Research Article

Expression of the bacterial *recA* gene impairs genetic recombination and sporulation in a *Saccharomyces cerevisiae* diploid strain

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Abstract

The *Escherichia coli* RecA protein (RecAp) has been demonstrated to induce mutagenesis in yeast cells, although there is still little information on the role of the RecAp in yeast recombination events. We evaluated spontaneous and induced general recombination in vegetative and meiotic cells of the XS2316 strain of the yeast *Saccharomyces cerevisiae* bearing the *recA* gene. We found that RecAp decreased reciprocal recombination, gene conversion and intrachromosomal recombination and promoted an increase in error-prone processes in both vegetative and meiotic cells, while its negative effect on meiotic recombination blocked ascospore formation.

Introduction

The bacterial *recA* protein (RecAp) is primarily involved in the repair of DNA damaged during recombination events but also functions in general recombination processes (Kowalczykowski *et al.*, 1994), these roles having been conserved during evolution and transferred to several proteins in eukaryotic cells. In the yeast *Saccharomyces cerevisiae* the *RAD*51, *RAD*55, *RAD*57 and *DMC1* genes are considered as *recA* gene homologues (Kanz and Mortimer, 1991; Bishop *et al.*, 1992; Shinohara *et al.*, 1992; Lovett, 1994), the Rad51 protein (Rad51p) having been demonstrated to form a RecAp-like nucleoprotein structure responsible for ATP-dependent homologous DNA pairing and strand exchange (Sung and Roberson, 1995).

When expressed in *S. cerevisiae*, RecAp has been shown to increase the resistance of wild-type cells to the killing effect of ionizing radiation (Brozmanová *et al.*, 1991), to partially restore induced reverse mutation in *pso*4 mutant (de Morais Jr *et al.*, 1994) and to partially complement the sensitivity of *rad*52 mutant to DNA damaging agents (de Morais Jr *et al.*, 1998). Although being very similar to Rad51p, RecAp failed to complement the sensitive phenotype of *rad*51 mutant (de Morais Jr *et al.*, 1998), probably due to lack of a structural domain in the RecAp N-terminal region or because of the different polarity of DNA strand transfer reactions mediated by RecAp and Rad51p (Milne and Weaver, 1993; Sung and Robberson, 1995). It appears that although RecAp is active in promoting DNA repair events in yeasts its effects seems to be closely dependent on the genetic background of the yeast strain, e.g. despite the fact that RecAp restored induced mutation in *pso4* mutant (de Morais Jr *et al.*, 1994) it drastically decreased the hypermutagenic effect of the *rad52* mutant (de Morais Jr *et al.*, 1998) and was unable to restore recombination repair in *pso*4-1 mutant (Slaninova *et al.*, 1997; Vlckova *et al.*, 1997). Analysis of single point missense mutations revealed that RecAp has functional domains that are responsible for its biological functions in the induction of SOS, recombination and error-prone repair (Sweasy *et al.*, 1990).

We previously demonstrated that error-prone RecAp repair is active in yeast cells (de Morais Jr *et al.*, 1994) but the involvement of RecAp in recombination was not clear and because of this we decided to investigate the effect of the expression of the *recA* gene on the general type of recombination which normally occurs in wild type haploid and diploid yeast cells. The results presented in this paper suggest that RecAp interacts with protein in the yeast recombinosome complex and that it acts as a dominant negative allele due to its high but opposite biological activity in regard to strand pairing and branch migration as compared to functionally homologous yeast proteins.

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Material and Methods

Strains and plasmids

The genotype of the diploid *Saccharomyces cerevisiae* strain XS2316 (de Morais Jr et al., 1994) is *MATa*/α +/*ade*6 *leu*1-12/*leu*1-12 *Trp*5-48/+ +/*cyh*2 +/*met*13 +/*lys*5-1 *his*1-1/ *his*1-1 and that of the haploid strain MB401-3c (Meira *et al.*, 1992) *MATa lys*2 *ura*3-52 (*his*4-39/260: pBR322: *his*4-1176/864), strain MB401-3c having duplicate *his*4 mutations cloned at orientation II into the *HIS*4 *locus* of chromosome III that can be reverted for His4 prototrophy after pop-out recombination. *Escherichia coli* strain DH5α (Stratagene, CA) was used for plasmid amplification, a pNF2*recA* plasmid being constructed using the pNF2 plasmid (which was also used as a control) as described by de Morais Jr *et al.* (1994). Yeast cells were transformed using the lithium chloride procedure of Ito *et al.* (1983). Transformed cells were incubated overnight in yeast-extract peptone dextrose (YEPD) broth ((%, w/v) yeast extract, 1; peptone, 2; dextrose, 2), before being selected using selective YEPD plates (YEPD broth containing 2% (w/v) agar and 600 μg/mL of the antibiotic Geneticin) for 4 days incubation at 28 °C. The immunoblot method using polyclonal anti-RecA antibody was used to detect *recA* gene expression (de Morais Jr *et al.*, 1998).

