Replication Protein A Is Required for Meiotic Recombination in *Saccharomyces cerevisiae*

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ABSTRACT

In *Saccharomyces cerevisiae*, meiotic recombination is initiated by transient DNA double-stranded breaks (DSBs). These DSBs undergo a 5′ → 3′ resection to produce 3′ single-stranded DNA ends that serve to channel DSBs into the *RAD52* recombinational repair pathway. *In vitro* studies strongly suggest that several proteins of this pathway—Rad51, Rad52, Rad54, Rad55, Rad57, and replication protein A (RPA)—play a role in the strand exchange reaction. Here, we report a study of the meiotic phenotypes conferred by two missense mutations affecting the largest subunit of RPA, which are localized in the protein interaction domain (*rfa1-t11*) and in the DNA-binding domain (*rfa1-t48*). We find that both mutant diploids exhibit reduced sporulation efficiency, very poor spore viability, and a 10- to 100-fold decrease in meiotic recombination. Physical analyses indicate that both mutants form normal levels of meiosis-specific DSBs and that the broken ends are processed into 3′-OH single-stranded tails, indicating that the RPA complex present in these *rfa1* mutants is functional in the initial steps of meiotic recombination. However, the 5′ ends of the broken fragments undergo extensive resection, similar to what is observed in *rad51*, *rad52*, *rad55*, and *rad57* mutants, indicating that these RPA mutants are defective in the repair of the Spo11-dependent DSBs that initiate homologous recombination during meiosis.

REPLICATION protein A (RPA) is the eukaryotic counterpart of the *Escherichia coli* single-stranded DNA-binding protein (SSB), which was initially identified as an essential factor for replication *in vitro* (for a review, see Wold 1997) and later shown by *in vivo* and *in vitro* approaches to be required for most aspects of eukaryotic DNA metabolism: specifically, RPA is required in nucleotide excision repair (Coverley *et al.* 1991; Guzder *et al.* 1995), telomere maintenance (Smith *et al.* 2000), and homologous recombination (Sung 1994; Smith and Rothstein 1999; for a review, see Wold 1997). RPA is also a component of the surveillance mechanisms that link the recognition of defects in DNA metabolism with cell cycle progression (Brush *et al.* 1996; Cheng *et al.* 1996; Lee *et al.* 1998; Kim and Brill 2001; Pellicioli *et al.* 2001).

In *Saccharomyces cerevisiae* as in other eukaryotes, RPA is composed of three subunits of 69, 36, and 13 kD that are encoded by the three essential genes *RFA1*, *RFA2*, and *RFA3*, respectively (Heyer *et al.* 1990; Brill and Stillman 1991). The Rfa1 subunit bears the major single-stranded DNA (ssDNA)-binding activity (Brill and Stillman 1989; Heyer *et al.* 1990; Philipova *et al.* 1996; Brill and Bastin-Shanower 1998). Biochemical studies have delineated three distinct domains of the protein: the N-terminal domain [amino acids (aa) 1–170], which is involved in interactions of RPA with other proteins, including Pol α (Kim *et al.* 1996) and Rfc4 (Kim and Brill 2001); two central DNA-binding subdomains, A and B (aa 180–416); and a C-terminal domain (aa 450–616), which includes a third DNA-binding subdomain, C (Brill and Bastin-Shanower 1998) and is required for binding the Rfa2 and Rfa3 subunits (for a review, see Wold 1997). Several genetic screens have led to the identification of *rfa1* mutations that confer different phenotypes. Some mutants are defective in intrachromosomal recombination (Longhese *et al.* 1994) and in the recombinational repair of induced double-stranded breaks (DSBs; Firmenich *et al.* 1995; Umezu *et al.* 1998), whereas others exhibit a stimulation of recombination among direct repeats (Smith and Rothstein 1995, 1999; Smith *et al.* 2000), UV irradiation and methyl methanesulfonate (MMS) sensitivities, growth thermosensitivity, and replication defects (Umezu *et al.* 1998; Kim and Brill 2001), or mutator phenotypes (Chen *et al.* 1998). Here, we have genetically and physically studied the phenotypes of two *rfa1* mutants and the role of RPA in meiotic homologous recombination.

In *E. coli*, the RecA protein catalyzes the strand exchange reaction between two homologous DNA molecules. In eukaryotic cells, several structural homologs of bacterial RecA have been described: Rad51 (Aboussekhra *et al.* 1992; Basile *et al.* 1992; Shinohara *et al.* 1992; Benson *et al.* 1994), Rad55 (Lovett 1994), Rad57

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(Kans and Mortimer 1991), and Dmc1, a meiosis-specific homolog (Bishop *et al.* 1992). However, thus far, only Rad51 has been shown to be a functional homolog of RecA with respect to strand exchange activity (Ogawa *et al.* 1993b; Sung 1994; Sung and Robberson 1995; Baumann *et al.* 1996; Baumann and West 1997; for a review, see Sung *et al.* 2000), although *in vitro*, the eukaryotic Rad51 proteins catalyze a rather inefficient strand exchange reaction. However, this reaction is strongly stimulated by the cofactors Rad52, RPA, and the heterodimer Rad55/Rad57 when they are added in the proper order and stoichiometry (Sung 1994; Baumann and West 1997; Sugiyama *et al.* 1997; Sung 1997a,b; Benson *et al.* 1998; New *et al.* 1998; Shinohara and Ogawa 1998; and for a review, see Sung *et al.* 2000). The role of Rad52 and Rad55/Rad57 as cofactors for the Rad51 recombinase activity is likely related to their ssDNA-binding properties (Mortensen *et al.* 1996; Sung 1997a) and their capacity to interact with Rad51 (Shinohara *et al.* 1992; Hays *et al.* 1995; Johnson and Symington 1995; Shen *et al.* 1996), which may facilitate Rad51-ssDNA nucleoprotein assembly in the presence of RPA that could compete with Rad51 for binding on ssDNA. Interestingly, immunostaining of budding yeast meiotic cells showed that DSB-dependent Rad51 focus formation requires the presence of the Rad52, Rad55, and Rad57 proteins and that Rad52 and RPA extensively colocalize (Gasior *et al.* 1998).

During meiotic recombination, all of the genes of the *RAD52* pathway (*RAD52*, *RAD50*, *RAD51*, *RAD55*, *RAD57*, *MRE11*, and *XRS2*) have essential but distinct roles (for a review, see Paques and Haber 1999). In *S. cerevisiae*, meiotic recombination is initiated by Spo11-dependent DSBs (Bergerat *et al.* 1997; Keeney *et al.* 1997), which form on one of a pair of homologous duplexes (Sun *et al.* 1989; Cao *et al.* 1990; Figure 1). The broken ends then undergo 5′ to 3′ processing leading to the production of 3′ single-stranded tails (Sun *et al.* 1991; Bishop *et al.* 1992; Vedel and Nicolas 1999). This key intermediate is the substrate used by recombination proteins to promote strand invasion of a homologous chromosome. *In vivo*, Rad51, Rad52, Rad55, Rad57, Rad54 (only in a *rdh54* background), and Dmc1 are all required for the formation of joint molecules (Schwacha and Kleckner 1997; Shinohara *et al.* 1997). In the corresponding deletion mutant strains, unrepaired DSBs accumulate in a hyperresected form (Bishop *et al.* 1992; Shinohara *et al.* 1992, 1997; Dresser *et al.* 1997; and this study).

The aim of the present study was to examine the effect of two RPA mutations (*rfa1-t11* and *rfa1-t48*) on the formation and/or stabilization of the 3′ single-stranded DSB tail. These mutants are UV and MMS sensitive and deficient in DSB-induced homologous recombination in mitotic cells, as shown by their sensitivity to HO endonuclease-induced DSBs and defects in mating-type switching or in single-stranded-annealing recombination induced by site-specific cleavage of the HO endonuclease between direct repeats (Umezu *et al.* 1998). Our physical analysis demonstrates that both *rfa1* mutants form normal levels of meiosis-specific DSBs and produce 3′-OH single-stranded DNA tails. This indicates that the RPA complex present in these *rfa1* mutants is functional in the initial steps of meiotic recombination. However, the 5′ ends of the broken fragments undergo extensive resection, similar to what is observed in *rad51*, *rad52*, *rad55*, and *rad57* mutants, indicating that these *rfa1* mutants are defective in the repair of the Spo11-dependent DSBs that initiate homologous recombination.

