Promoter elements of the *PHR1* gene of *Saccharomyces cerevisiae* and their roles in the response to DNA damage

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ABSTRACT

The *PHR1* gene of *Saccharomyces cerevisiae* encodes the apoenzyme for the DNA repair enzyme photolyase. *PHR1* transcription is induced in response to 254 nm radiation and a variety of chemical damaging agents. We report here the identification of promoter elements required for *PHR1* expression. Transcription is regulated primarily through three sequence elements clustered within a 120 bp region immediately upstream of the translational start site. A 20 bp interrupted palindrome comprises UAS_{PHR1} and is responsible for 80–90% of basal and induced expression. UAS_{PHR1} alone can activate transcription of a *CYC1* minimal promoter but does not confer damage responsiveness. In the intact *PHR1* promoter UAS function is dependent upon an upstream essential sequence (UES). URS_{PHR1} contains a binding site for the damage-responsive repressor Prp; consistent with this role, deletion or specific mutations of the URS increase basal level expression and decrease the induction ratio. Deletion of URS_{PHR1} also eliminates the requirement for UES_{PHR1} for promoter activation, indicating that the UES attenuates Prp-mediated repression. Sequences within UAS_{PHR1} are similar to regulatory sequences found upstream of both damage responsive and nonresponsive genes involved in DNA repair and metabolism.

INTRODUCTION

Altered expression of specific genes is one of the hallmarks of the DNA damage response in living cells and plays an essential role in cell survival and maintaining the stability of the genetic material. While damage-inducible genes have been identified in numerous organisms, regulation of the damage response has been most extensively characterized in *Escherichia coli* and *Saccharomyces cerevisiae*. In these organisms, exposure to far ultraviolet radiation or a variety of chemical agents which interfere with DNA replication leads to the temporally coordinated transcriptional induction of multiple unlinked genes involved in DNA repair, recombination, mutagenesis and cell cycle control. The primary

sensory network that directs this response in *E. coli* is comprised of two proteins, RecA and LexA (see ref. 1 for a review). RecA is the damage sensor and signal transducer of the network, while LexA, the common repressor of the damage-responsive genes, is the downstream target. Upon binding to single stranded DNA, RecA is activated to a form which interacts efficiently with LexA. This interaction stimulates the repressor's latent protease activity, leading to autoproteolysis of the repressor and consequent activation of transcription. While LexA-independent regulation has been reported for at least one UV-induced gene (2), it is clear that the large majority of UV-inducible genes in *E. coli* are regulated through this simple two component system.

Regulation of the damage response in *S. cerevisiae* is less well understood and is clearly much more complex. The 5' flanking sequences of the known damage responsive genes do not contain a single common sequence that could serve as the binding site for a damage-responsive global transcription regulator, suggesting that the sensory network acts upon multiple downstream targets (reviewed in ref. 3). Recent studies on the regulation of the ribonucleotide reductase (*RNR*) genes have revealed a multi-component pathway for transduction of the damage signal which operates on a subset of damage-responsive genes. Enhanced transcription of the *RNR2* and *RNR3* genes in response to hydroxyurea (HU) and methylmethane sulfonate (MMS) treatment requires protein kinases encoded by the *DUN1* and *SAD1* (*RAD53/SPK1/MEC2*) genes (4,5). *dun1* and *sad1* mutants fail to induce the *RNR* genes in response to these agents and are also UV sensitive. However, these mutants are not defective for induction of the UV- and MMS-responsive genes *UBI4* (polyubiquitin) and *DDR48* (4,5), suggesting the existence of at least two independent damage-response pathways (6). Pole has been implicated as a sensor of replication blocks and thus of certain types of damage during S-phase, because a class of *pol2* (*dun2*) mutants are defective in the activation of the S-phase checkpoint following HU treatment and fail to activate Dun1 kinase activity in response to MMS or HU treatment. However, in response to UV damage the same *pol2* mutants support activation of Dun1 kinase activity, significant *RNR3* induction, and activate the G1 and G2/M DNA damage checkpoints (6). Together these results suggest that in yeast and probably higher eukaryotes as well, there are multiple damage response pathways which employ at least some unique

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Figure 1. Plasmids used to define *PHR1* regulatory elements. The enlarged region below each plasmid shows the 5' flanking regions with restricted sites used in the construction of the reporter plasmids described in Materials and Methods. The black filled area indicated vector sequences, gray filled areas indicate yeast DNA from *PHR1* (pGBS116; 9) and *CYC1* (pLG669Z; 14), while the hatched boxes indicate the *CYC1* UASs. +1 is the first A in the translational start site of the fusion protein. 'T' indicates the positions of TATA boxes in the *CYC1* promoter (26). Bent arrows indicate translational start sites.

components to sense the damage, transduce the signal and target specific transcriptional regulators.

