Meiotic cells monitor the status of the interhomolog recombination complex

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During meiosis, mutations that cause defects at intermediate stages in the recombination process confer arrest at the end of prophase (e.g., pachytene). In yeast, mutations of this type include *rad50S*, *dmc1*, *rad51*, and *zip1*. Rad50 is likely part of a recombination initiation complex. *DMC1*, *RAD51*, and *ZIP1* encode two RecA homologs and a synaptonemal complex protein, respectively. We report here the effects of mutations in two other (meiosis-specific) genes, *RED1* and *MEK1/MRE4*, that encode a chromosome structure component and a protein kinase, respectively. A *red1* or *mek1/mre4* mutation alleviates completely *rad50S*, *dmc1*, *rad51*, and *zip1* arrest. Furthermore, the *red1* and *mek1/mre4* mutations define a unique, previously unrecognized aspect of recombination imposed very early in the process, during DSB formation. Finally, the *red1* and *mek1/mre4* mutations appear to alleviate prophase arrest directly rather than by eliminating, or permitting bypass of, the *rad50S*, *dmc1*, *rad51*, or *zip1* defects. These and other observations suggest that a meiosis-specific regulatory surveillance process monitors the status of the protein/DNA interhomolog recombination machinery as an integral entity, in its proper chromosomal context, and dependent upon its appropriate Red1 and Mek1/Mre4-promoted development. We speculate that a properly developed recombination complex emits an inhibitory signal to delay progression of meiotic cells out of prophase until or unless the recombination process has progressed, at least past certain critical steps, and perhaps to completion.

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Meiosis involves a single round of DNA replication followed by two rounds of chromosome segregation. At the unique meiosis I division, homologous chromosomes segregate to opposite poles. This process requires a physical connection between homologs that is usually provided by one or a few crossovers in conjunction with intersister cohesions (Carpenter 1994; Roeder 1995; Kleckner 1996).

In *Saccharomyces cerevisiae*, most or all meiotic recombination is initiated by double-stranded breaks (DSBs), which are resected rapidly to give 3' single-stranded tails. DSBs are then converted to double Holliday junctions, at about the time that synaptonemal complex (SC) forms between the two homolog structural axes. Mature recombinants, both crossovers and non-crossovers, arise at about the end of pachytene, the stage when SC is full-length. Immediately after SC dissolution, mother and daughter spindle pole bodies (SPBs) separate to form a short spindle (e.g., Shuster and Byers 1989; Padmore et al. 1991). The chemical events of recombination occur within an elaborate protein/DNA assembly, often observable cytologically as a prominent

nodular structure, which undergoes progressive morphogenesis as the recombination process proceeds. In addition, the recombination complex is juxtaposed physically to the structural axes of the interacting homologs throughout the process and important functional interplay between the two features seems likely (Kleckner 1996).

An interesting question is how meiotic cells coordinate the events of prophase to maintain a high fidelity of genetic transmission. In mitotic cells, the appropriate order of cell-cycle events is maintained by regulatory mechanisms; specific checkpoint systems monitor DNA replication and chromosome segregation and prevent the progression of cell cycle beyond critical stages if errors in these processes are detected (Hartwell and Weinert 1989). Not surprisingly, some somatic surveillance systems are conserved in meiotic cells. In yeast, *RAD9*-dependent checkpoints ensure that chromosome replication does not occur on damaged DNA during meiosis (Thorne and Byers 1993), as during vegetative growth, and also restrain commitment to recombination when meiotic S phase fails to complete (Weber and Byers 1992).

Previous observations suggested the existence of a checkpoint that monitors meiotic prophase chromosome metabolism (Bishop et al. 1992; Rose and Holm

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1993). One study analyzed a mutant lacking a meiosis-specific homolog of *Escherichia coli* RecA protein, Dmc1 (Bishop et al. 1992). A *dmc1Δ* mutant makes DSBs but is defective in strand exchange (Bishop et al. 1992; A. Schwacha and N. Kleckner, unpubl.) and, correspondingly, in crossover formation. SC formation is delayed, but substantial SC forms ultimately (this study; Bishop et al. 1992; Rockmill et al. 1995b). Intriguingly, *dmc1Δ* cells also fail to undergo SC disassembly, SPB separation, or meiotic divisions. These latter defects are similar to those conferred by mutations in *CDC28* (Shuster and Byers 1989), which encodes the catalytic subunit of maturation-promoting factor (MPF). Also, there is no indication that SPB separation and subsequent steps of meiosis are mechanically dependent on the chromosomes. Thus, it was proposed that proper execution of prophase chromosome metabolism is required for initiation of meiotic division, with prophase arrest resulting from a regulatory mechanism that blocks MPF activation (Bishop et al. 1992). The same could be true for meiosis in all organisms (e.g., Baker et al. 1996; Edelman et al. 1996).

Meiosis-specific chromosome metabolism must at least go through its initial stages for regulatory monitoring to come into play. Mutants that fail to make DSBs or to initiate SC formation still proceed in a timely and efficient fashion through two divisions and spore formation, although resultant spores are dead because of chromosome missegregation (Klapholz et al. 1985; Alani et al. 1990; Malone et al. 1991). Moreover, such “early block” mutations eliminate completely the arrest conferred by *dmc1Δ* (Bishop et al. 1992). Thus, although “relief of dependence” by loss-of-function mutations is generally considered to be evidence for the existence of a checkpoint (Hartwell and Weinert 1989), alleviation of arrest in these cases is attributable to an upstream block rather than to bona fide relief of dependence.

A number of other “intermediate block” mutations are known. For example, a *rad50S*, *rad51*, or *zip1* mutation each causes a defect in recombination after DSBs are formed, and confers arrest or delay in SPB separation that is alleviated by an early block mutation. Rad50 is likely part of a recombination initiation complex that promotes DSB formation (e.g., Raymond and Kleckner 1993; Johzuka and Ogawa 1995; Keeney and Kleckner 1995); *rad50S* alleles permit DSBs to form but not to progress (Alani et al. 1990; Keeney and Kleckner 1995; see below). Rad51 is another RecA homolog related to Dmc1; *rad51* and *dmc1* mutants exhibit similar but not identical defects (this study; Shinohara et al. 1992; D.K. Bishop and A. Shinohara, pers. comm.). Zip1 is an SC central region component but is required for normal recombination independent of its SC role (Sym et al. 1993; Sym and Roeder 1994; Storlazzi et al. 1996).

Several factors point to recombination as the process specifically subject to checkpoint surveillance. Dmc1 and Rad51 appear to be involved directly in the recombination reaction (Bishop 1994) and both are required for formation of interhomolog Holliday junctions (Schwacha 1996); also, a *dmc1* mutation affects the mor-

phology of interhomolog association sites observed in chromosomes lacking SC (Rockmill et al. 1995b). In addition, since successful completion of interhomolog crossing-over is a crucial *raison-d'être* of meiotic prophase, recombination would be an appropriate target for regulatory surveillance (Bishop et al. 1992). Alternatively, however, arrest might be triggered by defective SC formation (Sym et al. 1993) or by chromosomal interlocks (Rose and Holm 1993) although interlocks have not been detected in yeast (Sym et al. 1993).

In considering how regulatory surveillance of meiotic chromosome metabolism might occur, we were intrigued by the existence of certain meiotic mutants (e.g., *red1* and *mek1/mre4*) that did not fit into either the early block or intermediate block categories. Red1 is a major structural component of meiotic chromosomes (Roeder 1995), whereas Mek1/Mre4 has homology to protein kinases (Rockmill and Roeder 1991; Leem and Ogawa 1992). *red1* and *mek1/mre4* mutations confer a partial defect in formation of recombination products, similar to intermediate block mutations, but do not confer any arrest or delay in the onset of the two meiotic divisions (Rockmill and Roeder 1990, 1991; Leem and Ogawa 1992). Interestingly, the two mutations confer dramatically different effects on chromosome structure. In a *red1* mutant neither axial elements nor tripartite SC are detectable (Rockmill and Roeder 1990), whereas a *mek1/mre4* mutant makes nearly normal SC (Rockmill and Roeder 1991).

These phenotypes suggested to us that *red1* and *mek1/mre4* mutations might define aspects of the recombination process required to make its progression monitorable by the checkpoint system. In particular, checkpoint monitoring might require appropriate development of the meiotic recombination complex as promoted by Red1 and Mek1/Mre4. The experiments presented below investigate this possibility by examining the effects of *red1* and *mek1/mre4* mutations on prophase arrest conferred by intermediate block mutations and on the recombination process. Additional experiments determine whether meiotic prophase cells retain the capacity to arrest in response to nonspecific DNA damage and investigate whether timely and efficient completion of recombination requires SC or SC disassembly.

