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Replication Factor A Is Required In Vivo for DNA Replication, Repair, and Recombination

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Replication factor A (RF-A) is a heterotrimeric single-stranded-DNA-binding protein which is conserved in all eukaryotes. Since the availability of conditional mutants is an essential step to define functions and interactions of RF-A in vivo, we have produced and characterized mutations in the *RFA1* gene, encoding the p70 subunit of the complex in *Saccharomyces cerevisiae*. This analysis provides the first in vivo evidence that RF-A function is critical not only for DNA replication but also for efficient DNA repair and recombination. Moreover, genetic evidence indicate that p70 interacts both with the DNA polymerase α-primase complex and with DNA polymerase δ.

The identification of eukaryotic replication proteins has relied mainly on the use of in vitro replication systems. For example, the simian virus 40 system allowed the isolation of a number of replication proteins from human cell extracts and the reconstitution of an in vitro replication system with purified components (10, 31, 52, 55). However, the answer to the question of whether these proteins function in vivo to replicate chromosomal DNA requires the production of mutations in the corresponding genes and the characterization of the obtained mutant strains. The use of the yeast *Saccharomyces cerevisiae* has been particularly useful for this purpose (8, 9).

The human single-stranded-DNA-binding protein (also known as replication protein A or replication factor A [RF-A]), originally identified as a protein factor required for simian virus 40 DNA replication in vitro, is a heterotrimer of 70-, 34-, and 11-kDa subunits (19, 57, 58). The p70 polypeptide is sufficient for DNA binding (6, 32, 59), while the function of the p34 and p11 subunits is still unknown. The p34 polypeptide is phosphorylated in a cell cycle-dependent manner (14, 17, 20, 22), suggesting that RF-A might be a target of the regulatory mechanisms driving the G_1-to-S phase transition during the cell cycle.

The RF-A complex is highly conserved in all eukaryotes (6, 39, 58), and the three RF-A genes in budding yeast cells are essential for cell viability (7). Nevertheless, yeast RF-A only partially substitutes for human RF-A in the in vitro replication of simian virus 40 (6), indicating that species-specific interactions between RF-A and other replication proteins are important for its biological activity. Similarly, the DNA polymerase α (pol α)-primase complex is involved in determining the species specificity of the DNA replication process (42, 47, 48). The RF-A and pol α-primase complexes are quite peculiar, since these two protein complexes are required during both the initiation and elongation steps of DNA replication (11, 53, 55, 56). In vitro studies indicate that they interact with each other as well as with T antigen (15, 16, 37, 41, 43), suggesting a pivotal role for these proteins in DNA replication. Moreover, the function of the RF-A complex might not be limited to the replication process, since human RF-A seems to be required for the nucleotide excision repair of UV-damaged DNA in vitro (12), and both yeast and human RF-As stimulate in vitro strand exchange proteins (2, 28, 29, 40).

In this report, we describe the production and characterization of mutations in the *RFA1* gene, encoding the p70 single-stranded-DNA-binding subunit of the *S. cerevisiae* RF-A complex. We demonstrate that RF-A has a critical role in DNA replication, repair, and recombination in vivo. Moreover, by double-mutant analysis, we show that RF-A likely interacts in vivo not only with the pol α-primase complex but also with DNA polymerase δ (pol δ).