Media for growth and selection of Yeasts

For mitotic growth we used YEPD broth, pre-sporulation (PS) medium (containing (%, w/v) yeast extract, 0.250; potassium acetate, 0.1; dextrose 1) being used to synchronize diploid cells and sporulation (SPA) medium (containing 2% (w/v) potassium acetate supplemented with amino acids and bases) to induce sporulation. Selective yeast extract nitrogen base (YNB) medium (containing (%, w/v) YNB, 0.67; dextrose, 2; ammonium sulfate, 0.5; agar, 2; supplemented with appropriate acids and bases) was used to detect reverse mutation and pop-out recombination (selective YNB lacking histidine) and gene conversion (selective YNB lacking leucine) and YEPD supplemented with 200 μg/mL of cycloheximide for reciprocal recombination analysis. All media having been described in de Morais Jr *et al.* (1998) and da Silva *et al.* (1995).

Growth conditions

Both transformed and untransformed XS2316 or MB401-3c strains were cultivated overnight in YEPD broth at 28 °C with agitation (200 rev.min^{-1}) until they reached the stationary phase (~2 x 10^8 cells.mL^{-1}). For experiments using exponential phase cells, cultures were diluted 1:100 in fresh YEPD and allowed to grow for additional four hours. In both cases, the cells were washed twice and resuspended in 0,9% (w/v) NaCl to 1-2 x 10^7 cells.mL^{-1} and exposed to mutagens as described below.

For return-to-growth experiments (da Silva *et al.*, 1995), overnight YEPD broth cultures of strain XS2316, prepared as described above, were diluted with PS medium to 8 x 10^6 cells.mL^{-1} and incubated for further 21 h at 28 °C, after which the cells were harvested by centrifugation, washed, re-suspended in SPA medium and re-incubated at the same temperature for up to 7 days. Samples were taken after 1, 3, 5 and 7 days incubation, in each case the cells were scored for spore formation using phase-contrast microscopy, washed twice in 0.9% (w/v) NaCl, resuspended to 1-2 x 10^7 cells.mL^{-1} and submitted to mutagen treatment as described below.

Mutagenesis

Cell suspensions were incubated in Petri dishes at 4 °C for 15 min in the presence of 50 mM of 8-Methoxypsoralen (8-MOP, Sigma, USA), after which were irradiated with 365 nm ultra violet A (UVA) using a 9/7 W Osram lamp at an intensity of 1 kJ.m^{-2}.min^{-1}. Treatment with 254 nm ultra violet C (UVC) used a 15 W Phillips TUV germicidal lamp at an intensity of 37.5 Jm^{-2}.min^{-1}. For both treatments the doses were as indicated in the figures. After appropriated dilution, cell suspensions were plated onto non-selective YEPD agar for cell survival or 100 to 300 μL was directly plated onto YNB medium to detect reverse mutation, reciprocal recombination and gene conversion (de Morais Jr. *et al.*, 1998).

Ascospore formation

Yeast cells were synchronized for sporulation before incubation in SPA medium. At specific periods during incubation cells were collected and plated onto selective YNB medium to asses reciprocal recombination and gene conversion. Cells were also evaluated for ascospore formation using optical microscopy and stained with 0.5% (w/v) malachite green and 0.5% (w/v) safranin to differentiate mitotic (red) from meiotic (green) cells (Streiblová, 1988).

Results

Yeast transformation and recA gene expression

Haploid and diploid yeast cells were transformed with the pNF2 and pNF2*recA* plasmids and the transformants selected using geneticin-supplemented YEPD agar, low transformation efficiency being found for both plasmids with no significant difference in frequency between the two plasmids. Immunoblot analysis showed that the 38 KDa RecAp was efficiently produced by both haploid and diploid transformed yeast cells, with cellular growth rate appearing to be unaffected by expression of the *recA* gene (see de Morais Jr *et al.*, 1998).

### Mitotic DNA repair in recA-expressing cells

Exponential and stationary growth phase transformed cells were tested for DNA repair, exponential phase diploid

cells being found to be more resistant to killing by 8-MOP+UVA than stationary phase cells (Figure 1) with *recA* expression not affecting the survival of plasmid-bearing cells (Figure 1a). There was more 8-MOP+UVA induced reciprocal recombination in non-dividing stationary phase cells than in exponential phase cells, the presence of RecAp resulting in a 3 to 4-fold reduction of reciprocal recombination in the stationary phase cells to the level occurring in exponential phase cells, which were unaffected by the presence of RecAp (Figure 1b). Gene conversion frequency showed the same induction pattern irrespective of growth phase, while the presence of RecAp reduced gene conversion frequency for both stationary and exponential phase cells, stationary phase cells showing the greater reduction (Figure 1c). In cells expressing the *recA* gene there was a slight increase in 8-MOP induced reverse mutation in

exponential phase cells compared to stationary phase cells (Figure 1d).