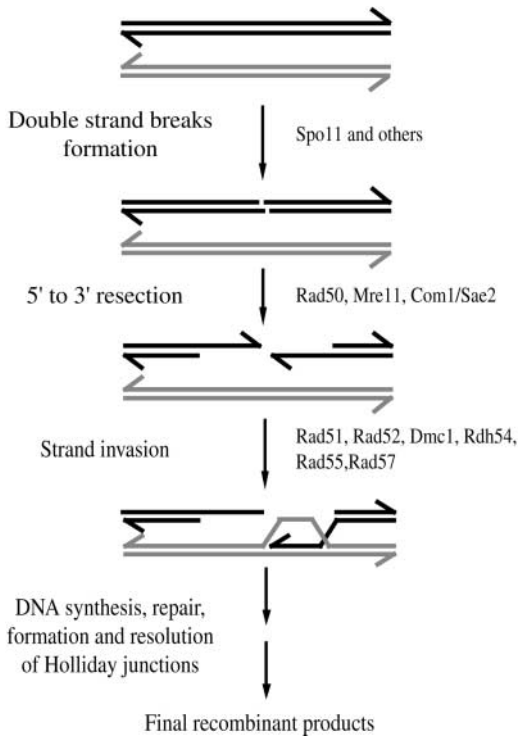

Figure 1.—Initial steps of meiotic recombination (as originally proposed by Szostak *et al.* 1983) and the main proteins involved.

MATERIALS AND METHODS

Plasmids and oligonucleotides: A *Sal*I-*Bam*HI fragment from the plasmid pKU2-*rfa1-t11*, which bears the *rfa1-t11* allele, was cloned into the same sites of pRS406 (Umezu *et al.* 1998), a *URA3* vector (Sikorski and Hieter 1989). The plasmid pRS(t48) was constructed by introduction of the pKU1-*rfa1-t48 Sal*I-*Bam*HI fragment bearing the *rfa1-t48* allele (Umezu *et al.* 1998) into pRS306. The plasmid pSTL11 (a gift from S. Lovett) contains the *RAD55* gene disrupted by insertion of a *Sal*I-*Xho*I *LEU2* fragment into the internal *Sal*I site (Lovett and Mortimer 1987). The *Hin*dIII fragment from pSTL11 was used for transformation. The plasmid p51::*LEU2* (a gift from F. Fabre) was created by cloning a *Bam*HI fragment bearing a *RAD51* disruption cassette into the *Bam*HI site of the vector pTZ18. The *RAD51* disruption was created by the insertion of a *Hpa*I fragment of *LEU2* into the *RAD51 Stu*I-*Nru*I sites, and the *Bam*HI fragment of p51::*LEU2* was used

TABLE 1

Yeast strains used in this study

Strain	Relevant genotype[a,b]	Source
MGD131-2C	*MAT*α *arg4*Δ*2060 leu2-3, 112 ura3-52 trp1-289 cyhr*	Rocco *et al.* (1992)
MGD131-102A	*MAT***a** *arg4*Δ*2060 his3-*Δ*1 ura3-52 trp1-289 ade2*	Rocco *et al.* (1992)
ORT2615	*MAT*α *arg4*Δ*2060 rad51::LEU2*	This study
ORT2618	*MAT*α *arg4*Δ*2060 rad55::LEU2*	This study
ORT2622	*MAT*α *arg4*Δ*2060 rad57::URA3*	This study
ORT2915.4	*MAT*α *arg4*Δ*2060 rad52::LEU2*	H. Debrauwère
ORT2641	*MAT*α *arg4*Δ*2060 rfa1-t11*	This study
ORT2659	*MAT***a** *arg4-Erv rfa1-t48*	This study
ORT2656	*MAT*α *arg4-Ebg rfa1-t48*	This study
ORD2130	*MAT***a** *arg4-Erv*	Rocco and Nicolas (1996)
	*MAT*α *arg4-Ebg*	
ORD2410	*MAT***a** *arg4-Erv rad50S-KI81-URA3*	Rocco and Nicolas (1996)
	*MAT*α *arg4-Ebg rad50S-KI81-URA3*	
ORD3200	*MAT*α *arg4-Ebg rad51::LEU2*	This study
	*MAT***a** *arg4-Erv rad51::LEU2*	
ORD3204	*MAT*α *arg4-Ebg rad55::LEU2*	This study
	*MAT***a** *arg4-Erv rad55::LEU2*	
ORD3233	*MAT*α *arg4-Erv dmc1::URA3*	This study
	*MAT***a** *arg4-Ebg dmc1::URA3*	
ORD3243	*MAT***a** *arg4-Erv rad57::URA3*	This study
	*MAT*α *arg4-Ebg rad57::URA3*	
ORD3284	*MAT***a** *arg4-Ebg rad52::LEU2 rad50S-KI81-URA3*	This study
	*MAT*α *arg4-Ebg rad52::LEU2 rad50S-KI81-URA3*	
ORD3285	*MAT***a** *arg4-Ebg rad52::LEU2*	This study
	*MAT*α *arg4-Erv rad52::LEU2*	
ORD3257	*MAT*α *arg4-Ebg rfa1-t11 rad50SKI81-URA3*	This study
	*MAT***a** *arg4-Ebg rfa1-t11 rad50SKI81-URA3*	
ORD3256	*MAT***a** *arg4-Erv rfa1-t11*	This study
	*MAT*α *arg4-Ebg rfa1-t11*	
ORD5402	*MAT***a** *arg4-Erv rfa1-t48*	This study
	*MAT*α *arg4-Ebg rfa1-t48*	

[a] The haploid strains correspond to the primary transformant and are derived from the MGD131-2C and MGD131-102A haploid strains.

[b] The *rad50S-KI81* mutation is marked *in cis* by an *URA3* insertion (Alani *et al.* 1990).

for transformation. The plasmid pAS263 (a gift from N. Kleckner) was derived from pAS264, which contains a *rad57::TRP1* construct (Schwacha and Kleckner 1997), by insertion of the *URA3* gene at the *Bgl*II site. The *Bam*HI-*Sph*I fragment of pAS263 was used for transformation. The *Eco*RV-*Bgl*II *ARG4* internal fragment was used as a probe in Southern blotting experiments. It was isolated from pNPS104, which was created by the insertion of a 3.3-kb *Pst*I fragment bearing the *ARG4* gene into the vector pMLC12. The "up 015" (135096–135116) and "down 015" (136235–136255) oligonucleotides amplify a part of the *YHR015* open reading frame (ORF). The coordinates are according to the published sequence of chromosome VIII (Johnston *et al.* 1994). All DNA fragments used in transformations or as probes were purified with the Qiaex kit (QIAGEN, Chatsworth, CA).

Strains and media: *E. coli* strains were either DH5α or NPS-RK2 and were grown in standard media [Luria broth (LB) and LB containing 1% thymidine, respectively]. All yeast strains used in this study are listed in Table 1 and were derived from the haploid parental strains MGD131-2C (*MAT*α *arg4-*Δ*2060 leu2-3,112, ura3-52 trp1-289 cyhr*) and MGD131-102A (*MAT***a** *arg4-*Δ*2060 his3-*Δ*1 ura3-52 trp1-289 ade2*). All strains were transformed by electroporation (Ausubel *et al.* 1987) with a Gene Pulser (Bio-Rad, Hercules, CA) with 200 ng of linear plasmid DNA fragments by the one-step gene replacement technique (Rothstein 1983), except for the *rfa1* mutations. The *rfa1-t11* mutation was introduced into MGD131-2C by transformation with *Nhe*I-linearized pKU2-*rfa1-t11* (pop-in) and selection for the plasmid-borne *URA3* marker, followed by selection on 5-fluoroorotic acid (5-FOA) plates for colonies that had lost the plasmid sequences (pop-out; Boeke *et al.* 1984). Transformants with a replacement of the *RFA1* allele by the *rfa1-t11* allele were identified by screening for sensitivity to MMS and UV irradiation. Among nearly 200 5-FOA^r clones tested, two candidate strains were identified by their sensitivity to 0.015% MMS and to UV irradiation (750 ergs). Haploid strains bearing the *rfa1-t11* mutation were then crossed to haploid strains bearing the *arg4-E* construct (an inversion of the *Eco*47III-*Sna*BI fragment of the *ARG4* gene) and the *rv* or *bg* markers at the *ARG4* locus (Rocco and Nicolas 1996). The *rfa1-t48* mutant strain was similarly constructed by direct introduction of *Nhe*I-linearized pRS-t48 into haploid strains