The results presented provide evidence that a meiosis-specific (Red1- and Mek1/Mre4-dependent) regulatory surveillance system monitors the status of a properly developed interhomolog recombination complex with Red1 and Mek1/Mre4 required for such development. We speculate that such a complex emits a signal to inhibit SC disassembly and SPB separation until recombination is complete, or nearly so. Possible relationships between meiosis-specific surveillance of recombination and mitotic checkpoint systems that monitor DNA replication and sense DNA damage are discussed.

Results

Prophase arrest and alleviation of such arrest

In cultures of a wild-type SK1 strain undergoing synchro-

nous meiosis, 90–95% of cells have completed meiosis I (MI) by 12 hr after transfer to sporulation medium (Fig. 1A); 5–10% of cells probably never enter meiosis (Padmore et al. 1991).

A *dmc1Δ* mutant exhibits permanent prophase arrest; only ~1% of cells have undergone MI by $t=24$ hr (Fig. 1A). *rad51Δ* and *zip1Δ* mutants both exhibit “mixed arrest”. Sixty to 70% of cells arrest permanently and 30–40% of cells arrest for several hours and then undergo MI (Fig. 1A); about two-thirds of the latter cells make spores (data not shown).

All three of these mutants arrest with duplicated but unseparated SPBs (Bishop et al. 1992; Shinohara et al. 1992; Sym et al. 1993). *dmc1Δ* and *rad51Δ* cells exhibit a delay in SC formation but substantial amounts of SC forms eventually (Fig. 1; Bishop et al. 1992; Rockmill et al. 1995b; D.K. Bishop, unpubl.). A *zip1Δ* mutant lacks SC central regions (Sym et al. 1993; Sym and Roeder 1994).

The *red1Δ* and *mek1/mre4Δ* mutants carry out two meiotic divisions and form spores with normal kinetics

Figure 1. Prophase arrest conferred by mutations in *DMC1*, *RAD51*, or *ZIP1* or methylmethane sulfonate treatment. (A) Isogenic SK1 strains of wild-type (NKY1113), *dmc1Δ* (NKY1455), *rad51Δ* (NKY2580), and *zip1Δ* (NKY2515) were taken through synchronous meiosis. Occurrence of meiotic divisions was monitored by DAPI staining and fluorescence microscopy. Plotted is the percentage of cells that had undergone one or both nuclear divisions (MI±MII) at various time throughout sporulation. (B) Sporulating cultures of wild-type (NKY611), *spo11Δ* (NKY648), and *rad50Δ* (NKY2712) strains were divided into two portions 3 hr after initiation of meiosis. To one portion 0.05% methylmethane sulfonate (MMS) was added, and the other portion served as a control. Two subcultures of each strain were allowed to continue sporulation. The kinetics of meiotic divisions in the presence and absence of MMS was monitored. (C–E) Formation of synaptonemal complexes (SCs) was examined in both wild-type and *dmc1Δ* strains in the experiment shown in A. Plotted is the percentage of nuclei containing pachytene SCs at various time during meiosis. Examples of nuclear morphology under light microscope are shown in D (wild-type at $t=5$ hr) and E (*dmc1Δ* at $t=8$ hr).

Figure 2. Effects of *red1Δ* and *mek1Δ* mutations on meiotic prophase arrest. (A–C) Isogenic SK1 strains with indicated genotypes were taken through synchronous meiosis. Occurrence of meiotic divisions was monitored. (A-i) NKY1113 (wild type), NKY1455 (*dmc1Δ*), NKY2682 (*red1Δ*), and NKY2685 (*dmc1Δ red1Δ*); (A-ii) NKY1551 (wild type), NKY1876 (*rad51Δ*), NKY2548 (*red1Δ*), and NKY2688 (*rad51Δ red1Δ*); (A-iii) NKY1551 (wild type), NKY2515 (*zip1Δ*), NKY2548 (*red1Δ*), and NKY2570 (*zip1Δ red1Δ*); (B-i) NKY1113 (wild type), NKY1455 (*dmc1Δ*), NKY2694 (*mek1Δ*), and NKY2697 (*dmc1Δ mek1Δ*); (B-ii) NKY1113 (wild type), NKY2580 (*rad51Δ*), NKY2694 (*mek1Δ*), and NKY2700 (*rad51Δ mek1Δ*); (B-iii) NKY1551 (wild type), NKY2515 (*zip1Δ*), NKY2702 (*mek1Δ*), and NKY2705 (*zip1Δ mek1Δ*). (C) NKY2559 (*rad50S*), NKY2955 (*red1Δ rad50S*), and NKY2721 (*mek1Δ rad50S*). (D,E) *red1Δ* and *mek1Δ* mutants were analyzed for MMS-induced meiotic prophase arrest as in Fig. 1B. NKY611 (wild type), NKY869 (*red1Δ*), NKY2711 (*mek1Δ*), NKY2712 (*rad50Δ*), NKY2713 (*rad50Δ red1Δ*), and NKY2714 (*rad50Δ mek1Δ*).

and proficiency (Fig. 2; Rockmill and Roeder 1990, 1991; Leem and Ogawa 1992).

A *red1Δ* or *mek1/mre4Δ* mutation alleviates completely the prophase arrest conferred by a *dmc1Δ*, *rad51Δ*, or *zip1Δ* mutation. Double mutants carrying one mutation of each type (*red1Δ* or *mek1/mre4Δ*, plus *dmc1Δ*, *rad51Δ*, or *zip1Δ*) all carry out two meiotic divisions and spore formation with kinetics and efficiency very similar to wild type (Fig. 2; data not shown). A *red1Δ* mutation also alleviates *zip1* arrest in the BR strain background (data not shown) where arrest is more severe (Sym et al. 1993).

A *rad50S* mutant also exhibits a delay in the onset of MI; furthermore, *red1Δ rad50S* and *mek1/mre4Δ rad50S* double mutants undergo MI with normal efficiency and timing (Fig. 2C). Thus, alleviation of arrest by *red1Δ* or *mek1/mre4Δ* mutations occurs with four different intermediate block mutants.

red1Δ and *mek1Δ/mre4Δ* mutations define a new and unique function required for normal DSB formation

Analysis of DSBs DSB formation was analyzed at the *HIS4LEU2* locus as described previously (Cao et al. 1990; Xu and Kleckner 1995; Fig. 3). DSBs occur at this locus at two specific sites I and II, each revealed by a corresponding diagnostic restriction fragment.

In a wild-type culture, DSBs can be observed as early as 2 hr after initiation of meiosis ($t=2$ hr), are at their maximal level at $t=3-4$ hr, begin to disappear at $t=5$ hr, and are essentially not detectable by $t=6$ hr (Fig. 4). The average lifespan of a DSB is 15–30 min (Padmore et al. 1991; Schwacha and Kleckner 1994); occurrence of DSBs over a longer time span reflects residual asynchrony in the culture (Padmore et al. 1991). The 5' termini of DSBs are resected ~600 nucleotides but with some variability; the diagnostic fragment thus gives a somewhat fuzzy appearance (Bishop et al. 1992; Fig. 4).

Early block mutations eliminate completely DSB formation (see introduction section). A *rad50S* mutation specifically blocks both DSB resection and turnover with resultant accumulation of DSBs in a sharp band on a Southern blot (Alani et al. 1990; Keeney and Kleckner 1995; Fig. 5).

dmc1, *rad51*, and *zip1* mutations affect recombination after the formation of resected DSBs. In *dmc1* and *rad51* mutants, DSB turnover is impeded and resection is not controlled properly: Both mutants accumulate higher than normal levels of hyperresected DSBs. In a *dmc1Δ* strain, DSBs are hyperresected modestly and persist indefinitely; in *rad51Δ*, DSBs are hyperresected dramatically and persist into late times but disappear eventually

(Bishop et al. 1992; Shinohara et al. 1992; Fig. 4). In *zip1Δ*, DSB formation and disappearance are essentially normal except that a low level of normally resected DSBs is seen at aberrantly late times (e.g., $t=8$ hr; Fig. 4D; A. Schwacha and N. Kleckner, unpubl.), one indication that *ZIP1* function is also executed at the transition from DSBs to later forms (Storlazzi et al. 1996).

The *red1Δ* and *mek1Δ/mre4Δ* mutations confer yet another DSB phenotype. In each single mutant, DSBs appear and disappear with wild-type kinetics and exhibit a normal degree of resection, but the steady-state level of DSBs is severalfold lower than in a wild-type strain at $t=3$ and 4 hr and at most other time points (Fig. 4). The *red1Δ* and *mek1Δ/mre4Δ* mutants exhibit steady-state DSB levels of ~25% and ~15% of wild type, respectively. The maximal level of DSBs at site I, as a percentage of total DNA, is 5–6% in wild type, 1.4% in *red1Δ* and 0.85% in *mek1Δ/mre4Δ*. The reduced steady-state DSB level in *mek1Δ/mre4Δ* was also observed previously (Leem and Ogawa 1992).