To further define the damage response pathway in yeast, we have undertaken a detailed study of the transcriptional regulation of the *PHR1* gene. *PHR1* encodes the apoenzyme of the DNA repair enzyme photolyase (7). This enzyme carries out the light-dependent repair of cyclobutane pyrimidine dimers, the most frequent lesion induced in DNA by far UV radiation, and in addition stimulates the light-independent removal of dimers via the nucleotide excision repair pathway (for a review see ref. 8). *PHR1* transcription is induced in response to far UV radiation as well as by exposure to MMS, 4NQO, MNNG, and bleomycin (9), however the enzyme does not repair DNA lesions induced by the latter chemical agents. Unlike the majority of damage inducible genes characterized in yeast (10), the only known function of the *PHR1* gene and photolyase is DNA repair. Thus study of the regulatory mechanisms that control *PHR1* transcription should shed light on damage responsive regulation specifically. We have previously demonstrated that the response of *PHR1* to UV and MMS is mediated, at least in part, through a damage responsive transcriptional repressor which we call Prp. Prp binds to the *PHR1* promoter region in the absence of DNA damage and binding rapidly disappears following damage (11). In the current

study we sought to identify additional promoter elements that contribute to the *PHR1* damage response and to further define the Prp binding site.

MATERIALS AND METHODS

Yeast strains, growth conditions and transformations

All experiments were performed using yeast strain GBS76 [*MATa rad2 phr1-1 ura3-52 leu2-3, 112* (9)] which was cultured at 30°C in synthetic complete (SC) medium lacking uracil (12). Transformations were carried out using a lithium acetate procedure (13).

Construction of *PHR1* promoter deletions and mutations

Plasmid pGBS116 is a yeast-*E.coli* shuttle vector carrying 352 bp of *PHR1* 5' flanking sequence as well as 282 bp of coding sequence joined in-frame to the *E.coli lacZ* gene (Fig. 1; 9). pGBS145 is a derivative of pGBS116 in which the region footprinted by Prp (–116 to –96 relative to the translational start site) has been deleted (11). Derivatives of pGBS116 and pGBS145 were constructed as follows. pGBS256 was constructed by *Bal31* digestion beginning at the *NarI* site of pGBS116, followed by intramolecular ligation. All other derivatives were constructed using the PCR and oligonucleotide primers carrying the desired deletions or mutations. Sequences of the oligonucleotide primers are shown in Table 1. Unless stated otherwise, pGBS116 was used as template for the PCR. Plasmids pGBS265, pGBS270, pGBS273, pGBS277 and pGBS279 carry deletions or mutations between the *NarI* and *BglII* sites only and were constructed using primers incorporating the *NarI* site for top strand synthesis and oligonucleotide PHRBgl, which includes the *BglII* site at +46 to +51, as the bottom strand primer. The resulting PCR products were digested with *NarI* and *BglII* and ligated into *NarI*-*BglII* digested pGBS116 thereby replacing the wild type fragment. pGBS265 and pGBS270 were constructed using oligonucleotides D2 and M1 respectively. pGBS273 and pGBS277 were produced using oligonucleotides M1 and M3 respectively and pGBS145 as template, while pGBS279 was constructed using oligonucleotide M3 with pGBS270 as template. Plasmids containing mutations incorporating or upstream from the *NarI* site were constructed in two sequential PCRs in which the product of the first reaction was purified from a nondenaturing polyacrylamide gel and used as one of the primers for the second PCR. Plasmids pGBS263, pGBS267 and pGBS271 were constructed using oligonucleotide PHRBgl in conjunction with oligonucleotides D1, D3 or M2 respectively in the first PCR; the second PCR utilized as top strand primer oligonucleotide PHRXba, which incorporates the *XbaI* site at –329 to –324 in the *PHR1* 5' flanking sequence. The product of the second PCR was digested with *XbaI* and *BglII* and ligated into *XbaI*-*BglII* digested pGBS116. Plasmids pGBS282, pGBS283, pGBS285 and pGBS288 were constructed using a similar strategy in which oligonucleotide PHRXba was used in conjunction with oligonucleotides D4, D5, D6 or M4 respectively in the first synthesis reaction, then the purified products were used as primers in conjunction with oligonucleotide PHRBgl in the second PCR. pGBS292 was constructed using a strategy identical to that used for pGBS288 except that pGBS145 was used as template in the second PCR. The nucleotide sequence of the entire PCR-amplified region was determined for each of the constructs.

Plasmids carrying point mutations within the Prp binding site were constructed using the PCR and oligonucleotide primers

PrpMut and PHRBgl. Following digestion with *BfrI* and *BglII* the PCR products were ligated into *BfrI*-*BglII* digested pGBS116 to produce a library of plasmids containing mutations at -67, -63, -62 and -58. Individual clones were isolated from transformed *E.coli* and sequenced, and mutants were identified by DNA sequencing.

