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MINI-REVIEW

Molecular mechanisms of yeast tolerance and in situ detoxification of lignocellulose hydrolysates

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Abstract Pretreatment of lignocellulose biomass for biofuel production generates inhibitory compounds that interfere with microbial growth and subsequent fermentation. Remediation of the inhibitors by current physical, chemical, and biological abatement means is economically impractical, and overcoming the inhibitory effects of lignocellulose hydrolysate poses a significant technical challenge for lower-cost cellulosic ethanol production. Development of tolerant ethanologenic yeast strains has demonstrated the potential of in situ detoxification for numerous aldehyde inhibitors derived from lignocellulose biomass pretreatment and conversion. In the last decade, significant progress has been made in understanding mechanisms of yeast tolerance for tolerant strain development. Enriched genetic backgrounds, enhanced expression, interplays, and global integration of many key genes enable yeast tolerance. Reprogrammed pathways support yeast functions to withstand the inhibitor stress, detoxify the toxic compounds, maintain energy and redox balance, and complete active metabolism for ethanol fermentation. Complex gene interactions and regulatory networks as well as co-regulation are well recognized as involved in yeast adaptation and tolerance. This review presents our current knowledge on mechanisms of the inhibitor detoxification based on molecular studies and genomic-based approaches. Our improved understanding of yeast tolerance and in situ detoxification provide insight into phenotype-genotype relationships, dissection of tolerance mechanisms, and strategies for more tolerant strain development for biofuels applications.

Keywords Aldehyde inhibitors · Gene regulatory networks · Genomic adaptation · Lignocellulose-to-ethanol conversion · Reprogrammed pathways · Stress tolerance

Introduction

Pretreatment of lignocellulosic biomass generates inhibitory compounds that interfere with microbial growth and fermentation and poses a significant challenge for economical cellulosic biofuels production. Remediation of inhibitory compounds by physical and chemical means has been determined to be too expensive for use in practice (Liu and Blaschek 2010). A bioabatement method was able to remove aldehyde inhibitors such as 2-furaldehyde (2-furancarbaldehyde; furfural); however, additional sugar and carbon source were consumed, and most abatement agents lack fermentation capability (Nichols et al. 2010). Tolerant ethanologenic yeast strains were found to be able to convert furfural and 5-(hydroxymethyl)-2-furaldehyde [5-(hydroxythyl)-2-furancarbaldehyde; 5-(hydroxymethyl)-2-furfural; HMF], representative inhibitors for biomass pretreatment, into less toxic compounds furanmethanol (FM) and furan-2,5-dimethanol (FDM; 2,5-*bis*-hydroxymethylfuran) while producing normal yields of ethanol (Liu et al. 2004; 2005; 2008b; Liu 2006; Talebnia and Taherzadeh 2006; Martin et al 2007). The identification and clarification of FM and FDM as metabolic conversion products of furfural and HMF suggested that the attached aldehyde

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functional group on the furan ring is the toxic cause but not the furan since numerous furan compounds are not toxic to yeast (Liu et al. 2004; 2008b; Liu 2006). Commonly termed as furan inhibitors inherited by historical reasons, furfural and HMF are in fact aldehyde inhibitors. The conversion of the aldehyde functional group into an alcohol form reduces the chemical toxicity. This clarification has led to an attempt to classify the inhibitors by the chemical functional groups to facilitate mechanism studies of the in situ detoxification (Larsson et al 1999; Klinke et al 2004; Liu and Blaschek 2010). The current classification of inhibitors contains aldehydes, ketones, phenols, and organic acids commonly associated with lignocellulose hydrolysates and biomass pretreatment procedures (Fig. 1). The new classification of the inhibitors has facilitated discoveries of new genes and new functions of known genes. For example, a newly described aldehyde reductase enzyme encoded by *ARI1*, a previously uncharacterized ORF *YGL157W* of *Saccharomyces cerevisiae*, possessed reduction capabilities toward at least 14 aldehydes including common lignocellulose-derived inhibitors such as furfural, HMF, vanillin, and cinnamaldehyde (Liu and Moon 2009). Since the discovery of the in situ detoxification of the fermentation inhibitors by tolerant yeast, significant progress has been made in understanding mechanism of the detoxification in the last decade (Palmqvist and Hahn-Hägerdal 2000; Liu 2006; Liu et al 2008a). This review presents our current knowledge of molecular mechanisms involved in the yeast tolerance and in situ detoxification of inhibitors, mainly aldehydes, derived from lignocellulosic hydrolysates. Improved understanding of yeast tolerance and the detoxification provides insight into dissection of tolerance mechanisms and the strategies for more tolerant strain development for economical biofuels production.

Reduction enzymes

Collective functions of multiple reduction enzymes

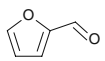
Understanding the importance of detoxification of the aldehyde functional group has allowed recognition of numerous enzymes possessing aldehyde reductase activities that contribute to the detoxification of the aldehyde inhibitors associated with lignocellulose pretreatment such as furfural, HMF, cinnamaldehyde, and vanillins. It is well established that the detoxification of the aldehyde inhibitors is attributed to multiple enzymes involved in reduction activities coupled with cofactors nicotinamide adenine dinucleotide phosphate (NADPH) and/or nicotinamide adenine dinucleotide (NADH) (Morimoto and Murakami 1967; Nemirovskii et al 1989; Villa et al. 1992; Wahlbom and Hahn-Hägerdal 2002; Liu et al. 2004; 2008b; Nilsson et al 2005; Liu 2006; Petersson et al. 2006; Liu and Moon 2009; Almeida et al. 2008; Liu and Blaschek 2010; Moon and Liu 2011; Fig. 2). Most in vitro enzyme assays for reduction of furfural and HMF were evaluated using whole-cell protein extracts; however, a few examples using partially purified proteins are available (Liu and Moon 2009; Moon and Liu 2011). Some notable enzymes such as alcohol dehydrogenase *ADH7*, *ADH6*, and *ADH1*, aldehyde dehydrogenase *ALD4*, and methylglyoxal reductase *GRE2* and *GRE3* have been demonstrated to possess efficient aldehyde reduction activities (Table 1). Comparative proteomic analysis of an industrial yeast strain suggested Adh5p and Adh1p as the catalytic agents for furfural reduction (Lin et al. 2009a). Protein extracts from individual gene clones often show distinct cofactor preference. However, whole-cell protein extracts from a tolerant ethanologenic yeast display strong aldehyde reduction activities with either NADH or NADPH and do not appear to have a strong cofactor preference (Liu et al. 2008b). A single gene deletion of the related reductase does not appear to significantly affect the detoxification capacity in yeast. It is likely that yeasts are able to respond globally and at multiple complex levels in biotransformation of the aldehyde inhibitors. Transcriptome analysis indicated many reductase genes were immediately induced by the toxic treatment such as *ADH7*, *ARI1, GRE2*, and *ALD4* (Ma and Liu 2010). Among these, *ADH7* can have a greater than 30- to 80-fold increase in transcript abundance after the HMF addition at 10 min and 1 h, respectively. As demonstrated by ^{13}C-labeled metabolic flux and transcription study, *ADH7* and ORF *YKL071W*, and possibly four other reductases, are associated with the yeast resistance to the furfural challenge (Heer et al. 2009).

Reduction functions of known genes

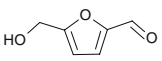
Important new functions of aldehyde reduction have been discovered for previously reported enzyme-coding genes. For example, yeast clones overexpressing *ADH6* and *ADH7* displayed high reduction capabilities toward furfural and HMF (Petersson et al. 2006; Liu et al. 2008b). Although they were characterized as alcohol dehydrogenases, the kinetic study of ADH6 and ADH7 showed that their reductive reactions were 50- to 100-fold more efficient than the corresponding oxidations (Larroy et al 2002a, b, 2003). It is possible that ADH6 or ADH7 act as an aldehyde reductase rather than as an alcohol dehydrogenase as their major metabolic function. Cell protein extracts of mutated *ADH1* containing yeast strains also showed significant aldehyde reductase activities (Almeida et al.

Fig. 1 A classification of inhibitory compounds to fermentative microorganisms derived from biomass pretreatment and lignocellulose hydrolysates based on chemical functional groups of aldehydes, ketones, organic acids, and phenols

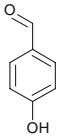
Aldehydes



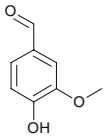
2-Furaldehyde
(2-furancarbaldehyde; furfural)
MW 96.09



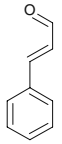
5-(Hydroxymethyl)-2-furaldehyde
[5-(hydroxymethyl)furan-2-carbaldehyde; 5-(hydroxymethyl)furfural; HMF]
MW 126.11



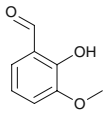
4-hydroxybenzaldehyde
(HBA)
MW 122.12



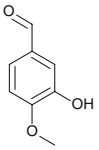
4-hydroxy-3-methoxy-benzaldehyde (vanillin)
MW 152.15



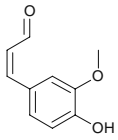
(2*E*)-3-phenylprop-2-enal
(cinnamaldehyde)
MW 132.16



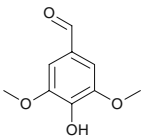
3-hydroxy-4-methoxybenzaldehyde
(isovanillin)
MW 152.15



2-hydroxy-3-methoxybenzaldehyde
(ortho vanillin)
MW 152.15

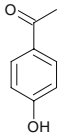


(2*Z*)-3-(4-hydroxy-3-methoxyphenyl) -prop-2-enal
(coniferyl aldehyde)
MW 178.18

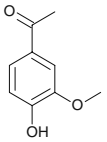


4-hydroxy-3,5-dimethoxybenzaldehyde
(syringaldehyde)
MW 182.17

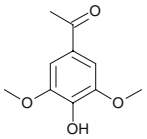
Ketones



1-(4-hydroxyphenyl)ethanone
(4-hydroxyacetophenone)
MW 136.15



1-(4-hydroxy-3-methoxyphenyl)ethanone
(acetovanillin)
MW 166.17

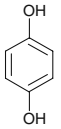


1-(4-hydroxy-3,5-dimethoxyphenyl)ethanone
(acetosyringone)
MW 196.2

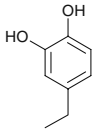
Phenols

phenol
MW 94.11

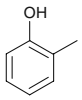
benzene-1,2-diol
(catechol)
MW 110.11



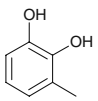
benzene-1,4-diol
(hydroquinone)
MW 110.11



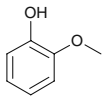
4-ethylbenzene-1,2-diol
(ethylcatechol)
MW 138.16



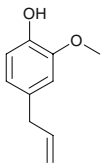
2-methylphenol
MW 108.14



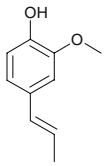
3-methylbenzene-1,2-diol
(methylcatechol)
MW 124.14



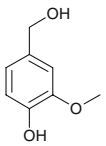
2-methoxyphenol
(guaiacol)
MW 124.14



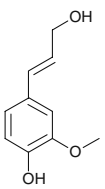
2-methoxy-4-(prop-2-en-1-yl) phenol
(eugenol)
MW 164.2



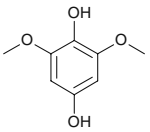
2-methoxy-4-[(1*E*)-prop-1-en-1-yl] Phenol (isoeugenol)
MW 164.2



