MOLECULAR AND CELLULAR BIOLOGY, May 1996, p. 2091–2100 Vol. 16, No. 5
0270-7306/96/$04.00+0
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The *Saccharomyces cerevisiae IMP2* Gene Encodes a Transcriptional Activator That Mediates Protection against DNA Damage Caused by Bleomycin and Other Oxidants

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Received 20 December 1995/Returned for modification 5 February 1996/Accepted 26 February 1996

Bleomycin belongs to a class of antitumor drugs that damage cellular DNA through the production of free radicals. The molecular basis by which eukaryotic cells provide resistance to the lethal effects of bleomycin is not clear. Using the yeast *Saccharomyces cerevisiae* as a model with which to study the effect of bleomycin damage on cellular DNA, we isolated several mutants that display hypersensitivity to bleomycin. A DNA clone containing the *IMP2* gene that complemented the most sensitive bleomycin mutant was identified. A role for *IMP2* in defense against the toxic effects of bleomycin has not been previously reported. *imp2* null mutants were constructed and were found to be 15-fold more sensitive to bleomycin than wild-type strains. The *imp2* null mutants were also hypersensitive to several oxidants but displayed parental resistance to UV light and methyl methane sulfonate. Exposure of mutants to either bleomycin or hydrogen peroxide resulted in the accumulation of strand breaks in the chromosomal DNA, which remained even after 6 h postchallenge, but not in the wild type. These results suggest that the oxidant hypersensitivity of the *imp2* mutant results from a defect in the repair of oxidative DNA lesions. Molecular analysis of *IMP2* indicates that it encodes a transcriptional activator that can activate a reporter gene via an acidic domain located at the N terminus. Imp2 lacks a DNA binding motif, but it possesses a C-terminal leucine-rich repeat. With these data taken together, we propose that Imp2 prevents oxidative damage by regulating the expression of genes that are directly required to repair DNA damage.

Bleomycin (BLM) is used extensively to treat a variety of cancers, including those of the lungs, testicles, head, and neck (41, 61, 62). The antitumor effect of BLM is exerted through oxidative lesions in chromosomal DNA, formed via a free radical-reactive complex that is produced when BLM binds to iron and oxygen in vivo (6, 64). The activated Fe · BLM complex takes a hydrogen atom from the 4′-carbon of deoxyribose, resulting in two types of lesions: (i) oxidized apurinic/apyrimidinic (AP) sites and (ii) DNA single-strand breaks that terminate with 3′-phosphoglycolate (16, 44, 64). Noncoding AP sites lead to the incorporation of incorrect nucleotides by DNA polymerase (29, 34). While 3′-phosphoglycolate is known to block DNA synthesis, the mutagenic effect of this adduct is unknown (9, 20, 31, 56). Activated Fe · BLM complex can also produce double-strand breaks at certain DNA sequences, such as CGCC (1, 58). In any case, the lesions produced by BLM are toxic and are considered to be mutagenic (4).

In *Escherichia coli*, the bifunctional enzyme endonuclease IV plays a direct role in repairing BLM-induced DNA lesions (7, 31, 47). This enzyme possesses two enzymatic activities, AP endonuclease and 3′-diesterase, that, respectively, cleave the bond on the 5′-side of AP sites and remove a variety of modified 3′ termini, including 3′-phosphoglycolate esters in damaged chromosomal DNA (8). Cells deficient in endonuclease IV are hypersensitive to BLM and accumulate unrepaired DNA strand breaks in their chromosomal DNA (31). A homolog of endonuclease IV, Apn1, is present in the budding yeast *Saccharomyces cerevisiae* (40). However, *apn1* deletion mutants retain wild-type resistance to BLM treatment, indicating that Apn1 is not critical for the repair of BLM-caused DNA lesions in yeast cells (48).

In yeast cells, Rad6 and Rad52 are the only known DNA repair proteins that are involved in the repair of BLM-induced DNA lesions (23). These proteins are also required to repair a broad array of DNA lesions that are produced, for example, by ionizing radiation, UV light, and alkylating agents (13). At least 12 BLM-sensitive yeast mutants that are assigned to seven complementation groups, *BLM1* to *BLM7*, have been previously described (39). To date, the genetic defects of these mutants have not been identified, and whether the *RAD6* or *RAD52* gene belongs to any of the complementation groups is unclear.

We are also interesting in understanding the processes that are involved in repairing BLM-induced DNA lesions in eukaryotic cells. We have chosen *S. cerevisiae* as a model to take advantage of the fact that there is a high degree of functional conservation between *S. cerevisiae* and human DNA repair genes (27, 42, 60). In this study, we exploited the high spontaneous mutation rate of the yeast *apn1* deletion mutant to isolate mutants that are hypersensitive to BLM (7, 48). We show that one of these mutants bears a mutation in a previously identified gene, *IMP2* (10), which hitherto had no identified role in defense against BLM-induced toxicity.

MATERIALS AND METHODS

Strains, media, and plasmids. The *S. cerevisiae* strains used in this study are described in Table 1. The *E. coli* strains used for plasmid maintenance were HB101 and DH5α. Yeast cells were grown in either complete YPD medium or minimal synthetic (SD) medium (57). When necessary, nutritional supplements were added at 20 μg/ml to SD medium.

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TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype	Source or reference
DBY747	*MAT***a** *leu2-3,112 his3-Δ1 trp1-289a ura3-52*	Laboratory stock
DRY370	Isogenic to DBY747, except *apn1Δ::HIS3*	48
DRY211c	Isogenic to DBY747, except *apn1Δ::HIS3 imp2-1*	This work
DRY211	Isogenic to DBY747, except *imp2-1*	This work
DRY212	Isogenic to DBY747, except *imp2Δ::LEU2*	This work
DBY747 (*rad1Δ*)	Isogenic to DBY747, except *rad1Δ::URA3*	This work
DBY747 (*rad2Δ*)	Isogenic to DBY747, except *rad2Δ::URA3*	This work
DBY747 (*rad6Δ*)	Isogenic to DBY747, except *rad6Δ::URA3*	This work
DBY747 (*rad9Δ*)	Isogenic to DBY747, except *rad9Δ::URA3*	This work
DBY747 (*rad52Δ*)	Isogenic to DBY747, except *rad52Δ::URA3*	This work
DBY747 (*sod1Δ*)	Isogenic to DBY747, except *sod1Δ::URA3*	This work
DBY747 (*ctt1Δ*)	Isogenic to DBY747, except *ctt1Δ::LEU2*	This work
CTY10-5d	*MAT***a** *ade2 trp1-Δ901 leu2-112 his3-Δ200 gal4 gal80 ura3::lexAop-lacZ*	Dan Gietz
CTY10-5d (*imp2Δ*)	Isogenic to CTY10-5d, except *imp2Δ::LEU2*	This work
FY86	*MATα his3Δ200 ura3-52 leu2Δ1 GAL*$^+$	Fred Winston
DRY214	Isogenic to FY86, except *imp2Δ::LEU2*	This work
SM6210	*MATα leu2::*pTEP9 *ura3 his3 lys2 trp1*	65
SM12	Isogenic to SM6210, except *yap1-Δ1::HIS3*	65
SM6212	Isogenic to SM6210, except *imp2Δ::LEU2*	This work
W303α	*MATα ade2-1 his3-11 leu2-3,112 trp1-1 ura3-1 can1-100*	36
W303α (*blh1Δ*)	Isogenic to W303α, except *blh1::URA3*	36
W303α (*imp2Δ*)	Isogenic to W303α, except *imp2Δ::LEU2*	This work
YKH12a	*MAT***a** *ade2-n::URA3::ade2-a leu2-3,112 his3-11,15 can1-100 ura3-1 trp1-1*	22
DRY216	Isogenic to YKH12a, except *imp2Δ::LEU2*	This work
EG103	*MATα leu2-3,112 his3Δ1 trp-289a ura3-52 GAL*$^+$	33
SL103	Isogenic to EG103, except *atx1Δ::LEU2*	33

