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The FK506 Binding Protein Fpr3 Counteracts Protein Phosphatase 1 to Maintain Meiotic Recombination Checkpoint Activity

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Summary

The meiotic recombination checkpoint delays gamete precursors in G2 until DNA breaks created during recombination are repaired and chromosome structure has been restored. Here, we show that the FK506 binding protein Fpr3 prevents premature adaptation to damage and thus serves to maintain recombination checkpoint activity. Impaired checkpoint function is observed both in cells lacking *FPR3* and in cells treated with rapamycin, a small molecule inhibitor that binds to the proline isomerase (PPIase) domain of Fpr3. *FPR3* functions in the checkpoint through controlling protein phosphatase 1 (PP1). Fpr3 interacts with PP1 through its PPIase domain, regulates PP1 localization, and counteracts the activity of PP1 in vivo. Our findings define a branch of the recombination checkpoint involved in the adaptation to persistent chromosomal damage and a critical function for FK506 binding proteins during meiosis.

Introduction

FK506 binding proteins (FKBPs) share a common proline isomerase (PPIase) domain that catalyzes the interconversion between the *cis* and *trans* peptidyl proline bonds in vitro and acts as a receptor for two clinically important drugs, FK506 and rapamycin. The best-studied aspect of FKBP biology is the ability of FKBP12 to bind and inhibit calcineurin or TOR kinase, when bound to FK506 or rapamycin, respectively, thereby mediating the immunosuppressive and antiproliferative effects of these drugs (reviewed in Hamilton and Steiner, 1998; Heitman et al., 1992). Less is known about the physiological roles of FKBPs. Deletion of all four yeast FKBPs does not affect cell proliferation under standard conditions (Dolinski et al., 1997). In humans, FKBP12, through its PPIase domain, acts as a modulator of several different receptors (reviewed in Breiman and Camus, 2002), and the yeast FKBP12-homolog *FPR1* is required for feedback control in aspartate homeostasis (reviewed in Arevalo-Rodriguez et al., 2004). Interestingly, *Fkbp6*$^{-/-}$ mice and *as/as* (*Fkbp6*) rats exhibit a spermatogenesis defect (Crackower et al., 2003), and *shu* (Fkbp) mutant flies are defective in oogenesis (Munn and Steward, 2000). Although the basis of these defects is not understood, these observations raise the possibility that a role of FKBPs in gametogenesis is conserved across species.

Meiosis, a central event in gametogenesis, is a specialized cell division where two rounds of chromosome segregation, meiosis I and meiosis II, follow a single round of chromosome duplication, leading to the separation of homologous chromosomes and sister chromatids, respectively. Faithful segregation of homologous chromosomes requires their physical connection through interhomolog recombination. Recombination is initiated by the introduction of DNA double-strand breaks (DSBs) by the transesterase Spo11 (Keeney, 2001). DSBs are subsequently repaired using the homologous chromosome as a template because repair off of the sister chromatid is blocked (reviewed in Petes and Pukkila, 1995; Zickler and Kleckner, 1999). One important factor for meiotic DSB repair is Dmc1, a homolog of the bacterial DNA strand invasion factor RecA, which serves to direct nascent DSB toward the homologous chromosome (Bishop et al., 1992; Schwacha and Kleckner, 1997). Absence of Dmc1 leads to the accumulation of unrepaired DSBs and a checkpoint-dependent delay in meiotic G2 (Bishop et al., 1992).

If broken chromosomes persist, a conserved meiotic surveillance mechanism called the recombination or pachytene checkpoint delays cell cycle progression in meiotic G2 (Lydall et al., 1996; Roeder and Bailis, 2000). In budding yeast, the G2 delay is brought about by the inhibition of cyclin dependent kinase (CDK) activity (Roeder and Bailis, 2000) and by preventing activation of the transcription factor Ndt80, which induces the expression of factors necessary for meiotic chromosome segregation and spore formation (Pak and Segall, 2002; Tung et al., 2000). Factors implicated in the recombination checkpoint in yeast include components of the mitotic DNA damage signaling machinery (Mec1, Rad24, Rad17, Mec3, and Ddc1), several meiosis-specific chromosomal proteins (Red1, Hop1, and Mek1), and a number of nucleolar proteins (Pch2, Sir2, and Glc7; reviewed in Roeder and Bailis, 2000). Yeast protein phosphatase 1 (PP1), Glc7, is not a checkpoint component per se but is thought to promote resumption of the cell cycle after recombination checkpoint-dependent delay, by reversing phosphorylation events put in place by the checkpoint kinase Mek1 (Bailis and Roeder, 2000).

In a systematic search for novel recombination checkpoint components, we identified the yeast FKBP Fpr3 as being required for continued cell cycle arrest. Using point mutants and rapamycin, we demonstrate that the proline isomerase domain but not its PPIase activity is required for the protein's checkpoint function. Our data also provide insight into the mechanism whereby Fpr3 functions in the recombination checkpoint. Fpr3 associates with protein phophatase 1 through its proline isomerase domain and inhibits PP1 function in vivo. We propose that Fpr3 acts as an inhibi-

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tor of PP1, thereby preventing premature adaptation to chromosomal damage.

Results

FPR3 Is Required for Continued Checkpoint Arrest

Loss of the strand invasion factor *DMC1* elicits a recombination checkpoint-dependent arrest in meiotic G2 and hence a failure to form spores (Bishop et al., 1992; Lydall et al., 1996). By screening the *S. cerevisiae* deletion collection (Figure S1A in the Supplemental Data available with this article online), we identified 15 deletions that allowed *dmc1Δ* cells to progress through meiosis and form spores. Fourteen deletions were previously known to alleviate the recombination checkpoint-mediated cell cycle delay (Figure S1B). One suppressor deletion was novel and eliminated the gene encoding the FK506 binding protein Fpr3 (Benton et al., 1994; Manning-Krieg et al., 1994; Shan et al., 1994).

We first compared the kinetics with which *dmc1Δ* and *dmc1Δ fpr3Δ* cells progressed through meiosis, using the timing of cyclin Clb3 protein accumulation and spindle pole body (SPB) separation as markers to assess progression out of meiotic G2 into prometaphase I. Clb3 accumulation was strongly delayed in *dmc1Δ* cells, compared to wild-type cells, and was accelerated when *FPR3* was deleted (Figure 1A). We note, however, that compared to wild-type cells and *fpr3Δ* mutants, a 3 hr delay in Clb3 accumulation persisted in *dmc1Δ fpr3Δ* cells (Figure 1A). The separation of SPBs occurred with similar kinetics as Clb3 accumulation. *dmc1Δ fpr3Δ* cells initiated SPB separation 7 hr after transfer into sporulation medium, whereas little separation occurred in *dmc1Δ* mutants (Figure 1B). Finally, sporulation efficiency increased dramatically when *FPR3* was deleted in *dmc1Δ* cells (Figure 1C). Our data show that deletion of *FPR3* allows *dmc1Δ* cells to escape the checkpoint-dependent G2 block and to complete the meiotic program.

The suppression of the checkpoint block in the absence of *FPR3* is not restricted to *dmc1Δ* mutants. Deletion of *FPR3* accelerated the timing of entry into meiosis I for *hop2Δ*, *rec8Δ*, *mer3Δ*, and *rad50S* cells (Alani et al., 1990; Klein et al., 1999; Leu et al., 1998; Nakagawa and Ogawa, 1999) (Figures 1D–1G). However, as observed in *dmc1Δ* cells, deletion of *FPR3* did not eliminate the G2 delay, suggesting that some aspects of the recombination checkpoint were still functional in the absence of *FPR3*. Interestingly, deletion of *FPR3* did not allow *zip1Δ* mutants (Sym et al., 1993) to exit the G2 block more effectively, but instead appeared to slightly exaggerate it (Figure 1H). The reason why some but not all blocks are bypassed by deleting *FPR3* is at present unclear. The situation is likely more complex as suggested by the recent finding that *zip1Δ* and *mer3Δ* mutants have very similar phenotypes at low (23°C) and high (33°C) temperatures but differ at the intermediate temperature (30°C) used in this study (Borner et al., 2004). Our findings nevertheless suggest that the prophase delay observed in *zip1Δ* cells (at 30°C) is qualitatively different from the delays caused by the deletions of *DMC1*, *HOP2*, *REC8*, or *MER3*.

We next asked whether overexpression of *FPR3* would affect the recombination checkpoint. Wild-type cells expressing *FPR3* from a 2-micron plasmid progressed through meiosis with kinetics indistinguishable from cells carrying an empty control plasmid (Figure 1I). However, high levels of *FPR3* dramatically exaggerated the checkpoint-dependent cell cycle delay observed in *dmc1Δ* cells. Our data indicate that high levels of *FPR3* lead to a maintained arrest in meiotic prophase in a DNA damage-dependent manner.

DSBs Form Normally and Persist in *dmc1Δ fpr3Δ* Cells

Why are *dmc1Δ fpr3Δ* double mutants able to progress through meiosis? Two possibilities we considered were that (1) DSBs are not formed in the absence of *FPR3* and that (2) the DNA damage caused by the absence of *DMC1* could be repaired once *FPR3* was eliminated. To test these hypotheses, we analyzed DSB formation and repair at the well-characterized *HIS4LEU2* hotspot (Storlazzi et al., 1995) as cells progressed through meiosis. DSBs appeared and were repaired in *fpr3Δ* cells with kinetics indistinguishable from that of wild-type cells (Figures 2A and 2C). Furthermore, DSBs formed and accumulated to the same extent in *dmc1Δ* and *dmc1Δ fpr3Δ* mutants (Figures 2A and 2C), indicating that the lack of *FPR3* did not affect DSB formation. DSBs appeared to be resected with comparable kinetics in both strains, because the DSB band increased in heterogeneity at a similar rate in both strains (Figures 2A and 2C). This analysis did not allow us to determine whether the drop in DSB signal was solely due to hyperresection of the breaks, or whether a subset of breaks were repaired from the sister chromatid. However, it was clear that no crossover repair products were formed in *dmc1Δ* and *dmc1Δ fpr3Δ* mutants (Figures 2A and 2D), suggesting that deletion of *FPR3* did not reactivate crossover repair in *dmc1Δ* mutants. The absence of crossover products also indicates that deletion of *FPR3* does not cause overactivation of *RAD51* or *RAD54*, which has previously been shown to bypass the requirement for *DMC1* in crossover repair (Bishop et al., 1999; Tsubouchi and Roeder, 2003).