4-(hydroxymethyl)-2-methoxyphenol
(vanillyl alcohol)
MW 154.16



4-[(1*E*)-3-hydroxyprop-1-en-1-yl]-2 -methoxyphenol (coniferyl alcohol)
MW 180.2



2,6-dimethoxybenzene-1,4-diol
(2,6-dimethoxy-hydroquinone)
MW 170.16

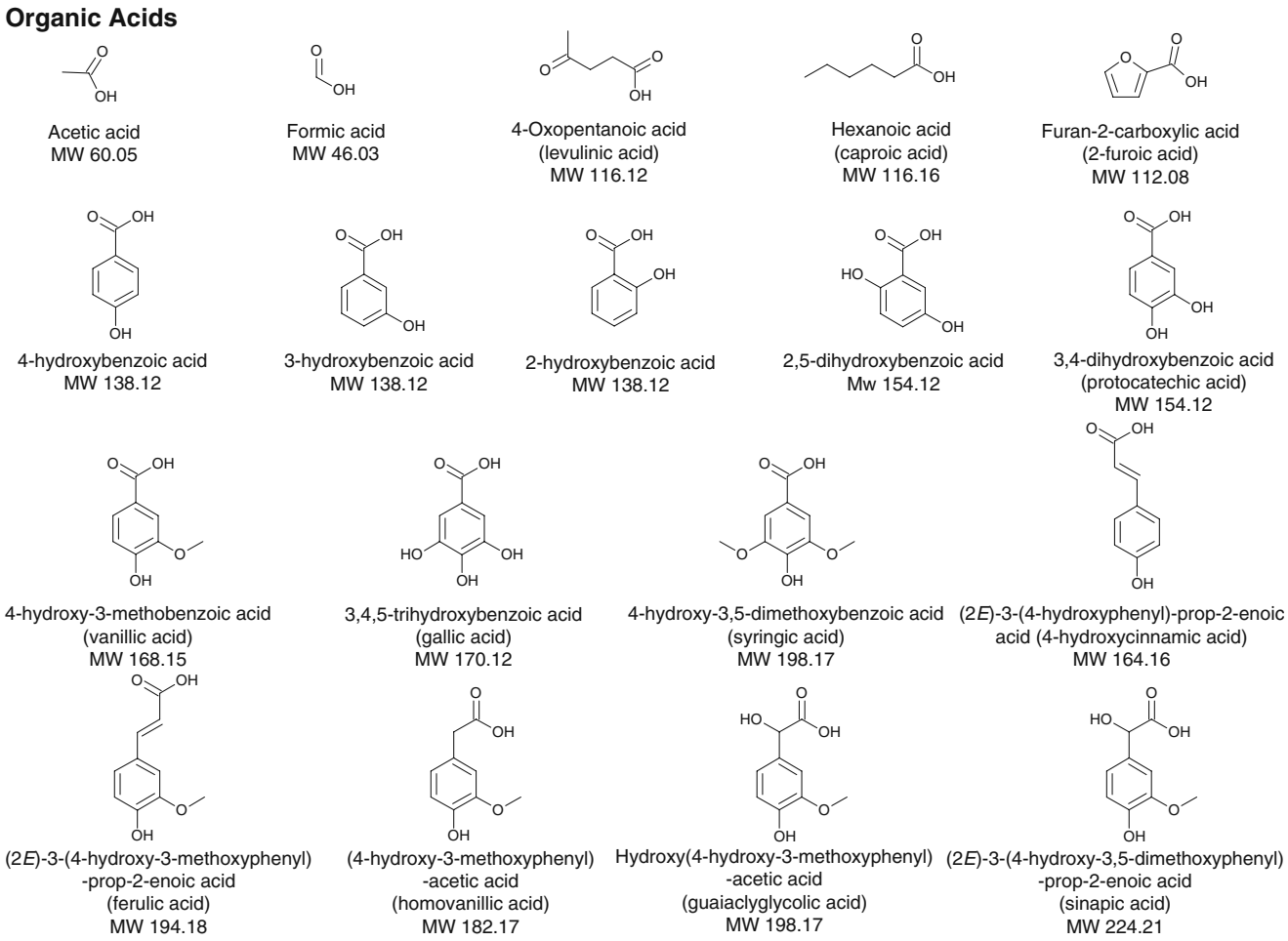


Fig. 1 (continued)

2008; Laadan et al 2008). ALD4 is a major mitochondrial aldehyde dehydrogenase that is required for growth on ethanol and the conversion of acetaldehyde to acetate using $NADP^+$ or NAD^+ as coenzymes. This enzyme is also able to reduce HMF and furfural utilizing NADH as a cofactor. Aldehyde dehydrogenase is known to play an important role in yeast acetaldehyde metabolism. Thus, aldehyde dehydrogenase could be another potential candidate gene for detoxification of aldehyde inhibitors. Similarly, aldo-keto reductase and methylglyoxal-related reductase GRE3 and GRE2 showed aldehyde reduction activities (Liu et al. 2008b; Liu and Moon 2009; Moon and Liu 2011). Xylose reductase from *Pichia stipitis* and expressed in *S. cerevisiae* also possessed reduction activities toward furfural and

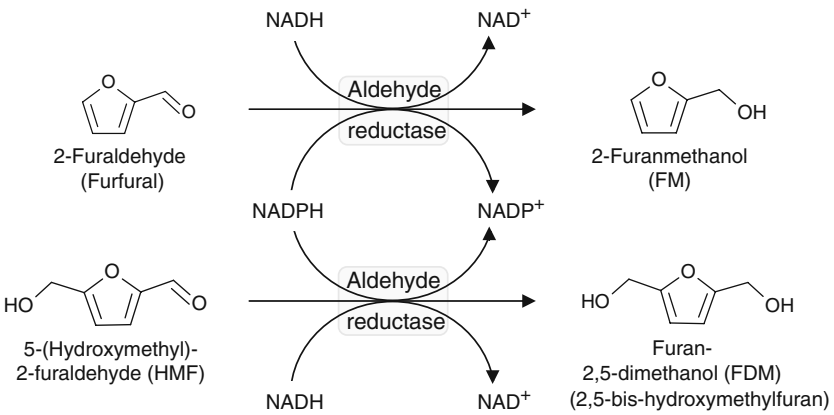


Fig. 2 Conversion pathways of 2-furaldehyde (furfural) and 5-(hydroxymethyl)-2-furaldehyde (HMF) into 2-furanmethanol (FM) and furan-2,5-dimethanol (FDM) coupled with NADH and/or NADPH and catalyzed by multiple reductases

Table 1 Genes encoding enzymes possessing aldehyde reductase activities examined by the enzyme specific activity using whole-cell protein extract or partially purified proteins exampled by furfural and HMF for ethanologenic strains of *S. cerevisiae*

Gene	Enzyme Commission number	Cofactor	Substrate	Enzyme specific activity (mU/mg protein)	Reference
ADH6	1.1.1.2	NADPH	Furfural HMF	98-4000 79-4000	Petersson et al, 2006; Liu et al, 2008b; Almeida et al. 2008 Petersson et al, 2006; Liu et al, 2008b
		NADH	Furfural	62-210	
			HMF	ns	
ADH7	1.1.1.2	NADH	Furfural HMF	86 158	Liu et al, 2008b
ALD4	1.2.1.5	NADH	Furfural HMF	67 93	Liu et al, 2008b
GRE3	1.1.1.- 1.1.1.21	NADH	Furfural HMF	115 157	Liu et al, 2008b
ADH1	1.1.1.1 1.1.1.190	NADH	Furfural HMF	~3,900 ~3,800	Almeida et al. 2008
ARI1	1.1.1.-	NADPH	Furfural HMF	4,290 580	Liu and Moon, 2009
GRE2	1.1.1.283	NADH	Furfural HMF	540 50	Moon and Liu, 2011
Y62	1.1.1.-	NADH	Furfural	349	Liu and Blaschek, 2010
Y76	1.1.1.-	NADH	Furfural	353	Liu and Blaschek, 2010

ns Not significant

HMF (Almeida et al. 2008). In addition to their significant involvement under stress conditions, these genes appeared to be important candidates facilitating inhibitor reduction. GRE3 has been deleted in an effort to reduce xylitol byproduct production to improve xylose utilization efficiency of yeast (Träff et al. 2001; Kuyper et al. 2005). Considering the significant interaction between inhibitor tolerance and efficient pentose utilization, it is worthwhile to clarify the roles and interplay among the important candidate gene groups for balanced metabolic function in biofuel conversions by yeasts.

New aldehyde reductase genes

A novel gene encoding NADPH-dependent aldehyde reductase, *ARI1*, was characterized recently (Liu and Moon 2009; Bowman et al. 2010; *Saccharomyces* Genome Database http://www.yeastgenome.org/). The product of *ARI1* is the first purified yeast protein reported as an aldehyde reductase involved in the detoxification of inhibitors of lignocellulose hydrolysates. As mentioned, it has reduction activities toward at least 14 aldehydes including those frequently identified during biomass pretreatment procedures. The optimum performance temperature of the enzyme is 25 °C at pH 7.0. The protein of *ARI1* has an approximate molecular mass of 38 kDa and is a member of the subclass "intermediate" of the short-chain dehydrogenase/reductase superfamily with the following typical characteristics: the conserved catalytic site lies at Tyr_{169}-X-X-X-Lys_{173}; an indispensable reduction catalytic tetrad at Asn_{106}, Ser_{131}, Tyr_{169}, and Lys_{173}; and an approved cofactor binding motif at Gly_{11}-X-X-Gly_{14}-X-X-Ala_{17} near the N terminus. The function of the gene was annotated by conserved functional sequence motifs, gene expression, protein expression, and partially purified protein assays. This newly described gene possibly represents a group of uncharacterized multiple functional genes such as other potential candidates *YKL071W*, Y62, Y76, Y81, and Y82 (Heer et al. 2009; Liu and Blaschek 2010; Liu and Moon 2009).

Detoxification pathways

Enhanced genetic background

A tolerant yeast is able to withstand challenges of high levels of furfural–HMF inhibitor complex and produce normal yields of ethanol while the parental strain fails to establish a viable culture under the same conditions (Liu et al. 2009). It is clear that the tolerant yeast possesses different genetic mechanisms for in situ detoxification of the toxic compounds that enable active metabolism for ethanol production. Characterization of gene expression dynamics of the tolerant yeast strain suggested that the tolerant yeast appeared to have an inheritable genetic

makeup that is distinct from its parental strain. At least 16 gene transcripts involved in glucose metabolism had significantly greater abundance in the inhibitor-tolerant yeast compared with its parental strain even without the inhibitor treatment (Liu et al. 2009). Many of these are key genes involved in glucose metabolic process, NAD(P)H metabolic and regeneration, and transferase activities such as *HXK1, HXK2, GLK1, TDH1, TDH3, LAT1, PDC6, ADH4, ALD2, ALD4, ZWF1, SOL3, RBK1, TAL1, NQL1*, and *PRS2* (Table 2). Some of these key genes displayed as high as four- to sixfold increased abundance, for example, the hexokinase-encoding genes *HXK1* and *HXK2*, glyceraldehyde-3-phosphate dehydrogenase gene *TDH1*, dihydrolipoamide acetyltransferase component (E2) of the pyruvate dehydrogenase complex gene *LAT1*, and major mitochondrial aldehyde dehydrogenase gene *ALD4* and *TAL1* that encodes transaldolase.

