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Expression of the Yeast *PHR1* Gene Is Induced by DNA-Damaging Agents

JOSEPH SEBASTIAN, BETSY KRAUS, AND GWENDOLYN B. SANCAR*

Department of Biochemistry, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7260

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The *PHR1* gene of *Saccharomyces cerevisiae* encodes a photolyase which repairs specifically and exclusively pyrimidine dimers, the most frequent lesions induced in DNA by far-UV radiation. We have asked whether expression of *PHR1* is modulated in response to UV-induced DNA damage and to DNA-damaging agents that induce lesions structurally dissimilar to pyrimidine dimers. Using a *PHR1-lacZ* fusion gene in which expression of β-galactosidase is regulated by *PHR1* 5′ regulatory elements, we found that exposure of cells to 254-nm light, 4-nitroquinoline-*N*-oxide, methyl methanesulfonate, and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine induced synthesis of increased amounts of fusion protein. In contrast to these DNA-damaging agents, neither heat shock nor exposure to photoreactivating light elicited a response. Induction by far-UV radiation was evident both when the fusion gene was carried on a multicopy plasmid and when it replaced the endogenous chromosomal copy of *PHR1*, and it was accompanied by an increase in the steady-state concentration of *PHR1-lacZ* mRNA. Northern (RNA) blot analysis of *PHR1* mRNA encoded by the chromosomal locus was consistent with either enhanced transcription of *PHR1* after DNA damage or stabilization of the transcripts. Neither the intact *PHR1* or *RAD2* gene was required for induction. Comparison of the region of *PHR1* implicated in regulation of its expression with other damage-inducible genes from yeast cells revealed a common conserved sequence that is present in the *PHR1*, *RAD2*, and *RNR2* genes and is required for damage inducibility of the latter two genes. These sequences may constitute elements of a damage-responsive regulon in *S. cerevisiae*.

Pyrimidine dimers (*cis,syn*-cyclobutane dipyrimidines) and other bulky adducts in DNA elicit a variety of physiological responses in living cells, including transient cell cycle arrest, increased mutagenesis of both damaged and undamaged DNA, and enhanced radioresistance. In *Escherichia coli*, these changes have been termed the SOS response and reflect the coordinated expression of a group of unlinked genes regulated at the level of transcription via the *recA* and *lexA* gene products. During normal growth, transcription of these genes is repressed by LexA protein, which binds to a common DNA sequence near or overlapping each promoter. In response to bulky DNA adducts such as pyrimidine dimers, activated RecA protein binds to LexA, thereby stimulating autoproteolysis by the repressor. Induction is the direct consequence of the reduced affinity of cleaved LexA for its cognate operator. Among the genes comprising this regulon are *lexA*, *recA* (required for recombination as well as activation of LexA and UmuD), *uvrA*, *uvrB*, and *uvrD* (required for nucleotide excision repair), *umuC* and *umuD* (required for damage-induced mutagenesis), and *sfiA* (involved in cell septation) (for a recent review, see reference 72).

Many of the physiological changes induced as part of the SOS response in *E. coli* are also displayed by eucaryotic cells following DNA damage; transient cell cycle arrest (26, 33, 69), increased mutability and recombination of both damaged and nondamaged templates (10, 12, 13, 27, 63), and enhanced levels of nucleotide excision repair (4, 35) have been reported, and in several cases it has been shown that these responses depend on RNA or protein synthesis following damage, suggesting an inducible component (4, 13, 27, 35). Increased amounts of specific transcripts following DNA damage have been observed in both mammalian and yeast cells (18, 38, 55), and several damage-inducible genes have been identified, including those encoding c-Fos, collagenase, and metallothionein in human cell lines (1–3) and the *RAD2*, *RAD7*, *RAD54*, *POL1*, *RNR2*, *CDC8*, *CDC9*, and *UBI4* genes of *Saccharomyces cerevisiae* (6, 9, 14, 32, 36, 48, 49, 53, 71, 73). However, in contrast to the situation in *E. coli*, in eucaryotes strong evidence for transcriptional control mediated by one or a few damage-responsive regulatory elements is lacking. This may reflect the absence of global regulation or the fact that the majority of damage-inducible genes identified to date in eucaryotes participate in metabolic processes other than or in addition to DNA repair and thus may respond to stimuli that are not damage specific (16, 19, 39, 71).

To further elucidate the mechanisms involved in regulating expression of genes whose products are involved specifically in DNA repair and damage tolerance in eucaryotes, we have characterized the expression of the *PHR1* gene of *S. cerevisiae*. *PHR1* encodes the apoenzyme of a DNA photolyase that catalyzes the light-dependent repair of pyrimidine dimers in DNA and stimulates nucleotide excision repair of these lesions in the dark (58, 61). The holoenzyme contains two noncovalently bound chromophores, $FADH_2$ and 5,10-methenyltetrahydrofolate (31, 62). The only known function of the enzyme is DNA repair. It has been previously reported that photolyase activity increases in vivo in response to photoreactivating light and that at least a part of this increase requires protein synthesis, suggesting that enzyme activity or concentration is regulated (22–24). Whether this increase reflects transcriptional, translational, or posttranslational modifications has been unclear, in part because a method of quantifying the number of photolyase molecules independent of photoreactivating activity was not available. We report here the construction of a *PHR1-lacZ*

* Corresponding author.

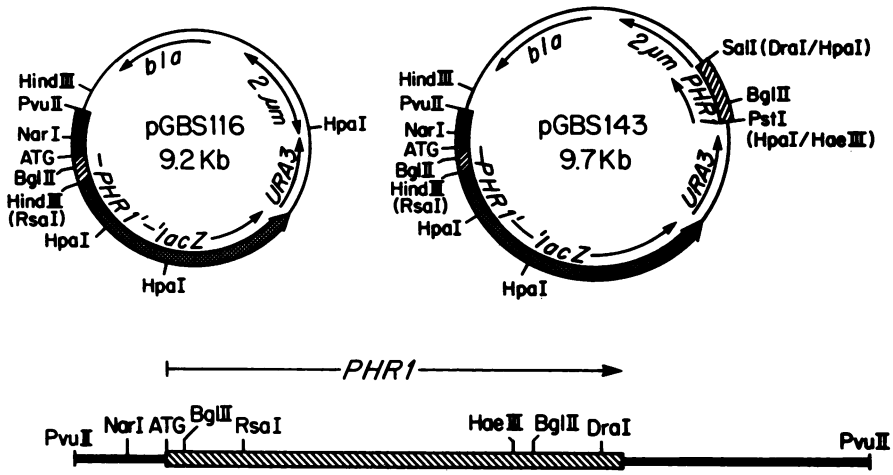

FIG. 1. Restriction maps of plasmids carrying the *PHR1-lacZ* fusion gene and of the 3.3-kbp *Pvu*II fragment of yeast genomic DNA carrying the *PHR1* gene (59, 64). Symbols: ■, *PHR1* 5′ and 3′ flanking sequences; ▨, *PHR1* coding sequences; ▩, locations of *lacZ* coding sequences. ATG indicates the translational start site of *PHR1*. Restriction sites in parentheses were destroyed during subcloning. Arrows indicate the direction of transcription. Plasmid constructions are described in Materials and Methods.

fusion gene that allows changes in photolyase apoenzyme to be quantified independent of repair activity. Using this construct, we have demonstrated that expression of a *PHR1-lacZ* fusion gene increases in a dose-dependent manner in response to 254-nm light as well as to a number of DNA-damaging agents that do not induce formation of pyrimidine dimers, but not after heat shock or exposure to photoreactivating light. Induction reflects an increase in the steady-state concentration of *PHR1* mRNA.

