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GENOME WIDE ANALYSIS IDENTIFIES SPHINGOLIPID METABOLISM AS A NEW TARGET OF VALPROIC ACID

by

SHYAMALAGAURI JADHAV

DISSERTATION

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

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for the degree of

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MAJOR: BIOLOGICAL SCIENCES

Approved By:

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Advisor Date

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DEDICATION

I would like to dedicate my dissertation to my late mother Chandrakala Jadhav who was a constant support and inspiration through the years.

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First and foremost I would like to thank my advisor, Dr. Miriam L. Greenberg for her intellectual, moral, financial, and emotional support throughout my graduate journey. The realization of this thesis would have been impossible without her constant encouragement and strong support. I would like to thank her for the confidence she had in my ability to think and design the project through; and for her guidance when needed. She stood by my side and gave me the scientific freedom to explore my research endeavors. This has helped me to develop into a scientist with the ability to think critically and independently. I have seen her not just as my dissertation advisor, but a mentor whose guidance and advice I shall cherish forever. I am grateful for all the time and effort she has invested in educating and training me towards achieving my professional goals.

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CHAPTER 1 INTRODUCTION

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Despite decades of studies, little is known about the molecular mechanisms underlying the neuropsychiatric disorders schizophrenia, depression, anxiety, and bipolar disorder (BD), which contribute about 14% of the global burden of disease (Prince et al., 2007). The development of effective treatments for these devastating illnesses is hampered by the failure so far to elucidate the molecular mechanisms underlying their pathologies. Recent findings suggest that sphingolipids may play an important role in the pathophysiology of psychiatric disorders. Because sphingolipids are abundant in the brain and are involved in the regulation of processes essential for normal brain function, sphingolipids and sphingolipid metabolites may be promising targets for new treatments of psychiatric disorders.

Lipids constitute about 50% of the dry weight of the brain, which has the second highest lipid composition after adipose tissue (Watkins et al., 2001; Morrel and Toews, 1993). The mammalian nervous system is rich in sphingomyelin, glycosphingolipids, and molecules with ceramide backbones (Cutler and Mattson, 2001; Tettamanati, 2004). Sphingolipids are a ubiquitous, highly conserved, diverse group of bioactive lipids, comprising about 10-20% of cellular lipids (van Meer et al., 2008). Technical advances in lipidomics and mass spectrometry in the past two decades have greatly facilitated the quantification and characterization of sphingolipids (Ejsing et al., 2009; Skevchenko and Simons, 2010). The yeast model has been invaluable in identifying and characterizing the genes and enzymes that mediate sphingolipid metabolism (Dickson and Lester, 2002), and elucidating the role of sphingolipids in the regulation of

essential cellular functions, including growth, endocytosis, protein trafficking, calcium homeostasis, aging, nutrient uptake, maintenance of lipid rafts, regulation of heat stress, actin cytoskeleton, and cell wall synthesis and repair (Dickson et al., 2006; Funato et al., 2002; Cowart and Hannun, 2004; Sims et al., 2004). Several of these functions have been associated with psychiatric disorders, including cellular signaling, endocytosis, inflammation, secretion, apoptosis, and proliferation (Breslow, 2013). The new lipidomic technologies, combined with the powerful yeast system to generate genetic and molecular models of sphingolipid functions that can be tested in relevant mammalian systems may offer promising new avenues for drug design.

We begin with a comparison of sphingolipid metabolism in yeast and mammals. The very high degree of conservation of sphingolipid biosynthetic enzymes and of the functions of individual sphingolipids and metabolites underscores the utility of yeast to elucidate molecular mechanisms underlying sphingolipid function. We then discuss studies associating perturbation of sphingolipids with schizophrenia, depression, anxiety and BD. Finally, we suggest avenues whereby yeast can be exploited further to design drugs that may be used to modulate sphingolipid targets that are implicated in these disorders.

1. Sphingolipid structure

Sphingolipids contain two hydrophobic tails, including a long chain base (LCB) and a fatty acid chain, linked to a hydrophilic polar head group. The fatty acid is linked to the C-2 of the LCB via an amide bond, and the polar head group is linked to C-1 of the LCB via an OH group (Kihara et al., 2007). The basic sphingolipid structure is shown in Fig. 1.1.

In the yeast *Saccharomyces cerevisiae*, LCBs are linear alkanes containing hydroxyl groups on C-1 and C-3 and an amino group on C-2. The two major LCBs found in yeast are dihydrosphingosine (DHS) and phytosphingosine (PHS). PHS contains an extra hydroxyl group on C-4 compared to DHS. DHS in yeast contains 16, 18 or 20 carbons, and PHS contains 18 or 20 carbons (Lester and Dickson 2001). Sphingosine, the major LCB of mammals, is not found in yeast. Mammals also have minor amounts of DHS (Dickson and Lester 2002). The mammalian LCB, sphingosine, contains a 4,5- trans double bond, which is not found in yeast (Dickson and Lester, 1999).

The fatty acid linked to the LCB in yeast is generally a C26 saturated fatty acid containing zero to three hydroxyl groups, whereas the fatty acids in mammalian sphingolipids vary in chain length, degree of hydroxylation and saturation (Dickson et al., 2006). The hydrophilic polar head group also varies. In yeast, these groups are myo-inositol containing namely, phosphorylinositol, mannosyl phosphorylinositol, or mannosyl diphosphorylinositol. In mammals, the polar groups are mostly phosphorylcholine (sphingomyelin), galactose (galactosylceramide) or glucose (glucosylceramide). Mammals contain more than 100 classes complex sphingolipids, while yeast contain only three (Kihara et al., 2007).

2. Comparative sphingolipid metabolism in yeast and mammals

Sphingolipids have long been known to play a structural role in cell membranes (Obeid et al., 2002). In addition, studies in yeast and mammals have shown that sphingolipids act as secondary messengers in several signaling pathways (Dickson et al., 1998). The sphingolipid synthesis pathway in yeast and mammals is highly

conserved through the synthesis of ceramide, with minor variations in chain length, hydroxylation and saturation (Epstein and Riezman, 2013). Ceramide is synthesized in the endoplasmic reticulum (ER) and transported to the Golgi, where complex sphingolipids are synthesized in both yeast and mammals (Breslow, 2013). The sphingolipid biosynthetic pathways of yeast and mammals are shown in Fig. 1.3.

2.1 Synthesis of long chain bases (LCBs)

The synthesis of LCBs, the precursors of sphingolipids, is highly conserved from yeast to mammals. Sphingolipid synthesis begins in the ER with the condensation of L-serine with fatty acyl-coenzyme A (CoA), primarily palmitoyl-CoA (but also stearoyl-CoA), catalyzed by serine palmitoyl transferase (SPT), which produces 3-ketodihydrosphingosine (KDHS) (Gable et al., 2003; Hanada, 2003; Lowther et al., 2012). The genes encoding these enzymes are shown in Table 1.1.

In the second reaction of sphingolipid synthesis, the short-lived product KDHS is reduced to DHS in an NADPH-dependent reaction catalyzed by 3-ketodihydrosphingosine reductase (Dickson 2008; Beeler et al., 1998; Kihara and Igarashi 2004). PHS is synthesized in yeast via hydroxylation of C-4 of DHS, catalyzed by sphinganine C4-hydroxylase (Dickson, 2008). The reverse reaction, conversion of PHS to DHS, does not occur. PHS is also found in the skin epidermis of mammals where it regulates important functions such as apoptosis, inflammation, and differentiation (Kim et al., 2014).

2.2 Ceramide synthesis

Ceramide is the central intermediate of sphingolipid metabolism. Interestingly, altered ceramide levels have been associated with schizophrenia, depression, and BD,

as discussed below (Schwartzet al., 2008; Kornhuber et al., 2005). Ceramide exists as dihydroceramide or phytoceramide. DHS is linked to mostly a C26 fatty acid via an amide linkage at the C-2 position to form dihydroceramide. The ER-localized ceramide synthase catalyzes this reaction. In yeast, dihydroceramide is hydroxylated to phytoceramide by sphinganine C4-hydroxylase. Phytoceramide can also be synthesized by hydroxylating DHS to PHS, which is amide-linked to a C26 fatty acid (Dickson 2008). The yeast ceramide synthases Lag1 and Lac1 have overlapping functions, as single mutants of Lag1 and Lac1 are viable, while the double mutant is not (Cowart and Obeid 2007). Mammals contain six ceramide synthases, CerS1-6, which exhibit specificity for fatty acyl-CoAs. CerS1 exhibits specificity for C18, CerS2 and CerS4 for C22 and C24, and CerS5 and CerS6 for C16, while CerS3 utilizes a broad range of substrates (Mizutani et al., 2006). In mammals, dihydroceramide is desaturated by Δ4-desaturase between C-4 and C-5 to phytoceramide (Ternes et al., 2002; Mizutani et al., 2004; Omae et al., 2004).

2.3 Fatty acid elongation pathway

Very long chain fatty acids required for ceramide synthesis are synthesized in the ER by the fatty acid elongation pathway. The fatty acid elongase complex adds two carbons to fatty acyl CoAs per cycle (Jakobsson et al., 2006). Each isoform of fatty acid elongase synthesizes a very long chain fatty acyl CoA of a characteristic length (Denic and Weissman 2007). The four steps of fatty acid elongation in yeast and mammals include condensation, reduction, dehydration, and a second reduction, as shown in Fig. 1.2. Malonyl-CoA is first condensed with acyl-CoA to synthesize 3-ketoacyl-CoA, catalyzed by the highly conserved fatty acid elongase (Jakobsson et al.,

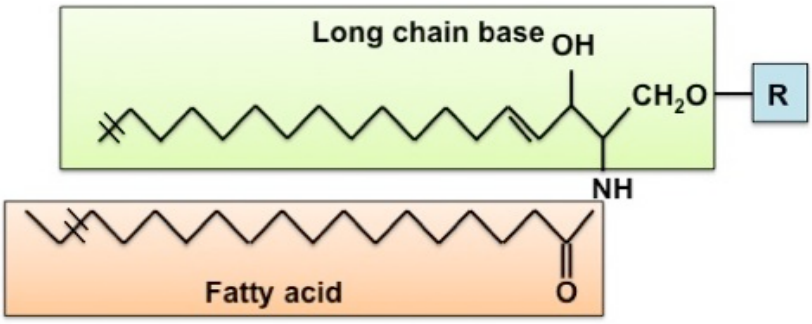

Figure 1.1. Sphingolipid structure. The sphingolipid backbone consists of a long chain base linked to a fatty acid chain via an amide link A polar head group R is linked to the OH of C-1 of the LCB via a OH bond R=H in a ceramide molecule; R= Phosphocholine in sphingomyelin; R= Sugar in glycosphingolipids. ⫽ Represents variations in the chain length.

2006). Three elongases in yeast exhibit specificity for fatty acids of specific chain length (Table 1.1). Elo1 catalyzes elongation of C14 to C16, Elo2/ Fen1 catalyzes elongation of C20 to C22 and C22 to C24, and Elo3/Sur4 catalyzes the elongation of C24 to C26 and, with a lower efficiency C22 to C24 (Oh et al., 1997). Mammalian fatty acid elongases are encoded by seven genes, ELOVL1-7, which also exhibit acyl chain specificity (Table 1.1).

ELOVL6 elongates saturated fatty acyl-CoA chains of C16 or shorter. *ELOVL3* and *ELOVL7* elongate both saturated and unsaturated fatty acyl-CoAs of C16 to C22 but have highest activity for C18 (Ohno et al., 2010). *ELOVL1* elongates saturated C18-C26 and monounsaturated C20:1 n-9 and C22:1 n-9 (Ohno et al., 2010). *ELOVL1* is the major elongase responsible for the synthesis of C24 containing sphingolipids, which are present in high amounts in mammals (Kihara, 2012). *ELOVL4* elongates very long saturated fatty acids or polyunsaturated fatty acids with chains longer than C26. *ELOVL2* and *ELOVL5* exhibit specificity for polyunsaturated fatty acyl-CoAs. *ELOVL2* elongates polyunsaturated fatty acyl CoAs with C22 and *ELOVL5* with C18, and both can elongate polyunsaturated fatty acyl CoAs with C20 (Kihara, 2012). 3-ketoacyl-CoA is reduced by 3-ketoacyl-CoA reductase to 3-hyroxyacyl-CoA, which is dehydrated to 2,3-trans-enoyl-CoA by 3-hydroxyacyl-CoA dehydratase. 3-hydroxyacyl-CoA undergoes another round of reduction catalyzed by enoyl reductase to yield an acyl-CoA with 2 more carbon chains than the original acyl-CoA (Kihara, 2012). The steps in the fatty acid elongation pathway as well as the fatty acid elongases are conserved from yeast to mammals (Kihara, 2012).

2.4 Complex sphingolipid synthesis

Complex sphingolipids differ from yeast to mammals primarily in their head groups. Mammals contain more than 100 different sphingolipid classes due to diverse head groups linked to ceramide, while yeast contain only three types of complex sphingolipids. Yeast sphingolipids are derived mostly from phytoceramide and, to a lesser extent, dihydroceramide (Perry et al., 2005; Vallee and Reizman 2005). In yeast, ceramides are transported to the Golgi via vesicular or nonvesicular ATP-independent transport (Funato et al., 2002). The ER and the Golgi membranes must be in contact with each other for transport to occur (Funato et al., 2002). It is proposed that an ATP-independent translocation protein transports ceramide to the Golgi at the contact sites (Funato et al., 2002). In the Golgi, a polar, hydrophilic head group is transferred to ceramide to form complex sphingolipids, which are transported to the plasma membrane where they are abundant (Dickson 2008; Sims et al., 2004; Funato et al., 2002). The three types of complex sphingolipids synthesized in the yeast Golgi are inositol phosphoceramide (IPC), mannose inositol phosphoceramide (MIPC), and mannose (inositol-P)$_2$ ceramide (M(IP)$_2$ C).

The synthesis of IPC, catalyzed by IPC synthase, occurs by the addition of phosphorylinositol to ceramide (Dickson 2008; Sims et al., 2004; Funato et al., 2002). MIPC is synthesized by the addition of mannose from guanosine diphosphate mannose to IPC via MIPC synthase. There are two MIPC synthase complexes in yeast, one containing Sur1p and Csg2p and the other composed of Csh1p and Csg2p (Dickson 2008; Sims et al., 2004). The most abundant complex sphingolipid, M(IP)$_2$C, is formed by the addition of a second phosphorylinositol to MIPC, catalyzed by inositolphosphotransferase (Dickson 2008; Sims et al., 2004).

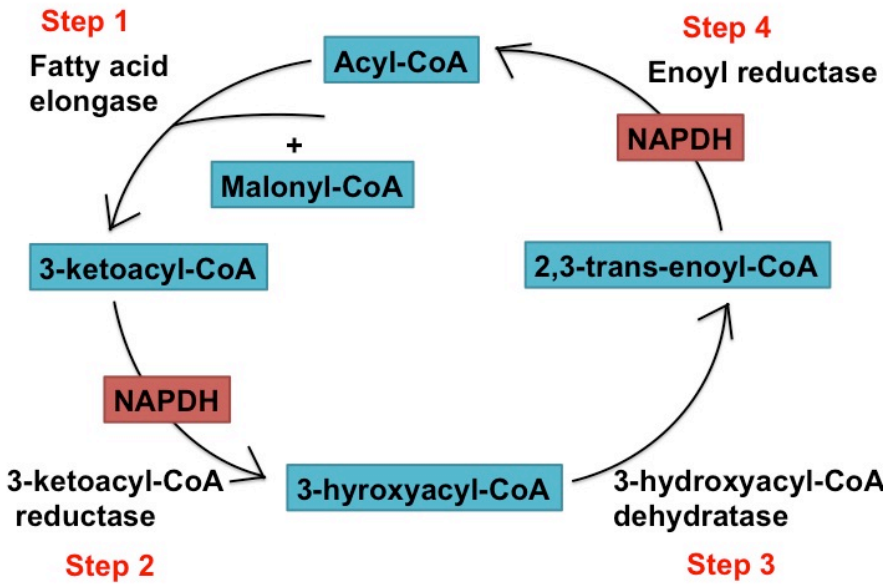

Figure 1.2. Fatty acid elongation pathway. Step 1: Malonyl-CoA is condensed with acyl-CoA to synthesize 3-ketoacyl-CoA. **Step 2:** 3-ketoacyl-CoA is reduced by 3-ketoacyl-CoA reductase to 3-hydroxyacyl-CoA. **Step 3:** 3-hydroxyacyl-CoA is dehydrated to 2,3-trans-enoyl-CoA by 3-hydroxyacyl-CoA dehydratase. **Step 4:** 3-hydroxyacyl-CoA undergoes a round of reduction catalyzed by enoyl reductase to yield an acyl-CoA with 2 more carbon chains than the original acyl-CoA.

In mammals, the C-1 position of ceramide is linked to a hydrophilic group such as phosphorylcholine, galactose, or glucose to form complex sphingolipids. Mammalian cells also exhibit both vesicular and non-vesicular ceramide transport. Vesicular transport involves coat protein complex II (COPII), which mediates vesicular transport of ceramides from the ER to the Golgi (Perry and Ridgway, 2005; Van Meer and Holthius, 2000). Non-vesicular transport of ceramides from the lumenal surface of the ER to the outer leaflet of the Golgi is mediated by the transporter ceramide transfer protein (CERT) (Hanada et al., 2003; Kumagai et al., 2005). Ceramide is primarily converted to sphingomyelin via the addition of a phosphorylcholine to the ceramide backbone by sphingomyelin synthase. There are two sphingomyelin synthases SMS1 catalyzes the synthesis of SM in the Golgi. SMS2 is mainly responsible for the synthesis of sphingomyelin in the plasma membrane. In addition to its activity in the plasma membrane, SMS2 functions redundantly with SMS1 in the Golgi (Hanada et al., 2007). Ceramide can also be galactosylated to produce galactosylceramide in the ER by galactosyltransferase (Holthuis et al., 2001). Galactosylceramide (galactocerebroside) is one of the predominant glycosphingolipid in the brain (Svennerholm et al., 1968).

Ceramide transported via vesicular or non-vesicular transport to the Golgi, is glucosylated to glucosylceramide via glucosylceramide synthase (Schulte and Stoffel 1993). Glucosylceramide and glycosylceramide act as precursors for the synthesis of glycosphingolipids, catalyzed by Golgi glycosyltransferases (Maccioni et al., 2011; Merill et al., 2011). These glycosyltransferases transfer a specific carbohydrate from a sugar nucleotide namely UDP-glucose, UDP-galactose, CMP-sialic acid to the

acceptor molecule, ceramide or the non reducing end of a growing carbohydrate chain attached to a ceramide molecule. Glucosylceramide, is galactosylated to produce lactosylceramide by galactosyltransferase (Perry and Ridgway, 2005; Hanada et al., 2007). Lactosylceramide acts as a precursor for the synthesis of a number of complex glycosphingolipids such as GA2 (Nagata et al., 1992; Hidari et al., 1994), GM3 (Ishii et al., 1998), Gb3 (Kojima et al., 2000); and Lc3 (Merrill, 2011; Biellmann et al., 2008). GA2, GM3, Gb3 and Lc3 are then precursors for the synthesis of other complex glycosphingosphingolipids (Merrill, 2011). In addition, galactosylceramide is sialylated to produce GM4 ganglioside, or sulfated to produce sulfatides (Merrill, 2011). Sulfate esters of galactosylceramide, or sulfatides are thought to play a role in the function of myelin. Perturbation of complex sphingolipid synthesis (especially GM1 and sulfatides) has been described in schizophrenia, as discussed below.

2.5 Long chain base phosphate (LCBP)

In yeast, the LCBs, DHS and PHS are phosphorylated to dihydrosphingosine phosphate (DHS-1P) or phytosphingosine phosphate (PHS-1P) by sphingolipid base kinases Lcb4 and Lcb5 (Nagiec et al., 1998). Lcb4 contributes about 97% of the kinase activity. Lcb5, which contributes about 3% of activity, is important for phosphorylation during heat stress (Nagiec et al., 1998; Ferguson-Yankey et al., 2002). Although the double mutant lacking both kinases has aberrant sphingolipid base content, it is viable (Cowart and Obeid, 2007).

In mammals, sphingosine bases are phosphorylated at C-1 by two sphingosine kinases that are homologous to the yeast enzyme. Sphingosine-1-phosphate (S1P) is

the major product of the kinase but DHS-1P also exists (Kihara et al., 2007). S1P is an important signaling molecule, as discussed in 3.11.2.

2.6 Ceramide-1- phosphate (C1P)

In mammals, ceramides are phosphorylated at the C-1 position by the calcium-stimulated ceramide kinase (CERK) to form ceramide-1-phosphate (C1P). C1P is a bioactive signaling molecule involved in macrophage migration, inflammation and vesicular transport (Chalfant and Spiegel, 2005; Misutake et al., 2005; Kihara et al., 2007). Ceramide kinase and C1P are not found in yeast (Sugiura et al., 2002).

2.7 Catabolism of complex sphingolipids

Isc1, the only sphingomyelinase (SMase) identified in yeast, hydrolyzes all three complex sphingolipids to phytoceramide. Isc1 is homologous to mammalian neutral SMase (Sawai et al., 2000). In mammals, five types of SMases degrade complex sphingolipids to ceramide. These include acid (aSMase), secretory (sSMase), alkaline (alk-SMase), Mg^{2+}-independent neutral (nSMase), and Mg^{2+}-dependent neutral SMases (Samet and Barenholz, 1999). A single gene, *ASM/SMPD1,* encodes both aSMase, which is targeted to the lysosomes, and sSMase, which is present in secretory vesicles (Wu et al., 2005; Duan, 2006). Alk-SMase is expressed in bile and intestinal tract (Wu et al., 2005; Duan, 2006). Very little is known about Mg^{2+}-independent neutral SMase, and the gene encoding this enzyme has not been identified. Three Mg^{2+}-dependent neutral SMases have been identified (Tomiuk et al., 1998, Hofmann et al., 2000; Krut et al., 2006).

2.8 Catabolism of ceramides

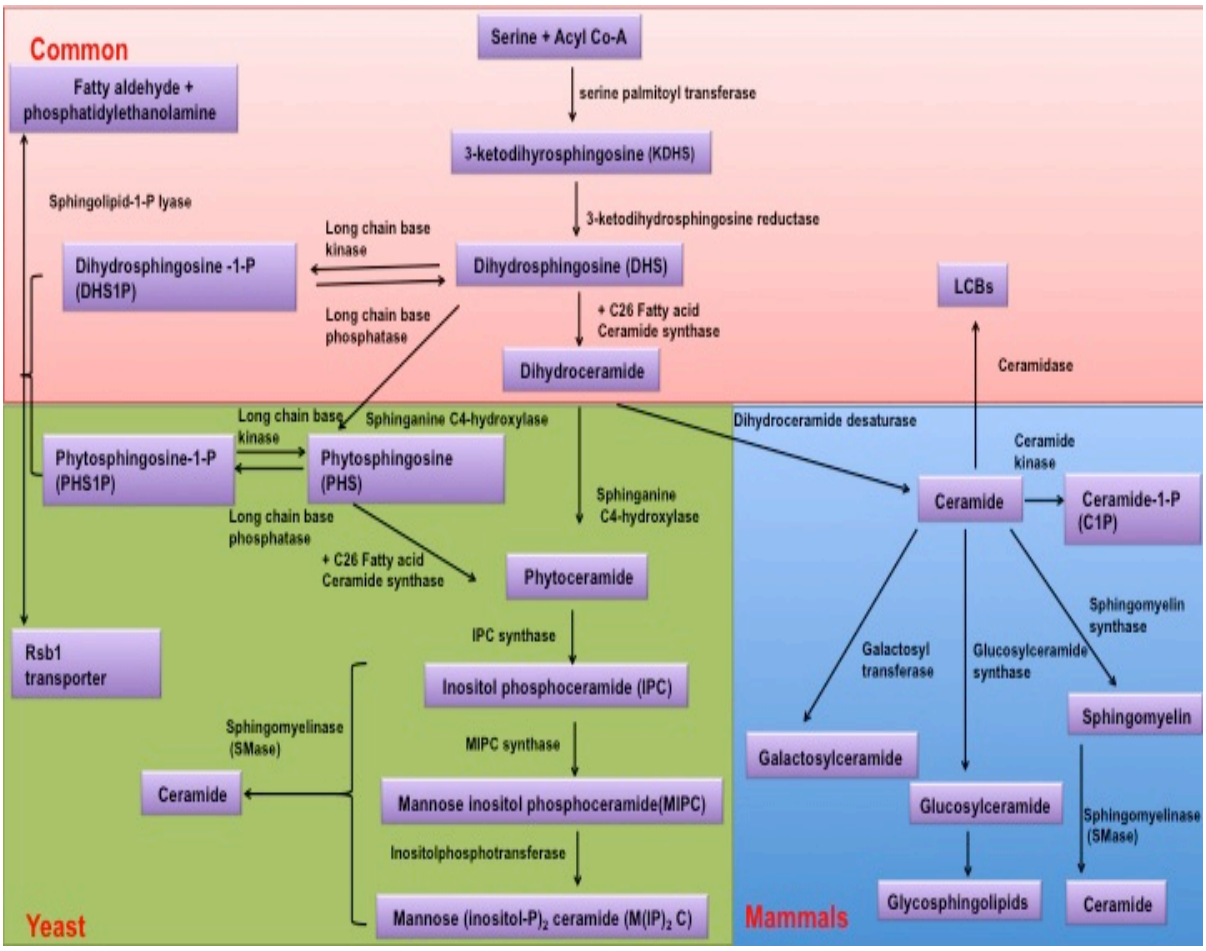

Figure 1.3. Synthesis and metabolism of sphingolipids in yeast and mammals. Common synthesis pathway: Sphingolipid synthesis begins in the ER in both yeast and mammals. Acyl-CoA is condensed with serine to form 3-ketohydrosphingosine, which is further reduced to dihydrosphingosine (DHS). C26 fatty acid is transferred to DHS to yield dihydroceramide. **In yeast:** DHS is hydroxylated to phytosphingosine (PHS), which is attached to a C26 fatty acid to synthesize phytoceramide. Dihydroceramide can also be hydroxylated to phytoceramide. Phosphatidylinositol is transferred to phytoceramide to produce inositolphosphorylceramide (IPC), which is mannosylated to mannoylinositolphosphorylceramide (MIPC). Another phosphatidylinositol group is added to MIPC to yield mannosyl(inositolphosphoryl)$_2$ ceramide [M(IP)$_2$C]. **In mammals:** DHS is N-acetylated to dihydroceramide, which is converted to ceramide. Different polar groups are added to the ceramide backbone to synthesize complex sphingolipids, including phosphorylcholine to produce sphingomyelin, and galactose and glucose to synthesize galactosylceramide and glucosylceramide, respectively. Glucosylceramide can be modified by the addition of sugars such as fucose, galactose, sialic acid, N-acetylglucosamine and N-acetylgalactosamine to produce several glycosphingolipids. **Common cleavage pathway:** Complex sphingolipids are catabolized to ceramide, which is cleaved to

LCBs. LCBs are phosphorylated to form LCBPs, which are dephosphorylated to LCBs or further cleaved to fatty aldehyde and phosphatidylethanolamine. In mammals, ceramide is phosphorylated to ceramide-1-phosphate (C1P). In yeast, Rsb1 transports sphingosine bases from the cytoplasmic side to the extra-cytoplasmic side of the membrane.

Ceramide that is synthesized de novo from sphingolipid bases or produced via the hydrolysis of complex sphingolipids is cleaved by ceramidases to LCBs, which can be phosphorylated or recycled for synthesis of ceramides (Ogretmen et al., 2002; Sultan e al., 2006). Reacylation of the cleaved sphingolipid bases to form ceramide is reported to be less efficient than the *de novo* synthesized sphingolipid bases, but it has been suggested that these cleaved bases can aid in the formation of ceramide with fatty acids of varying N-acyl chain length (Ogretmen et al., 2002; Sultan et al., 2006). Yeast contain two ceramidases, Ydc1 and Ypc1, while mammals contain three pH-dependent ceramidases. Acid ceramidases are located in the lysosomes (Koch et al 1996; Ferlinz et al., 2001). Neutral ceramidases are in the plasma membrane or secreted to the extracellular fluid following cleavage of the transmembrane region (Tani et al., 2003; Mao et al., 2001). Two alkaline ceramidases have been identified, one in the ER/Golgi, which selectively degrades phytoceramides (Mao et al., 2001), and one in the ER, which degrades N-acylsphingosine (Mao et al., 2003).

2.9 Catabolism of LCBPs

In yeast, DHS-1P and PHS-1P are cleaved by the sphingolipid lyase Dpl1 to generate ethanolamine phosphate and fatty aldehydes (Saba et al., 1997). This is the only known route whereby a sphingolipid can be converted to a non-sphingolipid. Ethanolamine phosphate is shuttled to synthesize phosphatidylethanolamine through the Kennedy pathway (Panwar and Scott- Moye Rowley, 2006). DHS-1P and PHS-1P are dephosphorylated to DHS and PHS. In mammals, S1P is catabolized to fatty aldehyde and phosphatidylethanolamine by S1P lyase encoded by SPL, which is

homologous to yeast *DPL1* (Kihara et al., 2007). S1P regulates cell survival, cell proliferation and migration, and neurotransmitter release (Okada et al., 2009).

2.10 Sphingolipid base transporter

In yeast, deletion of sphingolipid lyase *DPL1* leads to accumulation of sphingosine base phosphates and inhibits cell growth (Kim et al., 2000). In a screen to identify suppressors of the *dpl1* mutant growth defect caused by the accumulation of sphingosine phosphates, the ATP dependent sphingosine transporter Rsb1p was identified (Kihara and Igarashi 2002). Rsb1p is thought to be a transporter or flippase that transports sphingosine bases from the cytoplasmic side to the extra cytoplasmic side of the membrane in order to maintain normal intracellular levels of LCBs and membrane composition (Kihara and Igarashi 2002). Homologs of Rsb1p have not been found in mammals.

2.11 Sphingolipids regulate essential functions

Metabolites and intermediates of sphingolipid metabolism, including (but not limited to) LCBs, ceramide, S1P, and C1P, are signaling molecules that regulate essential cellular processes that are required for the regulation for neuronal processes (Hannun and Obeid, 2008). These include SNARE complex formation, synaptic vesicle exocytosis at nerve terminals, neurogenesis, neural tube closure, apoptosis, neurodegeneration, and neurotransmitter release (Okada et al., 2009; Shinghal et al., 1993; Kajimoto et al., 2007; Van Brocklyn et al., 2012; Mizugishi et al., 2005; Darios et al., 2009).

2.11.1 LCBs and sphingosine

LCBs regulate actin cytoskeleton, heat stress, and endocytosis (Hannun and Obeid, 2008). Heat stress causes a transient 2-3-fold increase in the levels C18 DHS and C18 PHS and a 100-fold increase in levels of C20 DHS and C20 PHS (Dickson et al., 1997; Jenkins et al., 1997). LCBs are required for proper actin cytoskeleton organization (Schmelze et a., 2002) and for the regulation of endocytosis (deHart et al., 2002). LCBs also play a role in inhibiting uptake of uracil and amino acids tryptophan, leucine, and histidine (Chung et al., 2001). It is not understood how LCBs regulate amino acid transport, but PHS is important for ubiquitin-mediated breakdown of the uracil transporter Fur4 (Chung et al., 2000).

Sphingosine activates synaptobrevin in synaptic vesicles, facilitating SNARE complex assembly, implicated in membrane fusion. In addition, sphingosine increases synaptic vesicle exocytosis in nerve terminals and neuromuscular junctions (Darios et al., 2009).

2.11.2 S1P

S1P regulates cell survival, cell proliferation, migration and neurotransmitter release (Okada et al., 2009; Kajimoto et al., 2007), and rescues cells from ceramide-induced apoptosis (Cuvillier et al., 1996). The effect of S1P depends on the level of neuronal differentiation. Thus, S1P induces proliferation in neural undifferentiated progenitor cells (Harada et al., 2004) but it increases apoptosis in well-differentiated neurons (Moore et al., 1999). In addition to its role in cell proliferation, S1P is important for neural and vascular development (Mizugishi et al., 2005). Sphingosine kinase mutants (*SphK1* and *SphK2)* do not show any abnormalities, but the double mutant

exhibits embryonic lethality, perturbed neurogenesis, neural tube closure, and angiogenesis in mice (Mizugishi et al., 2005).

2.11.3 Ceramide

Ceramides are anti-proliferative and promote apoptosis (Van Brocklyn and Williams, 2012). Increased levels of ceramide cause cell death (Obeid et al., 1993), while depletion of ceramide reduces progression of apoptosis (Bose et al., 2005; Santana et al., 1996). Altered ceramide levels are linked to cancer, neurodegeneration and inflammation (Echten-Deckert and Herget, 2006). Maintaining normal ceramide levels is crucial for brain function. Ceramide is also known to regulate cell senescence. The genes *LAG1* and *LAC1,* which encode ceramide synthase, regulate yeast life span (Schorling et al., 2005). The mammalian homolog of *LAG1 (CerS1)* is highly expressed in the brain (Pewzner-Jung et al., 2006). Overexpression of CerS1 leads to increased levels of C18 ceramide and induces apoptosis (Pewzner-Jung et al., 2006). CerS1-generated C18 ceramide negatively regulates the human telomerase reverse transcriptase promoter (Blasco et al., 1995; Menacarelli and Martinez-Martinez, 2012). Telomerase is expressed in neurons in the early stages of development, in neural stem cells and progenitors, but is downregulated in developed cells. Telomerase protects post-mitotic neurons from stress-induced apoptosis and is important for survival of neurons in the developing brain. Therefore, C18 ceramide very likely contributes to neuronal vulnerability in age related neurodegenerative disorders (Menacarelli and Martinez-Martinez, 2012). Ceramide affects several signal transduction pathways. The signaling molecules Akt serine/threonine kinase and PP2A are known targets of ceramide (Mora et al., 2002; Qin et al., 2012; Wolff et al., 1994). Ceramide was shown

Table 1.1. Yeast and mammalian genes involved in sphingolipid metabolism

Enzymes	Genes		References
	Yeast	Mammals	
Serine palmitoyl transferase	*LCB1, LCB2, LCB3*	*SPTLC1, SPTLC2, SPTLC3*	(Gable et al., 2003; Hanada 2003; Lowther et al., 2012)
3-ketodihydrosphingosine reductase	*TSC10*	*FVT-1*	(Dickson 2008; Beeler et al., 19998; Kihara and Igarashi 2004)
Sphinganine C4-hydroxylase	*SUR2*	-	(Dickson 2008)
Ceramide synthase	*LAG1, LAC1 LIP1*	*CERS1-6*	*(*Cowart and Obeid 2006; Mizutani et al., 2006*)*
Dihydroceramide desaturase	-	*DES1, DES2*	*(*Ternes et al., 2002; Mizutani et al., 2004; Omae et al., 2004)
Fatty acid elongases	*ELO1, ELO2/FEN1, ELO3 SUR4*	*ELOVL1-7*	*(*Jakobsson et al., 2006; Ohno et al., 2010; Kihara 2011; Kihara, 2012)
3-ketoacyl-CoA reductase	*Ybr159w, AYR1*	*KAR*	(Kihara 2011)
3-hydroxyacyl-CoA dehydratase	*PHS1*	*HACD1-4*	(Kihara 2011)
Enoyl reductase	*TSC13*	*TER*	(Kihara 2011)
IPC synthase	*AUR1*	-	(Dickson 2008; Sims et al., 2004; Funato et al., 2002)
Sphingolipid alpha-hydroxylase	*SCS7*	-	(Dickson 2008; Sims et al., 2004; Funato et al., 2002)
MIPC synthase	*SUR1, CSH1 and CSG2*	-	(Dickson 2008; Sims et al., 2004)
Inositolphosphotransferase	*IPT1*	-	(Dickson 2008; Sims et al., 2004)
Sphingomyelin synthase		*SMS1 and SMS2*	(Hanada et al., 2007)
Glucosylceramide	-	*GlcT-1*	(Schulte and

synthase			Stoffel 1993)
Galactosyltransferase	-	*CGT*	(Schulte and Stoffel 1993)
Long chain base kinase	*LCB4, LCB5*	*SPHK1 and SPKH2*	(Kihara et al., 2007)
Ceramide kinase		*CERK*	(Sugira et al., 2002)
Sphingomyelinase	*ISC1*	**aSMase, sSMase:** *ASM/SMPD1* ***alk-SMase****: NPP7* **Mg^{2+} dependent neutral SMase:** *SMPD3, SMPD4, SMPD5* **Mg^{2+} independent neutral SMase***: not known*	(Sawai et al., 2000; Wu et al., 2005; Duan, 2006; Tomiuk et al., 1998, Hofmann et al., 2000; Krut et al., 2006)
Ceramidase	*YDC1, YPC1*	**Acidic:** *ASAH1/AC* **Neutral***:* *ASAH2/NCDase* **Alkaline***: PHC, CER1/AAH3*	(Ogretmen et al., 2002; Sultan e al., 2006; Koch et al 1996; Ferlinz et al., 2001). Tani et al., 2003; Mao et al., 2001; Mao et al., 2003)
Sphingolipid-1-P lyase	*DPL1*	*SPL*	(Kim et al., 2000; Kihara et al., 2007)
Long chain base phosphatase	*LCB3, YSR3*	*SPP1, SPP2, LPP1-3*	(Panwar et al.,2006; Kihara et al., 2007).
Sphingolipid transporter	*RSB1*	-	(Kihara and Igarashi 2002).

to reduce Akt phosphorylation at Ser473; this dephosphorylation was reversed after treatment with antidepressants (Gulbins et al., 2013). Ceramide induces mitochondrial outer membrane permeabilization (MOMP), important for induction of apoptosis, by forming ceramide channels. In addition, ceramide enhances the autophosphorylation of the kinase suppressor of Ras (KSR), which activates the RAF1/MEK/ERK MAPK cascade, inducing apoptosis (Zhang et al., 1997; Basu et al., 1998). Several studies have demonstrated that ceramide activates the JNK pathway (Westwick et al., 1995; Verheij et al., 1996; Nica et al., 2008). JNK activation regulates proteins of the Bcl-2 family, involved in apoptosis (Bogoyevitch et al., 2006). Ceramide also activates PKC-zeta and inhibits PKC-alpha (Muller et al., 1995), phenotypes that are reversed by antidepressants imipramine and amitriptyline (Kornhuber et al., 2005). These findings suggest that antidepressants may restore normal signaling that is perturbed by increased ceramide levels.

2.11.4 Ceramide-1-phosphate (C1P)

Phosphorylation of ceramide produces C1P, which decreases apoptosis in several ways. C1P blocks ceramide synthesis by inhibiting acid sphingomyelinase (Gomez-Munoz et al., 2004) and SPT activity (Granado et al., 2009). C1P also enhances the activity of anti-apoptotic protein kinase B and *NF-kappaB* (*NF-kB*) (Granado et al., 2009). While the function of C1P has not been definitively elucidated, an early study proposed that it plays a role in synaptic vesicle functioning and release of neurotransmitters (Shinghal et al., 1993).

3. Sphingolipid metabolism in psychiatric disorders

As discussed above, sphingolipids affect essential cellular processes that are crucial not only for cellular homeostasis such as apoptosis, cell survival and development, but also for neuronal functions. In particular, sphingosine is required for activation of exocytosis of the synaptic vesicles at the nerve terminal. Ceramide, S1P, and C1P regulate apoptosis, neurogenesis, and neurotransmitter release. In view of the key role of sphingolipids in neurogenesis, neuronal cell death and neurotransmission. It is not surprising that perturbation of sphingolipid metabolism is observed in depression, anxiety, schizophrenia, and BD.

3.1 Depression

Depression is a chronic, severe, life-threatening disorder characterized by depressed mood, insomnia, weight loss, loss of interest or pleasure in normal activities, feelings of worthlessness, and a high risk of suicide. The worldwide prevalence of depression is 10% (Belmaker and Agam, 2008). Recent studies suggest that depression is linked to a change in the levels of neurogenic and apoptotic factors in the hippocampus, leading to hippocampal atrophy and neurodegeneration (Santarelli, et al., 2003; David et al., 2009).

A number of studies indicate that depression is associated with altered sphingolipid synthesis. A recent lipidomic analysis identified significant alterations in plasma sphingomyelin species in patients with depression (Demirkan et al., 2013). Several studies, summarized below, report increased levels of ceramide and/or aSMase in depression, and decreased levels of these components following antidepressant treatment.

3.1.1 Increased ceramide and acid sphingomyelinase in depression

Several studies suggest that increased ceramide levels are associated with depression. Increased aSMase activity was observed in peripheral blood mononuclear cells of patients suffering from major depressive disorder who were free of therapy for at least 10 days (Kornhuber et al., 2005). As discussed above, aSMase converts sphingomyelin to ceramide and phosphorylcholine (Gublins and Kolesnick 2003; Perrotta et al., 2010). Therefore, this finding suggested that increased levels of ceramide might be associated with depression. Consistent with this possibility, increasing the levels of ceramide by injecting C16 ceramide directly in the hippocampus or by administrating D,L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP, a glycosyltransferase inhibitor that increases ceramide levels by inhibiting glycosylation of ceramide) induced depressive disorder-like symptoms in mice (Gulbins et al., 2013). Plasma ceramide levels were analyzed in Alzheimer disease patients with recent depression, past depression and no depression. Interestingly, ceramide levels were elevated in patients with recent depression compared to patients with past or no depression (Garcia-Garcia et al., 2011). Indirect evidence associating ceramide with depression comes from a report of increased activity of phospholipase A2, which is stimulated by ceramide, in patients with major depression (Nopnenm et al., 1993).

3.1.2 Antidepressants inhibit aSMase activity and decrease ceramide levels

If ceramide plays a causal role in depression, as suggested by the correlational studies cited above, antidepressants may exert their therapeutic effects by inhibiting aSMase, thus decreasing the release of ceramide from sphingomyelin. Consistent with this, treatment with antidepressants imipramine and amitriptyline caused a rapid

reduction of aSMase activity in peripheral blood mononuclear cells of depressive patients (Kornhuber et al., 2005). In addition, a therapeutic concentration of antidepressants amitriptyline and fluoxetine inhibited aSMase activity in cultured neurons (Gulbins et al., 2013). Wild type mice treated with antidepressants amitriptyline (a tricyclic drug) or fluoxetine (a serotonin reuptake inhibitor) exhibited decreased aSMase protein levels and activity (Gulbins et al., 2013). The high levels of ceramide present in mice heterozygous for mutant ceramidase were also decreased by antidepressants amitriptyline and fluoxetine (Gulbins et al., 2013). These studies suggest that antidepressants inhibit aSMase activity via the following mechanism. The inner leaflet of the lysosomal membrane is negatively charged due to the presence of the anionic lipid bis(monoacylglycero)phosphate (Schulze et al., 2009). Positively charged aSMase adheres to the inner lysosomal membrane leaflet electrostatically (Kolzer et al., 2004). Lipophilic compounds and weak bases such as antidepressants in the neutral form can pass passively through the cell and lysosomal membranes and accumulate in the lysosome, where they become protonated thus, trapped in the lysososme, increasing the antidepressant concentration in the lysosome (Duve et al., 1974; Trapp et al., 2008). This changes the physiology of the lysosome. The lipophilic part of the drug adheres to the membrane, and the positively charged part of the drug is facing the lumen. Accumulation of the drug prevents the aSMase from adhering to the lysosomal membrane (Kolzer et al., 2004). This results in proteolytic cleavage of the enzyme, thus inhibition of the aSMase activity and increasing the levels of ceramide in the lysosome (Hurwitz et al., 1994). This suggests that antidepressants decrease levels of ceramide due to inhibition of aSMase activity via degradation of the aSMase protein,

which normalizes the reduced neurogeneisis and neuronal maturation caused by increased ceramide levels.

Interestingly, amitriptyline and fluoxetine increased neurogenesis, neuronal maturation and survival in wild type but not in aSMase-deficient mice, suggesting that the mechanism of action of the drugs is by affecting aSMase. Treatment of antidepressants amitriptyline and fluoxetine or the aSMase inhibitor fendiline attenuated corticosterone-induced depressive-like behavior in wild type mice but had no effect on corticosterone-treated aSMase-deficient mice (Gulbins et al., 2013). These studies suggest that increased levels of ceramide may play a causal role in the pathophysiology of depression, and that a potential therapeutic mechanism underlying antidepressant efficacy is the reduction of ceramide levels by inhibition of aSMase activity. Reducing ceramide levels could be the central goal for the development of new antidepressants.

3.2 Anxiety

Anxiety disorders are characterized by severe and prolonged distress and fear with physiological symptoms, which are manifested early and recur throughout life (Baxter et al., 2014). They are the sixth leading cause of disability in both low and high-income countries (Baxter et al., 2014). Several studies suggest that sphingolipids may play a role in the pathophysiology of anxiety disorder. In particular, increased levels of S1P are associated with anxiety-like behavior while increased levels of GM1 may reduce anxiety and cause neuroprotection.

3.2.1 Increased levels of S1P

Chronic stress is known to cause anxiety-like behavior. In several models of stress-induced anxiety in rats, including daily immobilization for 3 weeks or electric shock followed by immobilization, serum S1P levels were increased (Jang et at., 2011). In the former model, increased expression of protein markers of neurodegeneration were also observed, including iNOS (inducible nitric oxide synthase), GFAP (glial fibrillary acidic protein) and NR1 (N-methyl-D-aspartate receptor channel). Infusion of S1P into the lateral cerebroventricle also led to increased expression of these protein markers, suggesting that stress-induced anxiety causes increased S1P, which leads to degeneration (Jang et al., 2008). However, an alternative possibility comes from a study using an elevated plus maze model of anxiety, in which S1P-treated animals spent significantly less time in the open arms of the maze, suggesting that S1P induces anxiety (Jang et al., 2011).

3.2.2 Perturbation of glycosphingolipid levels

Precocious weaning is known to induce anxiety-like behavior in male mice, which is more pronounced than that observed in precocious weaned female or normal weaned mice (Ono et al., 2008). Interestingly, precocious weaned male mice exhibited an accumulation of galactosylceramide in the amygdala (but not in the hippocampus or prefrontal cortex), along with an increase in number and reduction in the diameter of myelinated axons at 5 weeks. Galactosylceramide and sulfatide contribute about 30% of the lipid content of the myelin sheath (Noton, 1984). The developmental time of myelin formation differs for each brain region (Akiyama et al., 2002). In normal-weaned male mice, galactosylceramide increases linearly until 8 weeks of age in the amygdala, and saturates by 5 weeks in the prefrontal cortex and hippocampus. Therefore, early

weaning may induce precocious myelination. Decreased axonal diameter could be caused by precocious myelination induced by early weaning. This can cause changes in axonal structure and neural circuts in basolateral amygdala, a site controlling anxiety like behavior (LeDoux, 1993; Schafe et al., 2001; Spiga et al.,2006). Therefore, precocious myelination of the amygdala in early-weaned male mice might affect the development of an anxiety-related network and thereby increase anxiety-related behavior.

GM1 (monoamine ganglioside) is a neuroprotective sialic acid-containing glycosphingolipid (Ariga et al., 2008; Wallis et al., 1995). An early study suggested that GM1 improved memory but not anxiety. GM1 was administered to male rats containing electrolytic lesions in the left cortex. (Electrolytic lesions produce transient behavioral deficits (Reeves et al., 1997; Loesche et al., 1977). Working memory was measured in a Morris water maze and anxiety levels were tested in a plus maze. GM1 treatment led to improvement in the working memory, but not in the anxiety levels (Glasier et al., 1995). However, GM1 was effective in blocking anxiety-related responses to ethanol withdrawal, including hypolocomotion, tremors and other anxiety-like behavior (Wallis et al., 1995). Pretreatment with GM1 or its constituent sialic acid was also effective in reducing effects of ethanol intoxication, such as nose-poke exploration, decreased locomotion, and anxiety (Wallis et al., 1995). Pretreatment with ganglioside or sialic acid also improved staircase climbing by 22%, consistent with lower anxiety (Wallis et al., 1995).

These studies suggest that cerebrosides may play a role in anxiety, as galactosylceramide accumulates in at least one model of anxiety. Galactosylceramide

and GM1 are required for proper myelination of the axon, thus exerting a protective role.

3.3 Schizophrenia

Schizophrenia is a debilitating mental disorder with drastic psychosocial consequences. It is characterized by defects in synaptic connectivity in the white matter tracts, leading to cognitive abnormalities (Fitzsimmons et al., 2013). Schizophrenic patients exhibit both positive symptoms (delusions and hallucinations) and negative symptoms (social withdrawal and loss of motivation) (Liddle, 1987). Schizophrenia is known to affect 1% of the world population (Capuano et al., 2002; Merikangas et al., 2007). Several neurochemical and neuropathlogical theories have been proposed to explain the pathology of schizophrenia, including perturbations in GABAergic (Lewis et al., 2005), dopaminergic (Carlsson, 1980), and glutamatergic signaling (Kim et al., 1980; Olney and Farber, 1985), and aberrations in neurodevelopment (Weinberger, 1987), prefrontal cortex function (Andreasen et al., 1986; Ingvar and Franzén, 1974), and inflammatory response (Lin et al., 1998; Rothermundt et al., 2001).

3.3.1 Perturbation of complex sphingolipids

Complex sphingolipids are required for myelination of neurons (Boggs et al., 2010). They play a role in carbohydrate-carbohydrate interactions between opposing membranes, cell-cell adhesion and transmembrane signaling, and communication between the axon and myelin sheath, which affects axonal and myelin functions and neurodegeneration (Boggs et al., 2008).

Decreased levels of complex sphingolipids, including sulfatides and cerebrosides were first observed in a postmortem schizophrenic brain in 1969 (Cherayil 1969). Subsequently, decreased levels of sphingomyelin and galactocerebrosides, which are required for myelin synthesis in the brain, were observed in post mortem brain samples of chronic schizophrenic patients (Schmitt et al., 2004). Consistent with decreased sphingomyelin, increased levels of phosphatidylserine, an activator of neutral sphingomyelinase, was observed in post mortem schizophrenic samples (Schmitt et al., 2004). Decreased levels of glycosphingolipids could result in decreased myelination and altered cell-cell adhesion and transmembrane signaling, leading to neurodegeneration. However, this conclusion is obscured by studies reporting increased levels of complex sphingolipids in schizophrenia, including glycosphingolipids, and gangliosides GM3 and GD3 (Haselhorst et al., 1988).

In a microarray analysis comparing post mortem brain samples of prefrontal cortex from schizophrenic patients and healthy controls, expression of a number of genes that encode enzymes of sphingolipid metabolism were observed. Changes were most notable in the early stages of the disorder (within 5 years of diagnosis) (Narayan et al., 2009). A significant reduction was observed in the expression of seven genes related to sphingolipid metabolism, including UDP glycosyltransferase 8 (*UGT8),* serine palmitoyltransferase, long-chain base subunit 2 (*SGPP1),* UDP-Gal:betaGlcNAcbeta1,4-galactosyltransferase polypeptide 6 (*B4GALT6),* galactosylceramidase *(GALC)*, galactose-3-O-sulfotransferase 1(*GAL3ST1),* serine palmitoyltransferase, long-chain base subunit 2 (*SPTLC2),* and N-acylsphingosine

amidohydrolase (acid ceramidase)1 (*ASAH)* (Narayan et al., 2009). *UGT8* encodes UDP glycosyltransferase, which converts ceramide to galactosylceramide. The *GAL3ST1*-encoded enzyme, galactose-3-O-sulfotransferase, catalyzing the synthesis of sulfatides, Sulfatides and galactosylceramide are components of the myelin sheath, and altered levels of these lipids may lead to impaired myelination. Postmortem studies of schizophrenic patients have revealed abnormalities in myelination of oligodendrocytes (Davis et al., 2003). Consistent with the hypothesis of impaired myelination, oligodendrocyte dysfunction was observed in schizophrenic patients (Kubicki et al., 2005). However, chronic treatment with haloperidol was associated with decreased expression of myelin oligodendrocyte related genes in the white matter of mouse brain samples (Narayan et al., 2007). Therefore, the role of abnormal myelination in schizophrenia remains to be elucidated.

3.3.2 Aberrant ceramide levels

Several reports indicate that ceramide levels are altered in schizophrenia, although a consistent pattern of alteration is not apparent. In a study comparing epidermal lipid profiles of drug naïve (first episode) schizophrenics and healthy controls, several classes of ceramide were altered (Smesny et al., 2012). A significant increase of α-hydroxy and 6-hydroxysphingosine with C26 or C28 and nonhydroxy 6-hydroxysphingosine/ α-hyroxysphingosine with C26 or C28 fatty acid chains, and a decrease in ceramide containing ω-hydroxylated fatty acid greater than C30 esterified to linoleic acid and nonhydroxy phytosphingosine with C26 or C28 were observed in patients compared to the healthy controls (Smesny et al., 2012).

A high throughput mass spectrometry profile of lipid levels in prefrontal cortex white and grey matter showed significant changes in the levels of ceramides in samples from schizophrenic vs. normal controls (Schwarz et al., 2008). Levels of ceramides were increased in the white matter. However, ceramide 34:1 was decreased in RBC samples from first onset patients (Schwarz et al., 2008). This study only analyzed one species of ceramide in RBCs, therefore the data of ceramide levels is not conclusive. In addition, ceramides were significantly increased with drug treatment (Schwarz et al., 2008). Cationic amphiphilic drugs used for the treatment inhibit acid ceramidase activity. Acid ceramidase catalyses the breakdown of ceramide. Inhibition by these drugs could lead to an increase in the ceramide levels (Elojemy et al., 2006).

The microarray analysis of postmortem brain samples of schizophrenic patients discussed in the previous section identified altered expression of genes that metabolize ceramide (Narayan et al., 2009). Interestingly, genes encoding enzymes that decrease ceramide levels were downregulated in chronic schizophrenia, including *UGT8*, *GAL3ST1,* and *ASAH1. ASAH1* was also found to have strong linkage to schizophrenia in a genome wide metascan study (Lewis et al., 2003). In addition, two single nucleotide polymorphisms in *ASAH1* were found to be associated with schizophrenia (Zhang et al., 2012). In summary, while some studies suggest that increased ceramide may play a role in schizophrenia, conflicting findings preclude a definitive conclusion.

3.4 Bipolar disorder (BD)

BD, which is characterized by depression and mania, is a severe mood disorder that affects 1-2% of the world population (Weissman et al., 1988). A high throughput lipidomics study of postmortem brain samples from bipolar patients and normal

controls revealed statistically significant increased levels of ceramide in the white matter, not accompanied by decreased levels of phosphatidylcholine, suggesting that the increase in ceramide results from increased synthesis rather than increased breakdown of sphingomyelin (Schwarz et al., 2008). While these studies are very preliminary, they suggest that BD may be associated with altered ceramide levels and ceramide signaling. However, whether ceramide plays a role in the development and progression of BD or is simply an outcome of the disease remains unknown.