Reprogrammed regulatory networks and redox balance

Glycolysis and pentose phosphate pathway are closely related pathways in yeast glucose metabolism. This close relationship is of such importance that the two pathways cannot be viewed separately when discussing yeast tolerance and detoxification of the lignocellulose inhibitors. Under the challenge of furfural–HMF complex, yeast is unable to grow, and most genes involved in these pathways are severely repressed. A tolerant yeast strain, on the other hand, demonstrated different expression dynamics and completed ethanol fermentation. Under inhibitor stress, high levels of expression by *HXK1, HXK2*, and *GLK1* appeared to secure the initiation stage of phosphorylation of glucose by these enzyme encoding genes (Liu et al. 2009). Then, the significantly induced expression of *ZWF1, SOL3, GND1*, and *GND2* as well as the repression of glycolytic enzyme phosphoglucose isomerase apparently drive the glucose metabolism toward pentose phosphate pathway. Gene deletion mutations of *ZWF1* and *GND1* are highly sensitive to furfural and HMF (Gorsich et al. 2006). The enhanced expression of *ZWF1* at an early step is key to shifting the glucose metabolism in favor of pentose phosphate pathway over glycolysis (Liu et al. 2009; Fig. 3). Consequently, all other cofactor NAD(P)H regenerating steps involving *ZWF1, GND1, GND2*, and *TDH1* were up-regulated in the tolerant yeast. Aldehyde reduction enzyme encoding genes *ALD4, ALD6, ADH6, ADH7*, and *SFA1* displayed significantly increased transcription at the early time points in the presence of furfural–HMF complex. These accelerated NAD(P)H-dependent reductions of acetaldehyde, furfural, and HMF would generate sufficient NAD^+ and $NADP^+$, in return, to provide necessary cofactors needed for oxidative reactions or NAD(P)H regenerations by Zwf1p, Gnd1p, Gnd2p, Tdh1p, and Ald4p. Redox metabolism, in the form of interconversion of the pyridine-nucleotide cofactors $NADH/NAD^+$ and $NADPH/NADP^+$, plays a key role in the yeast metabolism. NADH is required in respiration and fermentative pathway in conversion of pyruvate to CO_2 and ethanol. NADPH is mainly required for the synthesis of amino acids and nucleotides and a major source of NADPH production in yeast is through the oxidative phase of pentose phosphate pathway. The up-regulated *ZWF1, SOL3, GND1*, and *GND2* along with enhanced expressed *TDH1* are important for NAD(P)H regenerations to supply necessary cofactors needed for acetaldehyde conversion and reduction of furfural and HMF. Thus, a $NAD(P)^+$/NAD(P)H-dependent redox balance is well-maintained in the altered pathways for the in situ detoxification of furfural and HMF by the tolerant yeast.

Under the inhibitor challenge, tolerant yeast also appeared to be able to achieve NAD(P)H regeneration through a short path to the "TCA cycle": tricarboxylic acid cycle. This process involves many genes in amino acids metabolism pathways closely related to the TCA cycle, including both induced genes such as *CHA1*, *ALT1*, *PUT1*, *PUT2*, and *CAR1*, and repressed genes such as *ARG1*, *ARG3*, *ARG4*, *ARG5, ARG6*, *ARG7*, *ARG8*, *LYS4*, *LYS14*, and *LYS20* (Ma and Liu 2010). The accelerated catabolism of proline, serine, and alanine, together with the reduced biosynthesis of arginine, provides a shortcut for ATP regeneration via the TCA cycle. Thus, efficient energy metabolism can be maintained under the inhibitor stress. Apparently, enriched genetic background by aforementioned genes and a well-maintained redox balance through the reprogrammed expression responses involved in numerous pathways of the tolerant yeast strain are accountable for the acquired yeast tolerance and the detoxification of the inhibitors.

Integrated multiple gene interactions

Yeast exhibit an accelerated glucose conversion rate once they are recovered from the furfural and/or HMF challenges compared with what would normally occur without the inhibitors (Taherzadeh et al. 2000; Liu et al. 2004, 2005). The inhibition of glucose phosphorylation, together with repression of *PFK1*, *PFK2*, *PYK2*, and *CDC19* seemed responsible for the delayed glycolysis inhibited by furfural and HMF treatment (Liu et al. 2009). Such a delayed biological process in yeast can also be attributed to a lack of ATP, NAD(P)H, and intermediate metabolites necessary to support cell growth and reproduction (Wahlbom and Hahn-Hägerdal 2002; Fisk et al. 2006; Liu 2006). For the tolerant yeast, in addition to numerous induced expressions, gene transcription levels of *PGK1*, *ENO1*, *ENO2*, *PYK2*, *CDC19, PDA1*, and *PDB1* encoding varied enzymes for pyruvate metabolisms did not show repressed effect in

Table 2 Gene ontology (GO) categories and terms for significantly induced genes by HMF during the lag phase in *S. cerevisiae*

GO ID	GO term	Gene(s)
Cellular component		
GO:0005737	Cytoplasm	***SHP1***, ***ATG8***, *YBL107C*, ***HSP26***, ***NPL4***, ***CHA1***, *GPM2*, ***SNQ2***, ***RPN9***, ***SLF1***, ***SSA4***, ***OTU1***, ***RPN12***, ***PYC1***, ***ARI1***, ***YGR111W***, ***ECM29***, ***PUT2***, ***PRE3***, ***MET3***, ***MET14***, ***TPO1***, ***ALT1***, ***PUT1***, ***YAP1***, ***PGA3***, ***ERO1***, ***YNL155W***, ***PRE6***, ***GRE2***, *SGT2*, ***RSB1***, *YOR059C*, ***PDR5***, ***TPO4***, ***PRE10***, ***ALD4***, ***CAR1***
GO:0005634	Nucleus	***SHP1***, ***YBL100W-A***, ***HSP26***, ***RAD16***, ***RPT2***, ***RPN4***, ***YDR210W-B***, ***YDR316W-B***, ***YDR365W-B***, ***PRE1***, ***SSA4***, ***MAG1***, ***OTU1***, ***ARI1***, ***YGR111W***, ***ECM29***, *YKR011C*, ***YAP1***, ***YNL155W***, ***GRE2***, *YOR052C*, ***RPT4***
GO:0016020	Membrane	***ATG8***, ***NPL4***, ***SNQ2***, ***PDR15***, ***DDI1***, ***YOR1***, ***TPO1***, ***PGA3***, ***RSB1***, ***PDR5***, ***TPO4***, ***MCH5***, ***PDR12***, *PRM4*
GO:0005575	Cellular component unknown	*IMD1*, *YBR062C*, *YBR255C-A*, *YDR034W-B*, *YER137C*, *YGR035C*, ***YHR138C***, *YLL056C*, *ICT1*, *OYE3*
GO:0005886	Plasma membrane	***SNQ2***, ***DDI1***, ***YOR1***, ***TPO1***, ***PGA3***, ***RSB1***, ***PDR5***, ***TPO4***, ***MCH5***, ***PDR12***
GO:0005739	Mitochondrion	***CHA1***, ***SNQ2***, ***PUT2***, ***MET3***, ***ALT1***, ***PUT1***, ***PRE6***, ***PDR5***, ***ALD4***
GO:0005783	Endoplasmic reticulum	***NPL4***, ***PGA3***, ***ERO1***, ***RSB1***
GO:0005773	Vacuole	***ATG8***, ***TPO1***, ***TPO4***
GO:0005624	Membrane fraction	***SNQ2***, ***YOR1***
GO:0005933	Cellular bud	***TPO1***
GO:0005618	Cell wall	*TIR4*
GO:0012505	Endomembrane system	***NPL4***
GO:0030427	Cite of polarized growth	***CAR1***
Other	Other	***PRE7***, *ADH7*, ***RPT3***, ***PUP3***
Biological process		
GO:0008150	Biological process unknown	*IMD1*, *YBL107C*, *YBR062C*, *YBR255C-A*, *GPM2*, *YDR034W-B*, *YER137C*, ***ARI1***, *YGR035C*, *YKR011C*, *YLL056C*, ***YNL155W***, *TIR4*, *YOR052C*, *YOR059C*, *PRM4*, *OYE3*
GO:0044257	Cellular protein catabolic process	***PRE7***, ***SHP1***, ***RAD16***, ***NPL4***, ***RPT2***, ***RPT3***, ***RPN9***, ***PRE1***, ***PUP3***, ***DDI1***, ***RPN12***, ***PRE3***, ***PRE6***, ***RPT4***, ***PRE10***
GO:0006810	Transport	***ATG8***, ***PDR15***, ***SSA4***, ***DDI1***, ***YOR1***, ***TPO1***, ***PGA3***, ***RSB1***, ***PDR5***, ***TPO4***, ***MCH5***, ***PDR12***
GO:0006950	Response to stress	***ATG8***, ***HSP26***, ***RAD16***, ***RPN4***, ***SNQ2***, ***PRE1***, ***SSA4***, ***MAG1***, ***PRE3***, ***YAP1***, *SGT2*
GO:0042221	Response to chemical stimulus	***RPN4***, ***SNQ2***, ***PDR15***, ***YOR1***, ***MET14***, ***YAP1***, ***PDR5***
GO:0006519	Cellular amino acid and derivative metabolic process	***CHA1***, ***PUT2***, ***MET3***, ***MET14***, ***ALT1***, ***PUT1***, ***CAR1***
GO:0032196	Transposition	***YBL100W-A***, ***YDR210W-B***, ***YDR316W-B***, ***YDR365W-B***
GO:0006457	Protein folding	***HSP26***, ***SSA4***, ***ERO1***
GO:0006350	Transcription	***RPN4***, ***OTU1***, ***YAP1***
GO:0006464	Protein modification process	***RAD16***, ***OTU1***, ***ERO1***
GO:0030435	Sporulation resulting in formation of a cellular spore	***SHP1***, ***PRE1***, ***PRE3***
GO:0006259	DNA metabolic process	***RAD16***, ***RPN4***, ***MAG1***
GO:0016044	Membrane organization	***ATG8***, ***YHR138C***, ***RSB1***
GO:0007033	Vacuole organization	***ATG8***, ***YHR138C***
GO:0044262	Cellular carbohydrate metabolic process	***SHP1***, ***PYC1***
GO:0044255	Cellular lipid metabolic process	*ICT1*, ***GRE2***
GO:0006766	Vitamin metabolic process	***PYC1***, ***ALD4***
GO:0046483	Heterocycle metabolic process	***PUT2***, ***PUT1***
GO:0051186	Cofactor metabolic process	***PYC1***, ***ALD4***
GO:0016192	Vesicle-mediated transport	***ATG8***, ***DDI1***
GO:0051276	Chromosome organization	***RAD16***
GO:0016070	RNA metabolic process	***YAP1***
GO:0006412	Translation	***SLF1***
GO:0006091	Generation of precursor	***SHP1***

Table 2 (continued)

