Expression of the RAD1 and RAD3 genes of *Saccharomyces cerevisiae* is not affected by DNA damage or during the cell division cycle

Madan L. Nagpal, David R. Higgins, and Satya Prakash
Department of Biology, University of Rochester, Rochester, NY 14627, USA

**Summary.** The RAD1 and RAD3 genes of *Saccharomyces cerevisiae* are required for excision repair of UV damaged DNA. In addition, the RAD3 gene is essential since rad3 deletions are recessive lethals. We have examined the induction of the RAD1 and RAD3 genes by DNA damage and during the cell division cycle. We have made fusions of the RAD1 and RAD3 genes with the *Escherichia coli* lacZ gene encoding β-galactosidase, β-galactosidase activity was measured in a Rad⁺ yeast strain containing the RAD1-lacZ or the RAD3-lacZ fusion, either in a multicopy replicating plasmid or as a single copy integrant resulting from transformation with an integrating plasmid which transforms yeast by homologous recombination in the yeast genome. No induction of β-galactosidase activity occurred after ultraviolet light (UV) or 4-nitroquinoline-1-oxide (NQO) treatment. Haploid cells of mating type a were synchronized by treatment with α factor and β-galactosidase activity was measured in a Rad⁺ yeast strain containing the *RAD1-lacZ* fusion, either in a multicopy replicating plasmid or as a single copy integrant resulting from transformation with an integrating plasmid which transforms yeast by homologous recombination in the yeast genome. In the yeast *Saccharomyces cerevisiae*, a large number of DNA repair genes have been identified (Haynes and Kunz 1981) and several of these have been cloned recently. The RAD1 and RAD3 genes of *S. cerevisiae* are required for excision repair; mutants in these genes are highly defective in incision of DNA containing pyrimidine dimers (Wilson and Prakash 1981; Reynolds and Friedberg 1981) or interstrand DNA crosslinks (Miller et al. 1982; Jacobczyk et al. 1981; Magana-Schwenke et al. 1982). Various studies have established the utility of gene fusions with the *E. coli* lacZ gene for the study of gene regulation in *S. cerevisiae* (Rose and Botstein 1983; Guarente 1983). We have made fusions of the RAD1 and RAD3 genes with the lacZ gene and examined the regulation of the RAD1 and RAD3 genes of yeast following treatments with DNA damaging agents and during the cell division cycle.

**Materials and methods**

**Strains and plasmids.** *S. cerevisiae* strain 7799-4B, *MATa his4-17 ura3-52 RAD⁺", was used throughout this study. RAD1-lacZ and RAD3-lacZ fusions were constructed in the autonomously replicating, 2μ containing plasmid YEp24 (Botstein et al. 1979) and the integrating plasmid YIp5 (Scherer and Davis 1979). The 6.8-kb BamHI fragment of pMC931, containing the lacZ' and Y genes of *E. coli* (Casadaban et al. 1980), was inserted into the BglII site of plasmid pDH2, which contains the RAD1 gene of yeast inserted into YEp24 (Higgins et al. 1983b), thus creating a RAD1-lacZ fusion in the autonomously replicating plasmid, which was designated pDH25 (Fig. 1). The 12-kb BamHI/BglII DNA segment containing the RAD1-lacZ fusion was inserted into the BamHI site of the integrating plasmid YIp5 to generate the plasmid pDH29.

In the yeast *Saccharomyces cerevisiae*, a large number of DNA repair genes have been identified (Haynes and Kunz 1981) and several of these have been cloned recently. The RAD1 and RAD3 genes of *S. cerevisiae* are required for excision repair; mutants in these genes are highly defective in incision of DNA containing pyrimidine dimers (Wilson and Prakash 1981; Reynolds and Friedberg 1981) or interstrand DNA crosslinks (Miller et al. 1982; Jacobczyk et al. 1981; Magana-Schwenke et al. 1982). Various studies have established the utility of gene fusions with the *E. coli* lacZ gene for the study of gene regulation in *S. cerevisiae* (Rose and Botstein 1983; Guarente 1983). We have made fusions of the RAD1 and RAD3 genes with the lacZ gene and examined the regulation of the RAD1 and RAD3 genes of yeast following treatments with DNA damaging agents and during the cell division cycle.