Insertion of *PHR1* promoter elements into the *CYC1* promoter

pLG669Z is a yeast-*E.coli* shuttle vector containing the 5' regulatory region of the yeast *CYC1* gene and the translation start site fused in frame to *lacZ* (Fig. 1; 14). Plasmids containing various elements from the *PHR1* promoter inserted upstream from *CYC1* TATA sequences were constructed by digesting pLG669Z with *SmaI* and *SphI*, followed by ligation to the desired *PHR1* sequences to which *SmaI* and *SphI* compatible ends had been added. Hybridization of the complementary oligonucleotides PAL1 and PAL2 or MIXPAL1 and MIXPAL2 (Table 1) produced the wild type and mutant UAS_{PHR1} sequences inserted into pPAL and pMIXPAL respectively. The *PHR1* -322 to -125 region containing UES_{PHR1} was amplified from pGBS116 by PCR using oligonucleotides M5 and M6 (Table 1) to introduce *SmaI* and *SphI* sites, then ligated into these sites in pLG669Z, yielding plasmid pGBS289. All constructs were verified by nucleotide sequence analysis.

UV irradiation and β -galactosidase assays

Log phase liquid grown cells were collected by centrifugation, suspended in phosphate-buffered saline, irradiated and sampled as described (9), except that 1.5 ml aliquots of the cultures were collected in triplicate. UV fluences of 30 and 40 J/m² were used, which yielded 25–50% cell survival. β -galactosidase assays were performed as previously described except that the substrate was methylumbelligalactoside and the amount of 4-methylumbelliferone produced was determined by fluorescence spectroscopy. One unit of β -galactosidase activity is defined as 1 pmol of product per ml of culture per A₆₀₀ unit of cells in a 30 min assay. At least two isolates of each construct were tested, all assays were performed in triplicate and each construct was tested on at least two different occasions. Values obtained at the two UV fluences were averaged. While the standard deviation of the values obtained on any day was usually 10% or less, we noted up to 40% variation when comparing results of assays performed on different days. These variations were taken into account by expressing all values relative to the basal level expression of pGBS116/GBS76 assayed on the same day. The average basal level expression of pGBS116/GBS76 in 14 different experiments was 1002 units with a standard deviation of 281 units. All clones critical to the delineation of transcriptional regulatory regions were also assayed together at least once.

Table 1. Oligonucleotides used in this study

Name	Sequence ^a	Comments ^b
PHRBgl	TTCTATATCCAGCCGAGATCTCTTACTAGCATA	+66' to +34'
PHRXba	CTCGAAATTGTTATCTAGAAGTTTATGCATAATCAA	-24 to -307, M307
D1	TCCCGAACTACATCCACTTTTCTTCCTCG	-155 to -109, Δ (-142 to -125)
D2	CGCCACTTTTGTCAAATTAAACCTTAAGGG	-126 to -74, Δ (-116 to -94)
D3	TATCCCGAACCCTTTTCTTCCTCGTTTTTCG	-157 to -102, Δ (-147 to -125)
D4	GAGGAAGAAAAGTGGTAAAAGCGAGATATGTAGTTCGGG	-110' to -154', Δ (-130' to -125')
D5	AGGAAGAAAAGTGGGCGAGATATGTAGTTCGGG	-111' to -154', Δ (-135' to -125')
D6	AAAACGAGGAAGCGCCAGTAAAAGCGA	-106' to -139', Δ (-124' to -117')
M1	CTCGCTTTTACTGGCGCCACTTTTACCAACTATTTTTCGAGGA	-140 to -94, M (-116 to -109)
M2	CAATTATTTTATCCCAGGTTACATATCTCGCT	-167 to -135, M (-151 to -148)
M3	CTCGCTTTTACTGGCGCCACTTTTACCAACTATTTTTCGAGTACTCAGTCAAATTAACC	-140 to -79, M (-116 to -109), M (-103 to -96)
M4	CGAGGAAGAAAAGTGGTACTGTGCTGATGACCTCGTAAGTGGATGATAAAATAATTG	-109' to -167', M (-151' to -125')
M5	GTTTATGCAAAATCAAACTCCCCGGGTATCCAGAAATCATCA	-322 to -287, * (-302 to -301)
M6	TGTATGCATGCCCGCAGTAAAAGCGAGA	-125' to -139'
PAL1	GGGCTTTCCTCGTTTTTCGAGGAAGCATG	-116 to -94
PAL2	CTTCCTCGAAAAACGAGGAAGCCC	-96' to -116'
MIXPAL1	GGGATAGTGCAGCCGTAGTGCTGCAATG	
MIXPAL2	CAGGCACTACGGTCGCACTATCCC	
PrpMut	TCGACTTAAACCTTAAGGGGTGAAHTATHDTTADTTTGACA	-92 to -51, M (-67, -63, -62, -58)

^aAll sequences are shown 5'→3'. Italics indicate sequences added to introduce new restriction sites. H indicates C or A or T while D indicates A or G or T.

^bNumbers refer to locations, relative to the translation start sites, in the cloned *PHR1* gene (7). ' indicates location on the bottom strand. M, Δ , and * indicate the location of changed, deleted or inserted nucleotides relative to the gene sequence.

