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Functional Genomics Identifies Monopolin: A Kinetochore Protein Required for Segregation of Homologs during Meiosis I

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Summary

The orderly reduction in chromosome number that occurs during meiosis depends on two aspects of chromosome behavior specific to the first meiotic division. These are the retention of cohesion between sister centromeres and their attachment to microtubules that extend to the same pole (monopolar attachment). By deleting genes that are upregulated during meiosis, we identified in *Saccharomyces cerevisiae* a kinetochore associated protein, Mam1 (Monopolin), which is essential for monopolar attachment. We also show that the meiosis-specific cohesin, Rec8, is essential for maintaining cohesion between sister centromeres but not for monopolar attachment. We conclude that monopolar attachment during meiosis I requires at least one meiosis-specific protein and is independent of the process that protects sister centromere cohesion.

Introduction

The science of genetics was founded on Mendel's insight that germ cells contain both maternal and paternal genetic elements and that gametes inherit either one or the other element but never both (Mendel, 1865). Though many of the mysteries surrounding this process have been elucidated during the 100 years since the rediscovery of Mendel's work, the molecular mechanisms through which maternal and paternal centromeres are segregated away from each other during meiosis I remain obscure. Indeed, it constitutes one of genetics' major unsolved problems.

During meiosis, two rounds of chromosome segregation follow a single round of DNA replication, thereby producing haploid progeny (gametes) from diploid progenitors. This contrasts with mitosis, during which a single round of chromosome segregation follows each round of DNA replication, and the copy number of the genome remains constant at the end of each cycle.

Fundamental differences between the behavior of chromosomes during meiosis and mitosis are necessary to permit two rounds of chromosome segregation after only a single round of DNA replication (Moore and Orr-Weaver, 1998; Buonomo et al., 2000). The main reason for this is that chromosome segregation during mitotic divisions is crucially dependent on sister chromatid cohesion, which is only established during DNA replication (Uhlmann and Nasmyth, 1998; Tóth et al., 1999). This cohesion first facilitates the attachment of sister kinetochores to microtubules that extend to opposite poles (bipolar attachment) and then opposes the tendency of these microtubules to pull chromatids apart (Miyazaki and Orr-Weaver, 1994; Tanaka et al., 2000). Sister chromatid cohesion depends on a multisubunit complex called cohesin (Guacci et al., 1997; Michaelis et al., 1997; Losada et al., 1998; Tóth et al., 1999), whose removal from chromosomes is essential for sister chromatid separation (Ciosk et al., 1998).

During mitosis, cohesin is removed from chromosomes in two steps in many organisms. Most is removed by an unknown mechanism as chromosomes condense between prophase and prometaphase (Losada et al., 1998), but a residual fraction remains until metaphase and is removed as a consequence of proteolytic cleavage of its Scc1/Rad21 subunit by a cysteine protease called separase (Esp1 in yeast) (Uhlmann et al., 2000; Waizenegger et al., 2000). In yeast, where most Scc1 remains on chromosomes until metaphase, Scc1 cleavage is known to trigger the poleward segregation of chromatids at the metaphase to anaphase transition (Uhlmann et al., 1999, 2000). For much of the cell cycle, separase is bound by an inhibitor called securin (Pds1 in yeast), whose ubiquitination and proteolysis shortly before the onset of anaphase is a pre-condition for separase activation and thereby for chromosome segregation (Cohen-Fix et al., 1996; Funabiki et al., 1996; Ciosk et al., 1998).

There are three crucial differences between chromosome behavior during meiosis I and mitosis (Moore and Orr-Weaver, 1998; Zickler and Kleckner, 1998). First, crossovers between maternal and paternal sister chromatids ensure that sister chromatid cohesion (distal to crossovers) now holds not just sister chromatids together but also homologous chromosomes. The structures so produced are called chiasmata. Second, sister kinetochores always attach to microtubules from the same pole, which is known as monopolar attachment. This allows kinetochores from homologous chromosomes to attach to microtubules from opposite poles, which is called coorientation (Östergren, 1951). Due to these two innovations, cohesion between chromatid arms opposes the tendency of microtubules to disjoin homologous chromosomes during metaphase I and not sister centromeres as occurs during mitosis (Buonomo et al., 2000). The poleward segregation of chromosomes during meiosis I is thought to be triggered by destruction of cohesion along chromatid arms due to cleavage by separase of a meiosis-specific version of Scc1 called Rec8 (Buonomo et al., 2000). Monopolar attachment ensures that sister centromeres are now pulled to the same pole during anaphase I (Östergren, 1951).

The third major difference between meiosis I and mitosis is that cohesion between sister chromatids in the vicinity of centromeres survives until the onset of the

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second meiotic division (Moore and Orr-Weaver, 1998; Moore et al., 1998), presumably because Rec8 in this interval of the chromosomes is somehow protected from separase (Klein et al., 1999; Watanabe and Nurse, 1999; Buonomo et al., 2000). This residual cohesion subsequently has a crucial role in holding sister chromatids together when they come under tension from microtubules on the meiosis II spindle and its destruction presumably triggers anaphase II. Thus, meiotic cells pull off the remarkable feat of executing two rounds of chromosome segregation with only a single round of DNA duplication by using cohesion between chromatid arms and centromeres for the first and second divisions, respectively (Buonomo et al., 2000).

The notion that mitotic and both meiotic divisions might be triggered by a common mechanism, namely cleavage of Scc1 or Rec8 by separase, is consistent with the existence of two rounds of securin destruction during meiosis in yeast, shortly before the onset of anaphase I and II (Salah and Nasmyth, 2000). Indeed, destruction of securin is required for both mitotic and meiotic divisions (Cohen-Fix et al., 1996; Shonn et al., 2000). A single trigger for both meiotic divisions is also consistent with experiments with grasshoppers, showing that meiosis I bivalents transferred to the spindles of meiosis II cells disjoin at the same time as endogenous sister chromatids and that sister chromatids from meiosis II cells separate at the same time as meiosis I bivalents when transferred to the spindles of meiosis I cells (Paliulis and Nicklas, 2000).

Little is known about the mechanisms that ensure monopolar attachment of sister kinetochores and protect sister chromatid cohesion in the vicinity of centromeres during meiosis I. We describe here the identification, using a novel "genomics" approach, of a meiosis-specific protein called Mam1, which ensures that sister kinetochores attach to the same (monopolar) and not to opposite poles (bipolar attachment) during meiosis I in budding yeast. Cohesion between sister centromeres between anaphase I and anaphase II is not dependent on Mam1 but requires cohesin's meiosis-specific Rec8 subunit. Our results demonstrate that monopolar attachment and centromeric cohesion protection are independent properties of centromeric chromatin.

Results

A Novel Screen for Genes Needed for Meiotic Chromosome Segregation

The complete sequence of the yeast genome combined with DNA microarray technology has made it possible to measure the expression profiles of more than 6000 yeast genes during mitosis and meiosis (Cho et al., 1998; Chu et al., 1998; Spellman et al., 1998). We reasoned that genes needed for monopolar attachment or for protecting centromeric cohesion between meiotic divisions would be preferentially expressed during meiosis I. We therefore hand-picked 171 uncharacterized "meiosis-specific" genes (see Supplementary Data at http://www.cell.com/cgi/content/full/103/7/1155/DC1) using available microarray databases and deleted each one in a homothallic SK1 yeast strain. Pairing and segregation of chromosome V was analyzed in each mutant by viewing tetracycline repressor–green fluorescent protein (tetR-GFP) fusion protein bound to tandem repeats of Tet operators, which were integrated at the *URA3* locus (*URA3*-GFP dot), 35 kb from the chromosome V centromere (Michaelis et al., 1997). Our screen identified 4 genes essential for accurate chromosome segregation. This paper concentrates on the role of just one of these, a gene called *MAM1* (*m*onopolar microtubule *a*ttachment during *m*eiosis I, ORF: YER106W), whose deletion has no effect on vegetative growth but has extraordinary consequences during meiosis.

Mam1 Is Required for Accurate Chromosome Segregation during Meiosis

MAM1 encodes a 34 kDa protein with several α helical domains but little or no similarity to any other known protein. Deletion of *MAM1* reduces both the production of tetrads and the viability of spores still produced; only 5% of the spores from cells that produced tetrads are viable. The abnormal distribution of *URA3*-GFP dots among the four meiotic products (Figure 1a) suggests that chromosomes missegregate in most if not all *mam1*Δ cells, and this presumably accounts for their low spore viability.

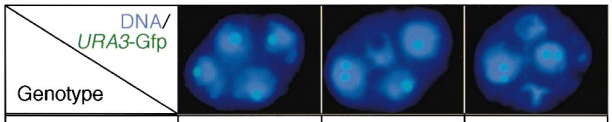
Mam1 Colocalizes with Kinetochores during Meiosis I

To detect Mam1 protein, we tagged the endogenous *MAM1* gene with 9 Myc epitopes at its carboxyl terminus. In situ immunofluorescence and Western blotting showed that Mam1-myc was absent from mitotic cells but started to accumulate within the nuclei of meiotic cells four hours after induction of sporulation (Figures 2a and 2b). Our observations suggest that Mam1 protein is present within nuclei from late pachytene until metaphase I (Figure 2c), disappears suddenly during or at the onset of anaphase I, and does not reappear during meiosis II (Figures 2a and 2c). Analysis of chromosome spreads showed that Mam1-myc was located at 10–20 discrete foci on synapsed chromosomes in 25% of Zip1 positive pachytene cells (Figure 2d). Mam1 persisted on chromatin after disappearance of the synaptonemal complex, but it was usually absent from chromatin by the end of anaphase I (data not shown). To test whether the Mam1 foci associated with pachytene chromosomes correspond to kinetochores, we analyzed chromosome spreads from a diploid strain that expressed both Mam1-myc9 and an HA-tagged version of the kinetochore protein Ndc10 (Cbf2). Mam1 foci largely colocalized with Ndc10 foci (Figure 2d). Many spreads contained Ndc10 but not Mam1 foci, suggesting that Mam1 associates with chromosomes after Ndc10. However, in those spreads clearly containing Mam1, most Ndc10 foci colocalized with Mam1 foci, and most Mam1 foci colocalized with Ndc10 foci. We conclude that Mam1 is a nuclear protein that associates with kinetochores from late pachytene till anaphase I.