MATERIALS AND METHODS

Reagents and materials. DNA-modifying enzymes, restriction endonucleases, oligonucleotide linkers, and RNA molecular weight markers were obtained from GIBCO-Bethesda Research Laboratories, Promega Corp. (Madison, Wis.), or New England BioLabs, Inc. (Beverly, Mass.), and were used as specified by the manufacturers. Working stocks of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and 4-nitroquinoline-*N*-oxide (4-NQO; Sigma Chemical Co., St. Louis, Mo.) were prepared either at 4.5 mg/ml in dimethyl sulfoxide and stored at 4°C (4-NQO) or at 20 mM in 10 mM sodium acetate (pH 4.8) and stored at −20°C (MNNG). Methyl methanesulfonate (MMS; Aldrich Chemical Co., Milwaukee, Wis.) was stored at −20°C and then diluted to 1% (wt/vol) in dimethyl sulfoxide immediately before use. Bleomycin sulfate was a gift from Bristol-Myers Corp. (Evansville, Ind.) and was dissolved in SC medium (see below) at 5 mg/ml immediately before use. Zymolyase 60000 and 100T were from Miles Laboratories, Inc. (Elkhart, Ind.) and ICN Pharmaceuticals (Irvine, Calif.), respectively; aprotinin, soybean trypsin inhibitor, leupeptin, and phenylmethylsulfonyl fluoride were from Sigma or Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Chlorophenolred-β-D-galactopyranoside (CPRG) was obtained from Boehringer Mannheim Biochemicals, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) was obtained from GIBCO-Bethesda Research Laboratories, [α-^{32}P]dCTP (>800 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, Ill.), and oligonucleotide primer labeling reagents were obtained from Pharmacia-LKB Biotechnology Inc.

Strains and media. Yeast strains for this study were constructed by standard techniques (66). DBY745 (α *ura3-52 ade1-101 leu2-3,112*) was obtained from David Botstein via Howard Fried. GBS43 (α *ura3-52*) is a haploid derivative from a genetic cross between DBY745 and X12-6B (a *rad1-1 ade2-1*; obtained from the Yeast Genetic Stock Center, Berkeley, Calif.). Constructions of GBS58 (a *rad18 ura3-52 leu1 his5 trp1*) and GBS76 (a *ura3-52 leu2-3,112 rad2 phr1*) have been previously described (60, 61). GBS77 (α *ura3-52 leu2-3,112 rad2*) is a product of the same genetic cross that produced GBS76. GBS112 is a derivative of GBS43 in which the *PHR1* gene has been replaced by a *Bgl*II fragment carrying a *PHR1-lacZ* fusion gene and *URA3* from pGBS143 (see below); as a result, GBS112 is Phr$^-$ Ura$^+$. For routine culture, yeast strains were grown in YPAD (66). Plasmid-containing strains and GBS112 were propagated in synthetic complete medium minus uracil (66). X-Gal plates were prepared as described previously (56). *E. coli* DH5α [φ80d *lacZ*ΔM15 Δ(*argF-lacZYA*)U169 *endA1 hsdR17 supE44 thi-1 recA1 gyrA96 relA1* λ$^-$] was used for routine propagation and amplification of plasmids containing the *PHR1-lacZ* fusion gene.

Plasmid constructions and transformations. Recombinant DNA techniques and transformation of yeast and *E. coli* strains were performed essentially as described elsewhere (25, 37, 54). Plasmid YEp357 is a 2μm *URA3* Amp^r yeast-*E. coli* shuttle vector containing a 5′ truncated *lacZ* gene immediately 3′ to a series of unique restriction sites (45). Expression of plasmid-encoded β-galactosidase in both *E. coli* and yeast cells depends on insertion of a promoter and translational start site 5′ to and in frame with *lacZ*. *PHR1* 5′ flanking sequences and the amino-terminal coding region were inserted into YEp357 as follows. *Hin*dIII linkers were ligated to a 1,148-base-pair (bp) *Rsa*I fragment containing (5′→3′) 515 bp of DNA from the coding region of the *cat* gene in pBR328 (*RsaI*$_{415}$-*Pvu*II$_{-101}$; 51), followed by 634 bp of yeast DNA extending from the *Pvu*II site 5′ to *PHR1* through the first 280 bp of the *PHR1* coding sequence (59; Fig. 1), and the fragment was inserted into YEp357 at the unique *Hin*dIII site. Colinear orientation of *PHR1* and *lacZ* coding sequences was confirmed by restriction mapping. The resulting plasmid, pGBS116 (Fig. 1), programmed synthesis of a fusion protein with β-galactosidase activity in

both *S. cerevisiae* and DH5α cells; presumably transcription of the *PHR1-lacZ* fusion gene in *E. coli* was due to the presence in the yeast DNA of a sequence with weak promoter activity (58). β-Galactosidase activity in extracts from yeast cells was not observed with YEp357 alone (45; our unpublished observations). In addition, removal of the pBR328 sequences from pGBS116 did not alter the pattern of expression of the fusion protein (K. Brust and G. B. Sancar, unpublished data).

For replacement of the chromosomal *PHR1* locus with the fusion gene, a 454-bp *Hae*III-*Dra*I fragment from the 3′ end of the *PHR1* coding sequence was inserted at a *Hpa*I site within the 2μm portion of pGBS116 as follows: pGBS116 was digested with *Hpa*I in the presence of ethidium bromide (47) such that on average only one of the three sites in the plasmid was cleaved, the *Hpa*I site was filled in with *E. coli* DNA polymerase I (Klenow fragment), and a *Sal*I-*Nru*I-*Pst*I linker was added via ligation. After transformation of DH5α, clones that still produced β-galactosidase were identified on X-Gal plates; plasmid pGBS142, containing a *Sal*I-*Nru*I-*Pst*I linker at the desired *Hpa*I site only, was identified by restriction mapping. pGBS142 was digested with *Nru*I, and the 454-bp *Hae*III-*Dra*I fragment was ligated into this site, yielding plasmid pGBS143 (Fig. 1). *Bgl*II digestion of pGBS143 produced a 5.1-kbp fragment carrying the *URA3* gene flanked by the *PHR1-lacZ* fusion gene on one side and the 3′ *PHR1* fragment on the other. This fragment was used to transform yeast strain GBS43 to uracil prototrophy, yielding strain GBS112. Replacement of the endogenous *PHR1* gene with a single copy of this fragment was confirmed by (i) Southern analysis of genomic DNA, (ii) mitotic stability of *URA3* under nonselective growth conditions, (iii) loss of the ability to photoreactivate UV-induced DNA damage, and (iv) 100% cosegregation of *URA3* and the Phr^- phenotype among the meiotic products of a cross between GBS112 and GBS58.