Survival curves. The sensitivity of the yeast strains to the antitumor drug BLM, hydrogen peroxide (H_2O_2), *tert*-butylhydroperoxide, paraquat, menadione, diamide, methyl methane sulfonate (MMS), and 4-nitroquinoline-1-oxide (4-NQO) was measured by using exponential-phase cultures. Overnight cultures grown to saturation at 30°C in YPD medium were diluted into fresh YPD medium to an optical density at 600 nm of 0.2 (~2×10^6 cells per ml) and incubated to an optical density at 600 nm of 0.8 to 1.0. Aliquots of the cultures were then treated with the indicated concentrations of BLM (generously provided by J. O'Sullivan, Bristol-Myers Squibb, Princeton, N.J.), hydrogen peroxide (Fluka Chemicals), *tert*-butylhydroperoxide, paraquat (ranging from 0 to 40 mM), menadione (ranging from 0 to 40 mM), diamide (ranging from 0 to 40 mM), MMS (ranging from 0 to 0.2%), and/or 4-NQO (ranging from 0.5 to 5 μg/ml) (all from Sigma, St. Louis, Mo.) at 30°C with shaking (250 rpm in an incubator shaker [New Brunswick Scientific Co., Inc., Edison, N.J.]) for 1 h. Survival was determined by diluting the samples in sterile 20 mM potassium phosphate buffer (pH 7.0) and plating them on YPD agar. Colonies were counted after 2 to 3 days of growth at 30°C. In the case of γ irradiation treatment, exponential-phase cells were irradiated in cold YPD medium with a ^{60}Co source at a dose rate of 55.5 rads/s (experiments carried out in the laboratory of Michael Resnick, National Institute of Environmental Health Sciences). Cells were immediately diluted and plated on YPD solid agar for 3 days at 30°C to determine the surviving fractions.

Isolation of the *IMP2* gene by complementation of DRY211. A yeast genomic library constructed in the single-copy vector YCp50, bearing the selective marker *URA3*, was generously provided by Fred Winston, Harvard University. The library was transformed into strain DRY211, and colonies were selected for growth on plates lacking uracil. Ura$^+$ transformants were replica plated onto YPD solid agar plates containing 2 μg of BLM per ml. Ura$^+$ colonies carrying potential BLM-resistant clones were traced to the master plate that contained no drug and were retested on YPD agar containing BLM. Of 6.0×10^4 Ura$^+$ colonies screened, 11 showed wild-type resistance to BLM. These 11 Ura$^+$ colonies were plated in the presence of 5-fluoroorotic acid (Sigma) to select for loss of the plasmid (18), and the resulting Ura$^-$ colonies regained their sensitivity to BLM.

Plasmids were isolated from 5 of the 11 positive colonies (pDR1012 to pDR1016) and amplified in *E. coli*. Retransformation with each of the five plasmids into strain DRY211 conferred resistance to BLM. All five plasmids contained the same 13-kb genomic fragment (see Fig. 2). The smallest genomic DNA from plasmid pDR1012 conferring BLM resistance to strain DRY211 was further characterized by subcloning 2.5-kb *Hin*dIII-*Hin*dIII, 3.0-kb *Hin*dIII-*Hin*dIII, 5.0-kb *Hin*dIII-*Hin*dIII, 5.0-kb *Eco*RI-*Eco*RI, and 2.0-kb *Sal*I-*Xba*I fragments into the single-copy vector YCp50 to produce, respectively, pDR1026, pDR1027, pDR1028, pDR1035, and pDR1051 (Fig. 2A). Plasmid pDR1051 contained the smallest fragment that conferred BLM resistance to DRY211. This fragment contained the *IMP2* gene as determined by DNA sequence analysis (10). The entire coding region of *IMP2* was isolated by PCR (45) with primers DR14 and JYM17 (primer sequence described below), with pDR1051 as a template, and then was subcloned into a 2μm multicopy vector, pYES2.0 (Clontech, Palo Alto, Calif.), bearing the galactose-inducible promoter *GAL1* to produce the Imp2 overexpression plasmid pDR1055. The same PCR protocol was used to isolate the native and defective *IMP2* genes, respectively, from the genomic DNA of the wild type and mutant strain DRY211.

Construction of the *imp2* deletion mutant. The 2.0-kb *Sal*I-*Xba*I fragment was subcloned into the vector pBluescript II (K/S). The resulting plasmid, pDR1049, was digested with *Pst*I, which removes 1,470 bp from bp −620 to +850 encompassing a major portion of the promoter region and 91% of the coding region. The released 1,470-bp fragment was replaced by a 3.8-kb fragment bearing the selective marker gene *LEU2*$^+$ to produce plasmid pDR1050. The latter plasmid was digested with *Hin*dIII and *Xba*I to isolate a 4.3-kb *imp2::LEU2* fragment flanked by ~200 and ~330 nucleotides of *IMP2* gene sequences, respectively, on the 5′ and 3′ sides of the *LEU2*$^+$ marker, which was used to transform parent strains to *LEU*$^+$ by one-step gene targeting (15, 51). Mutants bearing the deletion allele *imp2::LEU2* were confirmed by Southern blot analysis when probed with the ^{32}P-labeled *Sal*I-*Xba*I 2.0-kb fragment containing the entire *IMP2* gene and flanking sequences. While the parent strain produced a single 2.0-kb fragment upon digestion with *Sal*I and *Xba*I, the mutant produced the expected 4.3-kb fragment due to the insertion of the *LEU2*$^+$ selective marker gene.

Crude extract preparation. Log-phase cultures (typically 5 ml grown in selective medium or YPD medium) were centrifuged at 3,000 rpm for 5 min and washed twice with 20 mM potassium phosphate buffer (pH 7.0). To the pellet in a 0.5-ml Eppendorf tube was added 300 μl of yeast extraction buffer (50 mM Tris-HCl [pH 7.5]; 30 mM KCl; 10% glycerol; 1 μg of each of the protease inhibitors aprotinin, leupeptin, trypsin inhibitor, TPCK [tolylsulfonyl phenylalanyl chloromethyl ketone], and chymostatin per ml; 1 mM benzamidine; 1 mM phenylmethylsulfonyl fluoride) and 0.5-mm-diameter zirconium beads to fill the Eppendorf tube. Cells were broken by a mini-bead beater (Xymotech, Montreal, Canada) set at 5,000 rpm for six times at 20 s each with 1-min intervals on ice. Cell debris and beads were removed by centrifugation at 12,000 rpm for 5 min in an Eppendorf centrifuge at 4°C. The supernatant was collected, and the concentration of the crude protein extract was determined by the Bradford assay.

Alkaline sucrose density gradient. Yeast cells were lightly inoculated and allowed to grow overnight in 2 ml of SD medium containing 15 μl of [5,6-^{3}H]uracil (17 Ci/mmol; New England Nuclear) and with the required amino acids supplemented at 50 μg/ml and uracil supplemented at 5 μg/ml. The next day, the exponentially growing cells were washed twice in 20 mM potassium phosphate buffer (pH 7.0), resuspended at a density of ~1.0×10^7 cells per ml in YPD medium, and then either untreated or treated with 10 μg of BLM per ml for 1 h. All growth and treatments were performed at 30°C with shaking in an orbital shaker at 250 rpm. In the case of posttreatment incubation, cells were