Mam1 Is Required for the First Meiotic Division

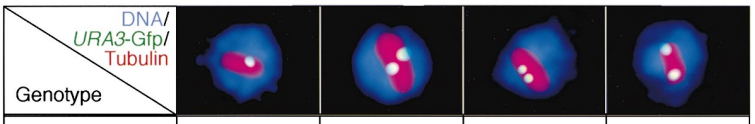
To clarify the chromosome segregation defect of *mam1*Δ cells, we compared wild-type and *mam1*Δ cultures as they progressed through meiosis (Figures 3a and 3b). FACS analysis showed that premeiotic DNA

a



Genotype			
WT	100	0	0
mam1Δ	21	46	33

b



Genotype				
WT	25	5	35	35
mam1Δ	58	25	13	4

Figure 1. Mam1 Is Required for Meiotic Chromosome Segregation and for Coorientation of Homologous Kinetochores during Meiosis I

(a) Segregation of the *URA3* locus in tetrads (marked on both homologs with GFP) produced by *MAM1* (K8409) and *mam1*Δ (K9496) homothallic strains expressing *Rec8-HA3*. Frequently, only 2 or 3 *URA3*-GFP dots were visible in *mam1*Δ cells, a situation that presumably arises due to chromosome missegregation and the close juxtaposition of more than one GFP dot within a single spore. When only 2 *URA3*-GFP dots were visible in a tetranucleate it was assumed that two of the four nuclei contained 2 unresolved *URA3*-GFP dots, because nuclei with more then 2 *URA3*-GFP dots were never observed. DNA was stained by DAPI (blue) and the *URA3* locus was marked by GFP (green).

(b) Orientation of homologous *URA3*-GFP dots in mononucleate cells containing short meiosis I spindles (red) and high levels of Pds1 (not shown) in *MAM1 PDS1-myc18* (K9498) and *mam1*Δ *PDS1-myc18* (K9497) cells. Samples were analyzed by in situ immunofluorescence using antibodies to α-tubulin and the myc epitope.

replication was unaffected by deletion of *MAM1* (data not shown). Recombination at the *LEU2* hotspot locus, as measured by Southern blotting, was also unaffected (data not shown). Pds1 and Rec8 appeared and disappeared with wild-type kinetics in *mam1*Δ cells and though first meiotic spindles appeared with wild-type kinetics, they never elongated and nuclear division was delayed. In wild-type cells, spindles elongate and nuclei divide soon after Pds1 degradation (Figure 3c, 1, top left). Thus, cells with short spindles and lacking Pds1 are rare in wild type. In contrast, *mam1*Δ cells accumulate transiently as mononucleate Pds1 negative cells with short spindles (Figure 3c, 2, top and bottom left); for example, 37% of mononucleate *mam1*Δ cells, but only 5% of mononucleate wild-type cells with short spindles lacked Pds1 at five hours. This implies that spindles neither elongate nor do nuclei divide during meiosis I after Pds1 disappears in *mam1*Δ cells.

Despite their failure to segregate chromosomes at meiosis I, *mam1*Δ cells nevertheless underwent a single nuclear division that coincided with the second meiotic division of wild-type cells (Figures 3a and 3b). This nuclear division was, however, highly abnormal. Instead of producing cells with two equal DNA masses, the one and only nuclear division in *mam1*Δ cells often resulted in the formation of four nuclei, two of which hardly contained any DNA. During this division, a seemingly normal elongated spindle usually spanned the two major DNA masses, whereas short or fragmented spindles usually colocalized with minor DNA masses (Figure 3c, 2, bottom right).

One explanation for this bizarre form of nuclear division is that the program of spindle pole duplication and spindle formation, like that of Pds1 destruction, proceeds normally in *mam1*Δ cells despite their failure to segregate chromosomes at the first meiotic division. According to this hypothesis, *mam1*Δ cells should possess four spindle pole bodies prior to their first actual division, which in reality corresponds to a second meiotic division. We therefore analyzed spindle pole body duplication in wild-type and *mam1*Δ cells whose spindle pole body protein Spc42 was tagged (at its C terminus) with GFP. Deletion of *MAM1* did not alter the kinetics of either the first or the second round of spindle pole body duplication during meiosis (Figures 4a and 4b).

Due to *mam1*Δ cell's delay in undergoing nuclear division, we frequently found that mononucleate cells contained four spindle pole bodies (Figure 4c, 2, left), a situation that never arises in wild type. Furthermore, almost all *mam1*Δ cells that had undergone a nuclear division (i.e., pseudo binucleates) contained four spindle pole bodies (Figure 4c, 2, right), whereas 40% of wild-type binucleate cells still had only two spindle pole bodies (Figure 4c, 1, left). The nuclear division cycle of mam1Δ cells is therefore clearly delayed relative to that of the spindle pole body (Figures 4b and 4c, 2). These findings confirm that the first meiotic division is never completed by *mam1*Δ cells, and their "first" nuclear division in fact corresponds to meiosis II. We conclude that Mam1 is needed for chromosome segregation and spindle elongation during meiosis I but not for progression through the meiotic cell cycle.

Sister Chromatids Separate Prematurely in *mam1*Δ Cells

We obtained an important, albeit mysterious clue as to what might be wrong with the first meiotic division in *mam1*Δ cells when we compared the kinetics of sister chromatid separation in wild-type and *mam1*Δ cells. We were able to measure this because only one of the chromosome V homologs of our strains was marked with GFP at the *URA3* locus. In wild-type cells, sister chromatid arms separate at the onset of anaphase I (Buonomo et al., 2000), but regions in the vicinity of centromeres like *URA3* only separate at the onset of anaphase II (Figure 3c, 1, top right). Thus, the curve for the fraction of cells with separated sister *URA3*-GFP dots coincides with that for the accumulation of tetranucleate cells (Figure 3a). In contrast, a sizeable fraction of *mam1*Δ cells separate *URA3*-GFP dots prematurely, before any nu-

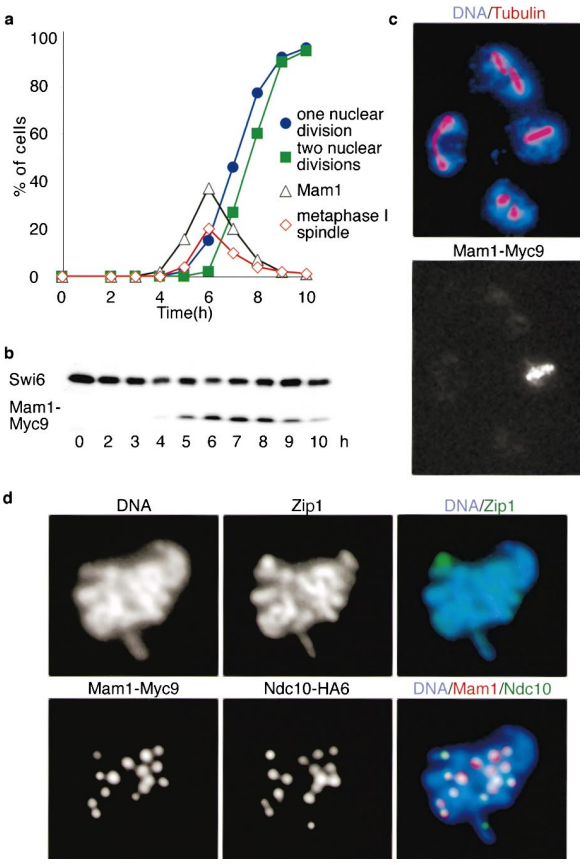


Figure 2. Mam1 Localization during Meiosis

Meiosis was induced in a diploid strain (K8936) that expressed Mam-myc9 and Ndc10-HA6.

(a) Shows the fraction of cells that underwent the first (blue filled circles) and a second (green filled squares) nuclear division, that had short metaphase I spindles (red diamonds), and that accumulated Mam1-myc9 in the nucleus (black triangles).

(b) The level of Mam1-myc9 and Swi6 measured by Western blotting.

(c) In situ immunofluorescence of cells from the 7 hr time point. Mam1-myc9 is present in the nuclei of metaphase I (middle right) but not anaphase I (middle left) cells. DNA was stained by DAPI (blue). Mam1-myc9 and meiotic spindles (red) were detected by antibodies to the myc epitope and α-tubulin, respectively.

(d) Chromosome spreads from the 6 hr time point. Mam1-myc9, Ndc10-HA3, and Zip1 were detected by antibodies to myc epitopes, and HA epitopes and Zip1, respectively.

clear division (Figure 3c, 2, top left); for example, sister GFP dots separated in nearly 20% of mononucleate *mam1*Δ cells from the 5 hr time point (Figure 3b). To address whether this precocious separation might be caused by defective sister chromatid cohesion, we asked whether sister centromere separation occurred before or after Pds1 destruction. A hallmark of cohesin mutants is that they separate sisters prior to Pds1 destruction. In *mam1*Δ cells, sister separation was never observed in mononucleate cells containing short spindles and high levels of Pds1 (Figure 3c, 2, top right) but was detected in 55% of mononucleate short spindle cells that lacked nuclear Pds1 (Figure 3c, 2, top left). This demonstrates that sister chromatid cohesion in *mam1*Δ cells is not impaired prior to Pds1 destruction.

