The Escherichia coli RecA protein complements recombination defective phenotype of the Saccharomyces cerevisiae rad52 mutant cells

Andrey Dudáš1, Eva Marková1, Danuša Vlasáková1, Ada Kolman2, Zdena Bartošová3, Jela Brozmanová1* and Miroslav Chovanec1*

1Department of Molecular Genetics, Cancer Research Institute, Slovak Academy of Sciences, Vlárská 7, 833 91 Bratislava 37, Slovak Republic
2Department of Molecular Biology and Functional Genomics, Stockholm University, SE-106 91 Stockholm, Sweden
3Department of Cancer Genetics, Cancer Research Institute, Slovak Academy of Sciences, Vlárská 7, 833 91 Bratislava 37, Slovak Republic

*Correspondence to: Miroslav Chovanec or Jela Brozmanová, Department of Molecular Genetics, Cancer Research Institute, Slovak Academy of Sciences, Vlárská 7, 833 91 Bratislava 37, Slovak Republic. E-mail: miroslav.chovanec@savba.sk or jela.brozmanova@savba.sk

Received: 10 October 2002
Accepted: 10 December 2002

Abstract

The Saccharomyces cerevisiae rad52 mutants are sensitive to many DNA damaging agents, mainly to those that induce DNA double-strand breaks (DSBs). In the yeast, DSBs are repaired primarily by homologous recombination (HR). Since almost all HR events are significantly reduced in the rad52 mutant cells, the Rad52 protein is believed to be a key component of HR in S. cerevisiae. Similarly to the S. cerevisiae Rad52 protein, RecA is the main HR protein in Escherichia coli. To address the question of whether the E. coli RecA protein can rescue HR defective phenotype of the rad52 mutants of S. cerevisiae, the recA gene was introduced into the wild-type and rad52 mutant cells. Cell survival and DSBs induction and repair were studied in the RecA-expressing wild-type and rad52 mutant cells after exposure to ionizing radiation (IR) and methyl methanesulphonate (MMS). Here, we show that expression of the E. coli RecA protein partially complemented sensitivity and fully complemented DSB repair defect of the rad52 mutant cells after exposure to IR and MMS. We suggest that in the absence of Rad52, when all endogenous HR mechanisms are knocked out in S. cerevisiae, the heterologous E. coli RecA protein itself presumably takes over the broken DNA. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: RecA protein; rad52 mutant cells; homologous recombination; heterologous expression; DNA double-strand breaks; pulsed-field gel electrophoresis

Introduction

DSBs are perhaps the most deleterious DNA lesions as they disrupt both DNA strands causing problems for all DNA transactions. They can be induced by exogenous as well as endogenous agents. Among exogenous agents, ionizing radiation (IR) is the most important one. In addition to IR, DSBs are generated by a wide range of other exogenous agents, including a number of chemicals such as bleomycin and etoposide (Nelson and Kastan, 1994; Olive and Johnston, 1997; Critchlow and Jackson, 1998) or methyl methanesulphonate (MMS) (Chlebowicz and Jachymczyk, 1979). DSBs are also introduced endogenously by free radicals — the products of oxidative metabolism (Wallace, 1998). The intermediates of some cellular processes also represent DSBs (Friedberg et al., 1995; Pastink and Lohman, 1999). The process of DNA replication itself is a predominant source of DSBs in dividing cells (Haber, 1999).

Cells recognize DSBs and act upon them rapidly and efficiently, because deleterious consequences can result if these are left unrepaired (Jackson, 1999). Two main repair mechanisms, HR and non-homologous end-joining (NHEJ), have evolved throughout evolution to deal with DSBs (Jeggo, 1998; Kanaar et al., 1998; Haber, 1999; Karran,
DSB repair through HR involves DNA exchange processes, in which the damaged chromosome retrieves genetic information from an undamaged homologue. Contrary to HR, NHEJ lacks a requirement for homologous DNA sequences and rejoins DSBs via direct ligation of DNA ends (Jeggo, 1998).

