Biochemical and Biophysical Research Communications 434 (2013) 42–47

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Salicylic acid resistance is conferred by a novel *YRR1* mutation in *Saccharomyces cerevisiae*

Naohiko Kodo [a,*], Toshiro Matsuda [b], Syuichi Doi [c], Hiroshi Munakata [a]

[a] Department of Biochemistry, Faculty of Medicine, Kinki University, 377-2, Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan
[b] Acute Medicine, Faculty of Medicine, Kinki University, 377-2, Ohno-Higashi,Osaka-Sayama, Osaka 589-8511, Japan
[c] Basic Medical Sciences, Faculty of Medicine, Kinki University, 377-2, Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan

ARTICLE INFO

Article history:
Received 12 March 2013
Available online 30 March 2013

Keywords:
Salicylic acid
YRR1
Saccharomyces cerevisiae
Pleiotropic drug resistance
ABC transporter

ABSTRACT

Yeast cells can extrude intracellular drugs through membrane-associated efflux pumps, such as ATP-binding cassette (ABC) transporters and members of the major facilitator superfamily. Gene expression of drug efflux pumps is regulated by several transcription factors involved in pleiotropic drug resistance (PDR). Salicylic acid (SA) possesses weak antifungal activity. Although the excretion mechanisms of some antifungal drugs have been revealed, the mechanism of SA extrusion remains unclear. To elucidate the mechanism of SA excretion, we screened SA-resistant mutants from random mutagenized *Saccharomyces cerevisiae* BY4741 cells. We successfully isolated 60 SA-resistant clones (KinSal001–060). KinSal052, one of the strongest SA-resistant clones, also exhibited resistance to 4-nitroquinoline-1-oxide and cycloheximide, indicating that it acquired the PDR phenotype. We identified a novel mutation in *YRR1* conferring SA resistance to KinSal052. *YRR1* encodes a $Zn(II)_2Cys_6$-type zinc-finger transcription factor that reportedly activates gene expression involved in PDR. Yeast cells carrying the *yrr1* allele (*yrr1-52*) activated expression of several efflux pump-encoding genes, including *YOR1*, *SNQ2*, *AZR1*, and *FLR1*. These results suggested that SA resistance in KinSal052 is conferred by the overexpression of efflux pumps constitutively activated by the mutant form of Yrr1p.

1. Introduction

Over recent years, fungal infections have emerged as significant clinical problems in patients with diabetes and immunodeficiency virus infection as well as those requiring organ transplantation and anticancer chemotherapy [1,2]. In proportion to the increase in mycotic infections, there has been an increase in the risk of acquired fungal pleiotropic drug resistance (PDR). In addition, the development of novel drugs against fungal infections is quite difficult since fungal cell structure and function are similar to mammalian cells, and only a few targets exist for designing new drugs [3]. One target is ergosterol, which is an important constituent of the fungal cell membrane, whereas cholesterol is a primary component of mammalian cell membranes. Azole antifungal drugs, such as ketoconazole, fluconazole, and miconazole, inhibit ergosterol biosynthesis, whereas polyene antifungal drugs, such as amphotericin B, bind to ergosterol in the fungal cell membrane, resulting in depolarization of the cell membrane and formation of pores [4]. Another target is the fungal cell wall. In recent years, several echinocandins, which inhibit the biosynthesis of 1,3-beta-D-glucan, an essential component of the fungal cell wall, have been developed [5]. Given these circumstances, it is necessary to elucidate the detailed mechanisms of fungal PDR to prevent further resistance to available drugs.

In *Saccharomyces cerevisiae*, PDR loci have been investigated genetically, and most of the involved genes have been cloned and characterized [6,7]. PDR genes are comprised of the following two main groups: those encoding membrane-associated drug efflux pumps and those encoding transcription factors that regulate gene expression involved in drug efflux and metabolism. PDR genes, including *PDR5*, *PDR10*, *PDR11*, *PDR12*, *PDR15*, and *PDR18*, encode the ATP-binding cassette (ABC) transporter [6], which functions as a pump to extrude a large number of structurally different chemicals, including toxins and drugs, out of the cell. Especially, *PDR5* has been extensively investigated and the gene product has been shown to be involved in extrusion of many antifungal reagents, including azole antifungal drugs [8]. *PDR1*, *YRR1*(*PDR2*), *PDR3*, and *PDR8* encode transcription factors containing $Zn(II)_2Cys_6$-type zinc-finger motifs that are regulators of PDR in *S. cerevisiae* [7,9]. Pdr1p and Pdr3p bind to PDR elements (PDREs) located in the promoter region of PDR-related genes and regulate gene expression [10]. Yeast cells carrying gain-of-function *PDR1* or *PDR3* mutations cause constitutive high expression of membrane-associated efflux pumps and consequent excretion of a wide variety of antifungal drugs [11–13]. Although it is unclear how

* Corresponding author. Fax: +81 72 366 0245.
E-mail address: kodo@med.kindai.ac.jp (N. Kodo).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.03.069

Pdr1p and Pdr3p regulate downstream gene expression, Thakur et al. recently reported that ketoconazole binds to Pdr1p as a ligand, and Pdr1p as a nuclear receptor, activates *PDR5* expression via this interaction [14].