We also investigated the killing effects of UVC on exponential and stationary phase diploid transformed cells, and found that expression of the *recA* gene did not change either cell survival or gene conversion rates (Figure 2 showing typical results for exponential phase cells) nor were reciprocal recombination or reverse mutation rates altered by the presence of RecAp.

Haploid yeast cells of strain MB401-3c harboring direct-repeat *his*4 alleles on chromosome III were used to evaluate intrachromosomal pop-out recombination, and we found that, similar to the case with diploid cells, the presence of the *recA* gene did not affected the survival of stationary phase cells treated with 8-MOP+UVA (Figure 3). In stationary phase haploid MB401-3c cells harboring the
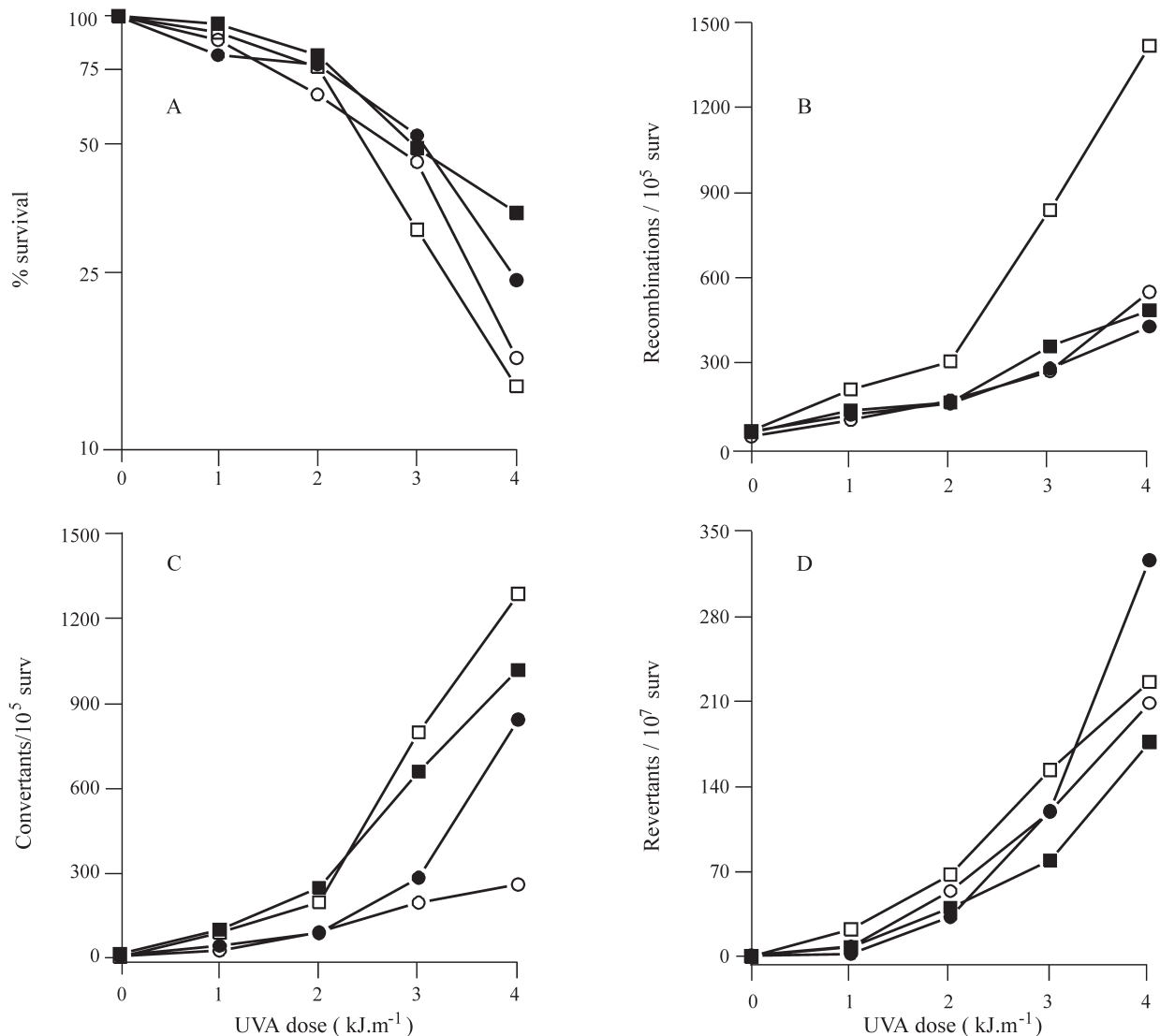