A pair of autonomously replicating and integrating plasmids was constructed to contain a RAD3-lacZ fusion and were designated pSP24 and pSP26, respectively. Plasmid pSP24 (Fig. 2) was constructed by inserting the 6.8-kb BamHI fragment of pMC931 into the BamHI site of pSP6, which contains the RAD3 gene of yeast inserted into YEp24 (Higgins et al. 1983b). The 9.2-kb SalI/BglII fragment of pSP24 was inserted into SalI/BamHI cut YIp5 to generate the plasmid pSP26.

**Media.** The strain 7799-4B containing the various plasmids described above was propagated in complete synthetic medium lacking uracil to maintain selection for the plasmid.
Synchronization of cells with alpha factor. Cells were grown to approximately $1 \times 10^7$ per ml at 30°C. alpha factor (Sigma) was added to 400 ng/ml. After 6 hours of incubation, alpha factor was removed by centrifugation and cells suspended at the same density in fresh medium lacking alpha factor, to initiate a synchronous cell division. Percent budded cells and cell number were monitored throughout as described previously (Peterson et al. 1985).

beta-Galactosidase assays. Cells were grown to mid exponential phase and one ml of cells was removed for determining levels of beta-galactosidase in untreated cells. The rest of the cells were UV irradiated in water with constant stirring at a dose rate of 1 J m$^{-2}$ s$^{-1}$. Cells were harvested by filtration, suspended in fresh medium, and incubated in the dark at 30°C. Culture aliquots were withdrawn at various times after UV irradiation and assayed for beta-galactosidase activity and cell density as described by Ruby and Szostak (1984). For strains containing an integrated RAD-lacZ fusion, cells were concentrated 5 to 10-fold for determination of beta-galactosidase activity. For NQO treatment, 0.05 ug NQO per ml was added to a mid exponential phase culture followed by incubation at 30°C. Samples were removed at various times and assayed for beta-galactosidase activity and cell density. beta-galactosidase activity was determined in untreated cultures as well. Units of beta-galactosidase specific activity were calculated as: OD$_{420}$ x 1000/t (h) x v (ml) x OD$_{600}$, where v is the volume of sample assayed and OD$_{600}$ is the cell density of the culture determined spectrophotometrically.

Results

beta-Galactosidase activity levels following UV irradiation or NQO treatment of yeast strains containing the RAD1-lacZ or RAD3-lacZ fusion. beta-galactosidase activity levels following DNA damaging treatments were determined in yeast cells containing either the RAD1-lacZ or the RAD3-lacZ fusion in a multicopy replicating plasmid or when integrated in the genome at the RAD1 or RAD3 sites, respectively, as determined by genetic analyses. The RAD1-lacZ fusion has the 6.8-kb BamHI lacZ cassette of plasmid pMC931 (Casadaban et al. 1980) inserted into the BgII site of the RAD1 gene (Fig. 1) and contains several kilobases of DNA upstream from the open reading frame (ORF) plus 782 amino acid residues of the RAD1 protein, as determined from the nucleotide sequence (unpublished results), and based on the assumption that the RAD1 protein is encoded beginning with the first ATG in the ORF. The lacZ cassette which is fused in frame with the RAD1 ORF is missing the lacZ 5' regulatory sequences for transcription and translation and the first 7 amino acids of beta-galactosidase (Casadaban et al. 1980). The RAD3-lacZ fusion has the 6.8-kb BamHI cassette of pMC931 inserted into the BamHI site of the RAD3 gene (Fig. 2) and contains the 5' regulatory sequences plus 148 amino acid residues of the RAD3 gene as determined from the nucleotide sequence (unpublished results).