MATERIALS AND METHODS

Plasmids. Plasmid pJM116 (a gift from B. Stillman, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) carries the *Pst*I-*Hin*dIII *RFA1* fragment cloned into the *Bam*HI-*Sal*I sites of plasmid YEp213 (44). Plasmid pML11 (*ARS1 CEN4 CDEIII(15-T) URA3 ADE3 RFA1*) was constructed by subcloning the *Bam*HI-*Sal*I *RFA1* fragment from pJM116, after filling in with Klenow enzyme, into the *Nru*I site of a pc2013 (13) derivative plasmid lacking the *CLN2 Sal*I fragment. To construct plasmid pML21, used for in vitro mutagenesis of the *RFA1* gene (see below), the *Bam*HI-*Sal*I fragment from pJM116 was inserted into the *Bam*HI-*Sal*I sites of the *ARS1 CEN6 TRP1* plasmid pLA411 (21); subsequently, the *Bam*HI site in plasmid pML21 was removed by addition of a 10-bp oligonucleotide, which generated a new *Sal*I site. The *ADE3 LEU2 CEN3* plasmids pDK243 and pDK368-7, carrying *ARS1* and *ARS1* plus seven copies of the H4 autonomously replicating sequence (ARS), respectively (30), were a gift from D. Koshland (Carnegie Institution of Washington, Baltimore, Md.).

Yeast strains and media. The genotypes of the yeast strains used in this study are listed in Table 1. Strain YLL99 was constructed from strain K2346 by transformation with plasmid pML11, followed by one-step replacement (46) of the 1,125-bp *Eco*RV-*Sac*I fragment in the chromosomal *RFA1* coding region (7) with a *Bam*HI fragment containing the *HIS3* gene. Strains YLL91 and YLL95 were derived from strain YLL99 by replacement of the chromosomal *PRI1* and *POL1* alleles, respectively, with the *pri1-2* (23) and *pol1-1* (36) alleles. Similarly, the *rfa1-M2* and *rfa1-M4* isogenic derivatives of strains K2346, CG378, TD28, and 344-115B2 were obtained by replacement of the *RFA1* chromosomal copy with the mutant alleles (46). The accuracy of all the gene replacements was

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TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype[a]	Source
K2346	*MAT***a** *ade2-1 ade3 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3*	K. Nasmyth
K2346-M2	*MAT***a** *ade2-1 ade3 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 rfa1-M2*	This study
YLL99	*MAT***a** *ade2-1 ade3 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 rfa1::HIS3* [pML11 *ADE3 URA3 RFA1*]	This study
YLL91	*MAT***a** *ade2-1 ade3 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 rfa1::HIS3* [pML11 *ADE3 URA3 RFA1*] *pri1-2*	This study
YLL95	*MAT***a** *ade2-1 ade3 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 rfa1::HIS3* [pML11 *ADE3 URA3 RFA1*] *pol1-1*	This study
CG378	*MAT***a** *ade5 can1 leu2-3,112 trp1-289 ura3-52*	L. Johnston
CG378-M2	*MAT***a** *ade5 can1 leu2-3,112 trp1-289 ura3-52 rfa1-M2*	This study
CG378-M4	*MAT***a** *ade5 can1 leu2-3,112 trp1-289 ura3-52 rfa1-M4*	This study
DGL2/1C	*MAT*α *met4 lys1*	Our laboratory
TD28	*MAT***a** *ura3-52 ino1 can1*	G. Fink
TD28-M2	*MAT***a** *ura3-52 ino1 can1 rfa1-M2*	This study
435/3B	*MAT***a** *cdc2-1 ade1 ade2 gal1-4 can1-11*	G. Simchen
DMP157/1A	*MAT*α *cdc2-1 lys1*	This study
19041	*MAT***a** *cdc2-3 ade2 ade3 his7 lys2 tyr1 ura1 can1 gal1*	J. Rosamond
DMP100/10A	*MAT*α *cdc2-3*	This study
344-115B	*MAT*α *his3-513::TRP1::his3-537 ura3-52 trp1 leu2-112::URA3::leu2-k*	H. Klein
344-115B2	*MAT*α *his3-513::TRP1::his3-537 ura3-52 trp1 leu2*	This study
344-115B2-M2	*MAT*α *his3-513::TRP1::his3-537 ura3-52 trp1 leu2 rfa1-M2*	This study

[a] Plasmids are indicated in brackets.

verified by Southern blot analysis. Strain 344-115B2 was derived from strain 344-115B (35) by reciprocal recombination at the *leu2-112::URA3::leu2-k* duplication on chromosome III. Strains DMP157/1A and DMP100/10A were obtained from strains 435/3B and 19041, respectively, which were first crossed to strain DGL2/1C followed by three rounds of backcrosses to strain TD28. Standard yeast genetic techniques and media were as described elsewhere (44). Selective media and procedures for the determination of mutation and recombination rates were as previously described (35).

