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Expression of the *denV* Gene of Coliphage T4 in UV-Sensitive *rad* Mutants of *Saccharomyces cerevisiae*

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A plasmid containing the *denV* gene from bacteriophage T4, under the control of the yeast alcohol dehydrogenase I (ADC1) promoter, conferred a substantial increase in UV resistance in the UV-sensitive *Saccharomyces cerevisiae* mutants *rad1-2* and *rad3-2*. The UV resistance of the *denV*$^+$ yeast cells was cell cycle dependent and correlated well with the level of the *denV* gene product as measured by immunoblotting and by a photoreversal assay for pyrimidine dimer-DNA glycosylase activity.

In *Saccharomyces cerevisiae* at least five proteins, encoded by the *RAD1*, *RAD2*, *RAD3*, *RAD4*, and *RAD10* genes, are thought to be involved in the process of making incisions at DNA helix distortions such as pyrimidine dimers in UV-damaged DNA (19, 24). The *RAD* gene products are believed to form a multiprotein complex, similar to the *Escherichia coli* Uvr protein complex that makes incisions at UV damage sites in DNA (3, 4, 7). A comparable UV repair complex has also been implicated in humans (3, 10). In contrast, bacteriophage T4 has evolved a capacity to incise specifically at pyrimidine dimers by using a single UV endonuclease, encoded by the *denV* gene. Endonuclease V combines both a DNA glycosylase activity specific for pyrimidine dimers and an apyrimidinic endonuclease activity in the same polypeptide molecule (6, 13, 15). No pyrimidine dimer DNA glycosylase activity has yet been detected in either yeast or human cells (4, 10).

To investigate whether the procaryotic *denV* gene product from phage T4 could replace the incision activity absent in *rad* mutants in vivo, we introduced the *denV* gene under the control of the yeast alcohol dehydrogenase I (*ADC1*) promoter in *rad1* and *rad3* mutants of *S. cerevisiae*. We report here the characterization of the *denV*$^+$ clones by UV survival experiments, immunoblotting, and an in vitro photoreversal assay.

A 457-base pair *Cla*I fragment from pdenV-52 (23) containing the *denV* gene coding sequence was blunt-end ligated into the *Hin*dIII site just downstream from the *ADC1* promoter of the yeast expression vector AAH5 (1) and cloned in *E. coli* MM294 (14). The correct orientation and sequence (2, 22) of the resulting construct, AAH5-denV, were confirmed by restriction analysis and by Maxam-Gilbert sequencing (12). AAH5-denV and AAH5 were introduced into yeast strains DH25-1B (*MATa his3-1 leu2-3 leu2-112 lys1-1 trp1-289 ura3-52 rad1-2*) and LP2649-1A (*MATα leu2-3 leu2-112 can1 ura3-52 rad3-2*) by the lithium acetate procedure of Ito et al. (8), and transformants were selected on minimal medium plates as described previously (1).

A UV survival experiment was performed as a first test for the expression of the *denV* gene (Fig. 1). Plasmid AAH5-denV (*denV*$^+$) enhanced the survival of log-phase *rad1-2* and *rad3-2* mutant strains, compared with control cells carrying AAH5 (*denV*$^-$). The *rad3-2* cells were rescued to a relatively higher level by the *denV*$^+$ plasmid than were the *rad1-2* cells. Neither of the *rad* mutants was rescued to *RAD*$^+$ levels of UV resistance. Stationary *rad1-2* cells were rescued by the *denV* gene to a lesser extent than were log-phase *rad1-2* cells, as expected from the known dependence of the *ADC1* promoter expression on the growth cycle (1).

To determine whether the *denV*$^+$ yeast cells contained a protein that reacted with antibodies against endonuclease V, crude protein extracts of *denV*$^+$ and *denV*$^-$ transformants of three types of cells (*rad1-2* and *rad3-2* yeast cells and *uvrA recA* and *E. coli*) were separated on a 15% sodium dodecyl sulfate-polyacrylamide gel (11), electroblotted onto a 0.45-μm nitrocellulose filter (20), and probed (9) with rabbit antiserum or a mouse monoclonal antibody raised against a protein A-endonuclease V fusion protein (16; Valerie et al., manuscript in preparation). Logarithmically growing *rad1-2* and *rad3-2* cells carrying AAH5-denV (*denV*$^+$) produce a protein indistinguishable from endonuclease V produced in *E. coli* (Fig. 2). The protein cannot be detected in *denV*$^+$ *rad1-2* cells isolated at stationary phase nor in logarithmically growing AAH5 (*denV*$^-$) cells. The amount of endonuclease V produced in AAH5-denV (log) cells can be estimated to be several percent of total protein, by laser scanning of the gel shown in Fig. 2A.

