MOLECULAR AND CELLULAR BIOLOGY, Nov. 1989, p. 5105–5112
0270-7306/89/115105-08$02.00/0

Vol. 9, No. 11

Evidence that Xeroderma Pigmentosum Cells from Complementation Group E Are Deficient in a Homolog of Yeast Photolyase

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Received 10 May 1989/Accepted 10 August 1989

Xeroderma pigmentosum (XP) patients are deficient in the excision repair of damaged DNA. Recognition of the DNA lesion appears to involve a nuclear factor that is defective in complementation group E (XPE binding factor). We have now identified a factor in the yeast *Saccharomyces cerevisiae* that shares many properties with XPE binding factor, including cellular location, abundance, magnesium dependence, and relative affinities for multiple forms of damaged DNA. Yeast binding activity is dependent on photolyase, which catalyzes the photoreactivation of pyrimidine dimers. These results suggest that yeast photolyase may also function as an auxiliary protein in excision repair. Furthermore, XPE binding factor appears to be the human homolog of yeast photolyase.

The environment contains a multitude of DNA-damaging agents, both naturally occurring, such as UV radiation, and man-made. In response, a variety of systems have evolved for the repair of damaged DNA. Some systems repair specific lesions in DNA, while other systems act on multiple forms of DNA damage. For example, the UV-induced cyclobutane pyrimidine dimer may be repaired either by lesion-specific or versatile damage repair systems.

Lesion-specific repair may be accomplished by the photoreactivating enzyme photolyase (reviewed in reference 8). The protein binds to the site of the pyrimidine dimer, absorbs energy from visible light through associated chromophores, and reduces the cyclobutane ring in situ to restore the DNA to its native state. Photolyase activity has been found in bacteria, plants, yeast, and animals, including marsupial mammals. However, its presence in placental mammals has never been established conclusively (20, 33).

Versatile repair of pyrimidine dimers and other lesions can be accomplished by excision repair pathways. In *Escherichia coli*, the initial events are carried out by the uvrA, uvrB, and uvrC proteins (reviewed in reference 26). These proteins act in concert to bind near the pyrimidine dimer and catalyze the endonucleolytic cleavage of the damaged DNA strand. Since the lesion itself is not the substrate for cleavage, the uvrABC complex can act on many different forms of DNA damage.

Although excision repair is well studied in bacteria, it remains poorly understood in humans. One approach is to study the autosomal recessive disease xeroderma pigmentosum (XP), which is characterized by defective excision repair (5). Cell fusion experiments have shown that patients fall into multiple complementation groups, implying that multiple gene products are involved in the excision repair pathway in humans (reviewed in reference 17). The pathway is capable of recognizing many different lesions, since XP cells are abnormally sensitive to a variety of DNA-damaging agents.

We have chosen to focus on the question: how do normal human cells recognize damaged DNA? The site of damage must be recognized reliably and efficiently, even though the lesion may have one of many different forms. Recently, we found that XP cells from complementation group E lack a nuclear factor that binds to damaged DNA. Denoted the XPE binding factor, it therefore appears to be involved in the recognition step of excision repair (4). To investigate further the nature of XPE binding factor, we searched for a similar factor in the yeast *Saccharomyces cerevisiae*. Such an approach might be fruitful, since many proteins involved in cellular metabolism are highly conserved between yeast and humans. Examples include genes involved in cell cycle control (34), gene transcription (32, 36), and, of particular relevance, DNA repair (35). Furthermore, a large number of DNA repair mutants have been generated in yeast, and many of the corresponding genes have now been cloned and sequenced (reviewed in reference 9).

By using the same gel electrophoresis binding assay that detects XPE binding factor in human cells, we have now found a factor in yeast that binds to damaged DNA and shares many characteristics with XPE binding factor. We have also found evidence that binding activity in yeast corresponds to photolyase, suggesting that XPE binding factor may be the human homolog of yeast photolyase.

MATERIALS AND METHODS

Human cell lines. The HeLa cell line and lymphoblastoid UC729-6 cell line were generously provided by Raul Berg and Marianne Dieckmann, Stanford University. UC729-6 is a human lymphoblastoid B-cell line which is hypoxanthine phosphoribosyltransferase negative (12). HeLa cells were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum. UC cells were grown in RPMI 1640 supplemented with 10% horse serum.

Yeast strains. The wild-type strain used was the homothallic diploid SK-1 (14). The following list of mutant strains indicates the relevant genotype in parentheses. The strains SFRAD1 (*rad1Δ*), SFRAD2 (*rad2::URA3*), αrad3-1 (*rad3-1*), LN116 (*rad3-2*), RF4-10S (*rad4::URA3*), CL79-6C (*rad6-1*), BJRAD10 (*rad10Δ*), LP26604A (*rad14-1*), X14-2A (*rad16-1*), CL493-1A (*rad18-2*), XS95-6C (*rad52-1*), SNM1-1 (*snm1-1*), 198a25 (*cdc8-1*), 244 (*cdc9-1*), WS8100-3A (*rev2-1*), and 16C-184 (*rev3-1*) were generously provided by Errol Friedberg and Wolfram Siede (Stanford University Medical School), and the strain GBS10 (*phr1-1*) was provided by Gwendolyn Sancar (University of North Carolina). The strains X16-9C (*rad2-1*), CMY135 (*rad6Δ*), LP2741-1B (*rad7Δ*), JG-8 (*rad8-1*), YJJ53 (*rad9Δ*), S211-1D (*rad23Δ1*),

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XKS255-1N (*rad24-1*), and XS217-5B (*phr1-1 rad2-1*) were obtained from the Yeast Genetic Stock Center (University of California at Berkeley).

