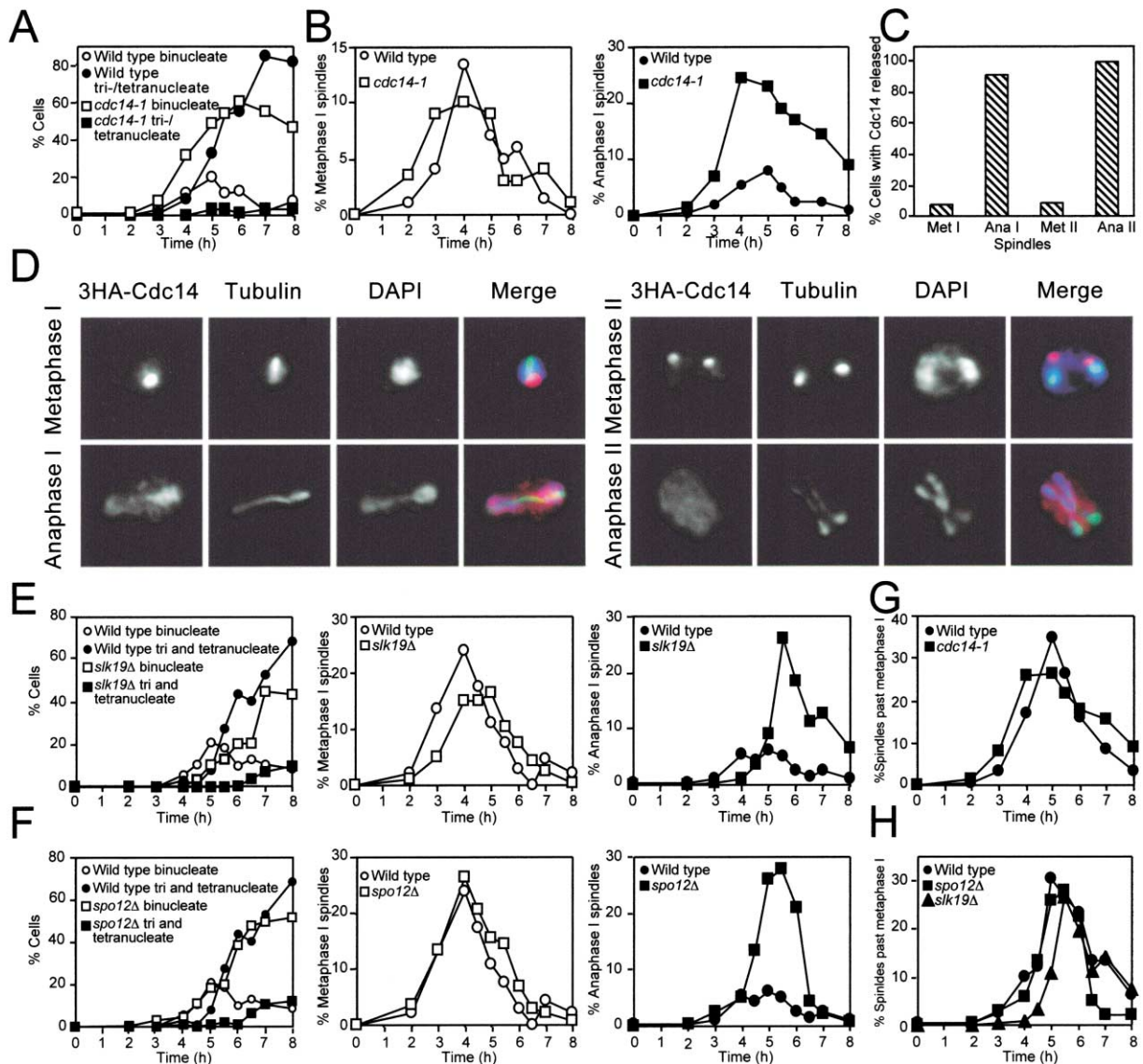
Figure 2. Cdc14 and FEAR Components Function in Meiosis

(A and B) Wild-type (A4199; circles) and cdc14-1 (A5423; squares) were induced to sporulate at 30°C. The percentages of binucleate (open symbols) and tri- or tetranucleate (closed symbols) cells (A) and the percentages of cells with metaphase I ([B], left graph, open symbols) or anaphase I spindles ([B], right graph, closed symbols) were determined at the indicated times after resuspension in SPO medium. cdc14-1 mutants exhibit a first cell cycle arrest in mitosis after shifting cells to 30°C (A.L.M., unpublished data), indicating that the cdc14-1 allele is largely inactive at this temperature.

(C) The percentages of metaphase I (MetI; n = 121), anaphase I (AnaI; n = 153), metaphase II (MetII; n = 81), and anaphase II (AnaII; n = 254) cells with Cdc14 either partially or fully released from the nucleolus.

(D) Representative images of Cdc14 localization in wild-type cells (A5067). 3HA-Cdc14, red; meiotic spindles, green; DNA, blue.

(E and F) Wild-type (A5067) and slk19Δ ([E]; A5071) or spo12Δ ([F]; A5069) cells were induced to sporulate at 30°C, and the percentages of bi- and tetranucleate cells (left graph) and cells with metaphase I (middle graph) and anaphase I (right graph) were determined.

(G and H) The percentage of cells that have progressed beyond metaphase I (anaphase I, metaphase II, and anaphase I spindles combined) is shown in (G) for the wild-type (A4199; circles) and cdc14-1 mutant (A5423; squares) and in (H) for the wild-type (A5067; circles) and spo12Δ (A5069; squares) and slk19Δ (A5071) mutants.

throughout the nucleus but also concentrated in the nucleolus) in 25% of anaphase I cells. In metaphase II cells, Cdc14 was resequestered, before being released upon anaphase II entry uniformly into the nucleus and cytoplasm (Figures 2C and 2D). Thus, Cdc14 undergoes two cycles of sequestration in, and release from, the nucleolus during meiosis.

Cdc14 release from the nucleolus was reduced in FEAR network mutants. Cdc14 was fully released in only about 20% of slk19Δ and spo12Δ cells and partially released in 25% of anaphase I cells. Cells lacking SLK19 or SPO12 were also delayed in anaphase I spindle disassembly and largely underwent a single meiotic division (Figures 2E and 2F). We noticed that the time cdc14-1,

Table 1. Sporulation Efficiency and Chromosome Segregation in cdc14-1, spo12Δ, and slk19Δ Mutants

Strain	Sporulation after 24 hr^a		Segregation of URA3-GFP Dots in Binucleate Cells^b,c		Segregation of CEN-GFP Dots in Binucleate Cells^c,d	
	Dyads	Tetrads	Reductional	Equational	Reductional	Equational
Wild-type	13	76	95	5	95	5
cdc14-1	1	0	84	16	87	13
slk19Δ	48	5	86	14	84	16
spo12Δ	46	1	74	26	73	27
P_HOP1-Clb2ΔdB	18	5	54	45	51	49

Strain	Segregation of URA3-GFP Dots in Binucleate Cells^c,e	
	Reductional	Equational
spo11Δ	98	2
spo11Δ cdc14-1	98	2
spo11Δ slk19Δ	100	0
spo11Δ spo12Δ	98	2
spo11Δ P_HOP1-Clb2ΔdB	56	43

a Wild-type (A4964), cdc14-1 (A5688) slk19Δ (A5628), spo12Δ (A5532), and P_HOP1-Clb2ΔdB (A4240) cells were grown at room temperature and induced to sporulate at 30°C in liquid culture. Cells were analyzed after 24 hr in sporulation medium.
b Strains used were A4199 (wild type), A5423 (cdc14-1), A4751 (slk19Δ), A4750 (spo12Δ), and P_HOP1-Clb2ΔdB (A4241).
c Strains were sporulated at 30°C, samples were taken every hour until 8 hr, and GFP dot segregation was analyzed. Reductional or equational segregation of GFP-marked chromosomes is shown as a percentage of all binucleate cells observed in this analysis (number of binucleates observed ranges from 67 [wild type] to 381). At least 130 binucleates were scored for each of the mutants.
d Strains used were A5811 (wild type), A5822 (cdc14-1), A5812 (slk19Δ), A5814 (spo12Δ), and A5813 (P_HOP1-Clb2ΔdB).
e Strains used were A3654 (spo11Δ), A5539 (spo11Δ cdc14-1), A5491 (spo11Δ slk19Δ), A5492 (spo11Δ slk19Δ), and A5597 (spo11Δ P_HOP1-Clb2ΔdB).