To determine whether the endonuclease V protein detected on immunoblots possessed enzymatic activity, UV-irradiated [^{3}H-dT]poly(dA)-poly(dT) was incubated with yeast cell extracts and then subjected to photoreversal by exposure to radiation at 254 nm (17), and the released [^{3}H]thymine was separated from phosphorylated thymidylate residues on a thin-layer chromatography plate (18). Purified endonuclease V gave the expected release of [^{3}H]thymine after photoreversal; poly(dA)-poly(dT) which had not been irradiated with UV and irradiated poly(dA)-poly(dT) which was not subjected to photoreversal did not release a significant amount of thymine (<0.2% above background) after incubation with endonuclease V (data not shown). Logarithmically growing *denV*$^+$ *rad1-2* cells contain pyrimidine dimer-DNA glycosylase activity not found in AAH5 (*denV*$^-$) cells (Fig. 3). The release of counts after photoreversal (Fig. 3A) was also observed when unirradiated substrate was used in the assay, indicating that the activity detected in *denV*$^-$ *rad1-2* cells is not directed against a UV photoproduct (data not shown). Protein extracts from stationary-phase *denV*$^+$ *rad1-2* cells have a much decreased

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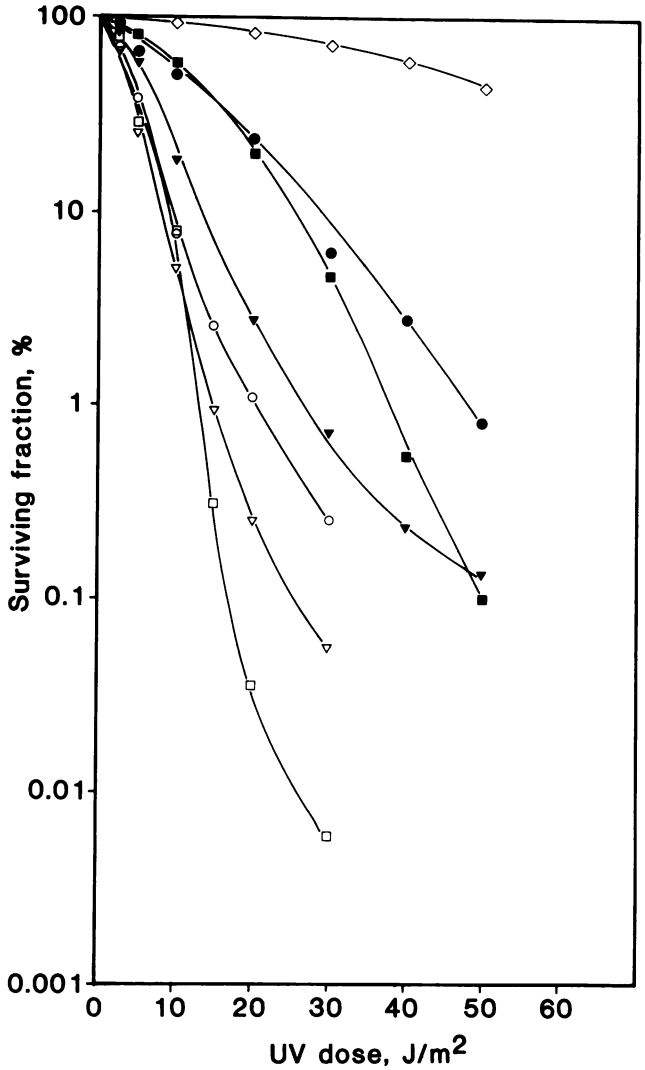

FIG. 1. UV survival of yeast cells. Serially diluted yeast cells, isolated from logarithmically growing (~125 Klett units) or stationary cultures (~300 Klett units) were exposed to UV light (at 254 nm) directly on yeast-peptone-dextrose plates at 0.5 W/m^2 and incubated for 3 to 4 days at 30°C without exposure to photoreversible light. Average counts from at least three different plates were used to calculate each percent survival. Yeast cells used include wild-type *RAD*$^+$ DH25-2A, (◇); DH25-1B *rad1-2*/AAH5 (logarithmic), (○); DH25-1B *rad1-2*/AAH5 (stationary), (▽); DH25-1B *rad1-2*/AAH5-denV (logarithmic), (●); DH25-1B *rad1-2*/AAH5-denV (stationary), (▼); LP2649-1A *rad3-2*/AAH5 (logarithmic), (□); and LP2649-1A *rad3-2*/AAH5-denV (logarithmic), (■).

level of activity, but it is above the background level of *denV*$^-$ extracts (data not shown).