Figure 1 - Effect of *recA* gene expression on mitotic diploid yeast cells treated with 8-MOP+UVA. Figure A, percentage cell survival; Figure B, reciprocal recombination; Figure C, gene conversion; Figure D, reverse mutation. Yeast transformants harboring the control pNF2 plasmid (○) or pNF2*recA* plasmid (□) were treated in the exponential (closed symbols) or stationary (open symbols) phase.
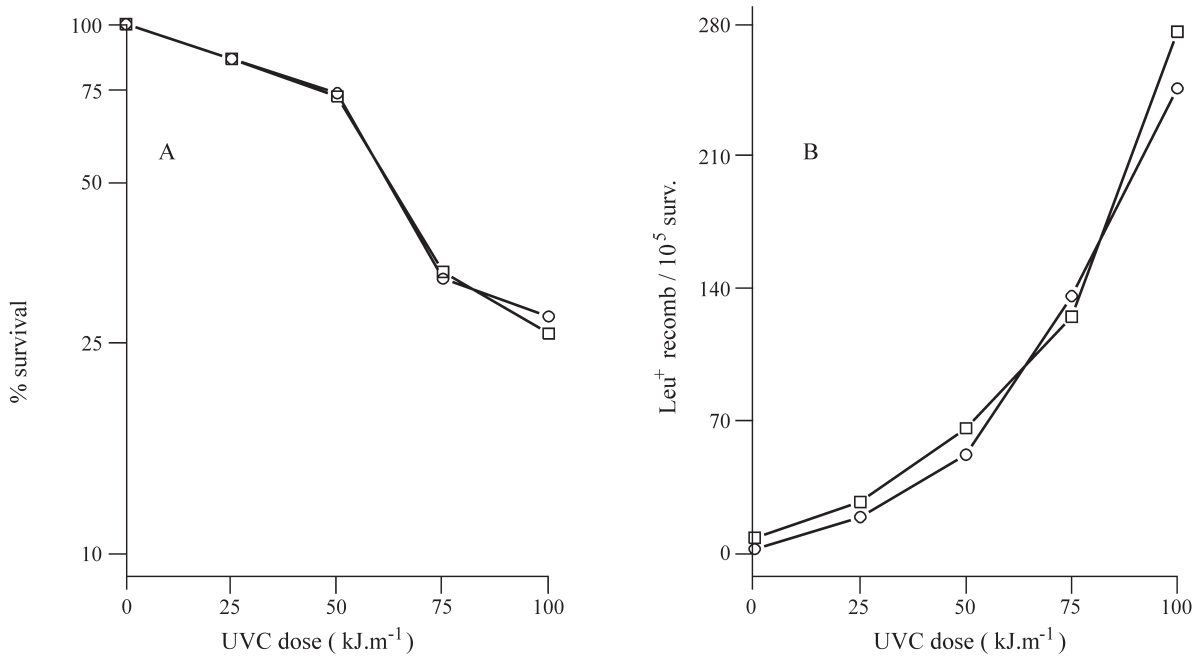
Figure 2 - Effect of UVC (254 nm) treatment on the percentage cell survival (Figure A) and gene conversion rate (Figure B) of stationary phase mitotic diploid yeast cells harboring the *recA* gene, symbols as in Figure 1.
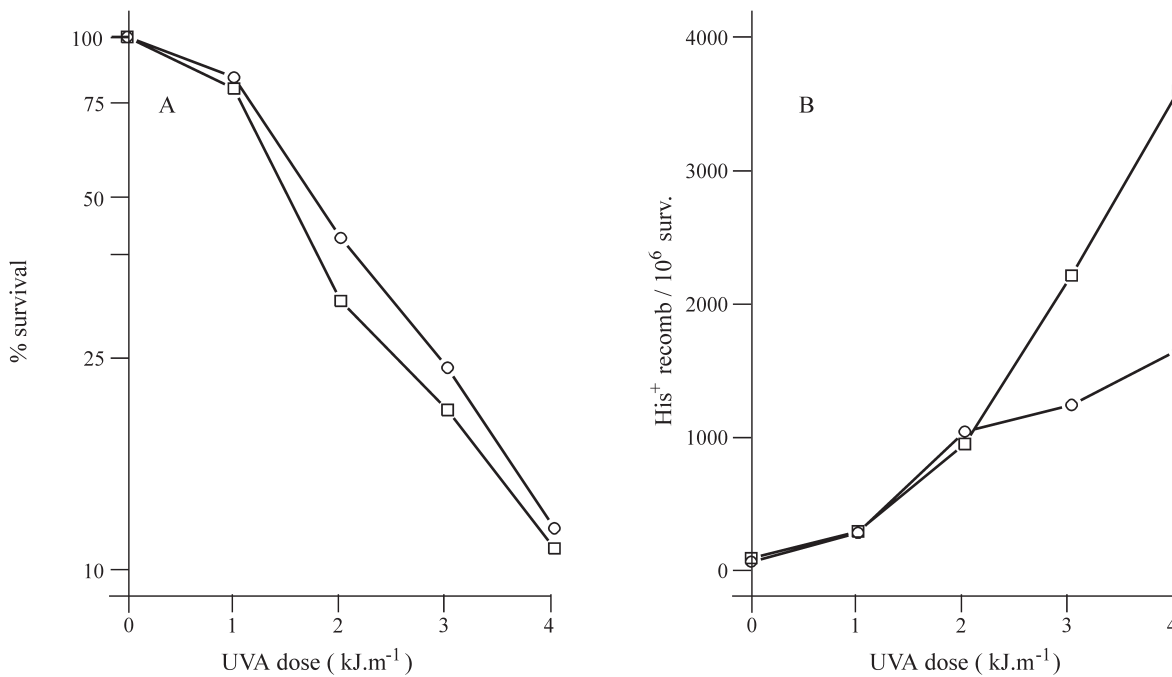
Figure 3 - Effect of 8-MOP+UVA (365 nm) treatment on the percentage cell survival (Figure A) and intrachromosomal recombination rate (Figure B) of stationary phase mitotic haploid yeast cells harboring the *recA* gene, symbols as in Figure 1.

