Recombination protein Tid1p controls resolution of cohesin-dependent linkages in meiosis in *Saccharomyces cerevisiae*

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Sister chromatid cohesion and interhomologue recombination are coordinated to promote the segregation of homologous chromosomes instead of sister chromatids at the first meiotic division. During meiotic prophase in *Saccharomyces cerevisiae*, the meiosis-specific cohesin Rec8p localizes along chromosome axes and mediates most of the cohesion. The mitotic cohesin Mcd1p/Scc1p localizes to discrete spots along chromosome arms, and its function is not clear. In cells lacking Tid1p, which is a member of the SWI2/SNF2 family of helicase-like proteins that are involved in chromatin remodeling, Mcd1p and Rec8p persist abnormally through both meiotic divisions, and chromosome segregation fails in the majority of cells. Genetic results indicate that the primary defect in these cells is a failure to resolve Mcd1p-mediated connections. Tid1p interacts with recombination enzymes Dmc1p and Rad51p and has an established role in recombination repair. We propose that Tid1p remodels Mcd1p-mediated cohesion early in meiotic prophase to facilitate interhomologue recombination and the subsequent segregation of homologous chromosomes.

Introduction

Chromosome structure and nuclear architecture are modified during meiosis to ensure that homologous chromosomes become connected and oriented to disjoin properly in the first meiotic division. These modifications likely begin with the establishment of meiosis-specific cohesin and condensin organization in premeiotic S-phase (Klein et al., 1999; Yu and Koshland, 2003). Additional meiosis-specific proteins create programmed DNA double strand breaks (DSBs) and regulate their repair during meiotic prophase so that a homologous chromatid is used as the template for repair rather than the sister chromatid. This process generates crossovers between the homologues. Sister chromatid cohesion that is distal to each crossover could be sufficient to provide stable interhomologue connection until release at the first meiotic anaphase (Maguire, 1995; Roeder, 1997; Nasmyth, 2001; Petronczki et al., 2003). How recombination repair of meiotic DSBs is prevented from using the sister chromatid, is directed to use the homologous chromosome, and is regulated to produce the appropriate connections remain important unanswered questions.

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Abbreviations used in this paper: DSB, double strand break; SC, synaptonemal complex; SPB, spindle pole body.

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In meiosis, the Mcd1p subunit of cohesin largely is replaced by the meiosis-specific subunit Rec8p, which localizes to the meiotic chromosome axes (Klein et al., 1999). Meiotic recombination is also associated with chromosome axes. In meiosis, several cohesin subunits are required both for axis formation and successful completion of DSB repair (Klein et al., 1999; Revenkova et al., 2004). However, close associations between sister chromatids near DSBs could be an impediment for interhomologue recombination. Several lines of evidence indicate requirements for cohesion and cohesins in the repair of DSBs in mitotic cells, where sister-biased recombination is the preferred pathway for the repair of DSBs (Kadyk and Hartwell, 1992). First, in several organisms, genes encoding proteins that are required for cohesion were described to be required for DNA repair (Huynh et al., 1986; Birkenbihl and Subramani, 1992; Denison et al., 1993). Second, mutations in genes encoding subunits of cohesin complexes lead to precocious sister chromatid separation and defective postreplicative repair in yeast (Sjogren and Nasmyth, 2001). Similarly, the depletion of chicken Rad21p/Mcd1p/Scc1p leads to premature sister separation, reduced efficiency of DSB repair, and reduced frequency of sister chromatid exchange (Sonoda et al., 2001). Third, the establishment of cohesion in the preceding S-phase is required

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for successful DSB repair in G2 (Sjogren and Nasmyth, 2001). Fourth, cohesins are recruited to the sites of induced DSBs both in yeast and in mammals (Kim et al., 2002; Strom et al., 2004; Unal et al., 2004). Newly loaded cohesins are able to hold sister chromatids together (Strom et al., 2004), suggesting that cohesins could promote mitotic DSB repair by ensuring close proximity of sister chromatids. Bias toward interhomologue recombination in meiosis would require disfavoring the sister chromatid as a donor for DSB repair (for example, by locally destroying the close association between sister chromatids). Thus, components of the recombination machinery could remodel cohesion around DSBs to ensure use of the homologue for DSB repair in meiosis.

Efficient interhomologue recombination during vegetative growth and during meiosis requires Tid1p, which is a member of the SWI2/SNF2 family of helicase-like chromatin-remodeling proteins and is a paralogue of the recombination repair protein Rad54p (Eisen et al., 1995; Shiratori et al., 1999). However, in mitotic cells, the major repair pathway for DSBs uses the sister chromatid as a template even when a homologue is present and requires Rad54p and an interacting strand exchange enzyme, Rad51p (Kadyk and Hartwell, 1992; Paques and Haber, 1999; Symington, 2002; Tan et al., 2003). Thus, although Tid1p may interact with Rad51p (Dresser et al., 1997) and can promote the strand exchange activity of Rad51p in vitro (Petukhova et al., 2000), the effect of *TID1* deletion on the repair of DSBs and on survival in mitotic cells is subtle unless sister chromatid–based repair is eliminated (Arbel et al., 1999; Lee et al., 2001; Signon et al., 2001; Ira and Haber, 2002).

During meiosis, most DSBs are repaired by a pathway that normally requires Dmc1p (a meiosis-specific paralogue of Rad51p; Bishop et al., 1992) in addition to Rad51p (Shinohara et al., 1992). Tid1p interacts with Dmc1p in the two-hybrid system (Dresser et al., 1997) and promotes Dmc1p colocalization with Rad51p on meiotic chromosomes (Shinohara et al., 2000), suggesting that Tid1p may serve together with Dmc1p during recombination repair to bias meiotic recombination specifically toward interhomologue repair (Dresser et al., 1997; Shinohara et al., 1997, 2003). The deletion of *TID1* causes a delay in the processing of meiotic DSBs and increased resection at the broken DNA ends (Shinohara et al., 1997). However, even though mature recombination products do eventually form, at least at an artificial DSB hot spot (Shinohara et al., 1997), the majority of *tid1Δ* cells fail to sporulate (Klein, 1997; Shinohara et al., 1997).

We describe a novel phenotype of the deletion of *TID1* that implicates Tid1p in the remodeling of chromosome structure (specifically sister chromatid cohesion). Tid1p is required for normal chromosome segregation in both meiotic divisions. The segregation defect in *tid1Δ* cells results from a failure of sister chromatid separation. In a *spo11Δ spo13Δ* background, there is no meiotic recombination, and the sole meiotic division requires that sister chromatids (not homologues) are separated. When *tid1Δ* is placed in the *spo11Δ spo13Δ* background, two thirds of the cells block in anaphase. This block is not suppressed by *REC8* deletion alone. However, in the *spo11Δ spo13Δ* background, the *tid1Δ* block is suppressed by *mcd1-1* (heat sensitive) if shifted to the nonpermissive temperature before or during meiotic prophase. It is also suppressed by *mcd1-1 rec8Δ* if shifted to the nonpermissive temperature at any time before cells begin to lose viability at the block. Moreover, both Mcd1p and Rec8p persist abnormally long in *tid1Δ* in an otherwise *wild-type* background. Our observations suggest that Tid1p plays a role in cohesion remodeling in meiotic prophase that is required for the successful separation of sister chromatids in anaphase.