These experiments show that endonuclease V is made in substantial amounts in logarithmically growing *denV*$^+$ yeast cells and suggest that endonuclease V activity is responsible for the increase in UV resistance seen in *denV*$^+$ *rad1-2* and *rad3-2* cells. It is very likely that the *denV* gene is under the control of the *ADC1* promoter, since endonuclease V levels are strongly dependent on cell cycling. The level of glycosylase activity and the amount of endonuclease V produced in yeast cells are not proportionate to the level of UV resistance, since stationary cells are more UV resistant than control cells but do not produce significant amounts of endonuclease V nor substantial in vitro glycosylase activity. We interpret this to indicate that the levels of endonuclease V being produced in *denV*$^+$ cells during logarithmic growth are well above levels needed for saturation. The incomplete restoration of UV resistance under these circumstances may simply be caused by the presence of lethal UV photoproducts which are not recognized by endonuclease V. Similar results have been obtained with UV-sensitive Chinese hamster ovary cells transformed with the *denV* gene (21).

Production of high levels of endonuclease V does not seem to have any deleterious effect on *denV*$^+$ yeast transformants. We have not detected any differences in growth characteristics or cell morphology between unirradiated *denV*$^+$ and *denV*$^-$ yeast cells, indicating that endonuclease V is not toxic to growth. In contrast, even moderate levels of endonuclease V appear to have a toxic effect on unirradiated *E. coli*, giving rise to filamentous growth under some circumstances (unpublished observations). The viability of *denV*$^+$ yeast transformants suggests that endonuclease V is not directly detrimental to DNA and that its interaction with undamaged DNA must be modulated by other cellular components. However, we cannot rule out the possibility that our *denV*$^+$ yeast transformants achieve only fairly low nuclear levels of endonuclease V, in spite of the high cytoplasmic levels, since the integrity of the yeast nuclear membrane is preserved throughout the cell cycle. Experiments are in progress to determine whether endonuclease V

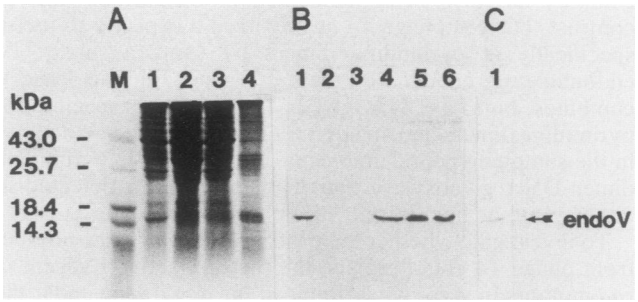

FIG. 2. Coomassie blue-stained protein gels and corresponding immunoblots of yeast and *E. coli* protein extracts. Yeast cells were vortexed with glass beads, and *E. coli* cells were sonicated in 10 mM Tris hydrochloride (pH 8.0)–100 mM NaCl–10 mM EDTA–1 mM phenylmethylsulfonyl fluoride. After centrifugation, supernatants were passed over a small DEAE column to remove nucleic acids (5). (A) Coomassie blue-stained 15% sodium dodecyl sulfate polyacrylamide gel of approximately 50 μg of protein per lane. Lanes: 1, *rad1-2*/AAH5-denV (logarithmically growing); 2, *rad1-2*/AAH5-denV (stationary); 3, *rad1-2*/AAH5 (logarithmic); 4, *E. coli* AB2480 (*uvrA recA*)/pTAC-denV (an endonuclease V overproducer). Prestained molecular mass markers (lane M; Bethesda Research Laboratories) were ovalbumin (43.0 kilodaltons [kDa]), α-chymotrypsinogen (25.7 kDa), β-lactoglobulin (18.4 kDa), and lysozyme (14.3 kDa). Endonuclease V (endoV) is indicated with an arrow at 16 kDa (15). (B) Immunoblot of a gel run in parallel with the gel in panel A and incubated with a mouse monoclonal antibody (clone I-C3) against T4 endonuclease V and detected with horseradish peroxidase conjugated to anti-mouse immunoglobulin G (Kirkegaard and Perry) followed by *O*-dianisidine and H_2O_2 (20). Lanes: 1–4, as in panel A; 5, *rad3-2*/AAH5-denV (logarithmic); 6, 1:1 mixture of extracts seen in lanes 1 and 4. (C) Immunoblot of a gel run in parallel with the gel in panel A, incubated with polyclonal rabbit antiserum against T4 endonuclease V and detected by successive incubation with an *E. coli* extract containing a λ *cro* protein-protein A-β galactosidase fusion protein and then a mixture of XGal and fast blue BB (Sigma Chemical Co.) chromogenic substrates. Lane 1: *rad1-2*/AAH5-denV (log).