Analysis of crossovers Formation of interhomolog crossovers is also monitored at *HIS4LEU2* by the appearance of diagnostic restriction fragments (R1 and R2; Fig. 3; Cao et al. 1990). In wild-type, crossovers are first detected at about $t=5$ hr and reach their maximal level at about $t=8$ hr, when recombinants make up 20–24% of total DNA (e.g., Fig. 6, leftmost lanes).

In *dmc1Δ*, *rad51Δ*, and *zip1Δ* strains crossovers occur at reduced level, ~10%, 15%, and 15% of wild-type, respectively at $t=8$ hr, as observed previously (Fig. 6; Table 1; Bishop et al. 1992; Shinohara et al. 1992; Storlazzi et al. 1996). In a *rad50S* mutant, crossovers are even further reduced (data not shown).

In *red1Δ* and *mek1Δ/mre4Δ* single mutants crossovers are also reduced, and to the same extent as steady-state DSB levels, 25% and 15% of the wild-type level, respectively (Fig. 6; Table 1). A *red1Δ* mutation also reduces the level of interhomolog noncrossover products to ~25% of wild-type (Storlazzi et al. 1996). The coordinate reduction in steady-state DSB and final product levels suggests that in these mutants all of the observable DSBs are converted into interhomolog recombination products with normal efficiency. In support of this conclusion, a *red1Δ* mutation reduces interhomolog Holliday junctions to about the same extent as both earlier and later species (Schwacha 1996).

Unique interplay between *rad50S* and *red1Δ* or *mek1Δ/mre4Δ* mutations All recombination-defective mutants analyzed previously exhibit comparable levels of DSBs in both *RAD50* and *rad50S* backgrounds. In early block mutants, DSBs are undetectable in both cases (e.g., Cao et al. 1990); in *mer1Δ* and *hop1Δ* mutants, DSBs occur at reduced levels in the presence or absence of a *rad50S* mutation (Storlazzi et al. 1995); and in *dmc1Δ* *rad50S*, *rad51Δ* *rad50S*, and *zip1Δ* *rad50S* strains, DSBs occur at the same level as in an otherwise wild-type *rad50S* strain (Bishop et al. 1992; Shinohara et al. 1992; Fig. 5B), as expected for mutations that affect steps after DSB resection. In contrast, whereas the *red1Δ* and *mek1Δ*

Figure 3. Physical analysis of meiotic recombination at *HIS4LEU2*. Shown is the *HIS4LEU2* locus on chromosome III, which specifies two DSB sites and promotes high levels of recombination during meiosis. Homologs A and B differ with respect to markers that flank site I; the presence of *his4X* allele and *URA3* insert on chromosome A eliminates two *XhoI* sites. Chromosome B carries either *his4B* or wild-type *HIS4*. In some cases, chromosome A harbors *MluI::BamHI* instead of the original *MluI* allele at site I (Xu and Kleckner 1995). DSB formation and crossing over can be assayed by *PstI* digestion/probe 291 and *XhoI* digestion/probe 155, respectively. In wild-type, recombinants account for ~20% (*MluI/MluI* homozygote) to 24% (*MluI::BamHI/MluI* heterozygote) of total DNA at this locus.

Figure 4. A *red1Δ* or *mek1Δ* mutation decreases the steady-state levels of DSBs. Isogenic SK1 strains of indicated genotypes were induced to undergo synchronous meiosis. DNA was isolated at indicated times after initiation of meiosis and assayed as in Fig. 3. Strains used are: (A) NKY1113 (wild type), NKY1455 (*dmc1Δ*), NKY2682 (*red1Δ*), and NKY2685 (*dmc1Δ red1Δ*); (B) NKY1113 (wild type), NKY1455 (*dmc1Δ*), NKY2694 (*mek1Δ*), and NKY2697 (*dmc1Δ mek1Δ*); (C) NKY1551 (wild type), NKY1876 (*rad51Δ*), NKY2548 (*red1Δ*), and NKY2688 (*rad51Δ red1Δ*) (at the bottom is the longer exposure of the DSB site I region); (D) NKY1551 (wild type), NKY2515 (*zip1Δ*), NKY2548 (*red1Δ*), and NKY2570 (*zip1Δ red1Δ*). Normal DSBs form a fuzzy signal on the Southern blot. Discrete, *rad50S*-like bands are sometimes observed at DSB sites at late times in this *HIS4LEU2* construct, for unknown reasons.

mre4Δ single mutants exhibit reduced steady-state DSB levels, *red1Δ rad50S* and *mek1/mre4Δ rad50S* double mutants exhibit the same level of DSBs as a *rad50S* strain (Fig. 5A).

red1-mek1/mre4 mutations and rad51-zip1-dmc1 mutations have substantially independent effects on DSBs and crossovers

RED1 and *MEK1/MRE4* functions are apparently executed at or during DSB formation (above; Discussion), whereas *DMC1*, *RAD51*, and *ZIP1* functions are executed after DSB resection. Thus, a *red1* or *mek1/mre4* mutation should further reduce the levels of DSBs and recombinant products in a *dmc1*, *rad51*, or *zip1* mutant background; in the simplest case, the double mutant phenotype should be the combination of the two component single mutant phenotypes.

A *red1Δ* or *mek1/mre4Δ* mutation and a *zip1Δ* muta-

tion do indeed appear to affect meiotic recombination independently. Steady-state levels of DSBs in a *zip1Δ red1Δ* strain or a *zip1Δ mek1Δ/mre4Δ* strain are much lower than in a *zip1Δ* strain and are similar to levels in *red1Δ* or *mek1Δ/mre4Δ* single mutants (see Fig. 4D; data not shown). Also, DSBs exhibit a normal degree of resection in the two double mutants as in the single mutants. Furthermore, low levels of DSBs appear to be present at late times in the *zip1Δ red1Δ* double mutant as in the *zip1Δ* single mutant but at the reduced level expected from the *red1Δ* mutation (Fig. 4D). The presence or absence of DSBs at late times in *mek1/mre4* single and double mutants is not yet known. Finally, the levels of crossovers observed in the two double mutant strains are much lower than in any of the component single mutants and are essentially those expected from the product of the two single mutant defects (Fig. 6; Table 1). The same is true for noncrossover recombinants (Storlazzi et al. 1996).

Figure 5. Effect of the *red1Δ*, *mek1Δ*, *dmc1Δ*, and *zip1Δ* mutations on levels of DSBs in a *rad50S* background. DNA was isolated at the indicated times after initiation of meiosis and analyzed for DSB formation (Fig. 3). (A) NKY2559 (*rad50S*), NKY2955 (*red1Δ rad50S*), and NKY2721 (*mek1Δ rad50S*). (B) NKY1392 (*rad50S*), NKY1593 (*dmc1Δ rad50S*), NKY2559 (*rad50S*), and NKY2525 (*zip1Δ rad50S*). NKY1392 and NKY1593 are homozygous for *MluI* at site I; other strains are *MluI/MluI::BamHI* (Fig. 3). Total DSBs at *HIS4LEU2* (sites I + II) in the three strains in A are 20.3%, 19.4%, and 19.8%, respectively. Total DSBs in the four strains in B are 18.8%, 19.2%, 23.1%, and 23.0%, respectively.

Similarly, a *red1Δ* or *mek1/mre4Δ* and a *rad51Δ* mutation also appear to act independently. A *red1Δ* mutation reduces the steady-state level of DSBs in a *rad51Δ* background severalfold, as in a *red1Δ* single mutant, but DSBs still exhibit the severe hyperresection, persistence at late times, and eventual disappearance at late time points seen in a *rad51Δ* single mutant. Although these

Figure 6. Crossover formation in the *dmc1Δ red1Δ*, *rad51Δ red1Δ*, and *zip1Δ red1Δ* double mutant strains. (A) Four isogenic strains were taken through synchronous meiosis: wild-type (NKY1113), *dmc1Δ* (NKY1455), *red1Δ* (NKY2682), and *dmc1Δ red1Δ* (NKY2685). In wildtype, maximal levels of mature crossover products (R1 and R2) are observed at about $t=8$ hr; a *dmc1Δ* mutant is defective in formation of crossover and only a very faint signal was present at the R2 position. A *red1Δ* strain makes ~25% wild-type level of recombinants. A *dmc1Δ red1Δ* double mutant hardly makes an observable level of recombinants. (B) Same as A except strains are wild-type (NKY1551), *rad51Δ* (NKY1876), *red1Δ* (NKY2548), and *rad51Δ red1Δ* (NKY2688). (C) Same as A except strains are wild-type (NKY1551), *zip1Δ* (NKY2515), *red1Δ* (NKY2548), and *zip1Δ red1Δ* (NKY2570).