Table 2. Nuclear Morphology in cdc14-1, slk19Δ, and spo12Δ Anaphase I Cells^a

Wild-type	71	27	2
slk19Δ	36	63	1
spo12Δ	12	81	8
cdc14-1	19	67	14
P_HOP1-Clb2ΔdB	28	70	2
spo11Δ	90	9	1
spo11Δ slk19Δ	91	8	1
spo11Δ spo12Δ	91	9	0
spo11Δ cdc14-1	85	15	0
spo11Δ P_HOP1-Clb2ΔdB	56	39	3

a Strains A4199 (wild-type), A5423 (cdc14-1), A4751 (slk19Δ), A4750 (spo12Δ), A4241 (P_HOP1-Clb2ΔdB), A3564 (spo11Δ), A5491 (spo11Δ slk19Δ), A5492 (spo11Δ spo12Δ), A5539 (spo11Δ cdc14-1), and A5597 (spo11Δ P_HOP1-Clb2ΔdB) were induced to sporulate at 30°C, and samples were taken on the hour for 8 hr. At least 150 cells with anaphase I spindles were scored for nuclear morphology. We scored approximately 50 anaphase I cells from all time points where anaphase I cells were readily observed.

slk19Δ, and spo12Δ mutants spent in anaphase I was comparable to the time it took wild-type cells to complete both meiotic divisions (Figures 2G and 2H), suggesting that eventual spindle breakdown is caused by the onset of late sporulation events, such as ingression of the spore membrane, which continue uninterrupted in the mutants. Like in the cdc14-1 mutant, nuclear division was impaired in slk19Δ and spo12Δ mutants, since many cells with an elongated spindle had not separated their DNA into two distinct masses (Table 2). Our results indicate that the FEAR network components SLK19 and SPO12 are required for the timely release of Cdc14 from the nucleolus, the disassembly of the anaphase I spindle, and efficient chromosome segregation.

Overexpression of CLB2 Lacking the Destruction Box during Meiosis Prevents Spindle Disassembly

In Xenopus oocytes cyclin B-dependent kinase activity declines to a low level toward the end of meiosis I (Gerhart et al., 1984, Iwabuchi et al., 2000), indicating that, in this organism, a transient decrease in meiotic CDK activity is associated with the meiosis I to meiosis II transition. As Cdc14's key function during mitosis is to trigger the inactivation of Clb-CDKs, we wished to investigate whether Cdc14 and its regulators were also required for the downregulation of Clb-CDKs during meiosis. Owing to the poor synchrony of meiotic yeast cultures and the short time period between the disassembly of the meiosis I spindle and reassembly of the meiosis II spindles, we were unable to determine whether Clb-CDK activity fell as cells exited meiosis I (data not shown), but we examined the fate of Clb1 protein by indirect in situ immunofluorescence. In wild-type cells, Clb1 protein declined during anaphase I and the protein was no longer concentrated in the nucleus (Figure 3A). Loss of Cdc14 function did not significantly alter the percentage of cells in which Clb1 was detectable (Figure 3B, middle panel). The decline of Clb1 in the nucleus during anaphase I was, however, impaired in the
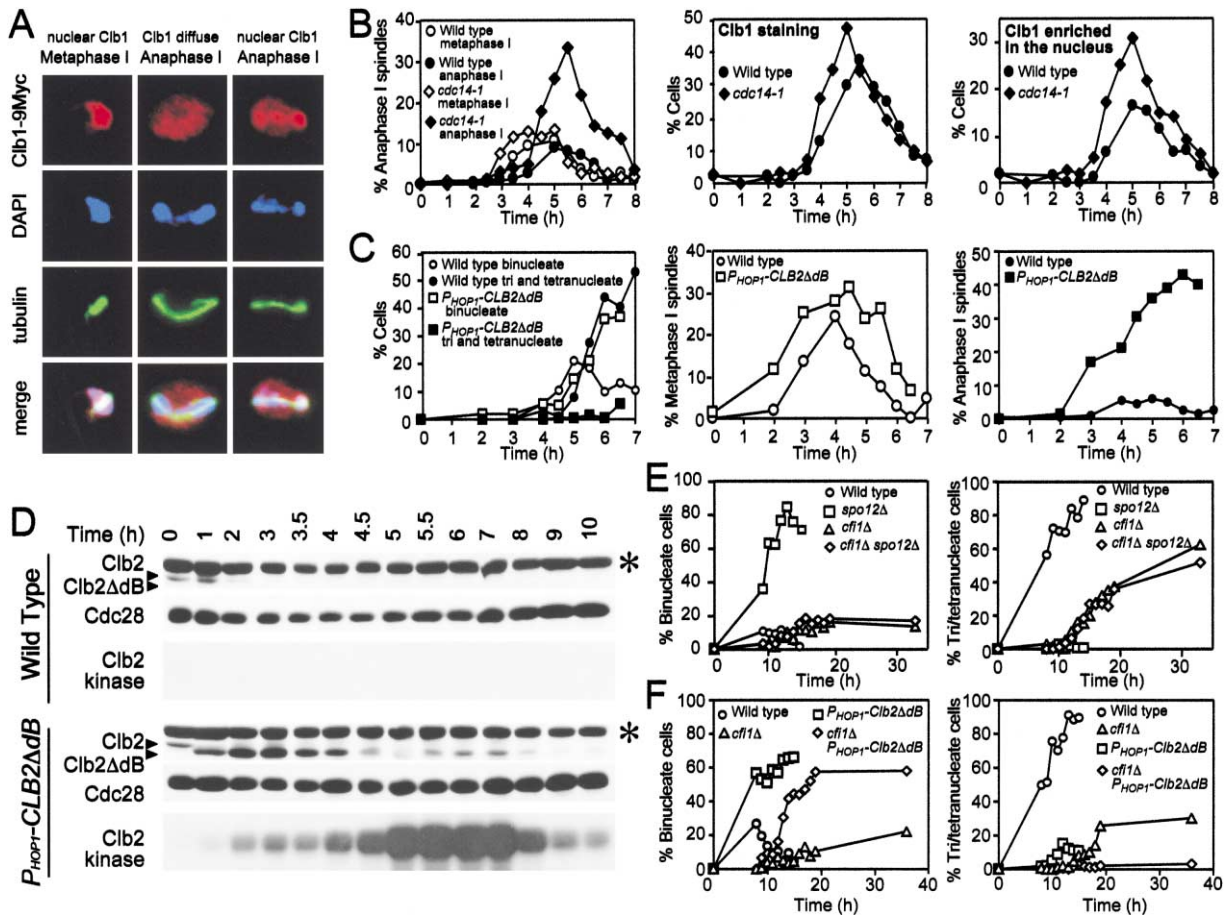
Figure 3. Cdc14 Regulates Meiosis by Antagonizing CDK Activity

(A and B) Wild-type (A7132) and *cdc14-1* (A7197) cells carrying a *CLB1-9MYC* fusion were induced to sporulate.

(A) Examples of Clb1 staining in wild-type (left and middle panel) and *cdc14-1* mutants (right panel).

(B) The percentages of cells with anaphase I spindles (left graph), with detectable Clb1 staining (middle graph), and with Clb1 enriched in the nucleus (right graph) were determined.

(C) Wild-type (A5067) and P_{HOP1}-Clb2ΔdB (A5075) cells were induced to sporulate and analyzed as described in Figures 2A and 2B.

(D) The amount of Clb2 protein and associated kinase activity in wild-type (A3685) and P_{HOP1}-Clb2ΔdB (A4240) cells undergoing meiosis. The asterisk indicates a crossreacting band. Cdc28 is shown as a loading control.

(E and F) Wild-type (A4199), *spo12Δ*, (A4750), *cfi1Δ* (A5235), *spo12Δ cfi1Δ* (A5663), P_{HOP1}-Clb2ΔdB (A4241), and *cfi1Δ P_{HOP1}-Clb2ΔdB* (A5112) cells were induced to sporulate at room temperature, and samples were taken to determine the percentages of binucleate (left graph) or tri- and tetranucleate (right graph) cells.

cdc14-1 mutant (Figure 3B, right graph) and in cells lacking the FEAR network components, Slk19 and Spo12 (Buonomo et al., 2003 [this issue of *Developmental Cell*]; our unpublished data). Our findings suggest that *CDC14* and the FEAR network are required for the timely decline of Clb1 in nuclei during anaphase I.

Next we determined whether lowering of Clb-CDK activity was actually a prerequisite for exit from meiosis I and/or II, by examining the consequences of high levels of Clb-CDKs on meiosis. To this end we expressed *CLB2* without the destruction box (*CLB2ΔdB*), a modification that causes stabilization of the protein (Amon et al., 1994), during meiosis by placing *CLB2ΔdB* under control of the meiosis-specific *HOP1* promoter (P_{HOP1}-*CLB2ΔdB*). *CLB2ΔdB* was expressed during meiosis and active as a kinase (Figure 3D), and it resulted in a dramatic cell cycle arrest. Cells underwent a single meiotic division and arrested with anaphase I-like spindles, and only a small percentage of cells packaged

spores to form dyads (Figure 3C; data not shown). We also noted that entry into meiosis I was consistently accelerated in cells expressing *CLB2ΔdB* (Figure 3C), typically by 2 hr. The finding that overexpression of *CLB2ΔdB* causes a delay in spindle disassembly similar to that of cells carrying a *cdc14-1*, *spo12Δ*, or *slk19Δ* mutation is consistent with the idea that persistence of the anaphase I spindle in these mutants is due to a failure to antagonize Clb-CDK activity.