Salicylic acid (SA) and acetylsalicylic acid, also known as aspirin, are nonsteroidal anti-inflammatory drugs that inhibit cyclooxygenase activity resulting in decreased prostaglandin synthesis in mammalian cells [15,16]. SA also induces mitochondrial dysfunction by opening the permeability transition pores located in the mitochondrial membrane [17,18]. In addition, SA has weak antifungal and keratolytic activities, hence is used as a dermatological agent to medicate tinea pedis (athlete's foot) caused by pathogenic fungi of the genus *Trichophyton* [19]. However, the pharmacological action and excretion mechanism of SA in fungal cells remain unknown. Hence, to reveal the mechanism of SA excretion, we isolated SA-resistant mutants from random mutagenized *S. cerevisiae* BY4741 cells and identified gene mutations that confer SA resistance.

2. Materials and methods

2.1. Strains, media, and drugs

S. cerevisiae BY4741 (*MAT his3*Δ *leu2*Δ *met15*Δ *ura3*Δ) and BY4742 (*MATα his3*Δ *leu2*Δ *lys2*Δ *ura3*Δ) strains used in the present study were purchased from the National Bio-Resource Project (NBRP) of the Ministry of Education, Culture, Sports, Science, and Technology (Tokyo, Japan) and strain *yrr1*Δ (on a BY4742 background) was purchased from Open Biosystems, Inc. (Huntsville, AL, USA). Yeast cells were grown in YPD (1% yeast extract, 2% peptone, 2% glucose) or synthetic dextrose (SD) minimal medium (0.67% yeast nitrogen base, 2% glucose) with appropriate amino acids. *Escherichia coli* XL1-Blue cells were used for plasmid propagation. Luria–Bertani medium (1% tryptone, 1% NaCl, 0.6% yeast extract) supplemented with 100 mg/L ampicillin was used to culture *E. coli* cells. When necessary, media were solidified with 2% agar. SA, 4-nitroquinoline-1-oxide (4-NQO), and cycloheximide were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and the stock solutions were prepared by dissolving in dimethyl sulfoxide (DMSO).

2.2. Drug susceptibility assay

Susceptibility to each drug was evaluated using a spot assay. Each strain was cultured overnight at 30 °C in YPD or SD medium with appropriate amino acids, and 10-fold serial dilutions of overnight cultures were spotted on YPD plates containing various drug concentrations. Plates were then incubated for 2–3 days at 30 °C.

2.3. Screening for SA-resistant mutants

To isolate SA-resistant mutants, BY4741 cells were mutagenized with ethyl methane sulfonate (EMS; Wako) as previously described with minor modifications [20]. In brief, logarithmic-phase yeast cells were washed twice with 0.2 M sodium phosphate buffer (SPB; pH 7.0), and culture density was adjusted to 1.0×10^8 cells/mL using the same buffer. One milliliter of cell suspension (1.0×10^8 cells) was transferred to a 50-mL conical tube, and then mutagenized by mixing with the following reagents in a draft chamber: 3.6 mL of 0.2 M SPB (pH 7.0), 0.25 mL of 4% glucose, and 0.15 mL of EMS. We prepared 37 tubes for screening. The reaction was incubated at 30 °C for 1 h with gentle shaking and was terminated by the addition of 5 mL of 10% sodium thiosulfate. Each tube was centrifuged and pelleted cells were resuspended in 5 mL of YPD medium. To calculate the cell number, serial dilutions of the cell suspension were prepared and plated on YPD plates. The cell suspensions (0.5 mL) were transferred to new sterile test tubes and shaken overnight at 30 °C. The next day, the cultures from each test tube were directly plated on YPD plates containing 15 mM SA and further incubated at 30 °C for 3–4 days. To avoid selecting cells from the same origin, only one colony was selected from each plate and transferred to a new YPD plate. Selected cells were further tested for drug susceptibility using a replicated YPD plate containing 15 mM SA. The surviving cells on the plates were selected as SA-resistant clones.