GO ID	GO term	Gene(s)
	metabolites and energy	
GO:0070271	Protein complex biogenesis	***RPN9***
GO:0007049	Cell cycle	***RPN4***
GO:0019725	Cellular homeostasis	***SLF1***
Other	Other	*ADH7*, ***YGR111W***, ***ECM29***
Molecular function		
GO:0016787	Hydrolase activity	***PRE7***, ***RAD16***, ***RPT2***, ***SNQ2***, ***YDR210W-B***, ***YDR316W-B***, ***YDR365W-B***, ***RPT3***, ***PDR15***, ***PRE1***, ***PUP3***, ***SSA4***, ***MAG1***, ***OTU1***, ***RPN12***, ***YOR1***, ***PRE3***, ***PRE6***, ***RSB1***, ***PDR5*** , ***RPT4***, ***PRE10***, ***PDR12***, ***CAR1***
GO:0003674	Molecular function unknown	*IMD1*, ***ATG8***, *YBL107C, YBR062C*, ***NPL4***, *YBR255C-A, GPM2, YDR034W-B, YER137C, YGR035C*, ***YGR111W***, *YKR011C, YLL056C*, ***PGA3***, ***YNL155W***, *SGT2, TIR4, YOR052C, YOR059C, PRM4*
GO:0008233	Peptidase activity	***PRE7***, ***RPT2***, ***YDR210W-B***, ***YDR316W-B***, ***YDR365W-B***, ***RPT3***, ***PRE1***, ***PUP3***, ***OTU1***, ***RPN12***, ***PRE3***, ***PRE6***, ***RPT4***, ***PRE10***
GO:0005215	Transporter activity	***SNQ2***, ***PDR15***, ***YOR1***, ***TPO1***, ***RSB1***, ***PDR5***, ***TPO4***, ***MCH5***, ***PDR12***
GO:0016491	Oxidoreductase activity	*ADH7*, ***ARI1***, ***PUT2***, ***PUT1***, ***ERO1***, ***GRE2***, ***ALD4***, *OYE3*
GO:0005515	Protein binding	***YBL100W-A***, ***HSP26***, ***YDR210W-B***, ***YDR316W-B***, ***YDR365W-B***, ***SSA4***, ***DDI1***, ***ECM29***
GO:0016740	Transferase activity	***YDR210W-B***, ***YDR316W-B***, ***YDR365W-B***, ***MET3***, ***MET14***, ***ALT1***, *ICT1*
GO:0003723	RNA binding	***YBL100W-A***, ***YDR210W-B***, ***YDR316W-B***, ***YDR365W-B***, ***SLF1***
GO:0016779	Nucleotidyltransferase activity	***YDR210W-B***, ***YDR316W-B***, ***YDR365W-B***, ***MET3***
GO:0003677	DNA binding	***RAD16***, ***RPN4***, ***YAP1***
GO:0016874	Ligase activity	***RAD16***, ***PYC1***
GO:0030528	Transcription regulator activity	***RPN4***, ***YAP1***
GO:0030234	Enzyme regulator activity	***SHP1***, ***YHR138C***
GO:0016829	Lyase activity	***CHA1***
GO:0005198	Structural molecule activity	***RPN9***
GO:0016853	Isomerase activity	*GPM2*

Genes in bold indicate their encoding proteins or enzymes are involved in more than one function

response to the inhibitor challenge at the early stage. This allowed a smooth flow of the central metabolic pathways. Since the tolerant yeast is able to in situ detoxify the aldehyde inhibitors, with the significant reduction in the concentration of the inhibitory aldehydes, more NADPH thus generated could shift from detoxification to accelerate biosynthesis processes and cell growth. In the meantime, alcohol dehydrogenase is favored for the conversion of acetaldehyde to ethanol with sufficient NADH supply, which contributes to the accelerated glucose consumption.

It should be pointed out that many genes that initially were repressed but were able to recover to their normal functional levels after the inhibitor challenges are necessary components in these globally integrated interactions under the stress. The functions of these genes allowed the tolerant yeast to maintain balanced biological processes to complete ethanol fermentation. In the absence of such reprogrammed transcription dynamics at the genome level, continued inhibition and repression by furfural and HMF, as demonstrated by a wild-type strain, led to loss of cell function and eventual death.

Global response

Gene expression profiling overview

Earlier studies on laboratory yeast strain response to environmental stimuli suggested a core set of genes are involved with the environmental stress response (Gasch et al. 2000; Gasch and Werner-Washburne 2002). For industrial ethanologenic yeast, 100 to 400 genes showed differential transcription expression response to furfural and HMF individually or in combination (Liu and Slininger 2005; Liu 2006; Liu and Slininger 2006; Liu et al. 2009; Ma and Liu 2010; Li and Yuan 2010). Unlike the laboratory strain showing mostly transient responses, ethanologenic yeast displays relatively persistent expression dynamics indicating different mechanisms may possibly be involved.

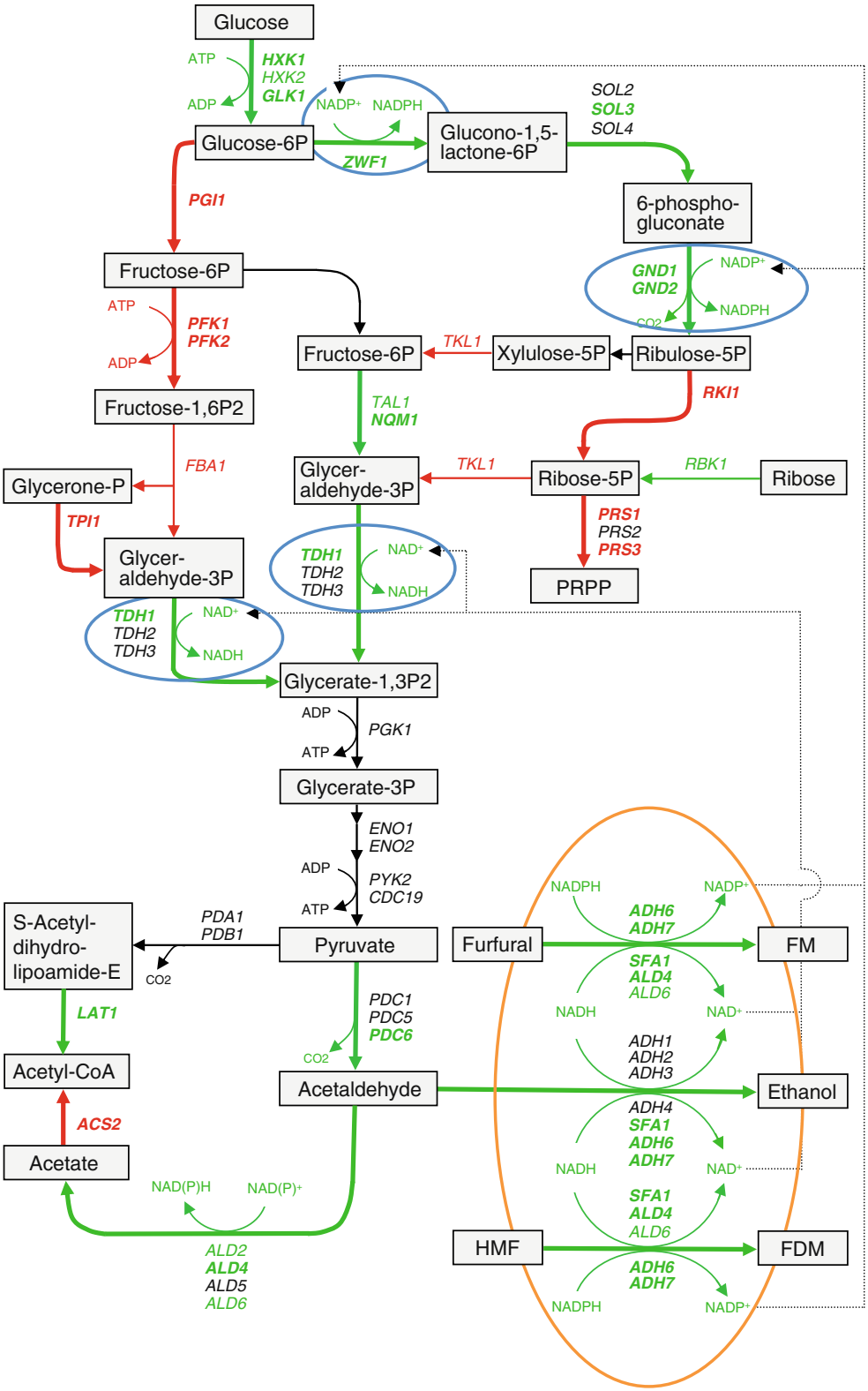


Fig. 3 A schematic illustration of glucose metabolic pathways and conversion of furfural and HMF by tolerant *S. cerevisiae* NRRL Y-50049 inferred by metabolic profiling analysis and quantitative mRNA expression analysis compared with its wild-type strain NRRL Y-12632. *Black arrowed lines* and *letters* indicate normal or near-normal levels of reactions, expressions, or pathways; *green* indicates enhanced, and *red* for repressed expressions, reactions, or pathways. *Bold lines* and *letters* indicate the levels of expression and pathways are statistically significant. Key steps of enhanced NAD(P)H regenerations are *circled in blue* and significant aldehyde reductions *circled in orange*. Interactions of cofactor regeneration and balanced utilization pathways are linked by *dotted lines*

Most studies on yeast response were characterized using a wild-type strain and differentially expressed genes distributed in a wide range of functional categories. Classic observations on stress-related high-osmolarity glycerol pathway, heat-shock protein genes, and transcription factors Msn2p/Msn4p are commonly observed (Lin et al. 2009a, b; Li and Yuan 2010; Ma and Liu 2010). Tolerant yeast responded to aldehyde inhibitors differently than a wild-type, although there are some overlappings during the early time point responses. In the remaining sections of this review, an emphasis is given

mainly to potential candidate genes in order to address global integration and interactions of the tolerance yeast.

Induced expression

The inhibitor-induced expression consists of only a small portion of genes responding to the challenge at the genome level. However, many of these genes have multiple functions. Some notable function categories involve cytoplasm, nucleus, membrane, mitochondrion, cellular protein catabolic process, transport, response to stress, amino acid and derivative metabolic process, hydrolase activity, peptide activity, oxidareductase activity, protein binding, protein fate, cellular transport, and several groups of unknown functions (Tables 2 and 3). At least seven transcription factor genes, *YAP1, YAP5, YAP6, PDR1, PDR3, RPN4*, and *HSF1*, were identified as key regulators for the induced expression response in yeast adaptation to HMF challenge (Song et al. 2009; Ma and Liu 2010). Most of these regulatory genes displayed greater than twofold increase of mRNA abundance after challenges by furfural and HMF. Protein binding motif analysis revealed that each of these transcription factor genes harbors multiple protein binding sites for Pdr3p, Yap1p, Yap5p, Yap6p, Rpn4p, and Hsf1p. For example, DNA binding motifs of Pdr1/3p are present in promoter regions of *PDR3*, *YAP5*, *YAP6*, and *RPN4* (Ma and Liu 2010; Fig. 4). DNA binding sites of Yap1p and Hsf1p exist in all five transcription factor genes except for *PDR1* having one Yap1p site and *PDR3*, two Hsf1p sites. Most transcription factor genes have multiple binding sites for multiple transcription factors. For example, *RPN4* has 13 binding sites of four transcription factors, and *PDR3* has six sites for two. These observations suggest potential interactions involving multiple transcription factors exist for inhibitor tolerance. High expression of *RPN4* by HMF treatment was suggested to be regulated by Yap1p, Pdr1p, Pdr3p, and Hsf1p based on ChIP-chip assay data, genome expression, and microarray assays of transcription factor mutations (Lee et al. 2002; Harbison et al. 2004; Hahn et al. 2006; Larochelle et al. 2006; Workman et al. 2006; Salin et al. 2008; Ma and Liu 2010). Numerous studies also demonstrated positive feedback of enhanced expression of *RPN4* to its regulators of Yap1p and Pdr1p (Harbison et al. 2004; Haugen et al. 2004; Salin et al. 2008). In addition, DNA binding motif of a transcription factors' own is present in its promoter region, such as *PDR3*, *YAP1*, and *HSF1* (Fig. 4). These suggest a possible self-regulated expression interaction involved in yeast tolerance response as well as co-regulation and interactions of multiple transcription factors under the stressed condition.