Results

Deletion of *TID1* prevents sporulation by arresting the majority of cells in pachytene and by blocking cells that progress past pachytene in anaphase I and II

To determine the kinetics of progression through meiotic divisions, we used a strain with a single *TUB1-GFP* allele to visualize the spindles. Cells were stained with DAPI to visualize DNA. The majority of *tid1Δ* cells are blocked as mononucleate

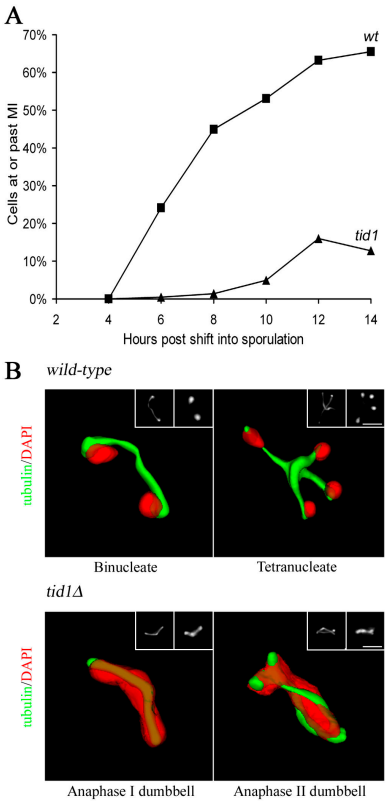

Figure 1. **Prophase arrest and anaphase block caused by *tid1Δ*.** (A) Kinetics of entry into the first meiotic division of *wild-type* and *tid1Δ*. Progression through the divisions is monitored by the behavior of the spindle (*TUB1-GFP*) and DAPI-stained chromatin. Cells at or past metaphase I are represented by cells with a single mass of chromatin with a spindle, by dumbbells, and by cells that have separated their chromatin into two (binucleates) or four masses (tetranucleates). *wild-type*, squares; *tid1Δ*, triangles. At least 200 cells were counted at each time point. (B) Three-dimensional images of chromatin (red, DAPI) and spindles (green, antibodies against tubulin) of dividing cells in *wild-type* (binucleate and tetranucleate) and *tid1Δ* (anaphase I and II dumbbells), displayed by isointensity surface extraction (and some rotation with respect to insets). The insets are gray-scale maximum intensity projections of the separate chromatin (right insets) and spindle signals (left insets) in the same cells. Bars, 4 μm.

cells with no spindle (Fig. 1 A) and remain blocked even 26 h after the shift into sporulation. At the same time, *tid1Δ* does not affect the kinetics of formation and the appearance of the synaptonemal complex (SC), suggesting that Tid1p is not required for synapsis. The accumulation of cells with fully formed SC in *tid1Δ* indicates that the deletion of *TID1* blocks cells in pachytene (Fig. S1, A and B, available at http://www.jcb.org/cgi/content/full/jcb.200505020/DC1). *tid1Δ* cells that were blocked in pachytene could be arrested by the pachytene checkpoint, which is triggered by unrepaired DSBs. Turnover of DSBs and formation of mature recombination products are delayed in *tid1Δ* (Fig. S1, C–F), confirming that pachytene arrest in *tid1Δ* is associated with the defective processing of DSBs.

The small percentage of *tid1Δ* cells that progress past pachytene (~16% of cells progress into divisions after an ~3-h delay; Fig. 1 A) reveals a new defect. Two thirds of these cells are blocked in anaphase I (single long spindle), whereas the remaining third enter the second division and are blocked in anaphase II (two long spindles). Chromatin in both types of blocked cells remains as a single stretched mass ("dumbbell"), indicating that the chromosomes stay largely unsegregated even though one or both divisions had occurred according to spindle behavior (Fig. 1 B). A very small fraction of cells (≤1%) successfully completes the meiotic divisions and segregates chromatin into four distinct masses. Consistent with the defect in chromatin segregation, the deletion of *TID1* increases the missegregation of chromosome *IV* in the *spo13Δ* background (Fig. S2, available at http://www.jcb.org/cgi/content/full/jcb.200505020/DC1).

Deletion of *SPO11* suppresses the *tid1Δ* pachytene arrest and allows anaphase I segregation

To prove that pachytene arrest of the majority of *tid1Δ* cells depends on DSB formation, the *SPO11* gene, which encodes the meiosis-specific endonuclease that is responsible for producing DSBs and chiasmata (Cao et al., 1990; Keeney et al., 1997), was deleted, and the progression of cells through meiotic divisions was monitored by staining chromatin with DAPI. *spo11Δ* suppresses the pachytene arrest of *tid1Δ*, allowing 68% of *spo11Δ tid1Δ* cells to enter the divisions without delay (Fig. 2 B), which supports the idea that the *tid1Δ* pachytene arrest is caused by unrepaired DSBs.

The deletion of *SPO11* also permits testing of whether or not the *tid1Δ* defect in chromosome segregation in the first meiotic division is caused by a failure to dissolve chiasmate connections between homologues. Chiasmata are produced as a result of interhomologue recombination and are stabilized by cohesion distal to crossover sites (see Introduction). s*po11Δ* eliminates interhomologue recombination and, thus, chiasmata and leads to random segregation of homologous chromosomes in the first meiotic division (Fig. 2 A; Klein et al., 1999). *spo11Δ* suppresses the anaphase I block of *tid1Δ*. In *spo11Δ tid1Δ*, most of the dumbbells are resolved after 10 h of sporulation, whereas in *tid1Δ*, they persist until their chromatin becomes fragmented after 16 h of sporulation (Fig. 2 C and not depicted). This suggests that the *tid1Δ* failure of segregation in the first division is caused by unresolved connections between homologues. Homologues may remain connected in *tid1Δ*

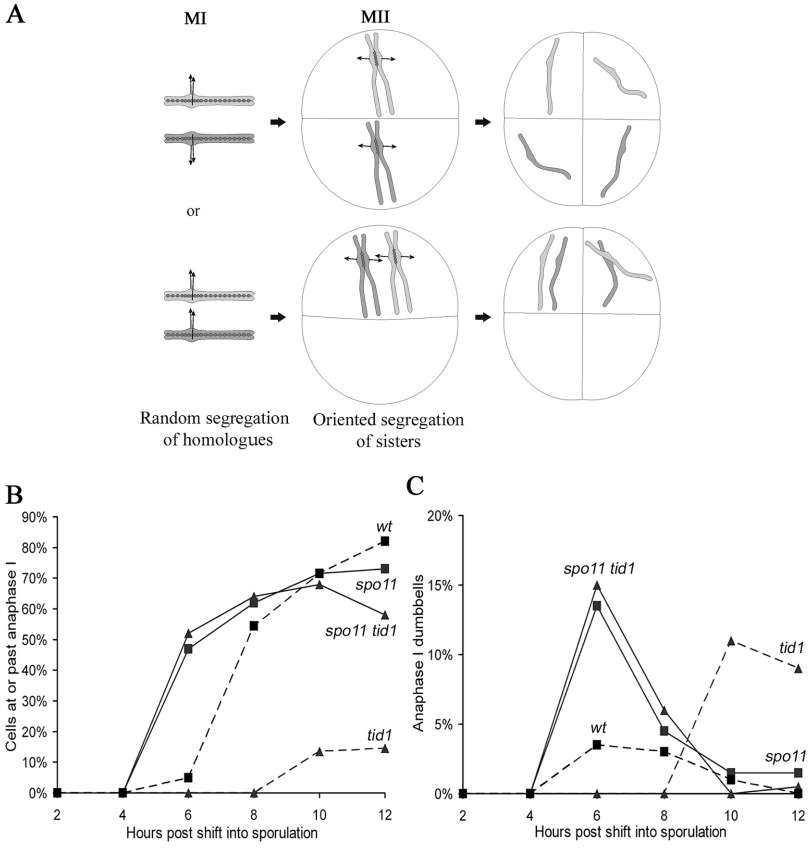

Figure 2. **Suppression of *tid1Δ* by the deletion of *SPO11*.** Progression of cells through the divisions was monitored by staining chromatin with DAPI. (A) Segregation of chromosomes in *spo11Δ*. Gray lines represent chromatids. Thickenings on gray lines are centromeres. Black dots are cohesins. Arrows represent the pulling forces of spindles. (B) Kinetics of entry into anaphase I. Cells at or past anaphase I are anaphase I and II dumbbells, binucleates, and tetranucleates. (C) Anaphase I dumbbells. At least 200 cells were counted at each time point.