UV sensitivity of yeast strains was monitored by using a "streak test" (10), and the photoreactivation phenotype was assayed as described previously (29).

Yeast strain XS217-5B was transformed by standard methods (30), with the *PHR1* gene (29) cloned in the yeast plasmid vector, YEp13 (1). The clone was a gift from David Schild (University of California at Berkeley). Transformants were selected by growth in minimal medium lacking leucine, since YEp13 carries the *LEU2* gene and this gene is mutated in XS217-5B.

Preparation of cellular extracts. Nuclear extracts from human cells were prepared as previously described (7). Yeast cell extracts were prepared by a modification of the methods described in reference 19. Yeast cells were grown either in YPD (2% bacto-peptone, 1% yeast extract, 2% glucose) or minimal medium containing 0.67% yeast nitrogen base supplemented with 2% glucose and amino acids, as required, to a density of 2×10^7 to 8×10^7 cells per ml. Spheroplasts were prepared by enzymatic digestion of the yeast cell wall by using standard methods (30). Spheroplasts were suspended in 2 to 5 ml (per 100-ml culture volume) of buffer (20% Ficoll–20 mM potassium phosphate [pH 6.5]–0.5 mM magnesium chloride–1 mM phenylmethylsulfonylfluoride). The cells were then disrupted by using a Wheaton Dounce homogenizer. The suspension was centrifuged at $5,000 \times g$ for 15 min, and the pellet was discarded. Nuclei were spun down at $25,000 \times g$ for 30 min. The supernatant representing the cytoplasmic fraction was dialyzed against buffer D, which contained 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid) (pH 7.9), 20% glycerol, 0.1 M KCl, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonylfluoride, and 0.5 mM dithiothreitol, and was clarified by centrifugation at $16,000 \times g$.

The nuclear pellet was suspended in 100 to 500 μl of buffer D and was either stored at −80°C or processed immediately. The nuclei were extracted by the addition of 3 M ammonium sulfate to a final concentration of 0.3 M and were then stirred for 20 min. The extract was centrifuged for 30 min at $16,000 \times g$, dialyzed against buffer D, and finally clarified by centrifugation at $16,000 \times g$. Extracts were stored in aliquots at −80°C.

Preparation of damaged DNA. The DNA probe used in all experiments was a 148-base pair *Pvu*II-*Hin*dIII fragment derived from the 5′ flanking and coding region of the bacterial chloramphenicol acetyltransferase gene (13). The fragment, referred to as f148, was end labeled with [α-^{32}P]dATP by using Klenow polymerase to fill in the 5′ overhang left by *Hin*dIII.

DNA was irradiated with 6,000 J/m^2 from a UV germicidal lamp while suspended in water at a concentration of 0.2 ng/μl for the f148 probe or 100 ng/μl for salmon sperm DNA. This dose of UV should convert about 6% of thymines to dimers (31). Cisplatin (7.2 μg/ml) was used to cross-link salmon sperm DNA (100 ng/μl) in phosphate buffer, by incubation at 37°C for 12 to 16 h, as previously described (3). Under these conditions, there should be about six cisplatin adducts per 100 nucleotides.

Assay for binding to damaged DNA. Unless otherwise stated, each binding reaction contained 0.2 ng of labeled probe and 1 μg of human nuclear extract or 6 μg of yeast nuclear extract. Variable amounts of the alternating copolymer poly(dI-dC)-poly(dI-dC) and salmon sperm DNA were added to mask nonspecific DNA binding. Reactions were carried out in a final volume of 10 μl at room temperature for 45 min in buffer containing 12% glycerol–12 mM HEPES (pH 7.9)–60 mM KCl–5 mM $MgCl_2$–4 mM Tris hydrochloride–0.6 mM EDTA–0.6 mM dithiothreitol, as previously described (2).

The products of the reaction were resolved by electrophoresis through 0.75-mm thick, 5% polyacrylamide gels in TBE buffer (89 mM Tris hydrochloride, pH 8, 89 mM borate, 2 mM EDTA) by using the Hoefer Scientific Instruments Mighty Small II electrophoresis apparatus at 150 volts. The gels were dried onto DE81 filter paper and autoradiographed.

Heparin agarose chromatography. Nuclear extracts (5 to 10 mg) were loaded onto a 1-ml heparin agarose column (Bethesda Research Laboratories, Inc.) equilibrated with low-salt buffer (0.1 M KCl–20 mM HEPES, pH 7.9–20% glycerol–0.5 mM EDTA–0.5 mM dithiothreitol), at a flow rate of 2 ml/h. Proteins were eluted from the column with a linear salt gradient from 0.1 to 0.5 M KCl. Fractions were stored at −80°C.

