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The *AUR1* Gene in *Saccharomyces cerevisiae* Encodes Dominant Resistance to the Antifungal Agent Aureobasidin A (LY295337)

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Aureobasidin A (LY295337) is a cyclic depsipeptide antifungal agent with activity against *Candida* spp. The mechanism of action of LY295337 remains unknown. LY295337 also shows activity against the yeast *Saccharomyces cerevisiae*. Generation of a mutant of *S. cerevisiae* resistant to LY295337 is reported. Resistance was found to reside in a dominant mutation of a single gene which has been named *AUR1* (for aureobasidin resistance). This gene was cloned and sequenced. A search for homologous sequences in GenBank and by BLAST did not elucidate the function of this gene, although sequence homology to an open reading frame from the *Saccharomyces* genome sequencing project and several other adjacent loci was noted. Deletion of *aur1* was accomplished in a diploid *S. cerevisiae* strain. Subsequent sporulation and dissection of the *aur1*/*aur1*Δ diploid resulted in tetrads demonstrating 2:2 segregation of viable and nonviable spores, indicating that deletion of *aur1* is lethal. As LY295337 is fungicidal and deletion of *aur1* is lethal, *aur1* represents a potential candidate for the target of LY295337.

Aureobasidins are antifungal cyclic depsipeptides isolated from *Aureobasidium pullulans* R106 (15, 25). Aureobasidin A (LY295337) is the major factor isolated from the fermentation broth of *A. pullulans* R106 (25). This compound has potent in vitro antifungal activity against *Candida albicans*, other species of *Candida*, and *Cryptococcus neoformans* (9, 26).

The model yeast *Saccharomyces cerevisiae* is susceptible to LY295337. The study of mutants of *S. cerevisiae* resistant to the action of LY295337 could lead to an understanding of its mechanism of action. In this study, generation of a dominant mutant of *S. cerevisiae* which is resistant to LY295337 has allowed the cloning of a gene, *ABR1*, which encodes resistance to LY295337 (12, 13). Hashida-Okado and coworkers and Okado et al. have recently isolated the same gene as *ABR1* through resistance to aureobasidin A (named *AUR1*) from *S. cerevisiae* (11, 16). The gene encoding resistance to aureobasidin A will hereafter be referred to as *AUR1*. *AUR1* is unique and essential for viability of *S. cerevisiae*. *AUR1* may encode the target for LY295337.

MATERIALS AND METHODS

Strains, media, plasmids, and transformations. *S. cerevisiae* strains used in this study are presented in Table 1. Cultivation, storage, and genetic manipulation of *S. cerevisiae* were carried out as previously described (19). The yeast shuttle plasmid pRS416 was used for construction of the yeast library, and pRS406 was used for construction of a deletion plasmid (24). Transformation of yeast strains was carried out by the method of Reddy and Maley (18). Transformants bearing the *URA3*-marked plasmids were cured by the use of 5-fluoroorotic acid (5-FOA) (4).

Selective medium for drug resistance was standard yeast-peptone-dextrose (YPD) agar with 5 μg of LY295337 per ml and 1% ethanol (19).

Library construction and propagation of plasmids were performed with *Escherichia coli* DH5α, grown and selected for by standard methods (21).

Determination of antibiotic sensitivity. Drug sensitivity of *S. cerevisiae* was determined by broth microdilution assays performed with YPD broth. The inoculum (10^4 cells per well) was prepared from fresh overnight cultures of *S. cerevisiae* strains in YPD. Plates were incubated at 30°C for 48 h, and growth was determined by A_{650} in a ThermoMax plate reader (Molecular Devices, Inc.).

Generation of mutant resistant to LY295337. The yeast strain YPH1 was made respiration incompetent by successive treatment with ethidium bromide. The resulting strain failed to grow on glycerol as a carbon source (7, 19).

The YPH1 [*rho*^{0}] strain (10^7 cells per plate) was mutagenized by exposure to UV irradiation (20% survival rate) directly on YPD plates containing 5 μg of LY295337 per ml. The plates were allowed to recover in the dark for 2 days and incubated for 3 more days at 30°C. Diploids formed by crossing the resistant mutants with the wild-type YPH2 strain were tested for their sensitivity to LY295337. The diploids were sporulated and dissected, and the resulting progeny were tested for resistance to LY295337.