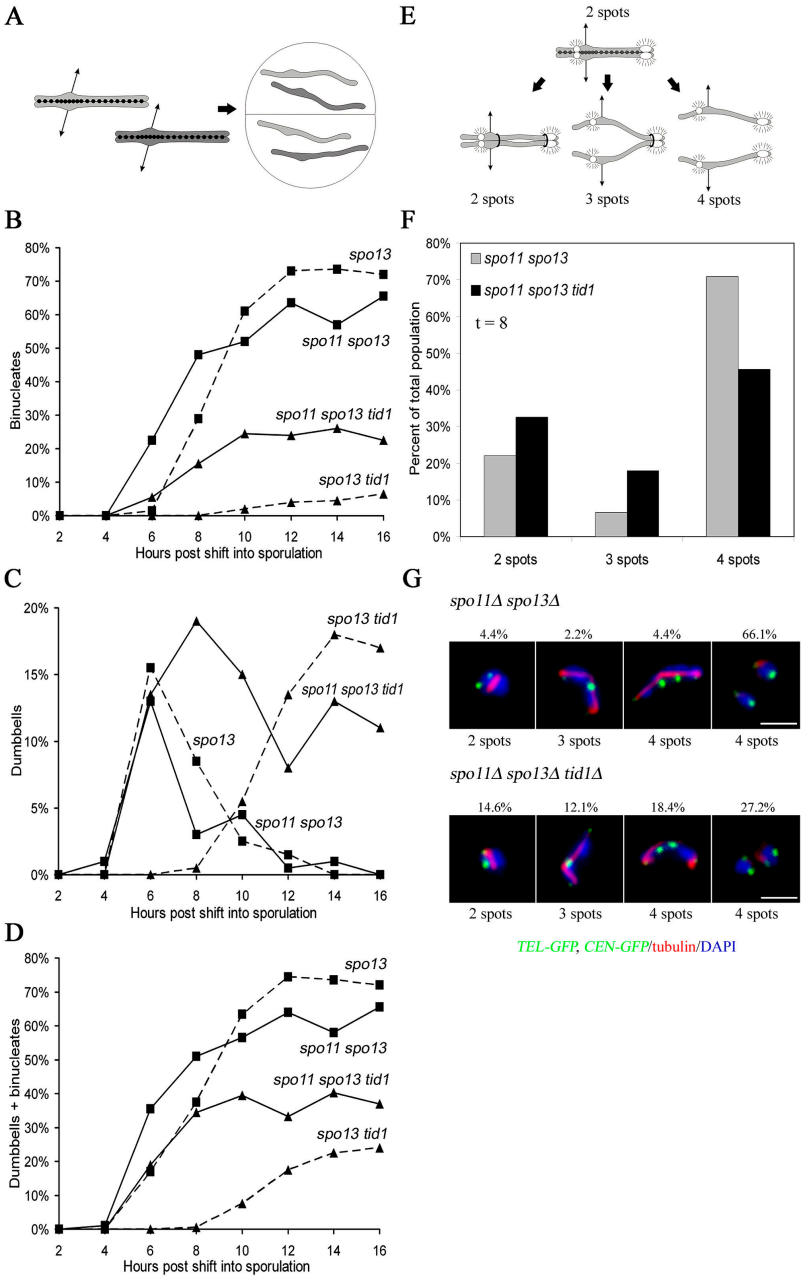

Figure 3. **Segregation defects of *tid1Δ* in the *spo11Δ spo13Δ* background.** (A) Segregation of sister chromatids in *spo11Δ spo13Δ*. Grey lines connected by black dots represent sister chromatids that are connected by cohesin complexes. Thickenings represent centromeres. Arrows represent the pulling forces of the spindle. (B) Kinetics of exit from the single meiotic division as marked by the appearance of binucleates. (C) Percentage of dumbbells. (D) Percentage of dumbbells and binucleates. The value at each point in graph B is derived by adding the values from the same time point in graphs C and D. 200 or more cells were scored at each time point. (E) Schematic representation of possible outcomes of segregation of sister chromatids labeled with telomere and centromere GFP spots in *spo11Δ spo13Δ tid1Δ*. White dots represent GFP spots. Black rings around sister chromatid represent connections persisting in *tid1Δ*. Only the labeled chromosome is shown. (F) Comparison of the segregation of chromosome *IV* sister chromatids in total populations of *spo11Δ spo13Δ tid1Δ* and *spo11Δ spo13Δ*. Differences between *spo11Δ spo13Δ tid1Δ* and *spo11Δ spo13Δ* are statistically significant ($P < 0.05$; X^2 test). (G) Segregation of chromosome *IV* sister chromatids labeled by centromere and telomere GFP spots in postprophase cells only. Data in F and G are results from the same experiment. Above the pictures are the percentages of each class of cells in the total population. Only classes of cells representing >3% of the total population are shown for *spo11Δ spo13Δ tid1Δ*, and only those same classes of cells are shown for *spo11Δ spo13Δ* as controls. Bars, 2 μm.

because of a failure to resolve sister chromatid cohesion that is distal to crossover sites. Even though *spo11Δ* suppresses the defects of *tid1Δ* in the first meiotic division, in *spo11Δ tid1Δ*, anaphase II dumbbells accumulate, and fewer cells finish the second meiotic division than in *spo11Δ* (this difference was seen in four independent trials; unpublished data). This raises the possibility that *tid1Δ* affects sister chromatid separation.

tid1Δ blocks sister chromatid segregation in the *spo11Δ spo13Δ* background

The effect of *TID1* deletion on the ability of cells to separate sister chromatids was tested in a background where sister chromatids segregate instead of homologues. The introduction of *spo13Δ* in the *spo11Δ* background, where meiotic recombination is eliminated, leads to biorientation of sister kinetochores and subsequent segregation of sister chromatids in the single meiotic division (Fig. 3 A; Klapholz et al., 1985; Shonn et al., 2002). In *spo11Δ spo13Δ tid1Δ*, 23% of cells complete the separation of chromatin into two masses (binucleates) compared with 66% of cells in *spo11Δ spo13Δ* (Fig. 3 B). Thus, even in the absence of interhomologue recombination, the deletion of *TID1* prevents some *spo11Δ spo13Δ* cells from successfully finishing the division, presumably because of the failure to segregate sister chromatids in the absence of Tid1p. Consistently, one reason for the reduction in the percentage of binucleates is the accumulation of dumbbells (Fig. 3 C), which begin degrading at ~10 h into sporulation (unpublished data). Another reason is an accumulation of cells with a single unstretched mass of chromatin (mononucleates) and a short spindle. In *spo11Δ spo13Δ tid1Δ*, only 40% of cells progress

past mononucleate stage (Fig. 3 D) compared with 70% in *spo11Δ tid1Δ* (Fig. 2 B), which is higher than the 15–25% in *tid1Δ* but lower than the 66% in *spo11Δ spo13Δ* (Fig. 3 B). Tagging of spindle tubulin with GFP reveals that the mononucleate cells consist of two classes: cells with a single spindle pole body (SPB) and cells with a short spindle. To determine what percentage of the population is represented by each class, we scored 50 *spo11Δ spo13Δ* mononucleate cells (29% of the total population is mononucleate at 8 h) and 68 *spo11Δ spo13Δ tid1Δ* mononucleate cells (53% of the total population is mononucleate at 8 h). The fraction of cells with a single SPB is similar in *spo11Δ spo13Δ* and *spo11Δ spo13Δ tid1Δ* (14% of each total population). However, the fraction of cells with a short spindle is lower in *spo11Δ spo13Δ* than in *spo11Δ spo13Δ tid1Δ*: 15 versus 39% of the total population, respectively. In addition, the fraction of cells with a short spindle subsequently decreases in *spo11Δ spo13Δ*, whereas it persists essentially unchanged in *spo11Δ spo13Δ tid1Δ* (unpublished data). Thus, the failure of *spo11Δ spo13Δ tid1Δ* cells to divide their chromatin into two masses can be attributed to a block with two phenotypes: dumbbells and mononucleate cells with a short spindle. The *tid1 K351R* allele, which is designed to eliminate ATP hydrolysis (Petukhova et al., 2000), causes a similar block in the *spo11Δ spo13Δ* background (unpublished data). A similar phenotype has been reported in other mutants where sister chromatids fail to segregate, leading to a block with a metaphase-length spindle (Toth et al., 2000; Clyne et al., 2003; Lee and Amon, 2003; Rabitsch et al., 2003).

Connections between sister chromatids persist in cells blocked in anaphase by the deletion of *TID1*

To examine the segregation of sister chromatids in greater detail, one of the homologues of chromosome *IV* was labeled with an array of *tetO* less than 2 kb away from the centromere (small GFP spot; He et al., 2000) and an array of *lacO* near the right telomere (~1,071 kb away from centromere *IV*; large GFP spot). These arrays bind GFP-linked *tetR* and *lacI*, respectively. Spots were visualized by using anti-GFP antibodies in fixed whole cells. Tubulin that was visualized with antitubulin antibodies and chromatin that was stained with DAPI were used to distinguish between different cell classes. Cells with two or four spots indicate unseparated or separated *IV* sister chromatids, respectively, whereas cells with three spots indicate the separation of chromatids at the centromeres but not at the telomeres (Fig. 3 E). To avoid scoring cells that have begun to degrade, samples were scored at the 8-h time point. The introduction of *tid1Δ* decreases the percentage of cells with four spots, whereas it increases the percentage of cells with two or three spots in the *spo11Δ spo13Δ* background (Fig. 3 F). Most of the increase in the percentage of cells with three GFP spots is accounted for by dumbbells, and most of the increase in the percentage of cells with two spots is accounted for by cells with a short spindle (cells with a single SPB are responsible for the high background of cells with two GFP spots in *spo11Δ spo13Δ* and *spo11Δ spo13Δ tid1Δ*; 14% in each; Fig. 3 G). In ~25% of the *spo11Δ spo13Δ tid1Δ* binucleates, the spots either segregate 4:0 (indicating missegregation) or 3:1 (suggesting breakage), whereas these classes are relatively rare in *spo11Δ spo13Δ* (~2%). The persistence of sister association in cells with short spindles suggests that the missegregation classes result from persistent connections between the chromosome *IV* sisters. Importantly, attachment of centromeres *IV* to the spindle appears unaffected by *tid1Δ*, as the centromeres are closer to the spindle pole than are the telomeres in all dumbbells, even when telomeres *IVR* are still unseparated. This observation makes it unlikely that a defect in kinetochore attachment is responsible for the *tid1Δ* failure of chromosome segregation. Altogether, these observations are consistent with persistent sister chromatid connections, which are variable in position and/or number, causing the *tid1Δ* block to chromosome segregation. Presumably, connections relatively far from centromeres allow spindle elongation and dumbbell formation, whereas connections closer to centromeres limit spindle length and prevent stretching of the chromatin mass.