recA gene, pop-out recombination induced by 8-MOP decreased after doses of 2 kJ.m^{-1} UVA. The cell survival and pop-out recombination rates of exponential phase cells were not affected by the presence of RecAp.

Analysis of the biological parameters tested at spontaneous level revealed that reciprocal recombination, gene conversion and pop-out recombination were significantly

decreased by a factor of 2 in *recA*-expressing cells, while reverse mutation showed a 2-fold increase (Table 1).

## Meiotic DNA repair and sporulation

Using the return-to-growth method we examined the effects of *recA* gene expression on recombination, gene conversion and reverse mutation of the sporulating cells of

Table 1 - Spontaneous frequences of reciprocal recombination (RC), gene conversion (GC), intrachromosomal pop-out recombination (IR) and reverse mutation (RM) in yeast mitotic cells expressing (pNF2recA) or not (pNF2) the recA gene.

Strain	RC Recombinants/10^5 cells	GC Recombinantes/10^5 cells	IR Recombinantes/10^6 cells	RM Mutants/10^7 cells
pNF2	608 ± 89	14.3 ± 4.9	115 ± 36	0.76 ± 0.66
PNF2recA	414 ± 71	6.2 ± 3.5	53 ± 19	1.74 ± 0.54

strain XS2316 after 8-MOP photoaddition (Figure 4). The cells were collected and treated with mutagens after one or three days of incubation in SPA medium, these periods being chosen because it was known that cell metabolism became directed towards sporulation within 24 h of incubation and that the highest rate of spore formation occurs after three days incubation (da Silva *et al.*, 1995). Yeast cells were more sensitive to the killing effect of 8-MOP+UVA during the metabolic change to meiosis than