Figure 2. The effects of deletions and mutations in the *PHR1* 5' flanking region on *PHR1* expression as monitored by β-galactosidase activity. The regulatory sites defined in this study are indicated on the map of the *PHR1* 5' flanking region: the black box indicates the region footprinted by Prp, the two open boxes indicate the palindromes within the UAS and the stippled boxes indicate the three similar sequences within the UES. For each plasmid construct, the solid line indicates the 5' flanking region present. M above the solid line indicates the approximate site and extent of a mutation, while deletions are indicated by interruptions in the solid line. The end points of deleted or mutated regions are indicated to the right. Where multiple blocks of mutated sequences are present, the leftward or rightward boundaries of each block are separated by /. Numbering is as in Figure 1.

RESULTS

Expression of *PHR1* is easily monitored using the reporter plasmid pGBS116 which carries a *PHR1-lacZ* fusion gene under the control of *PHR1* regulatory elements (Fig. 1). Changes in β-galactosidase synthesis programmed by pGBS116 parallel changes in mRNA synthesized from the endogenous *PHR1* gene, implying that the 352 bp of *PHR1* 5'-flanking sequence present on the plasmid are sufficient for regulated expression (7,9). In previous work we demonstrated that the region between -78 and -40 (relative to the *PHR1* translational start site) contains an upstream repressor sequence (URS) which is bound by the damage-responsive repressor PRP. Deletion of this region led to an increase in basal level expression and a decrease in the induction ratio following exposure of cells to UV and MMS (11). We now report the results of further deletion and mutational analyses of the *PHR1* promoter which have identified additional *PHR1* regulatory elements.

Identification of a UAS within the *PHR1* promoter

Sequences similar to CGAGGAAG(C/A)(C/A) have been noted in the 5' flanking regions of several yeast damage-inducible genes including *PHR1*, *RAD2*, *RAD6*, *RAD7*, *RAD23*, *RAD51*, *MAG*, *RNR2* and *RNR3* (9,15–25). However a role for these sequences in gene expression has been reported only in the cases of *RAD2* (15), *MAG* (21) and *RNR2* (22,23), and the precise effect on transcription varies with the promoter (see Discussion). In the

PHR1 5' flanking region this sequence forms the right half of an interrupted palindrome TTCTTCCTCGTTTTTCGAGGAAGCA extending from -118 to -94 (7). To assess the role of this sequence in *PHR1* expression we introduced deletions or mutations into this region of pGBS116. As can be seen in Figure 2, mutation or deletion of the left half of the palindrome (pGBS270 and pGBS265) reduced basal and induced levels of expression to ~50% of the level seen with pGBS116, however the induction ratio following UV exposure remained essentially unchanged. In contrast, mutation of both halves of the palindrome (pGBS279) reduced basal level and induced expression to 7 and 15% respectively compared to pGBS116. Thus the palindrome plays an important role in both basal and induced expression of *PHR1*, perhaps by serving as an upstream activating sequence (UAS).

To determine whether the interrupted palindrome can act as a UAS outside of the context of *PHR1*, we constructed a derivative of plasmid pLG669Z which contains the yeast *CYC1* 5' regulatory sequence and initiating ATG fused in frame to the *lacZ* gene. By digesting pLG669Z with *SmaI* and *SphI* we removed both *CYC1* UASs as well as all known *CYC1* regulatory sequences but left four of the five TATA sequences of the gene intact (Fig. 1; 14,26,27). A single copy of a synthetic oligonucleotide containing the putative *PHR1* UAS was then inserted at the *SmaI* and *SphI* sites, yielding pPAL. We used an identical strategy to construct pMIXPAL in which the sequence of the putative UAS was altered throughout. As can be seen in Figure 3, pPAL programmed a high level of

	β -Galactosidase Activity (Units)		PHR1 Sequences	Plasmid
	Basal	Induced
8,522	10,078	None	pLG669Z
10,199	13,507	-118 to -94	pPAL
344	463	M(-118 to -94)	pMIXPAL
347	416	-301 to -125	pGBS289

Figure 3. The effects of various *PHR1* promoter elements in the context of the *CYC1* promoter. Heavy lines and symbols indicate the *PHR1* promoter elements, while thin lines indicate *CYC1* 5' flanking sequences. Symbols and numbering are as in Figures 1 and 2 except that T indicates the cluster of four TATA elements 3' to the *SphI* site in the *CYC1* promoter.

β -galactosidase synthesis while pMIXPAL supported only ~3% of the level seen with pPAL. Neither plasmid supported significant induction following exposure to UV. We conclude that the interrupted palindrome is an authentic UAS which is not damage responsive outside of the context of the *PHR1* promoter. Hereafter we will refer to this region as UAS_{PHR1}.