Table 1. Relative levels of crossovers in various single and double mutant strains

	wt	<i>red1Δ</i>	<i>mek1Δ</i>
wt	100	25	15
<i>dmc1Δ</i>	10	3(2.5)	2(1.5)
<i>rad51Δ</i>	15	3(3.75)	2(2.25)
<i>zip1Δ</i>	15	3(3.75)	2(2.25)

Strains with indicated genotypes were induced to go through synchronous meiosis. At desired time ($t = 0$ and 8 hr), samples of $\sim 5 \times 10^8$ cells were taken from each sporulating culture and chromosomal DNA was isolated and analyzed as in Fig. 6. DNA fragments were quantitated on a Fuji BAS2000 PhosphorImager. The level of crossovers in a wild-type strain is defined as 100%. Each value presented represents the average of two independent experiment except for those of *rad51Δmek1Δ* and *zip1Δmek1Δ*, which are from a single experiment. The numbers in parentheses are the crossover levels predicted from the products of the crossover levels observed in the two types of single mutants.

features in combination make the DSB signal difficult to detect in the double mutant, they are all readily apparent in extra long exposures of autoradiograms (Fig. 4C, bottom portion). Thus, the *red1* DSB deficit and the *rad51* defect in DSB progression both remain in the double mutant. Furthermore, a double mutant exhibits a much lower level of crossover products than either single mutant (Fig. 6; Table 1). Also, DSBs occur at normal *rad50S* levels in a *rad51Δ red1Δ rad50S* triple mutant (data not shown). A *rad51Δ mek1Δ/mre4Δ* mutant has not been analyzed for DSBs.

The interplay between *red1Δ* or *mek1/mre4Δ* and *dmc1Δ* mutations is more complex. The DSB phenotype of a *dmc1Δ red1Δ* mutant is the same as that of a *red1Δ* single mutant with respect to both the steady-state levels of DSBs at various times and the absence of discernible hyperresection. This is in sharp contrast to the *dmc1Δ* single mutant, where DSBs accumulate to high levels and persist in a hyperresected form essentially indefinitely. These *dmc1Δ red1Δ* phenotypes are fully explained by the proposition that Red1 has two independent roles, a positive role in promoting DSB formation and a negative role in preventing DSB progression in the absence of Dmc1. Preliminary experiments suggest that the *dmc1Δ mek1Δ* double mutant behaves the same as the *dmc1Δ red1Δ* strain.

Although DSBs persist indefinitely in a *dmc1Δ*, in both *dmc1Δ red1Δ* and *dmc1Δ mek1/mre4Δ* double mutants, DSBs appear and disappear in a timely fashion; furthermore, hyperresection is substantially reduced or absent in the double mutants (see Fig. 4). Thus, in the absence of Red1 or Mek1/Mre4, efficient progression of the recombination process beyond the DSB stage no longer requires Dmc1. However, interhomolog crossovers are not restored in the double mutant (Table 1). Further observations (Schwacha 1996; Y. Nikolsky and D.K. Bishop, pers. comm.) demonstrate that in a *dmc1Δ red1Δ* mutant, most DSBs undergo strand exchange by a novel intersister bypass pathway and suggest additional aspects of Red1 function that affect post-DSB events, as discussed elsewhere.

Intermediate block recombination defects are not consequences of prophase arrest The *rad51*, *zip1*, and *dmc1* defects in interhomolog recombination persist in strains in which no cell cycle arrest occurs (discussion above). Thus, the recombination defects in these mutants cannot be an indirect consequence of cell cycle arrest, as proposed for *zip1* (Sym et al. 1993). Presumably Zip1, as well as Dmc1 and Rad51, influences the recombination process directly.

*Alleviation of *rad50S*-, *rad51*-, *dmc1*-, or *zip1*-induced prophase arrest by a *red1* or *mek1/mre4* mutation does not result from elimination or bypass of the recombination defect*

Combining a *red1Δ* or *mek1Δ/mre4Δ* mutation with an intermediate block mutation does not alleviate arrest by creating an early block phenocopy in which DSBs fail to occur; in all three types of double mutants DSBs and interhomolog recombination products still occur.

Alleviation of arrest also does not result from restoration of normal recombination in the double mutants. To the contrary, the levels of interhomolog recombination products observed in the double mutants are generally much lower than in any of the corresponding single mutants.

Alleviation of arrest could in principle occur because a *red1Δ* or *mek1Δ/mre4Δ* mutation provides an alternative pathway for recombination in intermediate block mutants. In double mutants containing a *zip1Δ* or *rad51Δ* mutation, there is no indication of such a bypass. Presumptive stalled intermediates (i.e., DSBs), which are present at late times in *zip1Δ* and *rad51Δ* single mutants, are also observed in *zip1Δ red1Δ* and *rad51Δ red1Δ* double mutants. Moreover, in the *zip1Δ* case, there is only a subtle defect in progression of the reaction through the DSB step. Instead, nearly all of the DSBs appear to progress to Holliday junctions whose kinetics are aberrant (Storlazzi et al. 1996), and the *red1Δ* mutation affects only the number of interhomolog products observed in a *zip1Δ* background, not their aberrant distribution between crossover and noncrossover. Thus, any alternative process could only change the rate of progression through Holliday junctions to products rather than redirecting DSBs to a default pathway.

In a *red1Δ rad50S* or *mek1/mre4Δ rad50S* strain, DSBs are recovered at high levels, just as in a *rad50S* mutant. These findings provide additional evidence that alleviation of arrest does not involve elimination of the recombinational defect (assuming that DSBs observed in the double mutants are present in vivo; see Discussion).

In a *dmc1Δ red1Δ* mutant, some type of alternative process clearly occurs (discussion above). But independent work by Y. Nikolsky and D.K. Bishop (pers. comm.) has shown that the existence of this bypass process is not responsible for alleviation of arrest. A *rad54Δ* mutation eliminates DSB progression in a *dmc1Δ red1Δ* background, with resultant permanent accumulation of resected DSBs, but there is still no arrest.

Thus, it seems likely that *red1Δ* and *mek1Δ/mre4Δ*

mutations alleviate arrest directly, that is, Red1 and Mek1/Mre4 are required for either the sensing of or signaling of a problem.

MMS treatment during prophase causes late prophase arrest that is independent of RED1 and MEK1/MRE4

The effects of nonspecific DNA damage were compared with the effects of *dmc1Δ*, *rad51Δ*, and *zip1Δ* mutations. Cells in meiotic prophase were treated with a radiomimetic agent methylmethane sulfonate (MMS). MMS was added to cultures at *t*=3 hr; at this time, essentially all cells had completed DNA replication (Williamson et al. 1983) and meiosis-specific DSBs were at their maximal level (Cao et al. 1990). This protocol avoids triggering the earlier RAD9 checkpoint, which is activated by lesions present before or during meiotic S phase (Weber and Byers 1992; Thorne and Byers 1993). MMS treatment causes a mixed arrest phenotype very similar to that seen in the *rad51Δ* and *zip1Δ* mutants; 60–70% of cells arrest permanently, whereas 30–40% of cells arrest for several hours and then proceed through divisions and, eventually, spore formation (see Fig. 1B). The amount of MMS required to observe arrest, 0.05%, is relatively low. At this concentration, vegetative growth of SK1 cells is only slightly inhibited (Alani et al. 1990).

Repair of DSBs in mitotic cells specifically requires genes in the RAD52 epistasis group, one member of which is RAD50 (Resnick 1987). When a *rad50Δ* mutant is treated with MMS during meiotic prophase, the arrest response is exaggerated over that observed in wild type; now, 100% of cells arrest permanently (see Fig. 1B).

MMS-induced arrest probably results from a checkpoint response as in mitotic cells. This view is supported most specifically by the similarity of the MMS-arrest phenotype with that resulting from a *rad51* or *zip1* mutation. The effects of mitotic checkpoint-defective mutations remain to be examined, however.

Meiotic arrest induced by MMS treatment is not affected by mutations that alleviate the *dmc1Δ*, *rad51Δ*, or *zip1Δ* arrest. *spo11Δ*, *red1Δ*, and *mek1/mre4Δ* mutants behave similarly as wild type; also, the *red1Δ rad50Δ*, and *mek1/mre4Δ rad50Δ* double mutants arrest completely, just like the *rad50Δ* single mutant (see Figs. 1B and 2D,E).

