RESEARCH ARTICLE

Yeast response to LA virus indicates coadapted global gene expression during mycoviral infection

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Abstract

Viruses that infect fungi have a ubiquitous distribution and play an important role in structuring fungal communities. Most of these viruses have an unusual life history in that they are propagated exclusively via asexual reproduction or fission of fungal cells. This asexual mode of transmission intimately ties viral reproductive success to that of its fungal host and should select for viruses that have minimal deleterious impact on the fitness of their hosts. Accordingly, viral infections of fungi frequently do not measurably impact fungal growth, and in some instances, increase the fitness of the fungal host. Here we determine the impact of the loss of coinfection by LA virus and the virus like particle M1 upon global gene expression of the fungal host *Saccharomyces cerevisiae* and provide evidence supporting the idea that coevolution has selected for viral infection minimally impacting host gene expression.

Introduction

Fungal viruses infect diverse fungi and are believed to be of ancient origin (Bruenn, 1993; Ghabrial, 1998). There are more than 90 known fungal viruses with either dsRNA or ssRNA genomes, categorized into 10 viral families (Fauquet *et al.*, 2005). All of these viruses share a trait: they have no extracellular phase to their life cycle, being transmitted via cell fusion or cell division (Wickner, 1991). With this direct vertical transmission, selection is expected to have minimized the viral impact on host fit ness because the virus can only propagate when the yeast cell reproduces (Fine, 1975). Accordingly, they are typi cally associated with asymptomatic infections (but see Nuss & Koltin, 1990; Ghabrial, 1994; Nuss, 2001). Some viruses have even been demonstrated to benefit their fun gal hosts (Ahn & Lee, 2001). The advantage can even be indirect. The fungus *Curvularia protuberata* resides in a plant host, *Dicanthelium lanuginosum*. However, when the fungus becomes infected with a mycovirus, both the fun gus and its plant host become thermo tolerant, allowing all three organisms to survive at otherwise inhospitable temperatures (Marquez *et al.*, 2007).

The most extensively characterized fungal virus is the LA virus, a member of the genus *Totivirus* (Buck & Ghabrial, 1991). LA virus is a double stranded RNA (dsRNA) virus that spreads vertically via mitosis and via cell cell fusion during the mating process. While the rela tive frequencies of each are unknown in natural popula tions, no extracellular route of infection is known (Wickner, 1996). Research on the LA virus has been motivated by the role it plays in the yeast killer pheno type. This phenotype was first discovered in 1963 (Bevan & Makower, 1963) when strains of *Saccharomyces cerevisiae* were observed to produce a toxin that killed sensitive cells of the same or related yeast genera. The killer phenotype has subsequently been observed in a wide range of yeast genera (Schmitt & Breinig, 2002). The phenotype is known to result from coinfection by the LA virus and an M virus like particle (vlp; Fig. 1). The LA virus genome contains two open reading frames, coding for the major capsid protein and the RNA dependent RNA polymerase. The viral genome replicates stably in cells without detec tably slowing their growth (Schmitt & Breinig, 2006). It can be inherited vertically, as well as be transmitted hori zontally during mating (Wickner, 1996). The relative

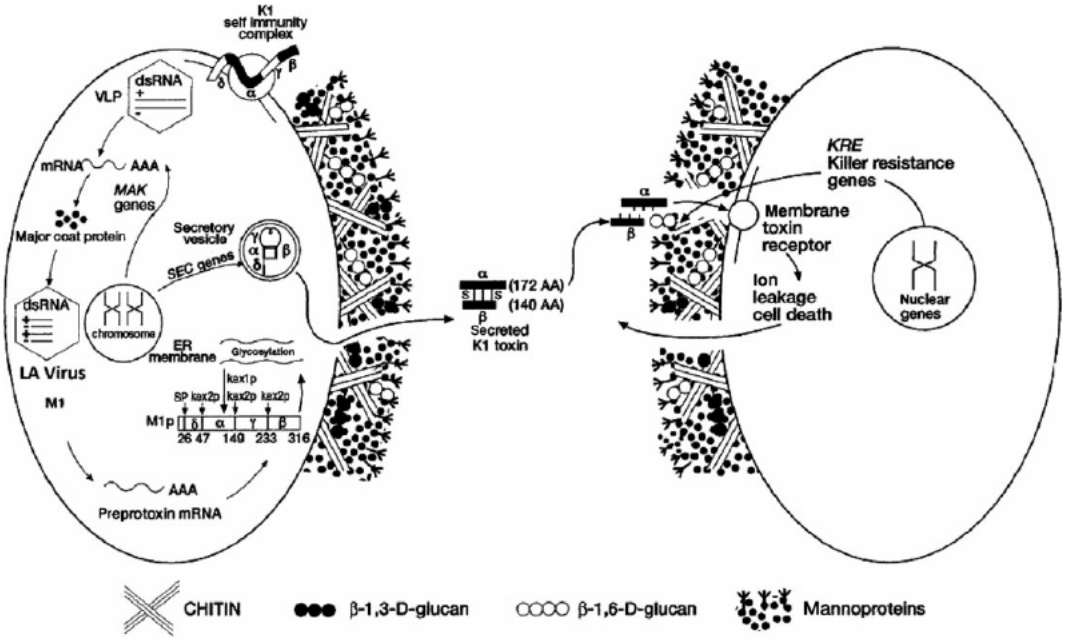

Fig. 1. Sketch of the genetics, toxigenesis, immunity, and activity of the *Saccharomyces cerevisiae* killer system. (modified from Magliani *et al.*, 1997).

importance of these two modes of transmission is unknown. However, *S. cerevisiae* is considered an infre quent mater in nature (Aa *et al.*, 2006). Uncertainty arises due to the unknown but usually presumed high level of inbreeding. Nevertheless, estimates of the frequency of mating vary from once every 1000 generations to once every 50 000 generations (Ruderfer *et al.*, 2006; Zeyl & Otto, 2007; Tsai *et al.*, 2008).

The coinfecting M vlp contains a single open reading frame coding for a precursor of the secreted toxin. M also confers immunity against the toxin to the host cell but is dependent on the LA virus for its replication, transcription, and encapsidation (Magliani *et al.*, 1997; Marquina *et al.*, 2002). In *S. cerevisiae,* three kinds of M vlps (M1, M2, and M28) have been discovered that code for specific toxins (K1, K2, and K28, respectively) and for components con ferring self immunity (Wickner, 1992). The toxin secreted by coinfected yeast is active at pH values that range between pH 4 and pH 5 (Bussey *et al.*, 1979; Palfree & Bus sey, 1979; Tipper & Bostian, 1984; Golubev & Shabalin, 1994; Marquina *et al.*, 2002). It has been shown that within this pH range toxin production can benefit toxin produc ing yeast, but this advantage is lost at pH levels outside of this range (Greig & Travisano, 2008; McBride *et al.*, 2008).

The killer phenotype is widespread in nature and con tributes to the structure of yeast communities (Starmer *et al.*, 1987; Ganter & Starmer, 1992; Abranches *et al.*, 2000; Pintar & Starmer, 2003). It has also has been coopted for many industrial and therapeutic applications. Killer yeast are used as starter cultures in industrial fermentation settings to exclude unwanted yeast contaminants (Salek *et al.*, 1992; Javadekar *et al.*, 1995; Kvasnikov, 1995;). They have also been proposed as natu ral food preservatives (Kono & Himeno, 1992), have shown potential as antifungals, and have been used in the biotyping of medically important pathogenic yeast (Theisen *et al.*, 2000; Buzzini & Martini, 2001; Selitrennikoff, 2001). Additionally, the killer toxin secretion system has played an important role as a successful model for enhancing our understanding of protein secretion in eukaryotic cells (Wickner, 1991; Schmitt & Breinig, 2002).

Despite the importance of the killer phenotype in both natural environments and in industrial, therapeutic, and scientific settings, little is known about how the LA virus and M vlp impact host gene expression. This knowledge gap is surprising considering the evolutionary significance of this interaction. These viruses are obligate endos ymbionts, whose reproductive success is intimately tied to that of their hosts. They have purportedly coevolved over evolutionary time with yeast, the result of which is a rather unique symbiosis (Ghabrial, 1998; Pearson *et al.*, 2009). Viruses are typically considered to inhabit the par asitic end of the parasitism mutualism continuum, but mycoviruses appear to occupy the other end of this con tinuum in the realm of commensalism or mutualism.