A potential source for the residual induced expression seen when UAS_{PHR1} is deleted is a second, weak, UAS within the *PHR1* promoter. The sequence AGAGGAATAA, found on the bottom strand of the *PHR1* regulatory region at positions -40' to -49', matches the consensus sequence noted above at eight out of 10 positions and lies within the region footprinted by the damage-responsive repressor Prp, that is within URS_{PHR1} (11). Thus one scenario, not addressed by our previous studies, is that binding by Prp physically blocks a UAS which is exposed upon dissociation of the repressor. In this case, deletion of URS_{PHR1} in the context of an inactivated UAS_{PHR1} should abolish residual induced expression. However, when both control elements were altered, basal level and induced expression increased relative to that seen when UAS_{PHR1} alone was mutated (Fig. 2, pGBS277 and pGBS279), ruling out a UAS within the Prp footprinted region as the sole source of residual induced expression. It is important to note that, compared to the expression levels seen with the URS deletion alone, inactivation of both the UAS and URS reduced basal level and induced expression to 6 and 11% respectively (Fig. 2, pGBS145 and pGBS277), in excellent agreement with the effect of mutation of the UAS in the context of an otherwise intact promoter (Fig. 2, pGBS116 and pGBS279). Thus, in the intact promoter, UAS_{PHR1} and URS_{PHR1} are the primary determinants of transcription activity.

Identification of a *PHR1* upstream essential sequence

We next asked whether sequences 5' to UAS_{PHR1} are involved in expression. To determine whether the region from -125 to -301 contains a UAS capable of acting outside of the context of the *PHR1* promoter, we replaced the *SmaI-SphI* fragment of pLG669Z with this region, yielding pGBS289. This region failed to activate

the reporter gene either in the absence of DNA damage or following UV irradiation (Fig. 3). In contrast, deletion analysis of the *PHR1* promoter in pGBS116 revealed the presence of an additional regulatory element(s). Deletion of 8 bp immediately 5' to UAS_{PHR1} had no effect on expression (Fig. 2, pGBS285), whereas deletions beginning 9 bp upstream of the UAS and extending various distances further upstream produced a progressive decline in both basal level and induced expression without significantly altering the induction ratio (Fig. 2, pGBS282, pGBS283, pGBS263, pGBS267 and pGBS256). Deletion of the entire region extending from -125 to -152 (Fig. 2, pGBS256) reduced basal level and induced expression to ~10% of that seen with the intact promoter. That specific sequences within the -125 to -152 region are required for *PHR1* promoter function is indicated by the results obtained when this region was replaced with a random sequence of the same length (Fig. 2, pGBS288). The random sequence reduced basal level and induced expression to 20 and 30% respectively compared to the intact promoter. In keeping with current nomenclature, this region qualifies as an upstream essential sequence, which we refer to as UES_{PHR1}.

The requirement for the -152 to -125 region for efficient *PHR1* expression was unexpected because, as demonstrated above, UAS_{PHR1} alone efficiently activates transcription when placed upstream of *CYC1* TATA sequences. We considered two models to explain these seemingly contradictory results: (i) the UES activates UAS_{PHR1} but the requirement for activation is dependent upon the context in which the UAS is placed; or (ii) the UES decreases the efficiency of repression by Prp. To distinguish between these two models we asked whether deletion of URS_{PHR1} relieves the requirement for the UES in UAS_{PHR1} activation. In contrast to results obtained with the intact promoter, mutation of the -152 to -125 region had no effect on expression when URS_{PHR1} was deleted (Fig. 2; pGBS145, pGBS292 and pGBS288). These results rule out a strict requirement for UES_{PHR1} as a coactivator of UAS_{PHR1} and strongly suggest that UES_{PHR1} acts instead as an antirepressor of Prp.

Close examination of the effects of specific deletions within the -125 to -152 region suggests the presence of multiple sequence

Figure 4. The effect of mutations within URS_{PHR1} on basal level and induced expression of *PHR1*. The sequence of the region footprinted by Prp (11) is shown below a map of the *PHR1* 5' flanking region. Symbols and numbering are as in Figure 2. The sites of base substitutions are shown in bold and are underlined, while the specific base changes in each plasmid are indicated below the sequence. The arrows show the location of the 22 bp palindrome previously postulated to constitute the Prp recognition sequence.

elements that affect expression. Deletions extending from -125 to -135, -147 and -152 reduced basal level expression by ~60, 80 and 90% respectively, and the effect on induced expression roughly paralleled this decline (Fig. 2). Each of these deletions removes part or all of the related sequences TTTACTGGC (-134 to -126), ATATCTCGC (-144 to -136) and TTATCCCGA (-158 to -150) (deleted sequences underlined). Additional evidence for a role for these sequences was obtained by mutation of sequences -151 to -148 in pGBS271; mutation leads to a 50% reduction in basal level expression and a 40% decrease in induced synthesis.