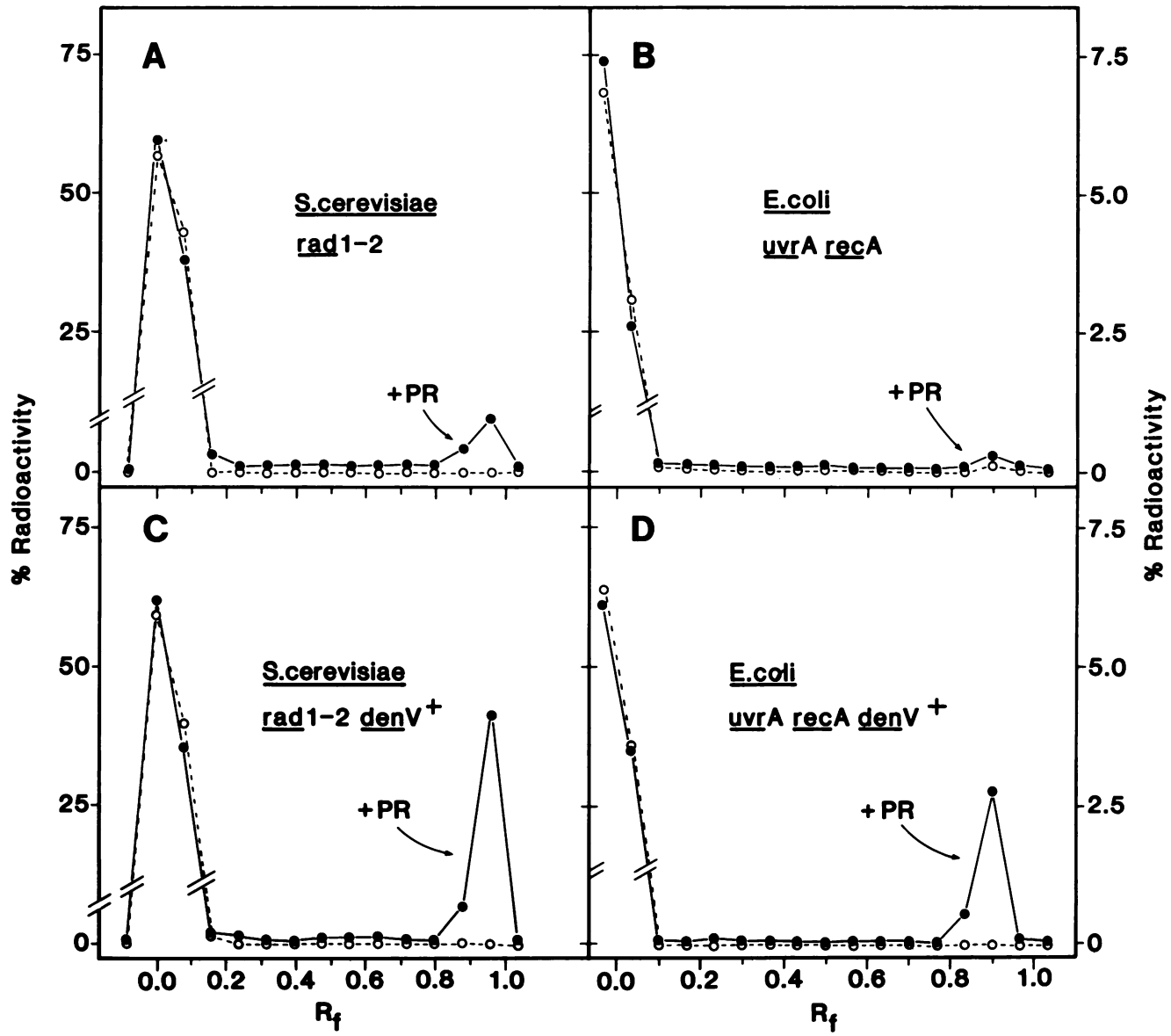

FIG. 3. Photoreversal of UV-irradiated [^{3}H-dT]poly(dA)-poly(dT) incubated with yeast and *E. coli* protein extracts. UV-irradiated (3,000 J/m^2, ~12% dimers) [^{3}H-dT]poly(dA)-poly(dT) was incubated in 5 mM Tris hydrochloride (pH 8.0)–5 mM EDTA–50 mM NaCl–0.5% bovine serum albumin at 37°C for 30 min with protein extracts from *S. cerevisiae rad1-2*/AAH5 (logarithmic growth) (A) or *rad1-2*/AAH5denV (logarithmic growth) (C) or extracts from *E. coli* AB2480 (B) or AB2480/pTAC-denV (D), dotted onto silica thin-layer chromatography plates, exposed to photoreversing UV light (+PR, ~9,000 J/m^2) (17), or left untreated. After ascending thin-layer chromatography was done as described previously (18), released thymine was scraped from the plate for scintillation counting.

in the *denV*$^+$ clones is specifically compartmentalized or randomly distributed within the cell.

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