Mam1 Is Not Required to Protect Cohesion at Centromeres

The premature separation of sister centromeres when *mam1*Δ cells embark on (but fail to undergo) the first meiotic division could be caused either by premature loss of cohesion between sister centromeres or by premature attachment of sister kinetochores to microtubules from opposite poles (bipolar attachment). In the first case, Mam1 would be required to delay cohesin's removal from centromeres until anaphase II, whereas in the second case, traction exerted by microtubules might pull sister centromeres to opposite poles even though cohesin (and cohesion) had persisted at centromeres. We therefore compared the localization of cohesin's scissile subunit Rec8 in chromosome spreads in wild-type and *mam1*Δ cells as they progressed through meiosis. Rec8 disappeared from chromatin with wild-type kinetics in *mam1*Δ cells, despite their abortive first division (data not shown). In wild-type cells, Rec8 disappears from the bulk of chromatin at the onset of anaphase I (Klein et al., 1999; Watanabe and Nurse, 1999) but persists in the vicinity of centromeres until anaphase II. As a result, small foci of Rec8 and Ndc10 protein are situated at opposite poles of bi-lobed DNA masses, which correspond to late anaphase I cells (Figure 3d, 1). Sister *URA3* sequences in the vicinity of the chromosome V centromere are separated in less than 10% of spreads of this type (Figure 3d, 1). They separate efficiently only when Rec8 is removed from centromeres at the onset of anaphase II (Klein et al., 1999; Watanabe and Nurse, 1999). In *mam1*Δ cells attempting anaphase I, Rec8 disappeared from the bulk of chromatin and Ndc10 foci appeared either along the axes of stretched chromatin or (occasionally) at opposite poles of chromosome spreads (Figure 3d, 2). Crucially, Rec8 was invariably associated with these Ndc10 foci, even though sister URA3 sequences were separated in 62% of the cases (Figure 3d, 2). Separated sister *URA3*-GFP dots were also usually associated with Rec8 and Ndc10 foci in these *mam1*Δ chromosome spreads. We therefore suspect, although we cannot be certain, that Rec8 persists even at those centromeres that have undergone precocious separation. Our observations suggest that Mam1 is not essential for Rec8's persistence at centromeres after the first meiotic division.

Another observation is consistent with the idea that premature sister centromere separation in *mam1*Δ cells is due to premature bipolar sister kinetochore attachment and is not due to defect in sister chromatid cohesion. Namely, sister *URA3* sequences are eventually segregated to different nuclei in 92% of all *mam1*Δ cells that complete sporulation. Indeed, when separated, sister centromere sequences are invariably situated at opposite ends of the single meiosis I spindle of *mam1*Δ cells that have degraded Pds1 during the first abortive division (Figure 3c, 2, top left). This implies that sister chromatids have not merely drifted apart but have been pulled toward opposite spindle poles during both meiosis I and II. It is hard to envisage how sister chromatids could segregate away from each other in *mam1*Δ cells if centromeric cohesion were not intact until bipolar sister kinetochore attachment is established either during the first abortive or during the second successful meiotic division.

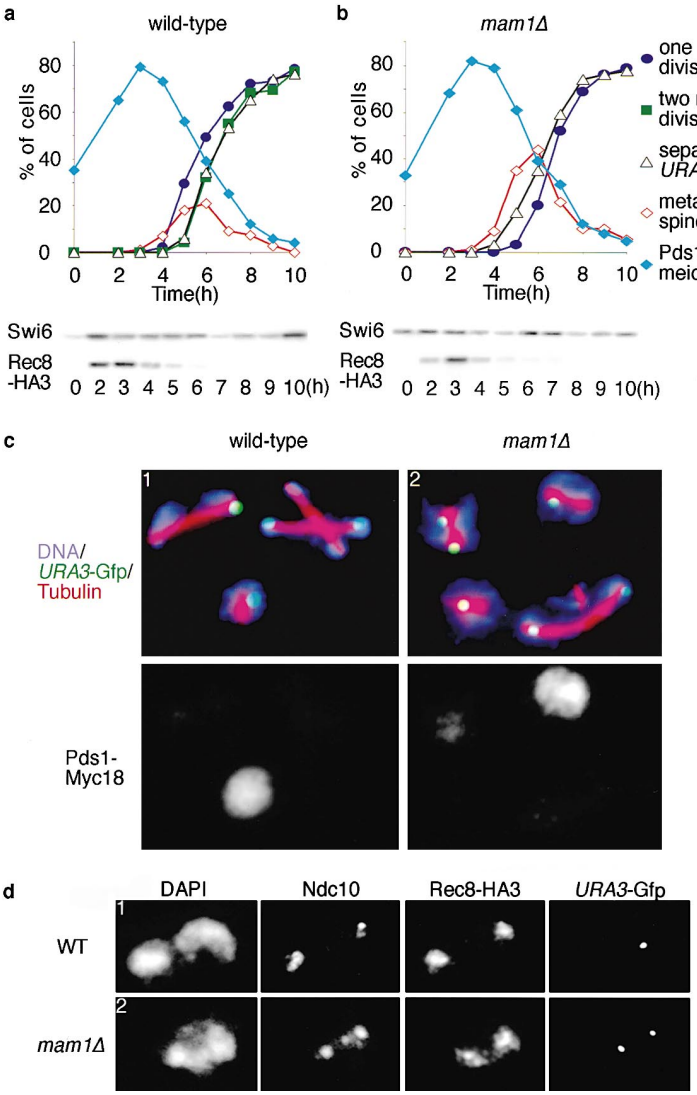


Figure 3. mam1Δ Cells Fail to Undergo the First Meiotic Division

Meiotic progression of (a) wild-type (K8925) and (b) *mam1Δ (K8923)* cells expressing Pds1-myc18 and Rec8-HA3 and with a single chromosome V homolog marked with GFP at the *URA3* locus. Shown are the fraction of cells that have undergone at least one meiotic division (blue filled circles), a second division (green filled squares), that contain a short spindle during meiosis I (red empty diamonds), that have separated sister URA3 sequences (black empty triangles), and that contained Pds1 in the nucleus during meiosis I (light blue filled diamonds). The level of Rec8-HA3 and Swi6 were measured by Western blotting.

(c) In situ immunofluorescence of (1) wild-type and (2) *mam1Δ* cells from the 7 hr time point. Spindles fail to elongate and nuclei fail to divide, but sister chromatids frequently separate after Pds1 is degraded during meiosis I in *mam1Δ* cells (top left). DNA was stained by DAPI (blue). *URA3* locus of chromosome V was visualized by GFP (green). Pds1-myc18 and meiotic spindles (red) were detected by antibodies to the myc epitope and α-tubulin, respectively.

(d) Chromosome spreads of (1) wild-type and (2) *mam1Δ* cells prepared from the 6 hr time point. DNA was stained by DAPI. *URA3* locus of chromosome V was visualized by GFP. Rec8-HA3 and Ndc10, were detected by antibodies to the HA epitope and to Ndc10.

Creating a Strain in which Cohesion between Sister Centromeres Is Lost at Meiosis I

If Mam1 were solely required to prevent bipolar attachment during meiosis I (Figure 8a), then sister chromatids in *mam1Δ* cells would be pulled in opposite directions by microtubules, but many if not most would be prevented from being pulled fully apart by cohesion/cohesin that persists in the vicinity of centromeres (Figure 8b). Spindle elongation and chromosome segregation would be prevented either by those centromeres that resist being split by spindle forces (40%–50% of the centromeres from chromosome V fail to be pulled apart) and/or by cohesion distal to centromeric regions that are split. This hypothesis makes the key prediction that the failure of *mam1Δ* cells to segregate chromosomes at the first meiotic division would be alleviated if cohesion/cohesin at centromeres were destroyed at the same time as arm cohesion/cohesin. To generate a mutant strain defective in protecting cohesion at centromeres, we investigated whether the ability to persist at centromeres after meiosis I might be a property unique to the meiotic form of cohesin, which contains Rec8 instead of Scc1. We therefore constructed a diploid yeast strain in which Scc1 (tagged with HA epitopes) and not Rec8 is expressed from the *REC8* promoter during meiosis (*rec8Δ::P_{REC8}SCC1-HA3*). This strain fails, however, to form synaptonemal complexes and is greatly delayed in undergoing meiotic divisions (S. B. C. B., unpublished data), probably because double strand breaks produced by Spo11 are not properly repaired in the absence of Rec8. This meant that we had to confine our studies to a version of this strain lacking Spo11.