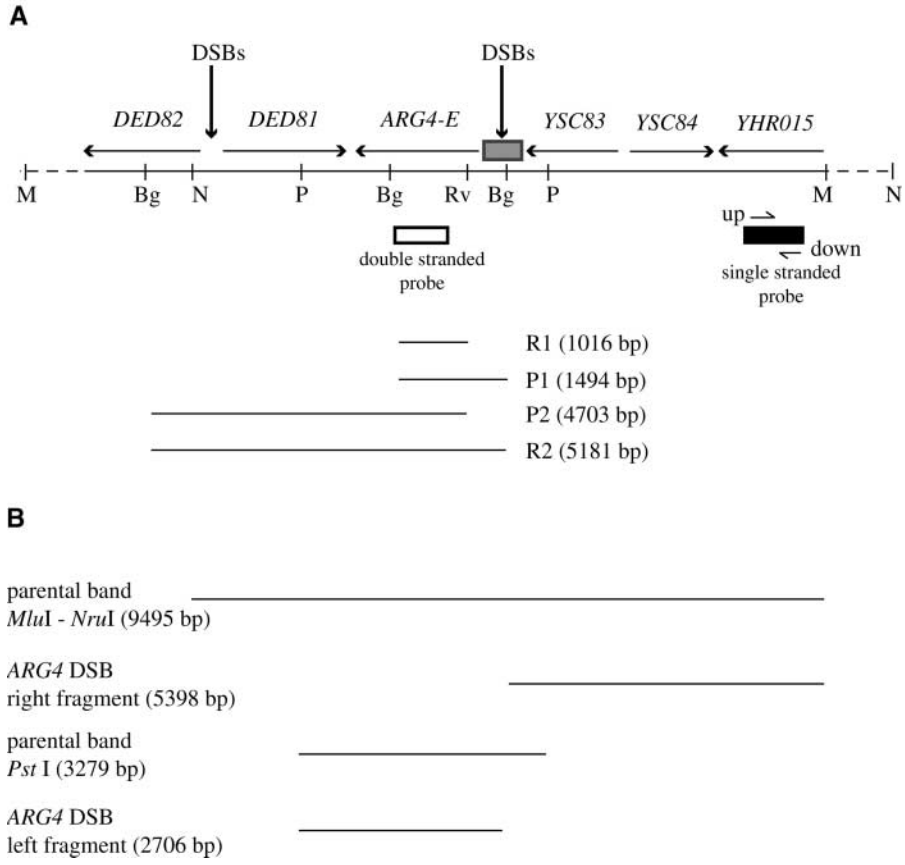

FIGURE 2.—Physical map of the *ARG4* region. (A) The *ARG4-E* construct is an *Eco*47III-*Sna*BI inversion of the *ARG4* gene with the poly1 sequence localized in the promoter region (shaded box; ROCCO and NICOLAS 1996). The mutations *rv* and *bg* (destroyed restriction sites) in the *ARG4* gene are indicated, as well as the sizes of the parental (P1 and P2) and recombinant fragments (R1 and R2) resulting from *Eco*RV + *Bgl*II digestion. The vertical arrows indicate the positions of the meiotic DSB sites (*DED81-82* and *ARG4*). The half-arrows termed "up" and "down" give the positions of the oligonucleotides used for the synthesis of the single-stranded DNA probe *YHR015*. The positions of the *YHR015* and *ARG4 Eco*RV-*Bgl*II probes are indicated by the solid and open boxes, respectively. The direction of transcription is indicated by the horizontal arrows. The relevant restriction sites are shown: *Mlu*I (M); *Nru*I (N); *Bgl*II (Bg); *Eco*RV (Rv); *Pst*I (P). (B) Sizes and localization of the parental (*Pst*I and *Mlu*I-*Nru*I) and meiotic DSB fragments ("right" and "left").

bearing the *arg4-E* construct with the *rv* and *bg* markers, ORT492 and ORT495, respectively (ROCCO and NICOLAS 1996). The *arg4-E* strains were chosen because they undergo a higher level of DSB formation and meiotic recombination than do wild-type strains (ROCCO and NICOLAS 1996). The *rad51*, *rad55*, and *rad57* strains were obtained by one-step transformation with the respective plasmids (as described above) into MGD131-2C (Table 1). The *rad52::LEU2* haploid strain (constructed by H. Debrauwère) corresponds to an insertion of a fragment from the pSM20 plasmid (D. Schild) in which a *LEU2* fragment is inserted into the internal *Bgl*II site of the *RAD52* gene. The *dmc1::URA3* diploid strain corresponds to an insertion by pop-in pop-out of a fragment from the pRNB92 plasmid (BISHOP *et al.* 1992). All the constructions were verified by Southern blot analysis. For both *rfa1* mutants, MMS and UV sensitivity tests for strain verification were performed on plates. Stationary liquid cultures in YPD medium were counted and diluted and drops of different dilutions were deposited on YPD and irradiated with a 254-nm UV lamp or deposited on YPD + MMS (0.015%). MMS and UV sensitivities were assessed after 3–4 days of incubation at 30° by comparison with untreated strains.

Standard media and culture conditions were used (AUSUBEL *et al.* 1987). Conditions for presporulation and sporulation were as previously described (RESNICK *et al.* 1983; DE MASSY and NICOLAS 1993). MMS plates were prepared by the addition of MMS to YPD medium to a final concentration of 0.015%; the plates were kept at 4° and used within 2 days after preparation. The entry into and progression through meiosis were monitored by light microscopic examination after staining with 4′,6-diamidino-2-phenylindole (DAPI).

Determination of recombination frequencies: Tetrad dissection and random spore analyses were performed by standard methods at 30° (AUSUBEL *et al.* 1987) using β-glucuronidase (Sigma, St. Louis), Zymolyase 20T (ICN), and an automated microdissector (Singer Instruments MSM). Tests for determination of the mating type or the presence of the specific *ARG4* alleles were performed as described using appropriate tester strains (NICOLAS *et al.* 1989). The return-to-growth assays were performed as described (SHERMAN and ROMAN 1963). Cells were induced to sporulate and at different times aliquots were counted, diluted, and plated onto YPD plates to test survival and onto selective medium plates to monitor the appearance of Arg^+ prototrophs. The frequency of Arg^+ recombinants was calculated as the ratio of Arg^+ colonies to total colonies on YPD, for each time point. Meiotic recombination was also examined physically by Southern blot analysis of DNA extracted from diploids heteroallelic for the *arg4-Erv* and *arg4-Ebg* markers, as previously described (ROCCO *et al.* 1992). Details are provided in the legends to Figure 2A and Figure 3B.

Detection of meiotic DSBs: Chromosomal DNA was extracted from meiotic cells as described (ROCCO *et al.* 1992), digested with appropriate enzymes, and fractionated by electrophoresis through 0.7% agarose gels. The DNA fragments were then transferred to a nylon membrane (Hybond-N+, Amersham, Buckinghamshire, UK) as described previously (ROCCO *et al.* 1992), with the exception that the DNA was fixed to the membrane by treatment with 0.4 N NaOH for 15 min and then rinsed in 0.5 M Na_2HPO_4, pH 7.3. The resulting membrane was prehybridized and hybridized for 24 hr according to CHURCH and GILBERT (1984). Labeling of the DNA probes was done by random priming according to the specifications of the manufacturer (ReadyPrime kit, Pharmacia, Piscataway, NJ) using 50 μCi of 3000 Ci/mM [α-^{32}P]dCTP (Amersham). The membrane was then exposed to a phosphor screen and quantified with a phosphorimager system using ImageQuant software analysis (Storm, Molecular Dynamics, Sunnyvale, CA).