Deletion of *CFI1/NET1* Suppresses the Phenotypes Caused by Deleting *SPO12*, but Not Those Caused by Overexpression of *CLB2*

The finding that the phenotypes associated with deleting FEAR network components are similar to those caused by inactivating *CDC14* suggests that it is the failure to release Cdc14 from the nucleolus that causes defects in meiotic cell cycle progression in these mutants. If this

were the case we predicted that abolishing the need for these genes in releasing Cdc14 from the nucleolus should rescue the phenotypes caused by deleting *SPO12* or *SLK19*.

Inactivation of *CFI1/NET1* causes Cdc14 to be released from the nucleolus throughout the mitotic and meiotic cell cycle (Shou et al., 1999; Visintin et al., 1999; data not shown) and led to cells progressing through the meiotic cell cycle slowly and with defects in spore packaging, but cells eventually underwent two meiotic divisions (Figures 3E and 3F; data not shown). When *CFI1/NET1* was deleted in *spo12Δ* cells, the double mutant underwent two meiotic divisions (Figure 3E). Because of the severe growth defect of *cfi1Δ slk19Δ* double mutants, we were not able to determine whether the inactivation of *CFI1/NET1* permitted *slk19Δ* cells to undergo two meiotic divisions (data not shown). Consistent with the idea that high levels of Clb-CDK activity counteract Cdc14 even when the protein is released from the nucleolus, deletion of *CFI1/NET1* did not allow cells expressing P_{HOP1}-*CLB2ΔdB* to undergo two meiotic divisions (Figure 3F). We conclude that defects in release of Cdc14 from the nucleolus are at least in part responsible for the *spo12Δ* phenotype.

Sister Chromatid Separation Does Not Occur Prematurely in *cdc14-1* and FEAR Network Mutants

One remarkable feature of *cdc14-1, slk19Δ*, and *spo12Δ* mutants is that chromosome segregation is mixed (Sharon and Simchen, 1990a, 1990b; Kamieniecki et al., 2000). To determine the reason for this peculiar chromosome segregation pattern, we analyzed the segregation behavior of chromosome V in *cdc14-1, slk19Δ*, and *spo12Δ* mutants and cells overproducing Clb2ΔdB. Chromosome V was marked either 35 kb (7 cM; URA3 dots) or 1.4 kb (CEN dots) from the centromere with tandem *tet* operator repeats. A green fluorescent protein fused to the TET repressor protein, which binds to these repeats, was introduced into these cells (heterozygous GFP dots; Michaelis et al., 1997; Tanaka et al., 2002). In a reductional segregation, sister chromatids stay together, so that a GFP dot is observed in just one nucleus after the first meiotic division (in binucleate cells). In contrast, if an inappropriate equational segregation occurs, sister chromatids separate, and binucleate cells with each nucleus containing a GFP dot are generated. In the majority of binucleate *cdc14-1, slk19Δ*, and *spo12Δ* cells, the GFP-labeled chromosome V was found in only one nucleus, indicating that chromosome V segregated in a reductional manner. In 14%–26% of binucleate cells, the GFP label was, however, found in both DNA masses (Table 1). This increase in binucleate cells with a GFP label in both DNA masses was not due to an increase in recombination, as the level of recombination in at least *slk19Δ* and *spo12Δ* cells is similar to that in wild-type cells (Klapholz and Esposito, 1980b; Kamieniecki et al., 2000; Zeng and Saunders, 2000). Furthermore, the centromere-linked GFP label on chromosome V showed a similar segregation pattern to the URA3 dots (Table 1). Overproduction of a stabilized version of Clb2 caused a more dramatic phenotype (Table 1). We have not been able to determine whether the

inappropriate segregation of sister chromatids of FEAR network mutants was rescued by the deletion of *CFI1/ NET1*, as cells lacking this gene also exhibited chromosome missegregation (data not shown).

The presence of binucleate cells with a GFP-labeled chromatid in each nucleus in *cdc14-1, slk19Δ*, and *spo12Δ* cells could be due to premature segregation of sister chromatids during meiosis I. To test this hypothesis we analyzed the kinetics of chromosome V separation in the absence of *CDC14, SLK19*, and *SPO12* or in P_{HOP1}-*CLB2ΔdB*-expressing cells. Figure 4 and Supplemental Figure S1 (see http://www.developmentalcell.com/ cgi/content/full/4/5/711/DC1) show that separation of URA3 dots, as judged by the presence of two GFP dots in the cell (whether they are in one, two, or four nuclei), did not occur prematurely in *spo12Δ, slk19Δ, cdc14-1*, and *CLB2ΔdB*-expressing cells (Figures 4A–4D; second panel). Similar results were obtained when the segregation behavior of CEN dots was analyzed (see Supplemental Figure S2 at http://www.developmentalcell.com/ cgi/content/full/4/5/711/DC1). The finding that sister chromatid separation did not occur prematurely raised the question as to when the binucleate cells with a GFP-labeled chromatid in each nucleus arise. To address this we determined when during the meiotic time course binucleate cells with a GFP label in one nucleus and binucleate cells with a GFP label in both nuclei were generated in *slk19Δ, spo12Δ*, and *cdc14-1* mutants and *CLB2ΔdB*-expressing cells. The third panel in Figure 4 and the fourth panel in Supplemental Figure S2 show that, in the *spo12Δ, slk19Δ*, and *cdc14-1* mutants, binucleate cells in which the labeled sister chromatids had segregated to one of the two nuclei were produced first. Only later did we observe binucleate cells with GFP dots in both nuclei. Both types of segregation patterns (GFP dots in one nucleus and GFP dots in both nuclei) were delayed compared with the timing of the two meiotic divisions in wild-type cells (Figure 4, third panel; see Supplemental Figure S2, fourth panel). In the *CLB2ΔdB*-expressing cells, however, both types of segregation pattern appeared, with delayed, but similar, kinetics. We conclude that loss of sister chromatid cohesion is not premature in *cdc14-1, slk19Δ, spo12Δ*, and *CLB2ΔdB*-expressing cells and that the reductional and equational segregations occur sequentially in *spo12Δ, slk19Δ*, and *cdc14-1* mutants.

Loss of Cohesion Occurs in a Stepwise Manner in *cdc14-1* and FEAR Network Mutants

To examine the kinetics with which sister chromatid cohesion is lost and to determine whether or not it occurs in a stepwise manner, we analyzed the localization of the cohesin subunit Rec8 on chromosome spreads. In wild-type cells, Rec8 was lost from chromosome arms during anaphase I (Klein et al., 1999; Figures 5A–5D, right graph). Centromeric Rec8 was not lost until the onset of anaphase II (Klein et al., 1999; Figures 5A–5D, right graph). In *spo12Δ, slk19Δ*, and *cdc14-1* mutants and P_{HOP1}-*CLB2Δdb* cells, loss of cohesion occurred in a stepwise manner, as in wild-type cells (Figures 5A–5D). However, loss of both arm and centromeric cohesion was delayed relative to anaphase I onset compared with
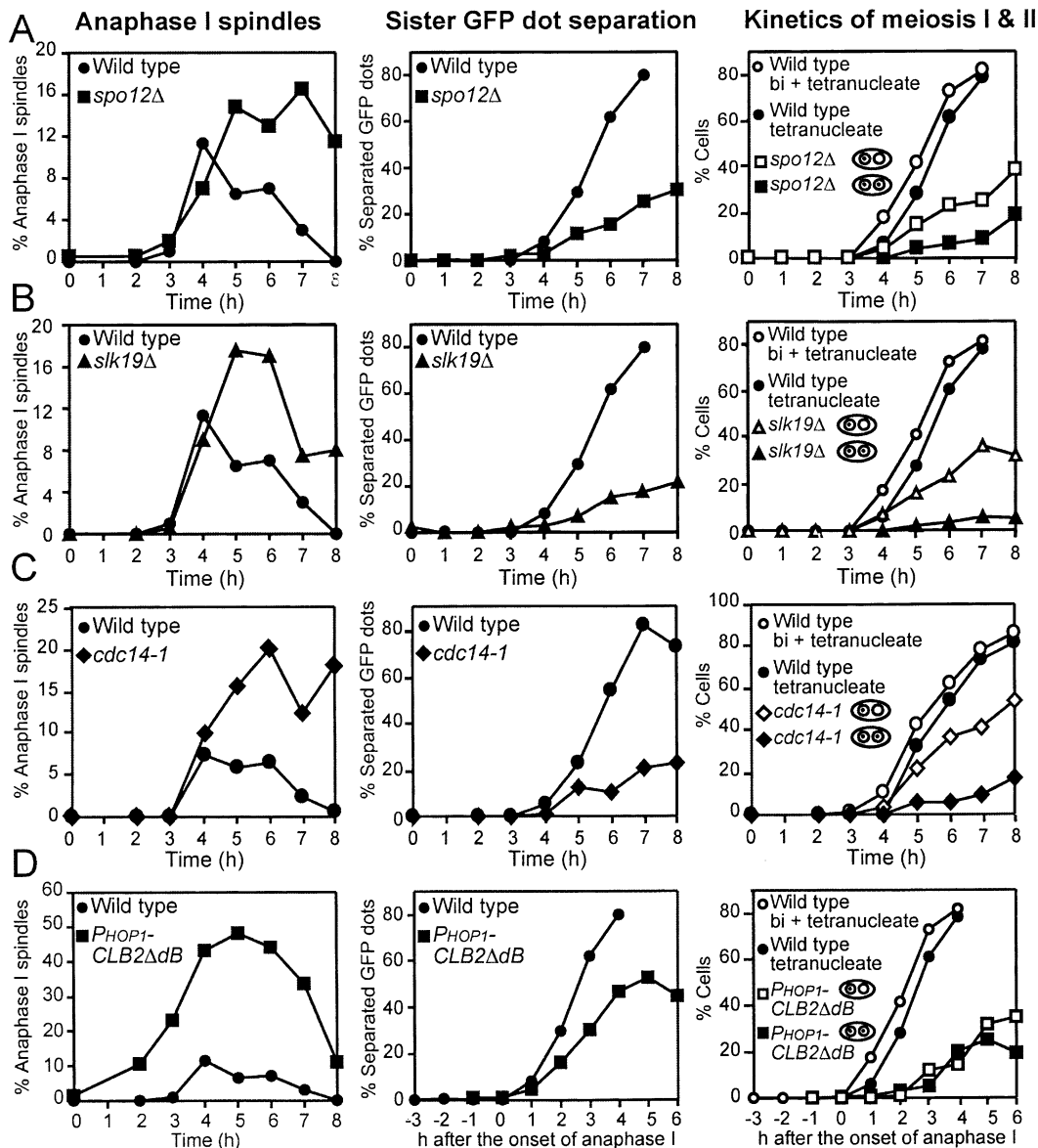
Figure 4. Sister Chromatid Separation Does Not Occur Prematurely in *slk19Δ*, *spo12Δ*, or *cdc14-1* Cells