Table I. **Percentage of Pds1p-positive and -negative mononucleate cells with a short spindle in the total population**

Genotype	Percentage of Pds1+	Percentage of Pds1−
spo11 spo13	6 (27)	9 (46)
spo11 spo13 tid1	10 (57)	29 (164)

Numbers in parentheses represent the total number of cells that were scored for a given class.

Pds1p turnover is not affected by *tid1Δ* in the *spo11Δ spo13Δ* background

Failure to separate sister chromatids in *tid1Δ* could result from an inhibition of the mechanism that is responsible for dissolving sister chromatid cohesion. Pds1p is down-regulated at anaphase I, and its loss is thought to enable cohesion dissolution by Esp1p/separase action (Petronczki et al., 2003). Therefore, we monitored the turnover of HA-tagged Pds1p in whole permeabilized cells by using anti-HA and antitubulin antibodies after 8 h of sporulation, when the *spo11Δ spo13Δ tid1Δ* and *spo11Δ spo13Δ* behaviors have just begun to diverge (Fig. 3 B) but before cell degradation. No Pds1p-positive cells with stretched dumbbell nuclei were seen either in *spo11Δ spo13Δ* (eight dumbbells were scored) or *spo11Δ spo13Δ tid1Δ* (52 dumbbells were scored). The increase in the percentage of cells with a short spindle that was observed in *spo11Δ spo13Δ tid1Δ* is almost exclusively accounted for by Pds1p-negative cells (Table I). This indicates that the short spindle arrest observed in *spo11Δ spo13Δ tid1Δ* occurs downstream of Pds1p destruction. Therefore, we conclude that the deletion of *TID1* in *spo11Δ spo13*Δ results in a block of two thirds of cells in anaphase (short spindle cells plus dumbbells). The kinetics of Pds1p turnover that was measured in whole cell protein extracts on Western blots is also nearly identical in *spo11Δ spo13Δ* and *spo11Δ spo13Δ tid1Δ* (unpublished data). Thus, the persistent sister chromatid association that is caused by *tid1Δ* does not arise from any mechanism that leads to the persistence of

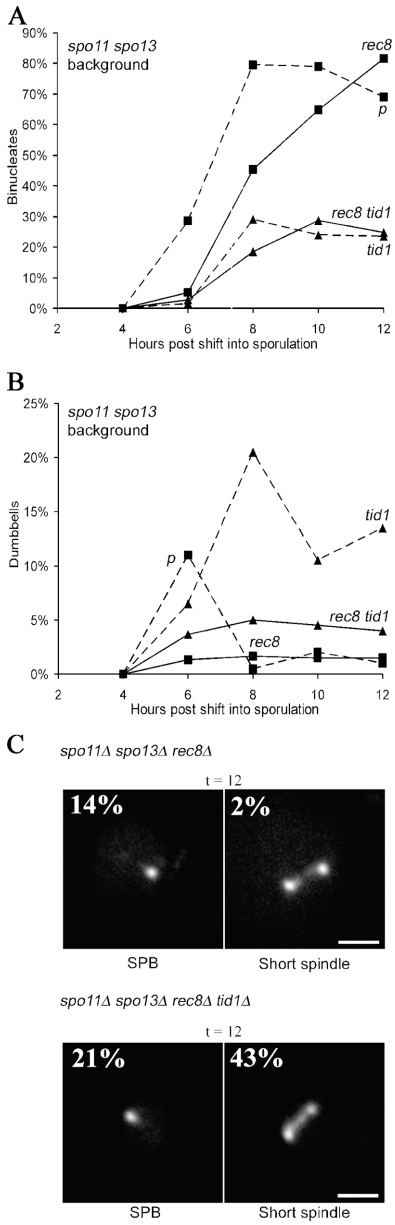

Figure 4. **Failure of *rec8Δ* to suppress the *tid1Δ* defect in sister chromatid separation in the *spo11Δ spo13Δ* background.** (A) Percentage of binucleates. (B) Percentage of dumbbells. The reduction in *rec8Δ tid1Δ*, as compared with *tid1Δ*, results from cells being blocked earlier (see C). (A and B) *spo11Δ spo13Δ*, dashed line with squares; *spo11Δ spo13Δ tid1Δ*, dashed line with triangles; *spo11Δ spo13Δ rec8Δ*, solid line with squares; *spo11Δ spo13Δ rec8Δ tid1Δ*, solid line with triangles. Progression of cells through the division is monitored using DAPI-stained chromatin. At least 200 cells were counted at each time point. (C) Spindle appearance in cells with a single unstretched mass of chromatin (as determined by DAPI staining; not depicted) in spread preparations of nuclei after 12 h of sporulation. SPB and short spindles labeled with antitubulin antibody are shown. Percentages shown are for cells of that appearance in the total population. At least 300 cells were scored for each genotype. Bars, 2 μm.

Pds1p. Note that in dumbbells, where there is no detectable Pds1p, a significant fraction nevertheless has tightly associated sisters at telomere *IVR* (Fig. 3 G, 3 spot class). This result suggests that the persistent connections occur in the presence of active Esp1p.

The sister chromatid segregation defect of *tid1Δ* is not rescued by the deletion of *REC8* in a *spo11Δ spo13Δ* background

The timely turnover of Pds1p in *spo11Δ spo13Δ tid1Δ* indicates that cells are not blocked in metaphase but, rather, progress into a defective anaphase in which sister chromatids fail to segregate. In meiosis, the maintenance of sister chromatid cohesion requires the meiosis-specific cohesin Rec8p, and its cleavage is required for chromosome segregation in anaphase (Klein et al., 1999; Buonomo et al., 2000). One possible reason for the failure of sister segregation in *tid1Δ* cells is the persistence of Rec8p-based cohesin complexes on chromosomes. To test this possibility, *REC8* was deleted in the *spo11Δ spo13Δ* background, where the effect of *tid1Δ* on sister chromatid separation is most obvious. The deletion of *REC8* does not rescue the defect of sister chromatid separation in *spo11Δ spo13Δ tid1Δ*. The percentage of cells that progress past anaphase (binucleates) is similar in *spo11Δ spo13Δ tid1Δ* and in *spo11Δ spo13Δ rec8Δ tid1Δ* (Fig. 4 A). The percentage of dumbbells is reduced in *spo11Δ spo13Δ rec8Δ tid1Δ* (Fig. 4 B) because more cells are blocked earlier with a single unstretched mass of chromatin (not depicted). To determine the fraction of cells that were blocked with short spindles, spread preparations of nuclei were prepared after 12 h of sporulation, and the spindle was visualized by immunostaining (Fig. 4 C). A large fraction of *spo11Δ spo13Δ rec8Δ tid1Δ* cells is arrested with short spindles (43% in *spo11Δ spo13Δ rec8Δ tid1Δ* vs. 2% in *spo11Δ spo13Δ rec8Δ* at 12 h of sporulation). Thus, the deletion of *REC8* fails to suppress the *tid1Δ*-mediated block to sister segregation.