2.4. Yeast genetic techniques and DNA manipulation

Standard yeast genetic techniques including cross, sporulation, tetrad dissection, and transformation were performed as previously described [20]. Recombinant DNA manipulations were performed by standard techniques [21] and the genomic DNA library was constructed as previously described [22]. In brief, genomic DNA was extracted from yeast cells and partially digested with *Sau*3AI. The DNA fragments were inserted into the *Bam*HI sites of the YCplac111 plasmid, which contains *ARS1*, *CEN4*, and *LEU2*. Wild-type and mutant *YRR1* coding regions containing upstream and downstream sequences were amplified by PCR with high fidelity DNA polymerase, KOD-FX (Toyobo Co., Ltd., Osaka, Japan). The primers used for this reaction were 5′-AATGGATCCTACTGGCAGAAATCATAGTG-3′ (forward primer) and 5′-AACGGATCCAGT GGGCTTGCCAAAATCT-3′ (reverse primer). The primers had *Bam*HI sites added to the 5′ termini. The resulting PCR products were digested with *Bam*HI and ligated into the corresponding site of the YCplac111 or YEplac181 plasmids (2 μm origin, *LEU2* marker), respectively, which were purchased from NBRP. DNA sequencing was performed using the ABI Prism 3130xl Genetic Analyzer and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA).

2.5. Quantitative real-time PCR (qPCR)

Total RNA was extracted from logarithmic-phase yeast cells, suspended in Isogen (Nippon Gene Co., Ltd., Tokyo, Japan), and broken by mixing with glass beads. cDNA was synthesized using the ReverTra Ace qPCR RT Kit (Toyobo). qPCR was performed using Thunderbird Syber qPCR Mix (Toyobo) and the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). All procedures were performed according to the manufacturer's protocols. The specific primers for the amplification of *ACT1*, *YRR1*, *YOR1*, *SNQ2*, *AZR1*, *FLR1*, and *PDR5* cDNA were as follows: 5′-CAAACCGCTGCTCAATCTTC-3′ and 5′-CTTCTGGGGCTCTGAATCTT-3′, 5′-TGATAGGCA GCGGGATCTCA-3′ and 5′-TTTCACCAGGCGAGGGTAAC-3′, 5′-AGTG CCGAAAGATTGGTAAC-3′ and 5′-CCATTGAGGGCCATGACTCT-3′, 5′-AAGGCTTCCCCATACACATA-3′ and 5′-TGAGCCGTTTGGTGGGTTGA-3′, 5′-GAAATCGGGTCGCTGATCTC-3′ and 5′-AATACCACAACCGCCGACTC-3′, 5′-ACAGAGTGCCCGTGAAGATG-3′ and 5′-CTATTAGGAC CGCATTCTAT-3′, and 5′-CGACTTGGGCGAAGGTTGTA-3′ and 5′-TGGCGTCAGCAGGGCATTTA-3′. *ACT1* mRNA was used as an internal control and each qPCR experiment was performed at least in triplicate. Each reported value is the mean and error bars represent the standard deviation. Statistical significance of the data was determined using the Student's *t*-test.

3. Results and discussion

3.1. Evaluation of SA susceptibility of S. cerevisiae BY4741 cells

Before screening for SA resistance, we estimated SA sensitivity of *S. cerevisiae* BY4741 cells using a spot assay. In brief, 10-fold

serial dilutions were prepared from the overnight cultures and spotted on YPD plates containing various SA concentrations (0, 7.5, 10, 12.5, and 15 mM). After incubation for 3 days, yeast cells were found on each plate containing 0–12.5 mM SA (Fig. 1A). However, cells exhibited growth defects dependent on SA concentration and did not grow on media containing 15 mM SA (Fig. 1A). Hence, we determined that 15 mM SA was an appropriate concentration for screening.

3.2. Isolation of SA-resistant mutants

BY4741 cells during the logarithmic phase were randomly mutagenized with EMS as described above. Overnight cultures of mutagenized cells were plated on YPD medium containing 15 mM SA and incubated for 3–4 days at 30 °C. In this screening, we selected 269 colonies as SA-resistant candidates from 365 plates (5.6×10^8 mutagenized cells). To retest for SA sensitivity, candidate clones were grown on YPD plates and replica plated on new YPD plates containing 0 and 15 mM SA. Finally, we obtained 60 resistant clones grown on 15 mM SA plates (Fig. 1B) and designated them as KinSal001–060, respectively. Several mutants exhibited resistance to not only SA but also 4-NQO and cycloheximide (Fig. 1C). These results indicated that some mutants acquired the PDR phenotype.