Scc1 Supports Sister Chromatid Cohesion and Monopolar Attachment during Meiosis I

To investigate whether Scc1 can support sister chromatid cohesion and monopolar attachment, we compared *spo11Δ REC8-HA3* and *spo11Δ rec8Δ::P_{REC8}SCC1-HA3* cells as they progressed through meiosis (Figure 5a). Both strains underwent two nuclear divisions with similar if not identical kinetics. Unlike wild type, both *spo11Δ* strains underwent a random division with reductional characteristics (i.e., monopolar attachment of sister kinetochores) and elongated their spindles before degra-

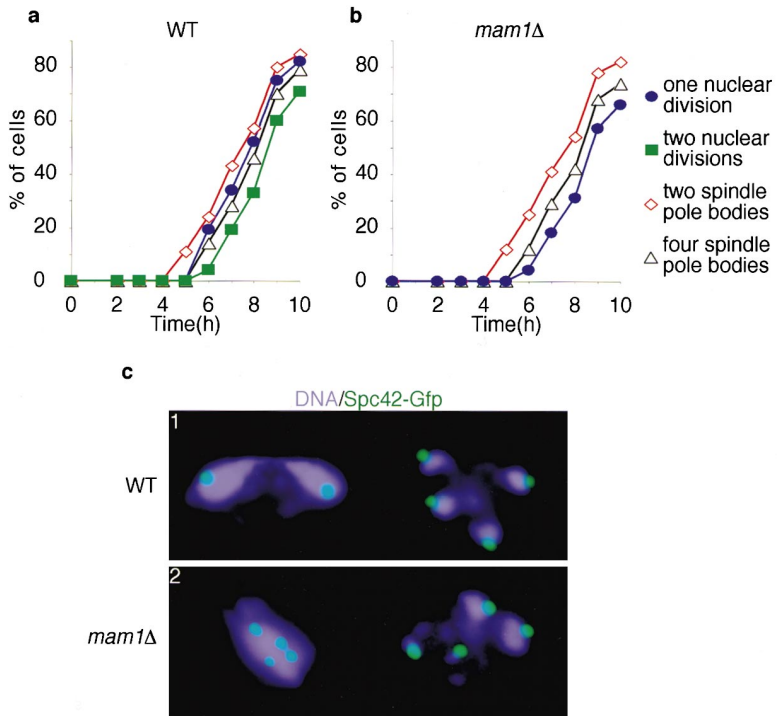


Figure 4. The Kinetics of Spindle Pole Body Duplication and Cell-Cycle Progression Is Normal in *mam1*Δ Cells

Meiosis in (a) wild-type (K9380) and (b) *mam1*Δ (K9379) strains expressing Pds1-myc18, Rec8-HA3, and Spc42-GFP (spindle pole bodies).

(a and b) Shows the fraction of cells that have undergone at least one meiotic division (blue filled circles) and a second nuclear division (green filled squares), that contained two spindle pole bodies (red empty diamonds), and that contained four spindle pole bodies (black empty triangles).

(c) Wild-type and *mam1*Δ cells from the 7 hr time point. Spindle pole bodies reduplicate in mononucleate *mam1*Δ cells. All the *mam1*Δ cells that undergo nuclear division and all the wild-type cells that undergo the second nuclear division contain four spindle pole bodies. DNA was stained by DAPI (blue). Spc42 was visualized by GFP (green).

dation of Pds1 and removal of Rec8 or Scc1 from the bulk of the chromatin (Figures 5b, 1 and 2, bottom, and 5d, 1 and 2, left). The reason for this is that neither separase activation nor cohesin cleavage are necessary to segregate maternal and paternal chromosomes to the poles if they have not previously been joined together by chiasmata (whose formation depends on Spo11) (Buonomo et al., 2000). Crucially, sister centromere (*URA3*) sequences remained tightly associated in both strains from premeiotic S phase till meiosis I and then always cosegregated to a single pole (Figure 5b, 1 and 2, bottom). Thus, Scc1 establishes and maintains (at least until destruction of Pds1) perfectly good sister chromatid cohesion, which is capable of supporting the attachment of sister kinetochores to the same spindle pole. The corollary is that Rec8 is not obligatory for monopolar attachment in *S. cerevisiae*.

Scc1 Disappears from Centromeres at Anaphase I and Cannot Support Cohesion after This Point

In *spo11*Δ *REC8-HA3 cells*, Rec8 disappears from bulk chromatin (i.e., chromosome arms) at the time of Pds1 degradation during meiosis I (Figure 5a). However, both Rec8 and cohesion persist at centromeres (Figure 5d, 1, right) until the second round of Pds1 degradation, when Rec8 completely disappears from the cells at the onset of anaphase II (Figure 5c, 1, middle). For example, Rec8 was always present, albeit in low amounts, and sister centromeres were associated in 95% of binucleate cells that had formed prophase II or metaphase II spindles but had not yet destroyed Pds1 in either nucleus (Figures 5b, 1, top and 5c, 1, right).

In *spo11*Δ *rec8*Δ*::P_{REC8}SCC1-HA3* cells, in contrast, neither Scc1 nor cohesion persists until metaphase II. Scc1 disappears completely from chromosome spreads around the time of Pds1 degradation during meiosis I (Figure 5a). For example, we never found chromosome spreads in which Scc1 was confined to centromeres; it was either present throughout the genome or completely absent from it. (Figure 5d, 2, left and right). Likewise, Scc1 was undetectable by in situ immunofluorescence in binucleate cells that had formed metaphase II spindles but had not yet destroyed Pds1 (Figure 5c, 2, left and right). As might be expected from Scc1's failure to persist at centromeres beyond meiosis I, sister centromeres separated prematurely in *spo11*Δ *rec8*Δ*::P_{REC8}SCC1-HA3* cells. Fifty percent of Pds1-positive cells that had formed second meiotic prophase or metaphase spindles contained separated *URA3*-GFP dots (Figure 5b, 2, top). We also noticed that meiosis II spindles started to elongate prematurely in these cells; that is, in the presence of Pds1 (Figure 5c, 2, right), which is consistent with the notion that sister chromatid cohesion normally resists the tendency of spindles to pull chromatids to the poles and delays spindle elongation. Finally, sister chromatid segregation during meiosis II was random in *spo11*Δ *rec8*Δ*::P_{REC8}SCC1-HA3* cells. Whereas sister chromatids segregated into different nuclei in all *spo11*Δ *REC8-HA3* cells at the second division, they only did so in 45% of *spo11*Δ *rec8*Δ*::P_{REC8}SCC1-HA3* cells. We conclude that cohesion between sister centromeres mediated by Scc1 is destroyed at the same time as that along chromosome arms, at the onset of the first meiotic division. Despite this "mitotic" behavior, cohesion mediated by Scc1 can nevertheless support monopolar attachment during meiosis I.

Precocious Loss of Sister Centromere Cohesion Allows *mam1*Δ Cells to Undergo a Fully Equational Division at Meiosis I

Armed with a strain (*spo11*Δ *rec8*Δ*::P_{REC8}SCC1-HA3*) that fails to protect centromeric cohesion but still possesses

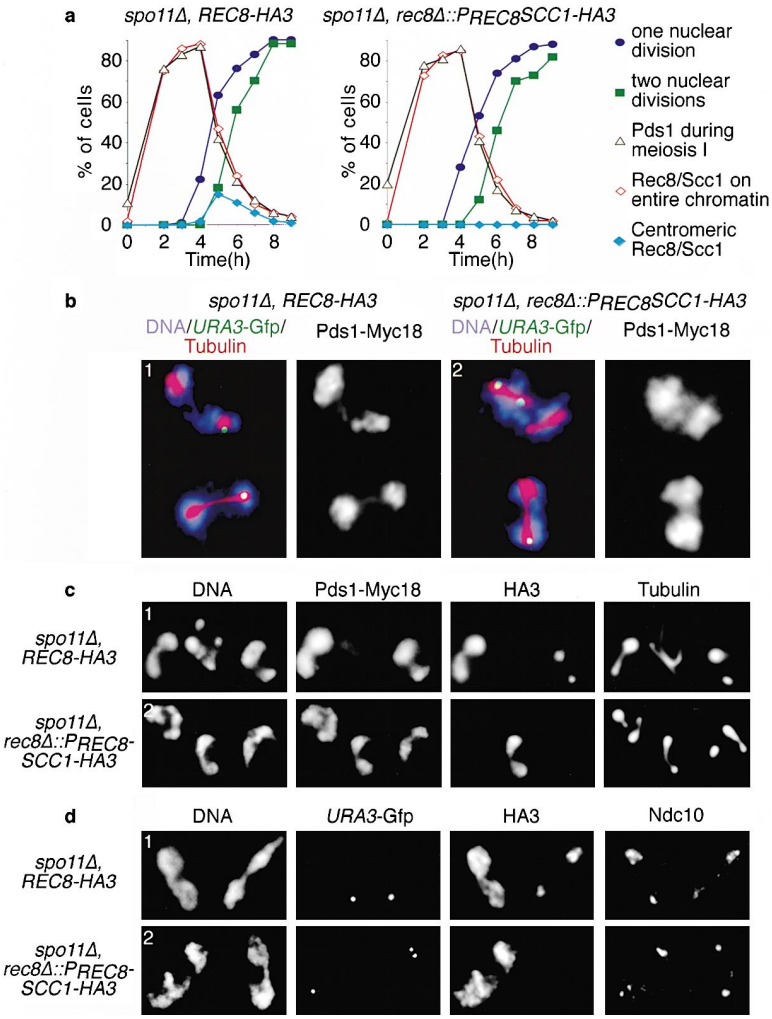


Figure 5. Rec8 Is Required for Persistence of Centromeric Cohesion but Not for Monopolar Microtubule Attachment of Sister Kinetochores during Meiosis I

Meiosis in *spo11Δ REC8-HA3* (K9109) and *spo11Δ rec8Δ::P_{REC8}SCC1-HA3* (K9110) strains expressing Pds1-myc18 and with a single chromosome V homolog marked with GFP at the *URA3* locus.

(a) Shows the fraction of cells that have undergone at least one meiotic division (blue filled circles), a second division (green filled squares), that contain Rec8 or Scc1 throughout the chromatin (red empty diamonds), that contain Rec8 or Scc1 in a restricted region around centromeres (light blue filled diamonds), and that contained Pds1 within nuclei during meiosis I (black empty triangles).