4. Future perspective: Exploiting yeast to elucidate sphingolipid functions and develop sphingolipid-modifying therapeutics

While the studies summarized above suggest that sphingolipid metabolism is perturbed in psychiatric disorders, the fundamental question – does perturbation of sphingolipid metabolism play a causal role, or is it a secondary effect of the disorder? – remains to be elucidated. The complexity of mammalian systems and the lack of suitable animal models that accurately reflect human psychiatric illness complicate the ability to effectively address this question. How, then, can a unicellular eukaryote shed light on these complex processes? The genetic tractability of yeast offers powerful advantages that can be exploited to elucidate the role of sphingolipids in essential and highly conserved cellular processes, as follows: 1) All the genes encoding the enzymes of the sphingolipid metabolism in yeast are well characterized, and most of the enzymes of sphingolipid metabolism have homologs or orthologs in mammals, as discussed above. The homology of yeast and mammalian genes has been exploited to clone mammalian sphingolipid genes by complementation of yeast mutants (Dickson, 2006; Modrak et al., 2006; Hannun and Obeid, 2002). The signaling pathways and

cellular processes that are controlled by sphingolipids are also conserved. Conservation of sphingolipid synthesis and function suggests that knowledge gleaned from the yeast system can shed light on similar processes in human cells. 2) A wealth of well-characterized yeast mutants can be exploited in *in vivo* experiments to address the role of specific sphingolipids in essential cellular processes, and to ascertain the global effects of targeted sphingolipid perturbations. 3) Yeast bioassays utilizing sphingolipid mutants can be developed to screen for drugs that target specific sphingolipid components. 4) Yeast genetic and metabolic databases can be utilized for *in vivo* and *in silico* experiments to determine the effects of sphingolipid perturbation on cell function.

4.1 Identifying inhibitors of ceramide and S1P synthesis

Increased levels of ceramide in depression, schizophrenia, and BD suggest that ceramide synthesis may be a potential therapeutic target. As discussed above, mammals have six different ceramide synthase enzymes that exhibit tissue specificity as well as specificity for fatty acyl CoAs (Levy et al., 2005). Creating and analyzing the effects of ceramide synthase knockouts would help to elucidate the role of specific ceramide species. Several knockout mice have already been described (Ebel et al., 2013; Imgrund et al., 2009). CerS6 knockout mice exhibited a decrease in C16:0 containing ceramide species relative to wild type mice. These knockout mice exhibit behavioural abnormalities, such as habituation deficit and clasping abnormalities of their hind limbs (Ebel et al., 2013). In addition, CerS2-deficient mice exhibit myelin sheath defects, cerebellar degeneration, and hepatocarcinomas (Imgrund et al., 2009).

Creating other CerS knockouts will enable the study of functional roles of the specific ceramide synthase and their products, ceramide species with fatty acyl CoA.

The available ceramide synthase inhibitors (fumonisin B, austalifugin) target the enzymes nonspecifically, but specific inhibitors would be useful in elucidating the functions of each enzyme. Yeast ceramide synthase mutants (*lag1, lac1*) exhibit growth defects that can be functionally complemented with mammalian homologs (Guillas et al., 2003). Therefore, inhibitors of specific ceramide synthases could be identified by inhibiting growth of yeast *lag1* or *lac1* mutants expressing the mammalian enzyme.

A similar approach could be used to identify inhibitors of S1P, the levels of which are increased in anxiety. FTY720, a sphingolipid analog, is currently approved for the treatment of the autoimmune disorder multiple sclerosis. In vertebrates, FTY720 is phosphorylated by sphingosine kinase 2 and acts as a S1P mimic (Hla et al., 2011). FTY720 inhibits sphingosine kinase 1 (Lim et al., 2011). Yeast was used to study the metabolism and possible cellular targets of FTY720 (Welsch et al., 2003). In yeast, FTY720 phosphorylation is not required for its action, suggesting that FTY720 may have other cellular effects, including mimicking sphingolipid bases, and controlling the ubiquitin pathway and amino acid permease trafficking (Lee et al., 2011; Welsch et al., 2004). In addition, FTY720 inhibits sphingosine phosphate lyase but it is not understood if this inhibition has a role in the mechanism of action of FTY720. In addition, siponimoid, a S1P1 agonist designed using FTY720 is currently completed phase 2 clinical trails for the treatment of relapsing-remiting multiple sclerosis (Pan et al., 2013). Identification of specific inhibitors of S1P kinase will help elucidate the cellular functions

of S1P. In addition, these molecules could act as potential drugs for the treatment of psychiatric disorders.

Therefore, there is a need to identify new inhibitors of sphingosine kinases or sphingosine phosphatases. Inhibitors of these enzymes may be identified by loss of rescue of growth defects of yeast *lcb4* and *lcb5* mutants expressing the mammalian homologs.

Inhibitors of these specific steps in sphingolipid metabolism would be powerful tools to elucidate the cellular roles of ceramide and S1P, just as studies with the SPT and ceramide synthase inhibitors myriocin and fumonisin B1, respectively, have contributed greatly to our understanding of the roles of sphingolipids in autophagy, heat stress, life span, and apoptosis (Daquinag et al., 2007; Huang et al., 2013).

4.2 Mathematical modeling the effects of perturbation of ceramide and S1P on yeast cell homeostasis

Mathematical modeling is a powerful tool to organize and analyze high throughput data pertaining to biochemical pathways (Alvarez-Vasquez et al., 2005). Mathematical modeling has been exploited to generate complex network maps of sphingolipid metabolism in yeast (Alvarez-Vasquez et al., 2004; 2005; 2007). For example, a recent study simulated the action of drugs affecting sphingolipid metabolism in the ER to determine the effects of the drugs on ergosterol in the plasma membrane (Alvarez-Vasquez et al. 2011). Simulation of inhibition of IPC synthase, a step inhibited by aerobasidin, suggested that vesicular co-transport of ergosterol and complex sphingolipids is impaired, resulting in redistribution of ergosterol from the outer to the inner leaflet of the plasma membrane. Similarly, simulations with drugs such as

myriocin, an inhibitor of serine palmitoyltransferase (SPT), suggested a decrease in vesicular ergosterol transport flux and an increase of the non-vesicular ergosterol flux. Mathematical modeling can be used to simulate perturbation of ceramide and S1P levels, generating *in silico* disease models in yeast. The predictions of these models can be validated easily in yeast and then translated to complex mammalian systems (Kemmmer et al., 2009).

4.3 Elucidating the global *in vivo* effects of perturbation of ceramide and S1P levels

Altered levels of ceramide or S1P in yeast can be achieved by genetic manipulation using mutants of ceramide synthase (*LAG1, LAC1*), sphingosine base kinases (*LCB4* and *LCB5*), and sphingosine base phosphate (*LCB3* and *YSR3*). The effects of perturbation can be analyzed in the context of the available yeast metabolome, transcriptome, proteome and interactome data (Ge et al., 2001; Humston et al., 2011; Ptacek et al., 2005; Schwikowski et al., 2000; Velculescu et al., 1997). Lipidomic analyses may identify specific (and unexpected) metabolites important in maintaining homeostasis that may be altered in disease conditions (Kemmer et al., 2009). Recent advances with mass spectrometric tools in the field of lipidomics have made it possible to identify and quantify several species of sphingolipids. In addition, recent developments in shotgun lipidomics enables the identification and quantification individual lipid species directly from lipid extracts of biological samples (Blanksby et al., 2010). Alterations in specific sphingolipid species may provide insights into the role in pathogenesis (Han et al., 2007). The results from these studies can be investigated and validated in mammalian systems.

5. Conclusion

There is a profound need to elucidate the molecular mechanisms underlying psychiatric disorders and to develop effective therapies to treat them. While many studies associate aberrant sphingolipid metabolism with the pathophysiology of psychiatric illnesses, the role of sphingolipids in the etiology of these disorders has not been established. The yeast model can be exploited to characterize the regulation of sphingolipid metabolism, the roles of specific sphingolipids in essential cell functions, and the global cellular consequences of perturbation of their synthesis. Because of the very high degree of conservation of function of these lipids between yeast and human cells, the data from studies in yeast can be used to generate testable hypotheses that can be experimentally addressed in appropriate mammalian systems. This knowledge has the potential to contribute to our understanding of the mechanisms underlying the pathophysiology of psychiatric disorders and to the development of new drugs that can be used in their treatment.

6. Project outline

The objective of the studies described in this thesis was to identify and characterize molecular targets of Valproic acid (VPA), a drug used for the treatment of BD, in order to gain insight into the therapeutic mechanism of action of the drug. Several pathways have been hypothesized to play a role in the therapeutic action of VPA, but the molecular mechanism is not understood. In order to accomplish this goal, I carried out the following studies:

Utilizing a genome-wide microarray analysis, I identified molecular targets and pathways that are affected by acute and chronic VPA treatment, as described in

Chapter 2. In this screen, I identified sphingolipid metabolism as a possible novel target of VPA, as further discussed in Chapters 3 and 4.

Studies in Chapter 3 focused on the chronic effects of VPA on sphingolipid metabolism. Chronic VPA-mediated inositol depletion increased expression of fatty acid elongases, which catalyze the synthesis of C24-C26 fatty acids utilized in ceramide synthesis. Consistent with this, phytoceramide with C24-C26 fatty acids were increased, and the expression of nutrient transporters decreased, inducing stress and activating the unfolded protein stress response (UPR) pathway. This study identified the UPR pathway as a possible new target of VPA, which could be important for the therapeutic action of the drug.

In experiments described in Chapter 4, I characterized the acute effects of VPA on sphingolipid metabolism. The gene *RSB1*, which encodes a transporter of the long chain bases (LCBs) dihydrosphingosine (DHS) and phytosphingosine (PHS), showed a striking high level of upregulation following acute VPA treatment. The *rsb1Δ* mutant exhibited sensitivity to PHS. This suggested that acute VPA causes accumulation of LCBs. In fact, I showed that *ino1Δ* starvation and acute VPA increase PHS levels, suggesting that VPA-mediated inositol depletion increases *de novo* synthesis of PHS.

LCB phosphates are lipid signaling molecules that are crucial for cell proliferation and differentiation. However, the role of these molecules in cellular functions is not understood. In the Appendix, I described a synthetic lethality screen with the major LCB kinase mutant, *lcb4Δ.* Among many synthetic interactions observed, an interesting category of mutants identified genes required for transport

from the endoplasmic reticulum (ER) to the Golgi, suggesting for the first time that LCB phosphates play a role in ER-Golgi transport.

The studies described in this dissertation identified sphingolipid metabolism as a potential new target of VPA, characterized effects of chronic and acute treatment of VPA on sphingolipid metabolism, and identified new genetic interactions with the sphingosine kinase, Lcb4. Many interesting questions emerged from my work and remain unanswered. Chapter 5 summarizes potential exciting directions for future studies.

CHAPTER 2 IDENTIFICATION OF GENES AND CELLULAR PATHWAYS AFFECTED BY ACUTE AND CHRONIC TREATMENT WITH VALPROIC ACID

INTRODUCTION

Bipolar disorder (BD) is a psychiatric disease characterized by recurrent manic and depressive episodes. The World Health Organization (Cheng et al., 2005) ranks BD as the sixth leading cause of disability worldwide. BD affects at least 1% of the population and leads to suicide in 15% of the cases (Bostwick and Pankratz, 2000). Valproic acid (VPA), an FDA approved drug used for the treatment of BD, is a short-branched chain fatty acid that was first synthesized by Burton in 1882. It was used as an organic solvent for about eighty years, and was serendipitously discovered to be an effective anticonvulsant (Meunier et al., 1963). VPA was first approved for the treatment of epilepsy in 1967, and since then has been used for the treatment of generalized and partial epilepsies. Later, VPA was found to be effective for the treatment BD (Calabresi et al., 2007; Emrich et al., 1981). VPA is effective in only 40-60% of BD cases and sometimes causes severe side effects, including hepatotoxicity and teratogenicity (Henry, 2003). The molecular targets underlying the therapeutic action of VPA are not known, nor are the underlying causes of the disease (Gould et al., 2004; Williams et al., 2002).

The inositol depletion hypothesis of the therapeutic action of drugs used for the treatment of BD was first proposed by Berridge, who showed that lithium inhibits inositol synthesis, possibly resulting in decreased inositol 1,4,5- triphosphate mediated signaling (Berridge, 1989). Our laboratory has shown that VPA, another drug used for the treatment of BD also decreases intracellular inositol in yeast and mammalian cells

(Vaden et al., 2001; Ye and Greenberg, 2015). The finding that both lithium and VPA, two structurally dissimilar drugs, cause inositol depletion suggests that this outcome may be important for the therapeutic action.

In yeast, VPA causes a decrease in intracellular inositol and a dramatic increase in expression of *INO1,* the gene encoding the enzyme catalyzing the rate-limiting step of *de novo* biosynthesis of inositol (Vaden et al., 2001). Interestingly, the drug exerts differential acute and chronic effects on *INO1* expression, although inositol depletion was apparent during both acute and chronic treatment (Ju and Greenberg, 2003). The expression of *INO1* decreased during acute treatment (30 minutes -1 hour), while chronic exposure to VPA resulted in increased expression of *INO1* (Ju and Greenberg, 2003). These data led me to speculate that acute and chronic treatment with VPA elicit differential effects on expression of target genes. I hypothesized that acute treatment affects the expression of signaling molecules while chronic treatment perturbs downstream pathways controlled by these signaling molecules.

In order to get a global picture of the effects of VPA, my study compared whole genome expression in response to acute and chronic treatment of VPA. To determine if the effect of VPA target gene expression was dependent on inositol depletion, the effects of VPA were determined in yeast cells grown in the presence or absence of inositol.

MATERIALS AND METHODS

VPA treatment

Wild type cells were pre-cultured in I+ (inositol-containing) medium, harvested, washed twice with sterile water, and grown in I+ medium until the cells reached the mid

log phase (A_{550}= 0.5). Cells were pelleted, washed twice with sterile water and inoculated in synthetic minimal I+ or I- to a final A_{550} of 0.05, and cultured until the cells reached the mid log phase (A_{550}= 0.5). Cells were then pelleted and suspended in fresh I+ or I- with or without 0.6 mM VPA and incubated for 30 minutes (acute treatment) or 5 hours (chronic treatment). Cells were pelleted and stored at -80°C. Each sample was processed in duplicate.

RNA extraction and microarray

Total RNA was isolated by hot phenol extraction (Kohrer and Domdey, 1991) and purified using an RNeasy kit from Qiagen. The quality of the RNA was determined using the Agilent 2100 Bioanalyzer. RNA was labeled using the Agilent Low Input Quick-Amp labeling kit (Agilent Technologies). Cy3 labeled cRNA was then hybridized to the 8x15K Agilent Yeast V2 Arrays (design ID 016322). Slides were scanned on an Agilent G2505B microarray scanner and the resulting image files were processed with Agilent Feature Extraction software (version 9.5.1). All procedures were carried out according to the manufacturer's protocols. Subsequent analysis was performed using the GeneSpring (v10.0) software. Microarray analysis was carried out at the Research Technology Support Facility in Michigan State University.

Data analysis

The Gene Spring 10.0 software package was used for data analysis. The fold change for each gene was calculated using the expression levels in the control (i.e. cells grown in medium without VPA under similar conditions) as the basal level. Genes that showed more than a two-fold change compared to the respective control were considered for further analysis. The gene list was subjected to a statistical Student T

test and genes were grouped based on their biological function using the GO Slim Mapper program available on the *Saccharomyces cerevisiae* genome database (SGD).

Validation by qRT-PCR

Total RNA was extracted using the hot phenol method (Kohrer and Domdey, 1991) and purified using an RNeasy mini plus kit (Qiagen, Valencia, CA). Complementary DNA (cDNA) was synthesized using the first strand cDNA synthesis kit from Roche Applied Science as described in the manufacturer's manuals. qRT-PCR reactions were done in a 20 µl volume reaction using Brilliant III Ultra-Faster SYBR Green qPCR master mix (Agilent Technologies, Santa Clara, CA). Each reaction was done in triplicate. RNA levels were normalized to *ACT1* levels (internal control). Relative values of mRNA transcripts are shown as fold change relative to that of the indicated controls. Primers were validated as suggested in the Methods and Applications Guide (Agilent Technologies). All primers used in this study had primer efficiency between 85 and 105%. Optimal primer concentrations were determined, and primer specificity of a single product was monitored by a melt curve following the amplification reaction. PCR reactions were initiated at 95°C for 10 min for denaturation followed by 40 cycles consisting of 30 s at 95°C and 60 s at 55°C.

RESULTS AND DISCUSSION

The microarray analysis confirmed our earlier findings that acute and chronic VPA exhibit differential expression of *INO1*, which showed downregulation with acute VPA and upregulation with chronic VPA (Ju and Greenberg, 2003). The findings suggested intriguing possible targets of VPA, including sphingolipid metabolism, the unfolded protein response (UPR) pathway, and glycolysis, as discussed below. This

study identified sphingolipid metabolism for the first time as a target of VPA and showed that VPA exerts differential effects on this pathway. Chronic VPA-mediated inositol depletion upregulates the UPR pathway, suggesting that this pathway may be a new target for future drug development. In addition, this screen suggests that VPA increases glycolytic flux, which suggests that it may inhibit inositol synthesis by channeling the common substrate, glucose-6-phosphate, towards glycolysis.

a. Sphingolipid metabolism

This study revealed for the first time that sphingolipid metabolism is altered by VPA. Upon acute exposure, the gene that was most strikingly upregulated was *RSB1,* a long chain base (LCB) transporter (Table 2.1), which showed a 158-fold upregulation in I- and 108-fold upregulation in I+. In the studies described in Chapter 4, I characterized the acute effects of VPA and showed that acute VPA-mediated inositol depletion increases the levels of PHS (phytosphingosine), a signaling lipid molecule. In response to chronic VPA, *FEN1* and *SUR4*, which code for fatty acid elongases, exhibited about 2-fold upregulation in both I+ and I- (Table 2.2). Further studies show that chronic VPA-mediated inositol depletion increases the intracellular levels of ceramide, which induces the UPR pathway, suggesting a possible new target for drug development (Chapter 3).

b. The UPR pathway

As described in Chapter 3, chronic VPA induces the UPR pathway by depleting intracellular inositol. Interestingly, the microarray data revealed that ER chaperone proteins *KAR2, JEM1, LHS1, EUG1, PDI1,* and *SEC63*, markers of the UPR pathway, exhibited 2- to 5-fold increased expression with chronic VPA treatment in I- medium, but not in I+, further supporting that UPR induction was likely due to inositol depletion.

Previously, Shulin Ju showed that overexpression of Ubi4, which ubiquitinylates misfolded proteins and targets them for degradation, conferred VPA resistance. These studies indicate that VPA could induce the UPR pathway by inhibiting degradation of unfolded proteins (PhD thesis 2005).

Several studies support that the UPR dysfunction might be involved in the pathophysiology of BD. Expression of ER stress proteins such as GRP78, GRP94 and calreticulin are upregulated in rat brain samples in response to chronic treatment with VPA (Shao et al., 2005). In addition, the UPR pathway induction is impaired in lymphoblastoid cell lines derived from bipolar patients (So et al., 2007). The expression of genes coding for proteins of the ubiquitin–proteasome system that ubiqutinlyate and mark misfolded proteins for degradation is greatly decreased in the hippocampus of bipolar patients (Hayashi et al., 2009). In summary, these studies suggest that the antibipolar drugs induce the UPR pathway by depleting intracellular inositol levels.

c. Glycolysis

Glycolysis genes *ERR2, ENO2, HXK2, LAT1, ENO1, PFK1, TPI1, ERR3, ERR1, PFK2, GPM1* showed increased expression with chronic VPA treatment, suggesting that chronic VPA may increase glycolysis flux. Several other studies in our laboratory support an effect of VPA on glycolysis. Shi et al. (2005) showed that VPA increases accumulation of two glycolytic intermediates, dihydroxyacetone phosphate (DHAP) and glyceraldehyde3-phosphate (G-3-P), which inhibit the activity of MIPS, decreasing inositol synthesis. In addition, a cDNA overexpression screen showed that overexpression of glycolytic genes increased sensitivity to VPA (Rania Deranieh, 2014). Based on these data, as well as his finding that VPA causes increased production of

ethanol (unpublished), Michael Salsaa' hypothesized that VPA affects the metabolic flux of glucose-6-P to glycolysis, thereby decreasing inositol synthesis and causing inositol depletion. These studies suggest that inhibiting inositol synthesis may be a regulatory mechanism whereby cells respond to an increased requirement for glycolysis.

d. Other pathways

After acute VPA treatment, 597 genes exhibited more than a two-fold change in gene expression in I-, and 545 genes in I+ (Table 2.1). A functional classification of genes showing more than 2-fold change in response to VPA using GO SLIM MAPPER (SGD) is shown in Fig. 2.3 (VPA I-) and Fig. 2.4 (VPA I+). Genes required for amino acid metabolism, transmembrane transport, lipid metabolism, protein modification, and the cell cycle, exhibited a significant change in expression with acute VPA. Further analysis showed that 129 genes were upregulated only in I-, 136 only in I+, and 273 in both I+ and I-. 129 genes were down regulated only in I-, 70 only in I+, and 66 genes in both I+ and I- (Fig. 2.1). The relatively large number of that genes exhibited altered expression only in I- likely represent pathways that are regulated by inositol. In addition, a large number of genes exhibited upregulation in both I+ and I- in response to VPA, but the upregulation was higher in I- than in I+, suggesting that expression is partially dependent on inositol.

After chronic VPA treatment, 413 genes exhibited more than a two-fold change in gene expression when grown in I-, and 324 genes in I+ (Table 2.2). Classification of genes showing more than 2 fold change using GO SLIM MAPPER (SGD) is shown in Fig. 2.5 (VPA I-) and 2.6 (VPA I+). The highest number of genes belonged to transport, cellular amino acid metabolic process, and cell cycle in I-, and cell cycle, DNA repair,

transport, and protein modification in I+. Further analysis showed that 108 genes were upregulated only in I-, 119 only in I+, and 60 in both I+ and I- and 184 genes were downregulated only in I-, 84 only in I+ and 64 genes in both, I+ and I- (Fig. 2.2).

In studies described in Chapters 3 and 4, I characterized the effects of VPA on sphingolipid metabolism. This study unraveled possible novel targets of VPA that can be further used to develop newer and better drugs for the treatment of BD.

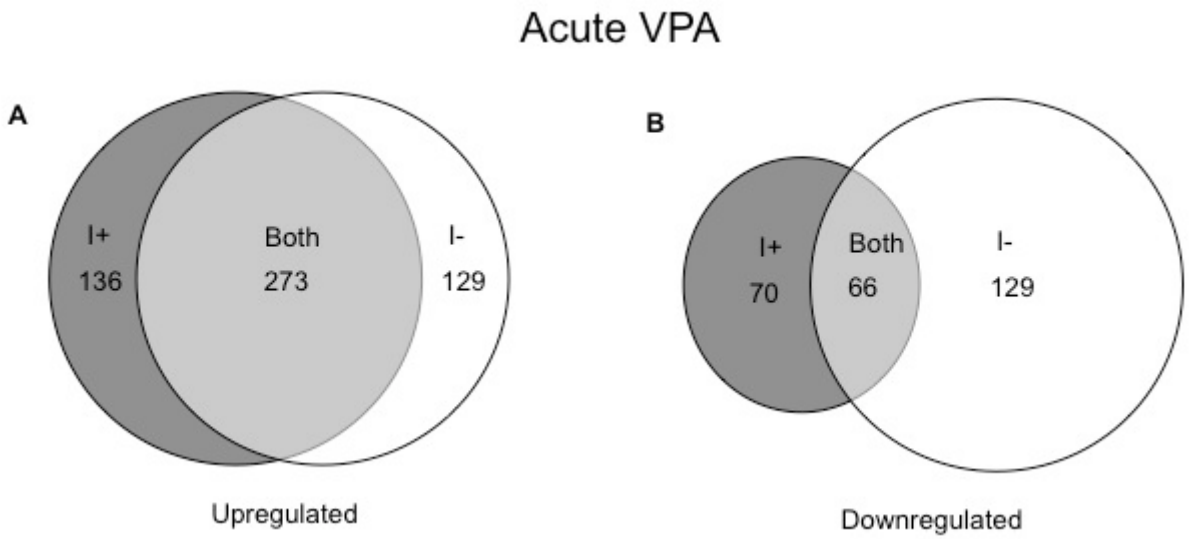

Figure 2.1. Differential effects on the regulation of gene expression in response to acute VPA treatment in the presence and absence of inositol (I). Genes showing differential expression (more than 2 fold), upregulated (A) and downregulated (B) in response to acute VPA treatment.

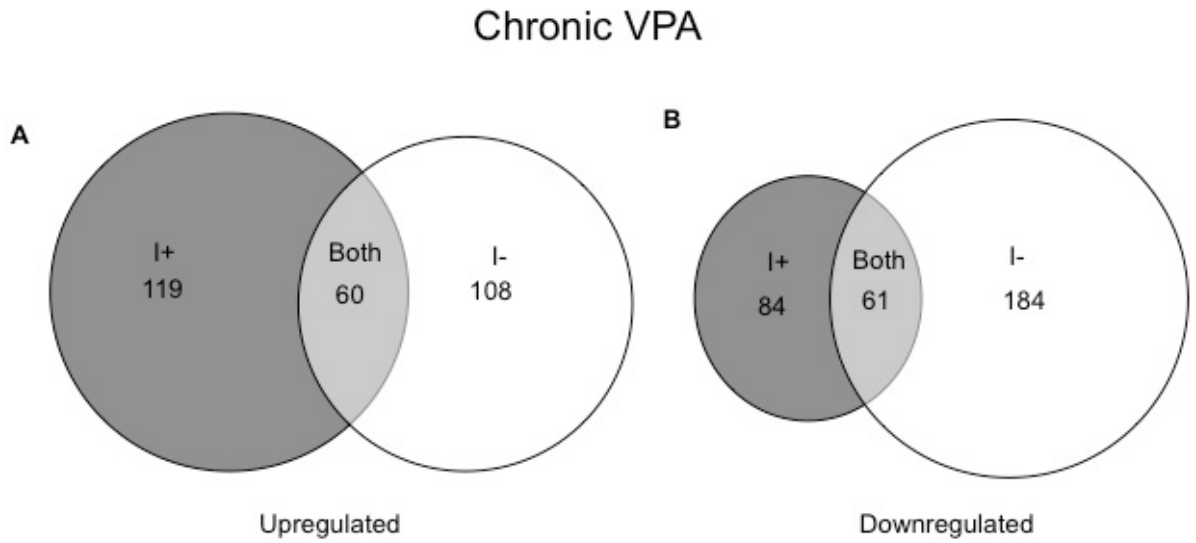

Figure 2.2. Differential effects on the regulation of gene expression in response to chronic VPA treatment in the presence and absence of inositol (I). Genes showing differential expression (more than 2 fold), upregulated (A) and downregulated (B) in response to chronic VPA treatment.

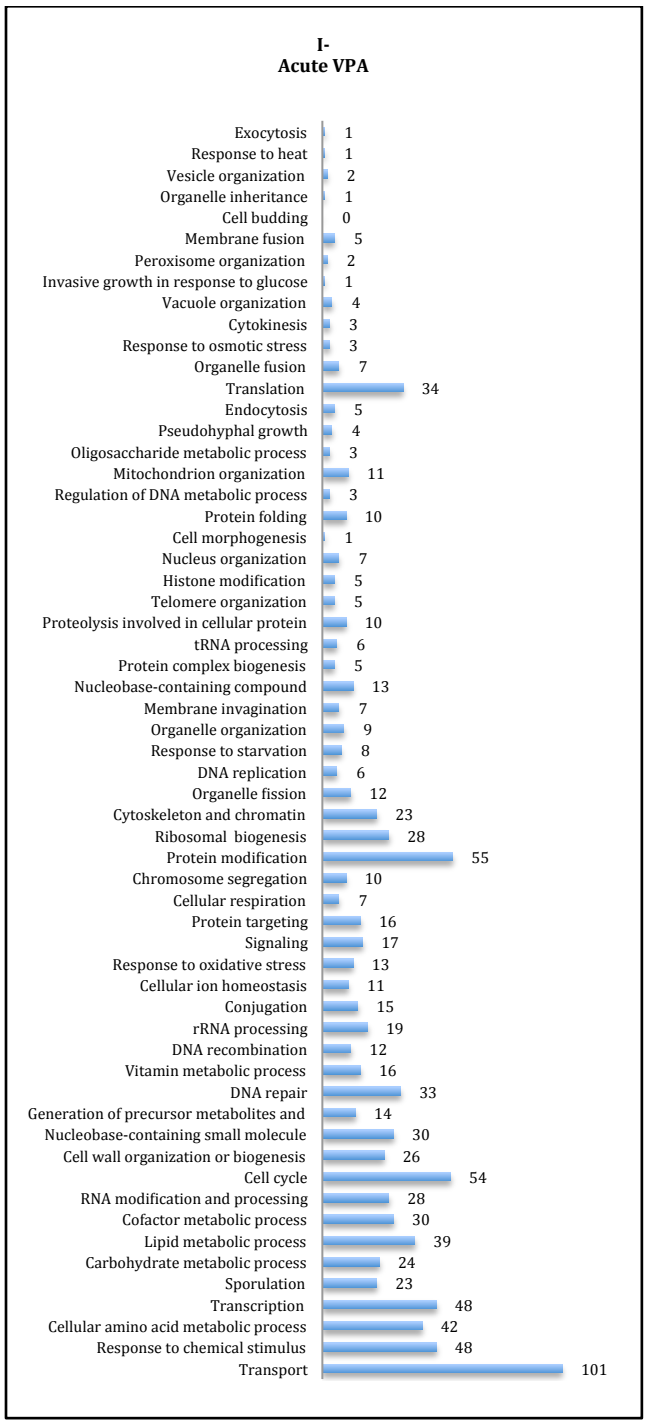

Figure 2.3. Genes that show more than 2 fold change in response to acute VPA treatment in I-

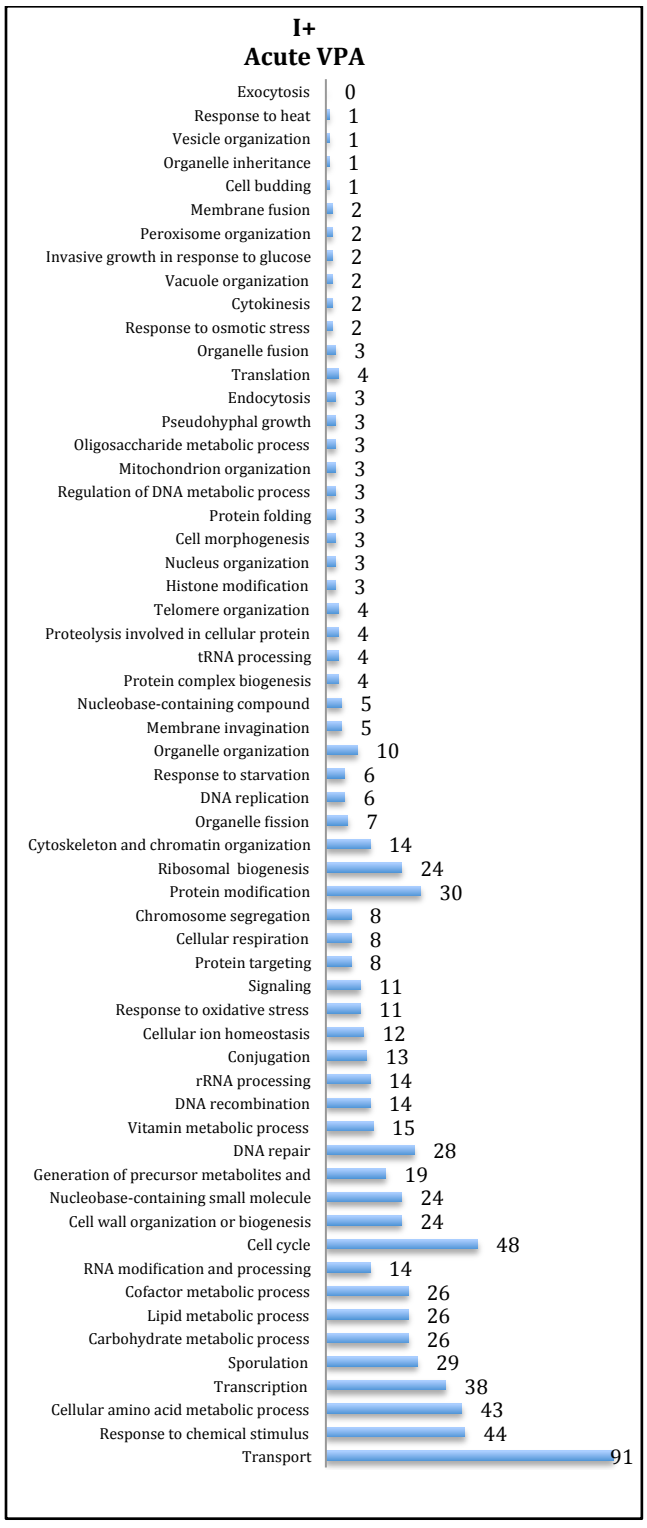

Figure 2.4. Genes that show more than 2 fold change in response to acute VPA treatment in I+

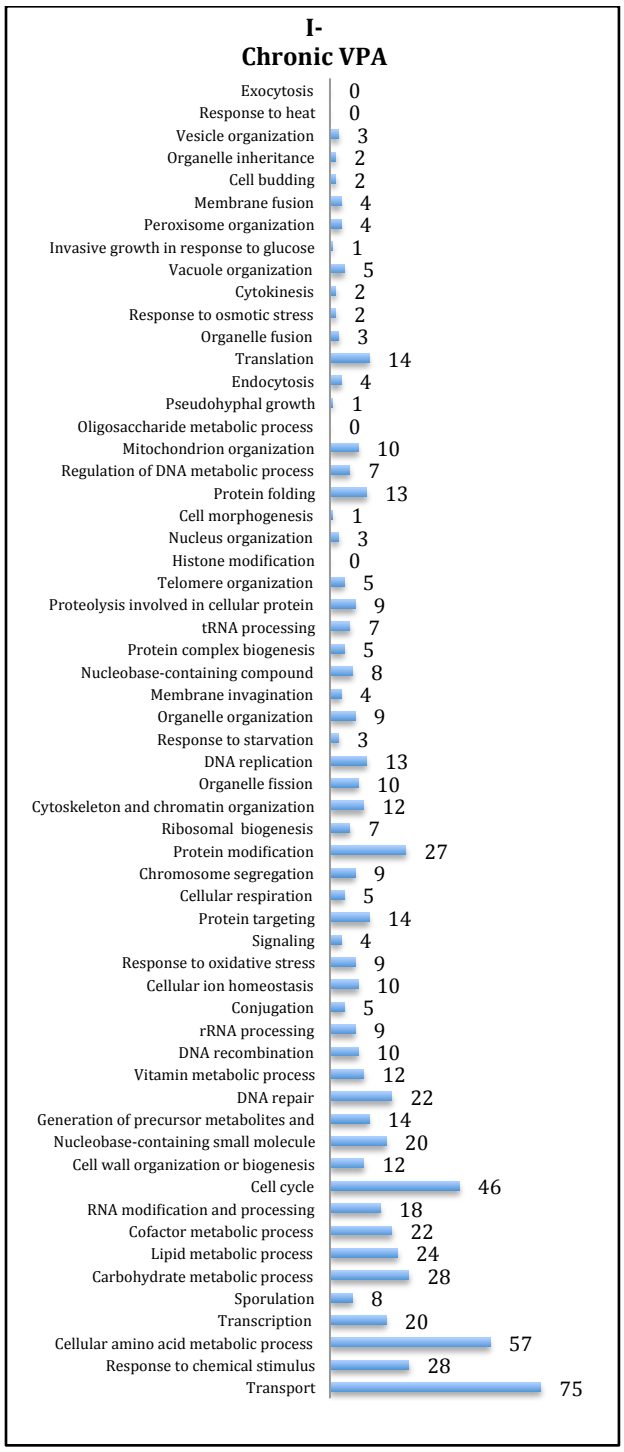

Figure 2.5. Genes that show more than 2 fold change in response to chronic VPA treatment in I-

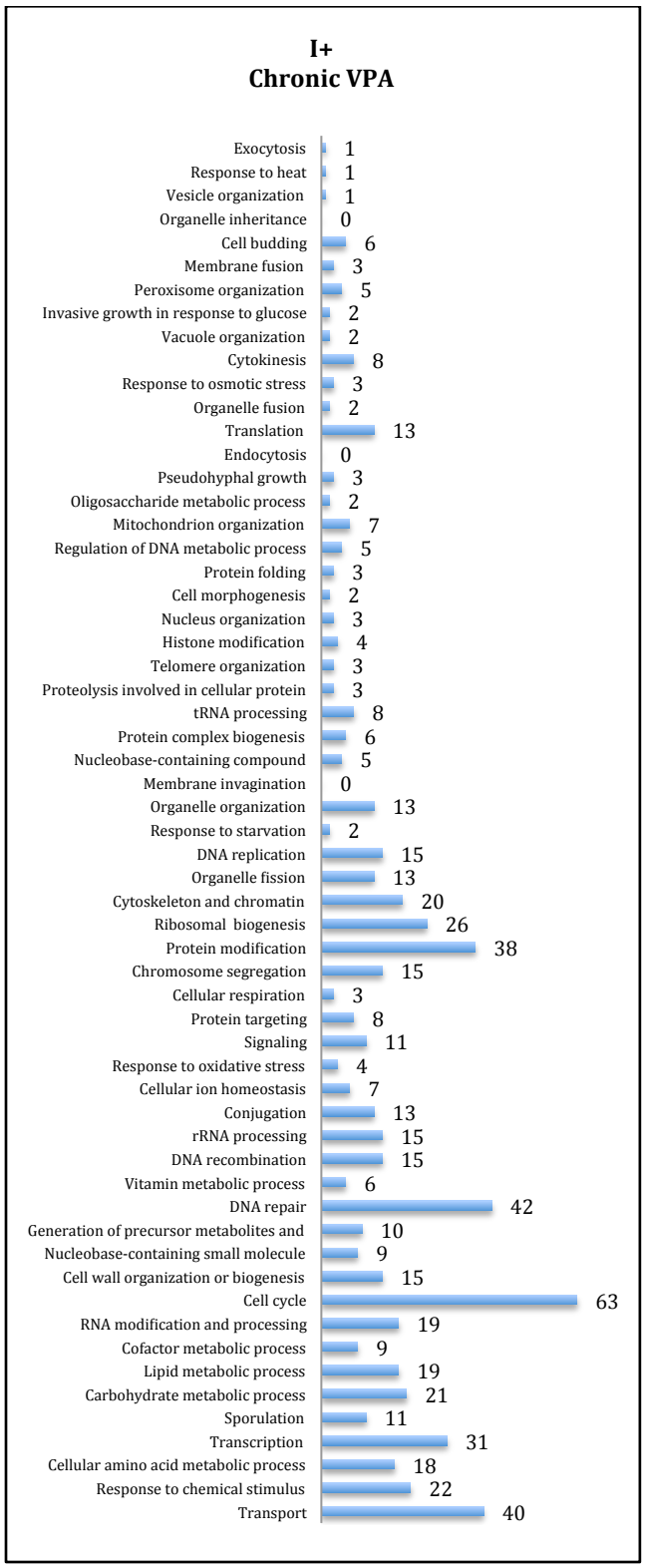

Figure 2.6. Genes that show more than 2 fold change in response to chronic VPA treatment in I+

Table 2.1. Genes showing more than 2 fold change in response to acute VPA treatment. (Gene descriptions are from Saccharomyces Genome Database).

	Gene name	Description	Fold change VPA+/VPA-	
			Inositol+	Inositol-
YOR049C	*RSB1*	Suppressor of sphingoid long chain base (LCB) sensitivity of an LCB-lyase mutation	108.14	158.2
YPL033C	*SRL4*	Protein of unknown function	70.44	68.69
YNR002C	*ATO2*	Putative transmembrane protein involved in export of ammonia	56	50.29
YAL018C	*YAL018C*	Putative protein of unknown function	45.39	25.76
YPL058C	*PDR12*	Plasma membrane ATP-binding cassette (ABC) transporter	36.14	34.12
YGR035C	*YGR035C*	Putative protein of unknown function, potential Cdc28p substrate	33.96	25.4
YCL026C-A	*FRM2*	Protein of unknown function, involved in the integration of lipid signaling pathways with cellular homeostasis	20.19	21.39
YGR224W	*AZR1*	Plasma membrane transporter of the major facilitator superfamily, involved in resistance to azole drugs such as ketoconazole and fluconazole	18.78	−
YPR001W	*CIT3*	Dual specificity mitochondrial citrate and methylcitrate synthase	18.78	31.46
YOR153W	*PDR5*	Plasma membrane ATP-binding cassette (ABC) transporter	18.78	19.34
YLR346C	*YLR346C*	Putative protein of unknown function found in mitochondria	18.78	13.93
YHR126C	*ANS1*	Putative protein of unknown function; transcription dependent upon Azf1p	18.78	−
YMR279C	*YMR279C*	Putative paralog of ATR1, but not required for boron tolerance	18.78	−
YBR040W	*FIG1*	Integral membrane protein required for efficient mating	18.78	−
YIR017C	*MET28*	Basic leucine zipper (bZIP) transcriptional activator in the Cbf1p-Met4p-Met28p complex, participates in the regulation of sulfur metabolism	11.35	13.04
YIL056W	*VHR1*	Transcriptional activator	10.74	13.74
YGR213C	*RTA1*	Protein involved in 7-aminocholesterol resistance	10.17	7.07
YLR099C	*ICT1*	Lysophosphatidic acid acyltransferase	10.06	9.41
YGL205W	*POX1*	Fatty-acyl coenzyme A oxidase, involved in the fatty acid beta-oxidation pathway; localized to the	9.55	6.08

		peroxisomal matrix		
YHR139C	*SPS100*	Protein required for spore wall maturation	9.40	19.68
YBL005W	*PDR3*	Transcriptional activator of the pleiotropic drug resistance network	9.30	10.21
YNR058W	*BIO3*	7,8-diamino-pelargonic acid aminotransferase (DAPA	8.54	10.49
YHR140W	*YHR140W*	Putative integral membrane protein of unknown function	8.05	9.14
YKL217W	*JEN1*	Lactate transporter	8.03	12.70
YBR047W	*FMP23*	Putative protein of unknown function	8.01	8.99
YBR045C	*GIP1*	Meiosis-specific regulatory subunit of the Glc7p protein phosphatase	7.96	8.62
YKL132C	*RMA1*	Putative dihydrofolate synthetase	7.91	8.92
YAL061W	*BDH2*	Putative medium-chain alcohol dehydrogenase with similarity to BDH1	7.89	12.72
YER024W	*YAT2*	Carnitine acetyltransferase	7.76	9.32
YGL184C	*STR3*	Cystathionine beta-lyase, converts cystathionine into homocysteine	7.71	−
YPR078C	*YPR078C*	Putative protein of unknown function	7.54	−
YOR100C	*CRC1*	Mitochondrial inner membrane carnitine transporter	7.41	13.67
YOR365C	*YOR365C*	Putative protein of unknown function	7.40	4.16
YLL028W	*TPO1*	Polyamine transporter that recognizes spermine, putrescine, and spermidine	7.14	9.33
YDR446W	*ECM11*	Non-essential protein apparently involved in meiosis	7.04	−
YBR180W	*DTR1*	Putative dityrosine transporter	6.86	3.34
YLR307W	*CDA1*	Chitin deacetylase	6.74	6.37
YOR388C	*FDH1*	NAD(+)-dependent formate dehydrogenase	6.66	2.83
YOR152C	*YOR152C*	Putative protein of unknown function	6.63	7.17
YBR250W	*SPO23*	Protein of unknown function	6.55	7.63
YGR281W	*YOR1*	Plasma membrane ATP-binding cassette (ABC) transporter	6.53	5.75
YHL042W	*YHL042W*	Putative protein of unknown function; member of the DUP380 subfamily of conserved, often subtelomerically-encoded proteins	6.49	−
YNR056C	*BIO5*	Putative transmembrane protein involved in the biotin biosynthesis pathway	6.40	8.21

YDR011W	*SNQ2*	Plasma membrane ATP-binding cassette (ABC) transporter	6.39	7.67
YOR351C	*MEK1*	Meiosis-specific serine/threonine protein kinase	6.19	5.64
YLR348C	*DIC1*	Mitochondrial dicarboxylate carrier	6.11	6.91
YMR322C	*SNO4*	Possible chaperone and cysteine protease	6.09	6.62
YPL280W	*HSP32*	Possible chaperone and cysteine protease	5.89	6.13
YKR015C	*YKR015C*	Putative protein of unknown function	5.86	6.24
YFR023W	*PES4*	Poly(A) binding protein, suppressor of DNA polymerase epsilon mutation, similar to Mip6p	5.85	7.40
YNR044W	*AGA1*	Anchorage subunit of a-agglutinin of a-cells, highly O-glycosylated protein with N-terminal secretion signal and C-terminal signal for addition of GPI anchor to cell wall, linked to adhesion subunit Aga2p via two disulfide bonds	5.77	−
YPL088W	*YPL088W*	Putative aryl alcohol dehydrogenase	5.77	5.78
YOL151W	*GRE2*	3-methylbutanal reductase and NADPH-dependent methylglyoxal reductase (D-lactaldehyde dehydrogenase	5.76	4.38
YHR214C-D	*YHR214C-D*	Putative protein of unknown function	5.75	5.21
YNL231C	*PDR16*	Phosphatidylinositol transfer protein (PITP) controlled by the multiple drug resistance regulator Pdr1p	5.73	6.13
YCR020C	*PET18*	Protein of unknown function, has weak similarity to proteins involved in thiamin metabolism; expression is induced in the absence of thiamin	5.62	6.23
YGL015C	*YGL015C*	Putative protein of unknown function	5.60	−
YDR406W	*PDR15*	Plasma membrane ATP binding cassette (ABC) transporter	5.55	7.67
YOR011W	*AUS1*	Transporter of the ATP-binding cassette family	5.47	7.02
YMR017W	*SPO20*	Meiosis-specific subunit of the t-SNARE complex	5.46	4.49
YGR087C	*PDC6*	Minor isoform of pyruvate decarboxylase	5.35	5.74
YJL160C	*YJL160C*	Putative protein of unknown function	5.20	6.79
YBR019C	*GAL10*	UDP-glucose-4-epimerase	5.19	−

YKL070W	*YKL070W*	Putative protein of unknown function	5.19	−
YER106W	*MAM1*	Monopolin, kinetochore associated protein involved in chromosome attachment to meiotic spindle	5.18	−
YBR018C	*GAL7*	Galactose-1-phosphate uridyl transferase	5.17	6.81
YCL027W	*FUS1*	Membrane protein localized to the shmoo tip	5.04	5.61
YPL250C	*ICY2*	Protein of unknown function	5.04	−
YNL036W	*NCE103*	Carbonic anhydrase	4.97	5.35
YAL064W	*YAL064W*	Protein of unknown function	4.95	4.87
YDR536W	*STL1*	Glycerol proton symporter of the plasma membrane	4.80	−
YER185W	*PUG1*	Plasma membrane protein with roles in the uptake of protoprophyrin IX and the efflux of heme	4.78	−
YNL279W	*PRM1*	Pheromone-regulated multispanning membrane protein involved in membrane fusion during mating	4.75	6.22
YDL138W	*RGT2*	Plasma membrane high glucose sensor that regulates glucose transport	4.72	5.59
YML066C	*SMA2*	Meiosis-specific prospore membrane protein	4.70	−
YDL114W	*YDL114W*	Putative protein of unknown function with similarity to acyl-carrier-protein reductases	4.70	3.18
YDR402C	*DIT2*	N-formyltyrosine oxidase	4.61	4.80
YJL037W	*IRC18*	Putative protein of unknown function	4.60	7.55
YIL060W	*YIL060W*	Putative protein of unknown function	4.41	3.74
YNR070W	*PDR18*	Putative transporter of the ATP-binding cassette (ABC) family, implicated in pleiotropic drug resistance	4.39	5.96
YHL043W	*ECM34*	Putative protein of unknown function	4.37	−
YAR029W	*YAR029W*	Member of DUP240 gene family but contains no transmembrane domains	4.34	4.15
YDR242W	*AMD2*	Putative amidase	4.32	−
YOR186W	*YOR186W*	Putative protein of unknown function	4.31	5.12
YKL071W	*YKL071W*	Putative protein of unknown function	4.29	−
YMR042W	*ARG80*	Transcription factor involved in regulation of arginine-responsive genes; acts with Arg81p and Arg82p	4.27	−

YPL264C	*YPL264C*	Putative membrane protein of unknown function	4.24	5.24
YGR059W	*SPR3*	Sporulation-specific homolog of the yeast CDC3/10/11/12 family of bud neck microfilament genes	4.24	−
YML116W	*ATR1*	Multidrug efflux pump of the major facilitator superfamily, required for resistance to aminotriazole and 4-nitroquinoline-N-oxide	4.22	5.05
YDL243C	*AAD4*	Putative aryl-alcohol dehydrogenase with similarity to P. chrysosporium aryl-alcohol dehydrogenase	4.21	−
YMR018W	*YMR018W*	Putative protein of unknown function with similarity to human PEX5Rp (peroxin protein 5 related protein)	4.18	5.45
YCR061W	*YCR061W*	Protein of unknown function	4.17	3.78
YIL164C	*NIT1*	Nitrilase, member of the nitrilase branch of the nitrilase superfamily	4.17	5.58
YJL071W	*ARG2*	Acetylglutamate synthase	4.16	5.35
YFL003C	*MSH4*	Protein involved in meiotic recombination	4.13	6.05
YNL277W	*MET2*	L-homoserine-O-acetyltransferase	4.13	7.01
YPR167C	*MET16*	3'-phosphoadenylsulfate reductase, reduces 3'-phosphoadenylyl sulfate to adenosine-3',5'-bisphosphate and free sulfite using reduced thioredoxin as cosubstrate, involved in sulfate assimilation and methionine metabolism	4.11	3.97
YER187W	*YER187W*	Putative protein of unknown function; induced in respiratory-deficient cells	4.04	7.16
YDR043C	*NRG1*	Transcriptional repressor that recruits the Cyc8p-Tup1p complex to promoters	4.03	3.73
YEL057C	*YEL057C*	Protein of unknown function involved in telomere maintenance	4.02	−
YDR403W	*DIT1*	Sporulation-specific enzyme required for spore wall maturation	4.01	4.74
YDR317W	*HIM1*	Protein of unknown function involved in DNA repair	4.00	−
YNL117W	*MLS1*	Malate synthase, enzyme of the glyoxylate cycle	4.00	11.00
YDL170W	*UGA3*	Transcriptional activator necessary for gamma-aminobutyrate (GABA)-dependent induction of GABA genes	3.99	4.85
YHR015W	*MIP6*	Putative RNA-binding protein, interacts with Mex67p, which is a component of the nuclear pore	3.99	4.00

		involved in nuclear mRNA export		
YLL056C	*YLL056C*	Putative protein of unknown function, transcription is activated by paralogous transcription factors Yrm1p and Yrr1p and genes involved in pleiotropic drug resistance (PDR	3.98	5.77
YOR393W	*ERR1*	Protein of unknown function, has similarity to enolases	3.96	−
YDL054C	*MCH1*	Protein with similarity to mammalian monocarboxylate permeases	3.96	4.43
YML083C	*YML083C*	Putative protein of unknown function	3.96	−
YJL089W	*SIP4*	C6 zinc cluster transcriptional activator that binds to the carbon source-responsive element (CSRE) of gluconeogenic genes	3.95	−
YPL281C	*ERR2*	Protein of unknown function, has similarity to enolases	3.93	4.29
YAR031W	*PRM9*	Pheromone-regulated protein with 3 predicted transmembrane segments and an FF sequence, a motif involved in COPII binding; member of DUP240 gene family	3.92	4.00
YJR155W	*AAD10*	Putative aryl-alcohol dehydrogenase with similarity to P. chrysosporium aryl-alcohol dehydrogenase	3.90	4.86
YGR251W	*YGR251W*	Essential protein required for maturation of 18S rRNA; green fluorescent protein (GFP)-fusion protein localizes to both the nucleus and the nucleolus	3.80	4.43
YDR223W	*CRF1*	Transcriptional corepressor involved in repression of ribosomal protein (RP) gene transcription via the TOR signaling pathway which promotes accumulation of Crf1p in the nucleus	3.80	−
YPR007C	*REC8*	Meiosis-specific component of sister chromatid cohesion complex	3.80	4.53
YNR071C	*YNR071C*	Putative protein of unknown function	3.79	4.25
YMR102C	*YMR102C*	Protein of unknown function	3.75	5.19
YIL165C	*YIL165C*	Putative protein of unknown function	3.74	5.28
YOR192C	*THI72*	Transporter of thiamine or related compound	3.71	4.03
YER085C	*YER085C*	Putative protein of unknown function	3.70	−
YML042W	*CAT2*	Carnitine acetyl-CoA transferase present in both mitochondria and	3.70	5.11

		peroxisomes		
YOL162W	*YOL162W*	Putative protein of unknown function	3.68	−
YJR130C	*STR2*	Cystathionine gamma-synthase, converts cysteine into cystathionine	3.64	4.17
YER174C	*GRX4*	Hydroperoxide and superoxide-radical responsive glutathione-dependent oxidoreductase	3.63	−
YDL020C	*RPN4*	Transcription factor that stimulates expression of proteasome genes	3.62	3.31
YMR118C	*YMR118C*	Protein of unknown function with similarity to succinate dehydrogenase cytochrome b subunit; YMR118C is not an essential gene	3.61	−
YLR004C	*THI73*	Putative plasma membrane permease proposed to be involved in carboxylic acid uptake and repressed by thiamine	3.61	4.43
YNL128W	*TEP1*	Homolog of human tumor suppressor gene PTEN/MMAC1/TEP1 that has lipid phosphatase activity and is linked to the phosphatidylinositol signaling pathway	3.61	3.39
YLL057C	*JLP1*	Fe(II)-dependent sulfonate/alpha-ketoglutarate dioxygenase	3.60	5.09
YOL024W	*YOL024W*	Putative protein of unknown function; predicted to have thiol-disulfide oxidoreductase active site	3.58	3.33
YDR534C	*FIT1*	Mannoprotein that is incorporated into the cell wall via a glycosylphosphatidylinositol (GPI) anchor	3.57	3.26
YOR269W	*PAC1*	Protein involved in nuclear migration, part of the dynein/dynactin pathway	3.56	4.21
YDR218C	*SPR28*	Sporulation-specific homolog of the yeast CDC3/10/11/12 family of bud neck microfilament genes	3.56	−
YER176W	*ECM32*	DNA dependent ATPase/DNA helicase belonging to the Dna2p- and Nam7p-like family of helicases that is involved in modulating translation termination	3.54	4.33
YNL095C	*YNL095C*	Putative protein of unknown function predicted to contain a transmembrane domain	3.52	4.36
YMR195W	*ICY1*	Protein of unknown function	3.52	4.64
YCR104W	*PAU3*	Member of the seripauperin	3.50	−

		multigene family encoded mainly in subtelomeric regions, active during alcoholic fermentation		
YOR391C	*HSP33*	Possible chaperone and cysteine protease with similarity to E. coli Hsp31 and S. cerevisiae Hsp31p, Hsp32p, and Sno4p	3.49	4.02
YEL048C	*TCA17*	Protein that interacts with subunits of the TRAPP complex and may play a role its assembly or stability	3.48	−
YLL046C	*RNP1*	Ribonucleoprotein that contains two RNA recognition motifs (RRM)	3.48	4.58
YKL187C	*YKL187C*	Putative protein of unknown function	3.45	−
YMR323W	*ERR3*	Protein of unknown function, has similarity to enolases	3.43	−
YHR022C	*YHR022C*	Putative protein of unknown function	3.41	3.86
YJR120W	*YJR120W*	Protein of unknown function	3.39	2.94
YNR068C	*YNR068C*	Putative protein of unknown function	3.39	3.96
YGR144W	*THI4*	Thiazole synthase	3.37	3.30
YDL245C	*HXT15*	Protein of unknown function with similarity to hexose transporter family members, expression is induced by low levels of glucose and repressed by high levels of glucose	3.36	−
YNL269W	*BSC4*	Protein of unknown function	3.35	4.07
YHL044W	*YHL044W*	Putative integral membrane protein	3.35	3.67
YBR104W	*YMC2*	Mitochondrial protein, putative inner membrane transporter with a role in oleate metabolism and glutamate biosynthesis	3.35	4.69
YGR142W	*BTN2*	v-SNARE binding protein that facilitates specific protein retrieval from a late endosome to the Golgi	3.34	3.36
YIL037C	*PRM2*	Pheromone-regulated protein	3.32	4.13
YJL106W	*IME2*	Serine/threonine protein kinase involved in activation of meiosis,	3.32	3.93
YCR045C	*RRT12*	Probable subtilisin-family protease with a role in formation of the dityrosine layer of spore walls	3.32	−
YHR048W	*YHK8*	Presumed antiporter of the DHA1 family of multidrug resistance transporters	3.30	−
YMR325	*PAU19*	Protein of unknown function	3.28	−

