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Genetics

Replication protein A binds to regulatory elements in yeast DNA repair and DNA metabolism genes

(*MAG* gene/3-methyladenine DNA glycosylase/gene expression)

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ABSTRACT ***Saccharomyces cerevisiae*** **responds to DNA damage by arresting cell cycle progression (thereby preventing the replication and segregation of damaged chromosomes) and by inducing the expression of numerous genes, some of which are involved in DNA repair, DNA replication, and DNA metabolism. Induction of the *S. cerevisiae* 3-methyladenine DNA glycosylase repair gene (*MAG*) by DNA-damaging agents requires one upstream activating sequence (UAS) and two upstream repressing sequences (URS1 and URS2) in the *MAG* promoter. Sequences similar to the *MAG* URS elements are present in at least 11 other *S. cerevisiae* DNA repair and metabolism genes. Replication protein A (Rpa) is known as a single-stranded-DNA-binding protein that is involved in the initiation and elongation steps of DNA replication, nucleotide excision repair, and homologous recombination. We now show that the *MAG* URS1 and URS2 elements form similar double-stranded, sequence-specific, DNA–protein complexes and that both complexes contain Rpa. Moreover, Rpa appears to bind the *MAG* URS1-like elements found upstream of 11 other DNA repair and DNA metabolism genes. These results lead us to hypothesize that Rpa may be involved in the regulation of a number of DNA repair and DNA metabolism genes.**

Alkylating agents covalently modify DNA to generate alkylated bases and are toxic, mutagenic, and carcinogenic. Some alkylated bases cause mutations because they miscode when replicated, and others cause cell death because they block DNA replication, preventing cells from proceeding properly through the cell cycle (1–3). If they are to avoid the mutagenic and cytotoxic effects of alkylating agents, cells must repair these alkylated bases before their DNA is replicated. Halting DNA replication when the genome is assaulted assures that DNA damage will be repaired before it has a chance to be encountered by the replication machinery.

Saccharomyces cerevisiae, like *Escherichia coli* and mammalian cells, responds to DNA damage by inhibiting DNA replication and arresting cell cycle progression, thus preventing the replication of damaged chromosomes (3–6). These cells also respond by inducing the expression of numerous genes, and many of these genes are involved in DNA repair and DNA metabolism (7, 8). The regulation of DNA damage-inducible regulons has been well characterized in *E. coli* (8–11). The SOS response is induced when single-stranded DNA (ssDNA), generated in the locale of DNA damage, activates RecA protein to facilitate the cleavage and inactivation of the LexA transcriptional repressor, derepressing a set of genes involved in DNA repair, mutagenesis, recombination, and cell division (8). Over 40 genes are regulated by OxyR and SoxRS in response to oxidative stress (9, 10), and four genes are regulated by the Ada DNA methyltransferase in response to alkylating agents (11). Methyl transfer from a methylphosphotriester lesion to the Ada Cys-69 residue converts the Ada protein into an efficient transcriptional activator for the induction of the following: (*i*) the Ada methyltransferase, which also repairs the potentially mutagenic O^6-methylguanine and O^4-methylthymine lesions; (*ii*) the AlkA 3-methyladenine (3MeAde) DNA glycosylase, which removes the replication blocking 3MeAde lesion plus various other lesions (11) and thus prevents cell death; and (*iii*) the AlkB and AidB proteins, whose precise functions are unknown.

The *S. cerevisiae* 3MeAde DNA glycosylase, the *MAG* gene product, displays homology to the *E. coli* AlkA 3MeAde DNA glycosylase (12). Like the *alkA* gene, *MAG* is induced when cells are exposed to alkylating agents (12–14), but unlike *alkA*, *MAG* induction is not specific for alkylating agents, does not involve DNA methyltransferase (14), and occurs upon exposure to several agents, including 4-nitroquinoline oxide, UV light, and γ irradiation (15). Several *S. cerevisiae* genes involved in DNA repair, DNA metabolism, and protein modification are induced by DNA-damaging agents—namely, *RAD2* (16), *RAD7* (17), *RAD18* (17), *RAD23* (18), *RAD51* (19), *RAD54* (20), *PHR1* (21), *RAD6* (18), *CDC9* (22), *CDC17/POL1* (7), *UBI4* (23), *RNR2* (24), and *RNR3* (25). Like *MAG*, each gene can be induced by a variety of DNA-damaging agents, irrespective of whether the gene product actually helps the cell to deal with the damage produced by those agents.