Identification of sequences involved in Prp binding

In the absence of DNA damage, URS_{PHR1} is bound by a protein called Prp; within 30 min following UV irradiation of cells, Prp binding at URS_{PHR1} disappears. We have previously demonstrated that Prp protects the region from -40 to -79 from attack by copper phenanthroline and have suggested that a 22 bp palindrome within the protected region may be the sequence recognized by Prp (11). To test this hypothesis we simultaneously introduced point mutations at positions -67, -63, -62 and -58 and assessed the effect on *PHR1* expression programmed by these pGBS116 derivatives. These sites were chosen because N7 of guanine is a frequent site of interaction between sequence-specific binding proteins and DNA. As can be seen in Figure 4, the mutant URS s can be divided into three groups with respect to their effects on *PHR1* expression. Group 1, carried by pGBS403 and 407, displayed a 50–60% reduction in basal level expression, a smaller decrease in induced expression, and an increased induction ratio. Thus, changing -67 and -63 from G to C and -58 and/or -62 from C to G reduced basal level expression, suggesting that these mutations enhance the affinity of Prp for the palindrome. In contrast, in the Group 2 mutants pGBS401 and pGBS406, replacement of all four sites with a mixture of A's and T's largely abolished repression. It is noteworthy that the fold increase in basal level and induced expression seen with pGBS401 and pGBS406 is similar to that seen when the entire 39 bp footprinted region is deleted (pGBS145, Fig. 2), suggesting that the effect on *PHR1* expression is mediated entirely through loss of Prp binding rather than through the fortuitous introduction of TATA-like elements

(28,29). The results obtained with the Group 3 mutants (pGBS400, 402 and 404) indicate that Prp binding is quite tolerant of some combinations of base changes; substitution of two or three positions with A or T has little effect on expression of *PHR1* as long as a G is present at either position -62 and/or -58 (compare pGBS402 and pGBS406, and pGBS400 and 401). It is particularly striking that, while pGBS402 and pGBS406 differ only at position -58, repression appears to be largely unaffected in the former construct but is strongly reduced in the latter. Together with the results obtained with the Group 1 and Group 2 mutants, these results indicate that the 22 bp palindrome extending from -73 to -52 is the recognition site for the damage-responsive repressor Prp.

DISCUSSION

The results reported here provide a framework for understanding the regulation of *PHR1* expression and its relationship to that of other damage-responsive genes in yeast. Basal level and damage induced transcription of *PHR1* are regulated primarily through three sequence elements clustered within a 120 bp region immediately upstream of the translational start site (Fig. 5). A 20 bp interrupted palindrome at -118 to -94 comprises UAS_{PHR1} and is responsible for 80–90% of basal level and induced expression of the gene. In the context of the intact *PHR1* promoter, UAS_{PHR1} function is strongly dependent upon the presence of a UES located at -125 to -152. Deletion or mutation of the UES reduces basal level and induced expression to levels similar to those seen with UAS_{PHR1} deletions or mutations. URS_{PHR1} , located at -78 to -40, contains a binding site for the damage-responsive repressor Prp (11); consistent with this role, deletion or specific mutations of the URS leads to a 7- to 10-fold increase in basal level expression and a 2.4-fold decrease in the induction ratio. Significantly, deletion of URS_{PHR1} also eliminates the requirement for UES_{PHR1} for activation of the UAS. The simplest interpretation of these results is that the UES attenuates repression of UAS_{PHR1} by Prp. This is consistent with the observation that UES_{PHR1} is not required for UAS_{PHR1} activity outside of the context of the *PHR1* promoter. Both the URS and the UAS bind proteins (11,30; D. Sweet and G. B. Sancar, in preparation) and it is likely that the same is true for the UES. The potential clearly

Figure 5. Sequence of the *PHR1* promoter region with the transcriptional regulatory elements boxed. Arrows within the boxes indicate palindromes. The three related sequences within the UES are underlined. Bent arrows indicate transcriptional start sites (F. W. Smith and G. B. Sancar, unpublished observation). Numbering is the same as in Figure 1.

exists for interactions between bound proteins to play an important role in regulation of *PHR1* transcription.

Sequences similar to the two palindromic half-sites of UAS_{PHR1} are also found upstream of a number of genes involved in DNA repair and damage tolerance. In the discussion that follows we will refer to these sequences as DNA repair consensus (DRC) elements; we do not imply by this distinction that DRC elements are necessarily limited to genes involved in DNA repair and damage tolerance. Thus far, DRC elements from *PHR1*, *RAD2*, *RAD23*, *RNR2*, *MAG*, *MGT1* and *RAD51* have been shown to play a role in transcriptional regulation *in vivo* (15,19,21–23,31; this work). Using these as a starting point, we have derived a consensus, (G/C)G(A/T)GG(A/C)RRNAN(A/T), for the DRC elements; a search of the GenBank and EMBL databases for similar sequences in the upstream regions of yeast DNA repair and metabolism genes revealed 24 examples of putative DRC elements among 19 genes. Alignment of these sequences is shown in Table 2. Our alignment differs from those previously published by Zheng and coworkers (32) and Xiao and coworkers (21) in that we have not inserted any gaps to optimize the alignment and in some cases we have aligned the sequences differently. Two important observations emerge from this comparison: (i) Functional and putative DRC elements are found in both damage-responsive and nonresponsive genes. Thus DRC elements do not appear to be intrinsically damage-responsive, a conclusion consistent with the fact that in the four cases (*MAG*, *MGT1*, *RAD23* and *PHR1*) in which an isolated DRC element has been moved into a heterologous promoter, it failed to confer damage-responsiveness on the test gene (21,31; S. Prakash, pers. comm.; this work). Clearly this does not rule out participation in the damage response via interactions with additional promoter elements unique to damage-inducible genes; (ii) DRC elements play diverse roles in transcriptional regulation. Deletion of DRC elements from the promoters of *PHR1* (this work) and *RAD2* (15) significantly reduces basal level and induced expression, while deletion of DRC elements from *MAG* (21), *MGT1* (33), *RNR2* (22,23) and *RAD51* (cited in ref. 21) enhances basal level expression. In this respect DRC elements are similar to the binding sites for the previously characterized bifunctional transcriptional regulators Rap1, Mcm1 and Abf1 (34–38). However there is an important mechanistic distinction. Isolated Rap1, Mcm1 and Abf1 binding sites from repressed or silenced promoters activate transcription when placed in the context of a heterologous promoter (34,36–38); in contrast, isolated DRC-URS elements from both *MAG* and *MGT1* continue to act as repressors when moved into the *CYC1* promoter (21,31). This suggests that DRC-URS and DRC-UAS elements are functionally distinct.