were mitotic cells, albeit they did not differ as they were at the beginning of sporulation or already sporulated (Figure 4a). When cells were incubated for one or three days in SPA medium there was a significant increase in survival to 8-MOP+UVA by cells expressing the *recA* gene (Figure 4a). We found a slight reduction in 8-MOP+UVA induced reciprocal recombination and gene conversion in *recA* transformed cells incubated in SPA medium, this effect being more pronounced after three days incubation when
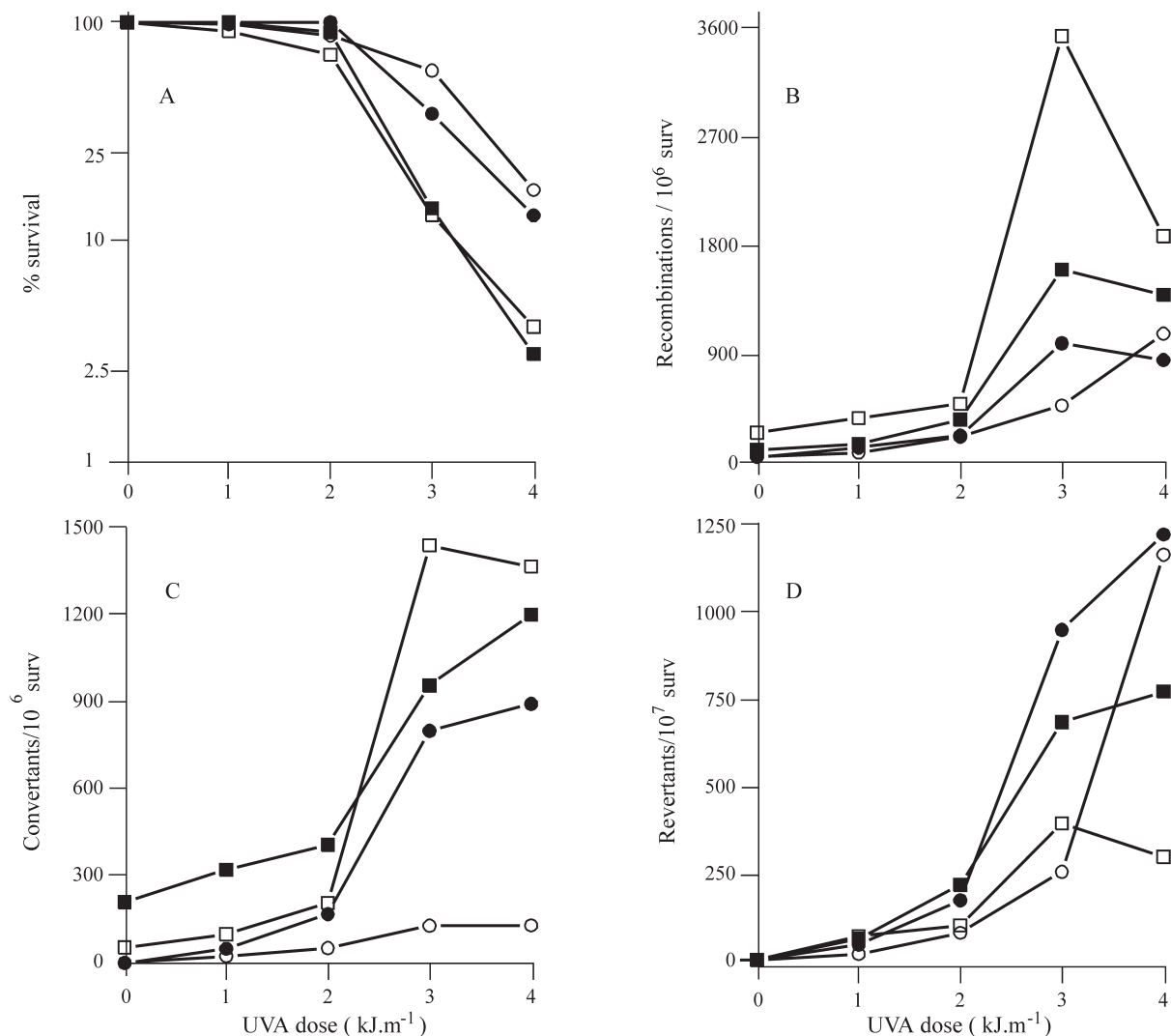
Figure 4 - Effect of *recA* gene expression on meiotic diploid yeast cells treated with 8-MOP+UVA. Figure A, percentage cell survival; Figure B, reciprocal recombination; Figure C, gene conversion; Figure D, reverse mutation. Yeast transformants harboring control pNF2 plasmid (○) or pNF2recA plasmid (□) were treated after one (closed symbols) or three (open symbols) days incubation in SPA medium.

there was almost complete inhibition of 8-MOP+UVA in- duced gene conversion (Figures 4b and 4c), inhibition again being more pronounced at UVA doses above 2 kJ.m^{-2}. Analysis of induced mutagenesis showed that one-day old cells were more error-prone than three-day old cells. Despite the fact that general recombination was re- duced the induced reverse mutation rate was increased by the presence of the *recA* gene in cells incubated for one and three days, mainly for those cells exposed to the highest UVA dose of 4 kJ.m^{-2} (Figure 4d). These results for cells undergoing meiosis are consistent with the spontaneous or induced response of mitotic cells (Table 1).

The above results demonstrated to us that recombina- tion repair in both mitotic and meiotic yeast cells was im- paired by the presence of RecAp and because of this we decided to investigate the influence of RecAp on the basic recombination mechanism responsible for chromosomal rearrangement and spore formation. We evaluated ascospore formation and genetic recombination during in- cubation in SPA medium and found that strain XS2316 lacking the *recA* gene (the control) started to produce asci after 24 h of incubation with 23% of cells exhibiting asci af- ter five days incubation, while the presence of RecAp pro- moted a five-fold reduction in ascospore formation with only 5% of cells containing asci after five days incubation (Figure 5a). Microscopy using differential staining showed that all mitotic vegetative cells expressing *recA* were red only (stained with safranin) while meiotic reproductive vector-bearing cells showed some red and some green structures (stained with malachite green and safranin). The *recA*-expressing cells also showed defective sporulation

with most of the asci having only two spores, possibly related to defective sporulation due to impaired meiotic re- combination. For *recA*-expressing yeast cells no meiotic in- duction of reciprocal recombination was observed (Figure 5b) and gene conversion was practically abolished (Figure 5c), showing that in these cells the defect in spore formation is due to the negative effect of RecAp on genetic recombi- nation.