To explore eukaryotic gene expression in response to DNA damage, we identified the *MAG* regulatory elements (15). The *MAG* upstream region contains one activating sequence (UAS) and two repressing sequences (URS1 and URS2). Moreover, *MAG* URS1-like sequences are present in at least 11 other DNA repair and DNA metabolism genes—namely, *MGT1, PHR1, RAD1, RAD2, RAD4, RAD10, RAD16, RAD51, DDR48, RNR2*, and *RNR3* (15). Here we show that replication protein A (Rpa), a multifunctional protein believed to participate in the initiation and elongation steps of DNA replication, in nucleotide excision repair, and in homologous recombination (26–28) is present in the protein–DNA complexes formed at the *MAG* URS1 and URS2 elements. Our results suggest an additional role for Rpa in regulating the transcription of DNA repair and metabolism genes, and we propose a model that links the DNA damage-induced inhibition of replication with DNA damage-induced gene expression.

MATERIALS AND METHODS

Strains, Media, and Molecular Biology Methods. Yeast strain DBY747 (*MAT***a** *his3-1 leu2-3,112 trp1-289 ura3-52*) was grown at 30°C in either YPD medium or SD medium (29). Yeast transformation was carried out as described (30, 31). β-Galactosidase assays were as described (15, 32). A 24-bp oligonucleotide containing 20 bp of the *MAG* URS2 region (−180 to −161; see Table 1) was cloned between the *Sal* I and

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Abbreviations: UAS, upstream activating sequence; URS, upstream repressing sequence; MeMes, methyl methanesulfonate; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA.

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Table 1. Oligonucleotide probes and competitors

Gene	Sequence
MGT1	GGCCGTTCA**GGTGGAGGCC**CAGAA-3′
	3′-CAAGT**CCACCTCCGG**GTCTTAGCT
RAD1	GGCCCTTAA**TGAGGTGGAA**AATGA-3′
	3′-GAATT**ACTCCACCTT**TTACTAGCT
RAD2	GGCCACCTC**CGTGGAGGCA**TTAAA-3′
	3′-TGGAG**GCACCACCGT**AATTTAGCT
RAD4	GGCCGAGAC**CGTGGATGAA**ACTGC-3′
	3′-CTCTC**GCACCTACTT**TGACGAGCT
RAD10	GGCCCGTTA**CGAGGAAGAA**TTGCA-3′
	3′-GCAAT**GCTCCTTCTT**AACGTAGCT
RAD16	GGCCCCCGC**CATGGTTGCC**AGGGA-3′
	3′-GGGCG**GTACCAACGG**TCCCTAGCT
RAD51	GGCCGTACG**CGTGGTGGGA**CCATA-3′
	3′-CATGC**GCACCACCCT**GGTATAGCT
RNR2	GGCCGGCAA**CGAGGTCGCA**CACGC-3′
	3′-CCGTT**GCTCCAGCGT**GTGCGAGCT
RNR3	GGCCTTGAA**CTAGGTAGCA**GAGCA-3′
	3′-AACTT**GATCCATCGT**CTCGTAGCT
DDR48	GGCCAGGTT**CGAGGATGAC**AAATC-3′
	3′-TCCAA**GCTCCTACTG**TTTAGAGCT
PHR1	GGCCTTTTT**CGAGGAAGCA**GTCAA-3′
	3′-AAAAA**GCTCCTTCGT**CAGTTAGCT
CAR1	CTTAGCGGT**AGCCGCCGA**GGGG-3′
	3′-ATCGCCA**TCGGCGGCT**CCCCAG
MAG URS1	TCGATATACTTTCTTA**TTCGACCTAC**TTTATATATC-3′
	3′-ATATGAAAGAAT**AAGCTGGATG**AAATATATAGAGCT
MAG URS2	GGCCTCTTT**TCGGTGGCGA**TGAAT-3′
	3′-AGAAA**AGCCACCGCT**ACTTAAGCT
NS1	pUC19
NS2	TCTCTCATCAATACTGGT-3′
	3′-GTTATGACCAGTCGAGGA
NS3	TCGAGATCAGTTAAAGCCATATCTTCACTGAC-3′
	3′-CTAGTCAATTTCGGTATAGAAGTGACTGAGCT