In E. coli, DSB repair occurs exclusively by HR and the RecA protein plays a pivotal role in the process because it participates in virtually all HR events (Kowalczykowski et al., 1994; Roca and Cox, 1997). In vitro, RecA performs two reactions that are prerequisite to HR: an ATP-stimulated DNA strand annealing between complementary single-strands of DNA and an ATP-dependent DNA strand invasion and exchange between single-stranded DNA (ssDNA) and a homologous sequence within double-stranded DNA (dsDNA) (Eggleston and Kowalczykowski, 1991; Kowalczykowski, 1991, 2000; Kowalczykowski et al., 1994; Kowalczykowski and Krupp, 1995; Bianco et al., 1998).

Because of importance of RecA for HR in E. coli, the identification of the proteins homologous to RecA in other organisms has been of a great interest. It has now been well established that the RecA-like proteins comprise a family of highly conserved proteins (Ogawa et al., 1993; Baumann and West, 1998; Thacker, 1999; Shinohara and Ogawa, 1999). In S. cerevisiae, the RAD52 gene product is considered to be a structural and functional homologue of RecA (Shinohara and Ogawa, 1995, 1999; Sung et al., 2000; Game, 2000), as it catalyses ATP-dependent strand exchange between ssDNA and a homologous linear DNA duplex (Sung, 1994; Sung and Robberson, 1995; Sung and Stratton, 1996; Namsaraev and Berg, 1997, 1998, 2000). Expectedly, due to impaired DSB repair (Contopoulou et al., 1987), the rad51 mutant cells are sensitive to the agents that induce DSBs (Moore, 1978; Petin, 1979; Ogawa et al., 1993; Abe et al., 1994; Grossmann et al., 2001), although another mutant from the RAD52 epistasis group, rad52, displays even higher sensitivity to certain DSB-inducing agents than rad51 (Morais et al., 1998; our unpublished results).

The yeast Rad52 protein shows DNA binding activity (Mortensen et al., 1996; Shinohara et al., 1998) and interacts with both Rad51 and yeast replication protein A (Hays et al., 1995, 1998; Song and Sung, 2000; Krejci et al., 2000). As shown by recent biochemical studies, it possesses two activities important to HR: the stimulation of Rad51-mediated DNA strand exchange (Sung, 1997; Benson et al., 1998; Shinohara and Ogawa, 1998; New et al., 1998) and the annealing of complementary ssDNA (Mortensen et al., 1996). In vivo, the RAD52 gene product appears to be necessary for HR in yeast, as all HR events are significantly decreased in the rad52 mutant cells (Dornfeld and Livingston, 1992; Schiestl et al., 1994; Rattray and Symington, 1995; Ivanov et al., 1996; Porter et al., 1996; You, 2000). In this respect, biological function of Rad52 resembles that of RecA.

Here, we have examined whether the E. coli RecA protein could complement the HR-defective phenotype of the rad52 mutant of S. cerevisiae. The present results show that recA expression leads to partial complementation of the sensitivity and full complementation of the DSB repair defect of rad52 mutant cells after exposure to IR and MMS. This suggests that in the absence of Rad52, when no HR event can occur in the yeast, heterologously expressed E. coli RecA protein can deal with induced DSBs.

Materials and methods

Materials

MMS, proteinase K, lyticase, N-laurylsarcosine and phenylmethylsulphonyl fluoride (PMSF) were obtained from Sigma (USA). Low melting point agarose (InCert®) and agarose for pulsed-field gel electrophoresis (PFGE) (FastLane® agarose) were purchased from FMC BioProducts (USA). Pefabloc®SC and β-mercaptoethanol (β-ME) were from Serva (Germany).

Strains and plasmids

The following strains were used in this study: F808 (MATa, ade1-100, his4-519, leu3,112, ura3-52) and F1 (rad52::LEU2 derivative of F808). The E. coli recA protein coding region was cloned into the pYEDP1/8-2 to generate the recombinant plasmid pYEDP1/8-2recA. pYEDP1/8-2 was described in detail elsewhere (Pompon, 1988). pYEDP1/8-2 contains a GAL10–CYC1 hybrid promoter composed of the upstream activating sequence of yeast GAL10 gene and the iso-1-cytochrome c
transcription initiation sequences. GAL10–CYC1 promoter is active in galactose-containing medium but repressed in glucose-containing medium.