Mutagenesis of the *RFA1* gene and plasmid shuffling. To obtain two-codon insertions (3, 21) in the *RFA1* gene (7), plasmid pML21 was linearized by partial digestion with *Taq*I, *Mae*II, *Hpa*II, and *Hin*pI, followed by ligation in the presence of the oligonucleotide 5′-CGGATC-3′, which generates a single *Bam*HI site. The ligation mixture was cut with *Bam*HI, and the 1.3-kb *Bam*HI fragment, containing the kanamycin resistance gene (Kan^r) isolated from plasmid pUC4-KISS (Pharmacia) was added before ligation and transformation of the kanamycin-sensitive strain *Escherichia coli* DH5α (Bethesda Research Laboratories). To obtain two-codon insertions, Kan^r insertion plasmids were digested with *Bam*HI and religated, leaving a *Bam*HI site between the cytosine and guanine nucleotides of each original *Taq*I, *Mae*II, *Hpa*II, and *Hin*pI sites. All of the two-codon insertions were verified by direct nucleotide sequencing. Plasmids were then used to transform yeast strains YLL99, YLL91, and YLL95. Transformants were selected on Ura- and Trp-deficient SC plates at 25°C and tested for the ability to grow on the same medium at different temperatures. No dominant phenotype was observed for any of the tested mutations. Transformants were assayed by the plasmid-shuffling procedure (49) for the ability to lose the *URA3 ADE3 RFA1* pML11 centromeric plasmid after growth in nonselective conditions at 25°C. Trp$^+$ Ura$^-$ clones, containing the different *rfa1* alleles on the pML21 derivative plasmids, were then assayed for the ability to grow when streaked on YPD plates incubated at different temperatures.

Fluorescence-activated cell sorting (FACS) analysis. Cells were grown in YPD medium, sonicated for 15 s, collected by centrifugation, and suspended in 70% ethanol for 16 h. Cells were then washed in 0.25 M Tris-HCl (pH 7.5) and suspended in the same buffer containing 2 mg of RNase A per ml. Samples were incubated for 12 h at 37°C and collected by centrifugation, and the pellet was resuspended in 0.5 M pepsin freshly dissolved in 55 mM HCl. Cells were then washed in 180 mM Tris-HCl (pH 7.5)–190 mM NaCl–70 mM $MgCl_2$ and stained in the same buffer containing 50 μg of propidium iodide per ml. Samples were then diluted 10-fold in 50 mM Tris-HCl (pH 7.8) and analyzed by using a Becton Dickinson FACScan.

RESULTS AND DISCUSSION

Production and characterization of *rfa1* mutant alleles. Despite all of the biochemical information available on RF-A and the finding that its function is essential for yeast cell viability, the definition of the in vivo role of this protein complex in DNA metabolism requires the production and characterization of conditional mutants.

As a first step to address this problem, we have mutagenized the *RFA1* gene, encoding the p70 subunit of RF-A in *S. cerevisiae* (7), by using the linker insertion procedure described in Materials and Methods. As summarized in Table 2, eight alleles, carrying two-codon insertions at different positions within the p70 coding region, were assayed by plasmid shuffling for the ability to complement at different temperatures the lethal effect of a deletion of the *RFA1* chromosomal locus. When the strain used for this assay did not carry any other mutation affecting DNA replication (*POL1 PRI1* in Table 2), the *T1* allele was lethal, the *M2* allele gave rise to a temperature-sensitive phenotype, the *M4* and *T2* alleles affected either severely (*M4*) or very slightly (*T2*) the growth rate at any temperature, while the remaining alleles did not cause any detectable growth defect.