RESULTS

Yeast and human cells contain factors that bind to UV-irradiated DNA. Nuclear extracts from yeast and human cells were incubated with a ^{32}P-labeled 148-base pair DNA fragment, denoted f148, derived from the 5′ end of the bacterial chloramphenicol acetyltransferase gene (13). This fragment was chosen because it did not show binding activity to sequence-specific binding proteins. To eliminate the effects of nonspecific binding, incubations were done in the presence of excess unlabeled salmon sperm DNA and alternating copolymer, poly(dI-dC)-poly(dI-dC). The products were then resolved by electrophoresis in a nondenaturing polyacrylamide gel.

The addition of human or yeast nuclear extract shifted the mobility of the UV-irradiated DNA probe into two new bands (Fig. 1). By contrast, no mobility shift was seen with the nonirradiated probe. Furthermore, the addition of up to a 50-fold excess of unlabeled, undamaged f148 DNA did not affect binding activity. Thus, binding in both humans and yeast was independent of DNA sequence and required the presence of UV-induced damage on the DNA probe. The binding activity in human extracts was previously shown to be missing in XP cells from complementation group E (4).

The shift in electrophoretic mobility of the DNA probe appeared to be due to the formation of protein-DNA complexes, since it disappeared if yeast or human extract was incubated with pronase. Moreover, binding did not require the presence of RNA, since incubation with RNaseA had no effect. Binding was also magnesium dependent, since the omission of magnesium from incubations with either human or yeast extract caused a greater than 10-fold reduction in binding activity (data not shown).

The mobility shifts from yeast extract were somewhat smaller than those from human extracts. Since the gel conditions are nondenaturing, this may represent differences between the yeast and human proteins in molecular weight, electrostatic charge, or conformation.

It is noteworthy that the pattern of mobility shift was very similar. In both organisms, two bands were observed, with the lower band significantly more prominent than the upper band. In human extracts, the proteins in the two bands, BH1 and BH2, could not be separated by a large number of fractionation methods, including ammonium sulfate precipitation, heparin agarose, DEAE Sephacel, DNA cellulose, or

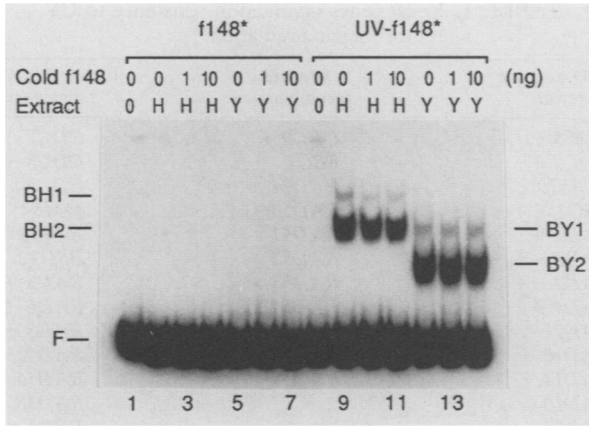

FIG. 1. Identification of factors which bind UV-damaged DNA. DNA probe labeled with ^{32}P was either intact (f148*) or UV-irradiated (UV-f148*) and incubated with HeLa cell nuclear extract (H), yeast nuclear extract (Y), or no extract (O). Binding reactions with HeLa extract contained 800 ng of poly(dI-dC)-poly(dI-dC) and 200 ng of salmon sperm DNA. Reactions with yeast nuclear extract contained 300 ng of poly(dI-dC)-poly(dI-dC) and 50 ng of salmon sperm DNA. Various amounts of cold, undamaged f148 DNA were also added, to compete for sequence-specific binding proteins. The products of the binding reaction were resolved by electrophoresis in a polyacrylamide gel. F indicates the position of free probe. BH1 and BH2 indicate protein-DNA complexes formed with human extract. BY1 and BY1 indicate complexes with yeast extract.

UV-irradiated DNA Sepharose chromatography (G. Chu and E. Chang, unpublished data). A plausible explanation is that a single protein in human extracts is responsible for both bands, forming two different complexes with DNA either as a monomer or as a dimer. The same result was found in yeast extracts, although characterization was not as extensive: the two bands, BY1 and BY2, could not be separated by either ammonium sulfate precipitation or heparin agarose chromatography.

The yeast and human binding factors have the same relative affinities for different forms of DNA damage. To study the affinity of the binding factors for damaged DNA, the extracts were incubated with UV-irradiated probe DNA in the presence of unlabeled competitor DNA in various forms (Fig. 2). Salmon sperm double-stranded DNA was used as a competitor because it is highly heterogeneous in sequence. It was added to the incubations in one of three forms: undamaged, UV-irradiated, or cisplatin cross-linked. In addition, competitor DNA from the bacteriophage M13 was added in four forms: double stranded, UV-irradiated double stranded, single stranded, or UV-irradiated single stranded.

The binding activity was quantified by scanning laser densitometry of the autoradiographs in Fig. 2, in order to estimate the amount of competitor required for a 50% reduction in binding (see Table 2). Thus, the competition experiments suggested that the yeast and human factors bound to five different DNA substrates with similar affinities. Their relatively high affinity for single-stranded DNA is noteworthy, suggesting that these factors might recognize any DNA adduct that disrupts the DNA duplex to form a local region of single-stranded DNA.