Genetic mapping. Linkage analysis was performed as described previously (23). Strains harboring *AUR1* were then crossed with multiply marked strains bearing centromere-linked markers for determination of linkage and chromosomal location (23).

Isolation of DNA. Yeast genomic DNA was isolated as previously described (17). Plasmid DNA was isolated from yeast cells as described previously (14). Isolation of plasmid DNA from bacterial cells was accomplished by alkaline lysis (21). Plasmid DNA was purified on Qiagen columns (Qiagen Co.) or Wizard Magic Miniprep columns (Promega) according to the manufacturer's instructions. A yeast genomic library was prepared from DNA isolated from strain SH1-1a. Size-fractionated DNA was obtained by partial digestion with *Sau*3A and purified on sucrose gradients and ligated to pRS416 by standard methods (17).

Cloning of *AUR1*. The yeast genomic library was transformed into *S. cerevisiae* YPH1. Transformed cells were selected on YPD agar with 5 μg of LY295337 per ml. Plasmid preparations were made from drug-resistant transformants and transformed into *E. coli*, and DNA was amplified and isolated as described above. Isolates were analyzed by restriction analysis and subsequent agarose gel electrophoresis. A plasmid-cured derivative of each isolate was obtained by growth on 5-FOA (4).

Plasmid pAUR1 was isolated from a resistant transformant and amplified in *E. coli*. Overlapping fragments of the original insert were generated by restriction enzyme digestion and subcloned into pRS416. Plasmids containing subcloned inserts were transformed into *S. cerevisiae*, and the transformants were tested for resistance to LY295337.

DNA sequencing analysis. Plasmid pAUR1-4X was used to construct a set of nested deletions by use of exonuclease III and mung bean exonuclease. Deletions were sequenced by the dideoxy method (22). The entire sequence of the *Xba*I fragment was analyzed with the Genetics Computer Group program, and comparison was done by BLAST analysis (1, 6).

Partial deletion of *ABR1* gene. Partial deletion of the *aur1* locus was accomplished by a two-step deletion-insertion method (20). pAUR1-4X was digested with *Bsa*BI. The linear plasmid was diluted and religated, forming pAUR1-4XΔ. Plasmid pAUR1-4XΔ was digested with *Kpn*I and *Xba*I, and the partial deletion fragment of *AUR1* was isolated by gel purification. Plasmid pRS406 was digested with *Kpn*I and *Xba*I and ligated with the pAUR1-4XΔ *Kpn*I-*Xba*I fragment. The resulting plasmid, pAUR1Δ, was digested with *Eco*RI and transformed as linear DNA into the diploid strain YPH1/YPH2. Transformants were selected on SC-ura (synthetic complete media without uracil) plates, and genomic DNA was prepared. Correct integration of the target sequence was determined by hybridization using the *Xba*I fragment from pAUR1-4X as a probe. Integrants bearing

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TABLE 1. Strains used in this study

Strain	Genotype	Source
YPH1	MAT**a** *ura3-52 lys2-801 ade2-101*	CSHL[a]
YPH2	MATα *ura3-52 lys2-801 ade2-101*	CSHL
K396-11b	MATα *ura3 ade1 his1 leu2 lys7 met3 trp5*	CSHL
1383-5c	MATα *his4-G met13 ura3 leu2 trp1 lys2 ho*::*LYS2*	CSHL
FW786	MAT**a** *ade8*	CSHL
GT153-6A	MATα *ade1 gal7 leu2 ura3 his2 leu1 arg4 his6 ilv3 met14 asp5 lys7 lys9 met2 ade2 aro7 ade*	YGSC[b]
GT160-34B	MAT**a** *ade1 leu2 his6 met14 lys9*	YGSC
GT161-31B	MAT**a** *ade2 leu1 his2 met2 lys7 asp5*	YGSC
GT160-45C	MATα *ade1 leu2 his6 met14 lys9 ura3*	YGSC
YPH1/2	YPH1 × YPH2	This study
YPH1 [*rho*^{0}]	YPH1 treated with ethidium bromide	W. L. Current[c]
M	LY295337-resistant mutant of YPH1 [*rho*^{0}]	This study
SH1	M × K396-11b	This study
SH1-2c	MAT**a** *ade leu2 ura3 ABR1*	This study
SH1-6d	MATα *ade his ura3 leu2 met3*	This study
SH2	M × 1383-5c	This study
SH2-3b	MATα *lys2 his4 ura3 ABR1*	This study
SH26	GT160-45C × SH1-2c	This study
SH28	FW786 × SH1-6d	This study