Since amino acid sequence conservation between yeast and human p70 is spread throughout the whole molecule (18), it is difficult to make a clear correlation between the position of the two-amino-acid insertions and the effect on p70 function. However, insertion of a proline residue in the very amino-terminal portion of the protein (*T1* allele) might severely perturb the α-helical structure predicted in that region by using the profile network method (45), giving rise to the observed lethal phenotype. Furthermore, the most dramatic growth defect is caused by the *M4* mutation, which results in a two-amino-acid insertion in the largest conserved region of the

TABLE 2. Growth phenotypes of *rfa1* mutants[a]

RFA1 allele	Growth[b]							Amino acid insertion[c]
	POL1 PRI1			*pri1-2*		*pol1-1*		
	25°C	28°C	37°C	25°C	28°C	25°C	37°C	
Wild type	++++	++++	++++	++	+++	+++	−	
T1	−	−	−	−	−	−	−	S-7**DP**R-8
M2	+++	+++	−/+	−	−	−	−	Y-96**GS**V-97
T2	+++	+++	+++	−/+	−/+	+	−	I-187**GSE**-188
M4	++	++	++	−	−	−	−	T-211**DPW**-212
T3	++++	++++	++++	++	+++	+++	−	F-290**GSD**-291
M5	++++	++++	++++	++	+++	+++	−	N-316**GSV**-317
Hp2	++++	++++	++++	++	+++	+++	−	P-430**GSG**-431
Hi2	++++	++++	++++	−/+	−/+	+	−	A-581**DPR**-582

[a] The isogenic strains YLL99 (*POL1 PRI1*), YLL91 (*pri1-2*), and YLL95 (*pol1-1*), all carrying a lethal *rfa1::HIS3* chromosomal disruption and the *RFA1 URA3 ADE3* pML11 plasmid, were transformed with *TRP1* pML21 derivative plasmids carrying the listed *RFA1* alleles. Trp^+ Ura^+ transformants were assayed for the ability to lose the pML11 plasmid (see Materials and Methods), and Trp^+ Ura^- clones were streaked on YPD plates and incubated at the indicated temperatures.

[b] −, no growth after 3 days. The number of plus signs indicates the extent of growth compared with the same strain carrying the *RFA1* allele on the *TRP1* plasmid. No growth at any temperature indicates that the corresponding Trp^+ Ura^+ transformant cannot lose the pML11 plasmid.

[c] The positions of the two-amino-acid insertions (boldface, one-letter code) are indicated by numbers.

protein (64% amino acid identity over a stretch of 50 residues) (18).

As shown in Fig. 1A, the *rfa1-M2* allele gave a clear temperature-sensitive phenotype also when used to replace the *RFA1* chromosomal copy in strain CG378. Although growth was not completely arrested at the restrictive temperature, mutant cultures significantly accumulated dumbbell-shaped cells (about 60%), with a single nucleus at the neck between mother and daughter cells (data not shown). FACS analysis showed that asynchronous CG378 *rfa1-M2* cell cultures contained more cells with DNA content between 1C and 2C (S-phase cells) after shift to 37°C (Fig. 1B). Together with the increase in the generation time, these results suggest that S phase is extended in the *rfa1-M2* cells at 37°C. Mutant cell cultures growing at 25°C contained fewer cells with 1C DNA content (G_1 cells), although the growth rate was very similar to