washed twice and allowed to recover in fresh YPD medium for 5 h. Untreated and treated cells were pelleted at 5,000 rpm for 2 min in an Eppendorf centrifuge and resuspended in 200 μl of buffer A (2% β-mercaptoethanol [vol/vol], 10 mM EDTA, 20 mM Tris-H_2SO_4 [pH 9.3]) for 10 min on ice. Cells were recovered and resuspended in 100 μl of buffer B (100 μg of Zymolyase per ml, 200 μg of RNase A per ml, 0.8% [vol/vol] Nonidet P-40, 10 mM EDTA, 50 mM potassium phosphate buffer [pH 7.0]) for 20 min at 30°C, and then 100 μl of buffer C (25 μg of proteinase K per ml, 1% [wt/vol] sodium dodecyl sulfate, 10 mM EDTA, 100 mM NaCl, 50 mM Tris-HCl [pH 8.5]) was added and incubated at 50°C for 1 h. The cell debris fraction was extracted once with 200 μl of phenol, and the aqueous phase was gently layered onto 100 μl of top layer (1 M NaOH, 20 mM EDTA) on top of a 4-ml 5 to 20% (wt/vol) alkaline sucrose gradient in buffer D (0.5 M NaCl, 10 mM EDTA, 0.2 M NaOH) (49). Each gradient contains DNA from $\sim 1.0 \times 10^7$ lysed cells. Prior to the making of the gradient, each polyallomer tube (Beckman) was precoated with 1 μg of salmon sperm DNA per ml sheared by 10 passages through an 18-gauge needle. The gradient contained a bottom layer cushion of 100 μl of buffer E (70% sucrose, 0.33 M NaOH). Gradients were spun in an SW56 Ti rotor at 35,000 rpm for 130 min and were calibrated with ^{3}H-labeled T7 DNA (molecular weight of 2.5×10^7), which was generously provided by Raymond Kim, Harvard University. Immediately after the run, fractions of 250 μl were collected in 0.5-ml Eppendorf tubes by siphoning with a 5-μl-capacity capillary tube immersed to the bottom of the gradient. In general, each gradient yielded 20 to 22 fractions. The fractions were allowed to sit for 4 to 5 h at room temperature to allow complete hydrolysis of RNA. The samples were processed by vacuum filtration onto 2.4-cm-diameter circular GF/C filters (Whatman) with a Millipore apparatus. Each filter was washed three times with 5 ml of 5% trichloroacetic acid, followed by a brief rinse with 95% ethanol. The dried filter was counted in 5 ml of scintillation fluid (Amersham), and counts in each fraction were expressed as a percentage of the total.

DNA polymerase I incorporation. Log-phase cells (10 ml) grown in YPD medium were treated with 20 μg of BLM per ml for 1 h. The cells were washed three times with 20 mM potassium phosphate buffer (pH 7.0) and either processed directly or resuspended in fresh growth medium for 5 h of additional growth before extraction of the chromosomal DNA, as described previously (47, 50). Incorporation of [*methyl*-^{3}H]dTMP into the DNA was performed as previously described (47).

Assay. β-Galactosidase activity was determined from crude extracts as previously described (38).

Immunoblotting. Anti-LexA antibodies (gift of Erica Golemis, The Fox Chase Cancer Center, Philadelphia, Pa.) were used at a dilution of 1:2,000 in 10 mM Tris-HCl (pH 7.5)–150 mM NaCl–5% powdered milk (14); 10 ml of this mixture was used to probe the nitrocellulose blots (8 by 10 cm) overnight at 4°C. The secondary antibody was goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase (Amersham), which was used at a 1:5,000 dilution and detected by the enhanced chemiluminescence system (Dupont, NEN).

Northern (RNA) blot. Total RNA was prepared by the rapid method (55). The nitrocellulose blot was probed with a random-primed ^{32}P-labeled 0.9-kb fragment derived from the coding region of the *IMP2* gene.

Construction of *lexA-IMP2* fusions. The following oligonucleotide primers were used for the construction of *lexA-IMP2* gene fusions: DR14, 5′-GAGTTC GACGGAATTCAGATGGAA-3′; JYM4, 5′-GGGAGGGAGGATCCTTGCT TTAGAC-3′; JYM12, 5′-GAGCAGATGGAATTCGGCCACAGGG-3′; JYM17, 5′-GGTCACTCGCGGATCCCCTTGCGCT-3′; JYM18, 5′-GGTAA CACTTGGATCCCCCAGATCGAT-3′; and JYM19, 5′-GCCCTGACCGAATT CATGGGCGCA-3′. The *Bam*HI and *Eco*RI restriction sites are underlined. The primer pairs JYM12 and JYM4, DR14 and JYM17, JYM12 and JYM18, and JYM19 and JYM4, respectively, amplified 1,200-, 966-, 378-, and 615-bp fragments of the coding region of the *IMP2* gene, except for the 1,200-bp fragment, which also contains 200 bp of 3′-untranslated DNA sequence, by PCR (45) and with plasmid pDR1051 as a template. The fragment bearing the entire *IMP2* coding region and 3′-untranslated region was subcloned into plasmid pBTM116 (generously provided by D. Gietz, University of Manitoba, Winnipeg, Manitoba, Canada) to produce the plasmid pN-lexA-Imp2(1–312); the N-lexA indicates that *lexA* is fused to the N-terminal end of the *IMP2* gene. The 966-bp fragment bearing the entire *IMP2* coding region was subcloned into pClexA (generously provided by Ian York, Dana-Farber Cancer Institute, Boston, Mass.), generating plasmid pC-lexA-Imp2(1–312); the C-lexA indicates that *lexA* was attached to the C-terminal end of the *IMP2* gene. The remaining two fragments, 378 and 615 bp in size, were also subcloned into pBTM116 to produce the plasmids pN-lexA-Imp2(1–116) and pN-lexA-Imp2(117–312), respectively. The DNA sequences of all constructs were confirmed by the dideoxy chain termination method (54). The plasmids were transformed into either the parent yeast strain CTY10-5d or the isogenic strain bearing a deletion (*imp2*::*LEU2*) of the *IMP2* gene. The lexA(1–87)-GAL4 plasmid was provided by Mark Ptashne (Harvard University).

Construction of the *IMP2* promoter fusion to *lacZ*. Primers JYM6 (5′-AA TCTTGTCGACGAACCA-3′) and JYM15 (5′-CCCTGTGGCCGAATTC**CAT** CTGCTC-3′), containing, respectively, the restriction sites *Sal*I and *Eco*RI (underlined), were used to amplify a 695-bp fragment consisting of the promoter region of the *IMP2* gene from positions −695 to +17. The fragment was subcloned into the multicopy vector YEP356R (generously provided by Fred Winston, Harvard Medical School, Boston, Mass.), such that the ATG of the *IMP2* gene forms the initiation codon for the *E. coli* reporter *lacZ* gene encoding β-galactosidase.

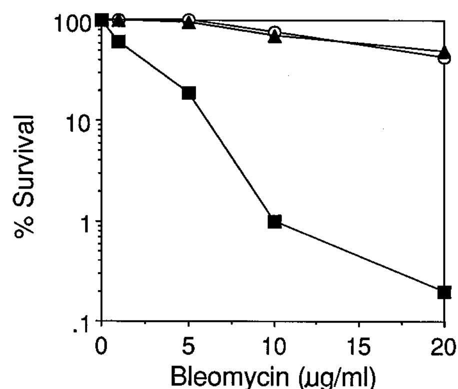

FIG. 1. Sensitivity of yeast strains challenged with BLM. ○, wild-type parent strain DBY747 (*APN1*$^+$); ▲, strain DRY370 (*apn1*Δ::*HIS3*); ■, the BLM-sensitive mutant strain DRY211 (*APN1*$^+$). Points represent the means of two independent determinations.

RESULTS

Isolation, characterization, and phenotypes of the yeast mutant DRY211. We have developed a screen to identify *S. cerevisiae* mutants that are hypersensitive to BLM. We took advantage of the finding that *apn1* deletion mutant DRY370, which lacks the major AP endonuclease, displays a high rate of spontaneous mutation (29, 48). This mutator effect in DRY370 was thus exploited to search for mutants that were hypersensitive to BLM. This was accomplished by plating DRY370 for independent single colonies that were subsequently streaked onto rich medium plates (YPD medium) with and without 2 μg of BLM per ml. From 15,000 colonies screened, three were found to be sensitive to BLM. The three mutants exhibited different sensitivities to BLM, but only the most sensitive mutant, DRY211-c, was chosen for further studies. The mutant strain DRY211-c was crossed to DBY747 (*APN1*$^+$; BLM resistant) to produce the diploid DBY747 × DRY211-c. The diploid was resistant to BLM, indicating that the genetic defect carried by DRY211-c was recessive. Tetrad analysis of the diploids resulted in a 2:2 BLM-resistant-to-BLM-sensitive segregation, suggesting that BLM sensitivity was due to a single mutation. A haploid strain, DRY211 (*APN1*$^+$; BLM sensitive), derived from the cross presented above, was used for further analysis.