Despite the lack of crossover repair, *dmc1Δ fpr3Δ* cells entered meiosis I after only a 2 hr delay compared to wild-type and *fpr3Δ* single mutants, and 3–4 hr before significant spindle formation could be observed in *dmc1Δ* mutants (Figure 2B). Consistent with the lack of DNA repair, we found that deletion of *FPR3* also did not rescue the defect of *dmc1Δ* cells in synaptonemal complex formation (Figure S2). Our results indicate that deletion of *FPR3* neither eliminates DSB formation nor allows crossover repair of DSBs from the homolog in *dmc1Δ* cells, while still allowing progression through meiosis.

FPR3 Is a Checkpoint Factor

DSB repair using the sister chromatid as a template is largely inhibited during meiotic recombination, making homologous chromosomes the preferred repair template (Petes and Pukkila, 1995; Schwacha and Kleckner, 1997). We took several approaches to test the possibility that deletion of *FPR3* allows repair of DSBs from the sister chromatid in *dmc1Δ* mutants. First, repair of

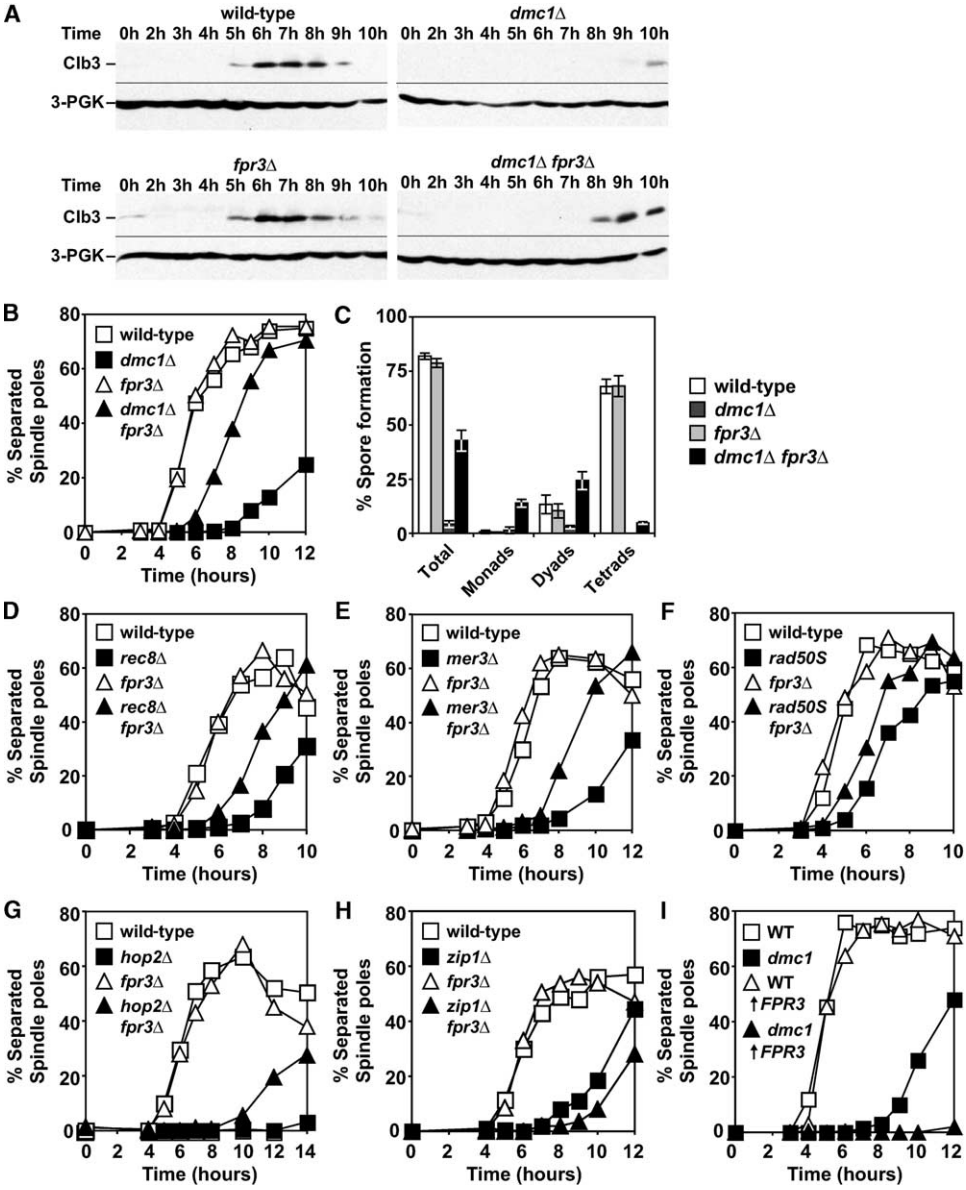

Figure 1. *FPR3* Is Required for Maintenance of the Recombination Checkpoint Block

(A) Synchronous meiotic cultures of wild-type (WT; A10125), *dmc1Δ* (A10122), *fpr3Δ* (A10124), and *dmc1Δ fpr3Δ* (A10123) were analyzed by Western blot at the indicated times for the amount of Clb3-HA protein. 3-Pgk served as loading control.

(B) Synchronous meiotic cultures of WT (A9671), *dmc1Δ* (A9669), *fpr3Δ* (A9672), and *dmc1Δ fpr3Δ* (A9670) were analyzed at the indicated times for the percentage of cells with separated SPBs.

(C) Sporulation efficiency of WT (A6871), *dmc1Δ* (A6872), *fpr3Δ* (A6924), and *dmc1Δ fpr3Δ* (A6683) cells. Asci were classified as containing one (monads), two (dyads), or three/four spores (tetrads). Error bars indicate standard deviation in three independent experiments.

(D–I) Synchronous meiotic cultures were analyzed at the indicated times for the percentage of cells with separated SPBs. The following strains were used for this analysis: (D) WT (A9621), *rec8Δ* (A9619), *fpr3Δ* (A9620), and *rec8Δ fpr3Δ* (A9618). (E) WT (A11014), *mer3Δ* (A11012), *fpr3Δ* (A11015), and *mer3Δ fpr3Δ* (A11013). (F) WT (A9617), *rad50S* (A8990), *fpr3Δ* (A9615), and *rad50S fpr3Δ* (A8989). (G) WT (A8342), *hop2Δ* (A8339), *fpr3Δ* (A8345; this strain also harbored *fpr4Δ*), and *hop2Δ fpr3Δ* (A8360). (H) WT (A9697), *zip1Δ* (A9037), *fpr3Δ* (A9700), and *zip1Δ fpr3Δ* (A9119). (I) WT + *YEp352* (A13749), *dmc1Δ* + *YEp352* (A13751), WT + *YEp352-FPR3* (A13750), *dmc1Δ* + *YEp352-FPR3* (A13752).

DSBs is expected to improve the spore viability of *dmc1Δ* mutants. We performed this experiment in a *spo13Δ* background because cells lacking *SPO13* undergo a single round of chromosome segregation, which partially alleviates the requirement for crossover recombination and chiasma formation (Wagstaff et al., 1982). Thus, if repair of DSBs were to occur from the sister chromatid, *spo13Δ fpr3Δ dmc1Δ* spores ought to exhibit increased viability over *spo13Δ dmc1Δ* spores. This, however, was not the case (Table S1), indicating that deletion of *FPR3* does not allow significant repair off the sister chromatid in *dmc1Δ* mutants. Because

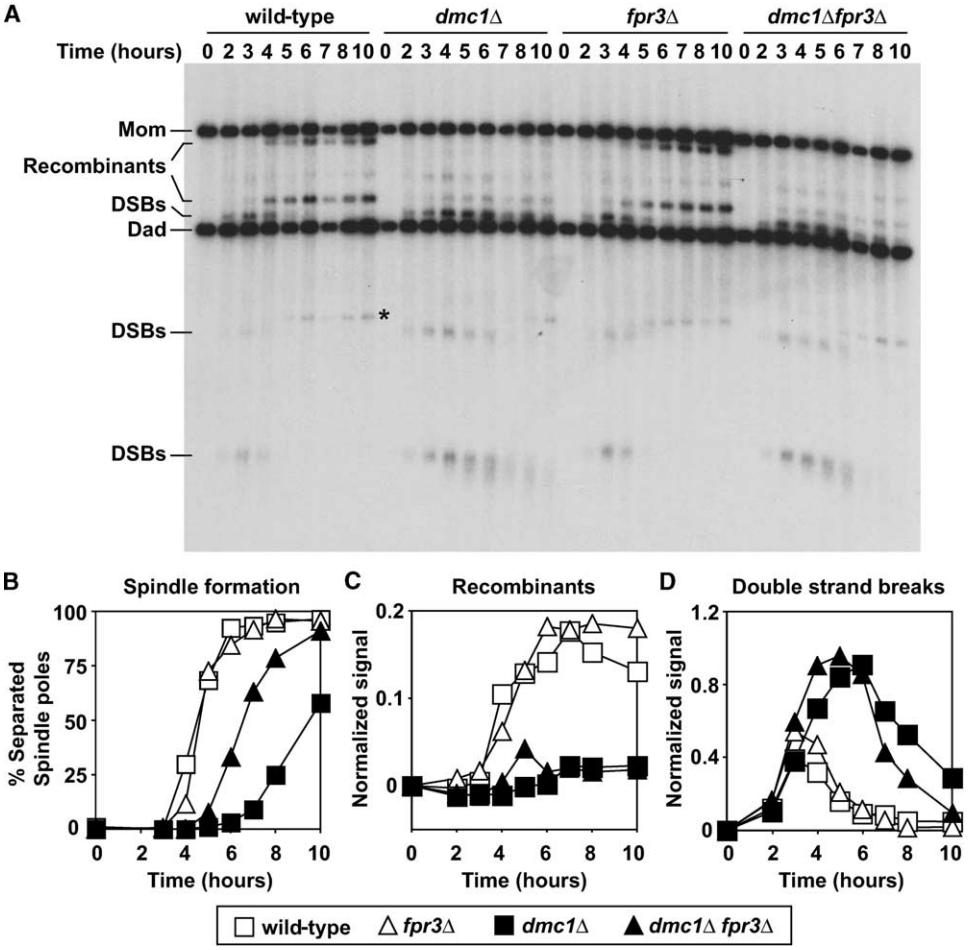

Figure 2. DSBs Form Normally in the Absence of *FPR3*

Synchronous meiotic cultures of WT (A7883), *dmc1Δ* (A7884), *fpr3Δ* (A7878), and *dmc1Δ fpr3Δ* (A7877) were analyzed by Southern blot at the indicated times for recombination at the *HIS4LEU2* hotspot (A), and for the percentage of cells with separated SPBs (B). The Southern blot was probed with probe A. * indicates a *RAD52*-dependent DNA fragment that is likely the result of an ectopic recombination event. Quantification of the slower migrating recombinant band (C) and of the fastest migrating DSB band (D) are shown. Measured signals were normalized to the signal of the parental band. The value of the 0 hr time point was then subtracted from all later time points to eliminate nonspecific signal.