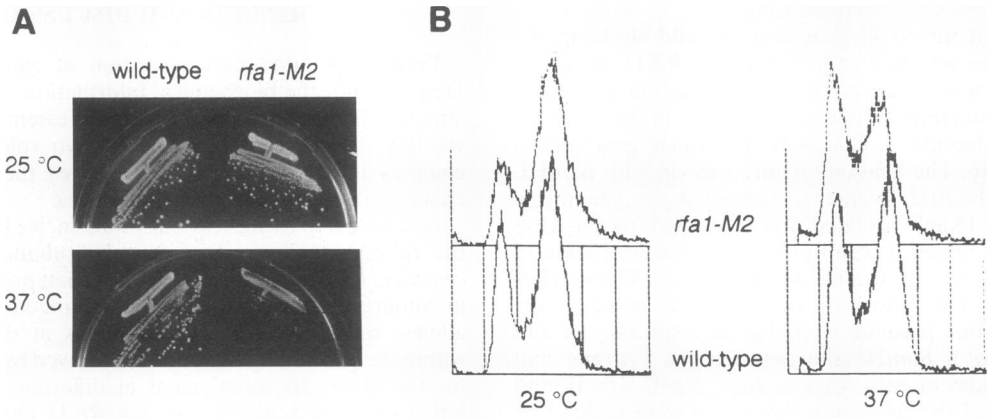

FIG. 1. The *rfa1-M2* mutant is defective in DNA replication. (A) Growth phenotypes of strain CG378 (wild type) and its isogenic *rfa1-M2* stable derivative CG378-M2. (B) Strains in panel A were analyzed by FACS analysis. Samples were taken from YPD cultures logarithmically growing at 25°C (left) or 2.5 h after a shift to 37°C (right). (C) Mitotic loss of pDK243 (single ARS) or pDK368-7 (multiple ARSs) in strain K2346 (wild type) and its isogenic *rfa1-M2* stable derivative K2346-M2. Both strains were transformed with the two plasmids; for each transformed strain, 10 independent colonies grown from single *ADE3 LEU2* cells on YPD at 25°C were resuspended in 1 ml of water and spread on three YPD plates (2×10^2 cells per colony per plate). Plates were incubated at 25°C, and the frequency of *ade3 leu2* colonies was estimated after 6 days. The rates of mitotic loss were calculated by the method of the median from fluctuation analysis as described by Lea and Coulson (34); the standard deviation for *m* values was always less than 0.4.

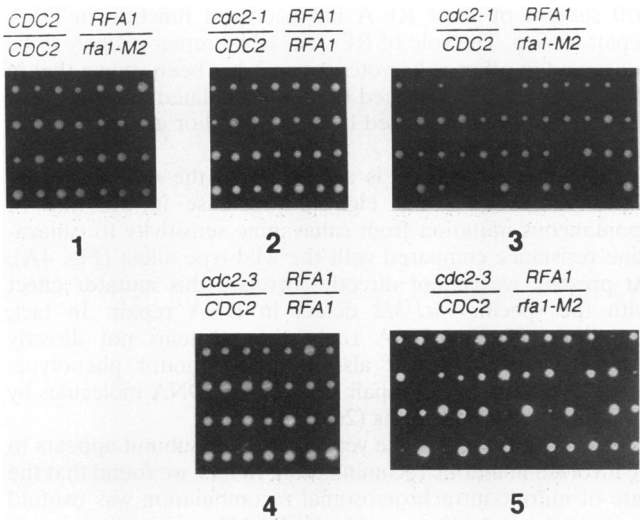

FIG. 2. Synthetic phenotypes of *rfa1 cdc2* double mutants. Strains DMP157/1A and DMP100/10A were crossed to strain TD28 to obtain diploid strains 2 and 4, respectively, and to strain TD28-M2 to obtain diploid strains 3 and 5, respectively. As a further control, strain TD28-M2 was also crossed to strain DGL2/1C to obtain diploid strain 1. Diploid strains with the indicated genotypes were allowed to sporulate, and tetrads were dissected on YPD plates and then incubated at 25°C for 3 days. Segregation of the temperature-sensitive phenotype and of any other heterozygous markers was tested on the viable spores, and the presence of the *cdc2* and/or *rfa1* alleles was assayed by a complementation test. By this analysis, all spores were viable and markers segregated properly in tetrads from the control diploid strains 1, 2, and 4, while no viable *cdc2-1 rfa1-M2* spores were found from diploid strain 3 and all of the inviable spores from the same strain were expected to be *cdc2-1 rfa1-M2* double mutants. In fact, all tetrads with four viable spores were parental ditypes (PD), all tetrads with three viable spores were tetratypes (TT), and all tetrads with two viable spores were nonparental ditypes (NPD). By the same analysis, all of the inviable spores and the spores forming very small colonies from diploid strain 5 were *cdc2-3 rfa1-M2* segregants. χ^2 analysis confirmed that the expected 1 PD:1 NPD:4 TT ratio was found among tetrads from both diploid strain 3 ($P = 1$) and diploid strain 5 ($P = 0.2$).