The yeast and human factors are localized to the nucleus and have the same abundance in relation to genome size. In the preparation of extracts from either yeast or human cells, there was a centrifugation step in which the nuclei were sedimented, leaving a soluble cytosolic fraction. Binding activity could then be measured in both nuclear and cytoplasmic compartments of the cell. In humans, XPE binding factor was found exclusively in the nucleus (4). Likewise, the yeast factor was found entirely in the nucleus (Fig. 3, lanes 1 through 5).

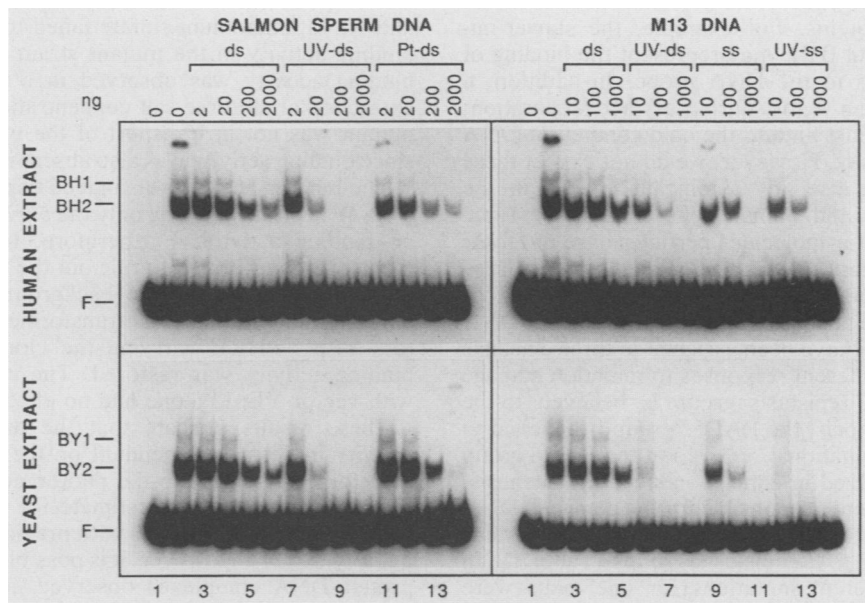

FIG. 2. Specificity of binding factors for different forms of damaged DNA. HeLa cell nuclear extract together with 1,000 ng of poly(dI-dC)-poly(dI-dC) (upper panels) or yeast nuclear extract with 300 ng of poly(dI-dC)-poly(dI-dC) (lower panels) were incubated with UV-irradiated ^{32}P-labeled f148 DNA. Competitor DNA was added as undamaged double-stranded DNA (ds), undamaged single-stranded DNA (ss), UV-irradiated DNA (UV-ds and UV-ss), or cisplatin cross-linked (Pt-ds) DNA. Lane 1 in each panel represents a control which contained no extract.

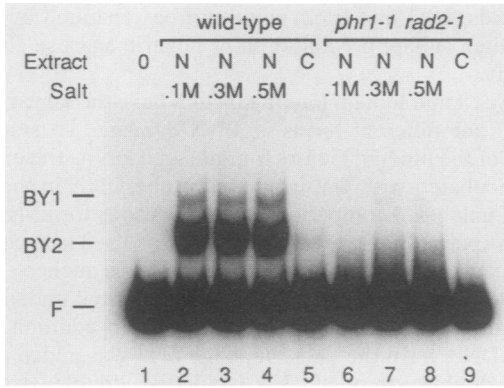

FIG. 3. Binding activity is localized in the nucleus and absent in a repair deficient yeast mutant. Nuclei (N) from yeast strains SK-1 (wild type) and XS217-5B (*phr1-1 rad2-1*) were extracted with ammonium sulfate at concentrations of 0.1, 0.3, and 0.5 M. Cytoplasmic extracts (C) were also prepared from each strain. In all binding reactions except the zero extract control, 6 μg of yeast extract was incubated with UV-irradiated f148 probe DNA in the presence of 300 ng of poly(dI-dC)-poly(dI-dC) and 50 ng of salmon sperm DNA.

A lower limit for the abundance of the binding factors can be calculated by assuming a 1:1 binding stoichiometry to the DNA probe. Thus, there are approximately 10,000 molecules of XPE binding factor in human cells (4). An analogous calculation yields an estimate of about 50 molecules of yeast binding factor per cell. The presumed function of these binding factors is to search the genome for DNA damage. Since the human diploid genome contains about 6,000 megabase pairs and the yeast genome contains about 28 megabase pairs (21), the abundance of the binding factors is about 1.7 molecules in humans and 1.8 molecules in yeast per megabase of genomic DNA. Thus, the yeast and human factors have almost identical abundances with respect to genome size.

Our calculations may underestimate somewhat the actual abundance of the proteins. For example, the slower-migrating bands, BH1 and BY1, may represent the binding of two protein molecules to the DNA probe. In addition, a small amount of protein is lost during extract preparation, and some molecules must bind to the cold competitor DNA used in the binding assay. However, we do not expect these sources of error to cause any significant change in the conclusion that yeast and human cells contain the same number of binding factor molecules per megabase of DNA.