[a] CSHL, Cold Spring Harbor Laboratory yeast genetics course.
[b] YGSC, Yeast Genetic Stock Center, Department of Molecular and Cellular Biology, University of California at Berkley.
[c] Department of Infectious Disease Research, Lilly Research Laboratories.

*aur1*Δ were then selected for reversion on 5-FOA (4). Revertants were examined as described above for integration of *aur1*Δ. Appropriate heterozygous diploids (*aur1*/*aur1*Δ) were subjected to sporulation and dissection (19).

RESULTS AND DISCUSSION

Generation of mutants resistant to LY295337. Attempts to generate spontaneous mutants resistant to the action of LY295337 failed. Treatment with the mutagen ethyl methanesulfonate to increase the frequency of mutations resulted in isolation of solely petite mutants which exhibited a phenotype of resistance to LY295337. This result may have been due to the combination of mutagenesis and drug selection, conditions which are known to enhance the formation of [*rho*$^-$] mutants (7). The susceptibility of a [*rho*^{0}] strain to LY295337 was found to be similar to that of the [*rho*$^+$] parent strain, however, suggesting that the target for this compound was not encoded by mitochondrial DNA. Other factors mediated by mitochondrial function, however, may affect or modulate the sensitivity of *S. cerevisiae* to LY295337.

Genetic characterization of *AUR1*. Strain M, isolated from plates containing 5 μg of LY295337 per ml, was crossed with the wild-type strains K396-11b and 1383-5c. The resultant SH1 and SH2 diploids were resistant to LY295337 (>20 μg/ml), indicating that the mutation encoding resistance was dominant. The diploids were sporulated and dissected. Dissection of 40 tetrads from these crosses resulted in a 2^S:2^R segregation pattern when the tetrads were replica plated on drug plates, indicative of resistance to LY295337 residing in a single genetic locus (data not shown). No other phenotypic differences were noted in the segregants bearing the resistance mutation. A single resistant segregant from this cross (SH1-6d) was then mated with FW786 to create the diploid SH28. Sporulation and dissection of this diploid gave similar results (data not shown). This locus encoding dominant resistance to LY295337 was initially termed *ABR1* (for aureobasidin resistance) (12, 13). During preparation of this paper, Hashida-Okado et al. and Okado et al. reported cloning of the same gene, naming it *AUR1* (11, 16).

Linkage analysis of the tetrad data demonstrated that *AUR1* was centromere linked but not linked to the markers examined (Table 2). A segregant from one of the initial crosses (SH1-2c) was crossed with the centromere-marked mapping strain GT153-6A. Sporulation and dissection of this diploid indicated that *AUR1* was linked to either *met14* or *met2*. Further analysis with complementation tester strains GT160-34B and GT161-31B indicated that the linked marker was *met14* and not *met2*. This was confirmed by a direct cross of SH1-2C with GT160-45c and linkage analysis (Table 2). This demonstrated that *AUR1* was closely linked to *met14* and places *AUR1* on chromosome XI. The distance from *AUR1* to the centromere was calculated to be approximately 2.5 centimorgans.

Susceptibility of *AUR1* to antifungal agents. There are a number of known pleiotropic drug resistance loci in *S. cerevisiae* (2, 3). Segregants from wild-type outcrosses of *AUR1* were tested against a number of compounds and compared with parental strains. The other compounds tested showed no significant (greater than fourfold) increases in their MICs (Table 3). The MIC of LY295337 for strains bearing *AUR1* was >500 times the MIC for the wild type (>20 versus 0.04 μg/ml).