β-Galactosidase assays. Quantitative assays for β-galactosidase activity in yeast cells grown in liquid medium were carried out on 5-ml samples of cultures in duplicate or triplicate. Cells were collected by centrifugation at 1,600 × *g* for 5 min at 4°C; the cell pellet was washed once in Z buffer (41), resuspended in 0.5 ml of Z buffer, and frozen at −80°C. For assay, the suspension was thawed on ice, and Zymolyase (50 μg/ml) and a cocktail of protease inhibitors (10 μg of aprotinin, 10 μg of soybean trypsin inhibitor, and 4 μg of leupeptin per ml, 20 mM phenylmethylsulfonyl fluoride) were added. After incubation for 2 h at 35°C, the mixture was vortexed vigorously for 30 s, 100 μl of CPRG (4 mg/ml in H_2O) was added, and the mixture was incubated for 10 min to 2 h at 22°C. Cleavage of CPRG was halted by addition of 250 μl of 1 M Na_2CO_3, and cellular debris was subsequently removed by centrifugation at 18,000 × *g* for 15 min. The absorbance maximum of cleaved CPRG is at 574 nm; thus, β-galactosidase activity, corrected for culture density and scattering by residual cellular debris, was calculated in Miller units (41) as follows: Miller units per milliliter of culture = $[(A_{574} - A_{634})/(A_{600} \times 5\text{ ml} \times \text{minutes of reaction})] \times 1{,}000$. We found that compared with the more commonly used *o*-nitrophenyl-β-D-galactoside (ONPG), use of CPRG as a substrate increased the sensitivity of the β-galactosidase assay in these crude cell extracts by 10- to 20-fold. Variation between duplicate or triplicate assays performed on the same day was <15%. All datum points shown are the means of two to three independent assays.

Treatment of cultures with inducing agents. Strains to be treated with various chemical or physical agents were grown at 30°C to early log phase (A_{600} = 0.1) in SC minus uracil (plasmid-containing strains) or YPAD. DNA-damaging chemicals were added directly to the culture medium, and 5-ml samples were removed in triplicate at subsequent times for β-galactosidase activity measurements. Cell survivals were determined 4 h after addition of the chemical by plating dilutions of treated or nontreated cultures on YPAD. For studies on the effect of heat shock on photolyase expression, cultures were grown to early log phase at 22°C and then exposed to a 20-min heat shock at 37°C before the culture was returned to 22°C (39). For UV irradiation, cells from early-log-phase cultures were harvested by centrifugation, washed in phosphate-buffered saline at 4°C, and suspended to A_{600} = 0.3. The cell suspension was exposed with stirring to 254-nm radiation from a G8T5 germicidal lamp (General Electric Co., Schenectady, N.Y.) (61). When Phr^+ strains were used, all manipulations during and after irradiation were performed under yellow safe lights to prevent uncontrolled photoreactivation. After irradiation, the cells were suspended in fresh growth medium at the same density as before irradiation and incubated at 30°C, and samples were taken for β-galactosidase activity as described above. Control cultures were subjected to the same manipulations as irradiated cultures. For the irradiated cultures, zero-time samples consisted of cells harvested immediately after irradiation and before return to growth medium. Experiments to determine whether photoreactivating light induced *PHR1* expression were carried out in a similar manner except that cells were grown under yellow safe lights for at least 24 h before and at all times after exposure to 365-nm radiation from a Sylvania TF8-BLB black light bulb.

Determination of steady-state mRNA levels. Yeast cells were cultured, irradiated, and returned to growth medium as described above. Samples of 20 ml were removed immediately after irradiation as well as at subsequent times after return to growth medium, and total RNA was extracted as described previously (40). Purified RNA in H_2O was spotted onto nitrocellulose membranes, using a dot blot manifold (Bio-Rad Laboratories, Richmond, Calif.). Hybridization was carried out at 42°C for 16 h, using 25 ng of a ^{32}P-labeled 2.5-kbp *Pst*I fragment from plasmid pMC1871 (65) to detect *lacZ* mRNA. The relative intensities of the hybridized dots were quantified by using a Biomed Instruments scanning laser densitometer. For analysis of transcription from the chromosomal copy of *PHR1*, 10-μg samples of total RNA, isolated as described above and denatured in 50% formamide, were subjected to electrophoresis in 1% agarose gels containing 2.2 M formaldehyde (37). RNA in the gel was blotted onto Nytran 2000 Plus membranes, and hybridization was performed as described above except that the probe was 25 ng of a ^{32}P-labeled 2.4-kbp *Pvu*II-*Pst*I fragment from pCB8 containing the entire *PHR1* gene (60). After autoradiography, the *PHR1* probe was removed from the filter by boiling for 2 min in distilled H_2O, after which the filters were probed with a 2.3-kbp ^{32}P-labeled *Sph*I fragment from the yeast *CYH2* gene, encoding ribosomal protein L29 (kindly provided by Howard Fried). Quantitation of relative amounts of RNA was performed as described above.

RESULTS

Expression of *PHR1* is induced in response to DNA-damaging agents. To monitor expression of *PHR1*, we constructed plasmid pGBS116, which carries 354 bp of 5′ flanking sequence from *PHR1* followed by the first 280 bp of the *PHR1* coding sequence fused in frame to a truncated *lacZ*

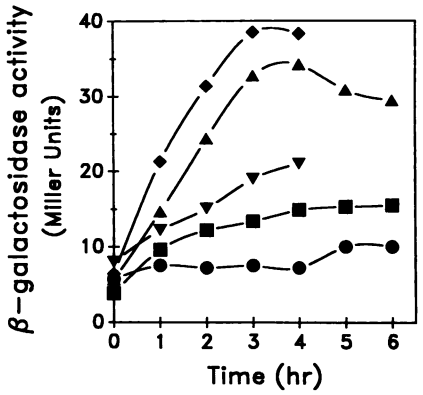

FIG. 2. Accumulation of the *PHR1-lacZ* fusion protein in response to various fluences of 254-nm light. Early-log-phase cells of strain GBS76(pGBS116) were irradiated, returned to growth medium, and assayed for β-galactosidase activity at various times after irradiation as described in Materials and Methods. Symbols: ●, nonirradiated control cells; ■, 2.5 J/m^2; ▼, 5 J/m^2; ▲, 7.5 J/m^2; ◆, 10 J/m^2.

gene from *E. coli* (Fig. 1). Plasmid pGBS116 thus encodes a hybrid *PHR1-lacZ* fusion protein, the expression of which is controlled by *PHR1* 5′ regulatory elements. Since *S. cerevisiae* does not normally produce β-galactosidase, any change in the enzyme activity should reflect solely changes in the amount of the fusion protein. Yeast genomic sequences upstream of those present on pGBS116 are not required for *PHR1* expression on a multicopy plasmid (60, 64). pGBS116 also carries the replication origin of the endogenous 2μm yeast plasmid and thus replicates to high copy number in yeast strains already containing complete 2μm plasmids. Because Phr1 is normally present at a level of only 75 to 150 molecules per cell in *S. cerevisiae* (21, 74), a multicopy plasmid was chosen for our initial studies to facilitate detection of the *PHR1-lacZ* fusion protein. In the absence of DNA damage, the *PHR1-lacZ* fusion protein accumulated at a constant rate until late log phase in strain GBS76(pGBS116) (Fig. 2).