Binding activity in yeast is dependent on photolyase. A large number of yeast mutants abnormally sensitive to UV or ionizing radiation have been generated for the study of DNA repair. The mutant loci have been assigned to three epistasis groups representing different responses to radiation damage (Table 1). The *RAD3* epistasis group is believed to be involved in excision repair, the RAD52 group is believed to be involved in recombinational repair, and the RAD6 group is believed to be involved in damage-induced mutagenesis. To identify the yeast gene responsible for the damaged DNA binding activity, we screened a large number of yeast strains with mutations in the *RAD* epistasis groups (Table 1). In some cases, two different mutations for one locus were examined. Because the *RAD3* epistasis group is associated with excision repair, our initial efforts concentrated on that group, and 13 of 15 loci were tested. Overall, a total of 23 mutants representing 20 independent *RAD* loci were examined, and binding activity was observed in each case.

TABLE 1. Yeast genes involved in resistance to UV or ionizing radiation[a]

RAD3 excision repair	*RAD52* recombination	*RAD6* mutagenesis
CDC8 +	*CDC9* +	*CDC7*
CDC9 +	*PSO1*	*CDC8* +
MMS19	*RAD24* +	*CDC40*
PSO2 +	*RAD50*	*MMS3*
RAD1 +	*RAD51*	*NGM2*
RAD2 ++	*RAD52* +	*PSO1*
RAD3 ++	*RAD53*	*RAD5* +
RAD4 +	*RAD54*	*RAD6* ++
RAD7 +	*RAD55*	*RAD8* +
RAD10 +	*RAD56*	*RAD9* +
RAD14 +	*RAD57*	*RAD15*
RAD16 +		*RAD18* +
RAD23 +		*REV1*
RAD24 +		*REV3* +
UVS12		*REV5*
		REV6
		REV7
		UMR1
		UMR2
		UMR3

[a] The table is adapted from reference 9. Genes are divided into the three epistasis groups thought to represent distinct responses to DNA damaging agents. Each epistasis group is named after a prominent gene in that group. A plus (+) sign represents a yeast strain carrying a mutation at the indicated locus, which was tested for binding activity. Two plus signs indicate that two different mutant alleles were tested in independent strains. Every strain tested in this table showed binding activity.

By contrast, binding activity was missing in a yeast strain (*phr1-1 rad2-1*) which carried mutations in *PHR1*, the gene encoding the photoreactivating enzyme photolyase, and *RAD2* (Fig. 3, lanes 6 through 9). Note that a strain carrying the *rad2-1* mutation alone had normal binding activity (Fig. 4, lane 3). The absent binding activity could not be recovered even when the nuclei were extracted over a wide range of salt concentrations. Nuclear extracts prepared at 0.1, 0.3, and 0.5 M ammonium sulfate failed to show any detectable binding activity in the mutant strain (Fig. 3). By contrast, binding activity was observed in a wild-type strain after extraction at all three salt concentrations. The defect in the mutant was not in transport of the protein to the nucleus, since binding activity was also absent in cytoplasmic extract.

The binding activity was indeed dependent on photolyase (Fig. 4). A strain carrying only the *phr1-1* mutation contained no binding activity. Furthermore, transformation experiments were performed to rule out the possibility of undetected differences in genetic background. When the *rad2-1 phr1-1* double mutant was transformed with a plasmid vector, YEp13-PHR1, carrying the cloned *PHR1* gene (29), binding activity was restored. The control transformation with vector YEp13 alone had no effect on binding activity.

These results indicate that the damaged-DNA binding activity in yeast is dependent on the *PHR1* gene and may therefore correspond to the photoreactivating enzyme photolyase. Moreover, our estimate for the abundance of the yeast binding factor agrees with previous estimates for yeast photolyase (11). However, it is possible that either of the two protein-DNA complexes observed in the gel assay might contain other proteins in tight association with photolyase.

Evidence that the binding factors have photoreactivating activity in yeast but not humans. The repair of pyrimidine dimers by photoreactivation occurs in two steps. First, photolyase binds to the pyrimidine dimer. Second, in the

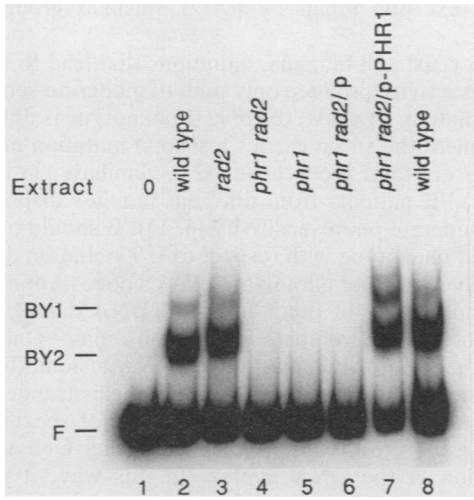

FIG. 4. Binding activity in yeast is dependent on the *PHR1* gene. Nuclear extracts were prepared from various yeast strains, the relevant genotypes of which are indicated (*phr1* and *rad2* are abbreviations for *phr1-1* and *rad2-1*). All reactions contained UV-irradiated f148 probe DNA, 300 ng of poly(dI-dC)-poly(dI-dC), and 50 ng of salmon sperm DNA. The extracts used in lanes 6, 7, and 8 were from the XS217-5B strain (*phr1-1 rad2-1*) transformed with either YEp13 (lane 6) or YEp13-PHR1 (lane 7). The extracts used for lanes 6 through 8 were prepared from yeast grown in minimal medium to select for the presence of the *LEU2* gene carried by YEp13. Only 3 μg of extract was used per binding reaction, compared with the normal 6 μg used in lanes 2 through 5.