Understanding how their evolutionary history has shaped the impact of infection on host patterns of gene

expression is important to a thorough understanding of this interaction. Our genome wide analysis represents a much broader study of this interaction than previously performed, with the potential to reveal unexpected differ ences. It also might provide key insights toward a more general understanding of how coevolving genomes can influence each other's gene expression patterns. Examin ing genome wide expression levels yields a larger, organ ism wide view of the impact of infection.

In this study, we examine how *S. cerevisiae* global gene expression is affected by the loss of infection with the LA virus and M vlp (M1). The current interdependency of LA virus and M vlp makes the study of each in isolation a less biologically relevant situation. Instead, we look at the impact of coinfection and explicitly examine *a priori* predictions regarding host genes that are known or are speculated to play an explicit role in the maintenance and expression of the killer phenotype, in particular the main tenance of killer (MAK) and suppression of killer (SKI) genes (Table 1). In addition, we examine the expression of other candidate genes whose expression might be affected by viral infection. These candidates were identi fied by previous studies seeking genes that moderate yeast sensitivity and resistance to killer toxin, genes that mod erate the interaction between mycoviruses and pathogenic fungi, and genes that moderate the interaction between fungi and plant viruses. We show that *S. cerevisiae* global gene expression is impacted by the loss of LA virus and M vlp infection and that these shifts produce small but significant and sometimes predictable changes in genes previously known or predicted to be involved in viral interactions. Moreover, we identify new genes previously not known or thought to be involved in viral interactions, which hint at a possible mechanism whereby virus infec tion might confer immunity to killer toxin.

Materials and methods

Strains and culture

Saccharomyces cerevisiae A364AXS7 killer strain, *MATa/ MATalpha ade/+ ade2/+ ura1/+ tyr1/+ his7/+ lys2/+ gal1/ gal1 [KIL k]*, was obtained from the American Type Cul ture Collection (ATCC 48161). To cure the strain, it was plated on Yeast Peptone Dextrose (YPD) and incubated overnight at 40 °C until colony formation was observed. The absence of the killer phenotype was tested by streak ing over a lawn of sensitive yeast on YPD plates contain ing 0.1 M citrate phosphate buffer pH 4.5 and 0.01% methylene blue. Absence of viral nucleic acids was

Table 1. A priori predictions of direction of expression changes of host genes in response to the killer phenotype

Areas of possible impact	Name	Host function	Viral function	Virus Δ exp.
Antiviral system	SKI	Involved in mRNA decay, degradation and translation inhibition of non poly(A) mRNAs and in 3′ 5′ RNA processing and degradation	Required for repressing propagation of dsRNA viruses	+
Control of dsRNA replication	MAK	Topoisomerase I, nuclear enzyme that relieves torsional strain in DNA, Involved in biogenesis of large (60S) ribosomal subunits	Involved in the replication and maintenance of LA and M	+
Protein processing	KEX	Protease involved in the processing of alpha factor precursor, involved in the activation of proproteins of the secretory pathway	Protease involved in the processing of killer toxin	+
Protien secretion	SEC	Genes involved in the secretory pathway	Required for the postranslational modification of toxin	+
Cell wall integrity signaling pathway	WSC, MID, MTL etc.	Genes involved in monitoring the integrity of the cell wall	Speculative	+
Apoptosis	YCA, DNM, etc.	Genes known to play a role in programed cell death pathways in yeast	Speculative	
Inhibitor of apoptosis	BIR, CIT, POR	Genes known to inhibit programmed cell death pathways in yeast	Speculative	+

confirmed by visual inspection of nondenaturing agarose gel electrophoresis of total nucleic acids alongside positive and negative controls. The yeast strains were cultured in Yeast Nitrogen Base (YNB) media: 6.7 g YNB (MP bio medical), 20 g dextrose and 10 g succinic acid L^{-1}. The pH was adjusted at 4.7 or 6.0 with sodium hydroxide. The cultures were grown at 30 °C shaken at 225 r.p.m. until the optical density reached 0.8 at 600 nm. To har vest, cells and media were rapidly transferred to 150 mL centrifuge bottles and pelleted by centrifugation at room temperature. Supernatant was discarded, and the cell pel let was flash frozen in the centrifuge bottle by immersion in liquid nitrogen.

RNA isolation

The total RNA was isolated from 100 mL yeast culture by phenol chloroform extraction using a modified protocol previously described (Schmitt *et al.*, 1990). The frozen pellet was re suspended in 4 mL AE buffer (50 mM sodium acetate pH 5.3 and 10 mM EDTA pH 8.0) fol lowed by the addition of 400 μL 10% SDS and 4 mL of saturated phenol pH 4.3. The samples were vortexed for 10 s, incubated at 65 °C for 30 min, and vortexed every 5 min, before cooling on ice and centrifugation for 5 min at 2500 *g*. The upper aqueous phase was collected and re extracted with 4 mL phenol, followed by a last extraction with 4 mL chloroform. The samples were precipitated with 2.5 volumes of ethanol and 1/10 volume of 3 M sodium acetate pH 5.3. The pellet was washed with 70% ethanol and re suspended in TE. Poly(A)$^+$ RNA was iso lated using Oligotex mRNA kit (Qiagen), following the Oligotex mRNA spin column protocol with the exception that the RNA samples were incubated at 27 °C for 60 min instead of 10 min.

Target mRNA preparation

A RNA mix of 2 μg poly(A)$^+$ RNA and 0.5 μg custom oligo $dT_{16\text{-}22}$ (Invitrogen) in a volume of 15.5 μL was incubated at 70 °C for 10 min and cooled at 4 °C for 10 min. Before an incubation at 42 °C for 2 h, the following reagents were added: 6 μL 5× first strand buffer, 3 μL 0.1 M DTT, 0.8 μL dNTP mix (25 mM dATP, dGTP, and dCTP, 10 mM dTTP, 15 mM amino ally dUTP from Sigma), along with 3 μL DEPC water (Invitrogen) and 1 μL Superscript II reverse transcriptase (200 units μL^{-1}, Invitrogen). The RNA template was degraded with 20 μL of alkaline hydrolysis solution (0.5 M NaOH and 0.25 M EDTA pH 8.0) and incubated at 65 °C for 15 min, then neutralized with 25 μL of 1 M HEPES pH 7.5. The cDNA was purified and concen trated to a volume of 40 μL using Microcon YM 30 columns (Amicon) and coupled with CyDye Post Labeling Reactive Dye Packs (GE Healthcare) according to manufacturer's pro tocol. The labeled cDNA was then purified with a QiaQuick PCR purification kit (Qiagen).

Hybridization

Competitive hybridization was performed using a proto col modified from Townsend & Taylor (2005). Cyanine 5 (Cy5) and Cyanine 3(Cy3) and labeled cDNA were con centrated to a volume of 40 μL instead of 20 μL. Buffer preparation, probe purification, and boiling were per formed according to Townsend and Taylor (2005) before hybridization on the spotted cDNA array The array was composed of PCR amplified whole open reading frames for all yeast genes as described in (Townsend, 2003). Additionally, spots composed of PCR amplified LA virus *gag* and *pol* genes were added to the array. The hybridized cDNA arrays were incubated at 60 °C for 16 18 h. Eight hybridizations were performed in total. Two dye swaps (four hybridizations) provided the key data comparing virus infected vs. uninfected expression. Two dye swaps were performed for each combination of strains to com pare virus infected expression when the viruses are pro ducing toxin at pH 4.7 vs. virus infected expression when the viruses are not producing toxin at pH 6.0. These two biological replicates were then combined in the statistical analysis (see below).

Wash and scan

The arrays were washed with a solution containing 0.3× SSC and 0.025% SDS, rinsed with 0.5× SSC, and then scanned with an Axon 4000B scanner (Axon Instruments, Foster City, CA) using GENEPIX 4.0 software (Axon Instruments).