**Media and growth conditions**

Media and growth conditions were the same as previously described (Brozmanová et al., 2001). Briefly, yeast cells were non-selectively grown in YPD medium (1% yeast extract, 2% bacto-peptone, 2% glucose, 2% agar for plates). For GAL10–CYC1-mediated recA induction, the transformed cells were cultivated in liquid YPGal medium (1% yeast extract, 2% bacto-peptone, 2% galactose). Synthetic complete (SC) medium (0.67% yeast nitrogen base, 1% ammonium sulphate and essential nutrients) lacking uracil and containing 2% glucose was used for selective growth of transformed cells. Prior to cultivation in YPGal medium, the transformed cells were first cultivated in i-SC medium containing 2% potassium acetate and 2% glycerol instead of 2% glucose. LB medium (0.5% yeast extract, 1% bacto-tryptone, 1% NaCl, 2% agar for plates) was used for growth of E. coli cells. Ampicillin was added at 100 µg/ml to LB medium for growth of bacterial cells harbouring plasmids.

**Cell extract preparation and immunological determination of the RecA protein**

Yeast cell extracts were prepared in lysis buffer (50 mM Tris–HCl, 1 mM EDTA, 3 mM dithiotreitol, pH 8.3) by sonication (Brozmanová et al., 2001). The Bradford (1976) method was used to determine the protein concentration in cell extracts using bovine serum albumin as a standard. Polyacrylamide gel electrophoresis and electroblotting were carried out according to Brozmanová et al. (2001). The anti-RecA polyclonal antibodies were used at a dilution of 1:40 000 in TBS buffer (20 mM Tris–HCl, 137 mM NaCl, 0.1% Tween-20, 5% low fat milk, pH 7.6) and Western blot was performed as previously described (Brozmanová et al., 2001).

**Cell survival**

MMS treatment was carried out as described earlier (Brozmanová et al., 2001). In case of survival after IR, the cells were cultivated as those for MMS treatment. When requested density was reached, the cells were resuspended in cold 0.1 M phosphate buffer (pH 7.0) and irradiated with 137Cs source (a model Gammacell 1000), providing a dose rate of 10 Gy/min. After irradiation, appropriate dilutions of the cells were prepared in physiological saline (0.9% NaCl) and diluted cell suspensions were plated onto YPD plates to determine cell survival.

**Pulsed-field gel electrophoresis**

PFGE experiments and computation of DSBs were performed according to our previous publication (Brozmanová et al., 2001). Briefly, the cells were after treatment washed twice with, and resuspended in, 50 mM EDTA (pH 7.5) at a density of 3.125 × 10^8 cells/ml. Thereafter, 5 × 10^7 cells were mixed with 40 µl SCE buffer (2 M sorbitol, 1 M citrate, 0.5 mM EDTA, pH 7.5) containing 10% β-ME and 5 µg lyticase (380 units/mg). 200 µl 1% low melting-point agarose in 0.125 M EDTA (pH 7.5) were added afterwards. The resulting cell suspension was then equilibrated at 42°C and then immediately transferred into the plug moulds and cooled until solidified. After removing them from the moulds, the plugs were incubated in 0.5 M EDTA, 0.4% β-ME and 0.01 M Tris–HCl (pH 8.0) for 2 h and lysed at 37°C overnight in 0.5 M EDTA, 0.01 M Tris–HCl, 1% N-laurylsarcosine and 0.5 mg/ml proteinase K. Next day, the plugs were incubated at 37°C for 2 h in a buffer consisting of 1 mM PMSF, 1 mM EDTA and 10 mM Tris–HCl (pH 7.0) and then rinsed twice with 50 mM EDTA (pH 7.5). The plugs were stored at 4°C in 1 mM PMSF, 10 mM EDTA and 10 mM Tris–HCl (pH 7.5) until used.

Before loading them into the wells, the plugs were equilibrated twice in a buffer composed of 10 mM Tris–HCl and 1 mM EDTA (pH 8.0). Loaded wells were covered with a 1% agarose gel. Electrophoresis was performed with a 1% agarose gel and TAE buffer (20 mM Tris acetate, 1 mM EDTA, pH 8.0) using a transverse alternating field electrophoresis system (Beckman Instruments) at 14°C as follows: (a) constant current of 170 V/mA for 30 min with 4 s pulse time; (b) constant current of 150 V/mA for 18 h with 60 s pulse time or for 20 h with 90 s pulse time. On completion, the gels were stained with ethidium bromide, destained, visualized and photographed.
Blotting of DNA from the gel onto the membrane (Hybond-N, Amersham) was carried out using a PosiBlot apparatus (Stratagene) at 75 mmHg for 1 h. 25 ng 1764 kb BamHI fragment of the HIS3 gene were labelled with [32P]-dCTP using a random primed DNA labelling kit (Boehringer). The labelled gene was used as a probe. Hybridization was carried out at 45 °C overnight. After hybridization, the membranes were stringently washed, wrapped in saran and exposed to X-ray film at −70 °C.