Discussion

In mitotic cells, homologous genetic recombination is the mechanism responsible for repairing double-strand breaks (DSB) induced by DNA cross-linking agents (*e.g.* 8-MOP and ionizing and UV radiation) and mono-func- tional alkylating agents such as Methyl-nitro-nitroso- guanidine (MNNG) and Methyl-methano-sulphonate (MMS) (reviewed by Henriques and Brendel, 1990). Hays *et al.* (1995) demonstrated that DSB repair in yeasts is cata- lyzed by the recombinosome complex which is thought to be formed by the interaction between the Rad51p and Rad52p proteins (Sung and Robberson, 1995), although DSB repair may also involve the RecAp homologous Rad55p (Lovett, 1994) and Rad57p (Kanz and Mortimer, 1991) proteins. Another RecAp homologous protein, Dmc1p, is specifically required for meiotic strand transfer and formation of the synaptonemal complex (Bishop *et al.*, 1992), other proteins that may be involved in DSB repair being reviewed by Petes *et al.* (1991).

Our results demonstrate that RecAp is able to change the frequency of general spontaneous and induced mitotic recombination in yeast cells, with gene conversion appear-
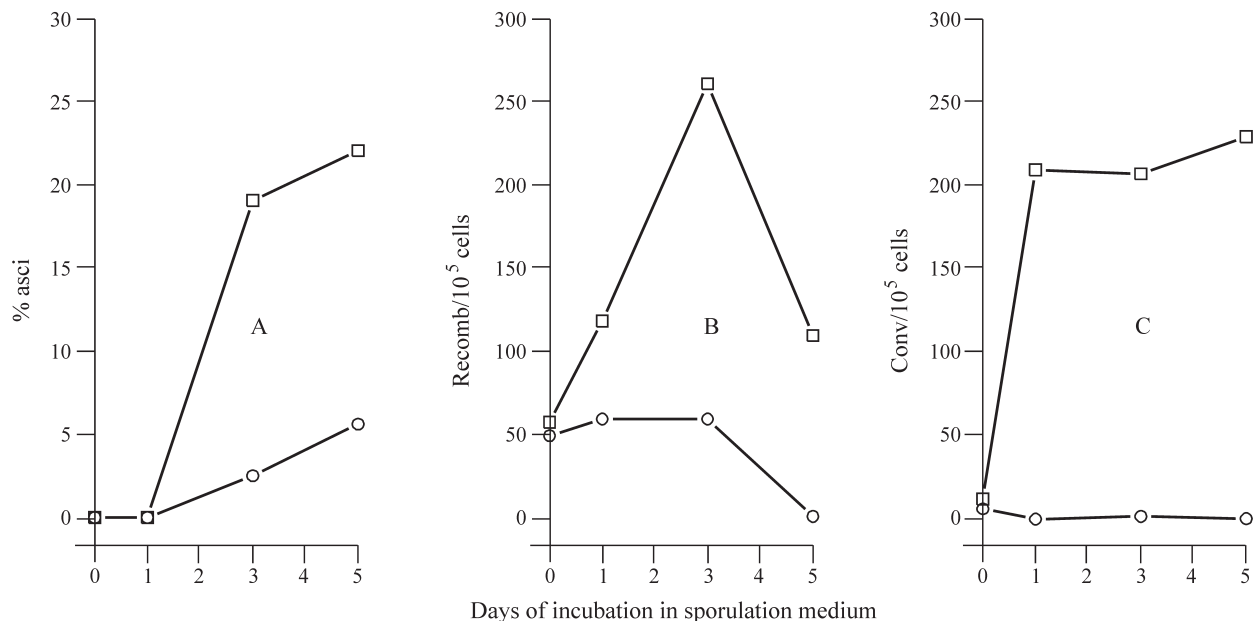
Figure 5 - Frequency of ascospore formation (Figure A), reciprocal recombination (Figure B) and gene conversion (Figure C) of diploid transformants harboring the control pNF2 plasmid (○) or pNF2*recA* plasmid (□).