Cloning of *AUR1*. The heterozygous diploid *AUR1*/*aur1* mutant demonstrated resistance to LY295337 at >20 μg/ml, suggesting that the mutation was dominant. A genomic library of a strain bearing the *AUR1* mutation was prepared in the plasmid vector pRS416 and transformed into the wild-type yeast strain YPH1, which was then plated on drug plates. Seven colonies were visible and were examined as described above.

TABLE 2. Linkage analysis of LY295337 resistance phenotype[a]

Diploid	Markers	Tetrad type[b]			Linkage
		PD	NPD	T	
SH1 and SH28	*ABR1-leu2*	36	31	17	Centromere
SH28	*ABR1-ura3*	25	29	12	Centromere
SH26	*ABR1-met14*	95	0	5	Centromere and linked to *met14*

[a] Segregants from crosses of strains resistant to LY295337 with wild-type cells demonstrated 2^R:2^S spores, indicative of *ABR1* residing in a single genetic locus. Resistance to LY295337 was tested at 5 μg/ml on YPD plates.
[b] PD, parental ditype; NPD, nonparental ditype; T, tetratype.

TABLE 3. Susceptibility testing of *ABR1* strains

Compound	MIC (μg/ml) for strain[a]:					
	YPH1 [*rho*^{0}]	M	SH1-1a	SH1-1b	SH1-1c	SH1-1d
LY295337	0.04	>20	>20	>20	0.04	0.04
Nystatin	5.0	2.5	10.0	5.0	10.0	5.0
5-FC[b]	20.0	20.0	>40	>40	>40	>40
Cycloheximide	0.63	0.63	0.63	1.25	0.63	0.63
280949[c]	0.63	0.63	2.5	1.25	2.5	1.25

[a] SH1 strains are derived from individual spores from a single tetrad from SH1.
[b] 5-FC, 5-fluorocytosine.
[c] 280949 is an echinocandin B analog with good in vitro activity against *S. cerevisiae.*

Three of the plasmid isolates contained inserts and appeared identical by digestion at a limited number of restriction sites. Plasmids containing inserts were retransformed into *S. cerevisiae* YPH1 and tested for prototrophy (Ura$^+$) and resistance to LY295337 (5 μg/ml). All three plasmids yielded transformants that were drug resistant. The transformants were then subjected to plasmid loss conditions as described above. The transformants returned to auxotrophy (Ura$^-$) and were sensitive to LY295337. The plasmids were digested with a number of restriction enzymes and appeared identical. The insert in plasmid pAUR1 was 7.7 kb (Fig. 1).

Subcloning and sequencing of *AUR1*. The 7.7-kb insert was fragmented with available restriction sites and subcloned into pRS416 (Fig. 1). The left-hand *Xba*I fragment was found to encode resistance to LY295337 (pAUR1-4X). Nested deletions of this fragment were prepared and sequenced. Analysis of all six reading frames revealed three open reading frames