Sporulating cultures were scored for the formation of anaphase I spindles (left graph) at the indicated times. Separation of sister chromatids (middle graph) was assayed by scoring the percentage of cells in which two GFP dots were visible, whether they were in separate nuclei or the same nucleus and irrespective of the number of nuclei in the cell (separated GFP dots). The right graph shows the kinetics by which GFP dots are segregated to one nucleus (open symbols) or both nuclei (closed symbols) for each of the mutants. In each case, the timing of the first and second meiotic divisions in the wild-type is shown as the percentage of bi- and tetranucleate cells (open circles) or tetranucleate cells (closed circles). For each of the mutants, the binucleate cells that have either one or two dots in the same nucleus (open symbols) or one dot in each nucleus (closed symbols) are expressed as a percentage of total cells at each time point. Strains used were as follows: A4199 (wild-type; [A–D]), A4750 (*spo12Δ*; [A]), A4751 (*slk19Δ*; [B]), A5423 (*cdc14-1*; [C]), and A4241 (P_{HOP1}-*CLB2ΔdB*, [D]). The reproducible early onset of anaphase in the P_{HOP1}-*CLB2ΔdB* cells (2 hr) is corrected for in (D) (middle and right graphs).

the wild-type. Consistent with this idea, we noticed an increase, albeit small, in cells in which loss of Rec8 from chromosome arms was incomplete in the mutants. A punctate pattern of Rec8 on noncentromeric chromosomal regions was detected in approximately 30% of anaphase I wild-type cells, but between 45%–70% of anaphase I *spo12Δ*, *slk19Δ*, and *cdc14-1* mutant cells and *CLB2ΔdB*-expressing cells (Figures 5E and 5F). We conclude that loss of Rec8 from chromosomes occurs

in a stepwise manner but that clearance of Rec8 from chromosomes does not occur efficiently in *spo12Δ*, *slk19Δ*, and *cdc14-1* mutants and P_{HOP1}-*CLB2ΔdB* cells.

The Chromosome Segregation Cycle and Meiotic Spindle Cycle Are Uncoupled in *cdc14-1* and FEAR Network Mutants

How can we reconcile the finding of binucleate cells with GFP labels in both nuclei with the observation that
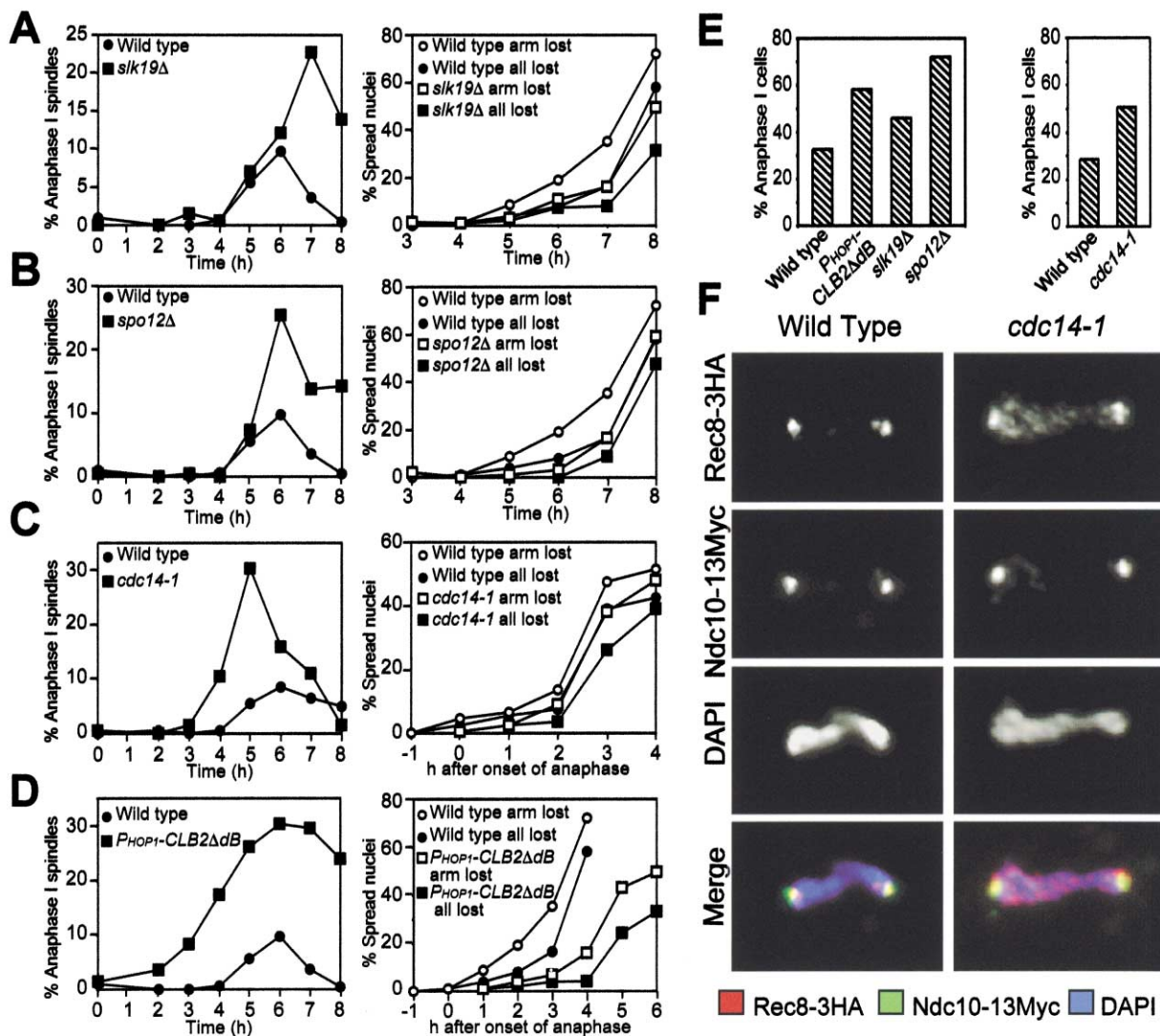
Figure 5. Loss of Cohesion Is Stepwise but Occurs Inefficiently in the Absence of Cdc14 and FEAR Network Function

(A–D) Wild-type (A4758) and *slk19Δ* (A4833; [A]), *spo12Δ* (A4834; [B]), *cdc14-1* (A5435; [C]), and *P$_{HOP1}$-CLB2Δdb* cells (A4757; [D]), all carrying *REC8-3HA* and *NDC10-13MYC* fusions, were induced to sporulate. The percentages of anaphase I spindles (left graph) and cells positive for the kinetochore marker Ndc10-13Myc, but with no visible Rec8 (closed symbols) or without Rec8-3HA staining on the chromosome arms (open symbols; note that this includes cells with no visible Rec8) were determined. The reproducible early onset of anaphase in the *cdc14-1* mutant (1 hr) and in *P$_{HOP1}$-CLB2ΔdB* cells (2 hr) is corrected for in (C and D) (right graph).

(E) Percentages of cells of genotypes described in (A) with two Ndc10-13Myc and Rec8-3HA clusters at opposing ends of the nuclei containing residual Rec8-3HA staining on chromosome arms. Numbers of cells analyzed were 92, 74, 68, 74, 125, and 134 for wild-type, *slk19Δ*, *spo12Δ*, wild-type, *cdc14-1*, and *P$_{HOP1}$-CLB2ΔdB* cells, respectively.