Analysis

The GENEPIX software package was used to perform com puter assisted manual location and circumscription of hybridized spots. Spot fluorescence intensity values were adjusted by subtracting background fluorescence from fore ground fluorescence for each spot. To eliminate signals that are most prone to estimation error, any spot was excluded from analysis if both the Cy3 and Cy5 fluorescence signals were within two standard deviations of the distribution of intensities of the background pixels for that spot. This pro cedure avoids artificially inflated measurements of expres sion due to near zero values in the denominator. Expression values were normalized by linear scaling of the Cyanine 5 values so that the mean Cy5 and Cy3 back ground corrected intensity values of nonexcluded spots were equal. Because the hybridizations were of uniformly high quality, this straightforward method yielded linear log

log Cy3 Cy5 intensity scatterplots for all hybridizations and no further manipulation of the data was necessary.

Normalized ratio data were analyzed using a Bayesian analysis of gene expression level (BAGEL; Townsend & Hartl, 2002). To estimate gene expression level across samples, BAGEL implements a Markov chain Monte Carlo integration of the likelihood of the data across expression levels and variances for each gene. Reconciling multiple cDNA microarray comparisons among multiple samples, BAGEL yielded estimates and credible intervals for gene expression level as well as *P* values for differential abundance of transcripts. A feature of this analysis is robustness to the selective absence of data for a gene due to low signal in a particular hybridization.

To assess the *post hoc* power of the experiment to detect differences of gene expression, a logistic regression of fold change magnitude as a predictor of statistical significance was performed (Townsend, 2004). The result was quantified by the GEL_{50}, the gene expression level at which there was a *post hoc* probability of 50% of calling a measured difference statistically significant. The 50% probability has been used as a measure of power in a gene expression study as it represents the gene expression level difference where an observation is as likely to be determined significant as insignificant (e.g. Johannesson *et al.*, 2006; see also Clark & Townsend, 2007). This usage is analogous to other summary statistics employing a 50% measure such as the median lethal dose (LD_{50}), where it has been viewed as the best indicator of the inflection point and as the best summary statistic for revealing where a continuous variable 'switches' among dichotomous outcomes. Lower GEL_{50} values indicate greater statistical power to detect because it reflects greater sensitivity to smaller fold changes.

To ascertain whether biological pathways were overrepresented with significantly differentially expressed genes, the term enrichment utility of the AMIGO program from the Gene Ontology project was applied (AMIGO version 1.8, amigo.geneontology.org). Up regulation and down regulation in infected vs. wild type strains were compared with the background set of genes for comparison: the 5280 genes for which the BAGEL analysis was able to calculate expression levels, which also had gene ontology annotations and which were found the in Saccharomyces Genome Database (www.yeastgenome.org). For a biological process to be included in the analysis, at least two genes had to be included at an alpha value for significance of 0.01.

Results

Most changes in host gene expression in response to the loss of viral infection were small (Fig. 2). With few exceptions, measured differences in expression all lay within a single fold change (Supporting information, Table S1). Despite the small magnitude of expression differences, the Bayesian analysis of gene expression level facilitated identification of many genes whose expression changed significantly (Fig. 2; Table S2; Townsend & Hartl, 2002). Our experimental technique and replicated experimental design were powerful enough that even changes in gene expression as small as 20% were detectable with an empirical probability of 50% (Fig. 3). This high sensitivity indicates that additional replicates in this study would provide little additional value, as additional statistically significant differences observed would be very small in magnitude (c.f. Clark & Townsend, 2007). The majority of significantly affected genes in the infection sample exhibited increased expression.

There were broad biological processes that exhibited differential expression between the wild type and infected strains. The 1408 annotated genes that were overexpressed in the infected strains yielded enrichment for a mitochondrial protein synthesis ($P = 0.0001$) and molecular catab

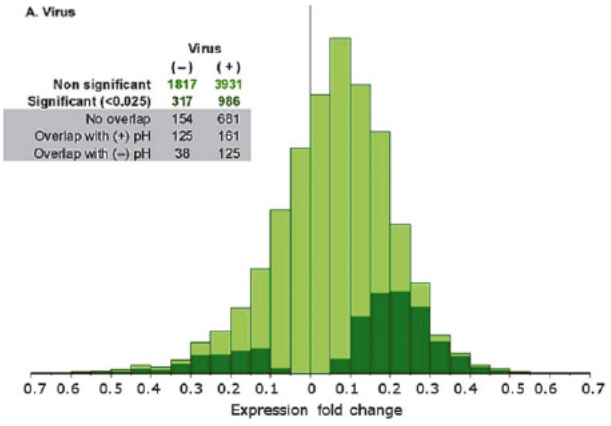

Fig. 2. Distribution of expression fold differences that result when wild type yeast is infected with the LA virus and M1 virus like particle.

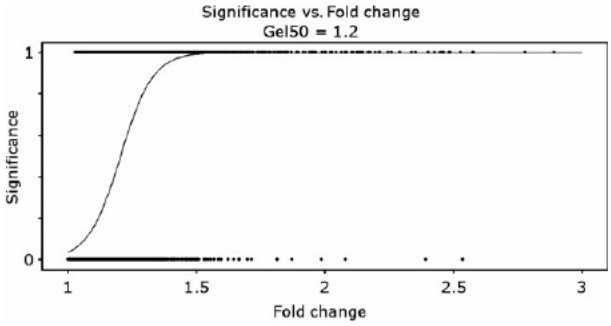

Fig. 3. GEL_{50} analysis of gene expression differences reveals a 50% chance of significance at a 20% expression difference. Twenty four data points are not shown with fold change > 3. All 24 were determined as significant.

olism ($P = 0.004$). In contrast, the 404 genes that were overexpressed in the uninfected relative to infected yeast yielded enrichment for metabolism of organic acids, including amino acid metabolism ($P = 0.008$).

Effects of LA virus and M vlp infection on *S. cerevisiae* gene expression

Host genes known to play a role in the maintenance and expression of the killer phenotype

A number of host genes are known to play a critical role in the maintenance and expression of the killer phenotype and many of these genes also play critical roles in normal host physiology (Wickner, 1991). Nevertheless, one would expect that infection would impact their expression in predictable ways. We detail these predictions in Table 1.

The maintenance of the killer phenotype is affected by two groups of host genes, the *SKI* 'Suppressor of *KIller*' genes and the *MAK* 'MAintenance of *Killer*'genes (Table 2). The *SKI* genes are part of the host antiviral system and are important in moderating viral replication (Sommer & Wickner, 1987; Matsumoto *et al.*, 1993). The products of the genes *SKI1 SKI8* repress the copy number of the M vlp, as well as repressing the translation of its mRNA, while *SKI2* plays a role in repressing the copy number of the LA virus (Ball *et al.*, 1984). The only *SKI* gene whose expres sion was significantly affected by the viral infection was *SKI6*, which exhibited increased expression and is known to be involved in repressing copy number of M vlps.

The *MAK* genes are necessary for the propagation and maintenance of the killer phenotype, as well as being important or essential for cell growth (Magliani *et al.*, 1997). Three of the more than 30 chromosomal *MAK* genes are needed for the propagation of the LA virus: *MAK3, 10,* and *PET18* (Fujimura & Wickner, 1987; Lee & Wickner, 1992; Tercero *et al.*, 1993). Many of the *MAK* genes are also responsible for the propagation of the M vlp, including: *MAK1, 7, 8, 11, 16, 18,* and *KRB1* (Wickner *et al.*, 1982; Icho & Wickner, 1988; Wickner, 1988; Schultz *et al.*, 1992; Carroll & Wickner, 1995; Ohtake & Wickner, 1995a, b). Of the three genes needed for propa gation of LA virus, only *MAK3* showed a significant increase in expression resulting from infection. Of the genes that are involved in the propagation of the M vlp, expression of *MAK7* and *MAK8* decreased significantly, and expression of *MAK16* significantly increased in expression as a consequence of infection.

The processing and secretion of the killer toxin is facil itated by *KEX* 'Killer EXpression defective' genes and *SEC* 'SECretory' genes (Table 3). *KEX1* and *KEX2* encode the proteases necessary for processing the protoxins as well as other yeast preproteins (Zhu *et al.*, 1992; Wickner, 1993). The *SEC* genes are involved in the secretion of the mature toxin, along with other proteins (Bussey *et al.*, 1983; Lolle & Bussey, 1986; Douglas *et al.*, 1988). No significant effects on the expression of *KEX* genes manifested, but a subset of *SEC* genes were significantly overexpressed (11/44) (*SEC12, 13, 15, 17, 28, 32, 34, 59, 62, 65, 66*) and two of these genes were significantly under expressed (2/44) (*SEC11, 61*).