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YOR394W	*PAU21*	Protein of unknown function	3.28	−
YJL077C	*ICS3*	Protein of unknown function	3.28	−
YPL282C	*PAU22*	Protein of unknown function	3.27	−
YPL095C	*EEB1*	Acyl-coenzymeA:ethanol O-acyltransferase	3.26	2.03
YGL192W	*IME4*	Probable mRNA N6-adenosine methyltransferase	3.26	−
YDR076W	*RAD55*	Protein that stimulates strand exchange by stabilizing the binding of Rad51p to single-stranded DNA	3.24	3.44
YNL211C	*YNL211C*	Putative protein of unknown function	3.23	3.94
YBL103C	*RTG3*	Basic helix-loop-helix-leucine zipper (bHLH/Zip) transcription factor that forms a complex with another bHLH/Zip protein, Rtg1p, to activate the retrograde (RTG) and TOR pathways	3.23	4.27
YAL067C	*SEO1*	Putative permease, member of the allantoate transporter subfamily of the major facilitator superfamily	3.23	−
YCR099C	*YCR099C*	Putative protein of unknown function	3.22	4.73
YCR100C	*YCR100C*	Putative protein of unknown function	3.22	4.53
YNR066C	*YNR066C*	Putative membrane-localized protein of unknown function	3.22	3.85
YOR273C	*TPO4*	Polyamine transport protein, recognizes spermine, putrescine, and spermidine	3.22	2.39
YNL270C	*ALP1*	Arginine transporter	3.21	3.95
YDL214C	*PRR2*	Serine/threonine protein kinase that inhibits pheromone induced signalling downstream of MAPK, possibly at the level of the Ste12p transcription factor	3.20	2.83
YDR114C	*YDR114C*	Putative protein of unknown function	3.19	−
YDR256C	*CTA1*	Catalase A, breaks down hydrogen peroxide in the peroxisomal matrix formed by acyl-CoA oxidase (Pox1p) during fatty acid beta-oxidation	3.18	−
YML007C-A	*YML007C-A*	Putative protein of unknown function	3.18	−
YOR130C	*ORT1*	Ornithine transporter of the mitochondrial inner membrane, exports ornithine from mitochondria	3.17	3.73

		as part of arginine biosynthesis		
YPL201C	*YIG1*	Protein that interacts with glycerol 3-phosphatase and plays a role in anaerobic glycerol production	3.16	4.15
YFR030W	*MET10*	Subunit alpha of assimilatory sulfite reductase	3.16	3.82
YOR302W	*YOR302W*	CPA1 uORF, Arginine attenuator peptide	3.13	3.08
YIR041W	*PAU15*	Protein of unknown function	3.13	−
YJR078W	*BNA2*	Putative tryptophan 2,3-dioxygenase or indoleamine 2,3-dioxygenase, required for de novo biosynthesis of NAD from tryptophan via kynurenine	3.10	−
YFR022W	*ROG3*	Protein that binds the ubiquitin ligase Rsp5p via its 2 PY motifs	3.09	3.55
YBR148W	*YSW1*	Protein required for normal prospore membrane formation	3.09	4.11
YCR023C	*YCR023C*	Vacuolar membrane protein of unknown function	3.08	3.48
YIR018W	*YAP5*	Basic leucine zipper (bZIP) transcription factor	3.07	−
YLL053C	*YLL053C*	Putative protein; in the Sigma 1278B strain background YLL053C is contiguous with AQY2 which encodes an aquaporin	3.06	2.56
YOR339C	*UBC11*	Ubiquitin-conjugating enzyme most similar in sequence to Xenopus ubiquitin-conjugating enzyme E2-C	3.06	2.94
YLR164W	*YLR164W*	Mitochondrial inner membrane of unknown function	3.05	3.80
YNR060W	*FRE4*	Ferric reductase	3.04	−
YPL188W	*POS5*	Mitochondrial NADH kinase	3.03	3.59
YOR328W	*PDR10*	ATP-binding cassette (ABC) transporter	3.03	3.47
YNL116W	*DMA2*	Protein involved in ubiquitination	3.01	3.39
YLL052C	*AQY2*	Water channel that mediates the transport of water across cell membranes, only expressed in proliferating cells, controlled by osmotic signals, may be involved in freeze tolerance	3.01	2.45
YNL311C	*YNL311C*	F-box protein of unknown function predicted to be part of an SCF ubiquitin protease complex	2.99	3.55
YAL037W	*YAL037W*	Putative protein of unknown function	2.96	3.46
YAR020C	*PAU7*	Member of the seripauperin multigene family, active during	2.96	−

		alcoholic fermentation, regulated by anaerobiosis, inhibited by oxygen		
YKR097W	*PCK1*	Phosphoenolpyruvate carboxykinase	2.95	2.76
YKL178C	*STE3*	Receptor for a factor pheromone	2.95	−
YHR044C	*DOG1*	2-deoxyglucose-6-phosphate phosphatase,	2.95	2.13
YBR046C	*ZTA1*	NADPH-dependent quinone reductase	2.94	3.94
YNR065C	*YNR065C*	Protein of unknown function	2.93	−
YNR057C	*BIO4*	Dethiobiotin synthetase	2.92	4.31
YMR201C	*RAD14*	Protein that recognizes and binds damaged DNA during nucleotide excision repair	2.90	3.28
YPL258C	*THI21*	Hydroxymethylpyrimidine phosphate kinase	2.90	3.55
YER184C	*YER184C*	Putative zinc cluster protein	2.88	4.15
YIR005W	*IST3*	Component of the U2 snRNP, required for the first catalytic step of splicing and for spliceosomal assembly	2.84	3.34
YJR111C	*YJR111C*	Putative protein of unknown function	2.83	3.51
YBL006C	*LDB7*	Component of the RSC chromatin remodeling complex	2.83	3.07
YHR122W	*YHR122W*	Protein of unknown function required for establishment of sister chromatid cohesion	2.83	3.14
YJL219W	*HXT9*	Putative hexose transporter that is nearly identical to Hxt11p, has similarity to major facilitator superfamily (MFS) transporters, expression of HXT9 is regulated by transcription factors Pdr1p and Pdr3p	2.80	2.30
YBR293W	*VBA2*	Permease of basic amino acids in the vacuolar membrane	2.80	2.99
YOR242C	*SSP2*	Sporulation specific protein that localizes to the spore wall	2.80	2.26
YDR523C	*SPS1*	Putative protein serine/threonine kinase expressed at the end of meiosis and localized to the prospore membrane	2.79	−
YGL116W	*CDC20*	Cell-cycle regulated activator of anaphase-promoting complex/cyclosome (APC/C)	2.79	−
YMR009W	*ADI1*	Acireductone dioxygenease involved in the methionine salvage pathway; ortholog of human MTCBP-1	2.78	3.55

YOL159C-A	*YOL159C-A*	Putative protein of unknown function	2.76	2.70
YJL043W	*YJL043W*	Putative protein of unknown function	2.75	2.87
YHL046C	*PAU13*	Protein of unknown function	2.75	−
YHR029C	*YHI9*	Protein of unknown function	2.74	3.02
YHR047C	*AAP1*	Arginine/alanine aminopeptidase	2.73	3.19
YER128W	*YER128W*	Putative protein of unknown function	2.72	3.67
YGL059W	*PKP2*	Mitochondrial protein kinase that negatively regulates activity of the pyruvate dehydrogenase complex by phosphorylating the ser-133 residue of the Pda1p subunit	2.71	−
YKL220C	*FRE2*	Ferric reductase and cupric reductase, reduces siderophore-bound iron and oxidized copper prior to uptake by transporters	2.71	−
YLR213C	*CRR1*	Putative glycoside hydrolase of the spore wall envelope	2.70	2.62
YMR096W	*SNZ1*	Protein involved in vitamin B6 biosynthesis; member of a stationary phase-induced gene family	2.70	4.64
YAL068C	*PAU8*	Protein of unknown function, member of the seripauperin multigene family encoded mainly in subtelomeric regions	2.69	2.21
YKL224C	*PAU16*	Protein of unknown function, member of the seripauperin multigene family encoded mainly in subtelomeric regions	2.65	−
YBL108C-A	*PAU9*	Protein of unknown function, member of the seripauperin multigene family encoded mainly in subtelomeric regions	2.65	−
YNL240C	*NAR1*	Component of the cytosolic iron-sulfur (FeS) protein assembly machinery	2.65	2.86
YDR249C	*YDR249C*	Putative protein of unknown function	2.65	3.19
YHR214C-E	*YHR214C-E*	Putative protein of unknown function	2.65	−
YGR286C	*BIO2*	Biotin synthase	2.64	3.55
YER040W	*GLN3*	Transcriptional activator of genes regulated by nitrogen catabolite repression	2.64	3.05
YDR542W	*PAU10*	Protein of unknown function	2.63	−
YGR131W	*YGR131W*	Protein of unknown function	2.63	2.51

YHR185C	*PFS1*	Sporulation protein required for prospore membrane formation at selected spindle poles, ensures functionality of all four spindle pole bodies during meiosis II	2.63	−
YGL224C	*SDT1*	Pyrimidine nucleotidase	2.62	2.60
YJL223C	*PAU1*	Member of the seripauperin multigene family encoded mainly in subtelomeric regions, active during alcoholic fermentation, regulated by anaerobiosis, negatively regulated by oxygen, repressed by heme	2.61	2.46
YOR381W	*FRE3*	Ferric reductase	2.60	3.21
YMR306W	*FKS3*	Protein involved in spore wall assembly, has similarity to 1,3-beta-D-glucan synthase catalytic subunits Fks1p and Gsc2p	2.60	2.79
YOR190W	*SPR1*	Sporulation-specific exo-1,3-beta-glucanase; contributes to ascospore thermoresistance	2.60	−
YBR240C	*THI2*	Zinc finger protein of the Zn(II)2Cys6 type, probable transcriptional activator of thiamine biosynthetic genes	2.60	2.83
YLR081W	*GAL2*	Galactose permease, required for utilization of galactose	2.60	−
YCR020W-B	*HTL1*	Component of the RSC chromatin remodeling complex	2.59	2.96
YNR030W	*ALG12*	Alpha-1,6-mannosyltransferase localized to the ER	2.59	2.38
YGR288W	*MAL13*	MAL-activator protein, part of complex locus MAL1	2.59	−
YDL177C	*YDL177C*	Putative protein of unknown function	2.58	3.64
YCR060W	*TAH1*	HSP90 cofactor; interacts with Hsp82p, Pih1p, Rvb1 and Rvb2	2.57	3.59
YGL039W	*YGL039W*	Oxidoreductase shown to reduce carbonyl compounds to chiral alcohols	2.57	3.66
YMR019W	*STB4*	Protein that binds Sin3p in a two-hybrid assay	2.56	2.94
YJR079W	*YJR079W*	Putative protein of unknown function; mutation results in impaired mitochondrial respiration	2.55	−
YIL176C	*PAU14*	Protein of unknown function	2.55	2.44
YBR068C	*BAP2*	High-affinity leucine permease	2.53	2.73
YER069W	*ARG5,6*	Protein that is processed in the mitochondrion to yield	2.53	2.28

		acetylglutamate kinase and N-acetyl-gamma-glutamyl-phosphate reductase		
YKL211C	*TRP3*	Bifunctional enzyme exhibiting both indole-3-glycerol-phosphate synthase and anthranilate synthase	2.52	2.77
YOL165C	*AAD15*	Putative aryl-alcohol dehydrogenase	2.52	2.77
YLL060C	*GTT2*	Glutathione S-transferase capable of homodimerization	2.50	−
YFR034C	*PHO4*	Basic helix-loop-helix (bHLH) transcription factor of the myc-family; binds cooperatively with Pho2p to the PHO5 promoter	2.49	3.33
YBL102W	*SFT2*	Non-essential tetra-spanning membrane protein found mostly in the late Golgi, can suppress some sed5 alleles; may be part of the transport machinery	2.48	2.85
YOL119C	*MCH4*	Protein with similarity to mammalian monocarboxylate permeases	2.48	3.28
YGR197C	*SNG1*	Protein involved in resistance to nitrosoguanidine (MNNG) and 6-azauracil (6-AU)	2.48	2.90
YIL117C	*PRM5*	Pheromone-regulated protein, predicted to have 1 transmembrane segment; induced during cell integrity signaling	2.48	2.31
YHR014W	*SPO13*	Meiosis-specific protein, involved in maintaining sister chromatid cohesion during meiosis I as well as promoting proper attachment of kinetochores to the spindle during meiosis I and meiosis II	2.47	−
YDL241W	*YDL241W*	Putative protein of unknown function	2.47	−
YDL037C	*BSC1*	Protein of unconfirmed function, similar to cell surface flocculin Muc1p	2.46	−
YIR035C	*YIR035C*	Putative cytoplasmic protein of unknown function	2.46	2.19
YKR069W	*MET1*	S-adenosyl-L-methionine uroporphyrinogen III transmethylase, involved in the biosynthesis of siroheme, a prosthetic group used by sulfite reductase; required for sulfate assimilation and methionine biosynthesis	2.45	3.45
YFL057C	*AAD16*	Putative aryl-alcohol dehydrogenase	2.45	2.94
YOL164W	*BDS1*	Bacterially-derived sulfatase required for use of alkyl- and aryl-sulfates as	2.45	2.45

		sulfur sources		
YLR363C	*NMD4*	Protein interacting with Nam7p, may be involved in the nonsense-mediated mRNA decay pathway	2.45	−
YBR301W	*PAU24*	Cell wall mannoprotein with similarity to Tir1p, Tir2p, Tir3p, and Tir4p; member of the seripauperin multigene family encoded mainly in subtelomeric regions	2.44	−
YOR303W	*CPA1*	Small subunit of carbamoyl phosphate synthetase	2.43	−
YPL272C	*YPL272C*	Putative protein of unknown function	2.42	−
YPL047W	*SGF11*	Integral subunit of SAGA histone acetyltransferase complex	2.41	2.81
YNL331C	*AAD14*	Putative aryl-alcohol dehydrogenase with similarity to P. chrysosporium aryl-alcohol dehydrogenase	2.40	2.82
YFL028C	*CAF16*	Part of evolutionarily-conserved CCR4-NOT regulatory	2.39	2.48
YDL142C	*CRD1*	Cardiolipin synthase; produces cardiolipin, which is a phospholipid of the mitochondrial inner membrane that is required for normal mitochondrial membrane potential and function	2.39	2.36
YMR062C	*ARG7*	Mitochondrial ornithine acetyltransferase, catalyzes the fifth step in arginine biosynthesis	2.39	2.36
YBR076W	*ECM8*	Non-essential protein of unknown function	2.39	2.19
YIL166C	*YIL166C*	Putative protein with similarity to the allantoate permease (Dal5p) subfamily of the major facilitator superfamily	2.38	2.84
YPR048W	*TAH18*	Conserved NAPDH-dependent diflavin reductase, component of an early step in the cytosolic Fe-S protein assembly (CIA) machinery	2.38	−
YNR073C	*YNR073C*	Putative mannitol dehydrogenase	2.38	−
YFL011W	*HXT10*	Putative hexose transporter, expressed at low levels and expression is repressed by glucose	2.38	−
YKL008C	*LAC1*	Ceramide synthase component, involved in synthesis of ceramide from C26(acyl)-coenzyme A and dihydrosphingosine or phytosphingosine, functionally equivalent to Lag1p	2.37	2.55

YBR256C	*RIB5*	Riboflavin synthase	2.37	2.62
YLL055W	*YCT1*	High-affinity cysteine-specific transporter with similarity to the Dal5p family of transporters	2.37	2.95
YGR294W	*PAU12*	Protein of unknown function	2.36	−
YGL154C	*LYS5*	Phosphopantetheinyl transferase involved in lysine biosynthesis	2.36	−
YGL249W	*ZIP2*	Meiosis-specific protein involved in normal synaptonemal complex formation and pairing between homologous chromosomes during meiosis	2.36	−
YFL020C	*PAU5*	Member of the seripauperin multigene family encoded mainly in subtelomeric regions	2.36	−
YKL072W	*STB6*	Protein that binds Sin3p in a two-hybrid assay	2.35	2.83
YKL086W	*SRX1*	Sulfiredoxin, contributes to oxidative stress resistance by reducing cysteine-sulfinic acid groups in the peroxiredoxin Tsa1p	2.34	2.70
YHL040C	*ARN1*	Transporter, member of the ARN family of transporters that specifically recognize siderophore-iron chelates	2.33	−
YPL165C	*SET6*	SET domain protein of unknown function	2.33	2.60
YGL180W	*ATG1*	Protein ser/thr kinase required for vesicle formation in autophagy and the cytoplasm-to-vacuole targeting (Cvt) pathway	2.33	2.49
YGL117W	*YGL117W*	Putative protein of unknown function	2.33	2.48
YGR110W	*CLD1*	Mitochondrial cardiolipin-specific phospholipase	2.33	2.21
YOR114W	*YOR114W*	Putative protein of unknown function; null mutant is viable	2.32	−
YIR013C	*GAT4*	Protein containing GATA family zinc finger motifs	2.32	−
YJR039W	*YJR039W*	Putative protein of unknown function	2.32	2.43
YMR187C	*YMR187C*	Putative protein of unknown function	2.31	−
YDR263C	*DIN7*	Mitochondrial nuclease functioning in DNA repair and replication, modulates the stability of the mitochondrial	2.31	2.07
YPL135W	*ISU1*	Conserved protein of the mitochondrial matrix	2.31	2.79

YEL070W	*DSF1*	Deletion suppressor of mpt5 mutation	2.31	−
YIL120W	*QDR1*	Multidrug transporter of the major facilitator superfamily, required for resistance to quinidine, ketoconazole, fluconazole, and barban	2.31	2.17
YJR095W	*SFC1*	Mitochondrial succinate-fumarate transporter	2.30	3.72
YKL128C	*PMU1*	Putative phosphomutase,	2.30	2.26
YEL029C	*BUD16*	Putative pyridoxal kinase	2.30	3.16
YKL188C	*PXA2*	Subunit of a heterodimeric peroxisomal ATP-binding cassette transporter complex (Pxa1p-Pxa2p)	2.30	2.58
YHR006W	*STP2*	Transcription factor, activated by proteolytic processing in response to signals from the SPS sensor system for external amino acids	2.30	2.37
YER039C	*HVG1*	Protein of unknown function, has homology to Vrg4p	2.29	2.65
YGR250C	*YGR250 C*	Putative RNA binding protein; localizes to stress granules induced by glucose deprivation; interacts with Rbg1p in a two-hybrid	2.28	2.46
YNL125C	*ESBP6*	Protein with similarity to monocarboxylate permeases, appears not to be involved in transport of monocarboxylates such as lactate, pyruvate or acetate across the plasma membrane	2.27	2.26
YPR057W	*BRR1*	snRNP protein component of spliceosomal snRNPs, required for pre-mRNA splicing and snRNP biogenesis	2.27	2.50
YNL335W	*DDI3*	Protein of unknown function; expression is induced over 100-fold by DNA damage	2.26	−
YHL047C	*ARN2*	Transporter, member of the ARN family of transporters that specifically recognize siderophore-iron chelates	2.26	−
YHR212W -A	*YHR212 W-A*	Putative protein of unknown function	2.26	−
YFL061W	*DDI2*	Protein of unknown function; expression is induced over 100-fold by DNA damage; induction decreased in rad6 and rad18 mutants	2.25	−
YLL038C	*ENT4*	Protein of unknown function, contains an N-terminal epsin-like	2.25	2.44

		domain		
YLR031W	*YLR031W*	Putative protein of unknown function	2.24	2.39
YKL222C	*YKL222C*	Protein of unknown function that may interact with ribosomes	2.23	2.39
YKL052C	*ASK1*	Essential subunit of the Dam1 complex (aka DASH complex), couples kinetochores to the force produced by MT depolymerization thereby aiding in chromosome segregation	2.23	2.50
YLR211C	*YLR211C*	Putative protein of unknown function	2.23	−
YGL248W	*PDE1*	Low-affinity cyclic AMP phosphodiesterase, controls glucose and intracellular acidification-induced cAMP signaling, target of the cAMP-protein kinase A (PKA) pathway	2.22	2.38
YLR461W	*PAU4*	Member of the seripauperin multigene family encoded mainly in subtelomeric regions	2.22	−
YLR318W	*EST2*	Reverse transcriptase subunit of the telomerase holoenzyme, essential for telomerase core catalytic activity, involved in other aspects of telomerase assembly and function	2.21	−
YOR389W	*YOR389W*	Putative protein of unknown function	2.21	−
YER065C	*ICL1*	Isocitrate lyase, catalyzes the formation of succinate and glyoxylate from isocitrate, a key reaction of the glyoxylate cycle	2.21	−
YKR102W	*FLO10*	Lectin-like protein with similarity to Flo1p	2.20	−
YMR251W	*GTO3*	Omega class glutathione transferase; putative cytosolic localization	2.20	−
YOR337W	*TEA1*	Ty1 enhancer activator required for full levels of Ty enhancer-mediated transcription	2.20	−
YLR099W-A	*YLR099W-A*	Putative protein of unknown function	2.20	2.18
YEL071W	*DLD3*	D-lactate dehydrogenase	2.20	2.99
YBR298C-A	*YBR298C-A*	Putative protein of unknown function	2.19	2.63
YCL066W	*HMLALPHA1*	Silenced copy of ALPHA1 at HML, encoding a transcriptional coactivator involved in the regulation of mating-type alpha-specific gene expression	2.19	2.14
YJR010W	*MET3*	ATP sulfurylase	2.19	2.57

YLR037C	*PAU23*	Cell wall mannoprotein with similarity to Tir1p, Tir2p, Tir3p, and Tir4p; member of the seripauperin multigene family encoded mainly in subtelomeric regions	2.19	−
YPR193C	*HPA2*	Tetrameric histone acetyltransferase with similarity to Gcn5p, Hat1p, Elp3p, and Hpa3p	2.18	2.84
YFL040W	*YFL040W*	Putative transporter	2.18	−
YFR012W-A	*YFR012W-A*	Putative protein of unknown function; identified by homology	2.17	−
YML005W	*TRM12*	S-adenosylmethionine-dependent methyltransferase of the seven beta-strand family	2.17	2.32
YMR066W	*SOV1*	Mitochondrial protein of unknown function	2.17	−
YCR107W	*AAD3*	Putative aryl-alcohol dehydrogenase with similarity to P. chrysosporium aryl-alcohol dehydrogenase	2.17	−
YIL015W	*BAR1*	Aspartyl protease secreted into the periplasmic space of mating type a cells, helps cells find mating partners, cleaves and inactivates alpha factor allowing cells to recover from alpha-factor-induced cell cycle arrest	2.17	2.01
YLR228C	*ECM22*	Sterol regulatory element binding protein, regulates transcription of sterol biosynthetic genes	2.16	−
YCR040W	*MATALPHA1*	Transcriptional co-activator involved in regulation of mating-type-specific gene expression	2.16	2.20
YOR245C	*DGA1*	Diacylglycerol acyltransferase, catalyzes the terminal step of triacylglycerol (TAG) formation, acylates diacylglycerol using acyl-CoA as an acyl donor, localized to lipid particles	2.16	2.61
YMR095C	*SNO1*	Protein of unconfirmed function, involved in pyridoxine metabolism;	2.16	3.92
YNL046W	*YNL046W*	Putative protein of unknown function; expression depends on Swi5p;	2.16	2.17
YGL261C	*PAU11*	Putative protein of unknown function and member of the seripauperin multigene family encoded mainly in subtelomeric regions	2.16	−
YCR089W	*FIG2*	Cell wall adhesin, expressed specifically during mating; may be involved in maintenance of cell wall	2.15	−

		integrity during mating		
YGL251C	*HFM1*	Meiosis specific DNA helicase involved in the conversion of double-stranded breaks to later recombination intermediates and in crossover control	2.15	−
YBR131W	*CCZ1*	Protein involved in vacuolar assembly, essential for autophagy and the cytoplasm-to-vacuole pathway	2.15	2.40
YMR175W	*SIP18*	Phospholipid-binding protein; expression is induced by osmotic stress	2.14	2.34
YJL088W	*ARG3*	Ornithine carbamoyltransferase (carbamoylphosphate:L-ornithine carbamoyltransferase), catalyzes the sixth step in the biosynthesis of the arginine precursor ornithine	2.14	2.51
YGL146C	*RRT6*	Putative protein of unknown function	2.14	−
YBR085C-A	*YBR085C-A*	Putative protein of unknown function	2.14	2.30
YDL067C	*COX9*	Subunit VIIa of cytochrome c oxidase	2.14	−
YDR530C	*APA2*	Diadenosine 5',5''-P1,P4-tetraphosphate phosphorylase II (AP4A phosphorylase), involved in catabolism of bis(5'-nucleosidyl) tetraphosphates; has similarity to Apa1p	2.13	2.55
YMR056C	*AAC1*	Mitochondrial inner membrane ADP/ATP translocator, exchanges cytosolic ADP for mitochondrially synthesized ATP	2.13	2.04
YOL015W	*IRC10*	Putative protein of unknown function	2.12	−
YDR222W	*YDR222W*	Protein of unknown function	2.12	−
YOR009W	*TIR4*	Cell wall mannoprotein of the Srp1p/Tip1p family of serine-alanine-rich proteins; expressed under anaerobic conditions and required for anaerobic growth	2.12	−
YPL187W	*MF(ALPHA)1*	Mating pheromone alpha-factor, made by alpha cells	2.11	−
YHR161C	*YAP1801*	Protein involved in clathrin cage assembly	2.11	−
YDR042C	*YDR042C*	Putative protein of unknown function; expression is increased in ssu72-ts69 mutant	2.11	−
YKR099W	*BAS1*	Myb-related transcription factor involved in regulating basal and	2.10	2.56

		induced expression of genes of the purine and histidine biosynthesis pathways		
YOL001W	*PHO80*	Cyclin, interacts with cyclin-dependent kinase Pho85p	2.10	2.14
YHR162W	*YHR162W*	Putative protein of unknown function	2.10	2.16
YMR041C	*ARA2*	NAD-dependent arabinose dehydrogenase	2.10	2.28
YPL278C	*YPL278C*	Putative protein of unknown function	2.10	2.36
YLR263W	*RED1*	Protein component of the axial elements of the synaptonemal complex	2.09	−
YFR029W	*PTR3*	Component of the SPS plasma membrane amino acid sensor system (Ssy1p-Ptr3p-Ssy5p), which senses external amino acid concentration and transmits intracellular signals that result in regulation of expression of amino acid permease genes	2.09	2.27
YMR106C	*YKU80*	Subunit of the telomeric Ku complex (Yku70p-Yku80p), involved in telomere length maintenance, structure and telomere position effect	2.08	2.57
YBL060W	*YEL1*	Guanine nucleotide exchange factor specific for Arf3p	2.08	2.41
YHR166C	*CDC23*	Subunit of the Anaphase-Promoting Complex/Cyclosome (APC/C	2.08	2.08
YMR020W	*FMS1*	Polyamine oxidase, converts spermine to spermidine	2.07	2.31
YLL063C	*AYT1*	Acetyltransferase; catalyzes trichothecene 3-O-acetylation	2.07	2.51
YPL277C	*YPL277C*	Putative protein of unknown function; localized to the membranes	2.07	2.19
YJR156C	*THI11*	Protein involved in synthesis of the thiamine precursor hydroxymethylpyrimidine (HMP)	2.07	−
YML097C	*VPS9*	A guanine nucleotide exchange factor involved in vesicle-mediated vacuolar protein transport	2.07	2.51
YGR239C	*PEX21*	Peroxin required for targeting of peroxisomal matrix proteins containing PTS2	2.06	−
YJR036C	*HUL4*	Protein with similarity to hect domain E3 ubiquitin-protein ligases, not essential for viability	2.06	2.14
YBR294W	*SUL1*	High affinity sulfate permease	2.06	−

YCL055W	*KAR4*	Transcription factor required for gene regulation in response to pheromones	2.06	2.03
YDR046C	*BAP3*	Amino acid permease involved in the uptake of cysteine, leucine, isoleucine and valine	2.06	2.47
YDR459C	*PFA5*	Palmitoyltransferase with autoacylation activity; likely functions in pathway(s) outside Ras	2.05	2.29
YJL045W	*YJL045W*	Minor succinate dehydrogenase isozyme	2.05	2.26
YOR377W	*ATF1*	Alcohol acetyltransferase with potential roles in lipid and sterol metabolism	2.05	2.31
YOR306C	*MCH5*	Plasma membrane riboflavin transporter	2.05	−
YDR481C	*PHO8*	Repressible alkaline phosphatase	2.05	−
YER130C	*YER130C*	Protein of unknown function	2.04	2.43
YKR071C	*DRE2*	Conserved component of an early step in the cytosolic Fe-S protein assembly (CIA) machinery	2.04	2.13
YDR082W	*STN1*	Telomere end-binding and capping protein, plays a key role with Pol12p in linking telomerase action with completion of lagging strand synthesis, and in a regulatory step required for telomere capping	2.04	−
YNL210W	*MER1*	Protein with RNA-binding motifs required for meiosis-specific mRNA splicing; required for chromosome pairing and meiotic recombination	2.04	2.88
YLR214W	*FRE1*	Ferric reductase and cupric reductase	2.04	2.25
YPR002W	*PDH1*	Mitochondrial protein that participates in respiration	2.03	−
YIL020C	*HIS6*	Phosphoribosyl-5-amino-1-phosphoribosyl-4-imidazolecarboxiamide isomerase, catalyzes the fourth step in histidine biosynthesis	2.03	2.07
YDR366C	*YDR366C*	Putative protein of unknown function	2.02	−
YNR076W	*PAU6*	Member of the seripauperin multigene family encoded mainly in subtelomeric regions, active during alcoholic fermentation, regulated by anaerobiosis, negatively regulated by oxygen, repressed by heme	2.02	−

YLL033W	*IRC19*	Putative protein of unknown function	2.02	−
YOL132W	*GAS4*	1,3-beta-glucanosyltransferase	2.02	−
YAR035W	*YAT1*	Outer mitochondrial carnitine acetyltransferase	2.01	2.12
YIL024C	*YIL024C*	Putative protein of unknown function	2.01	−
YCL069W	*VBA3*	Permease of basic amino acids in the vacuolar membrane	2.01	−
YGL144C	*ROG1*	Protein with putative serine active lipase domain	2.01	−
YDR531W	*CAB1*	Pantothenate kinase (ATP:D-pantothenate 4'-phosphotransferase	2.00	2.32
YPL241C	*CIN2*	GTPase-activating protein (GAP) for Cin4p	2.00	−
YMR272C	*SCS7*	Sphingolipid alpha-hydroxylase, functions in the alpha-hydroxylation of sphingolipid-associated very long chain fatty acids,	2.00	2.36
YEL066W	*HPA3*	D-Amino acid N-acetyltransferase,	-2.00	-2.35
YNL061W	*NOP2*	Probable RNA m(5)C methyltransferase	-2.01	-2.06
YFL037W	*TUB2*	Beta-tubulin; associates with alpha-tubulin (Tub1p and Tub3p) to form tubulin dimer, which polymerizes to form microtubules	-2.01	-2.48
YDL137W	*ARF2*	ADP-ribosylation factor, GTPase of the Ras superfamily involved in regulation of coated formation vesicles in intracellular trafficking within the Golgi	-2.01	−
YGR155 W	*CYS4*	Cystathionine beta-synthase, catalyzes synthesis of cystathionine from serine and homocysteine, the first committed step in cysteine biosynthesis	-2.02	−
YPL154C	*PEP4*	Vacuolar aspartyl protease (proteinase A), required for the posttranslational precursor maturation of vacuolar proteinases	-2.02	−
YEL039C	*CYC7*	Cytochrome c isoform 2, expressed under hypoxic conditions	-2.02	−
YOL126C	*MDH2*	Cytoplasmic malate dehydrogenase, one of three isozymes that catalyze interconversion of malate and oxaloacetate	-2.02	−
YIR038C	*GTT1*	ER associated glutathione S-transferase capable of homodimerization; expression induced during the diauxic shift and throughout stationary phase	-2.04	−

YLR223C	*IFH1*	Coactivator that regulates transcription of ribosomal protein (RP) genes;	-2.06	−
YDR516C	*EMI2*	Non-essential protein of unknown function required for transcriptional induction of the early meiotic-specific transcription factor IME1	-2.06	−
YHR148W	*IMP3*	Component of the SSU processome,	-2.06	−
YDR496C	*PUF6*	Pumilio-homology domain protein that binds the 3' UTR of ASH1 mRNA and represses its translation, resulting in proper asymmetric localization of ASH1 mRNA	-2.06	−
YDL022W	*GPD1*	NAD-dependent glycerol-3-phosphate dehydrogenase	-2.07	-2.97
YNL144C	*YNL144C*	Putative protein of unknown function	-2.07	−
YOR239W	*ABP140*	Nonessential protein that binds actin filaments and localizes to actin patches and cables, has similarity to S-adenosylmethionine (AdoMet)-dependent methyltransferases	-2.07	-2.69
YFL045C	*SEC53*	Phosphomannomutase, involved in synthesis of GDP-mannose and dolichol-phosphate-mannose; required for folding and glycosylation of secretory proteins in the ER lumen	-2.08	-2.94
YHR088W	*RPF1*	Nucleolar protein involved in the assembly and export of the large ribosomal subunit; constituent of 66S pre-ribosomal particles; contains a sigma(70)-like motif, which is thought to bind RNA	-2.09	−
YPL226W	*NEW1*	ATP binding cassette protein that cosediments with polysomes and is required for biogenesis of the small ribosomal subunit	-2.09	-2.34
YMR309C	*NIP1*	eIF3c subunit of the eukaryotic translation initiation factor 3 (eIF3), involved in the assembly of preinitiation complex and start codon selection	-2.10	-2.21
YDR155C	*CPR1*	Cytoplasmic peptidyl-prolyl cis-trans isomerase (cyclophilin	-2.10	−
YML060W	*OGG1*	Mitochondrial glycosylase/lyase that specifically excises 7,8-dihydro-8-oxoguanine residues located opposite cytosine or thymine residues in DNA, repairs oxidative damage to mitochondrial DNA,	-2.11	-2.05

		contributes to UVA resistance		
YNL102W	*POL1*	Catalytic subunit of the DNA polymerase I alpha-primase complex,	-2.12	−
YNR053C	*NOG2*	Putative GTPase that associates with pre-60S ribosomal subunits in the nucleolus and is required for their nuclear export and maturation	-2.13	−
YPL256C	*CLN2*	G1 cyclin involved in regulation of the cell cycle; activates Cdc28p kinase to promote the G1 to S phase transition	-2.13	−
YPR138C	*MEP3*	Ammonium permease of high capacity and low affinity	-2.14	−
YLR401C	*DUS3*	Dihydrouridine synthase, member of a widespread family of conserved proteins including Smm1p, Dus1p, and Dus4p; contains a consensus oleate response element (ORE) in its promoter region	-2.15	−
YJR074W	*MOG1*	Conserved nuclear protein that interacts with GTP-Gsp1p, which is a Ran homolog of the Ras GTPase family, and stimulates nucleotide release, involved in nuclear protein import, nucleotide release is inhibited by Yrb1p	-2.16	−
YKL109W	*HAP4*	Subunit of the heme-activated, glucose-repressed Hap2p/3p/4p/5p CCAAT-binding complex, a transcriptional activator and global regulator of respiratory gene expression	-2.17	−
YBR247C	*ENP1*	Protein associated with U3 and U14 snoRNAs, required for pre-rRNA processing and 40S ribosomal subunit synthesis	-2.17	−
YCL054W	*SPB1*	AdoMet-dependent methyltransferase involved in rRNA processing and 60S ribosomal subunit maturation; methylates G2922 in the tRNA docking site of the large subunit rRNA and in the absence of snR52, U2921	-2.19	-2.06
YER158C	*YER158 C*	Protein of unknown function, has similarity to Afr1p; potentially phosphorylated by Cdc28p	-2.19	-2.91
YDR101C	*ARX1*	Shuttling pre-60S factor; involved in the biogenesis of ribosomal large subunit biogenesis	-2.20	-2.09
YML008C	*ERG6*	Delta(24)-sterol C-methyltransferase,	-2.20	-2.23

		converts zymosterol to fecosterol in the ergosterol biosynthetic pathway by methylating position C-24		
YKR077W	*MSA2*	Putative transcriptional activator, that interacts with G1-specific transcription factor, MBF and G1-specific promoters	-2.21	−
YCR034W	*FEN1*	Fatty acid elongase, involved in sphingolipid biosynthesis; acts on fatty acids of up to 24 carbons in length; mutations have regulatory effects on 1,3-beta-glucan synthase, vacuolar ATPase, and the secretory pathway	-2.21	-3.47
YGL171W	*ROK1*	ATP-dependent RNA helicase of the DEAD box family; required for 18S rRNA synthesis	-2.21	−
YFL052W	*YFL052W*	Putative zinc cluster protein that contains a DNA binding domain	-2.21	−
YJL079C	*PRY1*	Protein of unknown function	-2.22	−
YGR271C-A	*EFG1*	Essential protein required for maturation of 18S rRNA	-2.22	−
YBR078W	*ECM33*	GPI-anchored protein of unknown function, has a possible role in apical bud growth; GPI-anchoring on the plasma membrane crucial to function; phosphorylated in mitochondria; similar to Sps2p and Pst1p	-2.22	-2.85
YLR056W	*ERG3*	C-5 sterol desaturase, catalyzes the introduction of a C-5(6) double bond into episterol, a precursor in ergosterol biosynthesis; mutants are viable, but cannot grow on non-fermentable carbon sources	-2.23	−
YIL136W	*OM45*	Protein of unknown function, major constituent of the mitochondrial outer membrane; located on the outer (cytosolic) face of the outer membrane	-2.24	−
YBL030C	*PET9*	Major ADP/ATP carrier of the mitochondrial inner membrane, exchanges cytosolic ADP for mitochondrially synthesized ATP	-2.25	-2.74
YGR079W	*YGR079W*	Putative protein of unknown function; YGR079W is not an essential gene	-2.26	−
YHR215W	*PHO12*	One of three repressible acid phosphatases, a glycoprotein that is transported to the cell surface by the secretory pathway; nearly identical to	-2.28	-2.49

		Pho11p		
YEL011W	*GLC3*	Glycogen branching enzyme, involved in glycogen accumulation	-2.28	−
YGR180C	*RNR4*	Ribonucleotide-diphosphate reductase (RNR), small subunit; the RNR complex catalyzes the rate-limiting step in dNTP synthesis and is regulated by DNA replication and DNA damage checkpoint pathways via localization of the small subunits	-2.28	-2.60
YNR016C	*ACC1*	Acetyl-CoA carboxylase, biotin containing enzyme that catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA	-2.29	-2.88
YPR112C	*MRD1*	Essential conserved protein that is part of the 90S preribosome	-2.29	−
YNL058C	*YNL058 C*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the vacuole	-2.29	-2.26
YKR081C	*RPF2*	Essential protein involved in the processing of pre-rRNA and the assembly of the 60S ribosomal subunit; interacts with ribosomal protein L11	-2.29	−
YHR052W	*CIC1*	Essential protein that interacts with proteasome components and has a potential role in proteasome substrate specificity	-2.30	−
YGR060 W	*ERG25*	C-4 methyl sterol oxidase, catalyzes the first of three steps required to remove two C-4 methyl groups from an intermediate in ergosterol biosynthesis	-2.30	-2.24
YGR271C -A	*EFG1*	Essential protein required for maturation of 18S rRNA; null mutant is sensitive to hydroxyurea and is delayed in recovering from alpha-factor arrest	-2.30	−
YOR101 W	*RAS1*	GTPase involved in G-protein signaling in the adenylate cyclase activating pathway, plays a role in cell proliferation	-2.35	-2.47
YJR070C	*LIA1*	Deoxyhypusine hydroxylase, a HEAT-repeat containing metalloenzyme that catalyzes hypusine formation	-2.35	-2.86
YPL093W	*NOG1*	Putative GTPase that associates with free 60S ribosomal subunits in the nucleolus and is required for 60S	-2.35	-2.14

		ribosomal subunit biogenesis		
YJL026W	*RNR2*	Ribonucleotide-diphosphate reductase (RNR), small subunit; the RNR complex catalyzes the rate-limiting step in dNTP synthesis and is regulated by DNA replication and DNA damage checkpoint pathways via localization of the small subunits	-2.35	-3.23
YDR234W	*LYS4*	Homoaconitase, catalyzes the conversion of homocitrate to homoisocitrate, which is a step in the lysine biosynthesis pathway	-2.36	-2.28
YER003C	*PMI40*	Mannose-6-phosphate isomerase, catalyzes the interconversion of fructose-6-P and mannose-6-P; required for early steps in protein mannosylation	-2.36	-3.87
YJL173C	*RFA3*	Subunit of heterotrimeric Replication Protein A (RPA), which is a highly conserved single-stranded DNA binding protein involved in DNA replication, repair, and recombination	-2.37	-2.95
YHR153C	*SPO16*	Meiosis-specific protein involved in synaptonemal complex assembly; implicated in regulation of crossover formation	-2.38	−
YKR075C	*YKR075C*	Protein of unknown function; similar to YOR062Cp and Reg1p	-2.38	−
YPL267W	*ACM1*	Pseudosubstrate inhibitor of the anaphase-promoting complex/cyclosome (APC/C), that suppresses APC/C [Cdh1]-mediated proteolysis of mitotic cyclins; associates with Cdh1p, Bmh1p and Bmh2p; cell cycle regulated protein	-2.39	−
YMR145C	*NDE1*	Mitochondrial external NADH dehydrogenase, a type II NAD(P)H:quinone oxidoreductase that catalyzes the oxidation of cytosolic NADH	-2.39	-2.72
YKL045W	*PRI2*	Subunit of DNA primase, which is required for DNA synthesis and double-strand break repair	-2.40	−
YCL025C	*AGP1*	Low-affinity amino acid permease with broad substrate range, involved in uptake of asparagine, glutamine, and other amino acids	-2.41	-2.46
YJR073C	*OPI3*	Phospholipid methyltransferase, catalyzes the last two steps in phosphatidylcholine biosynthesis	-2.42	−

YOR340C	*RPA43*	RNA polymerase I subunit A43	-2.43	−
YNL002C	*RLP7*	Nucleolar protein with similarity to large ribosomal subunit L7 proteins	-2.43	−
YHR128W	*FUR1*	Uracil phosphoribosyltransferase, synthesizes UMP from uracil; involved in the pyrimidine salvage pathway	-2.43	-2.84
YNL248C	*RPA49*	RNA polymerase I subunit A49	-2.45	-2.35
YPL186C	*UIP4*	Protein that interacts with Ulp1p, a Ubl (ubiquitin-like protein)-specific protease for Smt3p protein conjugates	-2.45	-2.63
YNL274C	*GOR1*	Glyoxylate reductase; null mutation results in increased biomass after diauxic shift	-2.46	-2.32
YMR196W	*YMR196W*	Putative protein of unknown function	-2.48	−
YLR134W	*PDC5*	Minor isoform of pyruvate decarboxylase, key enzyme in alcoholic fermentation, decarboxylates pyruvate to acetaldehyde	-2.51	-4.45
YDR497C	*ITR1*	Myo-inositol transporter with strong similarity to the minor myo-inositol transporter Itr2p, member of the sugar transporter superfamily	-2.52	-3.86
YNR014W	*YNR014W*	Putative protein of unknown function; expression is cell-cycle regulated, Azf1p-dependent, and heat-inducible	-2.52	−
YER145C	*FTR1*	High affinity iron permease involved in the transport of iron across the plasma membrane	-2.55	−
YDR097C	*MSH6*	Protein required for mismatch repair in mitosis and meiosis, forms a complex with Msh2p to repair both single-base & insertion-deletion mispairs; potentially phosphorylated by Cdc28p	-2.57	-2.01
YMR058W	*FET3*	Ferro-O2-oxidoreductase required for high-affinity iron uptake and involved in mediating resistance to copper ion toxicity, belongs to class of integral membrane multicopper oxidases	-2.60	−
YBL039C	*URA7*	Major CTP synthase isozyme (see also URA8), catalyzes the ATP-dependent transfer of the amide nitrogen from glutamine to UTP, forming CTP, the final step in de novo biosynthesis of pyrimidines	-2.69	-3.64

YDR453C	*TSA2*	Stress inducible cytoplasmic thioredoxin peroxidase	-2.70	-2.18
YER001W	*MNN1*	Alpha-1,3-mannosyltransferase, integral membrane glycoprotein of the Golgi complex, required for addition of alpha1,3-mannose linkages to N-linked and O-linked oligosaccharides, one of five S. cerevisiae proteins of the MNN1 family	-2.74	−
YOL084W	*PHM7*	Protein of unknown function, expression is regulated by phosphate levels	-2.77	−
YKL216W	*URA1*	Dihydroorotate dehydrogenase, catalyzes the fourth enzymatic step in the de novo biosynthesis of pyrimidines, converting dihydroorotic acid into orotic acid	-2.83	-4.34
YGR159C	*NSR1*	Nucleolar protein that binds nuclear localization sequences, required for pre-rRNA processing and ribosome biogenesis	-2.84	−
YGR121C	*MEP1*	Ammonium permease; belongs to a ubiquitous family of cytoplasmic membrane proteins that transport only ammonium (NH4+); expression is under the nitrogen catabolite repression regulation	-2.87	-2.69
YGR248W	*SOL4*	6-phosphogluconolactonase with similarity to Sol3p	-2.87	−
YBR029C	*CDS1*	Phosphatidate cytidylyltransferase (CDP-diglyceride synthetase	-2.89	-4.85
YIL094C	*LYS12*	Homo-isocitrate dehydrogenase, an NAD-linked mitochondrial enzyme required for the fourth step in the biosynthesis of lysine	-2.92	-2.90
YCL024W	*KCC4*	Protein kinase of the bud neck involved in the septin checkpoint,	-2.93	−
YHR183W	*GND1*	6-phosphogluconate dehydrogenase	-2.93	-3.42
YPR184W	*GDB1*	Glycogen debranching enzyme containing glucanotranferase and alpha-1,6-amyloglucosidase activities, required for glycogen degradation; phosphorylated in mitochondria	-2.94	−
YJL200C	*ACO2*	Putative mitochondrial aconitase isozyme	-2.94	−
YMR076C	*PDS5*	Protein required for establishment and maintenance of sister chromatid condensation and cohesion	-2.97	-2.54

YER091C	*MET6*	Cobalamin-independent methionine synthase	-3.08	−
YMR250W	*GAD1*	Glutamate decarboxylase	-3.12	-3.46
YMR120C	*ADE17*	Enzyme of 'de novo' purine biosynthesis containing both 5-aminoimidazole-4-carboxamide ribonucleotide transformylase and inosine monophosphate cyclohydrolase activities, isozyme of Ade16p; ade16 ade17 mutants require adenine and histidine	-3.14	−
YOR074C	*CDC21*	Thymidylate synthase	-3.15	-3.29
YOL109W	*ZEO1*	Peripheral membrane protein of the plasma membrane that interacts with Mid2p	-3.16	−
YNL112W	*DBP2*	Essential ATP-dependent RNA helicase of the DEAD-box protein family, involved in nonsense-mediated mRNA decay and rRNA processing	-3.24	−
YDL003W	*MCD1*	Essential subunit of the cohesin complex required for sister chromatid cohesion in mitosis and meiosis	-3.29	−
YFR015C	*GSY1*	Glycogen synthase with similarity to Gsy2p, the more highly expressed yeast homolog	-3.30	−
YER043C	*SAH1*	S-adenosyl-L-homocysteine hydrolase, catabolizes S-adenosyl-L-homocysteine which is formed after donation of the activated methyl group of S-adenosyl-L-methionine (AdoMet) to an acceptor	-3.37	-4.10
YER026C	*CHO1*	Phosphatidylserine synthase, functions in phospholipid biosynthesis; catalyzes the reaction CDP-diaclyglycerol + L-serine = CMP + L-1-phosphatidylserine, transcriptionally repressed by myo-inositol and choline	-3.40	-5.43
YDL204W	*RTN2*	Protein of unknown function	-3.54	−
YPR037C	*ERV2*	Flavin-linked sulfhydryl oxidase localized to the endoplasmic reticulum lumen, involved in disulfide bond formation within the ER	-3.65	-4.55
YLR183C	*TOS4*	Forkhead Associated domain containing protein and putative transcription factor found associated with chromatin	-3.76	-4.56

YBR208C	*DUR1,2*	Urea amidolyase, contains both urea carboxylase and allophanate hydrolase activities, degrades urea to CO2 and NH3	-3.79	−
YOR315W	*SFG1*	Nuclear protein, putative transcription factor required for growth of superficial pseudohyphae (which do not invade the agar substrate) but not for invasive pseudohyphal growth; may act together with Phd1p	-3.82	-5.06
YMR105C	*PGM2*	Phosphoglucomutase, catalyzes the conversion from glucose-1-phosphate to glucose-6-phosphate	-3.86	−
YLR327C	*TMA10*	Protein of unknown function that associates with ribosomes	-3.90	−
YLR180W	*SAM1*	S-adenosylmethionine synthetase, catalyzes transfer of the adenosyl group of ATP to the sulfur atom of methionine	-4.04	-2.26
YDL227C	*HO*	Site-specific endonuclease required for gene conversion at the MAT locus (homothallic switching) through the generation of a ds DNA break	-4.26	−
YFR053C	*HXK1*	Hexokinase isoenzyme 1, a cytosolic protein that catalyzes phosphorylation of glucose during glucose metabolism	-4.35	−
YBR291C	*CTP1*	Mitochondrial inner membrane citrate transporter, member of the mitochondrial carrier family	-4.42	-3.35
YOR348C	*PUT4*	Proline permease, required for high-affinity transport of proline	-4.47	−
YML128C	*MSC1*	Protein of unknown function; mutant is defective in directing meiotic recombination events to homologous chromatids	-4.73	−
YNL194C	*YNL194C*	Integral membrane protein required for sporulation and plasma membrane sphingolipid content; has sequence similarity to SUR7 and FMP45; GFP-fusion protein is induced in response to the DNA-damaging agent MMS	-4.95	-6.45
YOR375C	*GDH1*	NADP(+)-dependent glutamate dehydrogenase, synthesizes glutamate from ammonia and alpha-ketoglutarate; rate of alpha-ketoglutarate utilization differs from Gdh3p; expression regulated by nitrogen and carbon sources	-4.98	-7.99