(F) Example of Rec8-3HA at the centromere and with (right panel) or without (left panel) residual arm cohesion. Rec8-3HA, red; Ndc10-13Myc, green; DNA, blue.

loss of cohesion does not occur prematurely in *spo12Δ*, *slk19Δ*, and *cdc14-1* cells? One possible explanation for this apparent discrepancy is that the regulatory circuits controlling cohesion removal and kinetochore orientation function independently of the machinery that controls meiotic spindle disassembly. Meiosis I spindle disassembly is greatly delayed in *spo12Δ*, *slk19Δ*, and *cdc14-1* cells, but removal of centromeric cohesion occurs. If other meiosis II events were also to occur before meiosis I spindle breakdown, both meiotic chromosome

segregation phases could take place on the same meiotic spindle and binucleate cells with GFP labels in both nuclei would be generated.

A key prediction of this hypothesis is that other aspects of meiosis II chromosome segregation occur in *cdc14-1* and FEAR mutant binucleate cells. Pds1 undergoes two cycles of accumulation and destruction in wild-type cells. It is present in metaphase I and metaphase II cells, but not in anaphase I or anaphase II cells (Salah and Nasmyth, 2000; Figures 6A and 6B). In *slk19Δ*,
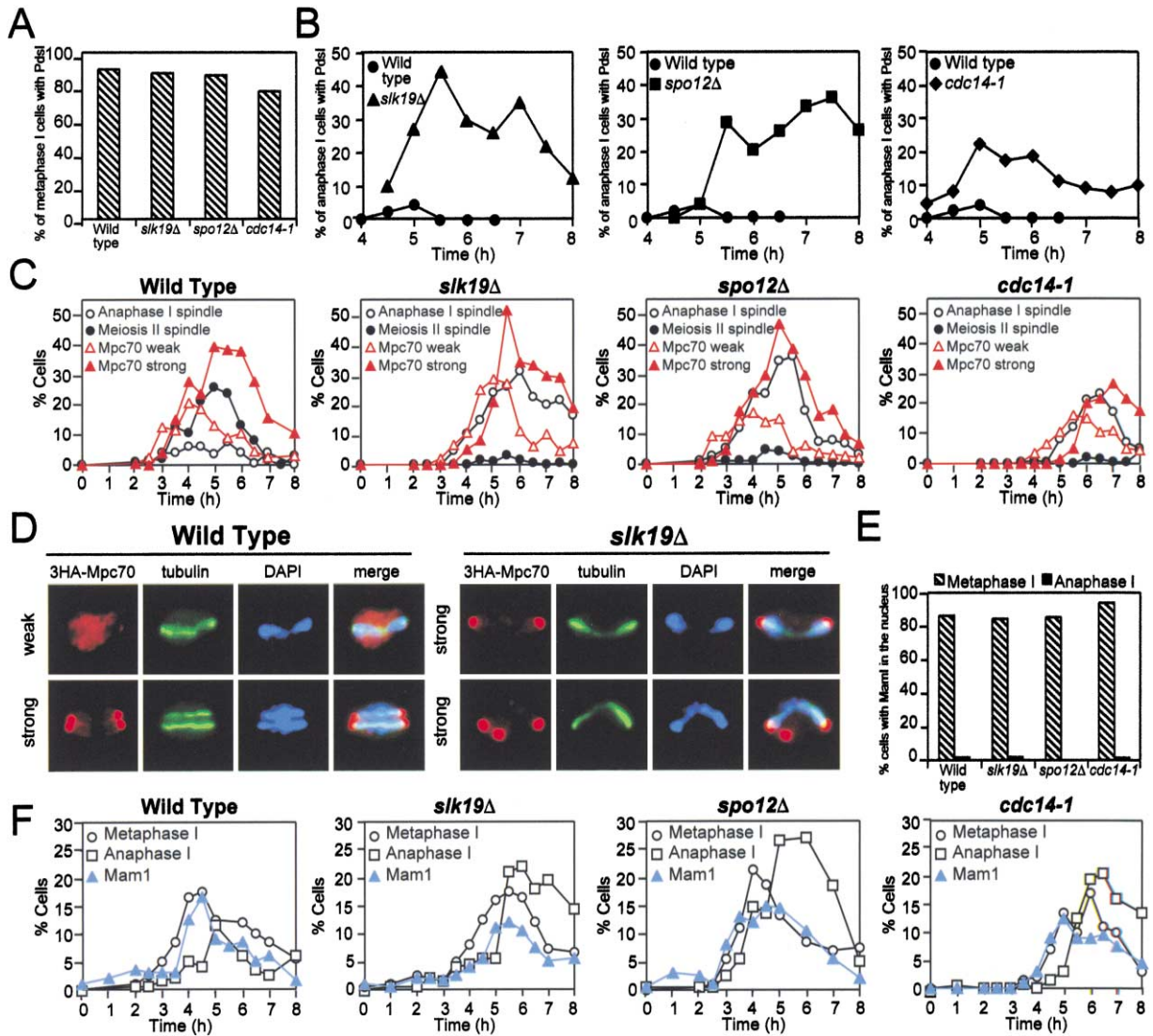
Figure 6. The Chromosome Segregation Cycle Is Uncoupled from the Meiotic Spindle Cycle in *cdc14-1* and FEAR Mutant Cells

(A and B) Wild-type (A4964), *slk19Δ* (A5628), *spo12Δ* (A5532), and *cdc14-1* (A5688) cells carrying a *PDS1-18MYC* fusion were sporulated.

(A) The percentage of cells with metaphase I spindles that are positive for Pds1.

(B) The percentage of cells with anaphase I spindles that are positive for Pds1 staining. Similar results were obtained for P_{HOP1}-*CLB2ΔdB* cells (data not shown).

(C and D) Wild-type (A6843), *slk19Δ* (A7195), *spo12Δ* (A7196), and *cdc14-1* (A7194) cells carrying a *3HA-MPC70* fusion were sporulated.

(C) The percentages of cells with weak (open triangles) or strong (closed triangles) Mpc70 staining (red lines) and the percentages of anaphase I or meiosis II (metaphase II and anaphase II) spindles were determined.

(D) Examples of Mpc70 staining in wild-type anaphase I and anaphase II cells and in *slk19Δ* anaphase I cells.

(E and F) Wild-type (A6102), *slk19Δ* (A6398), *spo12Δ* (A6401), and *cdc14-1* (A6514) cells carrying a *MAM1-13MYC* fusion were sporulated.

(E) Mam1 is present in metaphase I cells, but not in anaphase I cells.

(F) Kinetics of Mam1 appearance and disappearance from nuclei.

cdc14-1, and *spo12Δ* cells undergoing meiosis, Pds1 was also present in the nucleus of virtually all metaphase I cells (Figure 6A). In early stages of the meiotic time course, the percentage of anaphase I cells in which Pds1 staining was detected was low, as in wild-type cells (Figure 6B; see Supplemental Figure 3 at http://www.developmentalcell.com/cgi/content/full/4/5/711/DC1). These data indicate that the first cycle of Pds1 accumulation and destruction occurs normally in

slk19Δ, spo12Δ, and *cdc14-1* mutants. However, in contrast to wild-type cells, where Pds1 staining was almost never detected in anaphase I cells, a wave of Pds1 accumulation and decline was observed in anaphase I cells of *slk19Δ* and *spo12Δ* mutants (Figure 6B). In anaphase I cells of the *cdc14-1* mutant, Pds1 accumulation was slightly less pronounced for reasons that are currently unclear. We conclude that Pds1 undergoes two cycles of accumulation and destruction in *slk19Δ*,

spo12Δ, and *cdc14-1* mutants, despite the fact that the meiotic spindle cycle is halted in anaphase I.

We also examined the localization of Mpc70/Spo21, a spindle pole body protein required for prospore membrane formation (Knop and Strasser, 2000; Wesp et al., 2001). Mpc70 becomes detectable during late stages of meiosis I, most commonly as a diffuse signal throughout the cell or weakly associated with spindle pole bodies (Figure 6D; weak Mpc70). Mpc70 staining becomes much more pronounced (strong staining; Figure 6D) during late stages of meiosis II when the cell starts to assemble the prospore membrane (Knop and Strasser, 2000; Figure 6C, panel 1, and Figure 6D). In *cdc14-1* and FEAR network mutants, Mpc70 localization was indistinguishable from that in wild-type cells during metaphase I and during anaphase I in early time points (Figures 6C and 6D; data not shown). As cells continued to remain arrested in anaphase I, the characteristics of Mpc70 localization at SPBs became increasingly meiosis II-like as Mpc70 staining of SPBs became very pronounced (Figures 6C and 6D). Interestingly, we frequently observed up to two additional Mpc70 foci, which were not associated with microtubules in *cdc14-1* and FEAR network mutants, suggesting that some aspects of meiosis II SPB assembly also occur in these cells. We conclude that, during the anaphase I arrest, SPBs acquire meiosis II-like characteristics in *cdc14-1* and FEAR network mutants.