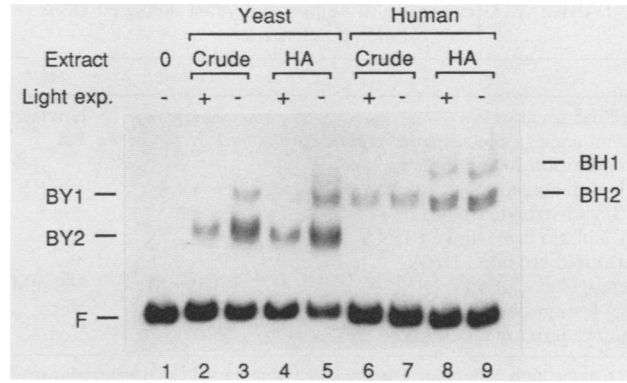

FIG. 5. Binding activity in yeast but not humans shows evidence for photolyase activity. A UV-irradiated f148 probe was incubated with yeast or human (UC cell) nuclear extract, which was either crude or partially purified by heparin agarose chromatography (HA). The binding reactions contained: 60 μg of yeast crude extract, 3 μg of poly(dI-dC)-poly(dI-dC), and 500 ng of salmon sperm DNA (lanes 2 and 3); 1 μg of yeast HA extract, 300 ng of poly(dI-dC)-poly(dI-dC), and 25 ng of salmon sperm DNA (lanes 4 and 5); 25 μg of human crude extract, 10 μg of poly(dI-dC)-poly(dI-dC), and 5 μg of salmon sperm DNA (lanes 6 and 7); 3.5 μg of human HA extract, 8 μg of poly(dI-dC)-poly(dI-dC), and 2 μg of salmon sperm DNA (lanes 8 and 9). In each reaction the extract was added last, under safelight illumination (Kodak OC filter). The reactions were then divided into two tubes: one tube was incubated in the dark and the other was exposed to ambient laboratory light. The dark samples were then loaded onto the gel and subjected to electrophoresis under a safelight.

presence of light, the dimer is repaired by cleavage of the cyclobutane ring, and the protein then dissociates from the DNA (reviewed in reference 8).

We reasoned that if the yeast and human binding factors contained photoreactivating activity, the level of binding should be reduced after exposure to light, compared with binding in the dark. To test for activity, the binding reactions were carried out with increased extract, so that more than half of the probe DNA was contained in a protein-DNA complex.

When the experiment was done with crude yeast nuclear extract, the binding activity was reproducibly reduced at least threefold in the presence of light (Fig. 5). Furthermore, photoreactivating activity copurified with binding activity after chromatographic fractionation of crude yeast extract on a heparin agarose column. Peak binding activity eluted at 350 mM KCl, yielding a 60-fold purification. Comparison between lanes 2 and 4 shows that photoreactivating activity was the same in both crude and partially purified yeast extracts, despite the addition of 60-fold less partially purified extract. This is evidence that yeast binding factor contains photoreactivating activity.

By contrast, both crude and partially purified human extracts showed no photoreactivating activity. Human extracts were subjected to heparin agarose chromatography in the same manner as the yeast extracts. Peak binding activity eluted at 310 mM KCl, yielding a 25-fold purification. Photoreactivation could not be observed in experiments with different light sources, including ambient fluorescent light and black light (General Electric blue-black bulbs). These light sources always showed photoreactivation in yeast extracts.

Thus, the gel assay was able to detect evidence for photoreactivation by the yeast binding factor. However, the same assay was not able to demonstrate photoreactivation by XPE binding factor. Therefore, although the yeast and human binding activities were similar in many ways, the human protein appeared to be incapable of photoreactivating DNA.

DISCUSSION

Yeast and human cells contain similar damaged-DNA binding factors. We have found that a gel electrophoresis binding assay with a UV-irradiated DNA probe detected factors in both yeast and humans which bound specifically to damaged DNA. In humans, the factor (XPE binding factor) was missing in XP cells from complementation group E (4).

There were many of similarities between the yeast and human factors (Table 2). In both organisms, the assay detected two bands representing two different protein-DNA complexes. In each case, the faster-migrating complex was much more abundant. In yeast and humans, the binding activity was dependent on the presence of magnesium, sensitive to protease but not RNase treatment, and localized to the nucleus.

The yeast and human factors were present in equal abundance: about 2 molecules of binding factor per megabase of genomic DNA, if 1:1 binding stoichiometry is assumed. Both factors had the same relative affinities for different forms of DNA. In particular, they bound to UV-irradiated DNA, undamaged single-stranded DNA, cisplatin cross-linked DNA, and undamaged double-stranded DNA with decreasing affinities.

XPE binding factor and yeast photolyase. An extensive survey of 23 yeast *rad* mutants representing 20 independent loci failed to reveal any abnormalities in binding activity. Notably, 15 mutants representing all but two loci from the *RAD3* epistasis group showed normal binding activity. This was particularly significant, since genes from that epistasis group are involved in excision repair.