Detection of single-stranded DNA intermediates: Meiotic chromosomal DNA was extracted, purified, and digested ex-

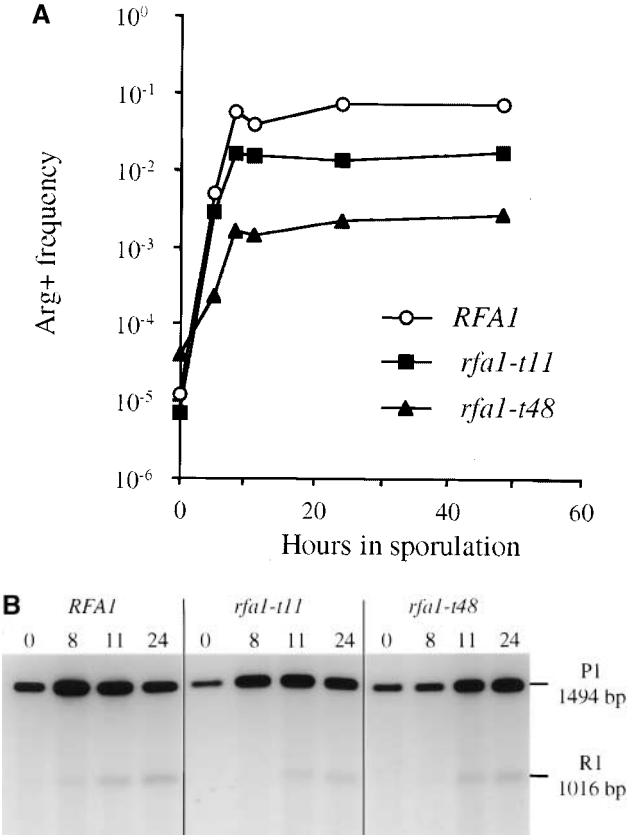

FIGURE 3.—(A) Meiotic recombination defects of *rfa1* mutants. Commitment to meiotic recombination in *RFA1* (ORD2130), *rfa1-t11* (ORD3256), and *rfa1-t48* (ORD5402) strains was followed throughout sporulation by a return-to-growth assay. After transfer to sporulation medium, aliquots of cells were taken at different times, diluted, and plated onto YPD plates and on plates lacking arginine to monitor the appearance of Arg^+ prototrophs. The frequency of recombination is calculated as the ratio of the number of Arg^+ colonies to the total number of colonies on YPD plates (see also Table 2). (B) Physical detection of recombinant molecules at the *ARG4* locus. Genomic DNA extracted from *RFA1* (ORD2130), *rfa1-t11* (ORD3256), and *rfa1-t48* (ORD5402) meiotic cells at the indicated times was digested with *Eco*RV and *Bgl*II and subjected to Southern blot analysis using as a probe an *Eco*RV-*Bgl*II fragment (1016 bp) internal to *ARG4*. The positions of the parental (P1) and recombinant (R1) bands are indicated.

actly as for neutral gels. The resulting fragments were electrophoresed through alkaline agarose gels as previously described (BISHOP *et al.* 1992). The DNA was then transferred to a Genescreen membrane (Dupont-NEN) by capillary blotting with 20× SSC buffer and fixed onto the membrane using a Stratalinker (Stratagene, La Jolla, CA). Prehybridization and hybridization were done as for the detection of DSBs, as described above. The synthesis and labeling by asymmetric PCR of single-stranded DNA probes were done according to DE MASSY *et al.* (1995). Exposure and quantification of the DNA fragments were done as for the detection of DSBs as described above.

RESULTS

Reduced sporulation and spore viability in diploids homozygous for the *rfa1-t11* and *rfa1-t48* mutations: To study the meiotic phenotypes conferred by the *rfa1-t11* and *rfa1-t48* mutations (UMEZU *et al.* 1998), we constructed diploids homozygous for these mutations (ORD3256 and ORD5402, respectively) by mating haploids containing the mutations of interest. These strains are derivatives of our standard haploid strains MGD131-2C and MGD131-102A (S288C background; see ROCCO *et al.* 1992). The genotypes of these and all other strains used in this study are indicated in Table 1. For the sake of simplicity, the homozygous diploids will be referred to hereafter as *RFA1*, *rfa1-t11*, and *rfa1-t48*. In this strain background, we observe that ~60% of *RFA1* diploid cells (ORD2130) sporulate after 2 days in sporulation medium (producing mainly four-spore tetrads), but that the *rfa1-t11* diploid has a reduced sporulation efficiency (25%, corresponding to a 2- to 3-fold decrease relative to the *RFA1* strain) while only 3% of *rfa1-t48* diploid cells form tetrads, representing a 20-fold decrease (Table 2). To examine meiotic progression, we stained cells with DAPI at different times during sporulation and counted the number of cells with one, two, or four nuclei to determine the percentage of cells that had undergone the first or the second meiotic division. The results show that *RFA1* and mutant (*rfa1-t11* and *rfa1-t48*) cells exhibit an increase in the number of binucleated cells at 11 hr after transfer to sporulation medium and an increase in the percentage of tetranucleated cells at 24 hr, with maximal frequencies of ~60% for the *RFA1* strain, 30% for the *rfa1-t11* strain, and 12% for the *rfa1-t48* strain. These results parallel the efficiency of sporulation as ascertained by light microscopy (Table 2). To test whether the sporulation defects of these mutants are accompanied by changes in spore viability, we dissected tetrads derived from each diploid. Up to 95% of *RFA1* spores are viable and in all *RFA1* tetrads three or four spores germinated and formed colonies. In contrast, we observed a severe reduction in spore viability for the *rfa1-t11* (18.7% viable spores among 142 four-spore tetrads dissected) and the *rfa1-t48* (7% viable spores among 84 four-spore tetrads) diploids (Table 2). For these mutants, in most tetrads either no spores or only a single germinating spore germinated, indicating that each meiotic cell is affected by the *rfa1* mutations. Microscopic examination of the dissection plates indicated that most of the spores did not germinate (nearly 90% for each mutant strain). The remaining spores that did not form visible colonies germinated but formed multiply budded structures with few cells, similar to what was previously described for *rfa1* null alleles (HEYER *et al.* 1990). We conclude, therefore, that *rfa1-t11* and *rfa1-t48* homozygous diploids are defective in progressing through meiosis and in sporulation and that they generally give rise to inviable spores.

Meiotic gene conversion is severely reduced in the *rfa1-t11* and *rfa1-t48* mutants: We next examined the proficiency of the *rfa1-t11* and *rfa1-t48* diploids for meiotic recombination by genetic and physical means. The *RFA1* (ORD2130), *rfa1-t11* (ORD3256), and *rfa1-t48*

TABLE 2

Meiotic phenotypes of the *rfa1* mutants

Strains	Sporulation phenotypes		Recombination (Arg^+ frequency)	
	Sporulation efficiency[a]	Spore viability[b]	Random spore analysis	Return-to-growth assay[c]
RFA1	60% (1)[d]	95% (1)	5.5×10^{-2} (1)	1.0×10^{-1} (1)
rfa1-t11	25% (0.41)	18.7% (0.2) [106/568]	8.0×10^{-3} (0.14)	1.4×10^{-2} (0.14)
rfa1-t48	3% (0.05)	7.1% (0.075) [24/336]	2.4×10^{-3} (0.043)	1.1×10^{-3} (0.011)

All the data are mean values of at least three experiments.

[a] Percentage of three- and four-spore tetrads among all cells.

[b] Percentage of spores giving rise to colonies. The numbers within brackets indicate the number of spores giving rise to colonies over the total number of spores dissected. Only four-spore tetrads were dissected.

[c] Values at 48 hr after transfer of diploids to the sporulation medium.

[d] Values within parentheses correspond to the ratio mutant over wild type (*RFA1*).