Inactivation of the mitotic cohesin complex subunit Mcd1p rescues the *tid1Δ* anaphase block in the *spo11Δ spo13Δ* background

In mitotic cells, the maintenance of cohesion between sister chromatids requires Mcd1p, which is a homologue of Rec8p (Guacci et al., 1997; Michaelis et al., 1997). Although Mcd1p has been reported not to have a major role in the maintenance of cohesion during meiosis in budding yeast (Klein et al., 1999), it does play a role in meiotic sister chromatid cohesion in fission yeast (Yokobayashi et al., 2003). Therefore, we tested whether Mcd1p is required for persistent sister chromatid connections in *tid1Δ* by using a heat-sensitive allele of *MCD1* (*mcd1-1*), which was previously shown to be defective in cohesion maintenance at nonpermissive temperature in mitotic cells (Guacci et al., 1997). Progression of cells through the division was monitored by using *TUB1-GFP* and DAPI-stained chromatin. Sporulating cells were shifted from permissive for *mcd1-1* temperature (21°C) to nonpermissive temperature (33°C) every 2 h, and binucleates were scored after at least 24 h of sporulation. Early shift to 33°C completely rescued the *tid1Δ* anaphase block (compare *spo11Δ spo13Δ mcd1-1* with *tid1Δ* vs. without *tid1Δ*; Fig. 5 A). However, this suppression disappears by metaphase (compare *spo11Δ spo13Δ mcd1-1 tid1Δ* in Fig. 5 A with *spo11Δ spo13Δ mcd1-1* in Fig. S3 C, in which cells with short spindles represent metaphase cells; available at

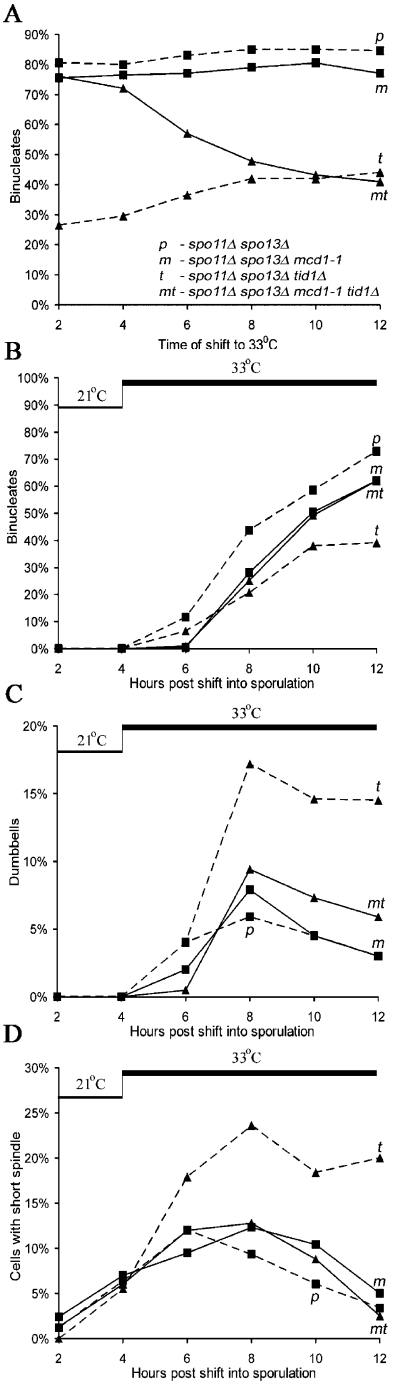

Figure 5. **Suppression of *tid1Δ* sister chromatid separation defect by *mcd1-1* in a *spo11Δ spo13Δ* background.** (A) Percentage of cells with two separated chromatin masses (binucleates) after a shift to nonpermissive (33°C) temperature at 2-h intervals. Cells were allowed to sporulate for at least 24 h after the shift to nonpermissive temperature before scoring. Fig. S3 (A and B; available at http://www.jcb.org/cgi/content/full/jcb.200505020/DC1) and (C) show the kinetics of the short spindle, dumbbell, and binucleate stages at 21°C during this experiment. (B and C) Percentage of binucleates (B) and dumbbells (C) after a shift from 21 to 33°C at 4 h. (D) Percentage of cells with short spindles after a shift from 21 to 33°C at 4 h. Progression of cells through the division is monitored by the behavior of the spindle (*TUB1-GFP*) and DAPI-stained chromatin. At least 200 cells were scored for each time point.

http://www.jcb.org/cgi/content/full/jcb.200505020/DC1). To more precisely determine the effect of Mcd1p inactivation on the *tid1Δ* anaphase block, the percentage of binucleates, dumbbells, and cells with a short spindle was compared in cells sporulating at 21°C and in cells shifted to 33°C at 4 h (Fig. 5, B–D, and Fig. S3, A–C), which is a time when *mcd1-1* completely suppresses *tid1Δ*. Consistently, identical levels of binucleates are reached by *spo11Δ spo13Δ mcd1-1* and *spo11Δ spo13Δ mcd1-1 tid1Δ* after a shift to 33°C at 4 h of sporulation. No accumulation of cells with a short spindle or of dumbbells was observed in *spo11Δ spo13Δ mcd1-1 tid1Δ* cells compared with *spo11Δ spo13Δ mcd1-1.* At 21°C, the phenotype of *tid1Δ* is unchanged by *mcd1-1*. These data suggest a direct involvement of Mcd1p in generating persistent connections in *tid1Δ*.

Deletion of *REC8* in *spo11Δ spo13Δ mcd1-1* allows late suppression of the *tid1Δ* sister chromosome segregation defect

Our data demonstrate that in the *spo11Δ spo13Δ* background, Mcd1p inactivation efficiently suppresses the *tid1Δ* anaphase block but only at early times in sporulation. This suggests that some other protein mediates persistent sister chromatid connections later in meiosis in the absence of Mcd1p. To determine whether the Mcd1p-independent sister connections are mediated by Rec8p, the effect of *rec8Δ* on the anaphase block of *tid1Δ* was studied in *spo11Δ spo13Δ mcd1-1*. Progression of cells through the divisions was monitored by using *TUB1-GFP* and DAPI-stained chromatin. Sporulating cells were shifted from permissive to nonpermissive temperature every 2 h, and binucleates were scored after 24 h of sporulation. The anaphase block of *tid1Δ* is suppressed by *mcd1-1 rec8Δ* at early times as well as at late times at 33°C (Fig. 6 A) despite the fact that cells with *tid1Δ* reach full anaphase block by 8–10 h of sporulation at 21°C (Fig. S3, D–F). A slight decrease in the level of suppression after 10 h coincides with the onset of chromatin fragmentation in a fraction of cells (not depicted). To confirm that persistent sister chromatid connections is the main reason for the anaphase block, *spo11Δ spo13Δ mcd1-1 rec8Δ tid1Δ* cells were allowed to block at anaphase (10 h of sporulation at 21°C) and were transferred to 33°C (Fig. 6, B–D). After a shift to 33°C, the turnover of anaphase-blocked cells (dumbbells and cells with a short spindle) in *spo11Δ spo13Δ mcd1-1 rec8Δ tid1Δ* occurs within 1 h, coinciding with an increase in binucleates. This quick and complete rescue indicates that both Mcd1p and Rec8p are required to maintain the sister chromatid connections that persist in *spo11Δ spo13Δ tid1Δ*.

Dissociation of cohesins Mcd1p and Rec8p from chromatin is delayed in *tid1Δ* in an otherwise *wild-type* background

Failure to segregate chromosomes in both divisions in *tid1Δ* is likely caused by Mcd1p- and Rec8p-dependent connections that persist in anaphase I and II. To confirm this possibility, the association of Mcd1p and Rec8p with chromatin was tested in both anaphase I and II in spread preparations of meiotic nuclei. Spindle behavior was used as an internal marker for progres-