3.3. Identification of a single base substitution in YRR1 conferring SA resistance to KinSal052 clone

Among the 60 clones, KinSal052 exhibited the PDR phenotype and was highly resistant to SA (Fig. 1C). Using this clone, we further identified the gene mutation conferring SA resistance. First, we crossed KinSal052 with BY4742, and the resulting diploid cells were tested for SA susceptibility using the spot assay. As shown in Fig. 2A, the diploid cells showed similar susceptibilities as the KinSal052 haploid cells, indicating that the mutation for SA resistance was genetically dominant. Following subsequent sporulation and dissection of the diploid cells, progeny cells showed a 2:2 segregation pattern for the SA resistant:SA sensitive phenotypes (data not shown), indicating that a single locus conferred SA resistance to KinSal052 cells.

To identify the dominant mutation, we constructed a genomic library derived from the KinSal052 clone. The partial *Sau*3AI-digested genomic DNA was cloned into the *Bam*HI site of the low-copy plasmid YCplac111. BY4741 cells were transformed with the genomic DNA library and Leu^+ colonies were replica plated on YPD plates containing 13 mM SA. After 3 days of incubation, several colonies grew on the plates. Plasmid DNA was recovered from *E. coli*. BY4741 cells transformed with the rescued plasmid DNAs and retested for susceptibility to 13 mM SA. Only one clone harboring a plasmid was able to grow on the plate and was designated YCp052N#8. This clone contained an 8-kb fragment as an insert, as measured by agarose gel electrophoresis (data not shown). DNA sequencing of both terminal regions of the insert was performed using M13 forward and reverse primers annealed to the multi-cloning site of YCplac111. BLAST search of the *Saccharomyces* Genome Database (www.yeastgenome.org) was performed using the resulting sequence data. As a result, the inserted DNA was presumed to be a fragment originating from chromosome XV at positions 637,510–646,001. The length of this chromosomal region was almost coincident with the insert size of YCp052N#8 and contained several open reading frames (Fig. 2B). We selected *YRR1* (*YOR162C*) as a candidate for further analysis, since this gene was

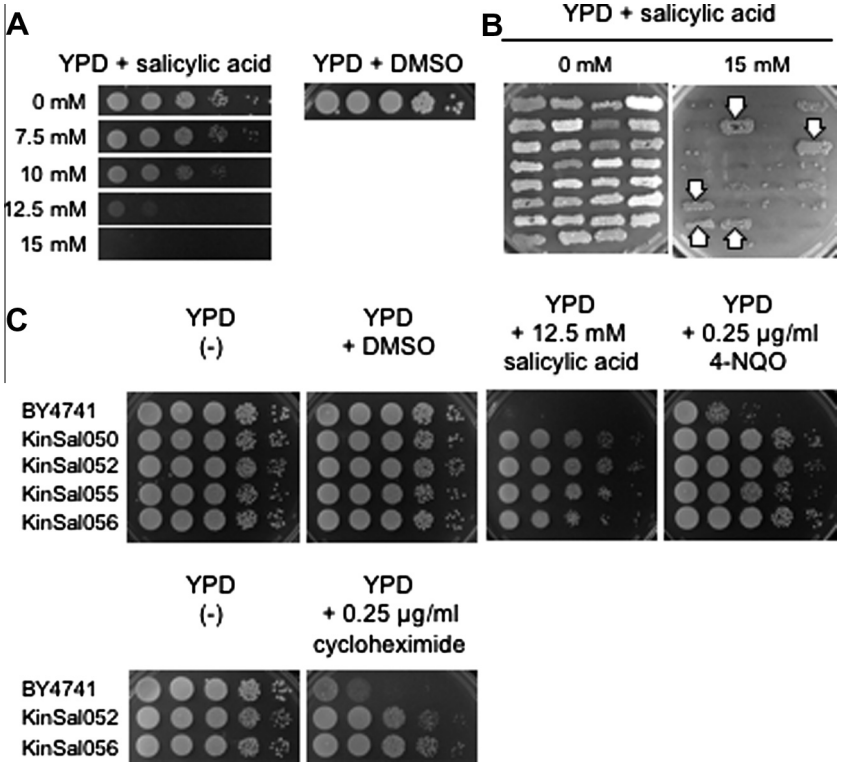

Fig. 1. Susceptibility of *S. cerevisiae* BY4741 and mutant cells to SA, 4-NQO, and cycloheximide. (A) Evaluation of SA sensitivity in *S. cerevisiae* BY4741 cells using the spot assay. Serial dilutions of overnight cultured yeast cells were spotted on YPD plates containing various SA concentrations and incubated for 3 days. DMSO was used as an SA solvent. (B) Isolation of SA-resistant mutants. Overnight cultures of randomly mutagenized BY4741 cells were plated on YPD containing 15 mM SA. Primary selected candidates (269 clones) were grown on new YPD plates and then replica plated on YPD plates containing 0 and 15 mM SA, respectively. Sixty clones (indicated by arrows) were grown on SA-supplemented YPD plates and selected as SA-resistant clones. (C) Other drug susceptibilities of SA highly resistant clones. Some SA-resistant clones were evaluated for susceptibility to 4-NQO and cycloheximide using the spot assay.