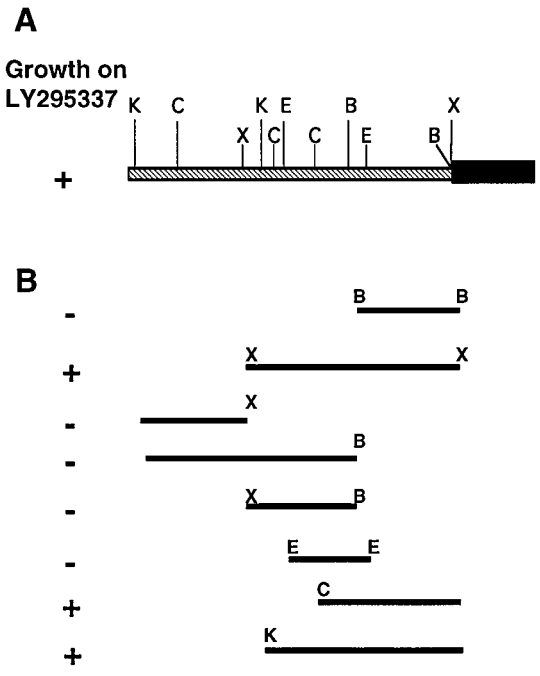

FIG. 1. Restriction map and subclones of *AUR1*. (A) Restriction map of original cloned insert from pAUR1 (7.7 kb). (B) Subcloned fragments of pAUR1 and their ability to maintain yeast cell growth on LY295337 (5 μg/ml on YPD plates). Restriction sites are as follows: K, *Kpn*I; B, *Bam*HI; C, *Cla*I; E, *Eco*RI; and X, *Xba*I.

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1     ATGGCAAACC CTTTTTCGAG ATGGTTTCTA TCAGAGAGAC CTCCAAACTG CCATGTAGCC
      M  A  N  P  F  S  R  W  F  L  S  E  R  P  P  N  C  H  V  A

61    GATTTAGAAA CAAGTTTAGA TCCCCATCAA ACGTTGTTGA AGGTGCAAAA ATACAAACCC
      D  L  E  T  S  L  D  P  H  Q  T  L  L  K  V  Q  K  Y  K  P

121   GCTTTAAGCG ACTGGGTGCA TTACATCTTC TTGGGATCCA TCATGCTGTT TGTGTTCATT
      A  L  S  D  W  V  H  Y  I  F  L  G  S  I  M  L  F  V  F  I

181   ACTAATCCCG CACCTTGGAT CTTCAAGATC CTTTTTTATT GTTTCTTGGG CACTTTATTC
      T  N  P  A  P  W  I  F  K  I  L  F  Y  C  F  L  G  T  L  F

241   ATCATTCCAG CTACGTCACA GTTTTTCTTC AATGCCTTGC CCATCCTAAC ATGGGTGGCG
      I  I  P  A  T  S  Q  F  F  F  N  A  L  P  I  L  T  W  V  A

301   CTGTATTTCA CTTCATCGTA CTTTCCAGAT GACCGCAGGC CTCCTATTAC TGTCAAAGTG
      L  Y  F  T  S  S  Y  F  P  D  D  R  R  P  P  I  T  V  K  V

361   TTACCAGCGG TGGAAACAAT TTTATACGGC GACAATTTAA GTGATATTTT TGCAACATCG
      L  P  A  V  E  T  I  L  Y  G  D  N  L  S  D  I  F  A  T  S

421   ACGAATTCCT TTTTGGACAT TTTAGCATGG TTACCGTACG GACTATTTTA TTTTGGGGCC
      T  N  S  F  L  D  I  L  A  W  L  P  Y  G  L  F  Y  F  G  A

481   CCATTTGTCG TTGCTGCCAT CTTATTCGTA TTTGGTCCAC CAACTGTTTT GCAAGGTTAT
      P  F  V  V  A  A  I  L  F  V  F  G  P  P  T  V  L  Q  G  Y

541   GCTTTTGCAT TTGGTTATAT GAACCTGTTT GGTGTTATCA TGCAAAATGT CTTTCCAGCC
      A  F  A  F  G  Y  M  N  L  F  G  V  I  M  Q  N  V  F  P  A

601   GCTCCCCCAT GGTATAAAAT TCTCTATGGA TTGCAATCAG CCAACTATGA TATGCATGGC
      A  P  P  W  Y  K  I  L  Y  G  L  Q  S  A  N  Y  D  M  H  G

661   TCGCCTGGTG GATTAGCTAG AATTGATAAG CTACTCGGTA TTAATATGTA TACTACAGCT
      S  P  G  G  L  A  R  I  D  K  L  L  G  I  N  M  Y  T  T  A

721   TTTTCAAATT CCTCCGTCAT TTTCGGTGCT TTTCCTTCAC TGCATTCCGG GTGTGCTACT
      F  S  N  S  S  V  I  F  G  A  F  P  S  L  H  S  G  C  A  T

751   ATGGAAGCCC TGTTTTTCTG TTATTGTTTT CCAAAATTGA AGCCCTTGTT TATTGCTTAT
      M  E  A  L  F  F  C  Y  C  F  P  K  L  K  P  L  F  I  A  Y