The only known substrates for DNA photolyases are *cis,syn*-cyclobutane dipyrimidines (pyrimidine dimers), which are the most frequent lesions introduced into DNA by far-UV light (λ_{max} = 200 to 300 nm). Therefore, we first asked whether exposure of log-phase GBS76 (*rad2 phr1*)(pGBS116) cells to 254-nm light altered the expression of the *PHR1-lacZ* fusion gene. We observed a dose-dependent increase in β-galactosidase activity that reached a plateau 3 to 4 h after UV challenge (Fig. 2). At the highest dose tested (10 J/m^2, 10% cell survival), the induction ratio (i.e., the ratio of activity in irradiated versus nonirradiated cultures at a particular time) was approximately 8, whereas two- to threefold induction was detected at fluences as low as 2.5 J/m^2 (80% survival).

We next asked whether the increase in *PHR1-lacZ* fusion protein was specific for UV-induced damage by testing the response of the fusion gene in GBS76(pGBS116) to treatment with the UV-mimetic agent 4-NQO, the alkylating agents MMS and MNNG, the γ-radiation mimetic agent bleomycin sulfate, and photoreactivating light (365 nm). Seven- to tenfold induction was seen at concentrations of MMS and MNNG that yielded cell survivals of 40 to 50%, comparable to the survival seen after exposure to a 5-J/m^2 dose of 254-nm light (Table 1). 4-NQO was much less

TABLE 1. Induction of Phr1–β-galactosidase fusion protein after treatment of strain GBS76(pGBS116) with various DNA-damaging agents

Agent	Dose	Induction ratio[a] at time (h):			
		1	2	3	4
None		1.0	1.0	1.0	1.0
UV radiation	5 J/m^2	4.4	5.2	6.6	7.2
MMS	2.3 mM	2.8	5.8	7.2	6.8
MNNG	2 μM	2.8	6.0	7.2	10.8
4-NQO	0.7 mM	1.1	2.0	3.3	3.0
	1.3 mM	2.8	6.0	8.8	9.0
Bleomycin	5 μg/ml	1.2	1.5	1.8	2.3
Heat shock	22°C→37°C	0.7	1.8	1.6	1.1
365-nm radiation	3 kJ/m^2	0.8	1.0	1.0	1.0

[a] Ratio of β-galactosidase activity in treated versus untreated cells. Only induction ratios of >2 are considered significantly different from uninduced levels.

effective as an inducing agent in that concentrations which yielded 40% cell survival (0.7 mM) produced only about twofold induction; β-galactosidase levels comparable to those seen at 40% survival with MMS and MNNG were seen only at 10% cell survival with 4-NQO (1.3 mM). Bleomycin treatment reproducibly led to little or no induction, and photoreactivating light failed to elicit increased amounts of fusion protein.

McClanahan and McEntee (39) have demonstrated that in *S. cerevisiae*, several DNA-damage-responsive (*DDR*) genes are also induced in response to a 20-min heat shock at 37°C. Similarly, Mitchell and Morrison (42, 43) have reported that heat shock induces increased resistance to UV-induced killing and, conversely, that UV irradiation increases the thermal tolerance of cells. These results suggest that some damage-inducible responses are in fact general responses to cell stress. To determine whether *PHR1* expression is also increased in response to thermal stress, GBS76(pGBS116) cells grown at 22°C were subjected to a heat shock according to the protocol described by McClanahan and McEntee (39). Induction of *PHR1-lacZ* fusion protein was not observed (Table 1).

Expression of *PHR1* in a Rad$^+$ Phr$^+$ background and from the *PHR1* chromosomal locus. The results presented in the preceding section establish that increased amounts of Phr1–β-galactosidase fusion protein accumulate after exposure of a *rad2 phr1* strain to various DNA-damaging agents. Because the specific biochemical roles of the *RAD* genes in nucleotide excision repair have not been defined and thus it is possible that one or more of these genes serve a regulatory function, we wanted to know whether the induction of *PHR1* was influenced by the presence of the *rad2* allele in GBS76. In addition, it is not known whether the Phr1 photolyase, in addition to its catalytic function, also acts as an autoregulator of *PHR1* expression; such dual activity for a repair protein is not unprecedented, as the *E. coli* Ada protein carries out repair of O^6-methylguanine in DNA and also acts as a positive regulator of the *ada-alkB* operon (68). To examine the roles of these genes in expression of *PHR1*, we introduced pGBS116 into DBY745, a strain with no known mutations in any of the *RAD* genes or in *PHR1* and with a wild-type level of UV resistance. After exposure to a 25-J/m^2 dose of 254-nm light, which yielded a cell survival of 45%, the amount of the Phr1–β-galactosidase fusion protein increased 8- to 10-fold, and the kinetics of induction were similar to that seen in the *rad2 phr1* background (data not

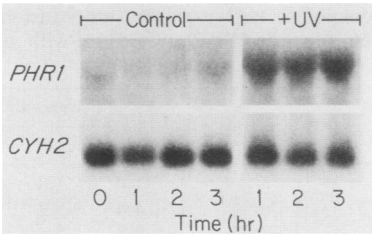

FIG. 3. Accumulation of *PHR1* and *CYH2* mRNAs after 254-nm radiation. Total cellular RNA was purified from GBS77 either before or at the indicated times after exposure to a 7.5-J/m^2 dose of 254-nm radiation or from a control, mock-irradiated culture, separated by agarose gel electrophoresis, blotted, and probed with ^{32}P-labeled *PHR1* DNA as described in Materials and Methods. After autoradiography, the probe was stripped from the filter, which was then exposed to ^{32}P-labeled *CYH2* DNA. The bands shown were the only ones apparent on the autoradiographs. The *PHR1* transcript migrated with size of 2.4 kb when the 18S and 25S rRNAs in the same lane were used as markers (7, 70). The autoradiograph of the *PHR1*-probed filter was obtained after a 7-day exposure; the *CYH2*-probed filter was exposed for 2 days. The *CYH2* RNA level was used to adjust for variations in the amount of RNA loaded on the gel.

shown). Thus, neither active Rad2 nor Phr1 is required for induction.