YPR192W	*AQY1*	Spore-specific water channel that mediates the transport of water across cell membranes, developmentally controlled; may play a role in spore maturation, probably by allowing water outflow, may be involved in freeze tolerance	-5.07	-3.08
YDR502C	*SAM2*	S-adenosylmethionine synthetase	-5.27	-14.85
YNL065W	*AQR1*	Plasma membrane multidrug transporter of the major facilitator superfamily, confers resistance to short-chain monocarboxylic acids and quinidine	-5.53	-3.28
YER070W	*RNR1*	Major isoform of the large subunit of ribonucleotide-diphosphate reductase	-5.60	-8.64
YPR160W	*GPH1*	Non-essential glycogen phosphorylase required for the mobilization of glycogen, activity is regulated by cyclic AMP-mediated phosphorylation,	-6.10	
YBR092C	*PHO3*	Constitutively expressed acid phosphatase similar to Pho5p	-6.21	-6.52
YNR050C	*LYS9*	Saccharopine dehydrogenase (NADP+, L-glutamate-forming	-6.39	-12.19
YNL142W	*MEP2*	Ammonium permease involved in regulation of pseudohyphal growth	-6.62	-3.89
YDR342C	*HXT7*	High-affinity glucose transporter of the major facilitator superfamily, nearly identical to Hxt6p	-8.35	−
YKL096W	*CWP1*	Cell wall mannoprotein that localizes specifically to birth scars of daughter cells, linked to a beta-1,3- and beta-1,6-glucan heteropolymer through a phosphodiester bond; required for propionic acid resistance	-8.71	-10.09
YDR343C	*HXT6*	High-affinity glucose transporter of the major facilitator superfamily, nearly identical to Hxt7p, expressed at high basal levels relative to other HXTs, repression of expression by high glucose requires SNF3	-9.44	−
YKR039W	*GAP1*	General amino acid permease	-11.78	-11.21
YFL014W	*HSP12*	Plasma membrane localized protein that protects membranes from desiccation	-16.71	−
YMR011W	*HXT2*	High-affinity glucose transporter of the major facilitator superfamily, expression is induced by low levels	-22.27	-58.00

		of glucose and repressed by high levels of glucose		
YLR012C	*YLR012C*	Putative protein of unknown function	−	10.42
YGL138C	*YGL138C*	Putative protein of unknown function	−	10.37
YGL183C	*MND1*	Protein required for recombination and meiotic nuclear division	−	6.18
YER053C-A	*YER053C-A*	Putative protein of unknown function	−	5.66
YNR062C	*YNR062C*	Putative membrane protein of unknown function	−	5.12
YJR149W	*YJR149W*	Putative protein of unknown function	−	4.93
YCR101C	*YCR101C*	Putative protein of unknown function	−	4.89
YOR298W	*MUM3*	Protein of unknown function involved in the organization of the outer spore wall layers	−	4.29
YJL072C	*PSF2*	Subunit of the GINS complex (Sld5p, Psf1p, Psf2p, Psf3p), which is localized to DNA replication origins and implicated in assembly of the DNA replication machinery	−	4.06
YOL131W	*YOL131W*	Putative protein of unknown function	−	4.00
YNR004W	*SWM2*	Putative protein of unknown function	−	3.89
YCR005C	*CIT2*	Citrate synthase, catalyzes the condensation of acetyl coenzyme A and oxaloacetate to form citrate, peroxisomal isozyme involved in glyoxylate cycle; expression is controlled by Rtg1p and Rtg2p transcription factors	−	3.80
YGR243W	*FMP43*	Putative protein of unknown function	−	3.68
YGR161C	*RTS3*	Putative component of the protein phosphatase type 2A complex	−	3.58
YCR106W	*RDS1*	Zinc cluster transcription factor involved in conferring resistance to cycloheximide	−	3.48
YIL029C	*YIL029C*	Putative protein of unknown function; deletion confers sensitivity to 4-(N-(S-glutathionylacetyl)amino) phenylarsenoxide (GSAO)	−	3.47
YJL213W	*YJL213W*	Protein of unknown function that may interact with ribosomes;	−	3.46
YLR266C	*PDR8*	Transcription factor; targets include ATP-binding cassette (ABC)	−	3.24

		transporters, major facilitator superfamily transporters, and other genes involved in the pleiotropic drug resistance (PDR) phenomenon		
YGL096W	*TOS8*	Homeodomain-containing protein and putative transcription factor found associated with chromatin	−	3.13
YGL170C	*SPO74*	Component of the meiotic outer plaque of the spindle pole body, involved in modifying the meiotic outer plaque that is required prior to prospore membrane formation	−	3.08
YGL219C	*MDM34*	Mitochondrial component of the ERMES complex that links the ER to mitochondria and may promote inter-organellar calcium and phospholipid exchange as well as coordinating mitochondrial DNA replication and growth	−	3.05
YGR287C	*YGR287C*	Major isomaltase (alpha-1,6-glucosidase) required for isomaltose utilization; has specificity for isomaltose, palatinose, and methyl-alpha-glucoside; member of the IMA isomaltase family	−	2.97
YHR184W	*SSP1*	Protein involved in the control of meiotic nuclear division and coordination of meiosis with spore formation; transcription is induced midway through meiosis	−	2.92
YOR177C	*MPC54*	Component of the meiotic outer plaque, a membrane-organizing center which is assembled on the cytoplasmic face of the spindle pole body during meiosis II and triggers the formation of the prospore membrane; potential Cdc28p substrate	−	2.91
YEL023C	*YEL023C*	Putative protein of unknown function	−	2.89
YOR012W	*YOR012W*	Putative protein of unknown function	−	2.88
YOR213C	*SAS5*	Subunit of the SAS complex (Sas2p, Sas4p, Sas5p), which acetylates free histones and nucleosomes and regulates transcriptional silencing; stimulates Sas2p HAT activity	−	2.87
YMR034C	*YMR034C*	Putative transporter, member of the SLC10 carrier family	−	2.86
YDL039C	*PRM7*	Pheromone-regulated protein,	−	2.86

		predicted to have one transmembrane segment; promoter contains Gcn4p binding elements		
YPL026C	*SKS1*	Putative serine/threonine protein kinase; involved in the adaptation to low concentrations of glucose independent of the SNF3 regulated pathway	−	2.83
YPL189W	*GUP2*	Probable membrane protein with a possible role in proton symport of glycerol; member of the MBOAT family of putative membrane-bound O-acyltransferases	−	2.83
YMR035W	*IMP2*	Catalytic subunit of the mitochondrial inner membrane peptidase complex, required for maturation of mitochondrial proteins of the intermembrane space	−	2.82
YGL114W	*YGL114W*	Putative protein of unknown function; predicted member of the oligopeptide transporter (OPT) family of membrane transporters	−	2.82
YER039C-A	*YER039C-A*	Putative protein of unknown function; YER039C-A is not an essential gene	−	2.77
YMR135C	*GID8*	Protein of unknown function, involved in proteasome-dependent catabolite inactivation of fructose-1,6-bisphosphatase	−	2.74
YJL058C	*BIT61*	Subunit of TORC2 (Tor2p-Lst8p-Avo1-Avo2-Tsc11p-Bit61p-Slm1p-Slm2p), a membrane-associated complex that regulates cell cycle-dependent actin cytoskeletal dynamics during polarized growth and cell wall integrity	−	2.69
YPL068C	*YPL068C*	Protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the nucleus and is induced in response to the DNA-damaging agent MMS	−	2.64
YIL009C-A	*EST3*	Component of the telomerase holoenzyme, involved in telomere replication	−	2.61
YLR205C	*HMX1*	ER localized heme oxygenase, involved in heme degradation during iron starvation and in the oxidative stress response	−	2.60
YGL162W	*SUT1*	Transcription factor of the Zn[II]2Cys6 family involved in sterol uptake; involved in induction of	−	2.60

		hypoxic gene expression		
YAL064C-A	*YAL064C-A*	Putative protein of unknown function; YAL064C-A is not an essential gene	−	2.60
YDL039C	*PRM7*	Pheromone-regulated protein, predicted to have one transmembrane segment; promoter contains Gcn4p binding elements	−	2.59
YNL314W	*DAL82*	Positive regulator of allophanate inducible genes; binds a dodecanucleotide sequence upstream of all genes that are induced by allophanate; contains an UISALL DNA-binding, a transcriptional activation, and a coiled-coil domain	−	2.53
YNR074C	*AIF1*	Mitochondrial cell death effector that translocates to the nucleus in response to apoptotic stimuli, homolog of mammalian Apoptosis-Inducing Factor, putative reductase	−	2.53
YPL148C	*PPT2*	Phosphopantetheine:protein transferase (PPTase), activates mitochondrial acyl carrier protein (Acp1p) by phosphopantetheinylation	−	2.52
YER073W	*ALD5*	Mitochondrial aldehyde dehydrogenase, involved in regulation or biosynthesis of electron transport chain components and acetate formation	−	2.50
YFR025C	*HIS2*	Histidinolphosphatase, catalyzes the eighth step in histidine biosynthesis; mutations cause histidine auxotrophy and sensitivity to Cu, Co, and Ni salts; transcription is regulated by general amino acid control	−	2.49
YPL024W	*RMI1*	Subunit of the RecQ (Sgs1p) - Topo III (Top3p) complex; stimulates superhelical relaxing and ssDNA binding activities of Top3p	−	2.48
YNL042W	*BOP3*	Protein of unknown function, potential Cdc28p substrate	−	2.45
YGL159W	*YGL159W*	Putative protein of unknown function	−	2.42
YIL003W	*CFD1*	Highly conserved, iron-sulfur cluster binding protein localized in the cytoplasm	−	2.41
YDL109C	*YDL109C*	Putative lipase; involved in lipid metabolism	−	2.41
YLR030W	*YLR030W*	Putative protein of unknown function	−	2.38

YIL152W	*YIL152W*	Putative protein of unknown function	−	2.37
YGR146C	*ECL1*	Protein of unknown function, affects chronological lifespan	−	2.35
YFR035C	*YFR035C*	Putative protein of unknown function, deletion mutant exhibits synthetic phenotype with alpha-synuclein	−	2.34
YOL133W	*HRT1*	RING finger containing subunit of Skp1-Cullin-F-box ubiquitin protein ligases (SCF); required for Gic2p, Far1p, Sic1p and Cln2p degradation; may tether Cdc34p (a ubiquitin conjugating enzyme or E2) and Cdc53p (a cullin) subunits of SCF	−	2.34
YJR094C	*IME1*	Master regulator of meiosis that is active only during meiotic events, activates transcription of early meiotic genes through interaction with Ume6p, degraded by the 26S proteasome following phosphorylation by Ime2p	−	2.33
YDL127W	*PCL2*	Cyclin, interacts with cyclin-dependent kinase Pho85p	−	2.33
YCL056C	*YCL056C*	Protein of unknown function	−	2.32
YOL025W	*LAG2*	Protein that negatively regulates the SCF E3-ubiquitin ligase by interacting with and preventing neddyation of the cullin subunit, Cdc53p; longevity determinant that is preferentially expressed in young cells; similar to mammalian Cand1	−	2.30
YLR326W	*YLR326W*	Putative protein of unknown function, predicted to be palmitoylated	−	2.30
YFR008W	*FAR7*	Protein involved in recovery from cell cycle arrest in response to pheromone, in a Far1p-independent pathway	−	2.30
YDR408C	*ADE8*	Phosphoribosyl-glycinamide transformylase, catalyzes a step in the 'de novo' purine nucleotide biosynthetic pathway	−	2.28
YOR179C	*SYC1*	Subunit of the APT subcomplex of cleavage and polyadenylation factor, may have a role in 3' end formation of both polyadenylated and non-polyadenylated RNAs	−	2.27
YCR007C	*YCR007C*	Putative integral membrane protein, member of DUP240 gene family; YCR007C is not an essential gene	−	2.27

YLR452C	*SST2*	GTPase-activating protein for Gpa1p, regulates desensitization to alpha factor pheromone	−	2.27
YOR201C	*MRM1*	Ribose methyltransferase that modifies a functionally critical, conserved nucleotide in mitochondrial 21S rRNA	−	2.26
YJR021C	*REC107*	Protein involved in early stages of meiotic recombination; involved in coordination between the initiation of recombination and the first division of meiosis; part of a complex (Rec107p-Mei4p-Rec114p) required for ds break formation	−	2.25
YPR083W	*MDM36*	Mitochondrial protein required for normal mitochondrial morphology and inheritance; proposed involvement in the formation of Dnm1p and Num1p-containing cortical anchor complexes that promote mitochondrial fission	−	2.24
YJR127C	*RSF2*	Zinc-finger protein involved in transcriptional control of both nuclear and mitochondrial genes, many of which specify products required for glycerol-based growth, respiration, and other functions	−	2.23
YJL163C	*YJL163C*	Putative protein of unknown function	−	2.23
YPL252C	*YAH1*	Ferredoxin of the mitochondrial matrix required for formation of cellular iron-sulfur proteins; involved in heme A biosynthesis	−	2.23
YMR059W	*SEN15*	Subunit of the tRNA splicing endonuclease, which is composed of Sen2p, Sen15p, Sen34p, and Sen54p	−	2.23
YBR107C	*IML3*	Protein with a role in kinetochore function, localizes to the outer kinetochore in a Ctf19p-dependent manner, interacts with Chl4p and Ctf19p	−	2.23
YMR065W	*KAR5*	Protein required for nuclear membrane fusion during karyogamy, localizes to the membrane with a soluble portion in the endoplasmic reticulum lumen, may form a complex with Jem1p and Kar2p; expression of the gene is regulated by pheromone	−	2.21

YJL100W	*LSB6*	Type II phosphatidylinositol 4-kinase that binds Las17p, which is a homolog of human Wiskott-Aldrich Syndrome protein involved in actin patch assembly and actin polymerization	−	2.21
YPL214C	*THI6*	Bifunctional enzyme with thiamine-phosphate pyrophosphorylase and 4-methyl-5-beta-hydroxyethylthiazole kinase activities, required for thiamine biosynthesis	−	2.20
YOR211C	*MGM1*	Chr XV from 741569-738924, reverse complement, Verified ORF, ""Mitochondrial GTPase, present in complex with Ugo1p and Fzo1p	−	2.20
YIL114C	*POR2*	Putative mitochondrial porin (voltage-dependent anion channel), related to Por1p but not required for mitochondrial membrane permeability or mitochondrial osmotic stability	−	2.19
YDR259C	*YAP6*	Putative basic leucine zipper (bZIP) transcription factor; overexpression increases sodium and lithium tolerance	−	2.19
YHL027W	*RIM101*	Transcriptional repressor involved in response to pH and in cell wall construction; required for alkaline pH-stimulated haploid invasive growth and sporulation; activated by proteolytic processing	−	2.17
YOR019W	*YOR019W*	Protein of unknown function that may interact with ribosomes, based on co-purification experiments	−	2.17
YLR193C	*UPS1*	Mitochondrial intermembrane space protein that regulates mitochondrial cardiolipin levels, null has defects in Mgm1p processing, integrity of mitochondrial inner membrane complexes, and mitochondrial morphology; ortholog of human PRELI	−	2.17
YFR005C	*SAD1*	Conserved zinc-finger domain protein involved in pre-mRNA splicing, required for assembly of U4 snRNA into the U4/U6 particle	−	2.17
YJR097W	*JJJ3*	Protein of unknown function, contains a J-domain, which is a region with homology to the E. coli DnaJ protein	−	2.16

YFL050C	*ALR2*	Probable Mg(2+) transporter; overexpression confers increased tolerance to Al(3+) and Ga(3+) ions; plays a role in regulating Ty1 transposition	−	2.16
YHR199C	*AIM46*	Putative protein of unknown function; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies; null mutant displays elevated frequency of mitochondrial genome loss	−	2.15
YFR036W	*CDC26*	Subunit of the Anaphase-Promoting Complex/Cyclosome (APC/C), which is a ubiquitin-protein ligase required for degradation of anaphase inhibitors, including mitotic cyclins, during the metaphase/anaphase transition	−	2.14
YBR063C	*YBR063C*	Putative protein of unknown function; YBR063C is not an essential gene	−	2.14
YML002W	*YML002W*	Putative protein of unknown function; expression induced by heat and by calcium shortage	−	2.13
YOR028C	*CIN5*	Basic leucine zipper (bZIP) transcription factor of the yAP-1 family, mediates pleiotropic drug resistance and salt tolerance	−	2.13
YKR106W	*YKR106W*	Protein of unconfirmed function; displays a topology characteristic of the Major Facilitators Superfamily of membrane proteins; coding sequence 98% identical to that of YCL073C	−	2.13
YJR136C	*TTI2*	Putative protein of unknown function	−	2.12
YGR230W	*BNS1*	Protein with some similarity to Spo12p; overexpression bypasses need for Spo12p, but not required for meiosis	−	2.11
YPR071W	*YPR071W*	Putative membrane protein; YPR071W is not an essential gene	−	2.11
YBR004C	*GPI18*	Functional ortholog of human PIG-V, which is a mannosyltransferase that transfers the second mannose in glycosylphosphatidylinositol biosynthesis; the authentic, non-tagged protein was localized to mitochondria	−	2.11
YAL015C	*NTG1*	DNA N-glycosylase and apurinic/apyrimidinic (AP) lyase	−	2.10

		involved in base excision repair; acts in both nucleus and mitochondrion; creates a double-strand break at mtDNA origins that stimulates replication in response to oxidative stress		
YKR019C	*IRS4*	EH domain-containing protein involved in regulating phosphatidylinositol 4,5-bisphosphate levels and autophagy	−	2.10
YOR383C	*FIT3*	Mannoprotein that is incorporated into the cell wall via a glycosylphosphatidylinositol (GPI) anchor, involved in the retention of siderophore-iron in the cell wall	−	2.10
YNR029C	*YNR029C*	Putative protein of unknown function, deletion confers reduced fitness in saline	−	2.10
YOR079C	*ATX2*	Golgi membrane protein involved in manganese homeostasis; overproduction suppresses the sod1 (copper, zinc superoxide dismutase) null mutation	−	2.10
YIL104C	*SHQ1*	Chaperone protein required for the assembly of box H/ACA snoRNPs and thus for pre-rRNA processing, forms a complex with Naf1p and interacts with H/ACA snoRNP components Nhp2p and Cbf5p; homology with known Hsp90p cochaperones	−	2.10
YFL051C	*YFL051C*	Putative protein of unknown function; YFL051C is not an essential gene	−	2.10
YOR183W	*FYV12*	Protein of unknown function, required for survival upon exposure to K1 killer toxin	−	2.09
YGR029W	*ERV1*	Flavin-linked sulfhydryl oxidase of the mitochondrial intermembrane space (IMS), oxidizes Mia40p as part of a disulfide relay system that promotes IMS retention of imported proteins; ortholog of human hepatopoietin (ALR)	−	2.09
YDR093W	*DNF2*	Aminophospholipid translocase (flippase) that localizes primarily to the plasma membrane; contributes to endocytosis, protein transport and cell polarity; type 4 P-type ATPase	−	2.09
YFL027C	*GYP8*	GTPase-activating protein for yeast Rab family members; Ypt1p is the	−	2.08

		preferred in vitro substrate but also acts on Sec4p, Ypt31p and Ypt32p; involved in the regulation of ER to Golgi vesicle transport		
YOL163W	*YOL163W*	Putative protein of unknown function; member of the Dal5p subfamily of the major facilitator family	−	2.07
YLR125W	*YLR125W*	Putative protein of unknown function	−	2.06
YAR028W	*YAR028W*	Putative integral membrane protein, member of DUP240 gene family;	−	2.06
YOR268C	*YOR268C*	Putative protein of unknown function	−	2.06
YPR061C	*JID1*	Probable Hsp40p co-chaperone, has a DnaJ-like domain and appears to be involved in ER-associated degradation of misfolded proteins containing a tightly folded cytoplasmic domain	−	2.05
YGL025C	*PGD1*	Subunit of the RNA polymerase II mediator complex	−	2.05
YDL236W	*PHO13*	Alkaline phosphatase specific for p-nitrophenyl phosphate	−	2.05
YOR107W	*RGS2*	Negative regulator of glucose-induced cAMP signaling	−	2.04
YMR303C	*ADH2*	Glucose-repressible alcohol dehydrogenase II, catalyzes the conversion of ethanol to acetaldehyde	−	2.04
YCR073C	*SSK22*	MAP kinase kinase kinase of the HOG1 mitogen-activated signaling pathway	−	2.04
YIL099W	*SGA1*	Intracellular sporulation-specific glucoamylase involved in glycogen degradation; induced during starvation of a/a diploids late in sporulation, but dispensable for sporulation	−	2.04
YGL179C	*TOS3*	Protein kinase, related to and functionally redundant with Elm1p and Sak1p for the phosphorylation and activation of Snf1p	−	2.03
YDR073W	*SNF11*	Subunit of the SWI/SNF chromatin remodeling complex involved in transcriptional regulation	−	2.03
YDR191W	*HST4*	Member of the Sir2 family of NAD(+)-dependent protein deacetylases; involved along with Hst3p in silencing at telomeres,	−	2.03
YPR058W	*YMC1*	Mitochondrial protein, putative inner	−	2.03

		membrane transporter with a role in oleate metabolism and glutamate biosynthesis		
YNL227C	*JJJ1*	Co-chaperone that stimulates the ATPase activity of Ssa1p, required for a late step of ribosome biogenesis; associated with the cytosolic large ribosomal subunit	−	2.02
YBR278W	*DPB3*	Third-largest subunit of DNA polymerase II (DNA polymerase epsilon), required to maintain fidelity of chromosomal replication and also for inheritance of telomeric silencing	−	2.01
YMR023C	*MSS1*	Mitochondrial protein, forms a heterodimer complex with Mto1p that performs the 5-carboxymethylaminomethyl modification of the wobble uridine base in mitochondrial tRNAs	−	2.01
YMR176W	*ECM5*	Non-essential protein of unknown function, contains ATP/GTP-binding site motif A; null mutant exhibits cellular volume up to four times greater than wild-type, also large drooping buds with elongated necks	−	2.00
YGR074W	*SMD1*	Core Sm protein Sm D1; part of heteroheptameric complex (with Smb1p, Smd2p, Smd3p, Sme1p, Smx3p, and Smx2p) that is part of the spliceosomal U1, U2, U4, and U5 snRNPs; homolog of human Sm D1	−	2.00
YPR013C	*YPR013C*	Putative zinc finger protein; YPR013C is not an essential gene	−	2.00
YML085C	*TUB1*	Alpha-tubulin; associates with beta-tubulin (Tub2p) to form tubulin dimer, which polymerizes to form microtubules	−	-2.01
YOL030W	*GAS5*	1,3-beta-glucanosyltransferase	−	-2.01
YKL027W	*YKL027W*	Protein of unknown function, localized to the mitochondrial outer membrane	−	-2.02
YHR123W	*EPT1*	sn-1,2-diacylglycerol ethanolamine- and cholinephosphotranferase	−	-2.02
YJL177W	*RPL17B*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl17Ap and has similarity to E. coli L22 and rat L17 ribosomal proteins	−	-2.02
YNL280C	*ERG24*	C-14 sterol reductase, acts in ergosterol biosynthesis	−	-2.02

YER031C	*YPT31*	Rab family GTPase, very similar to Ypt32p	−	-2.02
YML093W	*UTP14*	Subunit of U3-containing Small Subunit (SSU) processome complex involved in production of 18S rRNA and assembly of small ribosomal subunit	−	-2.02
YDR391C	*YDR391C*	Putative protein of unknown function	−	-2.03
YLR172C	*DPH5*	Methyltransferase required for synthesis of diphthamide	−	-2.03
YBR189W	*RPS9B*	Protein component of the small (40S) ribosomal subunit	−	-2.03
YNL151C	*RPC31*	RNA polymerase III subunit C31	−	-2.03
YKR059W	*TIF1*	Translation initiation factor eIF4A, identical to Tif2p; DEA(D/H)-box RNA helicase that couples ATPase activity to RNA binding and unwinding	−	-2.04
YBR137W	*YBR137W*	Protein of unknown function; localized to the cytoplasm	−	-2.04
YOR168W	*GLN4*	Glutamine tRNA synthetase, monomeric class I tRNA synthetase	−	-2.04
YHL033C	*RPL8A*	Ribosomal protein L4 of the large (60S) ribosomal subunit, nearly identical to Rpl8Bp and has similarity to rat L7a ribosomal protein; mutation results in decreased amounts of free 60S subunits	−	-2.04
YBR025C	*OLA1*	P-loop ATPase with similarity to human OLA1 and bacterial YchF; identified as specifically interacting with the proteasome	−	-2.04
YGL200C	*EMP24*	Integral membrane component of endoplasmic reticulum-derived COPII-coated vesicles, which function in ER to Golgi transport	−	-2.05
YMR307W	*GAS1*	Beta-1,3-glucanosyltransferase, required for cell wall assembly and also has a role in transcriptional silencing; localizes to the cell surface via a glycosylphosphatidylinositol (GPI) anchor	−	-2.05
YEL040W	*UTR2*	Chitin transglycosylase that functions in the transfer of chitin to beta(1-6) and beta(1-3) glucans in the cell wall	−	-2.05
YLR150W	*STM1*	Protein required for optimal translation under nutrient stress; perturbs association of Yef3p with ribosomes; involved in TOR signaling	−	-2.05

YEL031W	*SPF1*	P-type ATPase, ion transporter of the ER membrane involved in ER function and Ca2+ homeostasis	−	-2.05
YJL138C	*TIF2*	Translation initiation factor eIF4A, identical to Tif1p; DEA(D/H)-box RNA helicase that couples ATPase activity to RNA binding and unwinding	−	-2.05
YDL145C	*COP1*	Alpha subunit of COPI vesicle coatomer complex, which surrounds transport vesicles in the early secretory pathway	−	-2.05
YEL054C	*RPL12A*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl12Bp; rpl12a rpl12b double mutant exhibits slow growth and slow translation; has similarity to E. coli L11 and rat L12 ribosomal proteins	−	-2.06
YJR103W	*URA8*	Minor CTP synthase isozyme (see also URA7), catalyzes the ATP-dependent transfer of the amide nitrogen from glutamine to UTP, forming CTP, the final step in de novo biosynthesis of pyrimidines	−	-2.07
YJL050W	*MTR4*	ATP-dependent 3'-5' RNA helicase of the Dead-box family, involved in nuclear RNA processing and degradation both as a component of the TRAMP complex and in TRAMP independent processes	−	-2.07
YAL003W	*EFB1*	Translation elongation factor 1 beta; stimulates nucleotide exchange to regenerate EF-1 alpha-GTP for the next elongation cycle	−	-2.07
YDR002W	*YRB1*	Ran GTPase binding protein; involved in nuclear protein import and RNA export, ubiquitin-mediated protein degradation during the cell cycle	−	-2.07
YFR014C	*CMK1*	Calmodulin-dependent protein kinase; may play a role in stress response, many CA++/calmodulan dependent phosphorylation substrates demonstrated in vitro, amino acid sequence similar to Cmk2p and mammalian Cam Kinase II	−	-2.09
YGR214W	*RPS0A*	Protein component of the small (40S) ribosomal subunit, nearly identical to Rps0Bp; required for maturation of	−	-2.09

		18S rRNA along with Rps0Bp		
YHR113W	*YHR113W*	Cytoplasmic aspartyl aminopeptidase; cleaves unblocked N-terminal acidic amino acid residues from peptide substrates	−	-2.10
YPL247C	*YPL247C*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and nucleus	−	-2.10
YLR249W	*YEF3*	Gamma subunit of translational elongation factor eEF1B, stimulates the binding of aminoacyl-tRNA (AA-tRNA) to ribosomes by releasing eEF1A (Tef1p/Tef2p) from the ribosomal complex; contains two ABC cassettes; binds and hydrolyzes ATP	−	-2.10
YOR198C	*BFR1*	Component of mRNP complexes associated with polyribosomes; implicated in secretion and nuclear segregation; multicopy suppressor of BFA (Brefeldin A) sensitivity	−	-2.12
YGR123C	*PPT1*	Protein serine/threonine phosphatase with similarity to human phosphatase PP5; present in both the nucleus and cytoplasm	−	-2.12
YJL080C	*SCP160*	Essential RNA-binding G protein effector of mating response pathway, mainly associated with nuclear envelope and ER, interacts in mRNA-dependent manner with translating ribosomes via multiple KH domains, similar to vertebrate vigilins	−	-2.12
YMR260C	*TIF11*	Translation initiation factor eIF1A, essential protein that forms a complex with Sui1p (eIF1) and the 40S ribosomal subunit and scans for the start codon; C-terminus associates with Fun12p (eIF5B	−	-2.12
YJR115W	*YJR115W*	Putative protein of unknown function	−	-2.13
YJR133W	*XPT1*	Xanthine-guanine phosphoribosyl transferase, required for xanthine utilization and for optimal utilization of guanine	−	-2.13
YER110C	*KAP123*	Karyopherin beta, mediates nuclear import of ribosomal proteins prior to assembly into ribosomes and import of histones H3 and H4	−	-2.13
YLR133W	*CKI1*	Choline kinase, catalyzing the first	−	-2.14

		step in phosphatidylcholine synthesis via the CDP-choline (Kennedy pathway		
YOR230W	*WTM1*	Transcriptional modulator involved in regulation of meiosis, silencing, and expression of RNR genes; required for nuclear localization of the ribonucleotide reductase small subunit Rnr2p and Rnr4p; contains WD repeats	−	-2.15
YLR153C	*ACS2*	Acetyl-coA synthetase isoform which, along with Acs1p, is the nuclear source of acetyl-coA for histone acetylation	−	-2.15
YBL042C	*FUI1*	High affinity uridine permease, localizes to the plasma membrane; also mediates low but significant transport of the cytotoxic nucleoside analog 5-fluorouridine; not involved in uracil transport	−	-2.16
YER131W	*RPS26B*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps26Ap and has similarity to rat S26 ribosomal protein	−	-2.17
YAR071W	*PHO11*	One of three repressible acid phosphatases, a glycoprotein that is transported to the cell surface by the secretory pathway; induced by phosphate starvation and coordinately regulated by PHO4 and PHO2	−	-2.17
YPL211W	*NIP7*	Nucleolar protein required for 60S ribosome subunit biogenesis, constituent of 66S pre-ribosomal particles; physically interacts with Nop8p and the exosome subunit Rrp43p	−	-2.17
YOR293W	*RPS10A*	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps10Bp and has similarity to rat ribosomal protein S10	−	-2.18
YFL022C	*FRS2*	Alpha subunit of cytoplasmic phenylalanyl-tRNA synthetase	−	-2.18
YLR347C	*KAP95*	Karyopherin beta, forms a complex with Srp1p/Kap60p	−	-2.19
YIR036C	*IRC24*	Putative benzil reductase;(GFP)-fusion protein localizes to the cytoplasm and is induced by the DNA-damaging agent MMS	−	-2.19
YKL093W	*MBR1*	Protein involved in mitochondrial	−	-2.19

		functions and stress response; overexpression suppresses growth defects of hap2, hap3, and hap4 mutants		
YKL073W	*LHS1*	Molecular chaperone of the endoplasmic reticulum lumen, involved in polypeptide translocation and folding	−	-2.19
YOR099W	*KTR1*	Alpha-1,2-mannosyltransferase involved in O- and N-linked protein glycosylation; type II membrane protein	−	-2.19
YHR049W	*FSH1*	Putative serine hydrolase that localizes to both the nucleus and cytoplasm; sequence is similar to S. cerevisiae Fsh2p and Fsh3p and the human candidate tumor suppressor OVCA2	−	-2.19
YDL084W	*SUB2*	Component of the TREX complex required for nuclear mRNA export; member of the DEAD-box RNA helicase superfamily and is involved in early and late steps of spliceosome assembly	−	-2.19
YFR041C	*ERJ5*	Type I membrane protein with a J domain is required to preserve the folding capacity of the endoplasmic reticulum	−	-2.20
YAL040C	*CLN3*	G1 cyclin involved in cell cycle progression; activates Cdc28p kinase to promote the G1 to S phase transition; plays a role in regulating transcription of the other G1 cyclins, CLN1 and CLN2	−	-2.20
YOL107W	*YOL107W*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and colocalizes in a punctate pattern with the early golgi/COPI vesicles	−	-2.20
YCR084C	*TUP1*	General repressor of transcription, forms complex with Cyc8p, involved in the establishment of repressive chromatin structure through interactions with histones H3 and H4, appears to enhance expression of some genes	−	-2.20
YNL110C	*NOP15*	Constituent of 66S pre-ribosomal particles, involved in 60S ribosomal subunit biogenesis	−	-2.21

YHL034C	*SBP1*	Putative RNA binding protein	−	-2.21
YDL148C	*NOP14*	Nucleolar protein, forms a complex with Noc4p that mediates maturation and nuclear export of 40S ribosomal subunits	−	-2.23
YOR310C	*NOP58*	Protein involved in pre-rRNA processing, 18S rRNA synthesis, and snoRNA synthesis; component of the small subunit processome complex, which is required for processing of pre-18S rRNA	−	-2.24
YPL106C	*SSE1*	ATPase that is a component of the heat shock protein Hsp90 chaperone complex; binds unfolded proteins; member of the heat shock protein 70 (HSP70) family; localized to the cytoplasm	−	-2.24
YBR106W	*PHO88*	Probable membrane protein, involved in phosphate transport	−	-2.25
YNL300W	*TOS6*	Glycosylphosphatidylinositol-dependent cell wall protein, expression is periodic and decreases in respone to ergosterol perturbation or upon entry into stationary phase	−	-2.25
YPL111W	*CAR1*	Arginase, responsible for arginine degradation, expression responds to both induction by arginine and nitrogen catabolite repression	−	-2.25
YMR315W	*YMR315W*	Protein with NADP(H) oxidoreductase activity	−	-2.26
YOL012C	*HTZ1*	Histone variant H2AZ	−	-2.26
YEL042W	*GDA1*	Guanosine diphosphatase located in the Golgi	−	-2.27
YGL077C	*HNM1*	Choline/ethanolamine transporter	−	-2.27
YJL012C	*VTC4*	Vacuolar membrane polyphosphate polymerase	−	-2.28
YML056C	*IMD4*	Inosine monophosphate dehydrogenase	−	-2.30
YNL209W	*SSB2*	Cytoplasmic ATPase that is a ribosome-associated molecular chaperone,	−	-2.30
YCL036W	*GFD2*	Protein of unknown function, identified as a high-copy suppressor of a dbp5 mutation	−	-2.30
YMR199W	*CLN1*	G1 cyclin involved in regulation of the cell cycle	−	-2.30
YBR286W	*APE3*	Vacuolar aminopeptidase Y, processed to mature form by Prb1p	−	-2.30
YKL081W	*TEF4*	Gamma subunit of translational	−	-2.30

		elongation factor eEF1B		
YKR001C	*VPS1*	Dynamin-like GTPase required for vacuolar sorting	−	-2.35
YNL016W	*PUB1*	Poly (A)+ RNA-binding protein	−	-2.36
YOR176W	*HEM15*	Ferrochelatase	−	-2.37
YER145C	*FTR1*	High affinity iron permease involved in the transport of iron across the plasma membrane	−	-2.38
YDL229W	*SSB1*	Cytoplasmic ATPase that is a ribosome-associated molecular chaperone,	−	-2.38
YMR003W	*AIM34*	Protein of unknown function	−	-2.40
YKL069W	*YKL069W*	Methionine-R-sulfoxide reductase	−	-2.41
YER023W	*PRO3*	Delta 1-pyrroline-5-carboxylate reductase, catalyzes the last step in proline biosynthesis	−	-2.42
YJL012C	*VTC4*	Vacuolar membrane polyphosphate polymerase	−	-2.43
YLR432W	*IMD3*	Inosine monophosphate dehydrogenase	−	-2.45
YDL014W	*NOP1*	Nucleolar protein	−	-2.47
YNL169C	*PSD1*	Phosphatidylserine decarboxylase of the mitochondrial inner membrane	−	-2.47
YMR186W	*HSC82*	Cytoplasmic chaperone of the Hsp90 family	−	-2.50
YAL007C	*ERP2*	Protein that forms a heterotrimeric complex with Erp1p, Emp24p, and Erv25p	−	-2.52
YMR049C	*ERB1*	Constituent of 66S pre-ribosomal particles	−	-2.53
YJL091C	*GWT1*	Protein involved in the inositol acylation of glucosaminyl phosphatidylinositol (GlcN-PI) to form glucosaminyl(acyl)phosphatidylinositol (GlcN(acyl)PI)	−	-2.57
YAL005C	*SSA1*	ATPase involved in protein folding and nuclear localization signal (NLS)-directed nuclear transport	−	-2.58
YPL163C	*SVS1*	Cell wall and vacuolar protein	−	-2.58
YHL028W	*WSC4*	ER membrane protein involved in the translocation of soluble secretory proteins and insertion of membrane proteins into the ER membrane	−	-2.61
YHR096C	*HXT5*	Hexose transporter with moderate affinity for glucose	−	-2.64

YDL095W	*PMT1*	Protein O-mannosyltransferase	−	-2.66
YNL289W	*PCL1*	Cyclin, interacts with cyclin-dependent kinase Pho85p	−	-2.67
YKL182W	*FAS1*	Beta subunit of fatty acid synthetase	−	-2.70
YMR215W	*GAS3*	Putative 1,3-beta-glucanosyltransferase	−	-2.74
YNL246W	*VPS75*	NAP family histone chaperone	−	-2.75
YGR244C	*LSC2*	Beta subunit of succinyl-CoA ligase	−	-2.78
YPR069C	*SPE3*	Spermidine synthase	−	-2.79
YGL120C	*PRP43*	RNA helicase in the DEAH-box family	−	-2.85
YPR183W	*DPM1*	Dolichol phosphate mannose (Dol-P-Man) synthase of the ER membrane	−	-2.94
YMR290C	*HAS1*	ATP-dependent RNA helicase	−	-2.95
YNL135C	*FPR1*	Peptidyl-prolyl cis-trans isomerase (PPIase	−	-2.96
YGL255W	*ZRT1*	High-affinity zinc transporter of the plasma membrane	−	-2.96
YJL034W	*KAR2*	ATPase involved in protein import into the ER	−	-3.03
YAL023C	*PMT2*	Protein O-mannosyltransferase	−	-3.04
YDR399W	*HPT1*	Dimeric hypoxanthine-guanine phosphoribosyltransferase	−	-3.27
YNL195C	*YNL195C*	Putative protein of unknown function	−	-3.32
YJL073W	*JEM1*	DnaJ-like chaperone required for nuclear membrane fusion during mating	−	-3.37
YPL127C	*HHO1*	Histone H1, a linker histone required for nucleosome packaging at restricted sites	−	-3.46
YKL165C	*MCD4*	Protein involved in glycosylphosphatidylinositol (GPI) anchor synthesis	−	-3.46
YDL079C	*MRK1*	Glycogen synthase kinase 3 (GSK-3) homolog	−	-3.80
YNL044W	*YIP3*	Protein localized to COPII vesicles	−	-3.82
YHR216W	*IMD2*	Inosine monophosphate dehydrogenase,	−	-4.01
YDR034W-B	*YDR034W-B*	Predicted tail-anchored plasma membrane protein containing a conserved CYSTM module	−	-4.20
YGR157W	*CHO2*	Phosphatidylethanolamine methyltransferase (PEMT	−	-4.32
YNL141W	*AAH1*	Adenine deaminase (adenine aminohydrolase), converts adenine to hypoxanthine; involved in purine salvage	−	-4.43

YBR088C	*POL30*	Proliferating cell nuclear antigen (PCNA	−	-4.98
YDL227C	*HO*	Site-specific endonuclease required for gene conversion at the MAT locus (homothallic switching) through the generation of a ds DNA break	−	-5.27
YJL153C	*INO1*	Inositol-3-phosphate synthase	−	-21.48
YMR011W	*HXT2*	High-affinity glucose transporter of the major facilitator superfamily,	−	-58.00

Table 2.2. Genes showing more than 2 fold change in response to chronic VPA treatment. (Gene descriptions are from Saccharomyces Genome Database).

Systemic name	*Gene name*	Description	Fold change VPA+/VPA-	
			Inositol+	**Inositol-**
YCR034W	*FEN1*	Fatty acid elongase, involved in sphingolipid biosynthesis; acts on fatty acids of up to 24 carbons in length	2.46	2.86
YLR372W	*SUR4*	Elongase, involved in fatty acid and sphingolipid biosynthesis	2.77	2.47
YNR056C	*BIO5*	Putative transmembrane protein involved in the biotin biosynthesis pathway	−	-8.92
YCL025C	*AGP1*	Low-affinity amino acid permease with broad substrate range, involved in uptake of asparagine, glutamine, and other amino acids	−	-3.77
YKR039W	*GAP1*	General amino acid permease; localization to the plasma membrane is regulated by nitrogen source	−	-2.85
YBR068C	*BAP2*	High-affinity leucine permease, functions as a branched-chain amino acid permease involved in the uptake of leucine, isoleucine and valine	−	-6.22
YPL265W	*DIP5*	Dicarboxylic amino acid permease, mediates high-affinity and high-capacity transport of L-glutamate and L-aspartate	-2.22	-2.36
YDL210W	*UGA4*	Permease that serves as a gamma-aminobutyrate (GABA) transport protein involved in the utilization of GABA as a nitrogen source	−	-2.26
YEL063C	*CAN1*	Plasma membrane arginine permease	−	-2.31
YPL274W	*SAM3*	High-affinity S-adenosylmethionine permease, required for utilization of S-adenosylmethionine as a sulfur source	−	-13.79
YDR518W	*EUG1*	Protein disulfide isomerase of the endoplasmic reticulum lumen, function overlaps with that of Pdi1p	−	2.39
YJL073W	*JEM1*	DnaJ-like chaperone required for nuclear membrane fusion during mating, localizes to the ER membrane	−	5.81
YKL073W	*LHS1*	Molecular chaperone of the endoplasmic reticulum lumen, involved in polypeptide translocation and folding; nucleotide exchange factor for the ER lumenal Hsp70 chaperone Kar2p; regulated by the unfolded protein response pathway	−	3.50
YCL043C	*PDI1*	Protein disulfide isomerase, multifunctional protein resident in the endoplasmic reticulum lumen	−	2.83
YPL187W	*MF(ALPH*	Mating pheromone alpha-factor, made by alpha	11.29	−

	A)1	cells		
YOR153W	*PDR5*	Plasma membrane ATP-binding cassette (ABC) transporter, multidrug transporter actively regulated by Pdr1p	10.09	12.10
YLR307W	*CDA1*	Chitin deacetylase, together with Cda2p involved in the biosynthesis ascospore wall component, chitosan	8.54	8.31
YJL116C	*NCA3*	Protein that functions with Nca2p to regulate mitochondrial expression of subunits 6 (Atp6p) and 8 (Atp8p) of the Fo-F1 ATP synthase	7.43	−
YKL121W	*DGR2*	Protein of unknown function	5.55	2.68
YKL178C	*STE3*	Receptor for a factor pheromone, couples to MAP kinase cascade to mediate pheromone response	5.53	−
YBR040W	*FIG1*	Integral membrane protein required for efficient mating	5.37	−
YOL101C	*IZH4*	Membrane protein involved in zinc ion homeostasis, member of the four-protein IZH family, expression induced by fatty acids and altered zinc levels	5.35	5.88
YEL057C	*YEL057C*	Protein of unknown function involved in telomere maintenance	5.30	−
YNL289W	*PCL1*	Cyclin, interacts with cyclin-dependent kinase Pho85p	5.25	−
YJR078W	*BNA2*	Putative tryptophan 2,3-dioxygenase or indoleamine 2,3-dioxygenase, required for de novo biosynthesis of NAD from tryptophan via kynurenine	5.13	−
YGR225W	*AMA1*	Activator of meiotic anaphase promoting complex (APC/C	5.03	2.59
YOR315W	*SFG1*	Nuclear protein, putative transcription factor required for growth of superficial pseudohyphae (which do not invade the agar substrate) but not for invasive pseudohyphal growth	4.94	−
YDR317W	*HIM1*	Protein of unknown function involved in DNA repair	4.78	−
YML027W	*YOX1*	Homeodomain-containing transcriptional repressor, binds to Mcm1p and to early cell cycle boxes (ECBs) in the promoters of cell cycle-regulated genes expressed in M/G1 phase; expression is cell cycle-regulated	4.59	−
YGL055W	*OLE1*	Delta(9) fatty acid desaturase, required for monounsaturated fatty acid synthesis and for normal distribution of mitochondria	4.59	−
YER060W	*FCY21*	Putative purine-cytosine permease, very similar to Fcy2p but cannot substitute for its function	4.56	−
YPR119W	*CLB2*	B-type cyclin involved in cell cycle progression proteasome	4.54	−
YOL014W	*YOL014W*	Putative protein of unknown function	4.43	11.01

YHL028W	*WSC4*	ER membrane protein involved in the translocation of soluble secretory proteins and insertion of membrane proteins into the ER membrane	4.22	−
YLR183C	*TOS4*	Forkhead Associated domain containing protein and putative transcription factor found associated with chromatin	4.22	5.96
YBR021W	*FUR4*	Uracil permease, localized to the plasma membrane	4.20	−
YDL101C	*DUN1*	Cell-cycle checkpoint serine-threonine kinase required for DNA damage-induced transcription of certain target genes, phosphorylation of Rad55p and Sml1p, and transient G2/M arrest after DNA damage	4.19	−
YCL048W	*SPS22*	Protein of unknown function, redundant with Sps2p for the organization of the beta-glucan layer of the spore wall	4.12	2.53
YMR215W	*GAS3*	Putative 1,3-beta-glucanosyltransferase, has similarity to Gas1p	4.07	
YKL216W	*URA1*	Dihydroorotate dehydrogenase, catalyzes the fourth enzymatic step in the de novo biosynthesis of pyrimidines, converting dihydroorotic acid into orotic acid	3.93	4.66
YKR090W	*PXL1*	LIM domain-containing protein that localizes to sites of polarized growth, required for selection and/or maintenance of polarized growth sites, may modulate signaling by the GTPases Cdc42p and Rho1p	3.88	
YPL081W	*RPS9A*	Protein component of the small (40S) ribosomal subunit	3.88	3.15
YJL158C	*CIS3*	Mannose-containing glycoprotein constituent of the cell wall	3.85	3.06
YMR199W	*CLN1*	G1 cyclin involved in regulation of the cell cycle; activates Cdc28p kinase to promote the G1 to S phase transition	3.80	−
YPL256C	*CLN2*	G1 cyclin involved in regulation of the cell cycle; activates Cdc28p kinase to promote the G1 to S phase transition	3.80	−
YHL024W	*RIM4*	Putative RNA-binding protein required for the expression of early and middle sporulation genes	3.75	−
YMR076C	*PDS5*	Protein required for establishment and maintenance of sister chromatid condensation and cohesion, colocalizes with cohesin on chromosomes, may function as a protein-protein interaction scaffold	3.75	−
YHR184W	*SSP1*	Protein involved in the control of meiotic nuclear division and coordination of meiosis with spore formation	3.66	−
YLR012C	*YLR012C*	Putative protein of unknown function	3.64	−

YHR172W	*SPC97*	Component of the microtubule-nucleating Tub4p (gamma-tubulin) complex	3.56	−
YJL037W	*IRC18*	Putative protein of unknown function; expression induced in respiratory-deficient cells and in carbon-limited chemostat cultures	3.55	−
YLR103C	*CDC45*	DNA replication initiation factor	3.50	−
YHR154W	*RTT107*	Protein implicated in Mms22-dependent DNA repair during S phase, DNA damage induces phosphorylation by Mec1p at one or more SQ/TQ motifs	3.49	−
YHR153C	*SPO16*	Meiosis-specific protein involved in synaptonemal complex assembly	3.49	5.79
YDR451C	*YHP1*	One of two homeobox transcriptional repressors (see also Yox1p), that bind to Mcm1p and to early cell cycle box (ECB) elements of cell cycle regulated genes, thereby restricting ECB-mediated transcription to the M/G1 interval	3.37	−
YJL194W	*CDC6*	Essential ATP-binding protein required for DNA replication, component of the pre-replicative complex (pre-RC) which requires ORC to associate with chromatin and is in turn required for Mcm2-7p DNA association	3.36	−
YBR088C	*POL30*	Proliferating cell nuclear antigen (PCNA), functions as the sliding clamp for DNA polymerase delta	3.36	5.26
YJR053W	*BFA1*	Component of the GTPase-activating Bfa1p-Bub2p complex involved in multiple cell cycle checkpoint pathways that control exit from mitosis	3.34	2.67
YJR092W	*BUD4*	Protein involved in bud-site selection and required for axial budding pattern	3.33	−
YDR146C	*SWI5*	Transcription factor that activates transcription of genes expressed at the M/G1 phase boundary and in G1 phase	3.33	−
YDR042C	*YDR042C*	Putative protein of unknown function; expression is increased in ssu72-ts69 mutant	3.33	−
YPL231W	*FAS2*	Alpha subunit of fatty acid synthetase, which catalyzes the synthesis of long-chain saturated fatty acids	3.31	2.66
YMR101C	*SRT1*	Cis-prenyltransferase involved in synthesis of long-chain dolichols (19-22 isoprene units; as opposed to Rer2p which synthesizes shorter-chain dolichols	3.30	−
YNR030W	*ALG12*	Alpha-1,6-mannosyltransferase localized to the ER	3.30	−
YMR305C	*SCW10*	Cell wall protein with similarity to glucanases; may play a role in conjugation during mating based on mutant phenotype and its regulation by Ste12p	3.30	−

YOL151W	*GRE2*	3-methylbutanal reductase and NADPH-dependent methylglyoxal reductase (D-lactaldehyde dehydrogenase)	3.26	−
YNL301C	*RPL18B*	Protein component of the large (60S) ribosomal subunit, identical to Rpl18Ap and has similarity to rat L18 ribosomal protein	3.19	2.86
YNR073C	*YNR073C*	Putative mannitol dehydrogenase	3.17	−
YMR144W	*YMR14W*	Putative protein of unknown function; localized to the nucleus	3.11	−
YDL003W	*MCD1*	Essential subunit of the cohesin complex required for sister chromatid cohesion in mitosis and meiosis	3.10	3.70
YCL026C-B	*HBN1*	Putative protein of unknown function; similar to bacterial nitroreductases	3.05	2.68
YMR032W	*HOF1*	Bud neck-localized, SH3 domain-containing protein required for cytokinesis	3.03	−
YER187W	*YER18W*	Putative protein of unknown function	2.98	−
YJL092W	*SRS2*	DNA helicase and DNA-dependent ATPase involved in DNA repair, needed for proper timing of commitment to meiotic recombination and transition from Meiosis I to II	2.93	−
YBR078W	*ECM33*	GPI-anchored protein of unknown function, has a possible role in apical bud growth	2.91	−
YLR054C	*OSW2*	Protein of unknown function proposed to be involved in the assembly of the spore wall	2.89	2.17
YMR303C	*ADH2*	Glucose-repressible alcohol dehydrogenase II, catalyzes the conversion of ethanol to acetaldehyde	2.89	−
YDL114W	*YDL114W*	Putative protein of unknown function with similarity to acyl-carrier-protein reductases	2.82	−
YGR286C	*BIO2*	Biotin synthase, catalyzes the conversion of dethiobiotin to biotin, which is the last step of the biotin biosynthesis pathway	2.79	−
YOL132W	*GAS4*	1,3-beta-glucanosyltransferase, involved with Gas2p in spore wall assembly	2.77	−
YAL061W	*BDH2*	Putative medium-chain alcohol dehydrogenase with similarity to BDH1	2.76	−
YDR528W	*HLR1*	Protein involved in regulation of cell wall composition and integrity and response to osmotic stress	2.75	−
YIL123W	*SIM1*	Protein of the SUN family (Sim1p, Uth1p, Nca3p, Sun4p) that may participate in DNA replication, promoter contains SCB regulation box at -300 bp indicating that expression may be cell cycle-regulated	2.74	−
YJL080C	*SCP160*	Essential RNA-binding G protein effector of mating response pathway, mainly associated with nuclear envelope and ER	2.72	−
YBL031W	*SHE1*	Mitotic spindle protein that interacts with	2.71	2.20

		components of the Dam1 (DASH) complex, its effector Sli15p, and microtubule-associated protein Bim1p		
YBR092C	*PHO3*	Constitutively expressed acid phosphatase similar to Pho5p	2.71	−
YJL102W	*MEF2*	Mitochondrial elongation factor involved in translational elongation	2.70	3.61
YAR007C	*RFA1*	Subunit of heterotrimeric Replication Protein A (RPA), which is a highly conserved single-stranded DNA binding protein involved in DNA replication, repair, and recombination	2.68	2.97
YLR131C	*ACE2*	Transcription factor that activates expression of early G1-specific genes, localizes to daughter cell nuclei after cytokinesis and delays G1 progression in daughters, localization is regulated by phosphorylation	2.66	2.33
YER095W	*RAD51*	Strand exchange protein, forms a helical filament with DNA that searches for homology	2.66	−
YOR049C	*RSB1*	Suppressor of sphingoid long chain base (LCB) sensitivity of an LCB-lyase mutation	2.65	−
YDR503C	*LPP1*	Lipid phosphate phosphatase, catalyzes Mg(2+)-independent dephosphorylation of phosphatidic acid (PA), lysophosphatidic acid, and diacylglycerol pyrophosphate	2.62	−
YBR184W	*YBR18W*	Putative protein of unknown function	2.62	−
YPL248C	*GAL4*	DNA-binding transcription factor required for the activation of the GAL genes in response to galactose	2.58	−
YER043C	*SAH1*	S-adenosyl-L-homocysteine hydrolase, catabolizes S-adenosyl-L-homocysteine which is formed after donation of the activated methyl group of S-adenosyl-L-methionine (AdoMet) to an acceptor	2.57	2.62
YDR497C	*ITR1*	Myo-inositol transporter with strong similarity to the minor myo-inositol transporter Itr2p, member of the sugar transporter superfamily	2.57	−
YER032W	*FIR1*	Protein involved in 3' mRNA processing, interacts with Ref2p	2.56	2.28
YPL127C	*HHO1*	Histone H1, a linker histone required for nucleosome packaging at restricted sites	2.55	−
YER085C	*YER085C*	Putative protein of unknown function	2.54	−
YCR088W	*ABP1*	Actin-binding protein of the cortical actin cytoskeleton, important for activation of the Arp2/3 complex that plays a key role actin in cytoskeleton organization	2.54	−
YNR019W	*ARE2*	Acyl-CoA:sterol acyltransferase, isozyme of Are1p	2.52	2.74
YLR045C	*STU2*	Microtubule-associated protein (MAP) of the XMAP215/Dis1 family	2.51	−

YBR069C	*TAT1*	Amino acid transport protein for valine, leucine, isoleucine, and tyrosine, low-affinity tryptophan and histidine transporter	2.51	−
YEL070W	*DSF1*	Deletion suppressor of mpt5 mutation	2.50	−
YDR501W	*PLM2*	Forkhead Associated domain containing protein and putative transcription factor found associated with chromatin	2.50	−
YNL333W	*SNZ2*	Member of a stationary phase-induced gene family	2.50	2.15
YIR033W	*MGA2*	ER membrane protein involved in regulation of OLE1 transcription, acts with homolog Spt23p	2.48	2.54
YMR116C	*ASC1*	G-protein beta subunit and guanine nucleotide dissociation inhibitor for Gpa2p	2.48	2.55
YER070W	*RNR1*	Major isoform of the large subunit of ribonucleotide-diphosphate reductase	2.47	7.99
YAR018C	*KIN3*	Nonessential protein kinase with unknown cellular role	2.46	−
YDR507C	*GIN4*	Protein kinase involved in bud growth and assembly of the septin ring, proposed to have kinase-dependent and kinase-independent activities	2.46	−
YBR071W	*YBR07W*	Putative protein of unknown function	2.46	−
YEL040W	*UTR2*	Chitin transglycosylase that functions in the transfer of chitin to beta(1-6) and beta(1-3) glucans in the cell wall	2.46	−
YFL037W	*TUB2*	Beta-tubulin; associates with alpha-tubulin (Tub1p and Tub3p) to form tubulin dimer, which polymerizes to form microtubules	2.46	2.34
YML085C	*TUB1*	Alpha-tubulin	2.45	2.30
YBL063W	*KIP1*	Kinesin-related motor protein required for mitotic spindle assembly, chromosome segregation, and 2 micron plasmid partitioning	2.44	−
YPL163C	*SVS1*	Cell wall and vacuolar protein, required for wild-type resistance to vanadate	2.43	−
YOR025W	*HST3*	Member of the Sir2 family of NAD(+)-dependent protein deacetylases	2.43	−
YNL135C	*FPR1*	Peptidyl-prolyl cis-trans isomerase (PPIase), binds to the drugs FK506 and rapamycin	2.43	2.31
YJR118C	*ILM1*	Protein of unknown function	2.41	2.05
YML028W	*TSA1*	Thioredoxin peroxidase, acts as both a ribosome-associated and free cytoplasmic antioxidant	2.41	3.43
YJL045W	*YJL045W*	Minor succinate dehydrogenase isozyme; homologous to Sdh1p, the major isozyme reponsible for the oxidation of succinate and transfer of electrons to ubiquinone	2.40	−
YKL107W	*YKL107W*	Putative protein of unknown function	2.38	2.30
YLR413W	*YLR413W*	Putative protein of unknown function	2.38	4.85