DMC1 is required for recombination, we also analyzed the effect of *fpr3Δ* on a prophase delay when the recombination machinery was intact. Haploid cells that harbor mating type information for both a and α can be induced to undergo meiosis and form viable offspring if *SPO13* is deleted and if the meiotic inhibition of sister chromatid repair is eliminated (De Massy et al., 1994; Wagstaff et al., 1982). If the inhibition of sister chromatid repair is maintained, *MATa/α* haploids accumulate DSBs and delay in meiotic G2 (De Massy et al., 1994). Consistent with *FPR3* having a checkpoint role, we observed that deletion of *FPR3* in *spo13Δ MATa/α* haploids resulted in the bypass of the G2 delay, but spore viability did not increase (Table S1, Figure S3). These results indicate that a role of *FPR3* in preventing meiotic DSB repair off the sister chromatid is, if it exists at all, limited.

To conclusively determine whether *FPR3* was indeed a bona fide checkpoint factor, we constructed a strain in which the homologous chromosome as well as the sister chromatid would be absent during meiotic G2, based on the premise that no homologous repair should be possible if all repair templates are removed. In this situation any observed bypass should be attributable to the checkpoint function of *FPR3*. To prevent cells from undergoing premeiotic DNA replication, we constructed a meiosis-specific knockdown allele of the prereplicative complex component *CDC6* (Cocker et al., 1996) by placing *CDC6* under the control of the mitosis-specific *SCC1* promoter (*cdc6*-meiotic null; *cdc6-mn*). *cdc6-mn* cells duplicated their DNA normally during mitotic growth but underwent little premeiotic DNA replication (Figure 3C and S4A). Nevertheless *cdc6-mn* mutants showed only a small delay in the progression through meiosis (Figure 3A, top right panel) and underwent DSB formation and meiotic recombination with almost wild-type kinetics and efficiency (Figure S4B). The observation that DSB formation occurs in cells de-

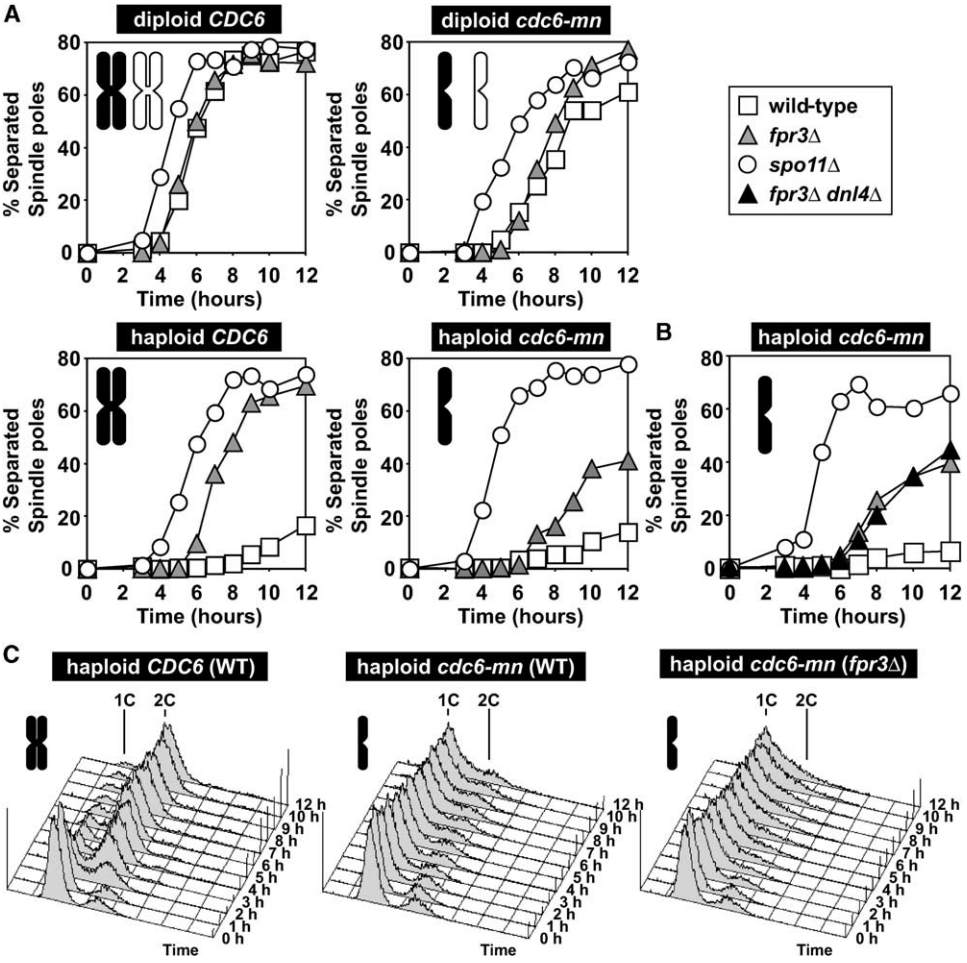

Figure 3. Fpr3 Is a Checkpoint Factor

(A and B) Synchronous meiotic cultures were analyzed at the indicated times for the percentage of cells with separated SPBs. The following strains were used: diploid *CDC6* strains: WT (A9671), *fpr3Δ* (A9672), *spo11Δ* (A12169); diploid *cdc6-mn* strains: WT (A9603), *fpr3Δ* (A9602), *spo11Δ* (A12168); MATa/α haploid *CDC6* strains: WT (A8873), *fpr3Δ* (A11288), *spo11Δ* (A10272); MATa/α haploid *cdc6-mn* strains: WT (A11550), *fpr3Δ* (A9723), *spo11Δ* (A12006), *fpr3Δ dnl4Δ* (A12007). Black and white chromosomes denote the C (complement) content of the strains.

(C) Flow cytometric analysis of DNA content. To improve the FACS profile quality, *LEU2* prototrophic versions of A8873, A11550, and A9723 were used.

pleted for Cdc6, in which DNA replication is absent, but not in cells lacking the S phase cyclins *CLB5* and *CLB6*, in which DNA replication also does not occur (Borde et al., 2000; Smith et al., 2001) raises the interesting possibility that Clb5/6-CDK activity is required for DSB formation.

When both *MATa* and *MATα* information was provided, haploid *cdc6-mn* cells initiated meiotic recombination with only a single copy of their genome (Figure 3A). These cells possessed the full meiotic repair machinery but lacked a template to repair the DSBs and exhibited a cell cycle delay. The delay was DSB dependent because deletion of *SPO11*, the enzyme that catalyzes DSB formation (Keeney, 2001), allowed *MATa/α cdc6-mn* haploids to progress through meiosis with kinetics indistinguishable from cells with a full set of repair templates (Figure 3A). If deletion of *FPR3* were to only activate repair from the sister chromatid, it would be expected to have no effect on the cell cycle progression of *MATa/α cdc6-mn* haploids. However, by 10 hr, ~40% of haploid *cdc6-mn fpr3Δ* cells had entered meiosis I as judged by SPB separation, even though little DNA replication had occurred by this time (Figures 3A [lower-right panel] and 3C). The bypass of the cell cycle block was also not due to DSB repair mediated by the nonhomologous end-joining pathway, because deletion of DNA ligase IV (*dnl4Δ*; Wilson et al., 1997b) did not affect the ability of *fpr3Δ* to bypass the delay of *MATa/α cdc6-mn* haploids (Figure 3B). As observed in cells lacking *DMC1*, *REC8*, *MER3*, or *HOP2* (Figure 1), deletion of *FPR3* allowed only partial bypass of the delay, indicating that some aspect of the recombination checkpoint is still functional in the mutant. Furthermore, the finding that haploid *CDC6* cells lacking *FPR3* progress through meiosis more efficiently than *cdc6-mn fpr3Δ* cells may indicate that *FPR3* also has a role in preventing DSB repair off the sister chromatid. Thus, while the analysis of meiosis in haploid sisterless cells

cannot exclude a role of *FPR3* in DSB repair, it clearly demonstrates a bona fide checkpoint role of *FPR3*.

Fpr3 Spreads from the Nucleolus into the Nucleoplasm during Meiosis

Unlike the checkpoint factors *PCH2, MEK1*, and *RED1*, *FPR3* did not appear to be developmentally regulated. Fpr3 protein levels remained constant during mitotic cell division and meiotic development (Figure S5 and data not shown). Localization studies found Fpr3 enriched in the nucleolus during vegetative growth (Benton et al., 1994; Shan et al., 1994). In fact, Fpr3 was directly associated with nucleolar chromatin because it remained localized to the low DAPI-staining regions in spread nuclei (Figures 4A and 4B). Surprisingly, Fpr3 did not colocalize with core nucleolar markers such as Nop1 and Cdc14. Rather, it localized to a subcompartment adjoining and frequently surrounding the Nop1- and Cdc14-positive nucleolar core structure (Figure 4B). In addition to the nucleolus, Fpr3 was also frequently localized to several foci on chromatin (Figures 4A and 4B, and S6), the nature of which is at present unclear.