It could also be argued that induction of *PHR1-lacZ* on pGBS116 is a consequence of its location on an autonomously replicating high-copy-number plasmid. To determine whether this was the case, we replaced the chromosomal *PHR1* locus in GBS43 with the *PHR1-lacZ* fusion gene, yielding strain GBS112. Consistent with reports that photolyase activity is low during normal log-phase growth (21, 74), the basal level of β-galactosidase activity was approximately 0.2 Miller units. Nonetheless, after irradiation with 254-nm light at 50 or 100 J/m^2, yielding 26 and 4% survival, respectively, β-galactosidase activity increased seven- to ninefold (data not shown). Thus, induction of *PHR1-lacZ* on pGBS116 is not a consequence of its extrachromosomal location.

Induction of *PHR1* is accompanied by an increase in the steady-state concentration of *PHR1* mRNA. Increased accumulation of the *PHR1-lacZ* fusion protein in cells treated with DNA-damaging agents could be regulated at the level of transcription, translation, or both. In addition, it is formally possible that the fusion protein and mRNA are subject to different pathways of degradation than are normal *PHR1* mRNA and protein. To further characterize this induction, we used RNA dot blots and Northern (RNA) analysis to examine the pattern of β-galactosidase mRNA accumulation in strain GBS76(pGBS116) and of mRNA from the intact *PHR1* locus in GBS77 (*rad2 PHR1*) after exposure to a 7.5-J/m^2 dose of 254-nm light. Induction was apparent, with the steady-state concentration of *PHR1* mRNA reaching a maximum approximately 1 h after UV treatment (Fig. 3 and Table 2). Similar kinetics were observed for accumulation of the *PHR1-lacZ* mRNA encoded by pGBS116 (Table 2). The temporal pattern of mRNA accumulation is consistent with the observation that the rate of fusion protein accumulation is at its greatest during the first 2 h after UV irradiation (Fig. 2). Densitometry indicated that the amount of *PHR1* mRNA from the chromosomal locus of GBS77 increased by a factor of 14 during the first hour after irradiation, whereas in GBS76(pGBS116) the *PHR1-lacZ* mRNA concentration increased by only a factor of 2 during the same time period (Table 1). In all cases, the induction ratio for the chromosomal gene exceeded that of the gene on the plasmid. This

TABLE 2. Induction ratios of *PHR1-lacZ* and *PHR1* mRNA after 254-nm irradiation

Strain	Induction ratio[a] at time (h):					
	0	0.5	1.0	2.0	3.0	4.0
GBS76(pGBS116) (*PHR1'-lacZ* fusion)	1.1	1.6	2.4	2.2	1.5	1.2
GBS77 (*PHR1*)	1.0	ND	13.7	6.4	3.7	ND

[a] Data are from densitometric scans of autoradiographs shown in Fig. 3. For pGBS116(GBS76), the induction ratio was calculated as the (peak area)$_{induced}$/(peak area)$_{uninduced}$. For GBS77, the induction ratio was similarly calculated except that the ratio of the areas *PHR1*/*CYC2* at each time was used to correct for differences in the amount of RNA loaded and transferred. ND, Not done.

finding suggests titration of a regulatory molecule by the multicopy plasmid and is consistent with our recent identification of a protein in nonirradiated cells which binds specifically to the 5′ region of *PHR1* (J. Sebastian and G. B. Sancar, unpublished observation). Thus, we conclude that enhanced expression of *PHR1* after DNA damage is due to an increase in the steady-state concentration of *PHR1* mRNA. Whether additional, translational controls are responsible for the differences between the induction ratios for mRNA (Table 2) versus fusion protein (Fig. 2) remains to be determined.

DISCUSSION

The results presented here demonstrate that expression of the *PHR1* gene of *S. cerevisiae*, which encodes the apoenzyme of a DNA photolyase, is modulated in response to irradiation of cells with far-UV light. Fusion of a 637-bp fragment, containing 354 bp 5′ to the *PHR1* translational start site and the first 283 bp of coding sequence, to an amino-terminal truncated copy of the *E. coli lacZ* gene rendered the encoded Phr1–β-galactosidase fusion protein UV inducible. The steady-state concentrations of both *PHR1-lacZ* mRNA and *PHR1* mRNA transcribed from the chromosomal copy of the gene increased after UV irradiation and preceded the peak in Phr1–β-galactosidase activity, consistent with utilization of this mRNA to synthesize photolyase apoenzyme. The simplest interpretation of these observations is that control of *PHR1* expression is exerted via enhanced transcription of the gene or stabilization of *PHR1* mRNA. Whether control is also exerted at the translational level remains to be determined.

Does induction of photolyase apoenzyme synthesis enhance the ability of cells to repair pyrimidine dimers in vivo? We have not demonstrated such enhancement in this work, as it is not possible at present to divorce photolyase induction from other damage-inducible processes which also increase survival (G. Sancar, unpublished observation). Direct demonstration of enhanced survival after UV irradiation will likely require comparison with a noninducible mutant. However, the fact that photolyase holoenzyme efficiently photoreactivates pyrimidine dimers in vitro in the absence of any additional cofactors or proteins (62) strongly suggests that enhancement of photoreactivation should occur in vivo provided both $FADH_2$ and 5,10-methenyltetrahydrofolate, the chromophores of the enzyme (31, 62), are also available. Replacement of sequences 5′ to the *PHR1* coding sequence with the *GAL10* promoter and upstream activation sequence leads to a 20- to 40-fold increase in the number of active photolyase molecules when cells containing this construct are grown in galactose-containing medium (60); this finding

indicates that the cellular concentrations of the chromophores are sufficient to saturate an amount of apoenzyme similar to that synthesized after UV induction.

In addition to UV light, treatment of *S. cerevisiae* cells with 4-NQO, MMS, and MNNG also resulted in increased expression of *PHR1*. This is a somewhat surprising result in view of the fact that the lesions produced by 4-NQO, MNNG, and MMS are neither bound nor repaired by photolyase (reviewed in reference 57); therefore, the signal for *PHR1* induction is not generation of its specific substrate but rather a more general metabolic response to DNA damage. That the signal is not produced by metabolic stress in general was shown in this work by the absence of increased *PHR1* expression after a brief heat shock at 37°C and is in marked contrast to the response of the *UBI4* (polyubiquitin), *DDRA2*, and *DDR48* genes of *S. cerevisiae*, which are induced by both DNA damage and heat shock (16, 39). The observation that several damage-inducible genes, including *RNR2* (ribonucleotide reductase subunit 2), *CDC8* (thymidylate kinase), *CDC9* (DNA ligase), and *POL1* (DNA polymerase I), are expressed only or primarily during S phase of the cell cycle during normal growth has led to the proposal that induction of some damage-responsive genes is due, at least in part, to cessation of cell cycling at a stage when these genes are normally expressed at high levels (14, 32, 49, 73). We consider such a regulatory mechanism unlikely in the case of *PHR1*; in contrast to the known damage-inducible, cell-cycle-regulated genes, photolyase does not play a role in nucleotide or DNA metabolism in nondamaged cells, and preliminary results indicate that *PHR1* expression is not limited to a particular phase of the cell cycle (Brust and Sancar, unpublished observations). Similar results have been reported for *RAD2* and *RAD54*, which are involved respectively in nucleotide excision repair and double-strand-break repair (9, 53). In addition, induction of *RNR2* and *POL1* by damaging agents can also occur when cells are arrested early in G_1, demonstrating that cell-cycle and damage-specific regulation can be uncoupled for at least some dually regulated genes (28, 32).