Host genes speculated to be affected by the acquisition of the killer phenotype

In addition to the propagation, maintenance, and secre tion pathways addressed previously, there are other path ways within which we expect infection induces significant changes in expression levels. While toxin producing cells are immune to their own toxin, they still bind toxin in normal amounts (Magliani *et al.*, 1997). The toxin adsorbs primarily to the ß 1, 6 D glucan components of the cell wall (Hutchins & Bussey, 1983). After binding, the K1 toxin is transferred to the cytoplasmic membrane, where it binds to the GPI linked C terminus of KRE1 'Killer toxin REsistant' and disrupts membrane function by forming cation specific ion channels that lead to cell death. However, in infected cells, this interaction is blocked via a mechanism that has been investigated but not fully elucidated (Zhu & Bussey, 1989; Martinac *et al.*, 1990; Schmitt & Breinig, 2006). Toxin binding might spe cifically impact cell wall integrity. One gene in this path way (*WSC3* 'cell Wall integrity and Stress response Component'), whose protein product resides in the plasma membrane, was significantly overexpressed in response to infection, and two of the genes (*MKK1* 'Mitogen activated protein Kinase Kinase' and *MLP1* 'Myosin Like Protein') downstream were significantly under expressed in response to infection (Fig. 4).

A common response to viral infection in multicellular organisms is the initiation of a programmed cell death pathway. The existence of these pathways in unicellular organisms until recently has been regarded with some skepticism (Ivanovska & Hardwick, 2005). A number of recent studies, however, have provided evidence for the existence of apoptotic pathways in yeast, as well as docu menting apoptotic pathways conserved in both yeast and mammals (Madeo *et al.*, 1997, 2002, 2009; Fabrizio *et al.*, 2004; Fannjiang *et al.*, 2004; Ahn *et al.*, 2005). Formation of cation specific ion channels by the killer toxin induces death at high toxin concentrations (Magliani *et al.*, 1997). At low concentrations, however, the toxin can induce apoptotic cell death in sensitive cells while at the same time providing the host with immunity to this death stimulus (Reiter *et al.*, 2005).

Table 2. *SKI* and *MAK* host genes that are known to play a critical role in the maintenance of the killer phenotype

Name	Host function	Viral function	ORF	Virus Δ exp.
SKI1	Involved in mRNA decay	Required for repressing propagation of dsRNA viruses	YGL173C	0.07
SKI2	Involved in degradation and translation inhibition of non poly(A) mRNAs	Required for repressing propagation of dsRNA viruses	YLR398C	−0.05
SKI3	Involved in degradation and translation inhibition of non poly(A) mRNAs	Required for repressing propagation of dsRNA viruses	YPR189W	0.15
SKI4	Involved in 3′ 5′ RNA processing and degradation	Required for repressing propagation of dsRNA viruses	YNL232W	0.15
SKI6	Involved in 3′ 5′ RNA processing	Required for repressing propagation of dsRNA viruses	YGR195W	0.21*
SKI7	Cytoplasmic protein required for degrading nonstop mRNAs	Required for repressing propagation of dsRNA viruses	YOR076C	0.14
SKI8	Degradation and translation inhibition of non poly(A) mRNAs	Required for repressing propagation of dsRNA viruses	YGL213C	0.10
MAK1	Topoisomerase I, nuclear enzyme that relieves torsional strain in DNA		YOL006C	0.16
MAK3	Catalytic subunit of N terminal acetyltransferase	Required for replication of dsRNA virus	YPR051W	0.25*
MAK5	Involved in biogenesis of large (60S) ribosomal subunits	Required for maintenance of M1 dsRNA virus	YBR142W	−0.03
MAK7	Ribosomal protein L4 of the large (60S) ribosomal subunit		YHL033C	−0.18*
MAK8	Protein component of the large (60S) ribosomal subunit	Involved in the replication and maintenance of LA	YOR063W	−0.25*
MAK10	Non catalytic subunit of N terminal acetyltransferase	Required for replication of dsRNA virus	YEL053C	−0.02
MAK11	Protein involved in 60S ribosomal subunit biogenesis	Essential for cell growth and replication of M vlp	YKL021C	0.08
MAK16	Essential nuclear protein, constituent of 66S pre ribosomal particles	Required for maintenance of M1	YAL025C	0.15*
MAK18	Protein component of the large (60S) ribosomal subunit	Required for propagation of M1	YHR141C	0.05
MAK21	Required for large (60S) ribosomal subunit biogenesis	Required for maintenance of dsRNA virus	YDR060W	0.12
PET18	Non catalytic subunit of N terminal acetyltransferase	Required for replication of dsRNA virus	YCR020C	0.14
MAK32	Interspecies interaction between organisms	Protein necessary for structural stability of LA	YCR019W	0.18
KRB1	Structural conbstituent of ribosome		YLL045C	0.19

*Significant differential expression ($P < 0.025$).

In mammals, mitochondria appear to play a central role in programmed cell death. In particular, the mam malian mitochondrial fission factor Drp1 promotes exces sive organelle fission and release of cytochrome c into the cytosol, where it can activate apoptotic pathways (Frank *et al.*, 2001; Breckenridge *et al.*, 2002). This role appears conserved in worms and yeast (Fannjiang *et al.*, 2004; Jagasia *et al.*, 2005). However, the expression of the yeast homolog of *DRP1* 'Dynamin Related Protein', *DNM1* 'DyNaMin related', is unaffected by infection with LA virus and M vlp. Another apoptotic pathway conserved in yeast is the pathway mediated by the yeast metacaspase *YCA1* 'Yeast metaCAspace' (Reiter *et al.*, 2005), expres sion of which was not significantly affected as a result of infection with LA virus nor M vlp.

A few genes are known to inhibit apoptosis in yeast, but the mechanism by which infection protects the infected host from cell death at high or low concentra tions of toxin is still unknown. Tracking down genes that inhibit apoptosis and whose expression is affected by viral infection moves us toward tracking down the mechanism by which infection provides immunity. *BIR1* 'Baculoviral IAP Repeat containing protein' is the most well known of these genes and is able to protect yeast cells against cell death by interfering with the ability of caspases to interact with their substrates (Riedl & Shi, 2004). Its expression was unaffected by infection with LA virus or M vlp. *CIT1* 'CITrate synthase' catalyzes the first step in the tricarbox ylic cycle, the condensation of acetyl CoA and oxaloace tate to form citrate (Owsianowski *et al.*, 2008), but also

Table 3. *KEX* and *SEC* host genes that are known to play a critical role in the processing and secretion of the killer toxin