YDR113C	*PDS1*	Securin, inhibits anaphase by binding separin Esp1p	2.36	−
YNL067W	*RPL9B*	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl9Ap and has similarity to E. coli L6 and rat L9 ribosomal proteins	2.35	−
YPL255W	*BBP1*	Protein required for the spindle pole body (SPB) duplication, localized at the central plaque periphery	2.35	2.17
YGL242C	*YGL242C*	Putative protein of unknown function; deletion mutant is viable	2.35	4.01
YJR047C	*ANB1*	Translation elongation factor eIF-5A, previously thought to function in translation initiation	2.33	−
YAL024C	*LTE1*	Protein similar to GDP/GTP exchange factors but without detectable GEF activity	2.32	−
YBR160W	*CDC28*	Catalytic subunit of the main cell cycle cyclin-dependent kinase (CDK	2.31	2.15
YMR179W	*SPT21*	Protein with a role in transcriptional silencing; required for normal transcription at several loci including HTA2-HTB2 and HHF2-HHT2, but not required at the other histone loci	2.30	−
YKL165C	*MCD4*	Protein involved in glycosylphosphatidylinositol (GPI) anchor synthesis	2.30	6.19
YLL002W	*RTT109*	Histone acetyltransferase critical for cell survival in the presence of DNA damage during S phase; acetylates H3-K56 and H3-K9	2.30	−
YJL091C	*GWT1*	Protein involved in the inositol acylation of glucosaminyl phosphatidylinositol (GlcN-PI) to form glucosaminyl(acyl)phosphatidylinositol (GlcN(acyl)PI), an intermediate in the biosynthesis of glycosylphosphatidylinositol (GPI) anchors	2.30	3.32
YML058W	*SML1*	Ribonucleotide reductase inhibitor involved in regulating dNTP production	2.29	2.11
YKR042W	*UTH1*	Mitochondrial outer membrane and cell wall localized SUN family member involved in cell wall biogenesis and required for mitochondrial autophagy	2.29	−
YBL035C	*POL12*	B subunit of DNA polymerase alpha-primase complex, required for initiation of DNA replication during mitotic and premeiotic DNA synthesis	2.29	−
YMR001C	*CDC5*	Polo-like kinase with multiple functions in mitosis and cytokinesis through substrate phosphorylation, also functions in adaptation to DNA damage during meiosis	2.27	−
YLR273C	*PIG1*	Putative targeting subunit for the type-1 protein phosphatase Glc7p that tethers it to the Gsy2p glycogen synthase	2.27	−

YHR061C	*GIC1*	Protein of unknown function involved in initiation of budding and cellular polarization, interacts with Cdc42p via the Cdc42/Rac-interactive binding (CRIB) domain	2.26	−
YGR260W	*TNA1*	High affinity nicotinic acid plasma membrane permease, responsible for uptake of low levels of nicotinic acid	2.26	−
YGR014W	*MSB2*	Mucin family member involved in the Cdc42p- and MAP kinase-dependent filamentous growth signaling pathway	2.24	−
YJL219W	*HXT9*	Putative hexose transporter that is nearly identical to Hxt11p, has similarity to major facilitator superfamily (MFS) transporters, expression of HXT9 is regulated by transcription factors Pdr1p and Pdr3p	2.24	−
YOR071C	*NRT1*	High-affinity nicotinamide riboside transporter; also transports thiamine with low affinity; shares sequence similarity with Thi7p and Thi72p	2.21	−
YAR008W	*SEN34*	Subunit of the tRNA splicing endonuclease, which is composed of Sen2p, Sen15p, Sen34p, and Sen54p	2.21	2.59
YGR254W	*ENO1*	Enolase I, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis	2.20	2.67
YDR368W	*YPR1*	NADPH-dependent aldo-keto reductase, reduces multiple substrates including 2-methylbutyraldehyde and D,L-glyceraldehyde, expression is induced by osmotic and oxidative stress; functionally redundant with other aldo-keto reductases	2.20	2.25
YGL038C	*OCH1*	Mannosyltransferase of the cis-Golgi apparatus, initiates the polymannose outer chain elongation of N-linked oligosaccharides of glycoproteins	2.19	−
YOL120C	*RPL18A*	Protein component of the large (60S) ribosomal subunit, identical to Rpl18Bp and has similarity to rat L18 ribosomal protein	2.19	−
YNL012W	*SPO1*	Meiosis-specific prospore protein; required for meiotic spindle pole body duplication and separation	2.19	−
YLR231C	*BNA5*	Kynureninase, required for the de novo biosynthesis of NAD from tryptophan via kynurenine; expression regulated by Hst1p	2.19	2.41
YGR049W	*SCM4*	Potential regulatory effector of CDC4 function, suppresses a temperature-sensitive allele of CDC4, tripartite protein structure in which a charged region separates two uncharged domains, not essential for mitosis or meiosis	2.19	−
YPR175W	*DPB2*	Second largest subunit of DNA polymerase II (DNA polymerase epsilon), required for normal	2.18	−

		yeast chromosomal replication		
YHR043C	*DOG2*	2-deoxyglucose-6-phosphate phosphatase, member of a family of low molecular weight phosphatases, similar to Dog1p, induced by oxidative and osmotic stress, confers 2-deoxyglucose resistance when overexpressed	2.18	3.70
YGR037C	*ACB1*	Acyl-CoA-binding protein, transports newly synthesized acyl-CoA esters from fatty acid synthetase (Fas1p-Fas2p) to acyl-CoA-consuming processes	2.16	2.74
YER154W	*OXA1*	Mitochondrial inner membrane insertase, mediates the insertion of both mitochondrial- and nuclear-encoded proteins from the matrix into the inner membrane, interacts with mitochondrial ribosomes	2.16	−
YJL115W	*ASF1*	Nucleosome assembly factor, involved in chromatin assembly and disassembly, anti-silencing protein that causes derepression of silent loci when overexpressed	2.15	−
YDL164C	*CDC9*	DNA ligase found in the nucleus and mitochondria, an essential enzyme that joins Okazaki fragments during DNA replication	2.15	−
YPL158C	*AIM44*	Protein of unknown function; GFP-fusion protein localizes to the bud neck	2.14	−
YGL226C-A	*OST5*	Zeta subunit of the oligosaccharyltransferase complex of the ER lumen, which catalyzes asparagine-linked glycosylation of newly synthesized proteins	2.14	2.22
YBR243C	*ALG7*	UDP-N-acetyl-glucosamine-1-P transferase, transfers Glc-Nac-P from UDP-GlcNac to Dol-P in the ER in the first step of the dolichol pathway of protein asparagine-linked glycosylation	2.14	−
YMR198W	*CIK1*	Kinesin-associated protein required for both karyogamy and mitotic spindle organization, interacts stably and specifically with Kar3p and may function to target this kinesin to a specific cellular role	2.14	−
YDR309C	*GIC2*	Redundant rho-like GTPase Cdc42p effector; homolog of Gic1p; involved in initiation of budding and cellular polarization	2.14	−
YLR187W	*SKG3*	Protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cell periphery, cytoplasm, bud, and bud neck	2.14	−
YKR010C	*TOF2*	Protein required for rDNA silencing and mitotic rDNA condensation	2.13	−
YDR009W	*GAL3*	Transcriptional regulator involved in activation of the GAL genes in response to galactose	2.12	−
YPL227C	*ALG5*	UDP-glucose:dolichyl-phosphate glucosyltransferase, involved in asparagine-linked glycosylation in the endoplasmic	2.11	−

		reticulum		
YGR156W	*PTI1*	Essential protein that is a component of CPF (cleavage and polyadenylation factor	2.11	−
YML078W	*CPR3*	Mitochondrial peptidyl-prolyl cis-trans isomerase (cyclophilin), catalyzes the cis-trans isomerization of peptide bonds N-terminal to proline residues	2.11	−
YNL246W	*VPS75*	NAP family histone chaperone; binds to histones and Rtt109p, stimulating histone acetyltransferase activity	2.10	−
YLR084C	*RAX2*	N-glycosylated protein involved in the maintenance of bud site selection during bipolar budding; localization requires Rax1p	2.10	−
YLR209C	*PNP1*	Purine nucleoside phosphorylase, specifically metabolizes inosine and guanosine nucleosides; involved in the nicotinamide riboside salvage pathway	2.09	−
YOL143C	*RIB4*	Lumazine synthase (6,7-dimethyl-8-ribityllumazine synthase, also known as DMRL synthase	2.07	3.24
YJL181W	*YJL181W*	Putative protein of unknown function; expression is cell-cycle regulated as shown by microarray analysis	2.07	−
YLR134W	*PDC5*	Minor isoform of pyruvate decarboxylase, key enzyme in alcoholic fermentation, decarboxylates pyruvate to acetaldehyde, regulation is glucose- and ethanol-dependent, repressed by thiamine, involved in amino acid catabolism	2.07	3.16
YGR292W	*MAL12*	Maltase (alpha-D-glucosidase), inducible protein involved in maltose catabolism	2.07	−
YAL062W	*GDH3*	NADP(+)-dependent glutamate dehydrogenase, synthesizes glutamate from ammonia and alpha-ketoglutarate	2.06	2.11
YFL045C	*SEC53*	Phosphomannomutase, involved in synthesis of GDP-mannose and dolichol-phosphate-mannose	2.06	−
YDR261C	*EXG2*	Exo-1,3-beta-glucanase, involved in cell wall beta-glucan assembly	2.04	−
YKL113C	*RAD27*	5' to 3' exonuclease, 5' flap endonuclease, required for Okazaki fragment processing and maturation as well as for long-patch base-excision repair	2.04	−
YLR293C	*GSP1*	Ran GTPase, GTP binding protein (mammalian Ranp homolog) involved in the maintenance of nuclear organization, RNA processing and transport	2.04	2.36
YNL072W	*RNH201*	Ribonuclease H2 catalytic subunit, removes RNA primers during Okazaki fragment synthesis and errant ribonucleotides misincorporated	2.03	2.49

		during DNA replication		
YHR149C	*SKG6*	Integral membrane protein that localizes primarily to growing sites such as the bud tip or the cell periphery; potential Cdc28p substrate	2.03	−
YPL208W	*RKM1*	SET-domain lysine-N-methyltransferase, catalyzes the formation of dimethyllysine residues on the large ribsomal subunit protein L23a (RPL23A and RPL23B)	2.03	−
YGL251C	*HFM1*	Meiosis specific DNA helicase involved in the conversion of double-stranded breaks to later recombination intermediates and in crossover control	2.03	−
YDL145C	*COP1*	Alpha subunit of COPI vesicle coatomer complex, which surrounds transport vesicles in the early secretory pathway	2.02	2.15
YGR221C	*TOS2*	Protein involved in localization of Cdc24p to the site of bud growth	2.02	2.09
YML009C	*MRPL39*	Mitochondrial ribosomal protein of the large subunit	2.01	−
YJR048W	*CYC1*	Cytochrome c, isoform 1	2.00	3.06
YLR272C	*YCS4*	Subunit of the condensin complex	2.00	−
YDR361C	*BCP1*	Essential protein involved in nuclear export of Mss4p, which is a lipid kinase that generates phosphatidylinositol 4,5-biphosphate and plays a role in actin cytoskeleton organization and vesicular transport	-2.01	−
YML099C	*ARG81*	Zinc-finger transcription factor of the Zn(2)-Cys(6) binuclear cluster domain type, involved in the regulation of arginine-responsive genes; acts with Arg80p and Arg82p	-2.01	−
YGR030C	*POP6*	Subunit of both RNase MRP, which cleaves pre-rRNA, and nuclear RNase P, which cleaves tRNA precursors to generate mature 5' ends	-2.01	−
YLL056C	*YLL056C*	Putative protein of unknown function, transcription is activated by paralogous transcription factors Yrm1p and Yrr1p and genes involved in pleiotropic drug resistance (PDR	-2.02	−
YPR086W	*SUA7*	Transcription factor TFIIB, a general transcription factor required for transcription initiation and start site selection by RNA polymerase II	-2.02	-2.18
YDL169C	*UGX2*	Protein of unknown function, transcript accumulates in response to any combination of stress conditions	-2.02	−
YBR293W	*VBA2*	Permease of basic amino acids in the vacuolar membrane	-2.03	-2.58
YDR465C	*RMT2*	Arginine N5 methyltransferase; methylates ribosomal protein Rpl12 (L12) on Arg67	-2.04	−

YNL314W	*DAL82*	Positive regulator of allophanate inducible genes	-2.04	−
YDL025C	*YDL025C*	Putative protein kinase, potentially phosphorylated by Cdc28p	-2.04	−
YPR157W	*YPR157W*	Putative protein of unknown function	-2.05	−
YPL199C	*YPL199C*	Putative protein of unknown function, predicted to be palmitoylated	-2.06	-2.56
YDR244W	*PEX5*	Peroxisomal membrane signal receptor for the C-terminal tripeptide signal sequence (PTS1) of peroxisomal matrix proteins, required for peroxisomal matrix protein import	-2.07	−
YNL004W	*HRB1*	Poly(A+) RNA-binding protein, involved in the export of mRNAs from the nucleus to the cytoplasm; similar to Gbp2p and Npl3p	-2.07	-2.15
YBR297W	*MAL33*	MAL-activator protein, part of complex locus MAL3; nonfunctional in genomic reference strain S288C	-2.07	−
YHR066W	*SSF1*	Constituent of 66S pre-ribosomal particles, required for ribosomal large subunit maturation	-2.07	-4.63
YMR265C	*YMR265C*	Putative protein of unknown function	-2.08	-2.35
YDL121C	*YDL121C*	Putative protein of unknown function	-2.08	-2.47
YPR112C	*MRD1*	Essential conserved protein that is part of the 90S preribosome	-2.08	−
YNL119W	*NCS2*	Protein required for thiolation of the uridine at the wobble position of Lys(UUU) and Glu(UUC) tRNAs	-2.11	−
YOR213C	*SAS5*	Subunit of the SAS complex (Sas2p, Sas4p, Sas5p), which acetylates free histones and nucleosomes and regulates transcriptional silencing; stimulates Sas2p HAT activity	-2.11	−
YER015W	*FAA2*	Medium chain fatty acyl-CoA synthetase, activates imported fatty acids; accepts a wide range of fatty acid chain lengths with a preference for medium chains, C9:0-C13:0; localized to the peroxisome	-2.11	-2.09
YOR179C	*SYC1*	Subunit of the APT subcomplex of cleavage and polyadenylation factor, may have a role in 3' end formation of both polyadenylated and non-polyadenylated RNAs	-2.11	−
YLR161W	*YLR161W*	Putative protein of unknown function	-2.11	−
YPR048W	*TAH18*	Conserved NAPDH-dependent diflavin reductase, component of an early step in the cytosolic Fe-S protein assembly (CIA) machinery	-2.11	−
YDR312W	*SSF2*	Protein required for ribosomal large subunit maturation, functionally redundant with Ssf1p	-2.11	−
YHR166C	*CDC23*	Subunit of the Anaphase-Promoting Complex/Cyclosome (APC/C), which is a	-2.13	-2.12

		ubiquitin-protein ligase required for degradation of anaphase inhibitors, including mitotic cyclins, during the metaphase/anaphase transition		
YOL136C	*PFK27*	6-phosphofructo-2-kinase, catalyzes synthesis of fructose-2,6-bisphosphate	-2.14	−
YOR101W	*RAS1*	GTPase involved in G-protein signaling in the adenylate cyclase activating pathway, plays a role in cell proliferation	-2.14	−
YDL223C	*HBT1*	Substrate of the Hub1p ubiquitin-like protein that localizes to the shmoo tip (mating projection)	-2.14	−
YNL113W	*RPC19*	RNA polymerase subunit AC19, common to RNA polymerases I and III	-2.14	−
YKL099C	*UTP11*	Subunit of U3-containing Small Subunit (SSU) processome complex involved in production of 18S rRNA and assembly of small ribosomal subunit	-2.15	−
YGL153W	*PEX14*	Peroxisomal membrane peroxin that is a central component of the peroxisomal protein import machinery	-2.15	−
YJL212C	*OPT1*	Proton-coupled oligopeptide transporter of the plasma membrane	-2.16	-3.25
YLR407W	*YLR407W*	Putative protein of unknown function	-2.18	−
YLR159W	*YLR159W*	Putative protein of unknown function	-2.18	−
YKR058W	*GLG1*	Self-glucosylating initiator of glycogen synthesis, also glucosylates n-dodecyl-beta-D-maltoside	-2.18	−
YJL112W	*MDV1*	Peripheral protein of the cytosolic face of the mitochondrial outer membrane, required for mitochondrial fission	-2.19	−
YCR100C	*YCR100C*	Putative protein of unknown function	-2.19	-2.34
YLL051C	*FRE6*	Putative ferric reductase with similarity to Fre2p; expression induced by low iron levels	-2.19	−
YGR202C	*PCT1*	Cholinephosphate cytidylyltransferase, also known as CTP:phosphocholine cytidylyltransferase, rate-determining enzyme of the CDP-choline pathway for phosphatidylcholine synthesis	-2.20	−
YDR030C	*RAD28*	Protein involved in DNA repair, related to the human CSA protein that is involved in transcription-coupled repair nucleotide excision repair	-2.20	−
YGR081C	*SLX9*	Protein required for pre-rRNA processing; associated with the 90S pre-ribosome and 43S small ribosomal subunit precursor	-2.20	-2.57
YIL113W	*SDP1*	Stress-inducible dual-specificity MAP kinase phosphatase, negatively regulates Slt2p MAP kinase by direct dephosphorylation, diffuse localization under normal conditions shifts to punctate localization after heat shock	-2.20	−

YPL202C	*AFT2*	Iron-regulated transcriptional activator	-2.21	−
YER037W	*PHM8*	Protein of unknown function, expression is induced by low phosphate levels and by inactivation of Pho85p	-2.22	−
YBL033C	*RIB1*	GTP cyclohydrolase II	-2.22	-2.34
YGR129W	*SYF2*	Member of the NineTeen Complex (NTC) that contains Prp19p and stabilizes U6 snRNA in catalytic forms of the spliceosome containing U2, U5, and U6 snRNAs	-2.22	−
YOR056C	*NOB1*	Essential nuclear protein involved in proteasome maturation and synthesis of 40S ribosomal subunits	-2.23	−
YOR262W	*YOR26W*	Protein of unknown function required for establishment of sister chromatid cohesion; contains an ATP/GTP binding site motif	-2.23	−
YPL212C	*PUS1*	tRNA:pseudouridine synthase, introduces pseudouridines at positions 26-28, 34-36, 65, and 67 of tRNA	-2.24	−
YMR185W	*YMR18W*	Putative protein of unknown function	-2.25	−
YER184C	*YER184C*	Putative zinc cluster protein	-2.25	-3.03
YIR031C	*DAL7*	Malate synthase, role in allantoin degradation unknown	-2.26	−
YJL072C	*PSF2*	Subunit of the GINS complex (Sld5p, Psf1p, Psf2p, Psf3p), which is localized to DNA replication origins and implicated in assembly of the DNA replication machinery	-2.26	-3.81
YKL106W	*AAT1*	Mitochondrial aspartate aminotransferase, catalyzes the conversion of oxaloacetate to aspartate in aspartate and asparagine biosynthesis	-2.26	−
YBR141C	*YBR141C*	Putative S-adenosylmethionine-dependent methyltransferase	-2.26	−
YNL221C	*POP1*	Subunit of both RNase MRP, which cleaves pre-rRNA, and nuclear RNase P, which cleaves tRNA precursors to generate mature 5' ends	-2.29	-2.07
YOR004W	*UTP23*	Essential nucleolar protein that is a component of the SSU (small subunit) processome involved in 40S ribosomal subunit biogenesis	-2.30	-2.09
YHR150W	*PEX28*	Peroxisomal integral membrane peroxin, involved in the regulation of peroxisomal size, number and distribution	-2.30	−
YKR024C	*DBP7*	Putative ATP-dependent RNA helicase of the DEAD-box family involved in ribosomal biogenesis	-2.31	−
YLR284C	*ECI1*	Peroxisomal delta3,delta2-enoyl-CoA isomerase, hexameric protein that converts 3-hexenoyl-CoA to trans-2-hexenoyl-CoA, essential for the beta-oxidation of unsaturated fatty acids, oleate-induced	-2.32	−

YDR421W	*ARO80*	Zinc finger transcriptional activator of the Zn2Cys6 family	-2.32	−
YER137C	*YER137C*	Putative protein of unknown function	-2.35	−
YOR338W	*YOR33W*	Putative protein of unknown function	-2.35	−
YCR107W	*AAD3*	Putative aryl-alcohol dehydrogenase with similarity to P. chrysosporium aryl-alcohol dehydrogenase	-2.35	−
YMR201C	*RAD14*	Protein that recognizes and binds damaged DNA during nucleotide excision repair	-2.37	−
YBL054W	*TOD6*	PAC motif binding protein involved in rRNA and ribosome biogenesis	-2.37	−
YBR257W	*POP4*	Subunit of both RNase MRP, which cleaves pre-rRNA, and nuclear RNase P, which cleaves tRNA precursors to generate mature 5' ends	-2.38	-2.59
YBR105C	*VID24*	Peripheral membrane protein located at Vid (vacuole import and degradation) vesicles	-2.39	-3.67
YKL072W	*STB6*	Protein that binds Sin3p in a two-hybrid assay	-2.39	-2.55
YGR121C	*MEP1*	Ammonium permease; belongs to a ubiquitous family of cytoplasmic membrane proteins that transport only ammonium (NH4	-2.39	-2.92
YNL014W	*HEF3*	Translational elongation factor EF-3; paralog of YEF3 and member of the ABC superfamily	-2.40	−
YPL230W	*USV1*	Putative transcription factor containing a C2H2 zinc finger	-2.40	−
YLR312C	*YLR312C*	Putative protein of unknown function	-2.40	-2.10
YLR023C	*IZH3*	Membrane protein involved in zinc ion homeostasis, member of the four-protein IZH family, expression induced by zinc deficiency	-2.42	−
YMR009W	*ADI1*	Acireductone dioxygenease involved in the methionine salvage pathway	-2.42	−
YMR145C	*NDE1*	Mitochondrial external NADH dehydrogenase, a type II NAD(P)H:quinone oxidoreductase that catalyzes the oxidation of cytosolic NADH	-2.43	−
YHR046C	*INM1*	Inositol monophosphatase, involved in biosynthesis of inositol and in phosphoinositide second messenger signaling	-2.44	-2.11
YEL029C	*BUD16*	Putative pyridoxal kinase, a key enzyme involved in pyridoxal 5'-phosphate synthesis, the active form of vitamin B6	-2.44	−
YFL030W	*AGX1*	Alanine:glyoxylate aminotransferase (AGT), catalyzes the synthesis of glycine from glyoxylate, which is one of three pathways for glycine biosynthesis in yeast	-2.49	-2.27
YKL023W	*YKL023W*	Putative protein of unknown function, predicted by computational methods to be involved in mRNA degradation	-2.50	-3.03
YOR192C	*THI72*	Transporter of thiamine or related compound; shares sequence similarity with Thi7p	-2.52	-3.27

YLR387C	*REH1*	Cytoplasmic 60S subunit biogenesis factor, associates with pre-60S particles	-2.52	−
YLL061W	*MMP1*	High-affinity S-methylmethionine permease, required for utilization of S-methylmethionine as a sulfur source; has similarity to S-adenosylmethionine permease Sam3p	-2.53	−
YBL069W	*AST1*	Peripheral membrane protein that interacts with the plasma membrane ATPase Pma1p and has a role in its targeting to the plasma membrane, possibly by influencing its incorporation into lipid rafts	-2.54	−
YPL017C	*IRC15*	Microtubule associated protein	-2.56	-2.27
YNL234W	*YNL234W*	Protein of unknown function with similarity to globins; has a functional heme-binding domain	-2.57	−
YOL165C	*AAD15*	Putative aryl-alcohol dehydrogenase with similarity to P. chrysosporium aryl-alcohol dehydrogenase; mutational analysis has not yet revealed a physiological role	-2.60	-2.72
YGR159C	*NSR1*	Nucleolar protein that binds nuclear localization sequences, required for pre-rRNA processing and ribosome biogenesis	-2.61	−
YDR120C	*TRM1*	tRNA methyltransferase; two forms of the protein are made by alternative translation starts	-2.65	−
YKL143W	*LTV1*	Component of the GSE complex, which is required for proper sorting of amino acid permease Gap1p	-2.68	−
YLR460C	*YLR460C*	Member of the quinone oxidoreductase family, up-regulated in response to the fungicide mancozeb	-2.71	−
YPR111W	*DBF20*	Ser/Thr kinase involved in late nuclear division, one of the mitotic exit network (MEN) proteins	-2.73	-3.36
YHR088W	*RPF1*	Nucleolar protein involved in the assembly and export of the large ribosomal subunit	-2.76	-2.13
YLL053C	*YLL053C*	Putative protein; in the Sigma 1278B strain background YLL053C is contiguous with AQY2 which encodes an aquaporin	-2.79	-2.85
YDR342C	*HXT7*	High-affinity glucose transporter of the major facilitator superfamily, nearly identical to Hxt6p, expressed at high basal levels relative to other HXTs, expression repressed by high glucose levels	-2.80	−
YFL061W	*DDI2*	Protein of unknown function; expression is induced over 100-fold by DNA damage	-2.87	-5.18
YLR297W	*YLR297W*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the vacuole	-2.88	−
YLL052C	*AQY2*	Water channel that mediates the transport of water across cell membranes, only expressed in proliferating cells, controlled by osmotic signals,	-2.89	-3.13

		may be involved in freeze tolerance		
YOR363C	*PIP2*	Autoregulatory oleate-specific transcriptional activator of peroxisome proliferation, contains Zn(2)-Cys(6) cluster domain, forms heterodimer with Oaf1p, binds oleate response elements (OREs), activates beta-oxidation genes	-2.90	−
YMR189W	*GCV2*	P subunit of the mitochondrial glycine decarboxylase complex, required for the catabolism of glycine to 5,10-methylene-THF	-2.90	-5.24
YKR099W	*BAS1*	Myb-related transcription factor involved in regulating basal and induced expression of genes of the purine and histidine biosynthesis pathways	-2.91	-4.06
YIL019W	*FAF1*	Protein required for pre-rRNA processing and 40S ribosomal subunit assembly	-2.94	−
YHR033W	*YHR03W*	Putative protein of unknown function	-2.95	−
YGR239C	*PEX21*	Peroxin required for targeting of peroxisomal matrix proteins containing PTS2	-3.02	-4.21
YDR343C	*HXT6*	High-affinity glucose transporter of the major facilitator superfamily, nearly identical to Hxt7p, expressed at high basal levels relative to other HXTs, repression of expression by high glucose requires SNF3	-3.02	−
YLR152C	*YLR152C*	Putative protein of unknown function	-3.03	-4.74
YIL003W	*CFD1*	Highly conserved, iron-sulfur cluster binding protein localized in the cytoplasm	-3.03	-3.22
YDR533C	*HSP31*	Possible chaperone and cysteine protease with similarity to E. coli Hsp31	-3.03	-2.24
YER176W	*ECM32*	DNA dependent ATPase/DNA helicase belonging to the Dna2p- and Nam7p-like family of helicases that is involved in modulating translation termination	-3.05	−
YDL170W	*UGA3*	Transcriptional activator necessary for gamma-aminobutyrate (GABA)-dependent induction of GABA genes (such as UGA1, UGA2, UGA4	-3.18	-5.69
YOL158C	*ENB1*	Endosomal ferric enterobactin transporter, expressed under conditions of iron deprivation	-3.21	-3.60
YDR019C	*GCV1*	T subunit of the mitochondrial glycine decarboxylase complex, required for the catabolism of glycine to 5,10-methylene-THF	-3.24	-4.41
YNL162W	*YNL162W*	Putative protein of unknown function	-3.25	−
YNL279W	*PRM1*	Pheromone-regulated multispanning membrane protein involved in membrane fusion during mating	-3.29	−
YKL155C	*RSM22*	Mitochondrial ribosomal protein of the small subunit	-3.63	−
YER145C	*FTR1*	High affinity iron permease involved in the transport of iron across the plasma membrane	-3.64	-5.70
YNL142W	*MEP2*	Ammonium permease involved in regulation of	-3.66	−

		pseudohyphal growth		
YMR107W	*SPG4*	Protein required for survival at high temperature during stationary phase	-4.07	−
YOR306C	*MCH5*	Plasma membrane riboflavin transporter	-4.11	−
YML123C	*PHO84*	High-affinity inorganic phosphate (Pi) transporter and low-affinity manganese transporter	-4.19	−
YNL112W	*DBP2*	Essential ATP-dependent RNA helicase of the DEAD-box protein family, involved in nonsense-mediated mRNA decay and rRNA processing	-4.19	−
YNR069C	*BSC5*	Protein of unknown function, ORF exhibits genomic organization compatible with a translational readthrough-dependent mode of expression	-4.47	−
YJL213W	*YJL213W*	Protein of unknown function that may interact with ribosomes; periodically expressed during the yeast metabolic cycle	-4.50	-13.17
YPL092W	*SSU1*	Plasma membrane sulfite pump involved in sulfite metabolism and required for efficient sulfite efflux	-4.79	-6.75
YGR230W	*BNS1*	Protein with some similarity to Spo12p	-4.87	-3.61
YIR032C	*DAL3*	Ureidoglycolate hydrolase, converts ureidoglycolate to glyoxylate and urea in the third step of allantoin degradation	-5.20	-4.19
YPR192W	*AQY1*	Spore-specific water channel that mediates the transport of water across cell membranes, developmentally controlled	-5.28	−
YHR048W	*YHK8*	Presumed antiporter of the DHA1 family of multidrug resistance transporters	-5.55	-6.38
YLL055W	*YCT1*	High-affinity cysteine-specific transporter with similarity to the Dal5p family of transporters; green fluorescent protein (GFP)-fusion protein localizes to the endoplasmic reticulum	-5.99	−
YIL165C	*YIL165C*	Putative protein of unknown function	-6.03	-7.59
YIL164C	*NIT1*	Nitrilase, member of the nitrilase branch of the nitrilase superfamily	-6.31	-7.94
YOR186W	*YOR18W*	Putative protein of unknown function; proper regulation of expression during heat stress is sphingolipid-dependent	-6.37	-6.03
YMR058W	*FET3*	Ferro-O2-oxidoreductase required for high-affinity iron uptake and involved in mediating resistance to copper ion toxicity, belongs to class of integral membrane multicopper oxidases	-6.64	-11.97
YFR055W	*IRC7*	Putative cystathionine beta-lyase; involved in copper ion homeostasis and sulfur metabolism	-6.82	-11.69
YDR242W	*AMD2*	Putative amidase	-7.12	-8.88
YBL043W	*ECM13*	Non-essential protein of unknown function; induced by treatment with 8-methoxypsoralen	-7.22	-9.22

		and UVA irradiation		
YHL016C	*DUR3*	Plasma membrane transporter for both urea and polyamines, expression is highly sensitive to nitrogen catabolite repression and induced by allophanate, the last intermediate of the allantoin degradative pathway	-7.66	-8.79
YPL095C	*EEB1*	Acyl-coenzymeA:ethanol O-acyltransferase responsible for the major part of medium-chain fatty acid ethyl ester biosynthesis during fermentation	-8.32	-3.60
YCL064C	*CHA1*	Catabolic L-serine (L-threonine) deaminase, catalyzes the degradation of both L-serine and L-threonine	-9.36	-12.61
YHR096C	*HXT5*	Hexose transporter with moderate affinity for glucose, induced in the presence of non-fermentable carbon sources, induced by a decrease in growth rate	-9.51	-8.23
YHR137W	*ARO9*	Aromatic aminotransferase II, catalyzes the first step of tryptophan, phenylalanine, and tyrosine catabolism	-16.37	-10.86
YKR034W	*DAL80*	Negative regulator of genes in multiple nitrogen degradation pathways; expression is regulated by nitrogen levels and by Gln3p	-22.06	−
YJL153C	*INO1*	Inositol-3-phosphate synthase, involved in synthesis of inositol phosphates and inositol-containing phospholipids	−	32.84
YER003C	*PMI40*	Mannose-6-phosphate isomerase, catalyzes the interconversion of fructose-6-P and mannose-6-P	−	8.04
YGR109C	*CLB6*	B-type cyclin involved in DNA replication during S phase; activates Cdc28p to promote initiation of DNA synthesis	−	6.29
YKL165C	*MCD4*	Protein involved in glycosylphosphatidylinositol (GPI) anchor synthesis	−	6.19
YGR256W	*GND2*	6-phosphogluconate dehydrogenase (decarboxylating), catalyzes an NADPH regenerating reaction in the pentose phosphate pathway	−	6.06
YOR387C	*YOR387C*	Putative protein of unknown function	−	5.85
YBR088C	*POL30*	Proliferating cell nuclear antigen (PCNA), functions as the sliding clamp for DNA polymerase delta	−	5.26
YML058W-A	*HUG1*	Protein involved in the Mec1p-mediated checkpoint pathway that responds to DNA damage or replication arrest, transcription is induced by DNA damage	−	5.03
YGR213C	*RTA1*	Protein involved in 7-aminocholesterol resistance	−	4.73
YNR016C	*ACC1*	Acetyl-CoA carboxylase, biotin containing	−	4.40

		enzyme that catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA		
YLR099C	*ICT1*	Lysophosphatidic acid acyltransferase, responsible for enhanced phospholipid synthesis during organic solvent stress	−	4.32
YGL253W	*HXK2*	Hexokinase isoenzyme 2 that catalyzes phosphorylation of glucose in the cytosol	−	4.18
YGR234W	*YHB1*	Nitric oxide oxidoreductase, flavohemoglobin involved in nitric oxide detoxification	−	3.97
YAL023C	*PMT2*	Protein O-mannosyltransferase, transfers mannose residues from dolichyl phosphate-D-mannose to protein Ser/Thr residues	−	3.84
YML070W	*DAK1*	Dihydroxyacetone kinase, required for detoxification of dihydroxyacetone (DHA	−	3.75
YOR388C	*FDH1*	NAD(+)-dependent formate dehydrogenase, may protect cells from exogenous formate	−	3.45
YML028W	*TSA1*	Thioredoxin peroxidase, acts as both a ribosome-associated and free cytoplasmic antioxidant	−	3.43
YKL182W	*FAS1*	Beta subunit of fatty acid synthetase, which catalyzes the synthesis of long-chain saturated fatty acids	−	3.41
YJL167W	*ERG20*	Farnesyl pyrophosphate synthetase, has both dimethylallyltranstransferase and geranyltranstransferase activities	−	3.41
YOL143C	*RIB4*	Lumazine synthase (6,7-dimethyl-8-ribityllumazine synthase, also known as DMRL synthase	−	3.24
YHR174W	*ENO2*	Enolase II, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis	−	3.20
YLR134W	*PDC5*	Minor isoform of pyruvate decarboxylase, key enzyme in alcoholic fermentation, decarboxylates pyruvate to acetaldehyde	−	3.16
YOR321W	*PMT3*	Protein O-mannosyltransferase, transfers mannose residues from dolichyl phosphate-D-mannose to protein serine/threonine residues	−	3.15
YHR183W	*GND1*	6-phosphogluconate dehydrogenase (decarboxylating), catalyzes an NADPH regenerating reaction in the pentose phosphate pathway	−	3.13
YLR354C	*TAL1*	Transaldolase, enzyme in the non-oxidative pentose phosphate pathway	−	3.10
YOR198C	*BFR1*	Component of mRNP complexes associated with polyribosomes	−	3.03
YOR393W	*ERR1*	Protein of unknown function	−	3.02
YFL011W	*HXT10*	Putative hexose transporter, expressed at low levels and expression is repressed by glucose	−	3.00

YPL281C	*ERR2*	Protein of unknown function	−	3.00
YMR323W	*ERR3*	Protein of unknown function	−	2.98
YOR288C	*MPD1*	Member of the protein disulfide isomerase (PDI) family	−	2.96
YLR259C	*HSP60*	Tetradecameric mitochondrial chaperonin required for ATP-dependent folding of precursor polypeptides and complex assembly	−	2.81
YMR214W	*SCJ1*	One of several homologs of bacterial chaperone DnaJ, located in the ER lumen where it cooperates with Kar2p to mediate maturation of proteins	−	2.76
YPL240C	*HSP82*	Hsp90 chaperone required for pheromone signaling and negative regulation of Hsf1p	−	2.76
YOR176W	*HEM15*	Ferrochelatase, a mitochondrial inner membrane protein, catalyzes the insertion of ferrous iron into protoporphyrin IX	−	2.75
YNR019W	*ARE2*	Acyl-CoA:sterol acyltransferase, isozyme of Are1p	−	2.74
YGR037C	*ACB1*	Acyl-CoA-binding protein, transports newly synthesized acyl-CoA esters from fatty acid synthetase (Fas1p-Fas2p) to acyl-CoA-consuming processes	−	2.74
YJR030C	*YJR030C*	Putative protein of unknown function; expression repressed in carbon limited vs carbon replete chemostat cultures	−	2.72
YLR304C	*ACO1*	Aconitase, required for the tricarboxylic acid (TCA) cycle and also independently required for mitochondrial genome maintenance	−	2.72
YOL031C	*SIL1*	Nucleotide exchange factor for the endoplasmic reticulum (ER) lumenal Hsp70 chaperone Kar2p, required for protein translocation into the ER; homolog of Yarrowia lipolytica SLS1	−	2.69
YKL121W	*DGR2*	Protein of unknown function	−	2.68
YBR011C	*IPP1*	Cytoplasmic inorganic pyrophosphatase (PPase), homodimer that catalyzes the rapid exchange of oxygens from Pi with water	−	2.67
YBR029C	*CDS1*	Phosphatidate cytidylyltransferase (CDP-diglyceride synthetase	−	2.62
YPL058C	*PDR12*	Plasma membrane ATP-binding cassette (ABC) transporter, weak-acid-inducible multidrug transporter required for weak organic acid resistance	−	2.60
YNL102W	*POL1*	Catalytic subunit of the DNA polymerase I alpha-primase complex, required for the initiation of DNA replication during mitotic DNA synthesis and premeiotic DNA synthesis	−	2.60
YLR234W	*TOP3*	DNA Topoisomerase III, conserved protein that functions in a complex with Sgs1p and Rmi1p to relax single-stranded negatively-supercoiled	−	2.56

		DNA preferentially		
YHR104W	*GRE3*	Aldose reductase involved in methylglyoxal, d-xylose, arabinose, and galactose metabolism; stress induced (osmotic, ionic, oxidative, heat shock, starvation and heavy metals)	−	2.53
YGL256W	*ADH4*	Alcohol dehydrogenase isoenzyme type IV, dimeric enzyme demonstrated to be zinc-dependent despite sequence similarity to iron-activated alcohol dehydrogenases	−	2.51
YOR073W	*SGO1*	Component of the spindle checkpoint, involved in sensing lack of tension on mitotic chromosomes	−	2.51
YDR148C	*KGD2*	Dihydrolipoyl transsuccinylase, component of the mitochondrial alpha-ketoglutarate dehydrogenase complex, which catalyzes the oxidative decarboxylation of alpha-ketoglutarate to succinyl-CoA in the TCA cycle	−	2.49
YKL103C	*LAP4*	Vacuolar aminopeptidase yscI	−	2.48
YOR247W	*SRL1*	Mannoprotein that exhibits a tight association with the cell wall, required for cell wall stability in the absence of GPI-anchored mannoproteins; has a high serine-threonine content	−	2.47
YOR385W	*YOR385 W*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm	−	2.43
YOR254C	*SEC63*	Essential subunit of Sec63 complex (Sec63p, Sec62p, Sec66p and Sec72p	−	2.42
YDR050C	*TPI1*	Triose phosphate isomerase, abundant glycolytic enzyme	−	2.41
YCL050C	*APA1*	Diadenosine 5',5''-P1,P4-tetraphosphate phosphorylase I (AP4A phosphorylase), involved in catabolism of bis(5'-nucleosidyl) tetraphosphates	−	2.40
YDL103C	*QRI1*	UDP-N-acetylglucosamine pyrophosphorylase, catalyzes the formation of UDP-N-acetylglucosamine (UDP-GlcNAc)	−	2.38
YDL022W	*GPD1*	NAD-dependent glycerol-3-phosphate dehydrogenase, key enzyme of glycerol synthesis, essential for growth under osmotic stress	−	2.38
YBL032W	*HEK2*	RNA binding protein involved in the asymmetric localization of ASH1 mRNA	−	2.38
YER103W	*SSA4*	Heat shock protein that is highly induced upon stress	−	2.38
YBR072W	*HSP26*	Small heat shock protein (sHSP) with chaperone activity	−	2.37
YJR143C	*PMT4*	Protein O-mannosyltransferase, transfers mannose residues from dolichyl phosphate-D-mannose to protein serine/threonine residues	−	2.37

YGR285C	*ZUO1*	Ribosome-associated chaperone, functions in ribosome biogenesis and, in partnership with Ssz1p and SSb1/2, as a chaperone for nascent polypeptide chains	−	2.36
YGL137W	*SEC27*	Essential beta'-coat protein of the COPI coatomer, involved in ER-to-Golgi and Golgi-to-ER transport; contains WD40 domains that mediate cargo selective interactions	−	2.34
YBL002W	*HTB2*	Histone H2B, core histone protein required for chromatin assembly and chromosome function	−	2.34
YLR131C	*ACE2*	Transcription factor that activates expression of early G1-specific genes, localizes to daughter cell nuclei after cytokinesis and delays G1 progression in daughters, localization is regulated by phosphorylation	−	2.33
YGR240C	*PFK1*	Alpha subunit of heterooctameric phosphofructokinase involved in glycolysis,	−	2.33
YKL152C	*GPM1*	Tetrameric phosphoglycerate mutase, mediates the conversion of 3-phosphoglycerate to 2-phosphoglycerate during glycolysis and the reverse reaction during gluconeogenesis	−	2.29
YLR132C	*YLR132C*	Essential protein of unknown function	−	2.28
YNL082W	*PMS1*	ATP-binding protein required for mismatch repair in mitosis and meiosis	−	2.28
YIL041W	*GVP36*	BAR domain-containing protein that localizes to both early and late Golgi vesicles	−	2.27
YHR064C	*SSZ1*	Hsp70 protein that interacts with Zuo1p (a DnaJ homolog) to form a ribosome-associated complex that binds the ribosome via the Zuo1p subunit	−	2.26
YDR508C	*GNP1*	High-affinity glutamine permease, also transports Leu, Ser, Thr, Cys, Met and Asn	−	2.26
YFL059W	*SNZ3*	Member of a stationary phase-induced gene family	−	2.25
YHL018W	*YHL018W*	Putative protein of unknown function	−	2.24
YOR083W	*WHI5*	Repressor of G1 transcription that binds to SCB binding factor (SBF) at SCB target promoters in early G1; phosphorylation of Whi5p by the CDK, Cln3p/Cdc28p relieves repression and promoter binding by Whi5; periodically expressed in G1	−	2.20
YLR133W	*CKI1*	Choline kinase, catalyzing the first step in phosphatidylcholine synthesis via the CDP-choline (Kennedy pathway)	−	2.19
YNL071W	*LAT1*	Dihydrolipoamide acetyltransferase component (E2) of pyruvate dehydrogenase complex, which catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA	−	2.19
YDL004W	*ATP16*	Delta subunit of the central stalk of mitochondrial F1F0 ATP synthase, which is a	−	2.18

		large, evolutionarily conserved enzyme complex required for ATP synthesis		
YPR183W	*DPM1*	Dolichol phosphate mannose (Dol-P-Man) synthase of the ER membrane, catalyzes the formation of Dol-P-Man from Dol-P and GDP-Man	−	2.17
YFL053W	*DAK2*	Dihydroxyacetone kinase, required for detoxification of dihydroxyacetone (DHA	−	2.16
YOR388C	*FDH1*	NAD(+)-dependent formate dehydrogenase, may protect cells from exogenous formate	−	2.16
YDL192W	*ARF1*	ADP-ribosylation factor, GTPase of the Ras superfamily involved in regulation of coated vesicle formation in intracellular trafficking within the Golgi	−	2.14
YGL175C	*SAE2*	Endonuclease that processes hairpin DNA structures with the MRX complex	−	2.14
YBR053C	*YBR053C*	Putative protein of unknown function	−	2.13
YDL140C	*RPO21*	RNA polymerase II largest subunit B220, part of central core	−	2.12
YDL219W	*DTD1*	D-Tyr-tRNA(Tyr) deacylase, functions in protein translation, may affect nonsense suppression via alteration of the protein synthesis machinery	−	2.12
YDR226W	*ADK1*	Adenylate kinase, required for purine metabolism	−	2.11
YHR068W	*DYS1*	Deoxyhypusine synthase, catalyzes formation of deoxyhypusine, the first step in hypusine biosynthesis	−	2.10
YMR205C	*PFK2*	Beta subunit of heterooctameric phosphofructokinase involved in glycolysis, indispensable for anaerobic growth, activated by fructose-2,6-bisphosphate and AMP, mutation inhibits glucose induction of cell cycle-related genes	−	2.09
YDR432W	*NPL3*	RNA-binding protein that promotes elongation, regulates termination, and carries poly(A) mRNA from nucleus to cytoplasm; required for pre-mRNA splicing	−	2.08
YNL233W	*BNI4*	Targeting subunit for Glc7p protein phosphatase, localized to the bud neck, required for localization of chitin synthase III to the bud neck via interaction with the chitin synthase III regulatory subunit Skt5p	−	2.07
YMR027W	*YMR027W*	Putative protein of unknown function	−	2.06
YDR111C	*ALT2*	Putative alanine transaminase (glutamic pyruvic transaminase)	−	2.06
YFL038C	*YPT1*	Rab family GTPase, involved in the ER-to-Golgi step of the secretory pathway)	−	2.05
YNR035C	*ARC35*	Subunit of the ARP2/3 complex, which is	−	2.04

		required for the motility and integrity of cortical actin patches		
YGL105W	*ARC1*	Protein that binds tRNA and methionyl- and glutamyl-tRNA synthetases (Mes1p and Gus1p), delivering tRNA to them, stimulating catalysis, and ensuring their localization to the cytoplasm	−	2.04
YNL010W	*YNL010W*	Putative protein of unknown function with similarity to phosphoserine phosphatases; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and nucleus	−	2.04
YPL032C	*SVL3*	Protein of unknown function, mutant phenotype suggests a potential role in vacuolar function	−	2.02
YDR510W	*SMT3*	Ubiquitin-like protein of the SUMO family, conjugated to lysine residues of target proteins	−	2.01
YDL185W	*TFP1*	Subunit A of the eight-subunit V1 peripheral membrane domain of the vacuolar H+-ATPase	−	2.01
YHL031C	*GOS1*	v-SNARE protein involved in Golgi transport, homolog of the mammalian protein GOS-28/GS28	−	2.01
YML054C	*CYB2*	Cytochrome b2 (L-lactate cytochrome-c oxidoreductase), component of the mitochondrial intermembrane space, required for lactate utilization	−	2.00
YGR279C	*SCW4*	Cell wall protein with similarity to glucanases; scw4 scw10 double mutants exhibit defects in mating	−	2.00
YOL164W	*BDS1*	Bacterially-derived sulfatase required for use of alkyl- and aryl-sulfates as sulfur sources	−	-2.00
YGR074W	*SMD1*	Core Sm protein Sm D1	−	-2.00
YMR126C	*DLT1*	Protein of unknown function, mutant sensitive to 6-azauracil (6AU) and mycophenolic acid (MPA)	−	-2.00
YLR266C	*PDR8*	Transcription factor; targets include ATP-binding cassette (ABC) transporters, major facilitator superfamily transporters, and other genes involved in the pleiotropic drug resistance (PDR) phenomenon	−	-2.01
YKR009C	*FOX2*	Multifunctional enzyme of the peroxisomal fatty acid beta-oxidation pathway	−	-2.01
YIL046W	*MET30*	F-box protein containing five copies of the WD40 motif, controls cell cycle function, sulfur metabolism, and methionine biosynthesis as part of the ubiquitin ligase complex	−	-2.03
YGR166W	*KRE11*	Subunit of TRAPPII, a multimeric guanine nucleotide-exchange factor for Ypt1p	−	-2.04
YBR212W	*NGR1*	RNA binding protein that negatively regulates growth rate; interacts with the 3' UTR of the mitochondrial porin (POR1) mRNA and enhances its degradation	−	-2.05

YDL231C	*BRE4*	Zinc finger protein containing five transmembrane domains	−	-2.05
YFR046C	*CNN1*	Kinetochore protein of unknown function; associated with the essential kinetochore proteins Nnf1p and Spc24p	−	-2.06
YMR132C	*JLP2*	Protein of unknown function, contains sequence that closely resembles a J domain (typified by the E. coli DnaJ protein)	−	-2.06
YNL221C	*POP1*	Subunit of both RNase MRP, which cleaves pre-rRNA, and nuclear RNase P, which cleaves tRNA precursors to generate mature 5' ends	−	-2.07
YDL215C	*GDH2*	NAD(+)-dependent glutamate dehydrogenase, degrades glutamate to ammonia and alpha-ketoglutarate	−	-2.08
YER015W	*FAA2*	Medium chain fatty acyl-CoA synthetase, activates imported fatty acids	−	-2.09
YOR004W	*UTP23*	Essential nucleolar protein that is a component of the SSU (small subunit) processome involved in 40S ribosomal subunit biogenesis	−	-2.09
YFR025C	*HIS2*	Histidinolphosphatase, catalyzes the eighth step in histidine biosynthesis	−	-2.10
YLL048C	*YBT1*	Transporter of the ATP-binding cassette (ABC) family involved in bile acid transport	−	-2.10
YER092W	*IES5*	Protein that associates with the INO80 chromatin remodeling complex under low-salt conditions	−	-2.11
YNR074C	*AIF1*	Mitochondrial cell death effector that translocates to the nucleus in response to apoptotic stimuli, homolog of mammalian Apoptosis-Inducing Factor, putative reductase	−	-2.11
YJR050W	*ISY1*	Member of NineTeen Complex (NTC) that contains Prp19p and stabilizes U6 snRNA in catalytic forms of spliceosome containing U2, U5, and U6 snRNAs, interacts with Prp16p to modulate splicing fidelity	−	-2.11
YIR013C	*GAT4*	Protein containing GATA family zinc finger motifs	−	-2.12
YLR193C	*UPS1*	Mitochondrial intermembrane space protein that regulates mitochondrial cardiolipin levels, null has defects in Mgm1p processing, integrity of mitochondrial inner membrane complexes, and mitochondrial morphology	−	-2.12
YGR280C	*PXR1*	Essential protein involved in rRNA and snoRNA maturation; competes with TLC1 RNA for binding to Est2p, suggesting a role in negative regulation of telomerase	−	-2.15
YPR200C	*ARR2*	Arsenate reductase required for arsenate resistance	−	-2.16
YDR249C	*YDR249C*	Putative protein of unknown function	−	-2.16

YOL044W	*PEX15*	Phosphorylated tail-anchored type II integral peroxisomal membrane protein required for peroxisome biogenesis, cells lacking Pex15p mislocalize peroxisomal matrix proteins to cytosol	−	-2.19
YBL049W	*MOH1*	Protein of unknown function, has homology to kinase Snf7p	−	-2.20
YOR184W	*SER1*	3-phosphoserine aminotransferase, catalyzes the formation of phosphoserine from 3-phosphohydroxypyruvate, required for serine and glycine biosynthesis	−	-2.20
YOL047C	*YOL047C*	Protein of unknown function	−	-2.21
YER040W	*GLN3*	Transcriptional activator of genes regulated by nitrogen catabolite repression (NCR), localization and activity regulated by quality of nitrogen source	−	-2.21
YPR168W	*NUT2*	Subunit of the RNA polymerase II mediator complex	−	-2.21
YLR130C	*ZRT2*	Low-affinity zinc transporter of the plasma membrane	−	-2.21
YKL220C	*FRE2*	Ferric reductase and cupric reductase, reduces siderophore-bound iron and oxidized copper prior to uptake by transporters	−	-2.22
YLR004C	*THI73*	Putative plasma membrane permease proposed to be involved in carboxylic acid uptake and repressed by thiamine	−	-2.22
YOL084W	*PHM7*	Protein of unknown function, expression is regulated by phosphate levels	−	-2.23
YNL046W	*YNL046W*	Putative protein of unknown function	−	-2.25
YDR257C	*RKM4*	Ribosomal lysine methyltransferase specific for monomethylation of Rpl42ap and Rpl42bp (lysine 55)	−	-2.29
YNL116W	*DMA2*	Protein involved in ubiquitination; plays a role in regulating spindle position and orientation	−	-2.30
YEL065W	*SIT1*	Ferrioxamine B transporter, member of the ARN family of transporters that specifically recognize siderophore-iron chelates	−	-2.35
YBR043C	*QDR3*	Multidrug transporter of the major facilitator superfamily, required for resistance to quinidine, barban, cisplatin, and bleomycin	−	-2.37
YNL129W	*NRK1*	Nicotinamide riboside kinase, catalyzes the phosphorylation of nicotinamide riboside and nicotinic acid riboside in salvage pathways for NAD+ biosynthesis	−	-2.37
YBL029W	*YBL029W*	Non-essential protein of unknown function	−	-2.38
YPR128C	*ANT1*	Peroxisomal adenine nucleotide transporter	−	-2.41
YBR166C	*TYR1*	Prephenate dehydrogenase involved in tyrosine biosynthesis, expression is dependent on phenylalanine levels	−	-2.41