Is *PHR1* a member of a larger set of damage-inducible genes that are regulated at the transcriptional level by a common effector? Recently 20- to 40-bp-long sequences required for the damage-specific transcriptional induction of *RAD2*, *RAD54*, and *RNR2* have been identified (8, 15, 67, 28), and in studies to be reported elsewhere (Sebastian and Sancar, unpublished data), we have found that all sequences required for damage-specific induction of *PHR1* lie 3′ to the *Nar*I site shown in Fig. 1. Comparison of the 5′ flanking sequences of these genes reveals a 10-bp sequence, CG(A/T)GG(A/T)NG(A/C)A, common to the damage-responsive regions of *RAD2* and *RNR2* and to *PHR1* (Fig. 4). Variations on this sequence are also found in the 5′ flanking regions of *RAD4* and *RAD10*, both of which are required for nucleotide excision repair (20). Although neither *RAD4* nor *RAD10* is induced by concentrations of 4-NQO sufficient to induce *RAD2* and *PHR1* (5, 17), the response of these genes to other DNA-damaging agents has not been addressed. No significant homologies were found between *PHR1* and a number of other genes known to be involved in DNA repair or in damage tolerance, including *RAD1*, *RAD3*, *RAD7*, *RAD9*, *RAD18*, *RAD52*, *RAD54*, *REV1*, and *REV3* (20, 34, 44), nor were homologies found to the upstream regions of the cell-cycle-regulated and damage-inducible genes *RAD6* (ubiquitin-conjugating enzyme; 29), *CDC9* (DNA ligase; 6, 30), and *POL1* (DNA polymerase I; 50). The absence of homology between the damage-responsive element of

PHR1	−103	CGAGGAAGCA
	−109′	CGAGGAAGAA
RAD2	−168	CGTGGAGGCA
RNR2	−424	CGAGGTCGCA
RAD10	−312′	CGAGGAAGAA
RAD4	−363	CGTGGATGAA
CONSENSUS		CG(A/T)GG(A/T)NG(C/A)A

FIG. 4. Nucleotide sequence homology in the 5′ flanking regions of *PHR1*, *RAD2*, *RAD4*, *RAD7*, and *RNR2*. The position given is relative to the first nucleotide in the first ATG codon in the open reading frame of each gene. Two regions of homology are found upstream of *PHR1*, one on each strand. Noncoding strands are indicated by a ′, and numbering is the same as on the coding strand. Sequences were taken from references 11, 15, 28, 36, 46, 52, and 59.

RAD54 and *PHR1* may reflect the presence of several elements in *S. cerevisiae* which respond to different classes of lesions; *rad54* mutants are primarily sensitive to the lethal and mutagenic effects of γ rays and agents, such as bleomycin, which introduce lesions normally repaired via mechanisms involving recombination (20). In contrast, *PHR1* does not play any known role in recombinational repair, bleomycin treatment of cells induces only very minor increases in *PHR1* expression, and the kinetics of induction are quite different from that seen with UV, MMS, 4-NQO, and MNNG (Table 1). It is particularly intriguing that among the *RAD* genes, the common decanucleotide sequence is found only among elements of the nucleotide excision repair pathway; coordinate regulation of these genes and *PHR1* would be consistent with the recent discovery that photolyase stimulates dimer repair by this pathway (61). Studies are now under way to ascertain the role of this conserved sequence in *PHR1* expression.

Previous studies have shown that growth of *S. cerevisiae* cells under photoreactivating light before exposure to 254-nm radiation enhances cell survival as a result of an apparent increase in the number of active photolyase molecules per cell (22–24). In contrast, we were unable to detect induction of Phr1–β-galactosidase fusion protein after exposure of dark-grown cells to fluences of photoreactivating light as great as 3 J/m^2, more than 10^4-fold greater than the smallest fluence of 254-nm light producing reproducible induction *PHR1*. These results appear to rule out rapid induction of photolyase apoenzyme synthesis in response to biologically significant fluences of photoreactivating light.

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LITERATURE CITED

1. **Angel, P., I. Baumann, B. Stein, H. Delius, H. J. Rahmsdorf, and P. Herrlich.** 1987. 12-*O*-Tetradecanoyl-phorbol-13-acetate induction of the human collagenase gene is mediated by an inducible enhancer element located in the 5′-flanking region. Mol. Cell. Biol. **7**:2256–2266.
2. **Angel, P., A. Poting, U. Mallick, H. J. Rahmsdorf, M. Schorpp, and P. Herrlich.** 1986. Induction of metallothionein and other mRNA species by carcinogens and tumor promoters in primary human skin fibroblasts. Mol. Cell. Biol. **6**:1760–1766.
3. **Angel, P., H. J. Rahmsdorf, A. Poting, and P. Herrlich.** 1985. c-fos mRNA levels in primary human fibroblasts after arrest in various stages of the cell cycle. Cancer Cells **3**:315–319.