Repressed response

Most differentially expressed genes show repressed response to inhibitor challenges regardless of the treatment methods used. The difference lies that repressed genes in certain

Table 3 Protein functional categories for significantly induced genes by HMF during the lag phase in *S. cerevisiae*

MIPS ID	Functionary category	*p* Value	Entries
01 Metabolism			
01.01.03.03.02	Degradation of proline	7.82E-04	PUT2, **PUT1**
01.01.03.05.02	Degradation of arginine	3.94E-04	**PUT1**, CAR1
01.02.03.01	Sulfate assimilation	3.54E-03	MET3, MET14
14 Protein fate (folding, modification, destination)			
14.07.11	Protein processing (proteolytic)	4.05E-09	**PRE7**, **ATG8**, **RPT2**, **RPT3**, **PRE1**, **PUP3**, **RPN12**, **PRE3**, **PRE6**, **RPT4**, **PRE10**
14.13	Protein/peptide degradation	3.97E-11	**PRE7**, SHP1, **ATG8**, NPL4, **RPT2**, RPN4, **RPT3**, RPN9, **PRE1**, **PUP3**, DDI1, OTU1, **RPN12**, ECM29, YHR138c, **PRE3**, **PRE6**, **RPT4**, **PRE10**
16 Protein with binding function or cofactor requirement (structural or catalytic)			
16.19.03	ATP binding	1.52E-03	**RPT2**, **SNQ2**, **RPT3**, **PDR15**, **YOR1**, **PDR5**, **RPT4**, **PDR12**
20 Cellular transport, transport facilities and transport routes			
20.01.27	Drug/toxin transport	4.70E-06	**SNQ2**, **YOR1**, TPO1, **PDR5**, TPO4, **PDR12**
20.03.22	Transport ATPases	3.68E-04	**SNQ2**, **YOR1**, RSB1, **PDR5**, **PDR12**
20.03.25	ABC transporters	1.44E-05	**SNQ2**, **PDR15**, **YOR1**, **PDR5**, **PDR12**
32 Cell rescue, defense, and virulence			
32.05.01.03	Chemical agent resistance	1.73E-05	**SNQ2**, MAG1, **YOR1**, YAP1, **PDR5**

Proteins in bold indicate functions involved in more than one category

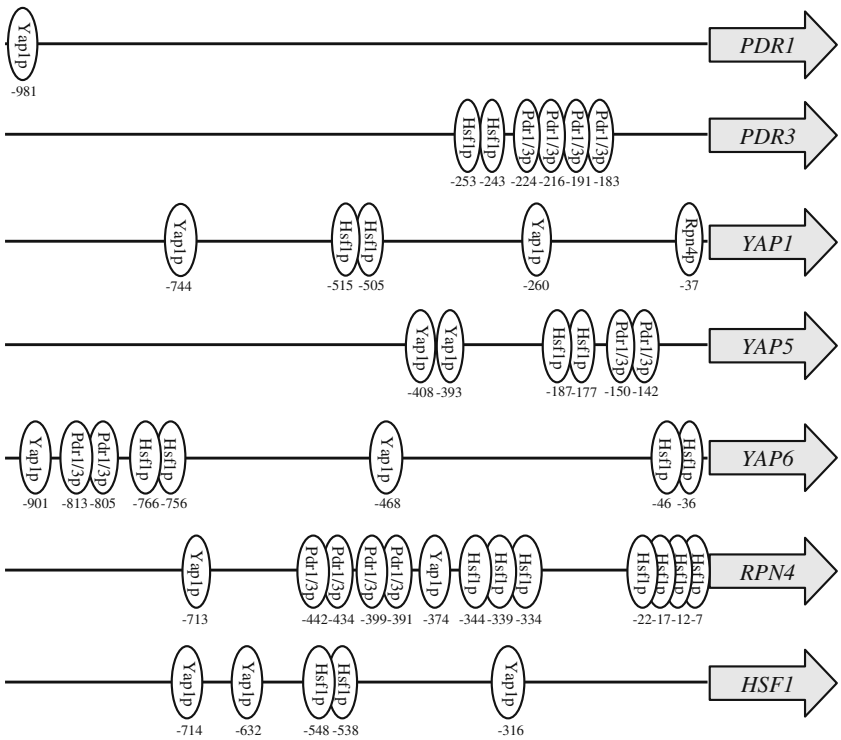


Fig. 4 DNA binding sites in the promoter regions from −1,000 to −1 for seven selective transcription factor genes *YAP1*, *YAP5*, *YAP6*, *PDR1*, *PDR3*, *RPN4*, and *HSF1* of *S. cerevisiae* showing overlapping and multiple binding sites that indicate gene co-regulation roles of key transcription factor genes

categories are able to recover over time while others remain repressed as demonstrated by comparative transcription dynamic analyses (Liu et al. 2009; Ma and Liu 2010). The importance of repressed genes is often neglected in contrast to overwhelmingly emphasized attention to the induced genes. In fact, many "overlooked" genes play necessary roles in yeast adaptation as they are able to recover and function under stress. As mentioned, the lack of such functional genes can result in non-viable biological processes including ethanol fermentation. Under certain conditions, down-regulated expression could be efficient means of energy utilization for economic pathway development (Ma and Liu 2010). The repressed genes are mainly involved in the functional categories of ribosome biogenesis, amino acid and derivative metabolic process, RNA synthesis, RNA metabolic process, transport, transcriptional and translation controls, mitochondrial, and others (Ma and Liu 2010; Li and Yuan 2010). For many repressed genes, at least five important regulatory genes including *ARG80, ARG81, GCN4, RAP1*, and *FHL1* were found to be involved in the significantly down-regulated expression. For example, *ARG1*, *ARG3*, *ARG4*, *ARG5, ARG6*, *ARG7*, and *ARG8* involved in arginine biosynthesis repressed by HMF were regulated by the transcription factor genes *ARG80* and *ARG81* as well as *GCN4* (De Rijcke et al. 1992; Natarajan et al. 2001; Ma and Liu 2010). In addition to regulation of arginine biosynthesis, *GCN4* regulates expression of many other genes related to amino acid biosynthesis such as a number of genes involved in biosynthesis of histidine, leucine, and lysine (Natarajan et al. 2001; Ma and Liu 2010). Among the many genes repressed by HMF, a large number of genes are involved in ribosome biogenesis and protein translation processes, which were predicted to be regulated by transcription factor genes *RAP1* and *FHL1*.

Genomic adaptation

YAP family- and YAP1-regulated oxidoreductase activity networks

The lag phase for cell growth in response to inhibitor challenges has been used as a measure of strain tolerance and to study the mechanisms of genomic adaptation (Liu et al. 2004; Liu 2006; Ma and Liu 2010). Recently, 365 candidate genes were identified as involved in yeast adaptation and tolerance to HMF (Ma and Liu 2010). The interventional networks and interplays are complex and comprehensive. However, at least three significant components are recognized by some key regulators. First, numerous functional encoding genes such as *ARI1, ADH6, ADH7*, and *OYE3*, as well as gene interactions involved in the biotransformation and inhibitor detoxification, are the direct driving force to reduce the HMF damage in cells. The yeast activator protein (YAP) family contains eight transcription factors with a b-ZIP protein at the DNA binding domain (Rodrigues-Pousada et al. 2010). Transcription factor Yap1p, the major oxidative stress regulator,

acts as a sensor for oxidative molecules and activates the transcription response of anti-oxidant genes by recognizing Yap1p response elements (YRE), 5'-TKACTMA-3', in the promoter region (Harbison et al. 2004; Fernandes et al. 1997; Dubacq et al. 2006). Under HMF challenged conditions, *YAP1* displayed consistently higher induced abundance of at least two- to threefold increase during the lag phase (Ma and Liu 2010). There are at least 41 HMF-induced genes possessing the YRE sequence in their promoter region. Many genes were confirmed to be regulated directly by *YAP1* or indirectly through *YAP5* and *YAP6* (Fig. 5). Most *YAP1*-regulated genes were classified in the functional categories of redox metabolism, amino acid metabolism, stress response, DNA repair, and others (Table 2). For example, the highly induced oxidoreductase genes *ADH7*, *GRE2*, and *OYE3* were found as regulons of *YAP1* (Lee et al. 2002; Haugen et al. 2004; Dubacq et al. 2006; Ma and Liu 2010). A recently characterized new aldehyde reductase gene, *ARI1*, was found to be regulated by Yap6p which is a regulon of *YAP1* (Harbison et al. 2004; Liu and Moon 2009; Ma and Liu 2010). *ADH7* and *GRE2*, two confirmed HMF-detoxification genes encoding reductase activities, were co-regulated by Yap5p and Yap6p (Harbison et al. 2004; Workman et al. 2006; Ma and Liu 2010). A few enzyme encoding genes, for example, *ALD4* and *GRE2*, were also co-regulated by Pdr1p. In addition, *YAP1* and other YAP gene family members were shown to co-regulate numerous genes in a wide range of functional categories such as PDR, heat-shock protein, chaperones, and amino acid metabolism (Fig. 5, Table 2). Multiple functions of a gene are commonly observed in yeast tolerance and co-regulation of numerous genes can be a reflection of the multi-functions of such genes.

Single YAP gene deletion mutations are able to grow normally without HMF treatment. However, in the presence of 15 mM HMF, mutations *Δyap1, Δyap4, Δyap5*, and *Δyap6* showed delayed growth compared with their parental strain (Ma and Liu 2010). Mutant *Δyap1* displayed a 4-day-long lag phase and high sensitivity indicating a profound defect function affected by the *YAP1* gene. The deletion mutation of *YAP1* also showed increased sensitivity and decreased reduction activity toward coniferyl aldehyde (Sundstrom et al. 2010). This evidence supports the significant role of the YAP gene family in adaptation and tolerance to HMF. Thus, *YAP1* regulated networks involving the functional reductase enzymes as described in a previous section is an important component for yeast tolerance and in situ detoxification of aldehyde inhibitors