When cells were starved to induce meiosis, the volume of both the Fpr3-positive and the Nop1-positive nucleolar compartment decreased dramatically, and a further loss in nucleolar volume was observed as cells progressed through the meiotic program (Figure 4C and data not shown). Concomitantly, Fpr3 lost its nucleolar chromatin association, such that by pachytene, when chromosomes were fully synapsed, Fpr3 staining was restricted to a single chromosome-associated focus (Figure 4C, bottom panels). Most of the cellular pool of Fpr3 became more diffusely distributed throughout the nucleus, as judged by whole-cell immunofluorescence of meiotic cells (Figure 4D). Our results indicate that at the time when the recombination checkpoint becomes active during meiosis, Fpr3 is present throughout the nucleus.

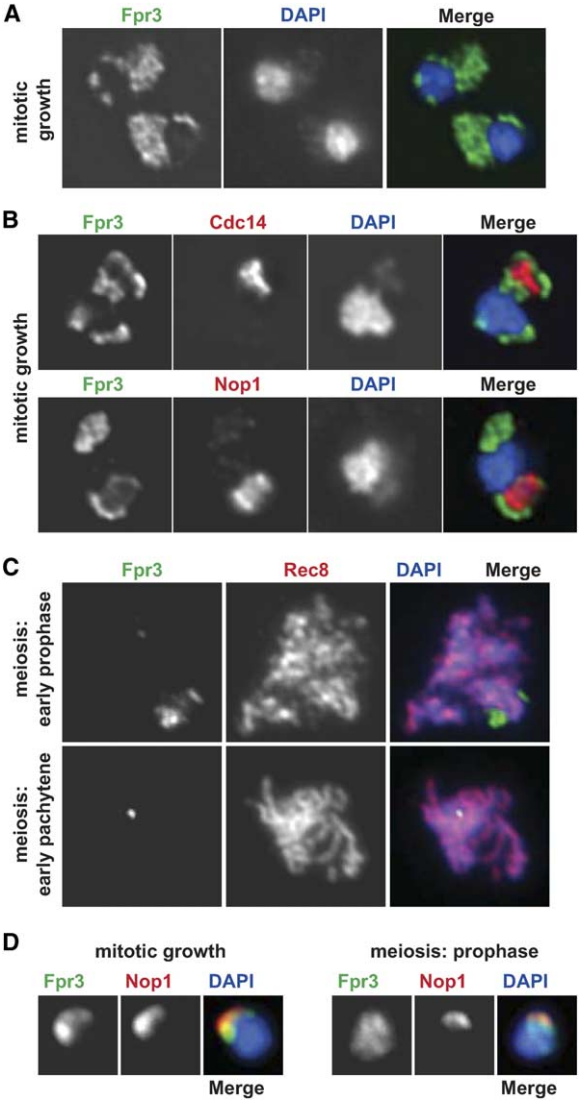

Figure 4. Meiotic Fpr3 Spreads from the Nucleolus into the Nucleus during Meiosis

(A) Deconvolved images of mitotic spreads of haploid WT (A10416) cells in late G1 (15 min after release from α-factor arrest). In the merge, Fpr3 is shown in green, DNA in blue.

(B) Deconvolved images of mitotic spreads of cycling diploid WT cells (top: A7872, carrying *CDC14-HA*; bottom: A9671). In the merge, Fpr3 is shown in green, Cdc14 or Nop1 in red, and DNA in blue.

(C) Images of meiotic spreads of diploid WT cells carrying Rec8-HA (A1972). Top: early prophase cell, bottom: early pachytene cell. Rec8-HA is shown in red and Fpr3 in green.

(D) Whole-cell immunofluorescence of WT (A6871) cells in exponential growth (left) or at 4 hr into meiosis (right). Fpr3 is shown in green and Nop1 in red.

FPR3 and *PCH2* Do Not Function Together in the Recombination Checkpoint

Pch2, like Fpr3, is found in the nucleolus (San-Segundo and Roeder, 1999), which raised the possibility that the two proteins act in a common pathway. However, the effects of deleting *FPR3* and *PCH2* on the G2 delays of recombination mutants were not identical. Deletion of *FPR3* alleviated the prophase delay of *dmc1Δ* but not *zip1Δ* mutants (Figure 1). Deletion of *PCH2* allowed *zip1Δ* mutants to enter meiosis I (San-Segundo and Roeder, 1999), whereas it enhanced the prophase block of *dmc1Δ* cells (Figures 5A and 5B). These results indicate that, at least at 30°C, the G2 delays of *zip1Δ* and *dmc1Δ* mutants are not caused by the same mechanism.

PCH2 may be a component of a checkpoint pathway acting in parallel to the checkpoint response defined by *FPR3*. However, several lines of evidence argue against this possibility and instead support a role for *PCH2* in DSB repair. First, *pch2Δ* cells themselves exhibited a two-hour delay in cell cycle progression (San-Segundo and Roeder, 1999) that could not be bypassed by the deletion of *FPR3* (Figure 5C). Second, while DSBs have largely disappeared by 4 hr in wild-type cells (Figure 2A), they persisted at least until the 6 hr time point in *pch2Δ* mutants (Figure S7). Accordingly, crossover repair products were also observed with a 2 hr delay. Moreover, the DSBs of *dmc1Δ pch2Δ* mutants did not get hyperresected as rapidly as in *dmc1Δ* cells (compare Figure S7 with Figure 2A). Together, these findings

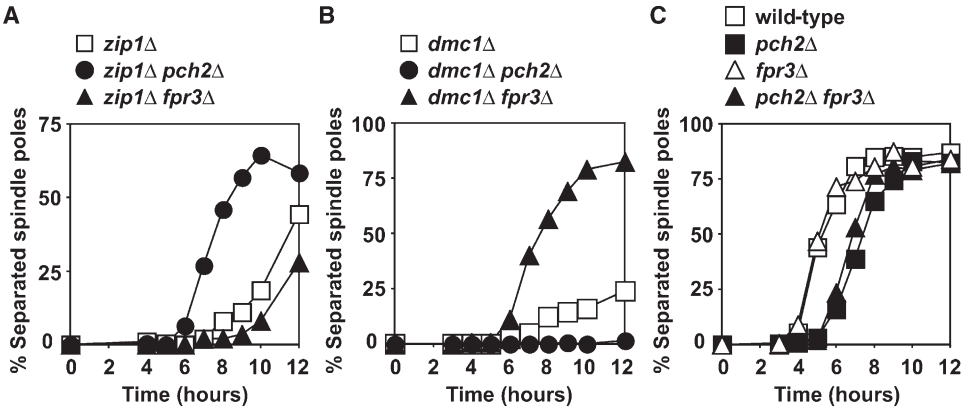

Figure 5. Distinct Functions of *FPR3* and *PCH2* in the Recombination Checkpoint

Synchronous meiotic cultures were analyzed at the indicated times for the percentage of cells with separated SPBs. The following strains were used:

(A) *zip1Δ* (A9037), *zip1Δ pch2Δ* (A9036), *zip1Δ fpr3Δ* (A9119).

(B) *dmc1Δ* (A7884), *dmc1Δ pch2Δ* (A10843), and *dmc1Δ fpr3Δ pch2Δ* (A10843).

(C) WT (A7883), *pch2Δ* (A11026), *fpr3Δ* (A7878), *fpr3Δ pch2Δ* (A10842).

indicate that *PCH2* has a role in the processing of DSBs in both wild-type and *dmc1Δ* mutants, which argues against a common role of *PCH2* and *FPR3* in the recombination checkpoint.

Fpr3 Associates with and Anchors Glc7/PP1 in the Nucleolus

PP1/Glc7 is a checkpoint factor (Bailis and Roeder, 2000) that, like Fpr3, is expressed during both mitosis and meiosis and found enriched in the nucleolus during mitotic growth (Bloecher and Tatchell, 2000). Furthermore, a large-scale affinity purification study showed that Fpr3 copurifies with a subset of nucleolar factors one of which is Glc7 (Ho et al., 2002). We therefore examined whether Fpr3 and Glc7 form a complex. Fpr3 forms a complex with Glc7 during mitosis (Figure 6C) and meiosis (Figure 6D) as evident from their ability to coimmunoprecipitate from both mitotic and meiotic extracts. Consistent with this, Glc7 co-localized with the nucleolar pool of Fpr3 on chromatin spreads of nuclei obtained from mitotically dividing and early meiotic cells (Figures 6A and 6B). Furthermore, Fpr3 was required for Glc7 association with the nucleolus in both mitotic and early meiotic cells (Figure 6B, and data not shown). The loss of Glc7 from the nucleolus was not due to a general disorganization of the organelle, as Nop1 localization was not affected by deletion of *FPR3* (Figure S8).

As cells enter the meiotic program, both Fpr3 and Glc7 leave their nucleolar compartment and spread throughout the nucleus (Figures 4D and 6B), such that at later stages in meiosis, when the nucleolar signal of Fpr3 becomes restricted to a single dot, Glc7 cannot be detected in the nucleolus anymore (Bailis and Roeder, 2000). Nevertheless, Glc7 and Fpr3 remained in a complex throughout meiosis (Figure 6D), indicating that they remained in the nucleoplasm as a complex. Interestingly, we observed a transient increase in coimmunoprecipitation efficiency in extract obtained from *dmc1Δ* cells as compared to wild-type cells around the time of DSB formation (3 hr time point, Figure 6D), which may point to a functional connection between these two proteins within the recombination checkpoint. Our attempts to reproduce the interaction between Fpr3 and Glc7 using recombinant proteins were not successful indicating either that Fpr3 and Glc7 do not interact directly, or that Fpr3 and/or Glc7 need to be modified in order to interact. Consistent with the latter idea is the observation that Glc7 appeared to preferentially associate with a slower migrating form of Fpr3 (arrow, Figure 6D) in meiotic extracts. Fpr3 has been shown to be both phosphorylated and sumoylated (Wilson et al., 1997a; Wohlschlegel et al., 2004). Whether the slower-migrating Fpr3 represents such a posttranslationally modified form is at present unclear. Our data suggests that as cells undergo meiotic recombination, a complex consisting of Fpr3 and Glc7 leaves the nucleolus and spreads throughout the nucleus.