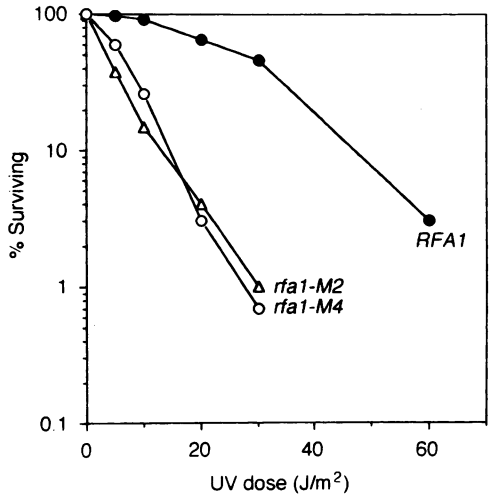

FIG. 3. UV sensitivity of *rfa1* mutant strains. Wild-type strain CG378 (closed circles) and its stable derivatives CG378-M2 (*rfa1-M2*; open triangles) and CG378-M4 (*rfa1-M4*; open circles) were used in this assay. Overnight saturated YPD cultures of wild-type and *rfa1* strains were diluted in sterile water; from each culture, 100 and 1,000 cells were spread on YPD plates, which were then exposed to the indicated dosages of UV radiation from a germicidal lamp. Plates were incubated at 25°C, and colonies were counted after 3 days.

the wild-type rate, suggesting that reduction of G_1 cells is likely due to a delay of S phase. A similar behavior was previously observed for mutations partially affecting the yeast origin recognition complex (38), which specifically binds to replication origins (ARSs) in *S. cerevisiae* (4).

To further investigate the role of RF-A in DNA replication, it was of interest to assay the stability of ARS1 centromeric plasmids in *rfa1* mutant strains. In fact, stability of these plasmids is severely affected in many cell division cycle (*cdc*) mutants, and this elevated mitotic loss can be rescued by the addition of several ARSs in *cdc6* mutant cells (30), which are considered to be defective in initiation of DNA replication (25, 27). As shown in Fig. 1C, the rate of mitotic loss of the centromeric plasmid pDK243 (30), carrying the *ARS1* replication origin, was eightfold greater in the *rfa1-M2* mutant than in the isogenic wild-type strain. The *rfa1-M2* effect on plasmid loss was instead abolished when seven additional copies of the H4 ARS were present on plasmid pDK368-7. These data strongly suggest a direct involvement of yeast RF-A in initiation of DNA replication at an origin. However, since in vitro data indicate that RF-A is required not only for initiation but also for the elongation step of DNA replication, the multiple ARSs effect observed in the *rfa1-M2* strain might be allele specific.

