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.

Keywords: DNA repair, genetic recombination, recA gene, sporulation, yeast.

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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 RAD51, RAD55, RAD57 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 nucleo-protein 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 pso4 mutant (de Morais Jr et al., 1994) and to partially complement the sensitivity of rad52 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 rad51 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 pso4-1 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.
Material and Methods

Strains and plasmids

The genotype of the diploid Saccharomyces cerevisiae strain XS2316 (de Morais Jr et al., 1994) is MATa/α, /ade6 leu1-12/leu1-12 Trp5-48/+ +/cyh2 +/met13 +/lys5-1 his1-1/ his1-1 and that of the haploid strain MB401-3c (Meira et al., 1992) MATa lys2 ura3-52 (his4-39/260: pBR322: his4-1176/864), strain MB401-3c having duplicate his4 mutations cloned at orientation II into the HIS4 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 pNF2recA 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-sporation (PS) medium (containing (% w/v) yeast extract, 0.250; potassium acetate, 0.1; dextrose 2) being used to synchronize diploid cells and sporation (SPA) medium (containing 2% (w/v) potassium acetate supplemented with amino acids and bases) to induce sporation. 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 10⁹ 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 10⁷ 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 10⁵ 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 10⁷ 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 they 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 Philips 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 sporation before incubation in SPA medium. At specific periods during incubation cells were collected and plated onto selective YNB medium to assess 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 pNF2recA 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 his4 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 affect the survival of stationary phase cells treated with 8-MOP+UVA (Figure 3). In stationary phase haploid MB401-3c cells harboring the

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**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 pNF2recA plasmid (□) were treated in the exponential (closed symbols) or stationary (open symbols) phase.
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 frequencies of reciprocal recombination (RC), gene conversion (GC), intrachromosomal pop-out recombination (IR) and reverse mutation (RM) in yeast mitotic cells expressing (pNF2 recA) or not (pNF2) the recA gene.

<table>
<thead>
<tr>
<th>Strain</th>
<th>RC Recombinants/10^6 cells</th>
<th>GC Recombinantes/10^6 cells</th>
<th>IR Recombinantes/10^6 cells</th>
<th>RM Mutants/10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>pNF2</td>
<td>608 ± 89</td>
<td>14.3 ± 4.9</td>
<td>115 ± 36</td>
<td>0.76 ± 0.66</td>
</tr>
<tr>
<td>PNF2 recA</td>
<td>414 ± 71</td>
<td>6.2 ± 3.5</td>
<td>53 ± 19</td>
<td>1.74 ± 0.54</td>
</tr>
</tbody>
</table>

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](image-url)

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 pNF2 recA 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 induced 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 reduced the induced reverse mutation rate was increased by the presence of the \(\text{recA}\) 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 recombination repair in both mitotic and meiotic yeast cells was impaired 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 incubation in SPA medium and found that strain XS2316 lacking the \(\text{recA}\) gene (the control) started to produce ascis after 24 h of incubation with 23% of cells exhibiting ascis after five days incubation, while the presence of RecAp promoted a five-fold reduction in ascospore formation with only 5% of cells containing ascis after five days incubation (Figure 5a). Microscopy using differential staining showed that all mitotic vegetative cells expressing \(\text{recA}\) 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 \(\text{recA}\)-expressing cells also showed defective sporulation with most of the ascis having only two spores, possibly related to defective sporulation due to impaired meiotic recombination. For \(\text{recA}\)-expressing yeast cells no meiotic induction 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 recombination.

**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-functional alkylating agents such as Methyl-nitro-nitrosoguanidine (MNNG) and Methyl-methano-sulphonate (MMS) (reviewed by Henriques and Brendel, 1990). Hays et al. (1995) demonstrated that DSB repair in yeasts is catalyzed 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-

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**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\(\text{recA}\) plasmid (□).
RecAp impairs yeast recombination

The presence of RecAp significantly decreases the frequency of reciprocal recombination and gene conversion in yeast cells. In experiments involving 8-MOP+UVA treatment, both reciprocal recombination and gene conversion were reduced, as shown in Figure 2. This observation supports the findings of Vlckova et al. (1994, 1997), who reported that RecAp inhibits these processes in diploid yeast cells. Interestingly, the presence of RecAp also decreases UVC-induced gene conversion, as reported by (De Morais Jr et al., 1994, 1997). This suggests that RecAp preferentially inhibits gene conversion in yeast cells.

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References

Alani et al. (1992)

De Morais Jr et al. (1994, 1997)

Vlckova et al. (1994, 1997)

Ray et al. (1998)

Sung and Robberson (1995)

Milne and Weaver (1993)

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References