Mam1 Is Lost from Nuclei with Wild-Type Kinetics in *cdc14-1* and FEAR Network Mutants

The idea that meiosis continues in *cdc14-1* and FEAR network mutants, despite a failure to disassemble the meiosis I spindle, also predicts that the switch from cooriented sister kinetochores to bioriented ones should occur in *cdc14-1* and FEAR network mutants. Several lines of evidence indicate that sister kinetochores acquire the competence to attach to microtubules emanating from opposite poles during anaphase I (Goldstein, 1981; Paliulis and Nicklas, 2000; Rufus et al., 1983, 1989). In budding yeast, Mam1, a protein required for coorientation, accumulates in the nucleus of late prophase I/metaphase I cells and becomes undetectable in early anaphase I, when it dissociates from kinetochores (Toth et al., 2000). The disappearance of Mam1 from meiotic nuclei thus serves as an indicator as to when sister kinetochores become competent to biorient. Accumulation and disappearance of Mam1 in *slk19Δ*, *spo12Δ*, and *cdc14-1* nuclei occurred with wild-type kinetics (Figures 6E and 6F). This analysis shows that proteins required for sister kinetochore coorientation are lost with wild-type kinetics in *cdc14-1* and FEAR mutants and suggests that the ability to biorient is acquired in these mutants. We conclude that anaphase I spindle disassembly is delayed in *cdc14-1* and FEAR network mutants but that events that denote meiosis II events continue to occur. The result of this uncoupling of meiotic events is that both meiotic chromosome segregation phases occur on the same anaphase I spindle. A model as to why only a fraction of chromosomes undergo this second meiotic division on the anaphase I spindle is presented below.

cdc14-1 and FEAR Network Mutants Exhibit Defects in Meiosis I Chromosome Segregation

During our analysis of chromosome segregation in *spo12Δ*, *slk19Δ*, and *cdc14-1* mutants and *CLB2ΔdB*-expressing cells, we observed cells that contained an anaphase I-like spindle, but in which the DAPI masses had not completely segregated. Instead, nuclei with stretched DAPI masses and cross-shaped nuclei with a large fraction of the DAPI masses at the center of the spindle were observed (Table 2; Figure 7A). Furthermore, we noticed that loss of Rec8 from chromosome arms was delayed in the mutants compared with the wild-type (Figures 5E and 5F).

The abnormal nuclear morphology could be due to difficulties in resolving homologs or to defects in kinetochore attachment. To distinguish between these possibilities, we analyzed the segregation of chromosome V, where both homologs carried a centromere-linked GFP label (CEN dot). If attachment of kinetochores were unaffected in the mutants, the GFP labels should be visualized as two separated dots toward the poles of the anaphase I nuclei. Figure 7B shows that, in *spo12Δ*, *slk19Δ*, and *cdc14-1* mutants, CEN-GFP dots were consistently found separated, though not always at opposite ends of anaphase nuclei, suggesting that kinetochore attachment was not impaired in the mutants. Other regions of the chromosome were, however, trapped in the center of the anaphase I spindle, as judged by DAPI staining (Figure 7A; Table 2). Our findings indicate that kinetochores are under tension in *spo12Δ*, *slk19Δ*, and *cdc14-1* mutants but that the mutants have difficulties segregating other chromosomal regions during meiosis I.

The Meiosis I Chromosome Segregation Defect of *cdc14-1* and FEAR Network Mutants Is Rescued by Preventing Recombination

Our findings indicated that homologous chromosomes are somehow entangled in *cdc14-1* and FEAR network mutants, leading to meiosis I chromosome segregation defects. If this were the case, preventing recombination should alleviate the defect because the absence of chiasmata eliminates physical linkages between the homologs. Cells lacking *SPO11*, the gene responsible for the introduction of double-strand breaks (Bergerat et al., 1997; Keeney et al., 1997), do not accumulate metaphase I spindles but form anaphase I spindles prematurely, since unlinked homologs can segregate immediately after kinetochore attachment (Shonn et al., 2000). Deletion of *SPO11* in *cdc14-1*, *spo12Δ*, and *slk19Δ* cells caused premature spindle elongation, as expected, and caused a slight amelioration of the meiosis I exit delay (Figure 7C; data not shown). Remarkably, preventing recombination in *cdc14-1* and FEAR mutants rescued the nuclear division defect in these mutants (Table 2). A reduction in the percentage of abnormally shaped anaphase I nuclei was also observed when recombination was prevented in *CLB2ΔdB*-expressing cells (Table 2). The finding that inactivation of *SPO11* rescued the meiosis I chromosome segregation defect of *slk19Δ*, *spo12Δ*, and *cdc14-1* cells suggests that defects in resolving bivalents are at least in part responsible for the nuclear morphology defects, but the delay in spindle
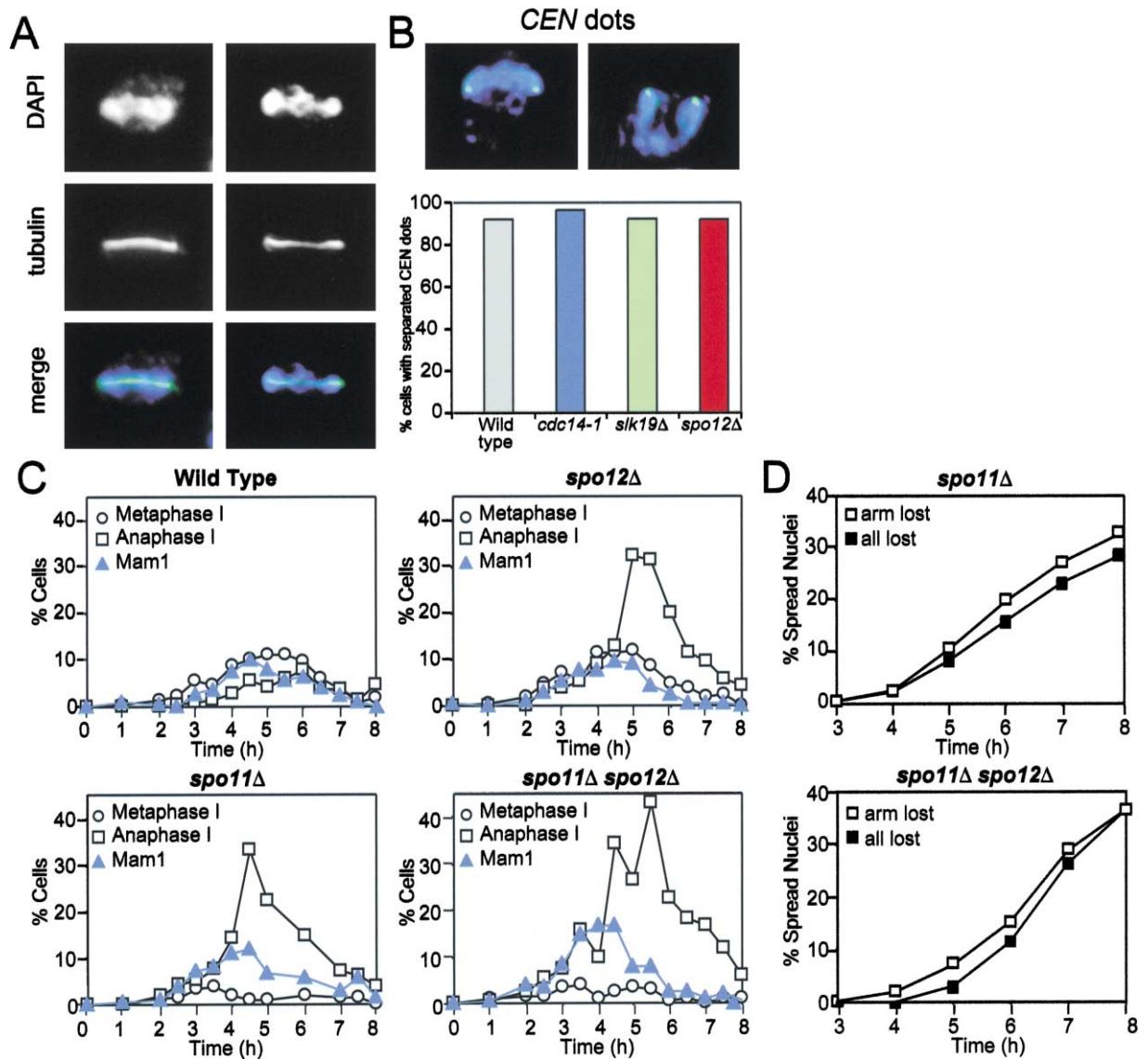
Figure 7. Meiosis I Chromosome Segregation Is Impaired in *cdc14-1*, *slk19Δ*, and *spo12Δ* Mutants

(A) Examples of the nuclear division defect in *cdc14-1*, *slk19Δ*, and *spo12Δ* cells. Shown are DAPI (top panel), tubulin staining (center panel), and merged (DAPI, blue; tubulin, green).

(B) Example of positions of homozygous CEN-GFP dots in the *slk19Δ* mutant (A5718) and quantification of the polar position of CEN dots (below). Both copies of chromosome V were marked at the centromere with GFP (wild-type [A5715], *slk19Δ* [A5718], *spo12Δ* [A5780], and *cdc14-1* [A5717]).