(ORD5402) diploids are heteroallelic for the *arg4-Erv* and *arg4-Ebg* mutations at the *ARG4* hot spot of meiotic recombination (Nicolas *et al.* 1989; Rocco *et al.* 1992; de Massy and Nicolas 1993), allowing us to determine the frequency of recombination by random spore analysis. In the *RFA1* strain, we observed a high frequency of Arg^+ prototrophs, up to 5.5% ($\pm 1.5 \times 10^{-2}$) of all spores. These recombinants are due mostly to gene conversion events (Nicolas *et al.* 1989). In contrast, the frequency of Arg^+ prototrophs was reduced by 7- and 23-fold, relative to the *RFA1* diploid, among the progeny of the *rfa1-t11* ($8.0 \pm 1.5 \times 10^{-3}$) and *rfa1-t48* ($2.4 \pm 0.2 \times 10^{-3}$) diploids, respectively (Table 2). Because of the poor spore viability of *rfa1* spores, which might make it impossible to accurately determine the frequency of cells that undergo recombination at the *ARG4* locus, we also performed a return-to-growth analysis. This assay allows for the recovery of cells in which meiotic recombination is initiated but not completed (Sherman and Roman 1963; for details see materials and methods). As shown in Figure 3A, in the *RFA1* strain we observed a nearly 10,000-fold increase in the frequency of meiotic Arg^+ recombinants, reaching $1.0 \pm 0.2 \times 10^{-1}$ 8 hr after transfer of the cells to sporulation medium. For the *rfa1-t11* and *rfa1-t48* diploids, we also observed an increase of the frequency of the Arg^+ prototrophs over the mitotic frequency but the maximal frequency is decreased by 10- ($1.4 \pm 0.3 \times 10^{-2}$) and 100-fold ($1.1 \pm 1.0 \times 10^{-3}$), respectively, relative to *RFA1* diploid (Figure 3A and Table 2). This large decrease of the meiotic recombination frequency in the mutant strains does not correlate with a loss of cell viability over the course of the return-to-growth experiment (at 24 hr, the cell viability is 100% for the *RFA1* strain, 90% for the *rfa1-t11* strain, and 80% for the *rfa1-t48* strain).

Finally, we examined the formation of recombinant molecules during meiosis at the *ARG4* locus by physical analysis. We monitored the appearance of the recombinant 1016-bp *Eco*RV-*Bgl*II restriction fragment (R1) by Southern blot analysis of DNA extracted from diploids at various times after transfer to sporulation medium. In the *RFA1*, *rfa1-t11*, and *rfa1-t48* diploids, we began to detect the recombinant fragment at 8 hr (Figure 3B). Quantification of the recombinant band at 24 hr indicates that it is reduced about fivefold in *rfa1-t11* mutants and fivefold or greater in *rfa1-t48* mutants, confirming that both mutants are defective for meiotic gene conversion as compared with wild-type strains. Although the *rfa1-t11* and *rfa1-t48* mutants can form recombinant products, albeit at a lower level, like the *rad51* and *rad52* deletion mutants (Bishop *et al.* 1992; Shinohara *et al.* 1992; Ogawa *et al.* 1993a) these events do not appear to be sufficient to ensure normal progression through meiosis, as manifested by the strong effects of the *rfa1* mutations on sporulation and spore viability. Consistent with its slower growth rate in vegetatively growing cells, the *rfa1-t48* diploid is more impaired than is the *rfa1-t11* diploid in meiotic events.

Meiotic DSBs form but undergo extended resection in the *rfa1-t11* and *rfa1-t48* mutants: To determine at which step meiotic recombination might be defective in *rfa1-t11* and *rfa1-t48* mutants, we monitored the appearance of the DSBs that initiate recombination. In wild-type diploids (*RAD50*), meiotic DSBs are detected as transient DNA fragments of heterogeneous size that reflect their processing (Sun *et al.* 1989, 1991; Cao *et al.* 1990). We first examined meiotic DSB formation in the 5′ intergenic region of the *ARG4* locus (Sun *et al.* 1989). As shown in Figure 4A, we performed a side-by-side comparison of DSB formation at the *ARG4* locus in *RFA1*, *rfa1-t11*, and *rfa1-t48* diploids. We found that meiotic DSBs form in the three diploids. In the MGD background, the meiotic DSBs are transiently detectable in the *RFA1* diploid between 5 and 11 hr after transfer to sporulation medium. Similarly, DSBs can be detected in the *rfa1-t11* strain. However, we note that DSB frag-

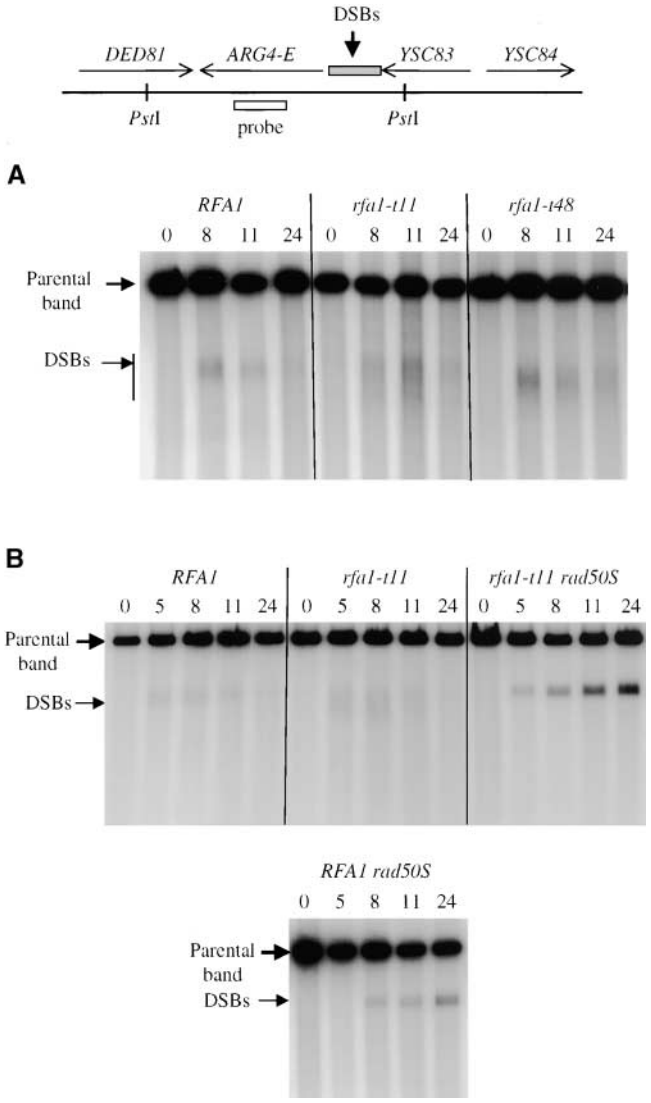

FIGURE 4.—Detection of meiotic DSBs at the *ARG4* locus in *rfa1* strains. Cells were taken at 0, 5, 8, 11, and 24 hr after transfer to sporulation medium and treated as described previously. Genomic DNA was extracted from meiotic cells, digested with *Pst*I, electrophoresed, transferred, and probed with a *Eco*RV-*Bgl*II fragment internal to *ARG4* (see Figure 2, A and B). Positions of the parental and *ARG4* DSB fragments are indicated by horizontal arrows. (A) Meiotic DSBs at *ARG4* in the *RFA1* (ORD2130), *rfa1-t11* (ORD3256), and *rfa1-t48* (ORD5402) strains. (B) Meiotic DSBs at *ARG4* in the *RFA1* (ORD2130), *rfa1-t11* (ORD3256), and *rfa1-t11 rad50S* (ORD3257) strains. A meiotic time course of the *RFA1 rad50S* strain (ORD2410), run independently, is shown in the bottom part of the figure.

ments are more heterogeneous in size in the *rfa1-t11* and the *rfa1-t48* mutants than in the *RFA1* strain (see below). Similar results were observed for DSB formation at the *CYS3* locus on chromosome I (data not shown). A close comparative examination of the characteristics of the DSB smears suggests that there are subtle differences in the range of fragment sizes: namely, the largest fragments appear to be of similar size in the *RFA1* and *rfa1-t11* diploids but greater in size than the largest fragments of the *rfa1-t48* strain. Moreover, the range of fragment sizes, as indicated by the width of the smear, is most restricted in the *RFA1* diploid (100–150 bp), intermediate in the *rfa1-t48* diploid (200–250 bp), and broadest (300 bp) in the *rfa1-t11* diploid (Figure 4A). Also, in both mutants, we noted that the overall level of DSB fragments only slightly decreases at late times and does not completely disappear (Figure 4, A and B), which correlates with the reduced production of Arg^+ cells and recombinant molecules (Figure 3, A and B).