p70 interacts in vivo with the pol α-primase complex and with pol δ. Combinations of conditional mutations in genes encoding for interacting proteins might result in a more dramatic defect than would the single mutations (24). Because of the dual role in initiation and elongation of DNA replication observed in vitro for RF-A, we tested the in vivo interaction of p70 with the pol α-primase complex and with pol δ by double-mutant analysis. As shown in Table 2, both the *rfa1-M2* and *rfa1-M4* alleles were lethal, in combination with the cold-sensitive *pri1-2* allele or the temperature-sensitive *pol1-1* allele, which affect the p48 primase subunit or the pol α polypeptide, respectively (23, 36). Moreover, both the *rfa1-T2* allele, which only slightly altered the cell growth of the *POL1 PRI1* strain, and the *rfa1-Hi2* allele, which did not cause per se any detectable growth defect, severely affected growth at the permissive temperature of both the *pri1-2* and *pol1-1* strains. Consistently, when we analyzed tetrads from double heterozygous diploid strains, in which one chromosomal copy of *RFA1* and *POL1* or *RFA1* and *PRI1* had been replaced with the *rfa1-M2* or *rfa1-Hi2* allele and the *pol1-1* or *pri1-2* allele, all of the *rfa1-M2 pol1-1* and *rfa1-M2 pri1-2* meiotic segregants were inviable, while all the *rfa1-Hi2 pol1-1* and *rfa1-Hi2 pri1-2* meiotic segregants grew very poorly at the permissive temperature (data not shown). The synthetic phenotype of the *rfa1 pri1* and *rfa1 pol1* double mutants is strong evidence for in vivo functional interaction between the p70 subunit of yeast RF-A and the pol α-primase complex, which is involved both in initiation of DNA replication at an origin and in discontinuous DNA synthesis on the lagging strand (55). Furthermore, these data substantiate the in vitro finding that RF-A physically interacts with the pol α-primase polypeptides and might explain the species-specific stimulation of pol α-primase activity by RF-A (15, 37, 43).

As shown in Fig. 2, a synthetic lethal phenotype was also found when we tested the effects of the *rfa1-M2* allele on

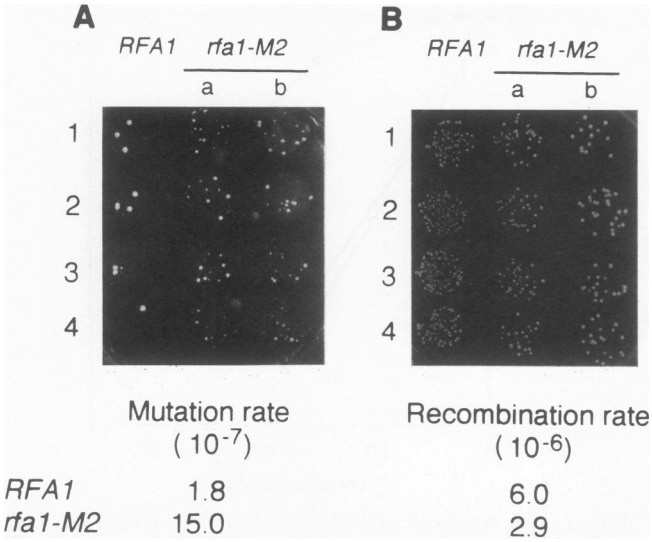

FIG. 4. Effects of the *rfa1-M2* allele on mutation and intrachromosomal mitotic recombination rates. Single cells from wild-type strain 344-115B2 and from two independent *rfa1-M2* isogenic stable derivatives (a and b) were allowed to form colonies on YPD plates at 25°C, and drops containing 5×10^6 cells from four independent colonies (1 to 4) for each strain were spotted on Arg-deficient, canavanine-containing SC plates (A) or His-deficient SC plates (B) (see text and reference 35 for details). Plates were incubated at 25°C, and pictures were taken after 4 days. Mutation and recombination rates as evaluated by plating on selective media 20 independent colonies of the wild-type and mutant strains are shown at the bottom. Rates (mutants or number of recombinants per cell per generation) were calculated by the method of the median from fluctuation analysis as described by Lea and Coulson (34); the standard deviation for *m* values was always less than 0.6 for forward mutation and less than 0.4 for recombination.

strains carrying two different temperature-sensitive alleles of the *CDC2* gene, encoding the catalytic subunit of pol δ (5, 50), which is considered the leading-strand DNA polymerase (8, 51, 56). In fact, all of the *cdc2-1 rfa1-M2* spores were inviable and all of the *cdc2-3 rfa1-M2* spores either were inviable or formed very small colonies, which could not be further propagated, when double heterozygous diploid strains were allowed to sporulate and tetrads were dissected. These data strongly indicate a functional interaction between p70 and pol δ in vivo, supporting the notion that RF-A stimulates the elongation step of DNA replication in vitro (32, 54).