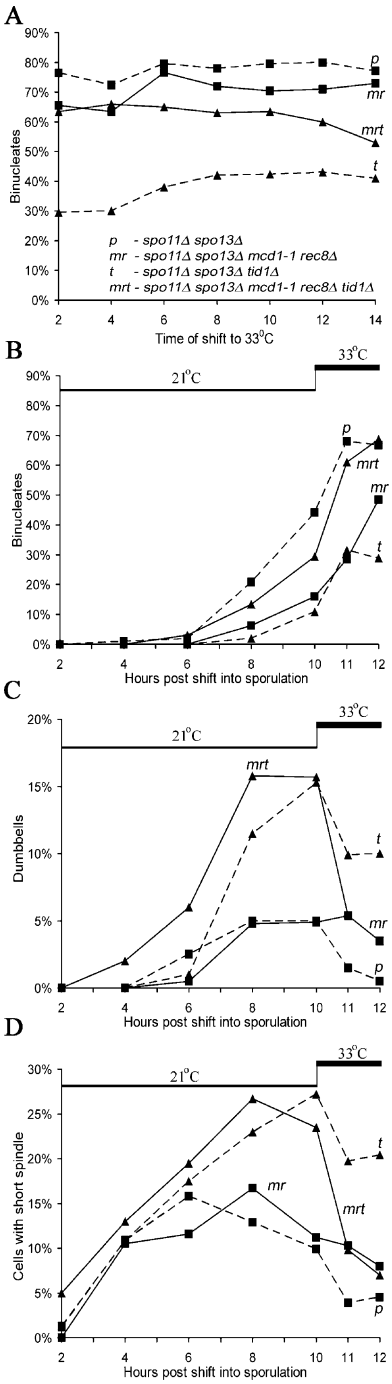

Figure 6. **Suppression of the *tid1Δ* sister chromatid separation defect by *mcd1-1 rec8Δ* in the *spo11Δ spo13Δ* background.** (A) Percentage of cells with two separated chromatin masses (binucleates) after a shift to nonpermissive temperature at 2-h intervals. Cells were allowed to sporulate for at least 24 h after a shift to nonpermissive temperature before scoring. Fig. S3 (D–F; available at http://www.jcb.org/cgi/content/full/jcb.200505020/DC1) show the kinetics of the short spindle, dumbbell, and binucleate stages at 21°C during this experiment. (B and C) Percentage of binucleates (B) and dumbbells (C) after a shift from 21 to 33°C at 10 h. (D) Percentage of cells with short spindles after a shift from 21 to 33°C at 10 h. Progression of cells through the division is monitored by the behavior of the spindle (*TUB1-GFP*) and DAPI-stained chromatin. At least 200 cells were scored for each time point.

sion through the meiotic divisions in order to compare cohesin dissociation from chromatin in *tid1Δ* with *wild-type*. Rec8p and Mcd1p were visualized by immunolabeling in spread nuclei of sporulating *tid1Δ* and *wild-type* cells. Although none of the *wild-type* cells have Rec8p that is associated with chromatin in anaphase II, Rec8p signal remains in 24% of *tid1Δ* cells that have two fully elongated spindles. Thus, the dissociation of Rec8p from chromatin is delayed in *tid1Δ* (Fig. 7, A and B). Aside from this delay, there were no obvious differences in the appearance of Rec8p in *wild-type* and *tid1Δ* cells (not depicted), which is consistent with the normal appearance of SCs in *tid1Δ* (Fig. S1 A).

Analogously, none of the wild-type cells have Mcd1p signal after metaphase I, whereas Mcd1p persists as spots on chromatin in *tid1Δ* through anaphase I (82% of cells) and II (90% of cells). Mcd1p foci in *wild-type* and *tid1Δ* are not apparently different in prepachytene nuclei (unpublished data). These data are consistent with a direct role for Mcd1p in the maintenance as well as in the establishment of persistent sister chromatid connections in *tid1Δ*.

Early suppression of *tid1Δ* anaphase block by *mcd1-1* in a *spo11Δ spo13Δ* background suggests that the misregulation of Mcd1p in the absence of Tid1p occurs before or during prophase. To confirm that *tid1Δ* has an effect on Mcd1p during prophase, Mcd1p was visualized by immunolabeling in spread nuclei of pachytene *tid1Δ* and *wild-type* cells, which were identified by the appearance of well-condensed chromosome bivalents. Mcd1p appears as spots on chromatin in spread preparations of pachytene cells (Klein et al., 1999; unpublished data). The number of Mcd1p spots on pachytene chromosomes in *tid1Δ* is significantly increased compared with *wild type* (Fig. 8), suggesting that the functional interaction between Mcd1p and Tid1p begins before or during pachytene. Thus, the misregulation of Mcd1p and, perhaps as a consequence, of Rec8p in or before prophase may lead to sister chromatid connections that persist through both anaphases and prevent chromosome segregation in both divisions of *tid1Δ*.

Discussion

Numerous observations indicate that Tid1p functions in conjunction with strand exchange enzymes in meiotic and mitotic cells, presumably to facilitate single strand invasion or subsequent processing during DNA DSB repair (see Introduction). However, a significant component of the *tid1Δ* phenotype is the failure of chromosome segregation during the meiotic divisions as a result of unresolved connections between sister chromatids. This *tid1Δ* phenotype provides a new perspective on the role of Tid1p in recombination repair.

Tid1p is required to remodel cohesion before the onset of divisions to allow severing of sister chromatid connections in anaphase

Our data indicate that persistent sister chromatid connections mediated by Mcd1p and Rec8p are the primary reason for the anaphase block in *tid1Δ*. We propose that Tid1p is required for

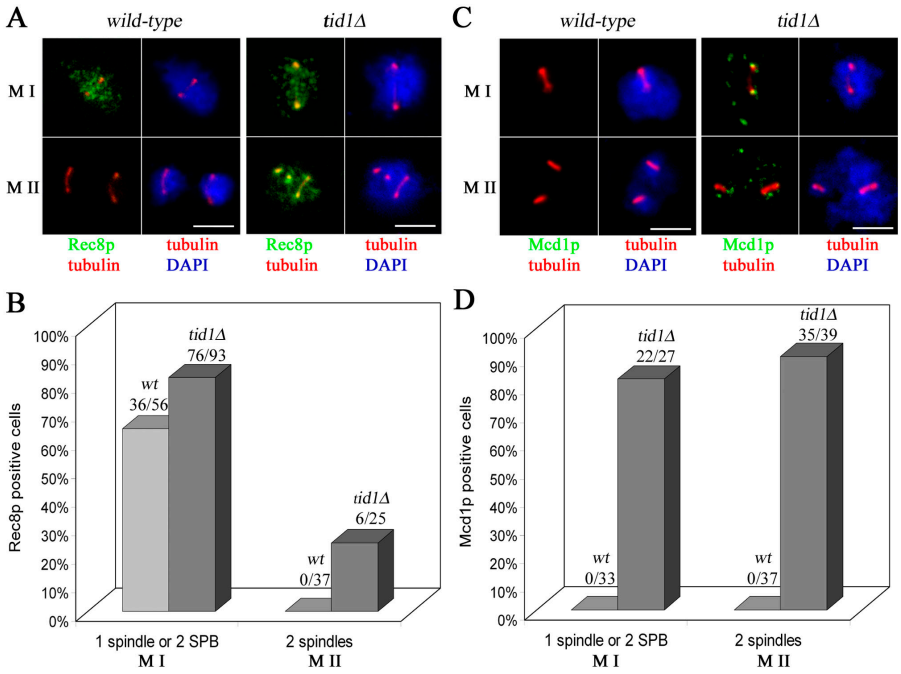

Figure 7. **Delayed dissociation of Mcd1p and Rec8p from chromatin in *tid1Δ*.** Spread preparations of sporulating cells were immunostained with antibodies against tubulin (red) and GFP (Rec8p-GFP, green) or Mcd1p (green). Chromatin is visualized with DAPI (blue). (A) Chromatin-associated Rec8p in *wild-type* and *tid1Δ* cells in the first (MI) and second (MII) division. (B and D) Quantification of Rec8p- (B) and Mcd1p-positive (D) cells in the first (1 spindle or 2SPB) and second (2 spindles) division. The differences in the numbers of cells that have Rec8p (B) and Mcd1p (D) associated with chromatin in *wild-type* and *tid1Δ* are statistically significant ($P < 0.05$; X^2 test) both for the cells with two spindles and for the cells with one spindle or two SPB. 1 spindle or 2 SPB, cells with long spindle or two SPBs on opposite sides of chromatin mass. Numbers above the bars represent Rec8p- or Mcd1p-positive cells/total number of cells counted in a given class. (C) Chromatin-associated Mcd1p in *wild-type* and *tid1Δ* cells in the first (MI) and second (MII) division. Bars, 4 μm.

Mcd1p (and possibly Rec8p) to be removed by an Esp1p-independent mechanism in prophase in preparation for or during DNA repair.

The timely progression into anaphase suggests that pachytene (DNA damage) and spindle checkpoints are not triggered by *tid1Δ* in the *spo11Δ spo13Δ* background. The involvement of a checkpoint that was triggered directly by DNA damage arising before the division (for example, in premeiotic S-phase) is possible but seems unlikely because no anaphase arrest was observed in the first division in *spo11Δ tid1Δ*.

A nucleosome remodeling function has been proposed for the Tid1p paralogue Rad54p (Alexeev et al., 2003; Jaskelioff et al., 2003; Wolner and Peterson, 2005). Another member of the SWI2/SNF2 family, SNF2hp, regulates cohesin loading on chromosomes, and ATPase activity is required for this process (Hakimi et al., 2002). We propose that a Tid1p chromatin remodeling function regulates Mcd1p and Rec8p association with chromosomes.