YLL027W	*ISA1*	Mitochondrial matrix protein involved in biogenesis of the iron-sulfur (Fe/S) cluster of Fe/S proteins, isa1 deletion causes loss of mitochondrial DNA and respiratory deficiency	−	-2.43
YGL146C	*RRT6*	Putative protein of unknown function	−	-2.45
YIL117C	*PRM5*	Pheromone-regulated protein, predicted to have 1 transmembrane segment	−	-2.46
YBL103C	*RTG3*	Basic helix-loop-helix-leucine zipper (bHLH/Zip) transcription factor that forms a complex with another bHLH/Zip protein, Rtg1p, to activate the retrograde (RTG) and TOR pathways	−	-2.46
YDR090C	*YDR090C*	Putative protein of unknown function	−	-2.46
YMR187C	*YMR187C*	Putative protein of unknown function	−	-2.46
YGR029W	*ERV1*	Flavin-linked sulfhydryl oxidase of the mitochondrial intermembrane space (IMS), oxidizes Mia40p as part of a disulfide relay system that promotes IMS retention of imported proteins	−	-2.47
YDL121C	*YDL121C*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the endoplasmic reticulum	−	-2.47
YCR089W	*FIG2*	Cell wall adhesin, expressed specifically during mating	−	-2.48
YER039C	*HVG1*	Protein of unknown function, has homology to Vrg4p	−	-2.52
YNL125C	*ESBP6*	Protein with similarity to monocarboxylate permeases, appears not to be involved in transport of monocarboxylates such as lactate, pyruvate or acetate across the plasma membrane	−	-2.53
YAL044C	*GCV3*	H subunit of the mitochondrial glycine decarboxylase complex, required for the catabolism of glycine to 5,10-methylene-THF	−	-2.53
YBL095W	*YBL095W*	Putative protein of unknown function	−	-2.54
YHR160C	*PEX18*	Peroxin required for targeting of peroxisomal matrix proteins containing PTS2	−	-2.54
YBR115C	*LYS2*	Alpha aminoadipate reductase, catalyzes the reduction of alpha-aminoadipate to alpha-aminoadipate 6-semialdehyde, which is the fifth step in biosynthesis of lysine	−	-2.55
YGL205W	*POX1*	Fatty-acyl coenzyme A oxidase, involved in the fatty acid beta-oxidation pathway	−	-2.56
YGL114W	*YGL114W*	Putative protein of unknown function	−	-2.62
YFL021W	*GAT1*	Transcriptional activator of genes involved in nitrogen catabolite repression	−	-2.64
YCR023C	*YCR023C*	Vacuolar membrane protein of unknown function	−	-2.64
YML097C	*VPS9*	A guanine nucleotide exchange factor involved in vesicle-mediated vacuolar protein transport;	−	-2.66

		specifically stimulates the intrinsic guanine nucleotide exchange activity of Vps21p/Rab5		
YOR221C	*MCT1*	Predicted malonyl-CoA:ACP transferase, putative component of a type-II mitochondrial fatty acid synthase that produces intermediates for phospholipid remodeling	−	-2.68
YKL033W-A	*YKL033W-A*	Putative protein of unknown function	−	-2.75
YLL057C	*JLP1*	Fe(II)-dependent sulfonate/alpha-ketoglutarate dioxygenase, involved in sulfonate catabolism for use as a sulfur source; contains sequence that resembles a J domain (typified by the E. coli DnaJ protein); induced by sulphur starvation	−	-2.76
YMR019W	*STB4*	Protein that binds Sin3p in a two-hybrid assay	−	-2.78
YBR248C	*HIS7*	Imidazole glycerol phosphate synthase (glutamine amidotransferase:cyclase), catalyzes the fifth and sixth steps of histidine biosynthesis and also produces 5-aminoimidazole-4-carboxamide ribotide (AICAR), a purine precursor	−	-2.82
YNR065C	*YNR065C*	Protein of unknown function	−	-2.84
YOR339C	*UBC11*	Ubiquitin-conjugating enzyme most similar in sequence to Xenopus ubiquitin-conjugating enzyme E2-C, but not a true functional homolog of this E2; unlike E2-C	−	-2.86
YOR008C-A	*YOR008C-A*	Putative protein of unknown function, includes a potential transmembrane domain	−	-2.87
YDR384C	*ATO3*	Plasma membrane protein, regulation pattern suggests a possible role in export of ammonia from the cell	−	-2.89
YBR249C	*ARO4*	3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase, catalyzes the first step in aromatic amino acid biosynthesis and is feedback-inhibited by tyrosine or high concentrations of phenylalanine	−	-2.91
YGR121C	*MEP1*	Ammonium permease; belongs to a ubiquitous family of cytoplasmic membrane proteins that transport only ammonium (NH4+)	−	-2.92
YPL052W	*OAZ1*	Regulator of ornithine decarboxylase (Spe1p), antizyme that binds to Spe1p to regulate ubiquitin-independent degradation	−	-2.95
YBL071W-A	*KTI11*	Zn-ribbon protein that co-purifies with Dph1 and Dph2 in a complex required for synthesis of diphthamide on translation factor eEF2 and with Elongator subunits Iki3p, Elp2p, and Elp3p involved in modification of wobble nucleosides in tRNAs	−	-2.96
YER055C	*HIS1*	ATP phosphoribosyltransferase, a hexameric enzyme, catalyzes the first step in histidine	−	-2.97

		biosynthesis; mutations cause histidine auxotrophy and sensitivity to Cu, Co, and Ni salts		
YIR034C	*LYS1*	Saccharopine dehydrogenase (NAD+, L-lysine-forming), catalyzes the conversion of saccharopine to L-lysine, which is the final step in the lysine biosynthesis pathway	−	-2.98
YER184C	*YER184C*	Putative zinc cluster protein	−	-3.03
YDR487C	*RIB3*	3,4-dihydroxy-2-butanone-4-phosphate synthase (DHBP synthase), required for riboflavin biosynthesis from ribulose-5-phosphate	−	-3.03
YDR076W	*RAD55*	Protein that stimulates strand exchange by stabilizing the binding of Rad51p to single-stranded DNA	−	-3.05
YNL311C	*YNL311C*	F-box protein of unknown function predicted to be part of an SCF ubiquitin protease complex; involved in regulating protein levels of sulfur metabolism enzymes	−	-3.11
YPL135W	*ISU1*	Conserved protein of the mitochondrial matrix, performs a scaffolding function during assembly of iron-sulfur clusters, interacts physically and functionally with yeast frataxin (Yfh1p	−	-3.13
YGR197C	*SNG1*	Protein involved in resistance to nitrosoguanidine (MNNG) and 6-azauracil (6-AU)	−	-3.13
YLR090W	*XDJ1*	Putative chaperone, homolog of E. coli DnaJ, closely related to Ydj1p	−	-3.14
YGL154C	*LYS5*	Phosphopantetheinyl transferase involved in lysine biosynthesis	−	-3.23
YLR348C	*DIC1*	Mitochondrial dicarboxylate carrier, integral membrane protein, catalyzes a dicarboxylate-phosphate exchange across the inner mitochondrial membrane	−	-3.25
YOR192C	*THI72*	Transporter of thiamine or related compound	−	-3.27
YJR010W	*MET3*	ATP sulfurylase, catalyzes the primary step of intracellular sulfate activation, essential for assimilatory reduction of sulfate to sulfide, involved in methionine metabolism	−	-3.33
YGR154C	*GTO1*	Omega-class glutathione transferase	−	-3.34
YHR208W	*BAT1*	Mitochondrial branched-chain amino acid aminotransferase, homolog of murine ECA39	−	-3.37
YER039C	*YER039C*	Putative protein of unknown function	−	-3.37
YGL186C	*TPN1*	Plasma membrane pyridoxine (vitamin B6) transporter; member of the purine-cytosine permease subfamily within the major facilitator superfamily	−	-3.38
YHR122W	*YHR12W*	Protein of unknown function required for establishment of sister chromatid cohesion	−	-3.41

YKL211C	*TRP3*	Bifunctional enzyme exhibiting both indole-3-glycerol-phosphate synthase and anthranilate synthase activities	−	-3.43
YPR078C	*YPR078C*	Putative protein of unknown function	−	-3.45
YDL054C	*MCH1*	Protein with similarity to mammalian monocarboxylate permeases, which are involved in transport of monocarboxylic acids across the plasma membrane	−	-3.51
YLR042C	*YLR042C*	Protein of unknown function; localizes to the cytoplasm	−	-3.58
YOR161C	*PNS1*	Protein of unknown function	−	-3.59
YJR155W	*AAD10*	Putative aryl-alcohol dehydrogenase with similarity to P. chrysosporium aryl-alcohol dehydrogenase	−	-3.59
YPL188W	*POS5*	Mitochondrial NADH kinase, phosphorylates NADH; also phosphorylates NAD(+) with lower specificity	−	-3.60
YGL059W	*PKP2*	Mitochondrial protein kinase that negatively regulates activity of the pyruvate dehydrogenase complex by phosphorylating the ser-133 residue of the Pda1p subunit	−	-3.63
YBR105C	*VID24*	Peripheral membrane protein located at Vid (vacuole import and degradation) vesicles	−	-3.67
YKR071C	*DRE2*	Conserved component of an early step in the cytosolic Fe-S protein assembly (CIA) machinery	−	-3.70
YBR104W	*YMC2*	Mitochondrial protein, putative inner membrane transporter with a role in oleate metabolism and glutamate biosynthesis	−	-3.74
YJL072C	*PSF2*	Subunit of the GINS complex (Sld5p, Psf1p, Psf2p, Psf3p), which is localized to DNA replication origins and implicated in assembly of the DNA replication machinery	−	-3.81
YDR158W	*HOM2*	Aspartic beta semi-aldehyde dehydrogenase, catalyzes the second step in the common pathway for methionine and threonine biosynthesis	−	-3.82
YPL273W	*SAM4*	S-adenosylmethionine-homocysteine methyltransferase, functions along with Mht1p in the conversion of S-adenosylmethionine (AdoMet) to methionine to control the methionine/AdoMet ratio	−	-3.83
YPL033C	*SRL4*	Protein of unknown function	−	-3.84
YCL030C	*HIS4*	Multifunctional enzyme containing phosphoribosyl-ATP pyrophosphatase, phosphoribosyl-AMP cyclohydrolase, and histidinol dehydrogenase activities	−	-3.85
YNL036W	*NCE103*	Carbonic anhydrase; poorly transcribed under aerobic conditions and at an undetectable level	−	-3.86

		under anaerobic conditions		
YBR045C	*GIP1*	Meiosis-specific regulatory subunit of the Glc7p protein phosphatase, regulates spore wall formation and septin organization	−	-3.88
YER056C	*FCY2*	Purine-cytosine permease, mediates purine (adenine, guanine, and hypoxanthine) and cytosine accumulation	−	-4.04
YMR321C	*YMR321C*	Putative protein of unknown function	−	-4.07
YHR018C	*ARG4*	Argininosuccinate lyase, catalyzes the final step in the arginine biosynthesis pathway	−	-4.10
YPR058W	*YMC1*	Mitochondrial protein, putative inner membrane transporter with a role in oleate metabolism and glutamate biosynthesis	−	-4.10
YOL141W	*PPM2*	AdoMet-dependent tRNA methyltransferase also involved in methoxycarbonylation	−	-4.15
YKL218C	*SRY1*	3-hydroxyaspartate dehydratase, deaminates L-threo-3-hydroxyaspartate to form oxaloacetate and ammonia	−	-4.31
YOL091W	*SPO21*	Component of the meiotic outer plaque of the spindle pole body, involved in modifying the meiotic outer plaque that is required prior to prospore membrane formation	−	-4.32
YDR253C	*MET32*	Zinc-finger DNA-binding protein, involved in transcriptional regulation of the methionine biosynthetic genes, similar to Met31p	−	-4.35
YPL252C	*YAH1*	Ferredoxin of the mitochondrial matrix required for formation of cellular iron-sulfur proteins	−	-4.39
YIL056W	*VHR1*	Transcriptional activator, required for the vitamin H-responsive element (VHRE) mediated induction of VHT1 (Vitamin H transporter) and BIO5 (biotin biosynthesis intermediate transporter) in response to low biotin concentrations	−	-4.41
YOR303W	*CPA1*	Small subunit of carbamoyl phosphate synthetase, which catalyzes a step in the synthesis of citrulline, an arginine precursor	−	-4.45
YDR354W	*TRP4*	Anthranilate phosphoribosyl transferase of the tryptophan biosynthetic pathway, catalyzes the phosphoribosylation of anthranilate	−	-4.58
YOR130C	*ORT1*	Ornithine transporter of the mitochondrial inner membrane, exports ornithine from mitochondria as part of arginine biosynthesis	−	-4.59
YER060W-A	*FCY22*	Putative purine-cytosine permease, very similar to Fcy2p but cannot substitute for its function	−	-4.59
YNR057C	*BIO4*	Dethiobiotin synthetase, catalyzes the third step in the biotin biosynthesis pathway	−	-4.68
YGL224C	*SDT1*	Pyrimidine nucleotidase; overexpression suppresses the 6-AU sensitivity of transcription elongation factor S-II, as well as resistance to	−	-4.89

		other pyrimidine derivatives		
YNL095C	*YNL095C*	Putative protein of unknown function predicted to contain a transmembrane domain	−	-4.94
YMR062C	*ARG7*	Mitochondrial ornithine acetyltransferase, catalyzes the fifth step in arginine biosynthesis	−	-4.99
YJL198W	*PHO90*	Low-affinity phosphate transporter; deletion of pho84, pho87, pho89, pho90, and pho91 causes synthetic lethality	−	-5.08
YOR302W	*YOR302W*	CPA1 uORF, Arginine attenuator peptide, regulates translation of the CPA1 mRNA	−	-5.18
YOL058W	*ARG1*	Arginosuccinate synthetase, catalyzes the formation of L-argininosuccinate from citrulline and L-aspartate in the arginine biosynthesis	−	-5.19
YBR256C	*RIB5*	Riboflavin synthase; catalyzes the last step of the riboflavin biosynthesis pathway	−	-5.28
YDL198C	*GGC1*	Mitochondrial GTP/GDP transporter, essential for mitochondrial genome maintenance	−	-5.29
YOR032C	*HMS1*	Basic helix-loop-helix (bHLH) protein with similarity to myc-family transcription factors	−	-5.29
YIR017C	*MET28*	Basic leucine zipper (bZIP) transcriptional activator in the Cbf1p-Met4p-Met28p complex, participates in the regulation of sulfur metabolism	−	-5.33
YLR089C	*ALT1*	Alanine transaminase (glutamic pyruvic transaminase)	−	-5.33
YJR111C	*YJR111C*	Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the mitochondria	−	-5.38
YJR154W	*YJR154W*	Putative protein of unknown function	−	-5.49
YJR137C	*MET5*	Sulfite reductase beta subunit, involved in amino acid biosynthesis, transcription repressed by methionine	−	-5.66
YNL104C	*LEU4*	Alpha-isopropylmalate synthase (2-isopropylmalate synthase	−	-5.87
YER174C	*GRX4*	Hydroperoxide and superoxide-radical responsive glutathione-dependent oxidoreductase; monothiol glutaredoxin subfamily member along with Grx3p and Grx5p	−	-5.88
YPR167C	*MET16*	3'-phosphoadenylsulfate reductase, reduces 3'-phosphoadenylyl sulfate to adenosine-3',5'-bisphosphate and free sulfite using reduced thioredoxin as cosubstrate, involved in sulfate assimilation and methionine metabolism	−	-5.95
YJR109C	*CPA2*	Large subunit of carbamoyl phosphate synthetase, which catalyzes a step in the synthesis of citrulline, an arginine precursor	−	-6.23
YER024W	*YAT2*	Carnitine acetyltransferase; has similarity to Yat1p, which is a carnitine acetyltransferase associated with the mitochondrial outer	−	-6.25

		membrane		
YNR068C	*YNR068C*	Putative protein of unknown function	−	-6.35
YKR069W	*MET1*	S-adenosyl-L-methionine uroporphyrinogen III transmethylase, involved in the biosynthesis of siroheme, a prosthetic group used by sulfite reductase	−	-6.40
YER069W	*ARG5,6*	Protein that is processed in the mitochondrion to yield acetylglutamate kinase and N-acetyl-gamma-glutamyl-phosphate reductase, which catalyze the 2nd and 3rd steps in arginine biosynthesis	−	-6.44
YKL029C	*MAE1*	Mitochondrial malic enzyme, catalyzes the oxidative decarboxylation of malate to pyruvate, which is a key intermediate in sugar metabolism and a precursor for synthesis of several amino acids	−	-6.47
YML116W	*ATR1*	Multidrug efflux pump of the major facilitator superfamily, required for resistance to aminotriazole and 4-nitroquinoline-N-oxide	−	-6.51
YGR065C	*VHT1*	High-affinity plasma membrane H+-biotin (vitamin H) symporter	−	-6.60
YJR130C	*STR2*	Cystathionine gamma-synthase, converts cysteine into cystathionine	−	-6.63
YLR092W	*SUL2*	High affinity sulfate permease; sulfate uptake is mediated by specific sulfate transporters Sul1p and Sul2p, which control the concentration of endogenous activated sulfate intermediates	−	-6.83
YMR108W	*ILV2*	Acetolactate synthase, catalyses the first common step in isoleucine and valine biosynthesis and is the target of several classes of inhibitors, localizes to the mitochondria	−	-6.90
YOL140W	*ARG8*	Acetylornithine aminotransferase, catalyzes the fourth step in the biosynthesis of the arginine precursor ornithine	−	-7.11
YPL264C	*YPL264C*	Putative membrane protein of unknown function	−	-7.16
YOL119C	*MCH4*	Protein with similarity to mammalian monocarboxylate permeases, which are involved in transport of monocarboxylic acids across the plasma membrane	−	-7.39
YPR027C	*YPR027C*	Putative protein of unknown function	−	-7.46
YGR224W	*AZR1*	Plasma membrane transporter of the major facilitator superfamily, involved in resistance to azole drugs such as ketoconazole and fluconazole	−	-7.62
YFR030W	*MET10*	Subunit alpha of assimilatory sulfite reductase, which converts sulfite into sulfide	−	-7.71
YHR071W	*PCL5*	Cyclin, interacts with and phosphorylated by Pho85p cyclin-dependent kinase (Cdk), induced by Gcn4p at level of transcription, specifically	−	-7.71

		required for Gcn4p degradation, may be sensor of cellular protein biosynthetic capacity		
YNR058W	*BIO3*	7,8-diamino-pelargonic acid aminotransferase (DAPA), catalyzes the second step in the biotin biosynthesis pathway	−	-8.23
YBR296C	*PHO89*	Na+/Pi cotransporter, active in early growth phase	−	-9.20
YHR029C	*YHI9*	Protein of unknown function; null mutant is defective in unfolded protein response	−	-9.30
YER175C	*TMT1*	Trans-aconitate methyltransferase, cytosolic enzyme that catalyzes the methyl esterification of 3-isopropylmalate, an intermediate of the leucine biosynthetic pathway, and trans-aconitate, which inhibits the citric acid cycle	−	-9.72
YGL117W	*YGL117W*	Putative protein of unknown function	−	-9.72
YGL184C	*STR3*	Cystathionine beta-lyase, converts cystathionine into homocysteine	−	-9.83
YBR148W	*YSW1*	Protein required for normal prospore membrane formation	−	-10.31
YBR047W	*FMP23*	Putative protein of unknown function	−	-12.46
YJL088W	*ARG3*	Ornithine carbamoyltransferase (carbamoylphosphate:L-ornithine carbamoyltransferase), catalyzes the sixth step in the biosynthesis of the arginine precursor ornithine	−	-14.12
YMR096W	*SNZ1*	Protein involved in vitamin B6 biosynthesis	−	-17.82
YPL250C	*ICY2*	Protein of unknown function	−	-18.06
YDR380W	*ARO10*	Phenylpyruvate decarboxylase, catalyzes decarboxylation of phenylpyruvate to phenylacetaldehyde, which is the first specific step in the Ehrlich pathway	−	-25.87
YMR095C	*SNO1*	Protein of unconfirmed function, involved in pyridoxine metabolism	−	-33.55
YBR294W	*SUL1*	High affinity sulfate permease	−	-39.15

CHAPTER 3 VALPROIC ACID INDUCES THE UNFOLDED PROTEIN RESPONSE BY INCREASING CERAMIDE LEVELS

INTRODUCTION

Bipolar disorder (BD), one of the most severe forms of mood disorder, is characterized by recurrent episodes of depression and mania (Belmaker, 2004; Goodwin and Jamison, 2007). BD is ranked as the sixth leading cause of disability worldwide. It affects about 1-2% of the total world population (Belmaker, 2004; Goodwin and Jamison, 2007, Cheng et al., 2005) and leads to suicide in 15% of cases (Bostwick and Pankratz, 2000). Valproic acid (VPA), a branched short-chain fatty acid, is one of the most widely used drugs for the treatment of BD. However, it is effective in only 40-60% of cases and results in serious side effects, including hepatotoxicity and teratogenicity (Henry et al., 2003). Although many hypotheses have been postulated to explain its efficacy, the therapeutic mechanism of the drug is not understood, nor is the underlying cause of the disease (Gould et al., 2004; Williams et al., 2002; Yu and Greenberg, 2016). This knowledge gap hampers the development of more effective drugs to treat BD.

The inositol depletion hypothesis has had a major impact on research in BD. Berridge proposed that lithium, widely used to treat BD, inhibits inositol monophosphatase (IMPase), causing inositol depletion and subsequently decreasing inositol 1,4,5-triphosphate mediated signaling (Berridge, 1989; Hallcher and Sherman 1980). Previous studies show that VPA, similar to lithium, causes a decrease in intracellular inositol in yeast and mammalian cells (Shaltiel et al., 2004; Vaden et al., 2001; Ye and Greenberg, 2015). VPA indirectly inhibits *myo*-inositol-3-phosphate synthase (MIPS), the enzyme responsible for the rate-limiting step of *de novo* synthesis

of inositol, suggesting that MIPS is post-translationally regulated (Ju et al., 2004). More recent findings show that yeast and human MIPS are phosphorylated, (Deranieh., et al 2013; Jesch et al., 2005) and that phosphorylation of conserved sites affects enzymatic activity (Deranieh., et al 2013). These findings suggest that the mechanism by which VPA causes inositol depletion is conserved in yeast and mammals, supporting the yeast model for genetic and molecular studies of the mechanism of the drug.

In yeast, supplementation of inositol triggers a change in the expression of hundreds of inositol-regulated genes, including genes for lipid synthesis (Cox et al., 1997; Chang et al., 2002; Jesch et al., 2010). Inositol containing lipids, including inositol phosphoinositides, glycosylphosphatidylinositol, and sphingolipids, play crucial structural and functional roles in regulating membrane biogenesis, membrane trafficking, cytoskeletal organization, and gene expression (Santiago and Mamoun, 2003; Jesch et al., 2005; Henry et al., 2014). Hence, inositol depletion exerts profound effects on cellular function (Deranieh and Greenberg, 2009). Inositol depletion not only alters lipid biosynthesis (Gaspar et al., 2006), but also activates stress response pathways, including the protein kinase C and unfolded protein response (UPR) pathways (Cox et al., 1997; Chang et al., 2002; Jesch et al., 2010). Cells grown in the absence of inositol exhibit induction of the UPR pathway (Cox et al., 1997; Promlek et al., 2011), which is reversed by inositol supplementation (Jesch et al., 2005). These studies suggest that decreasing the intracellular levels of inositol induces the UPR pathway by a mechanism not yet characterized.

Interdependence of the UPR pathway and ceramide synthesis has been demonstrated in yeast and mammals. Induction of the UPR increases ceramide levels

and viability in yeast (Han et al., 2010). In mammals, activation of the UPR increases the expression of ceramide synthase CerS6, leading to increased synthesis of ceramides containing C16 fatty acids (Han et al., 2010). Perturbation of *de novo* synthesis of sphingolipids activates the UPR in yeast (Mousley et al., 2008; Epstein et al., 2012), and defective ceramide homeostasis leads to UPR failure (Guenther, et al 2010). Ceramide also decreases the transcription of nutrient transporters, including amino acid transporter *mCAT-1*, glucose transporter *GLUT-1* (Payet, et al., 2013), glucose transporter *HXT4*, and uracil permease *FUR4* (Guenther, et al 2010). Therefore, the interrelationship between ceramide levels and the UPR pathway maintains cell homeostasis.

In the current study, I show for the first time that VPA mediated inositol depletion induces the UPR pathway by increasing *de novo* synthesis of ceramide, especially C24-C26 containing phytoceramide. These findings have implications for the therapeutic mechanism of VPA.

MATERIALS AND METHODS

Yeast strains, growth media and conditions

Strains used in this study are summarized in Table 3.1. Cells were maintained on YPD medium (2% glucose, 1% yeast extract, 2% bactopeptone). Deletion mutants were maintained on medium supplemented with G418 (200 µg/ml). Synthetic minimal medium without inositol (I-) contained all the essential components of Difco yeast nitrogen base (minus inositol), 2% glucose, 0.2% ammonium sulfate, vitamins, the four amino acids histidine (20 mg/liter), methionine (20 mg/liter), leucine (60 mg/liter), and lysine (20 mg/liter), and the nucleobase uracil (40 mg/liter). Where indicated, inositol (I)

was added at a concentration of 75 μM. For selection of plasmids, uracil was omitted. Liquid and solid media were supplemented with 0.6 mM and 1 mM VPA, respectively, when indicated. Fumonisin B1 (Sigma) and aureobasidin A (Clontech) were used at a concentration of 100 μM and 0.5 μg/μl, respectively. For solid media, 2% agar was added. Absorbance was measured at 550 nm to monitor growth in liquid cultures. All incubations were at 30°C.

VPA treatment

Wild type cells were pre-cultured in synthetic minimal medium with inositol (I+), harvested, washed twice with sterile water, and grown in I+ until the cells reached the mid log phase (A_{550}= 0.5). Cells were pelleted, washed twice with sterile water and inoculated in I+ or I- to a final A_{550} of 0.05 and cultured until the cells reached the mid log phase (A_{550}= 0.5). Cells were then pelleted and suspended in fresh I- or I+ medium with or without 0.6 mM VPA and incubated for 5 hours.

***ino1Δ* starvation**

ino1Δ cells were pre-cultured in I+, harvested, washed twice with sterile water, and grown in I+ until the cells reach mid log phase (A_{550}= 0.5). Cells were pelleted, washed twice with sterile water and transferred to fresh I- (inositol starvation) or I+ (control) for 3 hours.

Microarray analysis

Total RNA was isolated by hot phenol extraction Kohrer and Domdey, 1991) and purified using an RNeasy kit from Qiagen. Quality of RNA was determined using Agilent 2100 Bioanalyzer. RNA was labeled using the Agilent Low Input Quick-Amp labeling kit (Agilent Technologies). Cy3 labeled cRNA was then hybridized to the 8x15K Agilent

Yeast V2 Arrays (design ID 016322). Slides were scanned on an Agilent G2505B microarray scanner and the resulting image files were processed with Agilent Feature Extraction software (version 9.5.1). All procedures were carried out according to the manufacturer's protocols. Subsequent analysis was performed using GeneSpring (v10.0) software. Microarray analysis was carried out at the Research Technology Support Facility in Michigan State University.

Quantitative real time PCR (qRT-PCR) analysis

Total RNA was extracted using the hot phenol method Kohrer and Domdey, 1991) and purified using an RNeasy mini plus kit (Qiagen, Valencia, CA). Complementary DNA (cDNA) was synthesized using the first strand cDNA synthesis kit from Roche Applied Science as described in the manufacturer's manuals. qRT-PCR reactions were done in a 20 µl volume reaction using Brilliant III Ultra-Faster SYBR Green qPCR master mix (Agilent Technologies, Santa Clara, CA). Each reaction was done in triplicate. The primers used for the qRT-PCR reactions are listed in Table 3.2. RNA levels were normalized to *ACT1* levels (internal control). Relative values of mRNA transcripts are shown as fold change relative to that of the indicated controls. Primers were validated as suggested in the Methods and Applications Guide (Agilent Technologies). All primers used in this study had primer efficiency between 85 and 105%. Optimal primer concentrations were determined, and primer specificity of a single product was monitored by a melt curve following the amplification reaction. PCR reactions were initiated at 95°C for 10 min for denaturation followed by 40 cycles consisting of 30 s at 95°C and 60 s at 55°C.

Ceramide measurement

Cells were grown and treated with VPA as described above for 5 hours, pelleted and stored at -80°C. Extraction of lipids from yeast pellets and lipid quantification by LC/MS/MS was performed as previously described (Brice et al., 2009).

Western blot

Cells were broken in the presence of acid-washed glass beads in lysis buffer containing 50 mM Tris, 125 mM sodium chloride, 1% Nonidet P-40, 2 mM EDTA, and 1× protease inhibitor mixture (Roche Applied Science). Extracts were centrifuged twice for 5 min at 13,000 × g at 4°C to remove cell debris and glass beads. Protein concentration was determined using the Bradford assay (Pierce Protein), with bovine serum albumin as the standard protein. Proteins were separated on 10% SDS-PAGE and electrotransferred to a polyvinylidene difluoride (PVDF) membrane (Millipore). The membrane was incubated with antibodies (1:3000 anti-HA; 1:3000 anti-actin; 1:10000 appropriate secondary antibodies conjugated with HPR) and visualized using ECL Plus substrate (Pierce Protein), with α-actin as the loading control.

β-Galactosidase assays

Cells expressing the *UPRE-LacZ* reporter plasmid provided by Dr. Susan Henry (Chang et al., 2004) were precultured in Ura-I+, and grown in Ura-I+ to an A_{550} of 0.5, washed and transferred to Ura-I- medium with or without VPA for 5 hours at 30°C. Cells were harvested, and β-galactosidase was assayed as described (Fu and Xiao, 2006).

RESULTS

VPA increases the expression of fatty acid elongases

To determine candidate pathways that may be important for the therapeutic role of VPA, we performed a genome-wide microarray analysis of cells treated with 0.6 mM VPA for 5 hours in the presence or absence of inositol, as described under ”Material and methods” (Chapter 2). VPA treatment resulted in altered expression (>2-fold) of 324 genes in the presence of inositol and 413 genes in the absence of inositol (Table 2.2). Interestingly, fatty acid elongase genes *FEN1* and *SUR4* exhibited 2-fold increased expression in response to VPA (Table 2.2). qRT-PCR analysis of fatty acid elongase genes in wild type cells treated with VPA validated these findings. As seen in Fig. 3.1, mRNA levels of *FEN1* and *SUR4* were increased 6- and 4-fold in the absence of inositol, and to a lesser extent (3- and 2-fold) in the presence of inositol. Fen1 and Sur4 catalyze the synthesis of very long chain fatty acids, including C22 and C24 (Fen1), and C24 and C26 (Sur4) (Oh et al., 1997), which are used for the synthesis of ceramide. Mutants *fen1Δ and sur4Δ* exhibited sensitivity to VPA, as did the sphinganine C4-hydroxylase mutant, *sur2Δ* (Fig. 3.2). VPA sensitivity was partially rescued by inositol. The VPA-mediated increase in expression of these genes, and VPA sensitivity of Fen1, Sur4, and Sur2 mutants suggested that VPA induces an increase in ceramide containing C24-C26 fatty acids and PHS (phytosphingosine) (the product of Sur2).

VPA increases ceramide levels and downregulates amino acid transporters

To determine if VPA increases ceramide levels, DHC (dihydroceramide) and PHC (phytoceramide) ceramide species were analyzed by mass spectrometry. VPA increased PHC levels in wild type cells but did not significantly alter levels of DHC (Fig. 3.3). Interestingly, VPA treatment for 30 min upregulated the expression of *RSB1* (data

Table 3.1. Yeast strains and plasmids used in this study

Strains/Plasmid	Genotype/Description	Source/Ref.
Wild type	MATa, his 3Δ1, leu 2Δ0, met 15Δ0, ura3Δ0	Invitrogen
fen1Δ	MATa, his 3Δ1, leu 2Δ0, met 15Δ0, ura 3Δ0, fen1Δ::KanMX4	Invitrogen
sur4Δ	MATa, his 3Δ1, leu 2Δ0, met 15Δ0, ura 3Δ0, sur4Δ::KanMX4	Invitrogen
rsb1Δ	MATa, his 3Δ1, leu 2Δ0, met 15Δ0, ura 3Δ0, rsb1Δ::KanMX4	Invitrogen
ino1Δ	MATa, his 3Δ1, leu 2Δ0, met 15Δ0, ura 3Δ0, ino1Δ::KanMX4	Invitrogen
RSB1-HA	pRS316-RSB1Δ335–382-3×HA	Johnson et al., 2010
UPRE-LacZ	pJC104, containing UPRE-CYC-lacZ	Chang et al., 2004

Table 3.2. Real time PCR primers used in this study

GENE	**Primers**	**Sequence (5' to 3')**
ACT1	Forward	ACGTTCCAGCCTTCTACGTTTCCA
ACT1	Reverse	ACGTGAGTAACACCATCACCGGAA
FEN1	Forward	TGGGTTCAACAACTGCCACCTTTG
FEN1	Reverse	TCATTAACCTTTGCGGCAACACCG
SUR4	Forward	TGTTATGGTACTCAGGCTGCTGCT
SUR4	Reverse	AGTAGAAGAACCGGATGCAACGGA
RSB1	Forward	TTGCCCTCTCCAATGGCGTATTCT
RSB1	Reverse	ACATGATTGCCGGTTGTTGTGGAC
ELO1	Forward	AGAAAGCCTCTAGGTTTCGCCCAA
ELO1	Reverse	AAAGGCTGCTTCCCAACGGTAAAC
BIO5	Forward	GCATCCGGACTACGAGTTAAAG
BIO5	Reverse	GGGCAACGGAGTTGAATAAATG
AGP1	Forward	GAACGATCTTACGTCGGCTATC
AGP1	Reverse	GACCTGTATTAGCGCCTATGTT
GAP1	Forward	GTGACACTCCAGGTGCTAAA
GAP1	Reverse	GCAGCAAGACCAACCAATTC
BAP2	Forward	GAGGATGGCGTTGAGTCTATC
BAP2	Reverse	GTCCCAATACCTGTCCCTAAAG
DIP5	Forward	TCATTTCTTGGGCTGGTTACA
DIP5	Reverse	GGTCCTTCATTCTTCCCTCTTC
UGA4	Forward	TGGTGGTCCAGCAACATTAG
UGA4	Reverse	AGCGGTAGGAATGGAACTTG
CAN1	Forward	GAACGCTGAAGTGAAGAGAGAG
CAN1	Reverse	GTTGGTCAGAGGTGTGGATAAA
SAM3	Forward	GATGTATCTGCCTCTCCCTTTG
SAM3	Reverse	CACAACCGCAACAAGGATAAC
EUG1	Forward	TGGTCAAGTCTATCGCGGTGTCAA
EUG1	Reverse	CATTCAAGCCTGTCAAGCCTCTGT
JEM1	Forward	TGGGACAAGGTGCATCAGAAGGAT
JEM1	Reverse	GCGTTATGCGTAGCAGCTCAGAAA
KAR2	Forward	AAAGATGGGAAGCCCGCTGTAGAA
KAR2	Reverse	ACAGCATGGGTAACCTTAGTGCCT
LHS1	Forward	GCGCGGAAGTGCTTATCCAAACAA
LHS1	Reverse	ACGCAACTCCTGACGAGCACTTAT
SEC63	Forward	AGCAAAGGGCCTAACACCTGATGA
SEC63	Reverse	TGGGCCATCTGGATGACCGTATTT
PDI1	Forward	TGCCATCCACGACATGACTGAAGA
PDI1	Reverse	ACTCCAACACGATCTTGTCGCTCA

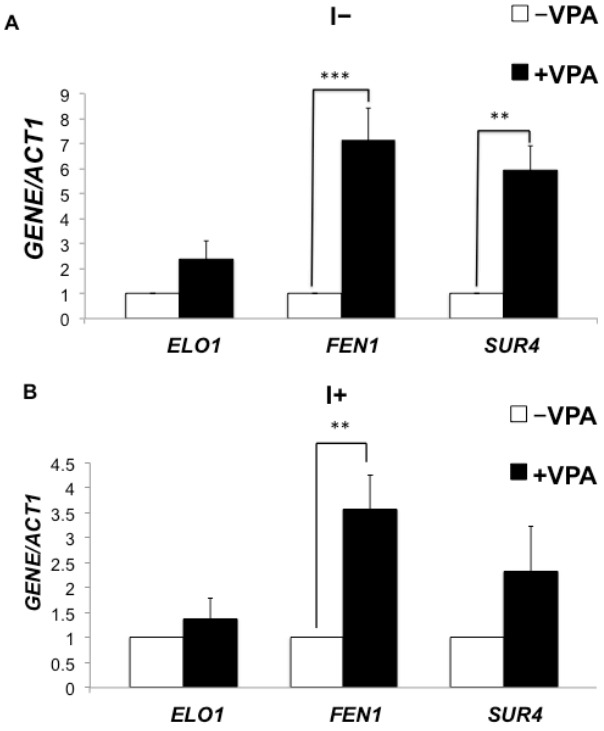

Figure 3.1. VPA upregulates expression of fatty acid elongase genes. Wild type cells were pre-cultured in I+ medium, harvested, washed twice with sterile water, and grown in I+ or I- medium until cells reached the mid log phase (A_{550}= 0.5). Cells were pelleted and suspended in fresh I- medium with or without 0.6 mM VPA and incubated for 5 hours. mRNA levels of fatty acid elongases were quantified by qRT-PCR in wild type cells grown in the presence or absence of VPA. Values are reported as fold change in expression in cells grown in VPA relative to cells grown without VPA, in the absence (A) or presence of inositol (B). Expression was normalized to the mRNA levels of the internal control *ACT1*. Data shown are mean ± SD (n=6) (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

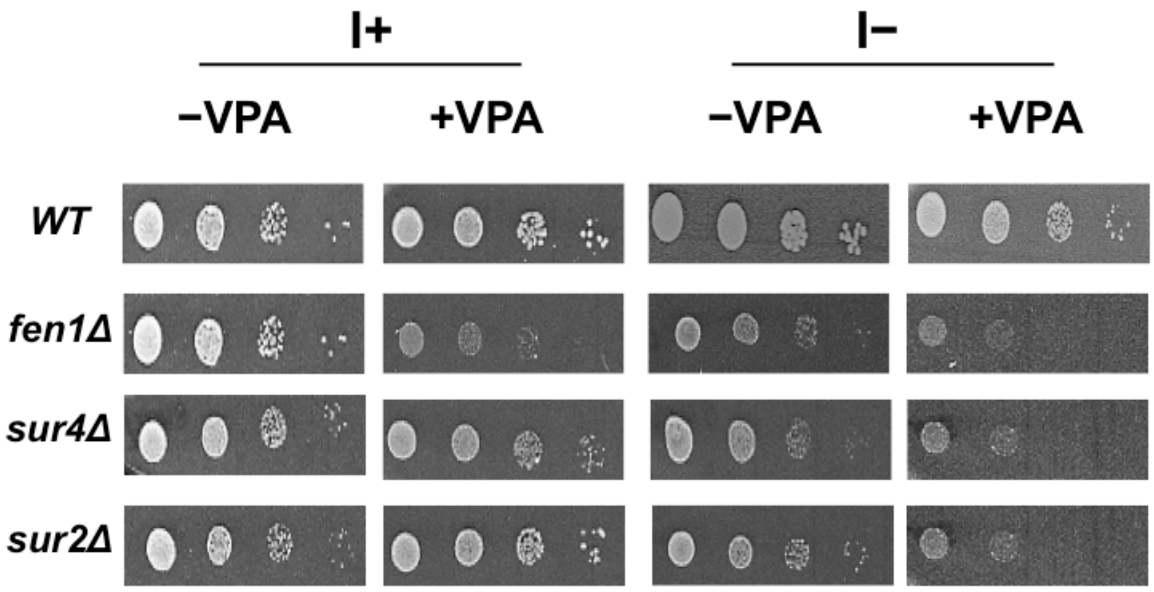

Figure 3.2. VPA sensitivity of fatty acid elongase and sphinganine C4-hydroxylase mutants. Cells were pre-cultured in I+, counted using a hemocytometer, and washed with sterile water. 3-µl aliquots of a series of 10-fold dilutions were spotted onto I+ or I− plates in the presence or absence of 1 mM VPA and incubated for 3 days at 30°C.

not shown), which transports LCBs, (long chain bases) (including DHS (dihydrosphingosine) and PHS (phytosphingosine) across the plasma membrane (Kihara and Igarashi, 2002). In wild type cells, upregulation of *RSB1* increases the transport of LCBs, reducing the intracellular levels of these ceramide precursors. Therefore, we measured the effect of VPA on ceramide levels in *rsb1Δ* cells. VPA increased both DHC and PHC in *rsb1Δ* (Fig. 3.3). Specific ceramide species were increased, including DHC with C16, C18:1, C20, C20:1, and C22, and PHC with C16, C18, C22, C22:1, C24, C26, C28 in *rsb1Δ* cells (Table 3.3). The highest increase was observed in C26 and C26:1 species of PHC, suggesting that VPA specifically increases levels of C26 containing PHC.

Previous studies have shown that increased levels of ceramide cause a decrease in the transcription of nutrient permeases leading to reduced intake of nutrients and induction of stress (Guenther et al., 2008; Payet et al., 2013). Consistent with this, the microarray analysis revealed decreased expression of amino acid transporter genes *BIO5, AGP1, GAP1, BAP2, DIP5, UGA4, CAN1,* and *SAM3* in response to VPA in the absence of inositol. The expression of these transporters was not altered in I+ medium (Table 2.2). Decreased expression of amino acid transporters was confirmed by qRT-pCR in both wild type and *rsb1Δ* cells (Fig. 3.4). Downregulation was greater in *rsb1Δ* than in wild type cells, consistent with the increase in ceramide levels. To determine if decreased expression of amino acid transporters resulted from increased ceramide, cells were treated with 100 μM fumonisin, a ceramide synthase inhibitor (Wu et al., 1995; He et al., 2006; Merill et al., 2000). Fumonisin reversed the downregulation of the amino acid transporters in both wild type and *rsb1Δ* cells (Fig.

3.4). Taken together, these findings suggest that VPA increases levels of specific ceramide species, resulting in decreased expression of nutrient permeases.

VPA induces the UPR by increasing ceramide levels

Decreased expression of nutrient transporters is expected to induce stress due to nutrient starvation (Guenther et al., 2008). In agreement with this, VPA increased the expression of ER chaperone genes *EUG1, JEM1, KAR2, LHS1, SEC63,* and *PDI1* in I- medium (Table 2.2 and Fig. 3.5), suggesting that the UPR pathway was induced. To test this, we analyzed the UPR response in wild type and *rsb1Δ* cells expressing a *UPRE-lacZ* reporter plasmid. Increased *lacZ* activity was observed in response to VPA in I- medium (Fig. 3.6A). The increase was more pronounced in *rsb1Δ* than in wild type cells. Fumonisin restored *lacZ* activity to normal levels. In contrast, aureobasidin, which inhibits the conversion of ceramide to complex sphingolipids, did not affect *UPRE* expression (Fig. 3.6B). Increased *UPRE* expression was not observed in I+ medium (Fig. 3.6C). These findings suggest that VPA induced the UPR pathway via increased *de novo* synthesis of ceramide, and not by inhibiting the conversion of ceramide to complex sphingolipids. Consistent with previous reports showing that inositol starvation induces the UPR pathway (Cox et al., 1997; Promlek et al., 2011), *UPRE* expression was increased in inositol-starved *ino1Δ* mutant cells (Fig. 3.7A). Treatment with fumonisin decreased *UPRE* expression to levels observed in control cells, indicating that inositol starvation of *ino1Δ* cells induces the UPR pathway by increasing ceramide levels.

Similar to VPA treatment*, ino1Δ* cells starved for inositol for 3 hours also exhibited decreased expression of amino acid transporter genes *BIO5, AGP1, GAP1,*

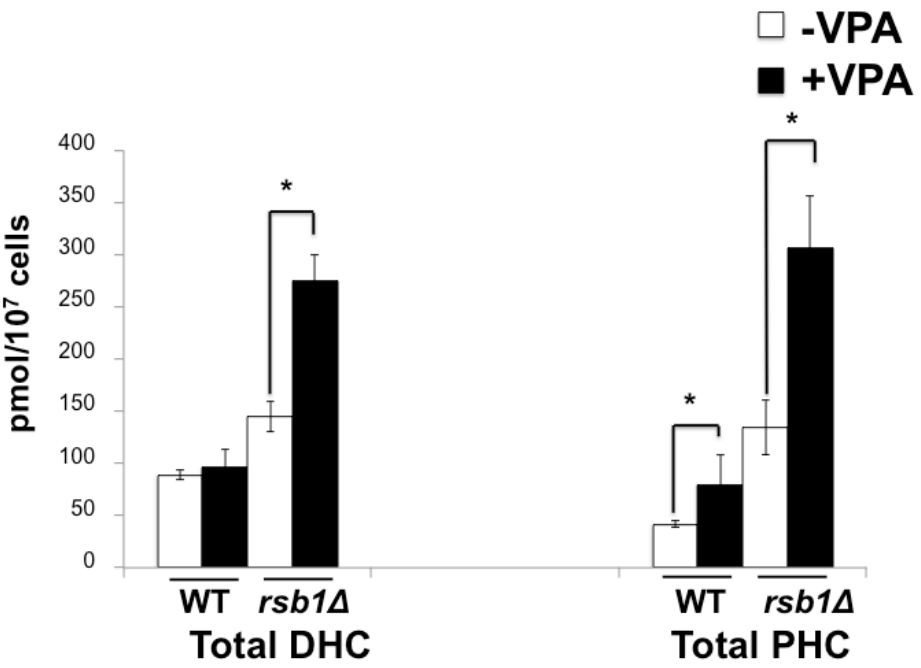

Figure 3.3. VPA increases levels of DHC and PHC. Wild type and *rsb1Δ* cells were grown as described in the legend of Fig. 3.1. Cells were pelleted and total DHC and PHC levels were quantified by mass spectrometry. Data shown are mean ± SD (n=6 *, $p < 0.05$).

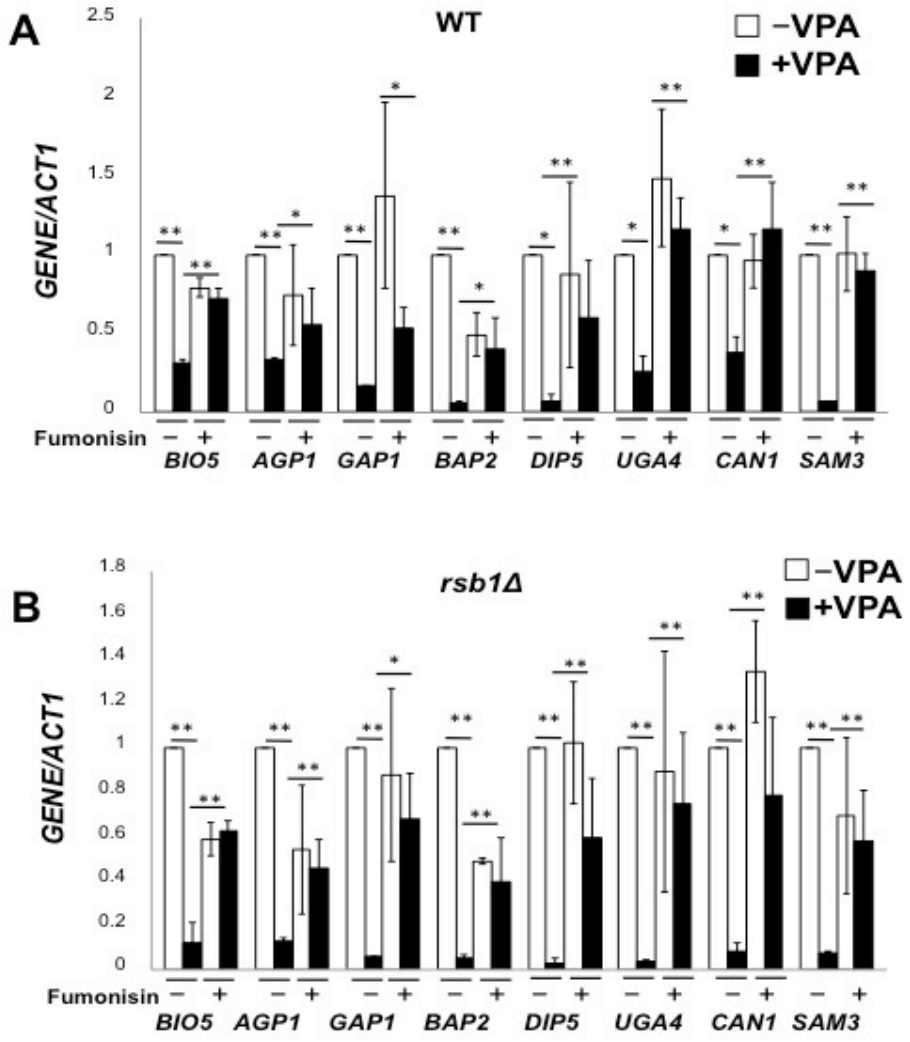

Figure 3.4. VPA downregulates expression of nutrient transporters via ceramide. mRNA levels of nutrient transporters were quantified by qRT-PCR in wild type (A) and *rsb1Δ* (B) cells grown in the presence or absence of VPA and fumonisin, as indicated. Values are reported as fold change in expression in cells grown in VPA relative to cells grown without VPA. Expression was normalized to the mRNA levels of the internal control *ACT1*. Data shown are mean ± SD (n=6) (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Table 3.3 A

	Wild type		***rsb1Δ***	
Dihydroceramide	**-VPA**	**+VPA**	**-VPA**	**+VPA**
dhC12-Cer	0.56 ± 0.23	0.82 ± 0.15	0.9 ± 0.15	1.68 ± 0.62
dhC14-Cer	0.78 ± 0.21	1.74 ± 0.29	1.78 ± 0.34	3.9 ± 1.03
dhC16-Cer	1.7 ± 0.11	2.48 ± 0.36	2.42 ± 0.43	5.51 ± 0.92
dhC18-Cer	12.4 ± 1.09	16.61 ± 2.8	23.77 ± 2.19	50.76 ± 3.26
dhC18:1-Cer	17.25± 0.98	13.73 ± 2.42	22.88 ± 0.25	37.3 ± 2.27
dhC20-Cer	28.7± 1.48	38.69 ±6.74	56.31 ± 9.72	111.24 ± 9.93
dhC20:1-Cer	25.04 ± 2.38	18.95 ±3.95	31.54 ± 3.24	55.81 ± 6.74
dhC22-Cer	1.02 ± 0.04	1.39 ± 0.21	1.61 ± 0.14	3.07 ± 0.32
dhC22:1-Cer	0.26 ± 0.04	0.31 ±0.09	0.67 ± 0.17	1.15 ± 0.12
dhC24-Cer	0.3 ± 0.16	0.45 ± 0.05	0.55 ± 0.07	0.84 ± 0.29
dhC24:1-Cer	0.02 ± 0.01	0.05 ± 0.031	0.05 ± 0.01	0.09 ± 0.05
dhC26-Cer	0.8 ± 0.18	1.11 ± 0.23	2.01 ± 0.23	2.87 ± 0.37
dhC26:1-Cer	0 ± 0	0 ± 0	0.03 ± 0.03	0 ± 0

Table 3.3 B

	Wild type		*rsb1Δ*	
Phytoceramide	**-VPA**	**+VPA**	**-VPA**	**+VPA**
PhytoC14-Cer	0 ± 0	0.15 ± 0.079	0.47 ± 0.12	0.79 ± 0.03
PhytoC16-Cer	1.76 ± 0.07	5.67 ± 1.63	5.05 ± 1.5	18.75 ± 4.24
PhytoC18-Cer	0.14 ± 0.14	0.81 ± 0.5	1.24 ± 0.43	5.45 ± 1.2
PhytoC18:1-Cer	0 ± 0	0.56 ± 0.33	0.44 ± 0.22	1.23 ± 0.48
PhytoC20-Cer	0.43 ± 0.22	0.93 ± 0.31	0.27 ± 0.19	1.09 ± 0.39
PhytoC20:1-Cer	0.83 ± 0.36	2.12 ± 0.95	1.31 ± 0.38	3.03 ± 1.25
PhytoC22-Cer	0.66 ± 0.34	1.29 ± 0.44	0.36 ± 0.2	1.55 ± 0.33
PhytoC22:1-Cer	0.92 ± 0.49	0.62 ± 0.33	1.28 ± 0.61	0.63 ± 0.63
PhytoC24-Cer	1.32 ± 0.32	1.56 ± 0.71	1.24 ± 0.4	3.45 ± 0.43
PhytoC24:1-Cer	0 ± 0	0.7 ± 0.5	0.84 ± 0.71	2.64 ± 1.03
PhytoC26-Cer	19.63 ± 0.86	36.52 ± 11.65	70.66 ± 14.26	139.67 ± 19.08
PhytoC26:1-Cer	9.16 ± 1.45	17.75 ± 8.32	38.5 ± 5.25	87.27 ± 16.92
PhytoC28-Cer	6.55 ± 1.22	10.29 ± 3.06	12.63 ± 4.82	39.36 ± 6.36
PhytoC28:1-Cer	0.25 ± 0.25	0.8 ± 0.63	0.14 ± 0.14	0.84 ± 0.42

Table 3.3. VPA increases C24-C26 ceramide species. Wild type and *rsb1Δ* cells were grown as described in the legend of Fig. 3.1. Cells were pelleted and DHC (A) and PHC (B) species with the indicated fatty acids were quantified by mass spectrometry. Data shown are mean ± SD (n=6).

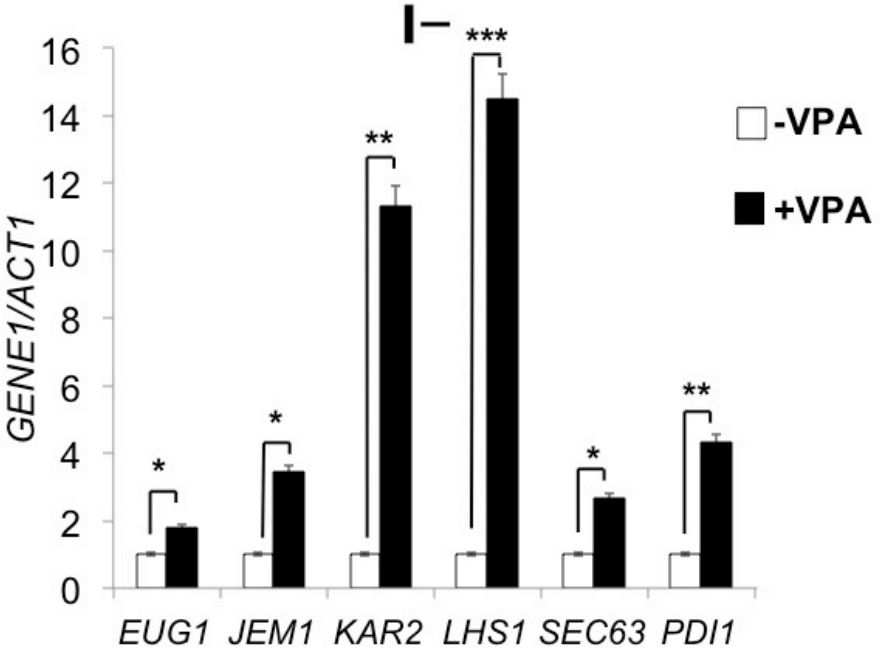

Figure 3.5. VPA increases expression of ER chaperones. Wild type cells were grown as described in Fig. 3.1. mRNA levels of chaperone genes were quantified by qRT-PCR in wild type cells grown in I- medium in the presence or absence of VPA. Values are reported as fold change in expression in cells grown in VPA relative to cells grown without VPA. Expression was normalized to the mRNA levels of the internal control *ACT1*. Data shown are mean ± SD (n=6). (*, $p < 0.05$; **, $p < 0.01$; ***,$p < 0.001$).

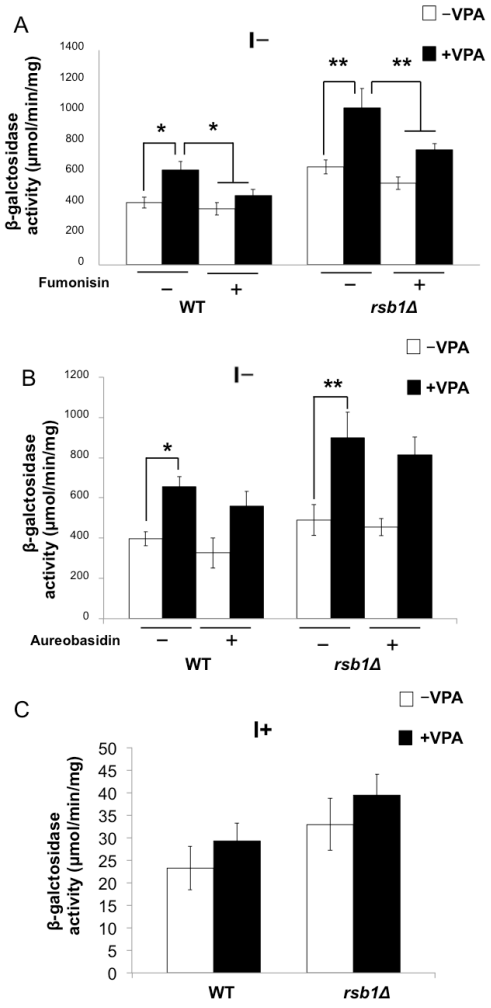

Figure 3.6. VPA upregulates *UPRE* expression. Wild type and *rsb1Δ* cells expressing a *UPRE-lacZ* plasmid were pre-cultured in Ura-I+ medium and then grown in (A,B) Ura-I- medium with or without 100 μM fumonisin or 0.5 μg/μl aureobasidin; or (C) Ura-I+ medium. 0.6 mM VPA was added when indicated. *LacZ* activity is quantified as indicated. Data shown are mean ± SD (n=6) (*, $p < 0.05$; **, $p < 0.01$).