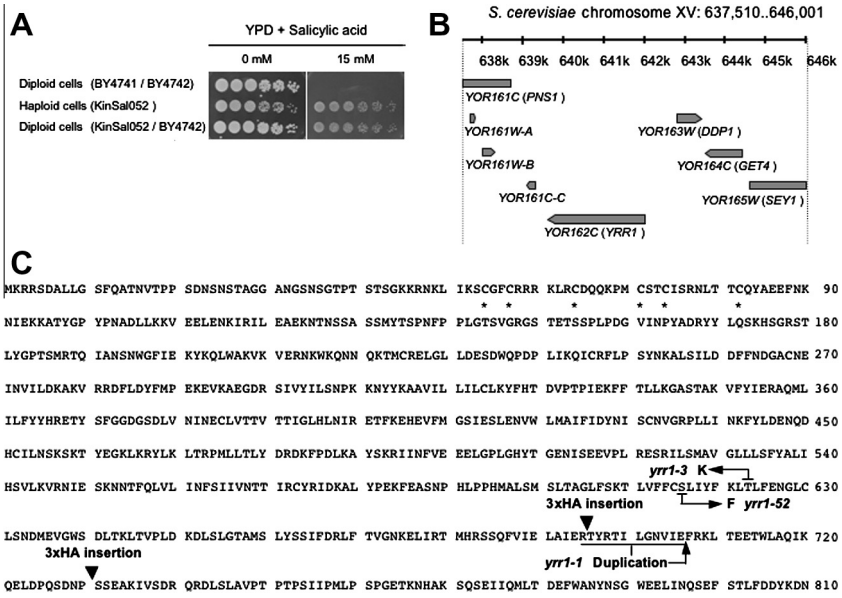

Fig. 2. SA resistance in KinSal052 cells was conferred by a dominant *YRR1* mutation. (A) SA susceptibility of haploid (KinSal052) and diploid cells (KinSal052/BY4742). KinSal052 cells were crossed with BY4742 cells and the resulting diploid cells were tested for SA susceptibility using the spot assay. (B) The inserted DNA fragment into the plasmid YCp052N#8, which conferred SA resistance to host cells, was obtained by screening the KinSal052 genomic library. Both ends of the inserted fragment were sequenced, and the identified the yeast genomic region was used for a BLAST search. (C) The amino acid sequence of *S. cerevisiae* Yrr1p. The amino acid substitution in this study (*yrr1-52*) and previously reported mutations (*yrr1-1*, *yrr1-3*, and 2 triple-HA-tagged insertions) are shown [23–25]. The six asterisks indicate cysteine residues conserved in the $Zn(II)_2Cys_6$-type zinc-finger motif.

previously implicated to be PDR [23]. Sequencing of the entire coding region of *YRR1* in the plasmid identified a single base substitution of C to T at position 1847, in which the A of the ATG start codon was at position 1. This base substitution caused an amino acid substitution, from serine to phenylalanine, in the C-terminal region (Fig. 2C). The same mutation was also confirmed in KinSal052 genomic DNA, but was not observed in the original BY4741 strain (data not shown). Next, we constructed a low-copy plasmid (YCplac111) and inserted it into wild-type and mutated *YRR1* and estimated SA susceptibility in *yrr1*Δ cells harboring each plasmid. Although *yrr1*Δ cells harboring the wild-type *YRR1* plasmid (*yrr1*Δ [YCp-YRR1] cells) barely grew on the medium containing 12.5 mM SA, *yrr1*Δ cells harboring the mutated *yrr1* plasmid (*yrr1*Δ [YCp-yrr1-52] cells) did grow on the medium (Fig. 3). In addition, *yrr1*Δ [YCp-yrr1-52] cells also exhibited 4-NQO resistance (Fig. 3). From the above experiments, we concluded that a single amino acid substitution in *YRR1* confers SA resistance and the allele was designated as *yrr1-52*.

3.4. Yeast cells harboring the yrr1-52 allele activated gene expression of several efflux pumps involved in PDR

YRR1 was originally isolated from the screening for reveromycin A resistance [23]. Several dominant drug-resistant mutants were isolated and exhibited resistance not only to reveromycin A but also to 4-NQO and oligomycin (Fig. 2C) [23–25]. Yrr1p, Pdr1p, and Pdr3p contain an N-terminal $Zn(II)_2Cys_6$-type zinc-finger motif, which acts as a transcriptional regulator [7,9]. Yrr1p binds to a specific DNA sequence, designated as YRRE, located in the PDR gene promoter regions [26].