(b and c) In situ immunofluorescence of (1) *spo11Δ REC8-HA3* (K9109) and (2) *spo11Δ rec8Δ::P_{REC8}SCC1-HA3* (K9110) cells. Sister *URA3* sequences are closely associated during metaphase II in cells that express Rec8, but tend to separate in cells where Rec8 is replaced by Scc1. (b) DNA was stained by DAPI (blue). Pds1 and meiotic spindle (red) were detected by antibodies to the myc epitope and α-tubulin, respectively. The *URA3* locus of chromosome V was visualized by GFP (green). (c) Rec8 is present, but Scc1 is not present in metaphase II cells. Pds1-myc18, Rec8-HA3 or Scc1-HA3, and meiotic spindle were detected by antibodies to the myc, HA epitope, and α-tubulin, respectively.

(d) Chromosome spreads from (1) *spo11Δ REC8-HA3* (K9109) and (2) *spo11Δ rec8Δ::P_{REC8}SCC1-HA3* (K9110) cells prepared from the 5 hr time point. DNA was stained by DAPI. Rec8-HA3 or Scc1-HA3 and Ndc10, were detected by antibodies to the HA epitope and to Ndc10p, respectively. *URA3* locus of chromosome V was visualized by GFP.

monopolar attachment at meiosis I, we were now in a position to test whether the lack of chromosome segregation during meiosis I in *mam1Δ* cells is due solely to the persistence of sister centromere cohesion in cells that have lost monopolar attachment. Since our "cohesion defective" strain lacked *SPO11*, our first step was to characterize the *mam1Δ* phenotype in *spo11Δ* cells. Deletion of *MAM1* produced a similar phenotype to that in *SPO11* cells, aborting the first meiotic division (Figure 6a) without affecting the kinetics of spindle formation, Pds1 destruction, and Rec8 disappearance from chromatin (data not shown). *mam1Δ spo11Δ* cells not only failed to divide their nuclei prior to Pds1 destruction (which occurs in *spo11Δ* cells) but did not even do so after the first round of Pds1 destruction (Figure 6b, 1, top and bottom), although at this stage approximately 60% of the cells separated sister *URA3* sequences (Figure 6b, 1, bottom). Nuclear division was delayed until residual Rec8 disappeared from centromeres and Pds1 was destroyed during meiosis II (Figure 6c, 1, middle).

Remarkably, replacing Rec8 with Scc1 (*rec8Δ::P_{REC8}SCC1-HA3*) enabled *mam1Δ spo11Δ* cells to segregate chromosomes at meiosis I (Figures 6a and 6b, 2, top and bottom right). Unlike the MAM1 *spo11Δ rec8Δ::P_{REC8}SCC1-HA3* strain, in which 70% of the cells underwent meiosis I in the presence of Pds1 (Figure 5a), the first meiotic division in *mam1Δ spo11Δ rec8Δ::P_{REC8}SCC1-HA3* cells only occurred after Pds1 had been destroyed (Figure 6b, 2). Strikingly, all of these cells underwent an equational instead of a reductional nuclear division following Pds1 degradation during meiosis I. Thus, sister centromeres were tightly associated in cells that still contained Pds1 but had segregated to opposite poles in all cells that had destroyed Pds1 at the onset of anaphase I (Figures 6a and 6b, 2). This equational meiosis I division was followed by an unequal second meiotic division (Figures 6a and 6c, 2, top right), during which individual chromatids presumably segregated at random. Spindle elongation during this second abnormal division often commenced before Pds1 destruction (Figure 6c, 2, bottom left).

If the first division in *mam1Δ spo11Δ rec8Δ::P_{REC8}SCC1-HA3* cells is truly equational, it should coincide with Scc1's dissociation from chromosomes, as occurs in mitotic cells. Analysis of chromosome spreads confirmed that this is indeed the case. Sister centromeres (*URA3*) never separated, while Scc1 was present on the chromatin of mononucleate *mam1Δ spo11Δ rec8Δ::P_{REC8}SCC1-HA3* cells (Figure 6d, 2, bottom), which contrasts with their frequent separation in the

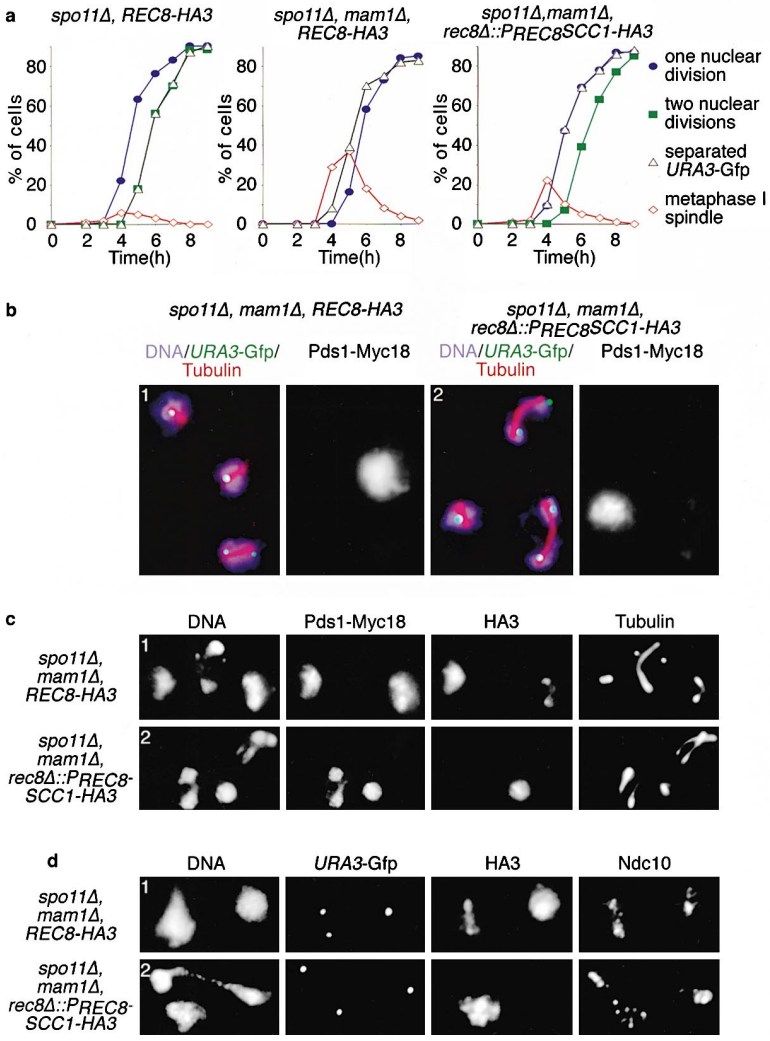


Figure 6. Replacement of Rec8 by Scc1 Permits *spo11Δ mam1Δ* Cells to Undergo an Equational Meiosis I Division

Meiosis in *spo11Δ REC8-HA3* (K9109), *mam1Δ spo11Δ REC8-HA3* (K9108), and *mam1Δ spo11Δ rec8Δ::P_{REC8}SCC1-HA3* (K9107) strains expressing Pds1-myc18 and with a single chromosome V homolog marked with GFP at the *URA3* locus.

(a) Shows the fraction of cells that have undergone at least one meiotic division (blue filled circles), a second division (green filled squares), that had short metaphase I spindle (red empty diamonds), and that have separated sister *URA3* sequences (black empty triangles).

(b and c) In situ immunofluorescence of (1) three *mam1Δ spo11Δ REC8-HA3* (K9108) and (2) three *mam1Δ spo11Δ rec8Δ::P_{REC8}SCC1-HA3* (K9107) cells. (b) Sister chromatids separate and segregate to opposite poles during meiosis I in *mam1Δ* cells if Rec8 is replaced by Scc1. Pds1-myc and meiotic spindles (red) were detected by antibodies to the myc epitope and α-tubulin, respectively, and DNA was stained by DAPI (blue). The *URA3* locus of chromosome V was visualized by GFP (green). (c) Pds1-myc18, Rec8-HA3 or Scc1-HA3, and meiotic spindles were detected by antibodies to the myc, HA epitopes, and α-tubulin, respectively.

(d) Chromosome spreads of (1) *mam1Δ spo11Δ REC8-HA3* (K9108) and (2) *mam1Δ spo11Δ rec8Δ::P_{REC8}SCC1-HA3* (K9107) cells. DNA was stained by DAPI. Rec8-HA3 or Scc1-HA3 and Ndc10, were detected by antibodies to the HA epitope and to Ndc10p, respectively. *URA3* locus of chromosome V was visualized by GFP.

presence of centromeric Rec8 in *mam1Δ spo11Δ REC8-HA3* cells (Figure 6d, 1, left). Thus, chromosome spreads containing Scc1 were never bi-lobed and always possessed a single *URA3*-GFP dot (Figure 6d, 2, bottom), whereas bi-lobed anaphase I spreads always lacked Scc1 and contained a GFP dot in each lobe (Figure 6d, 2, top). This pattern contrasts with that seen in spreads from *MAM1 spo11Δ rec8Δ::P_{REC8}SCC1-HA3* cells (Figure 5d, 2), where Scc1 was often present in bi-lobed anaphase I chromosome spreads (Figure 5d, 2, left) and where sister centromeres never segregated to opposite lobes of anaphase I nucleus once Scc1 had disappeared (Figure 5d, 2, right).