Crossovers occur with normal kinetics in the absence of SC and in cells arrested with full-length SC

Previous observations suggested that SC disassembly might be required for completion of crossing over (Padmore et al. 1991). The existence of a recombination checkpoint might predict oppositely that completion of recombination would be required for SC disassembly (see Discussion). Therefore, we examined the kinetics of crossover formation in the *red1Δ* mutant, which completely lacks SC (Rockmill and Roeder 1990), and in *cdc28*, which arrests with full-length SC (Shuster and

Byers 1989). The *cdc28-63* ts allele confers tight G_1 arrest in vegetative cells at 34°C (see Materials and Methods). A *cdc28-63* mutant (in both SK1 and BR2495 backgrounds) sporulates at the same proficiency as the corresponding wild-type strain at 20–22°C (data not shown). In contrast, at 34°C, all cells undergo permanent arrest before MI. Chromosome morphogenesis also arrests, as observed previously (Shuster and Byers 1989). In *cdc28-63* diploids of both strain backgrounds, the pachytene stage is about three times longer than that in the corresponding wild-type strain (Fig. 7B,E).

Crossovers appeared with wild-type kinetics in both *red1Δ* and *cdc28-63* mutant cases (Fig. 7A,C,F). The absolute level of crossing over is affected about twofold in both *cdc28* strains for reasons not yet known. Two-dimensional gel analysis shows that the crossover products formed in a *cdc28-63* mutant comprise intact DNA strands (Fig. 7D). These findings confirm and extend earlier indications that recombination occurs in pachytene-arrested cells (Rose and Holm 1993) and are further extended by the observation of normal crossover kinetics in an *ndt80Δ* mutant, which also exhibits pachytene arrest (Xu et al. 1995). Thus, SC disassembly is not required for completion of crossing over.

Discussion

Red1 and Mek1/Mre4 coordinately modulate a major mechanistic transition of meiotic recombination in a qualitatively unique way

A working model for yeast meiotic recombination is diagrammed in Figure 8. The process likely begins with assembly of components into a pre-DSB complex. Then, at a certain point, this complex undergoes a rapid series of changes that yield a recombination complex containing resected DSBs that are tightly held and poised for progression to the next stage. During cleavage, the two strands of the DNA duplex are cleaved at closely spaced positions and the nuclease remains covalently attached to the resultant 5' strand termini (de Massy et al. 1995; Keeney and Kleckner 1995; Liu et al. 1995). As one consequence of this feature, a DSB is likely to be chemically reversible until it has undergone resection. DSB formation and subsequent resection are tightly coupled both temporally (Alani et al. 1990) and functionally (de Massy et al. 1995; Keeney and Kleckner 1995; Liu et al. 1995).

This first transition is followed by further development of the recombination complex; large numbers of Rad51 and Dmc1 proteins likely join the complex at this

Figure 7. Crossovers occur with normal kinetics in the *cdc28* and *red1Δ* mutants. (A,B) Meiosis was initiated in wild-type (NKY175) and *cdc28-63* (NKY1780) SK1 strains (see Materials and Methods). The wild-type strain sporulated well under this condition; the *cdc28-63* strain failed to undergo any meiotic divisions and exhibited a delay in exit from pachytene at 34°C. Crossover formation was monitored by digesting meiotic DNA with *XhoI* and Southern blot analysis with probe 155 (A). Kinetics of recombinant formation are plotted in B. Recombinant levels in wild-type and *cdc28-63* at $t=12$ hr were each defined as 100%, and recombinant levels at other time points normalized to this maximal level for each strain. (C–E) Same as A and B except that strains used are of BR2495 background: LXY411 (wild type) and LXY417 (*cdc28-63*). Recombinant bands account for ~17% and ~30% of total DNA in wild-type and *cdc28-63* strains, respectively. A DNA sample from the *cdc28-63* mutant ($t=16$ hr) was also digested with *XhoI*, separated in a native gel in the first dimension, and then denatured and fractionated under alkaline condition in the second dimension (D). The fraction of DNA in the two recombinant bands along the diagonal is the same ($\pm 10\%$) as that observed in native gel (C). (F–G) Isogenic wild-type (NKY1380) and *red1Δ* (NKY1614) SK1 strains were taken through synchronous meiosis and the kinetics of crossover formation and meiotic divisions was examined as in A–E.

Figure 8. Model for the early stages of meiotic recombination. Entry of relevant gene products into the protein/DNA recombination complex and points at which relevant mutations confer defects are indicated, *above* and *below* the pathway, respectively. The homologous partner molecule, not indicated, may enter the reaction before DSB formation (for review, see Kleckner 1996).

stage. The resulting structure could correspond to cytologically observable "early recombination nodules" (RNs) (Bishop 1994). A second major transition then converts DSBs to double Holliday junctions by Rad51, Dmc1, and probably, Zip1 (see introductory section). Finally, double Holliday junctions are converted into mature products in what appears to be a third major transition (Kleckner 1996).

red1Δ and *mek1/mre4Δ* mutations confer qualitatively identical arrays of phenotypes. We infer that both mutations affect the same specific and previously undefined aspect of the recombination process. Genetic epistasis analysis is consistent with this view (Rockmill and Roeder 1991). The new aspect appears to modulate the first major transition of the recombination reaction rather than later steps. In *red1Δ* and *mek1/mre4Δ* mutants, the steady state level of DSBs is reduced but the breaks that are observed appear to be converted to subsequent intermediates and recombination products with normal efficiency and timing.

Red1 and Mek1/Mre4 must modulate DSB formation in a qualitatively unique way. Only two other mutations have been described that reduce DSB formation to lower, but still detectable levels: *mer1Δ* and *hop1Δ*; in neither case is the level of DSBs restored to normal by the *rad50S* mutation.

Previously analyzed mutations that reduce DSB levels likely affect either the assembly of a protein/DNA complex capable of making breaks or the DSB nuclease itself, with coordinate reduction of DSB levels in *RAD50* and *rad50S* backgrounds in such mutants reflecting a decrease in the potential for DSB formation. In support of this view, a *mer1* mutation reduces DSB formation (Storlazzi et al. 1995) because it results in a deficit of Mer2 protein (Engebrecht et al. 1990) and *mer2* is an early block mutant (Rockmill et al. 1995a).

The reduced steady-state level of DSBs in *red1Δ* and *mek1/mre4Δ* mutants is most directly explained if Red1 and Mek1/Mre4 are required for forming normal numbers of DSBs. In this case, the effects of the *rad50S* mutation can be explained in either of two ways. Because meiosis-specific DSBs are made by a topoisomerase-like

mechanism in which the protein that makes the breaks is covalently linked to the 5' strand termini, a DSB is chemically reversible until the 5' terminal protein has been removed and/or resection has begun (de Massy et al. 1995; Keeney and Kleckner 1995; Liu et al. 1995). If Red1 and Mek1/Mre4 affected the rate of this removal step (with concomitant resection of 5' termini), a *rad50S* mutation might suppress the absence of these proteins by increasing or decreasing the rates of DSB formation or protein removal, respectively (Fig. 8). Similarly, if the DSB transesterase activity is truly topoisomerase-like, pre-DSB complexes in *red1Δ rad50S* or *mek1/mre4Δ rad50S* mutant may be held in an "activated" conformation, with breaks provoked at maximal levels by exposure to proteases or detergent during DNA extraction.

Alternatively, Red1 and Mek1/Mre4 might be required for making a recombination complex that holds together during the DSB formation process. In that case, DSBs would form in normal numbers but would usually undergo rapid and uncontrolled resection such that they are invisible in a gel; moreover, such DSBs must no longer give interhomolog recombination products. The *rad50S* block to DSB resection would restore normal DSB levels by preventing such degradation. Because the DSBs do form a discrete signal in *red1* and *mek1/mre4* mutants and appear completely normal, this model would seem to require a binary choice between stability and instability.

Because Red1 is a prominent structural component of meiotic chromosomes (Roeder 1995), it could modulate recombination indirectly via the geometry or physical context of the recombination complex relative to the chromosome axes. Because Mek1/Mre4 is a protein kinase, it might work by phosphorylating Red1; alternatively, or in addition, it may be a local component of the recombination complex or another chromosome/chromatin structure protein.

Meiotic prophase cells monitor meiotic recombination specifically (and perhaps also chromosome pairing)

Recombination, not SC formation, is likely the monitored process The four intermediate block mutants, *rad50S*, *dmc1*, *rad51*, and *zip1*, all exhibit defects in SC formation as well as recombination. Thus, in principle, prophase arrest might arise from either defect. We strongly favor the view that arrest is triggered by a defect in the progress of recombination and that the SC defects in these mutants are not detected specifically. A strong argument comes from the fact that, for a *zip1* mutation, as examined in three different strain backgrounds, the severity of cell cycle arrest correlates directly with the severity of the recombinational defect, whereas identical SC phenotypes are observed in all three cases (Sym et al. 1993; Sym and Roeder 1994; Storlazzi et al. 1996). Similarly, as shown here, *red1* and *mek1/mre4* mutations both alleviate the arrest induced by a *rad50S*, *dmc1*, *rad51*, or *zip1* mutation, and both confer the same unique recombination phenotype, but the two mutations confer very different SC phenotypes (see introduc-

tory section). Formal proof of this view would require identification of a mutant situation in which recombination is defective (with concomitant cell cycle arrest) but SC formation is completely normal. This is likely to require a specialized type of mutation because it appears that progression of recombinational interactions is required for efficient initiation of SC formation in yeast (Roeder 1990; Kleckner et al. 1991; Rockmill et al. 1995b).