The underlined genes are induced by DNA-damaging agents. The underlined sequences contain partial *Eag* I and *Sal* I cloning sites and are not part of the gene. The bold sequences show the consensus sequence (15). The positions of the sequences relative to translational start sites are as follows: *MGT1*, −220 to −201; *RAD1*, −206 to −187; *RAD2*, −174 to −155; *RAD4*, −369 to −350; *RAD10*, −317 to −298; *RAD16*, −314 to −295; *RAD51*, −162 to −143; *RNR2*, −379 to −360; *RNR3*, −472 to −453; *DDR48*, −276 to −257; *PHR1*, −108 to −89; *CAR1*, −165 to −144; *MAG* URS1, −227 to −196; *MAG* URS2, −180 to −161. The primary references for the promoter sequences for listed genes can be found in ref. 15. The sequence of pUC19 can be found in ref. 33. Note that all the staggered ends were filled in to produce completely double-stranded DNA (dsDNA) for both labeled probes and nonradioactive competitor DNAs. NS, nonspecific.

Eag I sites of pNG22 (32), which contains the *CYC1* UAS–*lacZ* fusion and a polylinker 3′ to the *CYC1* UAS. pNG22 containing an unrelated sequence of the same size was created by digesting plasmid pNG22/MCS (15) with *Eag* I and self-ligating the vector.

Gel Mobility-Shift Assay. The oligonucleotides used as probes or as competitors are listed in Table 1. Labeled probes were prepared by annealing two complementary strands at 65°C for 3 min in Sequenase buffer (United States Biochemical) and slowly cooling to room temperature. Annealed oligonucleotides were radiolabeled with [α-^{32}P]dCTP by using Klenow DNA polymerase to fill the ends in order to ensure that all the labeled probe was double-stranded. Gel mobility-shift assays were carried out as described (15) with 0.2 ng of labeled probe and 1–2 μg of cell extract proteins. Supershift assays were carried out as follows: the indicated volumes of antisera (26) were preincubated with 2 μg of cell extract in buffer A (15) at 4°C for 30 min, after which the gel mobility-shift assay was conducted (15).

RESULTS

Deletion analysis of the DNA damage-inducible *S. cerevisiae* *MAG* 3MeAde DNA glycosylase gene (15) defined the presence of one UAS and one URS (URS1) and suggested the presence of a second URS (URS2). Deletion of URS1 alone or URS1 plus URS2 resulted in about a 30- and 60-fold increase in the basal level of transcription, respectively. The *MAG* URS1 behaved as an URS in a heterologous promoter and specifically bound proteins in yeast cell extracts (15). We now show that the *MAG* URS2 region also functions as an URS in the same heterologous promoter and that it too specifically binds yeast extract proteins.

Table 2. *MAG* URS2 mediates repression *in vivo*

Plasmid	β-Galactosidase, units/ml − MeMes	β-Galactosidase, units/ml + MeMes
pNG22	57.62 ± 4.97	63.83 ± 10.23
pNG22 + URS2	18.91 ± 1.63	21.08 ± 6.40
pNG22 + MCS	54.87 ± 11.06	59.87 ± 12.47

MeMes (0.05%) exposure was for 4 hr. Values are means ± SD of three determinations. MCS, multiple cloning site.