The haploid mutant strain DRY211 was 10-fold more sensitive to BLM than the wild type, at a dose that decreased survival by 50% (Fig. 1). Interestingly, DRY211 was also hypersensitive to the chemical oxidants hydrogen peroxide (H_2O_2) and *tert*-butylhydroperoxide and was modestly sensitive to γ rays compared with the wild type (see Fig. 3). However, strain DRY211 was no more sensitive than the wild type to other DNA-damaging agents, including the alkylating agent MMS, UV light, and the UV-mimetic agent 4-NQO (Table 2). Thus, this mutant appears to be hypersensitive only to oxidative DNA-damaging agents.

DRY211 exhibits unique phenotypes in response to DNA-damaging agents. A series of survival curves were examined to compare the drug sensitivity of strain DRY211 with those of a number of representative DNA repair-defective mutants belonging to the nucleotide excision (e.g., *rad1* and *rad2*), postreplication (e.g., *rad6* and *rad9*), and recombinational (e.g., *rad52*) DNA repair pathways. For simplicity, the results ob-

TABLE 2. Comparison of the drug sensitivities of the parent strain and various yeast mutants[a]

Strain	% Survival in response to[b]:					
	BLM (5 μg/ml)	H_2O_2 (1 mM)	*t*-BH (2 mM)[c]	MMS (0.05%)	γ rays (5 kilorads)	4-NQO (0.5 μg/ml)
DBY747 (parent)	94	76	80	88	97	95
DRY370 (*apn1*Δ)	96	55	60	49	94	97
DRY211	20	15	18	86	80	96
DBY747 (*rad1*Δ)	97	71	88	76	ND[d]	12
DBY747 (*rad2*Δ)	96	77	83	72	ND	15
DBY747 (*rad6*Δ)	60	ND	85	1	39	4
DBY747 (*rad9*Δ)	92	ND	80	78	ND	55
DBY747 (*rad52*Δ)	45	35	75	0.8	14	1

[a] All strains are isogenic to the parent DBY747, except for the deleted alleles shown in parentheses. Log-phase cells were treated with the indicated agents in YPD liquid medium as described in Materials and Methods.

[b] Values are averages obtained from data from three independent survival curves and represent the percent survival of each strain at a fixed dose of the DNA-damaging agent (see Materials and Methods).

[c] *t*-BH, *tert*-butylhydroperoxide.

[d] ND, not determined.

tained from a large number of survival curves are summarized in Table 2. Mutants defective in the nucleotide excision repair pathway were hypersensitive to 4-NQO, but strain DRY211 was not. Among other mutants tested, only *rad6* and *rad52* mutants displayed sensitivity to BLM (23). However, they were considerably less sensitive to BLM than DRY211 (Table 2). Both *rad6* and *rad52* mutants were also hypersensitive to a wide variety of other DNA-damaging agents, including MMS, γ rays, and 4-NQO (Table 2). Because of these phenotypic differences among the mutants in response to DNA-damaging agents, we infer that strain DRY211 harbors a distinct defect that is unrelated to mutants belonging to the nucleotide excision, postreplication, and recombinational DNA repair pathways. In addition, mutants that lacked either the oxidant defense genes *SOD1* (encoding superoxide dismutase), *CTT1* (encoding catalase T), *ATX* (encoding a putative copper transporter [33]), and *YAP1* (encoding a transcriptional activator [28, 65]) or *BLH1* (encoding the cysteine protease BLM hydrolase that degrades BLM [12, 36]) and mutants lacking functional mitochondria were not sensitive to BLM and thus were unrelated to DRY211 (data not shown).

Isolation of the gene *IMP2* that rescued the strain DRY211 defect. A plasmid-borne 13-kb DNA fragment was isolated from the *S. cerevisiae* single-copy genomic library that restored wild-type BLM resistance to strain DRY211 (Fig. 2A [see Materials and Methods]). The resistance was due to a small 2.0-kb *Sal*I-*Xba*I DNA segment subcloned from the larger fragment and carried by the single-copy plasmid pDR1051 (Fig. 2A and B [see Materials and Methods]). Partial DNA sequence analysis, followed by comparison with DNA sequences present in the GenBank database, revealed that the sequence of the 2.0-kb *Sal*I-XbaI fragment was identical to sequences starting from the left arm of chromosome IX. The corresponding fragment contained three open reading frames, two of which were truncated early by *Sal*I and *Xba*I restriction enzymes, but only one corresponded to the entire *IMP2* gene (accession number X61928). The *IMP2* gene was initially isolated by complementation of a yeast mutant, *CT/1*, which was unable to grow on galactose, maltose, and raffinose only when the mutant lacked functional mitochondria (10). The exact role of the *IMP2* gene product in utilization of these carbon sources has not been reported (10).

We examined whether the gene complementing the mutant was indeed *IMP2*. Restriction enzyme analysis revealed that the entire *IMP2* gene was located within the 2.0-kb *Sal*I-XbaI fragment. In addition, the precise *IMP2* coding region was

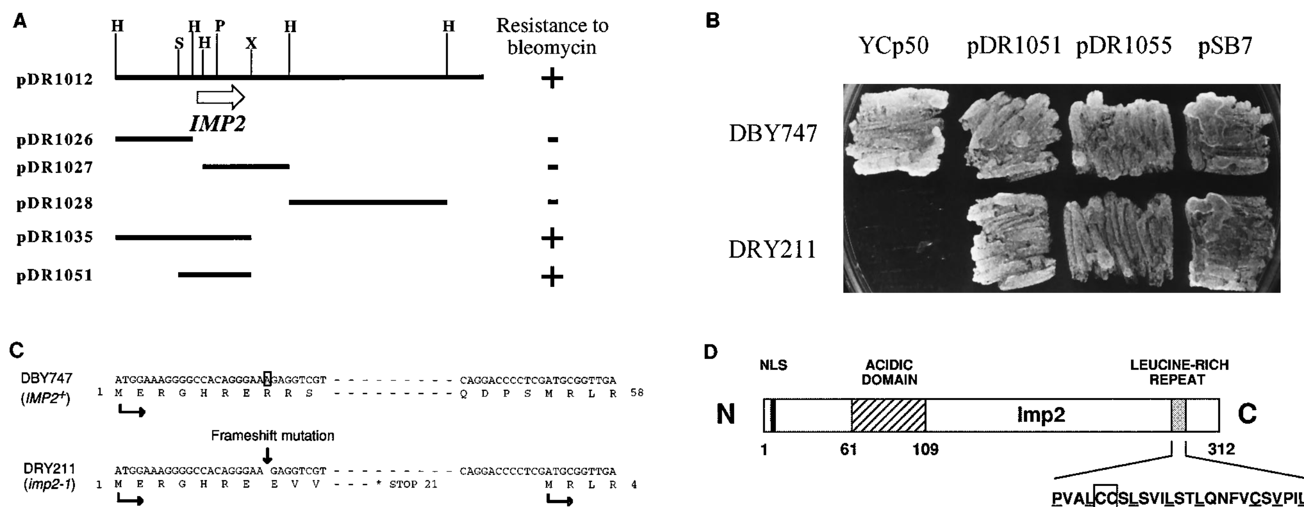

FIG. 2. Identification of the gene, *IMP2*, that restored BLM resistance to strain DRY211. (A) Physical map of the 13-kb genomic fragment and its derivatives subcloned into the single-copy vector YCp50. The letters indicate the following relevant restriction enzyme sites: H, *Hin*dIII; P, *Pst*I; S, *Sal*I; and X, *Xba*I. +, resistance to BLM; −, no resistance to BLM. (B) Plate assay of DBY747 and mutant DRY211 bearing the following vector or plasmid: vector YCp50; pDR1051, 2.0-kb *Sal*I-*Xba*I fragment subcloned into YCp50; pDR1055, the coding region of *IMP2* isolated from genomic DNA by PCR and subcloned next to the galactose-inducible *GAL1* promoter in the multicopy vector pYES2.0; and pSB7, which bears the entire *IMP2* gene with the 5′ and 3′ flanking untranslated DNA sequence (10). The plate contains solid YPD medium with 2 μg of BLM per ml. (C) Partial comparison of the DNA and predicted amino acid sequences of the *IMP2* gene isolated from DBY747 and the mutant DRY211. The entire *IMP2* gene with 5′ and 3′ flanking untranslated DNA sequences was isolated from the strains by PCR and subjected to DNA sequence analysis. The open box indicates the loss of an adenine nucleotide in the coding strand of the mutant allele, designated *imp2-1*, resulting in an early stop codon, represented by the asterisk. The arrow indicates the next in-frame start codon located at position +165 of the native gene *IMP2*$^+$ and its allele *imp2-1*. (D) Schematic representation of the structural features of the Imp2 protein. Full open box, the entire length of Imp2 protein from residues 1 to 312; vertical bar, putative nuclear localization signal (NLS) from residues 10 to 15; hatched box, acidic region spanning residues 60 to 109; shaded box, location of the LRR sequence, which is underlined.