Computation of DSBs was based upon the formula previously published by Frankenberg-Schwager et al. (1995):

$$N_{DSBs} = -\ln I/I_0$$

where $N_{DSBs}$ is the number of DSBs, $I_0$ is the density of band in untreated sample and $I$ is the density of the corresponding band in treated sample. As the HIS3 gene was used as a probe, $N_{DSBs}$ calculated from the formula represents the number of DSBs per chromosome XV, on which the HIS3 gene is located (Mortimer and Schild, 1985).

Density of bands was measured with a LKB 222/020 Ultrascan XL apparatus.

## Results

### Expression of the *E. coli* RecA protein

To confirm the expression of the *E. coli* RecA protein in yeast, Western blot analysis was performed using the cell extracts from the wild-type and rad52 mutant cells transformed with pYEDP1/8-2recA. As shown in Figure 1, both the wild-type and rad52 mutant cells grown in the presence of galactose expressed the RecA protein. On the other hand, there was no detectable level of the RecA protein in extracts prepared from the cells grown in glucose-containing medium.

### Cell survival after treatment with DNA damaging agents

To find out whether the recA expression can complement the recombination-defective phenotype of the rad52 mutant cells, the cellular response of the wild-type and rad52 mutant cells transformed with pYEDP1/8–2recA was examined after exposure to IR or MMS. As seen in Figure 2, the *E. coli* RecA protein indeed increased the resistance of the rad52 mutant cells to these agents, while it had no effect on the cellular response of the wild-type cells. Moreover, keeping the rad52 mutant cells for 2 h in non-growing conditions after IR importantly highlighted the effect of RecA, which was not the case for the wild-type cells (Figure 2B). Hence, the *E. coli* recA gene product can partially protect rad52 mutant cells from the cytotoxic effects of IR and MMS.

### DSBs repair after treatment with DNA damaging agents

It has already been shown that IR and MMS induce DSBs in yeast (Chlebowicz and Jachymczyk, 1979; Lobrich et al., 1993) and that the rad52 mutant cells are sensitive to these agents, due to impaired DSB repair via HR (Ho, 1975; Resnick and Martin, 1976). It has also been reported that the RecA protein participates in the majority of HR events in *E. coli* (Kowalczykowski et al., 1994; Roca and Cox, 1997). Therefore, we assumed that an increased survival of the rad52 mutant cells expressing the RecA protein (Figure 2) might be caused by more efficient DSB repair. Indeed, the recA gene product fully complemented the DSB repair defect of the rad52 mutant cells: the levels of the DSBs per chromosome XV in the wild-type and rad52 mutant cells, both expressing the RecA protein, were essentially the same (Figure 3B, C). Figure 4 shows a representative PFGE experiment after MMS treatment in recA-expressing rad52 mutant cells. Therefore, the RecA protein might possess some biochemical activity that is shared with Rad52 and that is important to DSB repair.
RecA complements recombination-defective phenotype of yeast rad52 mutants

Figure 2. Survival of the wild-type (circles) and rad52 mutant (triangles) cells transformed with pYEDP1/8–2recA after exposure to IR (A, 0 h post-incubation in non-growing conditions; B, 2 h post-incubation in non-growing conditions; see Section 2) and MMS (C). Open and closed symbols represent cells grown in the presence of glucose and galactose, respectively. Each data point represents the average of at least three independent experiments. Error bars represent standard deviation.

Figure 3. The number of DSBs per chromosome XV for the wild-type (circles) and rad52 mutant (triangles) cells transformed with pYEDP1/8–2recA after exposure to IR (A, 0 h post-incubation in non-growing conditions; B, 2 h post-incubation in non-growing conditions; see Section 2) and MMS (C). Open and closed symbols represent cells grown in the presence of glucose and galactose, respectively. Each data point represents the average of at least three independent experiments. Error bars represent standard deviation.