4. **Baker, T. I.** 1983. Inducible nucleotide excision repair in *Neurospora*. Mol. Gen. Genet. **190**:295–299.
5. **Bardwell, L., H. Burtscher, W. A. Weiss, C. M. Nicolet, and E. C. Friedberg.** 1990. Characterization of the *RAD10* gene of *Saccharomyces cerevisiae* and purification of the Rad10 protein. Biochemistry **29**:3119–3126.
6. **Barker, D. G., J. H. M. White, and L. H. Johnston.** 1985. The nucleotide sequence of the DNA ligase gene (*CDC9*) from *Saccharomyces cerevisiae*: a gene which is cell-cycle regulated and induced in response to DNA damage. Nucleic Acids Res. **13**:8323–8337.
7. **Bell, G. I., L. J. DeGennaro, D. H. Gefland, R. J. Bishop, P. Valenzuela, and W. J. Rutter.** 1977. Ribosomal RNA genes of *Saccharomyces cerevisiae*. I. Physical map of the repeating unit and the locations of the regions coding for 5 S, 5.8 S, 18 S, and 25 S ribosomal RNAs. J. Biol. Chem. **252**:8118–8125.
8. **Cole, G. M., and R. K. Mortimer.** 1989. Failure to induce a DNA repair gene, *RAD54*, in *Saccharomyces cerevisiae* does not affect DNA repair of recombination phenotypes. Mol. Cell. Biol. **9**:3314–3322.
9. **Cole, G. M., D. Schild, S. T. Lovett, and R. K. Mortimer.** 1987. Regulation of *rad54-* and *RAD52-lacZ* gene fusions in *Saccharomyces cerevisiae* in response to DNA damage. Mol. Cell. Biol. **7**:1078–1084.
10. **Cornelis, J. J., Z. Z. Su, and J. Rommelaere.** 1982. Direct and indirect effects of UV light on the mutagenesis of provirus H-1 in human cells. Proc. Natl. Acad. Sci. USA **75**:2378–2381.
11. **Couto, L. B., and E. C. Friedberg.** 1989. Nucleotide sequence of the wild-type *RAD4* gene of *Saccharomyces cerevisiae* and characterization of mutant *rad4* alleles. J. Bacteriol. **171**:1862–1869.
12. **DasGupta, U. B., and W. C. Summers.** 1978. Ultraviolet reactivation of herpes simplex virus is mutagenic and inducible in mammalian cells. Proc. Natl. Acad. Sci. USA **75**:2378–2381.
13. **Eckhardt, F., E. Moustacchi, and R. H. Haynes.** 1978. On the inducibility of error-prone repair in yeast, p. 421–423. *In* P. Hanawalt, E. Friedberg, and C. Fox (ed.), DNA repair mechanisms. Academic Press, Inc., New York.
14. **Elledge, S. J., and R. W. Davis.** 1987. Identification and isolation of the gene encoding the small subunit of ribonucleotide reductase from *Saccharomyces cerevisiae*: DNA damage-inducible gene required for mitotic viability. Mol. Cell. Biol. **7**:2783–2793.
15. **Elledge, S. J., and R. W. Davis.** 1989. Identification of the DNA damage-responsive element of *RNR2* and evidence that four distinct cellular factors bind it. Mol. Cell. Biol. **9**:5373–5386.
16. **Finley, D., E. Ozkaynak, and A. Varshavsky.** 1987. The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation, and other stresses. Cell **48**:1035–1046.
17. **Fleer, R., C. M. Nicolet, G. A. Pure, and E. C. Friedberg.** 1987. *RAD4* gene of *Saccharomyces cerevisiae*: molecular cloning and partial characterization of a gene that is inactivated in *Escherichia coli*. Mol. Cell. Biol. **7**:1180–1192.
18. **Fornace, A. J., Jr., I. Alamo, Jr., and M. C. Hollander.** 1988. DNA damage-inducible transcripts in mammalian cells. Proc. Natl. Acad. Sci. USA **85**:8800–8804.
19. **Fornace, A. J., Jr., D. W. Nebert, M. C. Hollander, J. D. Luethy, M. Papathanasiou, J. Fargnoli, and N. K. Holbrook.** 1989. Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. Mol. Cell. Biol. **9**:4196–4203.
20. **Friedberg, E. C.** 1988. Deoxyribonucleic acid repair in the yeast *Saccharomyces cerevisiae*. Microbiol. Rev. **52**:70–102.
21. **Fukui, T., H. Kotaro, and Y. Matsudaira.** 1978. Light-flash analysis of the photoenzymatic repair process in yeast cells. I. Determination of the number of photoreactivating enzyme molecules. Mutat. Res. **51**:435–439.
22. **Fukui, A., and W. Laskowski.** 1984. Modifying factors of the cellular concentration of photolyase molecules in *Saccharomyces cerevisiae*. I. Effects of temperature and light. Photochem. Photobiol. **39**:613–617.
23. **Fukui, A., and W. Laskowski.** 1984. Modifying factors of the cellular concentration of photolyase molecules in *Saccharomyces cerevisiae*. II. Effects of preillumination with light flashes. Photochem. Photobiol. **40**:227–230.
24. **Fukui, A., and W. Laskowski.** 1985. Light-illumination effects on the cellular concentration of photolyase molecules in yeast. Radiat. Environ. Biophys. **24**:251–258.
25. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. USA **75**:1929–1933.
26. **Hittleman, W. N., and P. N. Rao.** 1974. Bleomycin-induced damage in prematurely condensed chromosomes and its relationship to cell cycle progression in CHO cells. Cancer Res. **34**:3433–3439.
27. **Holliday, R.** 1975. Further evidence for an inducible recombination repair system in *Ustilago maydis*. Mutat. Res. **29**:149–153.
28. **Hurd, H. K., and J. W. Roberts.** 1989. Upstream regulatory sequences of the yeast *RNR2* gene include a repression sequence and an activation site that binds the Rap1 protein. Mol. Cell. Biol. **9**:5359–5372.
29. **Jentsch, S., J. P. McGrath, and A. Varshavsky.** 1987. The DNA repair gene *RAD6* encodes a ubiquitin-conjugating enzyme. Nature (London) **329**:131–134.
30. **Johnson, A. L., D. G. Barker, and L. H. Johnston.** 1986. Induction of yeast DNA ligase genes in exponential and stationary phase cultures in response to DNA damaging agents. Curr. Genet. **11**:107–112.
31. **Johnson, J. L., S. Hamm-Alvarez, G. Payne, G. B. Sancar, K. V. Rajagopalan, and A. Sancar.** 1988. Identification of the second chromophore of *Escherichia coli* and yeast photolyase as 5,10-methenyltetrahydrofolate. Proc. Natl. Acad. Sci. USA **85**:2046–2050.
32. **Johnston, L. H., J. H. M. White, A. L. Johnson, G. Lucchini, and P. Plevani.** 1987. The yeast DNA polymerase I transcript is regulated in both the mitotic cell cycle and in meiosis and is also induced after DNA damage. Nucleic Acids Res. **15**:5017–5030.
33. **Kupiec, M., and G. Simchen.** 1985. Arrest of the mitotic cell cycle and of meiosis in *Saccharomyces cerevisiae* by MMS. Mol. Gen. Genet. **201**:558–564.
34. **Larimer, F. W., J. R. Perry, and A. A. Hardigree.** 1989. The *REV1* gene of *Saccharomyces cerevisiae*: isolation, sequence, and functional analysis. J. Bacteriol. **171**:230–237.
35. **Lee, M. G., and G. T. Yarranton.** 1982. Inducible DNA repair in *Ustilago maydis*. Mol. Gen. Genet. **185**:245–250.
36. **Madura, K., and S. Prakash.** 1986. Nucleotide sequence, transcript mapping, and regulation of the *RAD2* gene of *Saccharomyces cerevisiae*. J. Bacteriol. **166**:914–923.
37. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **McClanahan, T., and K. McEntee.** 1984. Specific transcripts are elevated in *Saccharomyces cerevisiae* in response to DNA damage. Mol. Cell. Biol. **4**:2356–2363.
39. **McClanahan, T., and K. McEntee.** 1986. DNA damage and heat shock dually regulate genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**:90–96.
40. **McIntosh, E. M., and R. H. Haynes.** 1986. Isolation, sequence and expression of the dCMP deaminase gene (*DCD1*) of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**:1711–1721.
41. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
42. **Mitchell, R. E. J., and D. P. Morrison.** 1983. Heat shock induction of ultraviolet light resistance in *Saccharomyces cerevisiae*. Radiat. Res. **96**:95–99.
43. **Mitchell, R. E. J., and D. P. Morrison.** 1984. Is DNA damage the signal for induction of thermal resistance? Induction by radiation in yeast. Radiat. Res. **99**:383–393.
44. **Morrison, A., R. B. Christensen, J. Alley, A. K. Beck, E. G. Bernstine, J. F. Lemontt, and C. W. Lawrence.** 1989. *REV3*, a *Saccharomyces cerevisiae* gene whose function is required for induced mutagenesis, is predicted to encode a nonessential DNA polymerase. J. Bacteriol. **171**:5659–5667.
45. **Myers, M., A. Tzagoloff, D. M. Kinney, and C. J. Lusty.** 1986. Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of *lacZ* fusions. Gene **45**:299–310.
46. **Nicolet, C. M., J. M. Chenevert, and E. C. Friedberg.** 1985. The

RAD2 gene of *Saccharomyces cerevisiae*: nucleotide sequence and transcript mapping. Gene **36**:225–234.