amplified by PCR and placed under the control of the galactose-inducible *GAL1* promoter in the pYES2.0 vector to produce plasmid pDR1055 (Fig. 2B [see Materials and Methods]). This plasmid conferred full BLM resistance to strain DRY211 in the absence of induction by galactose (Fig. 2B [*GAL1* is not a tightly regulated promoter and can weakly express genes under glucose-grown conditions]). Subsequently, we obtained the plasmid pSB-7, which contained the *IMP2* gene under its native promoter (generously provided by C. Donnini, Parma, Italy), and found that it also conferred BLM resistance to the mutant (Fig. 2B), suggesting that the BLM-complementing gene and *IMP2* are the same. We then tested whether DRY211 contained a mutation in the *IMP2* gene. A 0.9-kb fragment that spanned the *IMP2* gene was isolated and completely sequenced in DRY211 (see Materials and Methods). A single base pair located 22 nucleotides downstream from the ATG start codon (the first adenine in codon 8, AGA) was deleted in *IMP2* (Fig. 2C). No additional mutation was found 565 bp upstream or 200 bp downstream, respectively, of the *IMP2* start and stop codons. The -1 frameshift mutation, which we designated *imp2-1*, resulted in an immediate stop codon (Fig. 2C).

The *IMP2* gene encodes a single open reading frame with a size of 936 nucleotides, which is predicted to encode a protein comprising 312 amino acid residues with a molecular mass of 35,182 Da and a calculated pI of 5.1. The deduced amino acid sequence indicated that the Imp2 protein has a putative nuclear localization signal, a highly acidic region comprising 29 aspartic and glutamic acid residues, and a single leucine-rich repeat (LRR) motif that is distinct from the heptad repeat characteristic of a leucine zipper (43) (Fig. 2D).

The *imp2* deletion mutant is hypersensitive to oxidative DNA-damaging agents. To directly test whether the *IMP2* gene is responsible for full cellular resistance to the oxidative DNA-damaging agents, a chromosomal deletion was constructed by replacing the entire coding region of the *IMP2* gene with the *LEU2* marker in the parent strain DBY747 to produce strain DRY212. The resulting deletion allele (*imp2*::*LEU2*) was confirmed by Southern blot analysis (see Materials and Methods). The *imp2* deletion mutant was viable, but grew slightly more slowly than the wild type. Moreover, the mutant displayed the following hypersensitivities (increase in sensitivity compared with that of the parent strain DBY747): BLM, 15-fold; hydrogen peroxide, 10-fold; *tert*-butylhydroperoxide, 10-fold, γ rays, 5-fold; paraquat, 4-fold; menadione, 6-fold; and diamide, 2-fold (Fig. 3 and data not shown). Consistent with hypersensitivity to the oxidative agents was the finding that bubbling of 95% oxygen for 2 h into exponentially growing cells did not kill the *imp2*::*LEU2* mutant but did severely hamper its ability to form colonies on solid YPD medium. As observed with the original mutant, strain DRY212 was no more sensitive to the alkylating agent MMS, UV, or 4-NQO than the parent strain DBY747 (data not shown). Thus, the *imp2* deletion mutant also appears to display hypersensitivities only to oxidative agents. Deletion of the *IMP2* gene in several other wild-type strains (Table 1), including FY86, CTY10-5D, W303α, and YKH12a, resulted in nearly identical drug hypersensitivities, as observed for strain DRY212. Thus, the phenotypes resulting from the loss of the *IMP2* gene are not strain specific. It is noteworthy that the *imp2* deletion mutant hypersensitivities to the oxidants were much more pronounced than those of strain DRY211 (Fig. 3). This finding suggests that expression of the Imp2 protein might be leaky in strain DRY211, which could be possible if there is some translational reinitiation at the next start codon located immediately downstream from the -1 frameshift mutation, i.e., at codon 55 of the *IMP2* gene (Fig. 2C).

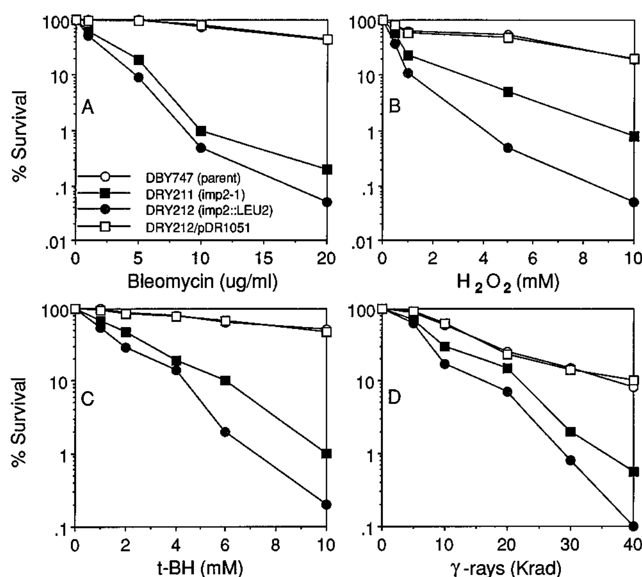

FIG. 3. Sensitivity of yeast strains challenged with the indicated DNA-damaging agents. t-BH, *tert*-butylhydroperoxide. The plasmid is the same as in Fig. 2. Points represent the average values from three independent experiments.

Transformation of plasmid pDR1051 into strain DRY212 restored full wild-type resistance to all of the oxidative agents (Fig. 3; data not shown for paraquat, menadione, and diamide). Increasing the dosage of the *IMP2* gene through the use of a multicopy plasmid, pDR1055, elevated the level of its message (see Fig. 6 below), as well as that of the Imp2 protein, but this did not bestow additional drug resistance to either the wild type or the mutant DRY212 (data not shown). The overproduction of the Imp2 protein was monitored by tagging Imp2 at its C terminus with the influenza virus hemagglutinin (HA1) epitope and was detected by antihemagglutinin antibodies (37). Thus, under normal growth conditions, the *IMP2* gene product is not limiting in the cell. We conclude from these findings that the *IMP2* gene is indeed necessary to specifically provide cellular resistance to oxidative agents. Because these oxidative agents can all produce lesions in the cellular DNA, we postulated that strain DRY212 may be defective in repairing oxidatively induced DNA lesions.