ing to be most negatively affected by the presence of Re-cAp. We found that 8-MOP+UVA induced reciprocal recombination and gene conversion were decreased (Figure 1) in XS2316 cells expressing the RecAp protein, which supports the findings of Vlckova *et al.* (1997) who reported that although MNNG produced no effect on reciprocal re-combination, after treatment with 8-MOP+UVA induced reciprocal recombination and gene conversion were de-creased. On the other hand, our UVC-induced gene conver-sion results seem to be in contrast to some other reports because we found no effect on stationary phased XS2316 cells containing RecAp (Figure 2), while Vlckova *et al.* (1994, 1997) found that UVC-induced gene conversion in exponential phase diploid D7 and XS2361 cells was in-creased by the presence of RecAp. Since all the experi-ments discussed above were carried out using the same plasmid, differences in the results could be due to genetic background or growth phase. The results in Figure 3 show that intrachromosomal recombination of direct repeated *his*4 mutant alleles induced by 8-MOP+UVA was de-creased by the presence of RecAp. Gene conversion in-duced by DSB has been considered to be the more frequent event during recombination of duplicated elements (Ray *et al.*, 1988), which reinforces the hypothesis that RecAp can preferentially inhibit gene conversion in yeast cells.

Interestingly, the results of our meiotic genetic re-combination experiments showed that the frequency of both meiotic 8-MOP+UVA induced reciprocal recombina-tion and gene conversion was decreased by the presence of RecAp, this effect being more pronounced in cells cultured for three days where practically no induction was observed and induced gene conversion was once again completely abolished (Figure 4). However, the frequency of spontane-ous reciprocal recombination and gene conversion reached a maximum (Figure 5). Genetic recombination is generated by strand exchange between homologous strands of DNA that are physically connected by the synaptonemal com-plex, this mechanism ensuring proper segregation of chro-mosomes at meiosis I. Strand exchange is favored by the formation of double-strand breaks in both of the homolo-gous DNA strands which are repaired by a group of genes (mainly those involved in the *RAD*52 pathway) also in-volved in recombination repair, recombination being indis-pensable for sporulation. Figure 5 shows that reciprocal recombination and gene conversion start spontaneously on the first day of incubation in SPA (before the appearance of asci) which shows that the cells were committed to sporulation, although the presence of RecAp inhibited gen-eral recombination and blocked ascospore formation.

Our results point to the interaction between RecAp and yeast proteins during spontaneous or induced recombi-nation events, this hypothesis being supported by two lines of evidence. Firstly, *in vitro* experiments have demon-strated interaction between RecAp and the yeast sin-gle-strand binding protein y-RPA (Alani *et al.*, 1992) and,

secondly, it has been reported (de Morais Jr *et al.*, 1998) that the presence of RecAp increased the survival rate of *rad*52 mutant cells exposed to 8-MOP+UVA and to MMS but not those exposed to UVC. This response is dependent on the presence of functional Rad51p as illustrated by the fact that no effect was observed in the double mutant *rad*51*rad*52 (de Morais Jr *et al.*, 1998) which suggests that when Rad52p is absent RecAp can bind to its homologue Rad51p, RecAp producing a nucleoprotein complex con-taining DNA (mainly single-stranded) responsible for DNA pairing and strand exchange of homologous DNA molecules. In *Escherichia coli,* these events are dependent on DNA-binding and catalytic proteins which are part of the recombination complex (Kowalczykowski *et al.,* 1994), a similar nucleoprotein structure having been demonstrated for yeast Rad51p (Sung, 1994). The 55 N-terminal amino acid residues present in Rad51p show ATP-dependent ho-mologous pairing and strand exchange activities also im-portant for the interaction of Rad51p with Rad52p (Sung and Robberson, 1995), but since RecAp lacks these amino acids it could not complement defective *rad*51 mutants (de Morais Jr *et al.*, 1998) but may interact with intact Rad51p through their homologous C-terminal regions to form a heteropolymeric spiral filament. RecAp was shown to pro-mote Holliday junction and branch migration between in-tact single-strand DNA and homologous damaged double-strand DNA, leading to the formation of intermedi-ate heteroduplex DNA which is processed to produce a gene conversion event (Kowalczykowski *et al.*, 1994). However, DNA strand transfer reactions mediated by either *RecA*p or Rad51p have been demonstrated to have different polarities (Sung and Robberson, 1995) because of which the putative chimeric structure formed by rad51p-RecAp in the yeast nucleus might interfere in the biochemical activity of the spiral filament by arresting branch migration and in-hibiting correct DNA strand transfer. RecAp should act as a dominant negative allele in this process, experiments using deleted forms of the *RAD*52 gene having demonstrated that Rad52p with deleted recombination activity but normal Rad51p binding competed with wild-type Rad52p for the Rad51-Rad52 protein complex and acted as a dominant negative allele inhibiting double-strand break repair (Milne and Weaver, 1993).

The evidence suggests that *RecA*p actively partici-pates in yeast DNA repair by interacting with yeast protein and binding directly to yeast chromosomes, although its bi-ological activity seems to be closely dependent on other key elements such as yeast proteins, type of DNA damage, the DNA repair pathway involved and the growth phase of the cell.

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