Recombination is likely monitored by a regulatory surveillance mechanism The notion that meiotic cells monitor chromosome metabolism through a checkpoint process (Bishop et al. 1992) is further supported by the current work. The *red1Δ* and *mek1/mre4Δ* mutations appear to alleviate cell cycle arrest without eliminating the defect that triggered arrest, and the existence of such a mutation is considered to be defining evidence for the existence of a checkpoint. Correspondingly, Red1 and Mek1/Mre4 are required directly for this monitoring process. Recent analysis of mitotic checkpoint mutations provides additional evidence for a recombination checkpoint (Lydall et al. 1996).

A recombination checkpoint might, in effect, also monitor chromosome pairing In yeast, chromosome pairing is thought to be independent of DSB formation; also, DSBs can occur at high levels in the absence of chromosome pairing, albeit not exactly normally (for review, see Kleckner 1996). Thus, if a particular pair of homologs failed to become properly colocalized and coaligned, and remained in separate parts of the nucleus, DSBs would form more or less as usual but might fail to progress for want of a partner. The presence of stalled DSBs would then be sensed by the recombinational surveillance mechanism exactly as in a *dmc1* mutant and the cell cycle would arrest. Thus, in effect, the process that monitors recombination would also detect failure in the earlier process of chromosome pairing. This possibility is raised and supported by analysis of haploid yeast cells tricked into entering meiosis (de Massy et al. 1994; B. de Massy, pers. comm.). In that situation, DSBs form at substantial levels and persist for a longer than normal period of time, presumably because no homolog is available and the sister chromatid is inaccessible, and, most important, the cell cycle arrests.

Fate of arrested yeast cells Yeast cells enter meiosis and form spores in response to nutrient deprivation. If nutrients are restored to meiotic cells before the first division, however, they return to vegetative growth. Yeast cells arrested by a defect in mid-prophase chromosome metabolism do not undergo SPB separation and return efficiently to a mitotic program if nutrients are provided (e.g., Bishop et al. 1992; Sym et al. 1993).

Generality Regulatory monitoring of meiotic recombination should be a general feature of meiosis in many or all organisms. Indeed, it has recently been found that the disruption of the mouse *MLH1* gene causes both a deficit

of crossovers and pachytene arrest (Baker et al. 1996; Edlmann et al. 1996). In a multicellular organism, in contrast to yeast, arrest might best be irreversible so as to preclude completely the formation of aneuploid gametes.

How do Red1 and Mek1/Mre4 contribute to checkpoint surveillance?

The *red1Δ* and *mek1/mre4Δ* mutations not only alleviate arrest, they also alter the recombination process itself. Moreover, alleviation of arrest does not involve a change in the nature of the DNA species present (at least in the *rad51* and *zip1* cases, and quite possibly in the *rad50S* case as well). These findings point to a model in which meiotic cells monitor the status of an appropriately developed meiosis-specific interhomolog recombination complex as it occurs in its normal chromosomal context, with the Red1- and Mek1/Mre4-promoted aspect (defined above) required for such development. Once this feature has been added, progression of the complex through subsequent steps of the recombination reaction can then be monitored.

Alternatively, *red1Δ* and *mek1/mre4Δ* mutations might alleviate arrest by modifying the basic cell cycle machinery. This seems unlikely because neither mutation affects MMS-induced arrest. Or, *red1Δ* and *mek1/mre4Δ* mutations might simply reduce the number of recombination complexes below some threshold level with any qualitative change in the complex being irrelevant. This possibility also seems unlikely because a *red1* mutation would only reduce the number of complexes to ~25% of normal.

Proposal: The recombination complex emits constantly an inhibitory signal until critical transition points are passed

Because interhomolog crossovers are essential for normal meiotic chromosome segregation, it seems appropriate that meiotic cells should monitor the progression of recombination as a way of coordinating the complex events of normal prophase, rather than only in response to a disaster. Therefore, we speculate that a properly assembled meiotic recombination complex constantly emits an inhibitory signal that blocks progression of the cell cycle beyond the end of meiotic prophase unless and until the formation of a DNA-based interhomolog connection is assured. This specific model is attractive because meiotic recombination process appears to occur in a series of discrete transitions, each separated by a significant delay (Kleckner 1996). If the complex emits a signal at all times, normal pauses in the recombination reaction would be accommodated automatically; the mechanism would not have to sense how much time has passed at each pause point.

The recombination checkpoint may be most important during the conversion of DSBs to double Holliday junctions. Many, and perhaps all, intermediate block mutations affect this step. Also, crossover interference,

which ensures that the number and distribution of cross-overs along each bivalent is appropriate, may be imposed at this step (Kleckner 1996; Storlazzi et al. 1996). Furthermore, a Holliday junction should already constitute an effective connection between homologs. On the other hand, it would seem biologically optimal for surveillance to continue through the completion of crossing over. Because completion of recombination is now known not to require SC disassembly (discussed above; Rose and Holm 1993), this possibility is clearly open.

Possible analogies between surveillance of meiotic recombination and surveillance of DNA replication

Regulatory surveillance of meiotic recombination as envisioned above would be logically analogous to surveillance of DNA replication. In both cases a cell must determine when an essential process has been completed successfully. In contrast, in the DNA damage checkpoint paradigm, surveillance is designed to detect an unprogrammed threat to genome integrity. Furthermore, components of the replication apparatus are known to participate directly in surveillance of replication (e.g., Navas et al. 1995) as proposed above for recombination. During replication, components of the regulatory machinery interact with the replication complex (Leatherwood et al. 1996). Perhaps Cdc28 or its putative meiosis-specific modulator Ndt80 (Xu et al. 1995) localizes to meiotic recombination complexes.

Relationship of the meiosis-specific recombination checkpoint to the mitotic DSB repair checkpoint

Mechanistically, the meiosis-specific recombination surveillance process is related to that used to monitor mitotic DNA damage. We show here that MMS treatment in prophase confers a characteristic mixed arrest/delay phenotype similar to that conferred by a *rad50S*, *rad51Δ*, or *zip1Δ* mutation. More directly, recent observations made concurrently with those described here demonstrate that genes required for the mitotic DNA damage response (*MEC1*, *RAD17*, and *RAD24*) are also required for *dmc1*- and *zip1*-induced meiotic prophase arrest (Lydall et al. 1996). Because meiotic recombination likely has evolved from mitotic DSB recombinational repair by the recruitment and meiosis-specific differentiation of the general repair machinery, the checkpoint monitoring process could similarly and concomitantly have been recruited and adapted to the sensing of defects in interhomolog recombination, with Red1 and Mek1/Mre4 contributing to that differentiation.

Meiosis-specific differentiation is likely essential because meiotic cells are concerned with the formation of interhomolog crossovers; monitoring of genome integrity alone may be insufficient. If a recombinational block were sensed only as DNA damage, a stalled intermediate might be repaired internally or completed through an interaction with a sister chromatid, as occurs preferentially in mitotic cells (Kadyk and Hartwell 1992); but

such repair would not restore interhomolog recombination, and thus normal meiotic chromosome segregation. Therefore, once meiosis resumed, aneuploid gametes would still result.

We suggest that meiotic regulatory mechanisms monitor the status of the protein/DNA recombination complex as an integral entity and as conditioned by its chromosomal context. Do these features also apply to the mitotic DNA damage checkpoint response?

Although attention has focused on single-stranded DNA as a primary signal in the DNA damage response, the observation that *rad9Δ cdc13ts* double mutant cells accumulate ssDNA even earlier than *cdc13ts* mutant cells (Lydall and Weinert 1995) raises the possibility that the entire DNA/protein repair complex participates in the checkpoint response (see also Lydall et al. 1996).

A role for chromosomal context in the DNA damage checkpoint has not been considered. The existence of such a connection would, however, be a novel explanation for the recent finding that the yeast *PDS1* gene is required both for normal sister chromatid association and for checkpoint-induced arrest in response to DNA damage (Yamamoto et al. 1996). Involvement of chromosome structure in the surveillance of both meiotic recombination, where interhomolog interactions are favored, and mitotic genome integrity, where recombinational interactions between sisters are strongly favored, would also fit nicely with the general thesis that meiotic interhomolog interactions are derived from basic mitotic intersister interactions (Kleckner 1996).