The *MAG* URS2 Region Functions as a Repressor Binding Site *in Vivo*. We used a *CYC1–lacZ*-containing vector (pNG22) described by Kovari *et al.* (32) to assay *MAG* URS2 function. A 24-bp oligonucleotide containing 20 bp of the putative *MAG* URS2 region was cloned 3′ of the *CYC1* UAS. Transformants containing the pNG22 vector expressed about 60 units of β-galactosidase both with and without exposure to the alkylating agent methyl methanesulfonate (MeMes) (Table 2). The *MAG* URS2 region produced about a 3-fold decrease in *CYC1* UAS function but expression was still unaffected by MeMes. The insertion of an unrelated sequence of identical length had no effect on *CYC1* UAS function. These results confirm that the *MAG* promoter contains an URS between −180 and −161 and demonstrate that MeMes-induced derepression cannot be achieved when the URS lies next to the *CYC1* UAS.

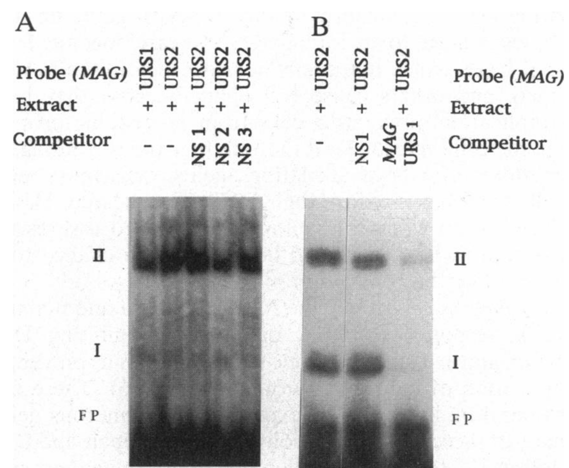

FIG. 1. Proteins which complex with *MAG* URS1 also complex with *MAG* URS2 elements. (*A*) Gel mobility-shift assay using *MAG* URS1 and URS2 probes. The sequences of the *MAG* URS1 oligonucleotides are described in Table 1 and they were labeled as described in *Materials and Methods*. For competition, double-stranded NS2 and NS3 were prepared by annealing the respective pairs of oligonucleotides and filling in the ends by use of Klenow DNA polymerase. NS1, pUC19; NS2 and NS3, nonspecific oligonucleotide competitor DNA as described in Table 1. A 100-fold weight excess of NS1 and a 100-fold molar excess of NS2 or NS3 competitor DNA was used. I and II, DNA–protein complexes; FP, free probe. (*B*) DNA–protein complex formation in the presence of unlabeled *MAG* URS1 DNA oligonucleotide. A 100-fold molar excess of competitor DNA was used. A set of oligonucleotides containing 20 bp of the *MAG* URS2 region or of the *MAG* URS1 was used to make the probe. NS1, nonspecific competitor pUC19 DNA.

The *MAG* URS1 and URS2 Elements Bind at Least One Protein in Common. In gel mobility-shift assays, the *MAG* URS2 region formed two DNA–protein complexes (Fig. 1). To our surprise, these two complexes exactly comigrated with the *MAG* URS1 complexes (Fig. 1*A*). A 100-fold excess of unlabeled plasmid DNA and two other nonspecific oligonucleotides of similar size to the *MAG* URS2 probe did not prevent the formation of the *MAG* URS2 complexes I and II, indicating that protein binding was specific (Fig. 1*A*). Formation of the *MAG* URS2 DNA–protein complexes was prevented by competition with excess unlabeled *MAG* URS2 probe (data not shown) and with excess *MAG* URS1 probe (Fig. 1*B*). We conclude that at least one protein which directly complexes with the *MAG* URS1 element also complexes with the *MAG* URS2 element.

Rpa Binds the *MAG* URS1 and URS2 Elements. The *MAG* URS2 region contains a sequence (−167 AGCCACCGC −175) that matches seven of the nine bases in the URS1 element of the *CAR1* arginase gene (−156 AGCCGCCGA −148) (34). Luche *et al.* (35) made the surprising observation that the *CAR1* URS1 element specifically binds the Rpa complex. Rpa is normally thought of as a ssDNA-binding protein that participates in the initiation and elongation steps of DNA replication, in nucleotide excision repair, and in homologous recombination (26–28). We tested whether Rpa also binds to dsDNA containing the *MAG* URS2 element.