DRY212 is defective in the repair of DNA damage produced by oxidants. To examine whether the drug hypersensitivity of the *imp2* deletion mutant could be a result of the accumulation of unrepaired DNA lesions, both the wild-type and mutant strains were either mock treated or treated with 10 μg of BLM per ml for 1 h. Chromosomal DNA was isolated from these strains and analyzed on alkaline sucrose density gradients. This analysis allows for the measurement of both DNA strand breaks and AP sites because AP sites are alkali labile and are rapidly converted to DNA strand breaks (48). Results from such analysis showed that the sedimentation patterns of chromosomal DNA obtained from either untreated parent or untreated *imp2* deletion mutant cells were indistinguishable (Fig. 4A). Upon treatment of cells, both the wild-type and mutant chromosomal DNAs sedimented much more slowly than the DNA isolated from untreated cells (Fig. 4B versus A). This slower sedimentation is consistent with the accumulation of a substantial amount of strand breaks in the chromosomal DNA. At this dose of BLM, the extent of strand breaks appeared to be similar in both the wild type and the *imp2* deletion mutant. When treated cells were washed free of BLM and resuspended

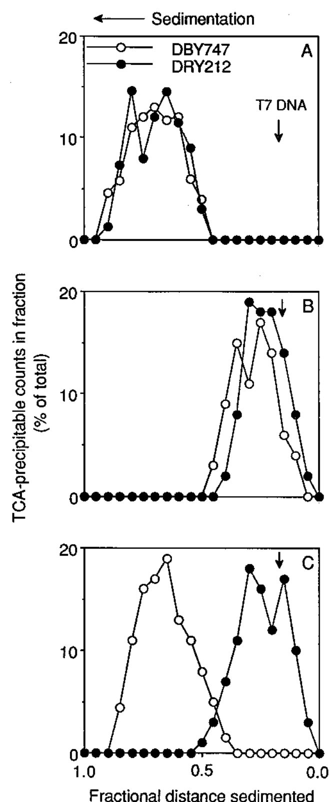

FIG. 4. Alkaline sucrose density gradient analysis of chromosomal DNA isolated from untreated and BLM-treated yeast cells. (A) Untreated. (B) Treated with 10 μg of BLM per ml for 1 h. (C) Treated as described for panel B, washed, and incubated posttreatment for 6 h in YPD medium. The sedimentation positions of bacteriophage T7 DNA are shown by vertical arrows. Points represent the means of two independent experiments. TCA, trichloroacetic acid.

in fresh YPD medium to allow repair of the damaged cellular DNA, the wild-type chromosomal DNA was fully repaired within 6 h of posttreatment incubation; no repair was detected in the mutant chromosomal DNA (Fig. 4C). Analogous results were obtained if cells were challenged with 10 mM H_2O_2 for 30 min (data not shown). In contrast, the mutant showed the same rate of DNA repair as the parent after treatment with 0.15% MMS for 1 h followed by 5 h of recovery (data not shown). These findings indicate that the *imp2* deletion mutant lacks a major function responsible for either directly or indirectly correcting BLM- or H_2O_2-induced DNA lesions.

Accumulation of 3′-hydroxyl ends in the chromosomal DNA of the treated mutant. The inability of the *imp2* deletion mutant to repair BLM-damaged DNA could be due to a defect in processing DNA strand breaks containing blocked 3′ termini. To test this possibility, we examined whether chromosomal DNA isolated from cells treated with BLM at 20 μg/ml for 1 h could allow in vitro DNA repair synthesis by *E. coli* DNA polymerase I (9, 20, 31, 47, 56). Chromosomal DNA obtained from untreated cells, of either the parent or the mutant strain, showed little incorporation of [*methyl*-^{3}H]dTMP by DNA polymerase I (Fig. 5A). In contrast, a substantial amount of label was incorporated into the damaged chromosomal DNA derived from either the wild-type- or the mutant-treated cells (Fig. 5A). This finding suggests that if single-strand-break-bearing 3′-phosphoglycolate is a major lesion produced in the chromosomal DNA by BLM, then this lesion is rapidly removed in both strains to create a free 3′-hydroxyl group during the 1-h treatment. Therefore, the mutant cannot be defective in removing 3′-phosphoglycolate ends from BLM-damaged DNA. The data also suggest that the two strains received the same initial level of DNA strand breaks upon exposure to BLM, as judged by the nearly identical levels of label incorporated into the damaged DNA. Thus, the defect of the mutant is unlikely to be a result of increased uptake of BLM leading to a higher level of DNA strand breaks. In fact, the mutant hypersensitivity to ionizing radiation cannot be explained by uptake (Fig. 3).

We further determined whether the strand breaks were repaired after the cells were washed free of BLM and reincubated in fresh YPD medium for 5 h. The extent of [*methyl*-^{3}H]dTMP incorporation into the DNA obtained from the treated and recovered parent cells was significantly reduced, from 25 pmol to 3 pmol (Fig. 5B), suggesting that strand breaks were efficiently repaired in the parent cells. In contrast, the level of [*methyl*-^{3}H]dTMP incorporation was unaltered in the mutant despite 5 h of posttreatment incubation (Fig. 5B), suggesting that the DNA strand breaks persisted and were not repaired. Collectively, these findings demonstrate that the *IMP2* gene plays an essential role in preserving the integrity of chromosomal DNA against the lethal effects of BLM, H_2O_2, and perhaps the other oxidative DNA-damaging agents.

Spontaneous mitotic recombination rate is elevated in the *imp2* deletion mutants. Since the *imp2* deletion mutant cannot tolerate exposure to agents that produce free radicals, we predicted that cells grown under normal aerobic conditions might spontaneously accumulate endogenous oxidative DNA lesions, such as DNA strand breaks, which, in turn, could induce recombination. We measured the rate of recombination in both the wild type and *imp2* deletion mutants by using a simple direct repeat recombination assay (22). The assay system consists of two mutated heteroalleles of the *ADE2* gene in a direct repeat orientation on a plasmid that was integrated at the *ADE2* locus of strain YKH12a and an isogenic strain, DRY216, carrying the *imp2*Δ::*LEU2* allele. One of the two heteroalleles bears a 2-bp insertion at the 3′ end of the *ADE2* gene (*ade2-n*), and the other contains a 4-bp deletion at the 5′ end of the gene (*ade2-a*) (22). The heteroalleles are separated by the selective marker gene *URA3*. Recombination between the two nonfunctional direct repeats *ade2-n* and *ade2-a* can produce a functional *ADE2* gene and can occur by a deletion mechanism resulting in the loss of the intervening *URA3* gene or by gene conversion, whereby the Ade$^+$ recombinant retains the *URA3*

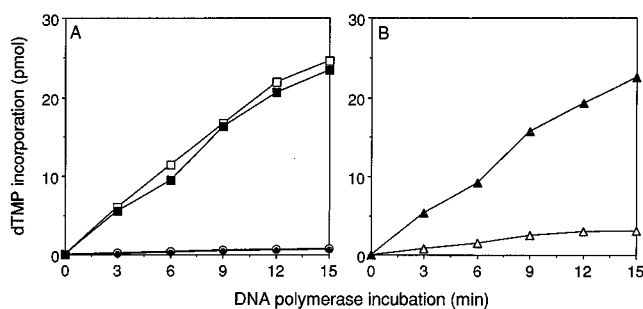

FIG. 5. Incorporation of [*methyl*-^{3}H]dTMP by DNA polymerase I into chromosomal DNA isolated from untreated and BLM-treated yeast cells. Open and solid symbols, respectively, represent parent strain DBY747 (*IMP2*$^+$) and mutant strain DRY212 (*imp2*Δ::*LEU2*). (A) ○ and ●, DNA isolated from untreated cells; □ and ■, DNA isolated from cells treated with 20 μg of BLM per ml for 1 h. (B) △ and ▲, DNA isolated from cells treated as described for panel A, washed twice, and incubated posttreatment for 5 h in YPD medium. Results are representative of three independent experiments.

TABLE 3. Spontaneous rate of recombination at the *ADE2* locus

Expt[a]	Rate of recombination/ cell/generation (10^{-7})[b]		Ura$^+$ colonies (% of Ade$^+$ colonies)[c]	
	YKH12a	DRY216	YKH12a	DRY216
1	4.7 ± 1.2	35.5 ± 4.1	16–21	76–87
2	3.2 ± 0.8	39.7 ± 5.7	18–26	78–85
3	4.1 ± 1.1	42.3 ± 4.8	21–26	82–91

[a] Each experiment represents the average of three independent determinations for a total of nine determinations.

[b] The rate of recombination, as measured by adenine prototroph formation, was determined as described previously (22).