Materials and methods

Plasmids, yeast strains, and genetic procedures

pOL145 (a gift from S. Reed, Scripps Research Institute, La Jolla, CA) harbors the *cdc28-63 ts* allele on a 1.6-kb *Bam*HI-*Pvu*II fragment. pRS424 is 2μ *TRP1* (Christianson et al. 1992). pNKY155 and pNKY291 are from Cao et al. (1990). NKY strains are isogenic derivatives of SK1 containing markers described previously (Alani et al. 1990; Cao et al. 1990; Rockmill and Roeder 1990; Bishop et al. 1992; Leem and Ogawa 1992; Shinohara et al. 1992; Sym et al. 1993; Sym and Roeder 1994; Xu and Kleckner 1995). NKY611 is *ho::LYS2/ho::LYS2 lys2/lys2 ura3/ura3 leu2::hisG/leu2::hisG*. The following strains are all the same as NKY611 but carry in addition the indicated allele(s): NKY648, *spo11Δ::hisG/spo11Δ::hisG*; NKY869, *red1Δ::LEU2/red1Δ::LEU2*; NKY2711, *mek1Δ::URA3/mek1Δ::URA3*; NKY2712, *rad50Δ::hisG/rad50Δ::hisG*; NKY2713, *rad50Δ::hisG/rad50Δ::hisG*, *red1Δ::LEU2/red1Δ::LEU2*; NKY2714, *rad50Δ::hisG/rad50Δ::hisG mek1Δ::URA3/mek1Δ::URA3*; NKY1392, *arg4-Bgl/ARG4*, *his4BLEU2-MluI/HIS4LEU2-MluI rad50-KI81::URA3/rad50-KI81::URA3*; NKY1593, *arg4-Bgl/arg4-Bgl HIS4LEU2-MluI/HIS4LEU2-MluI*, *dmc1Δ::LEU2/dmc1Δ::LEU2 rad50-KI81::URA3/rad50-KI81::URA3*; NKY2559, *his4XLEU2-MluI::Bam*HI-*URA3/his4BLEU2-MluI rad50-KI81::URA3/rad50-KI81::URA3*; NKY1113, *arg4-Bgl/arg4-Nsp his4XLEU2-MluI-URA3/his4BLEU2-MluI*; NKY1551, *arg4-Bgl/arg4-Nsp his4XLEU2-MluI::Bam*HI-*URA3/his4BLEU2-MluI*; NKY1380, *his4XLEU2-MluI::Bam*HI-*URA3/his4BLEU2-MluI*; NKY2175, *trp1::hisG/trp1::hisG his4XLEU2-MluI-URA3/his4BLEU2-MluI*. NKY2525, 2955, and 2721 are NKY2559 containing, respec-

tively, *zip1Δ::LEU2/zip1Δ::LEU2*, *red1Δ::LEU2/red1Δ::LEU2*, or *mek1Δ::URA3/mek1Δ::URA3*. NKY1780 is NKY2175 (above) also containing *cdc28-63/cdc28-63*. NKY1614 is NKY1380 (above) containing *red1Δ::LEU2/red1Δ::LEU2*. The following strains are all the same as NKY1113 (above) containing in addition the indicated allele(s): NKY1455, *dmc1Δ::ARG4/dmc1Δ::ARG4*; NKY2682, *red1Δ::LEU2/red1Δ::LEU2*; NKY2685, *dmc1Δ::ARG4/dmc1Δ::ARG4 red1Δ::LEU2/red1Δ::LEU2*; NKY2694, *mek1Δ::URA3/mek1Δ::URA3*; NKY2697, *dmc1Δ::ARG4/dmc1Δ::ARG4 mek1Δ::URA3/mek1Δ::URA3*; NKY2580, *rad51Δ::hisG/rad51Δ::hisG*; NKY2700, *rad51Δ::hisG/rad51Δ::hisG mek1Δ::URA3/mek1Δ::URA3*. The following strains are all the same as NKY1551 (above) containing in addition the indicated allele(s): NKY1876, *rad51Δ::hisG/rad51Δ::hisG*; NKY2548, *red1Δ::LEU2/red1Δ::LEU2*; NKY2688, *rad51Δ::hisG/rad51Δ::hisG red1Δ::LEU2/red1Δ::LEU2*; NKY2515, *zip1Δ::LEU2/zip1Δ::LEU2*; NKY2570, *zip1Δ::LEU2/zip1Δ::LEU2 red1Δ::LEU2/red1Δ::LEU2*; NKY2702, *mek1Δ::URA3/mek1Δ::URA3*; NKY2705, *zip1Δ::LEU2/zip1Δ::LEU2 mek1Δ::URA3/mek1Δ::URA3*. LXY411 is *ura3-1/ura3-1 leu2-27/leu2-3,112 arg4-8/ARG4 trp1-1/trp1-289, thr1-1/thr1-4 cyh10^R/CYH10 ade2-1/ade2-1 his4XLEU2-MluI-URA3/his4BLEU2-MluI*. LXY417 is LXY411 also containing *cdc28-63/cdc28-63*. *cdc28-63* was introduced by cotransformation with pRS424 (subsequently evicted). At 34°C, *cdc28-63* cells are unbudded, contain single undivided nuclei, and exhibit a pear-shaping morphology, all phenotypes characteristic of start mutants (Reed 1980). LXY411 and LXY417 are derived from BR1373-6D and BR1919-8B (Rockmill and Roeder 1990) by transformation and crosses. Yeast was grown in YPD, YP lactate (YPL), YP acetate (YPA), or drop-out medium (Sherman et al. 1986). For *trp1* strains, medium contained 100 mg/l tryptophan. Sporulation medium was either 0.3% potassium acetate plus 0.02% raffinose (SK1) or 2% potassium acetate (BR).

Meiotic time courses

For synchronous meiosis, frozen stocks of cells were thawed, grown on YPL plates for 12 hr and then streaked for single colonies on YPD plates. A single colony was inoculated in 5 ml of YPD broth and grown for 24 hr. Cells were diluted 100-fold in YPA and incubated with aeration at 30°C. The culture usually reached OD₆₀₀ of 1.5 after 13.5 hr. Special care was required for *rad51* mutant cultures, which grow slowly and fail to carry out robust meiosis if not pregrown properly. Cells were then collected by centrifugation, washed once with sterile water, transferred into sporulation medium, and incubated with aeration, with aliquots taken for analysis at desired times. For *cdc28-63* strains, pregrowth was carried out at 20–22°C. Single colonies of both *cdc28-63* and wild-type strains were inoculated in 5 ml of YPD broth, grown for 36 hr, diluted 20-fold (*cdc28-63*) or 40-fold (wild-type) in YPA and grown for 13.5 hr. Meiotic cultures were kept at 20–22°C for 2 hr and then shifted to 34°C.

Cytology

To monitor meiotic divisions and spore formation, cells were fixed in 50% ethanol, stained with 0.5 μg/μl of DAPI and examined by fluorescence/phase contrast microscopy (Alani et al. 1990). SCs were visualized in spheroplasted cells by phase contrast microscopy (Loidl et al. 1991).

DNA analysis

Yeast chromosomal DNA was isolated [Bishop et al. 1992] and analyzed according to standard protocols [Sambrook et al. 1989]

except random priming of ³²P-labeled DNA probes (Feinberg and Vogelstein 1984) and hybridization (Church and Gilbert 1984). Two-dimensional gel analysis is described by Xu and Kleckner (1995). DNA signals were quantitated on a Fuji BAS2000 PhosphorImager.

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References

- Alani, E., R. Padmore, and N. Kleckner. 1990. Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. *Cell* **61**: 419–436.
- Baker, S.M., A.W. Plug, R.A. Prolla, C.E. Bronner, A.C. Harris, X. Yao, D.-M. Christie, C. Monell, N. Arnheim, A. Bradley, T. Ashley, and R.M. Liskay. 1996. Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. *Nature Genet.* **13**: 336–342.
- Bishop, D.K. 1994. RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. *Cell* **79**: 1081–1092.
- Bishop, D.K., D. Park, L. Xu, and N. Kleckner. 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.
- Cao, L., E. Alani, and N. Kleckner. 1990. A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. *Cell* **61**: 1089–1101.
- Carpenter, A.T.C. 1994. Chiasma function. *Cell* **77**: 957–962.
- Christianson, T.W., R.S. Sikorski, M. Dante, J.H. Shero, and P. Hieter. 1992. Multifunctional yeast high-copy-number shuttle vectors. *Gene* **110**: 119–122.
- Church, G.M. and W. Gilbert. 1984. Genomic sequencing. *Proc. Natl. Acad. Sci.* **81**: 1991–1995.
- de Massy, B., F. Baudat, and A. Nicolas. 1994. Initiation of recombination in *Saccharomyces cerevisiae* haploid meiosis. *Proc. Natl. Acad. Sci.* **91**: 11929–11933.
- de Massy, B., V. Rocco, and A. Nicolas. 1995. The nucleotide mapping of DNA double-strand breaks at the *CYS3* initiation site of meiotic recombination in *Saccharomyces cerevisiae*. *EMBO J.* **14**: 4589–4598.
- Edelmann, W., P.E. Cohen, M. Kane, K. Lau, B. Morrow, S. Bennett, A. Umar, T. Kunkel, G. Cattoretti, R. Chaganti, et al. 1996. Meiotic pachytene arrest in MLH1-deficient mice. *Cell* **85**: 1125–1134.
- Engbrecht, J., J. Hirsch, and G.S. Roeder. 1990. Meiotic gene conversion and crossing over: Their relationship to each other and to chromosome synapsis and segregation. *Cell* **62**: 927–937.
- Feinberg, A.P. and B. Vogelstein. 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **137**: 266–267.