Name	Host function	ORF	Virus Δ exp.
KEX1	Protease involved in the processing of alpha factor precursor	YGL203C	0.07
KEX2	Involved in the activation of proproteins of the secretory pathway	YNL238W	−0.12
SEC61	Allows SRP independent protein import into ER	YLR378C	−0.33*
SEC62	Allows SRP independent protein import into ER	YPL094C	0.22*
SEC63	Allows SRP independent protein import into ER	YOR254C	0.22
SEC66	Allows SRP independent protein import into ER	YBR171W	0.28*
SEC72	Allows SRP independent protein import into ER	YLR292C	0.075
SEC9	Important for fusion of secretory vesicles with the plasma membrane	YGR009C	0.14
SEC13	Important for the formation of ER to Golgi transport vesicles	YLR208W	0.15*
SEC16	Important for the formation of ER to Golgi transport vesicles	YPL085W	−0.16
SEC23	Important for the formation of ER to Golgi transport vesicles	YPR181C	0.053
SEC24	Important for the formation of ER to Golgi transport vesicles	YIL109C	−0.027
SEC31	Important for the formation of ER to Golgi transport vesicles	YDL195W	−0.12
SEC12	Important for the initiation of transport vesicle budding	YNR026C	0.11*
SEC22	Involved in ER to Golgi and Golgi to ER transport	YLR268W	−0.02
SEC1	Involved in docking and fusion of exocytic vesicles	YDR164C	0.18
SEC21	Involved in ER to Golgi and Golgi to ER transport	YNL287W	0.16
SEC27	Involved in ER to Golgi and Golgi to ER transport	YGL137W	0.04
SEC26	Involved in ER to Golgi and Golgi to ER transport	YDR238C	0.12
SEC7	Involved in proliferation of the Golgi, intra and ER Golgi transport	YDR170C	0.08
SEC65	Involved in protein targeting to the ER	YML105C	0.24*
SEC14	Involved in regulating intracellular transport	YMR079W	−0.07
SEC20	Involved in ER to Golgi and Golgi to ER transport	YDR498C	−0.02
SEC34	Mediates fusion of transport vesicles to Golgi compartments	YER157W	0.27*
SEC35	Mediates fusion of transport vesicles to Golgi compartments	YGR120C	0.18
SEC36	Mediates fusion of transport vesicles to Golgi compartments	YGL223C	0.07
SEC37	Mediates fusion of transport vesicles to Golgi compartments	YNL041C	0.17
SEC38	Mediates fusion of transport vesicles to Golgi compartments	YPR105C	0.18
SEC32	Involved in ER to Golgi and Golgi to ER transport	YLR078C	0.23*
SEC39	Proposed to be involved in protein secretion	YLR440C	−0.01
SEC28	Involved in ER to Golgi and Golgi to ER transport	YIL076W	0.21*
SEC19	Regulates vesicle traffic in secretory pathways	YER136W	0.09
SEC18	Involved in ER to Golgi and Golgi to ER transport	YBR080C	0.12
SEC53	Required for folding and glycosylation of secretory proteins in the ER	YFL045C	−0.10
SEC17	Involved in ER to Golgi and Golgi to ER transport	YBL050W	0.18*
SEC59	Required for viability and for normal rates of lipid intermediate synthesis	YMR013C	0.23*
SEC11	Subunit of the Signal Peptidase Complex	YIR022W	−0.18*
SEC33	Surrounds transport vesicles in the early secretory pathway	YDL145C	0.10
SEC2	Essential for post Golgi vesicle transport	YNL272C	0.04
SEC4	Essential subunit of the exocyst complex	YER008C	−0.03
SEC15	Essential subunit of the exocyst complex	YFL005W	0.22*
SEC3	Essential subunit of the exocyst complex	YDR166C	−0.02
SEC5	Essential subunit of the exocyst complex	YIL068C	0.08
SEC6	Essential subunit of the exocyst complex	YPR055W	0.12
SEC8	Essential subunit of the exocyst complex	YLR166C	0.06
SEC10	Essential subunit of the exocyst complex	YGL233W	0.09

*Significant differential expression ($P < 0.025$).

plays a role in inhibiting apoptosis via its impact on ROS levels (Kispal *et al.*, 1988). *CIT1* displays significantly increased levels of expression upon infection. Another gene that has been shown to protect against apoptosis is *POR1* 'PORin', which exhibits increased expression and encodes a voltage dependent anion channel that functions to mitigate damage by acetic acid, H_2O_2, and diamide (Pereira *et al.*, 2007).

Genes known to moderate yeast sensitivity and resistance to killer toxin

A genome wide mutant screen for altered sensitivity to killer toxin in *S. cerevisiae* revealed numerous genes (186 genes) whose mutants showed increased resistance to killer toxin, as well as many genes (82 genes) whose mutants showed hypersensitivity to killer toxin (Page *et al.*, 2003).

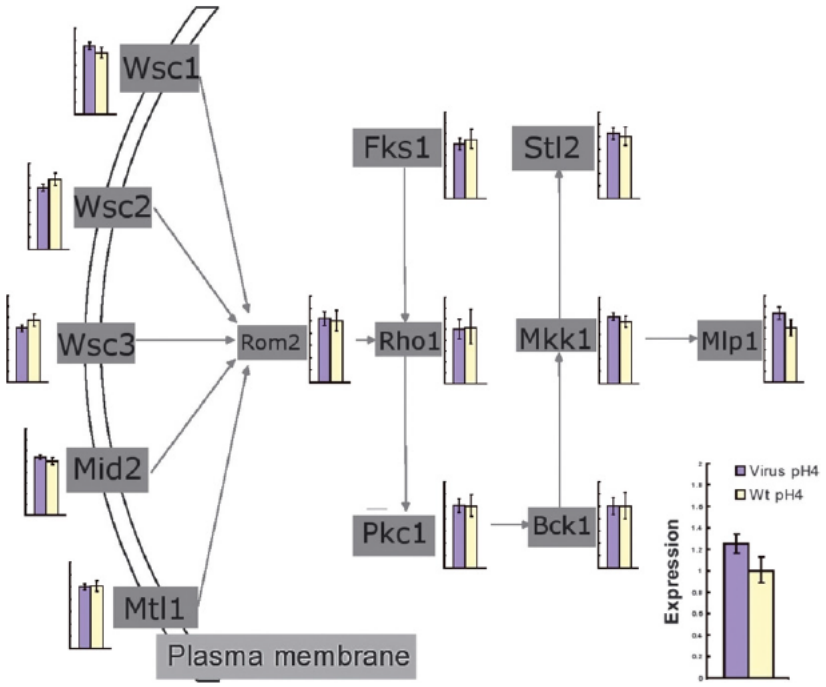

Fig. 4. Diagram of the cell wall integrity signaling pathway. Modified from Levin (2005). Gene names are in gray boxes. Bar graphs next to the gene names depict expression levels in wild type and virus infected cells.

Of the 186 genes implicated in increased toxin resistance, 21 experienced significant expression changes resulting from infection. There were 13 genes whose expression lev els increased, and eight whose expression levels decreased. The functional group of genes experiencing the largest frac tion (six, all increased expression) of these changes were genes involved in mitochondrial, respiratory and ATP metabolism (Table 4; c.f. Page *et al.*, 2003).

Of the 82 genes implicated in increased sensitivity to toxin, 15 experienced significant expression changes resulting from infection. Ten of these genes experienced increased expression, and five genes experienced decreased expression. The bulk of these significantly affected genes were ribosomal and translation initiation proteins (Table 5; c.f. Page *et al.*, 2003).

Genes known to moderate the interaction between mycoviruses and pathogenic fungi

A number of candidate genes could putatively be affected by viral infection. Cellular responses to stress classically include elevated expression of genes coding for Heat Shock Proteins (HSP*s*) and Glutathione S Transferases (GSTs). While HSPs are known to play a large role in protection from thermal stress, they are also known to be regulated by several animal and plant viruses (Aranda *et al.*, 1996; Glot zer *et al.*, 2000; Whitham *et al.*, 2003). Furthermore, while GSTs are known to play a role in the removal of reactive oxygen species via conjugation of glutathione with harmful ligands (Sheehan *et al.*, 2001), their expression might also represent a countermeasure to cellular apoptosis mediated defense responses (Tschopp *et al.*, 1998).

Previous studies examining the impact of hypovirus infection on the chestnut blight fungus, *Cryphonectria parasitica*, (Allen *et al.*, 2003; Allen & Nuss, 2004) revealed increased expression of homologs to *HSP70* and *GST* 'Glutathione S Transferase', suggesting that hypovirus infection induces these genes to facilitate viral functions. Interestingly, we find that a subset of HSP genes, i.e. *HSP42* and *HSP78*, are significantly overexpressed in response to viral infection, with the remainder of HSP genes not signif icantly affected. In contrast to the effect observed in *C. parasitica* in response to hypovirus infection, our assays indicated that all three GST genes, *GTT1*, *GTT2*, and *GTT3* in *S. cerevisiae*, decreased in expression (GTT is the accepted abbreviation for GST genes in yeast). Only one of them, *GTT3*, was statistically significantly down regulated.