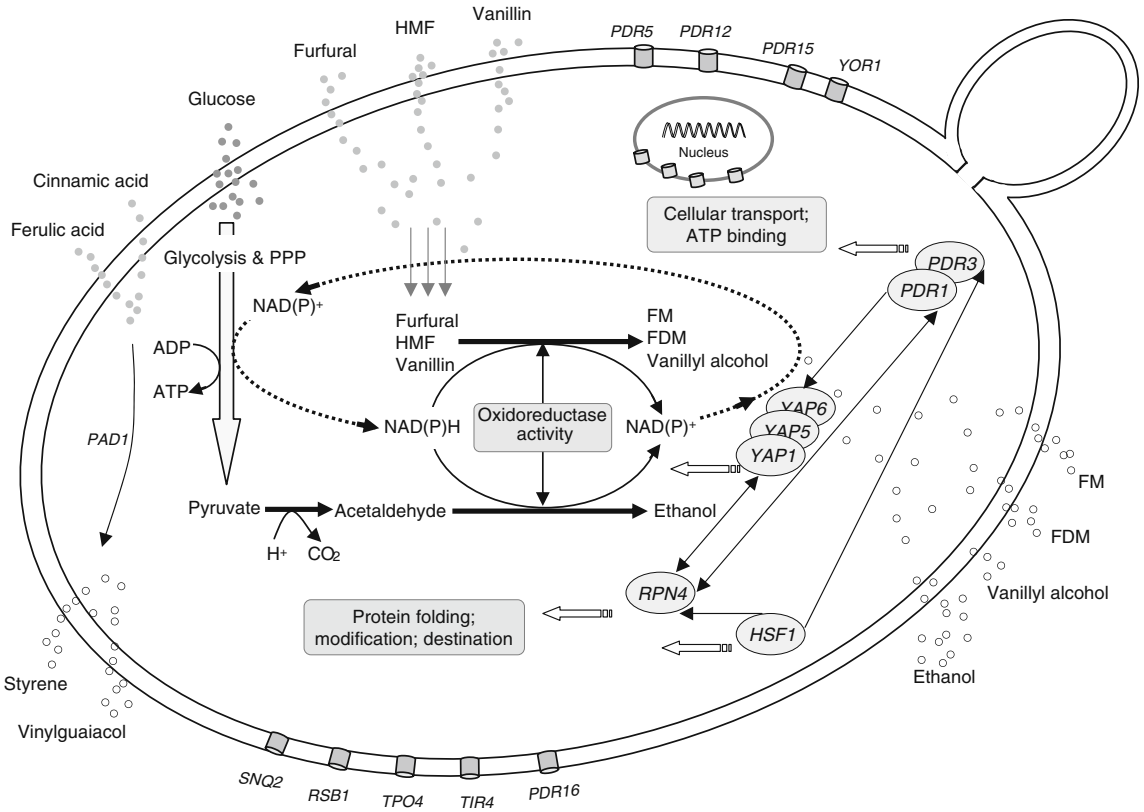


Fig. 5 A schematic diagram showing key gene regulatory elements involved in tolerance and in situ detoxification of lignocellulose hydrolysate inhibitors for *S. cerevisiae*. Important transcription factor genes and major functional gene categories are highlighted. See text for detailed descriptions

such as furfural, HMF, and coniferyl aldehyde. Excellent comprehensive reviews on Yap1p regulations involved in yeast stress response are available (Herrero et al. 2008; Rodrigues-Pousada et al. 2010).

PDR family and PDR1/3 involved cellular transport interactions

The second significant element for yeast tolerance and the in situ detoxification is the PDR gene family-centered functions that are regulated by Pdr1/3p as well as other co-regulator genes such as *YAP1* and *HSF1* (Fig. 5). The PDR genes encode plasma membrane proteins and function as transporters of ATP-binding cassette (ABC) proteins. These genes mediate membrane translocation of ions, and a wide range of substrates and often exhibit multiple functions in response to a large variety of unrelated chemical stresses (Mamnun et al. 2002; Moye-Rowley 2003; Jungwirth and Kuchler 2006; MacPherson et al. 2006). Many genes of the PDR family displayed consistent expressions of three- to 30-fold increases induced by furfural and HMF treatment (Liu et al. 2007; Song et al. 2009; Alriksson et al 2010; Ma and Liu 2010). Gene products of these increased transcripts are characterized in a broad range of protein categories such as drug/toxin transport for *TPO1* and *TPO4*, transport ATPase for *RSB1*, and ABC transporters for *PDR15* (Tomitori et al. 2001; Teixeira and Sá-Correia 2002; Ma and Liu 2010; Table 3). *SNQ2*, *YOR1*, *PDR5*, and *PDR12* encoding proteins shared functions of all these three categories. These genes are considered as a core set candidate genes promoting cellular survival and adaptation to the inhibitor stress. In addition, many PDR proteins have functions as ATP binding and chemical resistance agent.

Most of these genes have the pleiotropic drug response element (PDRE) in their promoter regions. HMF-induced transcription factor genes *PDR1* and *PDR3* regulate gene expression under a large variety of unrelated chemical stress conditions by binding to the PDRE of target genes (Mamnun et al. 2002; Moye-Rowley 2003; Jungwirth and Kuchler 2006; MacPherson et al. 2006). Both Pdr1p and Pdr3p recognize CGG triplets oriented in opposite directions to form an inverted repeat and able to form homodimers or heterodimers to activate target gene expression (Mamnun et al. 2002; Hellauer et al. 1996). Many induced genes regulated by Pdr1p and/or Pdr3p in this group are involved in export of both xenobiotic compounds and endogenous toxic metabolites using ABC transporters (Pdr5p, Pdr15p, Snq2p, and Yor1p), lipid composition of the plasma membrane (Rsb1p and Ict1p), export of polyamines by polyamine transporters (Tpo1p and Tpo4p), DNA repairing (Mag1p and Ddi1p), and other functions (Katzmann et al. 1995; Mahé et al. 1996; Wolfger et al. 1997; De Risi et al. 2000; Onda et al. 2004; Alenquer et al. 2006; Salin et al. 2008; Ma and Liu 2010). At least eight genes induced by HMF were regulated by both Pdr1p and Pdr3p. These two regulators also recognize and activate other subsets of genes. For example, Pdr3p participates in certain processes that do not involve Pdr1p, such as regulating DNA damage-inducible genes *MAG1* and *DDI1* (Zhu and Xiao 2004). Similarly, certain genes are only regulated by Pdr1p, such as *RSB1*, *ADH7*, and *PRE3* (Lee et al. 2002; Harbison et al. 2004; Kihara and Igarashi 2004). The *PDR3* promoter contains two PDREs that can be autoregulated by itself in addition to being a regulon of Pdr1p (Delahodde et al. 1995; De Risi et al. 2000). *PDR1* and *PDR3* also demonstrated regulatory connections with a broad range of functional category genes as well as most active regulatory genes.

Gene deletion mutation assays of *Δpdr1* displayed reduced transcriptional abundance for many genes including *PDR5, PDR10, PDR15, YOR1, SNQ2, ICT1, GRE2, TPO1, YMR102C*, and *YGR035C* compared with its parental strain (Ma and Liu 2010). The mutation *Δpdr3* appeared to have a similar regulatory effect but at a less degree except for a clear positive effect on *PGA3*. These results confirmed the influence of *PDR 1* and *PDR3* on the expression of their potential regulons. It is likely that ABC transporters play a key role to export excessive toxic compounds such as furfural and HMF, and endogenous toxic metabolites from intracellular environment brought about by the inhibitor damage. As mentioned above, the shortcut of the TCA cycle could provide energy for the pumping of HMF and toxic metabolites by ABC transporters under the stress.

RSB1 and *ICT1* are involved in phospholipid synthesis and transportation for membrane structure and functions that are responsible for yeast tolerance to organic solvents (Miura et al. 2000; Ghosh et al. 2008). It is possible that the induction of these PDR genes prevents the fast influx of HMF into cytoplasm and important organelles by membrane remodeling, thus, increasing the cell's tolerance to HMF. *MAG1* encodes a 3-methyladenine (3MeA) DNA glycosylase (Chen et al. 1990), which acts in the first step of a multistage base excision repair pathway for the removal of lethal lesions such as 3MeA and protects yeast cells from killing by DNA-alkylating agents (Fu et al. 2008). *DDI1*, located immediately upstream of *MAG1* and transcribed in an opposite direction, encodes an ubiquitin-related protein and is involved in a DNA-damage cell-cycle checkpoint (Clarke et al. 2001). Regulatory interactions of PDR gene family are complex, and many genes appeared to be regulated by multiple transcription factor genes involving *PDR1, PDR3, YAP1*, and *HSF1*. Regulatory roles of *PDR1* and *PDR3* to HMF challenge were suggested by computational modeling (Song and Liu 2007; Song et al. 2009).

Protein modification interplays mediated by RPN4, HSF1, and co-regulators

Another important component of the yeast tolerance and its capability for in situ detoxification involves degradation of damaged proteins and protein modifications such as *SHP1* and *SSA4*, regulated by transcription factor genes *RPN4* and *HSF1* as well as interplays with other closely related regulator genes such as *YAP1* and *PDR1* (Fig. 5). Chemical stress causes damage to protein conformation leading to protein unfolding and aggregation (Goldberg 2003). Small heat-shock proteins, acting as chaperones, assist in folding or refolding nascent or proteins and enzymes to maintain a functional conformation (Burnie et al. 2006). For example, *HSP26* and *SSA4* encoding chaperones were significantly induced to counteract the furfural–HMF complex damage to proteins. The deletion mutation of *SSA4* displayed a significant longer lag phase under the HMF challenge, indicating its important role in adaptation and tolerance to HMF (Ma and Liu 2010). While the presence of chaperones contributes protein protection, prolonged inhibitor stress may result in irreversible protein damages. Misfolded or damaged proteins, especially aggregated proteins, are highly toxic to cells (Goldberg 2003). Degradation of misfolded and damaged proteins by the ubiquitin-mediated proteasome pathway plays an important role in maintaining normal cell function and viability (Goldberg 2003; Wang et al. 2008; 2010). Denatured proteins are targeted via the covalent attachment of ubiquitin to a lysine side chain, and polyubiquitinated proteins are finally delivered to proteasome to be degraded. Strains with deletion mutations of these genes are sensitive to HMF such as *OTU1* and *SHP1*. It was suggested that the degradation of proteins by the ubiquitin-mediated proteasome pathway has regulatory roles on cell cycle, metabolic adaptations, gene regulation, development, and differentiation (Glickman and Ciechanover 2002).

At least 14 ubiquitin-related and proteasome genes (*PRE1, PRE3, PRE6, PRE7, PRE10, PUP3, RPN9, RPN12, ECM29, RPT2, RPT3, RPT4, SHP1*, and *OTU1*) for protein degradation were identified in relationship to HMF adaptation (Ma and Liu 2010). These genes encoding enzymes for degradation of damaged proteins maintain cell viability and functions under the inhibitor stress. The induction of these genes was predicted to be under the control of the transcription factor Rpn4p by binding to the proteasome-associated control element (PACE, 5'-GGTGGCAAA-3'), and the PACE was found in the promoter region of most ubiquitin-related and proteasome genes induced by HMF (Mannhaupt et al. 1999; Ma and Liu 2010). The expression of *RPN4* was persistently enhanced over time during the lag phase. Rpn4p levels are regulated by the 26S proteasome via a negative feedback control mechanism (Xie and Varshavsky 2001). It is also required for regulation of genes involved in DNA repair and other cellular processes, such as DNA damage-inducible genes *MAG1* and *DDI1* (Harbison et al. 2004; Zhu and Xiao 2004). Interestingly, Rpn4p is a feedback regulator of *YAP1* and *PDR1* (Salin et al. 2008). This was further demonstrated by the comparative performance of the deletion mutation response to HMF. While it was able to grow and establish a culture normally without HMF challenge, the strain harboring *Δrpn4* failed to recover in the presence of HMF (Ma and Liu 2010). These results confirmed the vital role of *RPN4* involvement in yeast tolerance. The enhanced expression of *HSF1* by HMF was consistent and statistically significantly greater. The up-regulated *HSP26* and *SSA4* for protein folding and refolding have been reported to be regulated by Hsf1p (Harbison et al. 2004; Ferguson et al. 2005; Ma and Liu 2010). Regulator gene *HSF1* is an essential gene and a positive regulator of other transcription factor genes *RPN4*, *PDR3*, *YAP5*, and *YAP6* (Lee et al. 2002; Harbison et al. 2004; Hahn et al. 2006; Workman et al. 2006). Therefore, the significant roles of *HSF1* involved in the complex co-regulation networks for the yeast tolerance cannot be underestimated.