BAP2, DIP5, UGA4, CAN1, and *SAM3* (Fig. 3.7B). This decrease was partialy ceramide dependent, as it was blocked by fumonisin. Decreased expression of amino acid transporter genes *BAP2, GAP1* and *UGA4* was partially rescued by fumonisin (Fig. 3.7B), suggesting that inositol depletion decreased the expression of amino acid transporter genes, at least in part, by increasing ceramide levels. To directly test the hypothesis that inositol depletion decreased the expression of amino acid transporters and induced the UPR by increasing the intracellular ceramide levels, *ino1Δ* cells were starved for 3 hours and ceramide levels were quantified (Table 3.4). Inositol starvation increased levels of total DHC and PHC (Fig. 3.7C). Most notable were increases in DHC with C20 and PHC with C24, C26, and C26:1 (Table 3.4). The highest increase was observed in C26 and C26:1 species of PHC, similar to the response to VPA (Table 3.3). Expression of fatty acid elongases *FEN1* and *SUR4* was also increased during inositol starvation (Fig. 3.7D). Taken together our findings indicate that inositol depletion mediated by VPA or by starvation of *ino1Δ* cells led to increased expression of fatty acid elongases (elongating C24-C26), resulting in increasing ceramide levels with C24-C26 fatty acids. Increased ceramide synthesis led to downregulation of transporters and induction of the UPR pathway. These findings are the first to show that VPA mediated inositol depletion induces the UPR by increasing ceramide levels.

DISCUSSION

In this study, we show that VPA induces the UPR by increasing ceramide levels via inositol depletion. Our specific findings indicated that VPA: 1) increased expression of fatty acid elongases synthesizing C24 and C26 fatty acids; 2) increased ceramide

Table 3. 4 A

Dihydroceramide	*ino1Δ* I+	*ino1Δ* I-
dhC12-Cer	1.08±0.02	0.92±0.28
dhC14-Cer	2.40±0.34	2.10±1.08
dhC16-Cer	2.07±0.40	1.72±0.02
dhC18-Cer	4.15±0.36	6.99±0.24
dhC18:1-Cer	2.85±0.27	3.51±0.16
dhC20-Cer	7.32±1.16	15.21±0.93
dhC20:1-Cer	4.14±0.47	4.69±0.39
dhC22-Cer	0.20±0.06	0.33±0.14
dhC22:1-Cer	0.05±0.01	0.064±0.02
dhC24-Cer	0.26±0.11	0.28±0.16
dhC24:1-Cer	0.04±0.03	0.046±0.01
dhC26-Cer	0.62±0.28	0.80±0.36
dhC26:1-Cer	0.03±0.02	0.02±0.008

Table 3.4 B

Phytoceramide	*ino1Δ* I+	*ino1Δ* I-
PhytoC14-Cer	0.11±0	0.23±0
PhytoC16-Cer	0.61±0.13	1.2±0.24
PhytoC18-Cer	0.09±0.05	0.11±0.10
PhytoC18:1-Cer	5.66±3.52	3.52±1.26
PhytoC20-Cer	0.26±0.14	0.85±1.13
PhytoC20:1-Cer	6.92±4.7	5.27±2.70
PhytoC22-Cer	0±0	0.02±0.01
PhytoC22:1-Cer	0.04±0	0.07±0.05
PhytoC24-Cer	0.086±0.06	0.1±0.02
PhytoC24:1-Cer	0.25±0.11	1.19±0.13
PhytoC26-Cer	4.39±0.77	8.41±1.51
PhytoC26:1-Cer	9.76±3.01	26.76±2.54
PhytoC28-Cer	0.01±0	0±0
PhytoC28:1-Cer	0.18±0	0±0

Table 3.4. Inositol depletion increases levels of ceramide species. *ino1Δ* cells were starved for inositol as described in materials and methods. Levels of DHC (A) and PHC (B) species were quantified by mass spectrometry.

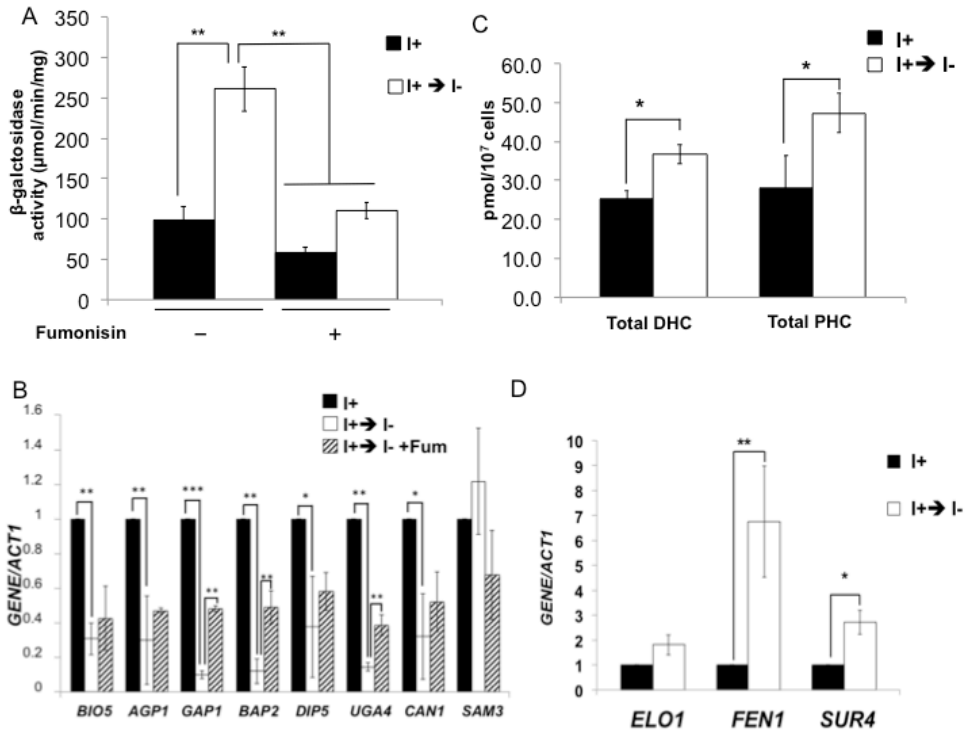

Figure 3.7. VPA induces the UPR by inositol depletion. *ino1Δ* cells expressing the *UPRE-lacZ* reporter plasmid were pre-cultured and grown in Ura-I+ medium until the cells reached mid log phase (A_{550}= 0.5). Cells were washed and transferred to Ura-I- medium with or without 100 μM fumonisin for 3 hours. (A) *LacZ* activity. Data shown are mean ± SD (n=6) (*, $p < 0.05$; **, $p < 0.01$; ***,$p < 0.001$). (B) Total DHC and PHC levels were quantified by mass spectrometry. Data shown are mean ± SD (n=6) (*, $p < 0.05$; **, $p < 0.01$; ***,$p < 0.001$). mRNA levels of nutrient transporters (C) and fatty acid elongases (D) were quantified by qRT-PCR as indicated. Values are reported as fold change in expression in cells grown in I- medium relative to cells grown in I+ medium. Expression was normalized to the mRNA levels of the internal control *ACT1*. Data shown are mean ± SD (n=6) (*, $p < 0.05$; **, $p < 0.01$; ***,$p < 0.001$).

levels, particularly PHC with C24 and C26 fatty acids; 3) decreased the expression of amino acid transporters; and 4) induced the UPR pathway. These outcomes were abrogated atleast partially by inhibition of *de novo* ceramide synthesis or by supplementation of inositol. Ceramide can be synthesized via two pathways, *de novo* synthesis by ceramide synthase or breakdown of complex sphingolipids by inositol phosphosphingolipid phospholipase C. Our findings suggest that VPA induces the UPR pathway by increasing *de novo* synthesis of ceramide (Fig. 3.6) as a result of increased expression of fatty acid elongases, as induction did not occur in the presence of the ceramide synthase inhibitor fumonisin (He et al., 2006; Merill et al., 2000). In contrast, aureobasidin, which inhibits inositol phosphorylceramide synthase, did not affect VPA-mediated induction of the UPR (Fig. 3.6C). Our findings are consistent with studies showing that inositol starvation induces the UPR. UPR target genes were found to be significantly upregulated in cells grown in the absence of inositol Jesch et al., 2005). In addition, inositol supplementation alters the expression of UPR pathway genes (Jesch et al., 2006). Although initial studies showed that accumulation of unfolded proteins in the ER induces the UPR pathway (Cox et al., 1993; Nikawa et al., 1996; Nikawa and Yamashita, 1992; Sidrauski et al., 1996, Villa-Garcia et al., 2011), it was since determined that Ire1p, the transmembrane kinase that senses ER stress (Cox and Walter, 1996; Mori et al., 2000), induces the UPR pathway in response to changes in membrane lipid composition (Cox and Walter, 1996; Mori et al., 2000). In this light, inositol starvation may trigger the UPR as a result of lipid related membrane changes in the ER, including perturbation of sphingolipid metabolism. The addition of inositol induces changes in the synthesis and levels of numerous lipids, including sphingolipids

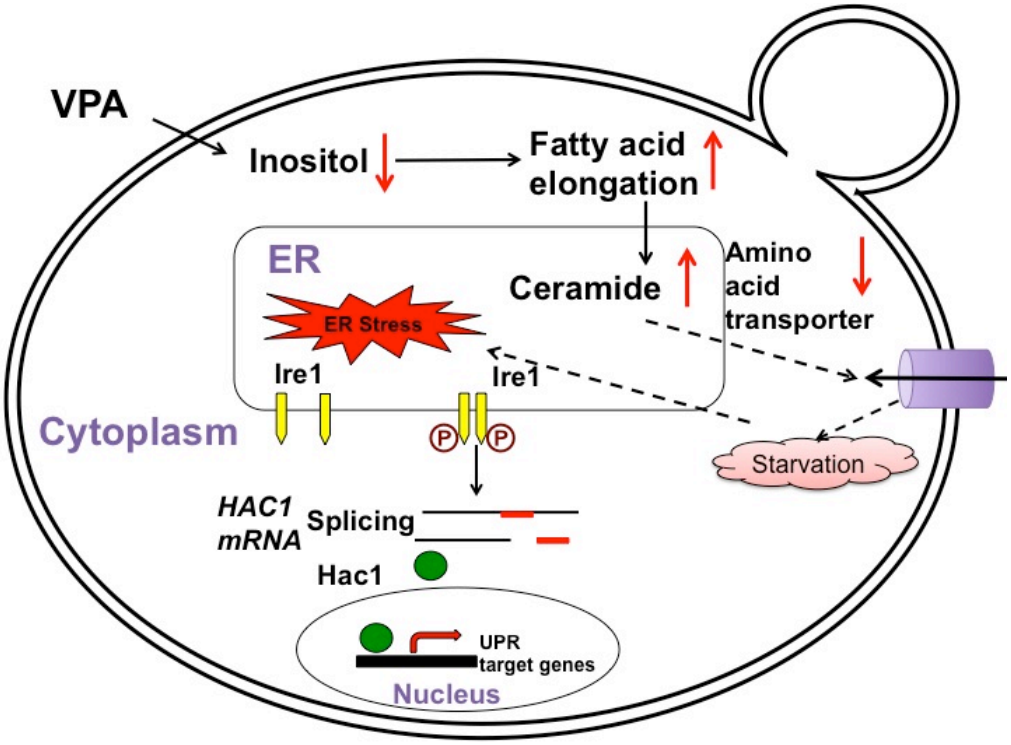

Figure 3.8. Model: VPA induces the UPR pathway by increasing intracellular ceramide levels. In the proposed model, VPA mediated inositol depletion leads to increased expression of fatty acid elongases and ceramide levels, especially PHC containing C24-C26 fatty acids. Increased ceramide levels decrease expression of nutrient transporters, stressing the cell due to lack of nutrients and inducing the UPR pathway.

(Alvarez-Vasquez et al., 2005; Jesch et al., 2010). Recent studies show that perturbation of sphingolipid metabolism may affect ER membrane homeostasis. Mutant cells lacking *ORM1* and *ORM2,* negative regulators of sphingolipid metabolism (Breslow et al., 2010; Han et al., 2010), exhibit constitutive induction of the UPR response (Han et al., 2010). In addition, *orm1Δ orm2Δ* cells contain elevated levels of sphingolipids (Breslow et al., 2010; Han et al., 2010) and are hypersensitive to stress induced by inositol starvation (Han et al., 2010). Similarly, UPR expression is constitutive in *isc1Δ* mutant cells, which contain elevated sphingolipids due to a block in sphingolipid turnover (Sawai et al., 2000; Gururaj et al., 2013). Conversely, inhibiting sphingolipid synthesis with myriocin treatment suppresses activation of the UPR induced by inositol starvation (Promlek et al., 2011). Elevated sphingolipids in the ER may lead to membrane aberrancy that activates the UPR pathway independent of accumulation of unfolded protein. The role of specific sphingolipids in activation of the UPR is not known. Our study suggests that accumulation of ceramide, specifically C24-C26 containing PHC, induces the UPR upon inositol depletion.

We have previously shown that VPA decreases intracellular inositol levels (Shaltiel et al., 2004; Vaden et al., 2001; Ye and Greenberg, 2015; Ju et al., 2004). VPA induction of the UPR occurred only in I- conditions, suggesting that the response to VPA was mediated by inositol depletion. Consistent with this, inositol starvation of *ino1Δ* cells caused similar effects, including increased expression of fatty acid elongases, increased intracellular levels of ceramides, especially PHC with C24 and C26, downregulation of nutrient transporters, and induction of the UPR. Based on these data, we propose the following model (Fig. 3.8). VPA decreases intracellular inositol levels causing an

increase in C24 and C26 PHC species as a result of upregulation of expression of fatty acid elongases (*FEN1* and *SUR4*). This results in increased ceramide synthesis, which causes downregulation of expression of nutrient transporters, inducing stress and the UPR.

Several studies suggest that the UPR pathway may be involved in the pathophysiology of BD. Lymphoblasts from bipolar patients show an aberrant ER stress response (Hayashi et al., 2009; So et al., 2007). Compared to cells from healthy controls, lymphoblasts from bipolar patients had lower expression of *XBP1* (mammalian homologue of yeast *HAC1*), the spliced form of which binds the *UPRE* element and induces the UPR pathway in response to ER stress inducers (So et al., 2007). A polymorphism (–116C→G) in the *XBP1* promoter that is associated with lower gene transcription was observed in BD lymphoblastoid cells (Kakiuchi et al., 2003). These findings suggest that the UPR pathway may be perturbed in bipolar patients, and activation of this pathway may be important for the therapeutic action of VPA. In this light, the UPR pathway may be a possible new target for BD therapy.

CHAPTER 4 ACUTE VALPROIC ACID TREATMENT INCREASES LEVELS OF PHYTOSPHINGOSINE VIA INOSITOL DEPLETION

INTRODUCTION

Valproic acid (VPA) is one of the most widely used drugs for the treatment of bipolar disorder (BD). Several targets are hypothesized to be important for the therapeutic action of VPA; however, the mechanism of action is not understood (Gould et al., 2004). Inositol depletion is one of the major hypotheses of the therapeutic action of VPA. Sir Michael Berridge first proposed the inositol depletion hypothesis in 1989, based on the finding that the antibipolar drug, lithium, causes inositol depletion by inhibiting inositol monophosphatase (IMPase) (Berridge, 1989). This was hypothesized to result in decreased levels of inositol, an increase in inositol phosphates, and subsequent downregulation of the phosphoinositide cycle.

Although many studies showed that antibipolar drugs lead to inositol depletion, this hypothesis was not universally accepted (Jope, 1999; Ikonomov et al., 1999; Agranoff and Fisher, 2001). Several findings argue against this hypothesis. First, although lithium depletes inositol with acute treatment, the therapeutic effects of the drug are observed with chronic administration (Pollack et al., 1994; Moore et al., 1999). However, this does not completely oppose the hypothesis, as acute inositol depletion could activate downstream signaling pathways important for the therapeutic efficacy. Second, studies show that lithium does not deplete inositol in the temporal lobe of human brain (Moore et al., 1999). Third, disruption of the sodium-dependent inositol transporter (SMIT1), which decreases intracellular inositol levels, in mice, does not mimic the effects of lithium (Shaldubina et al., 2001; Shaldubina et al., 2007). Finally, it remains unclear how inositol depletion could treat mania and depression, two opposing

phases of BD. To this end, Cheng et al, have shown that VPA inhibits prolyl oligopeptidase enzyme activity (Cheng et al., 2005), which enhances PI signaling and mimics the effect of addition of exogenous inositol (Willams et al., 2002). Based on this, they proposed a model for the dual action of VPA. According to their proposed model, VPA inhibits *de novo* synthesis of inositol, depleting intracellular inositol, which stabilizes mood in the manic phase, whereas, during the depressive phase it inhibits prolyl oligopeptidase enzyme activity, leading to enhancement of the PI cycle (Cheng et al., 2005). In summary, while many studies support inositol depletion as a therapeutic mechanism of action, conflicting reports challenge the significance of inositol depletion in the therapeutic action of BD.

Interestingly, VPA decreases intracellular inositol levels in yeast and mammalian cells, although not by inhibiting IMPase (Vaden et al., 2001; Shaltiel et al., 2004; Ye and Greenberg, 2015). Actually, VPA indirectly inhibits the enzyme catalyzing the *de novo* synthesis of inositol, *myo*-inositol-3-phosphate synthase (MIPS). This suggests that MIPS is post-translationally regulated in response to the drug (Ju et al., 2004). More recent findings show that yeast and human MIPS are regulated by phosphorylation (Deranieh et al., 2013). The finding that lithium and VPA, two structurally dissimilar drugs, cause inositol depletion, albeit by affecting different enzymes, suggests that inositol depletion may be important for the therapeutic mechanism of action.

In yeast, a decrease in intracellular inositol leads to a subsequent increase in the expression of *INO1,* which codes for MIPS (Hirsch and Henry, 1986; Henry et al., 2014).

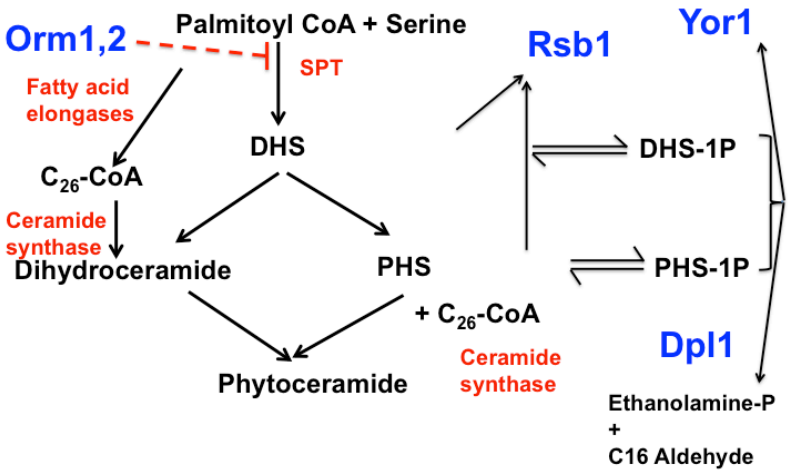

Figure 4.1 Sphingolipid metabolism in yeast. Serine is condensed with palmitoyl-CoA by serine palmitoyl transferase (SPT) to form 3-ketodihydrosphingosine, which is reduced to DHS. Orm1 and Orm2 negatively regulate SPT enzyme activity. DHS is hydroxylated on C-4 to produce PHS. Ceramide synthase catalyzes the linkage of very long chain fatty acids produced by fatty acid elongases to long chain bases (LCBs) DHS and PHS to form ceramide. DHS and PHS are also phosphorylated to DHS-1P and PHS-1P. Rsb1 and Yor1 transport LCBs and LCBPs, respectively, across the plasma membrane. DHS-1P and PHS-1P are metabolized by Dpl1 to generate ethanolamine phosphate and fatty aldehydes. (Abbreviations: SPT, Serine palmitoyl transferase; DHS, Dihydrosphingosine; PHS, Phytosphingosine; DHS-1P, Dihydrosphingosine-1-phosphate PHS-1P, Phytosphingosine-1-phosphate; LCB, Long chain base; LCBP, Long chainbase-1-phosphate)

Consistent with this finding, VPA causes a decrease in intracellular inositol and an increase in *INO1* expression (Vaden et al., 2001). Inositol depletion is apparent with acute VPA treatment (30 minutes - 1 hour) and continues to decrease (Ju and Greenberg, 2003). However, the normal response of UAS_{INO}-containing genes (target of Ino2-Ino4) to inositol depletion is abrogated with acute VPA, as expression of *INO1*, *INO2* and *CHO1* decreases with acute VPA even though inositol depletion is apparent (Ju and Greenberg, 2003). Conversely, chronic VPA increases the expression of UAS_{INO}-containing genes. These results suggest that VPA exerts differential acute and chronic responses.

Studies in this chapter focus on the acute response to VPA. In order to determine the acute effect of VPA on gene expression, I performed a genome wide microarray analysis (Chapter 2). In this study, I identified sphingolipid metabolism as a pathway that showed altered expression in response to VPA. *RSB1,* the transporter of long chain bases (LCBs), which are sphingolipid precursors, exhibited the highest upregulation in response to acute VPA.

Sphingolipids play a structural role in cell membranes (Obeid et al., 2002), and also act as second messengers in signaling pathways (Dickson et al., 1998). Sphingolipid synthesis (Fig. 4.1) begins in the endoplasmic reticulum with the condensation of L-serine with palmitoyl-CoA, catalyzed by serine palmitoyl transferase (SPT), to produce 3-ketodihydrosphingosine (KDHS), which is reduced to dihyrdrosphingosine (DHS) (Gable et al., 2002; Hanada, 2003; Lowther et al., 2012; Dickson 2008; Beeler et al., 1998; Kihara and Igarashi 2004). Orm1 and Orm2 are negative regulators that physically interact and inhibit serine palmitoyltransferase (SPT)

(Han et al., 2010; Breslow et al., 2010). Phytosphingosine (PHS) is synthesized in yeast via hydroxylation of C-4 of DHS, catalyzed by sphinganine C4-hydroxylase (Dickson, 2008). DHS and PHS are the two LCBs in yeast. Very long chain fatty acids synthesized by fatty acid elongases are linked to LCBs via an amide linkage at the C-2 position to form ceramide by ceramide synthase (Dickson 2008). LCBs are also phosphorylated by sphingolipid base kinases (Lcb4 and Lcb5) to DHS-1P and PHS-1P (Nagiec et al., 1998). Rsb1 and Yor1 transport LCBs and LCBPs, respectively, across the plasma membrane, which maintains normal intracellular levels of these metabolites (Kihara and Igarashi 2002; Boujaoude et al., 2001). DHS-1P and PHS-1P are cleaved by the sphingolipid lyase, Dpl1, to generate ethanolamine phosphate and fatty aldehydes (Saba et al., 1997).

There are no published studies to date indicating that VPA affects sphingolipids. The current study shows for the first time that acute VPA-mediated inositol depletion decreases levels of Orm proteins, inhibitors of *de novo* synthesis of LCBs and fatty acid elongases, thereby increasing intracellular PHS levels.

MATERIALS AND METHODS

Yeast strains, growth medium and conditions

Strains used in this study are summarized in Table 4.1. Cells were maintained on YPD medium (2% glucose, 1% yeast extract, 2% bactopeptone). Deletion mutants were maintained on medium supplemented with G418 (200 µg/ml). Synthetic minimal medium without inositol (I-) contained all the essential components of Difco yeast nitrogen base (minus inositol), 2% glucose, 0.2% ammonium sulfate, vitamins, the four amino acids histidine (20 mg/liter), methionine (20 mg/liter), leucine (60 mg/liter), and

lysine (20 mg/liter), and the nucleobase uracil (40 mg/liter). Where indicated, inositol (I) was added at a concentration of 75 μM. For selection of plasmids, uracil was omitted. Liquid and solid medium were supplemented with 0.6 mM and 1 mM VPA, respectively, when indicated. For solid media, 2% agar was added. Absorbance was measured at 550 nm to monitor growth in liquid cultures. All incubations were at 30°C.

VPA treatment

Wild type cells were precultured in synthetic minimal medium with inositol (I+), harvested, washed twice with sterile water, and grown in I+ until the cells reached the mid log phase (A_{550}= 0.5). Cells were pelleted, washed twice with sterile water and inoculated in I+ or I- to a final A_{550} of 0.05 and cultured until the cells reached the mid log phase (A_{550}= 0.5). Cells were then pelleted and suspended in fresh I- or I+ medium with or without 0.6 mM VPA and incubated for 30 minutes.

***ino1Δ* starvation**

ino1Δ cells were precultured in I+ medium, harvested, washed twice with sterile water, and grown in I+ until the cells reached the mid log phase (A_{550}= 0.5). Cells were pelleted, washed twice with sterile water and cultivated in fresh I- (inositol starvation) or I+ (control) for 30 minutes.

Microarray analysis

Total RNA was isolated by hot phenol extraction (Kohrer and Domdey, 1991) and purified using an RNeasy kit from Qiagen. Quality of RNA was determined using Agilent 2100 Bioanalyzer. RNA was labeled using the Agilent Low Input Quick-Amp labeling kit (Agilent Technologies). Cy3 labeled cRNA was then hybridized to the 8x15K Agilent Yeast V2 Arrays (design ID 016322). Slides were scanned on an Agilent G2505B

microarray scanner and the resulting image files were processed with Agilent Feature Extraction software (version 9.5.1). All procedures were carried out according to the manufacturer's protocols. Subsequent analysis was performed using GeneSpring (v10.0) software. Microarray analysis was carried out at the Research Technology Support Facility in Michigan State University.

Quantitative real time PCR (qRT-PCR) analysis

Total RNA was extracted using the hot phenol method (Kohrer and Domdey, 1991) and purified using an RNeasy mini plus kit (Qiagen, Valencia, CA). Complementary DNA (cDNA) was synthesized using the first strand cDNA synthesis kit from Roche Applied Science as described in the manufacturer's manuals. qRT-PCR reactions were done in a 20 µl volume reaction using Brilliant III Ultra-Faster SYBR Green qPCR master mix (Agilent Technologies, Santa Clara, CA). Each reaction was done in triplicate. The primers used for the qRT-PCR reactions are listed in Table 4.2. RNA levels were normalized to *ACT1* levels (internal control). Relative values of mRNA transcripts are shown as fold change relative to that of the indicated controls. Primers were validated as suggested in the Methods and Applications Guide (Agilent Technologies). All primers used in this study had primer efficiency between 85 and 105%. Optimal primer concentrations were determined, and primer specificity of a single product was monitored by a melt curve following the amplification reaction. PCR reactions were initiated at 95°C for 10 minutes for denaturation followed by 40 cycles consisting of 30 s at 95°C and 60 s at 55°C.

Long chain base measurement

Cells were grown and treated with VPA as mentioned in the above section for 30 minutes, pelleted and stored at -80°C. Extraction of lipids from yeast pellets and lipid quantification by LC/MS/MS was performed as previously described (Brice et al., 2009). LC/MS/MS experiments were performed by Dr. Ashley Cowart, Medical University of South Carolina.

Western blot

Cells were broken in the presence of acid-washed glass beads in lysis buffer containing 50 mM Tris, 125 mM sodium chloride, 1% Nonidet P-40, 2 mM EDTA, and 1× protease inhibitor mixture (Roche Applied Science). Extracts were centrifuged twice for 5 minutes at 13,000 × g at 4°C to remove cell debris and glass beads. Protein concentration was determined using the Bradford assay (Pierce Protein), with bovine serum albumin as the standard protein. Proteins were separated on 10% SDS-PAGE and electrotransferred to a polyvinylidene difluoride (PVDF) membrane (Millipore). The membrane was incubated with antibodies (1:3000 anti-HA; 1:3000 anti-actin; 1:10000 appropriate secondary antibodies conjugated with HPR) and visualized using ECL Plus substrate (Pierce Protein), with α-actin as the loading control.

RESULTS

Acute VPA increases the expression of *RSB1*

In order to identify pathways that are affected by acute VPA treatment, I carried out a genome wide microarray study of cells treated with 0.6 mM VPA for 30 minutes in the presence or absence of inositol (Chapter 2). After acute VPA treatment, 592 genes exhibited more than two-fold change in gene expression when grown in the absence of inositol and 542 genes in the presence of inositol, as summarized in Table 2.1. VPA

Table 4.1. Yeast strains and plasmids used in this study

Strains/Plasmid	Genotype/Description	Source/Ref.
Wild type	MATa, his 3Δ1, leu 2Δ0, met 15Δ0, ura3Δ0	Invitrogen
rsb1Δ	MATa, his 3Δ1, leu 2Δ0, met 15Δ0, ura 3Δ0, rsb1Δ::KanMX4	Invitrogen
ino1Δ	MATa, his 3Δ1, leu 2Δ0, met 15Δ0, ura 3Δ0, ino1Δ::KanMX4	Invitrogen
3xFlag-Orm1 / 3xHA-Orm2	BY4741; 3xFlag-ORM13xHA-ORM2	Breslow et al., 2010
3xFlag-Orm2 / 3xHA-Orm1	BY4741; 3xFlag-ORM2 3xHA-ORM1	Breslow et al., 2010
RSB1-HA	pRS316-RSB1Δ335–382-3XHA	Johnson et al., 2010
pRS316	pRS316	Johnson et al., 2010

Table 4.2. Real time PCR primers used in this study

GENE	**Primers**	**Sequence (5' to 3')**
ACT1	Forward	ACGTTCCAGCCTTCTACGTTTCCA
ACT1	Reverse	ACGTGAGTAACACCATCACCGGAA
FEN1	Forward	TGGGTTCAACAACTGCCACCTTTG
FEN1	Reverse	TCATTAACCTTTGCGGCAACACCG
SUR4	Forward	TGTTATGGTACTCAGGCTGCTGCT
SUR4	Reverse	AGTAGAAGAACCGGATGCAACGGA
RSB1	Forward	TTGCCCTCTCCAATGGCGTATTCT
RSB1	Reverse	ACATGATTGCCGGTTGTTGTGGAC
ELO1	Forward	AGAAAGCCTCTAGGTTTCGCCCAA
ELO1	Reverse	AAAGGCTGCTTCCCAACGGTAAAC
YOR1	Forward	TCTCCCAAGGCATCTGCTTCTTCA
YOR1	Reverse	TGCACACCAGTCAGTTGGGATGTA
DPL1	Forward	TTGTCGTAGGTTGTTGGGCCACTA
DPL1	Reverse	TTGCTGCACCGACTATTTCTTGGC
ORM1	Forward	GGCGTTCTTCCAGCATAATTTC
ORM1	Reverse	TTGGTCTACCCAAGTAGCATTC
ORM2	Forward	GACGGTCATCCAGCGTAATATC
ORM2	Reverse	CCCACGTAGCGTTCATGTT

treatment perturbed sphingolipid metabolism genes, as shown in Table 2.1. Interestingly, *RSB1* exhibited the highest upregulation in response to VPA, showing 158-fold and 108-fold upregulation in I- and I+ medium, respectively. qRT-PCR analysis of *RSB1* in wild type cells treated with VPA confirmed the increased expression, as mRNA levels of *RSB1* were increased 47-fold in I- (Fig. 4.2A), and 13-fold in I+ medium (Fig. 4.2B). The greater increase in I- than in I+ medium suggests that the induction was at least partially a response to inositol limitation. To determine if increased RNA levels resulted in increased protein, wild type cells harboring a plasmid expressing the *RSB1* gene, a transporter of LCBs with an HA tag were treated with VPA. Similar to the mRNA increase, Rsb1 protein levels were increased to a greater extent in I- than I+ (Fig. 4.2 C). Increased intracellular levels of LCBs are toxic to yeast cells, which respond by inducing Rsb1 (Kihara and Igarashi, 2002). Increased levels of Rsb1 suggest that VPA increases intracellular levels of LCBs.

Acute VPA increases PHS levels

Increased intracellular levels of LCBs are expected to increase sensitivity to added PHS. To determine if VPA increases the sensitivity to PHS, wild type and *rsb1Δ* cells expressing a plasmid containing *RSB1* or empty vector were grown in the presence or absence of VPA and plated on synthetic medium with or without 20 μM PHS. Wild type cells did not exhibit sensitivity to PHS in the presence of VPA, suggesting that Rsb1 maintains sub-toxic levels of PHS. However, VPA caused an increase in sensitivity of *rsb1Δ* cells to PHS, which was rescued by overexpression of *RSB1 (*Fig. 4.3). These findings are consistent with increased intracellular levels of PHS in VPA treated cells.

In order to directly determine if VPA increases the levels of LCBs, I measured LCBs and LCBPs in cells treated with VPA using mass spectrometry. Wild type cells showed a slight increase in PHS levels in response to VPA (Fig. 4.4A), and *rsb1Δ* cells exhibited an increase in PHS and PHS-1P. DHS-1P levels were decreased in *rsb1Δ* cells (Fig. 4.4B). These findings showed that levels of PHS increase in response to acute VPA, suggesting that VPA may increase *de novo* synthesis of PHS.

Acute VPA increases the expression of genes that aid in maintaining sub-toxic PHS levels

Acute VPA led to a small increase in PHS levels relative to the increase in Rsb1 levels. In addition to exporting LCBs by Rsb1, cells maintain low levels of LCBs by metabolizing them to other species and transporting them across the plasma membrane. Thus, as mentioned in the Introduction, LCBs are phosphorylated to LCBPs (DHS-1P and PHS-1P) by sphingolipid base kinases (Nagiec et al., 1998). LCBPs are cleaved by a lyase encoded by Dpl1 to generate fatty aldehyde and ethanolamine phosphate (Saba et al., 1997). Yor1, a transporter protein that belongs to the ATP binding cassette family, transports LCBPs across the plasma membrane (Fig. 4.1). Interestingly, VPA increased expression of *YOR1* and *DPL1* by 11-fold and 6-fold, respectively in I-. In the presence of inositol, the increase in expression was somewhat less, 6-fold and 3-fold in *YOR1* and *DPL1,* respectively (Fig. 4.5). This suggests that VPA induces expression of *YOR1* and *DPL1* in response to increased levels of LCBs to maintain low PHS levels.

VPA decreases ORMs and fatty acid elongases

Orm proteins negatively regulate LCB synthesis by physically interacting with and inhibiting SPT (Fig.4.1) (Han et al., 2010; Breslow et al., 2010). Phosphorylation of Orm proteins inhibits their association with SPT and increases the *de novo* synthesis of LCBs (Liu et al., 2012; Roelants et al., 2011). VPA decreased the expression of *ORM1* and *ORM2* in I-, and to a lesser extent in I+ (Fig. 4.6). In addition, VPA decreased the protein levels of Orm2 in I- (Fig. 4.6 C).

In addition to Orm negative regulators, LCB levels are also controlled by Fen1 and Sur4, which catalyze the synthesis of very long chain fatty acids of C24-C26 (Oh et al., 1997). Mutants of these fatty acid elongases (*fen1Δ* and *sur4Δ)* accumulate LCBs (Oh et al., 1997). As stated earlier (Chapter 3), *fen1Δ* and *sur4Δ* mutants exhibited VPA sensitivity. Here I showed that acute VPA downregulated the expression of *FEN1* and *SUR4* in I- medium, and to a lesser extent also in I+ (Fig. 4.7). Fatty acid elongases catalyze the synthesis of fatty acids that are linked to an LCB by ceramide synthase to synthesize ceramide. Downregulation of the fatty acid elongases by VPA may account for the observed increase in PHS (Fig. 4.4) as a result of decreased fatty acids required for ceramide synthesis. These results suggest that acute VPA decreases ORM levels and fatty acid elongases, which lead to an increase in PHS levels.

Inositol depletion downregulates the expression of *ORM* and fatty acid elongase genes and upregulates PHS and PHS-1P transporters

VPA affects the expression of sphingolipid metabolism genes to a greater extent in I- compared to I+, suggesting that VPA exerts effects on sphingolipid metabolism via inositol depletion. To test the effects of inositol depletion on sphingolipid gene expression, *ino1Δ* cells were cultured in I+ media, transferred to I- media for 30 minutes,

and gene expression was quantified. Acute inositol starvation of *ino1Δ* cells decreased expression of fatty acid elongases (*FEN1 and SUR4)* and Orm genes *(ORM1* and *ORM2)* (Fig. 4.7). In addition, inositol starvation of *ino1Δ* cells increased expression of *RSB1* and *YOR1*. These results suggest that the effects of VPA on sphingolipid metabolism genes can be explained by inositol depletion.

DISCUSSION

This study shows that acute VPA increases *de novo* synthesis of PHS by inositol depletion. Our specific findings indicated that acute VPA: 1) decreased expression of *ORM* and fatty acid elongase genes, 2) increased levels of PHS, and 3) increased expression of genes that transport *(RSB1, YOR1)* and metabolize *(DPL1)* PHS. These outcomes were partially dependent on supplementation of inositol.

My findings are consistent with studies showing that cells lacking *ORM1* and *ORM2* exhibit increased levels of the LCB, PHS and Rsb1 protein (Han et al., 2010). A greater increase in PHS levels in the *rsb1Δ* mutant compared to wild type (Fig. 4.3), and rescue of this phenotype by overexpression of *RSB1* (Fig. 4.2), further support a mechanism whereby VPA increases PHS levels and induces *RSB1* by decreasing *ORM* expression. Mutants of fatty acid elongases *fen1Δ* and *sur4Δ* are known to accumulate high levels of PHS (Oh et al., 1997). Decreased expression of *FEN1* and *SUR4* in VPA treated cells is also consistent with increased PHS levels. In summary, acute VPA increases PHS levels by downregulation of two key regulators of PHS, *ORM* and fatty acid elongase genes.

ORM2 is a UAS_{INO}-containing gene, whose expression is controlled by inositol (Han et al., 2010). Chronic VPA-mediated inositol depletion increases expression of

genes containing UAS_{INO} in their promoters (Ju and Greenberg, 2003). However, acute VPA-mediated inositol depletion decreases expression of UAS_{INO}-containing genes, including *INO1* and *INO2* (Ju and Greenberg, 2003). The current study shows that acute VPA downregulates the expression of *ORM* genes, similar to other UAS_{INO}-containing genes, via acute inositol depletion. This confirms the earlier finding that acute and chronic VPA-mediated inositol depletion differentially regulate expression of UAS_{INO}-containing genes (Ju and Greenberg, 2003).

Lithium, another antibipolar drug that also causes inositol depletion, does not decrease *INO1* expression in response to acute treatment. This suggests that the decrease in expression of UAS_{INO}-containing genes may be mediated via a mechanism other than inositol depletion. Ju and Greenberg (2003) proposed a mechanism to explain this abrogated expression in response to acute VPA. Their study showed that acute VPA causes a decrease in the level of phosphatidylserine, a protein kinase C activator. Decreased activity of PKC causes an increase in unphosphorylated Opi1 (active), which leads to a decrease in *INO1* expression (Sreenivas et al., 2001). Consistent with this, acute VPA does not affect expression of UAS_{INO}-containing genes in *opi1Δ* mutant cells. This model is consistent with my observations and suggests that acute VPA may downregulate expression of Orm2, a UAS_{INO}-containing gene, via Opi1.

Acute VPA decreases the expression of *ORM* and fatty acid elongase genes and increases the expression of *RSB1, YOR1,* and *DPL1.* The fold change of expression is greater in I- than in I+ medium, suggesting that the response to VPA was partially mediated by inositol depletion. Consistent with this, acute inositol starvation of *ino1Δ* cells caused similar effects, including decreased expression of *ORM, FEN1,* and *SUR4*

genes and increased expression of *RSB1* and *YOR1*. Based on these data, I propose that the VPA-mediated decrease in intracellular inositol levels causes a decrease in the expression of fatty acid elongase (*FEN1* and *SUR4*) and *ORM* genes, decreased Orm2 protein levels, and an increase in levels of PHS. To remediate the toxic effects of PHS, expression of *RSB1, YOR1,* and *DPL1* is increased, and PHS is either transported from the cell or degraded to other metabolites (Fig. 4.8).

This study shows for the first time that acute VPA-mediated inositol starvation increases intracellular levels of the signaling sphingolipid molecule, PHS, which regulates actin cytoskeleton, heat stress, and endocytosis (Hannun and Obeid, 2008). Heat stress induces a transient 2-3-fold increase in the levels of C18 PHS and a 100-fold increase in levels of C20 PHS (Dickson et al., 1997; Jenkins et al., 1997). PHS is required for regulation of actin cytoskeleton organization (Schmelze et al., 2002) and endocytosis (deHart et al., 2002). PHS also inhibits uptake of uracil and amino acids tryptophan, leucine, and histidine (Chung et al., 2001). The exact role of PHS in the regulation of amino acid transport remains unknown; however, PHS is important for ubiquitin-mediated breakdown of the uracil transporter Fur4 (Chung et al., 2000). Sphingosine, the mammalian equivalent of PHS, activates synaptobrevin in synaptic vesicles, facilitating SNARE complex assembly and membrane fusion. In addition, sphingosine plays a crucial role in increasing synaptic vesicle exocytosis at nerve terminals and neuromuscular junctions (Darios et al., 2009). In summary, PHS is an important signaling lipid crucial for maintaining actin cytoskeleton, heat stress, endocytosis and exocytosis, and neurotransmitter release in mammals. Perturbation of

PHS/sphingosine by VPA might affect neurotransmitter release and other processes that affect neural function and may, thus, be a novel mechanism of action of VPA.

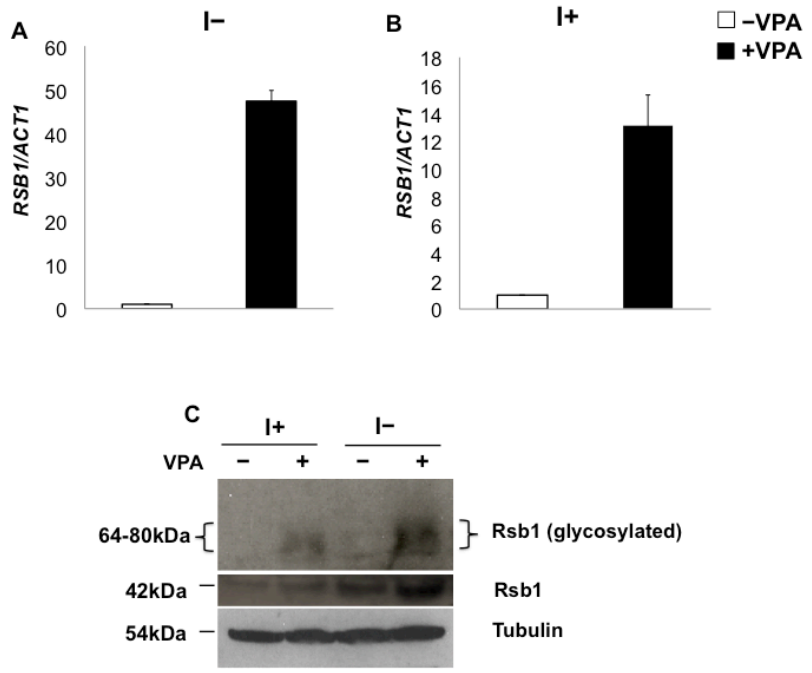

Figure 4.2. VPA increases the levels of *RSB1*. *RSB1* mRNA levels were quantified by qRT-PCR from wild type cells grown in the presence or absence of VPA for 30 minutes. Values are reported as fold change in expression of cells grown in the presence of VPA compared to cells grown in the absence of VPA, in I+ (A) or I- (B). Expression was normalized to the mRNA levels of *ACT1* (internal control). Data shown are mean of ± SD (n=6). C, Western blot analysis of Rsb1 protein. *rsb1Δ cells* expressing a plasmid containing *RSB1* tagged with HA were grown in the presence and absence of inositol and VPA, harvested and lysed for protein extraction. 30 µg total cell extract from each sample were subjected to Western blot analysis using 10% SDS-PAGE gel. Data shown are mean ± SD (n=6).

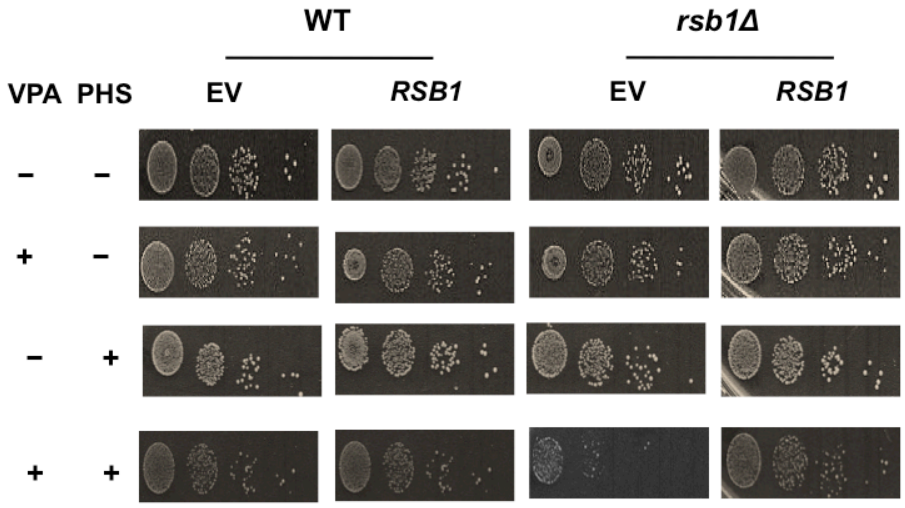

Figure 4.3. VPA increases sensitivity of *rsb1Δ* to PHS. Wild type and *rsb1Δ* cells expressing a plasmid containing *RSB1* or empty vector (EV) were precultured in I+ medium, diluted, and spotted on I- medium containing VPA (1mM) or PHS (20 μM) as indicated.

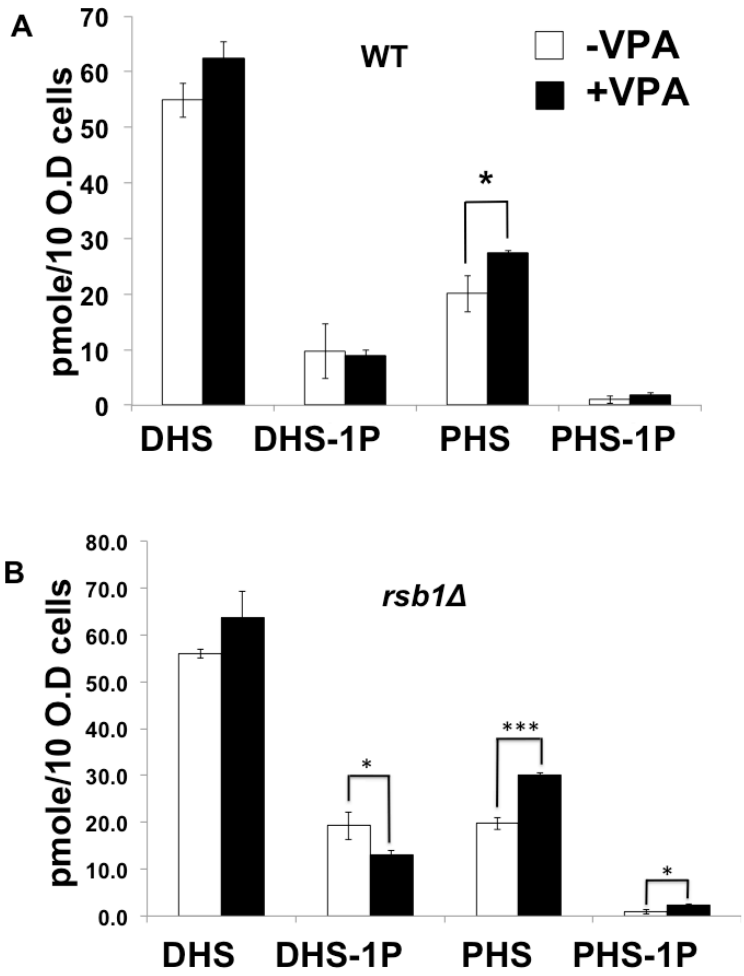

Figure 4.4. VPA increases levels of PHS. Wild type (A) and *rsb1Δ* (B) cells were pre-cultured in I+ medium, harvested, washed twice with sterile water, and grown in I- medium until cells reached the mid log phase (A_{550}= 0.5). Cells were pelleted and suspended in fresh I- medium with or without 0.6 mM VPA and incubated for 30 minutes. Cells were pelleted and DHS, PHS, DHS-1P, and PHS-1P levels were quantified by mass spectrometry. Data shown are mean ± SD (n=6 *, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

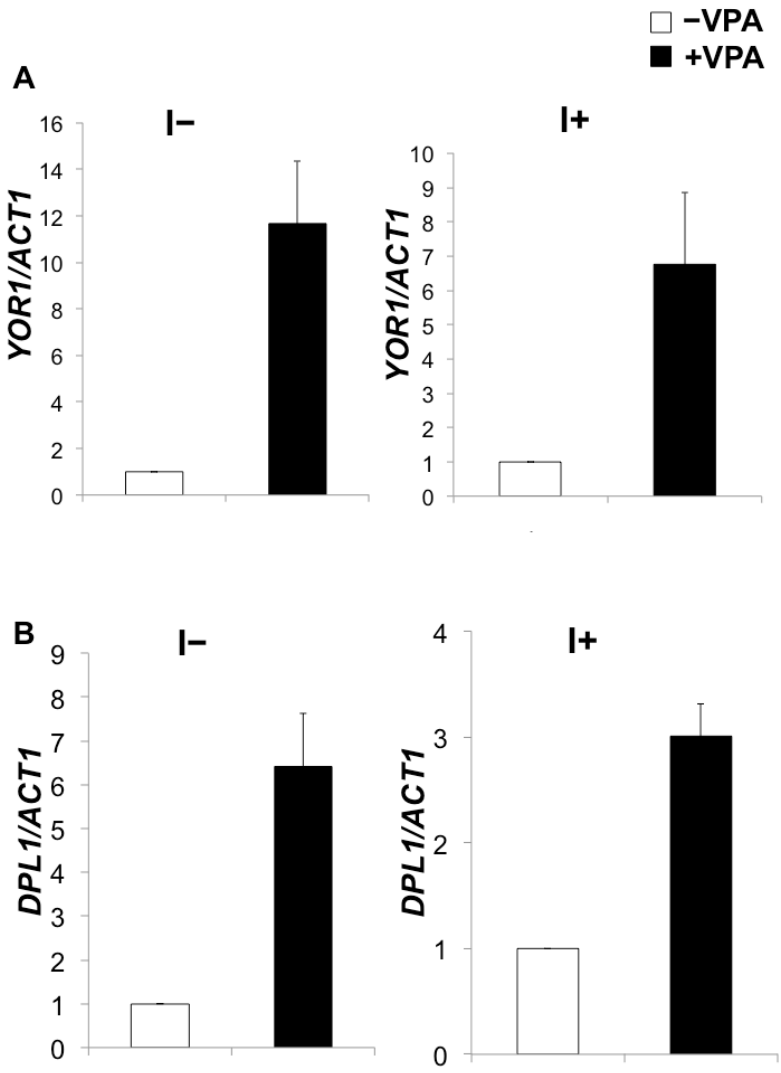

Figure 4.5. VPA increases the expression of *YOR1* and *DPL1.* mRNA levels were quantified by qRT-PCR from wild type cells grown in the presence or absence of VPA. Values are reported as fold change in expression in cells grown in the presence of VPA compared to cells grown in the absence of VPA, in I- and I+ medium, for *YOR1* (A) and *DPL1* (B). Expression was normalized to the mRNA levels of *ACT1* (internal control). Data shown are mean ± SD (n=6).

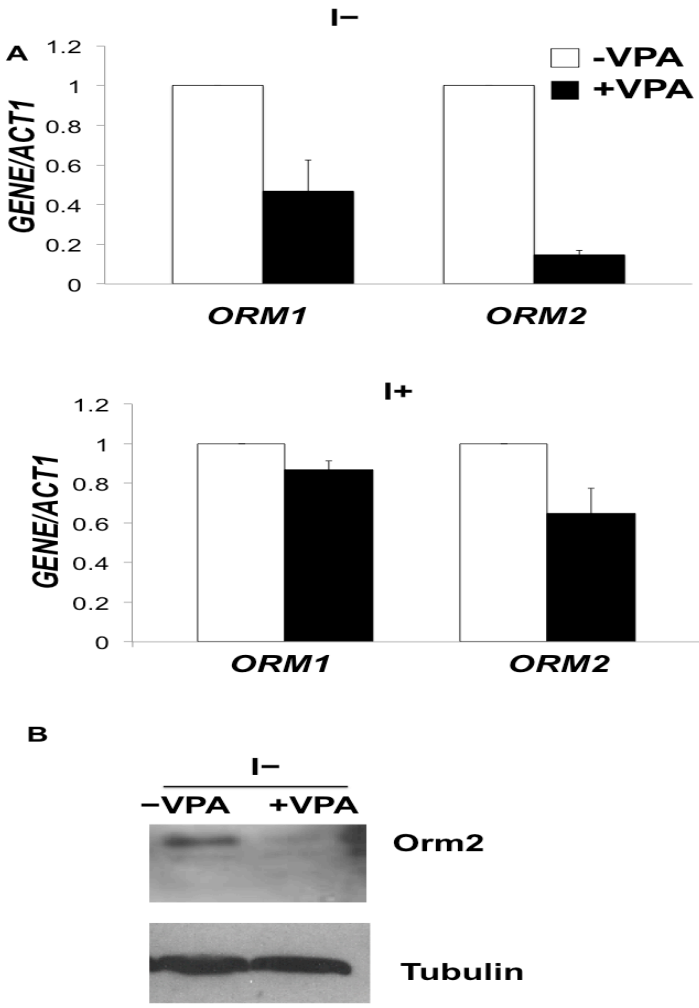

Figure 4.6. VPA affects ORM expression. (A) *ORM1* and *ORM2* mRNA levels were quantified by qRT-PCR from wild type cells grown in the presence or absence of VPA in I- (top) or I+ (bottom). Values are reported as fold change in expression in cells grown in the presence of VPA compared to cells grown in the absence of VPA. Expression was normalized to the mRNA levels of *ACT1* (internal control). Data shown are mean ± SD (n=6). (B) Western blot analysis of Orm2 protein levels in wild type cells expressing *ORM2* tagged with HA. Cells were grown in the presence and absence of VPA in I-, harvested and lysed for protein extraction. 60 µg total cell extract from each sample were subjected to Western blot analysis using 12% SDS-PAGE gel.