Allen *et al.* (2003) also revealed increased expression of S adenosyl L methionine synthetase and S adenosyl L homocysteine after hypovirus infection of *C. parasitica*, assessed via hybridization on cDNA microarrays and vali dation by real time RT PCR. The increased expression of S adenosyl L methionine might impact a number of metabolic and physiologic processes ranging from protein synthesis to membrane integrity (Allen *et al.*, 2003). The methionine metabolism pathway in *S. cerevisiae* responded

Table 4. Differentially expressed genes known to impact resistance to killer toxin

Description of gene product	Host function	ORF	Virus Δ exp.
Kinases, phosphatases, signal transduction	Cyclin dependent protein kinase	YPL031C	−0.05
	Guanine nucleotide binding regulatory protein	YER020W	−0.03
	Phosphotyrosyl phosphatase activator	YIL153W	−0.06
	Regulatory subunit for ser/thr phosphatase Glc7p	YMR311C	0.13
	Inorganic phosphate transporter	YJL117W	0.09*
	Phosphoprotein phosphatase	YPL179W	−0.16*
Transcription	Subunit of RNA Pol II elongator complex	YPL101W	−0.01
	CTD kinase, alpha subunit; RNA Pol II regulation	YKL139W	0.10
	Silencing regulatory protein	YKR101W	−0.12*
	Subunit of RNA Pol II elongator complex	YMR312W	0.12*
	TFIIF subunit (transcription initiation factor), 30 kD	YPL129W	0.28*
Actin organization	Subunit of the Arp2/3 complex	YLR370C	0.11
	Component of actin cortical patches	YOR181W	−0.08
Lipid/sterol synthesis	C 8 sterol isomerase	YER044C	−0.01
	IP5 kinase	YGL012W	−0.22*
	Similarity to human oxysterol binding protein (OSBP)	YLR372W	−0.08
Secretion/endocytosis	Suppressor of actin mutation, involved in vesicular transport	YAL042W	−0.24*
	Vesicular transport between Golgi and ER	YDR372C	0.17*
	Complex involved in vesicle transport to the Golgi	YLR262C	0.21*
	Vacuolar sorting protein	YLR148W	0.30*
	Required for endocytosis and cytoskeletal organization	YGL027C	0.04
Protein glycosylation	ER glucosidase I	YEL042W	−0.13*
	Glucosyltransferase	YOR067C	−0.02
Protein modification	Vacuolar protein sorting associated protein	YDR349C	0.07
Cell wall organization	cis Golgi glucanase like protein	YLR342W	−0.09
	Similarity to budding protein Sbe2p	YGR279C	−0.22*
Mitochondrial, respiratory, and ATP metabolism	Possibly involved in mitochondrial DNA maintenance	YJR121W	−0.18
	F1FO ATPase complex, F, 3 subunit	YCR071C	0.22*
	Required for integrity of mitochodrial genome	YLR382C	0.30*
	Leucine tRNA ligase precursor, mitochondrial	YKL134C	−0.01
	May be involved in mitochondrial function	YJL063C	0.07
	Ribosomal protein L17, mitochondrial	YJR144W	0.21*
	Mitochondrial genome maintenance protein	YKL170W	−0.01
	Required for mitochondrial shape and structure	YPR067W	0.18*
	Mitochondrial protein required for iron metabolism	YBR282W	0.31*
	Ribosomal protein YmL27 precursor, mitochondrial	YCR046C	−0.05
	Ribosomal protein, mitochondrial	YPL271W	0.07
	FIFO ATPase complex, F, epsilon subunit	YDR375C	0.16*
Ungrouped genes	P type calcium ATPase	YDR432W	−0.19*
	L threonine aldolase, low specificity	YAL020C	−0.12*
	Member of the nontransporter group of the ABC superfamily	YMR038C	0.03
Ungrouped or poorly characterized genes	Similarity to hypothetical *Arabidopsis thaliana* proteins	YLR270W	0.20*
	Hypothetical protein	YDR126W	0.08

*Significant differential expression ($P < 0.025$).

to viral infection (Fig. 5). There were nine genes in total affected by infection in this pathway. Seven of them experi enced increased expression, and two experienced decreased expression in response to viral infection.

Genes known to moderate the interaction between fungi and plant viruses

Saccharomyces cerevisiae has been increasingly used as a model to study the host virus interactions of plant viruses (Nagy, 2008). Many plant viruses, such as the Brome mosaic virus (BMV), the Tomato bushy stunt virus (TBSV), the Carnation Italian Ringspot virus, and others can complete most of the steps required for replication in yeast cells (Ishikawa *et al.*, 1997; Pantaleo *et al.*, 2003; Panavas *et al.*, 2005a). Genome wide screens implicate several genes that might be important to general host virus interactions (Noueiry & Ahlquist, 2003; Panavas *et al.*, 2005b). Noueiry & Ahlquist (2003) found 98 genes whose absence inhibited or stimulated BMV RNA replication

Table 5. Differentially expressed genes known to impact sensitivity to killer toxin

Description of gene product	Host function	ORF	Virus Δ exp.
Kinases, phosphatases, signal transduction	Ser/thr protein kinase of MAPK family	YLR113W	0.18*
	Ser/thr protein phosphatase 2A, regulatory chain A	YAL016W	0.21*
Transcription	RNA Pol II holoenzyme (SRB) subcomplex subunit	YHR041C	−0.29*
RNA processing	Required for mRNA splicing	YDR364C	0.32*
	Nuclear localization sequence binding protein	YGR159C	−0.01
	Involved in snRNP biogenesis	YPR057W	0.35*
	Large subunit of the nuclear cap binding protein complex CBC	YMR125W	0.30*
Ribosomal and translation initiation proteins	Ribosomal protein S16.e	YDL083C	−0.02
	40S small subunit ribosomal protein S19.e	YNL302C	0.08
	Ribosomal protein S10.e	YOR293W	0.09
	40S small subunit ribosomal protein	YMR116C	−0.34*
	Ribosomal protein S17.e.A	YML024W	−0.04
	Ribosomal protein S11.e	YDR025W	−0.07
	40S small subunit ribosomal protein S23.e	YPR132W	−0.42*
	Ribosomal protein S16.e	YMR143W	−0.02
	40S ribosomal protein p40 homolog B	YLR048W	−0.17*
	Similarity to human ubiquitin like protein/ribosomal protein S30	YOR182C	0.13*
	60S large subunit ribosomal protein L8.e	YIL018W	−0.25*
	Ribosomal protein	YKL006W	−0.06
N glycosylation	Required for complex N glycosylation	YPL050C	0.29*
New FYV genes	Kl toxin hypersensitivity phenotype	YNL133C	−0.11
	Kl toxin hypersensitivity phenotype	YLR068W	−0.001
	Kl toxin hypersensitivity phenotype	YOR183W	−0.03
Ungrouped or poorly characterized genes	Lipid and sterol metabolism	YLR242C	0.13*
	ER protein translocation complex subunit	YBR171W	0.28*
	Member of the major facilitator superfamily	YIL047C	0.06
	Gamma adaptin of clathrin associated AP 1 complex	YPR029C	0.20*

*Significant differential expression ($P < 0.025$).

and/gene expression. Of these genes, 16 were also signifi cantly affected by *LA* virus and *M* vlp infection; 15 expe rienced increased levels of expression, and one experienced decreased levels of expression (Table 6). Pan avas *et al.* (2005b) identified 96 host genes whose absence either reduced or increased the accumulation of the TBSV replicon. Of these genes, 15 were also significantly affected by *LA* virus and *M* vlp infection; 12 experienced increased levels of expression and three experienced decreased levels of expression (Table 7).

Discussion

We have demonstrated that the loss of coinfection of the yeast *Saccharomyces cerevisiae* by LA virus and the M virus like protein results in minimal change to host gene expression patterns. This minimal change was unexpected and might reflect the degree to which these two genomes are integrated and coadapted to function simultaneously and cooperatively. Alternatively, or perhaps in comple ment, this minimal change might also indicate that many of the host genes known to play a critical role in the maintenance and expression of the killer phenotype also play critical roles in normal host physiology. Lastly, this minimal change could reflect that yeast response to infec tion occurs via post transcriptional mechanisms. While the mRNA levels measured by our study are highly sug gestive, they are not exact indicators of biological activity. Post translational regulation of genes is very common. For example, protein products of genes are commonly regulated allosterically by metabolic products that are environmentally dependent. These gene environment interactions can be significant and merit consideration in addition to gene expression data (Hodgins Davis & Townsend, 2009).