Rpa is composed of three subunits of 70 kDa (Rpa1), 34 kDa (Rpa2), and 14 kDa (Rpa3) (26). Purified *S. cerevisiae* Rpa formed a complex with the *MAG* URS2 double-stranded oligonucleotide probe (Fig. 2*A*). In addition, purified Rpa complexed with the *MAG* URS1 double-stranded probe, as one would predict from the results described above (Fig. 1). As controls, Rpa formed a complex with the *CAR1* URS1 element and did not form a complex with a nonspecific probe (Fig. 2*A*). Similarly migrating complexes were produced when the URS elements were incubated with either cell extract proteins or purified Rpa (Fig. 2*B*) and a 50-fold excess of the unlabeled *CAR1* URS1 probe eliminated complex formation with the *MAG* URS2 probe (Fig. 2*C*). Moreover, we confirmed that Rpa was indeed present in the *MAG* URS2 complexes in yeast extracts because they could be supershifted with antisera specific for the Rpa1 and Rpa3 subunits (Fig. 2*D*). (Sufficient Rpa2 antiserum was unavailable.)

Because Rpa is known to bind ssDNA nonspecifically with extremely high affinity (26, 36), we confirmed that our labeled probes were indeed double-stranded and contained no significant ssDNA as judged from their migration in 20% polyacrylamide gels (data not shown). We next reasoned that ssDNA should be able to compete for binding to Rpa. To our surprise a 25- to 100-fold excess of an unrelated ssDNA oligonucleotide did not prevent the formation of complex II (Fig. 3*A*), although formation of complex I was prevented by excess ssDNA. In addition, we labeled each strand of the *MAG* URS2 probe and annealed it with an excess of the unlabeled complementary strand; a 50-fold excess of either unlabeled strand eliminated complex I but not complex II (Fig. 3*B*). We infer