[c] For each independent determination, 25 Ade$^+$ single colonies were streaked onto solid uracil omission SD medium to determine the number of Ura$^+$ colonies, which were expressed as a percentage of the Ade$^+$ colonies.

gene. The results of three independent experiments showed that the rate of recombination was elevated 7- to 12-fold in the *imp2* deletion mutant strain DRY216 compared with the rate of recombination in the wild type (Table 3). This is consistent with results obtained with other DNA repair-defective mutants that have also been shown to induce high rates of mitotic recombination because of the accumulation of DNA lesions that require repair by the recombination process (2).

We further examined whether the distribution of Ade$^+$ colonies that retained the *URA3* gene varied between the wild type and the mutant (Table 3). In the wild type, 16 to 26% of the Ade$^+$ colonies were Ura$^+$, suggesting that the major event in the parent leading to Ade$^+$ was through deletion. In contrast, 76 to 91% of the Ade$^+$ colonies obtained from the *imp2* deletion mutant were Ura$^+$, indicating that the Ade$^+$ colonies arose in the mutant perhaps through a gene conversion mechanism that retained *URA3*. Thus, the loss of *imp2* creates a situation that could favor recombination by gene conversion.

***IMP2* is not inducible by oxidative agents.** Several genes are induced at the transcriptional level when yeast cells are exposed to DNA-damaging agents (3). We examined whether the mechanism by which Imp2 protein provides resistance to the oxidants involves its own induction. Northern blot analysis revealed that the *IMP2* gene message was not abundantly expressed, but it was amply expressed by the multicopy plasmid pDR1055 (Fig. 6, lane 4). The mRNA level was not altered in the −1 frameshift mutant, DRY211, compared with that in the wild type (lane 2 versus lane 1). Thus, the strain DRY211 defect lies in the inability to express a functional Imp2 protein. As expected, no mRNA was expressed by the *imp2* deletion mutant (lane 3). In a separate experiment, when log-phase

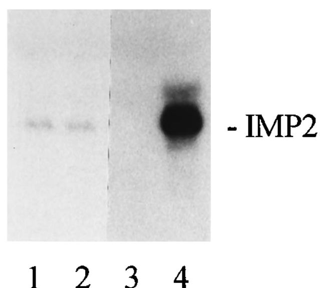

FIG. 6. Northern blot analysis probed with the coding region of the *IMP2* gene. Lanes 1 to 4 each contained 20 μg of total RNA prepared from, respectively, strains DBY747 (*IMP2*$^+$), DRY211 (*imp2-1*), DRY212 (*imp2*Δ::*LEU2*), and DRY212 harboring the multicopy plasmid pDR1055 bearing the *IMP2* gene. The intensities of actin (*ACT1* message) were the same in all lanes (data not shown).

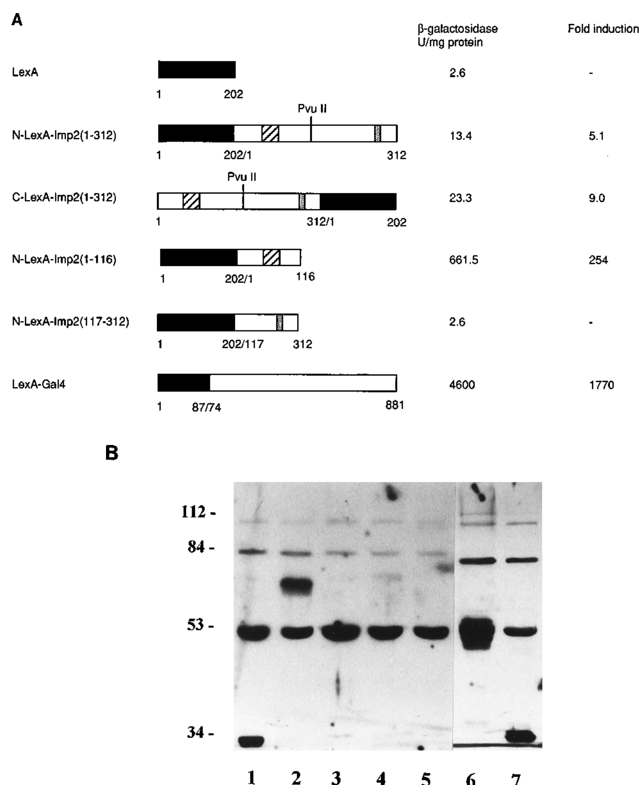

FIG. 7. Analysis of LexA-Imp2 fusion expression. (A) Activation by LexA-Imp2 fusions. Crude extracts derived from either strain CTY10-5d (*IMP2*$^+$) or CTY10-5d (*imp2*Δ::*LEU2*) harboring the indicated LexA-Imp2 fusion constructs were assayed for β-galactosidase. The results were the averages of three separate determinations. For each experiment, three independent single colonies bearing the indicated fusion construct were used to determine the β-galactosidase levels. (B) Immunoblot analysis of cell extracts with anti-LexA antibodies. Each lane contained 50 μg of crude protein extract prepared from strain CTY10-5d harboring various plasmids. Lanes: 1, pN-lexA plasmid; 2, pN-lexA-Imp2(1–312); 3, pC-lexA; 4, pC-lexA-Imp2(1–312); 5, no plasmid (showing the nonspecific polypeptides); 6, pN-lexA-Imp2(1–116); 7, same as lane 1. Lanes 6 and 7 were derived from extracts analyzed on a separate 10% polyacrylamide–SDS gel. The molecular mass standards (Bio-Rad) are indicated to the left in kilodaltons.

wild-type cells were treated with either increasing concentrations of BLM (ranging from 0 to 20 μg/ml) or H_2O_2 (ranging from 0 to 5 mM) for 30 min, no induction of the *IMP2* mRNA was observed relative to that of the untreated cells (data not shown). Consistent with this observation, a plasmid construct bearing the presumptive *IMP2* gene promoter from nucleotides −695 to +1 fused to the reporter gene *lacZ* showed basal-level expression of β-galactosidase of 114 U/mg of protein that was unaltered by drug treatment. These findings suggest that the mechanism by which the *IMP2* gene provides resistance to BLM or H_2O_2 may be dependent upon its constitutive expression.

***IMP2* encodes a transcriptional activator.** The presence in the Imp2 protein of an acidic domain, a structure found in a number of well-characterized transcriptional activators (21, 35, 53), hinted that it might carry out its biological function by acting as a transcriptional activator. We therefore tested directly whether Imp2 is a transcriptional activator by fusing the *IMP2* gene in frame with a portion of the bacterial *lexA* gene, which was engineered into two different plexA vectors, pN-lexA and pC-lexA, such that LexA can be fused to either the N- or C-terminal end of Imp2 to create, respectively, the N-LexA-Imp2(1–312) or C-LexA-Imp2(1–312) fusion protein (Fig. 7A).

The *lexA* gene used encodes a 202-amino-acid polypeptide bearing the DNA binding domain that recognizes and binds to the *lexA* operator constructed within the yeast *CYC* promoter. The *lexA-CYC* promoter is attached to a reporter gene, *lacZ*, encoding β-galactosidase, whose expression is dependent upon activation of the *lexA-CYC* promoter. This *lexA-CYC-lacZ* reporter cassette is integrated into the chromosome of strain CTY10-5d (Table 1).

To confirm that the N-lexA-Imp2(1–312) fusion construct expressed a heterologous protein, crude extracts were prepared from cells harboring either the N-lexA vector or the fusion construct and analyzed by immunoblotting with anti-LexA polyclonal antibodies (Fig. 7B). It should be noted that this antibody recognizes multiple nonspecific polypeptides in control extracts (Fig. 7B, lane 5). The N-lexA vector expressed a 31-kDa polypeptide (lane 1), whereas the N-lexA-Imp2(1–312) construct directed the expression of a stable chimeric polypeptide (~66 kDa) with the expected combined molecular mass of LexA and the predicted size (35 kDa) of Imp2 (lane 2). When the same extracts were quantitated for β-galactosidase activity, the level was fivefold higher from cells expressing N-LexA-Imp2(1–312) than from cells expressing LexA (Fig. 7A). This finding suggests that LexA, which cannot activate transcription, was able to bring the Imp2 protein into proper promoter context to activate β-galactosidase expression from the *lexA-CYC* promoter. The level of β-galactosidase activity was not affected by the *imp2*::*LEU2* deletion mutation or by the introduction into the cell of a single- or a multicopy plasmid bearing the *IMP2* gene (data not shown), thus eliminating the possibility that Imp2 influences the *lexA-CYC* promoter.