Conclusion and perspectives

It is clear that yeast tolerance and in situ detoxification of lignocellulosic hydrolysate inhibitors such as aldehydes, involve complex interplays of many genes at multiple levels at the genome scale. Functional reduction enzymes, largely involved in oxidoreductase activities, are the direct driving force in biotransformation of aldehyde inhibitors reducing the inhibitory damages. This group of genes and their interactions are regulated by members of the yeast activator protein gene family that is led by *YAP1*. These activities are closely related to the center metabolic pathways and ethanol fermentation. Tolerant yeast can be obtained with enhanced genetic background and reprogrammed pathways to overcome furfural–HMF stress. Identification of the inhibitor functional group and the use of structure–function strategy led to a better understanding of yeast tolerance and detoxification. Numerous members of the PDR gene family, showing consistent high levels of transcription abundance under the inhibitor stress, are considered as tolerance candidate genes. They are actively involved in exporting xenobiotic products and endogenous toxic metabolites and regulated mainly by *PDR1* and *PDR3*. These function-specific and multifunctional cellular transporters and ATP binding agents located at cell wall and nucleus membranes are critical for cell survival and adaptation in the presence of the inhibitors. Another necessary component of the yeast tolerance involves genes

functioning in protein folding, modification, and destination that is essential to reduce degraded protein toxicity and restore protein functions. Such genes are regulated by *RPN4, HSF1*, and other co-regulators. Furthermore, all regulators rolling these three basic components are co-regulatory and interactive. However, important elements of yeast tolerance are not limited to these outlined above. As indicated by recent transcriptome and proteomic studies, general stress response and several additional significant functional categories are recognized such as DNA repairing, oxidative stress, osmotic, and salt stress (Lin et al. 2009a, b; Ma and Liu 2010). While characterization and annotation of individual gene functions are necessary, identification of responsible functional categories and their interplays is of more importance from a global point of view. Time-course studies and temporal dynamic approaches reveal relevant and informative insight into a life response and should be used more widely for yeast tolerance mechanism studies. The snap-shot kind of method needs be limited and avoided. As demonstrated by transcription factor gene-linked regulatory interactions using systems biology approaches (Ma and Liu 2010), identification of major regulatory networks backboned with key regulators will further our understanding of the tolerance mechanisms in depth. Fortunately, having many advanced tools available in genomics, proteomics, metabolomics, biological engineering, and chemical engineering, more detailed understanding of molecular mechanisms and interplays of yeast tolerance at genome level is expected.

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References

Alenquer M, Tenreiro S, Sá-Correia I (2006) Adaptive response to the antimalarial drug artesunate in yeast involves Pdr1p/Pdr3p-mediated transcriptional activation of the resistance determinants TPO1and PDR5. FEMS Yeast Res 6:1130–1139

Almeida JRM, Roder A, Modig T, Laadan B, Liden G, Gorwa-Grauslund M (2008) NADH - vs NADPH-coupled reduction of 5-hydroxymethylfurfural (HMF) and its implications on product distribution in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 78:939–945

Alriksson B, Horváth IS, Jönsson LJ (2010) Overexpression of *Saccharomyces cerevisiae* transcription factor and multidrug resistance genes conveys enhanced resistance to lignocellulose-derived fermentation inhibitors. Process Biochem 45:264–271

Bowman MJ, Jordan DB, Vermillion KE, Braker JD, Moon J, Liu ZL (2010) Stereochemistry of furfural reduction by an aldehyde reductase from *Saccharomyces cerevisiae* that contributes to in situ furfural detoxification. Appl Envir Microbiol 76:4926–4932

Burnie JP, Carter TL, Hodgetts SJ, Matthews RC (2006) Fungal heat-shock proteins in human disease. FEMS Microbiol Rev 30:53–88

Chen J, Derfler B, Samson L (1990) *Saccharomyces cerevisiae* 3-methyladenine DNA glycosylase has homology to the AlkA glycosylase of *E. coli* and is induced in response to DNA alkylation damage. EMBO J 9:4569–4575

Clarke DJ, Mondesert G, Segal M, Bertolaet BL, Jensen S, Wolff M, Henze M, Reed SI (2001) Dosage suppressors of pds1 implicate ubiquitin-associated domains in checkpoint control. Mol Cell Biol 21:1997–2007

De Rijcke M, Seneca S, Punyammalee B, Glansdorff N, Crabeel M (1992) Characterization of the DNA target site for the yeast ARGR regulatory complex, a sequence able to mediate repression or induction by arginine. Mol Cell Biol 12:68–81

De Risi J, van den Hazel B, Marc P, Balzi E, Brown P, Jacq C, Goffeau A (2000) Genome microarray analysis of transcriptional activation in multidrug resistance yeast mutants. FEBS Lett 470:156–160

Delahodde A, Delaveau T, Jacq C (1995) Positive autoregulation of the yeast transcription factor Pdr3p, which is involved in control of drug resistance. Mol Cell Biol 15:4043–4051

Dubacq C, Chevalier A, Courbeyrette R, Petat C, Gidrol X, Mann C (2006) Role of the iron mobilization and oxidative stress regulons in the genomic response of yeast to hydroxyurea. Mol Genet Genomics 275:114–124

Ferguson SB, Anderson ES, Harshaw RB, Thate T, Craig NL, Nelson HC (2005) Protein kinase A regulates constitutive expression of small heat-shock genes in an Msn2/4p-independent and Hsf1p-dependent manner in *Saccharomyces cerevisiae*. Genetics 169:1203–1214

Fernandes L, Rodrigues-Pousada C, Struhl K (1997) Yap, a novel family of eight bZIP proteins in *Saccharomyces cerevisiae* with distinct biological functions. Mol Cell Biol 17:6982–6993

Fisk DG, Ball CA, Dolinski K, Engel SR, Hong EL, Issel-Tarver L, Schwartz K, Sethuraman A, Botstein D, Cherry JM (2006) *Saccharomyces cerevisiae* S288C genome annotation: a working hypothesis. Yeast 23:857–865

Fu Y, Pastushok L, Xiao W (2008) DNA damage-induced gene expression in *Saccharomyces cerevisiae*. FEMS Microbiol Rev 32:908–926

Gasch AP, Werner-Washburne M (2002) The genomics of yeast responses to environmental stress and starvation. Funct Integr Genomics 2:181–192

Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO (2000) Genomic expression program in the response of yeast cells to environmental changes. Mol Biol Cell 11:4241–4257

Ghosh AK, Ramakrishnan G, Rajasekharan R (2008) YLR099C (ICT1) encodes a soluble Acyl-CoA-dependent lysophosphatidic acid acyltransferase responsible for enhanced phospholipid synthesis on organic solvent stress in *Saccharomyces cerevisiae*. J Biol Chem 283:9768–9775

Goldberg AL (2003) Protein degradation and protection against misfolded or damaged proteins. Nature 426:895–899

Gorsich SW, Dien BS, Nichols NN, Slininger PJ, Liu ZL, Skory CD (2006) Tolerance to furfural-induced stress is associated with pentose phosphate pathway genes ZWF1, GND1, RPE1, and TKL1 in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 71:339–349

Glickman MH, Ciechanover A (2002) The ubiquitin proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev* 82:373–428

Hahn JS, Neef DW, Thiele DJ (2006) A stress regulatory network for co-ordinated activation of proteasome expression mediated by yeast heat shock transcription factor. Mol Microbiol 60:240–251

Harbison CT, Gordon DB, Lee TI, Rinaldi NJ, Macisaac KD, Danford TW, Hannett NM, Tagne JB, Reynolds DB, Yoo J, Jennings EG, Zeitlinger J, Pokholok DK, Kellis M, Rolfe PA, Takusagawa KT,

Lander ES, Gifford DK, Fraenkel E, Young RA (2004) Transcriptional regulatory code of a eukaryotic genome. Nature 31:99–104

Haugen AC, Kelley R, Collins JB, Tucker CJ, Deng C, Afshari CA, Brown JM, Ideker T, Van Houten B (2004) Integrating phenotypic and expression profiles to map arsenic-response networks. Genome Biol 5:R95

Heer D, Heine D, Sauer U (2009) Resistance of *Saccharomyces cerevisiae* to high concentrations of furfural is based on NADPH-dependent reduction by at least two oxireductases. Appl Environ Microbiol 75:7631–7638

Hellauer K, Rochon MH, Turcotte B (1996) A novel DNA binding motif for yeast zinc cluster proteins: the Leu3p and Pdr3p transcriptional activators recognize everted repeats. Mol Cell Biol 16:6096–6102

Herrero E, Ros J, Belli G, Cabiscol E (2008) Redox control and oxidative stress in yeast cells. Biotechnica Biophysica Acta 1780:1217–1235

Jungwirth H, Kuchler K (2006) Yeast ABC transporters – a tale of sex, stress, drugs, and aging. FEBS Lett 580:1131–1138

Katzmann DJ, Hallstrom TC, Voet M, Wysock W, Golin J, Volckaert G, Moyle-Rowley WS (1995) Expression of an ATP-binding cassette transporter-encoding gene (YOR1) is required for oligomycin resistance in *Saccharomyces cerevisiae*. Mol Cell Biol 15:6875–6883

Kihara A, Igarashi Y (2004) Cross talk between sphingolipids and glycerophospholipids in the establishment of plasma membrane asymmetry. Mol Biol Cell 15:4949–4959

Klinke HB, Thomsen AB, Ahring BK (2004) Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. Appl Microbiol Biotechnol 66:10–26

Kuyper M, Toirkens MJ, Diderich JA, Winkler AA, van Dijken JP, Pronk JT (2005) Evolutionary engineering of mixed-sugar utilization by a xylose-fermenting *Saccharomyces cerevisiae* strain. FEMS Yeast Res 5:925–934

Laadan B, Almeida JRM, Radstrom P, Hahn-Hagerdal B, Gorwa-Grauslund M (2008) Identification of an NADH-dependent 5-hydroxymethylfurfural-reducing alcohol dehydrogenase in *Saccharomyces cerevisiae*. Yeast 25:191–198

Larochelle M, Drouin S, Robert F, Turcotte B (2006) Oxidative stress-activated zinc cluster protein Stb5 has dual activator/repressor functions required for pentose phosphate pathway regulation and NADPH production. Mol Cell Biol 26:6690–6701

Larroy C, Fernadez MR, Gonzalez E, Pares X, Biosca JA (2002a) Characterization of the *Saccharomyces cerevisiae* YMR318C (ADH6) gene product as a broad specificity NADPH-dependent alcohol dehydrogenase: relevance in aldehyde reduction. Biochem J 361:163–172

Larroy C, Pares X, Biosca JA (2002b) Characterization of a *Saccharomyces cerevisiae* NADP(H)-dependent alcohol dehydrogenase (ADHVII), a member of the cinnamyl alcohol dehydrogenase family. Eur J Biochem 269:5738–5745

Larroy C, Rosario FM, Gonzalez E, Pares X, Biosca JA (2003) Properties and functional significance of *Saccharomyces cerevisiae* ADHVI. Chem Biol Interact 143–144:229–238

Larsson S, Palmqvist E, Hahn-Hägerdal B, Tengborg C, Stenberg K, Zacchi G, Nilvebrant N (1999) The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. Enzy Micro Technol 24:151–159

Lee TI, Rinaldi NJ, Robert F, Odom DT, Bar-Joseph Z, Gerber GK, Hannett NM, Harbison CT, Thompson CM, Simon I, Zeitlinger J, Jennings EG, Murray HL, Gordon DB, Ren B, Wyrick JJ, Tagne JB, Volkert TL, Fraenkel E, Gifford DK, Young RA (2002) Transcriptional regulatory networks in *Saccharomyces cerevisiae*. Science 298:799–804

Li Bz, Yuan Yj (2010) Transcriptome shifts in response to furfural and acetic acid in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 86:1915–1924

Lin FM, Qiao B, Yuan YJ (2009a) Comparative proteomic analysis for tolerance and adaptation of ethanologenic *Saccharomyces cerevisiae* to furfural, a lignocellulosic inhibitory compound. Appl Environ Microbiol 75:3765–3776

Lin FM, Tan Y, Yuan YJ (2009b) Temporal quantitative proteomics of *Saccharomyces cerevisiae* in response to a nonlethal concentration of furfural. Proteomics 9:5471–5483

Liu ZL (2006) Genomic adaptation of ethanologenic yeast to biomass conversion inhibitors. Appl Microbiol Biotechnol 73:27–36

Liu, Z.L., B.J. Andersh, and P.J. Slininger, (2007). Mechanisms of in situ detoxification of furfural and HMF by ethanologenic yeast *Saccharomyces cerevisiae*. 29th Symposium of Biofuels and Chemicals. Abs. 85.