Le Crom et al. investigated the up-regulated genes in yeast cells expressing gain-of-function Yrr1p inserted behind the 3× hemagglutinin epitope tag that is inserted into the coding sequence at the C-terminal region using microarray analysis [26]. Activated genes, including *AZR1*, *FLR1*, *SNG1*, *SNQ2*, *YOR1*, *YLR346C*, *YLL056C*, *YGR035C*, *YMR102C*, and *YPL088W*, all of which contain YRRE in their promoter regions, were observed in the mutant cells. We

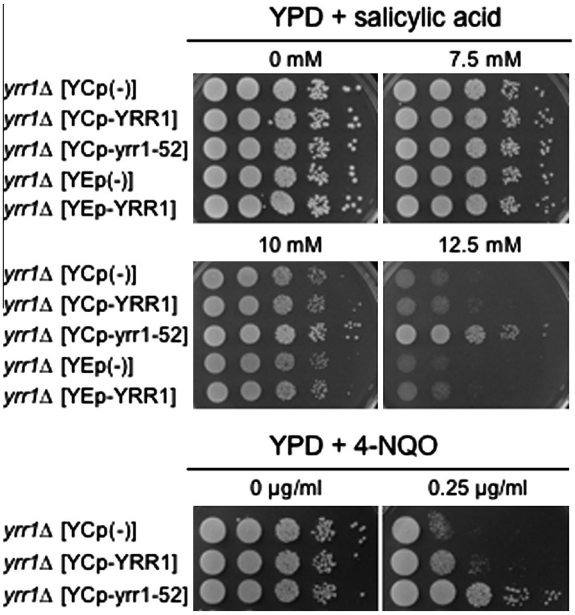

Fig. 3. Susceptibility to SA and 4-NQO of *yrr1*Δ cells harboring various plasmids. The *yrr1*Δ cells containing *YRR1* or *yrr1-52* on the low-copy plasmid YCp and *YRR1* on the high-copy plasmid YEp were evaluated for susceptibility to SA and 4-NQO using the spot assay.

evaluated some of the genes expressed in *yrr1*Δ cells harboring various plasmids during the logarithmic phase by qPCR (Fig. 4), which revealed that the expression of *SNQ2*, *YOR1*, *AZR1*, and *FLR1* in *yrr1*Δ [YCp-yrr1-52] cells was significantly up-regulated compared with that in *yrr1*Δ [YCp-YRR1] cells ($p < 0.05$). *SNQ2* encodes a plasma membrane ABC transporter involved in 4-NQO resistance [27]. Yeast cells carrying the *yrr1-1* allele showed elevated *SNQ2* expression and the drug resistance [23]. *SNQ2* expression in *yrr1*Δ [YCp-yrr1-52] cells is approximately fourfold higher