Discussion

In this paper, we have examined whether the E. coli RecA protein could complement the HR-defective phenotype of the rad52 mutant of S. cerevisiae. Therefore, cell survival and DSB induction and repair after treatment with agents inducing DSBs have been studied in the wild-type and rad52 mutant cells expressing the RecA protein. Here, we show that RecA partially complements the sensitivity and fully complements the DSB repair defect of the rad52 mutant cells after exposure to IR and MMS (Figures 2 and 3). The fact that the RecA protein is able to fully substitute Rad52 in...
DSB repair and that, despite this, rad52 mutant cells expressing RecA still remain sensitive to IR and MMS suggests that biological function of Rad52 might include some activity(ies) other than that important to DSB repair. Alternatively, the PFGE method is less sensitive than the survival assay and is not able to detect one unrepaired DSB per genome, which represents a lethal event in the cell survival assay.

How could the E. coli RecA protein operate in DSB repair in rad52 mutant cells? One of the explanations would have been that, in the rad52 background, RecA might have been able to replace Rad52 in the protein–protein interactions formed during DSB repair in yeast (Hays et al., 1995; Krejčí et al., 2001). Among all the interactions of Rad52 identified so far, the most important one seems to be interaction with Rad51 (Hays et al., 1995, 1998; Krejčí et al., 2001), although some others have been revealed as well (Hays et al., 1998; Davis and Symington, 2001). It has been shown that Rad51 catalyses the invasion of tailed duplex DNA into homologous covalently closed DNA and that this function of Rad51 is stimulated by Rad52 (McIlwraith et al., 2000). More recent data suggests that the stimulatory role of Rad52 resides in its ability to stabilize the Rad51 presynaptic filament (New and Kowalczykowski, 2002). Thus, the physical interaction of Rad51 with Rad52 is presumably necessary for Rad51-mediated DNA strand invasion and exchange, the two activities that are prerequisite to DSB repair by HR. To verify the possibility of replacing Rad52 with RecA in the Rad51–Rad52 complex, two-hybrid experiments have been performed; however, these showed no Rad51–RecA interaction. Nevertheless, it is worth mentioning that we have found RecA–RecA self-interaction (data not shown). This might suggest that the E. coli RecA protein expressed in yeast is able to form a multimeric complex and hence the nucleoprotein filament, the generation of which represents an early step of HR (Baumann and West, 1998). Thus, we prefer the explanation that, in the absence of Rad52, when all HR events are knocked out, RecA protein on its own presumably takes over broken DNA.

It has previously been shown that the RecA protein expressed in plants stimulated HR and repair of mitomycin C-induced DNA damage (Reiss et al., 1996). It also increased the frequency of sister chromatid exchange and the fidelity of DBS repair (Reiss et al., 2000). The stimulatory effect of bacterial RecA protein on HR in somatic mammalian cells has also been observed (Shcherbakova et al., 2000). All these results suggest that bacterial RecA may function in various HR events in higher eukaryotes. Our results showing the ability of the E. coli RecA protein to rescue impairment of the rad52 mutant cells in DSB repair is in agreement with previous observations.

The E. coli RecA protein possesses both ATP-stimulated DNA strand-annealing and ATP-dependent DNA strand-exchange activities (Eggleston and Kowalczykowski, 1991; Kowalczykowski, 1991, 2000; Kowalczykowski et al., 1994; Kowalczykowski and Krupp, 1995; Bianco et al., 1998). In budding yeast, DNA strand-annealing activity resides in the Rad52 protein, while Rad51 carries out DNA strand exchange. It seems that the two activities carried by one protein in bacteria have been scattered through evolution onto the two proteins in eukaryotic organisms.

Acknowledgements
We thank Drs D. Keszenman and I. Fridrichová for the generous gift of the strains and preparing the anti-RecA polyclonal antibodies, respectively. Dr M. Piršel

Figure 4. The representative PFGE analysis in the rad52 mutant cells transformed with pYEDP1/8–2recA after MMS treatment. (A) and (B) represent the cells growing in the presence of glucose and galactose, respectively.
is also acknowledged for critical reading and helpful discussion. This work was supported by Grants 2/7073/20 and 2/3091/23 from the VEGA Grant Agency of the Slovak Republic.

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