Liu ZL, Blaschek HP (2010) Lignocellulosic biomass conversion to ethanol by *Saccharomyces*. In: Vertes A, Qureshi N, Yukawa H, Blaschek H (eds) Biomass to biofuels: strategies for global industries. John Wiley & Sons, Ltd, West Sussex, UK, pp 17–36

Liu ZL, Moon J (2009) A novel NADPH-dependent aldehyde reductase gene from *Saccharomyces cerevisiae* NRRL Y-12632 involved in the detoxification of aldehyde inhibitors derived from lignocellulosic biomass conversion. Gene 446:1–10

Liu ZL, Slininger PJ (2005) Development of genetically engineered stress tolerant ethanologenic yeasts using integrated functional genomics for effective biomass conversion to ethanol. In: Outlaw J, Collins K, Duffield J (eds) Agriculture as a producer and consumer of energy CAB International. Wallingford, UK, pp 283–294

Liu ZL, Slininger PJ (2006) Transcriptome dynamics of ethanologenic yeast in response to 5-hydroxymethylfurfural stress related to biomass conversion to ethanol. In: Recent research developments in multidisciplinary applied microbiology: understanding and exploiting microbes and their interactions-biological, physical, chemical and engineering aspects (Mendez-Vilas A Ed) Wiley-VCH, pp679-684

Liu ZL, Slininger PJ, Dien BS, Berhow MA, Kurtzman CP, Gorsich SW (2004) Adaptive response of yeasts to furfural and 5-hydroxymethylfurfural and new chemical evidence for HMF conversion to 2, 5-bis-hydroxymethylfuran. J Ind Microbiol Biotechnol 31:345–352

Liu ZL, Slininger PJ, Gorsich SW (2005) Enhanced biotransformation of furfural and 5-hydroxymethylfurfural by newly developed ethanologenic yeast strains. Appl Biochem Biotechnol 121–124:451–460

Liu ZL, Moon J, Andersh AJ, Slininger PJ, Weber S (2008a) Multiple gene mediated NAD(P)H-dependent aldehyde reduction is a mechanism of in situ detoxification of furfural and HMF by ethanologenic yeast *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 81:743–753

Liu ZL, Saha BC, Slininger PJ (2008b) Lignocellulosic biomass conversion to ethanol by *Saccharomyces*. In: Wall J, Harwood C, Demain A (eds) Bioenergy. ASM Press, Washington DC, pp 17–36

Liu ZL, Ma M, Song M (2009) Evolutionarily engineered ethanologenic yeast detoxifies lignocellulosic biomass conversion inhibitors by reprogrammed pathways. Mol Genet Genomics 282:233–244

Ma M, Liu ZL (2010) Comparative transcriptome profiling analyses during the lag phase uncover *YAP1, PDR1, PDR3, RPN4*, and *HSF1* as key regulatory genes in genomic adaptation to lignocellulose derived inhibitor HMF for *Saccharomyces cerevisiae*. BMC Genomics 11:660

MacPherson S, Larochelle M, Turcotte B (2006) A fungal family of transcriptional regulators: the zinc cluster proteins. Microbiol Mol Biol Rev 70:583–604

Mahé Y, Lemoine Y, Kuchler K (1996) The ATP binding cassette transporters Pdr5 and Snq2 of *Saccharomyces cerevisiae* can mediate transport of steroids in vivo. J Biol Chem 271:25167–25172

Mamnun YM, Pandjaitan R, Mahé Y, Delahodde A, Kuchler K (2002) The yeast zinc finger regulators Pdr1p and Pdr3p control pleiotropic drug resistance (PDR) as homo- and heterodimers in vivo. Mol Microbiol 46:1429–1440

Mannhaupt G, Schnall R, Karpov V, Vetter I, and Feldmann (1999) Rpn4p acts as a transcription factor by binding to PACE, a nonamer box found upstream of 26S proteasomal and other genes in yeast. *FEBS Lett* 450:27–34

Martin C, Marcelo M, Almazan O, Jonsson LJ (2007) Adaptation of a recombinant xylose-utilizing *Saccharomyces cerevisiae* strain to a sugarcane bagasse hydrolysate with high content of fermentation inhibitors. Bioresour Technol 98:1767–1773

Miura S, Zou W, Ueda M, Tanaka A (2000) Screening of genes involved in isooctane tolerance in *Saccharomyces cerevisiae* by using mRNA differential display. Appl Environ Microbiol 66:4883–4889

Moon J, Liu ZL (2011) Protein engineering of GRE2 from *Saccharomyces cerevisiae* for enhanced detoxification of 5-hydroxymethylfurfural. (submitted)

Morimoto S, Murakami M (1967) Studies on fermentation products from aldehyde by microorganisms: the fermentative production of furfural alcohol from furfural by yeasts (part I). J Ferment Technol 45:442–446

Moye-Rowley WS (2003) Transcriptional control of multidrug resistance in the yeast *Saccharomyces*. Prog Nucleic Acid Res Mol Biol 73:251–279

Natarajan K, Meyer MR, Jackson BM, Slade D, Roberts C, Hinnebusch AG, Marton MJ (2001) Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. Mol Cell Biol 21:4347–4368

Nemirovskii V, Gusarova L, Rakhmilevich Y, Sizov A, Kostenko V (1989) Pathways of furfurol and oxymethyl furfurol conversion in the process of fodder yeast cultivation. Biotekhnologiya 5:285–289

Nichols NN, Dien BS, Cotta MA (2010) Fermentation of bioenergy crops into ethanol using biological abatement for removal of inhibitors. Bioresour Technol 101(19):7545–7550

Nilsson A, Gorwa-Grauslund MF, Hahn-Hagerdal B, Liden G (2005) Cofactor dependence in furan reduction by *Saccharomyces cerevisiae* in fermentation of acid-hydrolyzed lignocellulose. App Environ Microbiol 71:7866–7871

Onda M, Ota K, Chiba T, Sakaki Y, Ito T (2004) Analysis of gene network regulating yeast multidrug resistance by artificial activation of transcription factors: involvement of Pdr3 in salt tolerance. Gene 332:51–59

Palmqvist E, Hahn-Hägerdal B (2000) Fermentation of lignocellulosic hydrolysates II: inhibitors and mechanisms of inhibition. Bioresour Technol 74:25–33

Petersson A, Almeida JR, Modig T, Karhumma K, Hahn-Hägerdal B, Gorwa-Grauslund MF (2006) A 5-hydroxymethylfurfural reducing enzyme encoded by the *Saccharomyces cerevisiae* ADH6 gene conveys HMF tolerance. Yeast 23:455–464

Rodrigues-Pousada C, Menezes RA, Pimentel C (2010) The Yap family and its role in stress response. Yeast 27:245–258

Salin H, Fardeau V, Piccini E, Lelandais G, Tanty V, Lemoine S, Jacq C, Devaux F (2008) Structure and properties of transcriptional networks driving selenite stress response in yeasts. BMC Genomics 9:333

Song M, Liu ZL (2007) A linear discrete dynamic system model for temporal gene interaction and regulatory network influence in response to bioethanol conversion inhibitor HMF for ethanologenic yeast. Lect Notes Bioinfomatics 4532:77–95

Song M, Ouyang Z, Liu ZL (2009) Discrete dynamic system modeling for gene regulatory networks of HMF tolerance for ethanologenic yeast. IET Syst Biol 3:203–218

Sundstrom L, Larsson S, Jonsson LJ (2010) Identification of *Saccharomyces cerevisiae* genes involved in the resistance to phenolic fermentation inhibitors. Appl Biochem Biotechnol 161:106–115

Taherzadeh MJ, Gustafsson L, Niklasson C, Liden G (2000) Physiological effects of 5-hydroxymethylfurfural on *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 53:701–708

Talebnia F, Taherzadeh MJ (2006) In situ detoxification and continuous cultivation of dilute-acid hydrolysate to ethanol by encapsulated *S. cerevisiae*. J Biotechnol 125:377–384

Teixeira MC, Sá-Correia I (2002) *Saccharomyces cerevisiae* resistance to chlorinated phenoxyacetic acid herbicides involves Pdr1p-mediated transcriptional activation of TPO1 and PDR5 genes. Biochem Biophys Res Commun 292:530–537

Tomitori H, Kashiwagi K, Asakawa T, Kakinuma Y, Michael AJ, Igarashi K (2001) Multiple polyamine transport systems on the vacuolar membrane in yeast. Biochem J 353:681–688

Träff KL, Otero Cordero RR, van Zyl WH, Hahn-Hägerdal B (2001) Deletion of the GRE3 aldose reductase gene and its influence on xylose metabolism in recombinant strains of *Saccharomyces cerevisiae* expression the xylA and XKSI genes. Appl Environ Microbiol 67:5668–5674

Villa GP, Bartroli R, Lopez R, Guerra M, Enrique M, Penas M, Rodriquez E, Redondo D, Jglesias I, Diaz M (1992) Microbial transformation of furfural to furfuryl alcohol by *Saccharomyces cerevisiae*. Acta Biotechnol 12:509–512

Wahlbom CF, Hahn-Hägerdal B (2002) Furfural, 5-hydroxymethylfurfrual, and acetone act as external electron acceptors during anaerobic fermentation of xylose in recombinant *Saccharomyces cerevisiae*. Biotechnol Bioeng 78:172–178

Wang X, Xu H, Ju D, Xie Y (2008) Disruption of Rpn4-induced proteasome expression in *Saccharomyces cerevisiae* reduces cell viability under stressed conditions. Genetics 180:1945–1953

Wang X, Xu H, Ha SW, Ju D, Xie Y (2010) Proteasomal degradation of Rpn4 in *Saccharomyces cerevisiae* is critical for cell viability under stressed conditions. Genetics 184:335–342

Wolfger H, Mahé Y, Parle-McDermott A, Delahodde A, Kuchler K (1997) The yeast ATP binding cassette (ABC) proteins PDR10 and PDR15 are novel targets for the Pdr1 and Pdr3 transcriptional regulators. FEBS Lett 418:269–274

Workman CT, Mak HC, McCuine S, Tagne JB, Agarwal M, Ozier O, Begley TJ, Samson LD, Ideker T (2006) A systems approach to mapping DNA damage response pathways. Science 312:1054–1059

Xie Y, Varshavsky A (2001) RPN4 is a ligand, substrate, and transcriptional regulator of the 26S proteasome: a negative feedback circuit. Proc Natl Acad Sci USA 98:3056–3061

Zhu Y, Xiao W (2004) Pdr3 is required for DNA damage induction of MAG1 and DDI1 via a bi-directional promoter element. Nucl Acids Res 32:5066–5075