Mcd1p is required for local sister chromatid cohesion in budding yeast meiosis

In budding yeast, Mcd1p appears not to be essential for cohesion between sister chromatids in meiotic prophase, yet it forms discrete foci on pachytene chromosomes rather than localizing along their full lengths as with Rec8p (Klein et al., 1999). However, our results prove that Mcd1p can provide connections between sister chromatids in meiosis of *Saccharomyces cerevisiae*. Variability of the connections from cell to cell suggests that they could be independent of regular cohesin-binding sites. What leads Mcd1p to be loaded onto chromosomes in discrete, irregularly located spots (unpublished data) rather than along the chromosome axes (as it is in mitotic cells) and loaded as Rec8p is in meiotic cells is an important and unanswered question.

The Tid1p effect on recombination could be exerted through regulation of sister chromatid connections

We propose that Tid1p remodels Mcd1p-mediated cohesion to promote and regulate interhomologue recombination (Fig. 9). In this model, domains of Mcd1p would colocalize with DSBs that initiate meiotic recombination (and may be hot spots for DSBs; Petes, 2001). These domains would initially be prohibitive for impending interhomologue recombination for the following reason. In mitosis, cohesion between sister chromatids that was established during replication and de novo loading of cohesins at a DSB site are required for postreplicative DSB repair (Sjogren and Nasmyth, 2001; Strom et al., 2004; Unal et al., 2004). One of the roles proposed for cohesins in postreplicative repair in mitotic cells is to keep sister chromatids in proximity (Strom et al., 2004; Unal et al., 2004). If so, then in

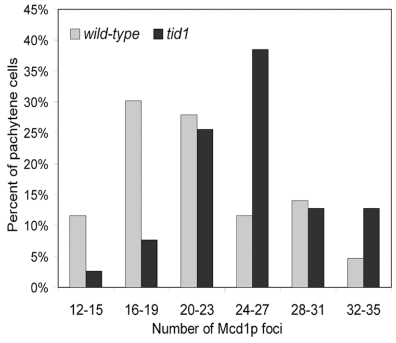

Figure 8. **Quantification of Mcd1p foci on pachytene chromosomes in *tid1Δ* and *wild-type*.** Mcd1p is visualized with anti-Mcd1p antibodies. Pachytene cells are identified as cells with clearly visible bivalents (visualized using DAPI) containing duplicated SPBs (visualized using antitubulin antibodies). Differences between *tid1Δ* and *wild-type* are statistically significant ($P < 0.05$; X^2 test).

meiosis, additional factors would have to be incorporated at the Mcd1p domains to guide DSB repair to lead to the separation of, rather than the alignment of, sister chromatids. We suggest that Tid1p specifically facilitates this separation, which allows interaction with one of the chromatids of the homologue, perhaps by promoting displacement from the loop to the axis (Blat et al., 2002). In the absence of this loop displacement, the DSB ends would be free to separate and could give rise to the separation of Dmc1p and Rad51p foci that were reported for *tid1Δ* (Shinohara et al., 2000). This two-step mechanism for control over the fate of multiple programmed DSBs in meiosis would provide an escape route through sister-based repair in case of a failure to initiate repair involving the homologue as a template. Remodeling of cohesins by Tid1p could be promoted by Tid1p interaction with Dmc1p, which is required for the interhomologue bias of recombination in meiosis (Bishop et al., 1992; Dresser et al., 1997). Thus, Tid1p would serve to remodel chromosome structure, which is promoted by but is not necessarily dependent on DSB metabolism. The model predicts that Mcd1p and proteins involved in DSB repair should colocalize to some degree depending on their relative times of activity and on whether Mcd1p is involved at all or at a subset of DSB sites.

According to this model, the defect in DSB processing and the failure to segregate chromosomes in *tid1Δ* arise as a result of the failure of the same Tid1p-dependent process. Proteins that were loaded in preparation for DSB processing could obscure the cleavage site of Mcd1p, creating an area of separase-resistant cohesion. The absence of Mcd1p remodeling in *tid1Δ* would cause Mcd1p-bound domains to persist and impede DSB repair. Recombination in *tid1Δ* would slowly overcome the obstacle if Mcd1p (and subsequently Rec8p) could be dislodged without Tid1p activity (for example, during the extended resection of DSBs that was observed in *tid1Δ*; Shinohara et al., 1997), thus resulting in a number of crossovers being flanked by a region of separase-resistant cohesion. It is interesting to note that flanking regions of separase-resistant sister chromatid cohesion could serve to stabilize chiasmata in *wild-type* cells and, thus, could provide a system for orientation that is distinct from the separase-regulated pathway.

Very little is known about how sister chromatid cohesion influences interhomologue recombination repair. However, it is clear that chromosome structure is adapted early to promote the programmed essential recombination repair that occurs in meiosis. Our results indicate that Tid1p is involved in this adaptation. Regulation of sister chromatid associations by Tid1p could similarly play a role during vegetative growth in the Tid1p-dependent subset of DSB repair that takes place between homologues in diploid cells (Klein, 1997; Shinohara et al., 1997; Arbel et al., 1999) and during break-induced replication (Signon et al., 2001; Ira and Haber, 2002). Structure–function genetic analysis by mutations in *TID1* and a biochemical description of the proposed remodeling activity of Tid1p will be required to establish the molecular functions of and the biological requirements for Tid1p in mitotic and meiotic cells.

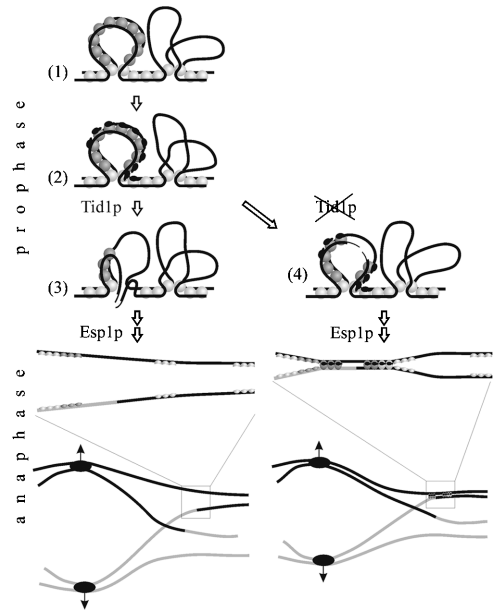

Figure 9. **Model of Tid1p regulation of sister chromatid cohesion.** Black and gray lines represent chromatids; black ovals represent centromeres. Dark gray spheres represent Mcd1p-dependent sister chromatid connections, and light gray spheres represent Rec8p-dependent connections. Spheres marked with black "hats" represent connections that are resistant to separase cleavage. Black arrows represent pulling forces of the spindle. Boxes indicate areas enlarged in the model. (1) Loading of Mcd1p occurs at a location that is permissive for DSBs. (2) Loading of additional factors marks the site for interhomologue recombination and renders Mcd1p and nearby Rec8p resistant to separase cleavage. (3) Remodeling/removal of cohesins by Tid1p results in local sister separation in an environment that promotes interaction of the broken chromatid with the homologue during repair. (4) In the absence of Tid1p, the loading of factors determining the fate of DSBs occurs normally, but local cohesion is not resolved, simultaneously creating an impediment for repair and persistent connections. Eventual inefficient dislodging of cohesins (for example, during resection or by Rad54p) allows the completion of recombination and results in a number of chiasmata that are flanked by areas of separase-resistant cohesion (or, in the absence of a DSB, separase-resistant connections alone). According to this model, the remodeling function of Tid1p in prophase would be promoted by but not dependent on DSB metabolism.

Materials and methods

Strains and plasmids

Diploid strains that were used in the assays were obtained by matings of MDY431 and MDY433 or their isogenic derivatives (Table S1, available at http://www.jcb.org/cgi/content/full/jcb.200505020/DC1). *spo13Δ* was made by single step replacement using p(spo13)16 (Wang et al., 1987). *spo11Δ* was introduced into strains by transformation with pGB518 (Giroux et al., 1989). The plasmid to transfer *tid1 K351R* into our strains was made by cloning the PstI–SalI fragment of *TID1* containing the *K351R* mutation from prdh54K/R.1 (Petukhova et al., 2000) into YIplac204. Integration of this construct into wild-type strains creates a partial duplication containing *tid1 K351R* and a truncation of *TID1* preceded by a plasmid sequence instead of the *TID1* promoter. *rec8Δ* was made by replacing *REC8* with a PCR product containing *TRP1* from pFA6a-TRP1 and small fragments of homology to flanking regions of the *REC8* gene (Longtine et al., 1998). The construct to replace *REC8* with *REC8-GFP* (pMDE820) was made by adding GFP coding sequence (EcoRI–XhoI) from pAFS135 (Straight et al., 1998) to the 3′ terminus of *REC8*, which was cloned by PCR from genomic template into pRS306. *MCD1* was replaced with *mcd1-1* by transformation of a fragment containing *mcd1-1* from pVG327 (provided by V. Guacci, Fox Chase Cancer Center, Philadelphia, PA). Spindles were marked by integrating *TUB1-GFP* at the *URA3* locus by transformation with pAFS125 (provided by A. Straight and A.W. Murray, Harvard University, Cambridge, MA) cut with

StuI. One additional copy of *PDS1-HA* was introduced into strains by transformation of one of the haploids with pVG319 (provided by V. Guacci) cut with KpnI. The presence of Pds1p-HA in these strains was confirmed on Western blots.