The reliability of these genetic data is further strengthened by the absence of synthetic lethality in double mutants carrying either the *rfa1-M2* allele in combination with mutations in genes likely to be involved in initiation of DNA replication, such as *cdc6-1* and *cdc7-4* (9), or the *cdc2-1* allele in combination with the *pol1-1* or *pri1-2* allele (data not shown).

***rfa-1* mutants are defective in DNA repair and in mitotic intrachromosomal recombination.** Increased sensitivity to DNA-damaging agents is typical of mutants defective in DNA repair. As shown in Fig. 3, the sensitivity to UV radiation of *rfa1-M2* and *rfa1-M4* strains, measured as a percentage of surviving cells at the same UV dosage, is about 50-fold greater than that of the isogenic wild-type strain. Both mutants are also very sensitive to methyl methanesulfonate, since cell survival on YPD plates containing 0.01% methyl methanesulfonate was less than 10^{-3} for *rfa1-M2* and *rfa1-M4* strains, while the wild-type strain was not affected in the same conditions. Therefore, besides being essential for DNA replication, the p70 subunit of yeast RF-A has a critical function in DNA repair in vivo. The role of RF-A in DNA repair is likely to be conserved in other eukaryotes, since it has been shown that in vitro repair of UV-damaged DNA is stimulated by addition of human RF-A and inhibited by an anti-p70 or anti-p34 monoclonal antibody (12).

A mutator phenotype is associated to the *rfa1-M2* allele, which causes an about eightfold increase in the rate of spontaneous mutation from canavanine sensitivity to canavanine resistance compared with the wild-type allele (Fig. 4A). At present, we cannot directly correlate this mutator effect with the specific *rfa1-M2* defect in DNA repair. In fact, mutations affecting DNA replication proteins not directly involved in DNA repair also cause a mutator phenotype, probably as a result of repair of damaged DNA molecules by error-prone repair systems (26, 35).

As shown in Fig. 4B, the yeast p70 RF-A subunit appears to be involved in mitotic recombination. In fact, we found that the rate of mitotic intrachromosomal recombination was twofold lower in the *rfa1-M2* strain 344-115B2-M2 than in the isogenic wild-type strain when we measured the rate of recombination events at the *HIS3* locus in strains carrying the *his3-513::TRP1::his3-537* heteroallelic duplication on chromosome XV (1, 35). This twofold difference is statistically significant, since rates were calculated by the method of the median from fluctuation analysis, and the standard deviation for *m* values was always less than 0.4 (34). Among the yeast DNA replication mutants so far analyzed, only the *rfa1-M2* mutant shows a decrease in mitotic recombination rate, which is instead increased in other yeast DNA synthesis mutants (1, 26, 35). In fact, defective DNA replication generates nicked and gapped DNA molecules, which are substrates for recombination events. Since DNA replication is impaired in the *rfa1-M2* mutant strain, the concomitant twofold decrease observed in the recombination rate indicates that the p70 RF-A subunit has a critical positive role in DNA recombination. Although this issue needs to be further investigated, also because of the complex interpretation of the data due to the opposite and possibly allele-specific effects of *rfa1* mutations on recombination, the genetic evidence provided here substantiates the observation that yeast p70 stimulates in vitro both yeast Sep1 and *E. coli* RecA strand exchange proteins (2).

To our knowledge, the data presented here provide the first evidence of multiple roles of RF-A in vivo. Similarly to what found for the *E. coli* single-stranded-DNA-binding protein (33), yeast RF-A is directly involved in DNA replication, recombination, and repair. Conservation of RF-A during evolution suggests that these properties might be extended to other eukaryotes.

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