- Hartwell, L.H. and T.A. Weinert. 1989. Checkpoints: Controls that ensure the order of cell cycle events. *Science* **246**: 629–634.
- Johzuka, K. and H. Ogawa. 1995. Interaction of Mre11 and Rad50: two proteins required for DNA repair and meiosis-specific double-strand break formation in *Saccharomyces cerevisiae*. *Genetics* **139**: 1521–1532.
- Kadyk, L.C. and L.H. Hartwell. 1992. Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae*. *Genetics* **132**: 387–402.
- Keeney, S. and N. Kleckner. 1995. Covalent protein-DNA complexes at the 5' strand termini of meiosis-specific double strand breaks in yeast. *Proc. Natl. Acad. Sci.* **92**: 11274–11278.
- Klapholz, S., C.S. Waddell, and R.E. Esposito. 1985. The role of the *SPO11* gene in meiotic recombination in yeast. *Genetics* **110**: 187–216.
- Kleckner, N. 1996. Meiosis: How could it work? *Proc. Natl. Acad. Sci.* **93**: 8167–8174.
- Kleckner, N., R. Padmore, and D.K. Bishop. 1991. Meiotic chromosome metabolism: One view. *Cold Spring Harbor Symp. Quant. Biol.* **56**: 729–743.
- Leatherwood, J., A. Lopez-Girona, and P. Russell. 1996. Interaction of Cdc2 and Cdc18 with a fission yeast ORC2-like protein. *Nature* **379**: 360–363.
- Leem, S.-H. and H. Ogawa. 1992. The *MRE4* gene encodes a novel protein kinase homologue required for meiotic recombination in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **20**: 449–457.
- Liu, J., T.-c. Wu, and M. Lichten. 1995. The location and structure of double-strand DNA breaks induced during meiosis: Evidence for a covalently linked DNA-protein intermediate. *EMBO J.* **14**: 4599–4608.
- Loidl, J., K. Nairz, and F. Klein. 1991. Meiotic chromosome synapsis in a haploid yeast. *Chromosoma* **100**: 221–228.
- Lydall, D. and T. Weinert. 1995. Yeast checkpoint genes in DNA damage processing: Implication for repair and arrest. *Science* **270**: 1488–1491.
- Lydall, D., Y. Nikolsky, D.K. Bishop, and T. Weinert. 1996. A meiotic recombination checkpoint controlled by mitotic DNA damage checkpoint genes. *Nature* **383**: 840–843.
- Malone, R.E., S. Bullard, M. Hermiston, R. Rieger, M. Cool, and A. Galbraith. 1991. Isolation of mutants defective in early steps of meiotic recombination in the yeast *Saccharomyces cerevisiae*. *Genetics* **128**: 79–88.
- Navas, T.A., Z. Zhou, and S.J. Elledge. 1995. DNA polymerase ϵ links the DNA replication machinery to the S phase checkpoint. *Cell* **80**: 29–39.
- Padmore, R., L. Cao, and N. Kleckner. 1991. Temporal comparison of recombination and synaptonemal complex formation during meiosis in *S. cerevisiae*. *Cell* **66**: 1239–1256.
- Raymond, W.R. and N. Kleckner. 1993. Rad50 protein of *S. cerevisiae* exhibits ATP-dependent DNA binding. *Nucleic Acids Res.* **21**: 3851–3856.
- Reed, S.I. 1980. The selection of *S. cerevisiae* mutants defective in the start event of cell division. *Genetics* **95**: 561–577.
- Resnick, M.A. 1987. Investigating the genetic control of biochemical events in meiotic recombination. In *Meiosis* (ed. P. Moens), pp. 157–210. Academic Press, New York, NY.
- Rockmill, B. and G.S. Roeder. 1990. Meiosis in asynaptic yeast. *Genetics* **126**: 563–574.
- . 1991. A meiosis-specific protein kinase homolog required for chromosome synapsis and recombination. *Genes & Dev.* **5**: 2392–2404.
- Rockmill, B., J. Engebrecht, H. Scherthan, J. Loidl, and G.S. Roeder. 1995a. The yeast *MER2* gene is required for chromosome synapsis and the initiation of meiotic recombination. *Genetics* **141**: 49–59.
- Rockmill, B., M. Sym, H. Scherthan, and G.S. Roeder. 1995b. Roles for two RecA homologs in promoting meiotic chromosome synapsis. *Genes & Dev.* **9**: 2684–2695.
- Roeder, G.S. 1990. Chromosome synapsis and genetic recombination: Their roles in meiotic chromosome segregation. *Trends Genet.* **6**: 385–389.
- Roeder, G.S. 1995. Sex and the single cell: Meiosis in yeast. *Proc. Natl. Acad. Sci.* **92**: 10450–10456.
- Rose, D. and C. Holm. 1993. Meiosis-specific arrest revealed in DNA topoisomerase II mutants. *Mol. Cell. Biol.* **13**: 219–242.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A laboratory manual*. 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Schwacha, A. 1996. "Isolation and characterization of branched meiotic recombination intermediates from both wild-type and mutant strains of the yeast *Saccharomyces cerevisiae*." Ph.D. thesis, Harvard University, Cambridge, MA.
- Schwacha, A. and N. Kleckner. 1994. Identification of joint molecules that form frequently between homologs but rarely between sister chromatids during yeast meiosis. *Cell* **76**: 51–63.
- . 1995. Identification of double Holliday junctions as intermediates in meiotic recombination. *Cell* **83**: 783–791.
- Sherman, F., G. Fink, and J. Hicks. 1986. *Methods in yeast genetics: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Shinohara, A., H. Ogawa, and T. Ogawa. 1992. Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. *Cell* **69**: 457–470.
- Shuster, E.O. and B. Byers. 1989. Pachytene arrest and other meiotic effects of the start mutations in *Saccharomyces cerevisiae*. *Genetics* **123**: 29–43.
- Storlazzi, A., L. Xu, L. Cao, and N. Kleckner. 1995. Crossover and noncrossover recombination during meiosis: Timing and pathway relationships. *Proc. Natl. Acad. Sci.* **92**: 8512–8516.
- Storlazzi, A., L. Xu, A. Schwacha, and N. Kleckner. 1996. Synaptonemal complex component Zip1 plays a role in meiotic recombination independent of SC polymerization along the chromosomes. *Proc. Natl. Acad. Sci.* **93**: 9043–9048.
- Sym, M. and G.S. Roeder. 1994. Crossover interference is abolished in the absence of a synaptonemal complex protein. *Cell* **79**: 283–292.
- Sym, M., J. Engebrecht, and G.S. Roeder. 1993. ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. *Cell* **72**: 365–378.
- Thorne, L.W. and B. Byers. 1993. Stage-specific effects of X-irradiation on yeast meiosis. *Genetics* **134**: 29–42.
- Weber, L. and B. Byers. 1992. A *RAD9*-dependent checkpoint blocks meiosis of *cdc13* yeast cells. *Genetics* **131**: 55–63.
- Williamson, D.H., L.J. Johnson, D.J. Fennell, and G. Simchen. 1983. The timing of the S phase and other nuclear events in yeast meiosis. *Exp. Cell Res.* **145**: 209–217.
- Xu, L. and N. Kleckner. 1995. Sequence non-specific double-strand breaks and interhomolog interactions prior to double-strand break formation at a meiotic recombination hot spot in yeast. *EMBO J.* **14**: 5115–5128.
- Xu, L., M. Ajimura, R. Padmore, C. Klein, and N. Kleckner. 1995. *NDT80*, a meiosis-specific gene required for exit from pachytene in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **15**: 6572–6581.
- Yamamoto, A., Guacci, V. and Koshland, D. 1996. PDS1p, an inhibitor of anaphase in budding yeast, plays a critical role in APC and checkpoint pathway(s). *J. Cell Biol.* **133**: 99–110.

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