Changes that were detected were not predicted by pre vious mycovirus infection studies. The LA virus is a yeast virus that is rarely found extracellularly (Elsherbeini & Bostian, 1987; Wickner *et al.,* 2008). Fungal viruses, whose reproductive success is intimately aligned with that of their host, should experience strong selection to mini mize deleterious effects. The implication of this coevolu tion is that the impact of infection on host global gene expression should be small to minimize disruption of highly evolved functionality. This hypothesis of coevolu tion is supported by the body of evidence demonstrating

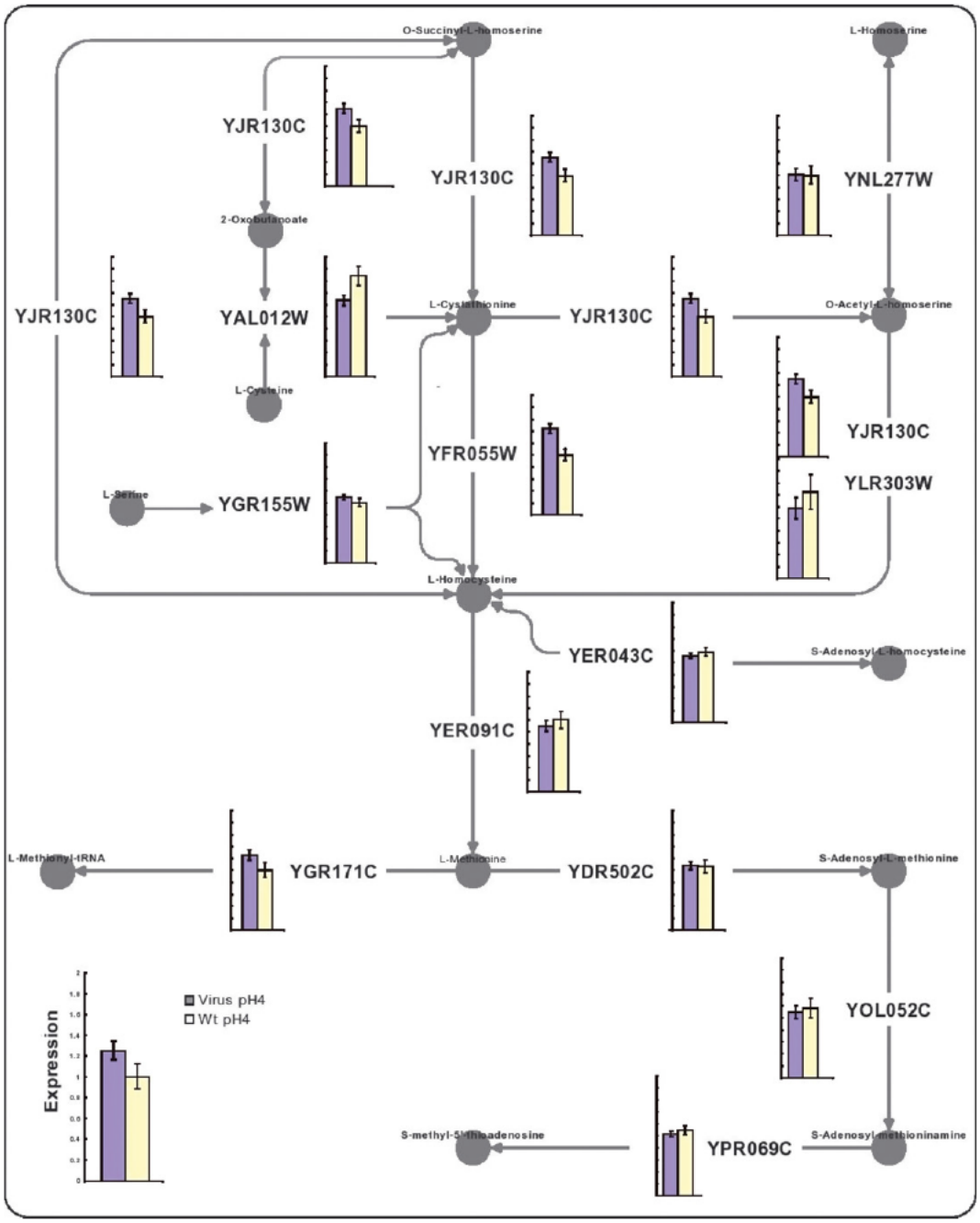

Fig. 5. Methionine metabolism pathway. Gene names are in bold, and substrate names are above gray circles. Bar graphs next to the gene names depict expression levels in wild type and virus infected cells.

few deleterious effects associated with fungal viral infec tions (Ghabrial, 1998). In this study, we provide general evidence that further supports this hypothesis.

Analysis of the gene ontology terms of genes that were differentially expressed in the two strains suggested that protein metabolism was up regulated. Macromolecular catabolism and mitochondrial protein translation func tion were enriched in the set of genes overexpressed in the infected strain. There might be a metabolic tradeoff with these processes. While macromolecular catabolism was up regulated, organic acid metabolism was enriched in the genes that were down regulated. This included amino acid, carboxylic acid, and oxoacid metabolism. These opposing metabolic shifts warrant further study and could point at general metabolic reactions to infection.

Table 6. Genes important in mediating host plant BMV interactions that are also important in mediating host virus interactions

Host function	ORF	Virus Δ exp.
Transcription elongation	YGR063C	0.26*
Proteasome activator	YBR173C	0.06
Glutathione synthesis	YJL101C	0.16*
Glutathione synthesis	YJL101C	0.16*
Nitrogen utilization	YNL229C	0.14*
Ubiquitin metabolism	YKL213C	0.15*
RNA polymerase I subunit	YJL148W	0.11*
RNA cap binding protein	YMR125W	0.30*
Ribose phosphate diphosphokinase	YHL011C	−0.04
Ubiquitin ligase	YGR184C	0.08
205 proteasome core α subunit	YGR135W	0.38*
Methionine biosynthesis	YGL125W	0.10*
Hypothetical ORF/unknown function	YNL056W	0.15*
Proteasome and cell cycle regulation	YDL020C	0.47*
Hypothetical ORF/unknown function	YGR001C	0.18*
Chromosome segregation	YPR046W	0.25*
Myoinositol metabolism	YER120W	−0.30*
Hypothetical ORF/unknown function	YML010W A	−0.06
mRNA catabolism/splicing	YDR378C	0.08
Protein tyrosine phosphatase activity	YNL099C	0.26*
Nuclear membrane/ER morphology	YHR004C	0.28*
Unknown function	YHR207C	0.17*
Chromatin modeling	YLR357W	0.28*
Transcription elongation	YPL101W	−0.01
Unknown function	YML017W	0.11*
Pol II transcription elongation	YMR312W	0.12*

*Significant differential expression ($P < 0.025$).

In addition to broad biological trends, quantitative study of gene expression differences showed that shifts in expression were slight but still significant. With regard to the yeast antiviral system (*SKI*), our expectation was that these genes should be significantly up regulated upon being infected with the virus. Only one of these genes was significantly overexpressed, suggesting that these genes are constitutively expressed in accord with their ordinary function, without modification due to a nearly ubiquitous distribution of mycoviruses in natural yeast populations. Alternatively, these genes could also play important roles in other cellular functions. The lone gene that was significantly overexpressed, *SKI6*, was found in the gene ontology analysis to be a part of a whole set of overexpressed genes generally associated with macromolecular catabolism. SKI6 up regulation might be partially or wholly an indirect result of infection as opposed to a direct response. SKI6 is a key subunit of the exosome and is essential to eukaryotic RNA processing (Mitchell *et al.*, 1997). While mutations in SKI6 do lead to accumulation of viral RNA, up regulation of the gene here could be indicative of the general up regulation of RNA processing as opposed to a specific antiviral function.

The genes responsible for maintaining the killer phenotype were also expected to be up regulated upon viral infection. The low number of genes whose expression was significantly affected by infection might again reflect the ubiquity of mycovirues or the fact that while these genes are required for viral maintenance. They also play other important cellular roles such as maintaining mitochondria.

The expression and secretion genes we looked at displayed a similar pattern to the *SKI* and *MAK* genes where only a small subset of the overall group of genes significantly changed expression levels. Both of these processes are involved not just with the processing and secretion of killer toxins, but other yeast proteins, too. The small numbers might reflect a subset that is more targeted toward killer toxin release than toward more general protein modification and secretion.