DRC elements from *RAD2*, *RAD7*, *RAD23*, *PHR1*, *MAG* and *MGT1* bind proteins in crude extracts from undamaged and damaged cells, and competition data indicate that the *RAD2*, *RAD23* and *RAD7* DRC binding proteins may be identical, as may at least some of the *MGT1* and *MAG* binding proteins (21,30). Indeed, Singh and Samson (39) have recently reported that *in vitro* the Rpa complex binds specifically to DRC elements from the promoters of *RAD1*, *RAD2*, *RAD10*, *RAD16*, *RAD51*, *RNR2*, *RNR3*, *PHR1*, *DDR48*, *MAG* and *MGT1*, while we have recently obtained evidence that the transcriptional regulator Ume6 binds to UAS_{PHR1} and URS_{MAG} (D. Sweet and G. Sancar, in preparation). *In vivo* the two classes of DRC elements may be recognized by different transcriptional regulators with similar, but non-identical, binding specificities and affinities. However, consistent sequence differences between DRC-URS and DRC-UAS elements which would permit binding discrimination are not apparent from the data in Table 2.

Among the promoter elements defined in this study, only URS_{PHR1} is clearly damage-responsive. This conclusion is based upon several observations. Mutation of either UAS_{PHR1} or UES_{PHR1} decreases both basal level and induced levels of expression, but not the induction ratio. Additionally, neither UAS_{PHR1} nor UES_{PHR1} , alone or together (data not shown), confer damage-responsiveness upon a heterologous promoter. In contrast, deletion of URS_{PHR1} from the intact *PHR1* promoter severely compromises induction and the element is capable of conferring damage-responsiveness on a heterologous promoter (11). The ability of URS_{PHR1} to mediate the damage response is almost certainly conferred by Prp. In intact cells, Prp- URS_{PHR1} binding activity rapidly disappears following DNA damage (11) and, as we have demonstrated here, point mutations within the 22 bp palindrome previously postulated to comprise the Prp binding site, have significant effects on both basal level and induced expression of *PHR1*. Surprisingly, multiple base changes within the palindrome are compatible with Prp-mediated repression. This suggests a previously unsuspected degree of degeneracy in the Prp recognition sequence, and may explain why database searches conducted at high stringency have failed to identify potential Prp binding sites in other yeast genes (11). The unusual location of URS_{PHR1} , only 15 bp downstream of the UAS and within 30 bp of the transcriptional start site, suggests that bound Prp may repress *PHR1* transcription by physically blocking access to the UAS or to the template by components of the preinitiation complex. This is not to say that steric hindrance alone is responsible for repression by Prp. The URS, when placed 80 bp downstream of the *CYC1* UAS1 [the primary active *CYC1* UAS under glucose repressing conditions; (27)], strongly inhibits expression (11). Both passive and active repression mechanisms are likely.

Table 2. Comparison of UAS_{PHR1} and upstream sequences from other genes involved in DNA repair and damage tolerance