TABLE 2. Comparison of human and yeast damaged DNA binding activities

	Human	Yeast
Cellular location	Nucleus	Nucleus
Abundance (molecules per megabase)	1.7	1.8
Competition for binding[a]		
UV-damaged DNA (ds or ss)	++++	++++
Single-stranded DNA	+++	+++
Cisplatin cross-linked DNA	++	++
Double-stranded DNA	+	+
Hep-ag salt elution[b]	310 mM	350 mM
Magnesium dependence	Yes	Yes
Photoreactivating activity	No	Yes

[a] Competition for binding was estimated from the gel electrophoresis assay, using 0.2 ng of probe DNA (see Fig. 2). The amount of competitor DNA required for a 50% reduction of binding activity was: 2 to 10 ng (++++); 10 to 100 ng (+++); 20 to 200 ng (++); 200 to 2,000 ng (+).

[b] Hep-ag salt elution represents the concentration of KCl at which the peak of binding activity eluted from a heparin agarose column.

By contrast, binding activity was not detectable in a photolyase mutant. Moreover, binding was restored by transformation of the mutant strain with the cloned photolyase gene *PHR1*. This is genetic evidence that the yeast binding activity is encoded by the *PHR1* gene. In support of this, photoreactivating activity and binding activity copurified from a heparin agarose column.

The extensive similarities between the yeast and human factors suggest that XPE binding factor is the human homolog of yeast photolyase. This is surprising because photoreactivating activity has not been established in placental mammals. Although there are reports of a weak photoreactivating activity in human cells (33), subsequent work has suggested that this activity might not represent enzyme-mediated photoreactivation (20).

The absence of photolyase in placental mammals is puzzling from an evolutionary point of view. Enzymatic photoreactivation is present in a wide variety of organisms, including bacteria, yeast, plants, insects, fishes, reptiles, birds, and even marsupial mammals (8). The findings in this report suggest an explanation for the disappearance of photolyase in placental mammals. The protein has retained the ability to bind UV-damaged DNA. However, it may have lost most, if not all, of its photoreactivating activity. Of course, demonstration that XPE binding factor is homologous to yeast photolyase must await isolation of the human gene.

Photolyase and excision repair. Our results suggest that photolyase has a second function in addition to photoreactivation. The XP syndrome arises from defective excision repair, and XP complementation group E patients display the dermatologic manifestations typical for that syndrome. Thus, XPE binding factor participates in excision repair. Because of its extensive similarity to XPE binding factor, we propose that yeast photolyase is also involved in excision repair. In fact, both yeast and human factors were capable of recognizing multiple forms of DNA damage, a property expected of a protein involved in the recognition step of excision repair.

Other laboratories have reported evidence for the involvement of photolyase in excision repair. In *E. coli*, the addition of purified photolyase causes a twofold stimulation of the repair of pyrimidine dimers by purified uvrA, uvrB, and uvrC proteins in vitro (25). Furthermore, genetic experiments in yeast suggest that expression of the *PHR1* gene enhances excision repair by *RAD3* epistasis group genes (27).

In both yeast and humans, mutations that lead to the loss of binding activity produce only mild to moderate sensitivity to UV radiation. In yeast, the *phr1-1* phenotype is difficult to detect, unless the strain carries a second mutation in one of the *RAD3* epistasis group genes (23). Similarly, in humans multiple XPE patients from different families display only mild to moderate photosensitivity (6, 15). It should be noted that a mild phenotype with respect to UV radiation does not necessarily imply that photolyase plays a nonessential role in the excision repair of other forms of DNA damage. How might photolyase participate in excision repair? One possibility is that photolyase may target DNA lesions for other excision repair proteins. Its affinity for single-stranded DNA is a clue to how it can have a broad range of specificity. It may not recognize the lesion itself but rather an associated deformation of the DNA duplex. In this way, it may be capable of recognizing any adduct that causes a local denaturation or bending of the double helix.

Photolyase and the leucine zipper motif. Photolyase and XPE binding factor may recruit other components of the excision repair system through protein-protein interactions. In this context, it is intriguing to note that the predicted amino acid sequences of yeast and *E. coli* photolyase contain features characteristic of the leucine zipper motif found in other DNA binding proteins (18). The motif, which is characterized by the periodic repetition of a leucine at every seventh residue, appears to stabilize the association of proteins into either homodimers or heterodimers (16, 28). Adjacent to the N terminus of the leucine zipper domain, many such proteins also contain a basic region. Dimerization appears to allow tighter binding by juxtaposing the two basic regions that contact the DNA. Synthetic peptides from yeast GCN4 form a leucine zipper as a coiled coil of two alpha helices (22). In fact, leucine zipper domains often contain a 4-3 heptad repeat of hydrophobic residues characteristic of coiled coil proteins.

Photolyase contains a repeat of four leucines in yeast and three leucines in *E. coli* (Fig. 6A). The fourth position in *E. coli* is a substitution with another hydrophobic residue, alanine. In both organisms, the leucine repeats are part of a 4-3 heptad of hydrophobic residues repeated over eight turns of the proposed helix, broken only by a lysine (Lys-68) in the last turn of the *E. coli* helix. The conserved proline next to the last repeated leucine probably indicates termination of the helix near that residue. Attesting to the possible functional significance in this region, 13 of 30 amino acids are exactly conserved. Furthermore, both proteins are strongly basic adjacent to the N terminus of the leucine repeats, with a net charge of +16 over 126 amino acids in yeast and +6 over 44 amino acids in *E. coli*. When the leucine repeat domains are displayed on a helical wheel, they are amphipathic (Fig. 6B). Two spokes of the wheel are hydrophobic, with one spoke containing the leucine repeats. Significantly, six of eight amino acids are conserved on this surface, which forms the proposed leucine zipper.