841   GTTTGCTGGT TATGGTGGTC AACTATGTAT CTGACACACC ATTATTTTGT AGACCTTATG
      V  C  W  L  W  W  S  T  M  Y  L  T  H  H  Y  F  V  D  L  M

901   GCAGGTTCTG TGCTGTCATA CGTTATTTTC CAGTACACAA AGTACACACA TTTACCAATT
      A  G  S  V  L  S  Y  V  I  F  Q  Y  T  K  Y  T  H  L  P  I

961   GTAGATACAT CTCTTTTTTG CAGATGGTCA TACACTTCAA TTGAGAAATA CGATATATCA
      V  D  T  S  L  F  C  R  W  S  Y  T  S  I  E  K  Y  D  I  S

1021  AAGAGTGATC CATTGGCTGC AGATTCAAAC GATATCGAAA GTGTCCCTTT GTCCAACTTG
      K  S  D  P  L  A  A  D  S  N  D  I  E  S  V  P  L  S  N  L

1081  GAACTTGACT TTGATCTTAA TATGACTGAT GAACCCAGTG TAAGCCCTTC GTTATTTGAT
      E  L  D  F  D  L  N  M  T  D  E  P  S  V  S  P  S  L  F  D

1141  GGATCTACTT CTGTTTCTCG TTCGTCCGCC ACGTCTATAA CGTCACTAGG TGTAAAGAGG
      G  S  T  S  V  S  R  S  S  A  T  S  I  T  S  L  G  V  K  R

1201  GCTTAA
      A
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FIG. 2. Nucleic acid sequence of *AUR1*. The coding sequence for *AUR1* determined from sequence analysis of the *Xba*I fragment of pAUR1-4X (underlined bases) and the sites of mutations from comparison of the sequence of *AUR1* with the wild-type sequence from YKL004w (8) (underlined codons) are indicated.

within the 4.5-kb *Xba*I fragment. Examination of the nested deletions for activity demonstrated that resistance to LY295337 was encoded by the middle open reading frame in the 4.5-kb *Xba*I fragment. The sequence of *AUR1* is 1,203 bp and contains no introns. It codes for a putative 401-amino-acid protein (Fig. 2). Homology searching identified an open reading frame sequenced as part of the *S. cerevisiae* genome sequencing project (Chr XI YKL004w [GenBank accession no. Z28004]) as well as by overlapping sequence homologies with the adjacent loci of YKL150 and *MRP17* (5, 8, 10). The homology of parts of the *Xba*I fragment to known sequences confirms the placement of *AUR1* on yeast chromosome XI and its close linkage to *met14* as determined by genetic analysis. No biological function has been assigned to the YKL004w open reading frame. No other known homologs of this gene from other species were found. In addition, functional analysis of the gene product by motif searching did not provide any further insight as to the possible functions of *AUR1*. Furthermore, there was no homology or overall structural similarity to pleiotropic drug resistance markers (2, 3).

Comparison of the sequence for *AUR1* with the chromosomal sequence for a wild-type strain revealed two potential amino acid changes in the putative translation product of this gene. In the mutant sequence, Leu-137 has been changed to a