Fpr3 Antagonizes Glc7 Function

FPR3 is required for maintained recombination checkpoint-induced cell cycle delay. In contrast, *GLC7* is required for the adaptation to DNA damage and overproduction of the phosphatase allows cells to bypass the recombination checkpoint delay (Bailis and Roeder, 2000). These findings together with our observation that the two proteins form a complex raise the possibility that Fpr3 functions as an inhibitor of Glc7. Consistent with this idea, we found that overexpression of *FPR3* suppressed the lethality caused by high levels of *GLC7* (Figure 6E). The suppression of the *GLC7*-induced lethality was not simply a result of lowering *GLC7* expression from the *GAL1-10* promoter, due to the presence of an additional copy of this promoter, because introduction of a *GAL1-10* promoter alone did not suppress the lethality associated with overexpressing *GLC7.*

Overexpression of *FPR3* also counteracted Glc7 ac-

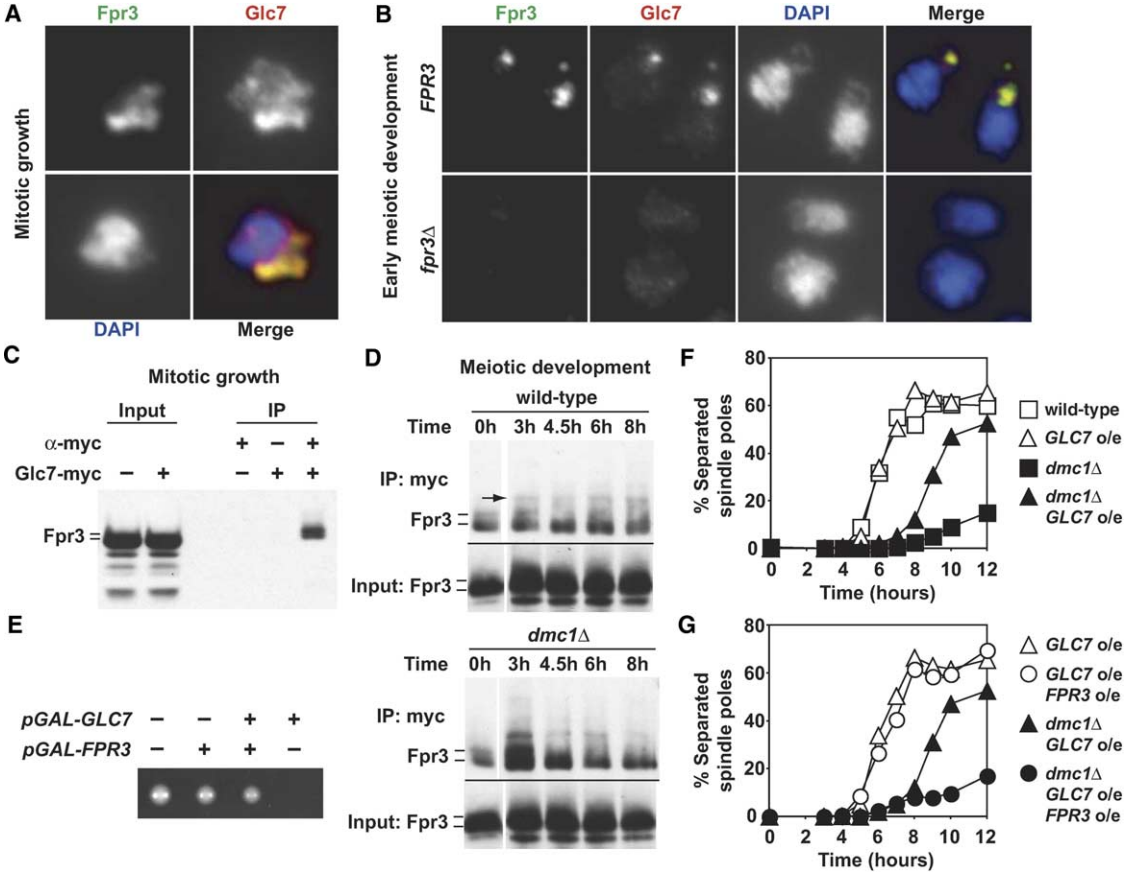

Figure 6. Fpr3 Interacts with and Counteracts Glc7

(A) Images of spread mitotic WT cell carrying *GLC7-myc* (A6030; haploid W303). Glc7 is shown in red, Fpr3 in green, and DNA in blue.

(B) Early meiotic (0h) *dmc1Δ* (A12445) and *dmc1Δ fpr3Δ* (A12443) cells carrying *GLC7-myc*.

(C and D) Western blots detecting Fpr3 after immunoprecipitation of Glc7-myc from (C) cycling mitotic cells (A6030) or (D) cells progressing through meiosis; WT (A12444), *dmc1Δ* (A12445). The arrow indicates a meiosis-specific modification of Fpr3.

(E) Segregants of a cross between *pGAL-GLC7* cells with *pGAL-FPR3* cells (A1631 × A12368, W303). Tetrads were micromanipulated on plates containing 2% galactose to induce overproduction of *GLC7* and *FPR3*.

(F and G) Synchronous meiotic cultures were analyzed at the indicated times for the percentage of cells with separated SPBs. The following strains were used for this analysis: (F) WT + *YEp352* (A13749), *dmc1Δ* + *YEp352* (A13751), *pHOP1-GLC7* + *YEp352* (A13753), *dmc1Δ pHOP1-GLC7* + *YEp352* (A13757). (G) *pHOP1-GLC7* + *YEp352* (A13753), *pHOP1-GLC7* + *YEp352-FPR3* (A13754), *dmc1Δ pHOP1-GLC7* + *YEp352* (A13757), and *dmc1Δ pHOP1-GLC7* + *YEp352-FPR3* (A13758).

tivity in the context of the recombination checkpoint. *GLC7* under the control of the strong meiotic *HOP1* promoter (*pHOP1-GLC7*) led to a partial bypass of the prophase delay in *dmc1Δ* (Figure 6F). This bypass was similar to that caused by the deletion of *FPR3* and only minimally accelerated by the deletion of *FPR3* (data not shown). Importantly, overexpression of *FPR3* prevented the *GLC7*-induced bypass of the cell cycle delay observed in *dmc1Δ* mutants (Figure 6G), indicating that Fpr3 counteracts Glc7 function in the recombination checkpoint. Interestingly, *GLC7* and/or *FPR3* overexpression only affected meiotic progression in *dmc1Δ* cells (when the recombination checkpoint is activated) but not in wild-type cells (Figures 1H and 6F), supporting a role for these two proteins in the cellular adaptation to persistent DNA damage. Our results indicate that Fpr3 associates with Glc7 to inhibit the phosphatase and maintain recombination checkpoint activity.

The PPIase Domain of *FPR3* Is Necessary for Complex Formation with Glc7

The carboxy-terminus of Fpr3 contains a proline isomerase domain that possesses PPIase activity in vitro (Benton et al., 1994; Manning-Krieg et al., 1994; Shan et al., 1994). To investigate whether the PPIase domain is required for the interaction between Fpr3 and Glc7, we created a series of point mutations in the hydrophobic pocket of the PPIase domain (Figure 7A), based on mutations that have previously been demonstrated to decrease PPIase activity (DeCenzo et al., 1996; Koser et al., 1993; Timerman et al., 1995). We furthermore analyzed a spontaneous mutation (T345A) that changed a threonine to alanine at a position frequently occupied by polar or charged residues in other FKBPs. Two mutant forms of Fpr3, Y386D and F341Y/D342V, were stable during mitosis (Figure S9A) but displayed reduced stability during meiotic development (Figure 7B). The

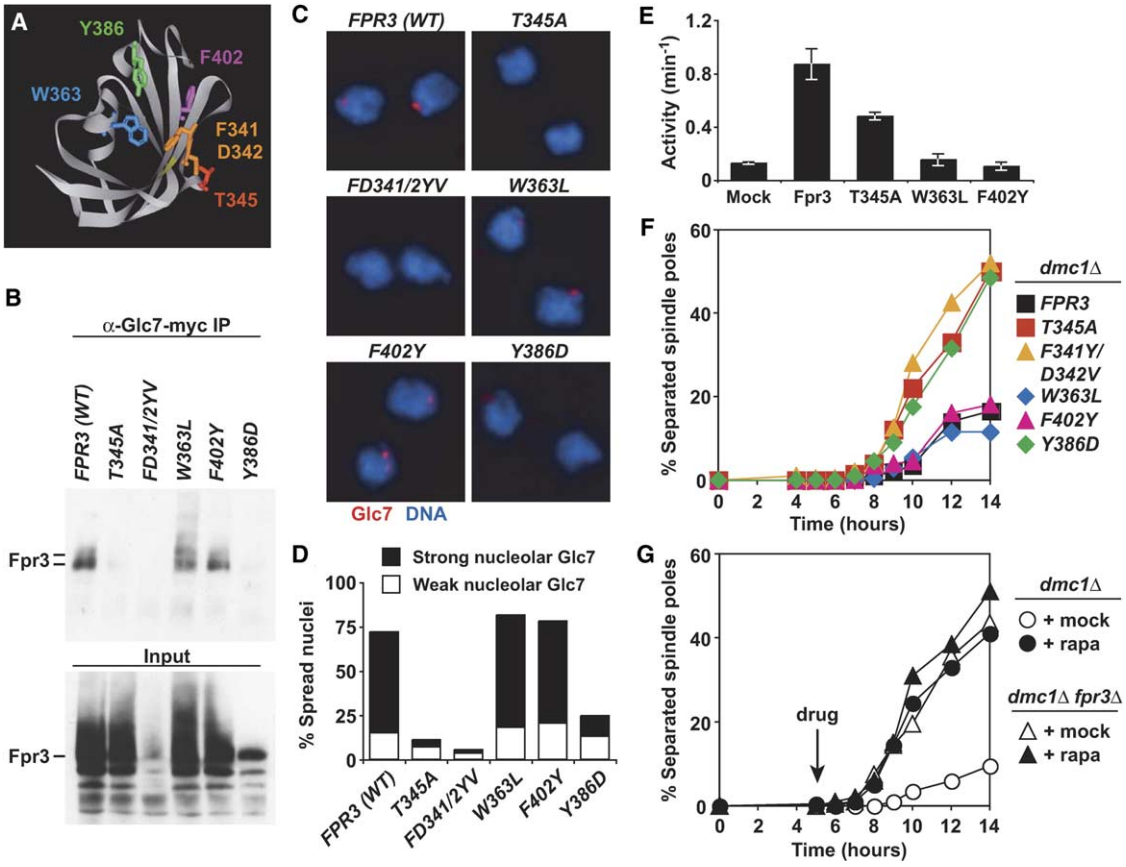

Figure 7. The PPIase Domain of Fpr3 Is Required for Checkpoint Function

(A) Predicted structure of the PPIase domain of Fpr3 to illustrate positions of mutated residues. WT residues are depicted. Colors correspond to color code in (E).