In summary, the abortive first meiotic division of *mam1Δ spo11Δ* cells is replaced by a fully equational division if, by replacing Rec8 by Scc1, sister chromatid cohesion is lost simultaneously along the entire length of chromosomes during meiosis I. Viewed from another angle, our results demonstrate that deletion of *MAM1* in *spo11Δ rec8Δ::P_{REC8}SCC1-HA3* cells is sufficient to transform its random, but in character reductional, first meiotic division into an equational one. These observations are best explained if we assume that loss of Mam1 causes sister kinetochores to attach to microtubules in a bipolar manner during what is otherwise a normal meiosis I division, and Rec8 but not Mam1 is needed for preserving cohesion at centromeres after meiosis I in wild-type cells.

Bipolar Attachment during Meiosis I in *SPO11 mam1Δ* Cells

For technical reasons, the experiments showing equational chromosome segregation (and by implication bipolar attachment) in *mam1Δ* cells during meiosis I were all performed on cells lacking recombination. The recent discovery that microtubules can split sister sequences immediately adjacent to centromeres before Pds1 destruction and Scc1 cleavage during mitosis suggests that it might be possible to detect bipolar attachment in recombination proficient *mam1Δ* cells by similar means (Goshima and Yanagida, 2000; He et al., 2000; Tanaka et al., 2000).

To detect "centromere splitting" during meiosis, we integrated tandem tetO repeats 1.4 kb away from the centromere of chromosome V in a cell expressing a tetracycline repressor-GFP fusion protein (Tanaka et al., 2000). We then compared the separation of these truly centromeric GFP dots in wild-type and *mam1Δ* cells

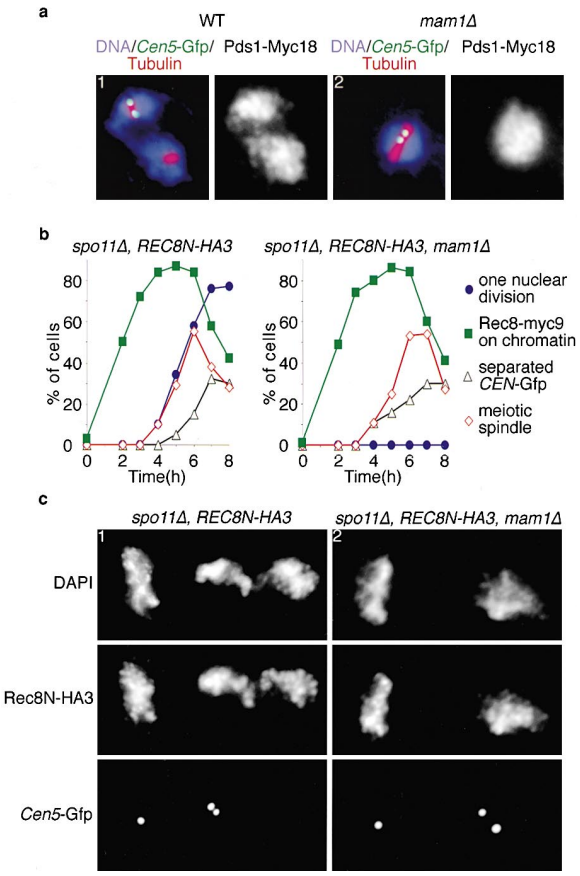


Figure 7. Deletion of *MAM1* Prevents Any Meiotic Divisions when Rec8 Cannot be Cleaved by Separase

(a) Immunofluorescence of (1) wild-type (K9106) and (2) *mam1*Δ (K9105) cells expressing Pds1-myc18 and with the centromere of a single chromosome V homolog marked with GFP (green). DNA was stained by DAPI (blue). Pds1-myc18 and meiotic spindles (red) were detected by antibodies to the myc epitope and α-tubulin, respectively.

(b) Meiosis in *spo11*Δ *MAM1 REC8-myc9 REC8N-HA3* (K9115) and *spo11*Δ *mam1*Δ *REC8-myc9 REC8N-HA3* (K9116) strains with a single chromosome V homolog marked with GFP at the centromere. Shown are the fractions of cells that underwent at least one of the meiotic nuclear divisions (blue filled circles), that separated sister centromeres (black empty triangles), that had meiosis I or meiosis II spindles (red empty diamonds), and that contained Rec8-myc9 on the chromatin (green filled squares).

(c) Chromosome spreads from (1) *spo11*Δ *MAM1 REC8-myc9 REC8N-HA3* (K9115) and (2) *spo11*Δ *mam1*Δ *REC8-myc9 REC8N-HA3* (K9116) cells. Sister centromeres frequently separate in mononucleate *mam1*Δ cells even in the presence of noncleavable Rec8. DNA was stained by DAPI. Rec8-HA3 was detected by antibodies to the HA epitope. Centromeres of one of the homologs of chromosome V were visualized by GFP.

undergoing meiosis. In wild type, centromere GFP dots never separated during meiosis I (data not shown) but separated in 40% of metaphase II cells; that is, before Pds1 destruction (Figure 7a, 1). In *mam1*Δ cells, by contrast, sister centromere sequences separated (along the spindle axis) in 15% of *mam1*Δ metaphase I cells (Figure 7a, 2) that had not yet destroyed Pds1. This observation suggests that deletion of *MAM1* causes sister centromeres to come under tension during metaphase I and confirms that Mam1 is required to prevent bipolar microtubule attachment of sister kinetochores during meiosis I in wild-type as well as in *spo11*Δ cells.

Mam1 Is Required for Coorientation of Homologous Centromeres

If *mam1*Δ mutants are truly defective in monopolar attachment, then they should be unable to pull homologous maternal and paternal sister centromeres toward opposite poles during metaphase I; that is, they should be defective in homolog "coorientation" (Östergren, 1951). In wild-type cells, maternal and paternal *URA3*-GFP dots are usually separated along the spindle axis, frequently at opposite poles, in cells that have not yet destroyed securin (Figure 1b). Furthermore, the line connecting homologous *URA3*-GFP dots is usually parallel with the spindle. In *mam1*Δ cells, in contrast, homologous centromeres either failed to separate or, if they did so, the line connecting them was usually not parallel to the spindle (Figure 1b). These data suggest that Mam1 is indeed required for the coorientation of homologous centromeres during meiosis I.

Mam1 Is Essential for the Nuclear Division of *spo11*Δ Cells that Cannot Cleave Rec8 and Prevents Sister Centromere Separation in Mononucleate Cells

To reduce the possibility that separation of sister centromeres in *mam1*Δ cells during metaphase I is due to precocious cleavage of Rec8, we investigated the effects of deleting *MAM1* in cells that express a version of Rec8 (*REC8-N*) whose two cleavage sites have been abolished by mutation (Buonomo et al., 2000). If precocious sister separation still occurs in cells that can no longer cleave Rec8, then it cannot be due to premature Rec8 cleavage. We chose to perform this experiment in cells lacking *SPO11*, because this also provided a yet more stringent test of the notion that Mam1 prevents bipolar attachment. Our rationale was the following: In wild type (*MAM1*), a single copy of *REC8-N* blocks both meiotic divisions, but deletion of *SPO11* allows cells to undergo the first, but not the second meiotic division. In the absence of crossovers, persistent sister chromatid cohesion due to *REC8-N* cannot hold homologs together and they segregate to the poles at random (Buonomo et al., 2000). However, this type of chromosome segregation should not be possible if sister kinetochores attach to opposite spindle poles. Thus, if *MAM1* is crucial for monopolar attachment, then its deletion should abolish chromosome segregation in *spo11*Δ *REC8-N* cells.

We used cells whose *CEN5* or nearby *URA3* sequences were marked with GFP and were heterozygous at the *REC8* locus; one copy expressed wild-type Rec8 protein tagged with Myc whereas the other expressed Rec8-N tagged with HA. Remarkably, deletion of *MAM1* completely abolished nuclear division even though it had no effect on the removal of wild-type Rec8 from chromosomes or on the timing of meiosis I and meiosis II spindles (Figure 7b). It also accelerated sister centromere separation, despite the lack of nuclear division (Figure 7b). Thus, sister centromeres separate in up to 30% of mononucleate *mam1*Δ *spo11*Δ cells (Figures 7b and 7c, 2, right). *URA3* sequences also separated, but

this occurred later (data not shown) and less efficiently (in no more than 10%–15% of cells). These data demonstrate that the precocious separation of sister centromeres in *mam1*Δ mutants still occurs when half the Rec8 protein cannot be cleaved and is therefore unlikely to be due to precocious Rec8 cleavage. Furthermore, deletion of *MAM1* completely blocks meiosis I division in *spo11*Δ *REC8-N* cells, just as predicted if Mam1's function is to prevent bipolar attachment.

Discussion

Genetics in the Postgenomic Era

The aim of this work was to understand one of the oldest problems in genetics, to elucidate the mechanisms that cause sister centromeres to segregate to a single pole at meiosis I and to opposite poles at meiosis II. These two properties combined with the lack of DNA replication between the two meiotic divisions permit the generation of haploid gametes from diploid germ cells and therefore form the basis for most sexual reproduction on this planet. It has long been appreciated that the extraordinary acrobatics of meiotic chromosomes are due to two key properties which are lacking in mitotic cells: the attachment of sister kinetochores to microtubules from a single pole during meiosis I (monopolar attachment) and the persistence of sister centromere cohesion at the first metaphase to anaphase transition. To shed new insight into these phenomena, we adopted a novel approach. We reasoned that some of the proteins involved must be specific to meiotic cells. We therefore screened for aberrant meiotic chromosome segregation caused by deletion mutations created in 171 candidate genes, selected merely on the basis of their higher expression during meiosis than during mitosis. Our approach is one that has been made possible by knowledge of an organism's entire genomic sequence and can therefore be considered a form of "functional genomics". Meiosis-specific genes are uniquely suitable for this sort of approach because they are not essential for vegetative growth. A vital but easily overlooked ingredient to our success was the decision to use a yeast strain specifically designed to study chromosome segregation. This allowed us to pinpoint *MAM1* as a key player at a very early stage of analysis. The application of different sophisticated tools would doubtless have identified important genes in other aspects of the meiotic or sporulation process.