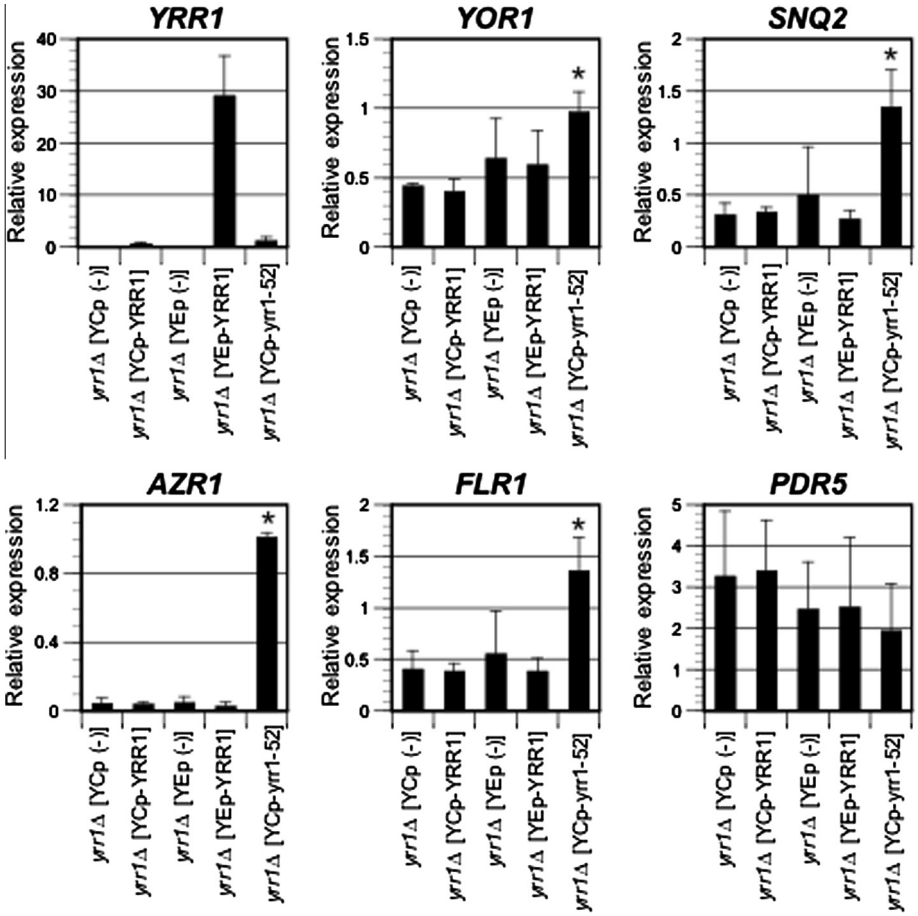

Fig. 4. Analysis of relative gene expression in *yrr1*Δ cells harboring various plasmids by qPCR. Total RNA was extracted from each yeast cell during the logarithmic phase. Each reported value is the mean and error bars represent standard deviations. Asterisks indicate a significant difference between *yrr1*Δ [YCp-yrr1-52] and *yrr1*Δ [YCp-YRR1] cells ($p < 0.05$).

than that in *yrr1*Δ [YCp-YRR1] cells, which coincided with 4-NQO resistance in *yrr1*Δ [YCp-yrr1-52] cells (Figs. 1C and 3). *YOR1* also encodes an ABC transporter involved in oligomycin efflux [28], and the *yrr1-52* allele might confer oligomycin resistance to yeast cells, but no significant difference existed in *PDR5* expression among all cells (Fig. 4). *PDR5* encodes an ABC transporter involved in efflux of several antifungal drugs [8] and contains PDRE sequences in the promoter region, hence the gene expression is controlled by Pdr1p and Pdr3p [10].

Next, we considered the possibility of whether over-expression of wild type *YRR1* confers SA resistance. We prepared *YRR1* constructs on the high-copy plasmid YEplac181 and *YRR1* or *yrr1-52* on the low-copy plasmid YCplac111. The *yrr1*Δ cells containing *YRR1* on high-copy plasmids (*yrr1*Δ [YEp-YRR1] cells) had approximately 30-fold higher *YRR1* expression compared with that of *yrr1*Δ [YCp-YRR1] or *yrr1*Δ [YCp-yrr1-52] cells (Fig. 4). However, there was no significant difference in expression of *YOR1*, *SNQ2*, *AZR1*, and *FLR1* between *yrr1*Δ [YEp-YRR1] and *yrr1*Δ [YCp-YRR1] cells (Fig. 4). In addition, *yrr1*Δ [YEp-YRR1] cells exhibited similar SA resistance as *yrr1*Δ [YCp-YRR1] cells, while *yrr1*Δ [YCp-yrr1-52] cells were more resistant than either of these cell lines (Fig. 3). These results suggested that SA resistance is conferred by the mutant form of Yrr1p and was not related to intracellular protein quantity.

3.5. Concluding remarks

To investigate the mechanism of SA excretion in yeast, we screened SA-resistant mutants and isolated 60 clones, which revealed a novel mutation in *YRR1* that conferred SA resistance to KinSal052. This mutation also caused the activation of some genes involved in PDR.

Acknowledgments

We wish to thank E. Honda, Y. Mine, and S. Kurashimo for their technical support.

References

[1] D.M. Dixon, M.M. McNeil, M.L. Cohen, et al., Fungal infections: a growing threat, Public Health Rep. 111 (1996) 226–235.
[2] M. Nucci, K.A. Marr, Emerging fungal diseases, Clin. Infect. Dis. 41 (2005) 521–526.
[3] R.D. Cannon, E. Lamping, A.R. Holmes, et al., Efflux-mediated antifungal drug resistance, Clin. Microbiol. Rev. 22 (2009) 291–321.
[4] A.H. Groll, S.C. Piscitelli, T.J. Walsh, Clinical pharmacology of systemic antifungal agents: a comprehensive review of agents in clinical use, current investigational compounds, and putative targets for antifungal drug development, Adv. Pharmacol. 44 (1998) 343–500.
[5] D.W. Denning, Echinocandin antifungal drugs, Lancet 362 (2003) 1142–1151.
[6] R. Ernst, R. Klemm, L. Schmitt, et al., Yeast ATP-binding cassette transporters: cellular cleaning pumps, Methods Enzymol. 400 (2005) 460–484.
[7] W.S. Moye-Rowley, Transcriptional control of multidrug resistance in the yeast Saccharomyces, Prog. Nucleic Acid Res. Mol. Biol. 73 (2003) 251–279.
[8] E. Balzi, M. Wang, S. Leterme, et al., PDR5, a novel yeast multidrug resistance conferring transporter controlled by the transcription regulator PDR1, J. Biol. Chem. 269 (1994) 2206–2214.
[9] S. MacPherson, M. Larochelle, B. Turcotte, A fungal family of transcriptional regulators: the zinc cluster proteins, Microbiol. Mol. Biol. Rev. 70 (2006) 583–604.
[10] D.J. Katzmann, T.C. Hallstrom, Y. Mahe, et al., Multiple Pdr1p/Pdr3p binding sites are essential for normal expression of the ATP binding cassette