(C and D) Wild-type (A6102), *spo12Δ* (A6401), *spo11Δ* (A7238), and *spo11Δ spo12Δ* (A7266) cells were treated as described in Figures 6E and 6F to determine Mam1 localization (C).

(D) *spo11Δ* (A7240) and *spo11Δ spo12Δ* (A7239) cells were treated as described in Figure 5 to determine Rec8-3HA localization on chromosome spreads.

breakdown that is observed in the mutants is largely independent of *SPO11*.

The Inhibition of Meiotic Recombination Prevents the Equational Chromosome Segregation Phase in FEAR Network and *cdc14-1* Mutants

In *slk19Δ, spo12Δ*, and *cdc14-1* cells, only a fraction of chromosomes undergo the second meiotic division, and their segregation is interdependent (both homologs either segregate reductionally or equationally; Sharon and

Simchen, 1990a, 1990b; Kamieniecki et al., 2000). A possible explanation for these observations came from our analysis of URA3 dots in FEAR network mutants lacking *SPO11*. Deletion of *SPO11* prevented the equational segregation of URA3 dots in binucleate cells (Table 1). Interestingly, deletion of *SPO11* did not inhibit the equational segregation in *CLB2ΔdB*-expressing cells (Table 1). This inhibition of the second meiotic chromosome segregation phase in *cdc14-1* and FEAR network mutants was not due to meiosis II events being prevented

in *spo11Δ* cells. Mam1 disappeared from nuclei with normal kinetics (Figure 7C), and Rec8 was lost in a stepwise manner in *spo11Δ* and *spo11Δ spo12Δ* mutants (Figure 7D). The lack of an equational segregation phase in the absence of recombination was also not due to release of Cdc14 from the nucleolus through a FEAR network-independent mechanism. Cdc14 was released from the nucleolus in 86% of wild-type anaphase I cells. In *spo11Δ* cells with anaphase I spindles, the percentage of Cdc14 release from the nucleolus was reduced (65% released). This is due to the fact that, in the absence of recombination, anaphase I spindle elongation occurs prematurely, so that some cells, which are biochemically in metaphase I, when Cdc14 is sequestered in the nucleolus, have anaphase I spindles (pseudoanaphase; Shonn et al., 2000). In *spo12Δ spo11Δ* mutants, Cdc14 was released from the nucleolus in only 31% of cells with anaphase I spindles, which is comparable to the release of Cdc14 in anaphase I *spo12Δ* cells (37%). We conclude that abolishing recombination and, thus, the defect in segregating homologs greatly diminishes the frequency with which chromosomes undergo the second meiotic division on the anaphase I spindle in *slk19Δ, spo12Δ*, and *cdc14-1* cells but does not do so in *CLB2ΔdB*-expressing cells, in which anaphase I spindle disassembly is much more delayed than in *cdc14-1* or FEAR network mutants. One interpretation of these findings is that the delay in anaphase I spindle disassembly combined with defects in homolog segregation results in some chromosomes undergoing equational segregation on the anaphase I spindle. Bivalents trapped in the center of the anaphase I spindle are more likely to capture microtubules and biorient on the anaphase I spindle when coorientation is lost. Thus, these bivalents are more likely to biorient and undergo a second round of segregation (see Discussion).

Discussion

Our studies of the role of the protein phosphatase Cdc14 and its regulators in meiotic cell cycle progression led to three conclusions. First, Cdc14 and the FEAR network are required for the timely exit from meiosis I, likely by antagonizing Clb-CDK activity. Second, our findings provide an explanation for the abnormal chromosome segregation pattern observed in *cdc14-1* and FEAR network mutants. The anaphase I spindle is not disassembled in *cdc14-1* and FEAR network mutants, but meiosis II events continue to occur. Finally, we present evidence to suggest that the combination of a delay in anaphase I spindle disassembly and defects in homolog segregation in *cdc14-1* and FEAR network mutants cause some, but not all, chromosomes to undergo a second chromosome segregation on the anaphase I spindle. Our studies suggest that meiosis II events are not inhibited in the absence of *CDC14, SPO12*, or *SLK19* or in the presence of high Clb-CDK activity. *CDC14* and its regulators thus play a key role in coordinating meiotic chromosome segregation. They not only ensure, presumably by antagonizing Clb-CDKs, that the meiosis I spindle is disassembled, but also create conditions that are incompatible with chromosome segregation. Thereby, Cdc14 and the FEAR network ensure that the two meiotic divisions occur on two sequentially formed meiotic spindles.

Control of Meiosis I Exit by Cdc14 and the FEAR Network

We can envision two mechanisms or a combination thereof whereby Cdc14 and the FEAR network promote exit from meiosis I. Cdc14 could promote exit from meiosis I by downregulating Clb-CDK activity. Two observations support this idea. (1) Cdc14 is required for the timely decline of Clb1 levels in the nucleus and the disassembly of the meiosis I spindle. (2) Overexpression of a nondegradable version of Clb2 also prevented meiosis I spindle disassembly, although we cannot exclude the possibility that this inhibition of spindle disassembly is unique to Clb2. An alternative model for Cdc14 action during meiosis I is that the phosphatase simply reverses phosphorylation put in place by Clb-CDKs, thereby counteracting Clb-CDK activity. Indeed, the meiosis I to meiosis II transition is a specialized one, as an intervening S phase is absent, and work in *Xenopus* oocytes has shown that a modest level of Clb-CDK activity must be retained to prevent DNA replication while promoting meiosis I exit (Iwabuchi et al., 2000).

Our data and that of Buonomo et al. (2003) and Lee and Amon (2003) also indicate that, as in mitosis, the FEAR network controls the activity of the protein phosphatase Cdc14. Inactivation of the FEAR network component *SLK19, SPO12, ESP1*, or *CDC5* leads to defects in Cdc14 release from the nucleolus and meiosis I spindle disassembly. Furthermore, deletion of the Cdc14 inhibitor Cfi1/Net1 suppressed the failure to undergo a second nuclear division associated with deletion of *SPO12*. Finally, we note that the meiosis I exit delay of FEAR network mutants is more pronounced than that during mitosis, suggesting that the FEAR network is primarily responsible for bringing about exit from meiosis I.

Interestingly, deletion of *SPO11* also slightly rescued the mitotic exit delay in *spo12Δ* cells, but it did not affect Cdc14 release from the nucleolus, at least as judged by indirect in situ immunofluorescence. This finding suggests that Spo11 in some way directly affects Clb-CDK activity. We speculate that, because progression through premeiotic S phase and prophase I is accelerated in cells lacking *SPO11* (Cha et al., 2000), Clb-CDK activity does not accumulate to levels as high as that in wild-type cells.

Defects in Meiosis I Chromosome Segregation in *cdc14-1* and FEAR Network Mutants

Several lines of evidence indicate that chromosome segregation during meiosis I, though not prevented, does not occur efficiently in *cdc14-1* and FEAR network mutants. First, the reductional segregation phase in these mutants is delayed compared with wild-type cells (Figure 4). Second, the nuclear morphology of anaphase I *cdc14-1* and FEAR network mutants is often abnormal, and nucleolar regions of the genome frequently are not separated (Figure 7; Table 2; Buonomo et al., 2003). These phenotypes are not observed when recombination is eliminated. Defects in losing arm cohesion, which is a prerequisite for resolving homologs linked by chiasmata (see Figure 1A), could therefore be responsible for the defect in meiosis I chromosome segregation in the mutants. Consistent with this idea, we found that Rec8

loss from chromosome arms is delayed in a fraction of *cdc14-1* and FEAR network mutant cells. Alternatively, defects in other aspects of meiotic chromosome segregation, such as chiasmata resolution, per se, or decatenation of sister chromatids could contribute to the meiosis I segregation problems in *cdc14-1* and FEAR network mutants. It is also possible that the pachytene checkpoint, which halts cell cycle progression in pachytene until the completion of recombination (reviewed in Roeder and Bailis, 2000), somehow interferes with homolog resolution during anaphase I. When *SPO11* is deleted, such interference would not occur. However, we note that the pachytene checkpoint has thus far not been implicated in the regulation of homolog segregation. Chromosome segregation defects have also been described for *cdc14* mutants during mitosis (Granot and Snyder, 1991; D. D'Amours and A.A., unpublished data). This finding raises the interesting possibility that *CDC14* and its regulators are not only important for efficient chromosome segregation during meiosis, but also in mitosis.

Meiosis II Is Not Inhibited in *cdc14-1* and FEAR Network Mutants

Perhaps our most surprising finding was that, despite a delay in disassembly of the anaphase I spindle, many aspects of progression through meiosis continue to occur in *cdc14-1* and FEAR network mutants. Pds1 protein levels continue to cycle. Loss of cohesion occurs in a stepwise manner. Mam1 disappears from nuclei with wild-type kinetics, and Mpc70 shows an increased association with SPBs in anaphase I cells, a feature characteristic of meiosis II spindles.