To more accurately quantify the extent of breakage in the *rfa1* mutants, we also examined DSB formation in diploids homozygous for the *rad50S* mutation, which accumulate DSB fragments. In *rad50S* mutants, DSB ends are neither resected nor repaired (ALANI *et al.* 1990) since the Spo11 transesterase remains covalently attached (KEENEY *et al.* 1997). In the *rfa1-t11 rad50S* diploid, DSBs form and accumulate as a discrete band similar to what is seen for a *RFA1 rad50S* diploid (Figure 4B). Quantitatively, the amount of DSB formation at 11 hr, measured as the ratio of the intensity of the DSB band to total DNA (parental + all DSB signals), is 11.6% for the *RFA1 rad50S* strain and 12.8% for the *rfa1-t11 rad50S* (ROCCO and NICOLAS 1996; this study). Altogether, these results demonstrate that the *rfa1-t11* and *rfa1-t48* mutants are not defective in meiotic DSB formation, but are affected in their processing, and that there are subtle differences between the *rfa1-t11* and *rfa1-t48* diploids.

The fate of DSB ends is the same in *RFA1*, *rfa1-t11*, and *rfa1-t48* diploids: Considering the ssDNA-binding properties of the RPA complex (BRILL and STILLMAN 1989; WOLD *et al.* 1989) and the above observation that the *rfa1-t11* and *rfa1-t48* diploids have an abnormal distribution of DSB fragments, we examined each of the two complementary DNA strands on both sides of the DSB in both *RFA1* and mutant diploids. For this purpose, *Mlu*I-*Nru*I-digested genomic DNA was resolved on alkaline denaturing gels and probed with a single-stranded probe specific to the *YHR015* ORF, near the *ARG4* locus (Figure 2, A and B). With this probe, we observed the 3′ end of the single-stranded DNA on the right side of the *ARG4* DSB site in the *RFA1 RAD50*, *rfa1-t11 RAD50*, and *rfa1-t48 RAD50* diploids as a discrete band of the same length as that found in *rad50S* diploids, indicating that this strand is not resected (Figure 5). Under the same experimental conditions, the 3′ single-stranded fragment on the left side of the *ARG4* DSB site was similarly detected with a probe specific to *ARG4* (data not shown). This unresected 3′ strand DSB fragment accumulates in the *rfa1-t11* and *rfa1-t48* mutants but not in the *RFA1* diploid. Altogether these results indicate that the deficiencies of the *rfa1-t11* and *rfa1-t48* mutants in DSB repair are not due to a defect in the formation or maintenance of the 3′ single-stranded

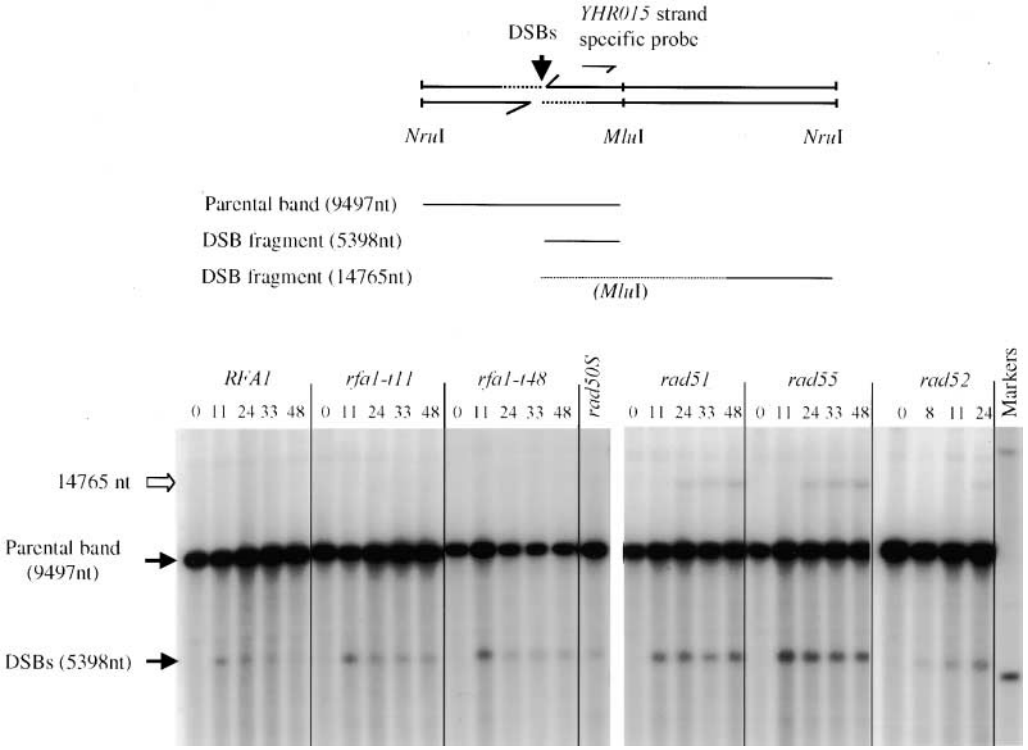

FIGURE 5.—Detection of the 3′ single-stranded end at the *ARG4* DSB site. Cells were taken at the indicated times; genomic DNA was extracted from *RFA1* (ORD-2130), *rfa1-t11* (ORD3256), *rfa1-t48* (ORD5402), *rad51* (ORD3200), *rad55* (ORD-3204), and *rad52* (ORD-3285) strains and digested with the restriction enzymes *Mlu*I and *Nru*I. Samples were subjected to alkaline electrophoresis and transferred by capillary blotting to nylon membranes as indicated in MATERIALS AND METHODS. DNA fragments were visualized by hybridization with a single-stranded DNA radiolabeled probe (*YHR015*, see Figure 2A). This probe was synthesized by asymmetric PCR with a single primer complementary to the 3′ strand. DNA from a *rad50S* strain (ORD-2410) is shown as a reference to compare the sizes of fragments generated by breaks in the different strains. The positions of the parental and 3′ single-stranded fragments are indicated by solid arrows. The top open arrow indicates the position of the higher molecular weight band seen in DNA from *rad51*, *rad55*, and *rad52* diploids.

DSB intermediate but more likely arise during the subsequent recombinational repair events.

Comparison of meiotic DSB formation in *rfa1-t11*, *rfa1-t48*, *rad51*, *rad52*, *rad55*, and *rad57* diploids: As RPA enhances the efficiency of the strand exchange reaction catalyzed by Rad51 with the help of the Rad52, Rad55, and Rad57 proteins (SUGIYAMA *et al.* 1997; SUNG 1997a,b; NEW *et al.* 1998; SHINOHARA and OGAWA 1998), we wished to compare DSB formation, processing, and repair in *rfa1-t11* and *rfa1-t48* diploids and in *rad51*, *rad52*, *rad55*, and *rad57* diploids, using mutant strains obtained in the MGD background (MATERIALS AND METHODS and Table 1). For strains with the *rad50S* mutation, we observed that the *RFA1*, *rad51*, *rad52*, *rad55*, and *rad57* diploids form and accumulate meiotic DSBs at a similar level, at both the *ARG4* (Figure 6B and data not shown) and *CYS3* loci (data not shown). Then we examined DSB formation and processing in the *RAD50* context, under nondenaturing conditions, using a *Pst*I digestion and the *ARG4 Eco*RV-*Bgl*II internal fragment as a probe. As previously reported for the *HIS4-LEU2* construct (BISHOP *et al.* 1992; SHINOHARA *et al.* 1992; SCHWACHA and KLECKNER 1997), we also observed that DSBs form at the *ARG4* (Figure 6A) and *CYS3* loci (data not shown) in the *rad51*, *rad55*, and *rad57* diploids and that for each mutant the DSB fragments are more heterogeneous in size than those in wild-type cells (*RFA1*). Using the same restriction digest, we were not able to detect DSB fragments at the *ARG4* locus in DNA prepared from *rad52* diploids. This is probably because the 5′ ends of the DSB fragments are extensively and rapidly degraded beyond the *Pst*I site in the absence of the Rad52 protein, which renders the single-stranded DSB fragments resistant to cleavage by the restriction enzyme used to digest the genomic DNA, as described previously (WHITE and HABER 1990).