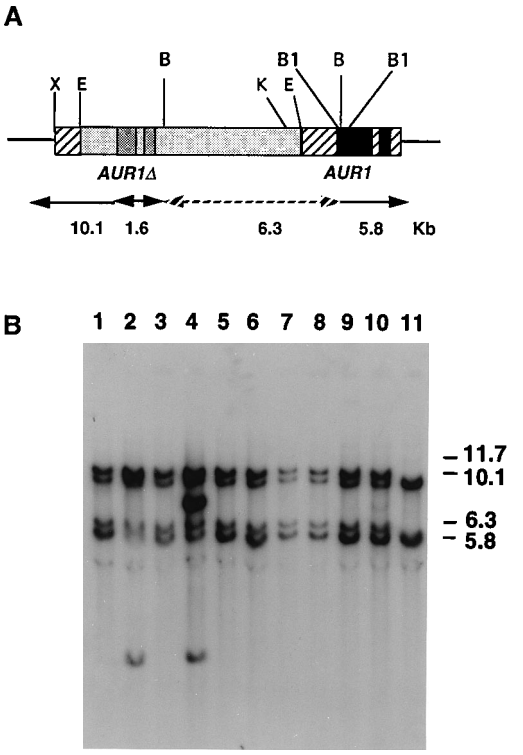

FIG. 3. Insertion of pAUR1Δ into YPH1/YPH2 diploid. (A) Map of tandem insertion of *aur1*Δ in the *aur1* locus. The sizes of the *Bam*HI fragments from the wild-type locus (single arrows), plasmid sequence (dashed double arrow), and *aur1*Δ sequence from the plasmid (solid double arrow) are indicated. Sizes are in kilobases. Restriction sites are as for Fig. 1; B1, *Bsa*BI. (B) Genomic DNA from integrants digested with *Bam*HI and probed with pAUR1-4X. Lanes 1 to 10, transformants; lane 11, YPH1/YPH2 wild-type diploid. Sizes are shown on the right (in kilobases).

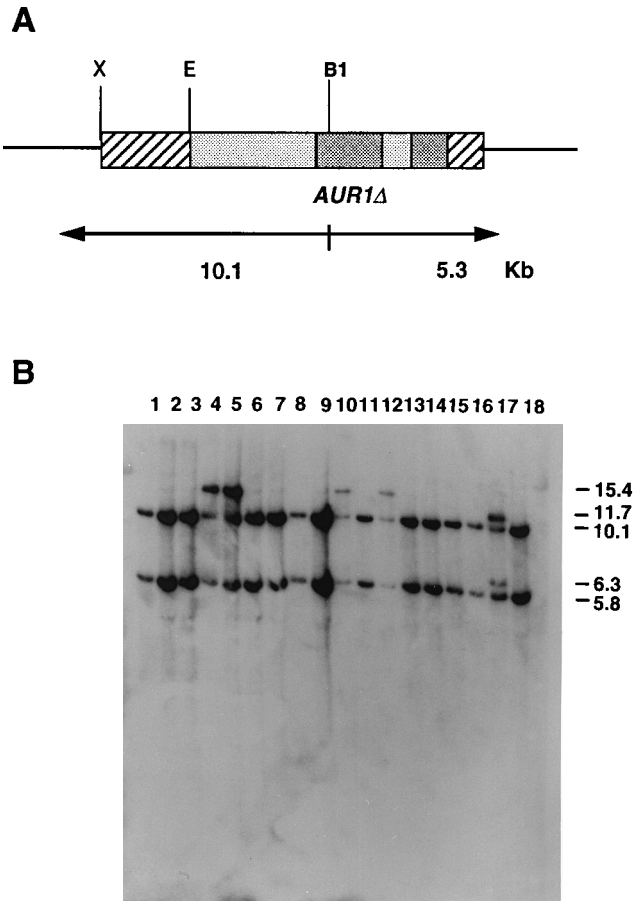

FIG. 4. Analysis of revertants from treatment with 5-FOA. (A) Map of *aur1*Δ locus after reversion on 5-FOA. Restriction sites as for Fig. 1 and 3. (B) Genomic DNA digested with *Bam*HI and probed as for Fig. 3. Lanes 1 to 16, individual clones from 5-FOA; lane 17, transformant 5 from Fig. 3; lane 18, YPH1/YPH2 diploid. Sizes are shown on the right (in kilobases).

phenylalanine and His-157 is changed to a tyrosine. Each change is the result of a single base pair alteration. The mutation at His-157 is adjacent to the amino acid mutated in the dominant resistance mutation in *aur1* of Okado and coworkers, in which residue Phe-158 was altered to a tyrosine (16). This region of the *AUR1* sequence thus appears important for dominant resistance to LY295337.