Gene	Location ^a	Sequence ^b												References
<i>Sequence has UAS activity</i>														
<i>PHR1</i> ^c	−103	C	G	A	G	G	A	A	G	C	A	G	T	(7; this work)
	−110′	C	G	A	G	G	A	A	G	A	A	A	A	
<i>RAD2</i> ^c	−166	G	G	A	G	G	C	A	<u>T</u>	T	A	A	A	(15)
<i>RAD23</i> ^c	−295	G	G	T	G	G	C	G	A	A	A	T	T	(19,40; S. Prakash, pers. comm.)
<i>Sequence has URS activity</i>														
<i>RNR2</i> ^c	−424	C	G	A	G	G	<u>T</u>	<u>C</u>	G	C	A	C	A	(22,23)
<i>MAG</i> ^c	−209′	G	G	T	<u>C</u>	G	A	A	<u>T</u>	A	A	G	A	(21,39)
	−173	G	G	T	G	G	C	G	A	T	<u>G</u>	A	A	
<i>MGT1</i> ^d	−207	G	G	A	G	G	C	<u>C</u>	A	G	A	A	T	(31)
<i>RAD51</i> ^c	−111	C	G	T	G	G	<u>T</u>	G	G	G	A	C	<u>C</u>	(20,21)
<i>Activity unknown</i>														
<i>RAD1</i> ^d	−448	G	G	A	G	G	<u>G</u>	A	A	G	A	A	T	(41–43)
<i>RAD4</i> ^d	−487	C	G	A	G	G	C	A	G	A	A	T	T	(43,44)
	−364	C	G	T	G	G	A	<u>T</u>	G	A	A	A	<u>C</u>	
<i>RAD6</i> ^c	−263	C	<u>A</u>	A	G	G	A	A	<u>C</u>	A	A	A	T	(17,19)
<i>RAD7</i> ^c	−121	G	G	A	<u>A</u>	G	C	A	A	A	A	A	T	(18)
<i>RAD9</i> ^d	−189′	C	G	A	G	<u>A</u>	A	A	G	T	A	T	A	(45)
<i>RAD16</i> ^c	−306′	C	G	A	G	G	A	G	<u>T</u>	A	A	C	A	(46,47)
<i>RAD23</i> ^c	−72′	<u>A</u>	G	A	G	G	A	A	G	G	A	A	A	(19,40)
<i>RAD26</i> ^e	−89	<u>A</u>	G	T	G	G	A	A	G	A	A	A	A	(48)
<i>RAD50</i> ^e	−107′	C	G	A	<u>T</u>	G	A	G	G	C	A	A	<u>G</u>	(49,50)
<i>RAD51</i> ^c	−488′	<u>A</u>	G	A	G	G	A	A	G	A	A	A	<u>G</u>	(20,21)
	−451′	G	G	T	G	G	C	A	A	A	A	A	A	
<i>RAD52</i> ^d	−194	C	G	T	G	G	A	<u>T</u>	<u>T</u>	C	A	A	<u>C</u>	(51)
<i>RNR3</i> ^c	−461	C	<u>T</u>	A	G	G	<u>T</u>	A	G	C	A	G	A	(24,25)
<i>SPK1 (RAD53, MEC2)</i> ^e	−230	C	G	T	G	G	<u>G</u>	<u>T</u>	A	G	A	C	T	(5,32)
<i>Consensus</i>		<u>G</u>	G	<u>A</u>	G	G	<u>A</u>	R	R	N	A	N	<u>A</u>	
		C		T			C						T	
Occurrence		<u>21</u>	<u>22</u>	<u>24</u>	<u>21</u>	<u>23</u>	<u>19</u>	<u>19</u>	<u>19</u>	<u>24</u>	<u>23</u>	<u>24</u>	<u>19</u>	
		24	24	24	24	24	24	24	24	24	24	24	24	

^aLocation relative to first A in the initiating ATG. ' indicates transcribed strand.^bDifferences from the consensus sequence are shown in bold and are underlined.^cGene damage-inducible.^dGene not damage-inducible.^eNo information available on damage-responsiveness.

An intriguing and unusual relationship between URS_{PHR1} and another promoter element, UES_{PHR1}, has been revealed in these studies. UES_{PHR1} functions to specifically antagonize Prp binding or its functional consequences. An attractive model is that the UES decreases the binding of Prp to URS_{PHR1}, either directly or through UES-bound protein. An important feature of any model to explain UES-mediated antirepression is that it operates on both basal level and induced expression. Equitoxic UV fluences induce expression from a promoter containing a mutated UES region to only 20% of the level seen with the intact promoter and this effect is alleviated by deletion of the URS. A reasonable model is that the UES (or a UES-bound protein) assists in removing Prp from the promoter during the damage response. Thus induction of *PHR1* transcription in response to damage reflects the interplay of two negative regulators rather than a single positive regulator. Such a model is consistent with our previous observation that, while

URS_{PHR1} strongly represses transcription in the context of the *CYC1* promoter, the damage response of the element is much weaker than that seen in the context of the intact *PHR1* promoter (11). The UES–URS interaction would also present another potential target for regulation of the damage response.

Our results suggest that UES_{PHR1} is comprised of three related sequence elements, TTTACTGGC (–134 to –126), ATATCTCGC (–144 to –136) and TTATCCCGA (–158 to –150). The effect of deletion of these elements on basal level and induced expression is additive, indicating that each element contributes to UES function. Comparison of these sequences to those of the known damage-inducible genes in yeast reveals similarity to sequences previously noted in the upstream regions of the *RAD6* and *RAD18* genes: (A/T)TTTCCCG(C/G) (19). Identification of the sequences involved in transcriptional regulation of *RAD6* and *RAD18* has not been reported, however the similarity to

sequences that are clearly important in *PHR1* promoter function is suggestive.

As is the case for promoters responding to a more complex array of physiological signals, combinatorial control, rather than a single regulatory element, determines the extent of the *PHR1* damage response. Given their relatively wide distribution and proven function in several promoters including *PHR1*, the DRC elements described here may well be important combinatorial control elements for a number of damage-responsive genes. Characterization of the proteins bound to the various *PHR1* promoter elements and delineation of the sites regulated through *DUN1* or other damage response pathways will be necessary to elucidate the dynamics of the induction process.

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