The cell wall integrity signaling pathway was not significantly impacted by infection, which includes exposure to toxin. The mechanism of immunity to high toxin concentrations might occur prior to the toxin interacting with the cell membrane. The toxin first binds to the cell wall and then is transferred to the cell membrane where it forms ion channels that result in cell death. If immunity prevents the toxin from interacting with the cell membrane, we would not expect to see a significant impact of exposure to toxin on the cell wall integrity signaling pathway.

Interestingly, a number of the genes that had previously been shown to be important in determining the sensitivity of uninfected yeast to killer toxin were also impacted by infection. These genes were identified in cells that were not infected by LA virus or M vlp, but were reacting to the K1 killer toxin encoded by the M1 vlp (Page *et al.*, 2003). They reflect a purely cellular response to toxin and provide clues to genes that interfere with toxin toxicity. This provides another suggested avenue for a mechanism of immunity.

We observed considerable overlap between the set of genes that moderate the interaction of hypoviruses and plant viruses with their hosts, and genes that moderate the interaction between the LA virus and M vlp and *S. cerevisiae*. This overlap suggests that despite the distant relatedness of these hosts, they potentially share fundamentally similar mechanisms for interacting with viruses. Interestingly, as with host response to hypovirus, we see a small subset of *HSP's* recruited. This small number indicates the absence of a general stress response and supports the contention that these genes play a role in facilitating viral functions.

Of the yeast genes homologous to those important for plant virus replication, we found that few of these genes significantly affecting gene expression overlapped with the

Table 7. Genes important in mediating host plant TBSV interactions that are also important in mediating host virus interactions

Description of gene product	Host function	ORF	Virus Δ exp.
Group 1: protein biosynthesis	Protein biosynthesis	YCR003W	0.31*
	Protein biosynthesis	YJL136C	0.05
Group 2: protein metabolism, posttranslation modification	Ubiquitin protein ligase	YDL074C	0.25*
	Protein amino acid acetylation	YPR051W	0.25*
	Uroporphyrin methyltransferase	YKR069W	0.28*
Group 3: RNA metabolism	mRNA binding	YDR432W	−0.19*
	RNA binding rRNA processing	YHR091C	0.14
Group 4: lipid metabolism	Sterol reductase	YGL012W	−0.22*
Group 5: vesicle mediated transport	Vesicle mediated transport	YNR051C	0.14*
	Transporter/membrane associated	YOR306C	0.09*
	Transporter/vacuolar membrane associated	YLR148W	0.30*
	t SNARE, v SNARE/nonselective vesicle fusion	YOL018C	0.25*
Group 6: protein vacuolar targeting	Protein vacuolar targeting	YGL124C	0.25*
	Protein vacuolar targeting	YPR087W	0.04
Group 7: membrane associated	ATPase/mitochondrial translocation	YGR028W	0.21
	Oligopeptide transporter	YJL212C	−0.12
	Potassium channel	YJL093C	−0.09
Group 8: stress related	Aldehyde reductase	YHR104W	−0.04
	Glutathione transferase	YIR038C	−0.11
	Ras GTPase activator	YOL081W	−0.04
	Glutamate catabolism	YBR006W	0.02
	Phosphatase activator	YNL197C	−0.04
Group 9: general metabolism	Cell wall organization and biogenesis	YER124C	0.34*
	Hydroxyacylglutathione hydrolase	YDR272W	0.15*
	Pseudohyphal growth	YKL043W	0.09
	S adenosylmethionine methyltransferase	YIL064W	−0.10*
Group 12: function unknown	Unknown	YDR275W	0.28*
	Unknown	YHR029C	−0.03
	Unknown	YIL090W	0.03

*Significant differential expression ($P < 0.025$).

BMV and TBSV studies (Fig. 6). There are only four genes in common between the BMV and TBSV studies. This overlap might be small because BMV and TBSV rep licate in association with cellular membranes of different intracellular compartments. BMV is a positive sense single stranded RNA (ssRNA) virus whose genome is rep licated within virus induced invaginations of the outer perinuclear ER membrane (Schwartz *et al.*, 2002). TBSV is also a positive sense ssRNA virus, but its genome repli cation is associated with membranes of the peroxisome (Panavas *et al.*, 2005b). Because LA virus has a dsRNA genome, little to no homology with the two ssRNA viruses is expected, and is consistent with the minimal gene overlap observed in our study.

Nevertheless, the insight that might be gained from gene expression studies into fungus virus interactions has significant implications for agriculture since many fungal viruses have the ability to affect interactions between fungi and plants. These plant fungus interactions pro foundly impact many natural ecosystems and crops. (Nuss, 2005). Fungal viruses have been used to varied effect in the context of combating plant pathogens such as the chestnut blight fungus *C. parasitica* (Heiniger &

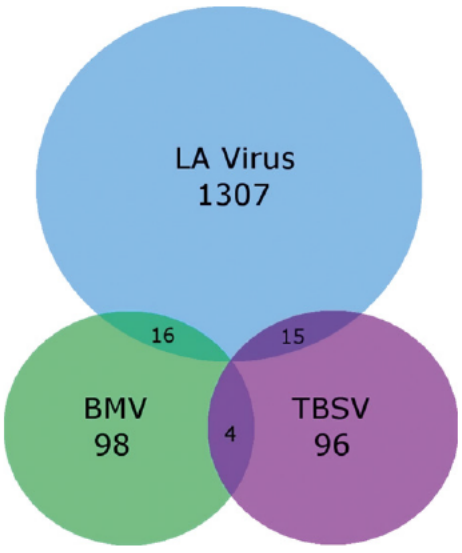

Fig. 6. Overlap of genes that were significantly impacted by infection of either the LA, BMV or TBSV viruses.

Rigling, 1994). A more complex level of interaction can be seen in the three way symbiosis among the panic grass *Dichanthelium lanuginosum,* the endophytic fungus *Curvularia protuberate,* and a fungal virus. The ability of the fungus to convey a thermo tolerant phenotype to its host plant was dependent on its infection by the virus (Marquez *et al.*, 2007). More subtle beneficial effects occur with other species and could underlie the wide spread occurrence and persistence of fungal viruses in many hosts (Pearson *et al.*, 2009).

The extent and complexity of these interactions has been recognized as providing great potential for the develop ment of fungal viruses as biocontrol agents against plant pathogens (Pearson *et al.*, 2009). However, this develop ment is dependent on understanding host virus interac tions more thoroughly. Specifically, genomic assays hold the potential to reveal key host virus interactions. Such studies gain more power when viewed in conjunction with strong molecular biological literature. They can lead to more powerful generalizations and facilitate the use of diverse fungal viruses and their hosts as biocontrol agents.

An understanding of the fundamental steps of virus life cycles can also lead insight into pathogenic viruses that infect humans and other animals. Accurate knowledge of these virus host interactions is essential for the design of novel effective antiviral strategies (Galao *et al.*, 2007). Viral effects on host cells tend to be negative, exerting selection pressure for co evolutionary arms races where cells evolve improved antiviral strategies and viruses respond by evolv ing better means of overcoming these defenses to usurp the translational machinery of the cell. However, in fungi the selective environment could be different, driving the evolu tion of mutualistic or commensal interactions between viruses and cells. Comparing and contrasting the effects of viruses on gene expression in fungal vs. non fungal hosts will illuminate how evolution transforms antagonistic rela tionships into cooperative ones.

To this end, reconstruction of phylogenies of both viruses and their hosts could resolve questions about the exact coevolutionary dynamics. One open question is the relative frequencies of horizontal (via mating) and vertical (via symmetric division) transmission of LA virus in yeast. Prior work has already demonstrated the feasibility of cophylogenetic reconstruction in RNA viruses with their hosts (Jackson & Charleston, 2004). It would also be interesting to estimate cophylogenies for yeast strains and the M vlp and LA virus individually. The majority of studies have looked at the combined interaction of the M vlp and LA virus since they are most biologically relevant when acting together. However, it is conceivable that their combined interaction is fairly recent in origin, so the M vlp and LA virus could have unique, separate his tories. Study of this coevolutionary process might inform the development of medical therapies which usefully reca pitulate benign interactions between viruses and cells molded by millions of years of evolution.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Normalized ratios of relative gene expression obtained from each two color microarray (columns). Zeros represent a relative expression that was not well measured.

Table S2. Relative gene expression across samples, confi dence intervals, and P values for differential expression from the Bayesian analysis of gene expression levels.