To confirm this hypothesis, we used an *Mlu*I-*Nru*I digestion instead of a *Pst*I digestion to obtain a parental genomic fragment of larger size covering the *ARG4* locus and used an internal fragment of the *YHR015* gene as a probe (see Figure 2, A and B). By using this restriction digest, we could consistently detect DSB signals as a faint smear that accumulates over the course of meiosis; the smallest fragments in the smear extend far below the unresected fragment observed in the *rad52 rad50S* diploid (Figure 6B). A comparison of the width of the DSB smear in the *rad51*, *rad55*, *rad57*, and *rfa1-t11* mutants was performed in the same experiment, with DNA from a *dmc1* mutant diploid included as a meiosis-specific control for the extent of meiotic DSB degradation (Figure 6A). This comparison, which was performed four times, suggests that the range of fragment sizes is the most restricted in the *RFA1* and *rfa1-t48* diploids, intermediate in the *rfa1-t11*, *rad51*, *rad55*, *rad57*, and *dmc1* diploids, and the most extensive in the *rad52* diploid. To confirm this conclusion, we examined the fate of the 3′ single-stranded DNA end in the *rad51*, *rad52*, *rad55*, and *rad57* mutant strains on denaturing alkaline

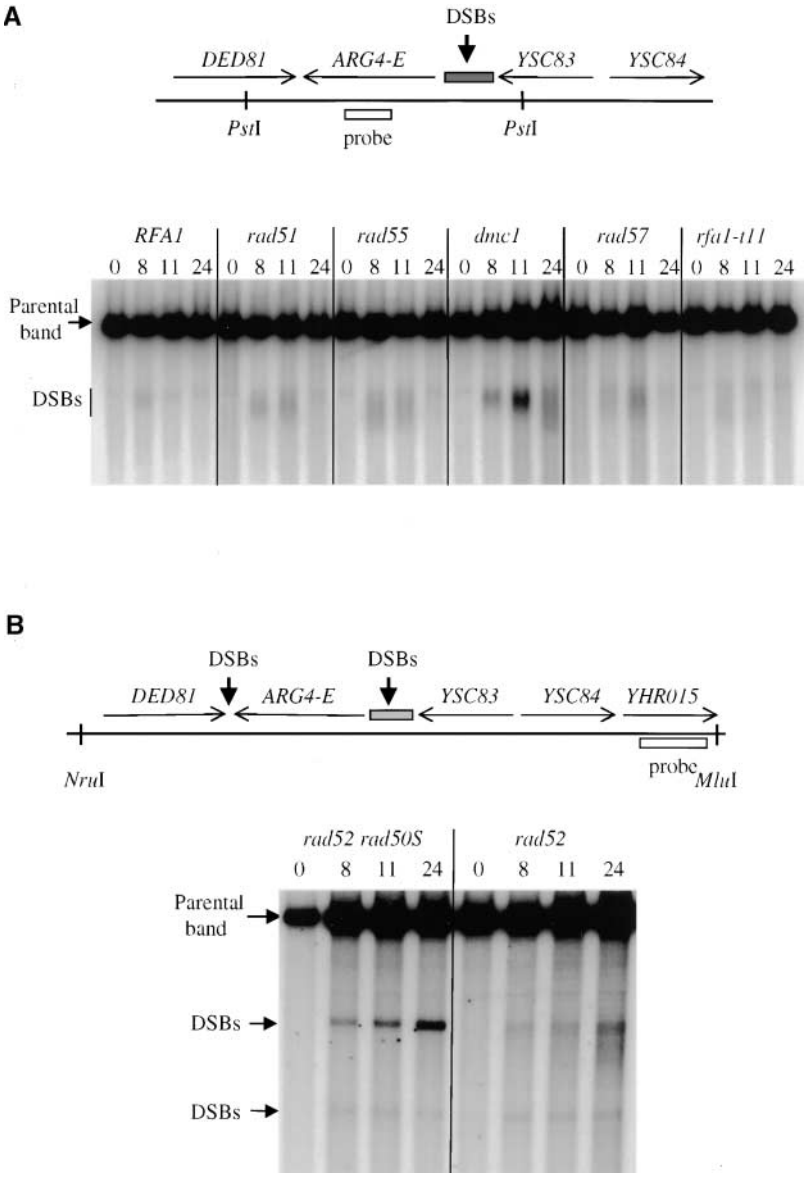

FIGURE 6.—Detection of meiotic DSBs at the *ARG4* locus in different mutant strains. (A) Samples of genomic DNA were extracted from *RFA1* (ORD2130), *rad51* (ORD3200), *rad55* (ORD3204), *dmc1* (ORD3233), *rad57* (ORD3243), and *rfa1-t11* (ORD3256) meiotic cells at the indicated times. DNA was digested with *Pst*I and probed with the internal *Eco*RV-*Bgl*II fragment of *ARG4*. (B) Samples of genomic DNA were extracted from *rad52 rad50S* (ORD3284) and *rad52* (ORD3285) strains, digested with *Mlu*I and *Nru*I, and probed with a part of *YHR015* as a probe. The positions of the parental and DSB fragments are indicated by horizontal arrows.

gels by using a single-stranded probe to detect *ARG4* DSB fragments. In all cases, as for the *RFA1* strain, we detected a meiosis-specific band of the expected size for the unresected 3′ single-stranded fragment (Figure 5 and data not shown). Noticeably, at late time points (24 hr and more), a fragment of higher molecular weight than that of the parental fragment is apparent in the *rad51*, *rad55*, and *rad52* diploids (Figure 5, open arrow). This band, never observed in *RFA1* and *rad50S* diploids, likely reflects the fraction of single-stranded DSB molecules that contain an undigested *Mlu*I restriction site downstream of the DSB site (see Figure 5). This therefore confirms that DSBs in these mutants undergo hyperresection, as seen under nondenaturing conditions (Figure 6). Close examination of the hybridization signals of the expected size for this higher molecular weight intermediate in all of our experiments involving the other mutants (Figure 5 and data not shown) indicates that the *rfa1-t11* strain sometimes exhibits a faint signal but that the *rad57* and *rfa1-t48* strains never do. These additional observations reinforce the conclusion that DSBs undergo extended resection in the *rfa1-t11* mutant, to a greater extent than in the *rfa1-t48* mutant (Figure 4), and that there is a subtle difference between the *rad55* and *rad57* mutants with respect to DSB processing. Altogether, we conclude that, as seen for *RFA1* cells, all of the mutant diploids studied here form the unresected 3′ single-stranded tails that are likely involved in the invasion of a homologous chromosome. However, all the mutants exhibit a more or less extensive resection of the 5′ strand.

DISCUSSION

Over the past years, the importance of the RPA heterotrimer in numerous aspects of DNA metabolism has become very compelling. Indeed, *in vivo* and *in vitro* studies have demonstrated its involvement in replication

(for review, see Wold 1997), in repair (Coverley *et al.* 1991; Guzder *et al.* 1995), in mitotic recombination (Longhese *et al.* 1994; Firmenich *et al.* 1995; Smith and Rothstein 1995, 1999; Umezu *et al.* 1998), and in surveillance mechanisms that link defects in DNA metabolism with cell cycle progression (Brush *et al.* 1996; Cheng *et al.* 1996; Lee *et al.* 1998). The isolation of a number of mutants has been instrumental in the characterization of the multiple functions of the RPA complex (Longhese *et al.* 1994; Firmenich *et al.* 1995; Smith and Rothstein 1995; Umezu *et al.* 1998; Smith and Rothstein 1999). The present study of two of these *rfa1* mutants adds insights into the role of RPA in meiotic recombination. We show here that homozygous *rfa1-t11* and *rfa1-t48* diploid strains have a low efficiency of sporulation and poor spore viability and that they are defective in the formation of recombinants. Physical analyses of early events in recombination indicate that both mutants form normal levels of meiosis-specific DSBs and produce 3′ single-stranded tails, indicating that the RPA complex present in these *rfa1* mutants does not impair the formation of this key early recombination intermediate. However, DSBs undergo extensive resection in both *rfa1* mutants. Similar phenotypes are observed for strains with mutations in the *RAD52* pathway and in the double mutants (*rad51 rfa1-t11*, *rad55 rfa1-t11*, *rad57 rfa1-t11*, and *rad52 rfa1-t11*; data not shown). The similarity of the phenotypes conferred by the *rfa1* mutations and the *RAD52* pathway mutations, in particular an accumulation of hyperresected DSB fragments, the formation of a residual level of recombinant molecules but a stronger defect in the formation of mature recombinant cells (Bishop *et al.* 1992; Shinohara *et al.* 1992; Ogawa *et al.* 1993a), suggests that these *rfa1* mutants are impaired in the formation of ssDNA nucleofilaments and/or in other post-DSB steps of homologous recombination.