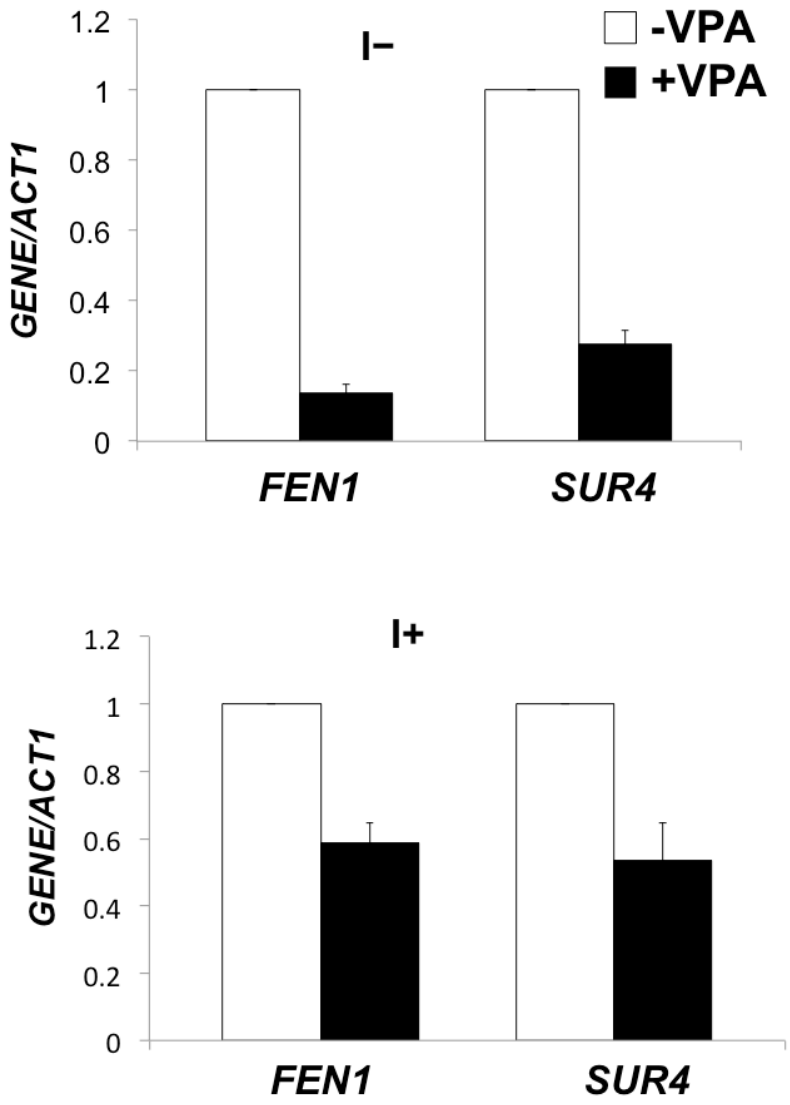

Figure 4.7. VPA decreases the levels of *FEN1* and *SUR4*. mRNA level*FEN1* and *SUR4* were quantified by qRT-PCR from wild type cells grown in the presence or absence of VPA in I- (top) or I+ (bottom), as described in Fig. 4.2.

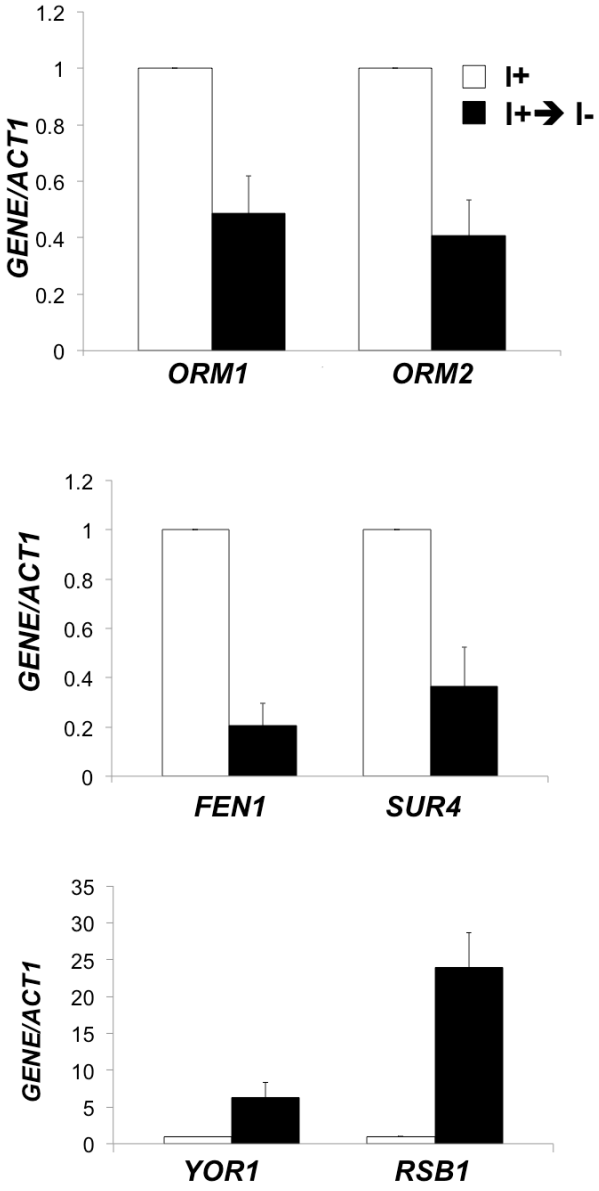

Figure 4.8. Inositol depletion downregulates *ORM* and fatty acid elongase genes and upregulates *RSB1* and *YOR1*. *ino1Δ* cells were precultured in I+ medium, harvested, washed twice with sterile water, and grown in I+ until the cells reached the mid log phase (A_{550}= 0.5). Cells were pelleted, washed twice with sterile water and cultivated in fresh I- (inositol starvation) or I+ (control) for 30 minutes. mRNA levels were quantified by qRT-PCR as described in Fig. 4.2.

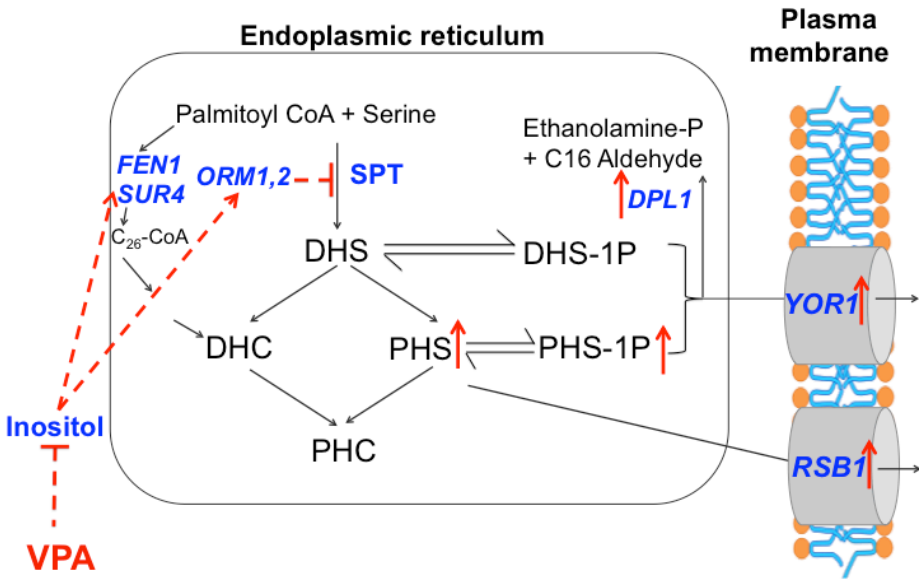

Figure 4.9. Proposed model. In the proposed model, acute VPA mediated inositol depletion leads to decreased expression of fatty acid elongases (*FEN1* and *SUR4*) and *ORM* genes. This increases the *de novo* synthesis and accumulation of the signaling sphingolipid molecule, PHS, which is toxic at high levels. Cells respond by increasing expression of *RSB1* (transporter of PHS), *YOR1* (transporter of PHS-1P), and *DPL1* (breaks down PHS-1P), which aid in maintaining non-toxic levels of PHS. (Abbreviations: SPT, Serine palmitoyl transferase; DHS, Dihydrosphingosine; PHS, Phytosphingosine; DHS-1P, Dihydrosphingosine-1-phosphate PHS-1P, Phytosphingosine-1-phosphate)

CHAPTER 5 FUTURE DIRECTIONS

The studies in this thesis identified and characterized sphingolipid metabolism as a new target of VPA, which could be important in the therapeutic action of the drug. While my work has shed light on the effect of VPA on sphingolipid metabolism, a number of fascinating unanswered questions remain to be explored. In this chapter, I suggest challenging and interesting directions for future studies that could lead to a better understanding of the mechanisms underlying the therapeutic action of VPA.

Does VPA induce the UPR pathway in mammals?

In Chapter 3, I showed that, in yeast, chronic VPA-mediated inositol depletion induces the UPR by increasing intracellular ceramide levels. This major finding identifies the UPR as a possible new target of VPA. However, It is not known if VPA induces the UPR pathway in mammals.

VPA is known to cause inositol depletion in yeast as well as mammalian cells (Vaden et al., 2001; Shaltiel et al., 2004; Ye and Greenberg, 2015). I suggest future experiments to determine if VPA induces the UPR pathway in mammalian cells, as determined by the effect of VPA on: 1) intracellular levels of ceramide, 2) induction of the UPR pathway, and 3) the role of ceramide in UPR induction.

My findings demonstrate that chronic VPA specifically increases levels of C24- and C26-containing phytoceramide. Future experiments should determine if VPA specifically increases C24-C26-ceramides in mammalian cells.

If experiments confirm the findings in yeast and reveal that VPA induces the UPR pathway in mammals, this will suggest that the UPR pathway is an important

target of VPA. Drugs that induce the UPR pathway can be tested for their antibipolar efficacy.

What is the mechanism that causes inositol depletion to increase ceramide levels and induce the UPR?

As stated previously, I showed that chronic VPA causes inositol depletion, which increases ceramide levels and induces the UPR pathway. However, the mechanism by which inositol depletion leads to increased ceramide levels remains unknown. VPA mediated inositol starvation increased the expression of fatty acid elongases *FEN1* and *SUR4,* which catalyze the synthesis of C24-C26 fatty acids and levels of C24- and C26-containing phytoceramide. I suggest future experiments to determine the effect of VPA and inositol starvation on the enzyme activity of fatty acid elongases and ceramide synthase. Solving this mystery will not only aid in elucidating the mechanism by which VPA increases ceramide levels, but also uncover a novel regulatory mechanism of ceramide synthesis.

What is the mechanism that causes acute VPA to increase PHS levels?

In Chapter 4, I showed that acute VPA-mediated inositol depletion increases *de novo* synthesis of PHS. However, the mechanism by which VPA increases PHS levels is not known. My studies revealed that acute VPA decreases expression of *ORM* and fatty acid elongases, suggesting that VPA increases *de novo* synthesis of PHS. I suggest further studies to determine if VPA: 1) increases the activity of serine palmitoyl transferase (SPT), the enzyme catalyzing *de novo* synthesis of PHS; 2) increases phosphorylation of Orm proteins, which regulate SPT activity; 3) decreases activity of

fatty acid elongases; and/ or 4) decreases ceramide synthase activity, which is expected to cause accumulation of the precursor, PHS.

PHS acts as a second messenger that controls a wide range of cellular processes, including growth, cell wall integrity, stress resistance, endocytosis, and aging (Liu et al., 2005). Future experiments should determine if VPA increases levels of sphingosine, the homolog of PHS in mammals. Further, characterizing the effect of acute VPA on: 1) sphingosine levels, 2) Orm protein and phosphorylation levels, 3) SPT activity, and 4) fatty acid elongation in mammalian cells would aid in determining if acute effects of VPA are conserved in yeast and mammals. Determining this will not only aid in elucidating the mechanism by which acute VPA mediated inositol depletion increases PHS levels, but may also uncover a novel regulatory mechanism of PHS synthesis via inositol.

Identify new targets of VPA that could play a role in the therapeutic action of the drug

In order to identify targets of VPA, I performed a genome wide microarray analysis with acute and chronic VPA treatment, as described in Chapter 2. Previous studies showed that acute VPA *decreases* and chronic VPA *increases* expression of *INO1* (Ju and Greenberg, 2003), which was confirmed by my microarray study. This indicates that VPA exhibits differential perturbation of gene expression in response to acute vs. chronic VPA treatment.

I focused my studies on the effect of VPA on sphingolipid metabolism. However, the screen revealed that VPA affects a number of pathways, including lipid metabolism, glycolysis, amino acid metabolism, and transmembrane transport as potential targets of

VPA. Further experiments to characterize the effect of VPA on these cellular pathways remain to be done. As an example, Michael Salsaa' has systematically analyzed this microarray data and determined that chronic VPA increases glycolytic genes in I-medium. His observations led to the exciting hypothesis that VPA-mediated inositol depletion diverts glucose-6-phosphate from inositol synthesis to glycolysis. I am certain that his hypothesis will result in a fascinating dissertation project. I invite my colleagues to continue to mine my microarray database to further explore the mechanism of action of VPA.

In conclusion, VPA is a drug that has been used for the treatment of BD for years, in spite of not understanding the molecular mechanism that elicits its therapeutic mode of action. The identification and characterization of cellular targets will aid in understanding the mechanisms of action of VPA and the pathophysiology of BD, which remains poorly understood.

Now that we are at the end of this thesis, we have certainly not finished identifying all the targets of VPA, nor do we completely understand the molecular mechanism of the drug. I am sure my lab mates will continue to explore and contribute to the understanding of VPA as a bipolar drug. I hope you enjoy the research and time in the lab as I did. Good luck!!!

APPENDIX

IDENTIFICATION OF SYNTHETIC GENETIC INTERACTIONS OF *LCB4*

INTRODUCTION

Sphingolipids are primarily found in the plasma membrane and can be metabolized to signaling molecules (Pitman et al., 2015). Among these, sphingosine-1-phosphate (S1P) is a signaling molecule that is known to play a role in the development of cancer, obesity, anxiety disorders, diabetes, and cardiovascular disorders (Pyne et al., 2016). S1P is synthesized by phosphorylation of sphingosine, catalyzed by sphingosine kinases SphK1 and SphK2 (Pitman et al., 2015). S1P regulates several important cellular functions, including cell proliferation, cell survival, cell migration, and neurotransmitter release (Okada et al., 2009; Zhang et al., 2001; Spiegel and Merrill, 1996; Spiegel et al., 1996; Meyer zu Heringdorf et al., 1999). The functional roles of S1P were discovered in the last several years and remain an exciting focus of current research.

Sphingolipid synthesis and metabolism are complex. The sphingolipid synthesis pathway is highly conserved in yeast and mammals (Epstein and Riezman, 2013). The two major yeast long chain bases (LCBs), dihydrosphingosine (DHS) and phytosphingosine (PHS), are homologous to mammalian sphingosine (Nagiec et al., 1998). DHS and PHS are phosphorylated to DHS phosphate (DHS-1P) or PHS phosphate (PHS-1P) by sphingolipid base kinases Lcb4 and Lcb5, which account for 97% and 3% of sphingolipid base kinase activity, respectively (Nagiec et al., 1998). In this study, I carried out a genetic screen to elucidate functions of *LCB4* by identifying synthetic lethal interactions of the *lcb4Δ* mutant. Synthetic lethality is a genetic

phenomenon in which deletion of two or more genes causes lethality, while deletion of only one gene is viable. Synthetic lethality suggests that the gene products carry out similar functions in a common pathway or in alternative pathways. The genetic screen known as Synthetic Genetic Array (SGA), currently possible only in yeast, identifies genetic interactions with all non-essential yeast genes (Tong et al., 2006).

Using the SGA approach, I identified genetically interacting mutants in several functional categories. Among these, especially interesting was ER-Golgi transport, suggesting for the first time that LCBPs generated by Lcb4 may play a role in this process. In addition to ER-Golgi transport, carbohydrate metabolism, cell cycle, cytoskeletal organization, cellular amino acid metabolic processes, and transmembrane transport mutants exhibited synthetic interactions with *lcb4Δ.* Synthetic interaction suggests a possible overlapping role of Lcb4 in these cellular functions.

MATERIALS AND METHODS

Growth medium

Synthetic complete medium contained adenine (20.25mg/l), arginine (20mg/l), histidine (20mg/l), leucine (60mg/l), lysine (200mg/l), methionine (20mg/l), threonine (300mg/l), tryptophan (20mg/l) and uracil (20mg/l), vitamin mix, yeast base (Difco), ammonium sulphate 5gm/l, glucose 20gm/l, 75μM inositol. Synthetic drop out medium contained all these components but lacked the indicated amino acid. G418 (400mg/ml) and 5-fluoroorotic acid (FOA) (2gm/l) were added when indicated. Sporulation medium contained yeast extract (1.25gm/l), glucose (1gm/l), potassium acetate (10gm/l), leucine (60mg/l), uracil 920mg/l), histidine (20mg/l), tryptophan (20mg/l) and methionine

(20mg/l). YPD medium contained yeast extract (1%), peptone (2%), and glucose (2%). Agar (2%) was added in solid medium.

Construction of the starting strain for the synthetic lethality screen

The starting strain was constructed in three steps: disruption of *LCB4*, confirmation of disruption by PCR, and genetic analysis of the phenotype. The disruption cassette containing the *URA* marker was amplified from the pUGK plasmid using forward primer 5'AGGTTATCAAGAACACAAAAGTCTAGCAGCGAAAAGTACGCGAAGAATCTACTAT AGATACAGCTGAAGCTTCGTACGC3' and reverse primer 5'TTTCGATGTAGTATTTCTTTTTACAAAAAAATCATTTTTGAAGGAAAATATAACG3' using polymerase chain reaction (PCR). The PCR product was electrophoresed on an agarose gel and cleaned using a Qiagen gel purification kit. The wild type SGA strain *Matαcan1Δ::STE2pr-LEU2 lyp1Δhis3Δleu2Δura3ΔmetΔ* (Tong and Boone, 2006) was transformed with the purified PCR product by electroporation and recombinants were selected on Ura^- medium. Disruption of the *LCB4* gene and insertion of the *URA* cassette were confirmed by PCR (Fig. A.1). The recombinants were also tested for mating type (*Matα*) and Ura^+, Leu^-, Met^-, and His^- phenotypes.

SGA design

The *MATα can1Δlcb4Δ* was crossed to the array of deletion mutants in the *MATa* background, in which the deletions are linked to the dominant selectable marker for geneticin resistance, *KanMx4.* Diploids were selected on Ura^- G418 medium and sporulation was induced. Haploid spore progeny were transferred to synthetic medium lacking leucine, which allowed for selective germination of *MATa* cells by virtue of the

STE2pr-LEU2 reporter. Following two rounds of selection on Leu$^-$ G418 medium, double mutant *MATa* progeny were selected on Leu$^-$ medium supplemented with G418. Synthetic interaction between *lcb4Δ* and deletion mutants was determined by examining growth of the double mutant on Leu$^-$ Ura$^-$ G418 compared to that of wild type and to single mutants on Leu$^-$ FOA. Mutants that exhibited growth on Leu$^-$ FOA but exhibited no growth or less growth on Leu$^-$ Ura$^-$ G418 were categorized as synthetically lethal or sick.

RESULTS AND DISCUSSION

An SGA screen was performed to identify mutants that synthetically interact with the *lcb4Δ* mutant in order to identify pathways that share a common or overlapping function with *LCB4*. As expected, the SGA screen identified a large number of synthetic interactions. Fifteen mutants showed a sick interaction with *lcb4Δ* at 30°C and one hundred and twenty four mutants at 37°C (Tables A.1 and A.2). Interacting genes were grouped based on their associated cellular processes (Tables A.3 and A.4) using the GO Slim Mapper program available on the Saccharomyces Genome Database (SGD).

Previous studies have shown that sphingosine kinase may play a role in ER-Golgi transport. Consistent with these studies, *lcb4Δ* interacted with genes involved in this process (Table A.5). *ECM21* was the only gene that showed a synthetic sick interaction at the optimal growth temperature of 30°C, suggesting that an essential function is not compensated by other genes in cells lacking Lcb4 and Ecm21. Mutants of *ERV15, VPS54, GGA1, EMP24, SLY41, ECM21, SUR7, CSR2, STP22, VPS54, and*

Figure A.1. PCR confirmation of the SGA query strain. Query strain DNA was subjected to PCR amplification using the following primers 1: primers for *LCB4;* 2: forward primer upstream and reverse primer downstream of LCB4; 3: forward primer upstream of *LCB4* and reverse primer within the gene; 4: forward primer upstream of the *LCB4* and reverse primer within the *URA* cassette; 5: primers for the *CAN1* gene.

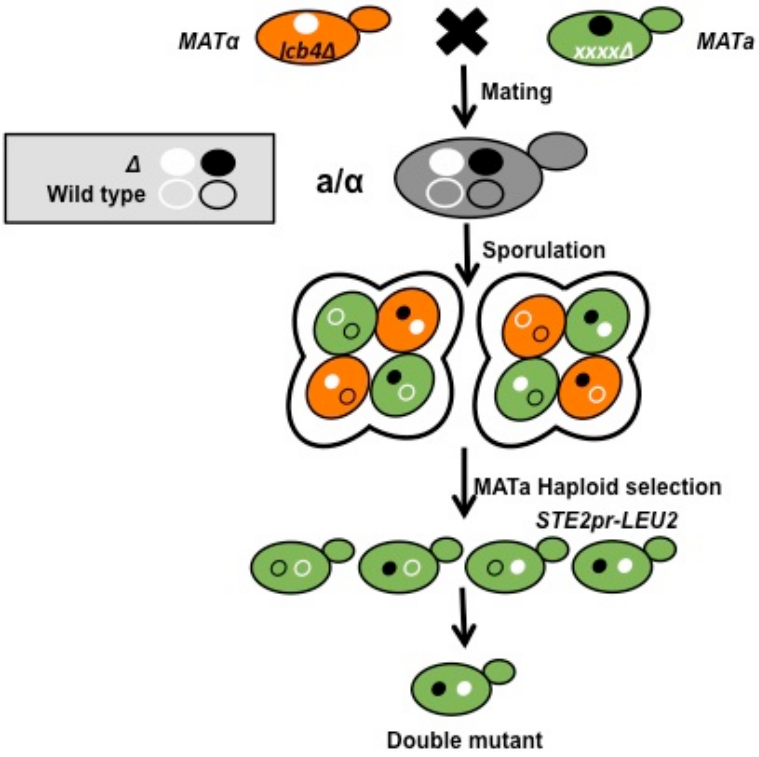

Figure A.2. Synthetic genetic array (SGA) (modified from Tong et al., 2001). *Matαcan1Δ::STE2pr-LEU2 lyp1Δhis3Δleu2Δura3ΔmetΔlcb4Δ::URA3* was crossed to about 4,800 *MATa* yeast deletion mutants, each carrying a deletion mutation linked to a kanamycin-resistance marker *(KanMX),* to form diploids. Haploid meiotic spores were formed following transfer of heterozygous diploids to sporulation medium. Haploid spore progeny were transferred to synthetic medium lacking leucine, which allows for selective germination of *MATa* cells because these cells express the *STE2pr-LEU2* reporter. The selected *MATa* meiotic progeny were transferred to Leu^- G418-containing medium. Leucine auxotrophy and kanamycin resistance identified the double mutant progeny.

GGA1 were synthetic sick at the stress temperature of 39°C. These genes function in ER-Golgi transport. Synthetic lethality of *lcb4Δ* and genes encoding for ER-Golgi transport proteins suggest that Lcb4 generated LCBP is required for efficient ER-Golgi transport.Ceramide is synthesized in the ER and transported to the Golgi by ER-Golgi transport proteins. Deletion of genes for ER-Golgi transport increases ceramide levels in the ER, resulting in ceramide induced apoptosis. S1P is known to rescue cells from ceramide-induced apoptosis (Cuvillier et al., 1996). In addition, overexpression of sphingosine kinase 1 (SphK1) rescues defective ER-Golgi protein trafficking (Veret at al., 2013), suggesting that S1P is required for efficient ER-Golgi transport. However, the mechanism by which S1P rescues defective ER-Golgi trafficking remains unknown. Lcb4 and ER-Golgi transport proteins may genetically interact because deletion of *LCB4* decreases S1P levels and deletion of ER-Golgi transport proteins increases ceramide levels inducing apoptosis. I hypothesize that S1P inhibits accumulation of ceramide in the ER by promoting its efficient transport to Golgi or by decreasing ceramide synthesis. This would identify a novel function of S1P.

Table A.1. Genes showing synthetic interaction with *lcb4Δ* at 30°C (Gene descriptions are from Saccharomyces Genome Database)

ORF	*GENE*	FUNCTION
YOR302W	*Orf verified*	CPA1 uORF; Arginine attenuator peptide, regulates translation of the CPA1 mRNA
YOR342C	*Orf verified*	Protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and the nucleus
YLR014C	*PPR1*	Zinc finger transcription factor; contains a Zn(2)-Cys(6) binuclear cluster domain, positively regulates transcription of URA1, URA3, URA4, and URA10, which are involved in de novo pyrimidine biosynthesis, in response to pyrimidine starvation; activity may be modulated by interaction with Tup1p
YDR132C	*S000002539*	Protein of unknown function; protein increases in abundance and relative distribution to the nucleus increases upon DNA replication stress
YLR384C	*IKI3*	Subunit of Elongator complex; Elongator is required for modification of wobble nucleosides in tRNA; maintains structural integrity of Elongator
YML094W	*GIM5*	Subunit of the heterohexameric cochaperone prefoldin complex; prefoldin binds specifically to cytosolic chaperonin and transfers target proteins to it
YPR060C	*ARO7*	Chorismate mutase; catalyzes the conversion of chorismate to prephenate to initiate the tyrosine/phenylalanine-specific branch of aromatic amino acid biosynthesis
YBL101C	*ECM21*	Protein involved in regulating endocytosis of plasma membrane proteins; identified as a substrate for ubiquitination by Rsp5p and deubiquitination by Ubp2p
YML027W	*YOX1*	Homeobox transcriptional repressor; binds to Mcm1p and to early cell cycle boxes (ECBs) in the promoters of cell cycle-regulated genes expressed in M/G1 phase; expression is cell cycle-regulated; potential Cdc28p substrate
YGL159W	*S00000317*	Putative protein of unknown function; deletion mutant has no detectable phenotype
YGL157W	*ARI1*	NADPH-dependent aldehyde reductase; utilizes aromatic and alophatic aldehyde substrates; member of the short-chain dehydrogenase/reductase superfamily
YGL156W	*AMS1*	Vacuolar alpha mannosidase; involved in free oligosaccharide (fOS) degradation

YER149C	*PEA2*	Coiled-coil 12S polarisome subunit; required for polarity establishment, apical bud growth, shmoo formation, filamentous differentiation; involved in Bni1p localization at sites of polarized growth, controlling polarized assembly of actin cables
YER109C	*FLO8*	Transcription factor; required for flocculation, diploid filamentous growth, and haploid invasive growth; forms a heterodimer with Mss1p that interacts with the Swi/Snf complex during transcriptional activation of FLO1, FLO11, and STA1
YER110C	*KAP123*	Karyopherin beta; mediates nuclear import of ribosomal proteins prior to assembly into ribosomes and import of histones H3 and H4

Table A.2. Genes showing synthetic interaction with *Icb4Δ* at 39°C (Gene descriptions are from Saccharomyces Genome Database)

ORF	*GENE*	FUNCTION
YLL032C	*ORF, verified*	Protein of unknown function
YLR046C	*ORF, Uncharacterized*	Putative membrane protein; member of the fungal lipid-translocating exporter (LTE) family of proteins
YLR062C	*BUD28*	Dubious open reading frame
YLR092W	*SUL2*	High affinity sulfate permease
YLR063W	*BMT6*	Methyltransferase required for m3U2843 methylation of the 25S rRNA
YML052W	*SUR7*	Plasma membrane protein of unknown function involved with endocytosis
YMR157C	*AIM36*	Protein of unknown function
YMR25W	*GTO3*	Omega class glutathione transferase; putative cytosolic localization
YMR282C	*AEP2*	Mitochondrial protein; likely involved in translation of the mitochondrial OLI1 mRNA;
YOL054W	*PSH1*	E3 ubiquitin ligase targeting centromere-binding protein Cse4p
YPL261C	*ORF, verified*	Dubious open reading frame; unlikely to encode a functional protein, based on available experimental and comparative sequence data
YPL254W	*HFI1*	Adaptor protein required for structural integrity of the SAGA complex
YPL244C	*HUT1*	Protein with a role in UDP-galactose transport to the Golgi lumen
YPL198W	*RPL7B*	Ribosomal 60S subunit protein L7B; required for processing of 27SA3 pre-rRNA to 27SB pre-rRNA during assembly of large ribosomal subunit; depletion leads to a turnover of pre-rRNA
YPL186C	*UIP4*	Protein that interacts with Ulp1p; a Ubl (ubiquitin-like protein)-specific protease for Smt3p protein conjugates
YPL192C	*PRM3*	Protein required for nuclear envelope fusion during karyogamy
YPL141C	*FRK1*	Protein kinase of unknown cellular role; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm
YBR197C	*ORF, Verified*	Protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and nucleus
YBR199W	*KTR4*	Putative mannosyltransferase involved in protein

		glycosylation
YBR210W	*ERV15*	Protein involved in export of proteins from the endoplasmic reticulum; ERV15 has a paralog, ERV14, that arose from the whole genome duplication
YOR307C	*SLY41*	Protein involved in ER-to-Golgi transport
YOR323C	*PRO2*	Gamma-glutamyl phosphate reductase; catalyzes the second step in proline biosynthesis
YOR343C	*ORF, verified*	Dubious open reading frame; unlikely to encode a functional protein, based on available experimental and comparative sequence data
YOL006C	*TOP1*	Topoisomerase I; nuclear enzyme that relieves torsional strain in DNA by cleaving and re-sealing the phosphodiester backbone
YOL007C	*CSI2*	Protein of unknown function
YOL014W	*YOL014W*	Putative protein of unknown function
YOL015W	*IRC10*	Putative protein of unknown function
YPL261C	*S000006182*	Dubious open reading frame
YPL244C	*HUT1*	Protein with a role in UDP-galactose transport to the Golgi lumen
YPL240C	*HSP82*	Hsp90 chaperone; redundant in function with Hsc82p
YPL178W	*CBC2*	Small subunit of the heterodimeric cap binding complex with Sto1p
YPL136W	*S000006057*	Dubious open reading frame
YPL106C	*SSE1*	ATPase component of heat shock protein Hsp90 chaperone complex; plays a role in determining prion variants
YPL192C	*PRM3*	Protein required for nuclear envelope fusion during karyogamy
YPL186C	*UIP4*	Protein that interacts with Ulp1p; a Ubl (ubiquitin-like protein)-specific protease for Smt3p protein conjugates
YDR358W	*GGA1*	Golgi-localized protein with homology to gamma-adaptin
YDR359C	*EAF1*	Component of the NuA4 histone acetyltransferase complex
YDR363W	*ESC2*	Sumo-like domain protein; prevents accumulation of toxic intermediates during replication-associated recombinational repair; roles in silencing, lifespan, chromatid cohesion and the intra-S-phase DNA damage checkpoint; RENi family member
YCL008C	*STP22*	Component of the ESCRT-I complex; ESCRT-I is involved in ubiquitin-dependent sorting of proteins into the endosome; prevents polyubiquitination of the arrestin-related protein Rim8p, thereby directing its monoubiquitination by Rsp5p
YLR219W	*MSC3*	Protein of unknown function
YKL177W	*S000001660*	Dubious open reading frame

YOR137C	*SIA1*	Protein of unassigned function
YGR055W	*MUP1*	High affinity methionine permease; integral membrane protein with 13 putative membrane-spanning regions; also involved in cysteine uptake
YOR258W	*HNT3*	DNA 5' AMP hydrolase involved in DNA repair; member of the histidine triad (HIT) superfamily of nucleotide-binding proteins
YJL170C	*ASG7*	Protein that regulates signaling from G protein beta subunit Ste4p
YLR357W	*RSC2*	Component of the RSC chromatin remodeling complex
YLR362W	*STE11*	Signal transducing MEK kinase
YLR384C	*IKI3*	Subunit of Elongator complex; Elongator is required for modification of wobble nucleosides in tRNA
YLR377C	*FBP1*	Fructose-1,6-bisphosphatase; key regulatory enzyme in the gluconeogenesis pathway, required for glucose metabolism
YLR308W	*CDA2*	Chitin deacetylase; together with Cda1p involved in the biosynthesis ascospore wall component, chitosan
YLR322W	*VPS65*	Dubious open reading frame; unlikely to encode a functional protein, based on available experimental and comparative sequence data
YGL196W	*DSD1*	D-serine dehydratase (aka D-serine ammonia-lyase)
YDR227W	*SIR4*	SIR protein involved in assembly of silent chromatin domains; silent information regulator (SIR) along with SIR2 and SIR3
YGL200C	*EMP24*	Component of the p24 complex; role in misfolded protein quality control
YGL214W	*S000003182*	Dubious open reading frame; unlikely to encode a functional protein
YGL222C	*EDC1*	RNA-binding protein that activates mRNA decapping directly; binds to mRNA substrate and enhances activity of decapping proteins Dcp1p and Dcp2p; has a role in translation during heat stress
YGL256W	*ADH4*	Alcohol dehydrogenase isoenzyme type IV; dimeric enzyme demonstrated to be zinc-dependent despite sequence similarity to iron-activated alcohol dehydrogenases
YPL088W	*S000006009*	Putative aryl alcohol dehydrogenase
YPL037C	*EGD1*	Subunit beta1 of the nascent polypeptide-associated complex (NAC); involved in protein targeting, associated with cytoplasmic ribosomes
YPL065W	*VPS28*	Component of the ESCRT-I complex; complex is involved in ubiquitin-dependent sorting of proteins into the endosome
YPL006W	*NCR1*	Vacuolar membrane protein

YBL053W	*S000000149*	Dubious open reading frame
YGL081W	*S000003049*	Putative protein of unknown function
YDR248C	*S000002656*	Putative gluconokinase; sequence similarity to bacterial and human gluconokinase
YKR033C	*S000001741*	Dubious open reading frame
YDR276C	*PMP3*	Small plasma membrane protein
YDR314C	*RAD34*	Protein involved in nucleotide excision repair (NER); homologous to RAD4
YDR335W	*MSN5*	Karyopherin; involved in nuclear import and export of proteins, including import of replication protein A and export of Far1p and transcription factors Swi5p, Swi6p, Msn2p, and Pho4p
YFL026W	*STE2*	Receptor for alpha-factor pheromone; seven transmembrane-domain GPCR that interacts with both pheromone and a heterotrimeric G protein to initiate the signaling response that leads to mating between haploid a and alpha cells
YFL054C	*S000001840*	Putative channel-like protein; similar to Fps1p
YOL095C	*HMI1*	Mitochondrial inner membrane localized ATP-dependent DNA helicase
YOL115W	*PAP2*	Non-canonical poly(A) polymerase; involved in nuclear RNA degradation as a component of TRAMP; catalyzes polyadenylation of hypomodified tRNAs, and snoRNA and rRNA precursors; required for mRNA surveillance and maintenance of genome integrity, serving as a link between RNA and DNA metabolism
YLL049W	*LDB18*	Component of the dynactin complex; dynactin is required for dynein activity
YMR312W	*ELP6*	Subunit of hexameric RecA-like ATPase Elp456 Elongator subcomplex; which is required for modification of wobble nucleosides in tRNA
YMR316C-A	*S000004933*	Protein of unknown function
YMR316C-B	*S000004934*	Dubious open reading frame
YML094W	*GIM5*	Subunit of the heterohexameric cochaperone prefoldin complex
YMR004W	*MVP1*	Protein required for sorting proteins to the vacuole; Mvp1p and Vps1p act in concert to promote membrane traffic to the vacuole; participates in transcription initiation and/or early elongation of specific genes
YMR121C	*RPL15B*	Ribosomal 60S subunit protein L15B; binds to 5.8 S rRNA; homologous to mammalian ribosomal protein L15, no bacterial homolog
YPR027C	*S000006231*	Putative protein of unknown function

YML117W-A	*S000004586*	Putative protein of unknown function
YPR030W	*CSR2*	Nuclear ubiquitin protein ligase binding protein; may regulate utilization of nonfermentable carbon sources and endocytosis of plasma membrane proteins
YPR075C	*OPY2*	Integral membrane protein that acts as a membrane anchor for Ste50p; involved in the signaling branch of the high-osmolarity glycerol (HOG) pathway and as a regulator of the filamentous growth pathway
YJL030W	*MAD2*	Component of the spindle-assembly checkpoint complex; delays onset of anaphase in cells with defects in mitotic spindle assembly; forms a complex with Mad1p
YJR108W	*ABM1*	Protein of unknown function
YLR436C	*ECM30*	Putative protein of unknown function
YAL047C	*SPC72*	Component of the cytoplasmic Tub4p (gamma-tubulin) complex
YDL243C	*AAD4*	Putative aryl-alcohol dehydrogenase; involved in oxidative stress response
YMR136W	*GAT2*	Protein containing GATA family zinc finger motifs
YDR042C	*S000002449*	Putative protein of unknown function; expression is increased in ssu72-ts69 mutant
YDR027C	*VPS54*	Component of the GARP (Golgi-associated retrograde protein) complex; GARP is required for the recycling of proteins from endosomes to the late Golgi, and for mitosis after DNA damage induced checkpoint arrest
YJL007C	*S000003544*	Dubious open reading frame
YJR035W	*RAD26*	Protein involved in transcription-coupled nucleotide excision repair; repairs UV-induced DNA lesions
YDL109C	*S000002267*	Putative lipase; involved in lipid metabolism; not an essential gene
YDL136W	*RPL35B*	Ribosomal 60S subunit protein L35B; homologous to mammalian ribosomal protein L35 and bacterial L29
YDL155W	*CLB3*	B-type cyclin involved in cell cycle progression; activates Cdc28p to promote the G2/M transition; may be involved in DNA replication and spindle assembly
YDL184C	*RPL41A*	Ribosomal 60S subunit protein L41A; comprises only 25 amino acids; rpl41a rpl41b double null mutant is viable; homologous to mammalian ribosomal protein L41, no bacterial homolog; RPL41A has a paralog, RPL41B, that arose from the whole genome duplication
YBL101C	*ECM21*	Protein involved in regulating endocytosis of plasma membrane proteins
YBR019C	*GAL10*	UDP-glucose-4-epimerase; catalyzes the interconversion of UDP-galactose and UDP-D-glucose in galactose

		metabolism
YBR030W	*RKM3*	Ribosomal lysine methyltransferase
YBR065C	*ECM2*	Pre-mRNA splicing factor
YBR069C	*TAT1*	Amino acid transporter for valine, leucine, isoleucine, and tyrosine
YIL110W	*HPM1*	AdoMet-dependent methyltransferase; involved in a novel 3-methylhistidine modification of ribosomal protein Rpl3p
YIL099W	*SGA1*	Intracellular sporulation-specific glucoamylase
YNL072W	*RNH201*	Ribonuclease H2 catalytic subunit
YNL097C	*PHO23*	Component of the Rpd3L histone deacetylase complex; involved in transcriptional regulation of PHO5
YFR013W	*IOC3*	Subunit of the Isw1a complex; Isw1a has nucleosome-stimulated ATPase activity and represses transcription initiation by specific positioning of a promoter proximal dinucleosome
YJL160C	*PIR5*	Putative protein of unknown function
YJL213W	*S000003749*	Protein of unknown function that may interact with ribosomes
YBR169C	*SSE2*	Member of the heat shock protein 70 (HSP70) family; may be involved in protein folding; localized to the cytoplasm; SSE2 has a paralog, SSE1, that arose from the whole genome duplication
YDL002C	*NHP10*	Non-essential INO80 chromatin remodeling complex subunit; preferentially binds DNA ends, protecting them from exonucleatic cleavage
YDL001W	*RMD1*	Cytoplasmic protein required for sporulation
YDL026W	*S000002184*	Dubious open reading frame
YDL027C	*S000002185*	Putative protein of unknown function
YDL041W	*S000002199*	Dubious open reading frame
YDL077C	*VAM6*	Vacuolar protein involved in vacuolar membrane fusion tethering
YDR440W	*DOT1*	Nucleosomal histone H3-Lys79 methylase
YGL101W	*S000003069*	Protein of unknown function
YGL109W	S*000003077*	Dubious open reading frame
YGL110C	*CUE3*	Protein of unknown function
YGL147C	*RPL9A*	Ribosomal 60S subunit protein L9A
YGL157W	*ARI1*	NADPH-dependent aldehyde reductase
YGL159W	*S000003127*	Putative protein of unknown function
YER149C	*PEA2*	Coiled-coil 12S polarisome subunit; required for polarity establishment, apical bud growth, shmoo formation, filamentous differentiation; involved in Bni1p localization at sites of polarized growth, controlling polarized assembly of actin cables

YDR483W	*KRE2*	Alpha1,2-mannosyltransferase of the Golgi
YJL028W	*S000003565*	Protein of unknown function
YIL066C	*RNR3*	Minor isoform of large subunit of ribonucleotide-diphosphate reductase
YML110C	*COQ5*	2-hexaprenyl-6-methoxy-1,4-benzoquinone methyltransferase

Table A.3. Genes classified based on the cell process showing synthetic interaction with *lcb4Δ* at 30°C

GO term	Frequency	Gene(s)
Transcription from RNA polymerase II promoter	33.30%	FLO8,PPR1,IKI3,YOX1,GIM5
Pseudohyphal growth	20%	FLO8,KAP123,PEA2
Carbohydrate metabolic process	13.30%	FLO8,AMS1
Mitotic cell cycle	13.30%	PEA2,YOX1
DNA-templated transcription, elongation	6.70%	GIM5
Cytoskeleton organization	6.70%	PEA2
Cytokinesis	6.70%	PEA2
Cellular amino acid metabolic process	6.70%	ARO7
Cell morphogenesis	6.70%	PEA2
RNA modification	6.70%	IKI3
Nucleobase-containing small molecule metabolic process	6.70%	PPR1
Protein complex biogenesis	6.70%	GIM5
Nuclear transport	6.70%	KAP123
Response to chemical	6.70%	PEA2
Invasive growth in response to glucose limitation	6.70%	FLO8
Cell budding	6.70%	PEA2
Endocytosis	6.70%	ECM21
Regulation of organelle organization	6.70%	PEA2
Oligosaccharide metabolic process	6.70%	AMS1
Conjugation	6.70%	PEA2
Regulation of translation	6.70%	YOR302W
tRNA processing	6.70%	IKI3
Protein targeting	6.70%	KAP123
biological process unknown	26.70%	YDR132C,ARI1,YGL159W, YOR342C

Table A.4. Genes classified based on the cell process showing synthetic interaction with *lcb4Δ* at 39°C

GO term	Frequency	Gene(s)
Chromatin organization	8.10%	NHP10,SIR4,EAF1,DOT1,IOC3,RAD26,RSC2,PHO23,TOP1,HFI1
Mitotic cell cycle	8.10%	SPC72,ERV15,CLB3,ESC2,DOT1,PEA2,MAD2,LDB18,BUD28,TOP1
Cellular response to DNA damage stimulus	7.30%	NHP10,RAD34,EAF1,ESC2,DOT1,RAD26,RSC2,PAP2,HNT3
Transcription from RNA polymerase II promoter	7.30%	RSC2,IKI3,GIM5,GAT2,ELP6,PHO23,TOP1,HFI1,CSR2
DNA repair	6.50%	NHP10,RAD34,EAF1,ESC2,DOT1,RAD26,RSC2,PAP2
Regulation of organelle organization	5.60%	SPC72,VAM6,CLB3,DOT1,PEA2,MAD2,HSP82
Protein targeting	5.60%	STP22,GGA1,VPS65,MVP1,EGD1,VPS28,HSP82
DNA recombination	5.60%	NHP10,ESC2,DOT1,MSC3,RSC2,TOP1,PAP2
Organelle fission	5.60%	SPC72,ESC2,DOT1,MAD2,MSC3,TOP1,PAP2
Regulation of cell cycle	4.80%	SPC72,CLB3,ESC2,DOT1,MAD2,OPY2
Conjugation	4.80%	PEA2,STE2,ASG7,STE11,PRM3,OPY2
Protein complex biogenesis	4.80%	SPC72,VAM6,EAF1,STE2,GIM5,HSP82
Meiotic cell cycle	4.80%	VPS54,DOT1,MSC3,CDA2,SUR7,PAP2
Ion transport	4.80%	TAT1,PMP3,MUP1,SUL2,SIA1,HUT1
Cytoskeleton organization	4.00%	SPC72,CLB3,PEA2,ABM1,LDB18
Carbohydrate metabolic process	4.00%	GAL10,KTR4,KRE2,SGA1,FBP1
Response to chemical	4.00%	PMP3,PEA2,STE2,STE11,OPY2
Cell wall organization or biogenesis	4.00%	VPS54,KRE2,CDA2,STE11,CSR2
Chromosome segregation	4.00%	SPC72,ESC2,MAD2,RSC2,TOP1
Proteolysis involved in cellular protein catabolic process	4.00%	STP22,GGA1,MAD2,PSH1,VPS28
Cytoplasmic translation	4.00%	RPL35B,RPL41A,RPL9A,RPL15B,RPL7B

Golgi vesicle transport	4.00%	ERV15,VPS54,GGA1,EMP24,SLY41
RNA modification	3.20%	BMT6,IKI3,ELP6,PAP2
Regulation of protein modification process	3.20%	STP22,CLB3,ESC2,STE11
Cellular amino acid metabolic process	3.20%	DSD1,ADH4,GTO3,PRO2
Protein folding	3.20%	SSE2,EGD1,SSE1,HSP82
Protein modification by small protein conjugation or removal	3.20%	STP22,ESC2,ELP6,PSH1
Protein phosphorylation	2.40%	CLB3,STE11,FRK1
Endosomal transport	2.40%	STP22,VPS54,GGA1
RNA catabolic process	2.40%	EDC1,RNH201,PAP2
DNA replication	2.40%	RNH201,TOP1,HSP82
Peptidyl-amino acid modification	2.40%	RKM3,HPM1,HFI1
Response to osmotic stress	2.40%	STE11,HSP82,OPY2
Cytokinesis	2.40%	ERV15,PEA2,BUD28
Protein alkylation	2.40%	RKM3,DOT1,HPM1
Generation of precursor metabolites and energy	2.40%	ADH4,SGA1,COQ5
Regulation of DNA metabolic process	2.40%	TOP1,PAP2,HSP82
Sporulation	2.40%	VPS54,CDA2,SUR7
Mitochondrion organization	2.40%	AEP2,HMI1,HSP82
tRNA processing	2.40%	IKI3,ELP6,PAP2
DNA-templated transcription, elongation	2.40%	RSC2,GIM5,TOP1
rRNA processing	2.40%	RPL35B,BMT6,RPL7B
Endocytosis	2.40%	ECM21,SUR7,CSR2
Signaling	2.40%	STE2,STE11,OPY2
mRNA processing	1.60%	ECM2,CBC2
RNA splicing	1.60%	ECM2,CBC2
Transmembrane transport	1.60%	TAT1,YFL054C
Histone modification	1.60%	DOT1,HFI1
Protein glycosylation	1.60%	KTR4,KRE2
Lipid metabolic process	1.60%	YDL109C,NCR1
Cell budding	1.60%	ERV15,PEA2
Nucleobase-containing compound transport	1.60%	MSN5,HUT1
Vesicle organization	1.60%	VAM6,EMP24
Pseudohyphal growth	1.60%	PEA2,STE11

Invasive growth in response to glucose limitation	1.60%	STE11,PHO23
Response to heat	1.60%	STE11,PHO23
Amino acid transport	1.60%	TAT1,MUP1
Ribosomal large subunit biogenesis	1.60%	RPL35B,RPL7B
Telomere organization	1.60%	NHP10,HSP82
Organelle fusion	1.60%	VAM6,PRM3
Membrane fusion	1.60%	VAM6,PRM3
Translational initiation	0.80%	AEP2
Nucleus organization	0.80%	PRM3
Cell morphogenesis	0.80%	PEA2
Membrane invagination	0.80%	VAM6
Nuclear transport	0.80%	MSN5
Transposition	0.80%	STE11
snoRNA processing	0.80%	PAP2
Response to starvation	0.80%	OPY2
Transcription from RNA polymerase I promoter	0.80%	PHO23
Cellular respiration	0.80%	COQ5
Vacuole organization	0.80%	VAM6
Mitochondrial translation	0.80%	AEP2
Oligosaccharide metabolic process	0.80%	KRE2
Cofactor metabolic process	0.80%	COQ5
Regulation of transport	0.80%	VAM6
Protein maturation	0.80%	MAD2
Protein acylation	0.80%	HFI1
Nucleobase-containing small molecule metabolic process	0.80%	RNR3
Regulation of translation	0.80%	EDC1
Biological process unknown	29.00%	YBL053W,YBR197C,RMD1,YDL026W,YDL027C,YDL041W,YDR042C,YDR248C,YGL081W,YGL101W,YGL109W,CUE3,ARI1,YGL159W,YGL214W,YJL007C,YJL028W,YJL160C,YJL213W,YKL177W,YKR033C,YLL032C,YLR046C,ECM30,YML116W-A,AIM36,YMR316C-A,YMR316C-B,CSI2,YOL014W,IRC10,YOR343C,YPL136W,UIP4,YPL261C,YPR0

		27C
Other	1.60%	AAD4,YPL088W

Table A.5. *LCB4* interacts with genes involved in ER-Golgi transport

Function	**Interaction at 30°C**	**Interaction at 39°C**
Golgi vesicle transport		*ERV15,VPS54,GGA1,EMP24, SLY41*
Endocytosis	*ECM21*	*ECM21,SUR7,CSR2*
Endosomal transport		*STP22,VPS54,GGA1*

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ABSTRACT

GENOME WIDE ANALYSIS IDENTIFIES SPHINGOLIPID METABOLISM AS A NEW TARGET OF VALPROIC ACID

by

SHYAMALAGAURI JADHAV

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Advisor: Dr. Miriam L. Greenberg

Major: Biological Sciences

Degree: Doctor of Philosophy

Bipolar disorder (BD), which is characterized by depression and mania, affects about 1% of the total world population. Current treatments are effective in only 40-60% of cases and cause severe side effects. Valproic acid (VPA), a branched short-chain fatty acid, is one of the most widely used drugs for the treatment of BD. Although many hypotheses have been postulated to explain the molecular mechanism of action of this drug in BD, the therapeutic mechanism is not understood. This knowledge gap has hampered the development of new drugs to treat this disorder. To identify candidate pathways affected by VPA, I performed a genome wide expression analysis in yeast cells grown in the presence or absence of the drug. Many genes and pathways showed altered expression in response to VPA. Among these, sphingolipid metabolism genes showed altered expression in response to both chronic and acute VPA treatment.

Chronic VPA caused upregulation of *FEN1* and *SUR4*, encoding fatty acid elongases that catalyze the synthesis of very long chain fatty acids (C24 to C26) required for the synthesis of ceramide. Interestingly, *fen1Δ* and *sur4Δ* mutants exhibited

VPA sensitivity. Consistent with this, VPA increased levels of ceramides, especially those that contain C24 and C26 fatty acids. As expected with an increase in ceramide, VPA decreased the expression of amino acid transporters, increased the expression of ER chaperones, and activated the unfolded protein response element (UPRE), suggesting that VPA induces the UPR pathway. These effects are rescued by supplementation of inositol and are similarly observed in inositol-starved *ino1Δ* cells. Starvation of *ino1Δ* cells increased expression of *FEN1* and *SUR4,* increased ceramide levels, decreased expression of nutrient transporters, and induced the UPR. These findings suggest that VPA-mediated inositol depletion induces the UPR by increasing the *de novo* synthesis of ceramide.

In response to acute VPA, the gene that exhibited the highest upregulation was *RSB1*, which encodes a transporter of the long chain bases (LCBs) dihydrosphingosine (DHS) and phytosphingosine (PHS). In addition to increased mRNA, acute VPA increased Rsb1 protein levels. The *rsb1Δ* mutant exhibited increased sensitivity to PHS in the presence of VPA, suggesting that VPA increases PHS levels. Consistent with this, acute VPA increased PHS levels, especially in *rsb1Δ* cells. LCBs are precursors of ceramide synthesis, which begins in the endoplasmic reticulum by the conversion of palmitoyl-CoA to PHS or DHS. These intermediates are converted to ceramide via ceramide synthase by addition of a fatty acid synthesized by the fatty acid elongation pathway. Orm proteins are negative regulators of de novo synthesis of PHS, which was shown to function as a signaling molecule. My findings indicate that acute VPA downregulates *ORM* and fatty acid elongases *FEN1* and *SUR4.* This leads to increased PHS levels and increased expression of *RSB1* as well as genes that transport and

metabolize PHS, including *YOR1* and *DPL1*. Inositol starvation of the *ino1Δ* mutant for 30 minutes increased expression of *RSB1* and *YOR1* and decreased expression of *FEN1, SUR4, ORM1,* and *ORM2.* This study shows for the first time that acute VPA-mediated inositol depletion increases levels of PHS.

In summary, I identified sphingolipid metabolism as a new target of VPA. My studies showed that VPA exerts inositol depletion-mediated differential effects on sphingolipid species. Chronic VPA increases ceramide levels and induces the UPR pathway, whereas, acute VPA increases the levels of PHS. These findings suggest that sphingolipid metabolism is a potential target of VPA that could be important for the therapeutic action of this drug.

AUTOBIOGRAPHICAL STATEMENT

SHYAMALAGAURI JADHAV

EDUCATION:

2009-2016: Ph.D. in Biology, Wayne State University, Detroit, USA

2006-2008: M.S in Life Sciences, Sophia College, University of Mumbai, Mumbai, India

2003-2006: B.S in Microbiology, Ramnarain Ruia College, University of Mumbai, Mumbai, India

HONORS AND AWARDS:

ASBMB 2016 Graduate/Postdoctoral Travel Award, 2016

Best poster (Second place), Biological Sciences Departmental retreat, WSU, 2015

Outstanding Graduate Research Assistant award, WSU, 2015

WSU Graduate Student Professional Travel Award, 2015

Best poster (Third place), 5th Annual Graduate School Research day, WSU, 2014

WSU Graduate Student Professional Travel Award, 2014

PUBLICATIONS:

Jadhav S and Greenberg M. L 2014, Harnessing the power of yeast to elucidate the role of sphingolipids in metabolic and signaling processes pertinent to psychiatric disorders. Clinical Lipidology, Vol. 9, No. 5, Pages 533-551.

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