(B) Glc7 was immunoprecipitated from meiotic extracts at 3 hr and probed for the presence of Fpr3. Strains carry point mutations at the endogenous *FPR3* locus and harbor a *GLC7-myc* fusion. The following strains were used: WT *FPR3* (A12658), *T345A* (A12659), *F341Y/D342V* (A12660), *W363L* (A12661), *F402Y* (A12662), and *Y386D* (A12663).

(C and D) Spread early meiotic cells (at the time of transfer into SPO medium) were analyzed for the presence of Glc7. Quantifications are shown in (D) and representative images in (C). Glc7 is in red, DNA in blue.

(E) Proline isomerase activity of recombinant Fpr3 (WT), Fpr3 T345A, Fpr3 W363L, and Fpr3 F402Y. Activity describes the reaction rate (change in OD395) when the data was fit to a first order reaction. Error bars show standard deviations from three experiments.

(F) Synchronous meiotic cultures were analyzed at the indicated times for the percentage of cells with separated SPBs. The following strains were used: WT *FPR3* (A9674), *T345A* (A9675), *F341Y/D342V* (A9676), *W363L* (A9677), *F402Y* (A9678), and *Y386D* (A9679).

(G) 5 hr after meiotic induction, *dmc1Δ* (A7594) and *dmc1Δ fpr3Δ* (A7593) cells were treated with 10 μM rapamycin or 1% methanol (mock) and the percentage of cells with separated SPBs was determined at the indicated times.

other point mutations did not affect protein stability (Figures 7A and 7B). Furthermore, all mutant proteins localized to the nucleolus normally in premeiotic cells (Figure S9B). We found that three different mutations of the PPIase domain (T345A, Y386D, and F341Y/D342V) caused a loss of the interaction between Fpr3 and Glc7 in both premeiotic and meiotic cells, as judged by immunolocalization studies on meiotic spreads (Figures 7C and 7D) and coimmunoprecipitation analysis (Figure 7B). Two other mutations in the isomerase domain (W363L and F402Y) did not affect the binding between Fpr3 and Glc7 (Figures 7B–7D). Analysis of the in vitro PPIase activity of recombinant Fpr3 point mutants showed that both W363L and F402Y mutants had lost PPIase activity (Figure 7E), consistent with observations in other FKBPs (DeCenzo et al., 1996; Timerman et al., 1995). The T345A mutation reduced Fpr3 PPIase activity to about half of wild-type levels (Figure 7E). Our observations show that the PPIase domain of Fpr3 is required for the association between Fpr3 and Glc7. The disparity between the in vitro isomerase activities and in vivo binding activity of the T345A, W363L, and F402Y mutants furthermore suggests that the proline isomerase activity itself is not required for the interaction between Fpr3 and Glc7.

The Proline Isomerase Domain of *FPR3* Is Necessary for *FPR3*'s Checkpoint Function

Fpr3's PPIase domain is essential for the checkpoint role of Fpr3. A C-terminal truncation of Fpr3 that removed the entire PPIase domain (amino acids 300–411) was unable to complement a deletion of *FPR3* (data

not shown). Furthermore, the same point mutations that exhibited a loss of interaction between Fpr3 and Glc7 (T345A, Y386D, and F341Y/D342V) also caused a loss of *FPR3* function in vivo as assayed by their inability to maintain a *dmc1Δ* arrest (Figures 7B and 7E). The two other mutations in the isomerase domain (W363L and F402Y) that did not affect Fpr3 binding to Glc7 also did not affect Fpr3 function in vivo. The strong correlation between the ability of Fpr3 to bind Glc7 and the checkpoint activity of Fpr3 suggests that the interaction between Fpr3 and Glc7 is important for Fpr3's checkpoint function.

As a final test of the importance of Fpr3's isomerase domain in the recombination checkpoint, we examined the effects of two well-characterized small molecule inhibitors of Fpr3, FK506 and rapamycin, on the recombination checkpoint-induced G2 delay. Treatment of *dmc1Δ* cells with either FK506 or rapamycin allowed them to progress through the meiotic divisions (Figures 7F and S10). Rapamycin exhibited its effect at substantially lower doses than FK506 (Figure S10). Selectivity for rapamycin has previously been observed for Fpr3 (Shan et al., 1994). Drug addition specifically affected Fpr3 and not other checkpoint factors because exposure to rapamycin bypassed the *dmc1Δ* arrest to levels similar to those observed when *FPR3* was deleted. Moreover, the effect of rapamycin was not enhanced by a deletion of *FPR3* indicating that rapamycin acted by inhibiting Fpr3 (Figure 7F). Taken together, the effects of Fpr3 point mutations and rapamycin on the recombination checkpoint indicate that the proline isomerase domain of Fpr3 but not its isomerase activity is required for the protein's checkpoint function.

Discussion

Fpr3 Is a Component of the Recombination Checkpoint

The recombination checkpoint is a conserved meiosis-specific surveillance mechanism (Roeder and Bailis, 2000). In the present study, we identified the FK506 and rapamycin binding protein Fpr3 as being required for maintained checkpoint arrest. Many meiotic checkpoint factors, in particular the components of the canonical mitotic DNA damage checkpoint machinery, Rad24, Rad17, Mec3, Ddc1, and Mec1, while being important sensors and transducers of the DNA damage signal in mitotic cells, have a poorly understood second role during meiosis in preventing DSB repair from the sister chromatid (Grushcow et al., 1999; Thompson and Stahl, 1999). Separating checkpoint and repair functions for these factors during meiosis has generally not been trivial. Here, we developed a tool to analyze the checkpoint contribution of any putative recombination checkpoint factor independently of its repair function. By constructing haploid cells that do not replicate their genome but still enter the meiotic program, we eliminated all homologous repair templates for meiotic recombination—the sister chromatid as well as the homologous chromosomes. This allowed us to unambiguously classify *FPR3* as a checkpoint factor. The same assay will be very helpful in evaluating the checkpoint roles of factors that also function to promote DSB repair.

What Is the Function of Fpr3 in the Recombination Checkpoint?

A role for PP1 in the exit from meiotic prophase has been observed in both budding yeast and *Xenopus*. In budding yeast, PP1 appears to counteract the activity of the checkpoint kinase Mek1 (Bailis and Roeder, 2000), while in *Xenopus*, it activates the cell cycle phosphatase Cdc25 (Margolis et al., 2003). Several lines of evidence suggest that Fpr3 functions at least in part through PP1. First, PP1 and Fpr3 influence the checkpoint arrest in opposing ways. PP1 is required for the exit from meiotic prophase (Bailis and Roeder, 2000; Margolis et al., 2003), whereas *FPR3* is necessary to inhibit premature exit from the checkpoint arrest. Second, Fpr3 and Glc7 share a similar nucleolar localization pattern and associate with each other in both mitotic and meiotic cells. This association can be abrogated by introducing point mutations into the proline isomerase domain of Fpr3. The same point mutations also cause loss of Fpr3's checkpoint activity. Finally, *FPR3* antagonizes *GLC7* function in vivo. In mitotic cells, the lethality associated with overexpression of *GLC7* was efficiently suppressed by high levels of *FPR3*. In meiotic cells, overexpression of *FPR3* prevented the bypass of the recombination checkpoint caused by high levels of *GLC7*.

Together, our data suggest a model in which *FPR3* maintains the checkpoint arrest by antagonizing *GLC7* function. This idea is consistent with our observation that the partial alleviation of the checkpoint delay in *dmc1Δ* cells by overexpression of *GLC7* is only insignificantly enhanced by the additional deletion of *FPR3*. The fact that inactivation of *FPR3* only bypasses the arrests of *dmc1Δ*, *hop2Δ*, *rec8Δ*, and *mer3Δ* mutants after an initial delay is also consistent with the above model. Because *FPR3* does not affect the checkpoint pathway itself, the checkpoint signal remains active in these mutants and could be responsible for the initial delay. Unrestrained Glc7 activity would however eventually override the checkpoint arrest and promote entry into the meiotic divisions. It has not escaped our attention that this model of Glc7 regulation is reminiscent of the regulation of the protein phosphatase Cdc14, which is kept inactive in the nucleolus by an inhibitory subunit Cfi1/Net1 (Stegmeier and Amon, 2004). The finding that the nucleolar structure occupied by Fpr3 and Glc7 differs from that occupied by Cdc14 furthermore raises the interesting possibility that distinct domains of the nucleolus may serve different signaling functions.

Based on our observations, we propose *FPR3* and *GLC7* function in the adaptation to persistent DNA damage. Adaptation, that is, continued cell cycle progression after an initial arrest even if the DNA damage remains, is a phenomenon that has been studied in yeast and vertebrates (Toczyski et al., 1997) and involves the inactivation of the checkpoint kinases Rad53 (the mitotic homolog of Mek1) and Chk1, respectively (Pellicioli et al., 2001; Yoo et al., 2004). In the absence of *FPR3* or upon overexpression of *GLC7*, adaptation may be accelerated. Indeed, our observations that some aspects of the checkpoint remain active in the absence of *FPR3*, as well as the fact that the effects of changing *FPR3* and *GLC7* levels can only be observed when the recombination checkpoint has been activated

by persistent chromosomal damage, are consistent with a role of the two proteins in adaptation. Intriguingly, one factor required for checkpoint adaptation is casein kinase II (CKII; Toczyski et al., 1997). Fpr3 has been identified as a physiological substrate of CKII (Wilson et al., 1997a). It will therefore be of interest to investigate the role of CKII phosphorylation of Fpr3 in the context of the recombination checkpoint. Conversely, the presence of both Fpr3 and Glc7 during the mitotic cell cycle raises the possibility that these two factors are also involved in the adaptation response outside of meiosis.