One of the challenges for systematic gene function analysis is how to combine the "systematic" with the "sophisticated". It is hard to recognize interesting phenotypes for what they are unless one looks in the right direction with the appropriate tools. The current fantasy that gene function can now be elucidated by systematic methods, as is now possible for genome sequencing and expression profiling, is a chimera that threatens to swallow huge resources in the field of genetic analysis. "Intelligent" screens that are directed at studying highly specific biological processes will continue, even in the post-genomic era, to provide greater insight into biological mechanisms than untargeted screens.

Monopolins: Meiosis-Specific Kinetochore Proteins that Prevent Bipolar Attachment of Sister Kinetochores during Meiosis I

It has long been recognized that sister kinetochores never attach to spindles from different poles during meiosis I (Östergren, 1951) and that this "monopolar" behavior permits kinetochores from homologous chromosomes to do so instead (which is somewhat confusingly called coorientation). The mechanism by which coorientation is achieved is now well understood (Nicklas, 1997; Moore and Orr-Weaver, 1998). Kinetochore–microtubule connections are stabilized by the tension generated when kinetochores of homologous chromosomes joined by chiasmata attach to opposite poles (Nicklas and Ward, 1994). The mechanism used to prevent sister kinetochores from attaching to spindles from different poles remains mysterious in contrast.

It is known that bivalents (homologous chromosomes joined by chiasmata) are segregated as if they were in meiosis I when micromanipulated into meiosis II cells (Paliulis and Nicklas, 2000). This suggests that monopolar sister kinetochore behavior is a property of the chromosome and not of the spindle or the state of the cell. Cytological studies in *Drosophila* suggest that sister kinetochores are fused in a single hemispherical structure during early prometaphase I, which soon thereafter splits to form a pair of discs, both of which attach to microtubules during metaphase (Goldstein, 1981). Similar observations have been made in grasshoppers (Suja et al., 1991).

No single meiosis-specific protein has thus far been implicated specifically in monopolar attachment. Spo13, whose expression is meiosis-specific, and Spo12 and Slk19, which are also expressed in mitotic cells, are clearly involved in yeast, but they are neither essential for monopolar attachment nor are they specifically involved in monopolar attachment. (Klapholz and Esposito, 1980a, 1980b; Klein et al., 1999; Kamieniecki et al., 2000; Zeng and Saunders, 2000). Our systematic deletion of genes preferentially expressed during meiosis I has finally revealed a single gene, called *MAM1*, that appears to be specifically concerned with monopolar attachment. Mam1 is therefore the first example of a new class of proteins (monopolins) whose role is to ensure that sister kinetochores do not form bipolar attachments during meiosis I.

Mam1 appears on kinetochores during pachytene and remains there until metaphase I, but disappears from this location during anaphase. We have been unable to locate Mam1 by in situ immunofluorescence after this point, but Western blotting suggests that some protein lingers on in post anaphase cells. Mam1's location at kinetochores before their attachment to spindles during meiosis I confirms the notion that monopolar attachment is conferred by the state of chromosomes (Paliulis and Nicklas, 2000).

The separation of an appreciable fraction of sister centromeres in *mam1*Δ cells soon after the formation of meiosis I spindles, even before securin destruction, indicates that sister kinetochores have attached to spindles from opposite poles and as a consequence have come under tension that tends to split them (Figure 8b). This never occurs in wild-type cells, where instead maternal and paternal sister centromeres remain tightly

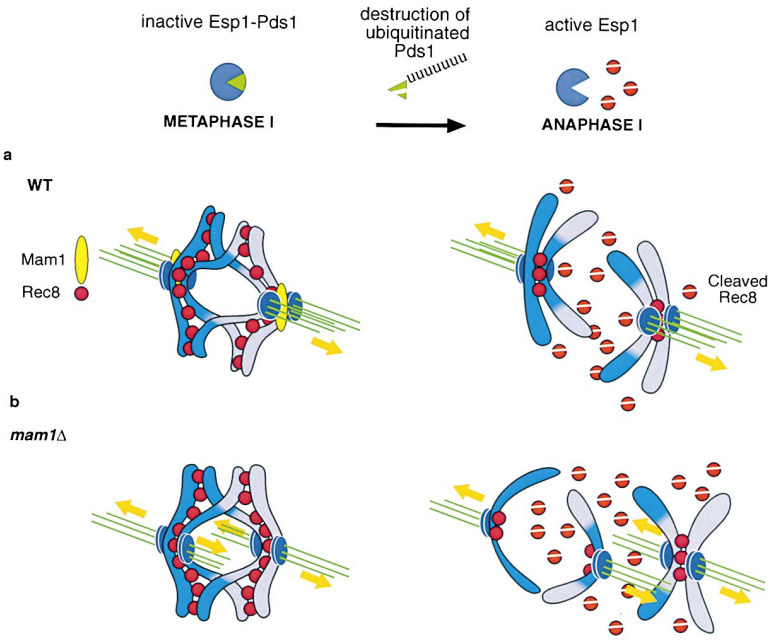


Figure 8. Model for Mam1 Function

(a) During meiosis I in wild-type cells, Mam1 enforces monopolar microtubule attachment of sister kinetochores, either by promoting fusion or copolarization of sister kinetochores or by inhibiting one of the two sister kinetochores. Homologs and not sister chromatids segregate to the opposite poles after separase cleaves Rec8 along chromosome arms at the onset of anaphase I. Cohesion is maintained at the centromeres of chromosomes until anaphase II, which enables bipolar attachment of sister chromatids in the absence of Mam1 during meiosis II.

(b) In *mam1*Δ cells, in contrast, sister kinetochores attach to microtubules emanating from the opposite poles of the cell during meiosis I, but Rec8 is still preserved at centromeres when it is cleaved by separase along chromosome arms at the onset of anaphase I. As a result, the pulling force of meiotic spindles is opposed by centromeric cohesion, chromosomes cannot segregate to the poles, and spindles cannot elongate. Nevertheless, some sister centromeres separate, probably because the spindle sometimes overwhelms cohesion and tears sisters apart. Only after centromeric Rec8 is destroyed at the onset of anaphase II can all of the chromosomes segregate to the poles.

associated and homologs move toward opposite poles (Figures 1b and 8a) during metaphase I. This bi- or coorientation of homologous kinetochores is greatly reduced if not entirely absent in *mam1*Δ cells (Figure 1b). Mam1 is therefore an essential part of the apparatus that generates coorientation during meiosis I (Figure 8a).

Unlike *spo13*Δ mutants, *mam1*Δ cells fail to segregate chromosomes either equationally or reductionally at the first meiotic division, even though spindle formation, Pds1 destruction, and Rec8's removal from the chromosomal arms all take place on schedule. Remarkably, this aborted meiosis I division can be turned into a perfect equational division when, by replacing Rec8 with its mitotic counterpart Scc1, cohesion between centromeres is destroyed at the same time as that between arms. Our explanation for this result is that Mam1 is required for monopolar attachment but not for protecting cohesion between sister centromeres after anaphase I. We cannot, however, exclude the possibility that centromeric cohesion is weakened in *mam1*Δ cells.

A uniquely pathological situation therefore arises in *mam1*Δ cells (Figure 8b). Spindles attempt to split sister chromatids but these remain held together by cohesion in the vicinity of centromeres. Under these "tug of war" conditions, microtubules sometimes manage to split sisters along at least part of an interval surrounding centromeres, but centromeric cohesion wins this battle and thereby blocks chromosome segregation and elongation of the spindle (Figure 8b). The meiotic cycle nevertheless rolls on, cohesion between sister centromeres becomes susceptible to separase, and all sister chromatids move to opposite poles when the second round of Pds1 destruction allows destruction of cohesion between sister centromeres during meiosis II. Were it not for the existence of two spindle axes during this second division, *mam1*Δ cells might merely produce viable dyads, but this does not occur, some chromosomes are segregated along a second spindle axis, and spore viability is therefore very low.

The phenotype of *mam1*Δ mutants suggests that both sister kinetochores of meiosis I cells are in principle capable of capturing microtubules from opposite poles but are specifically prevented from doing so by the Mam1 protein. How Mam1 performs this function is unclear. It will be important to establish whether sister kinetochores both attach to spindles but are somehow copolarized by Mam1 or whether Mam1 ensures that only one kinetochore from each sister pair attaches to microtubules. Cytological studies of spermatogenesis in *Drosophila* and *Arcyptera* suggest that the former occurs, at least in flies and grasshoppers (Goldstein, 1981; Suja et al., 1991; Paliulis and Nicklas, 2000).

Our work has also been revealing about proteins that were thought to be an integral part of the monopolar attachment apparatus. The observation that deletion of *REC8* from *S. pombe* permits cells to undergo an equational division during meiosis I has led to the suggestion that Rec8-like proteins are required for monopolar attachment (Watanabe and Nurse, 1999). Our observation that sister kinetochores move to the same pole in a monopolar fashion during meiosis I when Rec8 is replaced by Scc1 demonstrates that Rec8 does not possess any unique property that is necessary for monopolar attachment, at least in *S. cerevisiae*.