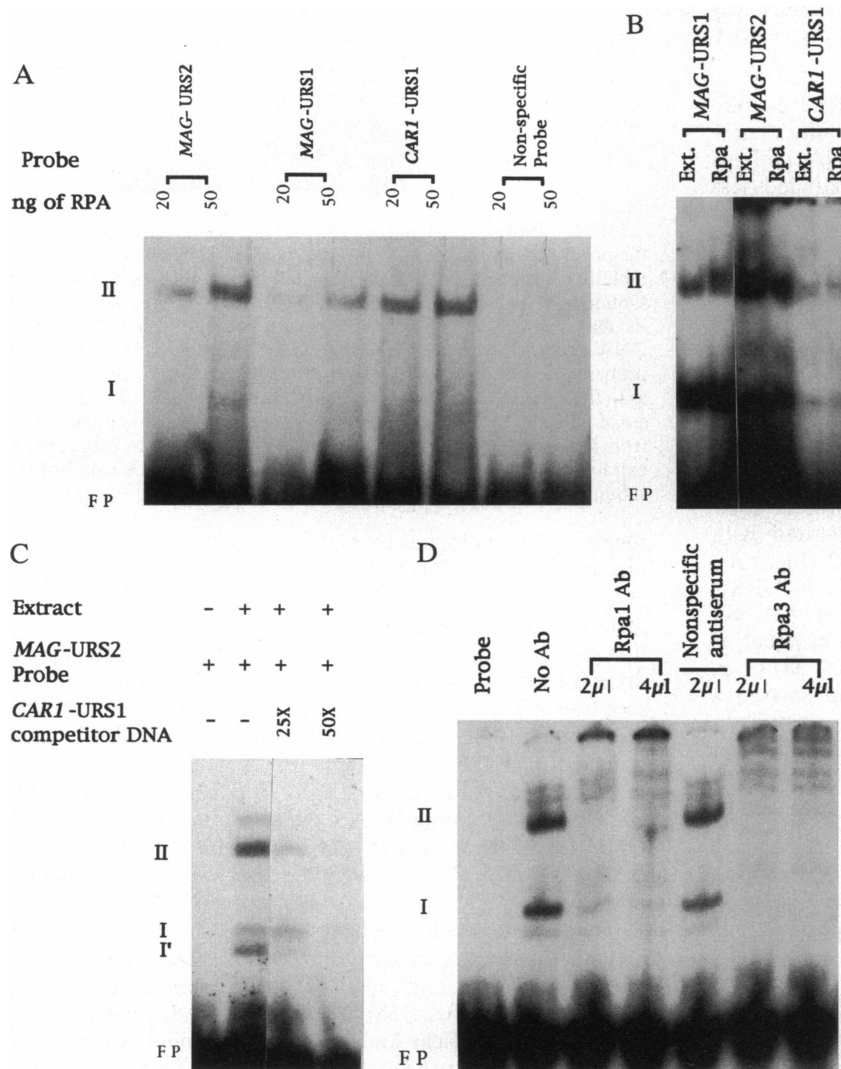

FIG. 2. Purified Rpa binds to the *MAG* URS1 and URS2 regions. (*A*) Purified yeast Rpa-mediated gel mobility-shift assay with *MAG* URS1 and URS2 probes, with the *CAR1* URS1 probe, and with a nonspecific probe, NS2 (see Table 1). Yeast Rpa was purified as in ref. 27 and was a gift from Richard Kolodner. The purity of the Rpa was checked by silver staining. I and II indicate DNA–protein complexes; FP, free probe. (*B*) *MAG* URS1, *MAG* URS2, and *CAR1* URS1 form similar DNA–protein complexes with yeast cell extract and purified yeast Rpa. Cell extract (2 μg) or pure Rpa (100 ng) protein was used to assay DNA–protein interactions. Note that at this high level of Rpa (100 ng) both complex I and complex II can be observed. (*C*) *CAR1* URS1 competes for protein binding to the *MAG* URS2 element. *MAG* URS2 was used as probe and the indicated fold excess of unlabeled *CAR1* URS1 was included in the DNA–protein binding reaction mixture. Complex I′ is perhaps due to proteolytic degradation of protein present in complex I. (*D*) Rpa antiserum supershifts *MAG* URS2 DNA–protein complexes. Ab, antibody.

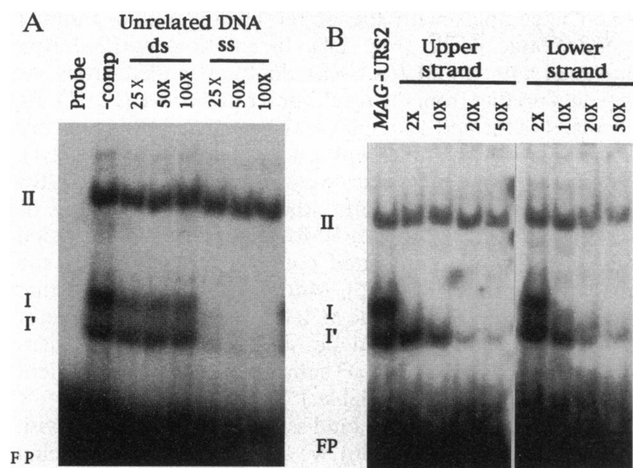

FIG. 3. ssDNA oligonucleotide competition. (*A*) Gel mobility-shift assay using the radiolabeled *MAG* URS2 probe and 2 μg of yeast cell extract proteins was carried out in the presence of the indicated excess of unlabeled double-stranded NS2 (Table 1) or single-stranded oligonucleotide (5′-TCT-CTC-ATC-AAT-ACT-GGT-3′). (*B*) Gel mobility-shift assay using the radiolabeled *MAG* URS2 probe and 1 μg of yeast cell extract proteins was carried out in the presence of the indicated unlabeled excess of either upper-strand or lower-strand single-stranded oligonucleotide used to make the *MAG* URS2 double-stranded probe. First the single-stranded oligonucleotide was labeled with [γ-^{32}P]ATP in the presence of T4 DNA kinase and then an excess of complementary strand was annealed, precipitated, and used in the binding reaction. I, I′, and II indicate DNA–protein complexes. I′ may be due to the proteolytic degradation of protein present in complex I. FP, free probe.

that (*i*) Rpa in complex II binds the *MAG* URS2 double-stranded oligonucleotide with a much higher affinity than it does ssDNA, or it has a reduced affinity for ssDNA, perhaps because it is modified, and (*ii*) the Rpa in complex I binds less tightly than that in complex II and can associate with both dsDNA containing *MAG* URS-like sequences and with ssDNA.