Potential role of RPA in the formation of a proper ssDNA nucleofilament: The mechanistic steps by which ssDNA-binding proteins act in recombination were originally described through studies of the *E. coli* SSB protein (for reviews, see Meyer and Laine 1990; Kowalczykowski *et al.* 1994). More recent studies with eukaryotic proteins showed that the strand exchange reaction catalyzed by Rad51 proteins is rather inefficient *in vitro* but is enhanced when the cofactors RPA, Rad52, and the Rad55/Rad57 proteins are added in the proper order and stoichiometry (Sung 1994, 1997a,b; Benson *et al.* 1998; New *et al.* 1998; Shinohara and Ogawa 1998; for a review, see Sung *et al.* 2000). A possible explanation for the requirement of the eukaryotic Rad51 proteins for those cofactors may stem from observations that, in contrast to RecA, Rad51 proteins can bind both double-stranded DNA (dsDNA) and ssDNA (Shinohara *et al.* 1992; Benson *et al.* 1994) and that RPA competes with Rad51 protein for binding ssDNA. Any interpretation of the phenotype of *rfa1* mutations should therefore consider the dual role of RPA in presynapsis and synapsis and should specifically take into account the role of the Rad52 and Rad55/Rad57 proteins as cofactors.

Two major biochemical functions of RPA could be differentially affected in the *rfa1* mutants described here: the ssDNA-binding activity and/or interactions with other proteins. Considering the first possibility, we initially envisaged that RPA could interact *in vivo* with 3′ single-stranded DNA, and we therefore examined in great detail this key recombination intermediate in the *rfa1* mutants. We found that both mutants form the resected intermediate with the same timing and strand polarity (3′ single-stranded tail) as does the *RFA1* strain. This result suggests that RPA has no role in the formation or in the stabilization of the DSB single-stranded tail. In this case other DNA-binding proteins such as Rad52 or the Rad55/57 heterodimer might substitute for RPA in coating the single-stranded DSB tail. Although this extreme hypothesis cannot be formally excluded, the *in vitro* properties of RPA, the abundance of the RPA complex throughout meiotic prophase (Plug *et al.* 1997, 1998), and the induction of DSB-dependent recombination foci that contain RPA (Gasior *et al.* 1998, 2001) argue that RPA has a role in the early steps of recombination. A hypothesis that may explain why RPA complexes containing the *rfa1-t48* mutation, localized within the conserved Rfa1-A ssDNA-binding domain (Philipova *et al.* 1996), retain ssDNA-binding activity is that RPA has four ssDNA-binding domains, an arrangement reminiscent of the tetrameric structure of SSB (Philipova *et al.* 1996). Another missense mutation (*rfa1-D228Y)* localized near the *rfa1-t48* mutation (amino acids 228 and 221, respectively) has been previously described (Smith and Rothstein 1995). This mutation confers a slow growth phenotype, UV but not gamma irradiation sensitivity, and a slightly reduced efficiency of heteroallelic recombination. Biochemical analysis of the RPA complex present in *rfa1-D228Y* strains indicates that the complex binds ssDNA but that its overall level is reduced twofold (Smith and Rothstein 1995). A tentative interpretation of the results obtained with the *rfa1-t48* strain, similar to what was concluded for the *rfa1-D228Y* mutant, is that the Rfa1-t48 protein complex binds DNA but interacts abnormally with it, thereby promoting subsequent defects in DSB processing and repair.

Alternatively, the *rfa1* mutants with alterations in the N-terminal part of the protein might be defective in interacting with other proteins. Two such *rfa1* alleles with overlapping phenotypes, *rfa1-44* (G77D; Firmenich *et al.* 1995) and *rfa1-t11* (K45E; Umezu *et al.* 1998), have been studied. Specifically, strains bearing these mutations are proficient for DNA replication, sensitive to UV and gamma irradiation, and strongly deficient in HO-induced recombination. The homozygous diploids have a reduced sporulation efficiency as well as severe

spore inviability (Firmenich *et al.* 1995; present results). A biochemical analysis of the purified RPA complex containing the Rfa1-t11 subunit indicates that it binds to ssDNA, but that Rad51 displaces the mutant RPA complex more slowly than the wild-type RPA complex and thereby impedes strand exchange (S. Kowalczykowski, personal communication). These mutations are localized in less conserved regions of the Rfa1 protein and are therefore more likely to be involved in species-specific protein-protein interactions. Evidence for this possibility is suggested by the observation that the mitotic phenotypes of *rfa1-44* cells are suppressed in a dose-dependent manner by *RAD52* (Firmenich *et al.* 1995). Studies of the interactions among the proteins of the *RAD52* pathway (by two-hybrid assay and co-immunoprecipitation analyses) indicates that RPA interacts with Rad52, which in turn interacts with Rad51. This scenario raises the possibility that the primary defect of the *rfa1-t11* mutant resides in the interaction of RPA with the Rad52 mediator protein (Hays *et al.* 1995, 1998; Park *et al.* 1996; Shinohara and Ogawa 1998). A defective interaction between Rfa1-t11-ssDNA and Rad51, which implicates the intermediation of Rad52, could result in a defect in the polymerization of Rad51 on ssDNA, thereby inhibiting the strand exchange reaction.

Potential role of RPA in post-ssDNA nucleofilament formation step(s): Once the ssDNA filament is properly formed, pairing between this presynaptic filament and a homologous dsDNA takes place and is followed by strand exchange, leading to the formation of a D-loop intermediate (for reviews, see Kowalczykowski *et al.* 1994; Sung *et al.* 2000). *In vitro,* the factors that function in the synaptic phase are the Rad51-ssDNA filament, the dsDNA, RPA, and the Rad54 and Rdh54/Tid1 proteins, which stimulate the formation of the D-loop by mediating an alteration of the duplex DNA conformation (Petukhova *et al.* 1998, 1999, 2000; Tan *et al.* 1999; Song and Sung 2000; VanKomen *et al.* 2000). Mutant analyses showed that the conversion of these intermediates to a double Holliday junction is dependent on the activity of the *RAD51*, *RAD52*, *RAD55*, *RAD57*, and *DMC1* gene products (Schwacha and Kleckner 1997). Strains with mutations in all of these genes, as well as our *rfa1* mutants, exhibit hyperresection of the 5′ ends of the break fragments (Bishop *et al.* 1992; Shinohara *et al.* 1992, 1997; Dresser *et al.* 1997; Schwacha and Kleckner 1997; present study, Figures 4 and 6). The origin of this hyperresection is not known. It may reflect an impairment in the initial mechanism of resection, which itself could be coupled to DSB formation and the assembly of a proper ssDNA recombination intermediate for strand invasion. Alternatively, these events may be uncoupled and therefore hyperresection would be due only to an independent mechanism of degradation that is activated when DSB repair is defective. This observation favors the view that this hyperresection is the consequence of the defect in repair, which of course could be due to the uncoupling of degradation and strand exchange but could also result from the improper assembly of an active Rad51 nucleofilament.

In conclusion, our study of the *rfa1-t11* and *rfa1-t48* mutants has further revealed the involvement of the RPA complex in the process of meiotic recombination. A future interest is to define the biochemical properties of the corresponding RPA complexes with respect to the formation and the activity of the single-stranded DNA nucleofilament in strand invasion, a key step in the repair of DNA double-stranded breaks by homologous recombination.

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