Thus, photolyase in yeast and *E. coli* contains a conserved domain with a leucine zipper motif which is especially striking in yeast. This region may mediate the formation of a heterodimer between photolyase and a second protein involved in the excision repair pathway. It is also possible that the study of protein interactions with the XPE binding factor may lead to the identification of the defects in other XP complementation groups.

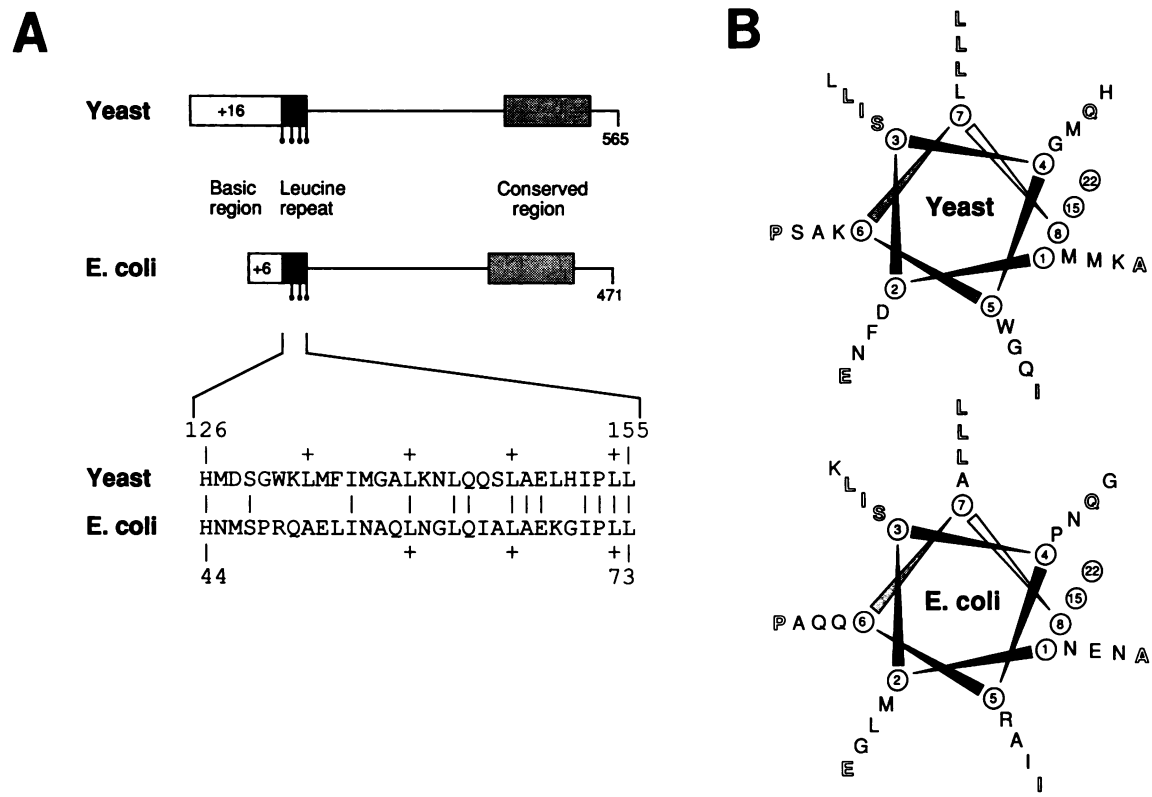

FIG. 6. Proposed leucine zipper domain in photolyase. (A) Photolyase in yeast and *E. coli*. The basic regions at the N terminus have the indicated net positive charges. The amino acid sequence indicates the leucine repeat with a plus sign (+). There is also a strongly conserved region near the C terminus. Sequence data is from references 24 and 37. (B) Helical wheels of the leucine repeat regions. Each position represents a rotation of 100 degrees around the axis of the helix. Every seven residues produce slightly less than two complete turns of the helix. The amino acids conserved between yeast and *E. coli* are indicated in block letters.

ACKNOWLEDGMENTS

We thank Rebecca Contopoulou, Jane Cooper, Errol Friedberg, Gwendolyn Sancar, and Wolfram Siede for providing yeast strains; David Schild for the *PHR1* yeast DNA clone; and Mingjie Cai for help with the preparation of yeast extracts. We thank Paul Berg, Elaine Chang, Ron Davis, Steve Elledge, Errol Friedberg, Ann Ganesan, Phil Hanawalt, Daniel Markowitz, and Susan Marqusee for helpful discussions; Pat Brown, Andy Buchman, Susan Klapholz, and Alan Sachs for critically reading the manuscript; and Phyllis Mayberg for help in the preparation of the manuscript.

M.P. is an EMBO postdoctoral fellow, and G.C. is a Rita Allen Foundation Scholar. This work was supported by Public Health Service grant CA44949 from the National Institutes of Health, American Cancer Society grant CD-332, and funds given in honor of Anna Nissen (G.C.).

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