Rpa Binds the *MAG* URS1-Like Elements from Other DNA Repair and DNA Metabolism Genes. We previously noted the existence of *MAG* URS1-like elements upstream of the *MGT1, RAD1, RAD2, RAD4, RAD10, RAD16, RAD51, RNR2, RNR3, DDR48*, and *PHR1* DNA repair and DNA metabolism genes (15, 21). (The underlined genes are known to be inducible by DNA-damaging agents.) The *MAG* URS1-like sequences from the upstream regions of each of these 11 DNA repair and metabolism genes formed one or more DNA–protein complexes; all 11 produced a complex that comigrated with complex II formed with *MAG* URS1 and URS2 (Fig. 4*A*). Deletions of the promoter regions containing the *MAG* URS1-like elements have been made for *RAD2* (37), *RNR2* (24, 38), *PHR1* (21) and *MGT1* (39); these regions function either as URSs for *MAG* (15), *MGT1* (39), and RNR2 (24, 38) or as UASs for *RAD2* (37) and *PHR1* (G. Sancar and R. Ferris, personal communication). The DNA–protein complexes formed with the *MAG* URS1-like sequences from *RAD2, RNR2, PHR1*, and *MGT1* did indeed contain Rpa, because they were supershifted with Rpa antiserum (Fig. 4*B*).

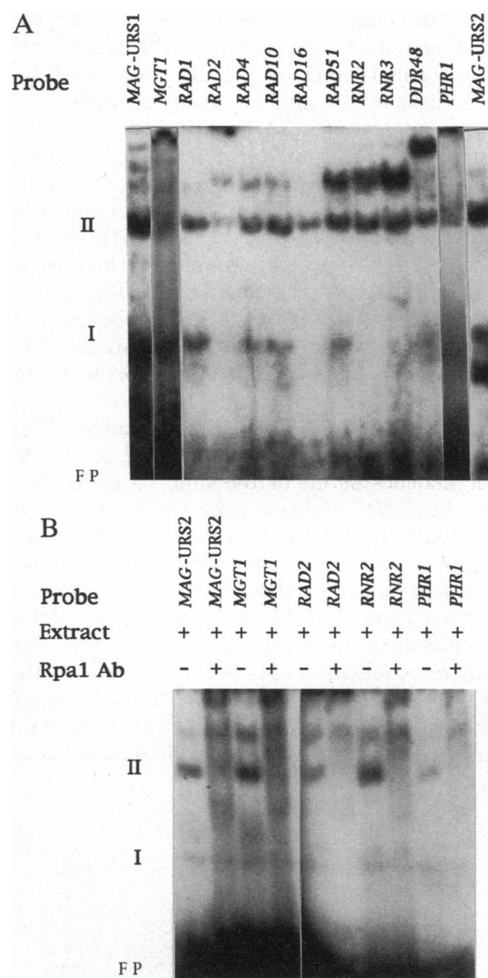

FIG. 4. Proteins bind to the *MAG* URS1-like sequences present in the promoters of 11 other DNA repair and metabolism genes. (*A*) Gel mobility-shift assay. Oligonucleotides containing the *MAG* URS1-like sequences from the indicated genes (Table 1) were labeled and incubated with 2 μg of yeast cell extract proteins. I and II indicate DNA–protein complexes; FP, free probe. The specific activities of probes varied by about 4-fold. Therefore no conclusion can be drawn as to the relative efficiencies of binding to each element. (*B*) Supershift assay. The oligonucleotides containing the *MAG* URS1-like sequences from the indicated genes were labeled and incubated with 2 μg of yeast extract that contained 2 μl of antiserum raised against the Rpa 70-kDa subunit (Rpa1 Ab).