We rationalized that the level of activation by N-LexA-Imp2(1–312) could be hindered by the location of LexA. As a result, LexA was attached to the C-terminal end of Imp2 with the vector pC-lexA to produce the chimeric protein C-LexA-Imp2(1–312) (Fig. 7A). However, the latter fusion was 15-fold less abundant than N-LexA-Imp2(1–312) (Fig. 7B, lane 4 versus lane 2), which could be due to an intrinsic property of the pC-lexA vector, since it only weakly expressed LexA (Fig. 7B, lane 3). Despite the weak expression of C-LexA-Imp2(1–312), the extract contained twofold more β-galactosidase activity than N-LexA-Imp2(1–312). This finding suggests that the position of LexA indeed affected the activation function of Imp2. Taken together, the data are consistent with a role for Imp2 as a transcriptional activator.

Because the C-LexA-Imp2 fusion was considerably less abundant, we used the N-LexA fusion to define the region of Imp2 that contains the activation domain. Two additional LexA fusion constructs were made, one bearing only residues 1 to 116 and the other containing the remaining 117 to 312 amino acid residues of the Imp2 protein (Fig. 7A). The N-lexA-Imp2(1–116) construct directed the expression of a mixed population of polypeptides, ranging from 49 to 55 kDa in size, that migrated to almost the same position as a nonspecific protein present in control yeast extracts (Fig. 7B, lane 6 versus lane 5). These polypeptides could result from minor proteolytic processing or from improper termination of the N-LexA-Imp2(1–116) polypeptide during translation. Interestingly, the N-LexA-Imp2(1–116) extracts contained a substantially high level of β-galactosidase activity, more than 250-fold higher than that of LexA alone (Fig. 7A). In contrast, N-LexA-Imp2(117–312) did not sustain activation of the reporter. We concluded that the N-terminal portion of Imp2 bears the activation domain, which is likely limited to the acidic domain (Fig. 2D).

DISCUSSION

In this study, we present the first direct evidence that the *IMP2* gene encodes a transcriptional activator, which plays a major role in the cellular defense against oxidative stress. The biological role of Imp2 is not, however, limited to the defense against oxidative agents, but it endows the cell with protection against other stressful conditions, such as osmotic shock and exposure to high temperatures (10, 37). In this respect, Imp2 could be a general stress-related transcriptional activator. We therefore propose that Imp2 activates or maintains constitutive expression of several proteins, including at least one target protein whose function is directly involved in the protection against oxidative damage to the cellular DNA.

We have not directly determined the type of strand breaks caused by BLM in yeast cells, but they are likely to be a mixture of single- and double-strand breaks, on the basis of known lesions that are produced by BLM (1, 58). In yeast cells, double-strand breaks are repaired by the recombinational DNA repair pathway involving the Rad52 protein, whose function is still unclear (13). *rad52* deletion mutants are exquisitely sensitive to double-strand breaks (13). The fact that *rad52* mutants are much less sensitive to BLM than *imp2* deletion mutants excludes the possibility that double-strand breaks are the predominant DNA lesions produced by BLM in yeast cells. Moreover, *rad52 imp2* double-deletion mutants are notably more sensitive to BLM than either of the single mutants (46). Thus, both *RAD52* and *IMP2* gene products have distinctive roles in the repair of BLM-induced DNA lesions. It should be emphasized that the facts that *imp2* mutants are also hypersensitive to H_2O_2 and that this oxidant is known to make very few double-strand breaks but makes mostly single-strand breaks (48, 63) argue strongly that the mutant has a defect in single-strand break rejoining.

Exactly how the DNA strand breaks induced by BLM are repaired remains to be elucidated. One possibility involves processing of the strand breaks by either a 3′-to-5′ exonuclease, a 5′-to-3′ exonuclease, or both, to create a gap allowing entry of DNA polymerase δ or ε to complete repair synthesis (5). Alternatively, the gap could be repaired by mitotic recombination, whereby one strand of another homologous duplex chromosome is transferred into the damaged duplex DNA and serves as a template for DNA repair synthesis (59). Because of the complexity underlying these DNA repair processes, due to the involvement of many proteins, it is difficult to ascertain which gene(s) could be under the positive regulation of the Imp2 protein. To date, we have isolated several additional mutants that are nearly as sensitive as the *imp2* deletion mutant to BLM (19). Whether any of these mutants contain a defective structural gene encoding a DNA repair protein that might be regulated by the Imp2 protein remains to be seen.

Our data indicate that the activation domain of Imp2 lies within the first 116 residues of the N terminus, consistent with the presence of a highly acidic region that spans amino acids 60 to 109. Acidic domains have been found in several well-defined transcriptional activators, including Gal4, Gcn4, and VP16 (21, 35, 53). Further experiments would be required to identify the exact sequence limit and the crucial amino acids involved in the activation domain of Imp2. The observed 250-fold activation of β-galactosidase by N-LexA-Imp2(1–116) parallels that reported for the potent yeast Snf5 transcriptional activator, which affects the expression of a broad array of genes (30), and suggests that Imp2 could also be a strong activator. Alternatively, the modest activation detected by the full-length protein N-LexA-Imp2(1–312), which is within the range expected of a weak transcriptional activator (17, 24), argues that Imp2 could

be a weak activator. However, the latter possibility could be misleading if N-LexA-Imp2(1–312) did not achieve its effective concentration in the nucleus, assuming that LexA or the larger size of the fusion protein impedes nuclear transport. The weak activation by N-LexA-Imp2(1–312) may also be explained if LexA(1–202), which is known to form a dimer (52), and the LRR motif, which could participate in protein-protein interaction (described below), together induce a structural change such that both the N and C termini of Imp2 are blocked, thereby limiting its access to the transcription complex. In the case of C-LexA-Imp2(1–312), a slightly better activator than N-LexA-Imp2(1–312), maximal activation may not have been attained by this protein because of its weak expression. If so, high-level expression of C-LexA-Imp2(1–312), perhaps through a promoter other than *ADH*, may strongly activate the reporter. Whether Imp2 is a strong or weak activator would be more evident when a target gene becomes available. Presently, additional studies are under way to identify Imp2 target genes by using differential display (32).

Possible mechanism of gene activation by Imp2. The Imp2 protein bears a single LRR motif that is 25 residues in length ($_{272}$Pro-Val-Ala-**Leu**-Cys-Cys-Ser-**Leu**-Ser-Val-Ile-**Leu**-Ser-Thr-**Leu**-Gln-Asn-Phe-Val-**Cys**-Ser-Val-Pro-Ile-**Leu**$_{296}$) (the boldface Cys is conserved in all LRRs). TheLRR motif ranges from 1 U to as many as 30 U, and each unit, 22 to 29 amino acid residues in length, is capable of forming a β-α hairpin structure (25). The LRR of Imp2 has a high degree of similarity to those present in a diverse group of proteins that function in DNA repair, signal transduction, cell adhesion, cell development, and RNA processing (25). In a few cases, the LRR has been clearly documented to mediate protein-protein interactions—for example, that of the yeast transcriptional activator Ccr4 (11, 26). Because Imp2 has no obvious DNA binding domain, it is conceivable that the C-terminal LRR interacts with a protein bound to the promoter element of target genes, thereupon permitting the N terminus containing the acidic domain to efficiently activate gene expression.

ACKNOWLEDGMENTS

We are grateful to Eric Alani, Elliot Drobetsky, Miriam Sander, and Michael Weinfeld for critically reviewing the manuscript.

Strain DRY211-c was initially isolated by D.R. in the laboratory of Bruce Demple, who was supported by a grant from NIH (ES03926). This work was supported by a grant to D.R. from the National Cancer Institute of Canada with funds from the Canadian Cancer Society. J.-Y.M. received a graduate student fellowship from the Fonds pour la Formation de Chercheurs et d'Aide à la Recherche, and D.R. is a scholar of the Fonds de la Recherche en Santé du Québec.

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