47. **Parker, R. C., R. M. Watson, and J. Vinograd.** 1977. Mapping of closed circular DNAs by cleavage with restriction endonucleases and calibration by agarose gel electrophoresis. Proc. Natl. Acad. Sci. USA **74**:851–855.
48. **Perozzi, G., and S. Prakash.** 1986. *RAD7* gene of *Saccharomyces cerevisiae*: transcripts, nucleotide sequence analysis, and functional relationship between the *RAD7* and *RAD23* gene products. Mol. Cell. Biol. **6**:1497–1507.
49. **Peterson, T. A., L. Prakash, S. Prakash, M. A. Osley, and S. I. Reed.** 1985. Regulation of *CDC9*, the *Saccharomyces cerevisiae* gene that encodes DNA ligase. Mol. Cell. Biol. **5**:226–235.
50. **Pizzagalli, A., P. Valsasnini, P. Plevani, and G. Lucchini.** 1988. DNA polymerase I gene of *Saccharomyces cerevisiae*: nucleotide sequence, mapping of a temperature-sensitive mutation, and protein homology with other DNA polymerases. Proc. Natl. Acad. Sci. USA **85**:3772–3776.
51. **Prentki, P., F. Karch, S. Iida, and J. Meyer.** 1981. The plasmid cloning vector pBR325 contains a 482 base-pair-long inverted duplication. Gene **14**:289–299.
52. **Reynolds, P., L. Prakash, D. Dumais, G. Perozzi, and S. Prakash.** 1985. Nucleotide sequence of the *RAD10* gene of *Saccharomyces cerevisiae*. EMBO J. **4**:3549–3552.
53. **Robinson, G. W., C. M. Nicolet, D. Kalainov, and E. C. Friedberg.** 1986. A yeast excision-repair gene is inducible by DNA damaging agents. Proc. Natl. Acad. Sci. USA **83**:1842–1846.
54. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–11.
55. **Ruby, S. W., and J. W. Szostak.** 1985. Specific *Saccharomyces cerevisiae* genes are expressed in response to DNA-damaging agents. Mol. Cell. Biol. **5**:75–84.
56. **Ruby, S. W., J. W. Szostak, and A. W. Murray.** 1983. Cloning regulated yeast genes from a pool of *lacZ* fusions. Methods Enzymol. **101**:253–268.
57. **Sancar, A., and G. B. Sancar.** 1987. DNA repair enzymes. Annu. Rev. Biochem. **57**:29–67.
58. **Sancar, G. B.** 1985. Expression of a *Saccharomyces cerevisiae* photolyase gene in *Escherichia coli*. J. Bacteriol. **161**:769–771.
59. **Sancar, G. B.** 1985. Sequence of the *Saccharomyces cerevisiae PHR1* gene and homology of the *PHR1* photolyase to *E. coli* photolyase. Nucleic Acids Res. **13**:8231–8246.
60. **Sancar, G. B., and F. W. Smith.** 1988. Construction of plasmids which lead to overproduction of yeast *PHR1* photolyase in *Saccharomyces cerevisiae* and *Escherichia coli*. Gene **64**:87–96.
61. **Sancar, G. B., and F. W. Smith.** 1989. Interactions between yeast photolyase and nucleotide excision repair proteins in *Saccharomyces cerevisiae* and *Escherichia coli*. Mol. Cell. Biol. **9**:4767–4776.
62. **Sancar, G. B., F. W. Smith, and P. F. Heelis.** 1987. Purification of the yeast *PHR1* photolyase from an *E. coli* overproducing strain and characterization of the intrinsic chromophores of the enzyme. J. Biol. Chem. **262**:15457–15465.
63. **Sarasin, A., and A. Benoit.** 1980. Induction of an error-prone mode of DNA repair in UV-irradiated monkey kidney cells. Mutat. Res. **70**:71–81.
64. **Schild, D., J. Johnston, C. Chang, and R. K. Mortimer.** 1984. Cloning and mapping of the yeast photoreactivation gene *PHR1*. Mol. Cell. Biol. **4**:1864–1870.
65. **Shapira, S. K., J. Chou, F. V. Richaud, and M. J. Casadaban.** 1983. New versatile plasmid vectors for expression of hybrid proteins coded by a cloned gene fused to *lacZ* sequences encoding an enzymatically active carboxy-terminal portion of β-galactosidase. Gene **25**:71–82.
66. **Sherman, F., G. L. Fink, and C. W. Lawrence.** 1978. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
67. **Siede, W., G. W. Robinson, D. Kalainov, T. Malley, and E. C. Friedberg.** 1989. Regulation of the *RAD2* gene of *Saccharomyces cerevisiae*. Mol. Microbiol. **3**:1697–1707.
68. **Teo, I., B. Sedgwick, B. Demple, B. Li, and T. Lindahl.** 1984. Induction of resistance to alkylating agents in *E. coli*: the *ada* gene product serves both as a regulatory protein and as an enzyme for repair of mutagenic damage. EMBO J. **3**:2151–57.
69. **Tobey, R. A.** 1975. Different drugs arrest cells at a number of distinct stages in G_2. Nature (London) **254**:245–247.
70. **Torczynski, R., A. P. Bollon, and M. Fuke.** 1983. The complete nucleotide sequence of the rat 18S ribosomal RNA gene and comparison with the respective yeast and frog genes. Nucleic Acids Res. **11**:4879–4890.
71. **Traeger, J. M., K. A. Heichman, and K. McEntee.** 1988. Expression of the yeast *UBI4* gene increases in response to DNA-damaging agents and in meiosis. Mol. Cell. Biol. **8**:1132–1136.
72. **Walker, G.** 1985. Inducible DNA repair systems. Annu. Rev. Biochem. **54**:425–457.
73. **White, J. H. M., D. G. Barker, P. Nurse, and L. H. Johnston.** 1986. Periodic transcription as a means of regulating gene expression during the cell cycle; contrasting modes of expression of DNA ligase genes in budding and fission yeast. EMBO J. **5**:1705–1709.
74. **Yasui, A., and W. Laskowski.** 1975. Determination of the number of photoreactivating enzyme molecules per haploid *Saccharomyces cerevisiae* cell. Int. J. Radiat. Biol. **28**:511–518.