The fact that a failure to breakdown the anaphase I spindle and to resolve some homologs does not cause a complete anaphase I arrest in *cdc14-1* and FEAR mutants suggests that checkpoint control mechanisms monitoring the successful execution of the meiosis I division are either absent or that *CDC14* and the FEAR network are part of a surveillance mechanism that prevents meiosis II when meiosis I has not been completed. The finding that high levels of Clb-CDK activity exhibit a phenotype similar to that of *cdc14-1* and FEAR network mutants argues for the former possibility. Even if the spindle checkpoint, which senses unattached kinetochores (reviewed in Gardner and Burke, 2000), were activated in the case that the switch from cooriented to bioriented sister kinetochores involved a detachment of kinetochores from microtubules, it is only transiently activated prior to the equational segregation phase occurring in *cdc14-1* and FEAR network mutants. Pds1, which is stabilized in response to checkpoint activation (Cohen-Fix et al., 1996), rises but declines again in anaphase I *cdc14-1* and FEAR network mutants. Thus, it appears that, once cells have entered anaphase I, surveillance mechanisms that prevent meiosis II events form occurring when meiosis I defects exist are only transiently active or absent.

A Model for the Unusual Chromosome Segregation Pattern of *cdc14-1* and FEAR Network Mutants

The inhibition of recombination with a *spo11Δ* mutant prevented an equational segregation of chromosome V on the anaphase I spindle in *cdc14-1* and FEAR network mutants. It is possible that activation of the pachytene checkpoint, which does not occur in *spo11Δ* mutants, regulates homolog segregation during meiosis I. While formally possible, a role for the pachytene checkpoint in controlling progression through meiosis past pachytene has not been identified. We favor the idea that the combination of defects in meiosis I chromosome segregation and anaphase I spindle disassembly are responsible for the mixed and interdependent nature of chromosome segregation in *cdc14-1* and FEAR network mutants (Figure 1B). We suggest that incomplete homolog resolution during meiosis I causes some bivalents to become trapped in the center of the anaphase I spindle and that it is these homologs that are more likely to undergo an equational segregation during meiosis II. We propose that the majority of homologs are segregated to opposite poles efficiently in meiosis I, which precludes their further (equational) segregation because chromosomes that have segregated all the way to the pole of the 8 μm-long anaphase I spindle in meiosis I are more likely to capture a microtubule emanating from the same pole, rather than the opposite pole. Chromosome trapping during meiosis I chromosome segregation could position the kinetochores of the trapped bivalents more centrally on the anaphase I spindle, increasing the probability that sister chromatids attach in a bipolar manner when coorientation is lost. Alternatively, it is possible that kinetochores of trapped chromosomes are, like kinetochores of nontrapped chromosomes, pulled toward the poles of the anaphase I spindle but that transient microtubule detachment occurs as the kinetochores gain the ability to biorient, causing the chromosome and kinetochores to spring back to a more central position on the anaphase I spindle. Our data regarding the localization of kinetochores in cells with anaphase I spindles are compatible with both possibilities. Centromeric regions of chromosome V were separated in more than 90% of cases, but their exact position with regard to the spindle poles is not known. Analysis of the positions of all cellular kinetochores by staining for the kinetochore component Ndc10 showed that kinetochores were found concentrated at the spindle poles but were also present along the anaphase I spindle (A.L.M. and B.H.L., unpublished data).

The relative efficiency of chromosome segregation in meiosis I and, thus, the extent of chromosome trapping could account for both the observation that the ability to undergo the equational segregation phase depends on the chromosome analyzed (Sharon and Simchen, 1990a, 1990b) and the fact that *cdc14* and FEAR mutants have a higher degree of equational segregation in other strain backgrounds (Sharon and Simchen, 1990a, 1990b; Kamieniecki et al., 2000). However, the mechanisms that cause some, but not all, chromosomes to be trapped in the center of the anaphase I spindle are elusive. We speculate that one of the following reasons or a combination thereof could be responsible. Resolution of homologs may be more efficient on some chromosomes than others. Alternatively, it has been shown that mixed segregation and interdependence of chromosome segregation depends on the centromeric region of a particular chromosome in *cdc14-1* mutants

(Sharon and Simchen, 1990b). Differences in the frequency of reciprocal exchange occurring near centromeric regions of individual bivalents may be responsible. Bivalents with chiasmata close to the centromere would be trapped in the center of the anaphase I spindle, since homologs would be connected to each other close to the point of tension exerted by the spindle. Bivalents with chiasmata distant from the centromere would be pulled close to the poles of the anaphase I spindle. It is, of course, also possible that chromosome trapping in the center of the anaphase I spindle is stochastic and that some kinetochores are simply better in capturing microtubules than others.

In cells expressing a stabilized version of *CLB2*, equational segregation occurs with high frequency, even when recombination is prevented by the deletion of *SPO11*. In these cells, spindle disassembly is greatly delayed compared with FEAR network and *cdc14-1* mutants. We therefore suggest that the length of time with which the anaphase I spindle persists also influences the frequency with which a second meiotic division occurs. An additional, but not mutually exclusive, possibility is that accelerated entry into meiosis in *CLB2ΔdB*-expressing cells prevents some kinetochores from becoming cooriented prior to attachment to the meiotic spindle. The finding that the reductional and equational segregation of GFP-labeled chromosomes occur concurrently in cells expressing a stabilized version of *CLB2* (Figure 4D) and the observation that deletion of *SPO11* only partially rescues the nuclear division defect of *CLB2ΔdB*-expressing cells (Table 2), together with the fact that Mam1 accumulates with wild-type kinetics in P_{HOP1}-*CLB2ΔdB* cells even though meiosis I spindle assembly is greatly accelerated (A.L.M, unpublished data), supports this idea.

Meiotic Chromosome Segregation Events Are Not Coupled

Our studies of *cdc14-1* and FEAR network mutants uncovered an unusual feature of the meiotic cell cycle. The various aspects of meiotic chromosome segregation, such as changes in kinetochore orientation, cohesin removal, and spindle pole body maturation during meiosis II are not linked, in the sense that their execution is not interdependent. We have observed uncoupling of meiotic events also in cells with high levels of Clb-CDKs, where the FEAR network is presumably active, and in metaphase I-arrested cells (B.H.L., unpublished data). These findings indicate that the FEAR network is not part of a checkpoint that couples meiosis II to the execution of meiosis I but that meiotic events occurring after the initiation of meiosis I are uncoupled. Thus, it appears that the meiotic cell cycle is fundamentally differently wired than the mitotic cell cycle. During the mitotic cell cycle, the spindle pole body duplication cycle, the Pds1 accumulation/destruction cycle, or the cohesin cycle does not continue when cells are arrested in mitosis. This finding of general uncoupling and, hence, absence of safeguard mechanisms after the initiation of the first meiotic division could also provide an explanation for the high percentage of defects in meiotic chromosome segregation and, hence, high frequency of spontaneous abortions in humans, which is estimated to be 10%–20% (Hassold and Hunt, 2001).

Experimental Procedures

Plasmids and Yeast Strains

All strains were derivatives of SK1. The *slk19Δ::kanᴿ* and *cdc14Δ::kanᴿ* deletions and the *NDC10-13MYC* and *MAM1-13MYC* tags were constructed by the PCR-based method described by Longtine et al. (1998). The *spo11Δ::URA3* and *REC8-HA* constructs were obtained from Klein et al. (1999). The *PDS1-18MYC* and *3HA-MPC70* constructs were obtained from Shonn et al. (2000) and Wesp et al. (2001), respectively. The Clb1-9Myc tag was a gift from K. Nasmyth. The *cfi1Δ::URA3* construct and *3HA-CDC14* constructs were described in Visintin et al. (1999) and Taylor et al. (1997), respectively. The *cdc14-1* allele was amplified from strain A1593 and integrated at the *CDC14* locus in SK1. The *spo12Δ::LEU2* disruption was obtained from Suzanne Prinz. The *HOP1-CLB2ΔdB* construct was made by fusing *CLB2ΔdB* (Surana et al., 1993) to 200 bp of the *HOP1* promoter shown to be sufficient for promoter activity (Prinz et al., 1995).

Sporulation

Strains were grown for 12 hr at 30°C or 24 hr at room temperature on YPG (3% glycerol) plates and then transferred onto YPD4 plates (4% glucose) for 24 hr. Cells were then grown in YPD to saturation (24 hr), diluted into YPA medium (1% yeast extract, 2% bactopeptone, and 1% potassium acetate) to $OD_{600} = 0.2$–0.3, and grown overnight to $OD_{600} > 1.8$. Cells were then resuspended in sporulation medium (0.3% Kac [pH 7] containing 0.02% Raffinose) to a final OD_{600} of 1.8–1.9. All sporulation experiments were performed at 30°C, unless otherwise specified.

Other Techniques

Immunoblot analysis of Clb2 and Cdc28 was performed as described by Cohen-Fix et al. (1996). Clb2 kinase activity was assayed as described by Surana et al. (1993). Fixing of cells for visualization of GFP-labeled chromosomes was performed as described by Klein et al. (1999). Indirect in situ immunofluorescence methods were as previously described (Visintin et al., 1999). Chromosome spreading was performed by the methods of Nairz and Klein (1997) and Loidl et al. (1998). Unless otherwise indicated at least 200 cells were counted at each time point.

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