transporter protein-encoding gene PDR5, J. Biol. Chem. 271 (1996) 23049–23054.
[11] J. DeRisi, B. van den Hazel, P. Marc, et al., Genome microarray analysis of transcriptional activation in multidrug resistance yeast mutants, FEBS Lett. 470 (2000) 156–160.
[12] A. Nourani, D. Papajova, A. Delahodde, et al., Clustered amino acid substitutions in the yeast transcription regulator Pdr3p increase pleiotropic drug resistance and identify a new central regulatory domain, Mol. Gen. Genet. 256 (1997) 397–405.
[13] E. Carvajal, H.B. van den Hazel, A. Cybularz-Kolaczkowska, et al., Molecular and phenotypic characterization of yeast PDR1 mutants that show hyperactive transcription of various ABC multidrug transporter genes, Mol. Gen. Genet. 256 (1997) 406–415.
[14] J.K. Thakur, H. Arthanari, F. Yang, et al., A nuclear receptor-like pathway regulating multidrug resistance in fungi, Nature 452 (2008) 604–609.
[15] A.J. Marcus, The eicosanoids in biology and medicine, J. Lipid Res. 25 (1984) 1511–1516.
[16] G.A. Higgs, S. Moncada, J.R. Vane, Eicosanoids in inflammation, Ann. Clin. Res. 16 (1984) 287–299.
[17] L.C. Trost, J.J. Lemasters, The mitochondrial permeability transition: a new pathophysiological mechanism for Reye's syndrome and toxic liver injury, J. Pharmacol. Exp. Ther. 278 (1996) 1000–1005.
[18] L.C. Trost, J.J. Lemasters, Role of the mitochondrial permeability transition in salicylate toxicity to cultured rat hepatocytes: implications for the pathogenesis of Reye's syndrome, Toxicol. Appl. Pharmacol. 147 (1997) 431–441.
[19] B. Chowdhury, M. Adak, S.K. Bose, Flurbiprofen, a unique non-steroidal anti-inflammatory drug with antimicrobial activity against Trichophyton, Microsporum and Epidermophyton species, Lett. Appl. Microbiol. 37 (2003) 158–161.
[20] D.C. Amberg, D.J. Burke, J.N. Strathern, Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual, Cold Spring Harbor Laboratory Press, New York, 2005.
[21] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, second ed., Cold Spring Harbor Laboratory Press, New York, 1989.
[22] M.D. Rose, P. Novick, J.H. Thomas, et al., A Saccharomyces cerevisiae genomic plasmid bank based on a centromere-containing shuttle vector, Gene 60 (1987) 237–243.
[23] Z. Cui, T. Shiraki, D. Hirata, et al., Yeast gene YRR1, which is required for resistance to 4-nitroquinoline N-oxide, mediates transcriptional activation of the multidrug resistance transporter gene SNQ2, Mol. Microbiol. 29 (1998) 1307–1315.
[24] X. Zhang, Z. Cui, T. Miyakawa, et al., Cross-talk between transcriptional regulators of multidrug resistance in Saccharomyces cerevisiae, J. Biol. Chem. 276 (2001) 8812–8819.
[25] J. Keeven, D. Ko, J. Shallom, et al., PDR2 Gain-of-function mutations eliminate the need for Pdr1 and require the UBP6 product for resistance to translational inhibitors, Curr. Genet. 41 (2002) 11–19.
[26] S. Le Crom, F. Devaux, P. Marc, et al., New insights into the pleiotropic drug resistance network from genome-wide characterization of the YRR1 transcription factor regulation system, Mol. Cell. Biol. 22 (2002) 2642–2649.
[27] J. Servos, E. Haase, M. Brendel, Gene SNQ2 of Saccharomyces cerevisiae, which confers resistance to 4-nitroquinoline-N-oxide and other chemicals, encodes a 169 kDa protein homologous to ATP-dependent permeases, Mol. Gen. Genet. 236 (1993) 214–218.
[28] D.J. Katzmann, T.C. Hallstrom, M. Voet, et al., Expression of an ATP-binding cassette transporter-encoding gene (YOR1) is required for oligomycin resistance in Saccharomyces cerevisiae, Mol. Cell. Biol. 15 (1995) 6875–6883.