Rec8 but Not Scc1 Is Protected from Separase during Meiosis I

Reductional chromosome segregation during meiosis I produces haploid gametes only if it is followed, without an intervening round of DNA replication, by an equa-

tional division at meiosis II. A crucial property of reductional meiosis I divisions that make the equational divisions at meiosis II possible is the retention of cohesion between sister chromatids in the vicinity of centromeres. Because Rec8 is present at centromeres during both meiosis I and meiosis II, this remarkable property of meiotic centromeres must be regulated either by a meiosis I specific cohesion "protector" or by a meiosis II specific cohesion "destructor" protein.

Although our screen has so far failed to identify such a protein, our experiments have nevertheless shed important insight into Rec8's role in this crucial aspect of centromere behavior. When expressed instead of Rec8 during meiosis, Scc1 mediated efficient sister chromatid cohesion, which was capable of supporting monopolar attachment of sister kinetochores when Mam1 was present and an equational division when absent. Despite this impressive performance, Scc1 disappeared from centromeres at the same time as it disappeared from arms (during anaphase I), it failed to hold sister centromeres together between meiosis I and meiosis II, and could not support an equational second division. These observations indicate that centromeric Scc1, unlike Rec8, cannot be protected from separase when this protease is activated at the first metaphase to anaphase transition. The implication is that resistance to separase cleavage is confined to a very specific class of cohesin bridges: those mediated by Rec8 in the vicinity of centromeres.

There are several possible mechanisms by which Rec8 might be maintained at centromeres after anaphase I. Rec8 in the vicinity of centromeres might simply be less susceptible to cleavage by separase than Rec8 on chromosome arms. In which case, Rec8 might eventually be removed from centromeres during anaphase II by a separase-independent mechanism. Another possibility is that centromeric Rec8 is very specifically protected from separase by a meiosis-specific "protector" that is neutralized or removed by anaphase II. We favor the second model, because Rec8 can replace Scc1 in supporting the proliferation of mitotic cells (Buonomo et al., 2000) and must presumably, therefore, be removed from centromeres during mitosis. It is interesting that Spo13 has many of the properties expected for the hypothetical protector protein; it is meiosis specific, disappears from cells after meiosis I (data not shown), and is possibly essential for protecting cohesion at centromeres (Klein et al., 1999).

We suggest that Rec8's separase resistance during meiosis I is propagated from centromeres with the aid of a meiosis I specific protein (possibly Spo13) and that this process is halted by the nearest cross over. Future studies must ascertain whether the centromere-specific signal is also meiosis specific. It is clear that Mam1, which is the only known meiosis-specific kinetochore protein, is not essential. The centromere/kinetochore proteins that initiate the propagation of separase resistance do not need to be specific to meiotic cells. Indeed, Mei-S332, which is essential for the persistence of centromeric cohesion until anaphase II in *Drosophila*, is located at centromeric heterochromatin during both meiosis and mitosis (Moore et al., 1998; Tang et al., 1998), as indeed, is Slk19 in yeast (Kamieniecki et al., 2000). The exact mechanism by which MEI-S332 helps to maintain centromeric cohesion during meiosis is still unclear. MEI-S332 might either form separase-resistant bridges between sister centromeres or protect other cohesive proteins like Rec8/Scc1 from separase.

Insufficiency of Rec8 and Mam1

An important goal in understanding meiosis is to determine the meiosis-specific proteins that distinguish it from mitosis. Reproducing meiotic chromosome behavior in mitotic cells is an important criterion for success in this venture. The question therefore arises whether Rec8 and Mam1, both of which are meiosis-specific proteins, can confer to mitotic cells separase-resistant sister centromere cohesion and monopolar attachment respectively. The answer is clearly "no". Rec8 can replace Scc1 in mitotic cells (Buonomo et al., 2000), at least when cells grow at low temperatures, whereas expression of Mam1 from the GAL promoter does not block vegetative proliferation (data not shown). This implies that centromere cohesion protection and monopolar attachment both require more than one meiosis-specific protein. An obvious candidate is Spo13, which is both meiosis-specific and somehow involved in both of these aspects of centromere behavior.

Conclusion

Attachment of sister kinetochores to the same spindle pole and the persistence of cohesion between sister centromeres during meiosis I are crucial aspects of meiotic chromosome behavior. Our work definitively identifies meiosis-specific proteins that are specifically required for one (Mam1) and the other (Rec8) property. The lack of sequence conservation in Mam1 (monopolin) has not yet permitted us to pinpoint homologs in other organisms, whereas Rec8-like proteins are found in most eukaryotic genomes. Our work confirms what had long been suspected, that monopolar attachment is conferred by a kinetochore associated protein, which we call monopolin. Future studies must identify other players in these two processes and elucidate the molecular mechanisms responsible for these two remarkable aspects of meiotic chromosome behavior. It is quite possible that defects in either process could contribute to the genesis of trisomy in humans, for example, Down's syndrome, or that inappropriate expression of proteins like Rec8 or Mam1 in mitotic cells could contribute to the high rates of chromosome loss in tumor cells.

Experimental Procedures

Plasmids, Yeast Strains, and the Screen

The list of deleted genes is included in the Supplementary Data (http://www.cell.com/cgi/content/full/103/7/1155/DC1). All gene deletions were performed by PCR-mediated gene replacement (Wach et al., 1994), exchanging the complete sequence of ORFs with the *HIS5* gene from *S. pombe*, complementing a his3Δ in *S. cerevisiae*. Gene deletions were obtained in the strain K8409 (SK1 *MATa/α HO, REC8-HA3::URA3, LEU2::tetR-GFP, URA3::tetO224, his3Δ, trp1Δ*). Heterozygous gene deletions were confirmed by PCR. For each gene deletion, we dissected five tetrads from sporulated cultures of three independent heterozygous transformants. By following the segregation of the *HIS5* gene from *S. pombe*, we were able to identify spore colonies that were homozygous for the gene deletion. (Note that the spores were *HO* and, therefore, were dip-

loidized after germination.) Homozygous gene deletions were confirmed by PCR in spore colonies. Cultures of homozygous deletion strains were sporulated on sporulation plates at 30°C. Sporulation efficiency and chromosome segregation were monitored after 36 hr in each homozygous gene-deletion strain. Three independent clones of each gene deletion were dissected, checked by PCR, and analyzed for defects in meiosis. All experimental strains were derivative of K8271 (SK1 *MATα ho::LYS2, lys2, ade2Δ, ura3Δ, leu2Δ, trp1Δ, his3Δ*) *REC8-N* mutants were obtained as previously described (Buonomo et al., 2000). The *REC8* promoter was amplified by PCR from genomic DNA with two oligonucleotides carrying NarI and XbaI restriction sites. The PCR product was cloned into the same sites within YIplac128 (Gietz and Sugino, 1988). The *SCC1-HA3* sequence was subcloned into the resulting plasmid into the XbaI and SphI sites. The construct P_{REC8}-*SCC1-HA3* was integrated using the *REC8* promoter upstream of the *rec8Δ::kanMX4*, following plasmid linearization with MluI. Single integration of the plasmid was confirmed by Southern blot.

Sporulation Procedures

Sporulation was induced at 30°C in cultures of diploid cells as described previously (Buonomo et al., 2000), except that we used 2% instead of 1% YEPA to grow cells prior to sporulation induction.

Chromosome Spreading

Chromosome spreading was performed according to procedures described previously (Nairz and Klein, 1997; Loidl et al., 1998). To detect Rec8-HA3, Rec8-N-HA3 and Ndc10-HA6, mouse anti-HA 16B12 (Babco) and rat anti-HA 3F10 (Babco) antibodies were used at 1:600 dilution. The secondary antibodies were goat anti-mouse Cy3 1:500 (CHEMICON) and, in the case of Ndc10-HA6, donkey anti-rat Cy5 1:100 (CHEMICON). Zip1 and Ndc10 were detected using anti-Zip1 and anti-Ndc10 polyclonal rabbit antibodies kindly provided by Shirleen Roeder and Tony Hyman, respectively. Goat anti-rabbit FITC 1:100 (CHEMICON) and goat anti-rabbit Cy5 1:100 (CHEMICON) were used as secondary antibodies for Zip1 and Ndc10 detection, respectively. Rec8-Myc9 and Mam1-myc9 were detected using rabbit anti-Myc 1:200 (Gramsch) and mouse anti-myc 1:5 9E10, respectively. Goat anti-rabbit Cy5 1:100 (CHEMICON) and goat anti-mouse Cy3 1:100 (CHEMICON) were used as the secondary antibodies.

Western Blotting

Cell extracts were prepared by TCA-precipitation as described previously (Piatti et al., 1996). Equal amounts of protein were analyzed by SDS-PAGE and blotting was performed according to standard procedures (Sambrook et al.., 1989). The HA epitope tag was detected using the mouse 16B12 antibody 1:10,000. The Myc epitope tag was detected using the mouse 9E10 antibody 1:200. Rabbit anti-Swi6 antibody was diluted 1:100,000 (Klein et al., 1999).

In Situ Immunofluorescence

In situ immunostaining was performed according to Piatti et al. (1996). α-tubulin staining was performed using rat anti-α-tubulin antibody 1:100 (Serotec). The secondary antibody was either goat anti-rat Cy3 1:100 (CHEMICON) or donkey anti-rat Cy5 1:100 (CHEMICON). Pds1-myc18 was detected using rabbit anti-Myc 1:200 (Gramsch). As a secondary antibody, we used either goat anti-rabbit FITC 1:100 (CHEMICON) or donkey anti-rabbit Cy3 1:100 (CHEMICON). Rec8-HA3 was detected using mouse anti-HA 16B12 1:600 (Babco). As a secondary antibody, we used either goat anti-mouse Cy3 1:500 (CHEMICON) or goat anti-mouse FITC 1:200 (CHEMICON).

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