The PPIase Domain of Fpr3 Is Required for Its Checkpoint Function

Fpr3 is one of four FKBPs in yeast. FKBPs are a highly conserved protein family, but the cellular roles of many FKBPs remain poorly understood (Hamilton and Steiner, 1998). This is particularly true of the proline isomerase domain. The PPIase domain of FKBPs is of interest not only because it acts as the receptor for rapamycin and FK506, two drugs of considerable clinical importance, but also because of a PPIase activity associated with this domain that has thus far remained an in vitro phenomenon (Hamilton and Steiner, 1998). Part of the problem to define an in vivo function for the FKBP PPIase activity is the lack of a suitable in vivo assay, and the generally transient nature of the isomerization event. However, even when targeted point mutations were analyzed that exhibited varying defects in PPIase activity in vitro, these variations often did not correlate with the functionality of the domain in vivo (Timerman et al., 1995). This has led to the speculation that the PPIase domain may function in some cases as a protein interaction domain rather than as an enzyme (Hamilton and Steiner, 1998). Fpr3, like other FKBPs, possesses PPIase activity in vitro (Benton et al., 1994; Manning-Krieg et al., 1994; Shan et al., 1994), and our analysis shows that Fpr3 checkpoint activity is lost when several residues in the PPIase domain are mutated. However, some point mutations that cause a complete loss of PPIase activity still exhibited wild-type function in the cell, whereas another point mutation that exhibits only a partial reduction in PPIase activity caused a complete loss of checkpoint function in vivo. It therefore appears that the PPIase activity of Fpr3 is not required for the protein's checkpoint function. It is however clear that the PPIase domain of *FPR3* is essential for its checkpoint function. Both point mutations in the PPIase domain and treatment of *dmc1Δ* cells with rapamycin led to a phenotype similar if not identical to that of deleting *FPR3*.

Is the Checkpoint Function of Fpr3 Shared by other FKBPs?

The yeast genome contains a close homolog of Fpr3 called Fpr4 that has a role in rDNA silencing (Kuzuhara and Horikoshi, 2004). *FPR3* and *FPR4* appear to share some common function since overexpression of either factor rescues the temperature sensitivity of a *tom1* mutant (Davey et al., 2000) and since double deletion of both genes causes a slight inhibition of cell proliferation in our strain background (unpublished data). However, even though *FPR4* is expressed at low levels in meiosis, inactivation of *FPR4* did not allow *dmc1Δ* or *hop2Δ* mutants to enter meiosis I, and the *fpr3Δ fpr4Δ* double mutant did not bypass the arrest significantly better than the *fpr3Δ* single mutant (unpublished data). Thus, if *FPR4* has a role in the recombination checkpoint, it is likely to be a very minor one.

Mouse *Fkbp6* is distantly related to *FPR3* and so far the only mammalian FKBP with a known role in meiotic progression. Male *Fkbp6*$^{-/-}$ mice show severe defects during meiotic G2, leading to an arrest prior to pachytene and to apoptosis (Crackower et al., 2003). Interestingly, disruptions and truncations of other mammalian checkpoint factors, such as *Atm*, *Brca1*, and *Brca2*, also cause infertility in mice (Baarends et al., 2001). Thus, although the *Fkbp6*$^{-/-}$ phenotype is quite different from the phenotype caused by the inactivation of *FPR3*, its similarity to the phenotypes of other checkpoint mutants in mouse raises the possibility of a role of FKBPs in mammalian recombination checkpoint signaling. If this were the case, the risks of defective gamete formation would have to be considered when using the immunosuppressive and antiproliferative drugs rapamycin and FK506.

Experimental Procedures

Yeast Strains and Plasmids

Unless otherwise noted, all strains were derivatives of SK1. Strains are listed in Table S2. Gene deletions, *CLB3-3HA* and *GLC7-13MYC* were constructed by one-step gene replacement (Longtine et al., 1998). *ZIP1-GFP*, *CDC14-3HA,* and *REC8-3HA* were described previously (Marston et al., 2003; White et al., 2004). *FPR3* point mutants were created by site-directed mutagenesis (QuickChange XL, Stratagene) and integrated at the *FPR3* locus. GST-Fpr3 expression vectors were constructed by cloning the *FPR3* ORF and the various point mutants into pGEX4T-1 (GE Healthcare).

Screen, Growth Conditions, and Drug Treatment

The screening procedure was based on a screen conducted by (Marston et al., 2004) and is described in detail in Figure S1. Conditions for α-factor release were as described (Visintin et al., 1999). Synchronous meioses were conducted as described (Marston et al., 2003).

Immunofluorescence and Spreads

Meiotic spreads and whole-cell immunofluorescence were performed as described by Marston et al. (2003). For spreads, monoclonal 4A6 α-myc antibody (Upstate Cell Signaling) or rabbit α-myc (Gramsch) were used at 1:150, N-terminal α-Fpr3 (Benton et al., 1994) at 1:2500, α-Zip1 antibody at 1:200, and α-Nop1 monoclonal 28F2 antibody (EnCor Biotechnology) at 1:2000. Conditions for visualizing Cdc14-HA and Rec8-HA have been described previously (Marston et al., 2003). For whole-cell IF, rat α-tubulin YOL1/34 (Oxford Biotechnology) was used at 1:200, α-Fpr3 at 1:150, and 28F2 at 1:250. For each time-point, 200 cells were scored. Where indicated, images were deconvolved from 0.2 μm z-stacks using the 3D restoration software of Openlab 3.1.5 (Improvision; 12-17 iterations).

Immunoprecipitation

Cell pellets were broken with glass beads in an equal volume of breakage buffer (50 mM potassium phosphate [pH = 7.4], 10 mM KCl, 10% glycerol, 0.01% NP40, 2.75 mM DTT, 2× complete protease inhibitors—EDTA [Roche]). Glc7 was immunoprecipitated from ~5 mg of total protein in 150 mM NaCl using monoclonal mouse α-myc 9E10 (Covance) and Protein G sepharose (Pierce).

Recombinant Fpr3 and PPIase Measurements

Recombinant wild-type and mutant Fpr3 were expressed as GST-fusion proteins at 30°C. Cells were lysed by sonication in 50 mM Tris (pH 7.4) (+0.5 mM DTT and complete protease inhibitors [Roche]). GST-Fpr3 was purified over Q sepharose (100 mM – 640 mM NaCl gradient). The peak fraction was applied to glutathione sepharose 4B (Amersham) and recombinant Fpr3 was released from the beads by thrombin cleavage at room temperature. PPIase activity of 50 μg recombinant Fpr3 was assayed following the procedure of Shan et al. (1994) using Suc-Ala-Leu-Pro-Phe-pNA (BACHEM) as a substrate. PPIase activity was observed at 4°C in a CARY 50 Bio UV-Vis Spectrophotometer (Varian) at 395 nm. The resulting data points were fit to first order kinetics.

Other Techniques

Southern blot analysis was conducted as described by Hunter and Kleckner (2001). Blots were quantified using ImageQuant software (Amersham Biosciences). Fpr3 was modeled on the crystal structures of homologous FKBPs using SwissModel (Guex and Peitsch, 1997; Peitsch, 1995; Peitsch, 1996) and visualized using DS Viewer Pro software (accelrys). Flow cytometric analysis of total cellular DNA content and Western analysis were performed as described in (Visintin et al., 1998). For Western analysis, C-terminal α-Fpr3 antibody was used at a dilution of 1:2500 and α-Cdc28 was used at 1:1000.

Supplemental Data

Supplemental Data include ten figures, two tables, and Supplemental References and can be found with this article online at http://www.cell.com/cgi/content/full/122/6/861/DC1/.

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References

Alani, E., Padmore, R., and Kleckner, N. (1990). Analysis of Wild-Type and rad50 Mutants of Yeast Suggests an Intimate Relationship between Meiotic Chromosome Synapsis and Recombination. Cell *61*, 419–436.

Arevalo-Rodriguez, M., Wu, X., Hanes, S.D., and Heitman, J. (2004). Prolyl isomerases in yeast. Front. Biosci. *9*, 2420–2446.

Baarends, W.M., van der Laan, R., and Grootegoed, J.A. (2001). DNA repair mechanisms and gametogenesis. Reproduction *121*, 31–39.

Bailis, J.M., and Roeder, G.S. (2000). Pachytene exit controlled by reversal of Mek1-dependent phosphorylation. Cell *101*, 211–221.

Benton, B.M., Zang, J.H., and Thorner, J. (1994). A novel FK506- and rapamycin-binding protein (FPR3 gene product) in the yeast Saccharomyces cerevisiae is a proline rotamase localized to the nucleolus. J. Cell Biol. *127*, 623–639.

Bishop, D.K., Nikolski, Y., Oshiro, J., Chon, J., Shinohara, M., and Chen, X. (1999). High copy number suppression of the meiotic arrest caused by a dmc1 mutation: REC114 imposes an early recombination block and RAD54 promotes a DMC1-independent DSB repair pathway. Genes Cells *4*, 425–444.

Bishop, D.K., Park, D., Xu, L., and Kleckner, N. (1992). DMC1: a meiosis-specific yeast homolog of E. coli recA required for recombination, synaptonemal complex formation, and cell cycle progression. Cell *69*, 439–456.

Bloecher, A., and Tatchell, K. (2000). Dynamic localization of protein phosphatase type 1 in the mitotic cell cycle of Saccharomyces cerevisiae. J. Cell Biol. *149*, 125–140.

Borde, V., Goldman, A.S.H., and Lichten, M. (2000). Direct Coupling Between Meiotic DNA Replication and Recombination Initiation. Science *290*, 806–809.

Borner, G.V., Kleckner, N., and Hunter, N. (2004). Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell *117*, 29–45.

Breiman, A., and Camus, I. (2002). The involvement of mammalian and plant FK506-binding proteins (FKBPs) in development. Transgenic Res. *11*, 321–335.

Cocker, J.H., Piatti, S., Santocanale, C., Nasmyth, K., and Diffley, J.F. (1996). An essential role for the Cdc6 protein in forming the pre-replicative complexes of budding yeast. Nature *379*, 180–182.

Crackower, M.A., Kolas, N.K., Noguchi, J., Sarao, R., Kikuchi, K., Kaneko, H., Kobayashi, E., Kawai, Y., Kozieradzki, I., Landers, R., et al. (2003). Essential role of Fkbp6 in male fertility and homologous chromosome pairing in meiosis. Science *300*, 1291–1295.

Davey, M., Hannam, C., Wong, C., and Brandl, C.J. (2000). The yeast peptidyl proline isomerases FPR3 and FPR4, in high copy numbers, suppress defects resulting from the absence of the E3 ubiquitin ligase TOM1. Mol. Gen. Genet. *263*, 520–526.