Partial deletion of *AUR1*. Partial deletion of the wild-type locus (*aur1*) was accomplished by deletion-insertion of the *AUR1* gene. A 549-bp deletion was constructed by digestion of pAUR1-4X with *Bsa*BI followed by religation. This deletion-religation removed the sites of the mutations in *AUR1* and also introduced a reading frame shift of a single base pair. The deleted fragment was inserted into pRS406, resulting in plasmid pAUR1Δ. This construct was digested with *Eco*RI and transformed into a YPH1/YPH2 diploid. After selection of transformants on SC-ura medium, colonies were picked for preparation of genomic DNA and Southern blots by using a labeled *Xba*I fragment from pAUR1-4X as a probe. Most transformants demonstrated a pattern consistent with a tandem insertion of a single disrupted allele of *aur1* with the wild-type locus (11.7 kb) compared with the nondisrupted alleles (10.1 kb) and showed the presence of plasmid sequences at 6.3 kb (Fig. 3). The lower band at 5.8 kb represents the 3′ end of the sequence from the wild-type sequence internal *Bam*HI site which is missing in the deletion construct. An additional band is seen at ~8 kb (Fig. 3B, lane 4). This probably represents an integration into another locus.

Transformants were picked and then selected for reversion to auxotrophy by being plated on 5-FOA. Loss of the Ura^+ marker was accompanied in some cases by recombination of tandemly inserted *aur1*Δ for the wild-type gene. Southern blots of the genomic DNA with the *Xba*I fragment of pAUR1-4X as a probe demonstrated two homologs of different sizes, one corresponding to the wild-type *aur1* gene (10.1 and 5.8 kb) and the second to *aur1*Δ (15.4 kb). The larger size of the deletion arises from the loss of the internal *Bam*HI site in the coding sequence of the deletion construct (Fig. 4). Excision of the integrant (loss of the Ura^+ marker) led in most cases to recovery of the wild-type allele for *aur1*.

A diploid carrying the *aur1*Δ/*aur1* loci was sporulated and dissected. The resulting spores demonstrated 2:2 segregation of viable to nonviable spores (data not shown). Microscopic examination of the plates revealed microcolonies of four to eight cells in which nonviable spores were located. Southern blots of genomic DNA from viable spores with the *Xba*I fragment of pAUR1-4X used as a probe demonstrated that viable colonies carried only the wild-type allele of *aur1* (10.1 and 5.8 kb), suggesting that the nonviable spores were *aur1*Δ (Fig. 5). The partial deletion of the *aur1* locus thus renders the cells capable of germination but unable to proceed through more than a few rounds of division and growth. Thus, *aur1* is essential for growth. In *Schizosaccharomyces pombe* and *S. cerevi-*

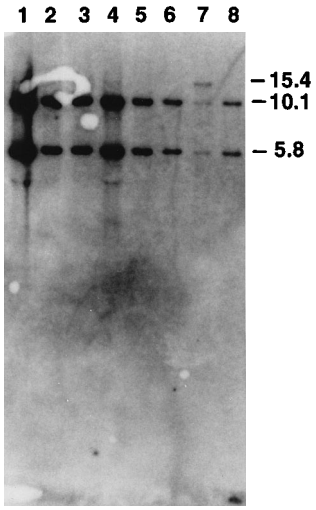

FIG. 5. Analysis of individual spores from the dissection of the sporulated diploid (*abr1*/*abr1*Δ). Genomic DNAs from individual spores and blots were prepared as described previously. Lanes 1 to 6, viable spore pairs from three tetrads; lane 7, the revertant of transformant 5 (Fig. 4); lane 8, the YPH1/YPH2 diploid. Sizes are shown on the right (in kilobases).

siae, similar results were found by gene disruption in *AUR1* (11, 16).

By inference, LY295337 perhaps disrupts this essential function and thereby causes cell death. Mutation of *ABR1* may therefore be expressed as resistance to LY295337 through alteration of the binding site and therefore the affinity of LY295337 for the *AUR1* gene product. Alternatively, LY295337 may bind to the *AUR1* gene product but is unable to inhibit the function of the mutated *AUR1* gene product. At present, there is no evidence for direct binding of LY295337 to the gene product of *AUR1*, and the biological role of *AUR1* in *S. cerevisiae* remains undefined and awaits further investigation.

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