Strains with a chromosome *IV* centromere and right telomere marked by GFP spots were constructed in several steps. To integrate a tandem array of 256 copies of the *lac* operator near the right telomere of chromosome *IV*, plasmid pMDE780 was constructed by cloning a HindIII–SphI fragment of chromosome *IV* (nt 1,521,038–1,522,195) into pAFS59 (Straight et al., 1996). P_{CYC1}-lacI/GFP was introduced by the integration of pAFS152 (provided by A. Straight and A.W. Murray) at *URA3* in derivatives of MDY431 and at *CYC1* in derivatives of MDY433. P_{DMC1}-lacI/GFP was introduced into strains at *LYS2* by the integration of pMDE798, which contains *lacI/GFP-NLS* from pAFS152 (provided by A. Straight and A.W. Murray) under control of the *DMC1* promoter in order to ensure expression during meiosis. To mark chromosome *IV* with a centromere GFP spot, pXH115 containing an array of 224 *tet* operator repeats was integrated into the chromosome. P_{URA3}-tetR/GFP was introduced by the integration of pXH123 at *LEU2* (He et al., 2000). Construction of strains with a paracentric inversion on chromosome *VIIR* have been described previously (Dresser et al., 1994). The presence of correct alleles in all strains was confirmed by genetic complementation and/or by PCR.

Cytological analysis

For all cytological experiments, cultures were prepared from freshly mated diploids that were grown in acetate-containing rich medium and were transferred to sporulation medium to sporulate at 8×10^7 cells + buds/ml at 30°C. For an analysis of progression through meiosis, cells were stained with 0.5 μg/ml DAPI in 50% ethanol and were examined by fluorescence microscopy. For experiments requiring the preservation of GFP fluorescence, cells were fixed in 1% PFA for 15 min at RT, washed with PBS and 30% ethanol, and stained with 5 μg/ml DAPI in PBS. Stained cells were embedded in 1% low melting point agarose (GIBCO BRL) in PBS to prevent flattening.

Pds1p-HA and tubulin were visualized in whole cells using antibodies against HA (12CA5; Babco) and tubulin (YL1/2; Abcam). Cells were fixed with 4% PFA in 50 mM phosphate buffer, pH 6.5, containing 0.5 mM magnesium chloride for 10 min and were washed twice with 0.1 M phosphate buffer containing 1.2 M sorbitol (immunofluorescence assay buffer B [IFB]). The cell wall was digested with 20 μg/ml Zymolyase-100T and was washed with IFB. Spheroplasts were permeabilized with 1% Triton X-100 in IFB, washed twice with IFB, and placed on coverslips that were treated with 1% polyethyleneimine. Nonspecific binding of antibodies was blocked by incubating the preparations in 4% nonfat dried milk (Bio-Rad Laboratories) in PBS for 20 min. Coverslips were incubated for 1 h with primary mouse anti-HA antibodies (1:500 final dilution) and rat antitubulin YL1/2 (1:1,000), washed three times with PBS, and incubated with secondary FITC-conjugated donkey anti–mouse and Rhodamine Red-X–conjugated donkey anti–rat antibodies (both 1:400 dilution; Jackson ImmunoResearch Laboratories). The preparations were then washed three times with PBS, stained with 10 μg/ml DAPI in PBS for 20 min, rinsed in PBS, and mounted on slides with 1.5% low melting point agarose (GIBCO BRL) in 70% glycerol in PBS. To slow fluorescence fading, Citifluor in glycerol (Ted Pella) was added under the coverslip before examination. This same method was used to visualize telomere and centromere GFP spots with primary rabbit anti-GFP antibodies (1:500; Invitrogen) and Cy2-conjugated donkey anti–rabbit secondary antibodies (1:400; Jackson ImmunoResearch Laboratories).

Spreads were prepared as described previously (Dresser and Giroux, 1988) on slides that were treated with 1% polyethyleneimine. Labeling with antibodies was performed on slides as described above with the exception of using TBS. To compare the percentage of cells that were arrested with a short spindle in *spo11Δ spo13Δ rec8 tid1Δ* with in *spo11Δ spo13Δ rec8Δ*, tubulin was visualized in spread preparations of meiotic nuclei using rat antitubulin YOL1/34 serum (Serotec) diluted 1:50 and Rhodamine Red-X–conjugated donkey anti–rat antibodies (Jackson ImmunoResearch Laboratories) diluted 1:400. To compare the dissociation of Rec8p from chromatin in *wild-type* with *tid1Δ*, Rec8p-GFP was visualized with chicken anti-GFP antibodies (Chemicon International) diluted 1:5,000 and FITC-conjugated donkey anti–chicken secondary antibodies (Jackson ImmunoResearch Laboratories) diluted 1:400. Tubulin was visualized as described above. Cross-reaction of the primary chicken anti-GFP antibodies with the rat antitubulin antibodies was noted but did not interfere with the assay. To compare the dissociation of Mcd1p from chromatin in *wild-type* with *tid1Δ*, Mcd1p was visualized with affinity-purified rabbit anti-Mcd1p (559–1) antibodies (provided by V. Guacci) diluted 1:4,000 and with Cy3-conjugated donkey anti–rabbit secondary antibodies (Jackson ImmunoResearch Laboratories) diluted 1:400. Tubulin was visualized using rat antitubulin YL1/2 antibodies (Abcam) diluted 1:1,000 and Rhodamine Red-X–conjugated donkey anti–rat antibodies (Jackson ImmunoResearch Laboratories) diluted 1:400.

All images were acquired at 12-bit depth at RT on a microscope (Axioplan 2ie; Carl Zeiss MicroImaging, Inc.) using a plan-Apochromat 100× NA 1.4 oil differential interference contrast objective (Carl Zeiss MicroImaging, Inc.) and a camera (Quantix *57*; Photometrics) driven by MetaMorph software (Universal Imaging Corp.). Stacks of images were produced by using either a nanopositioner (PolytecPI) or a stage (Carl Zeiss MicroImaging, Inc.) for focusing movements. Image processing was limited to scaling by using either MetaMorph software or AdobePhotoshop. The isointensity surface extraction that was used for three-dimensional reconstruction and maximum intensity projection images were generated by using OMRFQANT (written by M.E. Dresser).

EM

Silver-stained spread preparations of meiotic nuclei were prepared as described previously (Dresser and Giroux, 1988) and were examined in an electron microscope (model 1200EX; JEOL). Electron micrographs were acquired by using a high resolution digital camera (model ES4; Advanced Microscopy Techniques Corp.).

DNA DSB and recombination products analysis

The formation of DNA DSBs was assayed on CHEF gels (Gerring et al., 1991). Samples were run on an electrophoresis system (CHEF II; Bio-Rad Laboratories) in 1% FastLane agarose and 0.25× Tris-borate/EDTA electrophoresis buffer at 12°C and 6 V/cm by using a 20–40-s ramp over 24 h. Southern transfer was performed by alkaline capillary transfer (Chomczynski, 1992) onto ZetaProbe membrane (Bio-Rad Laboratories). DSB fragments were visualized by using a radioactively labeled probe that was complementary to a near-telomeric region of chromosome *III* (Dresser et al., 1997) and were quantified using a phosphoimager (PhosphorImager 425; Molecular Dynamics). The formation of mature recombination products was assayed in a strain that was hererozygous for a paracentric inversion on chromosome *VII* (Dresser et al., 1994).

Online supplemental material

Table S1 lists yeast strains that were used in this study. Fig. S1 shows the kinetics of SC formation, turnover of DSBs, and formation of mature recombination products in *wild-type* and *tid1Δ*. Fig. S2 shows the segregation of chromosome *IV* with a telomere GFP spot on both homologues in *spo13Δ* and *spo13Δ tid1Δ*. Fig. S3 shows control data for Figs. 5 and 6. More detailed information about these results is located in the supplemental text. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200505020/DC1.

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