De Massy, B., Baudat, F., and Nicolas, A. (1994). Initiation of recombination in Saccharomyces cerevisiae haploid meiosis. Proc. Natl. Acad. Sci. USA *91*, 11929–11933.

DeCenzo, M.T., Park, S.T., Jarrett, B.P., Aldape, R.A., Futer, O., Murcko, M.A., and Livingston, D.J. (1996). FK506-binding protein mutational analysis: defining the active-site residue contributions to catalysis and the stability of ligand complexes. Protein Eng. *9*, 173–180.

Dolinski, K., Muir, S., Cardenas, M., and Heitman, J. (1997). All cyclophilins and FK506 binding proteins are, individually and collectively, dispensable for viability in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA *94*, 13093–13098.

Grushcow, J.M., Holzen, T.M., Park, K.J., Weinert, T., Lichten, M., and Bishop, D.K. (1999). Saccharomyces cerevisiae checkpoint genes MEC1, RAD17 and RAD24 are required for normal meiotic recombination partner choice. Genetics *153*, 607–620.

Guex, N., and Peitsch, M.C. (1997). SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis *18*, 2714–2723.

Hamilton, G.S., and Steiner, J.P. (1998). Immunophilins: beyond immunosuppression. J. Med. Chem. *41*, 5119–5143.

Heitman, J., Movva, N.R., and Hall, M.N. (1992). Proline isomerases at the crossroads of protein folding, signal transduction, and immunosuppression. New Biol. *4*, 448–460.

Ho, Y., Gruhler, A., Heilbut, A., Bader, G.D., Moore, L., Adams, S.L., Millar, A., Taylor, P., Bennett, K., Boutilier, K., et al. (2002). Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry. Nature *415*, 180–183.

Hunter, N., and Kleckner, N. (2001). The single-end invasion: an asymmetric intermediate at the double-strand break to double-holliday junction transition of meiotic recombination. Cell *106*, 59–70.

Keeney, S. (2001). Mechanism and control of meiotic recombination initiation. Curr. Top. Dev. Biol. *52*, 1–53.

Klein, F., Mahr, P., Galova, M., Buonomo, S.B.C., Michaelis, C., Nairz, K., and Nasmyth, K. (1999). A Central Role for Cohesins in Sister Chromatid Cohesion, Formation of Axial Elements and Recombination during Yeast Meiosis. Cell *98*, 91–103.

Koser, P.L., Eng, W.K., Bossard, M.J., McLaughlin, M.M., Cafferkey, R., Sathe, G.M., Faucette, L., Levy, M.A., Johnson, R.K., Bergsma, D.J., et al. (1993). The tyrosine89 residue of yeast FKBP12 is required for rapamycin binding. Gene *129*, 159–165.

Kuzuhara, T., and Horikoshi, M. (2004). A nuclear FK506-binding

protein is a histone chaperone regulating rDNA silencing. Nat. Struct. Mol. Biol. *11*, 275–283.

Leu, J.-Y., Chua, P.R., and Roeder, G.S. (1998). The Meiosis-Specific Hop2 Protein of S. cerevisiae Ensures Synapsis between Homologous Chromosomes. Cell *94*, 375–386.

Longtine, M.S., McKenzie, A., 3rd, Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J.R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast *14*, 953–961.

Lydall, D., Nikolsky, Y., Bishop, D.K., and Weinert, T. (1996). A meiotic recombination checkpoint controlled by mitotic checkpoint genes. Nature *383*, 840–843.

Manning-Krieg, U.C., Henriquez, R., Cammas, F., Graff, P., Gaveriaux, S., and Movva, N.R. (1994). Purification of FKBP-70, a novel immunophilin from Saccharomyces cerevisiae, and cloning of its structural gene, FPR3. FEBS Lett. *352*, 98–103.

Margolis, S.S., Walsh, S., Weiser, D.C., Yoshida, M., Shenolikar, S., and Kornbluth, S. (2003). PP1 control of M phase entry exerted through 14–3-3-regulated Cdc25 dephosphorylation. EMBO J. *22*, 5734–5745.

Marston, A.L., Lee, B.H., and Amon, A. (2003). The Cdc14 phosphatase and the FEAR network control meiotic spindle disassembly and chromosome segregation. Dev. Cell *4*, 711–726.

Marston, A.L., Tham, W.H., Shah, H., and Amon, A. (2004). A genome-wide screen identifies genes required for centromeric cohesion. Science *303*, 1367–1370.

Munn, K., and Steward, R. (2000). The shut-down gene of Drosophila melanogaster encodes a novel FK506-binding protein essential for the formation of germline cysts during oogenesis. Genetics *156*, 245–256.

Nakagawa, T., and Ogawa, H. (1999). The Saccharomyces cerevisiae MER3 gene, encoding a novel helicase-like protein, is required for crossover control in meiosis. EMBO J. *18*, 5714–5723.

Pak, J., and Segall, J. (2002). Role of Ndt80, Sum1, and Swe1 as targets of the meiotic recombination checkpoint that control exit from pachytene and spore formation in Saccharomyces cerevisiae. Mol. Cell. Biol. *22*, 6430–6440.

Peitsch, M.C. (1995). Protein modeling by E-mail. Biotechnology (N. Y.) *13*, 658–660.

Peitsch, M.C. (1996). ProMod and Swiss-Model: Internet-based tools for automated comparative protein modelling. Biochem. Soc. Trans. *24*, 274–279.

Pellicioli, A., Lee, S.E., Lucca, C., Foiani, M., and Haber, J.E. (2001). Regulation of Saccharomyces Rad53 checkpoint kinase during adaptation from DNA damage-induced G2/M arrest. Mol. Cell *7*, 293–300.

Petes, T.D., and Pukkila, P.J. (1995). Meiotic sister chromatid recombination. Adv. Genet. *33*, 41–62.

Roeder, G.S., and Bailis, J.M. (2000). The pachytene checkpoint. Trends Genet. *16*, 395–403.

San-Segundo, P.A., and Roeder, G.S. (1999). Pch2 links chromatin silencing to meiotic checkpoint control. Cell *97*, 313–324.

Schwacha, A., and Kleckner, N. (1997). Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. Cell *90*, 1123–1135.

Shan, X., Xue, Z., and Melese, T. (1994). Yeast NPI46 encodes a novel prolyl cis-trans isomerase that is located in the nucleolus. J. Cell Biol. *126*, 853–862.

Smith, K.N., Penkner, A., Ohta, K., Klein, F., and Nicolas, A. (2001). B-Type Cyclins CLB5 and CLB6 Control the Initiation of Recombination and Synaptonemal Complex Formation in Yeast Meiosis. Curr. Biol. *11*, 88–97.

Stegmeier, F., and Amon, A. (2004). Closing Mitosis: The Functions of the Cdc14 Phosphatase and Its Regulation. Annu. Rev. Genet. *38*, 203–232.

Storlazzi, A., Xu, L., Cao, L., and Kleckner, N. (1995). Crossover and noncrossover recombination during meiosis: timing and pathway relationships. Proc. Natl. Acad. Sci. USA *92*, 8512–8516.

Sym, M., Engebrecht, J.A., and Roeder, G.S. (1993). ZIP1 Is a Synaptonemal Complex Protein Required for Meiotic Chromosome Synapsis. Cell *72*, 365–378.

Thompson, D.A., and Stahl, F.W. (1999). Genetic control of recombination partner preference in yeast meiosis. Isolation and characterization of mutants elevated for meiotic unequal sister-chromatid recombination. Genetics *153*, 621–641.

Timerman, A.P., Wiederrecht, G., Marcy, A., and Fleischer, S. (1995). Characterization of an exchange reaction between soluble FKBP-12 and the FKBP.ryanodine receptor complex. Modulation by FKBP mutants deficient in peptidyl-prolyl isomerase activity. J. Biol. Chem. *270*, 2451–2459.

Toczyski, D.P., Galgoczy, D.J., and Hartwell, L.H. (1997). CDC5 and CKII control adaptation to the yeast DNA damage checkpoint. Cell *90*, 1097–1106.

Tsubouchi, H., and Roeder, G.S. (2003). The importance of genetic recombination for fidelity of chromosome pairing in meiosis. Dev. Cell *5*, 915–925.

Tung, K.S., Hong, E.J., and Roeder, G.S. (2000). The pachytene checkpoint prevents accumulation and phosphorylation of the meiosis-specific transcription factor Ndt80. Proc. Natl. Acad. Sci. USA *97*, 12187–12192.

Visintin, R., Craig, K., Hwang, E.S., Prinz, S., Tyers, M., and Amon, A. (1998). The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. Mol. Cell *2*, 709–718.

Visintin, R., Hwang, E.S., and Amon, A. (1999). Cfi1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. Nature *398*, 818–823.

Wagstaff, J.E., Klapholz, S., and Esposito, R.E. (1982). Meiosis in haploid yeast. Proc. Natl. Acad. Sci. USA *79*, 2986–2990.

White, E.J., Cowan, C., Cande, W.Z., and Kaback, D.B. (2004). In vivo analysis of synaptonemal complex formation during yeast meiosis. Genetics *167*, 51–63.

Wilson, L.K., Dhillon, N., Thorner, J., and Martin, G.S. (1997a). Casein kinase II catalyzes tyrosine phosphorylation of the yeast nucleolar immunophilin Fpr3. J. Biol. Chem. *272*, 12961–12967.

Wilson, T.E., Grawunder, U., and Lieber, M.R. (1997b). Yeast DNA ligase IV mediates non-homologous DNA end joining. Nature *388*, 495–498.

Wohlschlegel, J.A., Johnson, E.S., Reed, S.I., and Yates, J.R., 3rd. (2004). Global analysis of protein sumoylation in Saccharomyces cerevisiae. J. Biol. Chem. *279*, 45662–45668.

Yoo, H.Y., Kumagai, A., Shevchenko, A., and Dunphy, W.G. (2004). Adaptation of a DNA replication checkpoint response depends upon inactivation of Claspin by the Polo-like kinase. Cell *117*, 575–588.

Zickler, D., and Kleckner, N. (1999). Meiotic Chromosomes: Integrating Structure and Function. Annu. Rev. Genet. *33*, 603–754.