DISCUSSION

When cells are exposed to DNA-damaging agents a transient arrest of DNA replication allows time for repair of the damage and thus minimizes cell death and mutation. DNA damage may also induce certain DNA repair and DNA metabolism genes, enabling cells to make an efficient recovery from damage. Others have presented *in vitro* evidence for the involvement of Rpa in DNA replication, nucleotide excision repair, and homologous recombination (26–28), and the data presented here lead us to hypothesize that Rpa may also play a regulatory role in the transcription of DNA repair and DNA metabolism genes. It is not unprecedented for DNA replication proteins to be diverted into acting as transcription factors; three T4 DNA replication accessory proteins stimulate transcription at T4 late promoters (40), and the eukaryotic RAP1, ABF1, and MCM1 proteins participate in both replication and transcriptional control (41–43).

Rpa appears to bind specifically to double-stranded oligonucleotides containing DNA sequences found upstream of 10 DNA repair genes (*MAG, MGT1, RAD1, RAD2, RAD4, RAD10, RAD16, RAD51, DDR48, PHR1*) and 2 DNA metabolism genes (*RNR2, RNR3*) from *S. cerevisiae*. For 5 of these sequences, *in vivo* evidence has confirmed that these sequences do indeed influence transcription (the others are untested); of these 5 elements, 3 act as URSs [in the *MAG, MGT1*, and *RNR2* genes (15, 39, 24, 38)], and 2 act as UASs [in *RAD2* (37) and *PHR1* (G. Sancar and R. Ferris, personal communication)]. Studies by Luche *et al.* (35) suggest that Rpa specifically

binds to sequences upstream of several genes involved in carbon and nitrogen metabolism, respiration, meiosis, and sporulation, and again these sequences function as either an UAS or an URS (35). How the regulation of these genes relates to the regulation of DNA repair and DNA metabolism genes, and exactly how the influence is exerted, probably depends upon Rpa modification in response to specific stimuli and probably requires the interaction of Rpa with other transcription factors upstream of each gene. Indeed, the introduction of the *MAG* URS1 and URS2 elements into a heterologous promoter does not confer MeMes inducibility (ref. 15 and Table 2), indicating that additional factors are required for DNA damage inducibility.

Rpa, in both yeast and mammalian cells, is phosphorylated as cells begin DNA replication in S phase (44). Moreover, human Rpa is phosphorylated when cells are exposed to DNA-damaging agents (45, 46). Exactly how the S phase and DNA damage-induced human Rpa modifications compare, and whether yeast Rpa undergoes DNA-damage induced phosphorylation, is not known. Human Rpa can interact with several transcription factors—namely, p53, VP16, and GAL4 (47–49)—raising the possibility that the Rpa in *MAG* URS–protein complexes is simply tethered to the complex via a transcription factor. However, the fact that purified Rpa complexed with the *MAG* URS probes argues against this. Furthermore, UV crosslinking of the *MAG* URS2–protein complexes bound a 70-kDa protein, suggesting that Rpa1 directly binds the URS element (data not shown).

It is tempting to propose that a DNA damage-induced alteration in Rpa function could simultaneously result in the inhibition of DNA replication and the transcriptional activation of DNA repair and DNA metabolism genes. A simple model would be that Rpa in DNA-damaged cells becomes targeted to sites of nucleotide excision repair and homologous recombination, becoming unavailable for normal replication forks and for repressing DNA repair and DNA metabolism genes. This could be achieved by Rpa binding to ssDNA at sites of excision repair and recombination (27, 28); alternatively, a DNA damage-induced modification of Rpa may decrease its affinity for *MAG* URS binding, or increase its affinity for binding to ssDNA, or both. It should be noted that Rpa is available for *MAG* URS binding in extracts from undamaged as well as DNA-damaged cells, but it is possible that Rpa dissociates from DNA during extract preparation, masking any prior sequestration (15). It should also be noted that at least in the case of *MAG*, we know that regulatory elements other than the URS1 and URS2 Rpa binding sites are required for DNA damage-induced transcription, because (*i*) the URS1 and URS2 elements do not confer DNA damage inducibility upon a heterologous promoter and therefore need to be situated in the *MAG* upstream region in order for induction to occur and (*ii*) the *MAG* UAS element upstream of the URS1 and URS2 region is actually required for DNA damage-induced *MAG* transcription (15). The modification state of Rpa in DNA-damaged cells and the interaction of *MAG* URS-bound Rpa with proteins at the *MAG* UAS element need to be explored.

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